TOX/2019/71

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

Discussion paper on soya drink consumption in children aged 6 months to 5 years of age.

Background

1. Soya drinks are a popular alternative to dairy products and their use is becoming more widespread. In 2018, 95.6 million litres of soya drinks were sold in the UK amounting to sales of £135.6 million pounds. They are commonly consumed by all sectors of the population including those wishing to avoid dairy products and individuals with an intolerance to lactose or another component of milk.

2. Soya products contain phytoestrogens (also known as isoflavones) which have been shown to have an effect on development and reproductive changes in animal studies, although human epidemiological studies have not produced conclusive results. The COT considered the safety of soya phytoestrogens in 2003[1] and in 2013[2]. This paper was considered at the October 2019 meeting, but revised and presented at the meeting in December, due to the identification of more appropriate analytical figures for isoflavone content of foods.

3. In the statement from 2013 (also attached as annex 1), the Committee on Toxicity concluded that:

> "Evidence from the few relevant epidemiological studies does not suggest important impacts of soya-based formula on later reproductive health in humans, although some studies have raised the possibility of subtle effects of uncertain clinical significance. However, animal studies where exposure to isoflavones was at levels similar to those reported in infants exclusively fed soya- based infant formula indicate some developmental and reproductive changes. There is thus some uncertainty about the safety of soya-based formula.
>
> There is no scientific basis for a change in the current government advice that there is no substantive medical need for, nor health benefit arising from the use of soya-based infant formula and it should only be used in exceptional circumstances to ensure adequate nutrition."

4. Levels of phytoestrogens in soya-based infant formula have been found to range from 18 - 46.7 mg/L. Levels in soya drinks have been found to be around 100 mg/L (BDA, 2017). The Department of Health and Social Care (DHSC), Public Health England (PHE) and the FSA are receiving an increasing number of enquiries

[1] Report available at: https://cot.food.gov.uk/sites/default/files/cot/phytoreport0503.pdf
[2] Statement available at https://cot.food.gov.uk/sites/default/files/cot/cotstaphytos.pdf

regarding the use of plant-based drinks in the diets of infants and young children. It therefore seemed appropriate for the COT to consider the potential health effects of soya drinks in the diets of children aged 6 months to 5 years of age given that the levels of phytoestrogens are greater than those found in soya-based infant formula. In the interests of completeness, the Secretariat have also carried out an exposure assessment on other soya-based products that are used as replacements for dairy products in this age group.

5. Current advice to parents on NHS Choices in relation to dairy products and soya alternatives can be found in annex 2 to this paper. The WHO state that soya-based drinks are unsuitable as a major source of nutrients in non-breastfed children aged 6-24 months of age but do not address safety or nutritional concerns (WHO, 2004).

Update to the literature since the 2013 COT review

6. Few original studies have been published since the Committee last reviewed the health effects of phytoestrogens in the diets of infants and young children in 2013. Described below are those relevant papers identified in an updated literature search focusing primarily on exposure post-weaning. Search terms can be found at the end of this paper in Appendix A.

Human studies

7. Fifty boys and 51 girls from a larger cohort study, who were either breast fed (n=35), fed cows' milk formula (n=32) or fed soya-based formula (n=34) underwent an ultrasound examination to assess volume and characteristics of breast buds, uterus, ovaries, prostate, and testes at 5 years of age. No differences were found between groups in volume or characteristics of the examined organs. Follow-up of this group through puberty is planned (Andres et al, 2015).

8. In a case-control study of 200 patients with Kawasaki disease (an acute vasculitis that can lead to heart disease in children) and 200 age-matched controls, intakes of isoflavones (OR 2.33 95% CI 1.37-3.96) or genistein (OR 2.46 95% CI, 1.46-4.16) were found to be associated with an increased risk for Kawasaki disease. No effect of maternal diet was observed (Portman et al, 2016).

9. Infant girls fed soya-based infant formula or cows' milk formula, were recruited to provide vaginal swab samples at 2 weeks and 4 weeks of age and monthly up to 9 months of age. These samples were analysed by pyrosequencing for methylation levels. Samples from girls receiving soya-based infant formula were found to have a significantly higher degree of methylation in vaginal epithelial cells compared to those receiving cows' milk formula. The long-term implications of this are currently unknown (Harlid et al, 2017).

