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

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

Introduction

1. The Committee on Toxicity (COT) has been asked to consider 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 soya phytoestrogens as a substantial amount of new evidence has become available.

2. A discussion paper on soya phytoestrogens (TOX/2012/39) was presented to Members in December 2012. Members agreed that further details on animal and epidemiological studies and data on differences in metabolism, including species and developmental differences, were needed. These points were addressed in a first draft statement (TOX/2013/11) in March 2013. The minutes of the discussion are included in Annex A.

3. The second draft statement in Annex B has been revised taking into account the previous discussions and incorporating details requested by Members in March. A table comparing receptor-binding potency of isoflavones in different species and further details on tabulated studies as well as results of an additional search for human studies have been included. Input from Members, who reviewed the literature and advised on the wording of relevant sections, has also been incorporated. Additional editorial changes have also been made.

Questions on which the views of the Committee are sought

4. Members are invited to comment on the text of the second draft statement.

Secretariat
September 2013

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

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Section of the minutes of the COT meeting of 26 March 2013

Item 6: First draft statement on the potential risks from high levels of soya phytoestrogens and soya products in the infant diet – TOX/2013/11

44. No interests were declared.

45. Soya phytoestrogens and soya products in the infant diet had been discussed at the meeting in December 2012. A first draft statement on potential risks from high levels of soya phytoestrogens and soya products in the infant diet (TOX/2013/11) had subsequently been prepared, incorporating details of epidemiological studies, discussion of differences in metabolism by age and between species, clarification of the term “absorption”, and tabulation of key data.

46. Members were content with the overall structure of the draft statement, but suggested addition of a table comparing receptor-binding potency of isoflavones in different species, and further details on tabulated studies. Members offered to review the relevant literature and advise on the wording of sections concerning the metabolism, absorption and bioavailability of isoflavones, animal studies and epidemiological studies. Relevant publications would be made available to them.

47. It was noted that soya-based infant formula and soya-based weaning foods were the main sources of isoflavone exposure in infants, and that it was plausible that exposures were at levels that could produce biological effects, although it was unclear whether these would be considered adverse. There was agreement that the priority was to address the toxicology of soya isoflavones based on available human studies – an additional search would be performed and relevant findings included in the next draft.

48. Members agreed that it was not possible to propose health-based guidance values for infants. Reasons for this included the difficulty in extrapolation from animals to humans because of differences in toxicokinetics, uncertainty with respect to differences between adults and infants (particularly those arising from development of the gut microflora), and the lack of dose-response data and the role of possible confounders in the available human studies. It was noted that the pig was the best animal model with respect to toxicokinetics, but no toxicity data were available for pigs.

49. These points would be addressed in a further draft of the statement for discussion at a future meeting.

These minutes are available at:
http://cot.food.gov.uk/pdfs/cotmins26mars13.pdf

Secretariat
September 2013

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

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

Background

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that bears on the Government's dietary recommendations for infants and young children. The review will identify new evidence that has emerged since the Government's current recommendations were formulated, and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years, but will be considered in two stages, focussing first on infants aged 0 – 12 months, and then on advice for children aged 1 to 5 years. SACN is examining the nutritional basis of the advice, and has asked that evidence on possible adverse effects of diet should be considered by other advisory committees with relevant expertise. In particular, SACN asked the Committee on Toxicity (COT) to review the risks of toxicity from chemicals in the infant diet.

2. This statement provides an overview of the potential risks from soya phytoestrogens and soya products in the infant diet. Soya-based infant formula and weaning food products containing soya are the main source of phytoestrogen exposure in newborns and infants. Soya formula is produced using soya protein isolate - a particularly rich source of three isoflavones: genistein, daidzein and to a lesser extent glycitein. It has been estimated that infants' daily intake of isoflavones from soya-based infant formula is approximately 4 mg/kg body weight/day (COT, 2003)[1].

3. The most recent evaluation of potential risks from soya-based formula conducted by the COT was published in 2003. The current statement summarises new literature concerning possible health effects from exposure of infants to soya isoflavones that has become available since the COT report on the topic in 2003, and considers the implications for introduction of soya products into the infant diet. Adopted search criteria are listed in Appendix A. The statement focuses on the exposure to the main phytoestrogens detected in soya, genistein, daidzein and glycitein, in humans and animals, with particular emphasis on pigs and non-human primates as the most relevant species. A number of studies performed in rodents are also included.

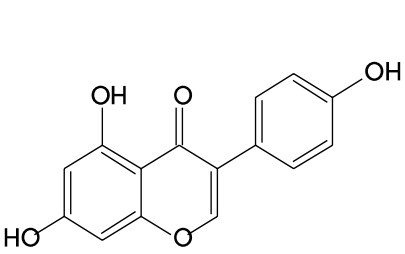
[1] COT Report – Phytoestrogens and Health (2003). Available at: http://cot.food.gov.uk/pdfs/phytoreport0503

Introduction

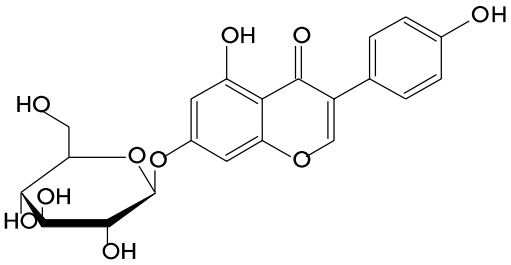
4. Phytoestrogens are chemicals of plant origin that have been shown to influence biological functions mainly through their structural similarities to oestrogens, and ability to bind to oestrogen receptors (ERs). The largest group of phytoestrogens are flavonoids, which can be further divided into three subclasses, coumestans, prenylated flavonoids and isoflavones.

5. The isoflavones, genistein, daidzein and glycitein (Figure 1) share a common structure with 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 soya isoflavone (NTP, 2010).

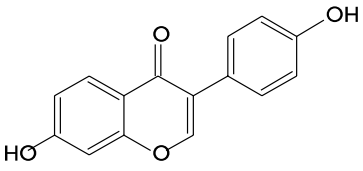
6. Soya isoflavones found in foods exist mainly as carbohydrate conjugates (glycosides), the major group being the glucose conjugates (glucosides), genistin (Figure 1), daidzin and glycitin. When β-glucosidic bonds of glucosides are hydrolysed, the biologically active aglucone forms are produced. Bacterial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



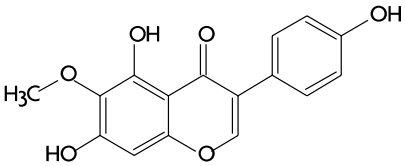
Genistein



Genistin (genistein glucoside)



Daidzein

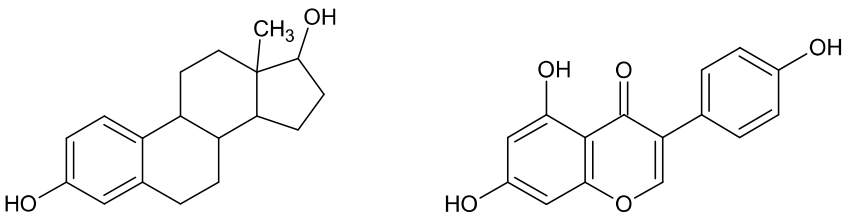


Glycitein

Figure 1. Chemical structures of the isoflavone aglucones genistein, daidzein and glycitein, and the glucoside genistin.

[2] Tofu, also called bean curd, is made by coagulation of soya juice and then precipitation of soya curd into blocks.

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



17β-oestradiol Genistein

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

Current UK Government recommendations in relation to infant diet

7. 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 soya-based infant formulas[4]. The Department of Health's Chief Medical Officer in 2004 advised doctors that soya-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 or galactosaemia and that they should only be used in exceptional circumstances to ensure adequate nutrition (DH, 2004).

Recommendations in other countries

8. 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 soya 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 soya-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).

9. In Israel, France and Germany consumption of soya-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).

[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

Absorption[5] and metabolism

10. 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. 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).

11. 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 conjugation pathway. 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.

12. The 2003 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).

13. In humans, the maximum concentrations of genistein and daidzein occur in plasma approximately 5.5 and 7.4 hours after oral 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 it has been shown that only 30 to 50% of human adults are equol producers (NTP, 2010). Specific bacteria responsible for equol production in humans have been identified e.g. *Lactobacillus sp*., *Enterococcus faecium*, *Bifidobacterium*

[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 parent molecules.

sp. or *Finegoldia magna* (reviewed by Setchell and Clerici, 2010). Newborns and infants exclusively fed soya infant formula from birth are lacking developed microflora necessary for equol production (Setchell *et al*., 1997 and 1998).

14. 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).

15. The bioavailability of genistein following oral administration of genistin (37.5 mg/kg bw/day) and genistein (37.5 mg/kg bw/day) has been investigated in neonatal mice using subcutaneously (sc) dosed genistein (50 mg/kg bw/day) as reported by Doerge *et al*. (2002) as the reference route of administration with assumed 100% bioavailability. The dose-adjusted area under the curve (AUC) for genistein measured in serum after oral exposure to genistin was 83% (total free plus conjugated genistein) and 48% (as free genistein). Following oral dosing of genistein the bioavailability was lower, at 12 and 15%, respectively. The authors also found that compared with controls approximately 20 - 33% more oral genistin (25 and 37.5 mg/kg bw/day) was needed to result in similar oestrogenic activity in female mouse neonates (increase in uterine wet weight) as that resulting from sc genistein (20 and 25 mg/kg bw/day), 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) using an intravenous reference route. The authors explained this by different absorption and metabolic behaviours of the two compounds and the assumption (supported by other evidence) that genistin can be absorbed in both its intact and aglucone forms (Kwon *et al*., 2007).

16. Bioavailability of soya phytoestrogens was investigated in Caucasian (n=12) and Asian (n=12) male volunteers consuming soya-based cheese (containing approximately 25 mg of genistein and 21 mg of daidzein) once a day. After a single intake Asians had higher isoflavone plasma concentrations and AUC. In contrast, ingestion for 10 days resulted in higher plasma concentrations in Caucasians, than in Asians, regardless of whether the background diet was Western or Asian (Vergne *et al.*, 2009).

17. 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).

Distribution and excretion

18. 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 fetal 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).

19. Isoflavones present in the mothers' diet can be transferred to babies *via* breast milk. Levels of isoflavones measured in urine samples of mothers who met the criterion of breastfeeding ≥80% of the time and their babies were similar after the mothers consumed soya protein beverages (Franke *et al*., 2006).

