Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

An Example of a Systematic Literature Review

following the Health Canada *Guidance Document for Preparing a Submission for Food Health Claims*

Food Regulatory Issues Division
Market and Industry Services Branch
Agriculture and Agri-Food Canada

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Canada

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Aussi offert en français sous le titre :

Un exemple d'une revue systématique de la littérature
suivant les Lignes directrices pour la préparation d'une demande d'approbation d'allégations santé relatives aux aliments *de Santé Canada*

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Background and How to Use this Resource

About the Food Regulatory Issues Division

The Food Regulatory Issues Division (FRID) in the Market and Industry Services Branch of Agriculture and Agri-Food Canada leads the Health Claims, Novel Foods, and Ingredients initiative under the Growing Forward policy framework. The goal is to foster innovation and competitiveness along the value chain and accelerate the market entry of innovative products in the growing category of foods with added health benefits by helping stakeholders understand and navigate Canada's science-based regulatory system.

Specifically, FRID:

- helps stakeholders determine plans and priorities with respect to health claims, novel foods, and ingredients;
- works with industry, the research community and other stakeholders to develop awareness of the science-based regulatory environment for innovative food products with added health benefits;
- assists stakeholders to prepare complete and comprehensive regulatory submissions in an effort to accelerate the approval process; and
- provides analysis and advice on domestic food policy and regulatory issues that impact investment, innovation and competitiveness based on information on market opportunities and the current state of science.

Health Canada's Guidance Document for Preparing a Submission for Food Health Claims

In March 2009, Health Canada released a *Guidance Document for Preparing a Submission for Food Health Claims* (HC Guidance Document). Its purpose is to ensure that health claims for foods are substantiated in a systematic, comprehensive and transparent manner. Petitioners who are preparing submissions for the use of new health claims on food products are required to follow the format set out in the HC Guidance Document.

The HC Guidance Document contains several sections:

- Section 1 provides background information and Section 2 and outlines submission requirements.
- Sections 3 and 4 help the petitioner to determine the specific health claim that is being sought, by characterizing the food of interest and the health effect of interest, respectively.
- Section 5 helps the petitioner to determine whether the quantity and quality of science available in the literature is adequate to support a food health claim. It leads the petitioner through 13 steps of a systematic literature review process required to filter the available literature. The steps are as follows:

    5.1.1 Step 1. Describe the Search Strategy for Literature Retrieval
    5.1.2 Step 2. Implement the Search Strategy for Literature Retrieval
    5.1.3 Step 3. Develop Inclusion and Exclusion Criteria to Filter the Literature Retrieved
    5.1.4 Step 4. Filter the Literature
    5.1.5 Step 5. Generate Reference Lists of Included and Excluded Studies
    5.1.6 Step 6. Tabulate Studies
    5.1.7 Step 7. Evaluate Study Quality
    5.1.8 Step 8. Tabulate Study Findings per Health Outcome
    5.1.9 Step 9. Assess Causality
    Step 9a. Rate Consistency
    Step 9b. Rate the Strength of the Association
    Step 9c. Discuss the Relationship between the Food Exposure and the Health Effect
    5.1.10 Step 10. Discuss Generalizability of the Data to the Target Population
    5.1.11 Step 11. Discuss the Physiological Meaningfulness of the Effect of the Food Exposure
    5.1.12 Step 12. Discuss the Feasibility of Consuming an Effective Amount of the Food
    5.1.13 Step 13. Make Conclusions

Purpose of this Resource

In 2009–2010, FRID commissioned systematic literature reviews in accordance with the HC Guidance Document to identify research gaps associated with promising food–health relationships. The reviews addressed the first nine steps of Section 5 of the HC Guidance Document (up to step 9b). As a systematic literature review can only be conducted on a clearly defined topic, Sections 3 and 4 of the HC Guidance Document—the characterization of the food and the characterization of the health effect—were also completed.

The experience gained by completing these literature reviews reinforced the technical and scientific nature of the requirements for substantiating health claims as outlined in the HC Guidance Document. Furthermore, different interpretations were possible for completing the steps in the literature review process. FRID concluded that agri-food stakeholders would benefit from a resource that illustrates how to present the findings of a systematic scientific literature review according to the specifications outlined in the HC Guidance Document.

The example selected to illustrate the process was a literature review on soy and cardiovascular disease, conducted by Nutrasource Diagnostics Inc. (NDI) for Agriculture and Agri-Food Canada in March 2010. As the resource is designed to serve as an example of the **process** for completing the tables required in the HC Guidance Document steps, only a subset of the full literature report has been shown. This resource is provided for illustration purposes only; it is not intended to comment on the state of the science or the relationship between soy and cardiovascular disease.

How to Use this Resource

The headings and section numbers of the excerpts in this resource correspond with those of the HC Guidance Document. In the original literature review, each of the 46 publications cited was carried through every step of the process. This resource includes a smaller but representative sample of the detailed tables and narrative, and does not include the original Appendices or the full reference list. This smaller sample adequately demonstrates what is required in the HC Guidance Document while allowing for a reasonable report size. Note, however, that the reported strength of association and consistency of effect still refer to calculations performed using all high quality intervention (n=43) and observational (n=1) publications that met the inclusion criteria at the full-text filtering stage. When information from the original literature review has been omitted, it is indicated by the symbol **(…)** and/or an explanation provided in **red text**. Furthermore, **red text within a box** offers additional information or tips to assist in following the HC Guidance Document.

It is important to note that a systematic literature review such as the example illustrated in this resource is only one part of a food health claim submission. A petitioner would need to complete all other sections of the HC Guidance Document, including steps 9c through 13 of Section 5, as well as provide a full list of references cited in the submission, and identify and fill any information or research gaps. Also note that systematic literature reviews used in a submission need to be current (i.e.completed within six months to one year prior to a submission).

FRID works directly with agri-food stakeholders to help them understand the science-based regulatory environment for innovative food products with added health benefits. FRID can advise stakeholders and sector groups on how to approach their systematic literature review as well as how to prepare complete submission packages. For further information, please contact FRID at:

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It is recommended that petitioners arrange a pre-submission meeting with Health Canada, to ascertain whether additional requirements apply or to clarify requirements on any part of an application for a food health claim submission. FRID can facilitate this type of meeting with Health Canada, or stakeholders can contact Health Canada directly.

Table of Contents*

*Section numbers correspond with those of the Health Canada Guidance Document (HC Guidance Document).

The headings and section numbers in this resource correspond to the numbering system in the HC Guidance Document. This resource shows examples of how to present the findings required in Sections 2, 3 and 4, and the first nine steps of Section 5 (up to step 9b).

Fillable form templates of each of the Tables shown in Sections 2 through 6 of the HC Guidance Document are available on Health Canada's website to make the preparation of submissions easier. See www.hc-sc.gc.ca/fn-an/legislation/guide-ld/subm-prep-soum-eng.php.

2.0 SUBMISSION REQUIREMENTS

2.2 DETAILS PERTAINING TO PROPOSED HEALTH CLAIM

Objective: *To communicate important aspects related to the health claim up front.*

It may be necessary to complete other sections of the HC Guidance Document to clarify some of the details required in Table 2. For example, the minimum effective intake of the food or bioactive substance will be determined at step 9c.

If the food or bioactive of interest is part of a broad category, or is present in different types and categories of products, the table should specify what has been reviewed. The proposed health claim should be as specific and detailed as possible as to the food, the dose, the effect, and the population if applicable. Note also that qualifying criteria are usually set to ensure that foods with high energy density and low nutritive value cannot carry a health claim. The product should reflect Canadian nutrition guidelines.

Table 2. Details pertaining to the proposed health claim

Item	Details	
Food/bioactive substance of interest	Soy protein	
Health outcome of interest	Reduced risk of cardiovascular disease	
Human studies used to support health claim	Intervention Studies X Yes No	Observational Studies X Yes No
Proposed health claim (claim wording)	In addition to exercise and a diet low in total and saturated fat and cholesterol, a diet containing soy protein may reduce the risk of cardiovascular disease.	
Voluntary submission	Yes No X	
Mandatory submission (for a claim that brings food under definition of a drug, or for any claim for use on infant formula)	Yes X No	
Minimum effective intake of the food/bioactive substance to obtain the claimed effect	To be determined	
Proposed daily intake of the food	To be determined	
Proposed qualifying criteria for food to carry a health claim	<3 g fat, <1 g saturated fat, <20 mg cholesterol, <480 mg sodium for individual foods; <720 mg sodium for a main dish; <960 mg sodium for a complete meal. Foods from whole soybeans can make the claim if they do not add any additional fat to what is naturally present in the soybean.	

Target population for the proposed claim	Healthy adults, with a specific emphasis on those with LDL-C levels >3.5 mmol/L (Genest et al., 2009)
Rationale for the target population	The Canadian Cardiovascular Society recently released revised guidelines for the diagnosis and treatment of dyslipidemia and prevention of CVD in adults. According to these guidelines, individuals with a Framingham Risk Score of 10%–19% and LDL-C levels >3.5 mmol/L are at a moderate risk level for developing CVD (Genest et al., 2009). Given that the Heart and Stroke Foundation of Ontario (2010) reports that nearly 40% of Canadians are classified as having high blood cholesterol levels, the effect of soy protein consumption in this specific population may be particularly beneficial.
Potential adverse effects related to food intake (from human studies)	Individuals with or at risk of an allergy to soy
Proposed restrictions on use of food (e.g. a subgroup of population, mode of consumption of food)	Those with an allergy to soy
Proposed risk management strategies to address adverse effects and/or restrictions on use of food (e.g. indicate wording of recommended warning statements)	Products containing soy or soy-based foods should include an allergen warning on the label.

2.3 Regulatory Status of The Health Claim in Other Jurisdictions

***Objective**: To understand the regulatory status of the health claim in other jurisdictions in addition to the claim wording and conditions for use of approved claims.*

It is important to ensure that any claims mentioned in this section are relevant to the food–health relationship suggested in the submission. Any relevant claims in other jurisdictions should be included, whether they are approved, pending, under review, rejected or withdrawn.

Table 3. Regulatory status of the health claim in other jurisdictions[1]

Country	Regulatory Body	Date of Submission	Status of Health Claim Application	Details for Approved Claims		
				Wording of approved claim	Conditions for use of the claim	Date of claim authorization
Brazil[2]	National Health Surveillance Agency	N/A	Approved	Daily consumption of at least 25 g of soy protein, as part of a diet low in saturated fat could help cholesterol reduction. Its consumption should be associated with a balanced diet and a healthy lifestyle.	N/A	2005

(…)

Table 3. Regulatory status of the health claim in other jurisdictions[1] (cont'd)						
Country	**Regulatory Body**	**Date of Submission**	**Status of Health Claim Application**	**Details for Approved Claims**		
				Wording of approved claim	**Conditions for use of the claim**	**Date of claim authorization**
Japan[4]	Ministry of Health, Labour and Welfare	N/A	Approved	This product contains isolated soya protein, which helps to decrease serum cholesterol level. It is designed to provide easy intake of soya protein, and it is helpful in improving the diets of those who like meat, but who are concerned about cholesterol.	Use of nutritionally appropriate ingredients (e.g. no excessive use of salt)	1996
(…)						
United States of America[8]	Food and Drug Administration	November 1998	Approved	25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of (name of food) provides ____ grams of soy protein.	≥6.25 g soy protein <3 g fat, <1 g saturated fat, <20 mg cholesterol, <480 mg sodium for individual foods <720 mg sodium for main dish, <960 mg sodium for complete meal Foods from whole soybeans can make the claim if they do not add any additional fat to what is naturally present in the soybean.	October 26, 1999

N/A: indicates that the information could not be found on the country's respective government websites or documentation available online and in English.
[1]Information regarding health claims in other jurisdictions was taken from Jew et al. (2008) and Xiao (2008); [2] National Health Surveillance Agency (2009); **(…)** [4]Ministry of Health, Labour and Welfare (2006); **(…)** [8]FDA (2009)

3.0 CHARACTERIZATION OF THE FOOD

Objective: *To understand the composition and manufacturing of the food/bioactive substance and to ensure it meets the quality standards and pre-defined specifications.*

This is an important section because of the need to clearly define what is being investigated—the whole food, a fraction or isolate, or a class of foods. Each choice has consequences as to how many publications will be available after the filtering process. Clearly defining the food constituent will make it easier to determine (in Section 5.1.3 step 3) whether the treatment in a particular publication conforms to this characterization.

Information required in this section is outlined in Table 4 of the HC Guidance Document. Information should be provided for each row that is relevant to the food / bioactive / component being investigated.

THE SOYBEAN

The **soybean** (*Glycine max*) is the food investigated in this systematic scientific literature review. The soybean is comprised of fleshy cotyledons (the part of the seed that forms the first plant leaves), the hypocotyl (the part of the axis of the plant embryo below the cotyledon) and the hull (the dry outer covering of the seed) (Price and Fenwick, 1985). It has been reported that soy protein comprises approximately 38% of the whole soybean (Torres et al., 2006). Other compounds of interest occurring in smaller concentrations in the soybean include phytoestrogens, tannins, saponins, and fibre (Rochfort and Panozzo, 2007). Epidemiological studies investigating the health effects of soy have identified lower incidences of cardiovascular disease among Asian populations who commonly consume soy (Adlercreutz et al., 1990). Clinical research has also provided evidence that consumption of soy protein, in place of animal protein, confers cardiovascular benefits (Anderson et al., 1995).

SOY PROTEIN

Soy protein has exceptional protein quality compared to other vegetable sources. After correcting for digestibility, protein from soybeans provides amino acids equal to or in excess of requirements. Protein from soybeans is also able to meet the proteins needs of infants and adults when consumed as the sole protein source at recommended levels of protein intake (Young, 1991). As such, soy protein is considered to be a “complete” protein as it provides all amino acids in sufficient quantities to maintain health. Studies in adult humans have shown that it has a high nutritional value, similar to that of egg and fish protein (Young, 1991). Animal studies have indicated that the amino acid profile of soy protein may contribute to reduced insulin:glucagon ratios, which affects the activity of transcription factors involved in the regulation of lipid metabolism. Soy protein has less lysine and more arginine than animal protein and it is thought that modulation of this amino acid ratio may contribute to some of the cardioprotective effects of soy protein (Torres et al. 2006). More recent research provides evidence that specific peptide products from soybeans may also contribute to the health effects of soy protein (Duranti et al., 2004; Manzoni et al., 2003).

Soy protein can be supplemented into the diet from different sources. These sources include whole soybeans, textured vegetable protein, soy protein concentrate and soy protein isolate. Each differs in its protein concentration as well as the content of other soy components (e.g. isoflavones, fibre) (Erickson et al., 1995).

Whole Soybeans

Whole soybean-based foods administered in clinical trials are derived from all components of the bean and include, but are not limited to: soy beverage, tofu, miso, soy nuts, soy cheese, tempeh, edamame and natto. Whole soybeans contain approximately 38% protein, while soy flour contains about 50% protein (Erickson et al., 1995; Soy 20/20, 2008).

Textured Vegetable Protein

Textured vegetable protein is made from defatted soybean flour, which is a by-product of oil extrusion. It contains 50% soy protein and is rehydrated prior to use (Erickson et al., 1995).

Soy Protein Concentrate

Soy protein concentrate contains about 70% protein and is made by removal of water soluble carbohydrate from dehulled and defatted soybeans. The soy globulins (the dominant storage proteins in soybeans, which account for 50%–90% of the total proteins (Zhang et al., 2007)) are immobilized while soluble carbohydrates, soy whey proteins and salts are leached from defatted soy flakes or flour. The protein can be retained by one of several treatments: leaching with 20%–80% aqueous alcohol (alcohol washed), leaching with aqueous acids (acid-washed) or leaching with chilled water or hot water (water washed) for heat-treated defatted soy meal/flour. This water washing process also retains most of the fibre of the original soybean (Erickson et al., 1995).

1. ***Water washed –*** protein is extracted with water (hot or chilled), retaining the isoflavones.
2. ***Ethanol washed –*** protein is extracted with alcohol and the isoflavones are lost. Other components associated with soy protein (e.g. saponins, phytic acid, and other alcohol-extractable phytochemicals) may also be removed during this processing technique (Dewell et al., 2006).
3. ***Acid-washed –*** defatted soybeans are treated at pH 4.2 to solubilise the sugars and cause the protein to coagulate (i.e. become insoluble).

Soy Protein Isolate

Soy protein isolate is a highly purified form of soy protein with a minimum protein content of 90% on a moisture-free basis. It is made from defatted soy flour that has had most of the non-protein components (e.g. fats and carbohydrates) removed. The aqueous extraction is carried out at pH <9. The extract is then clarified to remove any insoluble material and the supernatant is acidified to pH 4–5. The precipitated protein curds are then collected and further separated by centrifugation. The curd is usually neutralized prior to drying in order to facilitate solubility in water (Erickson et al., 1995).

There is growing evidence that specific soy protein products contribute to the cardio-beneficial effects of soy protein. The majority of soy protein is comprised of two types of storage globulins which are categorized based on their sedimentation rate: 11S and 7S (Ortiz et al., 2003). The ratio of 11S to 7S globulins in soybeans varies among cultivars and is about 0.5–1.7 (Utsumi et al., 1997; Wright, 1987). The 7S globulins of soybeans are classified into three major fractions with differing physicochemical properties. β-conglycitin is the most common type (accounting for 30%–50% of total seed proteins) and is a glycoprotein present as a trimer, with molecular mass of 150–200 kDa (Peng et al., 1984). The three subunits of the 7S globulins are: α, α’ and β, with the relative percentages of each being 34%, 35% and 20%, respectively (Maruyama et al., 1999). Glycitin is an 11S globulin with a hexamer structure and molecular mass of 300–380 kDa (Peng et al., 1984). Each subunit of glycitin is composed of an acidic polypeptide (A) with molecular mass of 35 kDa and a basic polypeptide (B) with molecular mass of 20 kDa. It is thought that these globulins, particularly the 7S globulins, may be responsible for the

cardioprotective effects of soy protein. Animal studies have indicated that the α' subunit of the 7S globulin lowers plasma lipids (Duranti et al., 2004), possibly through up-regulation of the LDL (Manzoni et al., 2003) and VLDL receptors (Duranti et al., 2004). The molecular conformation of soy globulins is dependent on pH and optimum functionality occurs at pH<5 (Utsumi et al., 1997). This may limit their applications in food products. More research into the biological effects of these globulins and their application in food is required before conclusions can be made regarding their health effects when supplemented in the diet through foods.

Soy protein is found inherently within the soybean and other soy products, and has been identified as a quantifiable bioactive substance that may be responsible for the reported biological effects associated with soy consumption. Therefore, for the purposes of this review, the soybean and other soy products will be characterized as *foods containing an inherent bioactive substance* (i.e. soy protein).

OTHER COMPONENTS OF SOYBEANS/SOY FOODS

As previously mentioned, soy foods are not a comprised of single chemical substance, but rather a collection of compounds found within the soybean. While the majority of soy research to date has been completed with isoflavones and proteins, a variety of other potentially biologically active compounds occurring in smaller concentrations in the soybean has also been studied, although less extensively. These compounds include protease inhibitors, soy lecithin, soy fibre, peptides, saponins, phytic acid, phytosterols, lignans, vitamins, minerals and other nutrients (Erdman, 2000; Klein et al., 2010). The type and proportion of these bioactive constituents vary depending upon plant genetics, growing and harvesting conditions, storage conditions, plant part used, and processing and extraction methods (Erdman, 2000; Klein et al., 2010). A variety of health benefits has been attributed to the ingestion of these soy-derived compounds, both alone and in combination. For example, there is increasing evidence that the biologically active compounds in soy listed below may provide cardiovascular benefits by helping to lower blood cholesterol levels.

Trypsin Inhibitors

While trypsin inhibitors are ubiquitous in foods, their activity is often destroyed in soy products due to the heat-treatment process (Erdman, 2000). However, hypercholesterolemic effects have been observed with small amounts of the heat-stable Boman-Birk inhibitor that acts by increasing the secretion of cholecystokinin. Bile acid synthesis from cholesterol is stimulated as a result, helping to eliminate cholesterol through the gastrointestinal tract (Erdman, 2000). While little research in humans on this topic has been investigated to date, animal studies have not demonstrated a hypercholesterolemic effect when trypsin inhibitor was added to the diet (Roy, 1981).

Phytic Acid

Phytic acid is found in all non-fermented soy protein products and is very stable during heating (Erdman, 2000). Phytic acid chelates zinc strongly in the intestinal tract, thus decreasing its absorption. This is an important characteristic, as a copper deficiency or a high ratio of zinc to copper results in a rise in blood cholesterol (Klevay, 1975; Zhou and Erdman, 1995). Therefore, it has been hypothesized that soy foods (which contain both copper and phytic acid) may lower cholesterol levels by decreasing the ratio of zinc to copper (Erdman, 2000). Further research in this area is necessary.

Fibre

Fibre has also been identified as one of the bioactive ingredients in various soy protein preparations. One publication reported the cholesterol-lowering effect of soy fibre in humans with hypercholesterolemia (Shorey et al., 1985). Another showed a reduction in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) as a result of the addition of soy cotyledon fibre to the diets

of hypercholesterolemic individuals (Lo, 1990). Conversely, two other publications did not find an increase in the cholesterol-lowering ability of soy protein when soy fibre was added to the diets of mildly hypercholesterolemic men (Bakhit et al., 1994; Potter et al., 1993). Results of these latter two studies indicate that soy protein plus soy fibre was no more effective than soy protein plus cellulose (an insoluble fibre that typically does not affect lipidemia) in lowering blood lipids in mildly hypercholesterolemic men (Bakhit et al., 1994; Potter et al., 1993). Furthermore, several studies have demonstrated the ability of isolated soy protein which contains virtually no soy fibre to significantly reduce TC and LDL-C (Anderson et al., 1995; Carroll, 1991). Thus, the fibre component of soybeans does not appear to be a major factor in the cholesterol-lowering effects of soy protein (Potter, 1995).

