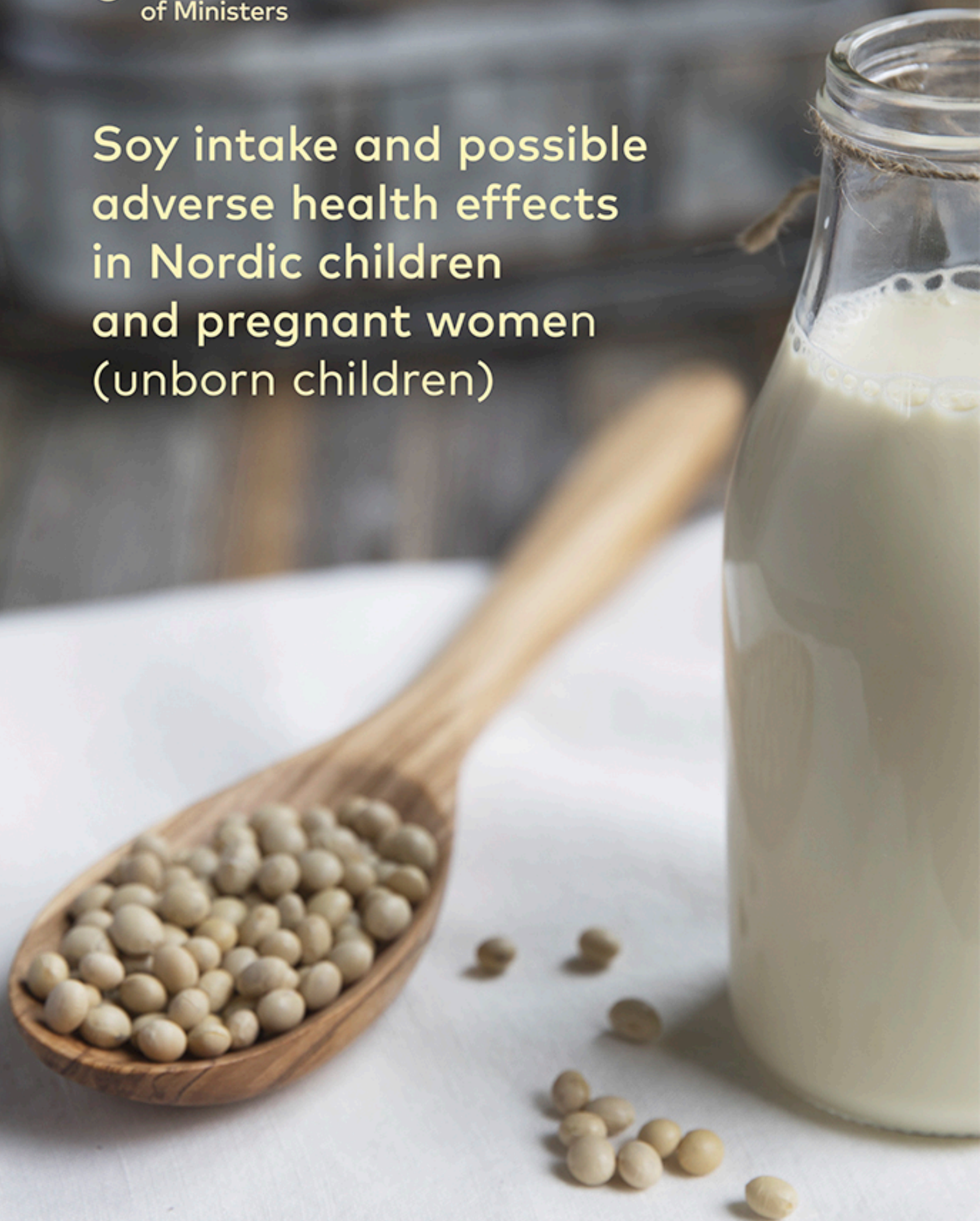


Soy intake and possible adverse health effects in Nordic children and pregnant women (unborn children)



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Preface

This report describes a project funded by the Nordic Working Group for Diet, Food & Toxicology (NKMT) on gathering expertise within dietary intake, nutrition and toxicology in a common evaluation of possible adverse health effects of soy intake among children and pregnant women (unborn children) in the Nordic countries.

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Short summary

The intake of plant-based foods is currently increasing in the Nordic countries and many of these products are based on soy. To meet the development of the population's food intake, there is therefore a need for risk assessments of isoflavones and evaluation of the nutritional impact of soy products to substantiate advice given by risk managers in the Nordic countries. The aim of this report is thus to explore dietary intake, nutrition and toxicology in a joint evaluation of possible adverse health effects of soy intake among children and pregnant women (unborn children) in the Nordic countries. The methodology used was 1) establishment of a dietary exposure scenario with substantial substitution of soy-based products in the diet of Danish women (age 18–45 years) and children (age 4–10 years) for the exploration of effects of soy-based products on dietary intake and nutrition and 2) a literature search for the exploration of animal and human studies relevant for a risk assessment of isoflavones. The results showed that when substituting with soy-based products in the diet, intake of soy is higher in children than in women, due to higher intake of milk products among children. Approximately 60% of the intake of soy is derived from dairy products (milk and cream products) substituted with soy-based drinks and cream among children. Only minor changes occurred in the intake of energy, protein, carbohydrates and fats among women and children, when substituting animal-based products and pulses with soy-based varieties. The intake of various micronutrients changed by this substitution, but did not change the degree of fulfilling recommended intake levels for most nutrients. Health-based guidance values (HBGVs) based on experimental animal data could not be determined for mixed soy constituents, but for genistein intake by pregnant women (unborn children) an HBGV of 0.09 mg/kg bw per day is suggested, and for children an HBGV of 0.07 mg/kg bw per day. Estimated exposure to genistein from a substantial soy-substituted diet is of no concern for pregnant women (unborn children) (mean intake 0.06 mg genistein/kg bw per day) but a potential health concern is indicated for children eating a substantial soy-substituted diet (mean intake 0.16–0.19 mg genistein/kg bw per day).

Kort sammendrag på dansk

Indtag af plantebaserede produkter stiger i øjeblikket i de nordiske lande, og mange af disse produkter er baseret på soja. For at imødegå udviklingen i befolkningens kost er der derfor et behov for risikovurderinger af isoflavoner, samt evaluering af ernæringseffekten af sojaprodukter. Dette skal underbygge de kostråd, der gives af risikohåndtørrerne i de nordiske lande. Formålet med denne rapport, er således at undersøge ernæringsmæssige og toksikologiske (sundhedsskadelige) konsekvenser af stigende sojaindtag blandt børn og gravide kvinder (ufødte børn) i de nordiske lande. De anvendte metoder var 1) etablering af et indtagsscenario, med væsentlig substitution af sojabaserede produkter i kosten hos danske kvinder (alder 18-45 år) og børn (4-10 år), og vurdering af afledte ændringer i indtag af makro- og mikronæringsstoffer og 2) en litteratursøgning, for at identificere relevante dyreforsøg og humane studier til forbedret risikovurdering af isoflavoner.

Resultaterne viste, at i en soja substitueret kost, er indtag af soja højere hos børn end hos kvinder, på grund af et højere indtag af mælkeprodukter blandt børn. Cirka 60 % af indtaget af soja stammer fra mejeriprodukter (mælk og flødeprodukter) erstattet med sojabaserede drikkevarer og fløde blandt børn. Kun mindre ændringer forekom i indtag af energi, protein, kulhydrater og fedt fra den substituerede kost, blandt kvinder og børn, ved erstatning af dyrebaserede produkter og bælgrugter med sojabaserede produkter. Indtag af forskellige mikronæringsstoffer ændrede sig i den substituerede kost, men ændrede ikke graden af opfyldelse af de anbefalede indtagniveauer. Der blev ikke fundet egnede data til at fastsætte toksikologiske grænseværdier for soja. For genistein kunne forsøgsdyrsdata anvendes til at foreslå toksikologiske grænseværdier for gravide kvinder (ufødte børn) på 0,09 mg/kg legemsvægt pr. dag og for børn på 0,07 mg/kg legemsvægt pr. dag. Det estimerede indtag af genistein fra en væsentligt sojasubstitueret kost, vurderes ikke at udgøre en sundhedsmæssig risiko for gravide kvinder (ufødte børn) (middel indtag 0,06 mg genistein/kg legemsvægt pr. dag), men en sundhedsmæssig risiko kan ikke udelukkes for børn, der spiser en væsentlig sojasubstitueret kost (middel indtag 0,16-0,19 mg genistein/kg legemsvægt pr. dag).

Introduction

The intake of plant-based foods is currently increasing in the Nordic countries and many of these products are based on soy. For example, soy-based products with appearances close to minced meat are marketed as substitutes for meat, and many soy-based milk alternatives are replacing cow's milk. The increased interest in such products can partly be attributed to the rise in persons identifying themselves as vegetarians or vegans (AHDB, 2018). In Denmark, it is estimated that 3% of the population are vegetarians, 1% are vegans, and the numbers are increasing (Stamer et al., 2018). In Sweden 2% of girls (17-18 years) are vegetarians and 2% vegans (Lemming et al., 2018). An increased intake of soy-based products as replacements of milk and meat may be beneficial to health and is considered to be a more sustainable option by many, but there is a concern for adverse health effects in certain population groups. The natural content of estrogen-like substances (e.g. isoflavones) in soy gives rise to concern for endocrine disrupting effects in children and unborn children (i.e. pregnant women). The data and information is limited and there are currently few assessments available. In a recent EFSA opinion on food supplements containing isoflavones focusing on three target organs, mammary gland, uterus and thyroid, the conclusion was that no health-based guidance values (HBGVs) or safe intake levels could be determined for the target group postmenopausal women (EFSA, 2015). The reasons were mainly due to lack of data on doses and duration. Such guidance values for children and pregnant women are currently not available either. The Danish Food and Veterinary Administration advises against giving soy milk to children under the age of two years but there is a pressing need for risk assessments of isoflavones and evaluation of the nutritional impact of soy products to substantiate advice that meets the development of the population's food intake.

Scope of this project

The aim of the project has been to 1) explore available data on soy intake, 2) propose intake scenarios for children and pregnant women from the Nordic countries with high intake of soy products, 3) estimate the nutritional impact of substituting conventional products of animal origin with soy products and 4) clarify whether HBGVs can be determined for isoflavones for children and women of childbearing age.

In a Nordic cooperative effort, we will therefore:

- Gather expertise within dietary intake, nutrition and toxicology in a risk assessment of adverse health effects of soy intake in the Nordic countries.
- Explore the available data on soy intake in Denmark, including recently published data on isoflavone content in different foods (Forslund & Andersson, 2017) and identifying knowledge gaps with the aim of future data collection.
- Propose intake scenarios for Nordic children and pregnant women with high intake of soy products (e.g. vegetarians or vegans) and compare with the intake in populations with a traditionally high dietary intake of soy, i.e. Asian countries

if data are available.

- Estimate the nutritional impact of substituting conventional products of animal origin, e.g. dairy and meat products, with soy products.
- Clarify whether HGBVs can be determined for certain isoflavones for children and unborn children, and to identify knowledge gaps relevant for future toxicological research.

Delimitations

This evaluation does not consider possible beneficial health effects of soy intake.

This project will only to a limited extent consider adverse nutritional health effects of replacement of meat and milk with soy/plant-based products as well as substituting dishes with pulses e.g. beans or chickpeas with soybeans. This topic will be based on Danish dietary data and will cover selected relevant nutrients. Similar data from other Nordic countries were not accessible within the allocated resources.

The Danish Food and Veterinary Administration already advises against giving soy milk to children under the age of 2 years and intake data is currently only available for children ≥ 4 years. The literature search on relevant toxicological studies in humans was thus limited to exposure of pregnant women (unborn children) and children old enough to get the majority of dietary intake from solid food (>1 years). This means that studies on soy infant formula was not included. The literature search on animal data was focused on exposures in gestation and infancy up to puberty.

The evaluation of whether HGBVs can be determined for soy products was based on previous risk assessments. Evaluation of animal studies followed up on conclusions from particularly a National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) evaluation from 2010.

The toxicological evaluation focuses on effects for which human relevance is clear. As human data on effects on the immune system, obesity and neurotoxicity was found to be scarce, the literature study on experimental animal data excluded these endpoints (see literature search strategy below).

Allergic reactions to soy or soy-based products cannot be used to identify a HGBV and is therefore considered out of scope for this project.

Effects of soy or isoflavones on pregnant women that do not affect the foetus (unborn child) is not included.

Possible adverse health effects of other constituents in soy (e.g. trace elements like nickel) are also considered out of scope for this project.

The high amount of references obtained in the literature search made it necessary to focus on the three major isoflavones from soy (genistein, daidzein and glycitein). Other constituents of soy (i.e. coumestrol, secoisolariciresinol, lariciresinol and pinoresinol) that were also included in the literature search were therefore not included in this project. The amount of literature relevant for a hazard characterization of these compounds were in general limited.

The literature search was performed without any restrictions regarding language. It

was later decided to only include publications in English to avoid bias in other languages included induced by the language capabilities of the working group.

Chemistry and occurrence

Isoflavones are phytoestrogens, which have been defined as plant constituents that can trigger biological responses in vertebrates and that can change or mimic the activities of endogenous estrogens, in particular 17β -estradiol, predominantly by binding to estrogen receptors (Forslund & Andersson, 2017). The structural similarities of 17β -estradiol and selected isoflavones are shown in Figure 1.

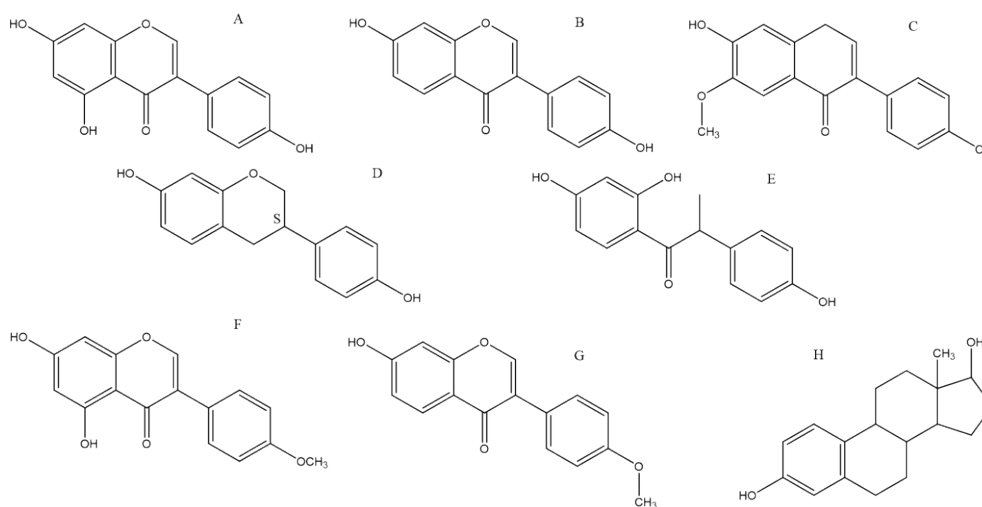


Figure 1. Structural formulas for selected isoflavones and 17β -estradiol. A: genistein, B: daidzein, C: glycitein, D: S-equol, E: O-DMA, F: biochanin A, G: formononetin, H: 17β -estradiol. Molecular structures drawn in ChemDraw Professional version 18.1.0.535 © 1998-2019 PerkinElmer Informatics, Inc.

Soy (*Glycine max* (L.) Merr.), belonging to the plant family Leguminosae, is the richest and most significant human dietary source of isoflavones (Coward et al., 1993; Wang & Murphy, 1994). The three main isoflavones are, in their unconjugated form or aglycones, genistein, daidzein and glycitein. Each of these isoflavones is also found as β -glucosides (genistin, daidzin and glycitin). In addition, the three β -glucosides can each be esterified with either malonic acid or acetic acid to 6''-O-malonylgenistin and 6''-O-acetylgenistin, 6''-O-malonyldaidzin and 6''-O-acetyldaidzin, and 6''-O-malonylglycitin and 6''-O-acetylglycitin, yielding a total of 12 different isomers. Formononetin and biochanin A are also found in soy and are methylated precursors of daidzein and genistein, respectively (Forslund & Andersson, 2017). The variation of isoflavone content in soybeans varies considerably and is influenced by factors such as maturity and origin. The most common isoflavones in soy expressed as aglycone are daidzein (47% of all isoflavones) and genistein (40% of all isoflavones), while glycitein only constitute a minor part (13% of all isoflavones) (Forslund & Andersson, 2017).

Studies have shown a large variability in concentration and composition of isoflavones among different soybeans and soy-products. This is a function of different cultivars, geographic and environmental conditions, and extent of industrial

processing of the soybeans (Eldridge & Kwolek, 1983; Wang & Murphy, 1994). The malonyl- and acetyl-glucosides are susceptible to heat and readily convert to their more stable glucosides. Therefore, depending on the extent of processing of the soybean, the relative proportions of these conjugates can vary considerably among different soy foods (Coward et al., 1993). While heat treatment appears to change the relative proportions of the different glucosides, the total isoflavone content is relatively unchanged by cooking (Barnes et al., 1994).

Previous risk assessments and risk management decisions

Only previous risk assessments/management decisions that included studies on adverse effects in children or pregnant women (unborn children) are listed here (in chronological order). None of these has identified a HGBV for soy intake or intake of any of the isoflavones (genistein, daidzein and glycitein) for pregnant women or children (not considering soy formula intake). A report by EFSA (EFSA, 2015) is also included as relevant parts on toxicokinetics and genotoxicity were reviewed but the toxicological data on adult exposures in that report was not within our scope and not considered further here. A more complete list of previous hazard and risk assessments on isoflavones is provided in VKM (2017).

Italian Health Authorities (2002) (in Italian)

The Italian Health Authorities have advised the general public (with no specific limitation to age or life-stage) to maintain daily intake of phytoestrogens as dietary supplements below 80 mg per day, expressed as the total amount of isoflavone isomers, referenced in Morandi et al. (2005), and Sirtori et al. (2005). This represents a maximal daily intake of phytoestrogens of about 1 mg/kg bw. (Cited from VKM (2017)).

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), Food Standards Agency, UK (COT 1996, 2003)

In 1996, the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) recommended that research should be undertaken as a matter of high priority to determine whether ingestion of soy-based formula carried any risk for infants, which was later done in 2003 (COT, 1996; 2003). The committee stated additionally, that as a result of further research, it might be necessary to consider the potential risk of soy products to other parts of the population. COT (2003) completed an extensive review on phytoestrogens and health, which included consumers of dietary supplements containing isoflavones. In the COT (2003) report, some concerns regarding the potential interference between phytoestrogens and thyroxine replacement therapy were noted and further research was recommended to monitor the plasma thyroxine levels of children and adults with hypothyroidism who consume large quantities of dietary phytoestrogens. (Cited from VKM (2017)).

AFSSA - L'Agence Française de Sécurité Sanitaire des Aliments (2005) Sécurité et bénéfices des phyto-estrogènes apportés par l'alimentation – Recommendations (in French)

The report indicated 1 mg/kg bw per day of isoflavone aglycone as a safe amount, i.e. 70 mg per day for a person with 70 kg body weight. This do not apply to children receiving formula or breast cancer patients or persons with a history of breast cancer in the family. (Cited from VKM (2017)).

