Review of potential risks from high levels of soy phytoestrogens in infant diet

Introduction

1. The COT has been asked to consider aspects related to the toxicity of chemicals in the infant diet, in support of a review by the Scientific Advisory Committee on Nutrition (SACN) of Government recommendations on complementary and young child feeding. An initial paper (TOX/2012/03), highlighting some of the areas requiring consideration was discussed by the COT in February, 2012. Members agreed that a comprehensive review of soy phytoestrogens in infant diet would be prepared as a substantial amount of new evidence had become available since the COT report on phytoestrogens and health was published in May 2003. In this paper, Members are invited to comment on the new information and advise on any toxicological implications for the health of infants.

Background

2. Phytoestrogens are chemicals of plant origin that have been shown to influence biological functions mainly due to their structural similarities to oestrogens, and ability to bind to oestrogen receptors (ERs). The largest group of phytoestrogens is formed by flavonoids, which can be further divided into three subclasses, coumestans, prenylated flavonoids and isoflavones. Isoflavones occur in a limited number of plants of which soy beans seem to be the richest source containing approximately 1 mg of isoflavones per gram fresh weight. Other foods with high amounts of total isoflavones are flaxseed, pistachios and walnuts and products that contain them (Thompson et al., 2006). Fletcher et al. reported concentrations of isoflavones in soy beans to be between 560 and 3810 mg/kg, in soy protein concentrates and isolates derived from soy beans: 466 – 615 mg isoflavones/kg, and in products such as soy milk, bean sprouts and bean curds: between 13 to 2030 mg isoflavone/kg (Fletcher, 2003).

3. Soy-based infant formula and weaning food products containing soy are the main source of isoflavone exposure in newborns and infants. This developmental stage of life is associated with low endogenous oestrogen

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levels and historically low exposure to isoflavones (Cooke et al., 2006). The Committee has recognised a need to re-evaluate new publications on the possible risks associated with the consumption of soy formula milk and soy-based weaning products by infants. Therefore the most prevalent dietary soy isoflavones, genistein, daidzein and glycitein, have been chosen for consideration in the current paper.

4. The isoflavones, genistein, daidzein and glycitein (Fig.1) share a common structure consisting of two aromatic rings linked through three carbons forming part of an oxygenated heterocyclic ring. The phenolic ring and two hydroxyl groups (and the distance between them) are key structural similarities between isoflavones and 17β-oestradiol that allow them to bind to oestrogen receptors (Fig.2).

5. Most of the isoflavones found in foods exist as glucoside conjugates (Fig.1). To become biologically active β-glucosidic bonds of glucosides must be hydrolysed e.g. during fermentation (Telang et al., 2010). There is only a small proportion of the active forms, known as aglucones, present in blood – 1-5% of the total isoflavones (Barnes, 2010).

Figure 1. Chemical structures of the isoflavone glucoside: genistin and aglucones: genistein, daidzein and glycitein.
Current UK Government recommendations in relation to infant diet

6. The COT report (2003) identified a number of population subgroups for whom the evidence suggested higher intake of phytoestrogens than the average consumer. This included vegetarians/vegans, consumers of dietary supplements or soy-rich diets and infants fed soy-based infant formula, the latter being likely to have the highest exposure to isoflavones (approximately 4 mg/kg bw/day). As the report provided evidence of potential health implications, the COT and the SACN expressed concerns about the use of soy-based formula. The SACN concluded that there was no substantive medical need for, nor health benefit arising from, the use of soy-based infant formulas.

7. Based on the COT and SACN advice, the Department of Health’s Chief Medical Officer in 2004 advised doctors that soy-based infant formulas should not be used as the first choice for the management of infants with proven cows’ milk sensitivity, lactose intolerance, galactokinase deficiency and galactosaemia and they should only be used in exceptional circumstances to ensure adequate nutrition (DH, 2004).

Recommendations in other countries

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 soy protein may help reduce heart disease risk. In correspondence to the FDA, Sheehan and Doerge expressed concern that the general public should also be aware of potential risks associated with isoflavones: toxicity in oestrogen sensitive tissues and possible higher sensitivities at some ages (Sheehan and Doerge, 1999).

9. In 2005 in Israel, Berger-Achituv et al. (2005) stressed that long-term effects of soy consumption on infants’ health were still unknown and the use of soy-based formula should be reserved for limited medical indications.

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(Berger-Achituv et al., 2005). The Israeli Health Ministry examined available evidence of soy consumption on adverse health effect related to fertility, thyroid functions and cancer risk and recommended to minimise consumption of soy foods in young children, and avoid it, if possible, in infants. Soy-based formula consumption in Israel is among the world’s highest per capita and the Ministry stated that babies should be fed with soy formula only as a last resort. Also day care centres and schools were advised to limit soy foods to no more than one serving per day and no more than three times per week (Siegel-Itzkovich, 2005).

10. In 2006 the French Food Safety Agency (AFSSA) recommended that food manufacturers should decrease the amount of isoflavones in soy infant formula to 1 ppm and introduce warning labels for customers. Soy consumers should be alerted to soy-related risk for children under three, children with hypothyroidism and women with a history of breast cancer (AFSSA, 2005).

11. In Germany soy food was found to be not suitable as a diet for healthy infants. The German Federal Institute for Risk Assessment (BfR) warned that soy-based infant formula should be only given in exceptional cases and following doctors recommendation due to no proven health benefits and possible health risks (BfR, 2007).

12. The American Academy of Pediatrics (AAP) considers soy protein-based formula as having no advantage over breastfeeding and cows’ milk-based formula unless infants have an indication for its use such as: galactosaemia, hereditary lactase deficiency and secondary lactose intolerance or preferences for a vegetarian diet. Infants with cows’ milk protein allergy are very likely to be also sensitive to soy protein and a specialised hydrolysed formula should be given (Bhatia and Greer, 2008). Soy protein-based formula could be given from the age of 6 months when infant’s organs are more mature and tolerance to soy protein could be established (Turck, 2007). Similar conclusions were reached by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Agostoni et al., 2006).

Absorption, distribution, metabolism and excretion

Absorption

13. 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).

14. Setchell et al. examined bioavailability of equol, which unlike the soy isoflavones genistein and daidzein, has a chiral centre and can occur as two diastereoisomers S- and R-equol. They established S-equol as the exclusive product of human intestinal bacterial synthesis found in human plasma and urine. S-equol has a high affinity for ER\(\beta\) (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 effects of orally administered genistin – the glucosylated form of genistein – have been investigated in mice. On postnatal days (PNDs) 1-5, Jefferson et al. dosed female mice oral genistin (6.25, 12.5, 25, or 37.5 mg/kg bw/day expressed as genistein equivalent doses), oral genistein (25, 37.5, or 75 mg/kg bw/day) or subcutaneous (sc) genistein (12.5, 20, or 25 mg/kg bw/day). Oral doses were administered using a pipette inserted inside pup’s mouth. The total and aglucone levels of genistein measured in serum as the dose-adjusted area under the curve (AUC) after oral exposure to genistin were 83% (total genistein) and 48% (as aglucones) of sc genistein (treated as 100%). Following oral dosing of genistein the bioavailability was lower, at 12 and 15%, respectively. Oral exposure to genistin resulted in oestrogenic activity in female mouse neonates similar to the response to sc genistein. The authors concluded that oral genistin, being predominantly found in most soy products including soy infant formula is rapidly hydrolysed and absorbed into the neonatal circulation in mice (Jefferson et al., 2009).

**Modulation of absorption**

16. 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).

17. Cassidy et al. (2006) observed that the type of food matrix affects the bioavailability of isoflavones in healthy adults, premenopausal (n=21, age: 18-53) and postmenopausal (n=17, age: 48-69) women and men (n=21, age: 18-55). In this random crossover trial three soy foods having different isoflavone composition: soy milk, textured vegetable protein (TVP) and fermented soy product - tempeh were studied. The proportion of aglucones (genistein and daidzein combined together) was <15% total isoflavones when samples of soy milk and TVP were analysed. Tempeh contained greater proportion of aglucones, approximately 50%. As stomach emptying occurs later after ingestion of solid foods, differences in time ($t_{\text{max}}$) needed to reach peak serum isoflavone concentration ($C_{\text{max}}$) were observed. $C_{\text{max}}$ of genistein and daidzein were reached 2 hours later for TVP comparing to soy milk ($t_{\text{max}} = 5.5 – 6.6$ h). Bioavailability of both isoflavones, was assessed by area under the concentration-time curve (AUC$_{(0-t)}$) reflecting the exposure of serum to isoflavone from time zero to time $t$ when the serum concentration returned to baseline (AUC$_{(0-t)}$ unit: µmol·h/L·mg dose) and presented as mean (SD). After consumption of soy milk serum total genistein concentrations were in a range from 50.01 (21.31) in premenopausal women to 54.06 (32.68) in postmenopausal women and for total daidzein: 22.08 (9.25) in men – 28.94 (10.02) in postmenopausal women. Levels detected after consumption of TVP were significantly lower: 18.35 (6.42) in premenopausal women to 22.98 (14.12) in men for total genistein and 15.28 (3.76) in premenopausal women to 16.29 (4.65) in men for total daidzein. When tempeh was ingested, the AUC$_{(0-t)}$ for serum total genistein and daidzein [26.91 (13.50) in men to 35.02 (14.52) in postmenopausal women and 19.79 (7.87) in men to 33.99 (16.66) in postmenopausal women, respectively] values were higher compared to TVP. The values were adjusted to take into account the differences in the proportion of isoflavones within each food. As such the values presented are adjusted to mg of each isoflavone ingested per kg bw. The soy milk, TVP rolls and tempeh provided an intake equivalent to 0.44 mg isoflavones/kg bw/day. It was concluded that consumption of tempeh resulted in higher serum peak levels of genistein and daidzein, compared with TVP. However, soy milk was absorbed faster and peak levels of isoflavones were attained earlier than with the other soy foods (Cassidy et al., 2006).

18. Effects of supplementation of soy milk with probiotics, such as *Lactobacillus sp* and *Bifidobacterium sp*, and prebiotics (e.g. fructooligosaccharides, inulin, pectin or mannitol) were investigated in vitro by Yeo and Liong (2010). Prebiotics increased the viability of probiotics and enhanced β-glucosidase activity. As a result an enhanced bioconversion of glucosides to bioactive aglucones, especially genistin and malonyl genistin to genistein, was observed (Yeo and Liong, 2010).