Isoflavones

Soy is the major food source of isoflavones, a subclass of the more ubiquitous flavonoids (Erdman, 2000). Isoflavones are phytoestrogens that are bioactive in humans and are widely present in all soy flours, concentrates and isolates produced by a water extraction process. Genistein, daidzein, and glycitein are the three hydrolyzed forms of isoflavones (aglycones) (Erdman, 2000).

Due to their structural similarities to mammalian estrogens and their ability to bind with estrogen receptors, it has been postulated that isoflavones may be responsible for the effects of soy protein on blood lipids (Kuiper et al., 1997). Extensive research evaluating the hypocholesterolemic effects of soy isoflavones have found that soy protein containing isoflavones lowered cholesterol significantly more than soy protein without isoflavones in humans (Cassidy et al., 1995; Crouse et al., 1999; Pelletier et al., 1995). However, while some have concluded that the cholesterol-lowering effect of soy protein was entirely attributable to isoflavones (Crouse et al., 1999), others have found no changes in plasma lipid levels in women consuming extracted soy isoflavones (without soy protein) (Nestel et al., 1997). These results suggest that some synergy between soy protein and isoflavones is necessary to reduce levels of cholesterol.

Further research into the mechanism(s) by which the aforementioned biologically active components in soy may be exerting its/their beneficial effects is warranted.

Soy protein can be supplemented into the diet from different sources, such as whole soybeans, textured vegetable protein, soy protein concentrate and soy protein isolate. Each differs in its protein concentration as well as the content of other soy components (e.g. isoflavones, fibre). It is also important to note that even when whole soy foods were used as a treatment in an intervention, the soy protein content of the whole soy food was always quantified and highlighted to be primarily responsible for the metabolic effects associated with the soy food consumption.

DIETARY INTAKE ESTIMATES

Actual consumption data is better than data on food disappearance or food availability by commodity. Food disappearance data are based on production, imports and exports and represent all food available for consumption. However, actual intake is much lower because of wastage, spoilage, and non-food uses. Statistics Canada's Food Stats (2009) and Canadian Community Health Survey Cycle 2.2 are useful sources of information on food consumption data.

It has been estimated that Canadians and Americans consume <1 g soy protein daily. In comparison, an intake of approximately 55 g/day soy protein is common in Japan (Messina et al., 1994; Nagata et al., 1998). It is estimated that the daily intake of soy protein in Hong Kong is 7 g, while 8 g is consumed in China daily (Ho et al., 2000; Lee, 2006; Nagata, 2000; Soy 20/20, 2008).

The **Canadian Soy Use and Attitude Study** was a survey developed, conducted and analyzed by BrandTrust on behalf of Soy 20/20. This online survey, which questioned a representative sample of 1008 Canadian adults, found that 8.3% of adults consumed soy or soy beverages on a daily basis. This survey also found that 10.9% of Canadian adults consume soy or soy beverages at least once a week and 6.3% consume soy or soy beverages at least once a month. Overall, this equates to 25% of Canadian adults consuming soy or soy beverages at least once per month. Additionally, this survey highlighted that 71% of Canadian adults rate soy products as healthy and 36% of consumers are interested in combinations of soy and conventional foods (Soy 20/20, 2008). Another factsheet published by Soy 20/20 identified that overall, Canadians are increasing their consumption of soy protein (e.g. textured soy protein, soy protein concentrate, soy protein isolate) (Soy 20/20, 2004). According to this report, Canadians in general consumed 0.68 g of soy protein per day in 2001, compared to U.S. consumption of 0.32 g/day (Soy 20/20, 2004).

Traditional Asian diets include soy in the form of soybeans, soybean sprouts, toasted soy protein flours, soy beverages tofu, and fermented soybean products such as miso, tempeh, soybean paste, natto and soy sauce (Coward et al., 1993; Wang and Murphy, 1996). However, most soy foods consumed in Western countries are in the form of processed soybean protein products, such as flours, grits, isolates, concentrates and textured soy proteins (Wang and Murphy, 1996). This may explain why the majority of clinical research tends to focus on the effect of soy protein products rather than traditional soy foods. Soy protein is found naturally in products such as tofu and soy beverages and is also added to food products to improve protein quality (Soy 20/20, 2008). Processing techniques used to derive soy protein from the soybean may, however, negatively affect the total isoflavone content within the extracted protein.

Canada's Food Guide recommends 2 to 3 servings of Meat and Alternatives be consumed each day, with a specific emphasis placed on meat alternatives such as beans, lentils and tofu. Tofu is an example of a soy food product made from the whole soybean. Please refer to **Supplementary Table S1** for the nutrient profiles (calories and levels of macronutrients and micronutrients) of numerous soy products that Canadians may consume (Health Canada, 2009).

When completing a systematic literature review, nutrient profile data should be included for any type of food that relates to the food of interest for the submission. Nutrition composition data from the Canadian Nutrient File (www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php) is preferred. U.S. data can be used in some instances if they are similar to Canadian data; the petitioner must be able to show how the data can be applied to the Canadian population.

Supplementary Table S1 provides an example of how to portray the nutrient profile for one soy product, using data obtained from the Canadian Nutrient File. The full systematic literature review from which this example was derived also presented the nutrient profile for enriched soy beverages, soybean cheese, soy protein concentrate, soy protein isolate, edamame, miso, dry boiled soybeans, dry roasted soybeans, tempeh and tofu.

Supplementary Table S1. Nutrient profile by product
Canadian Nutrient File (CNF)
a: Beverage, soy, fluid, unenriched Food Code: 5241

Nutrient name	Unit	Value per 100 g of edible portion	175 ml / 259 g (CFG) 0.5 FG Serving
Proximates			
Moisture	g	88.03	227.89
Ash	g	0.6	1.7
Protein	g	4.48	11.60
Total Fat	g	1.92	4.97
Carbohydrate	g	4.93	12.76
Alcohol	g	0.0	0.0
Energy (kcal)	kCal	52	135
Energy (kJ)	kJ	217	562
Other Carbohydrates			
Fibre, total dietary	g	0.5	1.3
Sugars, total	g	0.50	1.29
Minerals			
Calcium, Ca	mg	38	98
Iron, Fe	mg	1.10	2.85
Magnesium, Mg	mg	25	65
Phosphorus, P	mg	55	142
Potassium, K	mg	124	321
Sodium, Na	mg	55	142
Zinc, Zn	mg	0.44	1.14
Copper, Cu	mg	0.141	0.365
Manganese, Mn	mg	0.218	0.564
Selenium, Se	µg	4.8	12.4
Vitamins			
Beta carotene	µg	367	950
Alpha carotene	µg	0	0
Retinol	µg	0	0
Retinol activity	µg	31	80
Folacin, total	µg	16	41
Folic acid, synthetic form	µg	0	0
Folate, naturally	µg	16	41
Dietary folate	µg	16	41
Niacin	mg	0.289	0.748
Niacin equivalents	NE	1.122	2.905
Pantothenic acid	mg	0.518	1.341
Riboflavin	mg	0.051	0.132
Thiamin	mg	0.061	0.158
Vitamin B-6	mg	0.096	0.249
Vitamin B-12	µg	1.22	3.16
Vitamin C	mg	0.0	0.0
Vitamin D	µg	0.000	0.000
Vitamin K	µg	3.0	7.8
Tocopherol, alpha	mg	1	3

Amino Acids			
Tryptophan	g	0.050	0.129
Threonine	g	0.141	0.365
Isoleucine	g	0.149	0.386
Leucine	g	0.243	0.629
Lysine	g	0.172	0.445
Methionine	g	0.036	0.093
Cystine	g	0.000	0.000
Phenylalanine	g	0.148	0.383
Tyrosine	g	0.116	0.300
Valine	g	0.153	0.396
Arginine	g	0.245	0.634
Histidine	g	0.080	0.207
Alanine	g	0.136	0.352
Aspartic acid	g	0.377	0.976
Glutamic acid	g	0.637	1.649
Glycine	g	0.134	0.347
Proline	g	0.193	0.500
Serine	g	0.184	0.476
Lipids			
Fatty acids, saturated,	g	0.233	0.603
16:0	g	0.174	0.450
18:0	g	0.058	0.150
Fatty acids,	g	0.383	0.991
17:1	g	0.012	0.031
18:1	g	0.360	0.932
20:1	g	0.012	0.031
Fatty acids,	g	0.766	1.983
18:2	g	0.679	1.758
18:3	g	0.087	0.225
Cholesterol	mg	0	0
Other components			
Caffeine	mg	0	0
Theobromine	mg	0	0
Lutein and zeaxanthin	µg	0	0
Lycopene	µg	0	0
Beta cryptozanthin	µg	0	0

Canadian Nutrient File, 2007
(CFG) - Refers to the serving size based on *Eating Well with Canada's Food Guide*

Note: Nutrient profiles of additional foods presented in the full systematic literature review have been omitted to allow for reasonable report size.

QUANTIFYING SOY PROTEIN

Factors Affecting the Protein Content and Structure of Soyfoods

Protein can be found in the seed coat and aleurone layers of the mature soybean and the amount of protein is determined by genetic and environmental factors (Dhaubhadel et al., 2005; Luedders, 1977). For example, a study investigating the effects of 12 cultivars of soybeans grown in 4 locations varying in altitude, temperature and precipitation found that the same cultivar grown in one location had significantly different protein concentrations when grown in another location (Maestri et al., 1998). The protein contents of the soybeans ranged from 377–436 g/kg in one location and 329–375 g/kg in another location. Latitude was inversely correlated with protein content ($r=-0.617$, $p=0.01$) while altitude was positively correlated with soy protein ($r=0.795$, $p=0.01$). Precipitation was also inversely correlated with soy protein content of the beans ($r=-0.476$, $p=0.01$) (Maestri et al., 1998).

Different processing techniques can also affect soy protein content and structure. For example, heating at high temperatures can denature soy proteins as the globular structure opens up and the resultant long-chain proteins form insoluble aggregates (Arrese et al. 1991; Petrucelli and Anon, 1995). This denaturation may affect the biological activity of the proteins, as ultra-heat treatment of soy beverage has been shown to destroy the cholesterol-lowering effect of soy protein (Hoie et al., 2006). Furthermore, storage of soybeans at 84% relative humidity and 30^{o}C induced significant changes in protein structure, decreasing surface hydrophobicity and increasing in intramolecular disulfide bonds in both 7S and 11S globulins (Hou and Chang, 2004a & 2004b). A significant increase in α-helix content and decrease in β-sheet content were also found in the secondary structure (Hou and Chang, 2004a & 2004b). The impact of these changes on bioavailability and bioactivity of these proteins is still unknown.

A study completed in soy beverage and tofu production found that different processing methods and soybean cultivars resulted in different 11S:7S ratios in the finished product. It was concluded that a standardized processing method should be put in place to prevent variation in globulin content ratios between laboratories (Cai and Chang, 1999).

Methods for Measuring Soy Protein and Globulin Content

Extraction methods: Soybean protein extraction is completed using a number of solvents. To extract soy protein from defatted soybean flour or one of its products (e.g. TVP, soy protein concentrate and soy protein isolate) it is treated with Tris/HCl at pH 8.5 containing 200 mmol/L of NaCl and 0.02% 2-mercaptoethanol (Hill and Breidenbach, 1974).

To extract the soy 7S and 11S globulins, the protein extract resulting from the above process is treated with ammonium sulfate in order to “salt out” the globulins at their isoelectric precipitation point (Thanh et al., 1975).

Quantification methods: Soy proteins are typically quantified using the Bradford Method (Bradford, 1976). This method uses colorimetric protein assay based on absorbance shifts in Coomassie dye. When the dye is added to the solution it is red in colour. After binding to a protein, the dye changes and stabilizes to a blue colour. The amount of blue present in solution is then used as a measure of the protein concentration by use of an absorbance reading. The bound form of the dye (blue) has an absorption spectrum maximum at 595 nm and the increase in absorbance at 595 nm is proportional to the amount of bound dye, and thus to the concentration of protein in the solution (Bradford, 1976). Soy protein content can also be estimated using the Kjeldahl method (Maesteri et al., 1998). However, this method is not favoured as it gives a quantification of nitrogen content that is then used to estimate protein content.

One method of 7S and 11S globulin quantification was completed with soy beverage products (Gardner et al., 2007). In this study, the endothermic protein compositions of the soy beverages were compared to a standard soy isolate with known peaks for 7S and 11S globulins using differential scanning composition densitometry. The patterns of protein bands were identified using polyacrylamide gel electrophoresis (PAGE) and quantified using scanning densitometric techniques (SDS). Another study used two-dimensional electrophoresis (2-DE) to compare the globulin contents of soy flour extract and soy protein concentrate (Giannazza et al., 2003). Proteins were separated based on charge on a nonlinear pH 4–10 immobilized pH gradient in the presence of urea and then according to size by SDS-PAGE, similar to the previously mentioned study. Following SDS-PAGE, the proteins were electroblotted onto a nitrocellulose membrane and immunodectection was completed with a polyclonal *anti*-7S antibody. Samples were then prepared for matrix-associated laser desorption ionization (MALDI) and electrospray ionization mass spectronomy (MS) analysis. This final analysis was completed in order to identify specific structures in the globulins which would indicate the presence of certain globulin subunits.

4.0 CHARACTERIZATION OF THE HEALTH EFFECT

Objective: *The purpose of this section is to provide information on the health effect, the validity of biomarkers used, and the relevance of the health effect to the Canadian population.*

The choice of health effect and its relevant biomarker(s) is one of the most important parts of a health claim application. It will strongly influence the wording of the health claim, and dictate the criteria chosen to include or exclude publications from the body of evidence used to support the health claim.

The biomarker(s) must be substantiated as being appropriate and relevant to the health effect, and must have methods of measurement that are valid, reliable and generally accepted.

The health effect under consideration in this systematic scientific literature review is **cardiovascular disease**. Related narrative information regarding a rationale for the selection of biomarkers to be used, data on the prevalence of the health effect/its biomarkers in the Canadian population, a rationale on the cause for concern about the health effects/its biomarkers and the methodological and biological validity of the health effect/its biomarkers is also discussed.

Cardiovascular disease (CVD) is a term that refers to more than one disease of the circulatory system including the heart and surrounding blood vessels and those blood vessels affecting the lungs, the brain, kidneys or other parts of the body. Types of CVD include coronary heart disease (CHD), cerebrovascular disease and peripheral arterial disease (PAD) (Mensink et al., 2003). According to the World Health Organization, CVD is the number one cause of death globally, representing 30% of all fatalities (World Health Organization, 2008). The diseases that constitute CVD are among the leading causes of death in Canadian adults (Health Canada, 2009). Most CVD deaths are preventable through lifestyle alterations such as avoidance of cigarette smoking, participation in physical activity and consumption of a nutritionally balanced diet (Anderson and Major, 2002).

The aetiology of CVD is multi-factorial with many potential risk factors. Validated, applicable and generally accepted **biomarkers** of CVD include serum lipid profiles (TC, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels), and moderately high to high blood pressure levels (Mensink et al., 2003). Increased serum TC and LDL-C are well-established risk factors for ischemic heart disease (Garber et al., 1996). LDL-C, however, is considered to have greater specificity over TC as a

predictor of CVD risk (Tardif et al., 2008). Because a reduced level of HDL-C is an independent risk factor for CVD, its assessment in individuals is also highly recommended (Mensink et al., 2003). A high serum TG level may be an independent risk factor for CVD, especially in women (Jacobson et al., 2007), and it is associated with other CVD risk factors, such as obesity and diabetes (Health Canada, 2009). CVD risk can also be assessed by evaluating clinical endpoints such as myocardial ischemia (inadequate flow of blood to the heart), myocardial infarction (heart attack), atherosclerosis (cholesterol–lipid–calcium deposits in arterial linings) or cardiovascular death (Tardif et al., 2006; Thomas, 1997).

According to the Heart and Stroke Foundation of Ontario (2010), almost 40% of Canadian adults can be classified as having high blood cholesterol levels and it is estimated that as many as 10 million Canadian adults have cholesterol levels above the recommended target. This is an increase from the Canadian Heart Health Survey conducted in 1999, which found that 30% of the sample population had a TC level of 6.2 mmol/L or greater (i.e. the cut-off point used to assess risk of coronary heart disease (Canadian Consensus Conference on Cholesterol, 1988)) (Langille et al., 1999). High blood cholesterol levels have been identified as one of the primary risk factors attributable to the significant prevalence of heart disease in Canada (Heart and Stroke Foundation, 2010). The substantial prevalence of elevated cholesterol levels therefore supports the cause for concern regarding heart disease in the Canadian population.

The INTERHEART study (a global case–control study) identified the imbalance between atherogenic and atheroprotective lipoproteins as the most powerful potentially modifiable risk factor for the development of CVD (Yusuf et al., 2004), and the impact of a dietary intervention on biomarkers of CVD may be specifically relevant to the health of the Canadian population. To that end, evidence from clinical literature consistently indicates a link between regular soy consumption and favourable management of serum lipid concentrations, particularly LDL-C (Welty et al., 2007; Xiao et al., 2008).

LDL-CHOLESTEROL

LDL-C, which is comprised of low density lipoprotein and lipid-like cholesterol, is produced when cholesterol is packaged into lipoproteins in the form of cholesterol esters and delivered to tissues of the body that require it (Sharma *et al.*, 2009). Elevated LDL can cause cholesterol deposition in macrophages and smooth muscle cells in the arterial wall, which may promote smooth muscle proliferation, inflammation and calcification, and potentially produce an atherosclerotic plaque (Schaefer, 2000). Research pioneered by Steinberg et al. (1989) identified that *oxidized* LDL specifically plays a significant role in the development of atherosclerotic plaques. The susceptibility of LDL to become oxidized can vary considerably among individuals, and may be influenced by differences in the concentration of vitamin antioxidants, types of fatty acids, free radical contents of LDL, and the size of the LDL particles (Grundy, 1993). Low HDL concentrations, hypertriglyceridemia, hypertension, smoking and type II diabetes mellitus have also been proposed to be risk factors that may affect oxidation of LDL (Grundy, 1993).

Numerous epidemiological studies have shown that the risk of experiencing a cardiovascular event and/or cardiovascular mortality is positively correlated with LDL-C concentrations (Jones, 2004). Additionally, LDL-C and TC have been consistently associated with CHD risk in several clinical investigations (The National Cholesterol Education Program (NCEP) ATP (III), 2001). Furthermore, the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group (1998) and the Scandinavian Simvastatin Survival Study Group (1994) both reported reductions in CVD morbidity and mortality after a reduction in LDL-C concentration. Clinical research has also demonstrated that decreasing serum LDL-C with pharmacological agents can promote atherosclerotic plaque regression (Ballantyne et al., 1997). Overall, there is general consensus in the scientific community that changes in

LDL-C are directly related to changes in CVD risk (Mensink et al., 2003). This consensus is reflected in current risk-prediction instruments (Conroy et al., 2003; Wilson et al., 1998) and guidelines for CHD prevention (De Backer et al., 2003; NCEP ATP (III), 2001) that highlight the use of LDL-C and/or TC as the cornerstone for CHD risk assessment. Additionally, the National Cholesterol Education Program and American Diabetes Association both identify LDL-C as the primary therapeutic target in lipid management (Sharma et al., 2009).

A validated and reliable biomarker is specifically reflective of the presence of an identified clinical health effect and is sensitive to the effect of an intervention (dietary, drugs, surgery, vaccines) on that health effect (Biomarkers Definitions Working Group, 2001). LDL-C is present in the blood of humans at all times, in all physiological states. The level of LDL-C in one's system can vary as a function of environmental variables (such as a dietary intervention), thus providing support for its measurement as a biomarker of disease risk. Also, the chemical and biological stability of LDL-C supports that the accuracy of its measurement is not compromised, so long as plasma samples are properly stored. Additionally, the numerous analytical methods available for measuring LDL-C ensure excellent precision, validity and reproducibility (Mensink et al., 2003). These are all key characteristics of an acceptable measureable biomarker.

TOTAL CHOLESTEROL

Serum TC, a natural and abundant metabolite present in blood, is a measurement of all cholesterol subfractions (including LDL-C and HDL-C) (Anonymous, 2001). The quantitative variation of cholesterol is reflective of a range of metabolic states that are then used to predict CVD risk (Knopp, 1999). Although CVD has a multi-factorial aetiology, it is generally accepted that the absolute concentration of cholesterol in serum is mechanistically associated with its accumulation on artery walls, leading to the formation of atherosclerotic plaques (Mensink et al., 2003). The extensive epidemiological and clinical assessments that have been carried out across populations, cultures and age groups consistently demonstrate that populations with elevated serum cholesterol concentrations are at a greater risk of developing CVD than those with low serum cholesterol concentrations (Mensink et al., 2003). Furthermore, clinical studies have shown that successfully reducing serum cholesterol translates into improvements in disease outcomes, regardless of whether this reduction is achieved through the use of pharmacological agents (Shepherd et al., 1995), nutritional protocols (Mensink et al., 1992) or organ transplants (Cosio et al., 2002).