Japanese Food Safety Commission, Novel Foods Expert Committee (2006) Fundamental concepts in the safety assessment of foods containing soy isoflavones for the purpose of specified health use (in Japanese)

An intake from food of 70–75 mg per day of soy isoflavones was determined as the upper limit for a safe daily intake for adults. For foetuses, covered by the group pregnant women and potentially pregnant women, intake as a food for specified health use for these groups in addition to intake from standard foods cannot be recommended. This is based on considerations that there are no known benefits concerning intake of soy isoflavones by pregnant women, and that soy isoflavones may inhibit topoisomerase II (which maintains normal DNA structure), and potentially cause myeloid-lymphoid leukaemia gene abnormality in foetuses exposed *in utero*. For children, it is considered that intake as a food for specified health use for infants and small children and intake from standard foods cannot be recommended based on data suggesting various effects on reproductive function of neonate animals and immature animals through exposure to high concentrations of soy isoflavones and their estrogen-mediated effects. It is also stated that suggested by animal experiments, sensitivity to exogenous estrogen may possibly be higher in infants than in pre-menopausal women, because the homeostatic mechanisms of infants are not fully developed. (Cited from VKM (2017)).

NTP-CERHR 2010 (NTP-CERHR, 2010)

In 2010, the NTP-CERHR in USA presented a monograph on soy infant formula. This report presents conclusions on the '(1) strength of scientific evidence that soy infant formula or its isoflavone constituents are developmental toxicants based on data from *in vitro*, animal, or human studies; (2) extent of exposures in infants fed soy infant formula; (3) assessment of the scientific evidence that adverse developmental health effects may be associated with such exposures; and (4) knowledge gaps that will help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations.' The Expert Panel expressed minimal concern for adverse developmental effects in infants fed soy infant formula. This level of concern represents a "2" on the five-level scale of concern used by the NTP that ranges from negligible concern ("1") to serious concern ("5").

NTP-CERHR (2010) reviewed studies using soy treatment or individual isoflavones. They concluded 'clear evidence' of adverse effects on the female reproductive system following treatment with genistein during foetal development. The risk assessment was based mainly on animal data but as these were found insufficient the NTP-CERHR called for further work in the field.

The review of human data showed that the overall evidence was considered

insufficient to reach a conclusion on whether use of soy infant formula does or does not produce developmental toxicity with infant exposure in girls or boys at actual intake levels. The expert panel had several remarks related to the quality of the available information for specific endpoints.

The NTP-CERHR found that in some cases, blood levels in infants may exceed maximum concentrations of total genistein associated with dose levels of genistein that caused adverse developmental effects in rodents. Nevertheless, they concluded 'minimal concern for adverse effect' due to study limitations particularly for mixtures of isoflavones and for studies using exposure during lactation only.

The NTP-CERHR panel preferentially evaluated studies that used oral administration since this best mimics the human route of exposure. However, subcutaneous injection studies were considered when they used dose levels, which produced blood levels representative of those measured following human exposure. Most weight in the assessment was given to those studies that exposed developing animals to soy, or in most cases the specific isoflavone genistein, solely during the period from birth to weaning. The panel concluded that during this window of exposure, which approximates the timing of soy formula ingestion in infants, genistein may affect the following endpoints in developing animals: puberty onset, neural differentiation, reproductive tract morphology and mammary gland organization. In addition, the panel only found a limited number of studies where experimental animals were treated only during the relevant life stage of birth to weaning, and concluded that multigenerational studies do not permit discerning effects attributed to gestational or lactational exposure.

European Food Safety Authority (EFSA) 2015 (EFSA, 2005)

EFSA performed a risk assessment for peri- and post-menopausal women taking food supplements containing isolated isoflavones (EFSA, 2015). This risk assessment focused on three target organs: mammary gland, uterus and thyroid, and the conclusion was that no HGBV or safe intake levels could be determined for the target group postmenopausal women. The reasons were mainly due to lack of data on doses and duration. The toxicological data evaluation of adult exposures was not within our scope and is not considered further here.

The report contains a useful overview of kinetics and metabolism of isoflavones (mainly genistein and daidzein) in healthy adult women.

Based on numerous *in vitro* and *in vivo* studies mainly on genistein and daidzein, EFSA (2015) concluded that the use of isoflavones (as food supplements) is not of genotoxic concern. As use of soy-containing food products usually result in lower intake of isoflavones than use of food supplements, we conclude that soy containing food products are not of genotoxic concern.

Vitenskapskomiteen for mattrygghet (VKM) 2017 (VKM 2017)

VKM (2017) evaluated the intake of soy isoflavones added to food supplements and other foods and did not find sufficient data to draw any conclusions on potential adverse effects of isoflavones in supplements in children (aged 10 to <14 years).

For adolescents of both genders it was concluded that isoflavones as supplements in

doses of 40 or 80 mg per day for one to three months may represent a risk of adverse effects on hormone levels in adolescents, including menstrual function in girls. It was also concluded that these isoflavone doses (40 or 80 mg per day) do not appear to have other significant adverse effects on adolescents.

For premenopausal women (representing women of childbearing age) it was concluded that isoflavones as supplements in doses of 40 or 80 mg per day taken for one to three months may represent a risk of negative effects on hormone levels and/or menstrual function. It was also concluded that these doses do not appear to have other significant negative effects on pre-menopausal women.

Lessons from previous risk assessment reports

It was concluded that these reports were of limited use for identifying HBGVs for intake of soy products or isoflavones by pregnant women and children. Genotoxicity was not considered relevant based on conclusions drawn by EFSA (2015).

Due to the focus on effects induced during development, the toxicological evaluation in the NTP-CERHR 2010 report was considered useful even though its scope was infant formula and not soy-containing food products as in our project. Their comprehensive literature review was considered sufficient to cover the time-period up to 2010, and therefore our literature search was focused on the period 2010 to 2019 for studies in experimental animals.

Specifically, the NTP-CERHR applied animal data from an experimental study on genistein (NTP, 2008) for the risk assessment but concluded that further work using mixed isoflavones would be required to improve the risk assessment. Our literature study on animal data thus aimed to identify more recent studies addressing the data gaps identified by NTP-CERHR.

The NTP-CERHR report did not set a HBGV but focused on comparisons of internal exposures. In absence of useful human internal exposure data for the purpose of this report, the same animal data may however be applicable to set a preliminary HBGV for comparing human intake levels with effect levels in orally exposed experimental animals.

Dietary exposure to soy and certain isoflavones in Denmark

Intake scenarios for children and pregnant women with high intake of soy products

In general, information on consumption of soy-based products is low in many European dietary surveys, due to a relatively low consumption of these products. The working group considered available data from Nordic dietary surveys, but realised that the data are not suitable for a thorough analysis. Instead, the working group decided to use the Danish National Survey on Diet and Physical Activity 2011–2013 as a basis for intake scenarios of soy-based products.

Calculation of content of soy and nutrients

The basis of the calculations of intake of soy is the Danish National Survey of Diet and Physical Activity 2011–2013 (DANSDA); a nationwide, cross-sectional survey assessing diet and physical activity of the Danish population (Pedersen et al., 2015). For DANSDA 2011–2013, a representative sample of 7356 children and adults aged 4–75 years was randomly selected from the Danish Civil Registration System and data collection took place between April 2011 and August 2013. Danish citizenship was a criterion for inclusion; however, disabled individuals, residents of nursing homes and home-dwelling individuals receiving meals from outside the home were excluded from the sample to ensure that individuals had sufficient knowledge about their dietary intake.

Dietary intake was estimated by seven days consecutive dietary recording, using food diaries with pre-coded response categories and open answer options. In all, 3,946 participants in the age of 4–75 years had recorded their dietary intake. The dietary record was structured according to the typical Danish meal pattern (i.e. breakfast, lunch, dinner and in-between meals) and food items were categorized according to food groups. The pre-coded response options included food items and beverages most commonly consumed in Denmark, with the possibility of adding additional items in an open field. Portion sizes were estimated by predefined household measures (cups, glasses, spoons, slices, etc.) and portion-size pictures.

For each participant, the intake of different food items, and the content of energy and nutrients within, were estimated using the General Intake Estimation System (GIES) software version 1.000.i6 and the Danish Food Composition Databank version 7.0 (www.foodcomp.dk), both developed at the National Food Institute of the Technical University of Denmark (DTU Food).

In this report, data from a subgroup of women and children were used for the dietary analysis. The average intake of foods (g per day) and nutrients for the following groups was calculated:

Women 18–45 years (N=686)

Boys 4–10 years (N=251)

Girls 4–10 years (N=248)

The number of total foods in the survey was approximately 400. Firstly, the energy and nutrient intake in the diet in the three groups were calculated (Diet A in Table 4). This diet included six existing soy-containing foods (e.g. tofu, soybeans, soy drinks). The nutrient content of these products occurs in the food composition tables and was used in the calculation of nutrients in the diet:

- Soy beans, dry, raw
- Soy drink, not fortified
- Soy drink, fortified with calcium
- Flour of soy
- Soy sauce
- Tofu

Secondly, foods that realistically could be substituted by soy-containing food products were identified. Although soy is not botanically a pulse, soy is used like pulses as a contributor to plant protein. Therefore, soy-based products can substitute products based on plant or animal protein. In total, 37 foods were identified that could be substituted with plausible soy-based foods. After substitution, the energy and nutrient intake in the diet in the three groups were calculated (Diet B in Table 4). Many of these soy products do not occur in the Danish Food Composition table. Instead, products were constructed on basis of their list of ingredients. Soy products marked with an asterisk* are constructed products. The foods substituted were:

- All types of milk (whole milk, buttermilk, semi skimmed and skimmed milk) were substituted with a mixture of 50% fortified and 50% not fortified soy drink¹.
- Whole milk, semi skimmed and skimmed milk on breakfast cereals, including oats, were substituted with a mixture of 50% fortified (calcium) and 50% not fortified soy drink.
- Chocolate milk was substituted with cocoa-containing soy drink*
- Milk/cream on fruits and fruit desserts were substituted with a mixture of 90% soy drink and 10% soy "cream"*.
- Cream (38% fat and 9% fat) and sour cream were substituted with soy "cream". Yoghurts with fruits (fat content varies) were substituted with soy "yoghurt" with fruits*.
- Yoghurt naturel (fat content varies) were substituted with soy "yoghurt" naturel*.
- High protein fermented milk products with fruits (e.g. curd with fruits) were substituted with soy "yoghurt" with fruit.
- Ice cream was substituted with soy-based "ice cream"*.
- Sausages were substituted with soy "sausages"*.
- Minced beef patties were substituted with soy-based meat alternatives resembling minced beef.* The products were fried in margarine like beef patties.
- Meatballs (to put on bread or eat at dinner) were substituted with a mixture of

1. Denmark has voluntary fortification of milk and plant drinks, while e.g. Sweden has compulsory vitamin D fortification on dairy as well as soy drink and other vegetable milk replacement products.

two different soy-based meat alternatives.* The mixture was fried in margarine like meatballs.

- Dishes with minced meat (e.g. meatloaf) were substituted with 50% "soy-based meat alternatives resembling minced beef*" and 50% of a mixture of two different soy-based meat alternatives.*
- In the recipe for sauce Bolognese/ragu, the minced meat was substituted by soy-based meat alternatives resembling minced beef. No other ingredients in the recipe were changed.
- In the recipes containing dried pulses (dried beans/lentils and dishes with pulses), all pulses were substituted by soy beans.
- Sausage/mortadella was substituted with soy-based sliced plant sausage*.
- Liverpaste, paté etc. were substituted by vegan paste containing soy*.
- Cheese with 10–30% fat in dry matter was also substituted by vegan paste containing soy*. Only the low-fat cheese was substituted as only few cheese substitutes containing soy are on the Danish market (autumn 2019). It was therefore not considered realistic to substitute all cheese.

As no fish alternatives on the Danish market at the time of data collection (2019) were based on soy, no fish products were substituted with soy based fish alternatives.

As it has been necessary to construct many products, interpretation of intake of nutrients and isoflavones should be done with caution.

Calculation of average intake of soy in gram per day among children and women

The soy content in the selected products was estimated on basis of the ingredients. The ingredients based on soy were described as soy flour, soybeans, soy protein or rehydrated soy protein and all were considered to consist of 100% soy.

Table 1. Content of soy in the various products.

Food product	Content of soy (%)
Soy sauce	30
Tofu	13
Dishes with minced soy	42
Ice cream based on soy	2
Minced soy patties	58
Chocolate milk based on soy	6
Sausages based on soy to put on bread	18
Sauce Bolognese/ragu based on soy	20
Milk/cream based on soy on fruits	5
Dinner sausages based on soy	17
Dishes with pulses substituted with soy	13
Dried pulses substituted with soy	40
Soy drink	7
Soy yoghurt with fruit	7
Soy yoghurt naturel	8
Soy cream	2
Vegan paste	6
Meat balls based on soy	27

Table 2. Mean intake of soy if all possible products listed in Table 1 are substituted with soy-containing products.

Population group	Gram soy per day
Women 18-45 years	27
Boys 4-10 years	42
Girls 4-10 years	37

Table 3. Contribution to mean intake of soy from different food groups (%). Based on scenario calculations.

Food groups	Women 18–45 years	Boys 4–10 years	Girls 4–10 years
Meat products (substituted with soy based meat alternatives)	46	34	33
Milk and cream products (based on soy)	42	59	60
Yoghurt (based on soy)	9	6	6
All foods	97	99	99

RE: retinol equivalents, α -TE: alpha-tocopherol equivalents.

The total intake of soy-based foods in the intake scenario with substitution is higher in children compared to women, due to higher intake of milk products among children. Approximately 60% of the intake of soy is derived from milk and cream products among children.

Nutritional impact of substituting conventional products of animal origin with soy products

Table 4 shows the intake of nutrients among women and children in the diet without substitution of products (diet A) as well as with substituting possible products with soy-based alternatives (diet B). Regarding diet B, it is important to emphasize that since it has been necessary to construct many products, interpretation of intake of nutrients should be done with caution.

Table 4. Mean daily intake of energy and selected nutrients with and without substitutions.

	Diet A. Mean nutrient intake with no substitution.			Diet B. Mean nutrient intake with substitution of possible soy-containing products.			Difference (%). Diet with substitution compared with no substitution (diet B - diet A).		
	Women 18–45 years	Boys 4–10 years	Girls 4–10 years	Women 18–45 years	Boys 4–10 years	Girls 4–10 years	Women 18–45 years	Boys 4–10 years	Girls 4–10 years
No of participants	686	251	248	686	251	248	686	251	248
Nutrient, unit									
Energy, kJ	8576	8569	7731	8691	8751	7882	1	2	2
Protein, g	74	72	64	73	70	63	-1	-3	-2
Carbohydrates, g	227	252	226	228	252	227	-	-	-
Fats, g	82	79	72	85	83	75	4	5	4
Vitamin A, RE	1158	1305	1210	846	740	696	-27	-43	-42
Vitamin D, µg	3.3	2.7	2.5	3.2	2.4	2.3	-3	-11	-8
Vitamin E, α-TE	9.2	8.1	7.4	11.2	10.6	9.7	22	31	31
Thiamine, mg	1.3	1.4	1.2	1.3	1.3	1.2	0	-7	0
Riboflavin, mg	1.6	1.7	1.6	1.2	1.0	0.9	-25	-41	-44
Niacin, mg	17	13	12	17	13	12	0	0	0
Vitamin B6, mg	1.6	1.4	1.3	1.5	1.3	1.2	-6	-7	-8
Folate, µg	357	304	285	385	320	303	8	5	6
Vitamin B12, µg	5.2	5.5	5.1	3.5	2.4	2.3	-33	-56	-55
Vitamin C, mg	124	108	112	121	101	106	-2	-6	-5
Sodium, mg	3132	2950	2639	3214	3038	2711	3	3	3
Potassium, mg	3075	2773	2545	3153	2793	2560	3	1	1
Calcium, mg	1055	1049	964	877	739	684	-17	-30	-29
Magnesium, mg	327	291	263	371	354	318	13	22	21
Phosphorus, mg	1363	1401	1259	1231	1172	1054	-10	-16	-16
Iron, mg	9.7	9.4	8.4	10.8	11.1	9.9	11	18	18
Zinc, mg	10.5	10.0	9.1	9.4	8.6	7.9	-10	-14	-13
Iodine, µg	175	170	157	151	124	115	-14	-27	-27

Only minor changes occur in the intake of energy, protein, carbohydrates and fats among women and children, when substituting animal-based products (as well as substituting pulses) with soy-based varieties, while the intake of various micronutrients change when substituting. The intake of the following micronutrients was reduced by 20% or more among both women and children: vitamin A, riboflavin and vitamin B₁₂, while the intake of vitamin E increased more than 20% among women and children. Among children, the intake of calcium and iodine decreased 20% or more when substituting animal-based products with soy-based varieties, while the intake of magnesium increased. For intake of other nutrients, only minor changes occurred among women and children.

The intake of selected micronutrients was compared with the Nordic recommended intake (average daily intake over time to be used for planning diets for groups) (Table 5). The values include a safety margin accounting for variations in the requirement of the group of individuals and are set to cover the requirements of 97% of the group. The actual requirements are lower than the Nordic recommended intake for almost all individuals (Nordic Nutrition Recommendations (NNR), 2012). The micronutrient intake among women 18–45 years were compared to the recommended intake for women in the age group 18–30 years in NNR (2012). The dietary intake of the 18–45-year-old women were not compared with the recommended intake for pregnant women, as only very few women in the age group were categorised as pregnant at the time of recording their diets. Therefore, recommended intake during pregnancy has been included in Table 5 but is not addressed further. The micronutrient intake among boys and girls 4–10 years were compared to the recommended intake for children in the age group 6–9 years in NNR (2012).

The comparisons are only a rough indication of how the nutrient intakes change with substitution to soy products. A thorough statistical analysis based on individual intake of micronutrients is beyond the scope of this report.

Table 5. Recommended intake of selected nutrients expressed as the average daily intake over time for use in planning diets for groups.

Predefined groups (NNR, 2012)	Children 6–9 years	Women 18–30 years	During pregnancy
Vit. A, RE	300	700	800
Vit. D, µg	10	10	10
Vit. E, α-TE	6	8	10
Thiamine, mg	0.9	1.1	1.5
Riboflavin, mg	1.1	1.3	1.6
Niacin, NE	12	15	16–17
Vit. B6, mg	1.0	1.2	1.4
Folate, µg	130	400	500
Vit. B12, µg	1.3	2.0	2.0
Vit. C, mg	40	75	85
Potassium, g	2.0	3.1	3.1
Calcium, mg	700	800	900
Magnesium, mg	200	280	280
Phosphorus, mg	540	600	600
Iron, mg	9	15	Additional 40
Zinc, mg	7	7	9
Iodine, µg	120	150	175

NE: Niacin equivalents, RE: retinol equivalents, α-TE: alpha-tocopherol equivalents.

Both with and without substitution the intake of vitamin D is low among women and children when compared to the recommended intake for use in planning diets for groups. In addition, the intake of iron is low with or without substitution among women. For most of the other micronutrients, the intake is near or above the recommended intake for use in planning diets for groups both with and without substitution.

Estimated isoflavone intake

Based on the estimated soy food intake, estimated exposure to genistein, daidzein and glycitein have been calculated using data from Forslund & Andersson (2017). The authors have summarized the knowledge on the levels of isoflavone content, including genistein, daidzein and glycitein, in a wide selection of different foods, which provides a means to estimate the dietary exposure of these isoflavones in the diet described above.

The French consumer organisation UFC-Que Choisir published in 2019 test results from measurements of isoflavones in soy-based products (UFC-Que Choisir, 2019). The results showed that many of the measured products contain high levels of isoflavones. DANSDA does not contain information on consumption of these particular brands of soy-based products and the UFC-Que Choisir test results are not publicly available in mg/kg soy-based product. Therefore, the working group could not use these results for estimation of isoflavone intake.

