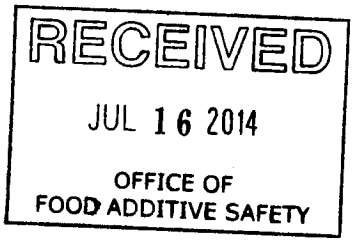
Original Submission

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
United States



GRN 000529

Attn: Dr. P. Gaynor

Dr. Gaynor,

Enclosed in this package, please find 3 binders containing 1 original and 2 copies of the following documents addressing the GRAS Exemption Claim for glabrous canary seed as a food cereal grain:

1) Letter of Notification from the Canaryseed Development Commission of Saskatchewan (CDCS)

2) GRAS Exemption Claim

3) Expert Panel Consensus Statement

4) Dossier: "Documentation supporting the Generally Recognized as Safe (GRAS) status of glabrous annual canary seed (*Phalaris canariensis* L) as a food cereal grain"

Respectfully,

(b) (6)

C.A. Patterson, PhD, PAg
On behalf of the CDCS

Notification letter

000003

Canaryseed
Development
Commission of
Saskatchewan

Bay 6A – 3602 Taylor Street East, Saskatoon, SK S7H 5H9
Telephone: 306.975.6624 Fax: 306.244.4497

July 8, 2014

Paulette Gaynor, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

In accordance with 21 CFR 170.36 (62 FR 18960; April 17, 1997), the Canaryseed Development Commission of Saskatchewan (CDCS) is hereby submitting notice of a claim that the use of annual glabrous canary seed in foods is generally recognized as safe (GRAS) based on scientific procedures, and that it is therefore exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act.

My contact information is provided below. Please feel free to contact me by phone or e-mail if you have any questions regarding this GRAS notice.

Sincerely,

(b) (6)

Kevin Hursh, Executive Director
Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street Saskatoon, SK Canada S7H 5H9
Tel: (306) 933-0138
Email: kevin@hursh.ca

Gross Exemption Claim

Canaryseed
Development
Commission of
Saskatchewan

Bay 6A – 3602 Taylor Street East, Saskatoon, SK S7H 5H9
Telephone: 306.975.6624 Fax: 306.244.4497

GRAS EXEMPTION CLAIM

The Canaryseed Development Commission of Saskatchewan (CDCS), hereby notifies the U.S. Food and Drug Administration that the uses of annual glabrous canary seed (*Phalaris canariensis* L) described below are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because CDCS has determined that such uses are generally recognized as safe (GRAS). CDCS made this GRAS determination based on scientific procedures in concert with an appropriately convened panel of experts who are qualified by their scientific training and experience. This finding is based on scientific procedures as described in the following sections, and the evaluation accurately reflects the conditions of the intended use of this substance in foods.

(b) (6)

Kevin Hursh, Executive Director
Canaryseed Development Commission of Saskatchewan

1.1. Name and Address of Notifier

Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street
Saskatoon, Saskatchewan, S7H 5H9
Canada

Contact Name: Kevin Hursh, Executive Director:
Telephone: (306) 933-0138
Facsimile: (306) 249-4869
Email: kevin@hursh.ca

As the notifier, Canaryseed Development Commission of Saskatchewan accepts responsibility for the GRAS determination that has been made for annual glabrous canary seed (*Phalaris canariensis* L) as described in the subject notification; consequently glabrous canary seed as described herein is exempt from pre-market approval requirements for food ingredients.

1.2. Name of GRAS Substance

Annual glabrous canary seed (*Phalaris canariensis* L) is commonly known as canary seed or annual canarygrass in North America and "alpiste" in European and South American countries. Dehulled glabrous brown and yellow coloured canary seed grain (also known as groats) and its milled products will be sold as food ingredients. In the US and Canada, the common name for annual glabrous canary seed will be "canary seed".

1.3. Conditions of Use

Glabrous canary seed groats (dehulled grain) either as a whole groat, whole meal, whole grain flour or a milled product are intended for use as an ingredient in various baked goods, breads, cereals and pasta products. The grain could also be used as a low fat substitute for sesame seed in bread and snack foods or in combination with other seeds as toppings or ingredients.

1.4. Basis for GRAS Determination

The CDCS GRAS determination for the intended uses of glabrous canary seed is based on scientific procedures as described under 2 1 CFR§170.30(b). Information provided by the CDCS and comprehensive searches of the literature through March 2014 conducted by The Pathfinders Research and Management Ltd and BMagnuson Consulting, served as the basis for preparation of a monograph summarizing the totality of the available information germane to determining the safety of the intended uses of glabrous canary seed.

Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 similar to other food cereal grains and pseudocereals. Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains.

It may be concluded that glabrous canary seed is safe under the intended conditions of use because the total exposure to glabrous canary seed and its constituents resulting from these uses is well within levels shown to be safe by both current levels of consumption of other cereal grains, which are compositionally very similar to canary seed, and animal safety studies. The estimated intakes of canary seed, even for the highest users, are below the level shown to have no adverse effects or nutritional hazards, based on nutritional composition comparisons and animal safety studies.

An Expert Panel determined the intended use of glabrous canary seed to be safe, and also GRAS, by demonstrating that the safety of this level of intake is based on publicly available and accepted information and is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances added to food.

Therefore, the intended uses of glabrous canary seed are determined to be safe and GRAS. Determination of the safety and GRAS status of glabrous canary seed for direct addition to food under their intended conditions of use was made through the deliberations of an Expert Panel consisting of Julie Miller Jones, PhD, Stephen Taylor, PhD, and John A. Thomas, PhD, who reviewed the information in this monograph as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They critically reviewed and evaluated the publicly available information, including the potential human exposure to glabrous canary seed resulting from the intended use of glabrous canary seed, and individually and collectively concluded that the available information on glabrous canary seed contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under the intended conditions of use.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion. Therefore, glabrous canary seed is GRAS by scientific procedures under the conditions of use described.

1.5. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of the Canaryseed Development Commission of Saskatchewan.

Consensus Statement

Expert Panel Consensus Statement Regarding the Generally Recognized as Safe (GRAS) Status of of Glabrous Annual Canary Seed

May 28, 2014

INTRODUCTION

At the request of the Canaryseed Development Commission of Saskatchewan (CDCS), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended use of glabrous annual canary seed is safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures.

The Panel consisted of: Julie Miller Jones, PhD, Distinguished Scholar and Professor Emerita, College of St. Catherine (CSC), St. Paul, Minnesota; Stephen Taylor, PhD, Professor, Co-Director Food Allergy Research and Resource Program, University of Nebraska-Lincoln, Lincoln, Nebraska; and John A. Thomas, PhD, A.T.S., Professor (adjunct), Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, IN.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on canary seed from the literature and other published sources through March, 2014, provided by CDCS. In addition, the Panel evaluated other information deemed appropriate or necessary. The information evaluated by the Panel included details pertaining to the method of development, compositional analyses, supporting analytical data, intended use-levels in specified foods, consumption estimates for intended use, and a comprehensive assessment of

the available scientific literature pertaining to the safety of glabrous annual canary seed (*Phalaris canariensis* L).

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that the intended uses described herein for glabrous annual canary seed (*Phalaris canariensis* L), meeting appropriate food-grade specifications as described in the supporting dossier [Documentation Supporting the Generally Recognized as Safe (GRAS) Status of Glabrous Annual Canary Seed (*Phalaris canariensis* L)] and produced according to current Good Agricultural Practices and Good Manufacturing Practice (GMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY

CDCS intends to market glabrous annual canary seed as a grain product for use as an ingredient in breads, flours, breakfast cereals, and pastas, as well as baked goods (e.g. biscuits, crackers, cookies, granola bars, nutrition bars, energy bars) and baking mixes (e.g. cakes).

Annual canary seed (*Phalaris canariensis* L) is an annual species of the genus *Phalaris* that has primarily been used in the birdfeed market. Canary seed has an excellent nutritional profile and is proposed for use as a human food ingredient. Canary seed can be considered a novel food crop as its history as a human cereal grain has not been well documented. Glabrous, or hairless, canary seed has been produced by selective breeding techniques.

Glabrous canary seed provides a source of protein, carbohydrate, essential fatty acids, dietary fiber, minerals and vitamins, as well as phytochemicals. The US Dietary Guidelines for Americans recommend 5-8 servings of grains per day, with at least half of these grains being whole grains. There is an opportunity for glabrous canary seed to be consumed as a whole grain in the diet and contribute to dietary eating habits. Canary seed would ideally, as a new whole grain food introduction, be consumed with the other available whole grain diet choices. Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 (Jones & Engelson, 2010) similar to other food cereal grains and pseudocereals consumed by humans.

Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains. Glabrous canary seed has a nutritional and compositional profile similar to other commonly consumed cereal grains being mainly comprised of protein (19-23%), starch (53-61%), fat (5.5-8%), dietary fiber (6-10%) and ash (1.9-2.4%). Similar to other cereals, the proteins in canary seed are deficient in

lysine but rich in cysteine, tryptophan, phenylalanine and arginine. Canary seed contains levels of trace minerals and B vitamins comparable to other cereal grains. Folate levels are similar to other grains. As in other cereal grains and legumes, phenolic acids, phytate, trypsin inhibitors and amylase inhibitors are found in the grain. Phytate is present at about twice the level found in Western Red Spring wheat, but at similar levels to other cereals, pulses and commonly consumed nuts and seeds. Growth and nutritional studies in swine and rodents confirmed the analytical results, demonstrating growth and food consumption rates comparable to other grains.

Levels of alkaloids, heavy metals, mycotoxins and microbial contamination in canary seed were similar or lower than reported in other cereal grains, and are not of toxicological concern. No evidence of allergenic potential of glabrous brown or yellow canary seed groats was identified from detailed assessments. Feeding glabrous brown or yellow coloured canary seed groats to rats for 90 days in detailed toxicological studies resulted in no adverse toxicological findings that could be attributed to consumption of glabrous canary seed groats. In this pivotal 90-day oral study, no adverse effects were observed with the highest doses tested of yellow and brown glabrous canary seed groats, which ranged from 5.1 to 5.7 g/kg/d.

Estimates for the intake of canary seed were based on the proposed food-uses and use-levels for canary seeds in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Optimistic projections for the replacement of currently-used grains and seeds with canary seed products in various food products were used to calculate the highest likely consumption levels of canary seed (i.e. worst case scenarios for intakes). Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of canary seed and the percentage of consumers were determined. On an all-user basis, the mean and 90th percentile intakes of canary seed by the total U.S. population from all proposed food-uses were determined to be 0.8 g/kg body weight/day and 1.7 g/kg body weight/day, respectively.

Thus the highest anticipated exposure levels for canary seed, based on the proposed intended uses and use levels, are well below the levels shown to be safe by both animal safety studies and current levels of consumption of other cereal grains, which are compositionally very similar to canary seed. The estimated intakes of canary seed, even for the highest users, are below the level shown to have no adverse effects or nutritional hazards, based on the animal safety studies and nutritional composition comparisons.

The entirety of the available scientific data and studies reviewed support the conclusion that glabrous brown and yellow coloured canary seed groats and milled products are nutritious and safe to consume for the US population. On the basis of the novel food safety assessment guidelines, glabrous canary seed groats and milled products would not be expected to cause adverse effects in humans under the conditions of intended use in foods.

Based upon the entirety of the available scientific data and summarized in this dossier, it is concluded that glabrous canary seed groats would be generally recognized as safe for consumption in their intended uses in food.

CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that the intended uses of glabrous canary seed, presented in the supporting dossier [Data supporting the Generally Recognized as Safe (GRAS) Status of Glabrous Canary Seed] and produced consistent with Good Agricultural Practices and Good Manufacturing Practices (GMP), are safe.

We further conclude that the intended uses of glabrous canary seed, meeting food grade specifications presented in the supporting dossier and produced consistent with current GMP are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

Julie Miller Jones, PhD — 6/15/14 Date

(b) (6)

Stephen Taylor, PhD — 6/18/14 Date

(b) (6)

John A. Thomas, PhD — 6/6/14 Date

REFERENCES

1. Jones, J.M., and Engleson, J. 2010. Whole Grains: benefits and challenges. Annu. Rev. Food Sci. Technol. 1:19-40.

2. CDC (2011). *National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/nhanes09_10.htm [Page last updated: November 7, 2011].

3. USDA (2012). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 07/16/2012].

GRAS Dossier

Canaryseed
Development
Commission of
Saskatchewan

Documentation Supporting the Generally Recognized as Safe (GRAS) Status of Glabrous Annual Canary Seed (*Phalaris canariensis* L.) as a Food Cereal Grain

March 17, 2014

Prepared for:

Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street Saskatoon, SK S7H 5H9

Prepared by:

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March 2014

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE STATUS OF GLABROUS ANNUAL CANARY SEED (PHALARIS CANARIENSIS L.) AS A FOOD CEREAL GRAIN

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Section 3.0 Background Information

Section 7.0 Intended Use

Section 8.0 History of Use

Section 9.0 Nutritional Considerations

LIST OF FIGURES

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GLABROUS ANNUAL CANARY SEED (*PHALARIS CANARIENSIS* L.) AS A FOOD CEREAL GRAIN

EXECUTIVE SUMMARY

The Canaryseed Development Commission of Saskatchewan (CDCS), on behalf of producers of canary seed in Canada, plans to introduce glabrous (hairless) hull varieties of brown and yellow coloured canary seed (*Phalaris canariensis* L.) as a new cereal food grain to be used as an ingredient in food products in the United States.

Canary seed provides a source of protein, carbohydrate, essential fatty acids, dietary fiber, minerals and vitamins, as well as phytochemicals. The US Dietary Guidelines for Americans recommend 5-8 servings of grains per day, with at least half of these grains being whole grains. There is an opportunity for glabrous canary seed to be consumed as a whole grain in the diet and contribute to dietary eating habits. Canary seed would ideally, as a new whole grain food introduction, be consumed with the other available whole grain diet choices.

The purpose of this dossier is to outline information respecting the development of glabrous canaryseed, details of potential manufacturing and processing methods, its intended use and directions for preparation, evidence of traditional use, data to establish glabrous canaryseed is safe for human consumption and estimations of its level of consumption by consumers.

Glabrous canary seed can be considered a novel food crop as its history as a human cereal grain has not been well documented. Glabrous canary seed has been produced by selective breeding techniques.

A major obstacle in developing annual canary seed as a food grain for human consumption was the presence of small silicified hairs (trichomes) or spicules covering the hull surface of commercial cultivars. Due to the increasing importance of canary seed production in Western Canada, a mutation breeding program was initiated at the University of Saskatchewan, Canada, in the 1990s to eliminate hull pubescence (hairy) in canary seed. The objectives in developing glabrous, annual canary seed cultivars were three fold:

a) To reduce the skin irritation encountered by farmers during the harvest process,
b) To eliminate any potential health concerns associated with the *Phalaris* silica trichomes due to their irritative properties (Rabovsky, 1995),
c) To develop cultivars suitable for human consumption (glabrous and yellow coloured grain).

The data and information contained in this report support the safety of consumption of annual canary seed (*Phalaris canariensis* L.) as a human food cereal grain. Glabrous canary seed groats (*i.e.* hull-free grain) are proposed for use as an ingredient in breads, flours, breakfast cereals, and pastas, as well as baked goods (e.g. biscuits, crackers, cookies, granola bars, nutrition bars, energy bars) and baking mixes (e.g. cakes).

Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains. *Phalaris canariensis* has a nutritional and compositional profile similar to other commonly consumed cereal grains being mainly comprised of protein (19-23%), starch (53-61%), fat (5.5-8%), dietary fiber (6-10%) and ash (1.9-2.4%). Similar to other cereals, the proteins in canary seed are deficient in lysine but rich in cysteine, tryptophan, phenylalanine and arginine. Canary seed contains levels of trace minerals and B vitamins comparable to other cereal grains. As in other cereal grains and legumes, phenolic acids, phytate, trypsin inhibitors and amylase inhibitors are found in the grain. Phytate is present at about twice the level found in Western Red Spring wheat, but at similar levels to other cereals, pulses and commonly consumed nuts and seeds. Growth and nutritional studies in swine and rodents confirmed the analytical results, demonstrating growth and food consumption rates comparable to other grains.

Levels of alkaloids, heavy metals, mycotoxins and microbial contamination in canary seed were similar or lower than reported in other cereal grains, and are not of toxicological concern. No evidence of allergenic potential of glabrous brown or yellow canary seed groats was identified from detailed assessments. Feeding glabrous brown or yellow coloured canary seed groats to rats for 90 days in detailed toxicological studies resulted in no adverse toxicological findings that could be attributed to

consumption of glabrous canary seed groats. In the pivotal 90-day study, no adverse effects were observed with the highest doses tested of yellow and brown glabrous canary seed groats, which ranged from 5.1 to 5.7 g/kg/d.

Estimates for the intake of canary seed were based on the proposed food-uses and use-levels for canary seeds in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Optimistic projections for the replacement of currently-used grains and seeds with canary seed products in various food products were used to calculate the highest likely consumption levels of canary seed. Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of canary seed and the percentage of consumers were determined. On an all-user basis, the mean and 90th percentile intakes of canary seed by the total U.S. population from all proposed food-uses were determined to be 0.8 g/kg body weight/day and 1.7 g/kg body weight/day, respectively. Thus the anticipated exposure levels for canary seed, based on the proposed intended uses and use levels, are far below the observed NOAEL of 5.1 to 5.7 g/kg/d in the 90-day rat study.

The entirety of the available scientific data and studies summarized in this dossier support the conclusion that glabrous brown and yellow coloured canary seed groats and milled products are nutritious and safe to consume for the American population. While two colors of canary seed are available, there is no significant nutritional or safety related differences between canary seed of different colors. Glabrous canary seed groats and milled products would not be expected to cause adverse effects in humans under the conditions of intended use in foods.

Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 (Jones & Engelson, 2010) similar to other food cereal grains and pseudocereals consumed by humans.

Based upon the entirety of the available scientific data and summarized in this dossier, it is concluded that glabrous canary seed groats are safe for consumption in its intended use in food.

1.0 Common Name

Annual canary seed (*Phalaris canariensis* L) is commonly known as canary seed or annual canarygrass in North America and "alpiste" in European and South American countries. Dehulled glabrous brown and yellow coloured canary seed grain (also known as groats) and its milled products will be sold as food ingredients.

In the US and Canada, the common name for annual canary seed will be "canary seed".

2.0 Principal Place of Business

Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street Saskatoon, SK Canada S7H 5H9
Executive Director: Kevin Hursh

Description of the Novel Food

3.0 Background Information

The Canaryseed Development Commission of Saskatchewan (CDCS), on behalf of producers of canary seed in Canada, wishes to introduce glabrous (hairless) hull varieties of brown and yellow coloured canary seed (*Phalaris canariensis* L.) as a new cereal food grain to be used as an ingredient in food products in the US.

Glabrous canary seed can be considered a novel food crop as its history of use in human foods has not been well documented and has been developed by selective breeding techniques. Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 (Jones & Engelson, 2010) similar to other food cereal grains and pseudocereals consumed by humans. Glabrous canary seed cultivars have the potential to be used as a whole groat (dehulled cereal grain) or as milled grain products in food products similar to the use of other cereal grains.

The gathering of information for the safety assessment of glabrous canary seed has proceeded in two discrete timeframes in the past fifteen years. The initial project

(Phase 1) (1992-2002) involved the development of glabrous canary seed and the identification of both brown and yellow coloured groats amongst the glabrous varieties. In Phase 1, the nutritional and chemical characteristics of glabrous, brown coloured canary seed groats (*P. canariensis*, CDC Maria) were compared to its pubescent (hairy) parent *P. canariensis,* cultivar "Keet" (also a brown coloured groat) and to a Western Red Spring (CHRS) common hard wheat (*Triticum aestivum* subsp. Vulagare[Vill. Host] Mackey), cultivar "Katepwa". The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals, as well as a 90-day rodent trial and two poultry feeding trials.

With the establishment of the Canaryseed Development Commission of Saskatchewan in 2006, the collection of levy funds and the securing of additional funding, the novel food project for glabrous canary seed was once again initiated in 2008. This second project (called Phase 2, 2008-2014) involved a comprehensive comparison of two glabrous yellow coloured cultivars (designated C05041 and C05091) to the glabrous brown coloured cultivar CDC Maria, which had been studied in the Phase 1 project. Nutritional, chemical, additional rodent feeding toxicology studies, and allergenicity studies were conducted. Comprehensive searches of the literature were conducted by C.A. Patterson and B. Magnuson from the initiation of the project through February 2014 for the preparation of the dossier and summation of all available information related to the safety of the consumption of canary seed. Other data were provided by the CDCS.

The purpose of this dossier is to outline information respecting the development of glabrous canaryseed, details of potential manufacturing and processing methods, its intended use and directions for preparation, its history of use, data to establish glabrous canaryseed is safe for human consumption and estimations of its level of consumption by consumers.

3.1 Current production and use of *P. canariensis*

Annual canary seed (*Phalaris canariensis* L), also known as annual canarygrass, is the only annual species of the genus *Phalaris* that has gained commercial importance as a specialty grain crop. Argentina, Morocco and Australia have been the traditional

world producers of annual canary seed as a source of birdfeed but Canada is now the world's largest producer and exporter of annual canary seed with Saskatchewan accounting for about 69% of the tonnage (ca. 125,000 tonnes) of the world canary seed exports in 2011.

Canary seed is primarily used in the birdfeed market as it is a major component in feed mixtures for pet and wild birds. However, Canadian producers are investigating other market opportunities for the glabrous canary seed to mitigate the risk of selling into one market.

Six annual canary seed cultivars are currently registered in Canada–Keet, Elias and Cantate have pubescent (hairy) hulls and CDC Maria, CDC Togo, and CDC Bastia have glabrous (hairless) hulls. All have brown coloured grain kernels. The glabrous cultivars were developed by the University of Saskatchewan in the 1990s. The Food Production and Inspection Branch, Seed Division, Variety Registration Office, Agriculture and Agri-Food Canada issued registration NO 4607 to CDC Maria on 12 June 1997, registration NO 5834 to CDC Togo on 10 June 2004 and registration NO 6259 to CDC Bastia on April 13, 2007.This is not intended to be an exhaustive list of food grade canary seed as addressed by this GRAS determination. Development of new glabrous cultivars is an ongoing process and new cultivars are appearing in Canadian production (Hucl, 2013).

3.2 Projected Uses

The introduction of glabrous canary seed into the human food market will require significant effort from the CDCS and a commercial champion to introduce this specialty crop to the food industry and gain acceptance by consumers. Thus, projecting a realistic dietary exposure to glabrous canary seed is based upon the following factors which will influence its market penetration:

1. Canary seed production volumes: In the last 3 crop years (2009, 2010, 2011) approximately 30-50% of the canary seed produced in Canada was of the glabrous hull brown seeded variety, an average of 74,000 tonnes of glabrous canary seed being grown each year. All of the current pubescent and glabrous canaryseed production goes to the birdfeed market. However, glabrous brown

canary seed could enter the human food market as soon as regulatory approval is gained.

2. Production of glabrous yellow coloured canary seed: Yellow canary seed varieties are not yet in commercial production, nor registered as a new canary seed variety. Thus it will be at least 1 to 2 years beyond regulatory approval before sufficient glabrous yellow coloured canary seed is available for commercial use as a food ingredient.

3.3 Definitions used in this Dossier

To aid the reader, the following explanations of terminology used in this dossier and accompanying reference literature are provided in Table 3-1.

Table 3-1 Terminology and definitions used in dossier and literature[1,2]

Term	Description	Also known as	In Dossier
Phalaris canariensis	Annual canarygrass	Canary seed Canarygrass Alpiste	Canary seed Annual canarygrass
Glumes	External covering of a cereal grain containing the lemma and palea. Glumes retained after harvesting	Husk Hull Covered grain	Hull or Hulled
Caryopsis	Parts of the cereal grain comprised of pericarp (bran) , endosperm and germ	Grain, Seed Kernel	Grain
Pubescent	Glume (lemma and palea) are covered with silicified trichomes (hairs)	Hairy	Pubescent Hairy
Glabrous	No silicified trichomes (hairs) on the glumes or palea	Hairless	Glabrous Hairless
Dehulling	The process of removing the glumes (outside covering or hull) of the cereal	Dehulling	Dehulling
Dehulled canary seed	Removal of the glumes of canary seed	Grain, kernel, groat	Groat
Whole grain	Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same rich balance of nutrients that are found in the original grain seed.		Whole grain canary seed
Conditioning	Water addition under specific conditions to optimize grain for further processing (e.g. grinding and milling)	Tempering	Tempering
Milling	Grain is mechanically processed under controlled conditions of breaking, reduction and separation resulting in separation of various grain components	Milling	Milled fractions to make whole grain flours, flakes, refined flours, brans etc

[1] Serna-Saldivar, 2012; [2] Jones & Engleson, 2010

4.0 CANARY SEED DEVELOPMENT INFORMATION

4.1 History of Organism

Note: The following information has been extracted from the publications by Putnam et al, (1996) and Abdel-Aal and Hucl (2005), which provide a comprehensive description of the history, genetics and breeding, agronomic characteristics, composition and physical properties and processing and utilization of pubescent (hairy) annual canary seed. Glabrous varieties were not commercially available until 1998.

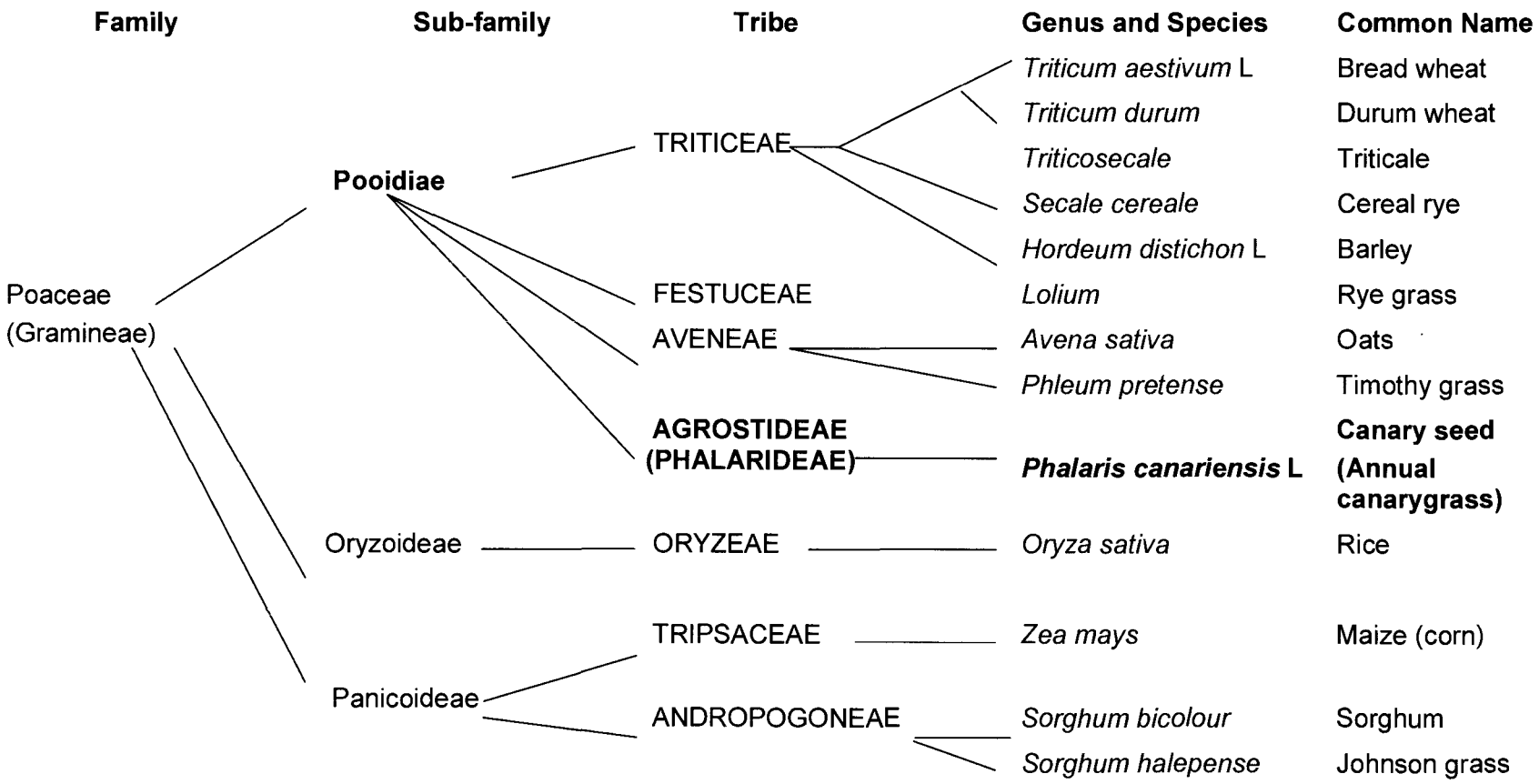
Note: Both "annual canary seed" and "annual canarygrass" are used in many publications referring to Phalaris canariensis.