Similar to LDL-C, TC shows significant responses to environmental variables, such as diet, drugs, health status and exercise (Mensink et al., 2003), thus providing support for its designation as a well-established biomarker of CVD risk. Additionally, the chemical and biological stability of TC ensure excellent precision, validity and reproducibility when measuring concentrations with a variety of established methodologies (Mensink et al., 2003).

HDL-CHOLESTEROL

High-density lipoprotein (HDL) enables lipids (such as cholesterol) to be transported back to the liver. HDL-C is representative of the HDL particle that contains a cholesterol ester (Sharma et al., 2009). It has been reported that HDL-C possesses a multitude of anti-atherogenic actions, including a role in reverse cholesterol transport and cellular cholesterol efflux, anti-inflammatory action, endothelial repair and vasodilatory activity. Additionally, HDL-C may have anti-infectious, anti-oxidative, anti-thrombotic and anti-apoptotic properties (Sharma et al., 2009). Atherogenic dyslipidemia is characterized by a low concentration of HDL-C, elevated TG and elevated small, dense LDL. This dyslipidemia is associated with a high risk of developing CVD (Lamarche et al., 1997; Sharma et al., 2009).

Although the relationship between HDL-C and cardiovascular disease is not entirely clear, the NCEP ATP (III) guidelines (2002) report that low HDL-C is a significant and independent risk factor for coronary heart disease (CHD) and is inversely related to CHD. Several large epidemiological studies also support HDL-C as a validated cardiovascular risk factor, independent of LDL-C (Assmann et al., 1998; Castelli, 1983; Cremer et al., 1997; Kannel, 1983). For example, the Gottingen Risk, Incidence and Prevalence study demonstrated that the risk for myocardial infarction increased five-fold in subjects with moderately elevated LDL-C concentrations (4 mmol/L or 150 mg/dL) but very low HDL-C levels (<0.8 mmol/L or 35 mg/dL) compared to the risk in those with elevated LDL-C and average HDL-C levels (Cremer et al., 1997). Furthermore, the Framingham Offspring Study found a continuous increase in CVD risk from highest to lowest HDL-C levels, especially in males with values below 1.2 mmol/L (45 mg/dL) and in females with values less than 1.4 mmol/L (55 mg/dL) (Kannel, 1983). It has been proposed that every 1 mg/dL (0.0258 mmol/L) increase in HDL-C corresponds to a 2%–3% decrease in the risk of developing coronary heart disease (Gordon et al., 1989).

Similar to LDL-C, HDL-C responds well to environmental stimuli and can be measured with excellent precision, validity and reproducibility using a variety of analytical methods (Mensink et al., 2003). These properties support the use of HDL-C as a biomarker for cardiovascular disease risk. It should be acknowledged, however, that because TC is comprised of approximately 75% LDL-C and only 20% HDL-C (MacLean et al., 1999), changes in LDL-C may more accurately reflect changes in TC in the blood. Changes in LDL-C may therefore be a more specific measure of the risk of developing CVD than changes in HDL-C.

TRIGLYCERIDES

Serum triglyceride/triacylglycerol (TG), fasting or postprandial, can be expressed in terms of its component triacylglycerol-rich lipoproteins derived from either intestinal (chylomicrons, chylomicron remnants) or hepatic (VLDL) sources (Mensink et al., 2003). These lipoproteins are metabolized by lipoprotein lipase in the bloodstream to form atherogenic remnant lipoproteins (Schaefer, 2000), which can directly damage the vascular endothelium, contributing to the formation of atherosclerotic plaques (Sattar et al., 1998). It has been suggested that exceeding the critical threshold of serum TG (1.5 mmol/L) can lead to the formation of small, dense LDL and a low HDL-C level (Griffin et al., 1994). Once bound to the arterial wall, small, dense LDL particles demonstrate relatively greater oxidative susceptibility than larger and lighter LDL subclasses. Oxidized LDL has been proposed to play a significant role in the development of atherosclerotic plaques, and reduced HDL-C may decrease reverse transport of cholesterol out of arterial lesions (Schaefer, 2000).

Elevated serum TG is positively correlated with the development of CHD (Grundy, 1993). Prospective studies, such as PROCAM (Assmann et al., 1996) and the Physician's Health Study (Stampfer et al., 1996), have elucidated a significant and independent association between serum TG and the incidence of major coronary events. Additionally, a meta-analysis performed by Hokanson and Austin (1996) found that elevated serum TG was positively associated with the risk of CHD.

Overall, elevated serum TG is associated with lipoprotein abnormalities, including a low HDL concentration and a prevalence of small, dense atherogenic LDL compounds (Mensink et al., 2003). In addition to its association with a 3-fold increase in young myocardial infarction survivors, this cluster of abnormalities has also been recognized as a common source of lipid-mediated risk in free-living populations (Austin et al., 1988). As a biomarker, TG can be measured with excellent precision, validity and reproducibility using a variety of analytical methods. A disadvantage of the fasting measurement of TG is that it can be confounded by acute fat consumption, which exaggerates the measurement.

However, TG measurement remains a preferred biomarker as its high stability allows for accurate measurement (Mensink et al., 2003).

BLOOD PRESSURE

Hypertension (defined as a measurement consistently ≥140/90 mmHg) is a major health concern in Canadians as it affects approximately 20% of the population (Heart and Stroke Foundation, 2010; Public Health Agency of Canada, 2009). Hypertension is a risk factor for stroke, myocardial infarction, congestive heart failure, kidney failure and peripheral vascular disease. It has been reported that risk of CVD doubles with each 20/10 mmHg increase in blood pressure beginning at 115/75 mmHg (Chobanian et al., 2003).

Hypertension is associated with hypertrophy of the resistance vessels. When intraluminal pressure is chronically elevated, it results in the synthesis of matrix proteins, causing the infiltration of growth factors and cytokinines which are secreted by the endothelium and the smooth muscles (Mensink et al., 2003). Hypertension has also been shown to exacerbate atherosclerosis, possibly due to the stress it exerts on the artery walls, subsequently resulting in proliferation of vascular smooth muscle cells and narrowing of the affected vessel (Mensink et al., 2003). Therefore, effects of hypertension include narrowing of the lumen, increased transport of lipoproteins, oxyradical formation and intravascular coagulation, which contribute to an increased risk for myocardial infarction, stroke and peripheral vascular disease. The effects of hypertension are widespread, affecting both the cerebral circulation (by impairing the tolerance to pressure changes and increasing the risk for transient ischemic attacks and strokes) and the heart (by causing left ventricular pressure overload resulting in left ventricular hypertrophy and ultimately dilatation and heart failure) (Mensink, 2003).

Pharmacological interventions that reduce blood pressure have been shown to modestly decrease the risk of stroke, myocardial infarction and mortality. However, as hypertension remains prevalent, additional treatment options are needed (Burt et al., 1995). For the last decade, hypertension has been the leading diagnosis for adult visits to physicians, and the proportion of total visits to a physician for hypertension is increasing (Hemmelgarn et al., 2008). Increased blood pressure is estimated to consume 10% of health care costs in developed countries like Canada (Gaziano et al., 2009). The World Health Organization has indicated that increased blood pressure is the leading risk for death (predicting an epidemic of hypertension) and is advocating for prevention and treatment programs as a priority (World Health Organization, 2010; Kearney, et al., 2005).

Hypertension is one of the most important modifiable risk factors for cardiovascular disease, and clinical data supports the effect of blood pressure reduction on CVD risk. For example, a review published by Collins et al. (1990) provided an overview of 17 randomized trials of antihypertensive treatment. Overall, these clinical trials demonstrated an average blood pressure reduction of 5–6 mmHg diastolic and 10–14 mmHg systolic was associated with 38% reduction in stroke, 16% reduction in CHD and 21% reduction in total mortality. A meta-analysis of prospective studies found that every 1 mmHg reduction in the mean population systolic blood pressure could prevent approximately 10 000 CHD deaths each year in the United States (Lewington et al., 2002). Additionally, Cook et al. (1995) reported that a 2 mmHg reduction in diastolic blood pressure resulted in a 6% reduction in coronary heart disease and a 15% reduction in stoke. Blood pressure is considered to be a surrogate endpoint for CVD and is accepted by both clinicians and regulators (Desai et al., 2006).

CHD VERSUS CVD

According to the World Health Organization (WHO), cardiovascular diseases are a group of disorders of the heart and blood vessels and include: coronary heart disease (disease of the blood vessels supplying the heart muscle); cerebrovascular disease (disease of the blood vessels supplying the brain); peripheral arterial disease (disease of blood vessels supplying the arms and legs); rheumatic heart disease (damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria); congenital heart disease (malformations of heart structure existing at birth); and deep vein thrombosis and pulmonary embolism (blood clots in the leg veins, which can dislodge and move to the heart and lungs) (World Health Organization, 2008). While this could arguably be considered a global definition, a discrepancy in the use of the term CVD in both the literature and among experts has been identified.

Although the term "**CVD**" has not appeared in any of the health claims for soy protein in other jurisdictions, the following few paragraphs provide an explanation supporting the use of the term CVD and its associated risk with elevated blood cholesterol.

Several published papers have identified levels of serum lipids (as well as lipoproteins, apolipoproteins and various derived ratios) to be good predictors of **CVD risk** (Genest et al., 2009; Mensink et al., 2003). According to a publication from the Nutrition Research Division of the Health Products and Food Branch of Health Canada, soy protein or associated isoflavones have been shown to have beneficial impacts on the risk factors of **CVD** in their ability to lower liver or blood TG, TC and LDL-C levels, increasing HDL-C and the ratio of HDL-C/LDL-C (Xiao, 2008). Furthermore, the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group (1998) and the Scandinavian Simvastatin Survival Study Group (1994) both reported reductions in **CVD** morbidity and mortality in response to reduced LDL-C concentrations. The most recent American Heart Association statement regarding soy protein and cardiovascular health indicates that LDL-C is the most studied risk factor for **CVD**, the primary criterion on which the National Cholesterol Education Program estimates risk and recommends therapy, and forms the basis for the FDA-approved health claim (Sacks et al., 2006). Additionally, according to the recently released Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of **CVD** in adults (2009), LDL-C >3.5 mmol/L is specifically emphasized as a CVD risk factor. While emphasis is placed on the fact that none of the traditional CVD risk factors or biomarkers reflects the actual presence or absence of CVD itself, the report does acknowledge their help in establishing CVD event risk. Finally, the Heart and Stroke Foundation of Canada indicates that high blood cholesterol is a major risk factor for heart disease and stroke. They state that the risk of circulatory problems, heart disease and stroke can be dramatically reduced by lowering cholesterol levels (Heart and Stroke Foundation, 2010). The terms "heart disease" and "stroke" both fall under the umbrella of **CVD**.

In addition to serum lipids, high blood pressure significantly increases the risk for stroke, ischemic heart disease, peripheral vascular disease and heart failure (Public Health Agency of Canada, 2010). As mentioned previously, the World Health Organization (WHO) includes stroke (cerebrovascular disease), ischemic heart disease (CHD), and peripheral vascular disease (peripheral arterial disease) under the definition of CVD (World Health Organization, 2008). Therefore, investigating blood pressure as another biomarker of interest in this systematic scientific literature review further warrants the use of the term "**CVD**", as opposed to the more restrictive term "CHD".

While CVD is the health outcome of interest in this investigation, the term CHD has been used in several instances. It should be noted that these two terms are intended to have two separate meanings, as defined above, and therefore should not be interpreted as interchangeable terms.

5.0 EVALUATION OF HEALTH CLAIM VALIDITY

Objective: *The purpose of this section is to guide the retrieval and evaluation of the totality of relevant evidence on the food/health relationship, to allow for an assessment of causality and generalizability, as well as the biological relevance of the health effect and the feasibility of consuming an effective intake of food.*

Step 5 of the HC Guidance Document consists of 13 steps. This resource outlines an example of steps 1 through 9b. A complete submission would need to include all other sections of the HC Guidance Document, including steps 9c through 13 of Section 5, as well as identify and fill any information or research gaps.

5.1 DETAILS OF THE STEPS

5.1.1 STEP 1. DESCRIBE THE SEARCH STRATEGY FOR LITERATURE RETRIEVAL

Objective: *To develop a relevant, comprehensive, and reproducible strategy that will be used to retrieve the totality of evidence from human studies of the food/health relationship.*

Table 5. Identification of databases and search parameters used for literature retrieval

A. Electronic Databases

- **List electronic databases used and identify fields searched within each database**

Database	Fields searched in database
Medline	All Fields
Food Science and Technology Abstracts	Title
Web of Science	Title
Current Contents Connect	Title
BIOSIS Previews	Title

B. Non-Electronic Methods/Sources • **State whether the below were conducted/considered**	
Hand Searching	☐ Yes X No
Unpublished Studies	☐ Yes X No
C. Humans • **State whether a search parameter was used to limit retrieval to human studies**	
X Yes (in databases when available) ☐ No	Search parameter used: Limits: Humans
D. Publication Years • **State the publication years considered for your electronic/non-electronic searches and justify the start date**	
Start date: 1980	**End date:** March 2010
Justification for start date: In order to evaluate the totality of scientific evidence, a start date of 1980 was chosen. This start date provides a 30-year time frame for investigation of published scientific literature, which should ensure that a full scope of available literature is considered. In order to include current and relevant studies (utilizing modern methodology) in this systematic literature review, literature published prior to 1980 was not considered.	
E. Languages • **State the languages considered for your electronic/non-electronic sources**	
Languages considered for search:	English

Table 6. Keywords and their combination used to retrieve literature on the food/health relationship from electronic databases

A. Food

Indicate keywords used:

- Soy
- Soybean
- *Glycine max*
- Soy isoflavones
- Soy protein (isolate)
- Soy phytoestrogens

B. Health effect(s)

1. Final health effect	**2. Biomarker/Surrogate marker of health effect**
Indicate keywords used: • CVD OR cardiovascular disease • CHD OR coronary heart disease • Myocardial ischemia • Myocardial infarction OR heart attack • Atherosclerosis • Hypercholesterolemia • Hyperlipidemia	Indicate keywords used: • Cholesterol OR cholesterol reduction • Lipids • Blood pressure • Homocysteine* • Total cholesterol OR TC • Triglycerides OR triacylglycerol OR TG • LDL cholesterol OR LDL-C • HDL cholesterol OR HDL-C

C. Combinations of keywords used

Indicate combinations of keywords used:
'food' (from section A) + 'final health effect OR biomarker/surrogate marker of health effect' (from section B)

D. Justification for exclusion of potentially relevant terms

Please specify and justify the disuse of relevant terms as keywords:
N/A

N/A = not applicable

Please note that although the search strategy initially included homocysteine as a biomarker/surrogate marker of CVD, further in-depth research highlighted the inconsistencies in the literature with respect to the specificity and sensitivity of the relationship between homocysteine and incidence of CVD. Consequently, it was decided that homocysteine would not be included in this investigation as a validated biomarker of CVD.

5.1.2 STEP 2. IMPLEMENT THE SEARCH STRATEGY FOR LITERATURE RETRIEVAL

Objective: *To implement the search strategy consistently across all electronic databases, to maintain a record of all literature retrieved prior to literature filtering and to organize the retrieval of the literature in a systematic way.*

The literature review must include the search history and search results for each database. Use the tools 'publish to PDF', 'save web page' or 'print screen' while conducting the database searches to avoid having to repeat them to obtain a record of the history.

Note that Appendix 1 containing the search history has not been included in this report.

Please note that a list of citations (Title, Author and Source), along with a copy of the search history of each database, can be found in **Appendix 1, Volumes 1–9**.

Table 7. Number of references retrieved from electronic and non-electronic sources

Source	# of References
A. Retrieved from Electronic Databases	48 905
B. Retrieved from Non-Electronic Databases (e.g. unpublished literature; hand searched)	0
C. Duplicates	34 247
TOTAL (A+B-C):	14 658

[1]Utilization of different combinations of the search strategy keywords outlined in **Table 6** yielded **11 880** references from the Medline database. Utilization of different combinations of the search strategy keywords outlined in **Table 6** yielded **37 025** references from the Web of Knowledge database, which simultaneously searched 4 individual databases for relevant references (Food Science and Technology Abstracts, Web of Science, Current Contents Connect, BIOSIS Previews). A total of **48 905** references were retrieved from the 5 electronic databases searched. Of the 11 880 references retrieved from the Medline database, 9 782 were duplicates. The literature search of PubMED and Medline retrieved a total of 2 098 unique references. Of the 37 025 references retrieved from Web of Knowledge, 22 367 were duplicates. The literature search of Web of Knowledge generated a total of 14 658 unique references. Following duplicate filtering of individual databases, the search strategy results from Medline and Web of Knowledge were combined. There were a total of **16 756** references. Of these 16 756 references, 2 098 were duplicates (as calculated following **combined** duplicate filtering). Overall, a total of **14 658** unique references were retrieved from the aforementioned electronic databases.

It may be necessary to revisit Table 7 after reviewing the literature. Reading of retrieved publications may uncover some cited references that did not appear in the electronic database search. If those publications are then added to the list of references, they would be considered 'hand-searched'.

5.1.3 Step 3. Develop Inclusion and Exclusion Criteria to Filter the Literature Retrieved

Objective: *To develop inclusion/exclusion criteria that will be applied to all references retrieved from electronic and non-electronic databases so that not relevant/non-useful references can be excluded.*

This is a critical step. Setting clear inclusion and exclusion criteria at this step provides the foundation of the search. Having clear criteria will eliminate inconsistency and the need to revisit criteria and redo the literature filtering.

Table 8a. Inclusion and exclusion criteria used for literature filtering

Factor	Inclusion Criteria	Exclusion Criteria
Source	Published in peer-reviewed journal	Published in non-peer reviewed media (e.g. internet reports, newspaper articles, magazine articles)
Report Type	• Full-text articles • Original research • Human data - human intervention studies - observational studies - cohort studies - nested case–control studies • Systematic reviews or meta-analyses (human data) • Authoritative statement position papers by a credible scientific body (e.g. WHO, IOM)	• Published abstracts, published opinion letters, anecdotal data, consumer testimonials • Non-human data - *in vitro* data - animal data • Review papers • Retrospective cohort, case–control, cross-sectional, ecological, time-series, or demographic studies
Language	• English	• All but English
Publication Year	• 1980 to date of search	• N/A
Duplicate	• N/A	• Publication is a duplicate

Table 8a. Inclusion and exclusion criteria used for literature filtering (cont'd)		
Factor	**Inclusion Criteria**	**Exclusion Criteria**
Treatment	• Observational studies in which food intake was calculated • Intervention studies in which food intake was quantified • Intervention studies in which food was independently consumed (i.e. food was not administered in combination with drugs or with other foods) • Oral intake • Selected biomarkers of the food–health relationship are biologically and/or methodologically relevant	• Observational studies in which food intake was not calculated • Intervention studies in which food intake was not quantified • Intervention studies in which food was not independently consumed (i.e. food was administered in combination with drugs or with other foods) • Non-oral intake • Selected biomarkers of the food–health relationship are not biologically and/or methodologically relevant
Control[1]	• The study used a control group and a control/placebo appropriate to study design	• The study did not use a control group or comparison group, or used an inappropriate control
	Appropriate control foods are project specific. In this case, some of the criteria for the control food were: 1) isocaloric to the treatment food; 2) similar macronutrient composition to the treatment food; 3) the quality and quantity of the protein. Identifying the appropriate control will depend on whether the submission is for a whole food, a food containing an inherent bioactive substance or a food containing an added bioactive substance.	
Route of Exposure	• Oral	• Non-oral (e.g. intravenous)
Health Effect	Direct • The health effect of interest has been accurately measured Indirect • Selected biomarkers of the health effect are biologically and/or methodologically relevant	Direct • The health effect of interest has not been accurately measured Indirect • Selected biomarkers of the health effect are not biologically and/or methodologically relevant

Table 8a. Inclusion and exclusion criteria used for literature filtering (cont'd)		
Factor	**Inclusion Criteria**	**Exclusion Criteria**
Population health status/ study setting	• Health status of the study population is representative of the desired target population - Generally healthy adults[2] - Free living *or* controlled feeding	• Health status of study population is not representative of health status of desired target population - Hospitalized or free-living sick or diseased individuals[2]
Ages	• Representative of target population: Individuals greater than 9 years of age[3]	• Not representative of target population: Individuals less than 9 years of age
Statistical significance	• Reported	• Not reported
Duration	• Treatment duration is adequate (e.g. ≥ 3 weeks exposure in intervention studies)[4] • Follow-up time (to measure health effect) adequate (cohort studies)	• Treatment duration is not adequate (e.g. <3 weeks in intervention studies) • Follow-up time (to measure health effect) not adequate (cohort studies)

[1]An appropriate control group represents study subjects who did not receive the treatment/substance. When an intervention study involves provision of a food or food component, the experimental and control diets should be similar enough that the relationship between the substance and disease can be evaluated (U.S. FDA, 2009a). Furthermore, relevant baseline data that may affect the endpoint being measured should not significantly differ between control and treatment groups (e.g. baseline lipid measurements, weight/BMI) (FDA, 2009).
[2]Please note, studies that utilized subjects with the metabolic syndrome and/or elevated cholesterol levels (specific cut-offs vary according to individual researcher) but who were otherwise healthy were included in this investigation. Studies that included subjects with familial or Type II hypercholesterolemia were excluded from this investigation. Studies that included individuals taking prescription drugs or that incorporated a weight-loss program into the experimental design were excluded from this analysis due to the potential for confounding.
[3] For the purpose of performing an inclusive scientific literature search, both male and female populations will be considered.
[4] The U.S. FDA has considered 3 weeks to be the minimum duration for evaluating the effect of an intervention on LDL-C concentrations (FDA, 2009). Three weeks will therefore be considered an adequate duration of food exposure for intervention studies included in this investigation.