19. Absorption of isoflavones may be also modulated by age although results of different studies are not conclusive. Bioavailability of isoflavones in human volunteers was determined in a study in which urinary excretion rates
were measured after consumption of soy nuts (0.28 g nuts/kg bw, equivalent to 0.615 ± 0.036 mg total isoflavones/kg). In school age children (n=19, age: 3-17) the urinary excretion rate was statistically significantly higher for daidzein (+39%), genistein (+44%), all non-metabolites (daidzein + genistein + glycitein) (+41%) and total isoflavones (+32%), when compared to adults (n=18) (Halm et al., 2007). In a study performed by Cassidy et al. (data presented in Paragraph 17) age and gender did not produce significantly different results and the data showed that pre- and postmenopausal women as well as men absorb isoflavones from a range of different soy-rich foods in a similar manner. However, when soy milk and tempeh were analysed, the bioavailability of genistein and daidzein, measured as AUC(0-t), was slightly increased in the postmenopausal group. Also higher $t_{1/2}$ for the elimination of isoflavones was observed in this group, compared to younger women. The authors concluded that genistein concentrations exceeded daidzein concentrations in serum and higher levels of isoflavones were detected in women and that there was no major influence of age. However children were not included in this study (Cassidy et al., 2006).

20. Franke et al. suggested that maturity of gut flora related to age as well as type of food may determine isoflavone uptake ability in infants and children (Franke et al., 2006). In their previous publication the authors had considered isoflavone glucuronides and sulphates (as present in breast milk of mothers eating soy) to be more available to infant than the β-glucoside conjugates (as in soy food), as they require mainly intestinal bacteria for hydrolysis and are less available in infants due to the immature gut flora (Franke and Custer, 1996). However, another study provided conflicting results where low isoflavone values were observed in body fluids of breast fed infants, whereas higher levels, exceeding those observed in adults eating soy products, were reported in weaning infants consuming tofu. Isoflavone levels measured in urine and plasma as well as concentrations of isoflavones in tofu and mothers’ diet, are presented in Paragraph 24. The authors commented that this finding was probably due to the very low isoflavone dose, but also to the lower ability of the nonmatured gut flora in breastfed infants (n=18; age: 2-45 weeks) 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 (9, 10 and 24 months; n=3) and it is possible that their gut flora attained ability to hydrolyse β-glucosides efficiently (Franke et al., 2006).

Distribution, metabolism and excretion

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

22. Excretion of isoflavones is rapid and occurs in approximately 24 hours. Hoey et al. investigated the urinary excretion of soy isoflavones and their metabolites in infants who had been fed soy-based infant formula (isoflavone content presented in Table 2) in early infancy. Subjects who had consumed cows’ milk formula as babies and who subsequently were not given soy products, were recruited as controls. Control group infants (>6 months only) were given a soy isoflavone challenge (soy yoghurt alternative product containing 4.8 g soy protein and on average 22 mg total isoflavones) to establish whether they were capable of converting daidzein to equol and/or O-DMA. It was shown that 4-6 month old infants (n=7) fed soy-based infant powder formula had significantly higher urinary concentrations of isoflavones compared to a control group (n=7). Genistein and daidzein were present in urine at mean (SD) concentrations 65 (53) and 60 (32) µg/mg creatinine (Cr), respectively. Glycitein levels were considerably lower: 15 (7) µg/mg Cr. The metabolites, O-DMA, 8 (16) and equol 0.05 (0.10) µg/mg Cr were detected in 75 and 25% of infants, respectively. In the control group (aged 4-6 months) only glycitein and O-DMA were detected, both at mean (SD) concentrations 0.02 (0.04) µg/mg Cr. Urine samples of 7-12 month old infants (n=7) fed soy-based infant formula had the mean (SD) values of genistein, daidzein, glycitein, O-DMA and equol as follows: 13 (12), 14 (10), 4 (3), 1 (2) and 0.03 (0.06) µg/mg Cr. In the control group receiving soy challenge these concentrations were respectively: 41 (53), 36 (38), 0.2 (0.2), 0.9 (0.2) µg/mg Cr and equol – not detected. In this older age group, O-DMA was detected in 75% (soy group) and 50% (control group) and equol in 19% (soy group) and 5% (control group) of samples. This study confirmed excretion of isoflavones and their effective absorption in infants suggesting that the hydrolytic ability develops by or before 4-6 months of age. In contrast, majority of older infants did not seem to be able to convert daidzein to equol, which indicates that there is no lasting effect of early life isoflavone exposure on isoflavone metabolism (Hoey et al., 2004).

23. Cao et al. measured genistein, daidzein and equol in urine, saliva and blood of infants (age: <2 days – 48 weeks) fed exclusively (although in older children relatively small amounts of other feedings were allowed) with breast milk (n=129), cows’ milk (n=128) formula or soy formula (n=125). Levels of isoflavones in each type of food were not measured. Most blood or saliva samples obtained from breast milk and cows’ milk-fed infants had undetectable genistein and daidzein concentration values. Based on 75th percentiles (medians were below the limit of detection, LOD) urine samples collected from infants fed cows’ milk formula had 6 to 10 time higher concentrations of genistein and daidzein when compared to breastfed children. In soy formula fed infants genistein and daidzein were above the LOD in at least 91 and 83% of blood and saliva samples, respectively, and in all urine samples. Infants fed soy formula, comparing to cows’ milk-fed group,
had 500 times higher median urinary concentrations of isoflavones. Concentrations of equol were below the LOD in 100% of blood and saliva samples and in 91% of urine samples in all three feeding groups. Limits of detection for genistein, daidzein and equol were 0.8, 1.6 and 3.3 ng/ml (in urine); 27, 10 and 12 ng/ml (in blood) and 1.4, 0.8 and 3.6 ng/ml (in saliva), respectively. These results indicate limited bacterial transformation in infants as equol was rarely detected. The authors did not find significant correlations between isoflavone concentrations and the levels of certain hormones in children fed soy formula (Cao et al., 2009). Limited detection of equol was also observed in plasma and urine samples of infants examined by other researchers in previous studies (Franke et al., 2006; Setchell et al., 1997) where high concentrations of genistein and daidzein were observed in plasma samples from infants fed soy-based formula (Setchell et al., 1997).

24. Franke et al. obtained urine samples from 7 mother-infant pairs and significant increases in isoflavone levels were observed in both, mothers and their children after soy protein beverage consumption by the mother. One daily serving of the soy beverage (55 mg isoflavones; daidzein:genistein:glycitein = 1:1:0.1) was consumed for 2-4 days. The mean (SE) values before and after the intervention were 18.4 (13.0) and 135.1 (26.0) nmol/mg Cr in mothers’ urine and 29.8 (11.6) and 111.6 (18.9) nmol/mg Cr for infants, respectively. When additional participant pairs, where mothers were consuming soy, provided urine samples, results were similar: 157.1 (18.5) nmol/mg Cr for mothers (n=9) and 186.1 (25.1) nmol/mg Cr for infants (n=5). In infants’ plasma mean isoflavone levels of 19.7 nmol/L (median: 2.5 and range: 0.2 – 148.5 nmol/L) were reported. In samples from 3 children aged 9-25 months consuming tofu (44 g tofu ~ 7.4 mg isoflavones), much higher mean values of isoflavones were observed: 299 nmol/mg Cr in urine (median: 145 and range: 61 – 482 nmol/mg Cr) and 1048.6 nmol/L in plasma (median: 663.1 and range: 629.1 – 1853.6 nmol/L). Although glycine values in breast milk and infants’ plasma were negligible, its contribution to the total isoflavone concentration in mothers’ and infants’ urine samples was 9 and 17%, respectively. Therefore, it appears that isoflavones in mothers’ diet are transferred to babies via breast milk, reaching similar systemic exposures to those of the mothers. However, the highest values are measured in infants after consumption of fermented soy products, such as tofu (Franke et al., 2006).

25. Irvine et al. examined urinary excretion of isoflavones by infants. No isoflavones were detected in the urine of infants fed cows’ milk-based formula (n=25). However, all infants fed soy formula (n=4) regardless of age had mean (SD) daidzein and genistein concentrations: 2.9 (0.3) mg/L and 1.5 (0.2) mg/L, respectively and daily excretion rates in the range of 0.37 (0.03) to 0.58 (0.06) mg/kg bw/day for daidzein and 0.15 (0.03) to 0.32 (0.04) mg/kg bw/day for genistein with the mean % of the daily isoflavone intake recovered in the urine of 38 ± 4% and 13 ± 3%, respectively. Isoflavones present in soy-based formulas were shown to be digested, absorbed and excreted by children as effectively as in adults (Irvine et al., 1998).
26. 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 (limit of quantification, LOQ: 0.5 ng/ml). The authors observed that amniotic fluid samples from women pregnant with female fetuses who reported the use of soy products were found to have significantly higher concentrations of both isoflavones compared to male pregnancies. Amniotic fluid genistein and daidzein concentrations from male pregnancies were 54 and 48% (0.55 ± 0.10 and 0.41 ± 0.07 ng/ml) of the concentrations from female pregnancies (1.04 ± 0.19 and 0.85 ± 0.16 ng/ml) respectively. 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 both, male and female infants had similar birth weights. The authors suggested that there may be a different metabolic handling of isoflavones during fetal life (Jarrell et al., 2012).

27. Gu et al. compared the isoflavone metabolic phenotype (as ability to produce equol from daidzein) of women consuming diet formulated with soy protein isolate (SPI) with that of experimental animals. Female monkeys (n=15) had a serum profile of equol more similar to that of female Sprague-Dawley rats (n=9), than to that of women (n=10). Rats and monkeys appeared to have intestinal bacterial composition favouring equol biosynthesis (77 and 52% of summed isoflavones measured in serum), whereas serum concentrations of equol in women and pigs (n=5) were undetectable and genistein and daidzein contributed to 88 and 91% of summed isoflavones, respectively. Monkey and rat urine contained high levels of aglucones (>85% and >32%, respectively), whereas pigs and women excreted isoflavone mainly in the form of glucuronides (>80%), with <1% as aglucones. The authors noted that the metabolic profile of pigs was closer to that of women than that of rats or monkeys suggesting that pigs may be a better animal model for studying the effects of isoflavones in humans (Gu et al., 2006).