20. Excretion of isoflavones is complete within approximately 24 hours. Hoey *et al.* found that 4-6 month old infants fed soya-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 soya formula in early infancy and those who were not, had similar levels 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 soya 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).

21. 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 soya products, were found to have significantly higher concentrations of both isoflavones compared to those with male pregnancies. This sex difference has not been previously reported. 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). Although sex differences in fetal levels of glucuronyltransferases have been reported in animals, parallel information is not available for humans. However, metabolic differences have been suggested to contribute to pharmacological differences in the fetus and neonate, without good supporting data.

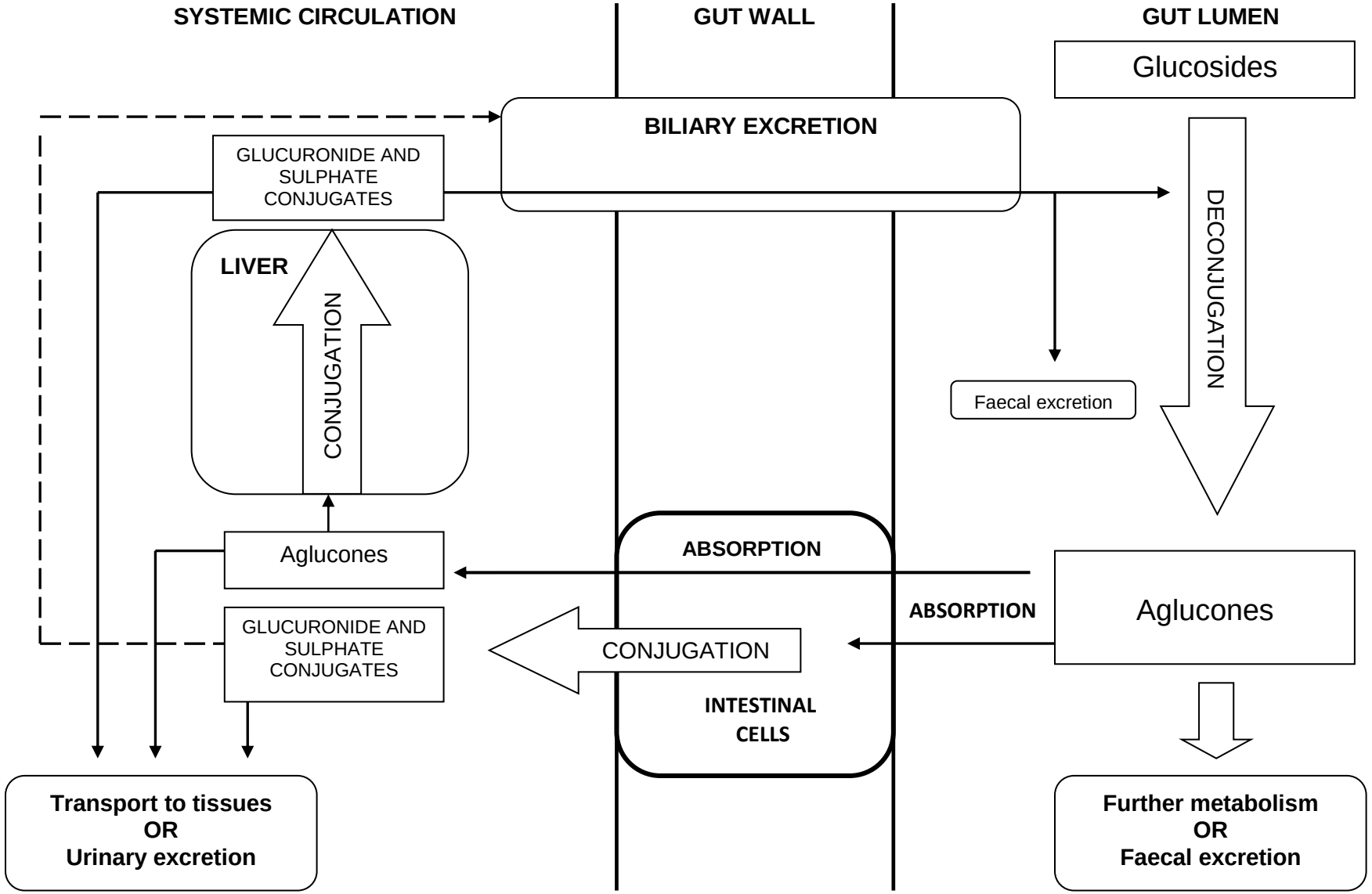


Figure 3. Schematic representation of isoflavone metabolism

Modulation of absorption and metabolism of isoflavones

22. 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

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

Pro- and pre- biotics

24. Effects of supplementation of soya 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). Using model systems, prebiotics have been found to increase growth of probiotics and enhance β-glucosidase activity and proteolysis. 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. Cohen *et al*. (2007) suggested that this organism may alter isoflavone metabolism by stimulating deconjugation and/or inhibiting degradation and hence increase circulating levels of isoflavones.

[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

25. 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 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 (soya 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 soya-rich foods to a similar degree. Children were not included in this study. Infants can effectively absorb isoflavones from breast milk, soya infant formula and food products containing soya and differences in metabolism when compared to adults may be due to the maturity of the intestinal flora and/or larger intake when adjusted for body weight. Infants also have decreased ability to glucuronidate isoflavones due to their lower expression of uridine diphosphate (UDP)-glucuronosyltransferases activity (NTP, 2010).

Maturity of gut flora

26. 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 develop gut microflora fully capable of metabolising isoflavones (COT, 2003). The main factors stimulating gut flora maturation process are: decreasing gut permeability, 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 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).

27. 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 soya) to be more available to the infant than the glucosides (as in soya 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 breast fed infants, and higher levels, exceeding those observed in adults eating soya 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 breast fed 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).

Species differences in isoflavone metabolism

28. Gu *et al*. compared the ability to produce equol from daidzein of women and experimental animals consuming soya 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% respectively of summed isoflavones. 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).

29. 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). Gender and species differences in tissue distribution of isoflavones were also observed by Gilani *et al*. (2011), who reported significantly higher concentrations of isoflavones in serum in male rats comparing to female rats fed isoflavone-supplemented diets.

30. There are inter-individual differences in the human ability to metabolise daidzein. It has been reported that approximately 30 to 50% of individuals are equol producers and 80 to 90% are able to produce *O*-DMA. Equol production has been associated with increased ingestion of diet rich in isoflavones, carbohydrates and fibre but low in fat (Atkinson *et al*., 2009; reviewed in NTP, 2010). Song *et al*. studied different daidzein metabolising patterns using a standardised soya challenge: daily consumption of 1 soya protein bar for 3 days containing approximately 38 mg of daidzein as aglucones equivalents. The prevalence of equol producers amongst American women of Korean background (51%, n=91) appeared to be higher than reported by Frankenfeld *et al*. (2004) for Caucasian women (36%, n=222). However, the prevalence of the *O*-DMA producer phenotype was lower (84 vs 92%). The Asian women consumed approximately 3 times more soya products than Caucasians, but soya consumption was not associated with equol producer phenotype (Song *et al*., 2006).

Hazard identification and characterisation

In vitro studies

31. *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

32. *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).

33. 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). The COT report noted that the single available *in vivo* study suggested that genistein at dietary levels was not mutagenic (COT, 2003). This is supported by the more recent study of McClain *et al*. (2006) who reported a lack of mutagenicity of genistein *in vivo* in the micronucleus assay in mice and rats.

Animal studies

34. Animal studies performed before 2003 indicated that intake of isoflavones can 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 sexual development (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 reproductive function, and the use of much higher doses or non-oral routes of administration (COT, 2003).

35. The few available studies evaluating effects of isoflavones exposure in primates were summarised in a previous COT report (COT, 2003). Treatment with isoflavones appeared to have no adverse effect on vaginal maturation in ovariectomised cynomolgus macaques, no effect on endometrial or mammary tissue in female macaques, no effect on plasma hormone concentrations and uterine, prostatic and testicular weights in rhesus monkeys. There was also no significant difference in maternal, fetal or placental weights in rhesus monkeys measured at the time of delivery. 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).

Oestrogenic potency of phytoestrogens

36. Oestrogenic effects have been reported in studies performed in juvenile or ovariectomised rodents treated with isoflavones through different oral and non-oral routes. Increased uterine weights, advanced vaginal opening, and changes in uterine and vaginal epithelium have been observed in mice and rats following oral exposure at doses starting from about 60-100 mg/kg bw/day (see Table 1). Irregular oestrus cycles have been additionally observed following sc exposure (10 mg genistein/kg bw/day) (Bateman and Patisaul, 2008).

Effects of phytoestrogens on fertility and development

37. A number of studies have been conducted to address potential adverse developmental health effects resulting from exposure to isoflavones in early life (see Table 1). Body weight, onset of puberty, changes in adipose tissue and reproductive organs were among measured endpoints. Observed effects in various studies investigating reproductive and developmental effects although variable appear to be consistent with interference in oestrogen mediated responses. The lowest dose of genistein of 0.42 mg/kg bw/day administered through the diet to pregnant and lactating rats increased thymus masses and subpopulations of T cells in male offspring, possibly explained by reduced gonadal steroid secretion. Reduced serum testosterone was seen in the offspring of treated mothers (Klein *et al*., 2002). Health effects observed after sc administration of genistein directly to offspring included dose-related increase in multi oocyte follicles (MOFs) (NOAEL = 5 mg/kg bw/day; LOAEL = 50 mg/kg bw/day) (Jefferson *et al*., 2002 and 2006) and uterine weights (NOAEL = 12.5 mg/kg bw/day; LOAEL = 20 mg/kg bw/day) (Jefferson *et al*., 2009) as well as advanced vaginal opening, prolonged and persistent oestrus cycles, abnormalities in uteri and ovaries (Kouki *et al*., 2003; Dinsdale *et al*., 2011 and Kaludjerovic *et al*., 2012). 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 (LOAEL = 37.5 mg/kg bw/day) and advanced vaginal opening and ovarian cycle abnormalities, decreased anogenital distance (AGD) and changes in body weight.

