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

Second draft statement on the effects of soya consumption on thyroid status.

Introduction

1. A 2003 Committee on Toxicity (COT) report on phytoestrogens and health identified individuals with hypothyroidism as a subgroup of the population of potential concern for adverse effects of phytoestrogens in soya, and made recommendations for research. During 2014 the Committee considered the results of several FSA-funded research studies on phytoestrogens including the third arm of T05029: “The effect of soya phytoestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism: a randomized double blind crossover study”. As a result the Committee considered that it would be timely to review evidence related to potential effects of phytoestrogens on thyroid function, which had become available since the 2003 COT report on phytoestrogens and health. The first draft statement (TOX/2014/41) summarising the new literature concerning potential risks from consumption of soya phytoestrogens on thyroid function, with particular emphasis on the risks to individuals with hypothyroidism was presented to Members in December 2014.

2. The second draft statement in Annex A has been revised taking into account previous discussion and incorporating details requested by Members in December. A table summarising effects on isoflavone exposure on thyroid function reported in human studies and further details on tabulated studies have been included. Certain sections of the document have been reworded. Additional editorial changes have also been made.

Questions on which the views of the Committee are sought

3. Members are invited to comment on the structure and text of the second draft statement.

Secretariat
January 2015

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

Second draft statement on the effects of soya consumption on thyroid status.

Background

1. A 2003 COT report on phytoestrogens and health[1] identified individuals with hypothyroidism as a subgroup of the population of potential concern for adverse effects of phytoestrogens in soya, and made recommendations for research. The Food Standards Agency subsequently commissioned research to address these recommendations and invited the Committee to consider the results. The Committee concluded that it would be timely to review evidence related to potential effects of phytoestrogens on thyroid function, which had become available since the 2003 COT report on phytoestrogens and health. This statement summarises the results of that review and the COT conclusions. The focus was principally on human studies because there were a number available and they were most relevant. The criteria that were employed in the literature search are set out in Annex 1.

Phytoestrogens

2. Phytoestrogens are naturally produced by some edible plants. They have been shown to influence biological processes mainly through their structural similarities to oestrogens, and their ability to bind to oestrogen receptors (ER) and interfere with the natural hormonal responses in humans and animals. The largest group of phytoestrogens are flavonoids, which can be further divided into three subclasses, coumestans, prenylated flavonoids and isoflavones (e.g. genistein, daidzein).

Thyroid gland

3. The thyroid gland is responsible for the production of hormones involved in regulating metabolism, bodyweight and oxygen requirements, as well as normal growth and development during childhood. The thyroid gland produces hormones thyroxine (T_4) and triiodothyronine (T_3), which are synthesised from iodine and the amino acid tyrosine. The production of T_3 and T_4 is controlled by thyroid stimulating hormone (TSH), secreted from the pituitary gland and regulated by the central nervous system (CNS).

[1] http://cot.food.gov.uk/cotreports/cotwgreports/phytoestrogensandhealthcot

4. Inhibition of T_3 and T_4 synthesis leads to increased secretion of TSH by the pituitary gland, and is one of the causes of enlargement of the thyroid gland, known as a goitre, which can occur in hyper- and hypothyroidism. Hypothyroidism is a condition that occurs when the thyroid gland is underactive and T_4 concentration is below the normal range. Hyperthyroidism results from over production and secretion of free thyroid T_3 and T_4.

Potential effects of phytoestrogens on thyroid function

5. Some phytoestrogens, notably genistein and daidzein, have a similar chemical structure to thyroid hormones. It has been hypothesised that they interact with the thyroid gland by a number of possible mechanisms which could interfere with its normal function.

6. *In vitro* and animal studies have shown that phytoestrogens can interact with and inhibit thyroperoxidase (TPO), an enzyme involved in the synthesis of T_3 and T_4, which would then lead to reduced concentrations of T_3 and T_4 and increased release of TSH (COT, 2003). Genistein and daidzein were shown to inhibit the formation of T_4 *in vitro* in a concentration dependant manner with IC_{50} values being for genistein approximately 2 µM (0.5 µg/mL) and for daidzein 8.8 µM (2.24 µg/mL) (Divi *et al*., 1997; BfR, 2007). However it is not clear if such concentrations would occur *in vivo*. Paul *et al*. (2014) developed a high-throughput screening TPO inhibition assay utilising rat thyroid microsomes and a fluorescent peroxidase substrate. Genistein and daidzein were shown to have TPO-inhibiting potential.

7. Ebmeier and Anderson (2004) reported that genistein and daidzein can also inhibit the activity of human intrathyroidal sulphotransferases, which are involved in the inactivation and elimination of thyroid hormones.

8. Transthyretin (TTR) is one of the thyroid hormone binding proteins and binds up to 20% of T_4 in serum. TTR is involved in the distribution of T_4 in the body and in preventing T_4 excretion in the kidneys. TTR is the most important thyroid hormone-binding protein in cerebral spinal fluid (CSF). In serum and CSF, genistein and related isoflavones are highly effective inhibitors of T_4 and T_3 binding to TTR (Kd = 40 nmol/L, equimolar to T_4 binding), with potential effects on the distribution of thyroid hormones in the body (Green *et al*., 2005; Radovic *et al*., 2006; BfR, 2007).

9. It has been hypothesised that phytoestrogens could potentially increase levels of thyroxine binding globulin (TBG), a plasma protein involved in the transport of T_3 and T_4. Such an increase in TBG concentration could transiently increase the binding capacity for thyroxine and result in lower free T_4 levels and the subsequent over production of TSH to compensate for this deficit (COT, 2003).

Previous conclusions

The COT Report on Phytoestrogens and Health (2003)

10. The report noted that animal studies showed that high levels of dietary soya and isoflavones can affect thyroid function and may have a goitrogenic effect in rodents deficient in dietary iodine. Data from human studies suggested that isoflavones were unlikely to affect thyroid function in normal individuals with adequate iodine intake.

11. No data were found to indicate that maternal ingestion of phytoestrogens during pregnancy influence the development of the thyroid gland. However, the COT considered it possible that, together with low iodine intake, increased metabolic demands during pregnancy and increased need for thyroxine, maternal consumption of soya products could adversely influence the neurological development of the fetus.

12. A number of scientific publications evaluated by the Committee in 2003 reported cases in the 1950s and 1960s of altered thyroid function (mostly goitre) associated with consumption of soya-based formula and of increased faecal loss of orally administered thyroxine in an athyreotic hypothyroid patient when fed soya formula as compared with cows' milk formula. It was suggested that increased faecal mass and fibre content of the diet could alter entero-hepatic circulation of thyroxine. As a consequence, changes were made in the processing and formulation of infant formulae (supplementation with iodine and replacement of soya flour with soya protein isolate), and no further reports of goitre were published.

13. The COT identified individuals with hypothyroidism as a subgroup of the population of potential concern. The COT Report concluded that consumption of phytoestrogen supplements, or a soya-rich diet, may provide sufficient concentrations of phytoestrogens to interfere with T_4 replacement medication, which is given to patients with hypothyroidism. Although no adverse effects in hypothyroid children or adults were reported in the published literature, the Report recognised that research had not addressed this issue specifically. In view of the increasing availability of phytoestrogen-rich food and supplements in the UK, the Report recommended that research be conducted on the plasma T_4 levels of children and adults with hypothyroidism who consume large quantities of dietary phytoestrogens.