Annual canarygrass (*Phalaris canariensis*) is a crop belonging to the Poacea (Gramineae) family, Pooidiea subfamily and tribe Agrostideae. This places annual canarygrass in the same subfamily but different tribe as wheat (*Triticum aestivum* L), barley (*Hordeum vulgare* L) and rye (*Secale cereale* L) (all belonging to the Triticea tribe) or oat (Aveneae tribe). Thus, annual canarygrass is somewhat genetically related but completely reproductively isolated from these common cereal crops (Figure 4-1).

Annual canarygrass is of Mediterranean origin. Weedy species of *Phalaris* (e.g., *P. minor*) are found around the Mediterranean basin and farther east. The *P. minor* species (littleseed canarygrass) is a problem weed in wheat fields in Pakistan and India and in Mediterranean climates, including California. Littleseed canarygrass biotypes have developed resistance to a number of herbicides making this species a more problematic weed. Short-spiked canarygrass (*P. brachystachys*) is another problem weed in cereal crops in the Mediterranean basin. Paradoxagrass (*P. paradoxa*) is a major weed in winter wheat production in Australia.

Canarygrass was first domesticated in the Mediterranean region. However, no evidence currently exists to indicate specifically where this domestication took place. A number of seventeenth- and eighteenth century references allude to canary seed or to a morphologically similar species originating in the Canary Islands, in Spain, or in both areas, and being used to feed birds. Canarygrass was assumed to originate in the Canary Islands but it is not clear whether the crop is named after the islands or after the birds (*Serinus canarius*) that originated there. In any case, the grain was fed to canaries and the spread of the two outward from Spain to countries such as Belgium was linked.

Figure 4-1 Relationship between common cereals and grasses and *Phalaris canariensis



*Adapted from Baldo *et al*, 1980; Jones *et al*, 1995

000038

A mid-1700 dictionary indicates that *alpiste* is a Basque word suggesting annual canarygrass has a long history on the Iberian Peninsula.

Annual canarygrass is sometimes confused with reed canarygrass (*Phalaris arundinacea*), which is a commonly grown perennial forage grass and weed species. Although heads of both plants are panicles, annual canarygrass heads are spike-like and resemble club wheat. The seed of annual canarygrass is larger than reed canarygrass but smaller than wheat (Figure 4-2). The genus also includes Littleseed canary seed (*Phalaris minor* Retz.), a weedy grass also originating in the Mediterranean and which can be found in barley, wheat and seedling alfalfa fields or as a weed on marginal lands, particularly in the western United States. Of the annual species of this genus, *P. canariensis* is the only one that is grown as a grain crop, fitting best as a wheat replacement in a crop rotation.

Although the genus *Phalaris* traces its origins to the Mediterranean basin, the 15 species that make up the genus can be found over a wide range of latitudes. Annual canarygrass is grown in many areas of the world including Argentina, Australia, Netherlands, Hungary, North Africa, the Middle East, the United States and Canada. North American production is primarily in Saskatchewan, Manitoba and Alberta with small acreage in the Red River Valley of North Dakota and Minnesota.

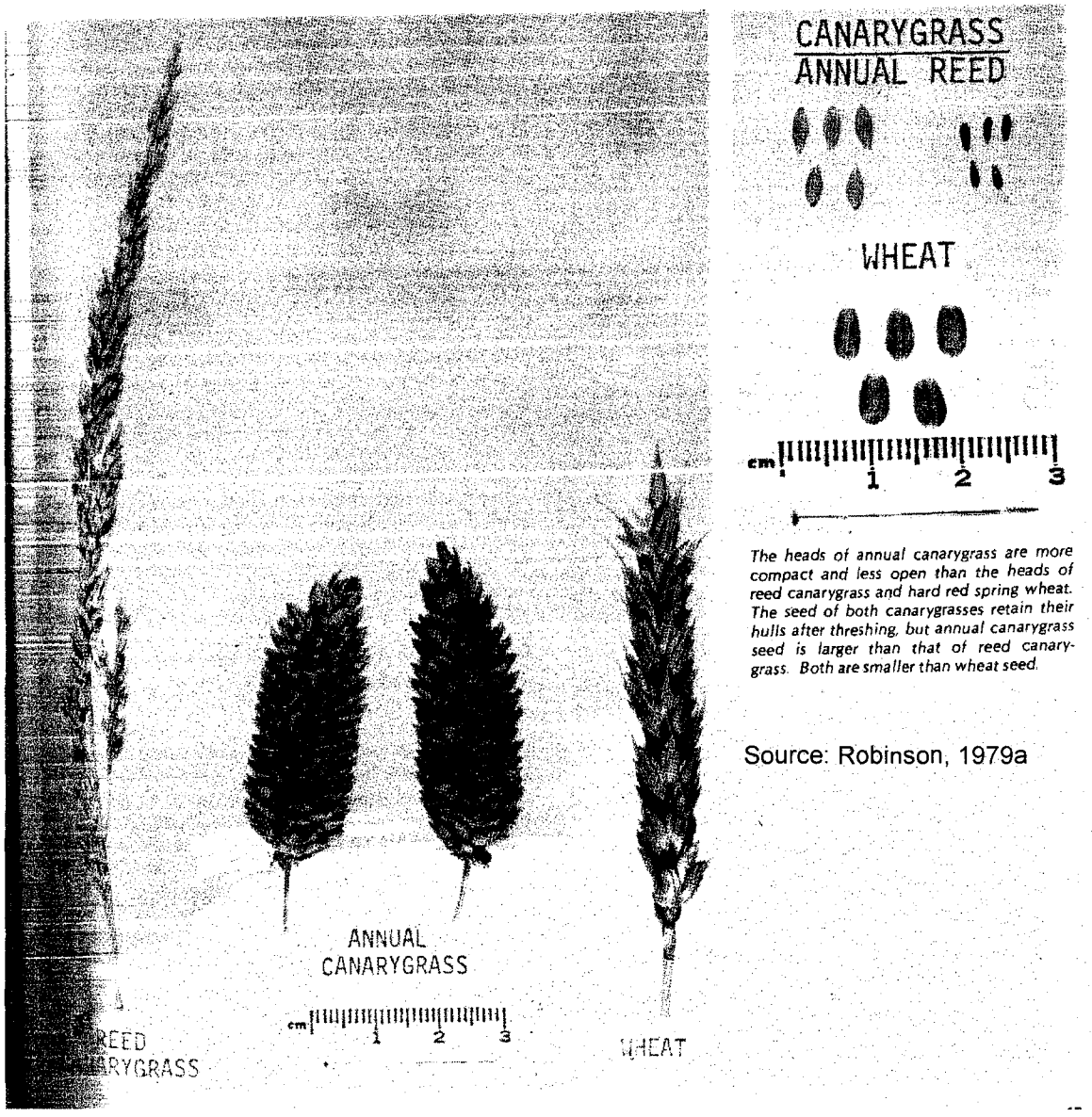
Annual canarygrass is a diploid with ($2n$ = 12), whereas most other *Phalaris* species (annual and perennial) have a basic chromosome number of x = 7. The only other species with $2n$ = 12 are the weedy annual *P. brachystachys* and the perennial *P. truncata* (Anderson, 1961). Based on isozyme and morphological analyses, *P. canariensis* and *P. brachystachys* are closely related (Matus & Hucl, 1999; Matus-Cádiz & Hucl, 2002). Taking into account the chromosome number homology between the two species, one can infer that *P. brachystachys* is probably the ancestral species from which annual canarygrass is derived.

The growth and development of annual canarygrass is quite similar to that of wheat (*Triticum aestivum* L) or oat (*Avena sativa*). It can be grown as either a spring-sown crop in regions with severe winter climates or as a winter-sown crop in Mediterranean climates. Generally, annual canarygrass required about 100-110 days to reach maturity, and is considered a cool-season crop preferring cool, moist conditions.

Even though it is less tolerant of heat and drought than hard red spring wheat, it has been grown successfully for several decades in semi-arid western Saskatchewan, one of the driest regions in Canada. It is frost tolerant and more tolerant of salinity and excess soil moisture than is wheat. Annual canarygrass is best adapted to heavy, moisture retentive soils due to its shallow rooting habit.

Canary seed produces small, elliptical grains with lengths and widths of approximately 4.0-5.1 and 1.5-2.0 mm, respectively (Abdel-Aal et al., 1997). The glabrous grain weighs approximately 7 mg, with an average test weight of 70 kg/hL (Hucl, 2009).

Figure 4-2 Comparison of the panicles and seed size of *P. canariensis*, *P. arundinacea* and hard red spring wheat



Reed Canarygrass | Annual Canarygrass | Wheat

4.2 Description of the Genetic Modification

4.2.1 Purpose of the Genetic Modification

Investigations in the 1970s first identified annual canary seed as a potential food grain crop (Robinson, 1978; 1979a,b). However, the presence of small silicified hairs (trichomes) or spicules covering the hull surface of commercial cultivars potentially prevented the use of canary seed as a food grain for human consumption..

Due to the increasing importance of canary seed production in Western Canada, a mutation breeding program was initiated at the University of Saskatchewan in the 1990s to eliminate hull pubescence (hairiness) and brown seed colour in canary seed. The rationale for this project was that exposure to trichomes from different *Phalaris* grass varieties had been proposed as a contributing factor to the high incidence of esophogeal cancer in certain geographical locations (O'Neill *et al.*, 1980). However, a mouse study found no evidence of damage due to consumption of trichomes from *Phalaris canariensis*, although dermal exposure promoted skin cancer in mice exposed to an initiating carcinogen (Bhatt *et al.*, 1984). The relationship between biogenic amorphous silicas in the trichomes and adverse health effects is not clear (Rabovsky, 1995). Thus, the absence of trichomes on glabrous canary seed eliminates concern associated with potential adverse health effects due to exposure. The selection for yellow coloured grain was to improve consumer appeal and acceptability of food products containing canary seed.

The objectives in developing glabrous, annual canary seed cultivars were three fold:

a) To reduce the skin irritation encountered by farmers during the harvest process,
b) To eliminate any potential health concerns associated with the *Phalaris* trichomes,
c) To develop cultivars suitable for human consumption (glabrous and yellow seed).

4.2.2 Pedigree and Breeding Method for the Glabrous Trait

Approximately 625,000 seeds of certified *P. canariensis* Keet (pubescent hull) were subjected to a 2-hour pretreatment soak in water prior to treatment with 1mM sodium azide for 12 hours (Faue *et al*, 1989). Seeds were subsequently flushed with

water and allowed to dry. (Note: Sodium azide is a commonly used agent for grain mutagenesis (Castillo *et al.*, 2001)).

Figure 4-3 provides a schematic of the breeding method for the glabrous and yellow seeded traits. The mutant (M) 1 and M2 populations were grown under field conditions and advanced as bulk samples. Ten kilograms of seed were harvested from the M1 plot. In the M2 and M3, a population size of approximately 80,000 plants in each generation was maintained.

Approximately 15,000 panicles were harvested from the M3 population growing under field conditions. Using a dissecting microscope, a single M3 glabrous panicle, possessing glabrous glumes and hulls, was identified from the M3 population. Ten M4 glabrous plants and their M5 progeny were grown in the greenhouse.

CDC Maria traces its origins to a single putative M4 seed. CDC Maria was selected based on agronomic field evaluation beginning in the M6 (Hucl *et al.*, 2001)

4.2.3 Performance

Since a registration test for annual canary seed did not exist, CDC Maria was evaluated during the years 1992-1996 in the University of Saskatchewan spring cereal testing system and Regional Variety Testing (RVT) system. Yield trials consisted of randomized complete block designs with three replications (Hucl, 2009).

CDC Maria is adapted to the traditional canary seed-growing region of Saskatchewan, the Brown, Dark Brown and Black soil zones.

4.2.4 Yellow Seeded Trait

The mutant populations of the above treated pubescent Keet seeds were also screened for the glabrous yellow seeded phenotype. Yellow-seeded line CY184 was selected from the same sodium azide-treated bulk population of Keet seed as was CDC Maria. CY184 was identified by de-hulling 3 million M4 seeds and subsequently sorting the dehulled seed using a color-sorter (Figure 4-3).

The CY184 breeding line is a pubescent, yellow-seeded line tracing its origin to a single putative M4 seed that breeds true in subsequent generations.

A CDC Maria - CY184 cross yielded brown, glabrous CC9007 (registered as CDC Bastia) and its sister line, glabrous yellow CC9005.

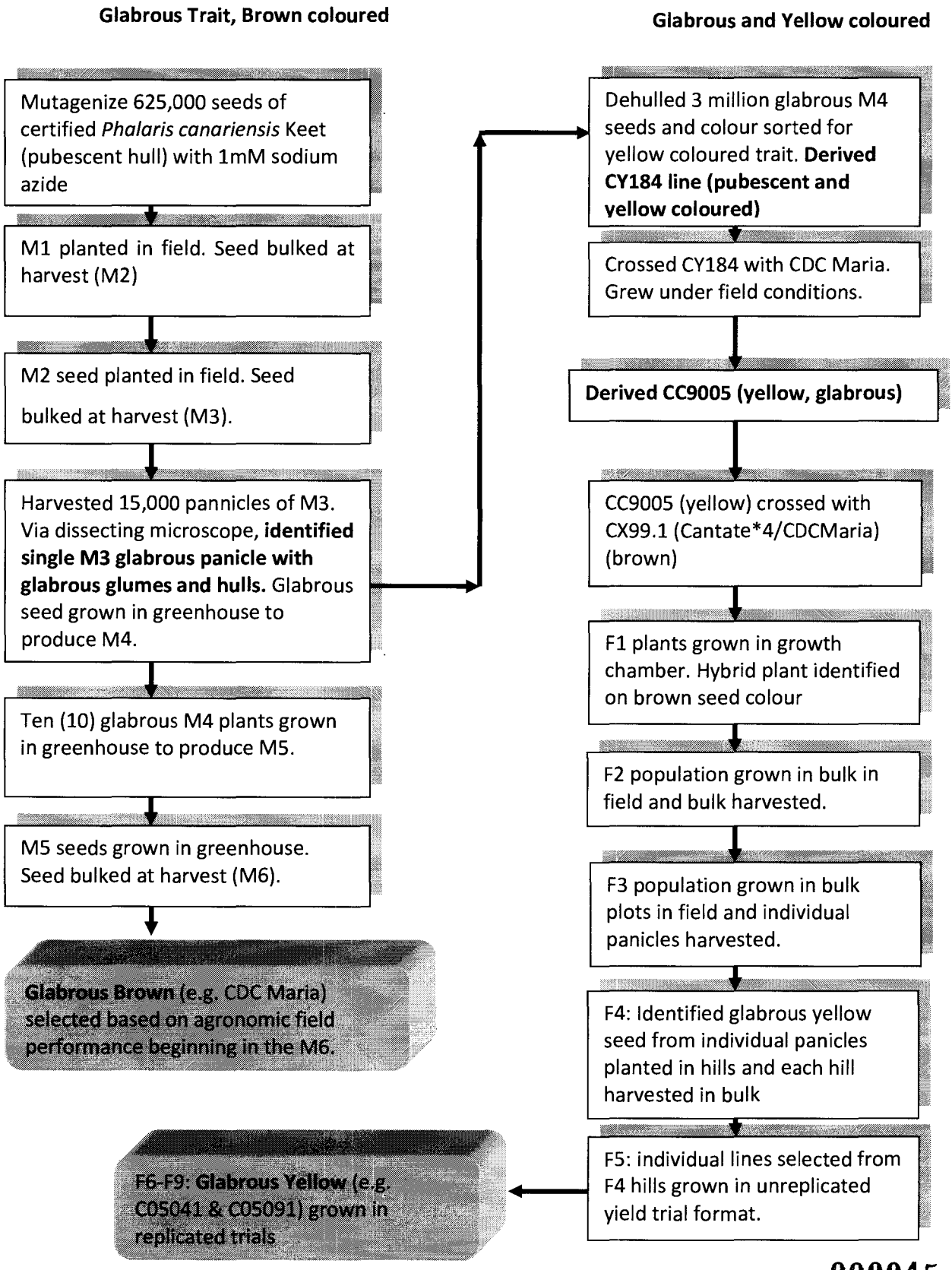
The yellow seeded hairless varieties used for this novel food petition were derived as follows (Figure 4-3). In 2000, CC9005 was crossed with CX99.1 (Cantate*4/CDC Maria) using the approach method in a field crossing nursery. The cross CX99.1 represented the third backcross of CDC Maria to Cantate in which the glabrous trait was selected. Cantate is a pubescent hull cultivar, registered in Canada.

Putative F1 plants were grown in a growth chamber and hybrid plants identified on the basis of brown seed colour. The F2 population was grown in a bulk plot in the field and bulk harvested. F2 families derived from each F1 plant were screened for segregation of hull pubescence and seed colour. The F3 population was grown in bulk plots in the field and individual panicles were harvested. Yellow seed from individual panicles were planted in hills and each hill harvested in bulk. Individual lines from the F4 hills were grown in an unreplicated yield trial format (F5). F6 to F9 generations of C05041 and C05091 were grown in replicated trials at five to six sites in Saskatchewan in the years 2006 to 2012.

The two glabrous, yellow seeded lines (C05041 & C05091) used for this novel food petition have the pedigree of CC9005//Cantate*4//CDC Maria.

The glabrous trait in canary seed is controlled by a single gene (Matus-Cadiz *et al.*, 2003) with the glabrous phenotype being recessive to the pubescent condition. The yellow seed colour is also recessive to the wild-type brown colour.

Figure 4-3 Breeding Program for Glabrous and Yellow Seeded Trait in *Phalaris canariensis*



5.0 METHOD OF MANUFACTURE

Annual canary seed will be processed using common cereal processing methods, the first two steps being harvesting and milling.

Annual canary seed is harvested after complete maturity is reached. Direct harvesting is used as canary seed is resistant to shattering. Once harvested, canary seed is stored in bins due to its low angle of repose (it flows quite easily) and to prevent rodent infestation. Canary seed is safe for storage at 12% seed moisture.

To avoid cross contamination of glabrous cultivars with pubescent cultivars, producers follow the quality management systems designed by the Canadian Seed Growers Association (CSGA) to ensure quality, identify preservation and traceability. Producers already provide documentation showing the canary seed variety. Documentation identifying varietal purity and guaranteeing a glabrous seed source will be critical to the quality chain.

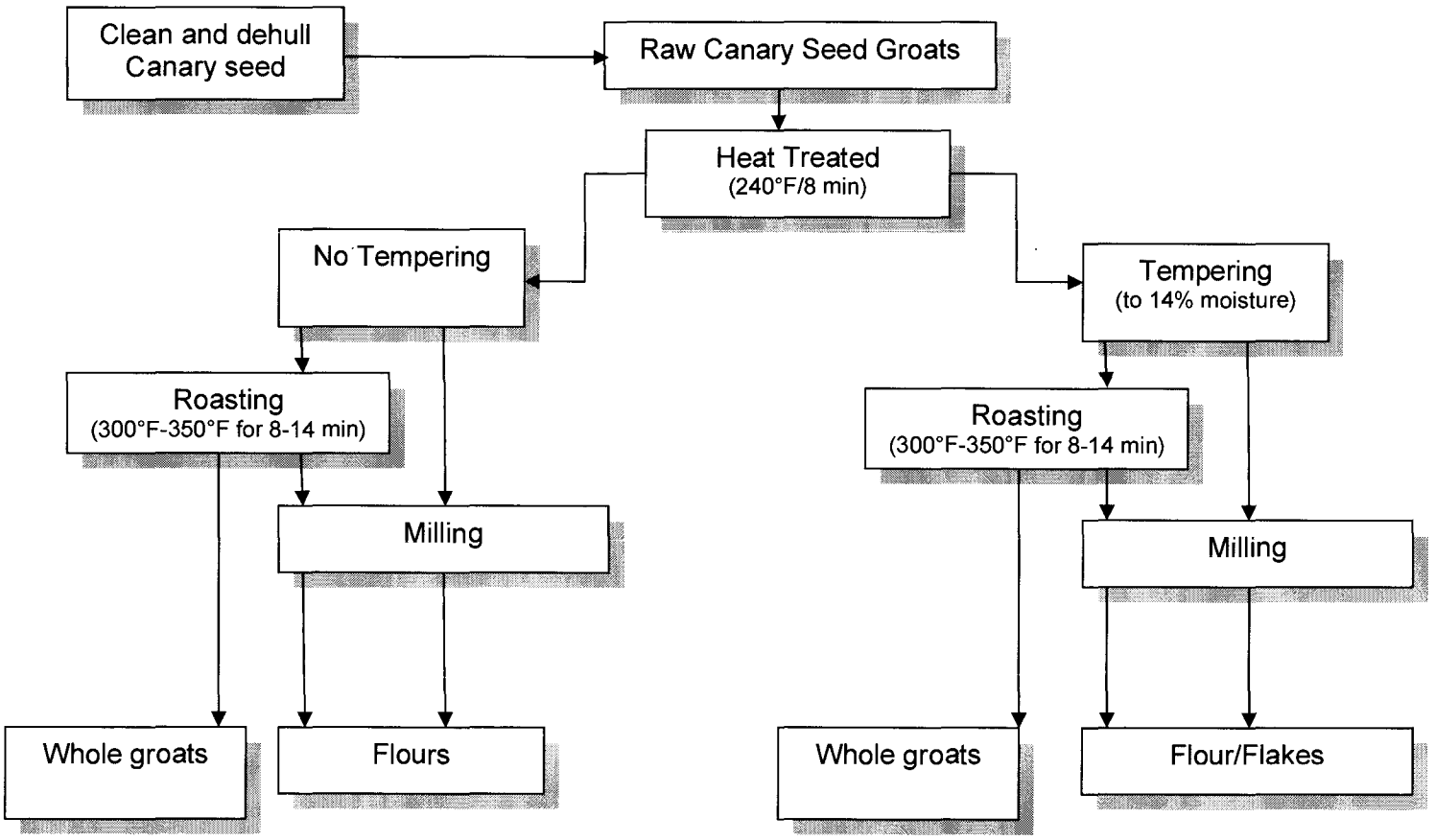
Canary seed processing involves the removal of debris and extraneous material from the harvested crop, removal of hulls, optional tempering of the groat to adjust moisture levels, and grinding and milling of the groats into whole meal flour, milled products or other forms (e.g. flakes). Canary seed groat products will then be sold as food ingredients.

Harvested glabrous canary seed destined as a food ingredient will be cleaned twice prior to dehulling. Dehulling is achieved via cone dehullers or plate dehullers that remove the glumes from the kernels via forced air and screen separation. Canary seed can be dehulled to >99% purity. Once dehulled the canary seed groats are then packaged into 50lb plastic or paper bags that are labeled, palletized and shrink-wrapped. Packaged dehulled canary seed is stored in forced air ventilated rodent-proof 40 foot containers until needed for shipment.

Currently there is no commercial manufacture of canary seed as a food ingredient or its incorporation into manufactured foods in Canada. There are a few canary seed producers/processors with the ability to dehull glabrous canary seed but they are awaiting novel food approval before targeting this niche market.

Processing methods and food products outlined in this submission are based on prototype products developed by the University of Saskatchewan and various Food Technology Centres in Canada. To facilitate processing, glabrous whole canary seed groats can be tempered to 14 % moisture. To enhance sensory properties and prolong shelf life, it can be roasted at 300°F to 350°F for 8-14 minutes and milled to produce whole grain flours or flakes and bran and white flour fractions (Abdel-Aal *et al*, 2010) that can be used directly in standard baking formulations (Figure 5-1). With increasing consumer interest in whole grain flours, the primary focus of product development has been on products containing roasted or unroasted whole groats or milled whole grain canary seed products.

Figure 5-1: Prototype processing methods for glabrous canary seed ingredients[1]



[1]Saskatchewan Food Industry Development Centre, Saskatoon, SK

6.0 DETAILS OF MAJOR CHANGE

The major change with glabrous annual *Phalaris canariensis* is the complete absence of trichomes (silicified hairs) from the glumes (palea and lemma) of canary seed and the selection of yellow coloured seeds in addition to the conventional brown coloured seeds. The presence and absence of hairs on the canaryseed glumes is illustrated in Figures 6-1a, b, respectively. Figure 6-2 shows the variation in canary seed groat colour.

Details relating to how this major change was achieved are outlined in *Section 4.2 Description of Genetic Modification*

Figure 6-1a Pubescent (hairy) hulled *Phalaris canariensis* (Keet)

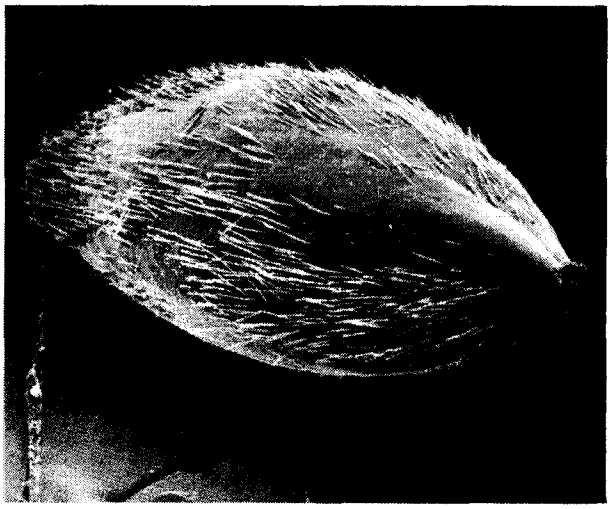
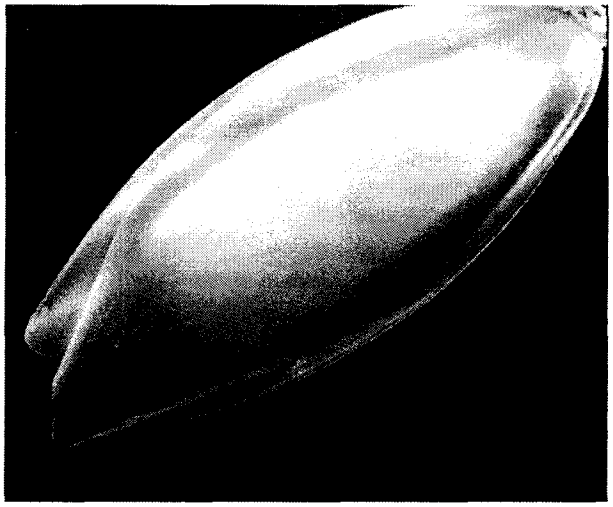
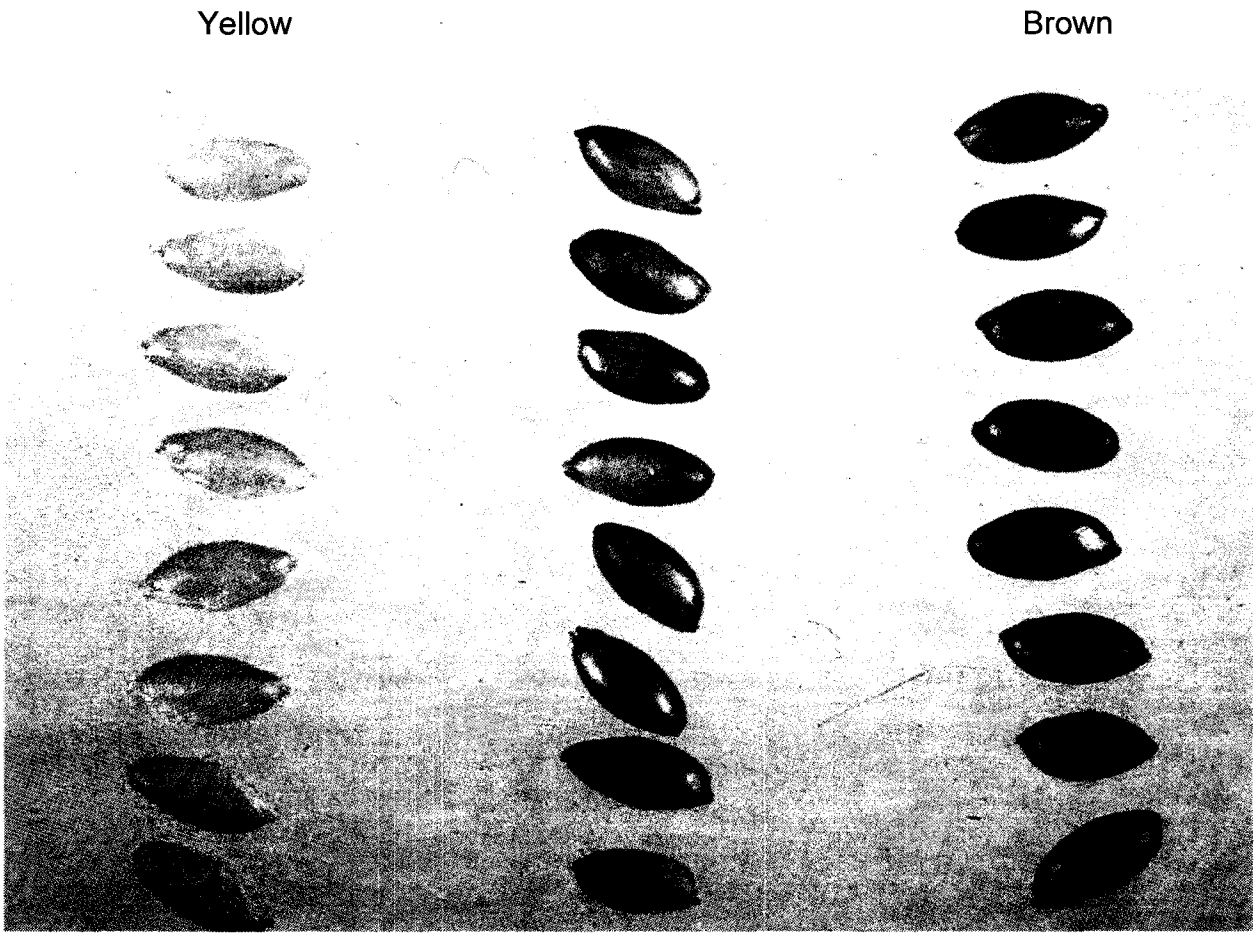


Figure 6-1b Glabrous (hairless) hulled *Phalaris canariensis* (CDC Maria)



(Photos courtesy of P. Hucl, University of Saskatchewan)

Figure 6-2 Canary seed groat colour



(Photo courtesy of P. Hucl, University of Saskatchewan)

7.0 Intended Use and Directions for Preparation

Canary seed groats (dehulled grain) either as a whole groat, whole meal, whole grain flour or a milled product are ideally suited for the bakery, cereal, pasta, snack and nutritional bar market. The grain could also be used as a low fat substitute for sesame seed (a common food allergen) in bread and snack foods or in combination with other seeds as toppings or ingredients.