38. Studies in monkeys reported no significant adverse reproductive health effects, though increased testicular weight, lower mean levels of testosterone and

higher number of Leydig cells were observed in monkeys treated with soya formula. Tan *et al*. (2006) performed a study in seven pairs of male marmoset monkey twins, in which one of each pair of twins was fed standard (cows') milk formula and the co-twin was fed soya formula milk (estimated isoflavone content: between 1.6 and 3.5 mg/kg bw/day) for 5-6 weeks. Observed effects in the monkeys fed soya formula included increased testis weight, and increased numbers of Sertoli and Leydig cells (32%; p=0.026). The increase in the number of Leydig cells was especially marked in males with low-normal testosterone levels indicating possible "compensated Leydig cell failure" subsequent to neonatal suppression of testosterone secretion (Tan *et al*., 2006). A 74% increase in Leydig cells accompanied by decreased levels of testosterone in marmosets fed soya formula for 4-6 weeks, was previously reported by Sharpe *et al*., (2002). In a recent study pubertal female cynomolgus monkeys were fed diet containing soya protein isolate, with a human equivalent dose of 120 mg isoflavones/day, for approximately 4.5 years. There were no changes in the onset of early menarche, growth and pubertal progression, oestradiol and progesterone levels. Treated animals had some changes in breast differentiation (increased numbers of differentiated large-sized lobular units and a lower proportion with immature ducts following menarche) (Dewi *et al*., 2013).

Phytoestrogens and behavioural studies

39. A number of animal studies investigated behavioural effects of oral exposure to diets containing isoflavones (see Table 1). Behavioural effects predominantly observed in treated animals included decrease in time spent in social interactions, increase in submissive behaviour, changes in sexual behaviour, increased activity and more frequent episodes of aggressive behaviour and also lower body weight and height. Concentrations of isoflavones in feed were in a range between 0.4 and 600 ppm, in some studies isoflavone content was not specified.

Phytoestrogens and immunosuppression

40. 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

41. 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).

42. Spontaneous mammary tumour development was investigated in female Tg.NK mice fed diet containing phytoestrogens (0, 11, 39, and 130 mg aglucones/kg diet). The highest concentration increased number and size of tumours ($p<0.05$). Increased degree of branching of the mammary tree was observed in all treated groups ($p<0.05$) (Thomsen *et al*., 2005).

43. 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 insulin-like growth factor 1 (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. Oestrogenic, developmental and behavioural effects reported in animal studies following oral exposure to isoflavones

Treated population	Dose/ compound	Route of administration/ duration	Endpoint	Observed health effects in treated animals/the offspring	Reference
Oestrogenic effects					
Mice					
25-27 day old C57/BL6 female mice	Genistein 300, 500, 1000 and 1500 ppm, equivalent to 60, 100, 200 and 300 mg/kg bw/day	Diet (starting a week after ovariectomy for 12 days)	Uterine weights, effects on adipose tissue: - body weight - fat pad weight - adipocyte circumference	• ↓ in parametrial fat pad weight ($p<0.05$) and adipocyte circumference at 100-300 mg/kg bw/day • ↑ uterine weights at all doses ($p<0.05$).	Naaz *et al*., 2003
Female CD-1 mouse pups (n=5/group)	Diets containing genistein and daidzein: **Group 1**: low (0-20 ppm) **Group 2**: medium (101-210 ppm) **Group 3**: high (270-370 ppm)	Diet (PND 15 to 30)	Vaginal opening	• Advanced vaginal opening at PND 24 in Group 3 (53 to 93%) compared to Group 1 (12 to 37%) ($p<0.05$)	Thigpen *et al*., 2003
Pre-pubertal CD-1 mouse pups (n=30)	Chow containing soya protein supplement (200,000 ppm)	Diet (PND 21 to 28)	Mammary gland morphology	• ↑ longitudinal gland growth (development beyond lymph node) ($p<0.05$)	Alston-Mills *et al*., 2011

Rats					
Juvenile female Wistar rats	Genistein 100 mg/kg bw/day; EE 30 µg/kg bw/day	Daily gavage for 3 days (starting 2 weeks after ovariectomy)	Uterine wet weight, uterine and vaginal epithelium	• Combination of both compounds led to increased uterine weight/height of the uterine epithelium/height of the vaginal epithelium compared to EE alone ($p<0.05$)	Schmidt *et al.*, 2006
Adult ovariectomi-sed female Wistar rats (n=8/group)	Soya extracts: 10; 50; 100; 300; 600 mg/kg bw/day corresponding to dosing of 4.3; 21.3; 42.6; 127.8; and 255.6 mg isoflavones/kg bw/day, respectively.	Daily gavage for 21 days (starting 1 month after ovariectomy)	Uterine weight, morphometric analysis	• ↑ uterine weights at soya extract >100 mg/kg bw/day • Changes in endometrial and myometrial morphometry at soya extract >300 mg/kg bw/day	Mosquette *et al.*, 2007
Female F334 rat pups (n=16-20 pups/diet)	Diets (genistein and daidzein): PMI5K96 (7 ppm); PMI5002 (98; 223; 431 ppm)	Diet (starting on PND19 until the time of vaginal opening)	Vaginal opening	• Advanced vaginal opening (5.5 day earlier) in group fed PMI5002 diet at 431 mg/kg compared to PMI5K96 ($p<0.05$)	Thigpen *et al.*, 2007
Female Sprague-Dawley rat pups (n=16-20 pups/diet)				• No significant difference	
Female F334 rat pups (n=16-20 pups/diet)	Diet AIN-76A spiked with genistein at 0 (control), 150, 300, and 450 ppm	Diet (PND 19 to 40)		• Advanced vaginal opening in group fed diet containing 300 (PND 34) and 450 (PND 26.8) mg/kg compared to control (PND 36.8) ($p<0.05$)	

Female Sprague-Dawley rat pups (n=16-20 pups/diet)				• Advanced vaginal opening in group fed diet containing 450 (PND 27.1) mg/kg compared to control (PND 29.6) ($p<0.05$)	
19 day old female Wistar rats (n=10)	5 samples of soya dry extract at doses: 125; 300; 720; 1730; 4150 mg/kg bw/day. Approximate contents: total isoflavone: 44-52%; genistein: 9-11% (not detected in samples 3-5); daidzein: 7-46%	Daily gavage for 3 days (starting on the 19th day of life)	Uterine weight	↑ uterine weights (significant at different points in all samples)	De Lima Toccafondo Vieira *et al*., 2008
12 week old adult female rats	Low isoflavone diet (daidzein and genistein<10 ppm) enriched with genistein at 700 ppm (corresponding to 42 mg/kg bw/day);	Daily oral administration (diet) for 12 weeks (starting 2 weeks after ovariectomy)	Uterine weights	No effects	Hertrampf *et al*., 2009
	High isoflavone diet (daidzein: 232 ppm; genistein: 240 ppm; corresponding to 14 mg genistein/daidzein/kg bw/day).				

Effects on fertility and development					
Mice					
C57BL/6 mice n≥9	Genistein 0.1, 0.5, 2.5, 10 mg/kg bw/day	Daily gavage of dams GD 12 to PND 21.	Body weight, AGD (PND 7 and 21), mammary glands (PND 49) in the female 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 mg/kg bw/day	Daily gavage of dams GD 12 to PND 21.	Body weight, AGD (PND 7 and 21), testis and seminal vesicle weight, sperm count and motility, *in vitro* fertilizing ability of sperm in the male 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 25, 37.5 or 75 mg/kg bw/day	Daily oral administration using a pipette (PND 1-5)	Uterotropic response	• No detected effects in uterine weights (slight ↑ at 75 mg/kg/day)	Jefferson *et al*., 2009
	Genistin 6.25, 12.5, 25 or 37.5 mg/kg bw/day (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 oestrous 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)	

Male mice		Daily oral administration (diet) from conception to adulthood	Testicular and reproductive functions	• ↓ proportion of haploid germ cells in testes • ↓ by 25% in epididymal sperm counts • ↓ by 21% in litter size • ↓ size of seminal vesicle • No changes in fertility and behaviour	Cederroth *et al*., 2010
Pups: C57BL/6 female and male mice n≤8	Genistein 50 mg/kg bw/day	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 months 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
Rats					
Pregnant and lactating Long-Evans hooded rats	**Study I:** soya milk = isoflavones (genistein:daidzein, 3:2). Single dose level estimated to be between 10-30 mg/kg bw/day	**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 mg/kg bw/day	**Study II**: daily gavage (GD 14 through PND 21)		• Significant ↑ in PR expression in glandular epithelial cells (20%) • Earlier onset of puberty in genistein treated males (LOAEL = 15 mg/kg bw/day)	
Part I: Pregnant and lactating Long Evans rats n=12 **Part II**: Male offspring	Genistein (5 and 300 ppm, corresponding approximately to 0.42 and 25 mg/kg bw/day)	**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 ($p<0.05$) and ↑ subpopulations of T cells in the spleen at both doses ($p<0.05$) ↓ testosterone concentrations at both doses ($p<0.05$) (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 mg/kg bw/day (male) and 0.5, 10, 51 mg/kg bw/day (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
Female Sprague-	Exposure to genistein as 5	Daily oral administration	AGD (measured on PND 2, 7, 14, 21),	Effects observed in male offspring: • ↓ mean AGD on PND 14 and 21	Ball *et al*., 2010

Dawley rats	ppm feed: **Group 1.** none (n=8) **Group 2**. during gestation and lactation (n=9) **Group 3.** during gestation (n=8) **Group 4**. during lactation (n=8)	(diet) for 2 weeks prior to mating	weight of reproductive organs, spatial learning and memory in the Morris water maze (latency to find a hidden platform and swim speed), cued and contextual fear conditioning (emotional learning)	(p=0.001) in Group 2 • ↓ mean body mass on PND 21 in Group 2 (p=0.02) and Group 4 (p<0.001) • No effect on organs weight measured on PND 70 • No impact on emotional learning • Impaired spatial learning in Group 2	
Pigs					
Piglets (gender not specified); n=8/group	Milk-replacer with low genistein content (1 mg/L) and high genistein content (14 mg/L)	Daily self-feeding from a nipple attached to tubing, from 48h to day 10 (approximately 360 ml/kg bw per day)	Body weight, small intestinal weight and length	• No significant differences in body weight and intestinal weight and length between different groups • ↓ intestinal cell proliferation and ↓ jejunal enterocyte migration	Chen *et al*., 2005
Postpubertal gilts – 180 days of age	Genistein injections: 50 (n=4); 100 (n=5); 200 (n=5); or 400 (n=7) mg/day	15 days post-ovariectomy gilts were assigned to intramuscular injections containing genistein (at 12 hours intervals for 10 days)	Uterine and cervical mass/weight; height of uterine epithelial cells; percentage of cells stained positive for progesterone receptor	• Dose dependent ↑ in uterine and cervical tissue mass • ↑ height of uterine and cervix epithelial cells at 400 mg/day • ↑ in % of cells staining positive for progesterone receptor in the uterine glands and cells lining the vaginal cervix	Ford *et al*., 2006