28. Circulating concentrations of unconjugated isoflavones in rodents and humans were also compared by Setchell et al. The mean (SE) steady state plasma concentrations expressed as ng/mL [and unconjugated fractions as a proportion of total (%)] of unconjugated genistein in the plasma of Sprague-Dawley rats and three strains of mice: C57BL/6 strain, the athymic (nude) mouse and transgenic AngptL4B6 mouse were: 6 (2) [4.0 ± 0.6%], 7 (1) [4.6 ± 0.6%], 35 (4) [11.6 ± 0.9%] and 52 (13) [30.1 ± 4.3%], respectively. For unconjugated daidzein the values were as follows: 7 (2) [8.1 ± 1.1%], 14 (3) [7.4 ± 0.7%], 63 (7) [16.1 ± 1.2%] and 52 (13) [32.7 ± 4.7%], respectively. All rodents had been maintained on the soy containing Purina 5008 or 5010 diet, but the concentrations of isoflavones were not provided. These concentrations were several fold higher than those in human plasma (n=20): 0.46 (0.12) for unconjugated genistein and 0.61 (0.15) for unconjugated daidzein (the mean percentages of genistein/daidzein were 0.2 ± 0.05/1.1 ± 0.02% in steady state). Human adults consumed 250 mL soy milk in the morning and evening for 3.5 days containing 9.5 mg genistein and 3.5 mg daidzein expressed as aglucone equivalents. Aglucone equivalents are glucosides converted on the basis of molecular weight and are used in calculating the exposure to a particular isoflavone. The total exposure is calculated as the sum of aglucones.
and aglucone equivalents. E.g. 1 unit of genistein is equal to 0.6 aglucone equivalents, based on the molecular mass ratio of genistein to genistin (270.24/432.38) (NTP, 2010). 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).

29. Although several studies investigating isoflavone distribution and metabolism in infants have been published since the previous COT review (COT, 2003), the amount of information on how these substances are handled in the newborns and infants is still limited. It has been confirmed that increased bioavailability of isoflavones can be expected when they are ingested as aglucones and that the gut microflora as well as diet greatly influence the exposure to ingested isoflavones. Infants can effectively absorb isoflavones from breast milk, soy infant formula and weaning food products containing soy and eventual differences when compared to adults are due to the maturity of the intestinal flora and/or larger intake when adjusted for body weight. Food matrix and age can also be determining factors. Transfer of isoflavones and their metabolites to body fluids as well as breast milk and foetal compartment have been shown. 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). Further information on these metabolic differences has been generated.

**Hazard identification and characterisation**

**In vitro studies**

30. *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 peroxidise 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. Although some genotoxic effects of isoflavones were also reported *in vitro*, the concentrations used were much higher than would be expected to be achieved *in vivo* following dietary exposure (COT, 2003).

31. Caco-2BBBe human intestinal cells were exposed to an increasing concentration of 0, 0.5, 1, 7, 14, and 30 mg/L genistein in the presence or absence of ICI, an oestrogen receptor antagonist. With the addition of ICI the high genistein concentrations reduced cell number by 40% and proliferation by 94% compared to control demonstrating that this effect was achieved through the oestrogen receptors (Chen and Donovan, 2004).
Animal studies

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

Oestrogenic potency of phytoestrogens

33. Naaz et al. (2003) examined the oestrogenic effects of genistein on adipose tissue in ovariectomised mice. Beginning a week after ovariectomy two groups of female mice: age matched 12-13 week old and 25-27 day old juvenile mice were sc injected daily with genistein (at 8-200 mg/kg bw/day for 21-28 days). To test effects of dietary genistein, 25 to 27 day old mice were fed diets containing 0-1500 parts per million (ppm) genistein for 12 days starting one week after ovariectomy. Adult mice showed 23 and 37% decreases in adipose tissue weight and circumference at higher doses (80 and 200 mg/kg bw/day over 21-28 days). Genistein treatment of juvenile mice at 20 and 80 mg genistein/kg bw/day for 28 days produced 36 and 47% decreases in parametrial fat pad weight and 18 and 25% decreases in adipocyte circumference, compared with controls. Juvenile female mice receiving dietary genistein (500, 1000 and 1500 ppm) displayed parametrial fat pad weight reduced by 37, 40 and 57%; increased uterine weights at 300, 500, 1000, 1500 ppm (oestrogenic effect) and reduced adipocyte circumference at 1000 and 1500 ppm. The dietary genistein (0, 300, 500, 1000, and 1500 ppm) resulted in circulating serum genistein concentrations of 0.08 ± 0.02, 1.02 ± 0.14, 1.79 ± 0.32, 2.55 ± 0.18, and 3.81 ± 0.39 µM, respectively, comparable to concentrations reported in humans: 2.53 ± 1.64 µM (Setchell et al., 1997).

Effects of phytoestrogens on fertility and development

34. Long-term reproductive effects of gestational and lactational genistein exposure on male and female mice were assessed by Fielden et al. (2002; 2003). Pregnant dams were treated by daily gavage with genistein at levels comparable to (0.1 and 0.5 mg/kg bw/day) or greater than (2.5 and 10 mg/kg
bw/day) human dietary exposure from gestational day 12 to PND 20. The offspring were weaned on PND 21. Female offspring were examined on PND 49 and no effect on mammary gland morphology at any dose was observed (Fielden et al., 2002). No significant treatment related effects on male offspring body weight or anogenital distance (measured on PNDs 7 and 21) were reported. Also, no adverse effects on sperm quality were observed when assessed on PND105 and 315 (Fielden et al., 2003).

35. Outbred CD-1 mice and ERβ knockout mice were treated with genistein by sc injection at doses of 1, 10 or 100 µg/pup/day (approximately 0.5, 5 or 50 mg/kg bw/day) on postnatal days (PND) 1-5 (day of birth = PND 1). Neonatal exposure to genistein induced expression of oestrogen receptor α (ERα). Histological evaluations showed a dose-related increase in multi-oocyte follicles (MOFs). This effect was not observed in mice lacking ERβ (Jefferson et al., 2002). Following sc treatment with genistein (50 mg/kg/day), altered ovarian differentiation during neonatal development was reported indicating that MOFs resulted from incomplete breakdown of oocyte nests (Jefferson et al., 2006). In a subsequent study Jefferson et al. showed that oral exposure to genistin (6.25, 12.5, 25, or 37.5 mg/kg/day presented as genistein equivalent doses) by gavage in female CD-1 mice on PNDs 1-5 resulted in oestrogenic activity similar to the response to sc genistein (12.5, 20, or 25 mg/kg/day). Both sc genistein and oral genistin treatment led to increased uterine wet weight. Other effects caused by orally administered genistin included altered ovarian differentiation (increased MOFs), delayed vaginal opening, abnormal oestrus cycles, decreased fertility and delayed parturition (Jefferson et al., 2009).

36. Neonatal mice were dosed by oral gavage from PND 1 to 5 at 50 mg genistein/kg bw per day, which produced serum genistein concentrations of 3 µM (approx. 810.72 ng/mL) mimicking the median genistein concentration of 890.70 ng/mL in soy formula-fed human infants reported by Cao et al. (2009). Observed results included a 28% decrease in thymic weight relative to body weight as well as 41% increased uterine weight and 3-fold increase in the number of multioocyte follicles. Early genistein exposure resulted in adult ovarian cycle abnormalities at 6 months of age. Despite changes in oestrous cycle length, fertility was not compromised, at least at 6 months (Cimafranca et al., 2010).

37. Neonatal exposure to isoflavones in mice was investigated by Dinsdale et al. The F1 female offspring of CD-1 mice was treated either with 7 mg of soy isoflavones/kg bw/day (2 mg daidzein/kg bw/day + 5 mg genistein/kg bw/day) or corn oil from PND 1 to 10 or from PND 1 to 21. Mice were subsequently mated with control males on PND 56 to obtain F2 females. The F1 female mice had increased body weight (~15%) during week 4-8 and reduced fertility, which was associated, inter alia, with abnormal oestrus cycles, fewer corpora lutea in ovaries and increased incidence of hyperplasia and atypia in the uteri. The F2 female offspring of isoflavone-treated F1 mice had also ~15% higher body weight during week 8-16 of age, compared to controls. The fertility of F2 female mice was normal (Dinsdale et al., 2011).
38. Kaludjerovic et al. analysed body and organ weights, histology of ovaries and uteri as well as bone metabolism in adult mice following an early life exposure to isoflavones (Kaludjerovic and Ward, 2009; Kaludjerovic et al., 2012). Neonatal female mice were randomised to different treatment groups: control (corn oil), daidzein (2 mg/kg bw/day), genistein (5 mg/kg bw/day) or daidzein and genistein combined together. From PND 1 to 5 treatments were administered daily via a single sc injection. The isoflavone treatment improved bone development in female mice at young adulthood (Kaludjerovic and Ward, 2009). However, when development of reproductive organs was analysed, and the same treatment conditions were extended to the first 10 days of life, higher body weights from 6 days to 4 months of age, as well as at adulthood, and hyperplasia in the oviduct were observed compared to controls. Both studies had similar long lasting adverse effects in the structure of ovaries and uterus in adult mice (Kaludjerovic et al., 2012).

39. Hughes et al. examined reproductive outcomes after developmental exposure to isoflavones in Long-Evans rats. Two animal studies were conducted. Study I examined effects of soy milk exposure on reproductive tract development of male and female pups during lactation. Lactating dams received instead of drinking water, either rice milk or soy milk containing between 3.5 and 8.5 mg of isoflavones/day (approximately 10 to 30 mg isoflavones/kg bw/day) from PND 1 through PND 21. Pregnant and lactating rats in study II were treated by gavage from gestational day (GD) 14 through PND 21 with either: corn oil (control), genistein (15 mg/kg bw/day), diethylstilbesterol (chosen as the estrogenic control: low and high dose, 0.5 and 5.0 µg/kg bw/day, respectively) or a combination of the low dose of diethylstilbesterol and genistein. The soy milk treated female pups had significantly increased body weight and decreased anogenital distance index (AGDI), whereas in male pups the epididymal weight was decreased. However, in utero and lactational exposure to genistein increased weaning AGDI. The onset of puberty occurred earlier in genistein treated males, in contrast to the lack of effect detected in the soy milk study. In both studies, exposure to dietary levels of isoflavones induced a significant increase in expression of progesterone receptor (PR) in uterine glandular epithelium of the 2 month old pups (Hughes et al., 2004).

40. Genistein was shown to act as an oestrogen causing defeminisation of the brain in a study conducted by Kouki et al. in rats. Following 5 daily (from birth) subcutaneous doses of genistein (1 mg), daidzein (1 mg), oestradiol (100 µg) and oil (control), genistein and oestradiol treated females had advanced vaginal opening, persistent or prolonged oestrus, and smaller ovaries compared to other groups. The genistein treated group had lower mean lordosis quotients (LQ – number of lordosis behaviours displayed/number of mounts x 100) than controls, but higher than the oestrogen group (Kouki et al., 2003). Demasculinisation of the reproductive system in rat pups was observed after female rats were exposed to genistein (0, 5, and 300 ppm) during gestation and lactation. Observed changes resulting from genistein exposure in male offspring included also increased thymus masses, increased subpopulations of T cells in the spleen, smaller testis size and lower testosterone concentrations in adulthood (Wisniewski et
al., 2003; Klein et al., 2002). When the same authors extended genistein exposure past weaning (until 70\textsuperscript{th} day of age), no additional effect over perinatal exposure on endocrine or immune responses in adulthood was observed (Klein et al., 2002).