Canary seed groat products are intended for use as an ingredient in various baked goods, breads, cereals and pasta products. The intended foods and use levels are presented in Table 7-1.

Table 7-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Canary seed in the U.S. (2009-2010 NHANES Data)

Food Category	Proposed Food-Uses	Maximum Proposed Use Level (%)
Baked Goods and Baking Mixes	Bagels	25
	Biscuits	20
	Breads and Rolls	25
	Cakes	20
	Cookies	50
	Cornbread, Corn Muffins, and Tortillas	25
	Crackers	26
	Croissants and Pastries	25
	Doughnuts	25
	Flours and Brans (pre-packaged)	100
	Muffins	20
	Pancakes and Waffles	25
	Pies	10
Breakfast Cereals	Instant and Regular Hot Cereals	15
	Ready to Eat Breakfast Cereals	15
Grain Products and Pastas	Energy, Meal Replacement, and Fortified Bars	25
	Granola and Cereal Bars	25
	Macaroni and Noodle Products	15
	Pasta, Rice and Other Grains	15
Snack Foods	Savory Snacks	25
	Seed-based snacks	40

Intended use and use levels identified above were based upon product prototypes developed at the University of Saskatchewan, the Canadian International Grains Institute, Manitoba Food Processing Development Centre, Guelph Food

Development Centre and the Saskatchewan Food Industry Development Centre using brown and yellow canary seed groats and flours.

Table 7-2 Prototype products from whole canary seed groats or whole grain flour

Centre	Prototype Products
Canadian International Grains Institute (Winnipeg, MB)	Pan bread, pasta, muffins, crackers, cereal bars, tortillas, snaps Topping for: bread and buns, crackers
Manitoba Food Development Centre (Portage La Prairie, MB)	Nutrition bars
Guelph Food Technology Centre (Guelph, ON)	Muffins
Saskatchewan Food Industry Development Centre	Pan bread and cookies
University of Saskatchewan	Pan breads

In all foods tested, the canary seed whole grain flour or whole groat was used to replace and/or complement other ingredients, whether it was refined wheat flour in breads, crackers, pasta, tortillas, muffins, or cookies, quick cooking oats (nutrition bars) or sesame seeds (sesame seed snaps). In the test conditions, up to 50% of refined wheat flour or whole wheat flour was substituted with canary seed whole grain flour in baked good formulations. A 25-35% substitution level produced acceptable food products. One hundred per cent of conventional seed toppings or sesame seed used for bread toppings, crackers, snaps and cereal and fruit bars were substituted with whole roasted canary seed (brown or yellow) groats illustrating the potential to use whole canary seed groats as alternatives to seeds or nuts. Snaps contained 100% substitution for sesame seeds.

Whole grain canary seed flour can also be sold as a stand-alone flour product in the retail market.

All products with the exception of muffins were tested using standard commercial formulations and were prepared in pilot plants. Muffins were tested using a standard household size recipe.

The Technology Centres found that dehulled Canadian glabrous brown and yellow canary seed groats could be processed into flour or roasted as a whole groat to

produce a wide variety of bakery, pasta and snack based products. Few adjustments were required to product formulations or processing conditions when canary seed was used. The flavor of the canary seed was found to be neutral in that it did not contribute nor detract from the flavor of the other ingredients in the formulation. Canary seed did not appear to negatively affect the texture when used as either a flour or whole seed. While food products containing yellow canary seed were more visually appealing than products made with brown canary seed, all products were considered to be acceptable.

All Centres provided the CDCS with prototype formulas and processing methods. Formulations and photographs of these products can be found in Appendix 1.

It is anticipated that canary seed in its whole groat form or as whole grain flour or milled product will first be sold as a food ingredient to secondary processors, with direct sales to consumers being the responsibility of a food processor. The CDCS will endeavor to provide future processors with as much processing information as possible and foresees the development of future recipe books as part of its marketing plan for food grade glabrous canary seed.

8.0 History of Use

Annual canary seed may have been originally used as a human food, although its historical uses are somewhat obscure. It is unclear when it was first used as birdseed, but Linnaeus's original typification and the scientific name *Phalaris canariensis* implies that its use for caged birds was well established in the 16th century. (Anderson, 1961; Baldini and Jarvis, 1991).

A comprehensive literature search in AGRICOLA, PubMed and CABI databases for evidence of human use of *Phalaris canariensis* indicated that canary seed (or alpiste) was recognized as a food in Europe as far back as the late 1500's particularly in those countries bordering the Mediterranean Sea as well as in South America and Mexico. A summary of the literature search is outlined in Table 8-1.

From a North American context, *Phalaris canariensis* appears to have been introduced to this continent in the mid- to late 1800's (Usher, 1974) with the Canadian Ministry of Agriculture growing the annual *Phalaris canariensis* at its Indian Head (SK) Experimental Farm in the late 1890s (MacKay, 1892). The reason for growing was not reported. Pubescent (hairy) canary seed was commercially grown as a grain crop in the northern Great Plains in the Red River valley of North Dakota and Minnesota starting after World War II while commercial production of pubescent canary seed in Canada began in the 1960s in Manitoba and 1971 in Saskatchewan. The primary market has been for use as bird feed.

The seeds of *Phalaris canariensis* are also listed as a food used by the indigenous population of Canada but no further explanations of use were given (Kuhnlein and Turner, 1991).

Other references identify its use as a grain for bread and cereals (Hedrick, 1919; Prance and Nesbitt, 2005) as well as a base for whiskey manufacture (Halliday, 1992). However, no data could be found describing human consumption levels or frequency of consumption for these applications.

Internet searches have shown that ground hulled canary seed is being sold as a beverage powder called "Canary Seed Milk" in the retail markets of Mexico and southern United States, but this appears to be as a traditional medicine rather than as a

food (Estrada-Salas *et al.*, 2014). Whole hulled seed is being sold as a tea (Alpiste) in the food markets of Spain. No data could be found regarding consumption levels.

In 2006, the American Association of Cereal Chemists (AACC) International Whole Grain Working Group Task Force on *Defining Whole Grains in Food* submitted a letter to the United States Food and Drug Administration (FDA) in response to the FDA's announcement in the Federal Register (V71 (33), Feb. 17, 2006) on Whole Grains Label Statements: Availability (AACCI, 2006). This letter (referred to as Docket No. 2006D-0066) included canary seed in its list of edible whole grains. Unfortunately, AACC International used the wrong species name in the whole grains list (*Phalaris arundinacea* rather than P. *canariensis*). An erratum to this Docket now correctly identifying the species name of *Phalaris* as "*canariensis*" was filed with the FDA in June 2011 (AACCI, 2011). A copy of the AACCI Docket response and erratum letter can be found in Appendix 2a & 2b and at the FDA Internet site:

http://www.regulations.gov/#!documentDetail;D=FDA-2006-D-0298-0027.

Links to the appropriate documents can also be found on the AACC International website:

Whole Grain Response:

http://www.aaccnet.org/initiatives/definitions/Pages/WholeGrain.aspx.

The letter itself is located at:

http://www.aaccnet.org/initiatives/definitions/Documents/WholeGrains/WGWGErrataCanarySeedtoFDA.pdf

Table 8-1 References describing the use of canary seed as a food*

Author	Description of Food Use of *Phalaris canariensis*
Jones & Engleson (2010)	The American Association of Cereal Chemists International (AACCI) whole grain working group task force listed canary seed as a true cereal as it fits with the definition of a whole grain.
Prance &Nesbitt (2005)	The author indicated that canaryseed was used as one of many cereals to make a local dish known as "gofio" in the Canary Islands. No other information is given in the artilce.
Halliday (1992)	Halliday noted that canarygrass (alpiste) was used as an ingredient in the making of whiskey. No other details given.
Kuhnlein & Turner (1991)	Authors listed the seed and root of *Phalaris canariensis* as an edible plant food for Canadian Indigenous people (Ch. 5)
Usher (1974)	Usher prepared a dictionary of plants used by man. Indicated canary seed was sometimes used for human consumption in the Mediterranean area.
Hedrick (1919)	In this treatise on edible plants, the author notes that "In Italy, the seeds are ground into a meal and made into cakes and puddings and in the Canary Islands, they are used in the same manner and also made into groats for porridge". No additional information given regarding consumption levels, or frequency of consumption
Piper (1916)	Piper provided background on the historical cultivation and use of annual canarygrass in the Mediterranean region. Refers to canary seed being used as a human food but no further details are given.
Ward (1911)	The Grocer's Encyclopedia: Identified uses for canary seed: as a flour in the manufacture of fine cotton goods and silk stuffs, and as a food in the Canary Islands, Italy and North Africa

*Note: all references, excluding Jones & Engleson, refer to the consumption of hairy varieties of *Phalaris canariensis.*

SAFETY ASSESSMENT

9.0 NUTRITIONAL CONSIDERATIONS

9.1 Compositional Analysis of Canary Seed Groats

Section 3 (Background Information) described the two research programs (Phase 1 and Phase 2) completed to support the safety assessment of glabrous canary seed. In Phase 1 (1992-2002), the nutritional and chemical characteristics of glabrous, brown coloured canary seed groats "CDC Maria" were compared to its pubescent brown coloured parent "Keet" and to Canada Western Red Spring (CWRS) common wheat "Katepwa". The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals.

Phase 2 (2008-2014) involved a comprehensive comparison of two glabrous yellow coloured cultivars (designated C05041 and C05091) to the brown coloured cultivar CDC Maria, which had been studied in the Phase 1 project.

Analytical results from Phase 1 and Phase 2 will be presented simultaneously to permit comparisons between the glabrous brown (CDC Maria) and yellow varieties (C05041 and C05091), the pubescent parent (Keet) and the CWHS wheat. Comparisons to compositional values of commonly consumed cereal grains will also be made.

9.1.1 Methods

9.1.1.1 Source of Grain Materials for Composition and Safety Assessment

The University of Saskatchewan (UofS) Crop Development Centre (CDC) was responsible for growing the pubescent and glabrous *Phalaris canariensis* and wheat used to gather information for the composition and safety assessment.

Phase 1 (1992-2002)

The glabrous canary seed (*P. canariensis* L.), cultivar CDC Maria and the pubescent cultivar Keet were grown in three-replicate randomized complete block experiments in Saskatoon, Saskatchewan in 1996-1998. The CWRS common wheat

Katepwa was grown in plots adjacent to the canary seed field trials. Two replicates from each variety of canary seed and wheat were analyzed separately. The analytical results are expressed as means of two replicates. For heavy metal and mycotoxin testing, the same randomized design was used to obtain samples of the glabrous and pubescent brown canary seed and CWRS wheat from ten sites in Saskatchewan, Canada in 1998.

The hulls of the canary seed grains were removed on an abrasive dehuller followed by air aspiration to produce hull-free grains called groats.

Phase 2 (2008-2014)

Three varieties of glabrous canary seed (brown coloured CDC Maria, and two yellow coloured varieties, C05041 and C05091) were grown at 5 sites throughout the province of Saskatchewan. At each of the five sites, a randomized block design was utilized and three replicate plots of each variety were planted in each of two years (2007 and 2008), providing the project with thirty (30) samples of each of the three varieties for a total of ninety (90) samples for initial analysis. In 2008, the three varieties were also grown in larger plots at the UofS Kernan Farm to provide sufficient grain (~500 kg grain harvested) for food product development, and the rodent toxicology trials and poultry feeding trials.

Statistical analysis of the proximate composition data for the ninety samples indicated there was no statistical difference in proximate composition analysis amongst the 3 replicate blocks of each cultivar at each site location, so hand-harvested grain from the 3 replicate blocks of a single cultivar were combined for further detailed chemical analysis. Three of the five sites produced sufficient quantities of canary seed (6 composite samples for each cultivar for a total of 18 composite samples) to continue in-depth compositional analysis for nutrients, antinutritional factors, inorganic chemicals and mycotoxins.

9.1.1.2 Analytical Methods for Chemical and Nutritional Composition

Tables 9-1 and 9-2 provide a listing of methods used to determine the compositional, nutritional and chemical characteristics of canary seed. Copies of the relevant methods for each analysis can be found in Appendix 3.

The majority of analyses conducted during Phase 1 were performed in-house at the UofS, while analyses for Phase 2 were primarily outsourced to accredited commercial laboratories (POS Biosciences (SK), Silliker Canada Ltd (ON), ALS Laboratory Group (SK), University of Guelph Laboratory Services (ON), Intertek-Sunwest Laboratoratories (SK) and Labs-Mart (AB)) and research laboratories (Agriculture and Agri-Food Canada and University of Manitoba) across Canada. Where necessary, additional methodology details are provided in the body of this dossier.

Table 9-1 Summary of Methodologies Used for Analyses in Phase 1 (1992-2002)

Component	Description	Method	Laboratory	Reference
Proximate Analysis	Moisture	AACC 44-15A	University of Saskatchewan (UofS)	AACC, 1998
	Crude protein	AACC 46-11A		AACC, 1998
	Crude fat	AACC 30-20		AACC, 1998
	Total ash	AACC 08-03		AACC, 1998
Carbohydrate	Starch	AACC76-13		AACC, 1998
	Soluble, insoluble and total dietary fiber	Enzymatic gravimetric procedure, AACC 32-21	UofS	AACC, 1998
	Soluble sugars	Sugar derivatives by gas chromatography		Abdel-Aal *et al.*,1997b
Lipids	Total and purified			Fölch *et al.*, 1957.
	Fatty acid composition	FAME-GC	UofS	Abdel-Aal *et al.*,1997b
Proteins	Fractionation into albumin, globulin, prolamin, glutelin	Successive extraction method based upon Osborne		Sosulski & Bakal, 1969
	Amino acid composition	Reversed-phased HPLC	UofS	Abdel-Aal *et al.*,1997b
	Tryptophan	Spectrometric method		Concon, 1975
	Protein digestibility	Multienzyme technique		Pedersen & Eggum, 1983
Vitamins	Thiamine	AOAC, thiamine 942.23	FDC Northwest Laboratories	AOAC, 1995
	Riboflavin	AOAC, 981.15		AOAC, 1995

	Niacin	AOAC 975.41		AOAC, 1995
Minerals	Major and trace minerals	AOAC 985.01, Inductively coupled argon plasma	FDC Northwest Laboratories	AOAC, 1995
Heavy Metals	Silver, arsenic, bismuth, cadmium, mercury, molybdenum, lead, antimony, tellurium and tungsten	Inductively coupled plasma-atomic emission spectrometry (ICPES)	Saskatchewan Research Council (SRC, Saskatoon, Canada)	Internal method
Mycotoxins	Aflatoxin, vomitoxin	ELISA	Grain Research Laboratory, Winnipeg, MB	
Alkaloids	Phenol, indole and beta-carbolines	GLC/HPLC	UofS	Duynisveld *et al.*, 1990
	Dhurrin	GLC/HPLC		Gorz *et al.*, 1986.
Phenolics	Total	Prussian blue spectrophotometric method	UofS	Price & Butler, 1977
	Condensed tannins	Vanillin assay		Price *et al.*, 1978
	Phenolic acids	Reversed phase-HPLC		Hatcher and Kruger, 1997
Phytate		Anion exchange method, AOAC 32.5.18	UofS	AOAC, 1995
Enzyme Inhibitors	Trypsin inhibitor activity	Spectrophotometric method	UofS	Kakade *et al.* 1974
	Amylase inhibitor activity			Mulimani & Supriya, 1993.

Table 9-2 Summary of Methodologies Used for Analyses in Phase 2 (2008-2014) [1]

Component	Description	Method	Laboratory	Reference
Proximate Analysis	Moisture	AOCS Ba2a--38 (meal)	POS	AOCS 2009
	Crude protein	AOCS Ba 4e-93	Biosciences	AOCS 2009
	Crude fat	Swedish tube (internal method)		
	Total ash	AOAC Bc 5-49		AOAC 2003
Carbohydrate	Starch	AACC 76-13		AACC 2003
	Crude fiber	AOCS Ba 6-84		AOCS 2009
	Soluble and insoluble	AACC 32-21	POS	AACC 2003
	Total dietary fiber	AACC 32-05		AACC 2000
	Acid detergent and lignins	AOAC 973.18		AOAC 2003
	Neutral	AACC 32-20 (Modified)		AACC 2003
	Soluble sugars	AOAC 980.13	Sunwest Food Laboratories (Saskatoon)	AOAC 2003
Lipids	Fatty acid composition	AOAC 969.33 prep, AOAC 996.06 quant. modified		AOAC 2003
	Unsaponifiable matter	AOCS Ca 6a-40	POS	AOAC 2003
Proteins	Amino acid composition	Reversed-phased HPLC Waters Pico-Tag Method and Internal Method	POS	Internal Method
	Protein dispersibility index	AOCS Ba 10a-65		AOCS 2009
Vitamins	Thiamine (B1)	AOAC 942.23	Silliker Canada Co.	AOAC 2003
	Pyridoxine (B6)	AOAC 961.15 (USFDA 400)		AOAC 2003
	Riboflavin	AOAC 981.15		AOAC 2003
	Niacin	AOAC 975.41 (USFDA 340)		AOAC 2003
	Folic Acid	AACC 86-47.01	Labs-Mart (Edmonton, AB)	AACC 2013
Minerals	Microelement panel (Al, As, B, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se, tin, titanium, zinc)	Toxi-024- Metals in biological materials by ICP-OES	University of Guelph Laboratory Services	Internal method provided
	Macro element panel (Ca, Mg, P, K, Na, S, Fe)	Metals in biological metals by ICP-OES (Toxi-024)	University of Guelph Laboratory Services	Internal method provided
Heavy Metals	Arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr),	ICPMS Analysis of Metals in Foods (Toxi-064)	University of Guelph Laboratory Services	Internal method provided

	copper (Cu), iron (Fe), lead (Pb), manganese (Mn), molybdenum, (Mo) nickel (Ni), zinc (Zn),			
	Silver (Ag), arsenic (As), bismuth (Bi), cadmium (Cd), mercury (Hg), molybdenum (Mo), lead (Pb), antimony (Sb), tellurium (Te)and tungsten (w)	Metals in environmental matrices by Inductively Coupled Plasma Mass spectrometry (ICP/MS)	ALS Laboratory Group (Edmonton, AB)	Internal method provided
Mycotoxins	Vomitoxin	Vomitoxin ELISA IMC-411	University of Guelph Laboratory Services	Method provided
	Ochratoxin A	RIDASCREEN®FAST Ochratoxin A Test	Intertek-Sunwest (SK)	Internal method
	Fumonisins (total)	AOAC – 2001.06 RIDASCREEN® FAST Fumonisin: Total Fumonisin in Corn		,AOAC, 19th edition 2012
	Zearalenone	AOAC 994.01 RIDASCREEN®FAST Zearalenone Enzyme Immunoassay for Quantitative Determination of Zearalenone		AOAC,19th edition, 2012
Alkaloids	Phenol, indole and beta-carbolines Dhurrin	GLC/HPLC, UPLC – internal method developed by AAFC GLC/HPLC	Agriculture and Agri-Food Canada, Saskatoon, SK	Duynisveld *et al.*, 1990, Muir *et al*, 1992 Gorz *et al.*, 1986.
Phenolics	Total Condensed tannins Phenolic acid composition	Folin-Ciocalteau Vanillin assay Reversed phase-HPLC	University of Manitoba	Li *et al.*, 2010 Price *et al.*, 1977 Li *et al*, 2011
Phytate	Phytic acid determination	Anion exchange method	University of Manitoba	Latta & Eskin 1980
Enzyme Inhibitors	Trypsin inhibitor activity Amylase inhibitor activity	Spectrophotometric method	University of Manitoba	Kakade *et al.*, 1974 Deshpande *et al*, 1982.
Phytosterols	Sterols and tocopherols	Capillary gas chromatography	POS	Slover *et al.*, 1983

[1] Methods for proximate analysis were used on the 90 samples and on the 18 composite samples.

9.1.1.3 Statistical Analysis

Phase 1

All analyses were carried out using at least two separate determinations for each sample. Analysis of variance was performed to determine significant differences between cultivars for nutrients, minerals, and vitamins using Minitab Software (version 12, Minitab Inc., State College, PA, USA). Differences were examined using the least significant difference (LSD) method and were considered to be significant when $p < 0.05$.

Phase 2

All analyses were carried out using at least two separate determinations for each sample. For the individual 90 samples, analysis of variance was carried out to assess the variation amongst the canary seed samples to determine the amount of variability between cultivars for protein, oil, ash, moisture and carbohydrate and to determine whether test plots of a specific variety from one site could be combined. In this study, varieties were nested in subsamples, subsamples in blocks, blocks in locations, and locations in years.

The variance components analysis was performed to assess the variation within each level of the dataset for the ninety samples to determine 1) the amount of between-site variation, and 2) whether further statistical analysis should be conducted on individual subsamples or averaged subsamples.

The subsample displayed little variation, and implied strong consistencies within the laboratory analyses. Little variation attributable to the experimental blocks indicated consistent environments within each field site and thus enabled composite samples to be prepared from the replicate plots.

Mixed effects models (Hurlbert, 1984) were used to assess how the varieties differed from each other with year, location and block specified as random effects. These models were fit using the "lme" function in the "nlme" library in the R package. (Crawley, 2007).

Orthogonal contrasts were used to assess whether there was a difference between varieties. Contrasts were only performed on models after the initial mixed model indicated significant differences.

9.1.2 Nutrient Composition of Raw Canary Seed Groats

Hand-harvested samples from each of the test plots were dehulled and hand cleaned. The hulls of the canary seed grains were removed on an abrasive dehuller followed by air aspiration to produce hull-free grains called groats.

9.1.2.1 Chemical Composition

For the purposes of this dossier, chemical and nutrient values for the two glabrous yellow cultivars (C05041 & C05091) analyzed in Phase 2 have been combined to provide the mean and range of values for yellow canary seed. Similarly, values for the glabrous brown variety (CDC Maria) include results from Phase 1 and Phase 2. Nutrient values for pubescent brown canary seed (Keet) and the CWRS wheat (Katepwa) are from the Phase 1 study only.

Microstructure analysis of canary seed illustrated that canary seed is a true cereal similar to wheat, oats, barley and rice containing three main components: bran, the germ and the starchy endosperm (Abdel-Aal *et al.*, 2011a).

Glabrous brown and yellow canary seed cultivars have a proximate composition profile similar to the pubescent parent, Keet (Table 9-3). Glabrous varieties were slightly lower in crude fat content and higher in protein content but had similar ash content to the pubescent cultivar. All canaryseed varieties (glabrous or pubescent) were higher in ash, crude fat and protein than the Canadian Western Red Spring (CWRS) wheat (Table 9-3). Robinson (1978) reported that canary seed caryopses were much higher in nitrogen, ash, oil, phosphorous and potassium but lower in fiber than other grain crops. The nitrogen-to-protein conversion factor used for canary seed protein was 5.7 as recommended for cereals by Sosulksi & Imafidon (1990).

For comparative purposes, the chemical composition of glabrous canary seed groats (dehulled canary seed) is compared to commonly consumed cereal grains such as wheat, barley, oats and rye and, in some instances, to other specialty whole grains (e.g. sorghum, millet), pseudocereals (e.g. amaranth, quinoa and buckwheat) and brown rice (Jones & Engleson, 2010).

Table 9-3 Comparison of proximate chemical composition (% dry basis) of glabrous brown and yellow coloured canary seed groats to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed[1, 2]								Pubescent Canary Seed[1]			Wheat[1]	
	Brown				Yellow				Brown			CHRS	
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range	Mean	SD
			Min	Max			Min	Max					
Ash	2.4	±0.2	2.1	2.6	2.2	±0.2	1.9	2.4	2.1	±0.1	2.0-2.1	1.7	±0.1
Crude Fat	6.2	±0.3	5.5	6.6	6.2	±0.2	5.8	6.4	8.7	±0.3	8.4-8.9	2.3	±0.1
Protein (Nx5.7)	21.8	±0.7	20.8	23.1	21.0	±1.0	19.3	22.8	18.7	±2.7	15.6-20.3	15.0	±2.0
Carbohydrate (by difference)	69.3	±0.7	68.4	70.4	70.6	±0.9	69.3	72.1	70.5	NR	NR	65.7	NR

[1] Abdel-Aal *et al.*, 1997b
[2] Phase 2, CDCS study
NR: not reported

Protein concentrations for glabrous canary seed ranged from 19.3 % to 23.1 %. These protein values are higher than those found in wheat (10-16%) (OECD, 2004), barley (7.6-14.4%) (OECD, 2003) and oats (13.8 – 22.5 %) (McMullen, 2000). The protein level for glabrous canary seed is also higher than protein levels in other specialty cereals such as millet (8.8% db (N x 6.25), sorghum (12.1 % db (N x 6.25) (Ragaee *et al.*, 2006),amaranth (16.8% N x 5.85) (Bejosana & Corke, 1998), buckwheat (12.5% N x 5.7), brown rice (7.9% N x 6.25) (Rosell & Marco, 2008) and quinoa (14.5 %, N x 5.96) (Alvarez-Jubete *et al.*, 2010). Glabrous canary seed has a higher content of crude fat (~6%) compared to wheat and barley (2.31%), millet (4.22%), rye (2.53%) and sorghum (3.32%) (Chung & Ohm, 2000). The content of crude fat in canary seed is very similar to oats (3.1-11.6%), quinoa (5.01-5.95 %) and amaranth (6.56-10.3%) and higher than buckwheat (2.4-2.8%) (Schoenlechner *et al.*, 2008) and rice (2.9%) (Rosell & Marco, 2008). The ash content in canary seed groats ranged from 1.94 to 2.6% across all varieties and sites examined. This range is comparable to the range of ash content found in other common cereals such as wheat (1.17-2.96%) (OECD, 2004), barley (2.0-5.0%) (OECD, 2003) and field maize (1.1-3.9%) (OECD, 2002) and pseudocereals such quinoa (2.4-3.3%)(Schoenlechner *et al.*, 2008). Canary seed has a mineral content lower than amaranth (3.25%) but higher than buckwheat (1.37-1.67%) (Schoenlechner *et al.*, 2008) and rice (1.5%) (Rosell & Marco, 2008).