Pre-pubertal piglets (n=8/group)	Diet containing soya bean extract (approx. 48 mg daidzein, 22 mg genistein and 0.5 mg equol)	Daily oral administration (diet) for 6 weeks	Strength and density of bones, body weight, effects on genital tracts	• No effects on growth rate, body weight, plasma bone markers, bone mineral density and strength • Heavier ovaries and ↑ number of follicles. No effects on uterus weight.	De Wilde *et al.*, 2007
Male and female piglets n=4/group	Soy formula (content not specified).	Daily oral administration from 48h through 21st day	Body and testicular weight, bone mineral density	• No differences in testicular development and body weight in males • ↑ trabecular bone mineral density/total mineral content and cortical thickness (measured in tibia bone) in males and females	Badger *et al*., 2009
Monkeys					
Marmoset monkeys n=13 pairs	Isoflavones in soya formula (range: 1.6 – 3.5 mg/kg bw/day)	Daily oral administration (syringe) from PND 4-5 until PND 35-45	Body weight, organ weights, testosterone levels, testicular cell composition	• ↓ levels of testosterone at PND 35-45 ($p<0.004$) • ↑ numbers of Leydig cells (74%) ($p=0.006$) • No differences in numbers of Sertoli and germ cells	Sharpe *et al*., 2002
Marmoset monkeys n=7 pairs of twins	Isoflavones in soya formula (range: 1.6 – 3.5 mg/kg bw/day)	Daily oral administration (syringe) from PND 4-5 until PND 35-45	Body weight, organ weights, testicular cell counts	• ↑ testicular weight (14%) and numbers of Sertoli (7%) and Leydig cells (32%) ($p<0.05$)	Tan *et al*., 2006
Pubertal female cynomolgus	Diet containing soya protein	Daily oral administration (diet) for 4.5	Pubertal breast development, sex hormones, growth	• ↑ endometrial area • Changes in breast differentiation	Dewi *et al*., 2013

monkeys (n=17)	isolate: 120 mg isoflavones per day	years		• No effect on onset of menarche, growth, pubertal progression, levels of oestradiol and progesterone	
Behavioural effects					
Rats					
8 week old male Lister rats	Diet containing isoflavones at 150 ppm (genistein and daidzein)	Daily oral administration (isoflavone diet *ad libitum*) for 2 weeks (n=32) followed by further 2 weeks of isoflavone diet (n=16) and control diet (without isoflavones, n=16). Then the rats were housed singly for 4 days and examined	Social interaction; anxiety measured as entries into the open arms of the elevated plus-maze apparatus; level of corticosterone and vasopressin	• ↓ time spent in active social interaction (p=0.02) • ↓ number of entries onto the open arms of the plus-maze (p=0.002) indicating an increase in anxiety • ↑ level of stress-induced corticosterone (p<0.01) and plasma vasopressin (p<0.04)	Hartley *et al*., 2003
Sexually experienced Sprague-Dawley rats (n=20/group)	Treatment with saline or isoflavones (0.4 or 0.8 mg/kg)	Daily oral administration (gastric tube) for 40 days	Sexual behaviour	• No significant differences in sexual performance • ↑ intromission frequencies in group fed 0.8 mg isoflavones/kg (p=0.13) • ↓ LH and testosterone plasma levels (p<0.05)	Cicero *et al*., 2004
7-8 week old male and female	Diets: **Study 1**: 1. Cookies	Daily oral administration (diet *ad libitum*)	Interaction between stressors, diet, and sex. **Study 1**:	**Study 1**: • ↓ consumption of lab chow and	Liang *et al*., 2008

Sprague-Dawley rats Study 1: n=23 Study 2: n=72	2. Peanuts 3. Lab chow 4. Soya beans (isoflavone content not specified) **Study 2:** 1. Lab chow (n=36) 2. Mixture of cookies and soya beans (n=36)	**Study 1**: for 7 days before exposure to stressors rats (n=23) were fed mixture of food types in order to determine their food preference, then diet continued in all rats but stressors were applied in half of the animals **Study 2**: There were 2 groups of animals: exposed to stressors (n=36) and left undisturbed (n=36). Half of each group was fed lab chow and the other half received mixture of cookies and soya beans.	<u>Stressors</u> were applied singly or in combination for 25 days to half of the rats (loud static, flashing light, tilted cages, wet bedding, water deprivation, photoperiod reversal) **Study 2**: Behaviour of rats exposed to stressors (n=36) was analysed before and after the period of stressor exposure using following methods: dark avoidance activity, open field activity, elevated plus-maze test, forced swim test	peanuts in both sexes • ↑ consumption of soya beans in males before and after stressors comparing to females (p=0.0036) • Interaction between sex and exposure to stressors in the consumption of cookies (p=0.004) **Study 2**: • ↓ total caloric intake of all diets in both sexes exposed to stressors (in males p<0.08) • No significant influence of diet on open field rearing • ↑ attention to novelty among males exposed to stressors (p<0.05) correlated with diet • In control females mixed diet lead to ↓ time in the closed arm (p<0.01), ↑ time in the open arm (p<0.01) and ↑ moving time (p<0.05) compared to control females fed lab chow. • ↑ time of immobility in females fed the mixed diet (p=0.0065)	
5 week old Wistar rats (n=7/group)	Diet containing fermented	Daily oral administration (diet *ad libitum*):	Voluntary wheel running activity in rats measured over 9 weeks	• ↑ running activity (p<0.05; p<0.01) observed 2 and 3 weeks after starting a diet in FSM group	Sato *et al.*, 2010

	(FSM) or non-fermented (SM) soya milk (isoflavone content not specified)	weeks 1-3 and 7-9: normal diet; weeks 4-6: diet containing FSM or SM		• No changes in running activity in SM rats • ↑ in mounting behaviour (by 10 days) in FSM rats ($p<0.01$)	
Female Long-Evans rats (n=3/4 per treatment): **Study 3**: ovariectomy (OV) before day 45 **Study 4**: Ovariectomy on day 100	Low-soya (**LS**) and soya-rich (**HS**) diet containing 10 and 600 ppm of isoflavones. In Study 3 additionally medium soya (**MS**) diet was used – 200 ppm of isoflavones. **Equol** sc at 5 mg/kg bw/day	**STUDY 1**: LS diet (n=23; conception=day 0 to 120 days), then 50% switched to HS diet until day 200. Equol sc in LS group (days 194-200). **STUDY 2**: LS (n=8) or HS (n=7) diet from day 0 to 145. Equol sc in LS group (days 136-145) **STUDY 3**: MS (n=22; 0 to 45 day), LS (n=22; 46 to 85 days), HS (n=11; 86 to 100 days). **STUDY 4**: LS or HS diet	Depressive-like behaviours measured by PFST*, weight gain and white adipose tissue deposition (WAT)	**STUDIES 1-4:** **Behaviour:** • No differences in behavioural parameters between groups **Effects observed in HS group:** • ↓ body weight gain ($p<0.05$; in Study 1 after diet change; and Study 4 after OV) and ↓ WAT ($p<0.01$ to $p<0.001$) • ↓ body weight following ovariectomy ($p<0.01$) **Effects observed in LS group:** • ↓ body weight following equol sc **STUDY 5:** **Behaviour:** • ↑ mobility, swimming distance and speed ($p<0.005$) • ↑ mobility in LS group when compared to pre-equol injection values ($p<0.05$) **Effects observed in HS group:**	Blake *et al*., 2011

		(n=8/group; 0 to 200 days). Equol sc in LS group (days 197-200). **STUDY 5**: LS (n=6) or HS (n=8) diet from 0 to 365 days. Natural ovarian failure (NOF) occurred approximately on day 295.		• ↓ body weights ($p<0.05$; a month after NOF) and ↓ WAT ($p<0.05$) **Effects observed in LS group:** • ↓ body weight following equol sc	
Monkeys					
Male cynomolgus macaques (n=44)	Soy protein isolate diet containing 0.94 and 1.88 mg isoflavones/g protein	Daily oral administration (diet) for 15 months	Spontaneous social behaviour	Effects observed at highest concentration of isoflavones: • ↑ frequencies of intense aggressive (67%) and submissive (203%) behaviour ($p<0.05$) • ↓ time of physical contact (68%), time in proximity to other animals (50%)($p<0.02$) • ↑ time spent alone (30%)($p<0.02$)	Simon *et al*., 2004
Male newborn rhesus monkeys (n=8/group)	Diet: cows' milk formula, soya formula (SF), soya formula with Mn (SMF)	Daily oral administration (diet) from birth to 4 months	Body weight, motor, cognitive and social parameters, rest and activity cycles	• ↓ in weight ($p<0.01$) and height ($p<0.05$) in control vs SF group at 9 months of age • ↑ number of total behaviour initiations, climbing behaviour (SF) and walking initiations (SMF)(ns) • ↓ duration of wake periods at 8 months ($p<0.01$, SF) ($p<0.05$,	Golub *et al.*, 2005

				SMF) • ↓ total duration of play behaviours ($p<0.05$)	

#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)
*PFST – Porsolt forced swim test – quantifies animals' mobility (swimming speed, distance, time of activity) as a sign of a less depressive behaviour
AGD – anogenital distance; AGDI – AGD Index (ratio of AGD/BW); bw – body weight; CL – corpora lutea; EE – ethinyl oestradiol; LQ – number of lordosis behaviours displayed/number of mounts x 100); ER – oestrogen receptor; MOFs – multi-oocyte follicle; NOF – natural ovarian failure; ns – not significant; OV – ovariectomy; PR – progesterone receptor;

Human studies

44. 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 soya formula the reasons for introducing such products into their diet may influence observed health outcomes, but are generally not reported. Soya 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 soya intake also remains unknown (Bernbaum *et al*., 2008; Zung *et al*., 2008; Gilchrist *et al.*, 2010; Adgent *et al*., 2012).