Conclusions reached by organisations in other countries

14. A number of concerns were raised in a 2007 risk assessment by the German Federal Institute for Risk Assessment (BfR). The BfR reported that available toxicological studies showed that isoflavones, when consumed at high doses (for example by menopausal women), can impair the functioning of the thyroid gland. The BfR advised against long-term consumption of isoflavone-containing products made from soya (BfR, 2007).

15. The American Academy of Pediatrics (AAP) Committee on Nutrition concluded in its review that the evidence for adverse effects of dietary soya isoflavones on human development, reproduction or endocrine function is not conclusive (Bhatia *et al*., 2008). The National Toxicology Program (NTP) considered that health effects described in the literature were not sufficient to reach the

conclusion that consumption of soya infant formula produced adverse thyroid effects. Further studies in this area were recommended (NTP, 2010).

16. Taking into account concerns that have been raised with respect to potential negative effects of soya isoflavones on sexual, reproductive and neurobehavioral development, immune function and thyroid function, the European Food Safety Authority (EFSA) considered that concentrations of isoflavones in infant formula and follow-on formula should be kept as low as is feasible (EFSA, 2014).

Food Standards Agency-funded research

17. The aim of the randomised double-blind controlled crossover study, T05029: "The effect of soya phytoestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism", was to determine whether soya in the diet may be clinically important in patients with compensated thyroid function. The study was undertaken in three independent parts (details are specified in Table 1). Each part used the same protocol where treatments (different in each part) were administered daily for 2 months, followed by a two month wash out period, followed by the second alternative treatment in that part for a further two month period. The treatments in each part were:

- Part 1: 30 g isolated soya protein (isoflavone free) with 16 mg of isoflavones (representative of vegetarian diet) or 30 g isolated soya protein (isoflavone free) with 2 mg of isoflavones (representative of Western diet);
- Part 2: 30 g isolated soya protein (isoflavone free) with 60 mg of isoflavones (equivalent dose to ingestion of phytoestrogen supplements) or 30 g the isolated soya protein (isoflavone free) alone;
- Part 3: 30 g of isolated soya protein (isoflavone free) alone, or 30 g casein protein alone (as control).

18. Results from the three study parts appeared inconsistent. The first part suggested a threefold increase in the risk of developing overt hypothyroidism following dietary supplementation of 16 mg soya phytoestrogens in individuals with subclinical hypothyroidism (Sathyapalan *et al*., 2011), the second part used a higher dose of isoflavones (60 mg) and did not confirm those findings. Fewer patients developed overt hypothyroidism and the authors suggested that this was because of a higher drop out rate and difference in the gender ratios in the two arms. The Committee noted that although this was a possible explanation, the difference in transition to overt hypothyroidism, could also have occurred by chance. The third part was conducted to help to clarify whether effects associated with the consumption of soya are particularly due to a soya protein effect or a general protein effect. None of the patients in the third part developed overt hypothyroidism during the study.

19. The results of all three parts of the T05029 study indicate that soya protein alone does not have an effect on thyroid in patients with mild thyroid dysfunction. Thus, the effects observed in the first two parts of the study appeared to have been

caused by isoflavones. The Committee was informed by the researchers that in a previous study on cardiovascular risk factors in diabetes following administration of 132 mg of isoflavones alone for four weeks (Gonzalez *et al*., 2007), they had observed no effect on thyroid function, which was a secondary end point in the study. The study outcomes suggest a combination or matrix effect when isoflavones were administered in the presence of soya protein (Atkins personal communication).

20. Thyroid function has also been evaluated in two other FSA-funded randomised double-blind controlled crossover studies investigating the effects of soya in men with type 2 diabetes (three month treatment, T01057[2]) and women within two years of the onset of the menopause (six month treatment, T01060[3]) (Table 1). These studies were also undertaken by the same researchers. There was no effect of soya protein alone (without isoflavones). However, when soya protein was administered together with 66 mg isoflavones, a significant increase in TSH and reduction in thyroxine was observed in both studies. Although within the normal range, the consistency of the changes observed in thyroid hormone levels following consumption of soya protein containing phytoestrogens, both by women within 2 years after the onset of menopause, and by men with type II diabetes and subclinical hypogonadism, supported the possibility of risks from soya ingestion in people with subclinical or overt hypothyroidism.

Other new data

21. A summary of relevant publications emerging since the COT report was published in 2003 is provided below. Specific details related to all mentioned studies are further described in Table 1.

Studies of thyroid function in children

Children with thyroid dysfunction receiving thyroxine treatment

22. Conrad *et al*. retrospectively analysed the medical records of infants diagnosed with congenital hypothyroidism and seen at a hospital during their first year of life. Two groups of patients were considered: a soya diet group consuming exclusively soya infant formula, who started on levothyroxine (a synthetic derivative of T_4) treatment at a median age of 15 days (n=8), and a non-soya diet group, who started treatment at 17 days (n=70). There was no significant difference in serum levels of TSH and T_4 levels between the groups before the start of treatment with levothyroxine. There was a significant difference between the two groups in: time to TSH normalisation (p=0.02; four times longer in soya group), first TSH measured after treatment began (p<0.01; six times higher in soya group), percentage of infants with increased TSH at 4 months of age (p=0.01) and throughout the first year of life (approximately four times higher in soya group). The authors suggested that these

[2] The effect of soya protein with and without isoflavones in men with type 2 diabetes and subclinical hypogonadism – A randomized double blind parallel study. University of Hull.
[3] Soya protein with isoflavones reduce bone turnover markers in women during their early menopause – A randomised double blind placebo controlled parallel study. University of Hull.

soya fed infants need close monitoring of free thyroxine and TSH measurements as they may need increased levothyroxine to achieve normal thyroid function tests (Conrad *et al*., 2004).

23. Fruzza *et al*. (2012) described two patients with congenital hypothyroidism who, although on levothyroxine treatment, were persistently hypothyroid. The patients were 3 week old and 5 year old females, and had been consuming soya-based formula and soya milk, respectively (no details of isoflavone levels were available). When the consumption of soya-based products was discontinued and the dose of levothyroxine decreased, the thyroid function slowly normalised over the course of a few weeks. The authors made the recommendations for children requiring levothyroxine treatment to avoid the use of soya products if possible, and if there was no other alternative to monitor levels of thyroid hormones and adjust/increase dose of treatment as required (Fruzza *et al*., 2012).

24. A low iodine intake by a vegan mother was the suggested cause of hypothyroidism in her child (breastfed for the first 6 days, examined at 10 days of life). A normal plasma TSH level was achieved in the infant following thyroxine treatment (infant) and Lugol's iodine solution administration (mother) over a course of one week and one month respectively (Shaikh *et al.*, 2003). The authors were subsequently criticised by J. Borak for overlooking anti-thyroid effects of soya milk consumed by the vegan mother as one of the causative factors (Borak, 2005).