As discussed in Methods (Section 9.1.1.3), statistical analysis of the proximate composition (protein, ash, crude fat) on the ninety individual samples grown in Phase 2 indicated that glabrous canary seed from replicate plots at one location could be combined to provide an adequate volume of grain for more detailed compositional and nutritional analysis. Three of the five test sites produced sufficient quantities of grain to produce 6 composites of each variety (18 samples) for further in-depth analysis.

9.1.2.2 Protein and Amino Acid Composition

The protein content in the canary seed groats was higher than that reported in the literature for barley, oat or wheat (Gutierrez-Alamo *et al.*, 2008; Quinde *et al*, 2004).

Glabrous canary seed has an amino acid profile similar to that of its pubescent parent (Table 9-4); the notable difference being the lower lysine range of the pubescent

cultivar (1.1-1.4 g AA /100g protein) compared to the glabrous varieties (1.4-2.6 g amino acid (AA) /100g protein). The lysine content in canary seed is slightly lower than that found in wheat, barley and oats, but is comparable to maize (Table 9-5).

Compared to other cereals, canary seed proteins have higher contents of tryptophan, phenylalanine, and cysteine, the methionine-sparing amino acid (Table 9-5). Tryptophan is nutritionally important as it is a precursor for important metabolites such as serotonin and nicotinamide (WHO, 2007). Its content is low in cereals, especially maize. The range of tryptophan in glabrous canary seed (2.7 -3.1 g AA/100g protein) is twice as high as that found in many cereals and pseudocereals. Comai *et al* (2007) reported tryptophan levels (all as g AA/100g protein) in spelt, 1.17; wheat, 1.16; quinoa, 1.14; sorghum, 1.1; oat, 0.97; pearl millet, 0.97; barley, 0.96; rye 0.82 and maize, 0.49. The phenylalanine content in glabrous canary seed ranged from 6.2 to 6.7 g AA/100g protein, higher than reported for wheat (3.5-5.4 g AA/100g), barley (4.2-5.4 g AA/100g) and oats (5.3 g AA/100g). Canary seed groats had cysteine levels ranging from 2.4 to 3.4 g/100g higher than wheat, oats, and barley (Table 9-5).

While the range of total essential amino acids in canary seed protein is higher than those of wheat, the higher canary seed amino acid values are comparable to those of oats, barley and maize (Table 9-5). The values of the non-essential amino acids in canary seed were comparable to wheat, oats, barley and corn.

Table 9-4 Comparison of protein (%), non-protein nitrogenous material (%) and amino acid profile (gAA/100g protein) of glabrous brown and yellow canary seed compared to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed								Pubescent Canary Seed[1]			CWRS[1]
	Brown[1,2]				Yellow[2]				Brown			Wheat
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range	Mean
			Min	Max			Min	Max				
Protein (N x 5.7) (%)	21.8	±0.8	20.8	23.06	21.0	±0.2	1.9	2.4	18.7	±2.7	15.6-20.3	15.0
Non-protein nitrogen (%)	0.8	±0.1	0.7	0.90	0.8	±0.1	0.7	0.9				
Amino Acid Profile												
Alanine	4.5	±0.1	4.5	4.6	4.5	±0.1	4.4	4.6	4.1	±0.1	4.1-4.2	3.0
Arginine	6.5	±0.2	6.3	6.8	6.6	±0.2	6.3	6.9	6.9	±0.1	6.8-7.0	5.1
Aspartic acid	4.4	±0.2	4.1	4.7	4.5	±0.1	4.2	4.7	4.6	±0.1	4.5-4.6	4.4
Cystine	2.5	±0.1	2.2	3.4	2.5	±0.1	2.4	2.6	3.3	±0.1	3.2-3.3	2.3
Glutamic acid	26.1	±0.6	25.2	26.7	26.5	±0.4	25.6	27.0	30.6	±0.2	30.4-30.7	33.0
Glycine	3.1	±0.1	3.0	3.2	3.1	±0.1	2.9	3.2	3.0	±0.1	3.0-3.1	3.8
Histidine	1.7	±0.1	1.6	1.9	1.7	±0.1	1.6	1.8	1.8	±0.1	1.7-1.9	2.1
Isoleucine	3.9	±0.1	3.4	4.1	3.9	±0.1	3.8	4.1	3.5	±0.1	3.5-3.6	2.8
Leucine	7.6	±0.2	7.1	7.8	7.6	±0.2	7.4	7.8	7.0	±0.1	7.0-7.1	5.3
Lysine	2.6	±0.2	1.4	2.8	2.5	±0.1	2.5	2.6	1.4	±0.2	1.1-1.4	1.9
Methionine	1.9	±0.2	1.4	2.2	1.9	±0.2	1.7	2.2	1.4	±0.1	1.3-1.5	1.4
Phenylalanine	6.5	±0.1	6.3	6.7	6.5	±0.1	6.2	6.6	6.7	±0.4	6.4-7.1	5.4
Proline	6.2	±0.1	6.1	6.3	6.3	±0.1	6.1	6.4	5.4	±0.1	5.3-5.4	8.6
Serine	4.5	±0.1	4.5	4.5	4.5	±0.1	4.3	4.9	4.2	±0.1	4.1-4.2	4.3
Threonine	2.7	±0.1	2.7	2.8	2.8	±0.2	2.5	2.9	2.7	±0.1	2.7-2.8	2.8
Tryptophan	2.8	±0.1	2.7	2.9	2.9	±0.2	2.7	3.1	2.8	±0.3	2.6-3.1	1.2
Tyrosine	3.6	±0.1	3.3	3.8	3.6	±0.2	3.4	3.8	3.2	±0.1	3.2-3.3	3.5
Valine	4.8	±0.1	4.7	4.9	4.8	±0.1	4.7	4.9	4.6	±0.2	4.5-4.8	3.8
Total A. A.	95.9	±1.2	94.5	97.6	96.6	±1.2	94.9	97.5	97.2	±0.3	97.0-97.5	94.7

[1] Values from Abdel-Aal *et al.*, 1997b
[2] Values from Phase 2, CDCS study

000068

Table 9-5 Comparison of amino acid composition of glabrous canary seed to four common cereal grains

Amino Acid	Canary Seed[a] (g/100g protein)	Wheat[b] (% total protein)	Barley[c] (g/100 g protein)	Maize[d] (g/16gN)	Oats[d] (g/16g N)
Essential AA					
Methionine	1.4-2.2	1.3-1.7	1.4-3.2	1.8	2.5
Cysteine	2.2-3.4	1.7-2.7	1.0-1.8	1.1	1.6
Lysine	1.4-2.8	2.2-3.0	3.1-4.2	2.6	4.2
Tryptophan	2.7-3.1	1.0-2.7	1.5[d]	0.7	1.3
Isoleucine	3.4-4.1	3.0-4.3	3.1-3.9	3.7	3.9
Histidine	1.6-1.9	2.0-2.8	1.9-3.3	2.8	2.2
Valine	4.7-4.9	4.4-4.8	3.9-5.3	5.3	5.3
Leucine	7.1-7.8	5.0-7.3	5.4-7.1	13.6	7.4
Phenylanlanine	6.3-6.7	3.5-5.4	4.2-5.4	5.1	5.3
Tyrosine	3.4-3.8	1.8-3.7	1.9-2.8	4.4	3.3
Threonine	2.7-2.9	2.4-3.2	3.0-3.7	3.6	3.3
Total essential AA	**36.95-43.75**	**26.3-41.6**	**30.4-42.19**	**44.7**	**40.3**
Non-essential AA					
Alanine	4.4-4.6	3.4-3.7	4.4-4.6	7.9	5.0
Arginine	6.3-6.9	4.0-5.7	4.2-6.2	3.8	6.9
Aspartic acid	4.1-4.7	4.8-5.6	6.8-7.4	6.3	8.9
Glutamic acid	25.2-26.9	29.9-34.8	21.9-26.1	18.9	23.9
Glycine	2.9-3.2	3.8-6.1	4.2-5.1	3.4	4.9
Proline	6.1-6.4	9.8-11.6	11.4-12.4	8.3	4.7
Serine	4.3-4.7	4.3-5.7	3.7-5.4	4.8	4.2

[a] Data range canary seed analysis (Phase 1 and Phase 2, yellow and brown glabrous canary seed))
[b] From OECD, 2004
[c] From OECD, 2003, except for tryptophan (Lookhart & Bean, 2000 Table 2)
[d] From Lookhart and Bean, 2000 Table 2

9.1.2.3 Fatty Acid Profile

Glabrous and pubescent canaryseed groats contain approximately 3 to 4 times the amount of crude fat than the CWRS wheat. Crude fat levels in the parent pubescent canaryseed ranged from 8.4-8.9%, the glabrous brown ranged from 5.5-6.6%; and the glabrous yellow ranged from 5.8-6.4%. The CWRS wheat in the study contained 2.3% crude fat. Glabrous canary seed has a higher content of crude fat (~6%) compared to wheat and barley (2.3%), millet (4.2%), rye (2.5%) and sorghum (3.3%)(Chung & Ohm,

2000). The content of crude fat in canary seed is within the range of crude fat in oats (3.1-11.6%).

Like other cereal grains, the predominant fatty acids in glabrous brown and yellow canary seed are palmitic (range: 11.2-12.3%), oleic (range: 26.7-33.6%) and linoleic acids (range: 48.2-54.9%)(Table 9-6). These values are comparable to that of the pubescent canary seed parent Keet (10.7%, 29.8% and 55.4%, respectively) (Table 9-6 and Abdel-Aal *et al.*, 1997b) and consistent with fatty acid values (palmitic, 12%; oleic, 32%; and linoleic, 54%) in other tested pubescent canary seed cultivars (Malik & Williams, 1966).

As a relative percentage of fatty acids, palmitic acid was present in lower levels (11.0-13.3%) in canary seed than found in the CWRS wheat (~16%, Table 9-6), other wheat varieties (17-24%), barley (19-28%) and rye (12-19%)(Chung & Ohm, 2000). Canary seed contained a relatively higher level of oleic acid (28.7-35.5%) than these cereal grains [wheat (8-21%), barley (9-17%) and rye (12-17%)] with a very similar relative level to oats (22-39%) (Youngs and Püskülcü, 1976) and buckwheat (37%) (Taira *et al*, 1986). Linoleic acid is the major fatty acid in canary seed oil, constituting about 55% of the total fatty acids compared to 61% in wheat oil.

Canary seed contains approximately 85% unsaturated fatty acids, of which approximately 32% is monounsaturated and 55% are polyunsaturated fatty acids (Table 9-7). Canary seed has a higher unsaturated to saturated fat ratio (~85:13) than wheat, barley and oats (all about 75:25) but contains a lower percentage of polyunsaturated fatty acids (~55%) than wheat (~66%) and barley (~60%) but more than oats (~48%). Canary seed has been found to exhibit antioxidant properties for fats and oils primarily due to the presence of caffeic acid esters and phytosterols (Takagi & Iida, 1980). Canary seed groats contain about 2% omega-3 fatty acids (Table 9-7), similar to other cereal grains.

Table 9-6 Comparison of fatty acid composition (% total fatty acids) of brown glabrous and yellow canary seed to pubescent brown canaryseed and CWRS wheat

		Glabrous Canary Seed								Pubescent Canary Seed[1]		Wheat[1]
		Brown[1,2]				Yellow[2]				Brown		CWRS
Fatty Acid		Mean	SD	Range		Mean	SD	Range		Mean	SD	Mean
				Min	Max			Min	Max			
Crude Fat (%)		6.2	±0.3	5.5	6.6	6.2	±0.2	5.8	6.4	8.7	±0.3	2.3±0.1
Monounsaturated FA												
Hexadecenoic	C16:1	0.2	±0.0	0.2	0.2	0.1	±0.0	0.1	0.2	nr		nr
Oleic	C18:1	30.9	±2.1	28.7	33.6	29.9	±1.8	26.7	32.4	29.5	±0.8	16.6
Octadecenoic	C18:1	0.7	±0.0	0.7	0.8	0.6	±0.1	0.5	0.8	nr		nr
Eicosenoic	C20:1	1.0	±0.1	0.9	1.1	0.9	±0.2	0.1	1.1	nr		nr
Erucic	C22:1	0.1	±0.0	0.1	0.1	0.1	±0.0	0.1	0.1	0.1	±0.1	0.0
Polyunsaturated FA												
Linoleic	C18:2	51.1	±2.1	48.2	53.2	52.2	±1.8	49.8	54.9	55.4	±1.0	61.2
Linolenic	C18:3	2.2	±0.3	1.9	2.6	1.9	±0.5	0.0	2.4	2.7	±0.2	4.6
Saturated FA												
Myristic	C14	0.2	±0.0	0.2	0.2	0.2	±0.01	0.2	0.2	0.2	±0.1	0.2
Palmitic	C16	11.9	±0.2	11.8	12.3	11.6	±0.3	11.2	12.1	10.7	±0.3	15.8
Stearic	C18	1.3	±0.1	0.9	1.4	1.4	±0.1	1.3	1.5	1.0	±0.1	0.8
Arachidic	C20	0.1	±0.0	0.1	0.1	0.2	±0.0	0.0	0.2	0.1	±0.1	0.0
Behenic	C22	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.1	0.1	±0.1	0.2
Others		0.1	±0.0	0.0	0.2	0.1	±0.0	0.0	0.1	0.9	0.1	0.7

*nr: Not reported
[1]Abdel-Aal *et al.*, 1997
[2]Values from Phase 2, CDCS study

000071

Table 9-7 Comparison of the Fatty Acid Profile (% of total fatty acids) in glabrous brown and yellow canary seed groats

	Brown[1,2]				Yellow[2]			
	Mean	STDEV	Range		Mean	STDEV	Range	
			Min	Max			Min	Max
Saturates	13.7	±0.3	13.5	14.1	13.5	±0.4	13.0	14.1
Monounsaturates	32.9	±2.1	30.6	35.6	31.9	±1.7	29.5	34.3
Polyunsaturates	53.3	±2.3	50.2	55.8	54.6	±2.0	51.6	57.4
Omega 3	2.2	±0.3	1.9	2.6	2.0	±0.2	1.8	2.4
Omega 6	51.1	±2.1	48.2	53.2	52.5	±1.8	49.8	55.0
Omega 9	32.1	±2.1	29.7	34.8	31.0	±1.7	28.7	33.6

[1]Abdel-Aal *et al.*, 1997
[2]Values from Phase 2, CDCS study

9.1.2.3.1 Tocopherol and Phytosterol Composition

Tocol derivatives (tocopherols and tocotrienols) are responsible for the vitamin E activity in plant tissues and various combinations of all eight tocol derivatives are found among the cereal grains (Chung & Ohm, 2000).

Wheat has 4 major tocol derivatives (α-tocopherol, α-tocotrienol, β-tocopherol and β-tocotrienol) present and barley has all eight naturally occurring tocopherols. Oats contain six of the tocopherols derivatives (α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol, δ-tocopherol and trace of Δ-trienol) (Chung & Ohm, 2000).

In the Phase 2 study, α-tocopherol and δ-tocopherol were detected in both brown and yellow glabrous canary seed (Table 9-8). Phytosterols were not determined in the Phase 1 study. The total tocopherol range in canary seed (1.8-3.4 mg/100g) is somewhat less than the total tocopherol content reported in wheat (4.9-5.8 mg/100g), barley (4.22-8.0 mg/100g), but similar to the levels found in oats (1.3-3.0 mg/100g) (Peterson *et al.*, 2007).

Cereals are recognized as significant plant sterol sources. The most abundant sterols in plant sources, including oilseeds and fresh vegetables, are sitosterol, campesterol, stigmasterol, Δ5-avenasterol and Δ7-avenosterol where sitosterol is the predominant sterol (Piironen *et al.*, 2002). The total phytosterol contents of bread wheat grains have been reported to range from 0.67-0.96 mg/g (db) with the differences being attributed to genetic variation, environmental factors and analytical methods (Pirronen *et al.*, 2009). β-sitosterol comprises about 60% of the total sterols in barley and in wheat, 41-53% of the total sterols. Campesterol is the next most abundant sterol found in barley (OECD, 2003) and wheat (OECD, 2004). Canary seed groats have the same sterol profile as other common cereals with β-sitosterol as the primary sterol comprising about 41.5 to 43% of the total sterols in canary seed, followed by campersterol, stigmasterol and cholesterol. However, the range of total sterols (0.44-0.50 mg/g dm) is similar to oats (0.35-0.49 mg/g dm) (Maata *et al.*, 1999) but less than found in wheat (0.67-0.96 mg/g dm) (Piironen *et al.*, 2009) and barley (0.89-1.1 mg/g dm) (Andersson *et al.*, 2008).

Table 9-8 Comparison of the tocopherol (mg/100g) and sterol (mg/g) content of glabrous brown and yellow canary seed groats[1]

	Brown[1]				Yellow[1]			
	Mean	SD	Range		Mean	SD	Range	
			Min	Max			Min	Max
Tocopherols (mg/100g)								
α-tocopherol	2.2	±0.3	1.8	2.8	1.9	±0.2	1.6	2.4
δ tocopherol	0.6	±0.2	0.3	1.0	0.5	±0.2	0.1	0.8
Total Tocopherols	2.8	±0.5	2.3	3.4	2.2	±0.3	1.8	2.8
Sterols (mg/g)								
β-sitosterol	0.20	±0.01	0.18	0.21	0.20	±0.01	0.19	0.21
Campesterol	0.11	±0.01	0.10	0.12	0.11	±0.00	0.11	0.12
Stigmasterol	0.01	±0.00	0.00	0.01	0.01	±0.00	0.01	0.01
Cholesterol	0.001	±0.00	0.001	0.001	0.00	±0.00	0.000	0.00
Other Sterols	0.15	±0.01	0.14	0.16	0.14	±0.01	0.12	0.15
Total Sterols	0.47	±0.03	0.44	0.50	0.45	±0.01	0.43	0.48
Unsaponifiable Matter (%)	1.71	±0.10	1.55	1.88	1.64	±0.17	1.43	1.94

[1] Phase 2, CDCS Study

9.1.2.4 Carbohydrate Fraction

Cereal grains are considered an important source of starch (40-90% of their dry weight) as are pulses (30-70%) and tubers (65-85%) (Shelton & Lee, 2000). Glabrous canary seed contains about 55-59% starch (db) (Table 9-9). The pubescent parent canary seed, Keet, has been reported to contain 54-65% starch (Abdel-Aal *et al*, 1997a). The starch content in glabrous canary seed is less than that reported in wheat (63-72%), corn (65-78%) and sorghum (60-77%) but is within the range reported for oats (43-61%) and barley (57.6-59.5) (Shelton & Lee, 2000).

Abdel-Aal and co-workers (1997a) studied starch extracted from pubescent canary seed and found that more than 95% of the polygonal shaped canary seed starch granules were an average size of 2.0µm. Previous studies on pubescent canary seed starch have reported granule size ranges of 2.5-5.0 µm (Goering & Schuh, 1967). The granule size of amaranth (1-3 µm) (Capriles *et al.*, 2008) and quinoa starch (0.6 to 2.0 µm) (Lorenz, 1990; Lindeboom *et al.*, 2005) are comparable to canary seed. Wheat starch granules range from 1-40 µm and, like barley and rye starches, have a bimodal size distribution containing large lenticular granules (25-40µm) and small spherical granules (1-10µm) (Shelton & Lee, 2000).

The amylose content (16.2-19.5%) in canary seed starch was less than in wheat (22.7%) and corn (24.5%) but fit within the range for that found in eight quinoa lines (3-20%) (Lindeboom *et al.*, 2005). Canary seed starch has A-type starch crystals, characteristics of most cereal starches with a high degree of crystallinity (Abdel-Aal *et al*, 1997a).

Cereals contain small amounts of free sugars: wheat (1-2%), barley (2-3%), corn (1-3%), oats (1-2%) and rye (~3%)(Shelton & Lee, 2000). The free sugars vary among cereal grains with sucrose, glucose, and fructose being predominant. Other sugars have been reported in cereals including raffinose, stachyose, and arabinose. Glabrous canary seed cultivars contained 0.6 to 1.1% soluble sugars, while the pubescent cultivar contained 1.7% and the CWRS wheat control contained 2.9% soluble sugars (Abdel-Aal *et al*, 2011a). Individual free sugars were measured in the pubescent parent canaryseed cultivar with that cultivar containing about 0.8% sucrose, 0.1%fructose, and 0.1%

glucose (Abdel-Aal *et al.*, 1997b), similar to that found in the glabrous cultivars. Sucrose was the predominant sugar in glabrous and pubescent canary seed (Table 9-9). Arabinose was also detected but not maltose.

9.1.2.4.1 Dietary fiber

There is quite a wide range in the dietary fiber content of cereals, ranging from 9.3% (db) in millet (Pönte *et al.*, 2000) to 25% (db) in rye (Gebruers *et al.*, 2008). Durum wheat, spring wheat and winter wheat all differ in the ranges of dietary fiber content. The European HEALTHGRAIN diversity screen determined that winter wheat ranged from 11.5-18.3% (db), spring wheat 12.1-17.5% (db) and durum wheat 10.7 to 15.5% (db). The diversity screen also found that dietary fiber levels in barley, rye and oat samples were higher than in wheat, with values (db) from 15.0 to 23.7% in barley, 20.4 to 25.2% in rye and 10.6 to 23.4% in oats (Gebruers *et al.*, 2008.) The majority of dietary fiber in cereals is composed of insoluble dietary fiber ranging from 1.87 % in soft wheat to ~22% in barley. Barley and rye have been reported to have the highest levels of soluble fiber, 2.56% and 3.7% respectively (Ragaee *et al*, 2006) although high levels (4.1-4.9%) have also been reported in oats (Manthey *et al.*, 1999). In comparison with these cereals, canary seed groats contain less total dietary fiber (range 5.9 to10.2%) with the majority being insoluble and less than 1% being soluble (Table 9-10). The dietary fiber content in the pubescent canary seed ranged from 5.5-8.3%, comprised of about 1% soluble fiber and the remaining insoluble fiber (Abdel-Aal *et al.,* 1997b).

Canary seed has a dietary fiber content similar to buckwheat (~7% db) (Wijngaard & Arendt, 2006), lower than quinoa (12.88% db)) and amaranth (11.14%db) (Schoenlechner *et al.,* 2008) and higher than brown rice (3.5-4.6% db) (Rosell & Marco, 2008).

Table 9-9 Comparison of the starch (%db) and sugars (% db) content of glabrous brown and yellow canary seed groats to pubescent brown canary seed groats

	Glabrous Canary Seed								Pubescent Canary Seed[1]	
	Brown[1,2]				Yellow[2]				Brown	
	Mean	SD	Range		Mean	SD	Range		Mean	SD
			Min	Max			Min	Max		
Total Starch	56.1	±1.1	54.2	57.6	57.1	±2.7	53.0	61.2	60.0	±2.6
Arabinose	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.2	tr	
Fructose	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.2	0.1	±0.0
Glucose	0.2	±0.1	0.1	0.3	0.1	±0.0	0.0	0.1	0.1	±0.0
Maltose	Nd	-	nd	nd	nd	-	nd	nd	nd	Nd
Sucrose	0.6	±0.1	0.5	0.7	0.6	±0.1	0.4	0.8	0.8	±0.1
Unknown									0.79	±0.1
Total Sugars	0.9	±0.1	0.8	1.1	0.8	±0.2	0.6	1.1	1.75	±0.1

[1]Abdel-Aal *et al*, 1997
[2] Phase 2 CDCS study
nd-not detected ; tr: trace

Table 9-10 Comparison of the dietary fiber content (% db) of glabrous brown and yellow coloured canary seed groats to pubescent brown canary seed groats and CWRS wheat

	Glabrous Canary Seed[1]								Pubescent Canary Seed[2]		
	Brown				Yellow				Brown		
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range
			Min	Max			Min	Max			
Lignins (%)	0.6	±0.3	0.3	1.0	0.6	±0.2	0.3	0.9	ND		
Soluble Fiber (%)	0.3	±0.2	0.1	0.7	0.4	±0.3	0.1	1.1	0.9	±0.1	0.8-0.9
Insoluble Fiber (%)	8.1	±0.9	7.1	9.1	8.1	±1.1	5.5	10.0	5.1	±0.5	4.7-5.6
Total Dietary Fiber (%)	8.4	±0.9	5.9	9.3	8.6	±1.2	6.0	10.2	6.6	±1.0	5.5-8.3

[1]Phase 2, CDCS study
[2]Abdel-Aal *et al.*, 1997
ND-not determined

9.1.2.5 Micronutrient composition

9.1.2.5.1 Vitamins

Levels of the B vitamins thiamine, riboflavin and niacin were measured in glabrous and pubescent canary seed cultivars and the CWRS wheat in Phase 1. These three B vitamins plus pyridoxine and folate was measured in the canary seed cultivars in the Phase 2 study. Thiamine content in canary seed (0.7 mg/100g db) was almost twice that measured in the CWRS wheat (0.4 mg/100 g (db)) with riboflavin levels being very similar (0.1-0.2 mg/100 g (db)). However, the niacin content in canary seed (ca.1.0 mg/100 g db) was significantly less than the niacin measured in CWRS wheat (7.3 mg/100 mg db) (Table 9-11). Measured levels of pyridoxine in glabrous canaryseed from Phase 2 were approximately 0.2 mg/100 g (db).

The thiamine content range reported here for glabrous canaryseed was comparable to the ranges reported in wheat, barley, oats and maize (Table 9-12). Riboflavin values for glabrous canary seed were similar to reported values for wheat and oats and higher than reported values for barley and field maize. Pyridoxine content in canary seed (0.2 mg/100g (db)) was less than reported levels in wheat, barley and maize, but similar to oat. However, canary seed contains less niacin than reported for wheat, barley and field maize, and is more similar to oat (Table 9-12).

Total folate content in glabrous canary seed ranged from 0.07-to 0.12 mg/100g (db) for yellow and brown coloured varieties; higher than the folate values reported for wheat (0.02-0.09 mg/100g db), barley (0.019-0.03 mg/100g db), maize (0.017-0.045 mg/100g db) and oats (0.06-0.07 mg/100g db) (Bock, 2000; OECD, 2002, 2003, 2004) (Table 9-12). Folate content in canary seed was comparable to those values reported for the pseudocereals amaranth (0.05-0.73 mg/100g db) and quinoa (0.13 mg/100g db), and higher than buckwheat (0.02 mg/100) (Schoenlechner *et al.,* 2010) and rice flour (0.006 mg/100 g) (Yazynina *et al.,* 2008).

Table 9-11 Comparisons of B vitamins (mg/100g db) content of glabrous brown and yellow canary seed groats compared to pubescent canary seed groats and CWRS wheat

	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[2]	Wheat[2]
	Brown				Yellow				Brown	CWRS
Vitamin	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Thiamine (B_1)	0.7	±0.1	0.6	0.9	0.7	±0.1	0.6	0.8	0.8	0.4
Riboflavin (B_2)	0.1	±0.0	0.1	0.2	0.1	±0.0	0.1	0.1	0.2	0.2
Niacin (B_3)	1.3	±0.1	0.7	1.3	1.1	±0.2	1.0	1.4	0.9	7.3
Pyridoxine (B_6)	0.2	±0.0	0.2	0.2	0.2	±0.0	0.2	0.2	ND	ND
Folic Acid (B_9)	0.09	0.01	0.07	0.12	0.08	0.01	0.07	0.10	ND	ND

[1]Phase 2, CDCS study
[2]Abdel-Aal et al., 2011a
ND-not determined

Table 9-12 Comparison of B Vitamin contents (mg/100g) in four cereal grains

Vitamin	Wheat [a]	Barley[b]	Field Maize[c]	Oats[d]
Thiamine (B_1)	0.13-0.99	0.12-1.6	0.23-0.86	0.77
Riboflavin (B_2)	0.06-0.31	0.08-0.07	0.025-.056	0.18
Niacin (B3)	2.20-11.10	4.6-14.7	0.93-7.0	1.8
Pyridoxine (B_6)	0.09-0.79	0.27-1.15	0.46-0.96	0.13
Folic acid (B_9)	0.02-0.09	0.019-0.03	0.017-0.045	0.06-0.07

[a] OECD, 2004, wheat
[b] OECD, 2003, barley
[c] OECD, 2002, maize
[d] Bock, 2000 (pg 482, Table 5)

9.1.2.5.2 Mineral Content

Cereals make up a significant dietary source of minerals and trace elements with cereals and cereal products in a typical Western diet contributing about 50% of the dietary manganese and iron, about 30% of copper and magnesium and about 20% of the zinc and phosphorous (Piironen *et al.*, 2009).