The levels of genistein, daidzein and glycitein in food used to estimate isoflavone exposure are shown in Table 6. The range of values from Forslund & Andersson (2017) is reflected in the tables presented below as LOW or HIGH levels. For some food items only one level of isoflavone content is listed resulting in equal content in LOW and HIGH levels.

Table 6. Levels of genistein, daidzein and glycitein in food (µg/g fresh weight (fw)) used to estimate isoflavone exposure.

Soy-based food used for estimation of isoflavone exposure	Corresponding food item in Forslund & Andersson (2017)	Genistein (µg/g fw) LOW*	Genistein (µg/g fw) HIGH**	Daidzein (µg/g fw) LOW*	Daidzein (µg/g fw) HIGH**	Glycitein (µg/g fw) LOW*	Glycitein (µg/g fw) HIGH**
Soy sauce	Soy sauce	0.6	15	0.2	24	0.05	0.05
Tofu	Tofu	123	171	93	113	7.3	7.3
Dishes with minced soy	Mince, soy-based	190	190	82	82	14	14
Ice cream based on soy	Ice cream, soy-based	80	80	36	36	17	17
Minced soy patties	Mince, soy-based	190	190	82	82	14	14
Chocolate milk based on soy	Soy milk	19	92	9.2	130	1.7	4.4
Sausages based on soy to put on bread	Sausage, soy-based	26	26	11	11	2.4	2.4
Sauce Bolognese/ragu based on soy	Mince, soy-based	190	190	82	82	14	14
Milk/cream based on soy on fruits	Soy milk	19	92	9.2	130	1.7	4.4
Dinner sausages based on soy	Sausage, soy-based	26	26	11	11	2.4	2.4
Dishes with pulses substituted with soy	Soybean	27	888	22	677	5.7	28
Dried pulses substituted with soy	Soybean	27	888	22	677	5.7	28
Soy drink	Soy milk	19	92	9.2	130	1.7	4.4
Soy yoghurt with fruit	Yoghurt, soy-based	66	69	13	34	0.1	3.0
Soy yoghurt naturel	Yoghurt, soy-based	66	69	13	34	0.1	3.0
Soy cream	Soy milk	19	92	9.2	130	1.7	4.4
Vegan paste	Tofu	123	171	93	113	7.3	7.3
Meat balls based on soy	Mince, soy-based	190	190	82	82	14	14
Beans	Soybean	27	888	22	677	5.7	28

***LOW = lowest level reported in Forslund & Andersson (2017), **HIGH = highest level reported in Forslund & Andersson (2017).**

Estimated isoflavone exposures for women, girls and boys are shown in Tables 7, 8 and 9, respectively. The major dietary contributors in these estimated intake scenarios of genistein, daidzein and glycitein are milk when replaced with soy milk and minced meat (dishes, beef patties, sauce Bolognese/ragu and meat balls) replaced by soy-based minced meat for women, girls and boys. Beans are significant contributors of genistein and daidzein for women while dried pulses are significant contributors of genistein and daidzein for girls. In general, soy sauce and cheese are among the food items that are minor dietary contributors of isoflavone intake among women, girls and boys.

Table 7. Estimated daily isoflavone exposure (µg) for women aged 18–45 years (N=686).

Soy-based food used for estimation of isoflavone exposure	Estimated soy consumption (g per day)	Estimated genistein exposure LOW* (µg)	Estimated genistein exposure HIGH** (µg)	Estimated daidzein exposure LOW* (µg)	Estimated daidzein exposure HIGH** (µg)	Estimated glycitein exposure LOW* (µg)	Estimated glycitein exposure HIGH** (µg)
Soy sauce	0.02	0.01	0.3	0.005	0.5	0.001	0.001
Tofu	0.007	0.9	1.2	0.7	0.8	0.05	0.05
Dishes with minced soy	2.5	472	472	204	204	34	34
Ice cream based on soy	0.1	9.4	9.4	4.2	4.2	2	2
Minced soy patties	3	487	487	211	211	35	35
Chocolate milk based on soy	1.4	25	126	13	178	2	6
Sausages based on soy to put on bread	0.1	3.5	3.5	1.5	1.5	0.3	0.3
Sauce Bolognese/ragu based on soy	3.4	648	648	280	280	47	47
Milk/cream based on soy on fruits	0.007	0.1	0.6	0.06	0.9	0.01	0.03
Dinner sausages based on soy	0.8	21	21	9.0	9.0	2.0	2.0
Dishes with pulses substituted with soy	0.07	2.0	62	1.5	47	0.4	2.0
Dried pulses substituted with soy	0.589	16	523	13	399	3.4	17
Soy drink	9.9	183	907	91	1287	17	44
Soy yoghurt with fruit	1.3	83	87	16	42	0.2	3.7
Soy yoghurt naturel	1.1	74	79	15	38	0.1	3.4
Soy cream	0.02	0.4	2.0	0.2	2.9	0.04	0.1
Vegan paste	0.7	84	116	64	77	5	5
Meat balls based on soy	2.2	422	422	182	183	31	31
Beans	0.5	14	457	11	348	2.9	14
Total Women	27	2,544	4,422	1,117	3,315	182	246

***LOW = lowest level reported in Forslund & Andersson (2017), **HIGH = highest level reported in Forslund & Andersson (2017).**

Table 8. Estimated daily isoflavone exposure (µg) for girls aged 4–10 years (N=248).

Soy-based food used for estimation of isoflavone exposure	Estimated soy consumption (g per day)	Estimated genistein exposure LOW* (µg)	Estimated genistein exposure HIGH** (µg)	Estimated daidzein exposure LOW* (µg)	Estimated daidzein exposure HIGH** (µg)	Estimated glycitein exposure LOW* (µg)	Estimated glycitein exposure HIGH** (µg)
Soy sauce	0.005	0.003	0.08	0.001	0.1	0.0003	0.0003
Tofu	0.01	1.4	1.9	1.0	1.3	0.08	0.08
Dishes with minced soy	3.2	599	599	259	259	43	43
Ice cream based on soy	0.1	12	12	5.3	5.3	2.5	2.5
Minced soy patties	2.0	376	376	163	163	27	27
Chocolate milk based on soy	2	33	165	17	234	3.0	7.9
Sausages based on soy to put on bread	0.5	14	14	5.9	5.9	1.3	1.3
Sauce Bolognese/ragu based on soy	1.9	352	352	152	152	25	25
Milk/cream based on soy on fruits	0.01	0.1	0.7	0.1	1.0	0.01	0.03
Dinner sausages based on soy	1.0	25	25	11	11	2.3	2.3
Dishes with pulses substituted with soy	0.1	2.0	66	1.6	50	0.4	2.1
Dried pulses substituted with soy	0.3	9.3	305	7.6	233	2.0	9.7
Soy drink	20	373	1850	186	2625	34	89
Soy yoghurt with fruit	1.6	106	112	21	54	0.2	4.8
Soy yoghurt naturel	0.5	33	35	6.4	17	0.06	1.5
Soy cream	0.01	0.2	1.0	0.1	1.4	0.02	0.05
Vegan paste	0.8	93	130	71	86	5.5	5.5
Meat balls based on soy	2.9	559	559	242	242	40	40
Beans	0.0001	0.001	0.05	0.001	0.04	0.0003	0.002
Total Girls	37	2,588	4,602	1,149	4,142	188	263

***LOW = lowest level reported in Forslund & Andersson (2017), **HIGH = highest level reported in Forslund & Andersson (2017).**

Table 9. Estimated daily isoflavone exposure (µg) for boys aged 4–10 years (N=251).

Soy-based food used for estimation of isoflavone exposure	Estimated soy consumption (g per day)	Estimated genistein exposure LOW* (µg)	Estimated genistein exposure HIGH** (µg)	Estimated daidzein exposure LOW* (µg)	Estimated daidzein exposure HIGH** (µg)	Estimated glycitein exposure LOW* (µg)	Estimated glycitein exposure HIGH** (µg)
Soy sauce	0.008	0.005	0.1	0.002	0.2	0.0004	0.0004
Tofu	0.002	0.3	0.4	0.2	0.3	0.02	0.02
Dishes with minced soy	2.7	515	515	223	223	37	37
Ice cream based on soy	0.1	9.3	9.3	4.2	4.2	2.0	2.0
Minced soy patties	2.6	495	495	214	214	36	36
Chocolate milk based on soy	1.7	31	152	15	216	2.8	7.3
Sausages based on soy to put on bread	0.6	15	15	6.3	6.3	1.4	1.4
Sauce Bolognese/ragu based on soy	3.5	667	667	289	289	48	48
Milk/cream based on soy on fruits	0.01	0.3	1.2	0.1	1.8	0.02	0.1
Dinner sausages based on soy	1.1	30	30	13	13	2.7	2.7
Dishes with pulses substituted with soy	0.1	3.2	104	2.6	80	0.7	3.3
Dried pulses substituted with soy	0.2	5.9	193	4.8	147	1.2	6.1
Soy drink	23	430	2131	214	3024	39	102
Soy yoghurt with fruit	1.9	123	130	24	63	0.2	5.6
Soy yoghurt naturel	0.5	32	33	6.2	16	0.1	1.4
Soy cream	0.004	0.07	0.3	0.03	0.5	0.01	0.02
Vegan paste	0.8	103	143	78	95	6.1	6.1
Meat balls based on soy	2.9	544	544	236	236	39	39
Beans	0.004	0.1	4.0	0.1	3.0	0.03	0.1
Total Boys	42	3,003	5,168	1,331	4,632	217	299

***LOW = lowest level reported in Forslund & Andersson (2017), **HIGH = highest level reported in Forslund & Andersson (2017).**

Interestingly, boys (4–10 years old) was the group with the highest isoflavone exposure, when substituting relevant food from their diet with soy-based food, compared to girls in the same age group and women (18–45 years old). This can in large parts be explained by the boys' higher intake of milk (12 g/kg bw per day). The intake of milk by girls and women was 10 g/kg bw per day and 2 g/kg bw per day, respectively. The total estimated intake of isoflavones range from 0.05–0.1 mg/kg bw per day for women (corresponding to 3.8–8.0 mg per day), 0.1–0.3 mg/kg bw per day for girls (corresponding to 3.9–9.0 mg per day) and 0.2–0.4 mg/kg bw per day for boys (corresponding to 4.6–10 mg per day), (Table 10 and 11).

Table 10. Total estimated daily soy (g), genistein, daidzein, glycitein and total isoflavone intake (mg) among Danish women, girls and boys.

Target group	Estimated soy intake (g)	Estimated genistein exposure LOW* (mg)	Estimated genistein exposure HIGH** (mg)	Estimated daidzein exposure LOW* (mg)	Estimated daidzein exposure HIGH** (mg)	Estimated glycitein exposure LOW* (mg)	Estimated glycitein exposure HIGH** (mg)	Estimated total isoflavone exposure LOW* (mg)	Estimated total isoflavone exposure HIGH** (mg)
Total Women	27	2.5	4.4	1.1	3.3	0.2	0.2	3.8	8.0
Total Girls	37	2.6	4.6	1.1	4.1	0.2	0.3	3.9	9.0
Total Boys	42	3.0	5.2	1.3	4.6	0.2	0.3	4.6	10

***LOW = lowest level reported in Forslund & Andersson (2017), **HIGH = highest level reported in Forslund & Andersson (2017).**

Table 11. Total estimated daily soy, genistein, daidzein, glycitein and total isoflavone intake (mg/kg bw per day) among Danish women, girls and boys.

Target group	Body weight (kg)*	Estimated total soy consumption (mg/kg bw per day)	Estimated genistein exposure LOW** (mg/kg bw per day)	Estimated genistein exposure HIGH*** (mg/kg bw per day)	Estimated daidzein exposure LOW** (mg/kg bw per day)	Estimated daidzein exposure HIGH*** (mg/kg bw per day)	Estimated glycitein exposure LOW** (mg/kg bw per day)	Estimated glycitein exposure HIGH*** (mg/kg bw per day)	Estimated total isoflavone exposure LOW** (mg/kg bw per day)	Estimated total isoflavone exposure HIGH*** (mg/kg bw per day)
Total Women	71	385	0.04	0.06	0.02	0.05	0.003	0.003	0.05	0.1
Total Girls	28	1319	0.09	0.2	0.04	0.2	0.01	0.01	0.1	0.3
Total Boys	28	1508	0.1	0.2	0.05	0.2	0.01	0.01	0.2	0.4

***Average body weights based on measured data from DANSDA. **LOW = lowest level reported in Forslund & Andersson (2017), ***HIGH = highest level reported in Forslund & Andersson (2017).**

Hazard identification and characterization of soy and certain isoflavones for children and pregnant women (unborn children)

Kinetics and metabolism of isoflavones

The kinetics and metabolism of isoflavones (mainly genistein and daidzein) in healthy adult women have been reviewed several times and a short overview based on EFSA (2015) is presented here. In EFSA (2015) data from a literature search on the kinetics of genistein, daidzein and glycitein (among other isoflavones) in both humans and animals is described.

In general, the kinetics of isoflavones are complex and their bioavailability is, as for other flavonoids, affected by a myriad of exogenous and endogenous factors, such as the complexity of the food matrix, gender, age, health status, dose, early or chronic exposure, background diet, molecular structure of the isoflavone, extent of conjugation, microbial catabolism, oral antibiotic treatment, amount of co-ingested compounds, mucosal mass, intestinal transit time, rate of gastric emptying and protein-binding in blood and tissues (Mullen et al., 2008; Holst & Williamson, 2008; Scholz & Williamson, 2007; Rowland et al., 2003; Hollman et al., 1999; Franke et al., 2014).

Absorption and bioavailability

In humans, the absorption of isoflavones varies considerably between individuals (30–96% absorption) and the dose of isoflavones may be a poor indicator of actual internal exposure (EFSA (2015) and van der Velpen et al. (2014)). According to Franke et al. (2014) (cited from VKM (2017)), the bioavailability of isoflavones is higher in children than in adults, higher in healthy vs. non-healthy individuals, while during oral antibiotics therapy it is decreased in children, but increased in adults.

In experimental animals, absorption is relatively high but possibly lower in males than females. Absolute bioavailability varies from 9–14% for genistein and 29–34% for daidzein in rats. In mice, absolute bioavailability in males is 7% and 15% in females (EFSA, 2015).

Distribution

No data on the distribution of isoflavones in humans were identified in the literature search made for the EFSA (2015) publication, but genistein and daidzein were identified in breast, prostate and amniotic fluid (EFSA, 2015; Geer et al., 2015). Rat studies showed genistein in all organs including reproductive tissues.

Metabolism

Isoflavones are metabolised by endogenous phase I and phase II enzymes (e.g. UDP-glucuronosyltransferase and sulfotransferase) mainly in the gut and the liver, as well as by the intestinal microbiota. Conjugation and microbial transformation reactions are major pathways. Minor metabolites are hydroxylated derivatives formed by the action of cytochrome P450 enzymes.

It is known that in humans, as in other species, biochanin A is mainly demethylated to genistein and formononetin is demethylated to daidzein by phase I hepatic enzymes (Setchell et al., 2001; Howes et al., 2002). Besides glucuronidation and sulphation, transformation reactions catalysed by the intestinal microbiota play a crucial role in the metabolism of isoflavones.

Daidzein can be converted to dihydrodaidzein and subsequently to *O*-desmethylangolensin (*O*-DMA) and/or *S*-equol. The extent of microbial metabolism of genistein and daidzein as well as the resulting microbial metabolite profile varies greatly among individuals. The prevalence of equol producers ranges from 20–30% in the population of Western countries to 50–60% in Asian populations consuming soy-containing diets. In contrast, 100% of rats are equol producers. Dihydrogenistein, dihydrodaidzein and *O*-DMA are found in rat plasma at concentrations very much lower than *S*-equol. This difference in equol production between humans and rats implies that studies performed in rats are considered relevant mainly to a sub-population of humans.

Elimination

In humans, most ingested genistein and daidzein are excreted as phase II conjugates and as phase II conjugates of microbial-derived metabolites in the urine. Faecal elimination has been found to be a minor route. Likewise, rats excrete a high percentage of daidzein, genistein and *S*-equol in the urine as aglycones.

Key studies of the NTP-CERHR 2010 report

As our evaluation to some extent builds on conclusions from the NTP-CERHR 2010 report, because of lack of sufficient new data found in our literature search, we here briefly review conclusions on key studies from that report.

Human data

The review of human data showed that the overall evidence was considered insufficient to reach a conclusion on whether use of soy infant formula does or does not produce developmental toxicity with infant exposure in girls or boys at recommended intake levels. The expert panel had several remarks related to the quality of the available information for specific endpoints. 1) Soy infant formula may or may not cause reproductive toxicity in boys and girls based on current evidence. Preliminary data addressed in the panel's report do not allow firm conclusions on this effect. Limited retrospective data suggested that soy formula fed infants might demonstrate premature thelarche (the start of breast development at the beginning

of puberty). The only other study considered to have limited utility that included reproductive parameters, had not sufficient power to rule out increased risks. 2) Soy infant formula may or may not cause adverse effects on thyroid function in male or female infants and children. A special cohort of infants and children with congenital hypothyroidism (CH) fed soy infant formula demonstrated a delay of TSH levels to return to normal after adequate treatment; these children may need increased doses of levothyroxine and closer follow-up. However, the studies that specifically targeted infants and children with CH were case studies, which results in limited inferences.

Animal data

NTP-CERHR (2010) concluded 'clear evidence' of adverse effects on the female reproductive system following treatment with genistein during development. Clear evidence was seen both in studies using exposure during lactation (mainly subcutaneous exposure) and in multigenerational studies covering also the gestational and post-weaning period (dietary exposure). Also genistin, the glucoside form of genistein, had such adverse effects. The panel found only few studies on other isoflavones, and the authors considered the evidence 'insufficient' to determine whether daidzein or equol produces developmental toxicity or not. Likewise, the NTP-CERHR found 'insufficient evidence' to conclude on adverse effects in studies on soy protein isolate, soy-based diet or mixtures of isoflavones.

Data applied by NTP-CERHR for risk assessment included 74 studies of 'limited' or 'high' utility, almost all with genistein. A few studies on daidzein and its metabolite equol showed inconsistent findings and were considered insufficient for evaluation of reproductive effects in laboratory animals. Studies that demonstrated clear evidence of developmental toxicity for genistein involved treatment only during the period of lactation in rodents (postnatal day (PND) 1–21), as well as multigenerational studies that included exposure during gestation, lactation and post-weaning (NTP-CERHR, 2010).

A series of studies showing clear evidence of adverse effects used an experimental design where CD-1 mice were treated on PND 1–5 with genistein, typically by subcutaneous injection, and the reproductive system was assessed during late postnatal life or adulthood (Jefferson et al., 2009; Jefferson et al., 2005; Newbold et al., 2001; Padilla-Banks et al., 2006). The NTP-CERHR considered the blood profiles of unconjugated genistein as comparable in infant mice following treatment by the oral route or subcutaneous injection. This was seen in studies in neonatal mice using subcutaneous injection (Doerge et al., 2002) or oral exposure (Cimafranca et al., 2010). Adverse effects on female reproductive development (early vaginal opening, altered estrous cycle, fewer corpora lutea, decreased fertility) were also observed in rats exposed to genistein via subcutaneous injection or orally as neonates (Bateman & Patisaul, 2008; Kouki et al., 2003; Nagao et al., 2001). Early onset of vaginal opening was also observed in rodents exposed via lactation (Nikaido et al., 2005; Lewis et al., 2003).