10. Infants (n = 410) enrolled after parents had made the choice on primarily source of infant food, (although families were approached before this time) were

followed for 28 weeks (boys) or 36 weeks (girls). Infants exclusively receiving soya formula (n = 102), cow's milk formula (n = 111) or breast milk (n = 70) were included in the study. Primary visits were completed within 72 hours of birth and follow-up visits were carried out at 2, 4, 8, 12, 16, 20, 24, 28, 32 and 36 weeks for girls and at 2, 4, 8, 12, 16, 20, 24 and 28 weeks for boys. At all visits, adherence to feeding regime and anthropometry were assessed and urogenital epithelial cells were collected. From week 2, blood samples were taken at each visit. The reason for feeding choices were assessed by questionnaire at week 2. Ultrasound images of breast buds and uterus were taken at birth and at weeks 4, 16, 24 and 32. Blood samples were analysed for serum hormones.

11. Maternal demographics did not differ between soy- and cows'- milk formula, but they did differ between formula-fed and breast-fed infants. Following adjustment for confounders, vaginal-cell maturation index (MI) was significantly higher (P = 0.01) and uterine volume decreased more slowly (P = 0.01) in soy-fed girls compared with cows' milk–fed girls; however, their trajectories of breast-bud diameter and hormone concentrations did not differ. The study observed no significant differences between boys fed cow-milk vs soy formula with oestradiol undetectable. Breastfed infants differed from soy-formula–fed infants in vaginal-cell MI, uterine volume (P < 0.01), and girls' estradiol and boys' breast-bud diameter (P = 0.02) (Adgent et al, 2018).

12. Healthy children from Bogota, Columbia, were given a fruit juice supplemented with soya protein-based supplement (n=29) or not supplemented (n=22) for 12 months. A nutritional assessment was carried out (bodyweight, height, triceps skinfold thickness, mid-upper arm circumference), body mass index (BMI), upper arm muscle area, arm muscle circumference, upper arm area, upper arm fat area; age and gender-specific percentiles were used as reference. Sexual maturation was measured by Tanner stage. Height, BMI/age, weight/age and height/age were significantly different (P < 0.05) at 12 months between girls in the control and intervention groups. Statistically significant differences between groups by gender (P < 0.05) were found in boys in the control group for the triceps skinfold thickness and fat area. Nutritional status was adequate according to the World Health Organization parameters. On average, 0.130 mg/kg body weight/day of isoflavones were consumed by children. Consumption of soya protein supplement did not affect sexual maturation or the onset of puberty in prepubertal boys and girls; however, it may have induced an increase in height, BMI/age, height/age and weight/age of the girls, associated with variations in fat-free mass (Duitama et al, 2018).

13. Boys aged 12-18 (n=248), self-reported their age at the onset of puberty and completed a food-frequency questionnaire. Energy-adjusted mean intakes of soya phytoestrogens were 0.8-54.9 mg/d for total isoflavones, 0.4-22.1 mg/d for daidzein, and 0.4-28.0 mg/d for genistein. Moderate and high total soy isoflavone intake were significantly associated with earlier adjusted median age at the onset of puberty: 12.58 years [RR (95% CI): 1.58 (1.06, 2.36)] for moderate and 12.50 years [RR (95% CI): 1.63 (1.03, 2.60)] for high vs. 13.00 years for low consumers. Similarly,

daidzein and genistein consumption was also significantly associated with age at pubarche. No significant associations were found for facial hair for any of the isoflavones (Segovia-Siapco et al, 2018).

Animal studies

14. Reproductive effects in animal models have been fairly well established. Further studies have been published since the 2013 COT statement, but these do not appear to add significantly to the overall database.