5.1.4 STEP 4. FILTER THE LITERATURE

Objective: *To exclude references that based on their title, abstract, or full-text, meet the exclusion criteria/do not meet the inclusion criteria specified in* ***Table 8a****.*

This step is open to individual interpretation and is one of the more labour-intensive steps. Filtering the literature is much easier if the inclusion and exclusion criteria are well defined. If during the filtering process it is determined that the inclusion and exclusion criteria are not specific enough to make clear decisions as to whether to include or exclude a study, it may be useful to stop the process, go back to Table 8a to refine the inclusion and exclusion criteria, and re-initiate the filtering process using the newly refined criteria.

Two people should independently apply the inclusion/exclusion criteria. It is better to err on the side of over-inclusion at the title filtering stage to minimize the likelihood of excluding relevant/useful literature. This approach should also be applied to the abstract and full-text filtering stages. If important details relating to inclusion/exclusion criteria are not reported in a publication, it is acceptable to contact the author to attempt to acquire these details.

Table 9. Results of literature filtering

Factor	Number of References
References prior to applying inclusion/exclusion criteria	14 658[1]
References excluded at title-filtering stage	14 249 (409 abstracts ordered)[2]
References excluded at abstract-filtering stage	263 (146 publications ordered)
References excluded at full-text filtering stage	87
TOTAL References Excluded (after applying inclusion/exclusion criteria):	14 599
TOTAL References Included (after applying inclusion/exclusion criteria):	59[3] (45 intervention study publications, 1 observational study publication, 13 meta-analyses/ systematic reviews)

[1] A copy of all references retrieved from the systematic literature search is included in **Appendix 1, Volumes 1–9.** Search strategy results were electronically filtered for duplicate publications (please refer to **Step 2** for details).
[2] A copy of all abstracts retrieved from the literature search is included in **Appendix 2**.
[3] Full-text copies of each of the 59 pertinent references are included in **Appendix 3**.

A list of all references retrieved from the systematic literature search, a list of all abstracts retrieved from the literature search, and full-text copies of each of the pertinent references should be included in appendices. Note that Appendices 1 through 3 containing this information have not been included in this report.

5.1.5 STEP 5. GENERATE REFERENCE LISTS OF INCLUDED AND EXCLUDED STUDIES

Objective: *To indicate the references that met the inclusion criteria and those that met the exclusion criteria at the full-text filtering stage.*

It is best to use a consistent style for referencing. Adding the date of the database search is also helpful for context and for future updates to the literature review.

If a publication is excluded for more than one reason, it can be helpful to include the multiple reasons in Table 11 in case one of the criteria is changed at a later date and the publications need to be re-filtered.

Table 10. List of references that met the inclusion criteria at the full-text filtering stage (n=59)

Ref #	Full reference
Intervention Study Publications (n=45):	
1	Allen, J.K., Becker, D.M., Kwiterovich, P.O., Lindenstruth, K.A., and Curtis, C. 2007. Effect of soy protein-containing isoflavones on lipoproteins in postmenopausal women. Menopause: The Journal of the North American Menopause Society; 14(1): 106-114.
2	Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., Padyab, M., Hu, F.B. and Willet, W.C. 2007. Soy inclusion in the diet improves features of the metabolic syndrome: a randomized crossover study in postmenopausal women. American Journal of Clinical Nutrition; 85: 735-741.
3	Bakhit, R.M., Klein, B.P., Essex-Sorlie, D., Ham, J.O., Erdman, J.W., and Potter, S.M. 1994. Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. Journal of Nutrition; 124: 213-222.
(…)	
45	Welty, F.K., Lee, K.L., Lew, N.S. and Zhou, J-R. 2007. Effect of soy nuts on blood pressure and lipid levels in hypertensive, prehypertensive, and normotensive postmenopausal women. Archives of Internal Medicine; 167: 1060-1067.
Observational Study Publications (n=1):	
46	Zhang, X., Shu, X.O., Gao, Y.T., Yang, G., Li, Q., Li, H., Jin, F., and Zheng, W. 2003. Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. Journal of Nutrition; 133: 2874-2878.
Meta-analyses, Systematic Reviews, Authoritative Statements (n=13)	
47	Anderson, J.W., Johnstone, B.M., and Cook-Newell, M.E. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. New England Journal of Medicine; 333: 276-282.
48	Costa, R.L., and Summa, M.A. 2000. Soy protein in the management of hyperlipidemia. Annals of Pharmacotherapy; 34: 931-935.
49	Erdman, J.W. 2000. Soy protein and cardiovascular disease: a statement for healthcare professionals from the nutrition committee of the AHA. Circulation; 102: 2555-2559.
(…)	
59	Zhuo X.G., Melby, M.K., and Watanabe, S. 2004. Soy isoflavone intake lowers serum LDL cholesterol: a meta-anlysis of 8 randomized controlled trials in humans. Journal of Nutrition; 134: 2395-2400.

Table 11. List of references excluded at the full-text filtering stage and reason(s) for exclusion (n=81)		
Ref #	**Reference (Full citation)**	**Reason(s) for Exclusion[1]**
1	Allison, D.B., Gadbury, G., Schwartz, L.G., Murugesan, R., Kraker, J.L., Heshka, S., Fontaine, K.R. and Heymsfield, S.B. 2003. A novel soy-based meal replacement formula for weight loss among obese individuals: a randomized controlled clinical trial. European Journal of Clinical Nutrition; 57: 514-522.	Treatment (soy protein, fibre and phospholipids as a meal replacement, combined with a low calorie diet for weight loss in obese subjects)
(…)		
3	Aubertin-Leheudre, M., Lord, C., Khalil, A. and Dionne, I.J. 2007. Effect of 6 months exercise and isoflavone supplementation on clinical cardiovascular risk factors in obese postmenopausal women: a randomized, double-blind study. Menopause: The Journal of the North American Menopause Society; 14(4): 1-6.	Treatment (soy isoflavones provided in capsules)
4	Blumenschein, S., Torres, E., Kushmaul, E., Crawford, J. and Fixler, D. 1991. Effect of oat bran/soy protein in hypercholesterolemic children. Annals New York Academy of Sciences; 413-415.	Treatment (unable to quantify absolute soy protein treatment, 1 g soy protein given on a per kg basis); Study population (included children <9 years of age)
5	Bricarello, L.P., Kasinski, N., Bertolami, M.C., Faludi, A., Pinto, L.A., Relvas, W.G.M., Izar, M.C.O., Ihara, S.S.M., Tufik, S. and Fonseca, F.A.H. 2004. Comparison between the effects of soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia. Nutrition; 20: 200-204.	Control (considerably higher fibre and fat content (predominantly unsaturated fat) and lower carbohydrate content with the soy milk **treatment**, as compared to non-fat cow milk **treatment**)
6	Carroll, K. 1991. Review of clinical trials on cholesterol-lowering response to soy protein. American Dietetic Association; 91(7): 820-828.	Report Type (review paper)
(…)		
9	Desroches, S., Mauger, J-F., Ausman, L.M., Lichenstein, A.H. and Lamarche, B. 2004. Soy protein favourably affects LDL size independently of isoflavones in hypercholesterolemic men and women. Human Nutrition and Metabolism; 134: 574-579.	Duplicate publication
(…)		
14	Fumagalli, R., Soleri, L., Farina, R., Musanti, R., Mantero, O., Noseda, G., Gatti, E. and Sirtori, C.R. 1982. Fecal cholesterol excretion studies in Type II hypercholesterolemic patients treated with soybean protein diet. Atherosclerosis; 43: 341-353.	Population Health Status (Type II hypercholesterolemic subjects)

(…)

81	Zittermann, A., Geppert, J., Baier, S., Zehn, N., Gouni-Berthold, I., Berthold, H.K., Reinsberg, J. and Stehle, P. 2004. Short-term effects of high soy supplementation on sex hormones, bone markers, and lipid parameters in young female adults. European Journal of Clinical Nutrition; 43: 100-108.	Control (soy protein cookies and white wheat flour cookies not described as matched, nor was a macronutrient comparison between treatment and control foods reported); Treatment (soy protein within soy flour was not quantified)
Total number of studies excluded per reason		**Treatment** (n=24); **Population Health Status** (n=28); **Report Type** (n=3); **Duplicate** (n=3); **Control** (n=23); **Health Effect** (n=1); **Study Population** (n=6); Other (1)

[1] **Reason(s) for exclusion include**: Source, report type, language, publication, year, duplicate, treatment, duration, control, route of exposure, health effect, population health status/study setting, age, statistical significance

Note that only original research should be evaluated in the remaining steps. Systematic reviews and meta-analyses lack sufficient detail on individual studies to be used in these steps. However, systematic reviews, meta-analyses and authoritative statements, as well as animal studies, may be used in the narrative of steps 9c to 13 to support the findings of the systematic literature review and strengthen a health claim submission.

5.1.6 STEP 6. TABULATE STUDIES

Objective: *To provide a synopsis of the relevant information from intervention and observational studies in a standardized and objective manner.*

This section should include information for each intervention (Table 12a template) or observational (Table 12b template) study publication listed in Table 10 (publications that met the inclusion criteria at the full-text filtering stage).

It is easier to review and compare the publications if the information is always presented in the same order (e.g. in the 'exposure and duration' column, always list information for each publication in the order of food matrix, food dose, duration of intervention, design and/or duration of stabilization period, washout, follow-ups and so on). Furthermore, specifying which variables are not reported will prevent the reviewers from having to access the publication looking for the information.

Note that most clinical trials have at minimum two study groups; a control group and a treatment group. However, some study designs may have multiple treatment groups. These groups may be assigned different treatments (soy beverage vs tofu) or different doses of the same treatment. The original literature review included 45 intervention study publications that detailed 79 treatment groups.

In this resource, Table 12a provides an example of information for 3 of the 45 intervention study publications included in the original literature review and Table 12b provides information for the one observational study publication.

See **Table 12a** for a summary of **79 treatment groups** (**in 45 intervention study publications**) included in this systematic scientific literature review.

See **Table 12b** for a summary of the **1 observational study publication** included in this systematic scientific literature review.

Supplementary Table S2 is an optional table created by Nutrasource Diagnostics Inc. to provide details on multiple treatment groups of intervention study publications. Alhough this table is not required by the HC Guidance Document, it is a useful tool for presenting information in an organized manner when several publications include multiple treatment groups.

Supplementary Table S2.
Description of treatment groups of included intervention study publications (optional)

Ref No.	Publication	No. of Treatment Groups	Description of Treatment Groups
1	Allen et al., 2007	1	20 g/d soy protein + 96 mg/d aglycones
2	Azadbakht et al., 2007	1	15 g/d soy protein + 84 mg/d isoflavones
3	Bakhit et al., 1994	2	All subjects: 25 g/d SPI + 20 g cellulose
			Hypercholesterolemic: 25 g/d SPI + 20 g cellulose
4	Basaria et al., 2009	1	20 g/d soy protein powder + 96 mg/d aglycones
5	Baum et al., 1998	2	40 g/d SPI + 56 mg/d aglycones
			40 g/d SPI + 90 mg/d aglycones
6	Blum et al., 2003	1	25 g/d soy protein powder + 85 mg isoflavones
7	Borodin et al., 2009	1	30 g/d SPI
8	Crouse et al., 1999	8	Low LDL (<4.29 mmol/L): 25 g/d SPI + 3 mg/d
			Low LDL (<4.29 mmol/L): 25 g/d SPI + 27 mg/d
			Low LDL (<4.29 mmol/L): 25 g/d SPI + 37 mg/d
			Low LDL (<4.29 mmol/L): 25 g/d SPI + 62 mg/d
			High LDL (≥4.29 mmol/L): 25 g/d SPI + 3 mg/d
			High LDL (≥4.29 mmol/L): 25 g/d SPI + 27 mg/d
			High LDL (≥4.29 mmol/L): 25 g/d SPI + 37 mg/d
			High LDL (≥4.29 mmol/L): 25 g/d SPI + 62 mg/d
(…)			
44	Shidfar et al., 2009	1	130 g soybeans (50 g/d soy protein + 164 mg/d isoflavones)
45	Welty et al., 2007	2	Normotensive: 56 g (½ cup) soy nuts (25 g/d soy protein + 101 mg/d aglycones)
			Hypertensive: 56 g (½ cup) soy nuts (25 g/d soy protein + 101 mg/d aglycones)
		79	**Total Number of Treatment Arms**

SPI = soy protein isolate

Table 12a. Summary of intervention studies addressing the food/health relationship (example 1: Allen et al. 2007)

Reference and Quality Rating (Author, Year)	Aim of Study	Design • R (Randomized) • NR (Non-Randomized) • C (Control group) • SB (Single-Blind) • DB (Double-Blind) • P (Parallel) • CO (Crossover)	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # recruited • # randomized • # in final sample	Exposure and Duration • Food matrix • Food dose; method and frequency of consumption • Duration of intervention • Design and/or duration of stabilization period, washouts, follow-ups	Background Diet & Assessment Tool	Results & Statistics • Changes in health • Adverse effects	Relevant Authors' Conclusions
Allen, J.K., Becker, D.M., Kwiterovich, P.O., Lindenstruth, K.A., and Curtis, C., 2007. Quality: 15 *(See Quality Appraisal Tool)*	To determine the effects of isolated soy protein containing isoflavones on lipoproteins and lipoprotein subclasses in both African American and white post-menopausal women with borderline to moderate low-density lipoprotein cholesterol elevations	R, C, DB, P	United States Postmenopausal women with LDL-C ranging from 3.37 to 4.92 mmol/L or TGs >1.70 mmol/L Free-living Avg age: 56.8 yrs 191 Females 245 recruited 216 randomized 191 in final sample *7 drop-outs due to gastrointestinal side effects (side effects were not specific to treatment vs. placebo)	**Food Matrix:** Soy protein powder to be mixed with beverages. **Food Dose & Exposure**: Study participants were randomly assigned to 1 of the 2 treatment phases per day: Treatment (n=107): 20 g of soy protein containing 160 mg of isoflavones (approximately 96mg aglycones), including 64 mg of genistein, 63 mg daidzein and 34 mg glycitein; Control (n=109): 20 g whole milk protein containing the same nutrients as the soy supplement other than the isoflavones **Duration**: 12 wks **Stabilization period**: 4-wk placebo-controlled (casein-based) run-in **Washout period:** Not reported	**Background Diet:** Dietary intake was assessed by the Block 1998 revision of the Health Habits and History FFQ at baseline and again at 6 and 12 weeks. A trained dietary counselor counseled all participants on a low-fat diet and on incorporating the supplement into their diet by decreasing their protein intake to compensate for the extra protein in the supplement. **Assessment Tool:** Detailed dietary and physical activity data were collected via interview. Weight was measured on a standardized scale, and a first-voided morning sample of urine was collected to measure isoflavone metabolites to monitor compliance.	**Statistical Analysis:** Data were analyzed according to the intention-to-treat principle, including all original participants in the groups to which they were randomly assigned. Baseline measures were used for missing outcome data for those who had dropped out at 6 or 12 weeks. The major approach to analysis was multiple linear regression modeling, predicting change in the outcomes and adjusting for age, race, changes in body weight, dietary fat, and kilocalorie energy expenditure. Authors also examined whether the effect of soy differed across various subgroups, including thresholds for baseline levels of LDL-C cholesterol (<4.14 and ≥4.14 nmol/L), BMI (<30 and ≥30), age (<56 and ≥56 yrs), and racial groups (African American and white).	A daily consumption of 20g of soy protein containing 160 mg isoflavones in a beverage did not improve lipid profile in postmenopausal women with moderately elevated LDL-C. There were modest effects of soy protein on LDL particle number (1410.10 ± 341.14 nmol/L vs 1349/50 ± 283.64, placebo and soy, respectively) which may be clinically beneficial to postmenopausal women with moderately high cholesterol levels that do not qualify for medications. Using soy foods to replace foods high in animal protein that contain fat and cholesterol may help improve atherogenic lipid profiles and confer benefits to cardiovascular health in postmenopausal women. The modest effects of soy protein on LDL cholesterol and LDL particle number may be beneficial for heart health in postmenopausal women who do not qualify for pharmacotherapy.

Table 12a. Summary of intervention studies addressing the food/health relationship (example 1: Allen et al. 2007 (cont'd))

Reference and Quality Rating (Author, Year)	Aim of Study	Design • R (Randomized) • NR (Non-Randomized) • C (Control group) • SB (Single-Blind) • DB (Double-Blind) • P (Parallel) • CO (Crossover)	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # recruited • # randomized • # in final sample	Exposure and Duration • Food matrix • Food dose; method and frequency of consumption • Duration of intervention • Design and/or duration of stabilization period, washouts, follow-ups	Background Diet & Assessment Tool	Results & Statistics • Changes in health • Adverse effects	Relevant Authors' Conclusions
Allen, J.K., Becker, D.M., Kwiterovich, P.O., Lindenstruth, K.A., and Curtis, C., 2007. *Cont'd*						Mean (SD) of lipoprotein and lipid outcomes after 6 and 12 weeks of soy or milk protein supplementation (adapted from **Table 2,** Allen et. al., 2007)	

	Soy	Control
Total Cholesterol (mmol/L), mean (SD)		
Baseline	5.80 (0.68)	5.71 (0.64)
6 weeks	5.73 (0.64)[b]	5.80 (0.66)[a]
12 weeks	5.75 (0.69)	5.74 (0.72)
LDL-C (mmol/L), mean (SD)		
Baseline	3.67 (0.57)	3.60 (0.57)
6 weeks	3.56 (0.52)[b]	3.65 (0.58)[a]
12 weeks	3.55 (0.55)	3.60 (0.61)
HDL-C (mmol/L), mean (SD)		
Baseline	1.56 (0.37)	1.52 (0.32)
6 weeks	1.61 (0.36)	1.57 (0.31)
12 weeks	2.86 (1.13)	2.87 (1.36)
TG (mmol/L), mean (SD)		
Baseline	2.87 (1.17)	2.92 (1.37)
6 weeks	2.79 (1.18)	2.93 (1.54)
12 weeks	2.86 (1.13)	2.87 (1.36)

**Values with different supercripts are significantly different. Total cholesterol and LDL cholesterol decreased significantly compared to placebo at 6 weeks, although this effect was attenuated at 12 weeks. (Soy n=93, Control n=98).

Adverse Effects:
No adverse effects were noted. However, some women dropped out due to gastrointestinal side effects (n=7). There was no significant difference in drop-out rate between the two groups.

Table 12a. Summary of intervention studies addressing the food/health relationship (example 2: Ashton and Ball 2000)

Reference and Quality Rating (Author, Year)	Aim of Study	Design • R (Randomized) • NR (Non-Randomized) • C (Control group) • SB (Single-Blind) • DB (Double-Blind) • P (Parallel) • CO (Crossover)	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # recruited • # randomized • # in final sample	Exposure and Duration • Food matrix • Food dose; method and frequency of consumption • Duration of intervention • Design and/or duration of stabilization period, washouts, follow-ups	Background Diet & Assessment Tool	Results & Statistics • Changes in health • Adverse effects	Relevant Authors' Conclusions
Ashton, E. and Ball, M. 2000. Quality: 9 *(See Quality Appraisal Tool)*	To investigate the effects of replacing lean meat with a soy product, tofu, on serum lipoprotein concentrations	R, C, CO	Australia Healthy males Free-living Age range: 34–62 yrs 42 Males 45 recruited 45 randomized 42 in final sample *Subjects omitted from analysis due to non-compliance.	**Food Matrix:** Tofu added to diet in replacement of meat **Food Dose & Exposure**: Study participants were randomly assigned to and crossed-over for the following treatment phases per day: Treatment: 290 g of tofu (providing 35 g soy protein + 80 mg isoflavones) + 5 g butter + 5 g lard + 8 mL olive oil to adjust for dietary fat difference between meat and tofu. Control: 150 g (raw weight) lean red meat with all visible fats removed + 15 g polyunsaturated margarine. **Duration**: 4 wks per treatment phase **Stabilization Period**: none reported **Wash-out Period:** 2-wk wash-out between treatments	**Background Diet:** Diets were designed to be similar in energy, protein, fat, carbohydrate, alcohol and dietary fibre with only the source of protein changing from animal source to plant source. Subjects consumed similar vegetarian breakfasts, lunches and snacks on both diets. Dietary counseling was provided weekly to help with dietary manipulation and to assess and improve compliance. All subjects were instructed to maintain their usual exercise patterns for the duration of the study. **Assessment Tool:** Prior to commencing the dietary treatments, participants recorded their habitual dietary intake for 7 days including 2 weekend days (provided with scales and household measuring equipment). During the last week of the treatment phase, participants completed a 7-day diet record using accurate scales (provided by researchers). All diets were coded and analyzed using computerized nutrition software.	**Statistical Analysis:** Wilcoxon signed rank test was used to compare serum TG between the two diets. A general linear model was used to investigate the overall effect of the two diets on lipoprotein levels. Mean ± SE of lipoprotein and lipid outcomes after 1 month tofu or lean meat consumption (adapted from **Table 1**, Ashton et. al., 2000) **Baseline (mean ± SE)** / **Tofu (mean ± SE)** **TC (mmol/L)**: 5.79 ± 0.97[a] / 5.42 ± 1.02[b] **LDL-C (mmol/L)**: 3.68 ± 0.86[a] / 3.48 ± 0.27[b] **HDL-C (mmol/L)**: 1.25 ± 0.35 / 1.24 ± 0.27 **TGs (mmol/L)**: 1.96 ± 1.33[a] / 1.62 ± 0.99[b] Values with different letters within the same row are significantly different. TC, LDL and TGs were significantly different from baseline after tofu supplementation ($p<0.05$). HDL increased from baseline after lean meat supplementation; however, no other parameters were affected. Each group had n=42. **Adverse Effects:** None reported.	Results indicate that soy decreased TC and TG concentrations, but the mean LDL-C was only 2% lower on the tofu diet. Hypercholesterol-emic subjects did not show a greater response to the soy than those with normocholesterol-emia. This study revealed the mean HDL-C was 6% lower on the soy diet compared with the meat diet. While the effects of replacing meat in the diet on cholesterol levels were small, in reality, substituting meat with tofu would also affect dietary fat and have additional effects on lipoprotein levels.