A multi-generational study on reproductive effects of genistein in Sprague-Dawley (SD) rats provided information on effects in adults and offspring (NTP (2008) and peer-reviewed publications (Latendresse et al., 2009, Delclos et al., 2009)). In this study, animals were fed genistein at dose levels of 0, 5, 100 and 500 ppm through the diet for three generations (NTP, 2008). Six-week-old rats were assigned to dose

groups (N = 35/sex/group) using a stratified randomized procedure that resulted in similar body weights among groups. The rats were fed 5K96, a soy- and alfalfa-free diet to which genistein ($\geq 99\%$ purity) was added at 0, 5, 100 or 500 ppm. Mean genistein level in the control diet was measured at 0.417 ppm. Genistein doses for the entire feeding period in males were estimated by study authors at 0, 0.3, 7 and 35 mg/kg bw per day, and genistein doses for the entire feeding period in females were estimated at 0, 0.5, 10 and 51 mg/kg bw per day.

A number of effects related to growth and reproductive and developmental parameters were observed at 500 ppm. The experimental design using multigenerational exposure made it impossible to determine in which exposure periods these effects were induced. For example, it was not clear whether effects on estrous cyclicity were induced during adulthood or due to developmental exposure. The observed effects on early endpoints in offspring, such as anogenital distance (AGD) and puberty timing, can be attributed to developmental exposure, and are thus considered the most relevant for risk assessment for foetal and child exposures.

Information on internal dose levels in a multigenerational study using dietary exposure was obtained from Chang et al., (2000). Exposure levels of total genistein and aglycone in rats exposed to 500 ppm genistein were comparable to levels in human infants fed soy infant formula (NTP-CERHR, 2010; Chang et al., 2000; Cao et al., 2009). In addition, these blood levels in infants were found to exceed maximum concentrations of total genistein associated with dose levels that caused adverse developmental effects in rodents exposed to genistein by subcutaneous injection in early life (Jefferson et al., 2006; 2002; 2005; Padilla-Banks et al., 2006; Newbold et al., 2001).

Nevertheless, the NTP-CERHR report concluded that data from laboratory animal studies on single compounds (mainly genistein and daidzein) is insufficient for risk assessment of soy infant formula. Due to limitations in single compound studies, there is a data gap regarding the effects of mixtures of isoflavones and/or other components present in soy infant formula because these mixture studies would better replicate human infant exposures. Further, they noted that continued exposure during gestation, lactation and beyond weaning in some studies made it difficult to distinguish when effects had occurred.

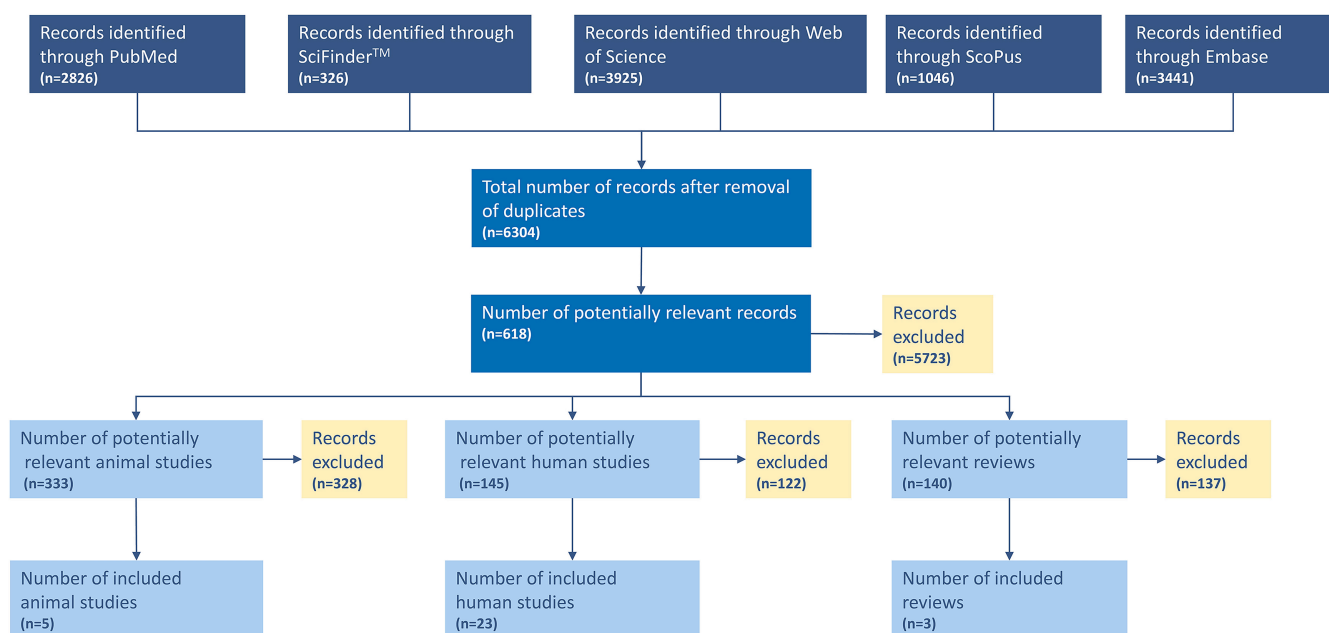
Literature searches

To improve the grounds for clarifying whether HBGVs can be determined for certain isoflavones for children and women of childbearing age, literature searches were performed on a) human data, and b) experimental animal data.

Literature searches were performed in Embase, PubMed, SciFinder®, Scopus and Web of Science in order to retrieve publications on adverse health effects caused by isoflavones from soy in relation to children and pregnant women (unborn children). All searches were performed on March 1, 2019, except for Embase, which was searched on February 22, 2019 (search strategies are shown in Appendix A). No limitation to publication year or language was initially applied. Patents were excluded from the SciFinder® search. All references were imported into EndNote™ and combined in one EndNote™ library.

The literature searches identified a total of 6,304 articles after removal of duplicates (see flow chart in Figure 2).

Figure 2. Flow chart for the literature search performed.



A primary crude screening was performed by one person to identify potentially relevant articles and for creating an overview of the published literature. In the secondary screening, titles and abstracts of all publications retrieved in the primary screening were screened by one person against the inclusion criteria checklist.

Inclusion criteria checklist

- Exposure of children (not to infant formula) and pregnant women (unborn children) and similar age groups in animal studies
- Endpoint is an adverse effect, and not a positive health effect or adaptive effect of exposure
- Route of exposure is oral
- Human studies are performed in apparently healthy individuals or patient groups assumed to have normal absorption and metabolism of the assessed substances or food items.

Articles that did not appear to meet the inclusion criteria based on title and abstract were excluded from further analysis. In cases where the relevance was uncertain, the articles were included for further screening. Full text versions of articles that passed the secondary screening was retrieved for a final assessment performed by one person.

The final assessment resulted in 5 animal studies, 23 human studies and 3 review papers, used for hazard identification and characterization.

Human studies from the literature search

To evaluate whether a HBGV for soy constituents can be set on the basis of data from human studies (cross-sectional, case-control, cohort and intervention-studies); a literature search was carried out as described above. The majority of studies found relevant for children and pregnant women (unborn children) included the endpoints described below: timing of puberty, breast cancer, hypospadias and thyroid function. Relevant data with only one published article identified (e.g. effects on adiposity, metabolic disturbances, Kawasaki disease and gender-role play behavior) were not included (Deierlein et al., 2017, Jeng et al., 2015, Portman et al., 2016, Adgent et al., 2011).

A total of 23 studies were found relevant for the current risk assessment and are described below (Tables 12–16).

Timing of puberty

Over the past decades, the age of onset of puberty in girls (menarche) has tended to decrease. This phenomenon may be partially attributed to the presence of environmental factors, including dietary isoflavones, and is of concern because of the association between earlier menarche and higher risk of breast and ovarian cancer (Messina et al., 2017). Due to their structural similarity to endogenous estrogens, dietary exposure to isoflavones has been proposed to be relevant for pubertal development. Specifically, isoflavones are capable of inhibiting the activity of CYP19 aromatase (the rate-limiting enzyme that converts androstenedione and testosterone to estrone and estradiol, respectively) and 17 β -hydroxysteroid dehydrogenase (which catalyzes the interconversion of the relatively inactive 17 β -keto steroids to active 17 β -hydroxyl sex steroids). In addition, they are capable of direct interaction with estrogen receptors (Cheng et al., 2012).

According to Segovia-Siapco et al. (2018) several morbidities such as diabetes, cardiovascular disease and reproductive cancers in adulthood are associated with earlier onset of puberty. Disordered eating behavior and depression can afflict adolescent males and females who enter adolescence either early or late. Specifically for males, late maturation may be associated with deviant behaviour or substance abuse during transition to adulthood as well as emotional and social difficulties or increased osteoporotic fractures later in life.

The included studies on effects on puberty are summarized in Table 12 for girls and Table 13 for boys.

Table 12. Human studies on exposure to soy/isoflavones during childhood/adolescence and effects on timing of puberty in girls.

Reference	Design	Number of subjects	Age during exposure and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Prenatal exposure						
Marks et al., 2017	Nested case-control study.	367 mother-daughter dyads.	Prenatal. United Kingdom.	Measured in maternal urine.	Median gestational urinary concentrations (µg/g creatinine) by age of menarche (tertile (T)): Genistein: Continuous, T2 (38.86–118.38), T3 (118.38–17916.60) Daidzein: Continuous, T2 (110.09–319.03), T3 (319.03–21880.45) Equol: Continuous, T2 (3.19–6.93), T3 (6.93–9005.85) O-DMA: Continuous, T2 (4.77–21.04), T3 (21.04–1631.58) Continuous represents natural log transformed values of isoflavone concentration.	Prenatal exposure to O-DMA associated with earlier age at menarche (OR (95% CI)): Continuous: 1.14 (0.99–1.30), P for trend = 0.06 T2: 1.51 (0.83–2.73), P-value = 0.18 T3: 1.89 (1.04–3.42), P-value = 0.04 P for trend = 0.03. When comparing those in the third tertile of O-DMA concentration to those in the lowest tertile, an 89% increase in the odds of early menarche was observed. No other statistically significant associations between prenatal exposure and age at menarche were observed.
Reference	Design	Number of subjects	Age during exposure and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Exposure between age 3–6 years						
Wada et al., 2011	Cross-sectional.	N=198.	3–6 years. Japan.	Estimated with 3-day dietary records.	Median soy intake (g per day): Q1 = 7.9, Q2 = 19.7, Q3 = 33.1, Q4 = 53.7 Median isoflavone intake (mg per day): Q1 = 4.7, Q2 = 10.2, Q3 = 16.4, Q4 = 24.0.	Statistically significant association between soy intake and higher urinary testosterone described by estimated geometric means (Q1 = 39.5, Q2 = 62.6, Q3 = 79.4, Q4 = 123.0, P-trend = 0.003) and 3β,17α-AED (Q1 = 54.4, Q2 = 66.4, Q3 = 76.4, Q4 = 92.4, P-trend = 0.027) excretion. Statistically significant association between isoflavone intake and higher urinary testosterone excretion (Q1 = 36.8, Q2 = 69.1, Q3 = 78.7, Q4 = 120.7, P-trend = 0.002). No statistically significant associations were observed between soy or isoflavonoid intake and urinary excretion of estrone, estradiol or DHEA.

Reference	Design	Number of subjects	Age during exposure and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Exposure between age 6–12 years						
Duitama et al., 2018	RCT (1 year).	Intervention N=16,	7–9 years. Colombia.	Measured in food supplement	Intake from supplement (mg per day): Total isoflavones: 3.49 ± 0.04 , genistein: 0.957 ± 0.06 , daidzein: 0.413 ± 0.03 , glycitein: 0.089 ± 0.01 , genistin: 1.494 ± 0.09 , daidzin: 0.540 ± 0.03 .	Association between isoflavone intake and sexual maturation: Growth velocity measured at 6 and 12 months showed no statistically significant differences between the control and intervention groups ($P > 0.05$). Likewise, no significant differences were found for bone age ($P > 0.05$). All participants were in Tanner stage 1* at the beginning and remained in this state until the end of the study.
Wolff et al., 2017	Prospective cohort study.	Control N=11.	6–8 years at enrolment. USA.	Measured in urine collected within a year of enrolment.	Median urinary biomarker concentrations ($\mu\text{g/g}$ creatinine) by age at menarche: Genistein: <11 years: 40 (16–141), 11–13 years: 42 (17–176), 13+ years: 59 (17–217), No menarche: 44 (14–136). Daidzein: <11 years: 90 (43–331), 11–13 years: 107 (36–416), 13+ years: 100 (36–380), No menarche: 99 (36–271).	No statistically significant relationship between urinary excretion of genistein or daidzein and age of menarche.
Wolff et al., 2015	Prospective cohort study.	N=1170.	6–8 years at enrolment. USA.	Measured in urine collected within a year of enrolment	Urinary concentrations ($\mu\text{g/g}$ creatinine): Genistein: Geometric mean = 58, 10th percentile = 9, 90th percentile = 602 Daidzein: Geometric mean = 126, 10th percentile = 19, 90th percentile = 1,226.	No statistically significant associations between urinary genistein or daidzein excretion and development of breasts or pubic hair.
Kim et al., 2011	Case-control.	N=108 cases and 91 controls.	6–10 years. Korea.	Measured in serum.	Total isoflavone , genistein and daidzein concentrations were divided into three exposure levels <30, 30–70 and ≥ 70 nmol/l.	Statistically significant association between total serum isoflavone (nmol/l) and risk of precocious puberty (OR (95%)): < 30: 1.0 (ref), 30–70: 4.39 (1.83–10.51), ≥ 70 : 5.22 (2.07–13.20), P for trend = 0.001. Mean serum concentrations of daidzein ($P=0.0202$) and genistein ($P=0.0021$) were higher in cases compared to controls. Odds ratios for the association between genistein and daidzein and risk of precocious puberty were not presented.
Cheng et al., 2010	Longitudinal (subcohort)	Dietary intake: N=108–119. Urinary measurements: N=51–55.	Mean ($\pm$ SD) age of 7.2 $\pm$ 1 years at baseline. Germany.	Estimated by FFQ. Measured in urine.	All ranges are divided into tertiles (minimum - maximum). Total dietary isoflavone intake ($\mu\text{g}/\text{dag}$): T1: 4.1–21.9, T2: 23.2–421.5, T3: 423.4–19,178 Total urinary isoflavones ($\mu\text{g}/\text{dag}$): T1: 26.2–120, T2: 121–237, T3: 246–1112 Median intake in dietary tertiles of genistein ($\mu\text{g}/\text{dag}$): T1: 8 (5, 11), T2: 33 (18, 102), T3: 644 (270, 2305). Median intake in dietary tertiles of daidzein ($\mu\text{g}/\text{dag}$): T1: 8 (5, 11), T2: 20 (16, 101), T3: 566 (243, 1833).	Statistically significant association between low dietary isoflavone intake at baseline and risk of earlier age at peak height velocity and Tanner stage* 2 for breast development compared to children with the highest isoflavone intakes. Lower isoflavone intakes also tended to be associated with an earlier growth spurt. Similar associations were observed for daidzein but not for genistein .

Wolff et al., 2008	Cross-sectional.	N=192.	9 years. USA.	Estimated from FFQ. Measured in urine.	Geometric means (SD) of urinary concentrations (µg/g creatinine): Genistein: Breast stage 1: 11.8 (3.4), Breast stage 2+: 5.6 (5.1), Pubic hair stage 1: 8.4 (3.7), Pubic hair stage 2+: 7.1 (6.2) Daidzein: Breast stage 1: 3.1 (6.6), X Breast stage 2+: 1.8 (5.3), Pubic hair stage 1: 2.8 (4.9), Pubic hair stage 2+: 1.5 (8.4)	Higher urinary excretion of daidzein was significantly associated with less developed breast stage. Prevalence ratios (95% CI) for breast stage 2+ vs. stage 1 = 0.89 (0.83-0.96). No other statistically significant associations between urinary isoflavone excretion and breast and pubic hair stages in girls were found.
Yum et al., 2013	Case-control.	N=150 cases and 90 controls.	6–12 years. Korea.	Measured in plasma.	Genistein (ng/ml): Control mean (min, max) = 3.04 (N.D., 23.92). Cases mean (min, max) = 8.12 (N.D., 124.92) Daidzein (ng/ml): Control mean (min, max) = 10.79 (N.D., 95.3), Cases mean (min, max) = 14.88 (N.D., 263.08) Equol (ng/ml): Control mean (min, max) = 0.46 (N.D., 26.29), Cases mean (min, max) = 0.59 (N.D., 11.39).	Concentration of genistein in the plasma of precocious puberty patients was 2.67 times higher than that of the control group (P=0.0008). No statistically significant differences were seen for daidzein (P=0.1339) or equol (P=0.6244).
Maskarinec et al., 2005	Intervention study (8 weeks).	N=17 (no control group).	10.7 ± 2.0 years. USA.	Estimated from three-day food record. Measured in urine.	One daily serving of sweetened plain soy milk (8.5 oz), honey-roasted soy nuts (1.0 oz) or tofu (6.7 oz), each providing approximately 30 mg isoflavone per day.	Statistical significant increase in urinary excretion of dehydroepiandrosterone from 0.03 ± 0.03 µg/g creatinine at baseline to 0.05 ± 0.03 µg/g creatinine at the end of the study of (P=0.007). No significant changes in urinary excretion of androsterone, 17β-dihydroandrosterone, androstenedione, testosterone, estrone, estradiol, estriol, 2-OH estrone, 16α-OH estrone, 4-OH estrone or pregnanediol between baseline and the end of the study.
Reference	Design	Number of subjects	Age during exposure and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Exposure between age 12–18 years						
Segovia-Siapco et al., 2014	Cross-sectional.	N=327.	12–18 years. USA.	Estimated from FFQ.	Total median soy intake (servings** per day) Q1 = 0.0, Q2 = 4.5, Q3 = 13.5, Q4 = 24.2.	No statistically significant associations between soy intake and age of menarche.

***Tanner stages: sexual maturity ratings. **There is no definition of serving size in the article by Segovia-Siapco et al., 2014. DHEA: dehydroepiandrosterone, FFQ: Food Frequency Questionnaire, N.D: not detectable, Q: quartile, RCT: randomised, controlled trial, SD: standard deviation.**

Table 13. Human studies on exposure to soy/isoflavones during childhood/adolescence and effects on timing of puberty in boys.