There are substantial differences in micronutrient concentrations in various grains depending upon type of grain, genotype, growing conditions and fertilizer application (Zhao *et al.*, 2009). In wheat, iron, zinc, copper and manganese contents are low. For many minerals (e.g. calcium, magnesium, copper, iron and selenium) the range in contents can be up to 10 fold (Piironen *et al.*, 2009). It appears soil type can cause more variation than the genotype or species. Table 9-13 provides examples of the micronutrient variation in four cereal grains–wheat, barley, field maize and oats.

Glabrous and pubescent canary seed cultivars had similar levels of major and trace minerals and all canary seed cultivars had significantly higher levels of phosphorous, sulphur, magnesium, calcium, iron, manganese and zinc than the CWRS wheat (Table 9-13). However, the values obtained for P, S, Mg, Ca, Fe, Mn and Zn are comparable to those reported in the literature for these nutrients in a number of wheat varieties (as given in Table 9-14). Glabrous canary seed exceeded oat and barley in phosphorous, magnesium and iron content.

Table 9-13 Comparison of the major mineral (mg/100g db) and trace mineral (mg/kg db) contents of glabrous brown and yellow canary seed to pubescent brown canaryseed and CWRS wheat

	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[1]	Wheat[1]
	Brown				Yellow				Brown	CWRS
	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Major Minerals (mg/100g)										
Phosphorous	645	±49	577	710	611	±38	540	660	590	430
Potassium	401	±33	349	443	370	±25	318	407	340	355
Sulfur	270	±23	242	305	270	±19	241	297	300	200
Magnesium	217	±10	200	233	210	±8	196	220	195	155
Calcium	32	±3	27	40	32	±5	24	41	40	20
Sodium	<MDL	-	<MDL	<MDL	<MDL	-	<MDL	<MDL	10	10
Trace Minerals (mg/kg)										
Iron	82	±16	64	110	77	±4	66	81	55	42
Manganese	51	±6	42	63	57	±6	48	68	71	59
Zinc	34	±2	30	39	30	±3	23	34	35	25
Copper	7	±1	5	22	6	±1	5	8	24	28
Nickel	3	±1	2	4	3	±1	2	3	3	0.3
Selenium	2	±1	2	4	2	±1	1	3	3	2

<MDL: less than method detection limit of 20 ppm

[1] Abdel-Aal *et al*, 2011a

[2] Phase 2, CDCS study

Table 9-14 Comparison of mineral content in four grain cereals

Mineral	Wheat[a]	Barley (whole grain)[b]	Field Maize[c]	Oats[b]
		Major Minerals (mg/100g, dry basis)		
Phosphorous	220-910	470	234-750	340
Potassium	280-730	630	320-720	460
Magnesium	20-220	140	82-1000	140
Calcium	10-80	90	3-100	95
Sodium	4.6	11.8	0-150	8.6
		Trace Minerals (mg/kg, dry basis)		
Iron	16-163	60	1.0-100	70
Manganese	10-90	18	NR	50
Copper	1.0-14.0	9	0.9-10	40
Selenium	0.4[d]	NR	0.01-1.0	NR
Zinc	15-102	40	12-30	39

*NR Information not reported in reference
[a]Piironen *et al.*, 2009
[b]Bock, 2000
[c]OECD, 2002
[d]Gawalko, 2002

9.1.2.6 Anti-nutrient Composition

Phytate, phenols, tannins, trypsin inhibitor, amylase inhibitor, glucosides and alkaloids may all be present in common cereal crops. The anti-nutrient composition of pubescent and glabrous canary seed cultivars compared to the CWRS wheat was evaluated with the following anti-nutrients being measured—phytate, total phenols, condensed tannins, trypsin inhibitor and amylase inhibitor (Table 9-15). Alkaloids are discussed in *Section 10, Chemical Considerations*.

Table 9-15 Comparison of anti-nutrient factor content in glabrous brown and yellow canary seed to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[1]	Wheat[1]
	Brown				Yellow				Brown	CWRS
	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Phytate (mg/g)	18.7	±3.4	14.1	22.3	18.2	±3.3	13.8	23.2	17.5	10.7
Total Phenols (mg/g)	1.79	±0.14	0.87	1.85	1.97	±0.07	1.89	2.09	0.83	0.81
Trypsin Inhibitor (TIU/mg)	0.50	±0.15	0.34	0.64	0.71	±0.10	0.60	0.90	0.51	0.47
Amylase Inhibitor (AIU/mg)	6.24	±2.45	2.8	10.06	5.56	±1.21	4.17	8.33	2.84	2.66
Condensed tannins	ND				ND				ND	ND

[1] Abdel-Aal *et al.*, 2011b; Li *et al*, 2010
[2] Phase 2, CDCS study
ND: not detected; TIU: Trypsin Inhibitor Units, AIU: Amylase Inhibitor units.

9.1.2.6.1 Phytate

Phytate (phytic acid and its salts) is found in the cotyledon of legumes and oilseeds or in the bran of cereal grains (Reddy & Sathe, 2002). It is considered an anti-nutrient due to its role in chelating mineral elements such as calcium and zinc in the human body. On the other hand, phytate is reported to have some potential beneficial effects such as its ability to lower blood glucose and its role in reducing plasma cholesterol and triacylglycerols, and cancer risk (Jenab & Thompson, 2002; Schlemmer *et al.*, 2009; Kumar *et al.*, 2010).

Glabrous and pubescent canary seed cultivars were found to contain about two times the phytate content of the control CWRS wheat. Phytate values for canary seed ranged from 13.8 to 23.2 mg/g (db) while the phytate content in the CWRS wheat was 10.7 mg/g (Table 9-15).

The content of phytate in cereals as reported by Anjum *et al.* (2002) and Hidvegi & Lasztity (2002) were 2.4-10.5 mg/g in wheat, 8.5-11.8 mg/g in barley and 9.0-14.2 g/mg in oat. As Table 9-16 illustrates, phytate levels in canary seed are within the range

found in common foods including whole grains, pulses, seeds and nuts. The amount of phytate can vary from 0.6 to 22 mg/g in cereals and 0.8 mg to 60 mg/g in cereal milled fractions and protein products (Reddy & Sathe, 2002). For instance, values reported for triticale are 5.0-18.9 mg/g; corn, 7.5-22 mg/g; wheat bran 25-58 mg/g; beans, 8.9-27 mg/g; and soybean 10-22.2 mg/g.

Other reported phytate values for common foods include edible nuts such as peanuts 1.7-44 mg /g (Schlemmer *et al.*, 2009); almonds 21.1 mg/g; cashews, 12.3 mg/g; pistachios, 28.4 mg/g and filberts 23.4 mg/g (Harland *et al*, 2004)].

Environmental fluctuations, growing location, soil type, fertilizer applications and year of growth influences the phytate content of seeds and grains (Reddy & Sathe, 2002).

Table 9-16 Phytate content of cereals, pulses and edible nuts			
Grain/Pulse/Edible Nuts	**Fraction**	**Phytate (mg/g)**	**Reference**
Canary seed	Whole groat	14.1-23.2	Abdel-Aal *et al.*, 2011b
Common Wheat	Whole grain	22	Harland & Oberleas, 1986
	Flour	3-8.24	Bos *et al.*, 1991, Tangkongchitr *et al.*, 1981
	Bran	25-58	Graf & Eaton, 1993
Durum wheat	Whole grain	9.8-14.3	Tabekhia & Donnelly, 1982
	Flour	4.5-7.2	
	Bran	23.3-43.2	
	Semolina	1.6-3.4	
Barley	Whole grain	9.8-11.5	Bos *et al.*, 1991
	Flour	6.93-8.45	Graf & Eaton, 1993
Oat		7.8-13.3	Miller *et al.*, 1980
Rice	Uncooked, ground	2.46-2.92	Harland & Oberleas, 1986
	Unpolished, cooked	12.7-21.6	Kumar *et al.*, 2010
Brown rice	Unpolished, uncooked	13.2	Moongngarm & Saetung, 2010
Sorghum	Pearled grain	2-13	Cilliers & Van Niekirk, 1986
	Whole grain flour	10.12	Garcia-Estepa et al., 1999
Rye		5.35-5.65	Kikunaga *et al.*, 1985.
Amaranth		5.2-6.1	Lorenz & Wright, 1984
		10.6-15.1	Kumar *et al.*, 2010
Buckwheat	Whole grain	9.2-16.2	Kumar *et al*, 2010
Pearl millet		1.79-3.06	Simwemba *et al.*, 1984. Kumar & Chauhan, 1993
Quinoa	Raw	10.5-13.5	Vega-Galvez et al., 2010
Soya	Dehulled	11.5	Thompson & Erdman, 1982
	Defatted	18.2	
Lentils	Whole	2.7-10.5	Reddy & Sathe, 2002
Peas	Whole	2.2-12.2	
Kidney beans	Whole	8.9-15.7	
Chickpeas	Whole	2.8-12.6	
Sesame seed	Roasted	39.3-57.2	Kumar *et al*, 2010
Peanut	Flour	15.6-19.4	Harland & Oberleas, 1986
	Whole	1.7-44	Schlemmer *et al*, 2009
Almonds	Oil roasted, blanched,	21.1	Harland *et al*, 2004
Cashews	Dry roasted	12.29	
Filberts	Shelled, dried	23.4	
Pistachios	Whole	28.35	

9.1.2.6.2 Total phenolics

Phenolic compounds are present in a variety of chemical forms in plants. The antinutritional properties of phenolics refer to their astringency and role in reducing the availability of certain minerals and amino acids. Conversely, phenolic compounds have antioxidant activity, which controls the oxidation of lipids (Naczk & Shahidi, 2006).

In Phase 1, canary seed groats (derived from the brown pubescent or glabrous cultivars) were found to have a total phenolic content (TPC) similar to that of the CWRS wheat, averaging 0.84 mg/g (Table 9-15).

In Phase 2, glabrous brown canary seed has significantly less total phenolic content (1.79 mg/g) than the glabrous yellow cultivar (1.95 mg/g) (Table 9-15) and the TPC levels in both brown and yellow cultivars in the Phase 2 study were about two times higher than those TPC values found in the Phase 1 for pubescent and glabrous cultivars tested. Variation in phenolic content is to be expected due to methodology (Zhou & Yu, 2004) and due to genotype and environmental effects (Moore *et al.*, 2006). A recent study on wheat phenolics showed that six Canadian wheat varieties grown in western Canada had mean total phenolics content ranging from 1.7-1.9 mg/g (db) (Mpofu *et al.*, 2006), higher than the CWRS wheat tested in Phase 1, but comparable to the TPC in the glabrous canary seed tested in Phase 2.

There is also a wide variation in total phenolics content amongst various grains. Whole grain rye ranges from 0.65-3.0 mg/g dm (Bondia-Pons *et al.*, 2009), barley ranges from 0.25-0.67 mg/g (db) (Andersson *et al.*, 2008), with millet containing 0.38 mg/g (dm), and sorghum 0.41 mg/g (dm) (Ragaee *et al.*, 2006). The HEALTHGRAIN study found differences between conventional wheats [spring (0.61mg/g); winter (0.66 mg/g db), durum (0.69 mg/g db)] and ancient wheats [spelt (0.58 mg/g db), einkorn (0.62 mg/g db) and emmer (0.78 mg/g db)] (Li *et al.*, 2008). TPC for wheat whole meal and wheat bran and flour ranged from 0.77 to 1.29 mg/g and 2.28 to 3.44 mg/g respectively. Thus the phenolic content of canary seed is within the range found in other food cereals.

The predominant phenolic acids in glabrous canary seed are ferulic, caffeic, sinapic and p-coumaric (Abdel-Aal *et al.*, 2011b; Li *et al*, 2010). Ferulic acid is the predominant phenolic acid in wheat and barley as well (Naczk & Shahidi, 2006).

Glabrous brown and yellow canary seed groats exhibit the same flavonoid profiles, being rich in flavonoid glycosides. High concentrations of O-pentosyl vitexin and O-pentosyl isovitexin were detected (Li *et al.*, 2011).

9.1.2.6.3 Condensed Tannins

Condensed tannins (proanthocyanidins) were not detected in glabrous canary seed as confirmed by analysis (Abdel-Aal *et al.*, 2011b; Li *et al*, 2011).

9.1.2.6.4 Other Phytochemicals

The carotenoid content of glabrous brown and yellow canary seed were determined in a project separate from the safety assessment but summary data are presented here to show the increased interest in investigating the attributes of canary seed as a new cereal food. Total carotenoid content in the whole meal canary seed ranged from 7.57 to 10.03 mg/kg with a mean of 9.21 mg/kg in the brown canary seed and ranged from 8.73 to 10.02 mg/kg with a mean of 9.34 mg/kg in the yellow varieties Li & Beta (2012). The major carotenoids detected in the glabrous brown and yellow varieties were β-carotene, lutein and zeaxanthin. On average, canary seed wholemeal contained 4946, 2316 and 530 µg/kg of β-carotene, lutein and zeaxanthin, respectively, in the brown cultivar, and 4974, 2238 and 440 µg/kg, respectively in the yellow cultivars. Li & Beta (2012) indicated that the total carotenoid content of glabrous canary seed was similar to the intermediate group of durum wheat (9.7-11.0 mg/kg), but that canary seed contained much higher levels of β-carotene compared to wheat (30-100 µg/kg), rice (66-150 µg/kg), and corn (49.2-458 µg/kg).

9.1.2.6.5 Enzyme Inhibitors

Trypsin and amylase inhibitors are found in raw cereal grains and legumes. These enzyme inhibitors have nutritional implications in human diet, but are typically not considered a problem because they are destroyed during the application of heat used in most cooking techniques.

Low levels of trypsin inhibitor were detected in pubescent (0.5 TIU/mg) and glabrous canary seed cultivars (0.51-0.71 TIU/mg) and the CWRS wheat (0.47 TIU/mg) (Table 9-15; Abdel-Aal *et al.*, 2011b) compared to 30.26 TIU/mg in soybean, a rich source of trypsin inhibitor (data not shown). Soybean was included as a sample check due to its high trypsin inhibitor activity.

In Phase 1, amylase inhibitor activity was measured in canary seed and wheat grains using soluble starch as a substrate and pure α-amylase with and without inhibitor extracts (Mulimani and Supriya, 1993). Canary groat and common wheat had similar α-amylase activities with means ranging from 2.66 AU/mg for wheat to 2.8 AIU/mg for canary seed (Table 9-15; Abdel-Aal *et al.*, 2011b).

In Phase 2, a slightly different method was used to determine alpha-amylase inhibitor activity. The inhibitory activity was measured by the decrease of α-amylase activity from the inhibitors using soluble starch as a substrate and pure α-amylase (from *Bacillus licheniformis*, Sigma) based on method by Deshpande *et al.* (1982). This is likely why slightly higher α-amylase inhibitor activities (5.47 to 6.24 AIU/mg) for the glabrous brown and yellow cultivars are being reported (Table 9-15).

9.1.3 Nutrient Composition of Processed Canary Seed Groats

As discussed in Section 5.0 *Manufacturing,* the Canaryseed Development Commission of Saskatchewan contracted with Food Technology Centres in Canada to optimize the post-harvest processing of dehulled canary seed, determine the nutritional composition and shelf stability of processed canary seed and develop prototype food products with canary seed ingredients.

Nutrient analysis was conducted on brown and yellow canary seed groats subjected to combinations of mild heat, tempering conditions and roasting treatments. Brown and yellow canary seed groats were subjected to the following treatments:

1) Treatment 1: No tempering, heat treatment: 240°F, 8 minutes;
2) Treatment 2: Tempering (to 14% moisture), heat treatment: 240°F, 8 minutes;
3) Treatment 3: No tempering, roasting 350°F, 10 minutes;
4) Treatment 4: Tempering (to 14% moisture), roasting 350°F for 8 minutes.

Nutrient composition results are presented in Table 9-17. The nutritional composition of processed glabrous canary seed was similar to that of the raw groats (as described in the previous section 9.1.2) indicating that processing does not change the nutritional profile of canary seed groats.

Phytate content was measured in the whole meal flours produced from processed canary seed groats. Compositional analyses on raw canary seed groats indicated phytate levels ranged from 14.1 mg/g to 23.2 mg/g (equivalent to 1.4% to 2.3%) (Table 9-15). Phytate levels in whole meal flours produced from treated canary seed groats ranged from 1.8% to 2.7% (Table 9-17), well within the range of phytate values for commonly consumed cereals, pulse and nuts (Table 9-16). Since phytates are heat stable and are not easily removed by cooking, autoclaving, roasting or other conventional heat processing methods (Venkatachalam & Sathe, 2002), a reduction in phytate levels in heat treated canary seed groats was not expected.

Table 9-17 Nutritional composition (per 100 g db) of processed glabrous brown and yellow canary seed groats

	Glabrous Canary Seed[1]							
	Brown				Yellow			
Nutrient	No tempering, heat treated	Tempering, heat treated	No tempering, Roasted	Tempering, Roasted	No tempering, heat treated	Tempering, heat treated	No tempering, Roasted	Tempering, Roasted
Calories, Total (per 100g, db)	431.9	431.5	436.8	438.5	432.2	429.3	434.4	434.7
Protein (g)	22.1	21.1	23.5	22.3	20.7	20.7	21.1	20.2
Carbohydrates (g)	65.9	67.4	64.8	65.4	67.8	68.7	68.0	68.5
Fat (g)	8.9	8.6	9.3	9.8	8.7	8.1	8.7	8.8
Ash (g)	2.8	2.6	2.9	2.8	2.7	2.7	2.7	2.8
Saturated Fatty Acids (g)	1.3	1.4	1.3	1.5	1.2	1.1	1.2	1.2
trans-Fatty Acids (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cholesterol (mg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dietary Fiber (g)	8.1	8.2	8.4	9.2	7.9	8.1	8.5	9.1
Sugars (g)	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.6
Fructose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Galactose/Glucose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sucrose (g)	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.6
Maltose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin A (IU)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin C (mg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sodium (mg)	1.1	1.6	1.5	1.6	1.0	1.8	1.4	1.5
Calcium (mg)	38.5	40.4	40.6	39.0	39.6	40.4	40.5	43.2
Iron (mg)	6.4	6.9	7.1	6.5	6.3	6.6	7.5	6.9
Canary Seed Flours								
Phytate (%)	2.0±0.2	1.9±0.2	2.3±0.2	2.2±0.1	2.1±0.2	1.8±0.2	2.3±0.2	2.7±0.1

1 Phase 2 CDCS study, not published

9.1.3.1 Nutrient Composition of Prototype Food Products

To provide examples of the nutrition composition of potential food products, nutrition fact tables (NFT) were generated for two products a) unbaked nutrition bars containing roasted canary seed groats incorporated at a 5%, 10%, 15%, 20% and 25% inclusion rates (Table 19-18); and b) muffins containing 7% roasted whole grain yellow canary seed flour (replaced 20% of the refined wheat flour in formulation) (Table 19-19).

Results of the canary seed chemical analyses (as shown in Table 9-17) were input into a Genesis® R & D SQL nutrient database to generate Canadian nutritional facts tables. Since there was little difference in the nutritional composition of the brown and yellow canary seed groats, NFTs were only generated for bars containing roasted glabrous brown canary seed groats. The nutritional composition of the bars as shown by the nutrition fact tables in Figure 19-1 was essentially unchanged by increased levels of canary seed. A NFT was generated for a muffin containing roasted yellow canary seed flour (Figure 19-2).

Table 19-18 Formulation (%) for prototype unbaked nutrition bar at differing inclusion levels of brown or yellow, roasted canary seed groats[1].

	Canary Seed Inclusion Level (%)				
Ingredients	**5**	**10**	**15**	**20**	**25**
Brown rice syrup	19.2	19.2	19.2	19.2	19.2
Honey	10.7	10.7	10.7	10.7	10.7
Canola oil	3.8	3.8	3.8	3.8	3.8
Monoglycerides	1.0	1.0	1.0	1.0	1.0
Canary seed	**5.0**	**9.9**	**14.9**	**19.8**	**24.8**
Quick oats	21.9	17.0	12.0	7.1	2.1
Oats #5	18.6	18.6	18.6	18.6	18.6
Rice crisps	4.4	4.4	4.4	4.4	4.4
Cranberries	7.6	7.6	7.6	7.6	7.6
Pecan pieces	7.6	7.6	7.6	7.6	7.6
Cinnamon	0.2	0.2	0.2	0.2	0.2

[1]Phase 2 CDCS study, not published

Figure 9-1 Nutritional fact tables for nutrition bars with differing inclusion level of roasted brown canary seed[1]

5% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	**6 %**
Saturated / saturés 0.5 g + Trans / trans 0 g	**3 %**
Omega-6 / oméga-6 0.8 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	**1 %**
Carbohydrate / Glucides 20 g	**7 %**
Fibre / Fibres 2 g	**8 %**
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	4 %

10% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	**6 %**
Saturated / saturés 0.5 g + Trans / trans 0 g	**3 %**
Omega-6 / oméga-6 0.9 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	**1 %**
Carbohydrate / Glucides 20 g	**7 %**
Fibre / Fibres 2 g	**8 %**
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

15% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	**6 %**
Saturated / saturés 0.5 g + Trans / trans 0 g	**3 %**
Omega-6 / oméga-6 0.9 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	**1 %**
Carbohydrate / Glucides 20 g	**7 %**
Fibre / Fibres 2 g	**8 %**
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

20% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 130	
Fat / Lipides 4 g	**6 %**
Saturated / saturés 0.5 g + Trans / trans 0 g	**3 %**
Omega-6 / oméga-6 1 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	**1 %**
Carbohydrate / Glucides 20 g	**7 %**
Fibre / Fibres 2 g	**8 %**
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

25% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 130	
Fat / Lipides 4 g	**6 %**
Saturated / saturés 0.5 g + Trans / trans 0 g	**3 %**
Omega-6 / oméga-6 1 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	**1 %**
Carbohydrate / Glucides 20 g	**7 %**
Fibre / Fibres 2 g	**8 %**
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

[1]Phase 2 CDCS Study, not published

Roasted, whole yellow canary seed flour replaced all purpose wheat flour at 10%, 15% and 20% in a muffin formula. The formula and the nutrition facts table for muffins containing ~ 7% roasted ground whole grain yellow canary seed flour (20% replacement of all purpose flour) is presented in Table 19-19 and Figure 19-2, respectively.

Table 9-19 Formulation (%) for prototype muffin containing roasted whole ground yellow canary seed flour at 20% replacement levels of all purpose flour[1]

Ingredient	%
Roasted Canary seed Flour	*7.10*
All purpose flour	*28.38*
2% Milk	*23.08*
Canola Oil	*10.60*
Sugar	*19.07*
Whole Egg	*9.46*
Baking Powder	*1.74*
Salt	*0.57*
TOTAL	*100.00*

[1]Phase 2 CDCS Study, not published

Figure 9-2 Nutritional facts table for prototype muffins containing ~7% roasted whole ground canary seed flour (Phase 2 CDCS Study, not published)

Nutrition Facts
Valeur nutritive
Per 1 muffin (43 g) / pour 1 muffin (43 g)

Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 170	
Fat / Lipides 6 g	**10** %
Saturated / saturés 0.5 g + Trans / trans 0 g	**4** %
Polyunsaturated / polyinsaturés 2 g	
Omega-6 / oméga-6 1.5 g	
Omega-3 / oméga-3 0.5 g	
Monounsaturated / monoinsaturés 3.5 g	
Cholesterol / Cholestérol 20 mg	
Sodium / Sodium 190 mg	**8** %
Carbohydrate / Glucides 23 g	**8** %
Fibre / Fibres 1 g	**3** %
Sugars / Sucres 9 g	
Protein / Protéines 4 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	6 %
Iron / Fer	8 %

9.1.3.2 Food Grade Specifications

Based upon the data provided in this dossier, the food grade specifications outlined in Tables 9-20 and 9-21 could be used as guidelines for the introduction of glabrous canary seed into the food market. It is expected that the values for proximate analysis may vary from those given in this table due to cultivar and environmental conditions, similar to that experienced in other cereal grains.

Table 9-20 Physical and chemical properties of canary seed

Physical Standard	Whole Groat	Milled
Appearance	Uniform brown or yellow colour	Uniform yellow colour/ uniform brown colour with or without darker flecks
Odour	No off odors	No off odors
Texture	Smooth, free-flowing granulation	Free-flowing powder
Bulk density	c. 65-70 kg/hl	c.41 kg/L (loose)
Particle sizes	Various depending upon size of kernel	Various

Table 9-21 Food Grade Specifications Whole Grain Canary Seed (dry basis)[a]			
Parameter	Unit	Specification Whole Groat	Specification Milled
Proximates			
Protein (N x 5.7)	(%)	18-25	18-25
Carbohydrates	(%)	68-72	68-72
Ash	(%)	1.9-2.6	1.9-2.6
Dietary fiber	(%)	5.9-10.2	5.9-10.2
Total Fat	(%)	5.5-6.4	5.5-6.4
Heavy metals[b]			
Lead	mg/kg	<0.2	<0.2
Cadmium	mg/kg	<0.2	<0.2
Mercury	mg/kg	<0.1	<0.1
Arsenic	mg/kg	<0.2	<0.2
Microbial[c]			
Aerobic plate count	CFU[d]/g	$<10^6$	$<10^6$
Coliforms	CFU/g	$<10^4$	$<10^4$
Yeast/Mold	CFU/g	$<5 \times 10^3$	$<5 \times 10^3$
Pathogens (*E.coli, Salmonella, S. aureus*	Absent	Absent	Absent

[a] Specifications defined based upon data presented in this dossier. Values may vary from year to year depending upon cultivar and environmental conditions
[b] As recommended by Codex Alimentarius, 2007
[c] As identified in ICMSF, 2005
[d] CFU: colony forming units

9.1.4 Nutritional Summary

From a nutritional perspective, glabrous canary seed would provide macro- (protein, starch, fat) and micronutrients (vitamins and minerals) at levels comparable to other cereal grains such as wheat, barley, oats and rye. Dietary fiber levels are similar to millet but lower than some of the other grains. Canary

seed contains approximately 19-22% protein, 5-7% crude fat, 2% ash, 55% starch and 6-10% dietary fiber. Similar to other cereal grains, the proteins in canary seed are deficient in lysine but rich in cystine, tryptophan, and phenylalanine, which could make them good complements to legumes. Canary seed contains the B vitamins, thiamine and riboflavin, at levels comparable to other cereals. Niacin levels are lower than in wheat and barley but similar to oat. Total folate content in canary seed is higher than the common cereals (wheat, barley and oats) and similar to the pseudocereals, amaranth and quinoa. Of the antinutritional compounds present in canary seed, the level of phytate was about two times higher than the tested CWRS wheat but still within the range of phytate content of other commonly consumed foods such as some cereals, pulses and edible nuts.

9.2 Nutritional Bioavailability

Canary seed is being introduced as a new cereal grain. As indicated in Section 8.0 *History of Use*, canary seed has limited history as a human food. Consequently there is limited information about its nutritional bioavailability in the scientific literature.

In vitro protein digestibility was evaluated as part of the Phase 1 study, and in Phase 2 the *in vitro* protein digestibility of thermally treated canary groats was studied. Two 90-day oral toxicity trials, one trial conducted in each Phase, and a 28-day study in Phase 2 evaluated the effect of consuming canary seed on growth of rats.

9.2.1 *In vitro* Protein digestibility

Digestibility of proteins is a factor that impacts nutritional value.

The *in vitro* digestibility of canary seed (84) is comparable with that of other plant protein sources (WHO, 2007) – [e.g. corn (87); wheat (86); oat (86); soy flour (86); and higher than other specialty grains such as millet (79) (WHO, 2007), amaranth (74) (Bejosana & Corke, 1998) and buckwheat (79.9) (Wijngaard & Arendt, 2005) but less than that of casein (95) (WHO, 2007).