Children with thyroid dysfunction not receiving thyroxine treatment/treatment not specified

25. An association between exposure to genistein, daidzein and equol, and the occurrence of congenital hypothyroidism was investigated by Jung et al. (2013). Two groups of infants took part in this study: infants with congenital hypothyroidism (n=39) and healthy infants (n=20). Equol (a metabolite of daidzein) and daidzein showed no significant difference between the groups, whereas the plasma concentration of genistein was significantly higher in healthy infants (11.15 ng/mL) than in patients (6.93 ng/mL), p=0.00026. The authors suggested that genistein may contribute to the improvement of congenital hypothyroidism.

26. Consumption of soya was investigated as a risk for goitre in primary school girls in Iran. There were no significant differences in consumption of soya between girls with goitre (n=284) and girls not presenting such symptoms (n=288). Loss of iodine from iodised salt stored in open containers was suggested as the major risk factor for goitre in the examined population, with medium socioeconomic family status, and place of residence being other potential risk factors (Mousavi *et al*., 2006).

Children with normal thyroid function

27. Thyroid hormones and thyroglobulin (TG) and TPO autoantibodies (markers for autoimmune thyroid dysfunction) were measured along with serum concentrations of genistein and daidzein in iodine-replete school children (n=268; age 8-15 years) without overt thyroid diseases. A significant positive association of genistein with thyroglobulin autoantibodies and a negative correlation with thyroid volume was reported by the authors. Higher levels of thyroxine were found in children (n=36) who had eaten some kind of soya-based product in the preceding 24 hours. However, as soya products were not part of the normal regular diet in participating children, it was suggested that even small differences in soya phytoestrogens consumption may have an impact on thyroid function, especially when accompanied by insufficient iodine intake (Milerova *et al*., 2006).

Studies in adults with normal thyroid function

28. In a randomised double-blind, placebo-controlled clinical trial post-menopausal women not on hormone replacement therapy (age 64-83 years) received a supplement (containing 90 mg of isoflavone) (n=22) or placebo (maltodextrin) (n=16) per day, plus a multi-vitamin and mineral supplement daily for six months. TSH, T_4 and T_3 were measured at baseline and after 90 and 180 days. No statistically significant differences in thyroid hormone measurements were recorded in this healthy iodine replete group of subjects (Bruce *et al.*, 2003).

29. In a randomised cross-over study healthy young men (20-40 years old) received diets supplemented with three protein powders: milk protein isolate, and low- and high-isoflavone soya protein isolates for 57 days, separated by 4 week washout periods. The low- and high-isoflavone soya protein isolates provided 1.64 ± 0.19 mg isoflavones/day (mean ± SD) and 61.7±7.4 mg isoflavones/day, respectively. Blood was collected on days 1, 29 and 57 and analysed for total and free T_3 and T_4, TSH and TBG, no significant changes were recorded in any thyroid parameters when low- and high-isoflavone groups were compared with group receiving milk protein isolate. Collected urine samples revealed a significantly ($p<0.0001$) higher excretion of isoflavones and their metabolites in subjects receiving the high isoflavone diet compared to the low isoflavone diet and milk protein isolate (Dillingham *et al.*, 2007).

30. Hampl *et al.* (2008) reported that short-term soya consumption had a modest and transitory effect on thyroid parameters, stating that 'some thyroid hormone parameters do correlate with actual isoflavone levels.' Male (n=32) and female (n=54) university students with normally functioning thyroid consumed unprocessed boiled natural soya beans (2 g/kg bw/day; containing approximately 1.2 – 4.2 mg isoflavones/g dry weight) for 7 days. TSH, free thyroid hormones, antibodies to TPO and TG, and actual levels of genistein and daidzein were measured in serum collected at the end and one week after completing the soya consumption. Levels of both isoflavones rose at the end of soya consumption and returned to background levels one week on. No significant changes in TSH and free T_3 and T_4 were found in the female group, while in males a transitory significant ($p<0.0001$) increase in TSH was recorded (Hampl *et al*., 2008).

31. The effect of a 3-year administration of genistein (54 mg/day) on thyroid function was investigated in osteopenic, postmenopausal women (n=40) compared with a control group (n=37) receiving placebo. Thyroid hormones (TSH, free T_3, free T_4) and thyroid-specific autoantibodies (TPO, TG and thyroid microsomal antigen [TMA]) were assessed following the 3-year treatment and were shown to be in the normal range (Bitto *et al*., 2010).

32. A lack of association between soya consumption during early pregnancy and development of thyroid dysfunction or autoimmunity was suggested by Li *et al*. (2011). Based on soya intake frequency, participants were divided into three groups: frequent (three or more times a week; 18.6%; n=94); conventional (twice per month>three times a week; 62.6%; n=316); and occasional consumers (two or less times per month; 18.8%; n=95). There was no significant difference between the three groups in free T_4, TSH, and TPO antibody levels. No marked difference was found in the prevalence of overt, subclinical hyper- and hypothyroidism; however, there was a non-significant increase in the percentage of subjects with subclinical hypothyroidism among frequent consumers. Urinary daidzein and genistein levels were determined in randomly selected frequent, conventional and occasional representative subjects (about 20% of the three groups: n=20, 59 and 16 respectively) and levels of both were significantly higher in frequent consumers.

33. Effects of isoflavones on thyroid functions (free T_3 and T_4, TSH, TBG and anti-TPO antibodies) were investigated in oophorectomised women in India (<55 years of age, with baseline TSH values): isoflavone (n=17) and placebo (n=17) groups. A tablet containing 75 mg of isoflavones was administered once a day orally at bedtime with 150 ml of water for 12 weeks. Participants were advised to avoid food products containing phytoestrogens during the study period. A modest reduction in serum free T_3 levels in the isoflavone group (p=0.02) in the absence of any significant effect on other thyroid parameters was reported (Mittal *et al*., 2011).

34. Healthy postmenopausal women (n=25; mean age 58 years) consumed seaweed capsules (475 µg iodine/day) or placebo (maltodextrose) for 7 weeks. Both treatments were supplemented by powdered soya protein isolate (141.3 mg isoflavones/day) during the 7^{th} week in a double-blinded crossover randomised study with a three week washout period between treatments. No changes in serum thyroid hormone concentrations were associated with isoflavone consumption (Teas *et al*., 2007).

35. Effects of soya consumption on thyroid function were investigated by several studies as an additional secondary outcome. Alekel *et al*. (2014) looked at effects of isoflavone treatment (80 and 120 mg/day) on endometrial thickness, circulating hormones and adverse events in postmenopausal women, over a 3 year period. There were no effects on circulating TSH concentrations in either of the treatment groups when compared to baseline levels. Lack of significant differences in levels of TSH and antibodies to TPO were also reported by Levis *et al.* (2011) in menopausal women receiving tablets containing 200 mg isoflavone/day for 2 years. Steinberg *et al.* (2011) reported slightly lower (p=0.052) free T_4 concentrations in menopausal women on isoflavone treatment for 2 years (80 mg/day, n=119; 120 mg/day, n=117) when compared to baseline. No significant changes in TSH concentrations were

reported (Steinberg *et al*., 2011). No significant effect of soya foods (approximately 36 mg isoflavones/day) on thyroid hormone status in premenopausal women was reported by Zhou *et al*. (2011).