Reference	Design	Number of subjects	Age during exposure, and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Exposure between age 3-6 years						
Wada et al., 2011	Cross-sectional	N=230	3-6 years. Japan.	Estimated with 3-day dietary records	Median soy intake (g per day): Q1 = 8.3, Q2 = 19.9, Q3 = 34.9, Q4 = 60.9 Median isoflavone intake (mg per day): Q1 = 5.1, Q2 = 10.6, Q3 = 15.6, Q4 = 26.5	Association between soy and isoflavone intake with urinary sex steroid levels. Soy intake Estrone: Q1 = 37.7, Q2 = 34.1, Q3 = 26.1, Q4 = 27.2 (P-trend = 0.013). Estradiol: Q1 = 7.5, Q2 = 7.1, Q3 = 4.9, Q4 = 4.3 (P-trend = 0.026). Isoflavone intake Estradiol: Q1 = 7.1, Q2 = 7.4, Q3 = 5.1, Q4 = 4.1 (P-trend = 0.037). No associations were seen between soy intake and excretion of testosterone, 3 β ,17 α -AED or DHEA. No associations were seen between isoflavone intake and excretion of estrone, testosterone, 3 β ,17 α -AED or DHEA. Separate analyses for genistein and daidzein showed similar results (data not shown); i.e. inverse associations between intake of each isoflavone with excretion of estrone and estradiol.
Exposure between age 7-10 years						
Duitama et al., 2018	RCT (1 year).	Intervention N=13, Control N=11.	7-9 years. Colombia.	Measured in food supplement	Supplement intake (mg per day): Total isoflavones: 3.49 $\pm$ 0.04, genistein : 0.957 $\pm$ 0.06, daidzein : 0.413 $\pm$ 0.03, glycitein : 0.089 $\pm$ 0.01, genistin : 1.494 $\pm$ 0.09, daidzin : 0.540 $\pm$ 0.03.	Association between isoflavone intake and sexual maturation: Growth velocity measured at 6 and 12 months showed no statistically significant differences between the control and intervention groups (P > 0.05). Likewise, no significant differences were found for bone age (P > 0.05). All participants were in Tanner stage* 1 at the beginning and remained in this state until the end of the study.
Cheng et al., 2010	Longitudinal (subcohort)	Dietary intake: N=96-108. Urinary measurements: N=51-56.	Mean ($\pm$ SD) age of 8.8 $\pm$ 0.9 years at baseline. Germany.	Estimated by FFQ. Measured in urine.	All ranges divided into tertiles (minimum - maximum). Total dietary isoflavone intake (μ g/dag): T1: 1.9-16.7, T2: 16.9-226.9, T3: 230-62,759. Total isoflavone urinary concentration (μ g/dag): T1: 16.1-108, T2: 109-209, T3: 212-927. Total isoflavone equals the sum of genistein, daidzein and equol. Median intake of genistein (μ g/dag): T1: 5 (3, 9), T2: 15 (11, 30), T3: 704 (273, 1572).	No statistically significant association between dietary or urinary total isoflavones or dietary daidzein and genistein , with ATO, APHV, age at Tanner stage* 2 for gonadal development and age at voice break in boys.

Median intake of **daidzein** (µg/dag): T1: 6 (4, 9), T2: 13 (12, 18), T3: 658 (211, 1452).

Reference	Design	Number of subjects	Age during exposure, and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Exposure between age 12–18 years						
Segovia-Siapco et al., 2018	Cross-sectional study.	N=248.	12–18 years. Male. USA.	Estimated from FFQ.	Low, Moderate and High total isoflavone consumption defined as <3 mg per day, 3–20 mg per day and >20 mg per day, respectively. Genistein (mg per day): Low: <1.5; Moderate: 1.5–10; High: >10. Daidzein (mg per day): Low: <1.2; Moderate: 1.2–8; High: >8.	Association between reaching pubarche (RR (95% CI)) by type of isoflavone and level of consumption (*P < 0.05): Total isoflavones : Low: 1.00 (ref); Moderate: 1.58 (1.06, 2.36)*; High: 1.63 (1.03, 2.60)*. Genistein : Low: 1.00 (ref); Moderate: 1.57 (1.05, 2.35)*; High: 1.68 (1.05, 2.69)*. Daidzein : Low: 1.00 (ref); Moderate: 1.54 (1.03, 2.29)*; High: 1.60 (1.00, 2.54)* Moderate or high consumers of total isoflavone, daidzein or genistein had a statistical significant higher probability (58–63%) of reaching pubarche earlier at any given point in time compared to the reference group (low consumers). Isoflavone consumption was not associated with occurrence of facial hair.

***Tanner stages: sexual maturity ratings. 3β,17α-AED: 5-androstene-3β, 17α diol, APHV: age at peak height velocity, ATO: age at take-off, DHEA: dehydroepiandrosterone, FFQ: Food Frequency Questionnaire, N.D: not detectable, Q: quartile, RR: relative risk, SD: standard deviation, T: tertile.**

A total of 12 human studies on the association between exposure to soy/isoflavones and effects on puberty in girls (11 studies) and boys (4 studies) ≤ 18 years were identified.

Four studies did not detect significant differences in the endpoints measured (sexual maturation, age at menarche (first menstruation) and first appearance of breasts or pubic hair development) (Duitama et al., 2018; Wolff et al., 2017; Wolff et al., 2015; Segovia-Siapco et al., 2014). These studies are presented in Table 12 and 13 but not evaluated further, since they are not of use for identifying a HBGV.

Two studies found associations between higher isoflavone exposure and lower risk of precocious puberty (Cheng et al., 2010; Wolff et al., 2008). These studies are presented in Table 12 and 13 but not evaluated further, since the present focus is on adverse and not beneficial effects.

A total of four studies found associations between higher isoflavone exposure and higher risk of precocious puberty (Kim et al., 2011; Marks et al., 2017; Segovia-Siapco et al., 2018; Yum et al., 2013). These studies are described below and summarised in Table 12 and 13.

Two of these studies (Yum et al., 2013; Kim et al., 2011) are case-control studies in girls with precocious puberty (cases) from Korea.

The case-control study in 6–12-year-old girls by Yum et al. (2013) reported mean genistein, daidzein and equol plasma concentrations of 3.04, 10.79 and 0.46 ng/ml in controls (N=90) and 8.12, 14.88 and 0.59 ng/ml in cases (N=150), respectively. For genistein, the mean plasma concentration in cases was 2.67 times higher than that of the control group ($P=0.0008$). No other statistically significant differences were observed. There does not seem to have been made adjustments for confounders in the study by Yum et al. (2013).

Similar observations were made in a case-control study with 6–10-year-old girls by Kim et al. (2011) including 108 cases with precocious puberty and 91 controls. Adjusted ORs (95% CI) were 1.0, 4.39 (1.83-10.51) and 5.22 (2.07-13.20) for total serum isoflavone concentrations of <30 , 30-70 and ≥ 70 nmol/l, respectively (P for trend = 0.001). Adjustments were made for age, height and body weight. Similar trends were seen for serum concentrations of genistein and daidzein, but reported without adjustment for confounders.

The association between prenatal exposure to genistein, daidzein, S-equol and O-DMA and risk of earlier age at menarche was investigated in a nested case-control study with 367 mother-daughter dyads from the United Kingdom (Marks et al., 2017). Prenatal exposure to O-DMA, measured in maternal urine, was associated with an 89% increased risk of earlier age at menarche when comparing the highest tertile (21.04-1631.58 μg O-DMA/g creatinine) to the lowest tertile (not quantified) ($P = 0.04$). No associations were observed for prenatal exposure to genistein, daidzein or S-equol and risk of earlier age at menarche.

The final study reporting an inverse association between isoflavone exposure and timing of puberty is an American cross-sectional study with 248 male subjects (age 12–18 years) (Segovia-Siapco et al., 2018). The dietary exposure of genistein (low: <1.5 ; moderate: 1.5–10; high: >10 mg per day) and daidzein (low: <1.2 ; moderate: 1.2–8; high: >8 mg per day) was compared with the timing of reaching pubarche (i.e. the beginning of puberty marked by the first growth of pubic hair) and facial hair

occurrence. Moderate and high consumers of daidzein and genistein had a statistically significantly higher risk ($P < 0.05$) of reaching pubarche earlier at any given point in time compared to the reference group (low consumers). No associations with occurrence of facial hair were identified.

Two studies assessed the association between isoflavone intake with urinary excretion of sex hormones and do therefore not directly provide information on effects on puberty (Wada et al., 2011; Maskarinec et al., 2005). These studies are described below and presented in Table 12 and 13.

In the eight-week soy food intervention study with 8–14-year-old American girls by Maskarinec et al. (2005), urinary excretion of dehydroepiandrosterone was statistically significantly higher at the end of the study ($0.05 \pm 0.03 \mu\text{g/g creatinine}$) compared to baseline ($0.03 \pm 0.03 \mu\text{g/g creatinine}$) ($P = 0.007$). The authors themselves described the result as a chance finding due to the limited sample size ($N = 17$, no control group) and the great variability in sex hormone excretion among girls. In addition, the numerical value for the difference was very small, and probably not biologically relevant.

In the cross-sectional study from Japan with pre-school children aged 3–6 years, several associations between soy/isoflavone intake and urinary sex hormone levels were identified (Wada et al., 2011). In boys ($N = 230$), soy intake (median intake in Q1 and Q4, 8.3 and 60.9 g soy per day, respectively) was inversely associated with urinary excretion of estrone ($P\text{-trend} = 0.013$) and estradiol ($P\text{-trend} = 0.026$). Isoflavone intake (median intake in Q1 and Q4, 5.1 and 26.5 mg isoflavone per day, respectively) was inversely associated with urinary excretion of estradiol ($P\text{-trend} = 0.037$). In girls ($N = 198$), soy intake (median intake in Q1 and Q4, 7.9 and 53.7 g soy per day, respectively) was positively associated with urinary excretion of testosterone ($P\text{-trend} = 0.003$) and 5-androstene- 3β , 17α diol ($P\text{-trend} = 0.027$). Isoflavone intake (median intake in Q1 and Q4 were 4.7 and 24.0 mg isoflavone per day, respectively) was positively associated with urinary excretion of testosterone ($P\text{-trend} = 0.003$). The study shows that soy/isoflavone exposure may affect pre-school boys and girls differently in relation to specific hormonal secretion but does not provide insight into the potential consequences of these changes.

Overall, no straight conclusion can be made for an association between exposure to dietary soy or isoflavones and risk of precocious puberty based on the identified studies for either girls or boys.

For girls, a total of three studies found adverse associations between exposure to dietary soy or isoflavones and age of puberty (Marks et al., 2017, Kim et al., 2011, Yum et al., 2013). The three-studies report exposure to isoflavones as either urinary excretion, or serum or plasma levels, which currently does not allow for direct extrapolation to dietary intake. Two of these studies are case-control studies with limited adjustment for confounders (Kim et al., 2011, Yum et al., 2013). The study by Marks et al. (2017) is a nested case-control study and the only study investigating the association between prenatal exposure of isoflavones and age of menarche. In this study, however, the only association between isoflavone exposure and risk of earlier age of menarche was found for O-DMA (not with genistein, daidzein or equol).

For boys, only one of three studies found associations between exposure to dietary isoflavones and age of puberty (Segovia-Siapco et al., 2018). However, earlier age of

puberty in boys is not as clearly associated with adversity as for girls and the study by Segovia-Siapco et al. (2018) is a cross-sectional study, which cannot be used to infer causal relationships.

Breast cancer

Breast cancer is an estrogen-dependent malignancy whose incidence is increasing in most Western societies. In Denmark, the risk of getting breast cancer before the age of 75 is 9.9% for women (2012–2016 data) (NORDCAN, 2019). The elevated incidence of breast cancer in women has been associated with prolonged exposure to high levels of estrogens as that occurring with early onset of menarche and late menopause. Due to the structural similarity between isoflavones and endogenous estrogens and the possible association between higher intake of isoflavones and risk of earlier menarche, there is a theoretical possibility that isoflavone intake is correlated with the risk of breast cancer.

A total of six human studies on the association between exposure to soy/isoflavones during childhood/adolescence and risk of breast cancer later in life were identified and are presented in Table 14. Four out of the six studies are with Asian populations (Korde et al., 2009; Lee et al., 2009; Wu et al., 2002; Shu et al., 2001) of which two are with Asian populations in the USA (Korde et al., 2009; Shu et al., 2001). The last two studies are from The Ontario Women's Diet and Health Study (OWDHS) in Canada (Anderson et al., 2013; Thanos et al., 2006).

Table 14. Human studies on exposure to soy/isoflavones during childhood/adolescence and risk of later development of breast cancer.

Reference	Design	Number of subjects	Age during exposure, gender and country	Exposure (estimated or measured)	Quantified isoflavone exposure	Results
Anderson et al., 2013	Population-based case-control study.	2,397 cases and 3,370 controls.	10–15 years. Female. Canada.	Estimated from non-validated retrospective FFQ.	Isoflavone exposure: 0–10, 11–20 and ≥ 21 μg per day. Isoflavone exposure included the sum of genistein, daidzein, glycitein and formononetin.	Among postmenopausal women, adolescent isoflavone intake was significantly associated with reduced breast cancer risk in the ER+PR- tumour subgroup (P for trend = 0.01). No associations were seen for premenopausal women or other postmenopausal tumor subgroups.
Korde et al., 2009	Population-based case-control study.	Subset with data from mothers: 99 cases and 156 controls. All study participants: 597 cases and 966 controls	5–11 years (mothers interviewed) and 12–19 years. Female. Asians in USA.	Estimated from retrospective FFQ.	Soy intake during childhood (mother interviewed): Range (times per week): Low = 0–0.75 Medium = 0.76–1.49 High = 1.50–8.8. Soy intake during adolescence (retrospective): Range (times per week): Low = 0–0.50 Medium = 0.51–1.9 High = 2.0–8.9.	For women in the highest and middle tertiles of childhood intake compared with the lowest tertile, risk of breast cancer was significantly reduced by ca. 60% (P=0.03). Adolescent soy intake was not associated with risk of breast cancer.
Lee et al., 2009	Prospective cohort study.	N=73,225 in total of which 594 were cases (305 premenopausal and 289 postmenopausal) Non-cases= 72,631.	13–15 years. Female. China.	Estimated from retrospective FFQ.	Soy protein and isoflavone exposure. Median values (and cut-offs) for the lowest to the highest quintile of soy protein intake (g per day): 1.73 (≤ 2.76), 3.76 (2.77, 4.70), 5.81 (4.71, 6.95), 8.86 (6.96, 11.32) and 15.16 (≥ 11.33). The corresponding numbers for soy isoflavones (mg per day): 4.31 (≤ 7.34), 10.04 (7.35, 12.82), 15.99 (12.83, 19.43), 24.11 (19.44, 31.28) and 42.26 (≥ 31.28).	Among postmenopausal women, adolescent isoflavone intake was moderately but significantly associated with higher risk for breast cancer RR Q5 vs. Q1=1.38, 95% CI 1.00, 1.91 (P-trend = 0.038). No other statistically significant associations between adolescent soy protein or isoflavone intake were observed.
Thanos et al., 2006	Population-based case-control study.	3,024 cases and 3,420 controls.	10–15 years. Female. Canada.	Estimated from retrospective FFQ.	The exposure to isoflavones is reported as scores according to the content of isoflavones in each food item. Score = 0: very low (0–50 $\mu\text{g}/100$ g); = 1: low (51–1,000 $\mu\text{g}/100$ g); = 2: high (1,001–10,000 $\mu\text{g}/100$ g); = 3: very high (>10,000 $\mu\text{g}/100$ g).	Higher isoflavone intake during adolescence was associated with a significant reduction in breast cancer risk (AOR (Q4 vs. Q1) = 0.81, 95% CI 0.71–0.94), P-trend = <0.01.

					100 g). Quartiles (scores) for isoflavone intake: Q1 (0-13), Q2 (14-17), Q3 (18-21), Q4 (≥22).	
Wu et al., 2002	Population- based case- control study.	501 cases and 594 controls.	12–18 years. Female. Asians in USA.	Estimated from retro- spective FFQ.	Tofu intake during adolescence: Less than monthly 1–3 times per month 1–3 times per week 4+ times per week.	Adolescent tofu intake was moderately but significantly associated with lower risk for breast cancer OR 'Less than monthly' vs. '4+ times per week' = 0.65, 95% CI 0.38-1.10 (P-trend = 0.04).
Shu et al., 2001	Population- based case- control study.	1,459 cases and 1,556 controls.	13–15 years. Female. China.	Estimated from retro- spective FFQ.	Quintile cut-offs for tofu intake (g per day): Quint2: 0.44 XQuint3: 0.88 XQuint4: 1.32 Quint5: 2.2 Quintile cut-offs for total soy food intake (g per day): Quint2: 2.20 Quint3: 4.41 Quint4: 6.61 Quint5: 11.01 Tertile cut offs for soy products other than tofu (g per day): T2: 3.52 T3: 5.28.	Adolescent soy food intake was significantly associated with lower risk for breast cancer. The ORs associated with soy food intake during adolescence were 0.75 (95% CI, 0.57–0.93), 0.69 (95% CI, 0.55–0.87), 0.69 (95% CI, 0.55–0.86) and 0.51 (95% CI, 0.40–0.65) for the second quintile to the highest quintile of soy food intake compared with the lowest quintile of intake (P-linear trend test = 0.0001). The dose-response relationship was observed for all three commonly consumed soy foods (tofu, soy milk (no exposure data), and soy products other than tofu) and in both pre- and postmenopausal women (except for soy milk).

AOR: age-adjusted odds ratio, CI: confidence interval, ER: Estrogen receptor, FFQ: Food Frequency Questionnaire, OR: odds ratio, PR: progesteron receptor, Quint: quintile, RR: relative ratio, T: tertile.

Most of the studies are case-control studies (five) with one prospective cohort study (Lee et al., 2009). All studies quantify soy/isoflavone exposure retrospectively. The women's age of exposure is in all studies adolescents (10–15 years) except for the study by Korde et al. (2009), which included children age 5–11 years by interviewing mothers. Two studies report estimated isoflavone exposure (Anderson et al., 2013, Lee et al., 2009), whereas the other studies report soy (Korde et al., 2009), soy protein (Lee et al., 2009), isoflavone score (Thanos et al., 2006), tofu (Wu et al., 2002; Shu et al., 2001) and soy product intake (Shu et al., 2001).

All six studies found associations between higher soy/isoflavone intake during childhood/adolescence and lower risk of breast cancer later in life for girls (Anderson et al., 2013; Korde et al., 2009; Lee et al., 2009; Thanos et al., 2006; Wu et al., 2002; Shu et al., 2001). These studies are therefore not considered for the risk characterisation, as they cannot be used for setting a HGBV. However, although not emphasized in the study with Chinese women by Lee et al. (2009), adolescent isoflavone intake was moderately but significantly associated with higher risk for breast cancer in postmenopausal women (N=289) RR Q5 vs. Q1=1.38, 95% CI 1.00, 1.91 (P-trend = 0.038). The median isoflavone intake was 4.31 (≤ 7.34) mg per day for Q1 and 42.26 (≥ 31.28) mg per day for Q5. Information on genotype of breast cancer subtype was not included in the study by Lee et al. (2009).

Overall, no association was found between early exposure to dietary soy or isoflavones and later risk of breast cancer based on the identified studies.

Hypospadias

Hypospadias is a relatively frequent urogenital anomaly in boys and its occurrence may be caused by imbalance of androgens and estrogens during early pregnancy (8th–14th week of gestation) (Michikawa et al., 2019). Due to their structural similarity to endogenous estrogens, isoflavones (consumed by pregnant women) may be related to the risk of hypospadias. In addition, studies in mice have shown that exposure to genistein *in utero* increases the occurrence of hypospadias in offspring (Michikawa et al., 2019).

The included studies on hypospadias are summarized in Table 15.

Table 15. Human studies on prenatal exposure to soy/isoflavones and risk of hypospadias.