Table 12a. Summary of intervention studies addressing the food/health relationship (example 3: Azadbakht et al. 2007)

Reference and Quality Rating (Author, Year)	Aim of Study	Design • R (Randomized) • NR (Non-Randomized) • C (Control group) • SB (Single-Blind) • DB (Double-Blind) • P (Parallel) • CO (Crossover)	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # recruited • # randomized • # in final sample	Exposure and Duration • Food matrix • Food dose; method and frequency of consumption • Duration of intervention • Design and/or duration of stabilization period, washouts, follow-ups	Background Diet & Assessment Tool	Results & Statistics • Changes in health • Adverse effects	Relevant Authors' Conclusions
Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., Padyab, M., Hu, F.B. and Williet, W.C., 2007 Quality: 10 *(See Quality Appraisal Tool)*	To determine the effects of soy consumption on components of the metabolic syndrome, plasma lipids, lipoproteins, insulin resistance and glycemic control in postmenopausal women with the metabolic syndrome	R, C, CO	Iran Postmenopausal women (metabolic syndrome according to the ATP III guidelines) Free-living Age: Not reported 42 Females 120 recruited 42 randomized 42 in final sample	**Food Matrix:** Soynuts or soy protein added to a DASH diet in replacement of one serving of red meat. **Food Dose & Exposure**: Study participants were randomly assigned to a sequence of the following treatment phases per day: Control: one serving of red meat within context of a DASH diet DASH+Soynuts: identical to control but red meat replaced with 30 g soynuts (providing 11.3 g soy protein +102 mg isoflavones) DASH+Soy protein: identical to control but red meat replaced with 30 g soy protein powder (providing 15 g soy protein + 84 mg isoflavones) **Duration**: 8 wks per treatment phase	**Background Diet:** Patients asked not to change their diet or physical activity levels. **Assessment Tool:** The participants were visited every 2 wks and were in touch with the study nutritionist daily by phone. For measuring food intake, 3-day diet records were used at baseline and during the intervention for each month. Every participant had to bring her 3-day diet record and physical activity records every month when they were reviewed by the study staff; these records were used for checking diet compliance. Each food and beverage in the diet records was then coded according to the prescribed protocol and analyzed for content of energy and the other nutrients by using computerized nutrition software.	**Statistical Analysis:** Three methods of analyses were used. First, the general linear models were used to compare the means of the metabolic variables at the end of the soynut, soy protein, and control phases. Then, Tukey's test was used as a post-hoc test for comparing the end of treatment values of each group with each other group. Groups were compared to each other using the percentage change in both repeated-measures analysis of variance and paired *t* test analyses. The mean percentage change differences was also determined, derived by calculating the differences in percentage change for each variable in pair-wise group comparisons. This parameter gives the most direct estimate of the difference in response in comparing groups. Interactions between soy intake and weight were not significant for any of the metabolic features. Period and treatment order effects were tested by using the appropriate general linear models. Pearson correlation coefficients were used to evaluate the relation between soy-derived phytoestrogens intake (calculated from self - reported soy intake in 3-day diet records) and plasma phytoestrogen concentrations. All results were considered significant if the two-tailed P value was <0.05.	The findings suggest that short-term soynut or soy protein consumption may reduce lipid concentrations in postmenopausal women with the metabolic syndrome. In the present study, soynut intake had more beneficial effects on metabolic risk factors than did soy protein intake.

Table 12a. Summary of intervention studies addressing the food/health relationship (example 3: Azadbakht et al. 2007 (cont'd))

Reference and Quality Rating (Author, Year)	Aim of Study	Design • R (Randomized) • NR (Non-Randomized) • C (Control group) • SB (Single-Blind) • DB (Double-Blind) • P (Parallel) • CO (Crossover)	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # recruited • # randomized • # in final sample	Exposure and Duration • Food matrix • Food dose; method and frequency of consumption • Duration of intervention • Design and/or duration of stabilization period, washouts, follow-ups	Background Diet and Assessment Tool	Results and Statistics • Changes in health • Adverse effects	Relevant Authors' Conclusions
Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., Padyab, M., Hu, F.B. and Williet, W.C. *Cont'd*				**Stabilization period:** 3-wk run-in on a usual diet prior to commencing the study **Washout period:** 4-wk wash-out periods		Mean ± SE of lipoprotein, lipid and blood pressure outcomes after 8 wks of a DASH diet (control), DASH diet + soy protein or DASH diet + soynuts (adapted from **Table 3**, Azadbakht et. al., 2007)	

	Soy Protein (mean ± SE)	Soynuts (mean ± SE)	Control (mean ± SE)
TC (mmol/L)			
Baseline	6.18 ± 0.02	6.15 ± 0.02	6.15 ± 0.26
End of Trial	5.61 ± 0.01[b]	5.40 ± 0.02[c]	5.90 ± 0.02[a]
LDL (mmol/L)			
Baseline	3.67 ± 0.02	3.54 ± 0.08	3.70 ± 0.02
End of Trial	3.28 ± 0.06[b]	3.05 ± 0.08[c]	3.47 ± 0.09[a]
HDL (mmol/L)			
Baseline	0.83 ± 0.01	0.83 ± 0.01	0.80 ± 0.01
End of Trial	0.88 ± 0.02	0.86 ± 0.01	0.86 ± 0.02
TG (mmol/L)			
Baseline	2.48 ± 0.01	2.46 ± 0.01	2.47 ± 0.01
End of Trial	2.37 ± 0.02	2.39 ± 0.02	2.40 ± 0.01
SysBP (mmHg)			
Baseline	136 ± 0.7	136 ± 0.7	136 ± 0.7
End of Trial	132 ± 0.7	131 ± 1.0	131 ± 1.2
DiaBP (mmHg)			
Baseline	87 ± 0.2	87 ± 0.2	87 ± 0.1
End of Trial	85 ± 0.5	85 ± 0.5	84 ± 0.5

Different letters within the same row indicate significant differences between values (P<0.01). End of trial values for LDL and TC were significantly different across treatment groups. Each group had n=42.

Adverse Effects:
1 person complained of feeling bloated during the soy protein period.

(…)

Abbreviations: avg (average); BMI (body mass index); BP (blood pressure); CVD (cardiovascular disease); FFQ (food frequency questionnaire); HDL-C (high density lipoprotein cholesterol); LDL-C (low density lipoprotein cholesterol); SPI (soy protein isolate); TC (total cholesterol); TG (triglycerides); yrs (years); VLDL-C (very low density lipoprotein cholesterol); wk (week)

Table 12b. Summary of observational studies addressing the food/health relationship (Zhang et al. 2003)

Reference and Quality Rating (Author, Year)	Aim of Study	Design • PROS (Prospective cohort) • Nested case–control within a cohort	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # in final sample	Exposure and Duration • Food exposure • Duration of follow-up (for measurement of health effects)	Diet Assessment Tool	Results & Statistics • Changes in health effect	Relevant Authors' Conclusions
Zhang, X., Shu, X-O., Gao, Y-T., Yang, G., Li, Q., Li, H., and Jin, F., 2003 Quality: 10 (*see Quality Appraisal Tool)*	To examine the relationship between soy food intake and incidence of CHD among Chinese women aged 40–70 yrs at the baseline survey that was conducted from 1997 to 2000	PROS	China (urban Shanghai) Healthy subjects (without previous diagnosis of CHD, stroke, or diabetes at baseline) Free-living Age range: 40-70 yrs; Avg. age = 51 yrs (at recruitment) Females 64 915 subjects in final sample	Median daily intake of total soy protein was 7.36 g Mean follow-up of 2.5 years	At baseline, in-person interviews were performed at participants' homes by trained interviewers using a comprehensive quantitative FFQ. The FFQ included tofu, soy beverage, fried bean curd, bean curd cake, and other soy products (covering nearly all soy foods consumed in this region). Intake was quantified by determining how often, on average, subjects had consumed a specific food or food group, as well as determining the average intake of that food group in grams per unit of time.	**Statistical Analysis:** The primary endpoint was incident of CHD, including nonfatal myocardial infarction and CHD death that occurred after baseline survey. Medical records were sought for all cases of self-reported myocardial infarction (MI) and reviewed by physicians who were unaware of the participant's exposure status. Deaths were ascertained through reports by the next of kin and linkage with the registry of vital statistics kept at the Shanghai Center for Disease Control and Prevention; underlying causes of death were established by reviewing medical records where possible. Person-years of follow-up for each participant were calculated from the date of the baseline interview to the date of the end point, death, or the follow-up survey, whichever came first. Quartiles were defined based on the distribution of total soy protein intake at baseline among the entire cohort, and the lowest quartile was treated as the reference group. Incidence rates were calculated by dividing the number of events by the person-years of follow-up in each category. Cox proportional hazards model was used to compute relative risks, rate ratios of each quartile vs. the reference quartile, and their 95% confidence interval for soy intake and CHD risk.	The major contributors to total soy protein intake were soy beverage, tofu and processed soy products other than tofu (collectively accounting for 81%). No association was found for soy intake and fatal CHD; this may have been due to a small number of outcomes. After a mean of 2.5 y (162 000 person-years) of follow-up, 62 incident cases of CHD (43 nonfatal MIs and 19 CHD deaths) were documented. Women in the highest quartile of soy protein intake had a 75% lower risk of total CHD and 86% lower risk of nonfatal MI than those in the lowest quartile, and this was independent of established CHD risk factors and other dietary factors. Authors note that a major concern for this observational study is the potential error in dietary assessment; however, the fact that the FFQs were externally validated does help to strengthen this potential confounder.

Table 12b. Summary of observational studies addressing the food/health relationship (Zhang et al. 2003 (cont'd))

Reference and Quality Rating (Author, Year)	Aim of Study	Design • PROS (Prospective cohort) • Nested Case–control within a cohort	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # in final sample	Exposure and Duration • Food exposure • Duration of follow-up (for measurement of health effects)	Diet Assessment Tool	Results & Statistics • Changes in health effect	Relevant Authors' Conclusions
Zhang, X., Shu, X-O., Gao, Y-T., Yang, G., Li, Q., Li, H., and Jin, F., 2003 *Cont'd*					Nutrient intake was obtained by multiplying the determined amount of food consumed by the nutrient contents per gram of food obtained from the Chinese Food Composition Table; total soy food intake was measured by summing up the soy protein intake for all soy food items. Study subjects were followed through biennial in-person interviews. FFQ was evaluated for its reproducibility and validity in assessing usual dietary intake in a random sample of 200 participants, and demonstrated an accurate representation.	Multivariate analysis adjusted for age, cigarette smoking, alcohol consumption, BMI (kg/m^2), waist-to-hip ratio, regular exercise, menopausal status, history of hypertension, attended education level, family income, season of recruitment, as well as intake of total energy, fat, fibre, fruit and vegetables. Adjustment for use of postmenopausal hormones, vitamin E supplement, multivitamins and other dietary intakes (e.g. meat, poultry, fish, egg, rice, and tea) did not materially alter the results; therefore these variables were not included in the final model. All *P*-values were two-sided. **Results:** Women with higher soy consumption were older, more likely to be postmenopausal, and have a history of hypertension, a higher BMI and waist-to-hip ratio relative to women with lower soy consumption. Higher soy consumption also correlated to increased levels of exercise, alcohol intake, and higher intakes of total energy, fat, fibre and vegetables relative to lower intakes of soy. ***Soy Protein Intake and CHD Risk:*** Over 162 277 person-years of follow-up, 62 incident cases of CHD were documented, including 43 nonfatal MIs and 19 deaths from CHD.	The short length of follow-up raised a concern that some subjects may have modified their diets because of early symptoms of undiagnosed CHD. A sensitivity analysis was conducted by excluding cases that occurred during the first 6 or 12 months of follow-up, and showed little change in the risk estimates.

Table 12b. Summary of observational studies addressing the food/health relationship (Zhang et al. 2003 (cont'd))

Reference and Quality Rating (Author, Year)	Aim of Study	Design • PROS (Prospective cohort) • Nested Case–control within a cohort	Sample Characteristics • Country • Health Status • Setting (metabolic unit, free-living subjects) • Age range • Gender (M, F) • # in final sample	Exposure and Duration • Food exposure • Duration of follow-up (for measurement of health effects)	Diet Assessment Tool	Results & Statistics • Changes in health effect	Relevant Authors' Conclusions
Zhang, X., Shu, X-O., Gao, Y-T., Yang, G., Li, Q., Li, H., and Jin, F., 2003 *Cont'd*						The age- and energy-adjusted risk of total CHD decreased with increasing total soy protein intake (*P* for trend = 0.03). Compared with women in the lowest quartile of total soy protein intake, the RR of CHD derived from the fully adjusted model was 0.25 (95% CI, 0.10–0.63, *P* for trend = 0.003) for women in the highest quartile of intake. A strong inverse association was observed for nonfatal MI with a multivariate RR for the highest quartile being 0.14 (95% CI, 0.04–0.48; *P* for trend = 0.001). Please refer to **Table 2** (adopted directly from Zhang et al., 2003), which reports relative risks of CHD by quartiles of soy protein intake. ***Soy Protein Intake and CHD Risk (other factors):*** In further analyses stratified by hypertension, BMI, waist-to-hip ratio, exercise, menopausal status and intakes of fat, fibre, fruit and vegetables, the inverse association between soy protein intake and CHD risk was observed consistently across all strata. The magnitude of the stratum-specific relative risks comparing the extreme tertiles did not differ substantially for all of these factors, indicating that there was no significant effect modification. Please refer to **Table 3** (adopted directly from Zhang et al., 2003), which reports relative risks of CHD by quartiles of soy protein intake stratified by selected factors.	

Abbreviations: avg (average); BMI (body mass index); BP (blood pressure); CVD (cardiovascular disease); FFQ (food frequency questionnaire); HDL-C (high density lipoprotein cholesterol); LDL-C (low density lipoprotein cholesterol); RR (relative risk); SPI (soy protein isolate); TC (total cholesterol); TG (triglycerides); yrs (years); VLDL-C (very low density lipoprotein cholesterol)

Table 12b. Summary of observational studies addressing the food/health relationship (Zhang et al. 2003 (cont'd))
Results and Statistics (cont'd)

Table 2 adopted directly from Zhang et al., 2003. (N=64 915).

TABLE 2

Relative risks (RR) and 95% CI of coronary heart disease (CHD) by quartiles of total soy protein intake, the Shanghai Women's Health Study (1997–2002)

	Quartiles of total soy protein intake				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Median intake, *g/(1000kJ · d)*	0.47	0.88	1.29	1.99	
Total CHD					
Person-years	41276	40699	40495	39807	
Events, *n*	19	17	16	10	
Age/energy adjusted RR	1.00	0.87 (0.45–1.68)	0.76 (0.39–1.51)	0.42 (0.19–0.94)	0.03
Multivariate RR model 1[1]	1.00	0.84 (0.43–1.62)	0.73 (0.37–1.45)	0.37 (0.16–0.84)	0.02
Multivariate RR model 2[2]	1.00	0.77 (0.39–1.49)	0.60 (0.30–1.22)	0.25 (0.10–0.63)	0.003
Nonfatal myocardial infarction					
Events, *n*	15	12	11	5	
Age/energy adjusted RR	1.00	0.78 (0.36–1.68)	0.68 (0.31–1.50)	0.28 (0.10–0.80)	0.02
Multivariate RR model 1[1]	1.00	0.73 (0.34–1.57)	0.62 (0.28–1.38)	0.24 (0.08–0.69)	0.007
Multivariate RR model 2[2]	1.00	0.65 (0.30–1.41)	0.49 (0.21–1.11)	0.14 (0.04–0.48)	0.001
CHD death					
Events, *n*	4	5	5	5	
Age/energy adjusted RR	1.00	1.21 (0.32–4.52)	1.11 (0.29–4.20)	0.91 (0.23–3.67)	0.80
Multivariate RR model 1[1]	1.00	1.24 (0.33–4.66)	1.18 (0.31–4.52)	0.89 (0.22–3.63)	0.77
Multivariate RR model 2[2]	1.00	1.19 (0.31–4.53)	1.09 (0.27–4.33)	0.73 (0.15–3.58)	0.61
Confirmed total CHD					
Person-years	41280	40701	40496	39808	
Events, *n*	10	12	13	5	
Multivariate RR model 2[2]	1.00	0.99 (0.42–2.32)	0.86 (0.36–2.06)	0.20 (0.06–0.70)	0.009
Confirmed nonfatal myocardial infarction					
Events, *n*	10	10	9	4	
Multivariate RR model 2[2]	1.00	0.81 (0.33–1.97)	0.59 (0.23–1.52)	0.16 (0.04–0.63)	0.006

[1] Model 1 adjusted for age (continuous), cigarette smoking (yes or no), BMI (quartile), waist-to-hip ratio (quartile), history of hypertension (yes or no), menopausal status (pre- or postmenopausal), regular exercise (yes or no), level of education (four categories), family income (four categories), alcohol consumption (yes or no), season of recruitment (four categories) and total energy intake (continuous).
[2] Model 2 additionally adjusted for intakes of fat, fiber, fruit and vegetables (all treated as continuous variables).

Table 3 adopted directly from Zhang et al., 2003. (N=64 915).

TABLE 3

Multivariate relative risks (RR) of coronary heart disease by tertiles of total soy protein intake, stratified by selected factors, the Shanghai Women's Health Study (1997–2002)[1]

	Tertiles of total soy protein intake			
Variable	Tertile 1	Tertile 2	Tertile 3	*P* for trend
Hypertension				
Yes	1.00	1.45 (0.66–3.18)	0.20 (0.06–0.71)	0.006
No	1.00	0.44 (0.16–1.19)	0.46 (0.16–1.35)	0.17
BMI, *kg/m*[2]				
<25	1.00	0.73 (0.35–1.56)	0.16 (0.05–0.54)	0.002
≥25	1.00	1.31 (0.52–3.33)	0.59 (0.18–1.91)	0.29
Waist-to-hip ratio				
<0.804 (median)	1.00	0.68 (0.22–2.13)	0.16 (0.03–1.03)	0.05
≥0.804	1.00	1.04 (0.53–2.05)	0.37 (0.15–0.92)	0.02
Menopausal status				
Premenopausal	1.00	1.16 (0.36–3.81)	0.23 (0.03–1.49)	0.11
Postmenopausal	1.00	0.84 (0.43–1.64)	0.31 (0.13–0.78)	0.01
Regular exercise				
Yes	1.00	1.00 (0.29–3.43)	0.18 (0.03–1.34)	0.08
No	1.00	0.88 (0.45–1.71)	0.34 (0.14–0.82)	0.01
Fiber, *g/d*				
<11	1.00	1.02 (0.51–2.04)	0.11 (0.01–0.85)	0.03
≥11	1.00	1.05 (0.33–3.37)	0.51 (0.16–1.65)	0.11
Fat[2]				
Low	1.00	1.02 (0.49–2.11)	0.32 (0.10–1.10)	0.08
High	1.00	0.79 (0.29–2.20)	0.28 (0.09–0.89)	0.02
Vegetables[2]				
Low	1.00	0.73 (0.34–1.58)	0.14 (0.03–0.72)	0.01
High	1.00	1.33 (0.51–3.51)	0.50 (0.17–1.50)	0.08
Fruit[2]				
Low	1.00	0.69 (0.32–1.47)	0.16 (0.05–0.54)	0.003
High	1.00	1.50 (0.56–4.03)	0.61 (0.19–1.97)	0.23

[1] Adjusted for age (continuous), cigarette smoking (yes or no), BMI (quartile), waist-to-hip ratio (quartile), history of hypertension (yes or no), menopausal status (pre- or postmenopausal), regular exercise (yes or no), level of education (four categories), family income (four categories), alcohol consumption (yes or no), season of recruitment (four categories), total energy intake, and intakes of fat, fiber, fruit and vegetables (all treated as continuous variables). When a variable was used for stratification, it was not included in the model.
[2] Median value was used as the cut-off point.

5.1.7 STEP 7. EVALUATE STUDY QUALITY

Objective: *To discriminate between studies that have a high or low internal validity and risk of bias.*

This section should include information for each intervention (Table 13a template) or observational (Table 13b template) study publication listed in Table 10 (publications that met the inclusion criteria at the full-text filtering stage). A copy of the completed quality appraisal, attached to a full-text copy of each publication, is required and easily organized in an appendix.

The number of reviewers used to assess quality should be stated in the narrative. It is suggested that 'supporting publications' (e.g. publications that may have state-of-the-art or different endpoints than the ones assessed, animal studies, or in vitro studies) also be assessed for quality as best they can. The resulting quality rating of the supporting publications should then be mentioned in the narrative.