15. Weanling male Sprague Dawley rats (10 per group) were allocated to groups as follows: 1) diet contained casein as the sole protein source, 2) diet contained soya protein isolate as the sole protein source, 3) casein plus 10 µg/kg bw/day estradiol via osmotic mini-pump and 4) soya protein isolate plus 10 µg/kg bw/day estradiol via osmotic mini-pump. Estradiol treatment reduced testis and prostate weights, and serum androgen concentrations ($P<0.05$). In contrast, soy protein isolate had no effect. Estradiol up-regulated 489 and downregulated 1237 testicular genes >1.5-fold ($P<0.05$). In contrast, soy protein isolate significantly up-regulated expression of 162 genes and down-regulated 16 genes. Soy protein isolate primarily affected pathways associated with macromolecule modifications including ubiquitination and histone methylation and not on reproductive processes per se (Ronis et al, 2018).

16. Female Wistar rats (10/group) were administered soya phytoestrogens via gavage at 0, 25, 50 or 100 mg/kg bw per day from weaning to sexual maturity at 12 weeks. Blood samples were taken at 3 months of age to determine circulating sexual hormone levels and at the oestrous phase of the oestrous cycle, rats were killed and their ovaries and uteruses examined. After soy isoflavones administration, a significant decrease (44%, $P<0.05$) in the serum estrodial levels of the high-dose (HD) group were observed. Cultured granulosa cells from the middle- (MD) and HD groups showed significantly reduced (31%, 45%, respectively; $P<0.05$) in vitro estradiol secretion, and those from the HD group showed significantly reduced progesterone (25%; P,0.05) secretion. Compared with the control group, the mRNA expression of the steroidogenic acute regulatory protein (Star), cytochrome P450 cholesterol side chain cleavage (Cyp11a1 and Cyp19a1), and hydroxysteroid dehydrogenase 3b (Hsd3b) genes also decreased. Real-time quantitative PCR and Western blotting revealed a significant ($P < 0.05$) decrease in key transcription factor steroidogenic factor-1 (SF-1) expression in the HD group. The detection of DNA methylation using bisulphite sequencing PCR (BSP) suggested a significantly ($P < 0.05$) increased total methylation rate in the proximal SF-1 promoter in the HD group. Further studies showed that treatment with soy isoflavones can significantly ($P < 0.05$) increase the mRNA expression of DNA methyltransferase (DNMT) 1 and DNMT3a (Wang et al, 2017). A similar study by the same author showed apoptosis in ovarian follicles following administration at the mid- and high-doses (Wang et al, 2014).

17. On postnatal day 1 (PND1), 50 mice received subcutaneous injections of 100 mg/kg genistein for 10 consecutive days, control mice received an equal volume of DMSO vehicle. On PND 3, 10, 21 and 90, groups of 10 treated and 6 control animals were killed, and their ovaries and uteri were collected. Histological evaluation

showed that the subcutaneous injection of genistein to neonatal mice induced the formation of multi-oocyte follicles and delayed the primordial follicle assembly in the ovaries. Genistein also significantly enlarged the cross-sectional area of the uterine cavity and wall and disrupted the regularity between the uterine stroma and myometrium. Proliferating activity was inhibited by genistein administration because fewer Ki67-positive nuclei were detected in ovarian and uterine cell populations compared to controls. Most ovaries from adult mice given neonatal genistein were without corpora lutea, and there appeared to be cystic follicles and hypertrophy of the theca, and cortical and medullary layers. Given that the observed effects are following intraperitoneal injection of high levels of genistein in an animal model, their relevance to humans is unclear (Wu et al, 2019).

18. Further studies have shown testicular development in rats to be impaired following consumption of a soya-based diet (Napier et al, 2014) and female rats dosed orally with 50 mg/kg bw genistein developed obesity, but males did not (Strakovsky et al, 2014). In contrast, a comparison between rats fed a diet containing casein as a protein source supplemented with 17β-estradiol (E2) and rats fed soy protein isolate showed a significant difference between gene expression of certain genes in ovary tissue. Microarray analysis showed that E2 treatment altered expression of 780 genes more than or equal to 2-fold ($P < 0.05$), whereas SPI feeding altered expression of 53 genes more than or equal to 2-fold (Ronis et al, 2012).