Reference	Design	Number of subjects	Age during exposure), gender and country	Exposure	Quantified isoflavone exposure	Result
Michikawa et al., 2019	Prospective cohort study.	Total of 41,578 mothers who delivered singleton live male births. Hypospadias cases: 51.	Prenatal. Male. Japan.	Estimated maternal exposure from FFQs and food composition tables.	Genistein (mg per day): ≤10 th percentile: ≤5.0; 11 th -89 th percentile: 5.1-36.1; ≥90 th percentile: ≥36.2.	OR (95% CI) for genistein intake and risk of hypospadias: ≤10 th percentile: 2.5 (1.1-5.6) 11 th -89 th percentile: Referent ≥90 th percentile: 1.0 (0.4-2.7). Low, but not high, maternal intake of genistein was associated with elevated risk of hypospadias.
Carmichael et al., 2013	Population-based case-control study.	Mothers of 1,250 infants with hypospadias and 3,118 controls.	Prenatal. Male. USA.	Estimated maternal exposure from FFQs and food composition tables.	Genistein (µg per day): ≤10 th percentile: ≤10.7; 11 th -89 th percentile: 10.8-41.7; ≥90 th percentile: ≥41.8 Daidzein : ≤10 th percentile: ≤4.9; 11 th -89 th percentile: 5.0-24.1; ≥90 th percentile: ≥24.2 Glycitein* : ≤10 th percentile: ≤3.7; 11 th -89 th percentile: 3.8-18.7; ≥90 th percentile: ≥18.8.	OR (95% CI) for intake of genistein , daidzein or glycitein* and risk of hypospadias (11 th -89 th percentile = referent): Genistein : ≤10 th percentile: 1.1 (0.9-1.4) ≥90 th percentile: 0.6 (0.4-0.9) Daidzein : ≤10 th percentile: 1.0 (0.8-1.3) ≥90 th percentile: 0.7 (0.5-1.0) Glycitein* : ≤10 th percentile: 1.0 (0.8-1.3) ≥90 th percentile: 0.7 (0.4- 1.0). High maternal intake of genistein , daidzein and glycitein* was associated with reduced risk of hypospadias, although for daidzein and glycitein* the confidence intervals included 1.0.
Pierik et al., 2004	Nested case-control study.	A total of 443 mother-child pairs: 78 cases of cryptorchidism, 56 cases of hypospadias, and 309 controls.	Prenatal. Male. The Netherlands.	Estimated from FFQ.	Soy protein intake (g per day): 0 >0-20 ≥20.	OR (95% CI) for soy protein intake and risk of hypospadias: 0 g per day: 1.0 (referent) >0-20 g per day: 1.1 (0.5-2.5) ≥20 g per day: 1.0 (0.5-2.2). No statistically significant association between maternal intake of soy protein and risk of hypospadias.
North & Golding 2000	Prospective cohort study.	N=6189 (soy milk during pregnancy) out of which 40 were cases and N=6189 (soy 'meat' during pregnancy) out of which 41 were cases.	Prenatal. Male. United Kingdom.	Estimated from FFQ.	Drank soy milk during pregnancy: Yes/No. Ate soy 'meat' during pregnancy: Never/Once in 2 weeks/>1 time per week.	OR (95% CI) for association between soy intake and risk of hypospadias: Soy milk Yes: 3.67 (0.87-15.44). No: 1.0 (ref). Soy 'meat' Never: 1.0 (ref). Once in 2 weeks: 1.01 (0.24-4.22) >1 time per week: 2.95 (0.90-9.68). No statistically significant association between maternal intake of soy milk or 'meat' and risk of hypospadias.

**Carmichael et al., 2013 writes glycitin, but based on context it is assumed to be glycitein. CI: confidence interval, FFQ: Food Frequency Questionnaire, OR: odds ratio.*

A total of four human studies on the association between exposure to soy/isoflavones and risk of hypospadias in boys were identified (Michikawa et al., 2019; Carmichael et al., 2013; Pierik et al., 2004; North & Golding, 2000).

Three studies did not detect significant differences in the endpoints measured (Carmichael et al., 2013; Pierik et al., 2004; North & Golding, 2000). Maternal exposure included genistein (≥ 41.7 μg per day), daidzein (≥ 24.1 μg per day), glycitein (≥ 18.8 μg per day), soy protein (≥ 20 g per day) and intake of soy milk (yes/no) or soy meat (>1 time per week) during pregnancy.

The fourth study found that low (≤ 5 mg per day), but not high (≥ 36.2 mg per day), maternal dietary genistein exposure was associated with elevated risk of hypospadias in a Japanese population (Michikawa et al., 2019).

Overall, although there may seem to be a theoretical correlation between *in utero* isoflavone exposure and risk of hypospadias, it has not been confirmed in the identified human studies.

Thyroid function

There are several possible mechanisms by which isoflavones may affect thyroid function. In the 1950s and 1960s, it was found that goitrogenic effects in children fed soy formula could be reversed with iodine supplementation. The goitrogenic effect of isoflavones was shown to be predominantly caused by the inhibition of thyroid peroxidase (TPO), an enzyme found in thyroid follicle cells that catalyzes two reactions required for thyroid hormone synthesis (Hampl et al., 2009). Genistein and daidzein, in particular, have been shown to inhibit TPO-catalysed iodination and coupling, thereby inhibiting thyroid hormone formation (Pearce & Braverman, 2009). The isoflavones may also influence thyroid hormone metabolism, via deiodinases, thyroid hormone transport and various thyroid hormone mediated signalling pathways (reviewed by Hampl et al. (2009)). In addition, soy may also interfere with the absorption of synthetic thyroid hormones used for medical purposes (Li et al., 2011).

The included studies on effects on thyroid function are summarized in Table 16.

Table 16. Human studies on exposure to soy/isoflavones during childhood/adolescence or as prenatal exposure and effects on thyroid function.

Reference	Design	Number of subjects	Age during exposure, gender and country	Exposure	Quantified isoflavone exposure	Results
Li et al., 2011	Cross-sectional.	505.	Pregnant women. Female. China.	Measured in spot urine samples (sub-fraction of 95 samples). Soy intake estimated from FFQ.	Urinary genistein (µg/mg creatinine): Frequent soy consumers Percentile (P): P25 = 0.32, P50 = 0.62, P75 = 1.66. Conventional soy consumers: P25 = 0.09, P50 = 0.23, P75 = 0.40. Occasional soy consumers: P25 = 0.04, P50 = 0.21, P75 = 0.68 Urinary daidzein (µg/mg creatinine): Frequent soy consumers P25 = 0.33, P50 = 0.57, P75 = 1.47. Conventional soy consumers: P25 = 0.10, P50 = 0.20, P75 = 0.55. Occasional soy consumers: P25 = 0.03, P50 = 0.22, P75 = 0.95.	No significant associations between isoflavone exposure and thyroid functions (serum fT ₄ and TSH level), prevalence of thyroid dysfunctions (subclinical and overt hyperthyroidism, subclinical and overt hypothyroidism and hypothyrocinemia), or anti-TPO antibody (TPOAb) during early pregnancy.
Milerová et al., 2006	Cross-sectional.	268.	8–15 years. N=139 for girls and 129 for boys. Czech Republic.	Estimated from questionnaire. Measured in serum.	Soy food eaten in the last 24 hours: Mean (SEM) (nmol/l) serum genistein : 1.068 (0.188), serum daidzein : 1.352 (0.362) No soy food eaten in the last 24 hours: serum genistein : 0.773 (0.081), serum daidzein : 0.664 (0.081). The difference in serum genistein and daidzein between the two groups was only statistical significant for daidzein (P = 0.0062), genistein (P = 0.0775).	Association between soy intake (isoflavone exposure) and thyroid parameters were statistically significant for T ₄ (16.48 pmol/l (SEM = 0.285) in 'soy food eaten in the last 24 hours' compared to 15.42 (SEM = 0.133) in 'no soy food eaten in the last 24 hours'. P = 0.0032). No significant associations were observed for T ₃ , TSH, urinary iodine, anti-Tg or anti-TPO. Statistically significant Spearman's correlations with serum genistein was observed with anti-Tg (positive, r = 0.2636) and thyroid volume (negative, r = -0.1695). Statistically significant Spearman's correlations with serum daidzein was observed with TSH (positive, r = 0.1873).

FFQ: food frequency questionnaire, fT₄: free T₄, SEM: Standard error of the mean, T₃: triiodothyronine, T₄: thyroxine, Tg: thyroglobulin, TPO: thyroperoxidase, TSH: thyroid-stimulating hormone.

Two relevant human studies on the association between exposure to soy/isoflavones and thyroid function were identified.

The associations between soy intake and maternal thyroid functions and anti-TPO antibody (TPOAb) production (marker for thyroid autoimmunity) during early pregnancy were investigated among 505 Chinese women (Li et al., 2011). In the early stages of pregnancy, maternal serum thyroxine (T_4) and a positive anti-TPOAb in maternal serum may influence foetal brain development. Both parameters are therefore of relevance for the unborn child. No significant associations were observed on maternal thyroid functions or TPOAb in the study by Li et al. (2011).

A cross-sectional study based in an iodine-deficient region of the Czech Republic with 268 subjects (139 girls and 129 boys) between 8 and 15 years of age studied the effect of isoflavones on selected parameters of thyroid function (Milerová et al., 2006). The children were euthyroid (i.e. with normal thyroid function) in terms of thyroid ultrasonography and free thyroid hormone levels but there were children with positive titres of anti-thyroid autoantibodies. According to the authors, these children were deliberately not excluded from the study so that the group would reflect as far as possible the European population, in which up to 10% of subjects display positive titres of anti-thyroid antibodies. The study showed modest but significant positive correlations of serum genistein levels (1.1 nmol/l after soy food eaten in the last 24 hours vs. 0.8 nmol/l after no soy food eaten in the last 24 hours) with thyroglobulin autoantibodies (anti-Tg) ($r=0.2636$) and a negative correlation with thyroid volume ($r=-0.1695$). For daidzein serum levels (1.4 nmol/l after soy food eaten in the last 24 hours vs. 0.7 nmol/l after no soy food eaten in the last 24 hours), a positive correlation with TSH was observed ($r=0.1873$). The authors concluded that although only modest associations were found between actual isoflavone levels and parameters of thyroid function, even small differences in soy isoflavone intake may influence thyroid function, which could be important when iodine intake is insufficient.

Overall, only two studies on adverse effects of isoflavones on thyroid function relevant for children and pregnant women (unborn children) were identified in the literature search. The study by Li et al. (2011) included Chinese women, which may respond differently to isoflavone exposure compared to Western populations due to the differences in metabolism. Based on the study by Milerová et al. (2006) adverse effects of isoflavones on thyroid function when iodine intake is insufficient cannot be excluded. Iodine deficiency is a problem that all Nordic countries has to deal with and fortification of table salt (Denmark, Finland, Sweden) or cow fodder (Finland, Iceland, Norway) has limited the occurrence of iodine deficiency to a large extent (Nyström et al., 2016). Although, according to Nyström et al. (2016), there are indications that pregnant and lactating women are mildly deficient in iodine in several of the Nordic countries.

Animal studies from the literature search

To facilitate the evaluation whether a HBGV for soy constituents can be set based on experimental animal data, a literature search was carried out as described above. Among the 333 studies identified in the primary literature search, the literature review was focused on the period 2010 to 2019, as a thorough literature evaluation

had been carried out by NTP-CERHR (2010) covering the period up to year 2010. The NTP-CERHR evaluation (2010) applied data from a dose-response study on multigenerational dietary genistein exposure (NTP 2008) and a series of studies using neonatal exposure by subcutaneous injections for their risk assessment. The NTP-CERHR (2010) report did not set a HBGV but focused on comparison of internal exposures. In absence of useful human internal exposure data for the purpose of this report, the same animal data may however be applicable to set a preliminary HBGV for comparing human intake levels with effect levels in orally exposed experimental animals (see below).

The aim of the literature review of animal data was to identify whether more recent studies would be relevant for determining a HBGV. The focus was therefore on two possibilities: a) identifying studies on genistein with potential to be used for determining a different point of departure than that determined using data from the multigenerational study (NTP 2008), and b) identifying studies on mixed isoflavones and/or studies on other isoflavones than genistein with potential to be used for determining a point of departure for setting a HBGV.

In total, 239 dietary and 61 gavage studies were identified in rodents, 4 in monkeys, 2 in mink, 3 in zebrafish and 24 in pigs. Studies including only one dose of a soy constituent and studies comparing two diets were omitted from further evaluation, as the aim was to determine point of departure for setting a HBGV. Of the 333 studies, 80 rodent studies fulfilled the following criteria: 1) examination of more than one dose group of genistein, daidzein, glycitein or a soy-containing extract/diet, 2) exposure by gavage or diet, and 3) use of a relevant exposure period. Specifically, we focused on studies in which exposure was limited to developmental periods, i.e. during gestation, lactation and/or the period from weaning to puberty. This was important to keep focus on effects of exposure during development of relevance to human foetal or child exposure. In studies using continued exposure after puberty, only effects seen in the neonatal to pubertal period were evaluated. Twenty papers were published after the NTP-CERHR review, i.e. in the period 2010–2019.

Of these 20 studies, we omitted studies examining immune parameters, lipid and glucose homeostasis, obesity, bone etc., as these endpoints did not reflect the human findings retrieved in the literature study above. These effects may be human relevant but sparse information was found in humans. The present analysis thus focused on reproductive and endocrine parameters, as these were clearly human relevant and signs of endocrine activity have been identified in humans. For the period 2010 to 2019, we identified four potentially relevant dose-response studies on genistein exposure (Musameh et al., 2014; Zin et al., 2013; Li et al., 2014; Zhang et al., 2013), one study on daidzein exposure (Talsness et al., 2015) and one dose-response study on mixed phytoestrogen exposure (Boberg et al., 2013). The latter study examined perinatal exposure to human relevant phytoestrogen mixtures in rats. This mixture included both soy estrogens genistein and daidzein but also lignans, which can be found in flaxseed and rye bread (Boberg et al., 2013). The investigated mixture was dominated by one lignan, secoisolariciresinol (82% of the mixture on a weight basis), which is only present in small amounts in soy products. Therefore, this study was not considered relevant for risk assessment of soy products and was omitted from the present review. A total of five animal studies were included from 2010–2019 from the literature search.

Below, we summarize the findings in these studies related to reproductive and

endocrine parameters: AGD, puberty timing, female reproductive tract development, male reproductive tract development and mammary development (Table 17). The NTP-CERHR 2010 evaluation based their conclusions mainly on a multigenerational study in rats showing effect at 500 ppm (NTP 2008). Below, this study is included, and the effect level of 500 ppm is compared with effect levels of the identified recent studies. Data is presented endpoint for endpoint following the structure of the human study descriptions above.

Table 17. Summary of five relevant animal studies identified from the period 2010-2019 and one study considered important by the NTP-CERHR (2010) evaluation (multigenerational study by NTP, 2008).

Reference	Animal model	N	Exposure period	Test compound and dose	Endpoints	Results (only endpoints clearly attributed to developmental exposure are considered relevant here due to the purpose of risk assessment of foetal and child exposure)
NTP (2008), Latendresse et al., 2009; Delclos et al., 2009	Sprague Dawley rats, multi-generation study.	35 per sex.	From six weeks of age of parental generation through F1, F2 and F3 generations. Exposure of F3 generation terminated at weaning.	Genistein in soy- and alfalfa-free diet 5K96. 0, 5, 100 and 500 ppm genistein (males: 0, 0.3, 7, and 35 mg/kg bw per day; females: 0, 0.5, 10, and 51 mg/kg bw per day for entire feeding period).	Growth and survival parameters, AGD, puberty timing, estorus cyclicity.	Earlier vaginal opening in F1 and F2 offspring at 500 ppm. In male mammary glands , an increased incidence of mammary hyperplasia was seen in adult F1, F2 and F3 offspring at PND 140. More marked effects were seen in the continuously exposed F1 and F2 generation than in F3 exposed until weaning only. This indicates an effect of adult exposure and not only developmental. Reduced AGD of male pups of F1 generation only and female pups of F1, F2 and F3 generations (highest dose). Decreased live litter size and total number of pups born in F2 generation (highest dose). No change in ovarian follicle counts or sperm parameters.
Musameh et al., 2014	Sprague-Dawley rats.	2 litters per dose group, 6 male pups per dose group.	Dams: gestation day 10 to 21 by gavage. Male pups: after birth to PND 21 by subcutaneous injection.	Genistein 0, 10, 100 mg/kg bw per day.	Growth, AGD, puberty timing, testis descent, testicular weight, testosterone level, testicular histology.	Reduced male AGD and body weight at PND 1, 8 and 15. AGD not weight adjusted. Delayed preputial separation at highest dose. Not considered a robust study. Reduced testis weight and reduced diameter of seminiferous tubule lumina, but no change of tubular diameters.
Zin et al., 2013	Sprague Dawley rats, female.	N=6.	PND 22 to 42.	Genistein 0, 10, 100 mg/kg bw per day.	Body weights, ovary and uterus histology and weight, plasma hormone levels evaluated at PND 43.	No change in body or organ weights. Reduced plasma lutenizing hormone at lowest dose. Reduced endometrial thickness, changes in ovarian histology (increased number of atretic follicles and reduced number of corpora lutea per unit area) at lowest dose mainly.
Zhang et al., 2013	Sprague Dawley rats.	N=6.	Gestation day 3 to PND 21.	0, 50, 400 mg/kg bw per day of genistein (with or without combination with DEHP 250 mg/kg bw per day).	Maternal weight gain, litter size, sex ratio, number of viable pups, weight adjusted AGD, testosterone level, testis histology and gene expression, reproductive organ weights.	No effects on these endpoints of genistein alone.
Talsness et al., 2015	Sprague Dawley rats, mated.	Unclear number of litters,	Gestational day 6 to 21.	Daidzein, 0, 5, 60 mg/kg bw per day.	Estrous cyclicity and ovary histology.	Altered estrous cyclicity : Prolonged estrous in high dose group, no change in cycle length.

AGD: anogenital distance, DEHP: diethylhexyl phthalate, PND: postnatal day.

Timing of puberty

Effects on puberty timing was seen in both male and female rats of the multigenerational study (NTP, 2008). At the highest dose level, females showed earlier vaginal opening in F1 and F2 generations (NTP, 2008). The high dose level of 500 ppm corresponds to ~35 mg/kg bw per day in males and ~51 mg/kg bw per day in females during the entire feeding period.

Earlier puberty was also identified in mice exposed from weaning onwards at doses of 100 and 500 ppm (Li et al., 2014). In that study, newly weaned female mice were fed 0, 5, 100 or 500 ppm genistein in the diet (N=35), and early vaginal opening was seen in the two highest dose groups. This effect seen at a lower dose level than in the multigenerational study in rats indicates a possibility that mice exposed up to puberty only may be more susceptible than rats exposed in a multigenerational study. The Li et al. (2014) study does not inform of intake in mg/kg, and EFSA guidance on default values is applied to convert from ppm. EFSA suggests using a conversion factor of 0.2 for subacute exposures of mice (EFSA, 2012). This means that 1 mg/kg in feed corresponds to 0.2 mg/kg bw per day. In this study, the Low Observed Adverse Effect Level (LOAEL) of 100 ppm for effect on puberty timing is thus estimated to 20 mg/kg bw per day.

This finding of earlier puberty timing support findings in other studies reported in the NTP-CERHR evaluation (2010) (Casanova et al., 1999; Delclos et al., 2001; You et al., 2002).

Puberty timing in females was not evaluated in genistein studies by Musameh et al. (2014), Zhang et al. (2013), Zin et al. (2013), or in the study on daidzein by Talsness et al. (2015).

In male rats, Musameh et al. (2014) detected delayed preputial separation. Time-mated Sprague-Dawley rats were exposed to genistein (0, 10 or 100 mg/kg bw per day) genistein by gavage from gestation day 10 to 21, and male offspring was exposed again from the day after birth to PND 21 by subcutaneous injection. This appears to be a very small study, using a total of 10 time-mated SD rats (N=2 per dose group), and 6 male pups per dose group. Therefore, these results are considered to be of limited utility.

Breast cancer

In the NTP mutigenerational study, male mammary glands were consistently affected (increased incidence of mammary hyperplasia) by genistein in all generations at the high dose of 500 ppm (NTP (2008), Latendresse et al., 2009). It was considered that both developmental and adult exposures contributed to the maintenance of these effects. Less marked effects were seen in the F3 generation exposed during gestation and lactation only, when compared to the continuously exposed F1 and F2 generation offspring.