In this resource, Table 13a provides an example of information for 3 of the 45 intervention study publications included in the original literature review and Table 13b provides information for the one observational study publication.

Each publication was rated independently by individual reviewers. Disagreements were resolved through discussion and a consensus quality appraisal score was assigned to each publication. As shown in **Tables 13a** and **13b** and summarized in **Supplementary Table S3**, of 46 publications (which included 79 treatment groups in 45 intervention study publications and 1 observational study publication), 43 intervention study publications were ranked as 'high quality', 2 intervention study publications were ranked as 'low quality' and the 1 observational study publication was ranked 'high quality' according to the quality appraisal tool.

Table 13a. Quality appraisal tool for intervention studies			
Assign a score of 1 for each "Yes", and a score of 0 for each "No/NR".			
Reference (Author, year): Allen, J.K., Becker, D.M., Kwiterovich, P.O., Linderstruth, K.A. and Curtis, C., 2007			
Item	**Question**	**Score**	
		Yes	**No/NR**
1. Inclusion/Exclusion Criteria	Were the inclusion and/or exclusion criteria for study participation reported?	1	
2. Group Allocation[1]	Was the study described as randomized?	1	
	Was the randomization method reported?	1	
	Was the randomization method appropriate?[2]	1	
	Was allocation concealed?[3]	1	
3. Blinding	Were the study subjects blinded to the intervention received?	1	
	Were the research personnel blinded to the intervention received by the subjects?	1	
4. Attrition	Was attrition numerically reported?	1	
	Were the reasons for withdrawals and dropouts provided?[4]	1	
5. Exposure/ Intervention	Was the type of food described (e.g. composition, matrix)?	1	
	Was the amount of food described (i.e. dose)?	1	
6. Health Effect	Was the methodology used to measure the health effect reported?	1	
7. Statistical Analysis	Was a between-group statistical analysis of the health effect conducted (i.e. control vs. intervention)?	1	
	Was an intention-to-treat analysis conducted?[5]	1	
8. Potential Confounders	Were potential confounders of the food/health relationship considered?[6]	1	
TOTAL SCORE (maximum of 15):		15	
Higher quality (Score ≥8)		X	
Lower quality (Score ≤7)			

Abbreviation: NR, not reported

[1] Studies without an appropriate control would be excluded at Step 3.

[2] Examples of appropriate randomization include the use of computer-generated random number table, while date of birth and alternate allocation are examples of inappropriate methods of randomization.

[3] Allocation concealment is not the same as blinding. Allocation concealment refers to the method used to implement the random allocation sequence, e.g. numbered envelopes containing the assignment. It protects the assignment sequence before and until allocation. Blinding protects the sequence after subjects have been allocated.

[4] If the study reported that there was no attrition (ie. no subjects were lost to follow-up, withdrew or were excluded) then reason for withdrawals/dropouts is a non-applicable factor. In such a circumstance, please check "yes" so as to not unfairly lose a point.

[5] If there was no subject attrition, a per-protocol analysis is appropriate and an intention-to-treat analysis is not applicable. In such a circumstance, please check "yes" so as to not unfairly lose a point.

[6] Confounder(s) considered: Subject Selection (inclusion criteria: healthy post-menopausal women of African American or white background younger than 79 with LDL between 3.37–4.92 nmol/L; exclusion criteria: history of diabetes, CVD, stroke, cancer, high blood sugar, high blood triglycerides, use of oral contraceptive agents or hormone therapy); Randomization (diet and physical activity measured and not significantly different between two)

Table 13a. Quality appraisal tool for intervention studies			
Assign a score of 1 for each "Yes", and a score of 0 for each "No/NR".			
Reference (Author, year): Ashton, E., and Ball, M., 2000			
Item	**Question**	**Score**	
		Yes	**No/NR**
1. Inclusion/Exclusion Criteria	Were the inclusion and/or exclusion criteria for study participation reported?	1	
2. Group Allocation[1]	Was the study described as randomized?	1	
	Was the randomization method reported?		0
	Was the randomization method appropriate?[2]		0
	Was allocation concealed?[3]		0
3. Blinding	Were the study subjects blinded to the intervention received?		0
	Were the research personnel blinded to the intervention received by the subjects?		0
4. Attrition	Was attrition numerically reported?	1	
	Were the reasons for withdrawals and dropouts provided?[4]	1	
5. Exposure/ Intervention	Was the type of food described (e.g. composition, matrix)?	1	
	Was the amount of food described (i.e. dose)?	1	
6. Health Effect	Was the methodology used to measure the health effect reported?	1	
7. Statistical Analysis	Was a between-group statistical analysis of the health effect conducted (i.e. control vs. intervention)?	1	
	Was an intention-to-treat analysis conducted?[5]		0
8. Potential Confounders	Were potential confounders of the food/health relationship considered?[6]	1	
TOTAL SCORE (maximum of 15):		9	
Higher quality (Score ≥8)		X	
Lower quality (Score ≤7)			

Abbreviation: NR, not reported

[1] Studies without an appropriate control would be excluded at Step 3

[2] Examples of appropriate randomization include the use of computer-generated random number table, while date of birth and alternate allocation are examples of inappropriate methods of randomization

[3] Allocation concealment is not the same as blinding. Allocation concealment refers to the method used to implement the random allocation sequence, e.g. number envelopes containing the assignment. It protects the assignment sequence before and until allocation. Blinding protects the sequence after subjects have been allocated.

[4] If the study reported that there was no attrition (ie. no subjects were lost to follow-up, withdrew or were excluded) then reason for withdrawals/dropouts in a non-applicable factor. In such a circumstance, please check "yes" so as to not unfairly lose a point

[5] If there was no subject attrition, a per-protocol analysis is appropriate and an intention-to-treat analysis is not applicable. In such a circumstance, please check "yes" so as to not unfairly lose a point

[6] **Confounder(s) considered:** Subject Selection (inclusion criteria: no prior diagnosis of CHD; exclusion criteria: use of medications that affect blood lipids or blood pressure, BMI >35, total cholesterol concentration >7.5 mmol/L, triglycerides >6.0 mmol/L)

Table 13a. Quality appraisal tool for intervention studies			
Assign a score of 1 for each "Yes", and a score of 0 for each "No/NR".			
Reference (Author, year): Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., Padyab, M., Hu, F.B. and Willet, W.C., 2007			
Item	**Question**	**Score**	
		Yes	**No/NR**
1. Inclusion/Exclusion Criteria	Were the inclusion and/or exclusion criteria for study participation reported?	1	
2. Group Allocation[1]	Was the study described as randomized?	1	
	Was the randomization method reported?		0
	Was the randomization method appropriate?[2]		0
	Was allocation concealed?[3]		0
3. Blinding	Were the study subjects blinded to the intervention received?		0
	Were the research personnel blinded to the intervention received by the subjects?		0
4. Attrition	Was attrition numerically reported?	1	
	Were the reasons for withdrawals and dropouts provided?[4]	1	
5. Exposure/ Intervention	Was the type of food described (e.g. composition, matrix)?	1	
	Was the amount of food described (i.e. dose)?	1	
6. Health Effect	Was the methodology used to measure the health effect reported?	1	
7. Statistical Analysis	Was a between-group statistical analysis of the health effect conducted (i.e. control vs. intervention)?	1	
	Was an intention-to-treat analysis conducted?[5]	1	
8. Potential Confounders	Were potential confounders of the food/health relationship considered?[6]	1	
TOTAL SCORE (maximum of 15):		10	
Higher quality (Score ≥8)		X	
Lower quality (Score ≤7)			

Abbreviation: NR, not reported

[1] Studies without an appropriate control would be excluded at Step 3.

[2] Examples of appropriate randomization include the use of computer-generated random number table, while date of birth and alternate allocation are examples of inappropriate methods of randomization.

[3] Allocation concealment is not the same as blinding. Allocation concealment refers to the method used to implement the random allocation sequence, e.g. numbered envelopes containing the assignment. It protects the assignment sequence before and until allocation. Blinding protects the sequence after subjects have been allocated.

[4] If the study reported that there was no attrition (ie. no subjects were lost to follow-up, withdrew or were excluded) then reason for withdrawals/dropouts is a non-applicable factor. In such a circumstance, please check "yes" so as to not unfairly lose a point.

[5] If there was no subject attrition, a per-protocol analysis is appropriate and an intention-to-treat analysis is not applicable. In such a circumstance, please check "yes" so as to not unfairly lose a point.

[6] **Confounder(s) considered:** Subject Selection (inclusion criteria: postmenopausal women with menstrual periods absent for >1 yr and FSH, serum LH, testosterone and estradiol levels measured to confirm their status; exclusion criteria: secondary causes of hyperglycemia, current or previous (last 6 mos) use of estrogen therapy, treatment with insulin or oral hypoglycemic agents, untreated hypothyroidism, smoking, kidney or liver diseases and breast malignancy or breast cancer)

Table 13b. Quality appraisal tool for prospective observational studies			
Assign a score of 1 for each "Yes", and a score of 0 for each "No/NR".			
Reference (Author, year): Zhang, X., Shu, X-O., Gao, Y-T., Yang, G., Li, Q., Li, H., and Jin, F., 2003			
Item	**Question**	**Score**	
		Yes	**No/NR**
1. Inclusion/Exclusion Criteria	Were the inclusion and/or exclusion criteria for study participation reported (e.g. age greater than 50 years, no history of heart disease)?	1	
2. Attrition	Was attrition numerically reported?	1	
	Were the reasons for withdrawals and dropouts provided[1]		0
3. Exposure	Was the methodology used to measure the exposure reported?	1	
	Was the exposure assessed more than once?		0
4. Health Outcome	Was the methodology used to measure the health outcome reported?	1	
	Was the health outcome verified (e.g. through assessment of medical records, confirmation by a health professional)	1	
5. Blinding	Were the outcome assessors blinded to the exposure status?	1	
6. Baseline Comparability of Groups	Were the subjects in different exposure levels compared at baseline?	1	
7. Statistical Analysis	Was the statistical significance of the trend reported?	1	
8. Potential Confounders	Were the key confounders related to subjects' demographics accounted for in the statistical analysis?[2,3]	1	
	Were key confounders related to other risk factors of the health outcome accounted for in the statistical analysis?[4]	1	
TOTAL SCORE (maximum of 12):		10	
Higher quality (Score≥7)		X	
Lower quality (Score≤6)			

Abbreviation: NR, not reported

[1] If the study reported no attrition (i.e. no subjects were lost to follow-up, withdrew, or were excluded) then reasons for withdrawals/dropouts is a "non-applicable" factor. In such a circumstance, please check "yes" so as to not unfairly lose a point.

[2] **Confounder(s) considered (overall):** Inclusion criteria (healthy women with no previous diagnosis of coronary heart disease, stroke or diabetes at baseline

[3] **Confounder(s) considered (related to subjects' demographics):** Age, level of attended education, family income and season of recruitment were adjusted for in the final model multivariate analysis.

[3] **Confounder(s) considered (related to other risk factors of the health outcome):** Cigarette smoking, alcohol consumption, waist-to-hip ratio, regular exercise, menopausal status, history of hypertension, and intake of total energy, fat, fibre, fruit and vegetables

Supplementary Table S3 is an optional table created by Nutrasource Diagnostics Inc. to present a summary of the quality scores. Although this table is not required by the HC Guidance Document, it is a useful tool to summarize the results of the quality appraisal.

Supplementary Table S3. Summary of quality scores (optional)	
Intervention Study Publications	
Reference	**Score**
High Quality (n=43)	
Allen et al., 2007	15/15
Crouse et al., 1999	14/15
Kreijkamp-Kaspers et al., 2005	14/15
Basaria et al., 2009	13/15
Gardner et al., 2007	13/15
Teede et al., 2001	13/15
Cuevas et al., 2003	12/15
Dent et al., 2001	12/15
Evans et al., 2007	12/15
Baum et al., 1998	11/15
Blum et al., 2003	11/15
Dalais et al., 2003	11/15

(…)

Low Quality (n=2)	
Shorey et al., 1981	7/15
Giovannetti et al., 1986	7/15
Observational Study Publications	
Reference	**Score**
High Quality (n=1)	
Zhang et al., 2003	10/12

5.1.8 STEP 8. TABULATE STUDY FINDINGS PER HEALTH OUTCOME

Objective: *To report the effect of the food exposure, per health outcome, in a consistent way across the studies and to summarize important elements of the studies.*

This section should include information on each biomarker (Table 14a template) or disease end-point (Table 14b template) of interest. All publications that measured the biomarker or disease end-point are to be included in each relevant table.

Health Canada has made a Magnitude of Effect Calculations Excel spreadsheet available to petitioners to assist them in the filing of submission information (www.hc-sc.gc.ca/fn-an/legislation/guide-ld/subm-prep-soum-eng.php). Calculations of the magnitude of effect for intervention publications are to be included as an Appendix in a submission.

In this resource, Table 14a provides an example of information for one biomarker and Table 14b provides an example for one disease endpoint.

See **Table 14a** and **14b** for study findings per health outcome.

See **Appendix 4** for the Excel™ spreadsheet used to assist with the calculations of the magnitude of effect for intervention publications.

Note that Appendix 4 from the full systematic literature review is not included in this report.

Table 14a. Summary of study findings from intervention studies for total cholesterol

Reference and Quality Score	Design	Sample Size	Outcome for which study was powered[1]	Duration of Exposure to soy	Food matrix	Food/Bioactive Intake per Day		Magnitude of Effect[2]			EOT or CFB	p-value[6]
						Soy Protein	Isoflavones	Number[3,4]	Percent[3,5,*]	Units		
Allen et al., 2007 Quality: 15/15	R, C, DB, P	191	NR	12 weeks	Beverage (protein powder)	20 g/day	96 mg aglycones	***0.01***	***0.17%***	mmol/L	EOT	NS (P=0.21)
Azadbakht et al., 2007 Quality: 10/15	R, C, CO	42	NR	8 weeks	SPI	15 g/day	84 mg/day	***-0.23***	***-5.20%***	mmol/L	EOT	P<0.05
Bakhit et al., 1994 Quality: 10/15	R, C, SB, CO	21 (all)	NR	4 weeks	SPI in muffins or beverage	25 g/day	NR	***0.21***	***3.67%***	mmol/L	CFB	NS
		11 (hyper-C)						***-0.24***	***-3.87%***			NS
Basaria et al., 2009 Quality: 13/15	R, C, DB, P	84	NR	12 weeks	Soy Protein Powder	20 g/day	96 mg/day aglycones	***-0.23***	***-4.09%***	mmol/L	EOT	NS (P=0.3210)
Baum et al., 1998 Quality: 11/15	R, C, DB, P	45	NR	24 weeks	SPI	40 g/day	56 mg/day aglycones	***0.10***	***1.64%***	mmol/L	EOT	NS
		43					90 mg/day aglycones	***0.05***	***0.82%***			NS
Blum et al., 2003 Quality: 11/15	R, C, DB, CO	24	NR	6 weeks	Soy Protein	25 g/day	85 mg/day	***0.05***	***0.81%***	mmol/L	EOT	NS (P=0.97)
Borodin et al., 2009 Quality: 10/15	R, DB, CO	28	NR	2 mos	SPI in a cookie	30 g/day	NR	***-0.43***	***-5.94%***	mmol/L	CFB	P<0.01 (within group

(…)

Abbreviations: NR: Endpoint not measured and/or not numerically reported; N/A: not applicable; R: randomized; NR: non-randomized; C: controlled; CO: crossover; SB: single blind; DB: double blind; P: parallel; CFB: change from baseline; EOT: end of treatment; NS: not significant

1 If the publication did not indicate an outcome for which it was powered, state N/A.

2 Excel spreadsheet that was used to derive these calculations is included in Appendix 4.

3 Computed values are italicized and bolded.

4 For studies with a control/comparison group, the effect was reported as: (Mean end-of-treatment - baseline) treatment group - (Mean end-of-treatment - Mean baseline) control group. For studies with a control/comparison group that do not report baseline values, the effect was reported as: Mean end-of-treatment treatment group - Mean end-of-treatment control group. For studies without a comparison group, the effect was reported as: Mean end-of-treatment - Mean baseline.

5 For studies with a control/comparison group, the effect was reported as: ((Mean end-of-treatment – Mean baseline)*100%treatment group – ((Mean end-of-treatment – Mean baseline)/Mean baseline)*100% control group. For studies with a control/comparison group that do not report baseline values, the effect was reported as: ((Mean end-of-treatment treatment group – Mean end-of-treatment control group)/Mean end-of-treatment control group)*100%. For studies without a comparison group, the effect was reported as: (Mean end-of-treatment – Mean baseline)/Mean baseline*100%.

6 Between group p-values. If between group p-values are not reported in the publication, within-group values are used with indication of the values that apply to within-group analyses.

Table 14b. Summary of study findings from prospective observational studies for risk of confirmed coronary heart disease

Reference and Quality Score	Design (Prospective cohort or Nested case–control)	Study Population and Final Sample Size	Quartile	Exposure (Dietary Intake/ Circulating Levels)	Incidence of Health Outcome	Multi-variate Adjusted Risk Ratios Between Different Centiles			
						Hazards Ratio	Relative Risk	95% CI	P trend
Zhang et al., 2003 Quality: 10/12	Prospective cohort	**75 000** Chinese postmenopausal women, aged 40 to 70 years in the study population **64 915** Chinese postmenopausal women (mean age of 60 years at recruitment) in final sample size	1st quartile of soy protein intake	<4.50 g/day	10	N/A	1.0		0.009
			2nd quartile of soy protein intake	4.50–7.35 g/day	12	N/A	0.99	0.42–2.32	
			3rd quartile of soy protein intake	7.36–11.18 g/day	13	N/A	0.86	0.36–2.06	
			4th quartile of soy protein intake	≥ 11.19 g/day	5	N/A	0.20	0.06–0.7	

Abbreviations: N/A: not applicable; NR: not reported

5.1.9 STEP 9. ASSESS CAUSALITY

5.1.9 STEP 9A. RATE CONSISTENCY

Objective: *To rate the consistency of findings across studies, per health outcome, with regard to the direction of effect of the food on the health outcome with consideration given to study quality.*

This step aims to determine whether most of the publications show the same effect of the food on health (whether positive, negative, or otherwise), or whether the results are inconsistent (i.e. completely different effect, or no effect). It is important to suggest plausible explanations for moderate or low consistency in the narrative part of this section, as well as to comment on the evidence related to study design (e.g. if observational designs tend to show an effect whereas interventions do not).

This section should include one Table 15 for each biomarker (Table 15a template) or disease end-point (Table 15b template) of interest. Each treatment group that measured the biomarker or disease end-point is to be included in the relevant table.

In this resource, Table 15a-1 provides an example for one biomarker and Table 15b-1 provides an example for one disease endpoint.

This systematic investigation examined a total of 46 publications, consisting of 45 intervention study publications (79 treatment groups) and 1 observational study publication to determine the effects of soy protein on biomarkers of CVD. Please refer to **Tables 15a-1** to **15a-6** and **Table 15b-1** to **15b-3** for an analysis of the consistency ratings.

Table 15a-1.
Rating of consistency in direction of effect for intervention studies, considering study quality

Health Outcome: Total Cholesterol							
A. Total number studies included: 76 studies (in 43 publications)							
Statistical Significance (SS)							
B1. # studies with a SS effect of exposure (p<0.05): **18**				**B2.** # studies with a non-SS effect of exposure (p>0.05): **57**			
Direction of Effect[1]							
C1. # studies from B1 with a SS favourable effect of the exposure: **18**		**C2.** # studies from B1 with a SS unfavourable effect of the exposure: **0**		**C3.** # studies from B2 with a non-SS favourable effect of the exposure: **32**		**C4.** # studies from B2 showing either a non-SS unfavourable effect or no distinguishable effect of the exposure: **26**	
Study Quality							
D1. # higher quality studies from C1: **17**	**D2.** # lower quality studies from C1: **1**	**D3.** # higher quality studies from C2: **0**	**D4**. # lower quality studies from C2: **0**	**D5.** # higher quality studies from C3: **31**	**D6.** # lower quality studies from C3: **1**	**D7.** # higher quality studies from C4: **25**	**D8.** # lower quality studies from C4: **1**
Consistency Rating on Direction of Favourable Effect							
(C1+C3)/A1 x 100% (18+32)/76 x 100% = **65.79%**				High (≥75%) Moderate (60%–74%) **X** Low (<60%)			
Consistency Rating on Direction of Favourable Effect in Higher Quality Studies							
(D1+D5)/(D1+D3+D5+D7)x100% (17+31)/(17+0+31+25)x100% = **66.76%**				High (≥75%) Moderate (60%–74%) **X** Low (<60%)			

[1] Direction of effect assesses whether the health outcome is changing in a favourable (i.e. beneficial) direction with exposure to the foods, or in an unfavourable (non-beneficial) direction, without regard to statistical significance; for total cholesterol, a decrease was interpreted as a favourable effect, while an increase was interpreted as an unfavourable effect.

Although it is not required by Health Canada's Guidance Document to include a detailed list of the studies included in each section of the table, it is useful to document which treatment groups were classified in which section of Table 15a, as this permits tracking of the treatment groups through the consistency of effect calculations. See the Supplement to Table 15a-1 for an example of a workchart summarizing this information.