Rajamohamed and coworkers (2013) examined the *in vitro* protein digestibility of canary seed under simulated gastrointestinal conditions and evaluated the impact of thermal treatment on protein digestibility. *In vitro* digestibility of yellow and brown canary seed proteins from raw groats, roasted groats (176°C (348.8°F), 12 min) and boiled (98°C (208.8° F), 12 min) flours under gastric, duodenal and sequential gastric–duodenal conditions was evaluated according to the method outlined in Rajamohamed *et al.*, (2013). The results indicated the canary seed proteins were digested more easily under sequential gastric–duodenal conditions than under gastric or duodenal conditions alone. Roasting of canary seeds altered the electrophoretic profile of the proteins and resulted in fainter bands compared to those observed for boiled and raw canary seeds. Thermal processing generally improved canary seed protein digestibility.

9.2.2 Rodents

The effect of consumption of glabrous canary seed on growth of rats was assessed in the Phase 1 90-day oral toxicity study and the Phase 2 28-day and 90-day oral toxicity studies (Magnuson *et al.,* 2014).

The objective of the Phase 1 study was to compare the growth and toxicological effects of glabrous (hairless) hulled and glabrous dehulled brown canary seed (CDC Maria) with the parent pubescent (hairy) hulled canary seed (Keet) and a common grain, Canada Western Red Spring (CWRS) wheat (CDC Teal), at maximal tolerable levels (50%) in the diet.

The objective of Phase 2 was to compare the growth and toxicological effects of the glabrous brown versus glabrous yellow canary seed cultivars. In Phase 2, the test diets included three concentration levels of dehulled yellow groats cultivar (C05041) and one concentration of dehulled glabrous brown (CDC Maria) compared to the AIN-76 rodent reference diet. Relevant nutritional information from the rodent studies is presented in the following summary tables (Phase 1, Table 9-22; Phase 2, Tables 9-25 and 9-26) and discussion.

Toxicological endpoints for these studies will be discussed in *Section 10: Toxicological Considerations*.

Table 9-22 Summary of 90-day rat study (CTR0012) (Phase 1)[1]

Objective: to compare the toxicological and growth effects of glabrous brown canary seed (CDC Maria) and pubescent brown parent (Keet) with that of CWRS (CDC Teal) wheat in rats

- Protocol followed OECD Test Guideline No.408
- 4-week old Sprague-Dawley rats (male and female); n=10/sex/group (total 80 rats)
- 4 diet groups:
  - Diet 1: 50% dehulled glabrous brown canary seed (CDC Maria)
  - Diet 2: 50% hulled glabrous brown canary seed (CDC Maria)
  - Diet 3: 50% hulled pubescent brown canary seed (Keet)
  - Diet 4: 50% CWRS wheat (CDC Teal) (control diet)
- All test diets provided the same amount of apparent metabolizable energy (AME) (3,500 kcal/kg) and crude protein (20%). Crude fat content of the diets varied 9.0%, 10.4%, 10.6% and 9.4% for Diets 1, 2, 3 and 4, respectively.
- Diets also contained corn and soybean meal in varying amounts.
- Water and test diet fed *ad libitum* for 90 days

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- Feed consumption data showed no difference among the various diet regimens
- Male rats had higher weight gain on dehulled glabrous canary seed than on the hulled glabrous canary seed or hulled pubescent Keet, but gain was similar to that of the CWRS wheat diet. There was no difference in weight gain among female rats on the diets.

Conclusions

- Values for feed consumption and body weight gain in rats fed diets containing 50% canary seed were comparable to values when fed a 50% wheat diet.

[1]Magnuson *et al.*, 2014

In Phase 1 a 90-day oral toxicity study was performed on Sprague Dawley rats using glabrous hulled canary seed and dehulled canary seed (groats) (CDC Maria), pubescent hulled canary seed (Keet) and CWRS wheat (CDC Teal) as the test ingredients according to OECD Test Guideline 408 for "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD, 1998). This study consisted of two identical trials staggered 8 days apart to make sample collection within a one-day period more manageable. Only one test ingredient concentration level (50%) was studied (limit test). The nutritional and compositional information on canary seed and its similarity to wheat composition did not show any potential

toxic elements (Section 9.1, Nutrient Composition). Complete Phase 1 study details are available in Appendix 4.

Four groups of animals, each consisting of 10 males and 10 females, were fed diets containing 50% CWRS wheat (control), 50% glabrous brown canary seed groats (CDC Maria); 50% glabrous hulled (CDC Maria) canary seed or 50% pubescent hulled canary seed cultivar (Keet). Diets (Table 9-23) were formulated to contain 3500 kcal/kg AME, 20.00% crude protein, 0.75% calcium, 0.15% sodium, 0.0781% choline, 1.20% lysine, 0.65% methionine and 0.80% threonine to meet or exceed the requirements for rat reproduction (National Research Council, 1995). All diets were provided in mash form.

Table 9-23 Specifications for formulation of rodent test diets[1]

	Diet Treatment			
Ingredients (%)	No. 1 Dehulled Glabrous canary seed	No. 2 Hulled Glabrous canary seed	No. 3 Hulled Pubescent canary seed	No. 4 CWRS wheat
Dehulled glabrous canary seed	50.0			
Hulled glabrous canary seed		50.0		
Hulled pubescent canary seed			50.0	
CWRS wheat				50.0
Corn	29.51	23.63	17.75	19.46
Soybean meal-48	11.22	14.90	21.20	18.41
Canola oil	4.65	6.83	6.73	7.73
Dicalcium phosphate[2]	0.72	0.79	0.83	0.88
Limestone	1.58	1.53	1.47	1.47
Sodium chloride	0.34	0.34	0.34	0.34
Vitamin/mineral premix[3,4]	0.50	0.50	0.50	0.50
Choline Chloride	0.15	0.15	0.15	0.15
DL-Methionine	.034	0.37	0.36	0.36
L-Threonine	0.24	0.25	0.17	0.17
L-Lysine HCl	0.74	0.71	0.48	0.53

[1]Magnuson *et al.*, 2014
[2]Dicalcium phosphate (15% Ca, 21% P)
[3]Supplied per kg diet: vitamin A (retinal acetate + retinyl palmitate), 11000 IU; vitamin D, 2200IU; vitamin E (dl-α-tocopherol acetate), 30 IU; menadione, 2.0 mg; thiamine, 1.5 mg, riboflavin, 6.0 mg; niacin, 60 mg;, pyridoxine, 4.0 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; biotin, 0.15 mg, ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[4]Supplied per kg feed: iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

Final body weight, weight gain and feed consumption are shown in Table 9-24. Total mean feed consumption data showed no difference between the various diet treatments for male or female rats; males consuming on average 1.9 to 2.1 kg and females 1.4 kg over the 90 day trial. Males fed the glabrous canary seed groats had higher final body weights and greater mean body weight change over the 90 days than those fed the glabrous hulled canary seed or the pubescent hulled canary seed, but were not statistically different from rats fed the control CWRS wheat diet. A similar trend was observed for females, but differences were not statistically significant. Higher weight gain in rats fed glabrous dehulled canary seed with similar intake as hulled canary seed is likely due to higher caloric value of feed per gram due to removal of hulls and lower indigestible fiber.

Males consumed 34, 33, 37 and 35 g per kg body weight per day of the CWRS wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively. Females consumed 43, 38, 42 and 42 g per kg body weight per day of the CWRS wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively.

Table 9-24: Summary of body weights, body weight changes and food consumption in the 90-day rat feeding study (Phase 1)[1]

		Body Weight Means[2] ±SD (g)				Body Weight Change (g)[3]	Total Feed Consumed (g)
Test diet	Sex	Day 1	Day 28	Day 56	Day 90		
50% dehulled glabrous canary seed[2]	M	97±5	329± 20	471 ±35	**572 [a] ±52**	**475 [a] ±51**	1994 ± 138
50% hulled glabrous canary seed[3]	M	98± 6	320± 31	431±29	**514 [b] ±42**	**416 [b] ± 38**	2058 ± 147
50% hulled pubescent canary seed[4]	M	97±12	302± 27	422± 35	**517 [b] ± 55**	**419 [b] ± 49**	1973 ± 188
Control - 50% Wheat[5]	M	97±7	316± 13	445± 15	**536 [ab] ± 21**	**439 [ab] ± 20**	1980 ± 100
50% dehulled glabrous canary seed	F	89 ±7	202±13	262± 40	290 ±25	201 ±26	1302 ± 82
50% hulled glabrous canary seed	F	89± 5	196± 8	238 ±16	271± 29	182± 28	1366 ± 117
50% hulled pubescent canary seed	F	90± 5	189± 21	236± 20	267± 29	178± 30	1372 ± 162
Control - 50% Wheat	F	88±9	199± 17	243± 24	265± 27	177± 28	1364 ± 88

[1]Magnuson *et al.*, 2014
[2]n=10
[3]Mean in the same column with different letters are significantly different at P<0.05.

000102

The Phase 2 rat studies evaluated the effects of consumption of glabrous yellow canary seed groats incorporated into diets at concentrations levels of 2.5%, 5% and 10% and of glabrous brown canary seed groats incorporated into diets at a concentration level of 10%. Rats were fed diets *ad libitum* over 2 time periods a) a 28-day period (Table 9-25) and b) a 90-day period followed by a 30-day recovery period (Table 9-26). Test diets were in the form of hard cold-pressed rodent chow pellets. The 28-day trial was used to establish testing parameters for the pivotal 90-day study. The studies were conducted by NucroTechnics and monitored by Cantox Intertek. The study reports for the 28-day and 90-day studies are described in Appendix 5a & 5b, respectively.

The rationale for the Phase 2 28-day and 90-day rodent study design was as follows:

- The objective of the novel food initiative is to obtain regulatory approval for use of glabrous brown and yellow cultivars in human foods.
- Glabrous yellow cultivars had not been evaluated in the Phase 1 rodent trial. There were no significant differences in the nutritional composition between brown and yellow glabrous canary seed, and only minor differences in antinutritional compounds, indicating high nutritional value and low toxicity.
- The Phase 1 90-day rat feeding study had shown no significant differences in growth or adverse toxicological effects in rats fed the glabrous brown cultivar or the pubescent parent brown cultivar as compared to CWRS wheat, when added to the diet at a level of 50%. Only one dose level of canary seed was evaluated.
- A standardized approach to the safety assessment of novel food ingredients is to determine the dose-response of any effects of consumption of the ingredients added to a standardized diet as compared to animals fed the standardized diet.
- Limited histopathology had been conducted in the Phase 1 90-day study, thus the brown cultivar was also included in the Phase 2 study.
- The dehulled form (groat) of glabrous canary seed is to be consumed by the human population, not the hulled form (with hull).
- Dietary levels of 2.5%, 5% and 10% were chosen for several reasons:

- When testing whole foods, using high concentrations presents the potential for inducing nutritional imbalances.
- Toxicology studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake (Health Canada, 2006).
- The high concentration, 10%, was chosen to reflect consumption levels higher than that targeted for the American population. Based upon the potential human consumption values obtained from the human dietary exposure assessment conducted in Phase 2 (Section 14 *Dietary Exposure*) using conservative and optimistic assumptions, the highest users (90^{th} percentile) in the general population were estimated to consume 1.7 g glabrous canary seed per kg body weight (BW) per day (Section 14 *Dietary Exposure*). The results of the Phase 1 90-day feeding study indicated that male and female rats consumed on average 33 g and 38 g glabrous dehulled canary seed/kg/day, respectively, when 50% of the test diet was canary seed. Using female intake (38 g/kg/d), if similar food intake occurs, a 10% concentration would result in consumption of 7.6 g/kg/day, which is about 5-fold the 90^{th} percentile intake expected by the human population. The lower doses were included to assess dose-response of any observed effects.

Table 9-25 Summary of the 28-day study in Sprague Dawley rats with brown and yellow canary seed groats (Phase 2)[1]

Objective: to compare the toxicological and growth effects of dehulled glabrous brown with dehulled glabrous yellow canary seed (groats)

- Protocol followed OECD Test Guideline N0. 408
- 5 groups of male and female Sprague-Dawley rats (25 male and 25 female/test diet), each group consisting of 5 male and 5 female rats (Strain: Crl:CD (SD)BR-Sprague-Dawley)
- 5 diet groups:
  - Diet 1: Control: AIN-76A (0% canary seed)
  - Diet 2: 2.5% dehulled glabrous yellow canary seed (C05041)
  - Diet 3: 5.0% dehulled glabrous yellow canary seed (C05041)
  - Diet 4: 10% dehulled glabrous yellow canary seed (C05041)
  - Diet 5: 10% dehulled glabrous brown (CDC Maria)
- Diets were formulated by Research Diets, Inc (New Jersey, U.S.A) to contain 20% protein and 5% fat and 3.9 kcal/g. Equivalent protein, carbohydrate, fat and fiber levels were achieved by varying levels of casein, corn starch, corn oil and cellulose. The diets were assessed for protein, fat, sugar profile, vitamin A and vitamin D_3 at the beginning and end of the experiment to confirm formulation and stability.
- Water and test diets fed *ad libitum* daily for 28 days.

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- No significant differences in body weight and body weight gains, gender matched, between the control and test diets
- No apparent differences in feed consumption between control and test diets.
- Slightly lower feed consumption was noted for males and females in the latter days of study, but body weights were not affected.

Conclusions:

- Rats fed diets containing 2.5%, 5% and 10% yellow and 10% brown canary seed showed no significant differences in body weight and body weight gains compared to the control diet throughout the study period indicating canary seed was nutritionally adequate.

[1]Magnuson *et al.*, 2014

Table 9-26 Summary of the 90-day study in Sprague Dawley rats with brown and yellow canary seed groats (Phase 2)[1]

Objective: to compare the toxicological and growth effects of dehulled glabrous brown canary seed (CDC Maria) with dehulled glabrous yellow canary seed (C05041) in rats

- Protocol followed OECD Test Guideline NO. 408
- 5 groups of male and female Sprague-Dawley rats (35 male and 35 female/test diet) consisting of 20 M/F in main group, 10M/F in satellite group and 5M/F in recovery group (30 days on control diet).
- 5 diet groups:
  - Diet 1: Control: AIN-76A (0% canary seed)
  - Diet 2: 2.5% dehulled glabrous yellow canary seed (C05041)
  - Diet 3: 5.0% dehulled glabrous yellow canary seed (C05041)
  - Diet 4: 10% dehulled glabrous yellow canary seed (C05041)
  - Diet 5: 10% dehulled glabrous brown (CDC Maria)
- Diets were formulated by Research Diets, Inc (New Jersey, U.S.A) to contain 20% protein and 5% fat and 3.9 kcal/g. Equivalent protein, carbohydrate, fat and fiber levels were achieved by varying levels of casein, corn starch, corn oil and cellulose. Each diet preparation was assayed for protein, fat, sugar profile, vitamin A and vitamin D_3 at the beginning and end of the experiment to confirm formulation.
- Water and test diets fed *ad libitum* daily for 90 days followed by a 30-day recovery period.

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- No significant differences in body weights and weight gain among diet groups, except for the body weights of male rats fed the 10% yellow canary seed; which were lower than controls at the end of the study.
- Food consumption for this group was also lower in the latter days of study
- Normalization of body weights at Day 91 per total feed consumption showed no differences between treatment groups and the control group.

Conclusions:

- Rats fed diets containing 2.5%, 5% and 10% glabrous brown canary seed showed no differences in body weight and body weight gains compared to the control diet throughout the study period. A reduction in food consumption in males fed and 10% glabrous yellow canary seed in the latter weeks of the study resulted in reduced body weight compared to controls. Overall the study indicated canary seed was nutritionally adequate.

[1]Magnuson *et al.*, 2014

In the 28-day study, there were no statistical differences in body weights and body weight gains, gender matched, among the control and test groups. Food consumption mirrored the body weight gains. There were no appreciable differences in food consumption amongst the groups (see study report, Appendix 5a). Food spillage did not indicate an excessive wastage of food. The feeding regimen of glabrous canary

seed at levels of 2.5%, 5% or 10% a*d libitum* for 28 days corresponded to average dose levels (gender combined) of 1.8, 3.6 and 7.0 g of yellow canary seed groat for the four concentration levels, respectively, and 6.9 g of brown canary seed groat per kg body weight per day.

In the Phase 2 90-day, statistical analysis of body weights and weight gain (ANOVA; p=0.05) showed no differences amongst the groups except the body weights of male animals in the group fed 10% yellow canary seed groat were lower at Day 85 (7% of control) and Day 90 (8% of control) (Table 9-27). Feed consumption mirrored the body weight gains. There were no apparent differences in total feed consumption amongst the groups, although feed consumption was significantly reduced in male animals fed the 10% yellow canary seed groat at during Days 78 to 85 (10% of control) and Days 85 to 90 (11% of control). Normalization of body weights at Day 91 per total feed consumption showed no differences between treatment groups and the control group. Furthermore, as is discussed in toxicological considerations (Section 10), the reduced body weight in males fed 10% yellow canary seed as compared to male rats fed the control diet was not associated with any adverse biochemical or histological effects. In contrast, male rats fed the 10% yellow canary seed diet has lower incidence and severity of liver lipidosis (fatty liver) as compared to male rats fed the control diet. Liver lipidosis is a common finding in laboratory rats that are fed *ad libitum* and tend to become obese. Thus, the slightly lower body weights and food consumption levels in male rats fed the 10% yellow canary seed diet at the end of the study period, are not considered adverse or to have toxicological effects.

Table 9-27 Summary of body weights, body weight changes and food consumption in the 90-day rat study (Phase 2) [1,2]

Group	Sex	Body Weight Means ± S.D. (g)				Mean Body Weight Change (Day 1 to 90) (g)	Mean Total Food Consumption (kg)
		Day 1[3]	Day 29[3]	Day 57[4]	Day 90[4]		
1. Control Diet	M	318 ± 17	492 ± 34	597 ± 49	668 ± 64	350 ± 56	2.4 ± 0.2
	F	220 ± 16	303 ± 26	346 ± 37	366 ± 46	146 ± 38	1.6 ± 0.2
2.Low Dose (2.5% Yellow canary seed)	M	311 ± 23	489 ± 44	596 ± 66	666 ± 84	355 ± 65	2.4 ± 0.3
	F	216 ± 14	297 ± 27	349 ± 28	373 ± 35	155 ± 28	1.6 ± 0.2
3.Mid Dose (5% Yellow canary seed)	M	316 ± 23	498 ± 44	602 ± 58	665 ± 81	352 ± 68	2.4 ± 0.2
	F	216 ± 16	295 ± 28	335 ± 38	351 ± 39	134 ± 32	1.5 ± 0.2
4.High Dose (10% Yellow canary seed)	M	310 ± 23	478 ± 36	560 ± 44	615* ± 56	311 ± 43	2.3 ± 0.2
	F	216 ± 14	296 ± 24	341 ± 31	359 ± 35	141 ± 28	1.6 ± 0.1
5.High Dose (10% Brown canary seed)	M	311 ± 21	491 ± 41	610 ± 47	687 ± 62	376 ± 52	2.5 ± 0.2
	F	216 ± 14	298 ± 30	340 ± 36	363 ± 41	148 ± 34	1.6 ± 0.2

[1] Magnuson *et al.*, 2014
[2] Prestudy body weights were also recorded but were not reported (they are recorded in the raw data)
[3] n = 35
[4] n = 25; as noted in Section 11, a satellite group of 10 rats per group were terminated after 6 weeks on diet.
* Statistically significant from Control Group ($p<0.05$).

Feed consumption in females was similar in the canary seed diet groups when compared to control animals with the exception of female rats in the mid-dose group experiencing statistically significant lower feed consumption during Days 64 to 71 (10% of control) and Days 85 to 90 (15% of control) and female rats in the low-dose group experiencing lower feed consumption (10% of control) during the period from Days 85 to 90. Given the fact that the body weights in these groups were not affected and no adverse effects were observed, this effect was considered to be of no toxicological significance. As observed in male rats, female rats fed the 10% canary seed diets also

displayed reduced incidence of liver lipidosis, further indicating that the lower feed consumption levels at the end of the study period did not adversely affect animal health.

The 90-day feeding regimen corresponded to average dose levels (gender combined) of 1.30, 2.54 and 5.15 g of yellow canary seed groats or 5.23 g of brown canary seed groats per kg per day, for the four dose levels, respectively.

Rodent Trials Summary

In summary, the results of the Phase 1 and Phase 2 rodent trials indicate that rodents fed diets containing 2.5%, 5% and 10% glabrous brown canary seed groats or diets containing 50% glabrous hulled or dehulled brown canary seed or pubescent hulled canary seed showed no differences in body weight and body weight gains compared to the control diets throughout the study periods. The only significant finding was a reduction in body weight of male rats fed 10% glabrous yellow canary seed, which was attributed to a reduction in food consumption during the latter period of the study. Overall, the results of these studies indicate that canary seed is nutritionally adequate.

9.2.3 Swine

Two studies evaluating canary seed as a potential feed for growing-finishing swine have been reported (Thacker, 2003; Qiao and Thacker, 2004). As the pig is considered to have very similar digestive system to man, these studies are particularly helpful in assessing the nutritional properties of canary seed as a human food. The first study, summarized in Table 9-28, evaluated growth of pigs fed graded levels of pubescent canary seed (cv. Elias) in the diet (Thacker, 2003).

Table 9-28 Summary performance of growing-finishing pigs fed diets containing graded levels of hulled pubescent brown canary seed (Thacker, 2003)

Objective: to determine the performance and carcass characteristics of growing-finishing pigs fed diets containing graded levels of pubescent canary seed (cv.Elias)

- Cross bred pigs; each diet fed to groups of 6 or7 gilts and 6 castrates each (n=12 or 13/diet)
- 5 diet groups:
  - Diet 1: 0 % canary seed (cv.Elias); 100% barley in diet,
  - Diet 2: 25% pubescent canary seed (cv.Elias), 75% barley in basal diet
  - Diet 3: 50% pubescent canary seed (cv.Elias), 50% barley in basal diet
  - Diet 4: 75% pubescent canary seed (cv.Elias), 25% barley in basal diet
  - Diet 5: 100% pubescent canary seed (cv.Elias) , 0% barley in basal diet
- Pigs were provided diets ad libitum for 30minutes, twice daily, during the growing period (34.4 to 84 kg) and the finishing period (84-107.8kg) (time not reported)
- Canary seed replaced 25 to 100% of barley ingredient in the basal diet.

Measured endpoints for growth evaluation

- Digestibility for dry matter, crude protein, and gross energy
- Performance parameters including daily weight gain and feed conversion
- Carcass traits including slaughter weight, carcass weight, dressing percentage, carcass value index, lean yield, loin fat and loin lean.

Results

- Decrease in dry matter digestibility with increasing canary seed level possibly due to higher fiber content of canary seed compared to barley. Increasing crude protein digestibility determined with increasing level of canary seed.
- Gross energy digestibility not affected by level of canary seed.
- Pigs fed diet containing 25% canary seed had highest weight gain; lowest weight gain observed on diets containing 100% canary seed.
- Feed intake and feed conversion not affected by level of canary seed
- Carcass traits not affected by canary seed inclusion in diet.

Conclusions

- Results for growth and feed intake of pigs suggest canary seed could be included up to 57% of the total diet (75% of cereal portion) without adversely affecting grower pig performance or altering carcass characteristics.
- Canary seed is palatable and nutrients can be effectively utilized.
- Canary seed did not appear to have any negative effect on pig performance.

This study (Thacker, 2003) on swine was conducted to determine the performance and carcass characteristics of growing-finishing pigs fed diets containing graded levels of pubescent hulled canary seed, cultivar Elias. Canary seed replaced the barley portion of the barley/soybean meal diet at levels of 25, 50, 75 or 100%. Thacker found that during the grower period, pigs fed the diet containing 25% canary seed had

the highest rates of gain (1.0 kg/day) and pigs fed the 100% canary seed diet had the lowest gain (0.90 kg/day). Pigs fed a diet containing 50% and 75% canary seed showed a daily gain of 0.98 kg/d and 0.97 kg/d, respectively, higher than the control diet where a daily gain of 0.93 kg was noted. In the finishing period, pigs fed the diet containing 50% canary seed had the highest gain (1.07 kg/d) while pigs fed the 100% canary seed diet showed the poorest growth (0.94 kg/d). Weight gains on the control diet (1.0 kg/d); 25% canary seed diet, (1.02 kg/d) and 75% canary seed diet (1.0 kg/d) were comparable. Daily intake and feed conversion during both periods were unaffected by level of canary seed. Canary seed diets were considered to be palatable and the nutrients effectively used. It appeared the canary seed did not contain any antinutritional factors at high enough levels to have a negative impact on pig performance. In general, Thacker found that canary seed could be included up to 57% of the total diet (75% of cereal portion) without adversely affecting grower pig performance or altering carcass characteristics.

The second swine study (Qiao & Thacker, 2004)(Table 9-29) focused on determining if a new method –mobile nylon bag technique (MNBT) – could accurately predict the digestible energy (DE) content of swine feed for use in ration formulation programs. The researchers evaluated 22 traditional (e.g. barley, corn, oats and wheat) and non-traditional feeds (e.g. low viscosity ryes, legumes, oilseeds and canary seed) to determine the potential of the MNBT as a tool to determine DE. Three varieties of canary seed (glabrous hulled CDC Maria, glabrous dehulled CDC Maria and pubescent hulled Keet) were evaluated as part of the study.

Table 9-29 Summary of determination of digestible energy content of traditional and non-traditional swine feeds (Qiao & Thacker, 2004)

Objective: to compare the dry matter and energy digestibility of swine feed ingredients using a mobile nylon bag technique.

- Crossbred pigs with duodenal cannuale were fed on a grower diet. After simulating gastric digestion, nylon bags containing feed samples were inserted into the duodenum. Bags were recovered for analysis of feces content.
- 22 traditional and non-traditional swine feed ingredients tested including 3 canary seed ingredients and the CDC Teal wheat

Canary seed samples tested;

- Dehulled glabrous canary seed (CDC Maria)
- Hulled glabrous canary seed (CDC Maria)
- Hulled pubescent canary seed (Keet)

Measured endpoints:

- Digestibility for dry matter, crude protein and gross energy
- Digestible energy

Results:

- Dry matter digestibility of hulled CDC Maria (75.2%) and Keet (76.3%) were similar to barley (74%) and less than CDC Teal wheat (84.9%)
- Greater dry matter digestibility for dehulled CDC Maria (92.4%) comparable to oat groats (92-94%)
- Similar pattern observed for energy digestibility

Analytical results for canary seed showed that glabrous dehulled CDC Maria had greater % dry matter digestibility, % energy digestibility, gross energy (MJ/kg) and higher digestible energy (MJ/kg) than either the glabrous or pubescent hulled canary seeds products. The glabrous dehulled canary seed had similar dry matter and energy digestibility values to the high fat oat groats; all being higher than the traditional cereal grains (barley, corn, oats and hard red spring wheat). The DE for dehulled CDC Maria was much higher (17.61 MJ/kg) than the traditional cereals (range: 11.25-14.26 MJ/kg) or secondary cereal grains (range: 13.53-16.95 MJ/kg) tested in the study. The hulled glabrous and pubescent cultivars had DE values slightly lower (13.76 and 13.82 respectively) than corn (13.89) and wheat (14.23), but higher than oats (11.25) and barley (12.40). Glabrous dehulled canary seed (CDC Maria) also showed higher digestible energy values than the CWRS wheat (CDC Teal) (DE, 14.62 MM/kg) which in turn was slightly higher than the DE values for hulled glabrous and pubescent cultivars.

These results showed that the digestible energy values for glabrous or pubescent canary seed cultivars were within the reported DE ranges of traditional and secondary cereal grains used as swine feeds.

9.2.4 Nutritional Bioavailability Summary

In general, the results of the animal nutritional studies (rodents & swine) support the conclusion that growth of animals on diets containing hairless canary seed (brown or yellow coloured groats) is as good or as better than growth of animals containing similar amounts of CWRS wheat in the control diets. No adverse effects on growth were noted during the study periods and the presence of the higher phytate levels in canary seed as compared to the CWRS wheat (Section 9.1.2.6.1) did not appear to negatively impact growth characteristics.