41. The National Toxicology Program (NTP) conducted a preliminary study on genistein in Sprague-Dawley rats to determine doses to be used in subsequent multigenerational and chronic studies. Feed concentrations of 0, 5, 100 and 500 ppm were identified as appropriate doses because they induced observable effects in the reproductive organs, such as hyperplasia of mammary glands in female and male pups, without severely impairing fertility in the F\textsubscript{1} generation. Groups of mated pairs of rats (for the F\textsubscript{0}, F\textsubscript{1}, F\textsubscript{2}, and F\textsubscript{3} generations) were fed diets containing genistein (concentrations as above) for 98 (F\textsubscript{0}), 161 (F\textsubscript{1} and F\textsubscript{2}) and 42 (F\textsubscript{3}, from conception to PND 21) days. F\textsubscript{4} generation received no dietary exposure to the test compound and was bred to produce an unexposed F\textsubscript{5} generation. Ingested genistein doses were approximately 0, 0.3, 7, or 35 mg genistein/kg bw/day for males and 0, 0.5, 10, or 51 mg/kg bw/day for females. Dietary exposure to 500 ppm genistein in females led to decreased body weights, accelerated vaginal opening, decreased anogenital distance, and altered oestrous cyclicity. In male rats observed changes included lower body weights and anogenital distance in F\textsubscript{1}. There was some evidence for reduced litter size in the F\textsubscript{1} and F\textsubscript{2} generations. Increased rates of mammary gland hyperplasia and calcification of renal tubes were observed in exposed 100 and 500 ppm males examined at 20 weeks of age. No evidence for a carryover of genistein effects into unexposed F\textsubscript{4} and F\textsubscript{5} generations was observed (NTP, 2008a).

42. Pregnant rats (n=9 per group) were treated daily from GD 11 until they gave birth with either tap water or skimmed cows’ milk containing low (LIM) or high (HIM) level of isoflavones. The isoflavone content of cows’ milk from Danish dairy farms varied with different feed ration composition. Cows fed a ration consisting of whole crop barley/pea silage (24%), clover grass silage (71%) and grains (5%) produced high isoflavone milk (HIM) where the total phytoestrogens content was 429 ± 11.9 ng/mL (daidzein: 5.8 ± 0.3 ng/mL). Cows fed horsebeans (24%), clover grass silage (66%) and grains (10%) produced low isoflavone milk (LIM) (101 ± 3.3 ng total phytoestrogens/mL; 1.7 ± 0.6 ng daidzein/mL). Authors were not able to detect genistein in milk, but expect the levels of genistein to be higher in HIM, when compared to LIM. The exposure from LIM and HIM was 16.9 ± 1.1 and 75.1 ± 2.2 µg phytoestrogens/kg bw/day, respectively. During the study the main diet consisted of phytoestrogen-free, semipurified diet ad libitum. Both female and male offspring of HIM dams tended to be lighter than the offspring of LIM dams (p=0.14). Maternal exposure to LIM caused an earlier puberty onset in the female offspring, determined by assessing the age of vaginal opening, compared with controls. No difference was seen between the HIM and control offspring. The age at which 50% of the rats showed vaginal opening was 33.6, 34.3 and 35.1 days for the LIM, HIM, and controls, respectively (Nielsen et al., 2011).
43. Tan et al. undertook a feeding study in marmoset monkeys to address concerns about feeding human male infants with soy formula. Co-twin neonatal males (n=7) were hand-fed with either cows’ milk formula or soy formula milk (intake estimated as 1.6-3.5 mg/kg bw/day) from PND 4 to 5 until PND 35-45 (1 twin was fed cows’ milk formula, 1 twin was fed soy formula milk). The milk formula was reconstituted from powder as per manufacturer’s instructions and given to infant marmosets through a syringe. The animals were exclusively fed milk formula during the day (one to four times daily), each time they were allowed to drink as much as they wanted (volumes were recorded). After the last feed of the day, marmosets were returned to the family cage and allowed to breastfeed until the following morning. After 80-104 days animals were culled. Body and organ weights (prostate, seminal vesicles, pituitary, thymus and spleen) were similar in co-twins. However, co-twins fed the soy formula had increased testicular weight (by 14%) and numbers of Sertoli (by 7%) and Leydig cells (by 32%) compared to control. No additional adverse reproductive effects were observed (Tan et al., 2006).

**Phytoestrogens and immunosuppression**

44. Genistein has been shown to inhibit cytokine enzymes involved in signalling pathways within the immune cells, such as interferon-γ (IFN-γ). These effects were noted to be observed only at non-physiological concentrations (approximately 100 µmol/L) unachievable through dietary consumption (Cooke et al., 2006). Effects of injected (8, 20, 80 mg/kg) and dietary (0, 1000, 1500 ppm) genistein on cell-mediated immune responses were also examined by Yellayi et al. in ovariectomised (one week prior to treatment) mice. Genistein injections produced 46-67% 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. Dietary genistein led to an almost 50% decrease in DTH response. The effects of genistein were reversible and the immune response returned to normal after cessation of the genistein treatments and period of recovery of 28 days (Yellayi et al., 2003). Curran et al. suggested that dietary genistein or soy could inhibit the amount of IFN-γ normally produced in response to a bacterial (Mycobacterium avium) reaction in mice (Curran et al., 2004).

45. Andres et al. used physiological isoflavone concentrations present in soy-based infant formulas to inhibit rotavirus (RV) infectivity using MA-104 cells (cell line derived from embryonic African green monkey kidney cells routinely used in RV infectivity experiments). The authors observed that genistein and mixture of isoflavonoids significantly reduced RV infectivity and highlighted the fact that epidemiological studies to evaluate this effect were warranted (Andres et al., 2007).
Phytoestrogens and cancer

46. The National Toxicology Program (NTP) performed studies to detect if exposure to genistein over the course of multiple generations could have any effect on development of cancer. Three studies were conducted in which animals were exposed to genistein from the time of conception and through weaning through their mothers, who were given genistein in their feed (5, 100, or 500 ppm). Study 1: feed was given to male and female rats from conception through two years, in study 2: up to 20 weeks following birth (followed by untreated feed for the remainder of 2 years), study 3: from conception through the process of weaning (then untreated feed for the remaining period of 2 years). In none of the three studies was there any evidence of carcinogenic activity of genistein in male rats. In female rats the rates of adenoma or adenocarcinoma of the mammary gland and pituitary gland adenoma and carcinoma were increased (study 1), or slightly increased in study 2 (pituitary gland adenoma or carcinoma) and study 3 (mammary gland adenoma or adenocarcinoma) (NTP, 2008b).

47. The effect of maternal exposure to cows’ milk having low (LIM) or high (HIM) levels of isoflavones was investigated by Nielsen et al. (see paragraph 42 for more details). Mammary tumorigenesis was induced by administering 10 mg of 7,12-dimethylbenz[a]anthracene (DMBA) orally on PND 50. Rats were examined for mammary tumours once a week starting from week 8 post DMBA-treatment until PND 126. Compared with water and HIM groups, of which 74% and 75% developed mammary tumours, respectively, maternal LIM intake during pregnancy result in the lowest incidence (60% mammary tumours). The number of mammary tumours per animal was higher in the HIM offspring (2.08) than in controls (1.42) or LIM (1.32) (p=0.19). Also the total tumour volume was 34% higher in the HIM group. The authors concluded that although the high isoflavone content seemed to prevent the effect on circulating oestradiol and IGF-1 levels, HIM increased DMBA-DNA adducts in the mammary gland and tended to increase mammary tumorigenesis. In contrast, offspring exposed to LIM in utero, did not exhibit increased breast cancer risk, despite having higher oestradiol and IGF-1 environment and consequently earlier puberty onset (Nielsen et al., 2011).

Human studies

48. 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).
**Oestrogenic potency of phytoestrogens**

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

50. Genistein and daidzein are the major contributors to the total oestrogenic effect of soy-based products. When infant diet is exclusively based on soy formula, daily genistein intake would be approximately 5 mg/kg bw/day. Isoflavones are considered to have relatively weak oestrogenic properties. Different *in vitro* studies reviewed by Chen and Rogan (2004) provided different estimates of the oestrogenicity of genistein, reporting it to be in a range between $10^{-5}$ and 0.4 times that of oestradiol. Based on the lower end of the range of these estimates the authors concluded that $10^{-3}$ to $10^{-5}$ relative oestrogenicity of genistein to oestradiol generates an equivalent intake of 0.05 to 5 µg of oestradiol per kg bw/day. This exposure can be compared to infants taking up to five contraceptive pills/day (daily oestrogen intake from contraceptive pills in women is approximately 0.4 - 1 µg/kg bw/day) (Chen and Rogan, 2004).

51. Basaria et al. evaluated the effects of high-dose isoflavones on self-reported quality of life, cognition, lipoproteins and androgen status in post-menopausal women. A powder containing 20 g of soy protein that consisted of 160 mg of total isoflavones (96 mg aglucones) was mixed with beverages and consumed daily. In this double-blind, randomized, placebo-controlled trial high doses of isoflavones have been shown to reduce testosterone and high-density lipoprotein levels and significantly improve vasomotor, physical and psychosexual aspects of life in post-menopausal women (n=84). However, isoflavones did not have any significant beneficial effects on cognition or lipids (Basaria et al., 2009).

**Effects of phytoestrogens on fertility and development**

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

53. Bernbaum *et al.* designed a study to establish whether soy formula can have an oestrogenic impact on infants development. They reported increased vaginal wall cell maturation in 1 – 6 month old girls fed exclusively soy-based formula in early life compared to girls of the same age on other diets. No changes were observed in breast and genital anatomy or milk secretion (Bernbaum *et al.*, 2008).

54. In Israel, 92 soy formula-fed female infants were monitored for a period of two years in order to evaluate a possible oestrogenic effect of phytoestrogens on breast development. Girls consuming soy-based formula had a higher prevalence of breast buds during the second year of life (p=0.02). A significant decline in the prevalence of breast buds was found between the first and second year in milk- but not soy-consuming infants (p<0.001). Diet based on soy formula appeared to have a preserving effect on breast tissue that had evolved in early infancy, leading to its slower waning in the 2nd year of life compared to groups fed breast milk and cow milk-based formula. The authors postulated that this effect was related to the oestrogenic effects of isoflavones present in soy, especially that in the second year of life, they may express mainly oestrogenic agonist effects, when endogenous oestradiol levels are low (Zung *et al.*, 2008). Consumption of soy-based formula was one of 3 environmental factors associated with a 3-fold increase in the prevalence of premature thelarche in girls younger than 2 years of age in Puerto Rico (Freni-Titulaer *et al.*, 1986).