Supplement to Table 15a-1:
Workchart of treatment groups classified by study quality, and statistical significance and direction of effect for calculation of consistency of effect for Total Cholesterol

D1: treatment groups from higher quality studies with a SS favourable effect of the exposure

Ashton & Ball, 2000 (Tofu)	Potter et al., 1993 (Soy flour)
Azadbakht et al., 2007 (SPI)	Shidfar et al., 2009 (Roasted soybeans)
Azadbakht et al., 2007 (Soy nuts)	Teixeira et al., 2000 (20 g soy protein)
Borodin et al., 2009 (SPI in a cookie)	Teixeira et al., 2000 (30 g soy protein
Crouse et al, 1999 (High LDL, 25 g soy protein + 37 mg isoflavones)	Teixeira et al., 2000 (50 g soy protein)
Crouse et al, 1999 (High LDL, 25 g soy protein + 62 mg isoflavones)	Thorp et al., 2008 (24 g soy protein)
Greany et al., 2004 (Normocholesterolemic)	Vigna et al., 2009
Lichtenstein et al., 2002 (LDL ≥4.14)	Wang et al., 2004
Potter et al., 1993 (SPI)	

D2 : treatment groups from lower quality studies with a SS favourable effect of the exposure

Shorey et al., 1981

D3 : treatment groups from higher quality studies with a SS unfavourable effect of the exposure

None

D4: treatment groups from lower quality studies with a SS unfavourable effect of the exposure

None

D5: treatment groups from higher quality studies with a non-SS favourable effect of the exposure

Bakhit et al., 1993 (Hypercholesterolemics)	Meinertz et al., 1990 (High cholesterol diet)
Basaria et al., 2009	Potter et al., 1998 (50 g soy protein + 56 mg aglycones)
Crouse et al., 1999 (High LDL, 25 g soy protein +3 mg Isoflavones)	Potter et al., 1998 (50 g soy protein + 90 mg aglycones)
Crouse et al., 1999 (High LDL, 25 g soy protein +27 mg Isoflavones)	Shige et al., 1998
Cuevas et al., 2003	Steinberg et al., 2003 (25 g soy protein + 1.82 mg isoflavones)
Dalais et al., 2003	Steinberg et al., 2003 (25 g soy protein + 107.67 mg isoflavones)
Dent et al., 2001 (40 g soy protein + 4.4 mg isoflavones)	Teede et al., 2001
Dent et al., 2001 (40 g soy protein + 80.4 mg isoflavones)	Teixeira et al., 2000 (40 g soy protein)
Greany et al., 2004 (Hypercholesterolemics)	Thorp et al., 2008 (12 g soy protein)
Higashi et al., 2000	Van Raaij et al., 1982 (53 g SPI)
Ma et al., 2005	Van Raaij et al., 1982 (55 g SPC)
Matthan et al., 2007 (Soybeans)	Wong et al., 1998 (Normocholesterolemics)
Matthan et al., 2007 (Soy flour)	Wong et al., 1998 (Hypercholesterolemics)
Matthan et al., 2007 (Soy beverage)	Welty et al., 2007 (Normotensives)
McVeigh et al., 2006 (32 g soy protein + 1.64 mg isoflavones)	Welty et al., 2007 (Hypertensives)
McVeigh et al., 2006 (32 g soy protein + 61.7 mg isoflavones)	

D6: treatment groups from lower quality studies with a non-SS favourable effect of the exposure

Giovannetti et al., 1986 (71 g soy protein + 38% fat)

D7: treatment groups from higher quality studies showing a non-SS unfavourable effect or no distinguishable effect of the exposure

Allen et al., 2007	Kurowska et al., 1997
Bakhit et al., 1994 (Normocholesterolemics)	Lichtenstein et al., 2002 (Normocholesterolemics)
Baum et al., 1998 (40 g soy protein + 56 mg aglycones)	Meinertz et al., 2002 (133 g soy protein + 14.6 mg aglycones)
Baum et al., 1998 (40 g soy protein + 90 mg aglycones)	Meinertz et al., 2002 (133 g soy protein + 317.9 mg aglycones)
Blum et al., 2003	Meinertz et al., 1990 (Low cholesterol diet)
Crouse et al., 1999 (Low LDL + 3 mg aglycones)	Nilausen & Meinertz, 1998
Crouse et al., 1999 (Low LDL + 27 mg aglycones)	Santo et al., 2008 (26 g soy protein + 56.2 mg aglycones)
Crouse et al., 1999 (Low LDL + 37 mg aglycones)	Santo et al., 2008 (26 g soy protein + 0.7 mg aglycones)
Crouse et al., 1999 (Low LDL + 62 mg aglycones)	Van Raaij et al., 1981 (SPI + casein)
Evans et al., 2007	Van Raaij et al., 1981 (soy protein only)
Gardner et al., 2001 (42 g soy protein + 80 mg aglycones)	West et al., 2005 (men)
Gardner et al., 2001 (42 g soy protein + 3 mg aglycones)	West et al., 2005 (women no HRT)
Gooderham et al., 1996	

D8: treatment groups from lower quality studies showing a non-SS unfavourable effect or no distinguishable effect of the exposure

Giovanetti et al., 1986 (71 g soy protein + 23% fat)

SS: statistical significance

INTERPRETATION

Total Cholesterol

Table 15a-1 shows that the proportion of treatment groups demonstrating a favourable direction of effect on TC following soy consumption, regardless of statistical significance and study quality, is MODERATE (65.8%). When considering only publications that were assigned high quality scores, the proportion of treatment groups demonstrating a favourable direction of effect on TC following soy consumption, regardless of statistical significance, remains MODERATE (66.8%). Thus, the results of 76 identified treatment groups (in 43 publications) indicate a moderately beneficial effect on TC following soy consumption.

This resource shows one sample table for a biomarker (Table 15a-1) as it adequately illustrates what would be required for such a table. Tables 15a-2 through 15a-6 have been omitted to allow for reasonable report size; however, the narrative that would accompany those tables is provided.

LDL Cholesterol

Table 15a-2 shows that the proportion of treatment groups demonstrating a favourable direction of effect on LDL-C following soy consumption, regardless of statistical significance and study quality, is HIGH (78.1%). When considering only publications that were assigned high quality scores, the proportion of treatment groups demonstrating a favourable direction of effect on LDL-C following soy consumption, regardless of statistical significance, remains HIGH (77.4%). Thus, the results of 64 identified treatment groups (in 37 publications) indicate a highly consistent beneficial effect on LDL-C following soy consumption.

HDL Cholesterol

Table 15a-3 shows that the proportion of treatment groups demonstrating a favourable direction of effect on HDL-C following soy consumption, regardless of statistical significance and study quality, is MODERATE (64.0%). When considering only publications that were assigned high quality scores, the proportion of treatment groups demonstrating a favourable direction of effect on HDL-C following soy consumption, regardless of statistical significance, is MODERATE (65.3%). Thus, the results of 75 identified treatment groups (in 45 publications) indicate a moderate beneficial effect on HDL-C following soy consumption.

Triglycerides

Table 15a-4 shows that the proportion of treatment groups demonstrating a favourable direction of effect on TG following soy consumption, regardless of statistical significance and study quality, is LOW (59.7%). When considering only publications that were assigned high quality scores, the proportion of treatment groups demonstrating a favourable direction of effect on TG following soy consumption, regardless of statistical significance, is MODERATE (62.3%). Thus, the results of 72 identified treatment groups (in 40 publications) indicate a moderate consistency for the effect on TG following soy consumption.

Systolic Blood Pressure

Table 15a-5 shows that the proportion of treatment groups demonstrating a favourable direction of effect on systolic blood pressure following soy consumption, regardless of statistical significance and study quality, is LOW (42.9%). Given that all of the publications in this category were assigned high quality scores, the proportion of treatment groups demonstrating a favourable direction of effect on systolic blood pressure following soy consumption, regardless of statistical significance, remains LOW (42.9%). Thus, the results of 14 identified treatment groups (in 9 publications) indicate a low consistency for the effect on systolic blood pressure following soy consumption.

Diastolic Blood Pressure

Table 15a-6 shows that the proportion of studies demonstrating a favourable direction of effect on diastolic blood pressure following soy consumption, regardless of statistical significance and study quality, is LOW (42.9%). When considering only publications that were assigned high quality scores, the proportion of studies demonstrating a favourable direction of effect on diastolic blood pressure following soy consumption, regardless of statistical significance, remains LOW (42.9%). Thus, the results of 14 identified studies (in 9 publications) indicate a low consistency for the effect on diastolic blood pressure following soy consumption.

Table 15b-1.
Rating of consistency in direction of effect for prospective observational studies, considering study quality

Health Outcome: Risk of Coronary Heart Disease					
A. Total number studies included: 1[1]					
Direction of Effect					
B1. # studies from A showing a trend for risk reduction (p<0.05): **1**		**B2.** # studies from A showing a trend for increase in risk (p>0.05): **0**		B3. # studies from A showing no effect (p>0.05): **0**	
Study Quality:					
C1. # higher quality studies from B1: **1**	**C2.** # lower quality studies from B1: **0**	**C3**. # higher quality studies from B2: **0**	**C4.** # lower quality studies from B2: **0**	**C5.** # higher quality studies from B3: **0**	**C6.** # lower quality studies from B3: **0**
Consistency Rating on Direction of Favourable Effect (Risk Reduction)		**Consistency Rating on Direction of Unfavourable Effect**		**Consistency Rating on No Effect**	
B1 x 100%= A **(1/1)x100% =100%**	High (≥75%) [X] Moderate (60%–74%) [ ] Low (<60%) [ ]	**B2 x 100%= A** **(0/1)x100% =0%**	High (≥75%) [ ] Moderate (60%–74%) [ ] Low (<60%) [X]	**B3 x 100%= A** **(0/1)x100%= 0%**	High (≥75%) [ ] Moderate (60%–74%) [ ] Low (<60%) [X]
Consistency Rating on Direction of Favourable Effect in Higher Quality Studies					
C1/ (C1+C3+C5) x 100% = **1/(1+0+0) x 100% = 100%**		High (≥75%) [X] Moderate (60%–74%) [ ] Low (<60%) [ ]			

[1] Zhang et al., 2003

This resource shows one sample table for a disease endpoint (Table 15b-1) as it adequately illustrates what would be required for such a table. Tables 15b-2 and 15b-3 have been omitted to allow for reasonable report size; however, the narrative that would accompany those tables is provided.

Risk for Coronary Heart Disease

Table 15b-1 demonstrates that the proportion of observational studies showing a decrease in risk for coronary heart disease following soy protein consumption, regardless of statistical significance and study quality, is HIGH (100%). Considering only those publications assigned high quality scores, regardless of statistical significance, the proportion of studies demonstrating a favourable direction of effect on risk for coronary heart disease remains unchanged (100%). Overall, the results of the consistency rating of one identified study indicate that the effect of soy protein consumption on risk for coronary heart disease may be consistently beneficial. However, considering that only one observational study was evaluated in this consistency rating, results should be interpreted with caution.

Risk for Myocardial Infarction

Table 15b-2 demonstrates that the proportion of observational studies showing a decrease in risk for myocardial infarction following soy protein consumption, regardless of statistical significance and study quality, is HIGH (100%). Considering only those publications assigned high quality scores, regardless of statistical significance, the proportion of studies demonstrating a favourable direction of effect on risk for myocardial infarction remains unchanged (100%). Overall, the results of the consistency rating of one identified study indicate that the effect of soy protein consumption on risk for myocardial infarction may be consistently beneficial. However, considering that only one observational study was evaluated in this consistency rating, results should be interpreted with caution.

Risk of Death from Coronary Heart Disease

Table 15b-3 demonstrates that the proportion of observational studies showing a decrease in risk of death from coronary heart disease following soy protein consumption, regardless of statistical significance and study quality, is LOW (0%). Considering only those publications assigned high quality scores, regardless of statistical significance, the proportion of studies demonstrating a favourable direction of effect on risk of death from coronary heart disease remains unchanged (0%). Overall, the results of the consistency rating of one identified study indicate that there is no effect of soy protein consumption on risk of death from coronary heart disease. However, considering that only one observational study was evaluated in this consistency rating, results should be interpreted with caution.

SUGGESTIONS FOR PLAUSIBLE EXPLANATIONS FOR MODERATE AND/OR LOW CONSISTENCIES

Processing of Soy Protein

When soy protein is degraded and digested in the intestine, the resulting peptides are absorbed into the portal circulation. Approximately 20%–55% of most conventional soy protein preparations are biologically active, water-soluble peptide fractions. These peptides are proposed to have a lipid-lowering effect in the liver and peripheral tissues. Both *in vitro* and clinical research has shown that the most prominent of the four known peptide fractions, 7S globulin, has the ability to enhance the activity of LDL receptors in liver cells. Innovations in soy processing techniques have led to an isolated soy protein that maintains up to 95% of its water solubility, resulting in an associated increase in cardiovascular benefit when compared to conventional soy protein preparations (Hoie et al., 2007).

While numerous studies have demonstrated that soy (protein) intake is related to significant beneficial decreases in serum levels of TC and LDL-C, the mechanism(s) behind such cardiovascular benefits associated with soy intake is (are) not yet fully understood. A potential confounding factor for differences in cardiovascular endpoints could be the degree of variability in soy protein preparations in terms of the presence or quantity of isoflavones, phospholipids, and cotyledon fibre, in addition to differences in protein structure and amino acid sequence. All of these factors are directly related to the type of processing used to isolate soy protein (Dewell et al., 2006; Hoie et al., 2005). Considerable variation in CVD-related endpoints following soy protein interventions have been noted when soy

protein is subject to alcohol extraction in processing (Dewell et al., 2006). This technique is known to significantly decrease the levels of isoflavones as well as other components of soy that may act to lower cholesterol, such as saponins, and phytic acid. Moreover, alcohol extraction is indicated in the degradation of protein and active peptides, which may influence the effects of soy protein on cholesterol metabolism (Dewell et al., 2006; Zhan and Ho, 2005).

Meinertz et al. (2002) compared the effects of alcohol-extracted soy protein (without isoflavones) to intact soy protein (with isoflavones) in normolipidemic women and found no effective difference between the treatments. Similarly, van Raaij et al. (1982) reported no significant differences between the effect of highly purified soy protein isolate compared to less refined soy protein concentrate on TC or HDL-C in healthy adults, although effects may have been confounded by weight loss. Conversely, Matthan et al. (2007) reported that soybeans and soy flour had no significant effect on LDL-C, while soy beverage significantly reduced LDL-C by 4.4%. The soy beverage treatment also significantly increased subjects' HDL-C levels compared to soybeans and soy flour. Soybean processing may have affected these three treatments differently, thus explaining the differences observed. Kurowska et al. (1997) reported no significant effect of 4 weeks of soy beverage consumption (containing 31 g soy protein) on TC, whereas Ashton and Ball (2000) found 4 weeks of tofu consumption (containing 35 g soy protein) to significantly reduce TC. Teede et al. (2001) administered soy protein isolate powder that was prepared by dehulling and defatting soybeans and blending for 45 minutes. This intervention significantly reduced systolic and diastolic blood pressure and TG levels, but had no significant effect on LDL-C, HDL-C or TC. Soybean processing and/or food form may have influenced results from these studies, making data difficult to compare accurately.

Overall, the processing techniques used to manufacture isolated soy protein and/or whole soy food treatments in many of the remaining included intervention studies could have varied, as many did not report on processing details. The methods of isolation (such as filtration or precipitation), wash steps (such as the type and quality of solvents), heat, enzyme treatment, and drying have all been reported to affect the composition of a soy product (Klein et al., 2010). This potential variability may have contributed to the low or moderate consistency ratings. Furthermore, processing and development of soy products have evolved over time, which may make it difficult to compare the soy protein isolate/concentrates used in earlier studies to more current investigations (Klein et al., 2010). As the magnitude of change in lipid parameters may differ depending on the overall structure and physicochemical properties of soy protein chosen for dietary intervention, future studies should consider processing techniques and their associated structural implications. This type of investigation will allow for a more relevant conclusion to be drawn about the mechanism of lipid reductions associated with soy protein intake.

Presence or Absence of Isoflavones

Although it has been supported in animal experiments that the serum lipid-lowering properties of soy protein may primarily be related to the presence or absence of isoflavones, this postulation has been met with conflicting views (Zhan and Ho, 2005). As discussed previously, processing techniques are largely influential on the presence of isoflavones within soy protein.

A 2005 meta-analysis of 23 randomized controlled trials published between 1995 and 2002 indicated that soy protein interventions with intact isoflavones were associated with significant decreases in serum TC, LDL-C, and TG, in addition to significant increases in HDL-C. These changes were related to subject sex and their baseline serum lipid concentrations. Larger reductions were seen in men than in women, as well as in subjects with initially high levels of serum lipids compared to subjects with relatively healthy initial levels (Zhan and Ho, 2005).

Approximately 53% of the studies included in this investigation involved soy protein or soy concentrate treatments containing isoflavones. The remaining studies (~47%) either did not report isoflavone content or involved a soy protein or soy concentrate treatment containing minimal isoflavone content. Some have attributed the positive changes in cardiovascular profiles associated with dietary soy intake directly to soy isoflavones. While there is some evidence to support this hypothesis in the literature reviewed herein, the studies in this review do not fully support that cardiovascular changes after dietary intervention with soy protein are dependent upon the presence or absence of isoflavones. This discrepancy in isoflavone concentrations could account for some of the inconsistency within the breadth of evidence investigated, especially considering that isoflavone dosages ranged from negligible amounts to over 300 mg/day.

In addition to the inconsistency due the presence or absence of isoflavones within a soy protein intervention, structural isomers of isoflavones and external factors affecting isoflavone bioavailability are also potential confounders.

Additional detailed information on isoflavones from the full systematic literature review (isomers of isoflavones, isoflavone bioavailability, equol production, gut microbiota and genetic polymorphisms) has been omitted to allow for reasonable report size. The remaining text adequately serves to demonstrate the type of narrative that should be included in such a literature review.

Background Diet

It has been demonstrated that dietary supplementation with fibre and other food components is more successful in the control of cholesterol levels than reducing the intake of dietary cholesterol itself (Hoie et al., 2007). While the breadth of evidence investigated in this systematic scientific literature review supports this finding, it must be noted that not all interventions adequately controlled for these other food components in the background diet of subjects. In order to ensure that the effects seen are attributable to soy protein specifically, an intervention study would have to exercise the highest level of control in terms of what other nutrients subjects are consuming and ensure that this consumption is similar between treatment and control groups. Of the 79 studies analyzed in this review, only 58 controlled for background diet, and ensured that all subjects consumed similar amounts of macronutrients. The methods of isolation (such as filtration or precipitation), wash steps (such as the type and quality of solvents), heat, enzyme treatment, and drying have all been reported to affect the composition of a soy product (Klein et al., 2010). This potential variability may have contributed to the low or moderate consistency ratings. Some nutrients can independently affect blood lipids and should therefore be kept consistent between treatment and control groups in order to accurately assess the effects of soy protein. Such nutrients include: fibre (amount and type), polyunsaturated fatty acids, saturated fats and cholesterol (FDA, 2009). In controlling for these nutrients, future research into this potential food–health association may attempt to better attribute changes in lipoprotein levels to soy protein and its components.

Dietary macronutrient intake can influence the microbial profile of the gastrointestinal system and consequently, an individual's ability to produce equol. There is mounting evidence that microbial populations, specifically populations of the large intestine, respond to dietary modifications. For example, the type and quantity of carbohydrate typically ingested alters the pH of the gut lumen, which can influence bacterial metabolism within the large intestine (Flint et al., 2007). The amount of soy consumed on a regular basis can also affect soy metabolism. Individuals who typically consume soy-derived foods on a regular basis (such as those in Asian countries, or individuals following a vegetarian-type diet) have been reported to have a greater capacity to produce equol (Setchell and Cole, 2006).

The frequency of equol producers among vegetarian populations has been reported to be 59%, similar to that reported in Japanese adults consuming soy. This frequency is much higher than for nonvegetarian adults (25%), suggesting that dietary components other than soy influence bacterial equol synthesis (Setchell and Cole, 2006). For example, it has been suggested that habitual dietary fat intake may decrease the capacity of gut microbial flora to synthesize equol (Rowland et al., 2000). Other studies investigating the influence of long-term dietary patterns on soy isoflavone metabolism have indicated that the ability to produce equol is increased by the intake of lipids from animal sources (Gardana et al., 2009; Hedlund et al., 2005; Wiseman et al., 2004). While most studies agree that increased soy intake is related to an increased capacity to produce equol, one study noted that this ability is up to 4.7 times higher in individuals who also consume animal meat regularly (Hedlund et al., 2005). It has also been suggested that the relationship of equol production and chronic soy consumption is due only to increased activity of β-glucosidase (Wiseman et al., 2004).

Finally, as the studies included in this investigation were conducted in a variety of countries (e.g. USA, Australia, Iran, Israel, Russia, Chile, Canada, Japan, Netherlands, Italy, and Denmark), it should be acknowledged that habitual background diets could have varied considerably. As 29 studies had subjects consume soy protein in the context of their habitual diet, this variability may have subsequently influenced the consistent beneficial effect of soy protein.

Influence of Exercise

It is important to note that few of the interventions included in this investigation accounted for the background exercise habits of subjects. Only two studies attempted to account for the exercise habits of subjects through the use of physical activity questionnaires. The importance of physical activity in cardiovascular research has been noted by many investigators, as exercise is known to differentially affect both postprandial lipemia as well as lipid parameters over long periods of time (Graham, 2004; Leung et al., 2008).

Additionally, regularly active individuals who abstain from exercise for 2 days prior to acute dietary challenges (e.g. oral fat tolerance tests) show increased fasting levels of both total and HDL-C, as well as detrimental changes in postprandial lipemia (Graham, 2004). This suggests that the metabolic impact of exercise on certain CVD risk factors may be attributed to the most recent bout of exercise rather than to habitual training (Graham, 2004). In any case, exercise has been shown to be directly related to the impact of consecutive meals on cardiovascular lipid pools, and therefore to overall cardiovascular health.