Exposure to phytoestrogens

Occurrence in foods

19. Table 1 below contains information on the isoflavone content of a variety of soya-based foods (data adapted from Kuhnle et al, 2009). Isoflavone content of soya-based foods and beverages is highly variable and these figures are a guide only

Food product	Concentration of phytoestrogens (µg/100g)
Soya milk, unsweetened	6028
Bread, Danish type	747
Burger, soya based	4430
Sausage, TVP/Soya	3994
Tofu, microwaved	10619
Bread, white, sliced	525
Potato, sweet, cooked	251
Yogurt, soya	8286
Mince, savoury, soya TVP based (high Soya content)	28758
Soya ice cream	13494
Soya mince granules, cooked	20850
Cheddar-like soya cheese[3]	6700

[3] Data on soya-based cheese taken from the Linus Pauling Institute, 2004 as no equivalent UK data were available.

Table 1: Data on the soya phytoestrogen content of a range of foods (adapted from data from Kuhnle et al, 2009)

Exposure

20. Potential exposures to isoflavones have been calculated using the isoflavone levels provided above and food consumption estimates taken from the UK Dietary and Nutrition Survey of Infants and Young Children (DNSIYC) (DH, 2013) for children up to 18 months of age and those between 18 months and 5 years from the National Diet and Nutrition Survey (NDNS) (Bates et al, 2014; 2016; Roberts et al, 2018).

21. Tables 2, 3 and 4 give the chronic exposure estimates for isoflavones in children between 6 months and 5 years of age, assuming: table 2: milk, yogurt and cheese are replaced with soya-based alternatives; table 3: all dairy is replaced with soya alternatives and table 4: meat is replaced with soya alternatives plus isoflavone contribution from tofu, vegetables and bread. For reference, the exposures to isoflavones from soya-based infant formula for infants aged 0-6 months are given in table 5 (taken from COT statement, 2013).

Drinking milk (excluding infant formula)

Drinking milk (excluding infant formula)				
Age group	Number of consumers	Mean	97.5th Percentile	Maximum
6-18 months	1826	1296.7	7351.8	4102.3
18 months - 5 years	1053	1095.3	3261.7	6313.5
Yoghurt				
6-18 months	1220	287.0	973.0	2387.6
18 months - 5 years	605	210.6	803.6	1368.2
Cheese				
6-18 months	1792	39.7	155.6	303.0
18 months - 5 years	866	42.1	139.6	316.9

Table 2: Estimated isoflavone exposure (µg/kg bw/day) of children aged 6 months to 5 years if consumption of milk, yogurt and cheese were replaced with soya-based alternatives.

Total diary (excluding infant formula)				
Age group	Number of consumers	Mean	97.5th Percentile	Maximum
6-18 months	2319	1196.3	4099.2	7454.2
18 months - 5 years	1112	1178.6	3334.9	6313.5

Table 3: Estimated isoflavone exposure (µg/kg bw/day) of children aged 6 months to 5 years if consumption of all dairy were replaced with soya-based alternatives (including ice-cream).

Meat products, tofu, sweet potato and bread				
Age group	Number of consumers	Mean	97.5th Percentile	Maximum
6-18 months	1935	135	784	1791
18 months - 5 years	1004	1	4	10

Table 4: Estimated isoflavone exposure (µg/kg bw/day) of children aged 6 months to 5 years if consumption of meat-derived burgers, sausages and mince were replaced with soya-based alternatives and contributions from bread (white Danish style loaf), sweet potato and tofu are taken into account.

Isoflavone level in mg aglycone equivalents/L	0-4 months (800mL)	0-4 months (1200 mL)	>4-6 months (800 mL)	>4-6 months (1200mL)
Soya-based formula 18-46.7 mg/L	2.4-6.3	3.7-9.5	1.8-4.8	2.8 - 7.2

Table 5: Estimated total isoflavone exposure (mg/kg bw/day) of infants fed exclusively soya-based infant formula.

Risk characterisation

22. There is a large body of literature which demonstrates effects of soya phytoestrogens on reproductive endpoints in animal studies at levels varying from 1.6-500 mg/kg bw/day (COT, 2003), although many of these studies used administration via the intravenous or subcutaneous injection. It is generally accepted by experts that soya-rich diets in animal models have a detrimental effect on reproductive systems which may be wholly or partly irreversible depending on the timing of exposure. It is also widely recognised that the levels of phytoestrogens found to cause these reproductive effects are similar to those consumed in a soya-rich western diet. What is not currently understood is the relative susceptibility of animals and humans to these effects.