10.0 CHEMICAL CONSIDERATIONS

10.1 Alkaloids

10.1.1 Alkaloids in *Phalaris* spp.

Prior to this novel food initiative on glabrous brown and yellow canary seed for human food use, there have been no reports of alkaloids present in the seeds (grain/groats) of any of the *Phalaris* species. Determination of alkaloids in *Phalaris* species has been entirely restricted to analysis of leaf material (Anderton *et al.*, 1999; Duynisveld *et al.,* 1990; Kalén *et al.,* 1992; Majak and Bose, 1977; Majak *et al.*, 1978; Ostrem, 1987; and Zhou *et al*, 2006.)

Alkaloids are nitrogen containing organic compounds that can be potentially toxic to humans. They are found in some families and species of higher plants, particularly in leguminosae, as byproducts of plant metabolism, as a reservoir for protein synthesis or as protective agents (Facchini, 2001).

Alkaloids may occur in the seeds of a number of species of interest for both animal and human consumption. Raw barley seeds, for instance, may contain small amounts of alkaloids. An examination of barley varieties used in the brewing industry for the presence of alkaloids showed that gramine was not detected in the seed of five barley cultivars tested. However, hordenine (0.7 µg/gm) was detected in one of the five cultivars and N-methyltyramine was detected in all five cultivars at levels ranging from 0.3 to 11.4 µg/gm (Poocharoen, 1983). Lupins (*Lupinus* spp.) accumulate significant quantities of quinolizidine alkaloids in their seeds; however in some cultivars of yellow lupin (*Lupinus luteus* L.), the indole alkaloid gramine, is the most abundant alkaloid. Gramine concentrations reported for this cultivar range from 166 to 1894 mg/kg (Jamroz & Kubizna, 2008; Wasilewko & Buraczewska, 1999). The ANZFA report of 2001 (ANZFA, 2001) provides a summary of the alkaloid profile and potential toxicity of the alkaloids in sweet lupins. The mean alkaloid content in sweet lupins is 130-150 mg/kg; however the varieties tested in this report did not contain gramine. The ANZFA report suggests a tolerable level of exposure of lupin alkaloids for humans of 35µg/kg/day.

10.1.2 Alkaloid Results

The analysis of alkaloids in pubescent and glabrous brown canary seed groats were conducted in Phase 1 (Abdel-Aal *et al.*, 2011b). The alkaloids, gramine, nonadecane, tryptamine and norharmane were determined in canary seed groats and CWRS wheat milling fractions by gas liquid chromatography (GLC) as described by Duynisveld and others (1990). HPLC was also used to confirm the alkaloids results as outlined by Muir and colleagues (Muir *et al.*, 1992). Detection of alkaloids was performed at 270 nm and a standard solution of gramine, tryptamine and β-carboline (5 mmol) was used for calibration and identification. No alkaloids were detected in the groats of the glabrous brown cultivar (CDC Maria) or its pubescent parent (Keet).

In Phase 2 of the canary seed project, a new method based upon the method of Muir *et al* (1992) was developed. In this Phase 2 study alkaloids were also evaluated in the grain (seed) of the perennial reed canarygrass (*P. arundinaceae* L) and compared to commercially grown samples of the annual glabrous *P. canariensis* cultivar, CDC Maria. The complete report outlining the methodology used and results of the alkaloid study for Phase 2 can be found in Appendix 7 (Muir *et al.*, 2010)

Seeds from three cultivars of *P. arundinacae* reed canarygrass (forage cultivars known to have significant foliar levels of the alkaloids gramine and hordenine) were used to develop a method for extraction and analysis of alkaloids from the seeds. Spike recovery experiments using gramine were undertaken during method development to ensure that the extraction and analytical process was appropriate to detect low levels of indole alkaloids in *Phalaris* seed samples.

The alkaloid content was evaluated in seeds of three cultivars of *P. arundinacea* reed canarygrass (Vantage, Rise and Rival) obtained from Plant Gene Resources of Canada (Agriculture Agri-Food Canada, Saskatoon) and a commercially available sample of glabrous CDC Maria (Crop Development Centre, University of Saskatchewan) (Table 10-1). Reference materials included gramine, 5-Methoxy-N,N-dimethyltryptamine, tryptamine, O-methylserotonin HCl, and tyramine. At the time of the analysis, no reference standard could be found for hordeine.

The major peak found in the seeds of all three reed canarygrass cultivars was the amine tyramine (15.12-18.96 µg/g) (Figure 10-1). Tyramine was essentially absent

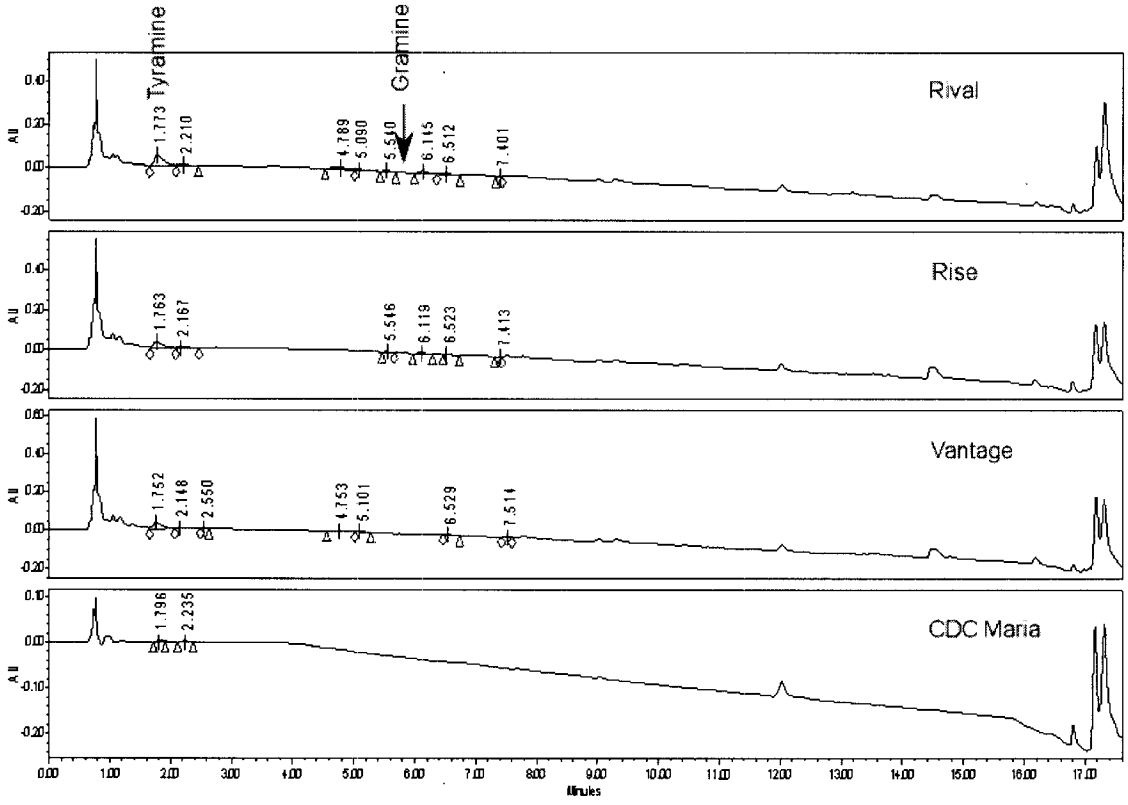
in the groat of commercially grown glabrous canary seed cultivar (CDC Maria). No peaks were found that co-chromatographed with gramine in any sample. A number of minor peaks were observed in the reed canarygrass grain extracts and examination of the UV spectra indicated that the peaks with retention times had UV spectra similar to gramine or related indole or phenylethylamine alkaloids for which reference standards were available. Mass spectral analysis also indicated the presence of nitrogen but the concentration was too low to obtain a positive identification of any compound. Because of the presence of nitrogen and a UV spectrum similar to gramine or tyramine, these peaks were presumed to be alkaloids or amines and the concentration was estimated using the external standard calibration curve for gramine.

Table 10-1. Tyramine and alkaloid-like compounds present in MeOH:NH_4OH (99:1) extracts of reed canarygrass and commercial glabrous canary seed (CDC Maria) seeds.[1]

	Tyramine µg/g sample (n=3)	**Total alkaloid-like compounds (excluding Tyramine)** µ/g sample (n=3)
CDC Maria canary seed	0.77	0.76
Vantage canarygrass	16.56	19.69
Rise canarygrass	15.12	10.53
Rival canarygrass	18.96	21.51

[1]Muir, 2010, report for CDCS, unpublished; Appendix 7

Figure 10-1. UPLC analysis (Symmetry C18 column) of seed extracts of reed canarygrass (*P. arundinacea*) and glabrous canary seed (*P. canariensis*) for the presence of alkaloids and amines. UV = 214 nm. The arrow indicates the retention time for gramine. [1]



[1] Muir, 2010, report for CDCS, unpublished; Appendix 7

Determination of the alkaloid content in glabrous hulled and dehulled canary seed.

The 18 composite samples of glabrous brown (6 composites of CDC Maria) and yellow canary seed (6 composites each of C05041 & C05091) were analyzed both as intact grain (with hulls) and dehulled grain (groats). All samples were extracted in triplicate and each lab sample was analysed in triplicate by Ultra Performance Liquid Chromatography (UPLC) (Table 10-2). Values reported are means of the 6 composite samples. The mean laboratory replicate values for each field replicate are reported in Appendix 7. Gramine was not detected in any of these samples. As reported above, the

major peak in all chromatograms was identified as tyramine and this is reported separately in Table 12-2. The criteria for considering a peak to be alkaloid-like included a UV spectrum similar to one of the reference standards and the presence of molecular or daughter ions indicating the presence of nitrogen in the molecule.

Table 10-2. Tyramine and alkaloid-like compounds present in MeOH:NH_4OH (99:1) extracts of glabrous hulled canary seed and dehulled canary seeds (n=6) [1]

	Tyramine µg/g	STDEV µg/g	Alkaloid-like compounds µg/g	STDEV µg/g
Glabrous Hulled				
Brown CDC Maria	3.50	±1.91	2.26	±0.67
Yellow C05041	21.19	±5.13	7.95	±0.70
Yellow C05091	20.80	±3.63	4.93	±0.96
Glabrous De-Hulled				
Brown CDC Maria	2.83	±0.61	1.23	±0.33
Yellow C05041	23.55	±6.09	5.69	±1.28
Yellow C05091	20.11	±6.56	7.07	±2.12

[1] Muir, 2010, report for CDCS, unpublished; Appendix 7

The commercially grown glabrous brown CDC Maria grain used in the comparison study with reed canarygrass appears to have lower concentrations of tyramine (0.77 µg/g) and alkaloid-like compounds (0.76 µg/g) (Table 12-1) than the glabrous brown CDC Maria grown in the small replicated plots for the Phase 2 study (tyramine, 2.83-3.5 µg/g; alkaloid-like compounds, 1.23-2.36 µg/g) (Table 12-2).

Both yellow coloured cultivars contained more tyramine (20.1-23.6 µg/g) and alkaloid-like compounds (5.7-7.1 µ/g) than the brown cultivar (2.8 µg/g and 1.2 µg/g), respectively (Table 12-2). While the tyramine levels in the glabrous yellow cultivars were similar to that measured in the reed canarygrass (Table 12-1), the alkaloid-like compounds were less.

The levels of both tyramine and alkaloid-like compounds were not significantly different between the hulled and dehulled grain indicating that most if not all of these compounds are residing in the embryo and cotyledon and not in the hull.

Levels of the biogenic amine tyramine detected in the grain of pubescent and glabrous canary seed were significantly below the level considered to have a biological

effect (e.g. >6000µg in two typical food servings sizes) (McCabe-Sellars et al., 2006). In all cases, the concentrations present were too low to allow positive identification of the individual compounds.

At the time of this alkaloid analysis for the novel food initiative, an authentic reference sample for hordeine could not be found. Consequently, the absence or presence of hordeine in the canary seed samples could not be confirmed. However, the researchers observed that of the unknown peaks that had enough absorbance to do a spectral analysis, no match for hordeine could be seen. This suggests that, if hordeine was present, it was below the detection threshold and below any level that could be quantified.

10.1.3 Alkaloid Summary

Gramine was not detected in any of the canary seed samples and the major peak in the chromatogram was identified as the amine tyramine. The glabrous yellow canary seed contained more tyramine and alkaloid-like compounds that the glabrous brown canary seed. However all detected levels of alkaloid-like compounds were too low to allow positive identification of the individual compounds. The concentrations of tyramine observed in both brown and yellow cultivars were also well below any level considered to have a biological effect (e.g. >6000µg) (McCabe-Sellars et al., 2001).

10.2 Heavy metals

Heavy metal concentrations in crops are dependent upon the environment, soil structure and agronomic practices (crop rotation, fertilizer application) as well as natural variation in the uptake and distribution of trace elements among crop species and among cultivars within species (Grant *et al.*, 2008). Heavy metals in plant foods represent a large group of constituents that are either essential or potentially toxic to human health.

In the Phase 1 study, samples of glabrous canary seed (CDC Maria), pubescent canary seed (Keet) and the CRSW wheat (Katepwa) obtained from ten sites in Saskatchewan were ground and wet digested using a mixture of nitric acid and perchloric acid for heavy metal analysis by inductively coupled plasma emission spectrometry (ICPES) at Saskatoon Research Centre (SRC), Saskatoon. In Phase 2, the heavy metal contents in 18 samples of glabrous canary seed (n=6 for each of CDC Maria (brown), C05041& C05091 (yellow)) from three sites in Saskatchewan were measured by inductively coupled plasma mass spectrometry (ICPMS) at ALS Laboratories (Saskatoon, SK).

Ten (10) heavy metals (molybdenum, antimony, tellurium, tungsten, arsenic, bismuth, cadmium, mercury, lead and silver) were measured in glabrous (CDC Maria) and pubescent (Keet) canary seed and compared with wheat as a traditional food in Phase 1 (Table 10-3). These same ten metals plus cobalt were measured in the brown and yellow glabrous varieties (CDC Maria, C05041, and C05091) in Phase 2 (Table 10-4)

The mean molybdenum concentration in glabrous canary seed samples ranged from 0.51 to 0.93 mg/kg, in pubescent canary seed, 0.41 mg/kg and in the CWRS wheat 0.93 mg/kg. However over all sites and for all crops, molybdenum values ranged from 0.10 to 2.40 mg/kg. These values are similar to those found in other cereal crops grown on the Canadian prairies: barley, 0.9 mg/kg; oats, 1.1 mg/kg; wheat, 1.0 mg/kg and rye, 0.6 mg/kg (McCartney *et al.*, 2006)

Three heavy metals with little or unknown effects on humans when ingested were measured in both project phases. This group is considered as neutral metals and

includes antimony, tellurium and tungsten. In Phase 1, there were no significant differences in the level of antimony (Sb), tellurium (Te) or tungsten (W) in hairless and hairy canary seed compared with wheat. In Phase 1, all these metals were present in very low amounts ranging between 0.1 and 0.29 mg/kg (Table 10-3). In Phase 2, the levels of these metals in the glabrous canary seed samples were all below the method detection limit for each metal (Sb, 0.05 mg/kg; Te, 0.50 mg/kg and W, 0.80 mg/kg) (Table 10-4).

The content of five heavy metals with potential toxicity for humans was also measured. Arsenic, bismuth, cadmium, lead and mercury were all detected at low concentrations in the pubescent and glabrous canary seed cultivars and the control wheat. Similar average concentrations of arsenic (0.2 mg/kg), bismuth (0.2 mg/kg), cadmium (0.1 mg/kg) and mercury (0.03 mg/kg) in both types of canary seed as well as wheat were found (Table 10-3) in Phase 1. In Phase 2, all canary seed sample results for bismuth, cadmium, lead, mercury, and silver were below the method detection limit for these metals (Bi, 0.30 mg/kg; Cd, 0.5 mg/kg; Pb, 0.1 mg/kg; Hg, 0.01 mg/kg and Ag, 0.08 mg/kg) (Table 10-4). Arsenic levels ranged from 0.06-0.10 mg/kg for the Phase 2 analysis, less than the 0.2 mg/kg average levels found during Phase 1 analysis.

In Phase 1 there was a slight, but insignificant difference between glabrous and pubescent canary seed in lead level (0.21 and 0.37 mg/kg, respectively) while wheat had only 0.13 mg/kg. In Phase 2, the lead content ranged from below the method detection limit of 0.02 mg/kg (in the yellow cultivars) to 0.059 ppm in the brown canary seed. The levels of lead obtained in both Phase 1 and Phase 2 studies were all within the range of 0.030-0.37 mg/kg reported in the literature for cereal crops (Cubadda *et al.*, 2003). The mean lead content in pubescent (0.43 mg/kg) and glabrous canary seed (0.21 mg/kg) analyzed in Phase 1 was slightly higher than the accepted 0.2 mg/kg (wet weight) for wheat and 0.1 mg/kg (wet weight) for other cereals (Codex, 2007). However, the range of lead in canary seed was wide, ranging from 0.10 to 1.20 mg/kg in pubescent canary seed and 0.1 to 0.7 mg/kg in glabrous canary seed suggesting that growing conditions and/or environmental factors may cause a high degree of fluctuation in lead content. Phase 2 canary seed lead values (<0.02 to 0.059 mg/kg) were all less than the Codex limit. Variations in lead content in Canadian grown barley (0.073-

0.21ppm), oats (0.110 to 0.130ppm) and wheat (0.087 to 0.18ppm) have also been reported (Dudas and Pawluk, 1977). Zook *et al.,* (1970) reported differences in lead content based upon wheat type [hard, 0.50 ppm (mg/kg); soft, 1.0 ppm (mg/kg); and durum (0.42 ppm (mg/kg)].

Reported literature values for arsenic, bismuth and cadmium in wheat were less than 0.05 mg/kg and mercury was less than 0.02 mg/kg on different soil types and under varying growth conditions (Cubadda *et al.*, 2003; Lavado *et al.*, 2001; Yager *et al.*, 2004). The cadmium level in hairless and hairy canary seed in Phase 1 was at the acceptable limit of 0.1 mg/kg set for cereals (other than buckwheat and quinoa) and less than the value set for wheat of 0.2 mg/kg (Codex, 2007), and in Phase 2, all samples results are reported as less than the method detection limit of 0.005 mg/kg (ppm). Cadmium levels in spring wheat, barley, oat and maize generally contain cadmium concentrations below 0.1 mg/kg (Grant et al., 2008). Reported literature values for cadmium levels in crops on the Canadian prairies has ranged from 0.05 mg/kg to 0.23 mg/kg for wheat durum (Clarke *et al*, 2002, Dudas & Pawluk, 1977), 0.30 to 0.12 mg/kg for barley and 0.04 to 0.065 mg/kg for oats (Dudas & Pawluk, 1977) but higher for flaxseed (0.2 to 0.4 mg/kg) (Clarke et al., 2010). Cadmium accumulation in a plant is dependent upon genotype and environment (Clarke *et al,* 2002; Grant *et al*, 1998).

Mercury levels in the Phase 1 canary seed samples and the control wheat were all less than 0.03 mg/kg while mercury levels in the Phase 2 glabrous samples were all less than 0.01 mg/kg. These values are similar to the reported literature values for oat (0.01-0.012 mg/kg), barley (0.006-0.012 mg/kg) and wheat (0.0053-0.01 mg/kg) grains (Dudas & Pawluk, 1977).

Due to the differences in methods and limits of quantification (LOQ) used in the two study phases, the arsenic levels in the Phase 1 canary seed analysis (where the LOQ = 0.2 mg/kg) were higher (0.2 mg/kg) than those values found in the Phase 2 analyses where all samples had arsenic levels less than the limit of quantification (<0.02mg/kg). Phase 2 arsenic results were well below the range (0.06-0.08 mg/kg) found in an extensive evaluation of cereal grains in Europe (EFSA, 2009) and less than some reports of arsenic levels found in wheat (0.17 mg/kg) (Raber *et al*, 2012) or rice

(0.02-0.36 mg/kg) (EFSA, 2007). Silver is reported for glabrous canary seed at levels less than the detectable limit of 0.08 mg/kg.

All heavy metals tested were within regulatory and/or acceptable levels.

Table 10-3. Comparison of heavy metal content (mg/kg) of glabrous and pubescent canary seed groats to CWRS wheat grown at 10 sites in Saskatchewan (Phase 1)[1]

Metal	Glabrous Brown Canary Seed			Pubescent Brown Canaryseed			CWRS Wheat		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Essential Metals									
Molybdenum (Mo)	0.51	0.71	0.10-2.20	0.23	0.41	0.1-1.4	0.64	0.94	0.10-2.40
Neutral Metals									
Antimony (Sb)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Tellurium (Te)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Tungsten(W)	0.2	0	0.2-0.2	0.22	0.04	0.2-0.3	0.2	0	0.2-0.2
Toxic Metals									
Arsenic (As)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Bismuth (Bi)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Cadmium (CD)	0.1	0	0.1-0.1	0.1	0	0.1-0	0.1	0	0.1-0.1
Mercury (Hg)	0.03	0	0.03-0.03	0.03	0	0.03-0.03	0.03	0	0.03-0.03
Lead (Pb)	0.21	0.23	0.10-0.70	0.37	0.42	0.10-1.20	0.13	0.09	0.10-0.40
Silver (Ag)	0.1	0	0.1-0.1	0.1	0	0.1-0	0.1	0	0.1-0.1

[1] Abdel-Aal, 2011b

Table 10-4 Comparison of heavy metal contents (mg/kg) of glabrous brown and yellow canary seed groats (Phase 2)[1]

		Glabrous Canary Seed							
		Brown				Yellow			
	Detection Limit (mg/kg)	Mean	SD	Range		Mean	SD	Range	
				Min	Max			Min	Max
Essential metals									
Molybdenum (Mo)	0.05	0.70	±0.32	0.41	1.15	0.93	±0.33	0.48	1.56
Neutral Metals									
Antimony (Sb)	0.05	<0.05	na	<0.05	<0.05	<0.05	na	<0.05	<0.05
Cobalt (Co)	0.50	<0.50	na	<0.50	<0.50	<0.50	na	<0.50	<0.50
Tellurium (Te)	0.50	<0.50	na	<0.50	<0.50	<0.50	na	<0.50	<0.50
Tungsten (W)	0.80	<0.80	na	<0.08	<0.08	<0.80	na	<0.80	<0.80
Toxic Metals									
Arsenic (As)	0.02	<0.02	na	<0.02	<0.02	<0.02	na	<0.02	<0.02
Bismuth (Bi)	0.30	<0.30	na	<0.30	<0.30	<0.30	na	<0.30	<0.30
Cadmium (Cd)	0.005	<0.005	na	<0.005	<0.005	<0.005	na	<0.005	<0.005
Lead (Pb)	0.02	<0.037	0.004	0.02	0.059	<0.02	na	<0.02	<0.03
Mercury (Hg)	0.01	<0.01	na	<0.01	<0.01	<0.01	na	<0.01	<0.01
Silver (Ag)	0.08	<0.08	na	<0.08	<0.08	<0.08	na	<0.08	<0.08

[1] CDCS Phase 2 study, unpublished

10.3 Pesticides

The following pesticides are registered for use on pubescent and glabrous canary seed *(Phalaris canariensis)* in Canada. Uses and application rates are similar to those of other cereal crops (wheat, barley, oats etc) grown in Canada and the US (CFR, 2013). One potential exception is the use of difenzoquat, which is currently under re-evaluation in both countries.

Table 10-5 Pesticides registered for use on canary seed in Canada[1]

	Product Name	Active Ingredient	Registrant
Herbicides	Avadex – granular formulation	Triallate	Gowan Co.
	Avenge	Difenzoquat	AmVac Crop/Syngenta
	Pardner, Koril, Bromotril, Brotex	Bromoxynil	Bayer
	Buctril M, Logic M, Mextrol 450, Badge	Bromoxynil + MCPA ester	Bayer
	Curtail M	Clopyralid + MCPA amine	NuFarm
	Banvel, Oracle, VMD 480 Dicamba	Dicamba + MCPA amine	BASF
	Target, Sword, Tracker XP	Dicamba +mecoprop+MCPA	Syngenta
	Prestige	Fluroxypyr + clopyralid + MCPA ester	Dow
	Trophy	Fluroxypyr + MCPA ester	NuFarm
Fungicides	Tilt, Bumper, Pivot	Propiconozole	Syngenta
Insecticides	Cygon, Lagon	Dimethoate	IPCO Cheminova UAP
	Malathion	Malathion	ICPO UAP

[1] Saskatchewan Guide to Crop Production 2014.

11.0 TOXICOLOGICAL CONSIDERATIONS

Although limited, there is some evidence of history of use of canary seed as human food in North America. This section describes all safety evaluation studies identified in the literature, as well as the studies conducted to support the GRAS determination.

Background: The gathering of information for the safety assessment of glabrous canary seed has proceeded in two discrete timeframes in the past fifteen years. The initial project (Phase 1) (1992-2002) involved the development of glabrous canary seed and the identification of both brown and yellow coloured groats amongst the glabrous varieties. In Phase 1, the nutritional and chemical characteristics of glabrous brown coloured canary seed groats (*P. canariensis*, CDC Maria) were compared to its pubescent parent *P. canariensis,* cultivar "Keet" (also brown coloured groat) and to a Canada Western Red Spring (CWRS) wheat. The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals, as well as a 90-day rodent trial.

Phase 2 (2008-2014) involved a comprehensive comparison of two yellow glabrous coloured cultivars (designated C05041 and C05091) to the brown coloured glabrous cultivar CDC Maria, which had been studied in the Phase 1 project. The toxicology studies conducted during Phase 1 and Phase 2, plus those in the published literature are summarized below.

11.1 Rodents

11.1.1 Mice

Bhatt *et al* (1984) investigated the carcinogenic promoting effect of the silica hairs from the pubescent hulls of *Phalaris canariensis.* Swiss mice were orally administered pubescent canary seed in one experiment, and in other experiments, dermal exposures to the silica hairs was undertaken. In all experiments, an initiator-promoter protocol was used. The initiator was 15,16-dihydro-11-methylcyclopental(a)phenanthren-17-one, which initiates skin cancer when injected intramuscularly or by dermal application. The tumor promoter was croton oil applied to

the skin. For oral administration of the pubescent canary seed, seeds were ground to a coarse meal, mixed with 50% by weight egg white, air-dried in a thin layer and broken into fragments. In the oral canary seed experiment, there were 5 treatment groups. Mice (20 male, 10 female) in Group 1 were injected with the initiator, and fed the canary seed mixture fragments in their food hoppers 4 days per week and standard mouse diet for the remaining 3 days per week. Group 2 (10 mice/sex) were fed the same dietary regime as Group 1, but did not receive the initiator. Group 3 (10 mice/sex) were injected with the initiator and fed the standard diet. Group 4 (10 mice/sex) received the initiator, standard diet and croton oil applications. Group 5 (10 mice/sex) received the standard diet and croton oil applications, but no initiator. Tumor incidence was assessed after 78 weeks (18 months). In the absence of the carcinogen initiator (Group 2), mice fed the pubescent canary seed were in normal health and 15% heavier than the control groups fed a standard mouse diet. No tumors were observed in mice in Group 2. Histopathological examination showed neither gross abnormalities in the oesophagus or stomach, or any significant incidence of internal tumours in any of the mice. The authors reported that no toxic effects were observed, and confirmed exposure as silica fibers on the grain hulls were recovered from the gut contents throughout its length and also from washed gut tissues. A promoting effect of dermal exposure to pubescent canary seed was demonstrated in Group 1 "initiated" mice fed canary seed. These mice developed tumors around in the facial trunk and ventral trunk. Most were benign squamous papilloma. The amount of canary seed consumed was not reported. Tumors were also observed in initiated mice fed the standard diet, (Groups 3 and 4). Subsequent experiments confirmed dermal contact of purified *P. canariensis* silica fibers promoted phenanthrene-induced skin tumors (Bhatt *et al*, 1984.)

11.1.2 Rats

The University of Saskatchewan and the Canaryseed Development Commission of Saskatchewan sponsored two 90-day oral sub-chronic rat studies using i) pubescent and glabrous canary seed, and ii) glabrous brown and glabrous yellow coloured canary seed varieties. A 28-day oral rat study was also conducted. The descriptions and results

of these studies have been published (Magnuson *et al.*, 2014), and are described in detail below.