Discussion

36. Since the 2003 COT report several human studies on the impact of soya consumption on thyroid function have been published. Although there is some indication that isoflavones have the potential to cause effects on thyroid function, especially if ingested at high levels by sensitive subgroups, such as iodine-deficient children or postmenopausal women, the currently available evidence is still inconclusive. Not all studies associate consumption of soya with negative health effects. Shaikh et al. (2003) suggested that insufficient iodine intake by a mother was a sole cause of hypothyroidism in her child and the fact that she consumed soya milk was not associated as causative factor. Based on higher genistein plasma concentrations in healthy controls compared to hypothyroid children, Jung *et al*., (2013) suggested that genistein may actually contribute to improvement of congenital hypothyroidism. There is a lack of long term studies in which ingested levels of isoflavones are assessed, addressing different soya-based foods and mixtures of isoflavones from sources other than soya.

37. A mechanism by which isoflavones exert their health effects has not been extensively analysed. However, it is known that interactions with soya and dietary fibre result in poor absorption of thyroid medications (levothyroxine) in both children and adults. Children with congenital hypothyroidism, who consume soya infant formula while receiving levothyroxine treatment were found as a group, which could require a close monitoring of thyroid hormones and increased levothyroxine treatment to achieve normal thyroid parameters (Conrad *et al*., 2004).

Conclusions

38. The Committee considered FSA-funded studies on the possible adverse effects of soya and concluded that it was timely to re-evaluate the interaction of soya-based food products containing isoflavones, and the thyroid gland, taking into account research published since the 2003 COT report.

39. In two of the three FSA funded randomised double-blind controlled crossover studies, a significant increase in TSH and reduction in thyroxine was observed in men with type II diabetes and subclinical hypogonadism, and in menopausal women following consumption of soya protein containing phytoestrogens. Although within the normal range, the consistency of the changes observed in thyroid hormone levels supported the possibility of risks from soya ingestion in people with subclinical or overt hypothyroidism.

40. The third FSA-funded study looked at the effects following administration of casein, soya protein alone or soya protein containing 2, 16 or 66 mg isoflavone for two months in individuals with subclinical hypothyroidism. The results suggested that when combined with soya protein, the isoflavones are likely to be responsible for the thyroid effect. Despite certain limitations (no clear dose response, low number of patients developing overt hypothyroidism and low group sizes, significant drop out rate) we consider that this study supported the 2003 conclusion that individuals with hypothyroidism were a subgroup of the population of potential concern.

41. Although there were some additional publications since 2003 the results reported were not always consistent due to differences in study design and comparators. In general isoflavone intake appeared to have an impact on thyroid hormone levels to a small extent and this was exacerbated in individuals with iodine deficiency. However, it was not possible to determine a dose response relationship from the data available nor to identify other risk factors (with the exception of iodine deficiency), which might influence this progression.

42. Overall the Committee concluded that there was consistently observed evidence that thyroid hormone levels were reduced following consumption of soya in different groups of the population. The changes in thyroid hormone level were small and remained within the normal range in the general population including in men with type 2 diabetes and women during their early menopause. Some individuals with compensated hypothyroidism developed overt hypothyroidism following administration of soya protein containing isoflavones. However, it was not possible to determine a dose response relationship from the data available nor to identify other risk factors (with the exception of iodine deficiency), which might influence this progression. Therefore it would still be prudent to consider all individuals with hypothyroidism a subgroup of the population of potential concern.

43. The Committee recommends that this population should be made more aware that increased consumption of soya in their diet or as supplements might exacerbate their condition. The Committee also recommends that general practitioners and endocrinologists should be informed that this group might require more frequent checks of their thyroid hormone status.

Secretariat
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Abbreviations

AAP	American Academy of Pediatrics
BfR	German Federal Institute for Risk Assessment
CNS	central nervous system
COT	Committee on Toxicity
CSF	cerebral spinal fluid
EFSA	European Food Safety Authority
ER	oestrogen receptors
FSA	Food Standards Agency
Kd	dissociation constant
NTP	National Toxicology Programe
SD	standard deviation
SPI	soya protein isolate
T_3	triiodothyronine
T_4	thyroxine
TBG	thyroxine binding globulin
TG	thyroglobulin
TMA	thyroid microsomal antigen
TPO	thyroperoxidase
TSH	thyroid stimulating hormone
TTR	transthyretin

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Table 1. Effects on thyroid function reported in human studies following oral exposure to isoflavones

Participants	Dose and duration of soya consumption	Dose (µg/kg bw/day) and duration of levothyroxine treatment	Baseline serum thyroid parameters TSH [mIU/L][1] total T_4/T_3 [nmol/L] free T_4/T_3 [pmol/L] Anti-Tg [U/ml] Anti-TPO [U/ml]	Serum thyroid parameters during/after treatment TSH [mU/L][1] total T_4/T_3 [nmol/L] free T_4/T_3 [pmol/L] Anti-Tg [U/ml] Anti-TPO [U/ml]	Observations	Reference
Children with thyroid dysfunction receiving thyroxine treatment						
Infants diagnosed with congenital hypothyroidism Group A: consuming soya infant formula (n=8; 4M and 4F) Group B: non-soya diet group (n=70; 29M and 41F)	*Soya infant formula* consumed throughout the first year of life; level of isoflavones not specified.	Group A: commenced at a median age of 15 days till 1 year. Starting median dose: 7.4 One year decrease in dose of: 3.3 Group B: commenced at a median age of 17 days till 1 year. Starting median dose: 9.3 One year decrease in dose of: 3.0	Group A: TSH median 428; tT_4 median 29.6 Group B: TSH median 229; tT_4 median 47.6 *Normal ranges: TSH 0.5-5 tT_4 81.1-321.8	After ~ 50 days of treatment Group A:TSH median 42.6 tT_4 median 153 Group B:TSH median 6.6 tT_4 median 188 Time to TSH normalisation Group A: median of 150 days Group B: median of 40 days	4 times longer time to TSH normalisation in soya formula group (p=0.02) 6 times higher TSH measured in soya formula group (p<0.01) 4 times higher % of infants with increased TSH at 4 months of age (p=0.01) and throughout the first year of life in soya formula group CIs not specified	Conrad *et al*., 2004