23. It is these uncertainties which prompted the COT to conclude that soya-based infant formula should not be generally consumed unless under exceptional circumstances.

24. Members are asked to review the likely exposures to isoflavones from soya-based drinks and other dairy alternatives and conclude on the suitability of these drinks for children aged 6 months to 5 years of age.

Questions for the Committee:

Members are asked to review the evidence available and consider the following questions:

i) Do the Committee think that intakes of phytoestrogens from consumption of soya drinks may be of concern in children aged 6 months to 5 years of age?
ii) Do the Committee have concerns over other soya-based products in the diets of children aged 6 months to 5 years of age?
iii) Does the Committee think that any potential concerns would be different between children aged 12-24 months and children aged over 24 months?
iv) Do the Committee consider it necessary to issue additional advice aimed at consumers?

Annexes to this paper

Annex 1: COT statement from December 2013

Annex 2: Current advice to parents on soya alternatives from NHS choices

Secretariat

October 2019

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Appendix A

Search strategy

Pubmed (2012-current) was searched using the following search terms:

- phytoestrogen effects, infants
- phyto-oestrogen, infants
- Isoflavone, infants
- Isoflavone, children

References that were already included in the statement from 2013 and in vitro studies were excluded.

TOX/2019/65/Annex 1

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

Discussion paper on soya drink consumption in children aged 6 months to 5 years of age.

This Annex contains the COT Statement on the potential risk from high levels of soya phytoestrogens in the infant diet

This annex is also available at:

https://cot.food.gov.uk/sites/default/files/cot/cotstaphytos.pdf

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

Discussion paper on soya drink consumption in children aged 6 months to 5 years of age.

This Annex contains the current advice on soya alternatives to milk and milk products for children taken from NHS Choices.

Available at: https://www.nhs.uk/conditions/pregnancy-and-baby/understanding-food-groups/

Milk and dairy products

Milk

Breast milk is the only food or drink babies need in the first 6 months of their life. It's best to carry on breastfeeding alongside an increasingly varied diet once you introduce solid foods.

Infant formula is the only suitable alternative to breast milk in the first 12 months of your baby's life. Whole cows' milk can be given as a main drink from the age of 1.

Whole milk and full-fat dairy products are a good source of calcium, which helps your child develop strong bones and teeth.

They also contain vitamin A, which helps the body resist infections and is needed for healthy skin and eyes.

Try to give your child at least 350ml (12oz) of milk a day, or 2 servings of foods made from milk, such as cheese, yoghurt or fromage frais.

Semi-skimmed milk can be introduced from the age of 2, provided your child is a good eater and growing well for their age.

Skimmed or 1% fat milk doesn't contain enough fat, so isn't recommended for children under 5. You can use them in cooking from the age of 1, though.

You can give your child unsweetened calcium-fortified milk alternatives, such as soya, almond and oat drinks, from the age of 1 as part of a healthy, balanced diet.

Toddlers and young children under the age of 5 shouldn't have rice drinks because of the levels of arsenic they contain.

If your child has an allergy or intolerance to milk, talk to your health visitor or GP. They can advise you on suitable milk alternatives.

And current advice on soya formula available at:
https://www.nhs.uk/conditions/pregnancy-and-baby/types-of-infant-formula/

Soya formula

Suitable from: six months, but only under medical supervision.

Soya formula is made from soya beans, not cows' milk. It's occasionally used as an alternative to cows' milk formula for babies who have cows' milk allergy.

There are some concerns about the fact that soya contains phytoestrogens. These are found naturally in some plants.

The chemical structure of phytoestrogens is similar to the female hormone oestrogen. Because of this there are concerns that they could affect babies' reproductive development, especially in babies who drink only soya-based infant formula.

Babies' lower body weight means that they take in much higher amounts of phytoestrogens than adults and older children who eat soya products as part of a mixed diet.

Also, because soya formula contains glucose instead of the milk sugar lactose, it's more likely to harm babies' teeth.

Only use soya formula if it has been recommended or prescribed by your health visitor or GP.