55. Three groups of infants fed soy formula, milk formula and breast milk were assessed by Gilchrist *et al.* as a part of “The Beginnings Study” at the Arkansas Children’s Nutrition Centre comparing health, growth and development of children. This was a longitudinal study in which children whose mothers reported no use of any soy products during pregnancy or lactation were fed cows’ milk formula or soy formula or breast milk. Of the soy-fed infants, 23% were exclusively fed soy formula from birth, 45% were switched to exclusive soy formula feeding within 4 weeks, and 32% switched between 4 and 8 weeks. No isoflavone levels in formula and breast milk were given in the study. Body weights of breast milk fed boys were greater than of soy-based formula fed boys (p<0.05). The study showed greater mean ovarian volume in girls fed cows’ milk-based formula when compared to breast fed or soy-based formula consuming girls (p<0.05). There was an increased number of follicles per cyst per ovary in girls fed cows’ milk or soy-based formula compared to breastfed infants. Lower testicular volume among soy and milk formula-fed boys was observed comparing to breastfed boys (p<0.04). The authors concluded that there was no evidence that soy-based formula had a deleterious effect on reproductive health. No differences were found in infant weight, length, or body surface area (Gilchrist *et al.*, 2010).

56. Adgent *et al.* assessed sexual dimorphism in young children by using the Pre-School Activities Inventory (PSAI), a psychometric test designed to observe gender-role play behaviours in 3,664 boys and 3,412 girls, born
between 1 April 1991 and 31 December 1992, at approximately 42 months of age enrolled in the United Kingdom Avon Longitudinal Study of Parents and Children (ALSPAC). In the UK the early life soy exposure (defined as the use of a soy formula occurring $\leq 4$ months of age) accounted for 2.4% of boys and 2.0% of girls ($n=54$). Mothers had to answer several questions related to their child playing behaviour, as well as maternal education, maternal age, presence of older brother or sister, and prenatal smoking. Each 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. Among girls and boys, the mean PSAI score was highest with early soy feeding (40.8 and 63.0, respectively) and lowest with early formula feeding for girls (36.7) and primarily breastfeeding for boys (61.3). However, the authors reported that the increase in PSAI scores among early soy-exposed participants were modest and did not place them outside the range of normal behaviour (Adgent et al., 2011). In a follow-up for the same study, authors investigated the association between exposure to soy as infants and age at menarche. Across the study sample, the median age at menarche was earliest for girls receiving an early soy diet (12.4 years), and latest among those who were primarily breastfed (12.8 years). Early soy-fed girls were at 25% higher risk of menarche throughout the course of follow-up, compared to girls fed non-soy-based or milk formula. The risk ratios for early menarche (< 12 years) were 1.53, 0.64 and 0.98 for early soy, late soy and primarily breast feeding exposures, respectively compared to the early formula reference group. The exact amount of soy intake in the ALSPAC study was unknown, so a true dose-response relationship could not be assessed (Adgent et al., 2012).

57. Sex differences associated with soy exposure were observed in the cross-sectional study in preschool children in Japan, aged 3-6 years, where soy intake (24.4 g/day for boys and 22.8 g/day for girls) was significantly negatively related to oestrone and oestradiol in boys ($n=230$) and positively related to testosterone and 5-androstene-3$\beta$,17$\alpha$-diol (3$\beta$,17$\alpha$-AED) levels in girls ($n=198$) measured in urine. The average levels of oestrone, oestradiol, testosterone, and DHEA in girls were higher than those in boys (Wada et al., 2011).

58. Strom et al. examined the association between consumption of soy formula during infancy and eventual health and development outcomes observed later on in adulthood. There was a slightly longer duration of menstrual bleeding (0.37 days) and increased discomfort reported by women who had been fed soy formula ($n=128$) compared to women who had been fed cows’ milk formula as infants ($n=563$). 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). The authors did not consider these findings to be biologically significant, and after adjustment for multiple comparisons, these findings were not statistically significant.
Effects of phytoestrogens on the thyroid gland and thyroid function

59. 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 (T₃) and thyroxine (T₄), with the potential to act through the inhibition of thyroperoxidase (TPO) or possible interactions with thyroid binding globulin (TBG)(COT, 2003).

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

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

62. Conrad et al. performed a retrospective analysis of medical records of infants diagnosed with congenital hypothyroidism and seen at the hospital during their first year of life. Two groups of patients were analysed: the soy diet group consuming exclusively soy infant formula (n=8) and the non-soy diet group (n=70). There was no significant difference in thyroid stimulating hormone (TSH) and thyroxine (T₄) levels in both groups prior to levothyroxine treatment initiation. Levels of T₄ measured on treatment were similar in both groups: median 153 nmol/L (soy group) and 188 nmol/L (non-soy group). However there was a difference in the first TSH measured between the soy and non-soy diet groups: median 42.6 mU/L (soy group) and 6.6 mU/L (range: 1.8 – 20.9), respectively. The soy diet group required a median of 150 days (range: 54 – 229) and non-soy diet group 40 days (range: 19 – 189) for the normalisation of TSH to levels < 10 mU/L. After two and four months of treatment, the percentage of patients with increased TSH was higher in soy diet group: 62.5 %, whereas in non-soy diet group these values were 35.7 and 17%, respectively. Overall, infants consuming soy-based formula had elevated levels of TSH and subsequently increased requirement for T₄. Prolonged increase of TSH was related to malabsorption and increased
faecal loss of levothyroxine (Conrad et al., 2004). This study was subsequently criticised due to differences in patient numbers and the fact that it was a retrospective study (Karadag et al., 2004).

**Allergy**

63. The Food Standards Agency (FSA) advises that: “Soy allergy is a common childhood allergy. Most children grow out of it by the age of two, but occasionally adults are allergic to soy. The symptoms of soy allergy are similar to milk allergy and they include rashes, diarrhoea, vomiting, stomach cramps and breathing difficulties. Some people with soy allergy might also react to milk. Very rarely soy can cause anaphylaxis. Infants with other allergic conditions, such as milk allergy, dermatitis etc, are also at higher risk of developing allergy to soy”

3. The health risks associated with infant feeding and the development of soy allergy will be included in the COT review of risks arising from the infant diet and the development of atopic and autoimmune disease. Details of the proposed scope and approach for the literature review were set in the initial paper (TOX/2012/27) and discussed by the COT in September 2012.

64. The Anaphylaxis Campaign, the UK charity supporting people with severe allergies, recommends that infants with increased susceptibility to allergy reactions (for instance through the family history) should not be fed soy formula without the advice of a health professional. The use of soy formula is not recommended for any child of six months or under. In their soy allergy factsheet, the charity informs that the allergy to soy is uncommon in the UK, and other allergies, such as milk, egg, peanut and fish allergy are more prevalent. It is also unclear how many children suffering from soy allergy reactions have chances of outgrowing it later on in life. Symptoms of soy allergy among children are frequently mild (rash) and rarely severe (breathing difficulties, anaphylaxis). There have been no fatalities triggered by soy in the UK. The soy allergy factsheet (2011) is available at: [www.anaphylaxis.org.uk](http://www.anaphylaxis.org.uk).

**Guidance values**

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

66. The US National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) panel in 2006 reviewed relevant scientific literature on the potential developmental effects of soy infant formula as well as its associated toxicities. The panel recommended that future research should focus on population studies with particular attention to

biomarkers of exposure and effects of concomitant ingestion and formula type, age of breast development, postnatal growth and neurobehavioral development, animal studies with adequate selection of animal and doses addressing prenatal outcomes as well as multigenerational studies with exposure continuing into adulthood and evaluation of both reproductive and postnatal developmental endpoints. After this first revision, the CERHR determined that updated evaluations of genistein and soy infant formula were needed (Rozman et al., 2006).

67. In 2010 the NTP-CERHR Expert Panel reviewed papers on soy infant formula published between February 2006 and August 2009 and concluded that there was no sufficient evidence to conclude that soy infant formula and soy-based diet posed a developmental toxicity risk in experimental animal studies considered to be relevant to the assessment of human risk. The authors considered evidence to be sufficient to conclude that there was “minimal concern” for adverse effects from consumption of soy infant formula by healthy full-term infants. This evaluation however did not include an assessment on the potential reproductive toxicity of genistein (McCarver et al., 2011). The Expert Panel acknowledged the fact that larger (in terms of sample size) and longer longitudinal, prospective cohort studies were needed, capturing for instance soy exposure from birth through puberty, addressing longer term endpoints such as cancer, bone mineral density or cognitive performance (NTP, 2010).

Occurrence

Levels of isoflavones in human breast milk

68. 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).

69. Table 1 summarises available data on concentrations of isoflavones in breast milk. Setchell et al. 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 omnivorous diet (Setchell et al., 1997; Setchell et al., 1998). Consumption of soy foods such as roasted soybeans has been shown to increase levels of isoflavones (Franke and Custer, 1996; MAFF, 1998b). In another study, mixed diet consumed by pregnant women led to lower values of isoflavones present in breast milk compared to other studies. The measured concentrations appeared to be higher in samples from women expecting boys (Jarrell et al., 2012).
70. In a study conducted in the US, milk samples were collected from breastfeeding mothers before and after consumption of a soy protein beverage (25 g soy protein/36.5 g of beverage containing 55 mg isoflavones: daidzein:genistein:glycitein = 1:1:0.1). The mean levels of isoflavones in breast milk after 2 – 4 days of daily consumption were 10-20 times higher than baseline values of $5.1 \pm 2.2$ nmol/L increasing to $70.7 \pm 19.2$ nmol/L. The values for this study shown in Table 1 have been converted to µg/L (Franke et al., 2006).

**Cows’ milk-based infant formula**

71. 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 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. Sample 1 had 1.2 mg/kg total isoflavones (0.7 mg genistein/kg and 0.5 mg glycitein/kg) and sample 2: 2.1 mg genistein/kg (Hoey et al., 2004).

**Soy-based infant formula**

72. Soy-based formulas available nowadays are free of cows’ milk protein and lactose and provide 67 kcal/dL. They contain 20% more calcium and phosphorus than cows’ milk-based formulas (Bhatia and Greer, 2008). The ratio of conjugated and unconjugated isoflavones is similar in all soy-based infant formulas. Conjugates of genistein account for >65% of the total isoflavones and only 3.2 – 5.8% exists as genistein and daidzein aglucones (Setchell et al., 1998).