Infant girls diagnosed with congenital hypothyroidism <u>Patient 1:</u> consuming soya infant formula <u>Patient 2</u>: consuming soya milk	*Soya infant formula* consumed every 2 hours, 1 hour before thyroxine treatment discontinued at 3 weeks of age *Soya milk* consumed 1 hour before thyroxine treatment between 3 and 4 year of age level of isoflavones in both products not specified	<u>Patient 1:</u> 15 (6 days of age – 3 weeks); 11 (3 – 6 weeks); 8 (6-10 weeks) <u>Patient 2</u>: 6 (3-5 years); 5 (5-5.5 years); 4 (5.5-6 years)	<u>Patient 1</u>: TSH 167 T_4 not specified <u>Patient 2:</u> TSH ~6 tT_4 ~161 *Normal ranges: TSH 1-20 tT_4 141-277 free T_4 9.1-23.8 ~ approximate values read from figure	<u>Patient 1</u>: TSH 216 and tT_4 51 (after 3 weeks); TSH ~25 and tT_4 ~219 (after 6 weeks); TSH ~8 and tT_4 ~203 (after 10 weeks) <u>Patient 2</u>: TSH 248 and free T_4 <5.2 (at 4 years); TSH 1.48 and tT_4 232 (at 5 years); TSH ~1-2 and tT_4 ~148 (at 5.5-6 years) ~ approximate values read from figure	discontinuation of soya-based formula and soya milk and decrease in the dose of thyroxine treatment led to normalisation of thyroid function CIs and p values not specified	Fruzza *et al.*, 2012
Infant girl (10 days old) with a small goitre	breastfed for the first 6 days of life by a vegan mother consuming soya milk (level of isoflavones not specified) formula fed (10	Dose of thyroxine not specified. Treatment was stopped at 2 weeks of age.	TSH 88 T_4 not specified *Normal ranges not specified	TSH 8.5 and fT_4 <5 (at 16 days of age); TSH 1.64 and fT_4 19.7 (at 149 days of age)	goitre disappeared by the age of 2 months presence of goitre was associated with mother's iodine deficiency CIs and p values not specified	Shaikh *et al.*, 2003

	µg iodine/100 mL) from 6 days onwards					
Children with thyroid dysfunction not receiving treatment/treatment not specified						
Mothers-infants pairs Group A: infants with congenital hypothyroidism (n=39) Group B: healthy infants (n=20)	Type of diet not specified.	Dose and type of treatment not specified.	Not measured.	Not measured.	level of genistein in serum in healthy infants was significantly 1.6 fold higher than in Group A (p=0.0026) CIs not specified	Jung *et al.*, 2013
Primary school girls Group A: with goitre (n=284) Group B: without goitre (n=288)	*Soya* (level of isoflavones and type of food not specified) was consumed during one month prior to questionnaire by 43.7% (Group A) and 45.5% (Group B) of participants	Dose and type of treatment not specified.	Not measured.	Not measured	no significant difference between groups in soya consumption during the last months (p=0.661; 95% CI 0.668-1.292) the major risk factors for goitre were storage of iodised salt in open containers (p<0.0001; 95% CI 1.412-3.428), medium socioeconomic situation (p=0.041; 95% CI 1.029-4.282), place of residence in Qom (Iran) (District 2: p=0.005; 95% CI 1.376-6.027 and	Mousavi *et al.*, 2006

					District 3: p=0.041; 95% CI 1.032-4.078).	
Children with normal thyroid function						
School children without overt thyroid disease (8-15 years old) Group A: consumed soya-based food within previous 24 h (n=36) Group B: no soya-based food eaten within previous 24 h (n=229)	*Soya-based food* consumed within 24 h prior to examination type of food and level of isoflavones not specified	No treatment	Not measured *Normal ranges: TSH 0.3-4.3 free T_4 12-22 free T_3 2.8-7.1	Group A: TSH mean 3.6 fT_4 mean 16.48 fT_3 mean 7.18 Anti-Tg 0.058 Anti-TPO 0.02 Group B: TSH mean 3.2 fT_4 mean 15.42 fT_3 mean 7.03 Anti-Tg 0.041 Anti-TPO 0.008	level of genistein and daidzein measured in serum in Group A was significantly 1.7 fold higher than in Group B (p=0.0025) level of free T_4 was significantly higher in Group A (p=0.0032) no other significant differences were found between the groups in all children there was a significant positive correlation of genistein/anti-Tg (p=0.000), daidzein and genistein/anti-Tg (p=0.0004), and daidzein/TSH (p=0.0027). A significant negative correlation of genistein with thyroid volume (p=0.0067). CIs not specified	Milerova *et al*., 2006

Adults with normal thyroid function						
Postmenopausal women (45-65 years old) <u>Group A:</u> consuming 80 mg of isoflavones/day <u>Group B</u>: consuming 120 mg of isoflavones/day <u>Group C:</u> consuming placebo	*Isoflavones tablets* provided: Group A: ~0.84-1.83 mg isoflavones/kg bw/day Group B: ~1.35-2.59 mg isoflavones/kg bw/day consumed as 3 compressed tablets/day ~ approximate values calculated based on specified body weights	No treatment.	Not specified for respective groups	Not specified for respective groups	serum TSH measurements as secondary outcome no effects on circulating TSH concentrations in treatment groups when compared to baseline levels	Alekel *et al*., 2014
Osteopenic postmenopausal women <u>Group A</u>: consuming genistein tablets (n=40) <u>Group B</u>:	*Genistein tablets* 54 mg/day duration of treatment was 3 years	No treatment.	<u>Group A</u>: TSH [µg/ml] mean 2.04; fT_4 mean 16.98 fT_3 mean 3.51 Anti-Tg 29.30 Anti-TPO 19.76	<u>Group A</u>: TSH [µg/ml] mean 2.02; fT_4 mean 17.50 fT_3 mean 3.41 Anti-Tg 29.00 Anti-TPO 19.35	no statistically significant differences in thyroid parameters (all within normal range) between both groups CIs not specified	Bitto *et al*., 2010

consuming placebo (n=37)			Group B: TSH [µg/ml] mean 1.69; fT_4 mean 17.37 fT_3 mean 3.60 Anti-Tg 32.90 Anti-TPO 20.33 *Normal ranges: TSH [µg/ml] 0.27-4.2 free T_4 11.96-21.87 free T_3 2.31-5.92	Group B: TSH [µg/ml] mean 1.7; fT_4 mean 17.88 fT_3 mean 3.47 Anti-Tg 30.50 Anti-TPO 18.34		
Postmenopausal women not on hormone replacement therapy (64-83 years) Group A: consuming isoflavone supplement (n=22) Group B: consuming placebo (n=16)	*Isoflavone supplement* -30 mg isoflavones consumed three times a day for 6 months participants were asked to maintain normal diet and limit intake of soya-based foods to one serving/week	No treatment.	Group A: TSH mean 3 tT_4 mean 149 tT_3 mean 1.53 Group B: TSH mean 3.35 tT_4 mean 145 tT_3 mean 1.55 *Normal ranges not specified	After 90 days: Group A: TSH mean 3.4 tT_4 mean 149.5 tT_3 mean 1.56 Group B: TSH mean 3.91 tT_4 mean 148 tT_3 mean 1.65 After 180 days: Group A: TSH mean 3.5 tT_4 mean 154.5 tT_3 mean 1.78	no statistically significant differences in thyroid parameters (all within normal range) between both groups CIs not specified	Bruce *et al.,* 2003