Oestrogenic potency of phytoestrogens

45. Cellular and molecular mechanisms of oestrogen action as well as estimated oestrogenic potency of phytoestrogens have been extensively described. 17β-Oestradiol binds with similar affinities to oestrogen receptors (ER) α and β. Although phytoestrogens are structurally similar to 17β-oestradiol and have both oestrogen agonist and antagonist activity, genistein and daidzein have been shown to have much higher affinity to ERβ (Kuiper *et al*., 1997 and 1998; COT, 2003)

46. The distribution of ERα and β varies between and within tissues of different species. Both subtypes have been found in humans and rodents in heart, uterus, ovary and bone tissue. They can also be found at different ratios in other tissues such as lung, kidney, prostate, testes, brain, bladder, liver or GI tract. In humans but not in rodents both receptors have been found in vascular, breast, endometrium tissues as well as vagina and fallopian tubes. Expression of both receptors in muscle and fat has been reported in rodents (COT, 2003).

47. Matsumura *et al.* (2005) determined ER binding of isoflavones in human breast cancer cells using radiolabelled oestrogen, [2,4,6,7-^{3}H]oestrogen at 16 x 10^{-10} M. Genistein inhibited [^{3}H]oestrogen binding by 50% at 1000- and equol at 4000-fold molar excess. Due to decreased solubility, only 40% inhibition by daidzein was assayed (10,000-fold molar excess) (Matsumura *et al*., 2005). ER-binding potencies of isoflavones have been analysed for each one separately but also in combinations in one and different species (Harris *et al.,* 2002; Mueller *et al.,* 2004; Zhao *et al*., 2009). In experiments with different isoflavones and their combinations, genistein appeared to have the maximum affinity to both receptors with approximately 60-fold higher binding preference for ERβ (Zhao *et al.,* 2009). Harris *et al.* tested selectivity of various phytoestrogens on human, rat and mouse ERs and reported that most compounds were non-selective. However, the authors did not expect big differences as ligand binding domains of both ERs were shown to be highly conserved among species (Harris *et al*., 2002). Comparison of different ER binding preferences of isoflavones is presented in Table 2.

Table 2. The ER binding potency of isoflavones in different species

Compound	ERα		ERβ		Fold selectivity for ERβ	Reference
	IC_{50} or [EC_{50}] (μM)	RBA (%)	IC_{50} or [EC_{50}] (μM)	RBA (%)		
Human						
17β-oestradiol	0.0020		0.0023		0.86	Harris et al., 2002
Diethylstilbestrol	0.0014		0.0011		1.27	
Genistein	0.3340		0.0066		50.60	
Daidzein	>5.0000		0.4100		>12.19	
17β-oestradiol	0.0043	107.00	0.0057	82.00	0.75	Mueller et al., 2004
Diethylstilbestrol	0.0046	100.00	0.0046	100.00	1.00	
Genistein	0.3000	1.00	0.0150	31.00	20.00	
Equol	1.5000	0.30	0.2000	3.00	7.50	
17β-oestradiol	[0.021 x 10^{-3}]	100.00	[0.11 x 10^{-3}]	100.00	0.19	Muthyala et al., 2004
Genistein	[0.0800]	0.02	[0.0066]	7.40	12.00	
Daidzein	[0.2500]	0.01	[0.1000]	0.04	2.50	
Equol	[0.2000]	0.20	[0.0740]	1.60	2.70	
17β-oestradiol		100.00				Kwok and Cheung, 2010
Diethylstilbestrol		100.00				
Genistein		1.50				
Mouse						
17β-oestradiol	0.0021		0.0025		0.84	Harris et al., 2002
Diethylstilbestrol	0.0002		0.0023		0.09	
Genistein	0.4000		0.0046		86.95	
Daidzein	4.9930		0.1670		29.89	
Rat						
17β-oestradiol	0.0018		0.0018		1.00	Harris et al., 2002
Diethylstilbestrol	0.0006		0.0011		0.54	
Genistein	0.2820		0.0058		48.62	
Daidzein	5.1790		0.3450		15.01	
17β-oestradiol	0.0250	100.00	0.0320	100.00	0.78	Zhao et al., 2009
Genistein	4.7350	0.53	0.0790	41.12	60.00	
Daidzein	26.6500	0.09	1.7380	1.87	14.27	
Equol	5.8800	0.43	0.5820	5.57	10.09	
G + D	9.8960	0.26	0.1570	20.62	62.87	
G + D + E	15.7100	0.16	0.1900	17.06	82.60	
17β-oestradiol	0.0009	100.00				Branham et al., 2002[a]
Genistein	0.2000	0.45				
Daidzein	4.0000	0.02				
Equol	0.6000	0.15				

IC_{50} – molar concentration of compound leading to a 50% inhibition of 17β-oestradiol binding to ER
EC_{50} – molar concentration of compound producing response equal to 50% of that observed with 17β-oestradiol
RBA – the Relative Binding Affinity of the compound as a percentage of the binding affinity of 17β-oestradiol (100%). Calculated as (IC_{50} of 17β-oestradiol)/(IC_{50} of test compound) x 100.
[a] ER type not stated, presumably ERα

48. Genistein and daidzein are the major contributors to the total oestrogenicity of soya-based products. When infant diet is exclusively soya formula, daily genistein intake would be approximately 5 mg/kg bw/day. Isoflavones are considered to have relatively weak oestrogenic properties. Chen and Rogan (2004) reviewed *in vitro* studies providing different estimates of the oestrogenicity of genistein, reporting a range between 10^{-5} and 0.4 times lower than that of oestradiol. From this range the

authors concluded that genistein intake would be equivalent to 0.05 to 5 µg of oestradiol per kg bw/day, which they equated to infants taking up to five contraceptive pills/day, assuming daily oestrogen intake from contraceptive pills in women as approximately 0.4 - 1 µg/kg bw/day (Chen and Rogan, 2004). However, it is not clear whether authors based their comparisons on oestradiol or the actual active ingredient used in contraceptive pills, ethinyl oestradiol which has 100 times greater oral oestrogen potency than oestradiol (Fritz and Speroff, 2010).

49. Table 2 shows a comparison of ER binding potency of isoflavones in human, mouse and rat. In all species the oestrogenic binding potency of isoflavones is much weaker comparing to oestradiol and several times higher molar concentrations are needed to achieve 50% inhibition of oestradiol binding to ERα and β. In the case of genistein such concentrations would be in a range of ≥ 100 fold (ERα) and ≥ 2 fold (ERβ) compared to corresponding molar concentrations of 17β-oestradiol. The Relative Binding Affinity (RBA) presented in Table 2 shows that isoflavone binding preference is for ERβ.

Effects of phytoestrogens on fertility and development

50. 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 soya-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).

51. Strom *et al.* examined the association between consumption of soya 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 soya formula (n=128) compared to women who had been fed cows' milk formula as infants (n=268). 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).

52. Studies performed after 2003 investigating the potential oestrogenic impact of exclusive soya formula consumption on infants' development when compared to breast fed or cows' milk formula fed infants are summarised in Table 3. Investigated outcomes include vaginal cell maturation (Bernbaum *et al*., 2008), prevalence of infantile breast tissue in the 2nd year of life (Zung *et al.*, 2008), reproductive health (Gilchrist *et al*., 2010), sexual dimorphism in gender-role play (Adgent *et al*., 2011),

risk of early menarche (Adgent *et al*., 2012) and also behavioural development and bone mineral content (Andres *et al*., 2012 and 2013).

53. The COT concluded that the evidence from the small number of epidemiological studies was not suggestive of important impacts of soy formula infant feeding on reproductive health and development. Four studies raised the possibility of minor subtle effects of uncertain clinical significance (early life breast development and minor differences in menarche/menstruation, gender related play behaviour) but the findings were not conclusive and may have been due to chance or issues with study design. Impacts have only been assessed to early adulthood, not to later life (e.g. cancers of reproductive organs).

Table 3. Human studies of reproductive and developmental outcomes associated with *e*xposure to isoflavones.

Participants (Reference)	Isoflavone exposure	Endpoint	Observed health outcome	Comments
Children <48 hours to 6 months of age (7 age intervals), 37 boys and 35 girls – 2 boys and two girls in each age interval and three feeding regimes: breast milk, cows' milk-based formula and soya formula AIM: to study whether soya formula prolongs maternal oestrogenisation of newborns (Bernbaum *et al*., 2008)	Cross-sectional pilot study. One-third of children exclusively fed soya-based infant formula (as reported by parents). Reasons for preference for soya formula unknown.	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 maturation in newborns and older girls (the curvature was statistically significant: $p<0.0001$; feeding regimen influenced the trajectory of maturation index: $p=0.07$). The lowest maturation index of vaginal wall cells was observed in 1 month olds. • No changes in breast and genital anatomy over the time	Reported change was based on approximately 2 girls per feed type. The results could have been influenced by 2 higher values in girls fed soya-based formula at the 6 months period, which could be a chance finding. This study does not allow conclusions regarding possible adverse effects

Female infants aged 3 to 24 months AIM: to evaluate the oestrogenic effect of soya-based formulas in female infants (Zung *et al*., 2008)	Cross-sectional study. At least 3 months of soya-based formula feeding either exclusively (n=92) or in combination with breastfeeding or cows' milk formula (n=602) (as reported by parents). Reasons for preference for soya formula unknown.	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 (p<0.02; 95% CI 1.11 – 5.39) • Preserving effect on infantile breast tissue and slower waning in the 2nd year of life compared to children fed breast milk or cows' milk formula (p<0.001)	This study raises the possibility that early life soya in an early life may affect natural development of breast tissue in a small percentage of children. The significance is not clear as changes were subtle rather than pathological. Findings would need to be confirmed in further studies.
Infants fed soya formula (n=39), cows' milk formula (n=41) and breast milk (n=40). Approximately 20 boys and 20 girls per diet group. AIM: to determine differences in hormone-sensitive organ size between infants on different diets. (Gilchrist *et al*., 2010)	Nested study within longitudinal cohort Soya formula, cows' milk formula or breastfeeding either exclusively or for the majority of the first 4 months of life, as reported by parents. No obvious selection for feeding decision.	Measurements of body weights, ovaries, number of follicles, testicular volume	• ↓ body weight in boys (comparing to cows' formula fed boys) (p<0.05) • ↓ ovarian volume in girls (comparing to cows' formula fed girls) (p<0.09) • ↑ number of follicles per cyst per ovary compared to breastfed girls (p<0.01) • ↓ testicular volume compared to breastfed boys (p<0.04) • No differences in infant weight, length, or body surface area	Well designed and conducted study with thorough statistical analysis. Investigators were blind as to diet group. Moderately small number of children in each group. No adverse effects of soya feeding on reproductive organ development were demonstrated by age 4 months.