73. COT (2003) noted that reported isoflavone levels in soy-based formulas were in the range of 18-41 mg aglucone equivalents/L (made up formula ready for consumption) (MAFF, 1998a). Levels of isoflavones in other soy infant formulas obtained in the UK were also measured by other researchers. All soy based infant formulas analysed by Hoey et al. contained between 34.00 and 46.70 mg aglucone equivalents/L as fed where genistein comprised $63 \pm 5\%$, daidzein $27 \pm 1\%$ and glycitein $10 \pm 5\%$, of the total (Hoey et al., 2004). Kuhnle et al. reported the total isoflavone content of the soy infant formula as 1000 times higher than non-soy formula (Kuhnle et al., 2008). Detailed values are given in Table 2. Concentrations of isoflavones were higher in powdered soy formula (46 – 47 mg/L) than in liquid formula (32 – 45 mg/L) (Setchell et al., 1998). Estimated isoflavone levels as aglucone equivalents as fed are shown in Table 3.

74. Total isoflavone concentrations as aglucone equivalents in soy-based infant formulas were also measured in other countries and were reported to be in the range of 81 – 92 mg/kg for genistein and 44 – 55 mg/kg for daidzein (Irvine et al., 1998) and in another study as 89.5 – 155.7 mg/kg for genistein,
52.7 – 101.6 mg/kg for daidzein and 12.8 - 24 mg/kg for glycitein (Franke et al., 1998). Comparison of isoflavone content in infant soy formulas from different countries as well as the conversion to ready to drink basis is shown in Table 3.
### Table 1. Levels of isoflavones measured in human breast milk

<table>
<thead>
<tr>
<th>Diet</th>
<th>Isoflavone content</th>
<th>LOQ</th>
<th>n</th>
<th>Genistein (μg/L) Mean ± SE (SD)</th>
<th>Range</th>
<th>n</th>
<th>Daidzein (μg/L) Mean ± SE (SD)</th>
<th>Range</th>
<th>n</th>
<th>Total (genistein + daidzein) (μg/L) Mean ± SE (SD)</th>
<th>Range</th>
<th>Cohort</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese diet + tofu soup once a day</td>
<td>unknown</td>
<td>1</td>
<td></td>
<td>8.11 – 13.51*</td>
<td></td>
<td>1</td>
<td>20.34 – 27.96*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>US</td>
<td>Franke and Custer, 1996</td>
</tr>
<tr>
<td>Omnivorous diet + additional intake of 5, 10, and 20 g of roasted soybeans at times 0, 24, and 72 h, respectively</td>
<td>Genistein: 913 mg/kg; Daidzein: 830 mg/kg</td>
<td>1</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>US</td>
<td>Franke and Custer, 1996</td>
</tr>
<tr>
<td>Omnivorous</td>
<td>unknown</td>
<td>14</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK</td>
<td>MAFF, 1998a</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>unknown</td>
<td>14</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK</td>
<td>MAFF, 1998a</td>
</tr>
<tr>
<td>Vegan</td>
<td>unknown</td>
<td>11</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK</td>
<td>MAFF, 1998a</td>
</tr>
<tr>
<td>Omnivorous</td>
<td>unknown</td>
<td>14</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK</td>
<td>MAFF, 1998a</td>
</tr>
<tr>
<td>Omnivorous</td>
<td>unknown</td>
<td>11</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK</td>
<td>MAFF, 1998a</td>
</tr>
<tr>
<td>Omnivorous + soy protein beverage daily for 2-4 days</td>
<td>36.5 g serving contained approx. 28.5 g genistein and 24.3 g daidzein</td>
<td>2.5</td>
<td></td>
<td>8.11 – 18.91*¹</td>
<td></td>
<td>1</td>
<td>3.8 – 15.25*¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>US</td>
<td>Franke et al., 2006</td>
</tr>
<tr>
<td>Soy diet</td>
<td>unknown</td>
<td>9</td>
<td></td>
<td>1.6 ± 1.9</td>
<td>0.6 – 6.4</td>
<td>9</td>
<td>3.1 ± 1.8</td>
<td>1.0 – 6.1</td>
<td>9</td>
<td>4.7 ± 4.4</td>
<td>1.6 – 13.6</td>
<td>US</td>
<td>Satchell et al., 1998</td>
</tr>
<tr>
<td>Mixed diet</td>
<td>unknown</td>
<td>9</td>
<td></td>
<td>1.6 ± 1.9</td>
<td>0.6 – 6.4</td>
<td>9</td>
<td>3.1 ± 1.8</td>
<td>1.0 – 6.1</td>
<td>9</td>
<td>4.7 ± 4.4</td>
<td>1.6 – 13.6</td>
<td>US</td>
<td>Satchell et al., 1998</td>
</tr>
<tr>
<td>(sex of foetus: male)</td>
<td>unknown</td>
<td>84</td>
<td></td>
<td>0.61 ± 2.20</td>
<td>0.13 – 8.7</td>
<td>89</td>
<td>0.25 ± 1.03</td>
<td>0.10 – 2.8</td>
<td>89</td>
<td>0.86 ± 1.73</td>
<td>0.30 – 3.94</td>
<td>Canada</td>
<td>Jarrell et al., 2012</td>
</tr>
<tr>
<td>(sex of foetus: female)</td>
<td>unknown</td>
<td>42</td>
<td></td>
<td>0.87 ± 0.44</td>
<td>0.14 – 1.2</td>
<td>43</td>
<td>0.36 ± 0.21</td>
<td>0.17 – 0.7</td>
<td>46</td>
<td>0.77 ± 0.27</td>
<td>0.17 – 0.8</td>
<td>Canada</td>
<td>Jarrell et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td></td>
<td>0.36 ± 0.19</td>
<td>0.19 – 0.6</td>
<td>46</td>
<td>0.16 ± 0.07</td>
<td>0.07 – 0.4</td>
<td>46</td>
<td>0.33 ± 0.15</td>
<td>0.13 – 0.60</td>
<td>Canada</td>
<td>Jarrell et al., 2012</td>
</tr>
</tbody>
</table>

LOQ – Limit of Quantification; SE – standard error; SD – standard deviation. *Franke et al., 2006 presented total isoflavone concentration as nmol/L stating that the daidzein to genistein ratio was on average 0.6. Therefore 60% of the total isoflavones in breast milk was daidzein and 40% - genistein. The units from nmol/L to μg/L were converted using molar mass of genistein as 270.24 g/mol and daidzein as 254.23 g/mol. The same approach was followed for Franke and Custer study (1996). ¹ The average values estimated based on a graph (Franke and Custer, 1996)
This is a background paper for discussion. It does not reflect the views of the Committee and should not be cited.

Table 2. Comparison of isoflavone content in soy infant formulas obtained in the UK

<table>
<thead>
<tr>
<th>Formula type</th>
<th>n*</th>
<th>n**</th>
<th>in mean aglucone equivalents as sold (mg/kg)</th>
<th>in mean aglucone equivalents as fed (mg/L)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genistein</td>
<td>Daidzein</td>
<td>Glycitein</td>
</tr>
<tr>
<td>Powder formula, reconstituted</td>
<td>7</td>
<td>8</td>
<td>24.60</td>
<td>14.40</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>19.40</td>
<td>12.90</td>
<td>1.70</td>
<td>34.00</td>
<td>19.40</td>
</tr>
<tr>
<td></td>
<td>13.60</td>
<td>7.80</td>
<td>1.60</td>
<td>23.00</td>
<td>13.60</td>
</tr>
<tr>
<td></td>
<td>17.00</td>
<td>12.10</td>
<td>1.90</td>
<td>31.00</td>
<td>17.00</td>
</tr>
<tr>
<td></td>
<td>23.80</td>
<td>15.20</td>
<td>2.00</td>
<td>41.00</td>
<td>23.80</td>
</tr>
<tr>
<td></td>
<td>19.70</td>
<td>10.80</td>
<td>2.50</td>
<td>33.00</td>
<td>19.70</td>
</tr>
<tr>
<td></td>
<td>10.40</td>
<td>6.70</td>
<td>0.90</td>
<td>18.00</td>
<td>10.40</td>
</tr>
</tbody>
</table>

| Powder, not reconstituted     | 3  | ?   | 232.00   | 93.00    | 21.00     | 346.00 | 31.30     | 12.50    | 2.80      | 46.70 |
|                               | 146.00 | 67.00 | 39.00    | 252.00    | 19.70     | 9.00  | 5.30      | 34.00     |
|                               | 217.00 | 98.00 | 23.00    | 338.00    | 29.30     | 13.20 | 3.10      | 45.60     |
|                               | 120.00 | 63.00 | 19.00    | 192.00    | 7.90      | 1.10  | 0.20      | 6.00      |

Adapted from the final CERHR Expert Panel Report on Soy Formula, 2010. The CERHR calculated concentrations of isoflavones in soy powdered not reconstituted formulas as fed based on the assumption that 0.135 kg of powder is used to reconstitute 1 L of formula. For reconstituted formulas, CERHR converted between mg/kg and mg/L because the density of prepared formula is similar to water. These recommendations are consistent with infant formula manufacturers’ advice in the UK.

*number of soy-based formula brands used for analysis

**number of samples of each soy-based formula brand used to calculate the mean
Table 3. Comparison of isoflavone content as fed in soy infant formulas from different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>Estimated ranges of mean isoflavone levels as aglucones equivalents as fed (mg/L) (percent of total)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genistein</td>
<td>Daidzein</td>
</tr>
<tr>
<td>US</td>
<td>20</td>
<td>12.1 – 31.2 (57.9 – 66.3%)</td>
<td>7.1 – 13.5 (28.8 – 34%)</td>
</tr>
<tr>
<td>UK</td>
<td>11</td>
<td>10.4 – 31.3 (58 – 67%)</td>
<td>6.7 – 15.2 (32.5 – 37%)</td>
</tr>
<tr>
<td>Australia</td>
<td>4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>New Zealand</td>
<td>5</td>
<td>10.9 – 18 (55 – 65.4%)</td>
<td>5.9 – 15 (34.6 – 45%)</td>
</tr>
<tr>
<td>Brazil</td>
<td>7</td>
<td>5.9 – 16.2 (59 – 59.1%)</td>
<td>2.4 – 8.6 (24 – 31.3%)</td>
</tr>
</tbody>
</table>

Adapted from the final CERHR Expert Panel Report on Soy Formula, 2010. The CERHR calculated concentrations of isoflavones in soy powdered not reconstituted formulas as fed based on the assumption that 0.135 kg of powder is used to reconstitute 1 L of formula. For reconstituted formulas, CERHR converted between mg/kg and mg/L because the density of prepared formula is similar to water. These recommendations are consistent with infant formula manufacturers’ advice in the UK.
Weaning products

Soy–based weaning products

75. Processed soy products have been recommended by advisory health web sites and organisations as high-quality protein and nutrient-dense foods that could be used to enrich weaning foods. It is claimed that children who are weaned on soy-based foods, grow better and stay healthier compared to other children. Various soy ingredients have been recommended to be used in weaning foods recipes as a replacement for water, corn meal or wheat flour such as corn soy blend, defatted soy flour or soy milk. However, it is unclear whether these claims are substantiated (WISHH, 2006).