Accelerated female mammary gland development (increased mammary gland duct length and area) was seen in female mice exposed from weaning onwards at doses of 100 and 500 ppm in feed (Li et al., 2014). As noted above, this LOAEL of 100 ppm for effect on mammary development is estimated to 20 mg/kg bw per day. As for puberty timing, the effective dietary exposure level was lower in this mouse study than in the multigenerational study in rats (NTP, 2008).

Mammary gland development was not evaluated in genistein studies by Musameh et al. 2014, Zhang et al. 2013, Zin et al. 2013, or in the study on daidzein by Talsness et al. 2015.

The findings of altered mammary development in males and females reported by NTP (2008)/ Latendresse et al. (2009) and Li et al. (2014) support several studies covering the developmental period reported in NTP-CERHR (2010). Increased numbers of Terminal End Buds (TEBs) in mammary glands are a marker of increased susceptibility to carcinogens, and changes in TEB numbers and increased susceptibility to carcinogen-induced mammary tumorigenesis were seen in several studies on genistein exposure reported in NTP-CERHR (2010) (Hilakivi-Clarke et al., 1998; Hilakivi-Clarke et al., 2002; Hilakivi-Clarke et al., 1999, Latendresse et al., 2009; Thomsen et al., 2006).

Hypospadias

None of the identified relevant studies after 2010 examined hypospadias frequency. Only one experimental study reviewed in the NTP-CERHR (2010) report found a higher incidence of hypospadias in genistein exposed mice. This study exposed pregnant mice to only one dose of genistein alone and in combination with vinclozolin (Vilela et al., 2007), and the finding is therefore of limited importance.

Thyroid function

The literature search did not identify any dose-response studies on effects on the thyroid hormone system. According to the NTP-CERHR (2010) evaluation, several targets of isoflavones in the thyroid hormone system have been identified. A number of *in vitro* and *in vivo* studies show that soy isoflavones can act as competitive substrates for TPO (reviewed in Doerge et al., 2002). The predicted effects *in vivo* from TPO inhibition would be reduced concentrations of thyroid hormones T₃ and T₄ and increased production of TSH. NTP-CERHR (2010) found that the predicted decreases in T₃/T₄ and increased TSH were not observed in either the genistein or soy-fed rats. In addition, no differences in thyroid weight or histopathology were observed at the highest dose tested in a separate study with the same experimental design. These findings, coupled with studies by other investigators using iodine-deficient rodent models, suggest that impaired thyroid function, e.g. iodine deficiency, is necessary for soy to exert anti-thyroid effects *in vivo*.

Fertility and markers of impaired reproductive development

The AGD is a hormone-sensitive endpoint found useful as a biomarker of endocrine disturbance during development. For genistein, shorter AGDs at birth were seen at the high dose of 500 ppm in male and female rat pups of the F1 generation of the

NTP (2008) multigenerational study, also when corrected for body weight differences. In females, reduced AGDs were also seen in the F2 and F3 generations. Although little is known on the predictive value of AGD in females, a change may be considered evidence of altered hormone balance during a sensitive developmental period.

In the same study, genistein treatment was associated with decreased live litter size and total number of pups born (NTP, 2008). Decreased live litter size and decreased total number of pups born were observed in the F2 generation of the high-dose group. A significant linear exposure concentration trend for decreased live litter size and decreased total number of pups born in the F1, F2 and F3 generations was also reported. Ovarian follicle counts were not observed to be affected by genistein treatment, and in male rats, genistein had no detected effect on sperm parameters. Thus, the reduced litter size can be considered to be due to impaired maternal reproductive function during pregnancy. This study consistently showed effects on estrous cyclicity across generations but it is unclear whether this is induced during adulthood or related to developmental exposure. Thus, these findings are of limited use for assessing risk for the offspring with foetal or postnatal exposure.

After dietary exposure of mice to genistein from weaning onwards, Li et al. (2014) reported longer time in estrous stage during the first 10 days after vaginal opening (N=6) at all doses of 5, 100 and 500 ppm. The two highest dose groups had a reduced number of animals showing cyclicity (sign of delayed onset of estrous cycle). Also from 5 to 8 weeks of age, prolonged time in estrous was seen in the high dose group but almost all females were cycling. Mating of high dose mice (500 ppm) showed no significant adverse effects on ovulation, fertilization, embryo transport or preimplantation embryo development. This study indicated potential delay in ovarian development, which is in contrast to the observed early puberty and mammary development, as described above. Effects at the low dose were modest but at the two high dose levels marked changes were seen. As exposure continued until termination in adulthood, the effects in mature animals cannot be attributed to developmental (pubertal) exposure only.

The study by Zin et al. (2013) evaluated post-weaning exposure to genistein. Eighteen female Sprague Dawley (SD) rats were exposed daily from PND 22 to 42 to 0, 10 or 100 mg/kg bw per day genistein by gavage (N=6). Histological examination of uteri and ovaries was carried out at PND 43. No statistically significant changes in body weights or weight of uterus or ovary were seen. Plasma luteinizing hormone (LH) was reduced by the low but not the high dose, of genistein. This group also had reduced thickness of endometrium, and both groups showed changes in ovarian histology (increased number of atretic follicles and reduced number of corpora lutea per unit area). This may reflect delayed or impaired maturation of reproductive organs with peri-pubertal exposure, but as effects were not as marked in the high dose group as in the low dose group and as it is not clear if only one section per animal was evaluated, these results are considered to be of limited utility.

Studies by Zhang et al. (2013) and Musameh et al. (2014) in male rats pointed to effects on male reproductive development. As noted above, the study by Musameh et al. (2014) is considered of limited utility due to small study size and using a combination of subcutaneous and oral exposure (see Table 17 for results). In the study by Zhang et al. (2013), pregnant SD rats (N=6) were treated by daily gavage

from gestation day 3 to PND 21 with peanut oil (vehicle control), diethylhexyl phthalate (DEHP) 250 mg/kg bw per day, genistein 50 or 400 mg/kg bw per day, or a combination of DEHP 250 mg/kg bw per day and genistein 50 mg/kg bw per day, or a combination of DEHP 250 mg/kg bw per day and genistein 400 mg/kg bw per day. There were no significant effects on maternal weight gain, litter size, sex ratio, number of viable pups or weight adjusted AGD at any dose tested. Genistein alone did not affect testosterone levels, testicular histology or gene expression at PND 22 or reproductive organ weights but these endpoints were affected by DEHP and genistein in combination.

A study on prenatal exposure of rats to daidzein showed subtle, persistent effects on adult ovarian histology and estrous cyclicity (Talsness et al., 2015). Six groups of mated SD rats were exposed from gestational day 6 to 21 by gavage to 0, 5 or 60 mg/kg bw per day of daidzein, or 0.002 mg/kg bw per day of ethinyl estradiol in 2% cornstarch (vehicle). The number of litters per dose group is unclear. Estrous cycle changes and ovarian histology were evaluated in adulthood. The percentage of offspring with prolonged estrous was significantly increased in groups exposed to ethinyl estradiol or 60 mg/kg bw per day of daidzein (N=39–40 offspring per dose group). There was no change in estrous cycle length. Ovarian histology was examined in a subset of offspring comparing each dose group with a separate control group (N=10). Daidzein appeared to reduce the number of secondary and tertiary follicles at both doses, and to increase the number of atretic follicles at the high dose. Five sections from the middle part of the ovary were used for quantification. This study is potentially relevant but has several limitations, e.g. uncertainty about number of litters included per dose group, and the use of different control groups for each dose group in the histological evaluation

According to NTP-CERHR (2010), effects on female reproductive tract were seen in studies using typically subcutaneous exposure of mice in the neonatal period. Genistein exposure of mice from PND 1-5 led to changes in ovarian and uterine histology and lower number of pups per litter, when the neonatally exposed females were mated in adulthood (Padilla-Banks et al., 2006; Newbold et al., 2001; Cimafranca et al., 2010; Jefferson et al., 2005, reported in NTP-CERHR 2010). Altered estrous cyclicity and signs of early reproductive senescence were reported (Jefferson et al., 2005). In rats, similar effect patterns were seen including effects on estrous cycling and age of vaginal opening (Bateman & Patisaul 2008; Kouki et al., 2003; Nagao et al., 2001; Lewis et al., 2003, reported in NTP-CERHR 2010). In contrast, some studies in mice showed delay in vaginal opening or no effect after neonatal genistein exposure (Cimafranca et al., 2010; Jefferson et al., 2009). Except for the study by Nagao et al. (2001), these studies all used subcutaneous exposure and are of limited use for direct comparison with human dietary exposure. Nagao et al. (2001), administered four doses of genistein from PND 1-5 by gavage (N=5–7) and this study was considered of high usefulness by NTP-CERHR (2010). This study showed possible effect on fertility index at 12.5 mg/kg bw per day, whereas clear ovarian histological changes were seen at 100 mg/kg bw per day with a NOAEL of 50 mg/kg bw per day. This NOAEL for ovarian effects is higher than the LOAEL for 500 ppm (35 mg/kg bw per day) seen in the NTP (2008) study, and is therefore of limited relevance for HBGV determination.

Relevance of data for HBGV derivation

For genistein, mainly effects on pubertal timing and early mammary development are considered relevant for HBGV derivation for risk assessment of human foetal and child exposure. These effects are likely caused by foetal or lactational exposure and thus relevant for the target groups. Findings reported by NTP (2008) /Latendresse et al. (2009) and Li et al. (2014) are considered relevant in this regard; see Discussion section below.

The observations of altered reproductive development after genistein exposure support findings described in the NTP-CERHR (2010) evaluation, but are not sufficient to derive an HBGV due to the limitations listed for each study. For example, effects on early markers such as AGD are not consistent, and effects on adult endpoints may be caused by adult exposure and the importance of developmental exposure is unclear.

For daidzein, the study by Talsness et al. (2015) is potentially relevant, but due to limitations, we find that it is not sufficient to change the conclusion of the NTP-CERHR (2010) that data on daidzein are insufficient for evaluation of reproductive effects in laboratory animals; see Discussion section below.

Discussion

Intake scenarios for children and pregnant women from Nordic countries with high intake of soy products

It was decided to use the Danish National Survey on Diet and Physical Activity as basis for the intake scenarios, as suitable data from the other Nordic countries were not available or it would be too labour intensive to calculate intake scenarios on dietary surveys from more than one country. Thus, in this report the calculation of intake of food is based on Danish 7-day food records.

The use of 7-day food records is considered a strength for describing the dietary intake. However, the intake of soy-based products depends upon how precise the dietary method measures the intake of soy containing products, and which data are available on content of soy in these products. The availability of information on soy-based products in Denmark is rather sparse and only few products have been analysed chemically. Knowledge on the Danish market for plant-based milk and meat alternatives was used for constructing the intake scenarios. In addition, information from Sweden on consumption of plant-based milk and meat alternatives from the recent dietary survey among youth (Riksmaten Ungdom) was used to complement the Danish data.

Therefore, the calculation of soy intake in the population is based on interpretation of available data and construction of products on basis of their ingredients.

The food intake is based on data collected in 2011–13, which must be considered a limitation in respect to being up to date. The market for plant-based products have increased since 2013, and the intake of soy-based products might be higher in 2020 than 2013. However, the market also experiences a trend towards products based on plant proteins from local crops like peas, lupine, fava beans, oats, etc. away from soy-containing products. Despite a possible increase in plant-based products, the consumption is still considered low. In a newly conducted nationwide survey on approximately 1300 Danish adults (18–65 years), very few individuals consume e.g. plant-based cheese alternatives and the intake of plant-based milk or meat alternatives is low compared to their conventional counterparts. The intake is higher among women and the younger part of the population (unpublished data, DTU Food (2020)).

Too few pregnant women participated in the dietary survey and therefore a proxy for pregnant women has been women 18–45 years old. This introduces a minor uncertainty in the data. The nutritional intake of the 18–45-year-old women was not compared to recommendations for pregnant women, which might underestimate the potential gap between actual and recommended intake for this group. The nutritional calculations are based on dietary intake of Danish women, and as pregnant women in Denmark are recommended to take dietary supplements (folic acid, vitamin D and iron), the nutritional intake calculations should have included the nutrient intake from dietary supplements. The resources for the analysis were not sufficient for the inclusion of dietary supplements.

The substitution of conventional products of animal origin or products based on pulses with soy-based products was done where it was considered plausible. It must, however, be emphasized that the substitution, and thus the intake scenario, is theoretical and introduces some uncertainty. The intake scenarios are primarily based on Danish data and Danish markets shares of plant based products. As fortification is different in the Nordic countries, outcome of the intake scenarios could have changed if e.g. Swedish plant based products were used in the substitution. In Denmark, fortification of foods is voluntary and especially in case of plant based milk alternatives, many products are organic and thus not fortified. In Sweden, plant based milk alternatives are compulsory fortified with vitamin D.

When comparing the nutritional intake of the normal diet (diet A) with the diet with substitution (diet B), many of the results are expected due to substituting animal-based products with soy-based products. One result was, however, unexpected, as diet B had a bit higher content of iron than diet A. The higher content of iron may be due to the fact that many of the soy-based products used for substitution were constructed. In many of the recipes for these constructed products, soy flour was used as a source of soy. Comparison of the content of iron in soy flour, beef and pork shows a higher amount of iron per 100 g in soy flour (Table 18), thus making it plausible with a higher content of iron in diet B (see Table 4).

Table 18. Iron content of soy flour, beef and pork.

Food item	Iron, mg/100 g
Soy flour.	4.0
Beef, unspecified medium fat.	2.2
Pork, minced, 10-15% fat.	0.9

Comparison with other studies

Comparison with other studies with similar age groups as in our study is however very limited. It has not been possible to find comparable studies with soy intake in Western diets. However, two Japanese studies can be used for comparison. The intake of soy in diet B (with substitution) among children 4–10 years of age are 42 g per day among boys and 37 g per day among girls. In a study by Wada et al. (2011), children aged 3–6 years have a daily intake of soy on 25 g among boys and 23 g among girls. Due to the younger Japanese age group, it is expected that the intake is lower than among the Danish children (age 4–10 years). The soy intake of the children seems comparable. Among adults, the daily intake of soy in a study of pregnant Japanese women shows an intake of 48 g (Miyake et al, 2005). An intake of 27 g soy per day in the Danish substituted diet among women represent a high intake of soy but the intake is expected to be lower than among women in Japan, taking into consideration that dietary habits in Asia (Japan) and West (Denmark) are quite different.

Use of human data for health-based guidance value determination

No critical effect of isoflavones on children or pregnant women (unborn children) was identified among the four included endpoints: timing of puberty, breast cancer, hypospadias and thyroid function in human studies.

With regard to timing of puberty, three out of 11 studies found adverse associations between exposure to dietary soy or isoflavones and age of puberty for girls (Marks et al., 2017, Kim et al., 2011, Yum et al., 2013). However, two of these studies are case-control studies in Asian populations with limited adjustments for confounders (Kim et al., 2011, Yum et al., 2013) and are therefore not considered suitable for HBGV determination for a Nordic population. The third study by Marks et al. (2017) is a nested case-control study and the only study investigating the association between prenatal exposure of isoflavones and age of menarche. However, the only association between isoflavone exposure and risk of earlier age of menarche was found for *O*-DMA (not with genistein, daidzein or equol). For boys, only one of three studies found associations between exposure to dietary isoflavones and age of puberty (Segovia-Siapco et al., 2018). However, earlier age of puberty in boys is not as clearly associated with adversity as for girls, and the study by Segovia-Siapco et al. (2018) is a cross-sectional study, which cannot be used to infer causal relationships.

No associations were found between early exposure to dietary soy or isoflavones and later risk of breast cancer or hypospadias based on the identified studies. However, in the study by Michikawa et al. (2019) a maternal intake of 5 mg genistein per day, which was considered a low intake, was associated with elevated risk of hypospadias. If a body weight of 70 kg is used as default, this will correspond to an intake of 0.07 mg genistein/kg bw per day. The estimated genistein exposure in the soy-substituted diet of Danish women (aged 18–45 years) is 0.04 - 0.06 mg genistein/kg bw per day. The study by Michikawa et al. (2019) is in a Japanese population and as such presumably not relevant for the majority of the Nordic population.

With regard to effects of isoflavones on thyroid function, the study by Milerová et al. (2006) showed that adverse effects of isoflavones on thyroid function with concomitant insufficient iodine intake cannot be excluded. Iodine deficiency is a problem that all Nordic countries has to deal with and fortification of table salt (Denmark, Finland, Sweden) or cow fodder (Finland, Iceland, Norway) has limited the occurrence of iodine deficiency to a large extent (Nyström et al., 2016). Based on the intake-scenario described in our report, the intake of iodine from a diet substituted with possible soy-containing products (shown in Table 4) is considerably lower for Danish women (-14%), girls (-27%) and boys (-27%) compared to a diet without substitution. However, the intake of iodine from a diet substituted with possible soy-containing products still provides iodine above or close to the Nordic recommendations for intake of iodine (NNR 2012) (Table 5) for Danish women, boys and girls. Nevertheless, according to Nyström et al. (2016), there are indications that pregnant and lactating women are mildly deficient in iodine in several of the Nordic countries.

Use of animal data for health-based guidance value determination

Risk assessments using experimental animal data are based on comparisons of human exposure values with a HBGV derived from NOAELs or LOAELs in an experimental animal study. Alternatively, a comparison of internal doses in humans with effective doses in experimental animals may be carried out. In that case, internal doses may be reflected as blood or organ levels of a substance.

Internal dose evaluation for risk assessment

In the NTP-CERHR (2010) report, blood levels of experimental animals exposed to genistein by dietary exposure in multi-generation studies or by subcutaneous exposure in the neonatal period were compared to blood levels of human infants fed soy formula, and the results indicated that human infant blood levels may exceed the internal doses measured in mice or rats at dose levels causing adverse effects. In addition, when comparing levels of unconjugated genistein it was concluded that human blood levels are similar to levels where adverse effects are seen in rat or mouse studies.

In the current project, focus is on risk assessment for pregnant women (unborn children) and children above the age of 3 years. For this purpose, a comparable approach to that applied by NTP-CERHR (2010) is theoretically possible. In practice, risk assessment of foetal exposure requires information on internal dose of pregnant rats at a dose level causing effects on developing offspring, as well as information on internal dose of a pregnant (or non-pregnant) women on a soy-containing diet. Risk assessment of child exposure requires information on internal dose of infant rats at doses causing health effects, and information on internal dose of children on a soy-containing diet. We do not find sufficient information to determine a HBGV for soy food products as such. Data from the NTP-CERHR (2010) report on internal doses of genistein in infant and adult rats associated with adverse effects could be applied for the purpose of setting a genistein-specific HBGV. These figures could be compared to data on adult or infant blood levels, if available. This requires further comparisons of toxicokinetics between humans and experimental animals and is not within the scope of this report.

External dose comparison for risk assessment

An alternative approach is to derive a HBGV using oral (external) doses for comparison with human dietary intake doses. This would assume that differences between humans and experimental animals are within the uncertainties expected for other chemical risk assessments in general. The derivation of a HBGV can thus be based on a NOAEL or LOAEL in an animal study.