Preschool boys (n=230) and girls (n=198), aged 3-6 years (Wada *et al.*, 2011)	Mean soya intake was recorded by parents over 3 days (24.4 g/day for boys and 22.8 g/day for girls)	Sex differences	• Soya intake negatively related to oestrone and oestradiol in boys ($p<0.05$) • Soya intake positively related to testosterone and 5-androstene-3β,17α diol (3β,17α-AED) levels in girls ($p<0.01$) • The average levels of oestrone, oestradiol, testosterone, and DHEA in girls were higher than those in boys	Significant results show biological activity of soya in food in this age group. The relevance to infants and soya formula is unclear
ALSPAC study* 42 month old children (3,664 boys and 3,412 girls). (Adgent *et al*., 2011)	Prospective, cohort study. Early-life feeding plan was reported by parents at 1, 6, 15 and 24 months postpartum as primarily breast (n=1428), early formula (n=5185), early soya (n=157) and late soya (n=306) Reasons for soy feeding were not given.	Sexual dimorphism in gender-role play behaviours assessed between infant feeding and scores of the Pre-School Activities Inventory (PSAI) test. Mother's response was scored on a 5-point Likert scale ("never" to "very often"). Higher scores indicated masculine typical behaviour, and lower scores indicated feminine typical behaviour.	• Modest increase in masculine typical behaviour among girls at 42 months of age fed soya in early life (but not 30 or 57 months). Mean [CI] PSAI score: 40.8 [38.6, 43.0] in early soya feeding and 36.7 [36.4, 37.1] in early formula feeding. • No significant effects in boys • Authors noted that the results were within the range of normal behaviour.	Bias or residual confounding by social class may explain the soya findings. There may be residual confounding by social class (better educated mothers were more likely to report both masculine and feminine traits in children). Dividing play into masculine and feminine could also be socially determined
ALSPAC study* Girls (n=2920) for	Prospective, cohort study. Early-life	Timing of menarche in relation to infant feeding	• Median age at menarche was earliest for girls	The study found a non-statistically significant

whom at least one puberty questionnaire was available between the ages of 8 and 14.5 years if age (at approximately 8, 9.5, 10.5, 11.5, 13 and 14.5 years of age) (Adgent *et al*., 2012)	feeding plan was reported by parents at 1, 6, 15, and 24 months either as primarily breast (n=631), early formula (n=2124), early soya (n=54) and late soya (n=111)		receiving an early soya diet (149 months; IQR 140-159), and latest among those who were primarily breastfed (154 months; IQR 145-165) • Early soya-fed girls were at 25% higher risk of early menarche throughout the course of follow-up (up to 14.5 years of age) (HR 1.25 [95% CI 0.92, 1.71]), compared to girls fed non-soya-based or milk formula	trend for slightly earlier menarche. This contrasts with study by Strom *et al*. (2001) described above, which found no difference in reported age at menarche between 128 females fed soya and 268 fed cows' milk formula.
Infants (n=391) fed breast milk (BF), cows' milk formula (MF), or soya formula recruited between ages 1 and 2 months. Assessment at age 3, 6, 9, and 12 months. (Andres *et al*., 2012)	One-third (n=129) of children exclusively fed soya-based infant formula (SF)	Infant behavioural development (Bayley Scales: MDI** and PDI***; PLS-3****)	• ↓ MDI at 6 months (compared to BF and MF) and at 9 and 12 months (compared to BF) ($p<0.05$) • ↓ PDI at 6 months compared to BF infants ($p<0.05$) • NS differences between different diets	Although significant the differences in the MDI were very small and within expected normal range. PLS-3 were the lowest in the MF group – with small differences and all within range.
Infants (n=207) fed breast milk (BM), cows' milk formula (CMF), or soya formula during 1st	One-third of children exclusively fed soya-based infant formula (SF)	Growth, fat mass and bone mineral content	• ↑ fat-free mass at 6 and 9 months compared to CMF ($p<0.001$) • ↑ bone mineral content by 12 months (lower at 3	

year of life. Assessment at age 3, 6, 9, and 12 months. (Andres *et al*., 2013)			months comparing to BM)	

*ALSPAC study – Avon Longitudinal Study of Parents and Children; ongoing study that enrolled pregnant women residing in the Avon region of the UK expecting to deliver between 1 April 1991 and 31 December 1992. During pregnancy 14 062 livebirths were recruited into the study.
**MDI – Mental Developmental Index measures performance in sensory perception, knowledge, memory, problem solving and early language with tasks adapted to age
***PDI – Psychomotor Development Index assesses fine and gross motor development with tasks adapted to age
****PLS-3 – Preschool Language Scale-3 assesses receptive, expressive language skills and language precursors
CI – confidence interval; DHEA – dehydroepiandrosterone; IQR – interquartile range; HR – hazard ratio

Effects of phytoestrogens on growth, allergy and immune responses

54. The Food Standards Agency (FSA) advises that: "*Soya allergy is a common childhood allergy. Most children grow out of it by the age of two, but occasionally adults are allergic to soya. The symptoms of soya allergy are similar to milk allergy and they include rashes, diarrhoea, vomiting, stomach cramps and breathing difficulties. Some people with soya allergy might also react to milk. Very rarely soya can cause anaphylaxis. Infants with other allergic conditions, such as milk allergy, dermatitis etc, are also at higher risk of developing allergy to soya*"[8]. The health risks associated with infant feeding and the development of soya 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.

55. The 2003 COT report noted that although there was one study indicating that infant exposure to soya-based infant formula could lead to lower antibody responses, there were no differences observed in immune response in infants fed soya formula in two other more recent studies (COT, 2003).

56. Klemola *et al*. performed a study in which infants diagnosed with cows' milk allergy were assigned to hydrolysed formula and soya-based formula. The follow-up period was 2 and 4 years. The authors reported good tolerance of soya formula by more than 70% of children. The remaining children had adverse reactions suspected by parents. However, some of them were doubtful and many were not supported by IgE and skin test data (Klemola *et al*., 2002). Slightly increased risk of sensitisation to soya formula was reported at age 4 (Klemola *et al*., 2005).

57. No adverse effects on growth and no differences in height and weight in a follow-up study in adults following consumption of soya formula as infants was reported (Strom *et al*., 2001; COT, 2003; Merritt and Jenks, 2004). In more recent study, infants fed soya formula appeared to have a lower weight gain compared to casein hydrolysate and rice hydrolysate fed groups (Agostoni *et al*., 2007), Similarly, a smaller increase in weight was observed in infants with cows' milk allergy fed soy formula compared to infants fed extensively hydrolysed whey formula (Seppo *et al.,* 2005). However, another study suggested that soya-based formula consumption could increase chances of being overweight (Stettler *et al*., 2005).

Effects of phytoestrogens on the thyroid gland and thyroid function

58. 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).

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

59. The 2003 COT report noted that animal studies showed that dietary soya or isoflavones can affect thyroid function and have a goitrogenic effect in rodents deficient in dietary iodine. Data from human studies suggested that isoflavones are unlikely to affect thyroid function in normal individuals with adequate iodine intake (COT, 2003).

60. A number of scientific publications evaluated by the Committee in 2003 noted the possibility that soya-based infant formula may be able to affect thyroid function in infants. Cases of goitre associated with consumption of soya formula, and a case of increased loss of orally administered thyroxine in an athyreotic hypothyroid patient when fed soya formula compared to cows' milk formula were reported in the 1950s and 60s. As a result changes in processing and formulation of infant formula were made (supplementation with iodine, replacement of soya flour with soya 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, the COT considered it possible that together with low iodine intake, increased metabolic demands during pregnancy and increased thyroxine need, maternal consumption of soya products could adversely influence the neurological development of the fetus (COT, 2003).

61. 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 soya diet group consuming exclusively soya infant formula started on treatment at a median age of 15 days (range: 11 – 22) (n=8) and the non-soya 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 (soya group) and 188 nmol/L (non-soya group). However, after initiation of treatment there was a difference in the first TSH measured at 50 days between the soya and non-soya diet groups: median 42.6 mU/L (range: 30.6 – 63.1) and 6.6 mU/L (range: 1.8 – 20.9), respectively. The soya diet group required a median of 150 days (range: 54 – 229) and non-soya 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 soya diet group: 62.5 %, whereas in non-soya diet group these values were 35.7 and 17%, respectively. Overall, infants consuming soya-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).

Cancer

One study has considered the relationship between consumption of soya-based infant formula and breast cancer risk in later life. History of feeding was obtained from mothers of women diagnosed with breast cancer (n=372) and controls without breast cancer (n=356). There was no statistical association between those two factors. Multivariate Odds Ratio (OR) and 95% CI in groups exclusively fed soya formula were as follows: during the first 4 months of life of cases (n=5) and controls

(n=10) OR=0.42 (95% CI, 0.13-1.40) and during the first 5-12 months of life of cases (n=7) and controls (n=8) OR=0.59 (95% CI, 0.18-1.90) (Boucher *et al*., 2008).

Guidance values

62. Soya 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 soya isoflavones.

63. In 2010 the NTP-CERHR Expert Panel concluded that there was no sufficient evidence to conclude that soya infant formula and soya-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 soya 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 soya exposure from birth through puberty, addressing longer term endpoints such as cancer, bone mineral density or cognitive performance (NTP, 2010).

64. The COT similarly concluded that it was not possible to propose health-based guidance values for infants. Reasons for this included the difficulty in extrapolation from animals to humans because of differences in toxicokinetics, uncertainty with respect to differences between adults and infants (particularly those arising from development of the gut microflora), and the lack of dose-response data and the role of possible confounders in the available human studies. It was noted that the pig was the best animal model with respect to toxicokinetics, but few toxicity data were available for pigs.

Occurrence

Levels of isoflavones in human breast milk

65. 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: 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).

66. 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 soya foods such as roasted soya beans has been shown to increase levels of isoflavones in breast milk from vegans up to 32 µg/L (Franke and Custer, 1996; MAFF, 1998b).

67. In a study conducted in the US, milk samples were collected from breastfeeding mothers before and after consumption of a soya protein beverage (25 g soya 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 of isoflavones in breast milk samples were 0.87 µg/L (genistein) and 0.36 µg/L (daidzein) from women with male infants and 0.36 µg/L (genistein) and 0.16 µg/L (daidzein) from women with female infants (Jarrell *et al*., 2012).

Cows' milk-based infant formula

68. 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).