76. Levels of isoflavones in a group of ready-to-eat and instant weaning foods have previously been reported by the COT (Table 4). Minor quantities of isoflavones in various foods, such as vegetables, fruit, cheese or meat products, typical of Western diets and possibly consumed by infants have also been reported by others (see Table 4 for range of values that were reported by Thompson et al., 2006; Kuhnle et al., 2008). However, since these minor sources were based on a very limited number of poorly described samples, and given the uncertainty about the extent of their consumption by infants in the MAFF 1986 survey of British infants aged 6-12 months (Mills and Tyler, 1992), the exposure assessment presented here is focused on ready-to-eat and instant weaning foods and tofu only (see section “Weaning products – exposure assessment”).

77. High levels of isoflavones have also been detected in soy milk in a range of 130 – 200 mg/L (COT, 2003). However, it is unclear whether these values from products designed for adults or infants (as infant formula). Therefore these data were not used in an exposure assessment. Furthermore, consumption of soy and cows’ milk (in non-formula form) is not recommended for infants.

Table 4. Isoflavone levels in foods included in infant diet

<table>
<thead>
<tr>
<th>Food type</th>
<th>Total isoflavone levels as µg/kg of foods as consumed</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-eat and instant weaning foods</td>
<td>Range: 18,000 – 78,000</td>
<td>COT, 2003</td>
</tr>
<tr>
<td>Firm tofu</td>
<td>275,000*</td>
<td>COT, 2003</td>
</tr>
<tr>
<td>Other foods with minor levels of isoflavones**</td>
<td>Range: 1 - 390</td>
<td>Irvine et al., 1998 Thompson et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kuhnle et al., 2008</td>
</tr>
</tbody>
</table>

*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 39 µg total isoflavones/100 g was detected in green and white beans (Thompson et al., 2006).
Exposure

78. Infants’ and children’s exposure to isoflavones, genistein and daidzein occurs through consumption of soy-based infant formula and weaning products containing soy as well as breast milk of mothers. It has also been shown in animal studies that placental transfer may be another route of developmental exposure (Doerge et al., 2001). Exposure to isoflavones is the sum of aglucones (unconjugated form of isoflavones) and respective conjugated glucoside compounds, which are converted to active forms during fermentation, for instance in the gut. As many studies do not clarify whether reported isoflavone levels are normalised on aglucone basis, the total calculated intake may be overestimated (McCarver et al., 2011).

79. The EFSA has concluded that 800 mL and 1200 mL are reasonable estimates of average and high-level daily consumption of breast milk or infant formula before weaning (e.g., EFSA, 2012) and these values have been used in exposure calculations. The mean bodyweights used for calculation of exposures are those reported by the Department of Health (DH) (1994) as these included weights for infants younger than 6 months, required for infant formula, weaning food and breast milk exposure estimates. These bodyweights 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 (DH, 1994).

Breast milk

80. Based on the upper end of the range of reported total isoflavone levels, 32 µg/L in breast milk from mothers (n=11) consuming a vegan diet in the UK (MAFF, 1998a), infant isoflavone exposure was calculated for average (800 mL) and high level (1200 mL) breastfed infants (Table 5). The total isoflavone concentrations reported in breast milk samples were expressed as a sum of genistein and daidzein as µg aglucone/kg and subsequently converted to µg/L. The estimated exposures are up to 6.5 µg/kg bw/day but likely to be lower in infants whose mothers consume omnivorous diet or infants who are not exclusively breastfed (e.g. cows’ milk formula is introduced). However, concurrent consumption of soy-based infant formula and high volumes of breast milk from mothers following a vegetarian/vegan diet could lead to much higher exposures.

Table 5. Infant total isoflavone exposure (µg/kg bw/day) from breast milk calculated for average and high level consumption

<table>
<thead>
<tr>
<th>Consumption</th>
<th>Infant age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 3</td>
</tr>
<tr>
<td>Average (800 mL)</td>
<td>4.3</td>
</tr>
<tr>
<td>High level (1200 mL)</td>
<td>6.5</td>
</tr>
</tbody>
</table>
Cows' milk-based infant formula

81. Infants' exposure was estimated using the maximum level of isoflavone reported in the Hoey et al. study (2.1 mg aglucone equivalents/kg, adjusted to 0.28 mg/L to take account of levels following reconstitution) and the default values of 800mL and 1200 mL for average and high-level consumers of formula milk (Table 6). The estimated average and high level exposures from cows’ milk-based infant formula are up to 38 and 57 µg/kg bw/day, respectively. However, since isoflavones were not detected in most samples of cows’ milk-based formula, the exposure in infants exclusively fed this kind of formula, is likely to be lower.

Table 6. Infant total isoflavone exposure (µg/kg bw/day) from cows’ milk formula calculated for average and high level consumption

<table>
<thead>
<tr>
<th>Consumption</th>
<th>Infant age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 3</td>
</tr>
<tr>
<td>Average (800 mL)</td>
<td>38</td>
</tr>
<tr>
<td>High level (1200 mL)</td>
<td>57</td>
</tr>
</tbody>
</table>

Soy-based infant formula

82. The total isoflavone intake from soy-based infant formula in the US was estimated to be 28 – 50 mg/day (approximately 6 - 9 mg/kg bw/day) (Setchell et al., 1997; Setchell et al., 1998). Isoflavone levels measured in soy-based infant formulas in New Zealand (17 - 33 mg isoflavones/L as aglucone equivalents as fed) were used to calculate mean daily isoflavone intake in infants as a mean (SD) daily dose of 3.2 (0.2) mg/kg bw/day (range between 2.9 and 3.8 mg/kg bw/day) (Irvine et al., 1998). Similar conclusions were made by other researchers.

83. Based on the mean (SD) 526 (110) ml/day consumption of soy-based formula by 4-6 month old infants (n=7) Hoey et al. estimated that the mean daily intake of total isoflavones from soy infant formula available in the UK was 28 mg isoflavones as aglucone equivalents/day equating to 3.6 (1.2) mg/kg bw/day for 8 kg infants (Hoey et al., 2004). For comparison, mean daily exposure for adults consuming 50 – 100 mg of isoflavones was 0.7 – 1.4 mg/kg bw/day.

84. Based on estimated isoflavone levels in reconstituted soy-based infant formulas from the UK (presented in Table3) the isoflavone exposure values have been calculated (Table 7).
Table 7. Infant mean total isoflavone exposure (µg/kg bw/day) from soy-based infant formula in the UK calculated for average and high level consumption

<table>
<thead>
<tr>
<th>Consumption</th>
<th>Infant age (months)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 3</td>
<td>4 - 6</td>
<td>7 - 9</td>
<td>10 - 12</td>
</tr>
<tr>
<td>Average (800 mL)</td>
<td>2.4 – 6.3</td>
<td>1.8 – 4.8</td>
<td>1.6 – 4.2</td>
<td>1.5 – 3.8</td>
</tr>
<tr>
<td>High level (1200 mL)</td>
<td>3.6 – 9.5</td>
<td>2.8 – 7.3</td>
<td>2.4 – 6.3</td>
<td>2.2 – 5.7</td>
</tr>
</tbody>
</table>

Weaning products – exposure assessment

85. Mean- and high-level exposure estimates for the isoflavone levels reported for weaning foods were derived (and reported in Table 8) by applying consumption data from the MAFF 1986 infant study to the reported occurrence data in Table 4. The ready-to-eat and instant weaning food group mainly comprised of commercial baby foods (including cereal-based foods and desserts). In the absence of survey data on isoflavone content of specific weaning food categories, it was assumed that the foods selected for the exposure assessment are similar to those analysed for isoflavone content and reported in the 2003 COT report (Table 4).

86. An exposure assessment for tofu (based on a portion size approach) was included as a scenario to allow comparison of exposures between a potentially rich source of isoflavones such as tofu with those estimated for ready to eat and instant weaning foods (a portion size approach was adopted for tofu as consumption data were not available from the MAFF 1986 survey for this product). The isoflavone content of some soy-based products was reported in isolated literature reports (Thompson et al., 2006; Kuhnle et al., 2008). Tofu was selected as representing these due to availability of more recipe information about its uses in weaning foods compared to other soy-based products. The 2003 COT report suggested that the isoflavone content of tofu was relatively higher than in other soy-based products.

87. Although mean exposure to isoflavones from consumption of tofu appears to be nearly 100 fold higher compared to ready-to-eat instant weaning foods, it should be noted that exposure was estimated from a portion size approach. Therefore, the actual consumption rate of tofu as used in the recipe may be much lower as it is unlikely that infant will be consuming this type of product (banana and tofu puree) daily over prolonged periods.
Table 8. Chronic exposure to isoflavones by UK infants from weaning foods (in µg/kg bw/day)

<table>
<thead>
<tr>
<th>Product category</th>
<th>Isoflavone level (µg/kg food)</th>
<th>Consumption rate (g/kg bw/day)</th>
<th>Exposure (µg/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>P97.5</td>
<td>Mean</td>
</tr>
<tr>
<td>Weaning Foods*</td>
<td>Range: 18,000 – 78,000</td>
<td>6</td>
<td>Range: 11 - 46</td>
</tr>
<tr>
<td>Tofu**</td>
<td>275,000</td>
<td>10</td>
<td>n/a</td>
</tr>
</tbody>
</table>

* Weaning foods group comprises foods reported in the MAFF British Infants Survey (Mills and Tyler, 1992) such as: instant weaning foods and ready-to-eat foods – most are commercial/retail baby foods (including cereal-based foods). Examples: egg/cheese-based meal dried; rice/semolina/chocolate instant puddings; yoghurt-based dried meals.