The NTP-CERHR (2010) used effects in a multi-generational study in rats showing developmental effects at 500 ppm of genistein (NTP, 2008) for the internal dose comparison. We consider the same data useful for external dose comparison, and for the purpose of HBGV determination for genistein, 500 ppm can be considered a LOAEL. The middle dose of 100 ppm can be considered a NOAEL. Our literature review has identified one additional study that may also be relevant for HBGV determination. Earlier puberty was identified in mice exposed from weaning onwards at doses of 100 and 500 ppm of genistein (Li et al., 2014) (N=35), thus 100

ppm is considered the LOAEL for this endpoint. Other parts of the same study showed changes in ovary and mammary development in the same dose groups but with a smaller number of animals per age group (N=6–10). In this study, the LOAEL is 100 ppm, and the NOAEL 5 ppm, for early puberty as well as changes in ovary and mammary development. Both studies (NTP, 2008 and Li et al., 2014) are of sufficient quality, and the post-weaning study data from Li et al. (2014) are useful for risk assessment of child exposure mainly. The data from the multi-generation study (NTP, 2008) is applicable for both foetal and child exposure.

For risk assessment of genistein for children, an overall LOAEL of 100 ppm can be set as a conservative choice based on these two studies. Due to a large dose interval in the Li et al. (2014) study, we consider that using this study NOAEL of 5 ppm may be too conservative. Instead, we suggest using 100 ppm as a LOAEL and add an extra uncertainty factor of 3. The Li et al. (2014) study does not inform of intake in mg/kg bw per day, and EFSA guidance on default values is applied to convert from ppm (EFSA, 2012). EFSA suggests using a conversion factor of 0.2 for subacute exposures of mice. This means that 1 mg/kg in feed corresponds to 0.2 mg/kg bw per day. In this study, the LOAEL of 100 ppm is thus estimated to 20 mg/kg bw per day. Using default uncertainty factors of 10 for differences between humans, 10 for differences between species, and 3 for use of LOAEL instead of NOAEL, we derive a HBGV of $20/300$ mg/kg bw per day = 0.067 mg/kg bw per day. Alternatively, the NTP (2008) study could be applied using a NOAEL of 100 ppm corresponding to 8.9 mg/kg bw per day in non-lactating females. Using default uncertainty factors of $10 \times 10 = 100$, this would result in a HBGV of $8.9/100$ mg/kg bw per day = 0.089 mg/kg bw per day. This corresponds well with the HBGV derived from the study by Li et al. (2014). As a conservative choice, the HBGV of **0.067 mg/kg bw per day is suggested for risk assessment for children, as a rounded value of 0.07 mg/kg bw per day of genistein**. This corresponds to 2.1 mg genistein per day for a person weighing 30 kg.

For risk assessment for pregnant women, an exposure dose of 100 ppm genistein in rats from the multi-generation study can be used as a NOAEL. Study data show that this corresponds to 8.9 mg/kg bw per day in non-lactating females. Using default uncertainty factors of $10 \times 10 = 100$, this results in a HBGV of $8.9/100$ mg/kg bw per day = **0.089 mg/kg bw per day which is suggested for risk assessment for pregnant women as a rounded figure of 0.09 mg/kg bw per day of genistein**. This corresponds to 6.3 mg genistein per day for a person weighing 70 kg.

Risk characterization

The estimated LOW and HIGH genistein exposures of Danish women (age 18–45), calculated after substituting possible products with soy-based alternatives is 0.036 and 0.062 mg/kg bw per day, respectively (Table 11). For girls (age 4–10 year) these figures are 0.09 and 0.16, and for boys (age 4–10 year) 0.11 and 0.19 mg/kg bw per day, respectively (Table 11).

The HBGV for genistein suggested for children derived from the mouse study (Li et al., 2014) is 0.07 mg/kg bw per day is suggested for risk assessment for children.

The HBGV for genistein suggested for pregnant women (unborn children) derived from the multi-generation study in rats (NTP, 2008) is 0.09 mg/kg bw per day is suggested for risk assessment for pregnant women.

Under these premises, there is no concern for pregnant women (unborn children) in relation to intake of genistein from a diet with high intake of soy-based products, since both the LOW and HIGH values of total genistein intake were below the HBGV. For girls and boys (age 4–10 year), on the other hand, the HBGV for genistein is slightly exceeded after intake of a diet with either LOW or HIGH intake of soy-based products, indicating a potential health concern for children eating a substantial soy-substituted diet.

Uncertainties

Uncertainties in relation to human exposure calculations

In relation to dietary exposure to soy and certain isoflavones in Denmark

The intake of soy-based products depends upon how precise the dietary method measures the intake of soy containing products and which data are available on content of soy in these products. The availability of information on soy-based products in Denmark is rather sparse and only few products have been analysed chemically. Thus, the calculation of soy intake in the population is based on interpretation of available data and construction of products on basis of their ingredients. The following dietary exposure of soy and isoflavones should thus be interpreted with caution.

In relation to dietary exposure in human studies in general

Many of the human studies estimate isoflavone exposure by food frequency questionnaires (FFQs), which are subjective, and food composition databases, which typically are incomplete. This way of estimating exposure is therefore more prone to uncertainties in relation to exposure than studies using objective biomarkers such as urine or serum/plasma levels. Variability in isoflavone content of food, as shown by Forslund & Andersson (2017), as well as inter-individual genotypes and microflora affecting isoflavone metabolism further add to this uncertainty.

Lack of information on the composition of the individual isoflavones (i.e. genistein, daidzein, glycitein) when total isoflavone exposure is reported or lack of information on the extent of conjugation (aglycone, glucoside etc.) are also contributing to uncertainties in the exposure assessment in these studies.

Uncertainties in relation to human epidemiological studies of adverse effects

Several of the human studies have been conducted in Asian populations (in Asian countries or in USA), including the Chinese study by Li et al. (2014) used in the risk characterization for children. There is some uncertainty as to whether Asian and non-Asian individuals respond similarly to the effects of isoflavones due to differences in e.g. bioavailability and metabolism (e.g. higher prevalence of equol producers among the Asian populations). In addition, Asians generally have a much higher average isoflavone intake throughout their life compared to Western populations and are generally exposed to isoflavones from an early age via the intake of traditional soy foods, whereas exposure in Western populations usually begins later in life.

Only a limited amount of relevant studies was identified assessing isoflavone exposure with adverse effects in pregnant women (unborn children) and children. It is therefore uncertain if all potential adverse effects have been detected, especially for boys.

Uncertainties in relation to animal studies of adverse effects

In our literature search, we omitted studies using only one dose level, and left out most studies on soy protein isolates and other mixed soy-containing diets. It is possible that this could have led to identification of studies relevant for determining whether or not such diets have other adverse effects on the developing animal. However, such studies would be difficult to use for risk assessment due to the lack of dose-response information. Therefore, the decision to omit these studies is not likely to have affected our overall conclusions.

We focused the animal data search on endpoints considered relevant for humans, and for that reason only studies on reproductive and endocrine targets were included. Studies on immune parameters, obesity or bone health may be potentially relevant despite lack of clear human relevance but were not addressed here. However, since there were few data on these endpoints, the decision to omit these studies is not likely to have affected our overall conclusions.

We were able to derive an HBGV for risk assessment of genistein intake, but not for soy products as such. Typically, there is somewhat more genistein/genistin and daidzein/daidzin in soybeans and soy foods, whereas glycitein/glycitin comprises a minor part of the total isoflavone content (Forslund & Andersson, 2017). However, the lack of information about the type of isoflavones and their individual chemical forms (aglycones or glycosides) and amounts in the various soy food products on the market adds to uncertainty of the relevance of our risk assessment.

Knowledge gaps

- Forslund & Andersson (2017) provides a thorough listing of isoflavone content in a high variety of food items. It is however clear, that for several food commodities, more data is needed for gaining knowledge of the variation in isoflavone content (type and amounts) of individual food items.
- There is a need for consumption data on actual soy food intake in the general population, but specifically for small children, pregnant women, vegetarians and vegans.
- There is a need for human studies with well-defined dietary exposure and serum measurement of isoflavones in order to compare animal and human data for risk assessment of isoflavones.
- There is a need for well-designed human studies focusing on prenatal effects of exposure to isoflavones and soy.
- Most human studies have been performed in Asian populations. Studies on Western populations are needed for determining the effect of the differences among these populations concerning metabolism and intake.
- Information about the distribution of *S*-equol producers and non-producers, affecting daidzein metabolism, in the Nordic population is lacking.

Conclusions

We were able to address all aims of the project: 1) explore available data on soy intake, 2) propose intake scenarios for children and pregnant women from Nordic countries with high intake of soy products, 3) estimate the nutritional impact of substituting conventional products of animal origin with soy products and 4) clarify whether HGBVs can be determined for isoflavones for children and women of childbearing age.

- Expertise within dietary intake, nutrition and toxicology in a risk assessment of adverse health effects of soy intake in the Nordic countries has been gathered and the knowledge obtained is presented in this report.
- The available data on soy intake among the general population in Denmark was explored by analysing data from the dietary survey and conducting desk research regarding available soy-based products on the Danish market. Online shopping pages were analysed in order to check available products on the market, and products sold in retail stores were checked by visiting stores. The publication of Forslund & Andersson (2017) was used for estimation of isoflavone intake; although it is clear that more studies on analysis of isoflavone content (types and amounts) of the food items is needed.
- We estimated intake of soy in women and children with high intake of soy in scenarios using substitution of foods that realistically could be substituted by soy-containing products. The results showed that intake of soy is higher in children than in women, due to higher intake of milk products among children. Approximately 60% of the intake of soy is derived from dairy products (milk and cream products) substituted with soy-based drinks and cream among children.
- Only minor changes occurred in the intake of energy, protein, carbohydrates and fats among women and children, when substituting animal-based products and pulses with soy-based varieties, while the intake of various micronutrients changed by substituting. Intake of vitamin A, riboflavin and vitamin B₁₂ was reduced 20% or more among both women and children, while the intake of vitamin E increased more than 20% among women and children. Among children, the intake of calcium and iodine decreased 20% or more when substituting animal-based products with soy-based varieties, while the intake of magnesium increased. This does, however, not change the degree of fulfilling recommended intake levels, as the intake is near or above recommended intake both with and without substitution for most substances, except vitamin D and iron, which is low regardless of substitution or not.
- No HGBVs for soy or isoflavone intake for pregnant women (unborn children) or children (not considering soy formula) were identified from previous risk assessments. Our literature search did not identify new human epidemiological data or experimental animal data that could be used to derive a HBGV for soy intake. Epidemiological studies showed effects on reproductive and endocrine endpoints, and to ensure human relevance, our literature search in experimental animals was focused on these endpoints. For genistein specifically, results from a multi-generational study in rats were used for HBGV derivation for genistein exposure of pregnant women (0.09 mg/kg bw per day corresponding to 6.3 mg

per day for a person weighing 70 kg), and a study on post-weaning exposure of mice was used for HBGV derivation for exposure of children (0.07 mg/kg bw per day corresponding to 2.1 mg per day for a person weighing 30 kg).

The HGBVs for genistein identified in the current project is considerably lower than the HGBVs identified for the total amount of isoflavones by e.g. the Italian and French authorities. This highlights the necessity for consideration of vulnerable sub-groups in the population. Data on low and high genistein intake for children and women with high soy food intake were used for risk assessment. There was no concern for pregnant women (unborn children) in relation to intake of genistein from a diet with either low or high intake of soy-based products, since both values of total genistein intake were below the HBGV established from animal experiments. For girls and boys (age 4–10 years), on the other hand, the HBGV for genistein was slightly exceeded after intake of a diet with either low or high intake of soy-based products, indicating a potential health concern for children eating a substantial soy-substituted diet. Knowledge gaps were identified for intake of soy-based products specifically for small children and pregnant women and for well-conducted human studies with well-defined dietary exposure and serum measurement of isoflavones in order to compare animal and human data for risk assessment of isoflavones.

Glossary

Euthyroid	The state of having normal thyroid gland function.
Goitrogenic	Substances that can affect thyroid function by inhibiting synthesis of thyroid hormones, resulting in enlargement of the gland (goiter).
Menarche	The beginning of the menstrual function.
Pubarche	The beginning of puberty marked by the first growth of pubic hair.

Abbreviations

3 β ,17 α -AED	5-androstene-3 β , 17 α diol
AGD	anogenital distance
APHV	age at peak height velocity
ATO	age at take-off
CH	congenital hypothyroidism
CI	confidence interval
COT	the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DANSDA	Danish National Survey of Diet and Physical Activity 2011–2013
DEHP	diethylhexyl phthalate
DHEA	dehydroepiandrosterone
DTU food	Technical University of Denmark, National Food Institute
FFQ	food frequency questionnaire
fT4	free thyroxine

GIES	general intake estimation system
HBGV	health-based guidance value
LH	luteinizing hormone
LOAEL	lowest observed adverse effect level
NE	niacin equivalents
NNR	Nordic Nutrition Recommendations
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OR	odds ratio
PND	postnatal day
Q	quartile
RE	retinol equivalents
RR	relative risk
T	tertile
T3	triiodothyronine
T4	thyroxine
Tg	thyroglobulin
TPO	thyroid peroxidase
TPOAb	thyroid peroxidase antibody
TSH	thyroid-stimulating hormone
VKM	vitenskapskomiteen for mattrygghet

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Appendix A. Literature searches

Database: PubMed

Date of search: March 1, 2019

Total number of hits: 2826

Restrictions: Searches limited to Title/Abstract.

Search #	Searches	Number of hits
1	(isoflavone*[Title/Abstract] OR soybean*[Title/Abstract] OR soy bean*[Title/Abstract] OR soyabean*[Title/Abstract] OR soy[Title/Abstract] OR soya[Title/Abstract] OR glycine max[Title/Abstract] OR phytoestrogen*[Title/Abstract] OR daidzin[Title/Abstract] OR genistin[Title/Abstract] OR glycitin[Title/Abstract] OR daidzein[Title/Abstract] OR genistein[Title/Abstract] OR glycitein[Title/Abstract] OR secoisolariciresinol[Title/Abstract] OR lariciresinol[Title/Abstract] OR pinoresinol[Title/Abstract] OR coumestrol[Title/Abstract])	66,757
2	(risk*[Title/Abstract] OR safe* OR adverse[Title/Abstract] OR sideeffect*[Title/Abstract] OR hazard*[Title/Abstract] OR harm[Title/Abstract] OR harmful[Title/Abstract] OR negative[Title/Abstract] OR contraindicat*[Title/Abstract] OR toxic*[Title/Abstract] OR effect*[Title/Abstract] OR affect*[Title/Abstract] OR endocrine[Title/Abstract] OR disrupt*[Title/Abstract])	10,087,815
3	(pregnant[Title/Abstract] OR fetus[Title/Abstract] OR foetus[Title/Abstract] OR fetal[Title/Abstract] OR foetal[Title/Abstract] OR prenatal[Title/Abstract] OR perinatal[Title/Abstract] OR postnatal[Title/Abstract] OR gestation*[Title/Abstract] OR child*[Title/Abstract] OR boy*[Title/Abstract] OR girl*[Title/Abstract])	2,010,504
4	#1 AND #2 AND #3	1,924
5	((woman[Title/Abstract] OR women[Title/Abstract])) NOT (menopaus*[Title/Abstract] OR postmenopaus*[Title/Abstract])	1,000,557
6	#1 AND #2 AND #5	1,057
7	#4 OR #6	2,826

Database: Web of Science

Date of search: March 1, 2019

Total number of hits: 3925

Restrictions: None.

Search #	Searches	Number of hits
1	TS= (isoflavone* OR soybean* OR soy bean* OR soyabean* OR soy OR soya OR glycine max OR phytoestrogen* OR daidzin OR genistin OR glycitin OR daidzein OR genistein OR glycitein OR secoisolariciresinol OR lariciresinol OR pinoresinol OR coumestrol)	158,881
2	TS= (risk* OR safe* OR adverse OR sideeffect* OR hazard* OR harm OR harmful OR negative OR contraindicat* OR toxic* OR effect* OR affect* OR endocrine OR disrupt*)	16,753,754
3	TS= (pregnant OR fetus OR foetus OR fetal OR foetal OR prenatal OR perinatal OR postnatal OR gestation* OR child* OR boy* OR girl*)	2,380,550
4	#1 AND #2 AND #3	2,525
5	TS= (woman OR women) NOT TS=(menopaus* OR postmenopaus*)	1,189,312
6	#1 AND #2 AND #5	1,641
7	#4 OR #6	3,925

Database: SciFinder®

Date of search: March 1, 2019

Total number of hits: 326

Restrictions: Patents not included

CAS RN 552-66-9 = daidzin, CAS RN 529-59-9 = genistin, CAS RN 40246-10-4 = glycitin, CAS RN 486-66-8 = daidzein, CAS RN 446-72-0 = genistein, CAS RN 40957-83-3 = glycitein, CAS RN 29388-59-8 = secoisolariciresinol, CAS RN 27003-73-2 = lariciresinol, CAS RN 487-36-5 = pinoresinol, CAS RN 479-13-0 = coumestrol

Search #	Searches	Number of hits
1	CAS RN 552-66-9	2,339
2	CAS RN 529-59-9	2,414
3	CAS RN 40246-10-4	941
4	CAS RN 486-66-8	7,526
5	CAS RN 446-72-0	18,828
6	CAS RN 40957-83-3	1,594
7	CAS RN 29388-59-8	718
8	CAS RN 27003-73-2	490
9	CAS RN 487-36-5	1,492
10	CAS RN 479-13-0	1,238
11	Categorize #1: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	66
12	Categorize #2: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	66
13	Categorize #3: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	10
14	Categorize #4: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	456
15	Categorize #5: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	1,605
16	Categorize #6: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	55
17	Categorize #7:	29

	Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	
18	Categorize #8: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	10
19	Categorize #9: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	15
20	Categorize #10: Category Heading = Biology Category = Substances in adverse effects Index terms = Select all	190
21	Combine #11-20	1,821
22	#21 Refine topic: pregnant	121
23	#21 Refine topic: fetus	71
24	#21 Refine topic: foetus	0
25	#21 Refine topic: fetal	71
26	#21 Refine topic: foetal	71
27	#21 Refine topic: prenatal	39
28	#21 Refine topic: perinatal	25
29	#21 Refine topic: postnatal	101
30	#21 Refine topic: gestation	51
31	#21 Refine topic: child	17
32	#21 Refine topic: boy	3
33	#21 Refine topic: girl	4
34	#21 Refine topic: woman	99
35	#21 Refine topic: women	99
36	Combine #22-35	326

Database: Embase

Date of search: February 22, 2019

Total number of hits: 3445

Restrictions: 1974 to 2019 February 22

Search #	Searches	Number of hits
1	(isoflavone* OR soybean* OR soy bean* OR soyabean* OR soy OR soya OR glycine max OR phytoestrogen* OR daidzin OR genistin OR glycitin OR daidzein OR genistein OR glycitein OR secoisolariciresinol OR lariciresinol OR pinoresinol OR coumestrol).tw,kw.	74,186
2	(risk* OR safe* OR adverse OR sideeffect* OR hazard* OR harm OR harmful OR negative OR contraindicat* OR toxic* OR effect* OR affect* OR endocrine OR disrupt*).tw,kw.	12,549,083
3	(pregnant OR fetus OR foetus OR fetal OR foetal OR prenatal OR perinatal OR postnatal OR gestation* OR child* OR boy* OR girl*).tw,kw.	2,426,775
4	#1 AND #2 AND #3	2,867
5	((woman OR women) NOT (menopaus* OR postmenopaus*)).tw,kw.	1,341,683
6	#1 AND #2 AND #5	1,485
7	#4 OR #6	4,107
8	limit #7 to (conference abstracts or embase)	3,492
9	#8 after removal of duplicates	3,441

About this publication

Soy intake and possible adverse health effects in children and pregnant women (unborn children)

Lea Bredsdorff, Sisse Fagt, Julie Boberg, Kirsten Pilegaard, Anneli Widenfalk and Inger-Lise Steffensen

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