Soya-based infant formula

69. COT (2003) noted that reported isoflavone levels in soya-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 soya infant formulas obtained in the UK were also measured by other researchers. All soya 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 soya infant formula as 1000 times higher than non-soya formula: 25.90 mg aglucone equivalents as fed (Kuhnle *et al*., 2008). Concentrations of isoflavones were higher in powdered soya formula (46 – 47 mg/L) than in liquid formula (32 – 45 mg/L) (Setchell *et al*., 1998). Conjugates of genistein accounted for >65% of the total isoflavones and only 3.2 – 5.8% existed as genistein and daidzein aglucones (Setchell *et al*., 1998).

70. Total isoflavone concentrations as aglucone equivalents in soya-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

Soya–based products

71. Levels of isoflavones in samples of ready-to-eat and instant foods for infants were previously reported by the COT (Table 4). Table 4 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 in this statement is based on the data presented in the COT report (2003).

Table 4. 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

72. 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].

Breast milk

73. The data for breast milk samples collected in the UK (see paragraph 65) have been used to estimate exposure of exclusively breast fed infants, based on the reported levels of isoflavones (expressed as a sum of genistein and daidzein) in mothers consuming different diets (Table 5). The estimated exposures range from

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

0.0001-0.0002 mg/kg bw/day for infants whose mothers consume an omnivorous diet up to 0.0065 mg/kg bw/day for infants whose mothers consume a vegan diet.

Table 5. Estimated total isoflavone exposure (mg/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	0.0001	0.0002	0.0001	0.0002
Vegetarian diet Mean = 4µg/L	0.0005	0.0008	0.0004	0.0006
Vegan diet Mean = 11µg/L	0.0015	0.0022	0.0011	0.0017
Vegan diet Maximum = 32µg/L	0.0043	0.0065	0.0033	0.0049

Cows' milk-based infant formula

74. 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: 0.135 kg of powder used to prepare 1 L of liquid formula (NTP, 2010). The estimated average and high level exposures from cows' milk-based infant formula are up to 0.038 and 0.057 mg/kg bw/day, respectively (Table 6). 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 6. Estimated total isoflavone exposure (mg/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)	0.038	0.029	0.025	0.023
High level (1200 mL)	0.057	0.044	0.038	0.034

Soya-based infant formula

75. Based on reported isoflavone levels in reconstituted soya-based infant formulas from the UK (range: 18 – 46.7 mg aglucone equivalents/L) (see paragraph

69) the isoflavone exposure of infants exclusively fed on soya-based infant formula is up to 9.5 mg/kg bw/day (Table 7).

Table 7. Estimated total isoflavone exposure (mg/kg bw/day) of infants fed exclusively soya-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)	2.4 – 6.3	1.9 – 4.9	1.6 – 4.2	1.5 – 3.8
High level (1200 mL)	3.7 – 9.5	2.8 – 7.3	2.4 – 6.3	2.2 – 5.7

Complementary feeding products – exposure assessment

63. Infants' exposure to total isoflavones from infant foods (Table 8) has been estimated based on the levels reported for infant foods (Table 4) 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 4 mainly comprised commercial baby foods (including cereal-based foods and desserts).

76. 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 8. Estimated exposure of UK infants to isoflavones from complementary foods (in mg/kg bw/day)

Product category	Isoflavone level (µg/g food)	Consumption rate (g/kg bw/day)		Exposure (mg/kg bw/day)	
		Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
Weaning Foods*	Range: 18 – 78	6	22	Range: 0.108 – 0.468	Range: 0.396 – 1.716
Tofu**	275	5	n/a	1.375	n/a

* 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

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

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 soya-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

77. Soya isoflavones are not classified as essential nutrients and soya-based infant formula is recommended for consumption by infants only in exceptional circumstances following medical advice. A TDI value has not been established.

78. Numerous animal studies have reported oestrogenic, reproductive and developmental effects in the offspring of treated animals, juvenile animals and mature animals exposed to soya formula or other soya-based food products during their life time. In experimental animals dietary administration of isoflavones has resulted in effects on body weight (observed at 7 mg 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 mg genistein/kg bw/day), ovarian cycle abnormalities and increased expression of progesterone receptor (observed at 10-30 mg 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. The most important effects include increased uterine weight, advanced vaginal opening, irregular oestrus cycles, changes in uterine and vaginal epithelium, changes in body and organ weights as well as behavioural changes such as decreased social interactions, increased mobility or frequencies of aggressive behaviour. 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.

79. 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.

80. Observations in children fed soya-based formula as infants were: increased vaginal cell maturation, higher prevalence of breast buds and risk of menarche,

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

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 soya 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 soya isoflavones.

81. 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 0.0065 mg/kg bw/day. Isoflavone exposure from infant formula for exclusively cows' formula-fed infants was up to 0.057 mg/kg bw/day. The highest exposure was estimated for infants exclusively fed soya-based infant formula reaching values up to nearly 9.5 mg/kg bw/day. Limited information is available regarding infants' exposure to isoflavones from complementary foods, but it is potentially in the range of 0.1 – 1.7 mg/kg bw/day.

82. The oestrogenicity of isoflavones and their influence on reproductive organs have been identified as the main concern in relation to soya formula consumption by infants. Other potential concerns are effects of soya 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 soya formula, as aglucone equivalents, is formed by genistein. Therefore estimated isoflavone exposure from consumption of soya 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 soya 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. The duration of infant formula feeding and its exclusive character may additionally increase risk of health effects, such as developmental and reproductive abnormalities. It should be noted that some of those effects could be unnoticed for many years and present themselves only in later life.

Conclusions

83. Infants can be exposed to soya isoflavones mainly through consumption of breast milk, soya-based infant formula and complementary infant feeding products containing soya.

84. Soya 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 soya isoflavones.

85. The COT concluded that it was not possible to propose health-based guidance values for infants. Reasons for this included the difficulty in extrapolation from animals to humans because of differences in toxicokinetics, uncertainty with respect to differences between adults and infants (particularly those arising from

development of the gut microflora), and the lack of dose-response data and the role of possible confounders in the available human studies. It was noted that the pig was the best animal model with respect to toxicokinetics, but few toxicity data were available for pigs.

86. The COT concluded that the evidence from the small number of epidemiological studies was not suggestive of important impacts of soya formula infant feeding on reproductive health and development. Four studies raised the possibility of minor subtle effects of uncertain clinical significance (early life breast development and minor differences in menarche/menstruation, gender related play behaviour) but the findings were not conclusive and may have been due to chance or issues with study design. Impacts have only been assessed to early adulthood, not to later life (e.g. cancers of reproductive organs).

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.

Agostoni C, Fiocchi A, Riva E, Terracciano L, Sarratud T, Martelli A, Lodi F, D'Auria E, Zuccotti G, Giovannini M (2007) Growth of infants with IgE-mediated cow's milk allergy fed different formulas in the complementary feeding period. *Pediatric allergy and immunology* **18**(7):599-606.

Alston-Mills B, Lepri JJ and Martin CA (2011) Modulation of mammary gland development in pre-pubertal mice as affected by soya and milk protein supplements. *Br J Nutr* **106**(4):502-9.

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

Andres A, Cleves MA, Bellando JB, Pivik RT, Casey PH and Badger TM (2012) Developmental status of 1-year-old infants fed breast milk, cow's milk formula, or soy formula. *Pediatrics* **129**(6):1134-40

Andres A, Casey PH, Cleves MA and Badger TM (2013) Body fat and bone mineral content of infants fed breast milk, cow's milk formula, or soy formula during the first year of life. *The Journal of Pediatrics* **163**(1):49-54

Atkinson C, Newton KM, Aiello Bowles EJ, Lehman CD, Stanczyk FZ, Westerlind KC, Li L, Lampe JW (2009) Daidzein-metabolizing phenotypes in relation to mammographic breast density among premenopausal women in the United States. *Breast Cancer Res Treat* **116**:587-594

Badger TM, Gilchrist JM, Pivik RT, Andres A, Shankar K, Chen JR, Ronis MJ (2009) The health implications of soy infant formula. *The American Journal of Clinical Nutrition* **89**(suppl):1668S-72S.

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.

Ball ER, Caniglia MK, Wilcox JL, Overton KA, Burr MJ, Wolfe BD, Sanders BJ, Wisniewski AB and Wrenn CC (2010) Effects of genistein in the maternal diet on reproductive development and spatial learning in male rats. *Hormones and behaviour* **57**(3): 313-22

Bateman HL, Patisaul HB (2008) Disrupted female reproductive physiology following neonatal exposure to phytoestrogens or estrogen specific ligands is associated with decreased GnRH activation and kisspeptin fiber density in the hypothalamus. *NeuroToxicology* **29**:988-997

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.

Blake C, Fabick KM, Setchell KDR, Lund TD and Lephart ED (2011) Neuromodulation by soy diets or equol: anti-depressive & anti-obesity-like influences, age & hormone-dependent effects. *BMC Neuroscience* **12**:28

Boucher BA, Cotterchio M, Kreiger N, Thompson LU (2008) Soy formula and breast cancer risk. *Epidemiology* **19**(1):165-166

Branham WS, Dial SL, Moland CL, Hass BS, Blair RM, Fang H, Shi L, Tong W, Perkins RG, Sheehan DM (2002) Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. *Biochemical and Molecular Action of Nutrients* **132**:658-664

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

Cederroth CR, Zimmermann C, Beny JL, Schaad O, Combepine C, Descombes P, Doerge DR, Pralong FP, Vassalli JD and Nef S (2010) Potential detrimental effects of a phytoestrogen-rich diet on male fertility in mice. *Molecular and Cellular Endocrinology* **321**(2):152-160

Chen A, Berhow MA, Tappenden KA, Donovan SM (2005) Genistein inhibits intestinal cell proliferation in piglets. *Pediatric Research* **57**(2):192-200.

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.

Cicero AF, Derosa G and Arletti R (2004) Effect of oral chronic isoflavones supplementation on male rat sexual performances and sexual hormone plasma levels. *Phytotherapy research* **18**:849-852

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.

De Lima Toccafondo Vieira M, Ferreira Duarte R, Moreira Campos LM, de Aguiar Nunan E (2008) Comparison of the estrogenic potencies of standardized soy extracts by immature rat uterotrophic bioassay. *Phytomedicine* **15**:31-37

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

Dewi FN, Wood CE, Lees CJ, Wilson CJ, Register TC, Tooze JA, Franke AA, Cline JM (2013) Dietary soy effects on mammary gland development during the pubertal transition in nonhuman primates. *Cancer Prev Res (Phila)* **6**(8):832-42.