Other researchers have noted that habitual physical activity also plays a major role in the maintenance of a favourable cardiovascular profile with respect to lipoprotein levels, and is further correlated with age (Lara et al., 2010). This has been specifically demonstrated in postmenopausal women, who comprised a large proportion of the subject population among the included interventions. Impaired cardiovascular characteristics associated with age correlate with a reduced capacity for aerobic exercise in healthy individuals as well as those with existing CVD (Parker et al., 2010). This interplay between age, gender and physical activity, in reference to CVD risk, highlights the need to account for these factors in future investigations.

Exercise is also positively associated with endothelial and vascular smooth muscle function through the regulation of genes mediating oxidative metabolism, inflammatory cytokines, apoptosis, and cellular growth and proliferation (Leung et al., 2008). Endurance training causes the release of nitric oxide and prostacyclin, which decrease vascular permeability of plasma lipoproteins and reduce platelet aggregation (Whyte and Harold Laughlin, 2010). Vasculature remodelling is also influenced by physical activity, through the formation of new capillary networks (angiogenesis) and the growth of collateral

arterioles, which are capable of compensating for the loss of function of occluded arteries (arteriogenesis) (Leung et al., 2008). Thus, exercise levels should be measured in all future cardiovascular research as they so strongly relate to overall vascular health.

Body composition has also been shown to correlate with lipid parameters in both physically active and sedentary individuals. For example, a cross-sectional study of 113 non-smoking men compared the cardiovascular risk factor profile of individuals who exercised habitually to that of sedentary individuals (O'Donovon et al., 2005). This study also investigated subgroups to specifically look at lean exercisers and lean sedentary individuals. Results indicated that the levels of total and LDL-C were lower in lean exercisers than in lean sedentary men, supporting an influence of exercise on these risk factors. Linear regression models indicated that time spent in vigorous activity was a significant predictor of these two lipid parameters. Although this study noted very little influence of exercise on levels of HDL-C and TG, levels of these two parameters were favourable in both lean subgroups as compared to their heavier counterparts. Future research should thus measure waist circumference in addition to accounting for subject exercise habits for a more accurate reflection of the potential effect of soy protein on cardiovascular disease.

Population Health Status – Hypercholesterolemic vs. Normocholesterolemic Subjects

When investigating the effects of dietary interventions aiming to beneficially alter cholesterol profiles and their associated impact on risk for cardiovascular disease, it is easier to quantify these biochemical changes in subjects who have initially high cholesterol levels at the beginning of intervention.

A meta-analysis of 23 randomized controlled trials published between 1995 and 2002 indicated that soy protein interventions with intact isoflavones were associated with significant decreases in serum TC, LDL-C and TG. Significant increases in HDL-C were also reported. These changes were directly related to baseline serum lipid concentrations. Larger reductions were seen in men than in women, as well as in subjects with initially high levels of serum lipids compared to subjects with relatively healthy initial levels (Zhan and Ho, 2005).

Studies investigating the effect of incorporating unprocessed soy protein into the diet have looked at both normocholesterolemic and hypercholesterolemic subject groups, and supported greater reductions in cholesterol parameters in groups with initially high levels of cholesterol (Dewell et al., 2006). The study completed by Greany et al. (2004) investigated the effects of soy protein on lipid levels in individuals who were either normo- or hypercholesterolemic. When subjects were stratified by serum TC levels, those who were hypercholesterolemic had significant reductions in total and LDL-C as well as TG, accompanied by increases in HDL-C compared to the milk protein control period. There were no significant effects of soy protein supplementation in the group with normocholesterolemic levels. These results appear to suggest that the cholesterol lowering effects of soy protein are limited to hypercholesterolemic individuals (Greany et al., 2004).

A second study investigated the effects of increasing isoflavone dosages with 25 g of soy protein in hyper- and normocholesterolemic subjects (Crouse et al., 1999). Following 9 weeks of treatment, only hypercholesterolemic individuals consuming 25 g of soy protein with 37 mg or 62 mg of aglycone isoflavones had significant decreases in total and LDL-C compared to the hypercholesterolemic control group consuming casein. The hypercholesterolemic individuals consuming 25 g soy protein with 62 mg of aglycone isoflavones also saw a significant reduction in serum TG, compared to the hypercholesterolemic control group consuming casein.

Another study investigating the effects of soy protein in hyper- and normocholesterolemic individuals saw significant effects of soy protein on plasma lipids in both populations (Wong et al., 1998). Subjects were administered 50 g soy protein isolate for 5 weeks and decreases in LDL-C and the LDL:HDL cholesterol ratio were seen in both groups. Conversely, Bahkit et al. (1994) reported no significant effect of 25 g soy protein isolate for 4 weeks on TC, HDL-C, LDL-C or TG levels, even when results were stratified to include only hypercholesterolemic subjects. Perhaps variable dose and duration could also explain the differences between the aforementioned studies.

Age, Gender and Menopausal Status

It is well documented that levels of lipid parameters tend to destabilize with increasing age; however, the mechanisms of this age-related dyslipidemia remain to be characterized. It has been proposed that this dyslipidemia may be related to hormonal changes, a decline in fractional clearance of LDL, decreased activity of the rate-limiting enzyme in bile acid synthesis (cholesterol 7α-hydroxylase (C7αOH)) and an associated reduced ability to remove cholesterol (Trapani et al., 2010).

These age-related changes in lipid parameters and the associated CVD risk are different between men and women. As women age, they undergo greater declining changes in cardiac and autonomic characteristics at a resting state than do men (Parker et al., 2010). Greater benefits with respect to decreased serum TC, LDL-C and TG and increased HDL-C were noted in men than in women in a 2005 meta-analysis of soy protein interventions with intact isoflavones (Zhan and Ho, 2005).

Three publications investigated the effect of soy (protein) and commented on the effect of gender (Meinertz et al., 2002; Teede et al., 2001; West et al., 2005). Various metabolic responses to soy protein were similar between men and women. Males and pre- and postmenopausal females ranging from 20–84 years of age were included in the studies comprising this investigation. Of the 45 publications, 17 investigated the effect of soy protein in postmenopausal women, 1 in young premenopausal women and 1 in perimenopausal women, 12 in men alone, and 14 in a mixed population of men and women. Variation in age, gender and menopausal status among the plethora of subjects considered in this systematic scientific literature review may have contributed to the low/moderate calculated inconsistencies. These factors and their associated implications for lipoprotein levels and CVD risk should always be considered in future investigations into the potential benefits of soy-related dietary modifications.

Conclusions

Taken together, it is clear that many factors are involved in the potential relationship between soy consumption and cholesterol levels. As a result, there are a large number of potential confounders which may or may not have contributed to the inconsistencies seen in the literature investigated here. Although a 2006 review of soy protein and isoflavone supplementation in the control of plasma cholesterol supports the overall findings of the current systematic review, results of human clinical trials have been largely inconsistent, and raise substantial concerns regarding the clinical relevance of the proposed relationship between soy consumption and cardiovascular disease (Dewell et al., 2006).

COMMENT ON EVIDENCE RELATED TO STUDY DESIGN

While the consistency of the beneficial effect of soy consumption appears to vary according to specific health outcome, the one observational study included in this systematic scientific literature review demonstrated a consistent beneficial relationship between soy protein intake and the relative risk for coronary heart disease and nonfatal myocardial infarction (see **Table 15b-1** and **Table 15b-2**). The researchers concluded that this soy food (containing soy protein) consumption was significantly and inversely associated with the risk of coronary heart disease among Chinese women (Zhang et al., 2003).

It has been reported that a major distinction between epidemiological and clinical investigations is that population-based studies examine soy intake from whole soy foods while clinical research is typically based on administration of isolated constituents (Klein et al., 2010). Additionally, this observational study (possibly due to its long-term prospective design and Chinese sample population) may have been able to demonstrate a consistent beneficial effect on health outcomes of CVD that the intervention studies (involving European, Russian, Australian, South American, Japanese, Middle Eastern and North American women and shorter study duration) did not. Overall, the diet and lifestyle differences (e.g. tobacco use, alcohol intake, activity level) between the different cultures may be largely responsible for the notable differences in consistency ratings between observational and intervention studies. Whether soy consumption is inversely associated with risk for coronary heart disease or nonfatal myocardial infarction among Western women has yet to be investigated in a well-designed prospective cohort observational study.

5.1.9 STEP 9B. RATE THE STRENGTH OF THE ASSOCIATION

Objective: *To assess the strength of the association between the food and health outcome by considering the proportion of studies that showed statistical significance at p<0.05 among all included studies.*

The systematic investigation examined a total of 46 publications, including 45 intervention study publications (79 treatment groups) and 1 observational study publication, to determine the effects of soy protein on biomarkers of CVD. The calculations below were performed based on values obtained in **Table 15a-1** to **Table 15a-6** and **Table 15b-1** to **Table 15b-3**, which rated consistency in direction of effect for intervention and observational studies, considering study quality.

Total Cholesterol

Among treatment groups which showed statistical significance (D1+D2/A), 18 of 76 treatment groups (23.7%) supported a statistically significant decrease in TC following soy consumption. Thus, the results of 76 identified treatment groups (in 43 publications) indicate that there is a LOW strength of association between soy consumption and TC.

Considering studies of higher quality presented in the consistency rating (**Table 15a-1**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, 23.3% of the treatment groups from high quality studies were found to show a statistically significant favourable effect on TC.

LDL-Cholesterol

Among treatment groups which showed statistical significance (D1+D2/A), 20 of 64 treatment groups (31.3%) supported a statistically significant decrease in LDL-C following soy consumption. Thus, the results of 64 identified treatment groups (in 37 publications) indicate that there is a LOW strength of association between soy consumption and LDL-C.

Considering studies of higher quality presented in the consistency rating (**Table 15a-2**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, only 32.3% of the treatment groups from high quality studies were found to show a statistically significant favourable effect on LDL-C.

HDL Cholesterol

Among treatment groups which showed statistical significance (D1+D2/A), 13 of 75 treatment groups (17.3%) supported a statistically significant increase in HDL-C following soy consumption. Thus, the results of 75 identified treatment groups (in 45 publications) indicate that there is a LOW strength of association between soy consumption and HDL-C.

Considering studies of higher quality presented in the consistency rating (**Table 15a-3**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, 16.7% of the treatment groups from high quality studies were found to show a statistically significant favourable effect of soy consumption on HDL-C.

Triglycerides

Among treatment groups which showed statistical significance (D1+D2/A), 11 of 72 treatment groups (15.3%) supported a statistically significant decrease in TG following soy consumption. Thus, the results of 72 identified treatment groups (in 40 publications) indicate that there is a LOW strength of association between soy consumption and TG.

Considering studies of higher quality presented in the consistency rating (**Table 15a-4**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, 15.9% of the treatment groups from high quality studies were found to show a statistically significant favourable effect on TG.

Systolic Blood Pressure

Among treatment groups which showed statistical significance (D1+D2/A), 3 of 14 treatment groups (21.4%) supported a statistically significant decrease in systolic blood pressure following soy consumption. Thus, the results of 14 identified treatment groups (in 9 publications) indicate that there is a LOW strength of association between soy consumption and systolic blood pressure.

Considering studies of higher quality presented in the consistency rating (**Table 15a-5**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, 21.4% of the treatment groups from high quality studies were found to show a statistically significant favourable effect on systolic blood pressure.

Diastolic Blood Pressure

Among studies which showed statistical significance (D1+D2/A), 3 of 14 treatment groups (21.4%) supported a statistically significant decrease in diastolic blood pressure following soy consumption. Thus, the results of 14 identified treatment groups (in 9 publications) indicate that there is a LOW strength of association between soy consumption and diastolic blood pressure.

Considering studies of higher quality presented in the consistency rating (**Table 15a-6**), an equation was performed (D1/(D1+D3+D5+D7)) to determine whether all or most of the treatment groups from higher quality studies showed a statistically significant favourable effect. Using this equation, 21.4% of the treatment groups from high quality studies were found to show a statistically significant favourable effect on diastolic blood pressure.

Risk for Coronary Heart Disease

Among studies that showed statistical significance (B1/A), **Table 15b-1** demonstrates that the proportion of studies showing a significant decrease in risk for coronary heart disease following soy protein consumption, regardless of study quality, is HIGH (100%).

Considering only those studies assigned high quality scores (C1/(C1+C3+C5)), the proportion of studies demonstrating a statistically significant favourable direction of effect on risk for coronary heart disease remains unchanged (100%). Overall, the results of the consistency rating of one identified study indicate that there is a HIGH strength of association between soy protein consumption and decreased risk for coronary heart disease. However, considering that only one observational study was included in this investigation, results should be interpreted with caution.

Risk for Myocardial Infarction

Among studies that showed statistical significance (B1/A), **Table 15b-2** demonstrates that the proportion of studies showing a significant decrease in risk for myocardial infarction following soy protein consumption, regardless of study quality, is HIGH (100%).

Considering only those studies assigned high quality scores (C1/(C1+C3+C5)), the proportion of studies demonstrating a statistically significant favourable direction of effect on risk for myocardial infarction remains unchanged (100%). Overall, the results of the consistency rating of one identified study indicate that there is a HIGH strength of association between soy protein consumption and decreased risk for myocardial infarction. However, considering that only one observational study was included in this investigation, results should be interpreted with caution.

Risk of Death from Coronary Heart Disease

Among studies that showed statistical significance (B1/A), **Table 15b-3** demonstrates that the proportion of studies showing a significant decrease in risk of death from coronary heart disease following soy protein consumption, regardless of study quality, is LOW (0%).

Considering only those studies assigned high quality scores (C1/(C1+C3+C5)), the proportion of studies demonstrating a statistically significant favourable direction of effect on risk of death from coronary heart disease remains unchanged (0%). Overall, the results of the consistency rating of one identified study indicate that there is a LOW strength of association between soy protein consumption and decreased risk of death from coronary heart disease. However, considering that only one observational study was included in this investigation, results should be interpreted with caution.

DISCUSSION OF FACTORS THAT MAY HAVE CONTRIBUTED TO THE LACK OF STATISTICAL SIGNIFICANCE

Methodological characteristics of clinical investigations, such as **sample size**, **treatment dose and duration**, **food form**, and **population health status**, may have affected the variability in strength of the association between soy protein and the health outcomes outlined in **Tables 15a-1** to **15a-6**. Studies that vary in terms of their design, such as those included in this investigation, may influence biomarkers of CVD differently, making overall comparisons difficult.

Sample Size and Power Calculation

The sample sizes used in the 45 intervention study publications ranged from 10 to 191 subjects. It is notable that out of 45 publications and 79 treatment groups therein, only a minority of researchers reported a proper power analysis as rationale for number of subjects included. The studies in three publications were powered to detect statistical significance in TC (Crouse et al., 1999, Shidfar et al., 2009; van Raaij et al., 1981); the studies in two publications were powered to detect statistical significance in LDL-C (Crouse et al., 1999; Gardner et al., 2001); and the study in one publication was powered for the endpoint of flow-mediated dilation (Kreijkamp-Kaspers et al., 2005).

Shidfar et al. (2009) determined that 21 subjects per group were required to detect a 10% reduction in TC concentration between groups at 90% and two-tailed significance of 0.05. van Raaij et al. (1981) calculated that 24 subjects per group were required in order to detect a change of 0.25 mmol/L in TC at a power of 0.80 and two-tailed significance of 0.05. As an absolute change may represent a different reduction in risk depending on serum TC levels, the 10% reduction from baseline power test yielding 21 subjects was used to investigate whether other studies were sufficiently powered to detect a reduction in TC. According to this point of reference, at least 21 subjects in a crossover design or 42 subjects (21 subjects per group) in a parallel design rendered a study as sufficiently powered for detection of changes in TC. Therefore, of the high quality studies that showed a favourable effect on TC regardless of statistical significance, 14/48 (36%) treatment groups were insufficiently powered to detect a statistically significant change in TC. Eleven of these 14 treatment groups did not support a statistically significant reduction in TC assessment. It is possible that insufficient power may have been a factor contributing to the lack of statistical significance in these studies.

Ten (in 2 publications) of the 79 treatment groups (in 45 publications) reported a power calculation pertaining to a decrease in LDL-C concentrations (Crouse et al., 1999 and Gardner et al., 2001). Crouse et al. (1999) determined that 30 participants per group would need to be recruited to evaluate 25 individuals (assuming drop outs) to detect a 6% relative change in LDL-C between groups with 95% confidence at the 2-sided significance level of 0.05. Gardner et al. (2001) determined that a sample size of 30 per group was required to detect a 10% change in LDL-C with a power level of 0.8 and significance levels of 0.05.

Both Gardner et al. (2001) and Crouse et al. (1999) determined that a sample size of 25 would be required in each treatment group. This number was used to evaluate whether the other 64 studies that investigated LDL-C were sufficiently powered to assess changes in LDL-C. According to this point of reference, at least 25 subjects in a crossover design or 50 subjects in a parallel design rendered a study as sufficiently powered for detection of changes in LDL-C. Therefore, of the high quality studies that showed a favourable effect on LDL-C regardless of statistical significance, 23/50 (46%) treatment groups were insufficiently powered to detect a statistically significant change in LDL-C. It is possible that insufficient power may have been a factor contributing to the lack of statistical significance in these studies.

Finally, it is unknown whether or not the studies under investigation had a sufficient sample size to detect statistically significant changes in HDL-C, TG and diastolic or systolic blood pressure as none of the studies were powered based on these specific biomarkers. Future studies must similarly incorporate statistical requirements for sample size in order to add support to the strength of association between dietary soy consumption and reduced risk for CVD.

Duration

The duration of intervention studies investigated within this breadth of evidence ranged from 3 weeks to 24 weeks. Minimum treatment duration of 3 weeks was chosen as an inclusion criterion based on recommendations from the U.S. FDA, which has considered 3 weeks to be the minimum duration for evaluating the effect of an intervention on LDL-C concentrations (FDA, 2009).

In a meta-analysis published by Zhan and Ho (2005), the investigation of 23 randomized controlled trials giving soy protein with isoflavones revealed that the strongest decreases in TC, LDL-C, and TG in subject circulation occurred within a short period of time after beginning intervention. Conversely, this study highlighted that within the same 23 trials, the largest magnitude of effect for increased HDL-C levels associated with intake of isoflavone-containing soy protein occurred only after 12 weeks of intervention (Zhan and Ho, 2005).

Treatment Dose

Variable dosages administered to participants in the experimental setting may have also influenced the consistency ratings. The dose of total soy protein used in the included clinical investigations ranged from 11.3 to 154 g/day and the quantity of isoflavones consumed varied from negligible amounts to >300 mg/day. While comment has been given in this report with respect to a dose–response relationship, it is clear that this relationship should be further investigated in future studies to support clear recommendations for soy consumption and reduced risk for CVD.

Food Form

The food form in which soy protein was delivered is another important variable to consider when evaluating the inconsistencies seen in clinical literature. For example, soy protein isolate and/or soy protein concentrate were incorporated into beverages and a variety of everyday food products such as cookies, muffins, meat analogues and soups. Approximately 15% of the included studies also investigated the effects of tofu, soy nuts, roasted soybeans, soy beverage, and soy flour on various biomarkers of CVD. Differences in physico-chemical properties and nutrient profiles of all soy vehicles considered in this investigation make it difficult to derive clear and consistent results for the relationship between soy consumption and reduced risk of CVD.

Population Health Status

It is possible that grouping together studies that investigated the effect of soy consumption on biomarkers of CVD in both normo- and hypercholesterolemic subjects may have diluted results derived from **Table 15a-1** to **Table 15a-6**. For example, although only 9 of the 23 (39.1%) treatment groups investigating the effect of soy consumption in hypercholesterolemic subjects found a significant improvement in LDL from baseline compared to control, the strength of the relationship between soy consumption and LDL-C increased by almost 12%. Six of the publications specifically investigated the effect of soy protein on LDL-C in hypercholesterolemic individuals compared to normocholesterolemic subjects (Bakhit et al., 1994; Crouse et al., 1999; Greany et al., 2004; Lichtenstein et al., 2002; Wong et al., 1998; Welty et al., 2007). The studies by Bakhit et al. and Wong et al. found no significant effect of baseline cholesterol status, while the other four studies had significant or favourable results in the hypercholesterolemic subjects only. Given the small number of clinical trials that have looked at this

effect specifically (compared to the number of clinical trials that included both normo- and hypercholesterolemic subjects), it is difficult to comment on the effects of baseline cholesterol status on the relationship between soy protein and LDL-C. Several meta-analyses (which utilized a much more rigorous statistical analysis than that performed herein) have indicated that the effects of soy protein on serum LDL-C reduction are strongly related to initial cholesterol concentrations (Anderson et al., 1995; Messina et al., 2009; Sirtori et al., 2007; Zhan and Ho, 2005).

This resource has shown an example of how to present the findings of a systematic literature in terms of the information required in Sections 2, 3 and 4, and the first nine steps of Section 5 (up to step 9b) of the HC Guidance Document (www.hc-sc.gc.ca/fn-an/legislation/guide-ld/subm-prep-soum-eng.php). It has been provided as an illustration of the process required and makes no claims as to the state of the science.

A complete food health claims submission would need to include all other sections of the HC Guidance Document, including steps 9c through 13 of Section 5, as well as provide a full list of references cited in the submission, and identify and fill any information or research gaps.