				Group B: TSH mean 3.63 tT_4 mean 154 tT_3 mean 1.75		
Healthy men (n=35; 20-40 years) <u>Arm 1:</u> Milk Protein Isolate (MPI) <u>Arm 2</u>: Low-isoflavone Soya Protein Isolate (low-iso SPI) <u>Arm 3:</u> High-isoflavone Soya Protein Isolate (high-iso SPI)	*Low-iso SPI* mean ± SD 1.64 ± 0.19 mg isoflavones/day *High-iso SPI* mean ± SD 61.7 ± 7.4 mg isoflavones/day all participants took part in 3 arms (57 days each) separated by 28 days of washout period participants were asked to maintain normal diet and limit intake of soya-based foods	No treatment	<u>Arm 1:</u> TSH mean 2.14 (95% CI 1.97-2.33) tT_4 mean 80.4 fT_4 mean 17.3 tT_3 mean 1.78 fT_3 mean 4.76 <u>Arm 2</u>: TSH mean 2.14 (95% CI 1.97-2.33) tT_4 mean 79.2 fT_4 mean 17.1 tT_3 mean 1.76 fT_3 mean 4.79 <u>Arm 3</u>: TSH mean 2.11 (95% CI 1.94-2.30) tT_4 mean 78.8 fT_4 mean 16.9 tT_3 mean 1.76 fT_3 mean 4.77	After 29 days: <u>Arm 1:</u> TSH mean 2.04 (95% CI 1.87-2.22) tT_4 mean 78.9 fT_4 mean 16.3 tT_3 mean 1.79 fT_3 mean 4.9 <u>Arm 2:</u> TSH mean 1.82 (95% CI 1.67-1.98) tT_4 mean 75.5 fT_4 mean 16.3 tT_3 mean 1.75 fT_3 mean 4.69 <u>Arm 3</u>: TSH mean 1.91 (95% CI 1.76-2.08) tT_4 mean 78.5 fT_4 mean 16.6 tT_3 mean 1.80 fT_3 mean 4.75 After 57 days: <u>Arm 1</u>: TSH mean 1.99	no significant differences in serum concentrations of thyroid parameters among groups during the study urinary excretion of isoflavones was 19 (daidzein) and 18 (genistein) folds higher in Arm 3 when compared to Arm 2, and 26 and 28 folds higher when compared to Arm 1 respectively ($p<0.0001$ for all comparisons).	Dillingham *et al.*, 2007

			*Normal ranges not specified	(95% CI 1.82-2.18) tT_4 mean 79.7 fT_4 mean 16.9 tT_3 mean 1.77 fT_3 mean 4.7 <u>Arm 2:</u> TSH mean 1.90 (95% CI 1.73-2.08) tT_4 mean 80 fT_4 mean 16.8 tT_3 mean 1.83 fT_3 mean 4.92 <u>Arm 3:</u> TSH mean 2.02 (95% CI 1.84-2.21) tT_4 mean 80.5 fT_4 mean 17.1 tT_3 mean 1.77 fT_3 mean 4.80		
University students without overt thyroid disease (n=86; 32M and 54F; 18-25 years old)	*Natural soya beans* unprocessed, boiled 2 g of soya beans/kg bw/day consumed for 7 days	No treatment.	<u>Men:</u> TSH ~1.4 fT_4 ~12.25 fT_3 ~5.25 <u>Women:</u> TSH ~1.5 fT_4 ~11.5 fT_3 ~4.8	<u>Men:</u> TSH ~1.8; fT_4 ~12.35; fT_3 ~5 (after 7 days of treatment); TSH ~1.6; fT_4 ~11.9; fT_3 ~5.12 (7 days after treatment termination)	levels of isoflavones significantly increased following soya consumption: for genistein (by 4 folds in men and ~3 folds in women) and for daidzein (by 8 folds in men and by 4 folds in women) ($p<0.0001$). Return to	Hampl *et al.*, 2008

	isoflavone content: approximately 1.2 to 4.2 mg per 1 g of dry weight		*Normal ranges: TSH 0.3-4.2 ~ approximate values read from figure	<u>Women:</u> TSH ~1.55; fT_4 ~11.7; fT_3 ~4.65 (after 7 days of treatment); TSH ~1.46; fT_4 ~11.8; fT_3 ~4.8 (7 days after treatment termination)	nearly initial values 7 days after treatment termination. no significant changes in thyroid parameters were found in women a significant increase in TSH ($p<0.0001$) in men following soya consumption CIs not specified	
Women within 5 years of menopause (45-60 years old) <u>Group A</u>: consuming soya isoflavone tablets (n=122) <u>Group B:</u> consuming placebo (n=126)	*Soya isoflavone tablets* 200 mg of isoflavones (91 mg genistein and 103 mg daidzein) corresponding to 2.05-4.50 mg/kg bw/day taken as 4 tablets daily (50 mg each) for 2 years	No treatment.	Not specified.	<u>Group A:</u> TSH increased by 0.32 (after 1 year treatment) and by 0.04 (after 2 year treatrment) <u>Group B</u>: TSH decreased by 0.15 (after 1 year treatment) and by 0.61 (after 2 year treatment)	thyroid parameters as secondary outcome no effects on TSH concentrations in treatment groups when compared to baseline levels mean total urinary isoflavones increased significantly by 56.5 pmol/µl in Group A ($p<0.001$), and by 2.9 pmol/µl in Group B after 2 years of treatment.	Levis *et al.,* 2011
Women in early	*Soya*	No treatment.	Not measured	<u>Group A</u>:	urinary levels of genistein	Li *et al*., 2011

stages of pregnancy (n=505) Group A: frequent soya consumers (n=94) Group B: conventional soya consumers (n=316) Group C: occasional soya consumers (n=95)	*consumption*: frequent – 3 or more times/week conventional – 2 times/month to 3 times/week occasional – 2 or fewer times/month type of food and level of isoflavones not specified		*Normal ranges: TSH 0.13-3.93 fT_4 12-23.34 fT_3 3.46-7.70	TSH mean 2.38 fT_4 mean 17.93 Group B: TSH mean 2.12 fT_4 mean 18.15 Group C: TSH mean 2.35 fT_4 mean 18.20	and daidzein (measured in 20% of participants) were significantly lower in conventional (n=59) and occasional (n=16) consumers when compared with frequent (n=20) consumers ($p<0.01$) no significant difference in TSH and free T_4 between all groups CIs not specified	
Oophorectomised women (<55 years old) Group A: consuming isoflavone tablet (n=17) Group B: consuming placebo tablet (n=17)	*Isoflavone tablet* contained 75 mg of soya isoflavones consumed once a day for 12 weeks participants were asked to avoid soya-based foods	No treatment.	Group A: TSH mean 1.87 fT_4 mean 13.88 fT_3 mean 4.05 Anti-TPO mean 42.06 Group B: TSH mean 2.04 fT_4 mean 13.45 fT_3 mean 4.21 Anti-TPO mean 98.25 *Normal ranges:	Group A: TSH mean 1.5; fT_4 mean 13.88; fT_3 mean 4.12; Anti-TPO mean 61.42 (after 6 weeks); TSH mean 3.28; fT_4 mean 13.61; fT_3 mean 3.76; Anti-TPO mean 63.75 (after 12 weeks) Group B: TSH mean 1.83; fT_4	significant decrease in free T_3 levels following treatment with isoflavones in group A ($p=0.02$) no other significant changes in thyroid parameters after 12 weeks of treatment between the groups CIs not specified	Mittal *et al.*, 2011