De Wilde A, Rassi CM, Cournot G, Colin C, Lacroix HC, Chaumaz G, Coxam V, Bennetau-Pelissero C, Pointillart A, Lieberherr M (2007) Dietary isoflavones act on bone marrow osteoprogenitor cells and stimulate ovary development before influencing bone mass in pre-pubertal piglets. *Journal of Cellular Physiology* **212**:51-59

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

Doerge DR, Twaddle NC, Banks EP, Jefferson WN, Newbold RR (2002) Pharmacokinetic analysis in serum of genistein administered subcutaneously to neonatal mice. *Cancer Letters* **184**(1):21-27

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.

Ford JA, Clark SG, Walters EM, Wheeler MB, Hurley WL (2006) Estrogenic effects of genistein on reproductive tissues of ovariectomised gilts. *Journal of Animal Science* **84**:834-842

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.

Frankenfeld CL, McTiernan A, Tworoger SS, Atkinson C, Thomas WK, Stanczyk FZ, Marcovina SM, Weigle DS, Weiss NS, Holt VL, Schwartz SM, Lampe JW (2004) Serum steroid hormones, sex hormone-binding globulin concentrations, and urinary hydroxylated estrogen metabolites in post-menopausal women in relation to daidzein-metabolizing phenotypes. *The Journal of Steroid Biochemistry and Molecular Biology* **88**:399-408

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

Gilani GS, Farmer C, Dyck M, Robertson P, Dahiya J, Sepehr E, Fan L, Nicolidakis H, Curran I (2011) Distribution of isoflavones in samples of serum, liver and mammary glands of rats or pigs fed dietary isoflavones. *Annals of Nutrition and Metabolism* **58**:171-180

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.

Golub MS, Hogrefe CE, Germann SL, Tran TT, Beard JL, Crinella FM, Lonnerdal B (2005) Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese. *Neurotoxicology and Teratology* **27**:615-627

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.

Harris HA, Bapat AR, Gonder DS, Frail DE (2002) The ligand binding profiles of estrogen receptors α and β are species dependent. *Steroids* **67**:379-384

Hartley DE, Edwards JE, Spiller CE, Alom N, Tucci S, Seth P, Forsling ML and File SE (2003) The soya isoflavone content of rat diet can increase anxiety and stress hormone release in the male rat. *Psychopharmacology* **167**(1):46-53.

Hertrampf T, Schleipen B, Offermanns C, Velders M, Laudenbach U, Diel P (2009) Comparison of the bone protective effects of an isoflavone-rich diet with dietary and subsutaneous administrtions of genistein in ovariectomized rats. *Toxicology Letters* **184**:198-203

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.

Klemola T, Vanto T, Juntunen-Backman K, Kalimo K, Korpela R, Varjonen E (2002) Allergy to soy formula and to extensively hydrolysed whey formula in infants with cow's milk allergy: a prospective, randomized study with a follow-up to the age of 2 years. *The Journal of Pediatrics* **140**(2):219-24.

Klemola T, Kalimo K, Poussa T, Juntunen-Backman K, Korpela R, Valovirta E, Vanto T. (2005) Feeding a soy formula to children with cow's milk allergy: the development of immunoglobulin E-mediated allergy to soy and peanuts. *Pediatric Allergy and Immunology* **16**(8):641-6.

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.

Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. *Endocrinology* **138**(3): 863-870.

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology* **139**(10):4252-4263.

Kwok KC, Cheung NH (2010) Measuring binding kinetics of ligands with tethered receptors by fluorescence polarization and total internal reflection fluorescence. *Analitical Chemistry* **82**:3819-3825.

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

Liang S, Byers DM, Irwin LN (2008) Sex and diet affect the behavioural response of rats to chronic mild stressors. *Physiology and behaviour* **93**:27-36

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

Matsumura A, Ghosh A, Pope GS, Darbre PD (2005) Comparative study of oestrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology* **94**:431-443.

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

Merritt RJ, Jenks BH (2004) Safety of soy-based infant formulas containing isoflavones: the clinical evidence. *The Journal of Nutrition* **134**:1220S-1224S.

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.

Mosquette R, de Jesus Simoes M, da Silva IDCG, Oshima CTF, Oliveira-Filho RM, Haidar MA, Simoes RS, Baracat EC, Junior JMS (2007) The effects of soy extract on the uterus of castrated adult rats. *Maturitas* **56**:173-183.

Mueller SO, Simon S, Chae K, Metzler M, Korach KS (2004) Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor α (ERα) and ERβ in human cells. *Toxicological Sciences* **80**:14-25.

Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, Katzenellenbogen BS, Helferich WG, Katzenellenbogen JA (2004) Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. *Bioorganic and Medicinal Chemistry* **12**:1559-1567.

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.

Patisaul HB, Luskin JR and Wilson ME (2004) A soy supplement and tamoxifen inhibit sexual behaviour in female rats. *Hormones and behaviour* **45**(4):270-277

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

Sato T, Shinohara Y, Kaneko D, Nishimura I and Matsuyama A (2010) Fermented soymilk increases voluntary wheel running activity and sexual behaviour in male rats. *Appl. Physiol. Nutr. Metab*. **35**:749-754

Schmidt S, degen GH, Seibel J, Hertrampf T, Vollmer G, Diel P (2006) Hormonal activity of combinations of genistein, bisphenol A and 17β-estradiol in the female Wistar rat. *Arch Toxicol* **80**:839-845.

Seppo L, Korpela R, Lonnerdal B, Metsaniitty L, Juntunen-Backman K, Klemola T, Paganus A, Vanto T (2005) A follow-up study of nutrient intake, nutritional status, and growth in infants with cow milk allergy fed either a soy formula or an extensively hydrolysed whey formula. *The American Journal of Clinical Nutrition* **82**:140-5

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

Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS and Walker M (2002) Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. *Human Reproduction* **17**(7):1692-1703

Simon NG, Kaplan JR, Hu S, Register TC and Adams MR (2004) Increased aggressive behaviour and decreased affiliative behaviour in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones. *Hormones and Behavior* **45**(4):278-284

Song KB, Atkinson C, Frankenfeld CL, Jokela T, Wahala K, Thomas WK, Lampe JW (2006) Prevalence of daidzein-metabolizing phenotypes differs between Caucasian and Korean American women and girls. *The Journal of Nutrition* **136**:1347-1351.

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

Stettler N, Stallings VA, Troxel AB, Zhao J, Schinnar R, Nelson SE, Ziegler EE, Strom BL. (2005) Weight gain in the first week of life and overweight in adulthood. *Epidemiology* **111**:1897-1903.

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.

Thigpen JE, Haseman JK, Saunders HE, Setchell KDR, Grant MG, Forsythe DB (2003) Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. *Comparative Medicine* **53**(6):607-615.

Thigpen JE, Setchell KDR, Padilla-Banks E, Haseman JK, Saunders HE, Caviness GF, Kissling GE, Grant MG, Forsythe DB (2007) Variations in phytoestrogen content between different mill dates of the same diet produces significant differences in the time of vaginal opening in CD-1 mice and F344 rats but not in CD Sprague-Dawley rats. *Environmental Health Perspectives* **115**(12):1717-1726.

Thomsen AR, Mortensen A, Breinholt VM, Lindecrona RH, Penalvo JL, Sorensen IK (2005) Influence of Prevastein, an isoflavone-rich soy product, on mammary gland development and tumorigenesis in Tg.NK (MMTV/c-neu) mice. *Nutrition and Cancer* **52**(2):176-88.

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

Vergne S, Sauvant P, Lamothe V, Chantre P, Asselineau J, Perez P, Dyrand M, Moore N, Bennetau-Pelissero C (2009) Influence of ethnic origin (Asian v. Caucasian) and background diet on the bioavailability of dietary isoflavones. *British Journal of Nutrition* **102**:1642-1653.

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.

Zhao L, Mao Z, Diaz Brinton R (2009) A select combination of clinically relevant phytoestrogens enhances estrogen receptor β-binding selectivity and nauroprotective activities in vitro and in vivo. *Endocrinology* **150**:770-783.

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)
AGD	anogenital distance
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
BW	body weight
CERHR	Center for the Evaluation of Risks to Human Reproduction
CI	confidence interval
COT	Committee on Toxicity
DH	Department of Health
DHEA	dehydroepiandrosterone
DMBA	7,12-dimethylbenz[a]anthracene
DTH	delayed-type hypersensitivity
EE	ethinyl oestradiol
ER	oestrogen receptor
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FDA	Food and Drug Administration
FSA	Food Standards Agency
GD	gestational day
GI	gastrointestinal
HIM	high isoflavone levels milk
HR	hazard ratio
IGF-1	insulin-like growth factor-1
IQR	interquartile range
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
MDI	Mental Developmental Index
MN	micronuclei
MOF	multi-oocyte follicles
NOAEL	no-observed-adverse-effect level
NOF	natural ovarian failure
NS	not significant
NTP	National Toxicology Program
O-DMA	*O*-demethylangolensin
PDI	Psychomotor Development Index
PLS-3	Preschool Language Scale-3
PND	postnatal day

PR	progesterone receptor
PSAI	Pre-School Activities Inventory
RBA	Relative Binding Affinity
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
TPO	thyroperoxidase
TSH	thyroid stimulating hormone
TVP	textured vegetable protein
UDP	urine diphosphate

Appendix 1
Search strategy

General isoflavones/genistein/daidzein exposure search

Websites interrogated

- EFSA
- COT
- FSA
- JECFA

Scientific publications literature search in PubMed

Specific search terms:

Isoflavone/phytoestrogens/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

Soya/soya formula/phytoestrogens AND animals AND diet AND oestrogenic effect
Search Dates (From May 2002 to present)
Exclusion Criteria:

- Studies in pre-menopausal females
- *In vitro* studies

Soya/soya formula/phytoestrogens AND animals AND diet AND behaviour
Search Dates (From May 2002 to present)
Exclusion Criteria:

- Studies where levels of isoflavones in the treatment are not given
- Studies where ways of administration are other than oral

Soya/soya formula/phytoestrogens AND animals AND diet AND reproduction
Search Dates (From May 2002 to present)*
*Some papers pre-May 2002 were included if they added value to the paper, particularly if studies published after May 2002 were sparse
Exclusion Criteria:

- Studies in pre-menopausal women and mature animals
- *In vitro* studies
- Studies performed in lower organisms, sheep and rabits
- Studies in undeveloped countries
- Co-treatment with other chemicals
- Studies investigating therapeutic effect of soya in relation to other diseases

Soya/soya formula/phytoestrogens 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 additional publications and latest government advice and opinions.

Secretariat
September 2013