**In the absence of consumption data on soy-based food product such as tofu from the MAFF Infant Survey, portion size data sourced from a popular baby food recipe website (50 g per person per day; recipes available at: http://www.annabelkarmel.com/recipes/babies-6-9-months/banana-tofu-puree) were used to estimate exposure levels. The EFSA default infant body weight⁴ (5kg) was used to estimate exposure from tofu on a bodyweight basis.

Risk characterisation

88. Based on maximum reported concentrations of isoflavones in breast milk from mothers following a vegan diet, the exposure of exclusively breastfed infants was estimated to be up to 6.5 µg/kg bw/day. Isoflavone exposure from infant formula for exclusively cows' formula-fed infants was up to 57 µg/kg bw/day. The highest exposure was estimated for infants exclusively fed soy-based infant formula reaching values up to 9500 µg/kg bw/day. The presence of isoflavones in ready-to-eat and instant weaning food and some soy-based products (e.g. tofu) have been reported previously by the COT in 2003.

89. Information on how soy phytoestrogens are absorbed, distributed, metabolised and excreted in newborns and infants is limited. Infants have been shown to effectively absorb isoflavones from breast milk, soy infant formula and weaning food products containing soy, and differences when compared to adults are due to the immaturity of the intestinal microflora (COT, 2003), limited bacterial transformation (Cao et al., 2009), lack of ability to hydrolyse β-glucosides efficiently (Franke et al., 2006) and/or larger intake when adjusted for body weight. Food matrix, diet, hygiene, stress, genetics and age can also be determining factors. Transfer of isoflavones and their metabolites to body fluids as well as breast milk has been shown, however more detailed tissue distribution studies are needed. 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. It was noted that the metabolic profile of pigs was closer to that of humans, whereas intestinal bacterial composition in rats was similar to that in monkeys (Gu et al., 2006). Humans were also shown to have much higher capacity to conjugate isoflavones compared to rodents (Setchell et al., 2011).

90. Dietary exposure levels reported in animals were associated with decreased or increased body weight (observed at $7 \times 10^3$ µg isoflavones/kg bw/day), mammary gland and oviduct hyperplasia, increased uterine wet weight, altered ovarian differentiation, decreased fertility or delayed parturition (observed at $6.25 - 37.5 \times 10^3$ µg genistein/kg bw/day), ovarian cycle abnormalities, increased expression of progesterone receptor (observed at $10-30 \times 10^3$ µg isoflavones/kg bw/day), increased thymus masses as well as subpopulations of T cells in the spleen (observed at $0.42 - 25 \times 10^3$ µg genistein/kg bw/day). Although the estimated dietary exposure of infants is in the range of the doses shown to have effects in experimental animals, effects observed in animals cannot be simply extrapolated to humans due to differences in absorption and capacity to conjugate isoflavones (Setchell et al., 2011).

91. Studies performed in animals are often based on exposure to a single compound, such as genistein, whereas in human studies the exposure is to the combination of the components of soy, including a mixture of isoflavones and other substances. The health effects reported in children fed soy-based formula were: increased vaginal cell maturation, higher prevalence of breast buds and risk of menarche, increased levels of TSH and requirement for T4, and increased discomfort and duration of menstrual bleeding in adulthood. None of these studies estimated isoflavone exposure as only estimated length of soy formula consumption and possible inclusion of other types of infant feeding were reported by parents. Therefore the available human studies do not support establishment of a health-based guidance or reference point for use in risk characterisation of soy isoflavones. Long-term studies involving larger number of participants are needed where exposure to soy formula would be recorded from birth through puberty (CERHR, 2010).

92. The oestrogenicity of isoflavones and their influence on reproductive organs have been identified as the main concern in relation to soy formula consumption by infants. The relative oestrogenicity of genistein to oestradiol was estimated to be between $10^{-5}$ and $10^{-3}$, indicating that exposure of 5 mg/kg bw/day corresponds to an intake of 0.05 to 5 µg of oestradiol per kg bw/day (daily oestrogen intake from contraceptive pills in women is approximately 0.4 to 1 µg/kg bw/day). Hoey et al. (2004) reported that approximately 63% of total isoflavones content of soy formula, as aglucone equivalents, is formed by genistein. Therefore estimated isoflavone exposure from consumption of soy formula, reaching up to 9.5 mg total isoflavones as aglucone equivalents/kg bw/day (of which approximately 5.7 mg/kg bw/day is genistein), may indicate that infants fed soy formula, can be exposed to levels equivalent to oestrogen exposure of 0.057 – 5.7 µg/kg bw/day, which would be in the range of pharmacologically active levels.
Questions for the Committee

102. Members are invited to comment on the information provided in this paper and to consider the following questions.

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

ii). Do Members consider the estimates of isoflavones intake indicate a concern for the health of infants in
   a) breast milk,
   b) formula
   c) dietary sources?

iii) Is the current UK government advice that soy-based infant formula should only be used in exceptional circumstances to ensure adequate nutrition of infants supported by the scientific evidence?

iv) Do Members have recommendations for additional information that should be obtained in order to reach conclusions?

Secretariat
November 2012
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
</tr>
<tr>
<td>ADME</td>
<td>absorption, distribution, metabolism and excretion</td>
</tr>
<tr>
<td>AFSSA</td>
<td>L’Agence française de sécurité sanitaire des aliments (French Food Safety Agency)</td>
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<tr>
<td>AGDI</td>
<td>anogenital distance index</td>
</tr>
<tr>
<td>ALSPAC</td>
<td>Avon Longitudinal Study of Parents and Children</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BfR</td>
<td>Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CERHR</td>
<td>Center for the Evaluation of Risks to Human Reproduction</td>
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<tr>
<td>COT</td>
<td>Committee on Toxicity</td>
</tr>
<tr>
<td>Cr</td>
<td>creatinine</td>
</tr>
<tr>
<td>DBPCFC</td>
<td>Double Blind Placebo Control Food Challenge</td>
</tr>
<tr>
<td>DH</td>
<td>Department of Health</td>
</tr>
<tr>
<td>DMBA</td>
<td>7,12-dimethylbenz[a]anthracene</td>
</tr>
<tr>
<td>DTH</td>
<td>delayed-type hypersensitivity</td>
</tr>
<tr>
<td>ER</td>
<td>oestrogen receptor</td>
</tr>
<tr>
<td>ESPGHAN</td>
<td>European Society for Paediatric Gastroenterology, Hepatology and Nutrition</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FSA</td>
<td>Food Standards Agency</td>
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<tr>
<td>GD</td>
<td>gestational day</td>
</tr>
<tr>
<td>HIM</td>
<td>high isoflavone levels milk</td>
</tr>
<tr>
<td>LIM</td>
<td>low isoflavone levels milk</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification</td>
</tr>
<tr>
<td>LQ</td>
<td>lordosis quotients</td>
</tr>
<tr>
<td>MAFF</td>
<td>Ministry of Agriculture, Forestry and Fisheries</td>
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<tr>
<td>MOF</td>
<td>multi-oocyte follicles</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program</td>
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<tr>
<td>O-DMA</td>
<td>O-demethylangolensin</td>
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<td>PND</td>
<td>postnatal day</td>
</tr>
<tr>
<td>PR</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>PSAI</td>
<td>Pre-School Activities Inventory</td>
</tr>
<tr>
<td>RDI</td>
<td>recommended daily intake</td>
</tr>
<tr>
<td>RV</td>
<td>rotavirus</td>
</tr>
<tr>
<td>SACN</td>
<td>Scientific Advisory Committee on Nutrition</td>
</tr>
<tr>
<td>sc</td>
<td>subcutaneous</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SHBG</td>
<td>sex hormone binding globulin</td>
</tr>
<tr>
<td>SPI</td>
<td>soy protein isolate</td>
</tr>
<tr>
<td>SPT</td>
<td>skin prick test</td>
</tr>
<tr>
<td>T4</td>
<td>thyroxine</td>
</tr>
<tr>
<td>TBG</td>
<td>thyroid binding globulin</td>
</tr>
<tr>
<td>TDI</td>
<td>tolerable daily intake</td>
</tr>
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</table>
This is a background paper for discussion.
It does not reflect the views of the Committee and should not be cited.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>TSH</td>
<td>thyroid stimulating hormone</td>
</tr>
<tr>
<td>TVP</td>
<td>textured vegetable protein</td>
</tr>
<tr>
<td>WISHH</td>
<td>World Initiative for Soy in Human Health</td>
</tr>
</tbody>
</table>
References


Ref Type: Report


Ref Type: Report

This is a background paper for discussion. 
It does not reflect the views of the Committee and should not be cited.


Ref Type: Report

Department of Health’s Chief Medical Officer. Advice issued on soya-based infant formulas. CMO Update 37. 2004.
Ref Type: Personal Communication


Fielden MR, Fong CJ, Haslam SZ and Zacharewski TR (2002) Normal mammary gland morphology in pubertal female mice following in utero and


Ref Type: Report

Ref Type: Report

Ref Type: Report


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.

Ref Type: Report


Sheehan D and Doerge D. Letter to Dockets Management Branch, Food and Drug Administration. 1999.
Ref Type: Personal Communication


Ref Type: Report


Search strategy

General isoflavones/genistein/daidzein exposure search

Databases interrogated –
- EFSA
- COT
- FSA
- JECFA

Scientific publications literature search

**Specific search terms:**

Isoflavone/genistein/daidzein/soy AND breast milk

*Search Dates (From/To) - From May 2002 to present*

*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified previous cases of chronic and acute isoflavone toxicity and where a dose which lead to toxicity was identifiable.*

**Exclusion Criteria** –
- Supplementation research in undeveloped countries
- Supplementation programs in undeveloped countries
- Deficiency related research

Isoflavone/genistein/daidzein/soy AND infant formula

*Search Dates (From/To) - From May 2002 to present*

*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified previous cases of chronic and acute isoflavone toxicity and where a dose which lead to toxicity was identifiable.*

**Exclusion Criteria** –
- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant formulas in non-EU countries

Isoflavone/genistein/daidzein/soy AND infant diet

*Search Dates (From/To) - From May 2002 to present*

*Some papers pre-2002 were included if it felt they added value to the paper, particularly with regards to papers which identified levels of isoflavones in foods*

**Exclusion Criteria** –
- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant diet in undeveloped countries
- Children’s diet (above >2 years) in developed countries

Isoflavone/genistein/daidzein/soy AND weaning
This is a background paper for discussion. It does not reflect the views of the Committee and should not be cited.

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

**Exclusion Criteria** –
- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Infant weaning in undeveloped countries
- Children’s diet (above >2 years) in developed countries

Retinol AND exposure

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

**Exclusion Criteria** –
- Supplementation studies in undeveloped countries
- Supplementation programs in undeveloped countries
- Deficiency related research

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