			TSH 0.5-5.5	mean 13.35; fT_3 mean 4.12; Anti-TPO mean 134.58 (after 6 weeks); TSH mean 2.72; fT_4 mean 13.55; fT_3 mean 4.07; Anti-TPO mean 131.53 (after 12 weeks);		
Menopausal women (40-60 years old) <u>Group A:</u> consuming 80 mg of isoflavones/day <u>Group B</u>: consuming 120 mg of isoflavones/day <u>Group C:</u> consuming placebo	*Isoflavones tablets* provided: <u>Group A</u>: mean ~1.16 mg isoflavones/kg bw/day <u>Group B</u>: mean ~1.77 mg isoflavones/kg bw/day ~ approximate values calculated based on specified body weights intake of soya food limited to one serving/week	No treatment.	<u>Group A:</u> TSH 2.2; fT_4 15.44 (n=135) <u>Group B</u>: TSH 2.4; fT_4 15.44 (n=134) <u>Group C</u>: TSH 2.8; fT_4 14.16 (n=134) *Normal ranges: TSH 0.35-5.5 fT_4 10.29-23.16	<u>Group A</u>: TSH 2.5; fT_4 15.44 (after 1 year treatment, n=122) and TSH 2.4; fT_4 14.16 (after 2 years of treatment, n=119) <u>Group B</u>: TSH 2.6; fT_4 15.44 (after 1 year of treatment, n=123) and TSH 2.2; fT_4 14.16 (after 2 years of treatment, n=117) <u>Group C:</u> TSH 2.6; fT_4 14.16 (after 1 year of treatment, n=128)	thyroid parameters as secondary outcome slightly lower (p=0.052) free T_4 levels in Group A and B when compared to placebo no significant changes in TSH concentrations after 1 and 2 years of treatment between the groups CIs not specified	Steinberg *et al*., 2011

				and TSH 2.4; fT_4 15.44 (after 2 years of treatment, n=126)		
Postmenopausal women (mean age 58 years) <u>Arm 1:</u> consuming seaweed capsules for 7 weeks (+high isoflavone powder in the 7th week of treatment) <u>Arm 2:</u> consuming placebo capsules for 7 weeks (+high isoflavone powder in the 7th week of treatment)	*High isoflavones powder* 2 mg of isoflavones/kg bw/day consumed once a day for 7 days in the last week of treatment all participants took part in 2 arms (7 weeks each) separated by 3 week washout period	No treatment	Not specified	<u>Arm 1</u>: TSH mean 2.19; tT_4 mean 85; tT_3 mean 1.97 (after 6 weeks with seaweed only); TSH mean 1.94; tT_4 mean 86; tT_3 mean 1.94 (after 7 weeks when isoflavones were added for one week) <u>Arm 2</u>: TSH mean 1.69; tT_4 mean 85; tT_3 mean 1.91 (after 6 weeks with placebo only); TSH mean 1.64; tT_4 mean 87.5; tT_3 mean 1.93 (after 7 weeks when isoflavones were added for one week)	seaweed consumption significantly increased levels of TSH and urinary iodine excretion ($p<0.01$). This effect was not altered by isoflavone ingestion no significant changes in thyroid parameters following isoflavone consumption CIs not specified	Teas *et al*., 2007
Premenopausal women (18-28 years	*Soya foods*	No treatment.	<u>Group A:</u> TSH ~0.027	<u>Group A:</u> TSH ~0.029	thyroid parameters as secondary outcome	Zhou *et al*., 2011

old) <u>Group A:</u> consuming soya foods (n=31) <u>Group B:</u> consuming animal foods (n=32)	soya protein content ranged from 18 to 22 g/day isoflavone content was approximately 36 mg/day 2 to 3 servings per day during 10 weeks period participants were asked to restrict any additional soya foods and limit animal foods to one serving/day		fT_4 15.83 <u>Group B:</u> TSH ~0.030 fT_4 15.70 ~ approximate values read from figure *Normal ranges not specified	fT_4 15.70 <u>Group B:</u> TSH ~0.026 fT_4 15.44 ~ approximate values read from figure	no significant changes in thyroid parameters following soya food consumption significant difference in level of TSH between two groups ($p<0.05$) mean total urinary isoflavones increased significantly by 16 folds in Group A ($p=0.016$), and decreased by 1.4 fold in Group B CIs not specified	
Food Standards Agency-funded research "The effect of soya phytoestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism"						
PART ONE						University of Hull 2005-2015 PART ONE published by Sathyapalan *et al.*, 2011
Patients with subclinical hypothyroidism (n=60; 8M and 52F; 44-70 years old) <u>Arm 1:</u>	*Phytoestrogen material* 54% genistein, 35% daidzein and 12% glycitein	Thyroxine treatment was commenced after diagnosis with overt hypothyroidism	<u>Arm 1</u>: TSH mean 7.8 fT_4 mean 11.8 fT_3 mean 4.0 <u>Arm 2:</u> TSH mean 7.9	<u>Arm 1:</u> TSH mean 7.5; fT_4 mean 11.9; fT_3 mean 4.4 (after 3 months) <u>Arm 2:</u>	6 patients (10%; all females) developed overt hypothyroidism (defined as TSH>4.7 mU/L and fT_4 <9 pmol/L) after treatment with 16 mg isoflavones	

consumed 2 mg phytoestrogen with 30 g soya protein powder <u>Arm 2:</u> consumed 16 mg phytoestrogen with 30 g soya protein powder	participants were asked to avoid soya-based foods all participants took part in 2 arms (8 weeks each) separated by 8 week washout period		fT_4 mean 12.2 fT_3 mean 4.2 *Normal ranges: TSH 0.5-4.7 fT_4 9-24 fT_3 2.5-5.3	TSH mean 8.4; fT_4 mean 11.3; fT_3 mean 4.3 (after 3 months)	Anti-TPO were positive (>75 U/ml) in 38 (63.3%) patients no significant changes in thyroid parameters between two arms levels of isoflavones significantly increased following phytoestrogen supplementation: for genistein (by 4 folds in arm 1 and 19 folds in arm 2) and for daidzein (by 5.5 folds in arm 1 and 12 folds in arm 2) ($p<0.01$)	
PART TWO						
Patients with subclinical hypothyroidism (n=34; 17M and 17F; 23-80 years old) <u>Arm 1:</u> consumed 30 g soya protein powder (phytoestrogen free)	*Phytoestrogen material* 54% genistein, 35% daidzein and 12% glycitein participants were asked to avoid soya-	Thyroxine treatment was commenced after diagnosis with overt hypothyroidism	<u>Arm 1</u>: TSH mean 5.0 fT_4 mean 12.2 fT_3 mean 4.6 <u>Arm 2</u>: TSH mean 5.3 fT_4 mean 11.3 fT_3 mean 4.7	<u>Arm 1</u>: TSH mean 5.2; fT_4 mean 12.0; fT_3 mean 4.3 (after 2 months) <u>Arm 2</u>: TSH mean 5.5; fT_4 mean 11.9; fT_3 mean 4.7 (after 2	2 patients (6%; 1M, 1F) developed overt hypothyroidism (defined as TSH>4.7 mU/L and fT_4 <9 pmol/L) after treatment with 66 mg phytoestrogens Anti-TPO were positive (>75 U/ml) in 17 (50%)	

<u>Arm 2:</u> consumed 66 mg phytoestrogen with 30 g soya protein powder	based foods all participants took part in 2 arms (8 weeks each) separated by 8 week washout period		*Normal ranges: TSH 0.5-4.7 fT_4 9-24 fT_3 2.5-5.3	months)	patients statistically significant increase in TSH (p=0.004) and decrease in fT_3 (p=0.03) in males after treatment with 66 mg phytoestrogens no significant changes in thyroid parameters between two arms levels of isoflavones significantly increased following 66 mg phytoestrogen supplementation: by 19 folds for genistein and by 8 folds for daidzein (p=0.00) CIs not specified	
PART THREE						
Patients with subclinical hypothyroidism (n=42; 16M and 26F; 23-80 years old) <u>Arm 1:</u>	all participants took part in 2 arms (8 weeks each) separated by 8 week washout period	No treatment.	<u>Arm 1:</u> TSH 5.70; fT_4 12.60; fT_3 4.44 <u>Arm 2:</u> TSH 5.93;	<u>Arm 1:</u> TSH 5.86 (95% CI: -0.06-0.16); fT_4 11.06 (95% CI: -0.96-0.5); fT_3 4.37 (95% CI: -0.12-0.11)(after 3	none of the patients developed overt hypothyroidism during the study period no significant changes in thyroid parameters were	

consumed 30 g soya protein powder (phytoestrogen free) <u>Arm 2:</u> consumed 30 g casein protein powder			fT_4 12.11; fT_3 4.40 *Normal ranges: TSH 0.5-4.7 fT_4 9-24 fT_3 2.5-5.3	months) <u>Arm 2:</u> TSH 5.71 (95% CI: -0.71-0.60); fT_4 11.93 (95% CI: -0.36-0.12); fT_3 4.43 (95% CI: -0.14-0.20) (after 3 months)	observed after the treatment	
Food Standards Agency-funded research "A double blind placebo controlled parallel trial of soya isoflavones on markers of bone turnover in females in early menopause"						
Women within two years of the onset on menopause (50-55 years old) <u>Group A:</u> consumed 30 g soya protein powder with 66 mg phytoestrogen (n=101) <u>Group B:</u> consumed 30 g soya protein powder (phytoestrogen free) (n=99)	*Soya protein powder and isoflavones* consumed twice a day as a 15 g bar containing 33 mg isoflavones for 6 months		<u>Group A</u>: TSH median 1.5 fT_4 median 13 fT_3 median 4.6 <u>Group B:</u> TSH median 1.6 fT_4 median 13 fT_3 median 4.7	<u>Group A:</u> TSH ~2.9; fT_4 ~12 (after 3 months); TSH ~2.9; fT_4 ~11 (after 6 months) <u>Group B</u>: TSH ~1.65; fT_4 ~13 (after 3 months); TSH ~1.6; fT_4 ~13 (after 6 months) ~ approximate median values read from figure	significant increase (although within normal ranges) in AUC for TSH (2.34 vs 1.71 mU/L; p=0.004; 95% CI: 0.18-0.95) and significant decrease in AUC for fT_4 (12.2 vs 13.1 pmol/L; p=0.02; 95% CI: -1.8- -0.1) with Group A compared to Group B significant increase in AUC for daidzein (22.6 vs 4.3 ng/ml; p<0.001; 95% CI:11.3-25.3) and genistein (89.1 vs 8.4 ng/ml; p<0.001; 95% CI: 56.3-104.8) following	University of Hull 2010-2014

					consumption of phytoestrogens when Group A compared to Group B	
Food Standards Agency-funded research "A double blind placebo controlled parallel trial of soya phytoestrogens in patients with compensated hypogonadism"						
Men with type 2 diabetes and subclinical hypogonadism (45-75 years old) <u>Group A:</u> consumed 30 g soya protein powder with 66 mg isoflavones (n=107) <u>Group B:</u> consumed 30 g soya protein powder (isoflavone free) (n=103)	*Soya protein powder and isoflavones* consumed twice a day as a 15 g bar containing 33 mg isoflavones for 3 months participants were asked to avoid soya-based foods	No treatment.	<u>Group A</u>: TSH median 1.82 fT_4 median 12.62 fT_3 median 4.66 <u>Group B:</u> TSH median 1.81 fT_4 median 13.08 fT_3 median 4.64	<u>Group A</u>: TSH median 3.28; fT_4 median 11.08; fT_3 median 4.71 (after 3 months) <u>Group B:</u> TSH median 1.97; fT_4 median 12.71; fT_3 median 4.55 (after 3 months)	significant increase (although within normal ranges) in TSH level ($p<0.01$; 95% CI: -1.63- -1.28) and decrease in free T_4 ($p<0.01$; 95% CI: 0.96-2.12) following treatment with 66 mg isoflavones levels of isoflavones significantly increased following 66 mg isoflavone supplementation: by 20 folds for genistein and by 13 folds for daidzein ($p<0.01$)	University of Hull 2009-2015

[1] Except Bitto *et al*., 2010 where different technique was used to measure TSH, which levels were reported as µg/ml as opposed to mIU/L

TSH – thyroid stimulating hormone; fT_3 – free triiodothyronine; tT_3 – total T_3; fT_4 – free thyroxine; tT_4 – total T_4; CI – confidence interval; Anti-Tg – thyroglobulin autoantibodies; Anti-TPO – thyroperoxidase autoantibodies; SPI – soya protein isolate; MPI – milk protein isolate; AUC – area under the curve

Appendix 1

Search strategy

Websites of international authorities/advisory bodies interrogated

- COT
- EFSA
- FSA
- IARC
- JECFA

Scientific literature search

PubMed was interrogated to identify publications published until November 2014 and relevant to effects of soya consumption on thyroid status. Specific search terms and the number of hits associated with those queries are listed below. The list below does not take into account overlap of search results between search terms. These hits have been further refined via interrogation of the abstracts and the articles themselves, excluding articles that were investigating effects of soya-based food products/isoflavones/phytoestrogens in combination with other compounds, in individuals with health problems not relevant to thyroid, in individuals with concomitant illnesses, studies in animals and in vitro studies, studies mentioned in the 2003 COT report. A reasonable number of relevant studies investigating thyroid effects in humans have been found. Therefore they have been the only ones included in this statement. The final list of articles used in this document can be found in the references section.

Specific search terms	Hits in Pubmed
Flavonoids AND hypothyroidism	27
Flavonoids AND subclinical hypothyroidism	2
Genistein/Daidzein/Glycitein AND hypothyroidism	4/2/0
Genistein/Daidzein/Glycitein AND subclinical hypothyroidism	1/0/0
Genistein/Daidzein/Glycitein AND thyroid	93/30/1
Isoflavones AND hypothyroidism	20
Isoflavones AND subclinical hypothyroidism	2
Isoflavones AND thyroid	143
Phytoestrogens AND hypothyroidism	6

Phytoestrogens AND subclinical hypothyroidism	2
Phytoestrogens AND thyroid	98
Soy/Soya AND hypothyroidism	22/22
Soy/Soya AND subclinical hypothyroidism	1/1
Soy/Soya AND thyroid	124/134
Soy/Soya protein AND hypothyroidism	7/5
Soy/Soya protein AND subclinical hypothyroidism	1/0
Soy/Soya protein AND thyroid	71/52