11.1.2.1 90-day rat study on glabrous and pubescent canary seed (Phase 1)

In this Phase 1 90-day rat feeding study, a single concentration (50%) of either glabrous canary seed (CDC Maria) or pubescent canary seed (Keet) as test ingredient in the diet was compared to CWRS wheat (50%) as the control. Diets were formulated according to National Research Council (1995) specifications to ensure nutritional equivalency. The high level of test ingredient was chosen to represent an artificially high dose of canary seed in the human diet. The test ingredient results revealed no significant adverse effects in growth, behavior, hematology, clinical chemistry or gross pathology. Histological assessment consisted of examining 4 animals per sex per group. Thus, this study provides support for the safety of oral consumption of the novel food, glabrous canary seed.

Table 11-1 provides a summary of the objective, protocol, data collected and results for this trial. Full protocol details can be found in Appendix 4 and Magnuson *et al.*, 2014.

Table 11-1 Summary of the 90-day rodent study (CTR0012) (Phase 1) [1]

Objective: to compare the toxicological and growth effects of glabrous canary seed CDC Maria and pubescent canary seed Keet with that of CWRS wheat in rats

- Protocol followed OECD Test Guideline No.408 (repeated dose 90-day toxicity study in rodents)
- 4-week old Sprague-Dawley rats (male and female); n=10/sex/group (total 80 rats)
- 4 diet groups:
  - Diet 1: 50% dehulled glabrous CDC Maria canary seed
  - Diet 2: 50% hulled glabrous CDC Maria canary seed
  - Diet 3: 50% hulled pubescent Keet canary seed
  - Diet 4: 50% CWRS wheat (control diet)
- Diets were formulated with additions of corn, soybean, canola oil, amino acids, vitamins and minerals to meet or exceed minimum nutrient requirements for rats.
- All test diets provided the same amount of apparent metabolizable energy (AME) (3,500 kcal/kg) and crude protein (20%). Crude fat ranged from 9% to 10.5%.
- Water and test diet fed *ad libitum* for 90 days

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational battery, hematology, clinical chemistry, organ weights, urinalysis, and limited histopathology.

Results:
No toxicologically significant effects were observed in rats fed diets containing 50% glabrous hulled canary seed, 50% glabrous dehulled canary seed, or 50% pubescent hulled canary seed as compared to rats fed diets contain 50% CWRS wheat for 90 days.

[1]Magnuson *et al.*, 2014),

Four groups of 20 Sprague-Dawley rats (10 per sex) were fed diets containing 50% CWRS wheat (control), 50% glabrous canary seed groats (dehulled) (CDC Maria), 50% glabrous hulled CDC Maria or 50% pubescent hulled canary seed cultivar Keet. Diets were formulated with additions of corn, soybean, canola oil, amino acids, vitamins and minerals to meet or exceed minimum nutrient requirements for rats. Diets contained 3500 kcal/kg AME, 20% crude protein, 0.75% calcium, 0.15% sodium, 0.078% choline, 1.2% lysine, 0.65% methionine and 0.80% threonine to meet or exceed the requirements for rat reproduction (National Research Council, 1995). The test diet was provided in mash form for 90 days. Other details of the experimental protocol are found in Appendix 4 and Magnuson *et al.*, 2014. The results from this study will be summarized below, but most data are not shown. The study report is provided in Appendix 4.

Final body weight, weight gain and feed consumption are shown in Table 9-22. Males fed the glabrous canary seed groats had a greater mean body weight change over the 90 days than those fed glabrous hulled canary seed, the pubescent hulled canary seed or the control wheat diet. A similar trend was observed for females, but differences were not statistically significant. Higher weight gain in rats fed dehulled glabrous groats with similar food intake as other diets, is likely due to higher nutritional bioavailability of feed per gram due to removal of hulls and lower indigestible fiber. Total mean feed consumption data showed no difference between the various diet groups for male or female rats. Males consumed 34, 33, 37 and 35 g per kg body weight per day of the wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively. Females consumed 43, 38, 42 and 42 g per kg body weight per day of the wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed, respectively.

Organ weights are shown in Table 11-2 as both absolute and relative to final body weight. No differences in absolute organ weights were observed among diet groups, with the exception of liver weights in male rats. Male rats fed the diet containing dehulled glabrous canary seed had significantly higher liver weights as compared to male rats fed the diets containing hulled glabrous or hulled pubescent canary seed, but were similar to those fed the control wheat diet. As male rats fed the dehulled form of canary seed also had higher body weights than rats fed the hulled form, the difference in absolute liver weight is likely due to higher body weight. This is further illustrated by the lack of significant differences in liver weights relative to body weight among the diet groups.

Compared to male rats fed the hulled canary seed diets and the wheat diet, increased body weights of male rats fed dehulled glabrous canary seed groats diet resulted in slightly reduced testes weights relative to body weight. No other significant differences in organ weights in rats fed canary seed as compared to the wheat control were observed, although some differences were observed among the canary seed diets. Differences were not considered toxicologically significant.

Table 11-2 Organ weights, total (g) and relative (g/100 g BW) in the Phase 1 90-day study with male and female rats fed diets containing 50% various types of canary seed or wheat*

		Dehulled glabrous canary seed groat[1]	Hulled glabrous canary seed[2]	Hulled pubescent canary seed[3]	Wheat (control)[4]
Males					
Heart	(g)	1.44 ± 0.16	1.34 ± 0.12	1.34 ± 0.14	1.38 ± 0.6
	(g/100 g BW)	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.01
Spleen	(g)	0.84 ± 0.16	0.79 ± 0.15	0.84 ±0.11	0.84 ± 0.11
	g/100 g BW)	0.15 ± 0.02	0.15 ± 0.03	0.16 ± 0.02	0.16 ± 0.02
Liver	**(g)**	**22.4 ± 2.4[a]**	**19.4 ± 2.6[b]**	**18.74 ± 2.2[b]**	**20.4 ± 2.8[ab]**
	(g/100 g BW)	3.91 ± 0.12	3.77 ± 0.32	3.62 ± 0.19	3.67 ± 0.23
Adrenals	(g)	0.069 ± 0.026	0.077 ± 0.018	0.067 ± 0.011	0.082 ± 0.036
	(g/100 g BW)	0.12 ± 0.004	0.15 ± 0.003	0.013 ± 0.002	0.015 ± 0.007
Kidneys	(g)	3.52 ± 0.37	3.45 ± 0.52	3.33 ± 0.31	3.47 ± 0.33

	(g/100 g BW)	0.62 ± 0.06	0.67 ± 0.08	0.65 ± 0.035	0.65 ± 0.07
Epididymides	(g)	1.41 ± 0.22	1.45 ± 0.15	1.53 ± 0.15	1.51 ± 0.20
	(g/100 g BW)	0.25 ± 0.05^{b}	0.28 ± 0.04^{ab}	0.30 ± 0.06^{a}	0.28 ± 0.04^{ab}
Testes	(g)	3.22 ± 0.14	3.41 ± 0.30	3.42 ± 0.27	3.57 ± 0.33
	(g/100 g BW)	**0.57± 0.6** ^{a}	**0.67 ± 0.10** ^{b}	**0.67 ± 0.09** ^{b}	**0.67 ± 0.08** ^{b}
Brain	(g)	2.22 ± 0.09	2.18 ± 0.08	2.22 ± 0.10	2.24 ± 0.07
	(g/100 g BW)	0.39 ± 0.03^{b}	0.43 ± 0.03^{a}	0.43 ± 0.05^{a}	0.42 ± 0.02^{ab}
Thymus	(g)	0.79 ± 0.22^{a}	0.63 ± 0.11^{ab}	0.56 ± 0.16^{b}	0.65 ± 0.15^{ab}
	(g/100 g BW)	0.14 ± 0.03	0.12 ± 0.02	0.11 ± 0.03	0.12 ± 0.03
Females					
Heart	(g)	0.84 ± 0.07	0.85 ± 0.08	0.84 ± 0.10	0.84 ± 0.09
	(g/100 g BW)	0.29 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.03
Spleen	(g)	0.49 ± 0.09	0.48 ± 0.07	0.44 ± 0.05	0.47 ± 0.04
	(g/100 g BW)	0.17 ± 0.04	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.02
Liver	(g)	10.2 ± 0.88	9.76 ± 1.8	9.82 ± 1.04	9.85 ± 0.91
	(g/100 g BW)	3.55 ± 0.29	3.58 ± 0.35	3.67 ± 0.18	3.67 ± 0.23
Adrenals	(g)	0.075 ± 0.023	0.083 ± 0.022	0.085 ± 0.026	0.077 ± 0.020
	(g/100 g BW)	0.026 ± 0.007	0.031 ± 0.009	0.032 ± 0.010	0.029 ± 0.007
Kidneys	(g)	1.91 ± 0.16	1.87 ± 0.22	1.86 ± 0.16	1.88 ± 0.23
	(g/100 g BW)	0.66 ± 0.78	0.69 ± 0.06	0.70 ± 0.06	0.70 ± 0.06
Ovaries	(g)	0.12 ± 0.04	0.14 ± 0.04	0.12 ± 0.02	0.12 ±0.04
	(g/100 g BW)	0.040 ± 0.015	0.051 ± 0.015	0.046 ± 0.010	0.046 ± 0.019
Uterus	(g)	0.50 ± 0.10	0.58 ± 0.14	0.59 ± 0.17	0.55 ± 0.10
	(g/100 g BW)	0.17 ± 0.03^{b}	0.21 ± 0.05^{ab}	0.22 ± 0.05^{a}	0.21 ± 0.06^{ab}
Brain	(g)	1.98 ± 0.06	1.97 ± 0.08	1.98 ± 0.07	2.01 ± 0.05
	(g/100 g BW)	0.69 ± 0.07	0.73 ± 0.07	0.75 ± 0.09	0.75 ± 0.08
Thymus	(g)	0.47 ± 0.18	0.42 ± 0.12	0.46 ± 0.19	0.40 ± 0.21
	(g/100 g BW)	0.16 ± 0.06	0.15 ± 0.04	0.17 ± 0.07	0.15 ± 0.07

[1]n=10
[2] Glabrous brown canary seed (CDC Maria cultivar) groats
[3] Glabrous brown canary seed (CDC Maria), hulled.
[4] Pubescent hulled brown canary seed (Keet cultivar), hulled.
[5] Canadian Western Red Spring wheat
Means in the same row with different letters are significantly different at P<0.05
*Magnuson *et al.*, 2014

There were no significant differences among rats on the various diets for either daily or monthly FOB. There was no association of ophthalmology lesions with a canary seed diet. There was no hematology, serum chemistry or urinalysis findings considered to be diet-related. There were no significant differences related to diet in terms of prothrombin time and activated partial thromboplastin time (Magnuson *et al.*, 2014; data provided in study report in Appendix 4).

Serum chemistry values for rats fed canary seed were not significantly different from rats fed the wheat diet, except for ALT levels, which were significantly lower for both genders when fed the glabrous canary seed groat diet than with the other diets. However, all values were within the normal physiological ranges and were not toxicologically significant. No significant differences between genders or diets were noted in urinalysis data (Magnuson *et al.*, 2014). Data are not shown (study report in Appendix 4).

All rats underwent gross examination and no diet-related lesions were noted. The limitation of this study is that tissues from only 32 of the 80 rats (i.e. 4 out of 10 rats per treatment/sex) were assessed histologically. The few observed lesions did not appear to be associated with any diet and consisted of mostly very mild changes, including mild inflammatory lesions in various tissues. Data are not shown (study report in Appendix 4).

In summary, rats fed a diet containing 50% hulled or dehulled glabrous canary seed, or hulled pubescent canary seed for 90 days had similar or improved growth, hematological and clinical chemistry parameters, as rats fed a diet containing 50% CWRS wheat. No adverse effects were observed. Although the study had limited histology, these findings support the safety of glabrous canary seed as a human food. The NOAEL for glabrous canary seed ranged from 33 to 37 g/kg/d for males and 38 to 42 g/kg/d for females (Magnuson *et al.*, 2014).

11.1.2.2 Rodent studies on yellow and brown glabrous canary seed (Phase 2)

The Phase 2 (2008-2014) rat studies examined the effects of administering yellow or brown glabrous canary seed groats in the diet at concentrations levels of 2.5%, 5% and 10% canary seed groats to rats *ad libitum* over 2 time periods: a) a 28

day period and b) a 90-day period followed by a 30-day recovery period. The rationale for the Phase 2 28-day and 90-day rodent study design was outlined in Section 9.22. The studies were conducted by NucroTechnics and monitored by Cantox Intertek. The objectives, protocols and results of these studies are summarized in Table 11-3 (28-day study) and 11-4 (90-day study).

The experimental protocols and full results including summary tables and raw data are available in the accompanying study reports (28-day study, Appendix 5a; 90-day study, Appendix 5b). Only a few summary tables, when noted, are included in the body of this dossier. These studies have been published (Magnuson *et al.*, 2014).

In establishing whether individual or group values were "normal" or "abnormal", Nucro-Technics' historical data and Charles-River published data for Sprague- Dawley rats were used (Charles River, 1984). Additional references for interpretation of clinical pathology findings were also used (Car, 2006; Clapp, 1982; Levine, 2002; Lewis, 1996; Ramaiah, 2007).

The study was conducted in accordance to the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 407.

11.1.2.2.1 28-Day feeding study on yellow and brown glabrous canary seed in rats

The 28-day study was conducted in accordance to the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 407.

Table 11-3 Twenty-eight (28) day dose range finding study in Sprague Dawley rats fed brown and yellow canary seed groats (Phase 2)[1]

Objective: a) to assess the effects of 3 dose levels of glabrous yellow dehulled canary seed (yellow groats) and 1 dose level of glabrous dehulled brown canary seed (brown groats) and b) to validate the diet preparation process and stability/homogeneity of different components in the diet. Information to be used in the 90 day study.

- 5 groups of male and female Sprague-Dawley rats (5 male and 5 female/test diet)
- 5 diet groups:
  - Diet 1: Control: AIN-76A
  - Diet 2: 2.5% dehulled yellow canary seed
  - Diet 3: 5.0% dehulled yellow canary seed
  - Diet 4: 10% dehulled yellow canary seed
  - Diet 5: 10% dehulled brown canary seed (CDC Maria)
- Diets were formulated to ensure test diets contained similar macro- and micronutrients as the AIN-76A diet. All diets contained 20% protein and 5% fat with total Kcal/g of 3.9.
- Water and test diets fed *ad libitum* daily for 28 days

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational batteries, hematology, clinical chemistry, urinalysis, organ weights and gross necropsy.

Data Type	Results
Mortality	All animals survived to scheduled euthanasia/necropsy date
Hematology	No findings attributable to consumption of canary seed diets
Functional Observational Batteries	Normal.
Organ weights and Growth	No appreciable differences in body weights and body weight gains. No notable changes in absolute organ weights and relative organ weights (to brain/body weights) except for higher relative lung weight (relative to brain weight) in Gr. 3 males. Not considered biologically relevant as there was no dose-response relationship.
Plasma chemistry	No significant findings
Coagulation	No significant findings
Urinalysis	No significant findings
Gross Necropsy	No significant findings
Histopathology	No histopathological assessment carried out

[1] Magnuson *et al.*, 2014

This 28-day rodent study examined the safety (systemic toxicity and target organs for toxicity) of yellow and brown canary seed glabrous groats incorporated into a diet at concentration levels of 2.5%, 5% and 10% and administered to rats *ad libitum* over a 28-day period. This study was initiated to identify the baseline parameters for the pivotal 90-day study.

Five groups of rats were used in the study (1 control, 4 test). Each test and control group consisted of 5 male and 5 female rats (Strain: Crl:CD®(SD)BR-Sprague-Dawley).

Based on the test groups' average body weights and food consumption, male rats consumed 1.7, 3.4 and 6.6 and 6.5 g/kg body weight per day, and females consumed 1.9, 4.0, 7.8 and 7.6 g/kg body weight per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% (glabrous yellow canary seed) or 10% (glabrous brown canary seed), respectively, over a 28-day period. The gender-combined consumption was 1.8, 3.6 and 7.0 g of yellow canary seed groat or 6.9 g of brown canary seed groat per kg body weight per day, for the four dose levels, respectively (Magnuson, *et al.,* 2014).

Various endpoints were monitored as well as body weight assessment, food consumption, clinical pathology, organ weights, and gross pathology. Daily clinical observations and weekly physical examinations showed no diet-related toxicity over a 28-day treatment period in any of the groups of rats.

Animals from all diet groups consumed food and gained body weight over the treatment period. There were no statistical differences in food consumption and body weight gains between the control and test groups of animals.

There were no haematology, serum chemistry or urinalysis findings considered to be diet-related and gross necropsy and organ weights and organ weight ratios were unremarkable (Magnuson, *et al.,* 2014). Full study details are available in Appendix 5a.

In conclusion, this study including clinical observations, clinical pathology and gross necropsy revealed no toxicity in rats that consumed yellow or brown canary seed groats incorporated into diets at concentration levels of 2.5%, 5% or 10% *ad libitum* for a 28-day period. These dose levels were used for the subsequent Phase 2 90-day study.

11.1.2.2.2 90-Day rat feeding study on glabrous yellow and brown canary seed (Phase 2)

This 90-day study was conducted in compliance with the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and

subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 408 with the exception of the test diet formulation and preparations which were conducted by Research Diets, Inc., New Brunswick, New Jersey, U.S.A. Although the diets were not prepared under strict GLP conditions, the preparation of the diets was designed to be consistent with the requirements of GLP. Full study details are available in Appendix 5b.

Table 11-4 Ninety (90) day safety study in Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats (Phase 2) [1]

Objective: to compare the toxicological and growth effects of dehulled glabrous canary seed (brown groats) with dehulled glabrous yellow canary seed (yellow groats) in rats

- Protocol followed OECD Test Guideline N0. 408
- 5 groups of male and female Sprague-Dawley rats (35 male and 35 female/test diet) consisting of 20 M/F in main group, 10M/F in satellite group and 5M/F in recovery group (30 days on control diet).
- 5 diet groups:
  - Diet 1: Control: AIN-76A
  - Diet 2: 2.5% dehulled yellow canary seed
  - Diet 3: 5.0% dehulled yellow canary seed
  - Diet 4: 10% dehulled yellow canary seed
  - Diet 5: 10% dehulled brown canary seed
- Diets were formulated to ensure test diets contained similar macro- and micronutrients as the AIN-76A diet. All diets contained 20% protein and 5% fat with total Kcal/g of 3.9.
- Water and test diets fed *ad libitum* daily for 90 days followed by a 30 day recovery period on control diet.

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational batteries, ophthalmology, hematology, bone marrow analysis, coagulation, clinical chemistry, urinalysis, organ weights, gross pathology and complete histology.

Data Type	Results
Bone marrow	No significant findings
Hematology	No findings attributable to consumption of canary seed diets
Functional Observational Batteries	Normal
Ophthalmological Examination	No findings attributable to test article
Organ weights and Growth	No findings attributable to consumption of canary seed diets
Plasma chemistry	No significant findings
Coagulation	No significant findings
Urinalysis	No significant findings
Gross Necropsy	No significant findings
Histopathology	No findings attributable to consumption of canary seed diets

[1] Magnuson *et al.*, 2014

Based on the test groups' average body weights and food consumption, male rats consumed 1.23, 2.45 and 4.92 or 5.03 g/kg per day, and females consumed 1.41, 2.68, 5.53 or 5.57 g/kg per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% yellow canary seeds or 10% brown canary seeds, respectively, over a 90-day period (Magnuson *et al.*, 2014).

Various biomarkers were monitored as well as body weight, feed consumption, ophthalmology, clinical pathology, organ weights, gross pathology and histopathology.

Daily clinical observations and weekly physical examinations showed no test article related toxicity over the 90-day period as well as over the subsequent 30-day recovery period, in any of the diet groups (Data available in Appendix 5b).

Animals from all groups consumed feed and gained body weight over the treatment period. There were no differences in feed consumption and body weight gains between the control and test groups of animals, with the following exceptions: mean weights of male rats treated with 10% yellow canary seed groats were lower at Day 85 (7% of control) and Day 90, (8% of control). This finding was also mirrored with slightly reduced feed consumption in these rats during the same time period: Days 78-90. Normalization of the body weights at day 91 per total feed consumption showed no differences between control and treatment groups. There was no dose-response in reduced body weight or food consumption observed in male rats fed the yellow canary seed groats, and no differences in body weight or food consumption was observed in female rats fed 10% yellow canary seed groats (see Table 9-25, Section 9.2.2). Thus, the differences in feed consumption and body weight were considered to be of no toxicological significance.

Based on the test groups' average body weights and food consumption, male rats consumed 1.23, 2.45 and 4.92 or 5.03 g/kg per day, and females consumed 1.41, 2.68, 5.53 or 5.57 g/kg per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% yellow canary seeds or 10% brown canary seeds, respectively, over a 90-day period.

Ophthalmology

There was no apparent dose-dependency in observations, or findings specific to the test article groups, thus findings were not considered to be diet-related.

Clinical Pathology

There was no hematology, serum chemistry or urinalysis findings considered to be diet-related (Magnuson *et al.*, 2014). It should be however noted that in some rats (across all groups, both genders and including controls) cholesterol and triglyceride levels were increased and in some rats, as well as increases in total bilirubin and ALT. These findings were associated with hepatic lipidosis, which is not uncommon in well-fed obese rats (Medinsky *et al.*, 1986).

Hematology

Summary tables for hematology data are presented in Appendix 5b and in Tables 11-5 and 11-6. There were no hematology findings that were considered to be related to the consumption of the diets (Magnuson *et al.*, 2014).

RBC counts, reticulocytes, hemoglobin, (Hb), Hematocrit (Hct) and RBC indices (MCV, MCH and MCHC) were all within the normal physiological limits throughout the study. WBC counts and differential counts were also all within the normal historical ranges for both genders, for all groups, and test periods, with the following exceptions: large unstained cells (LUC's) (a part of lymphocyte lineage) was marginally increased in male rats fed 5% yellow canary seed groats and the control diet. This finding was not associated with dose-dependent increases and male control rats were affected as well, thus this finding was not considered to be diet-related. Platelet counts were also within the normal historical ranges for all groups, both genders and all time points.

Statistically significant differences were observed in the hematocrit and MCHC values between males fed the control diet and males fed various canary seed diets. In both cases, the values were well within the normal ranges and this effect was considered to be of no biological relevance.

Table 11-5 Hematology data for male Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

Parameters	Unit	Group Means ± S.D. (n = 20) Group 1 Control Diet (0%)	Group 2 Low Dose Yellow (2.5%)	Group 3 Mid Dose Yellow (5%)	Group 4 High Dose Yellow (10%)	Group 5 High Dose Brown (10%)	Normal Ranges
RBC	$x10^{12}$ / L	8.49 ± 0.54	8.72 ± 0.42	8.56 ± 0.37	8.59 ± 0.42	8.80 ± 0.43	6.06 -9.46
Hb	g / L	142 ± 9	144 ± 5	142 ± 6	146 ± 6	146 ± 6	120 -181
Hct	%	44.1 ± 2.7	45.0 ± 1.7	45.3 ± 1.6	46.1 ± 1.8 *	46.8 ± 2.1 *	37.3 -50.2
MCV	fL	52.0 ± 2.1	51.6 ± 1.4	52.9 ± 1.6	53.7 ± 2.3	53.3 ± 2.3	47.5 -66.1
MCH	pg	16.7 ± 0.7	16.5 ± 0.5	16.6 ± 0.6	17.0 ± 0.7	16.6 ± 0.7	15.8 -23.1
MCHC	g / L	322 ± 6	319 ± 7	314 ± 8 *	317 ± 7 *	311 ± 5 *	287 -401
Platelets	$x10^9$ / L	905 ± 234	982 ± 123	1040 ± 216	978 ± 205	1022 ± 107	579 -1641
WBC	$x10^9$/ L	8.86 ± 2.48	8.70 ± 2.32	10.54 ± 10.02	7.96 ± 2.53	8.72 ± 2.69	5.00 -15.28
Neutrophils	$x10^9$ / L	1.37 ± 0.52	1.19 ± 0.49	2.16 ± 4.58	1.20 ± 0.36	1.16 ± 0.29	0.05 -2.37
Lymphocytes	$x10^9$ / L	6.99 ± 2.02	7.07 ± 1.93	7.32 ± 3.09	6.33 ± 2.15	7.10 ± 2.40	1.67 -14.00
Monocytes	$x10^9$/ L	0.23 ± 0.13	0.19 ± 0.07	0.27 ± 0.21	0.21 ± 0.09	0.24 ± 0.10	0 -0.46
Eosinophils	$x10^9$ / L	0.12 ± 0.03	0.11 ± 0.05	0.58 ± 2.06	0.11 ± 0.03	0.11 ± 0.03	0 -0.21
Basophils	$x10^9$ / L	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0 -0.06
LUC	$x10^9$ / L	0.13 ± 0.12	0.11 ± 0.04	0.19 ± 0.33	0.09 ± 0.05	0.10 ± 0.05	0 -0.14
Reticulocytes	$x10^9$ / L	211.6 ± 45.1	203.9 ± 51.0	204.5 ± 37.2	182.0 ± 28.6	202.1 ± 36.4	100 - 400

[1] Magnuson *et al.*, 2014

*Statistically significant difference from Control Group ($p < 0.05$)

Table 11-6 Hematology data for female Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

Parameters	Unit	Group Means ± S.D. (n = 20) Group 1 Control Diet (0%)	Group 2 Low Dose C Yellow (2.5%)	Group 3 Mid Dose Yellow (5%)	Group 4 High Dose Yellow (10%)	Group 5 High Dose Brown (10%)	Normal Ranges
RBC	$x10^{12}$ / L	8.21 ± 0.30	8.12 ± 0.28	8.20 ± 0.41	8.21 ± 0.44	8.11 ± 0.36	6.16 -9.09
Hb	g / L	141 ± 4	141 ± 4	142 ± 5	141 ± 5	140 ± 5	127 -172
Hct	%	43.0 ± 1.3	42.9 ± 1.4	43.4 ± 1.4	43.4 ± 1.9	42.9 ± 1.6	35.3 -47.5
MCV	fL	52.3 ± 1.3	52.8 ± 1.6	53.0 ± 2.0	53.0 ± 1.9	52.9 ± 1.7	47.5 -64.0
MCH	pg	17.2 ± 0.4	17.4 ± 0.5	17.3 ± 0.7	17.3 ± 0.7	17.3 ± 0.5	17.9 -21.6
MCHC	g / L	328 ± 4	329 ± 5	327 ± 5	325 ± 6	327 ± 5	325 -385
Platelets	$x10^{9}$ / L	864 ± 156	878 ± 145	865 ± 113	889 ± 175	942 ± 125	526 -1648
WBC	$x10^{9}$ / L	5.61 ± 1.81	5.48 ± 1.46	5.06 ± 1.38	5.48 ± 0.98	5.43 ± 1.52	4.30 -13.00
Neutrophils	$x10^{9}$ / L	0.86 ± 0.39	0.73 ± 0.29	0.65 ± 0.20	0.80 ± 0.35	0.71 ± 0.27	0.10 -2.67
Lymphocytes	$x10^{9}$ / L	4.39 ± 1.40	4.43 ± 1.16	4.12 ± 1.25	4.37 ± 0.74	4.42 ± 1.31	0.33 -11.60
Monocytes	$x10^{9}$ / L	0.18 ± 0.09	0.15 ± 0.06	0.14 ± 0.05	0.14 ± 0.07	0.15 ± 0.06	0 -0.30
Eosinophils	$x10^{9}$ / L	0.08 ± 0.04	0.08 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.04	0 -0.20
Basophils	$x10^{9}$ / L	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0 -0.04
LUC	$x10^{9}$ / L	0.09 ± 0.06	0.08 ± 0.03	0.08 ± 0.03	0.11 ± 0.13	0.08 ± 0.03	0 -0.11
Reticulocytes	$x10^{9}$ / L	173.1 ± 34.3	164.9 ± 37.3	154.4 ± 27.7	160.6 ± 27.3	193.2 ± 72.5	100 - 400

[1] Magnuson *et al.*, 2014

* Statistically significant difference from Control Group ($p < 0.05$).

Blood Coagulation

There were no coagulation alterations that were attributed to consumption of canary seed. Individual coagulation data can be found in Appendix 5b of this report.

Serum Chemistry

There were no serum chemistry changes that were attributed to the consumption of the test diets. Summary tables for male and female rats from the main 90 day study are presented in Tables 11-7 and 11-8, respectively.

Total protein, albumin, globulin and A/G ratios were not affected by the test diets. BUN levels were not affected by the diets. In the main study, male rats in the groups of high dose yellow canary seed groats and brown canary seed groats, and female rats fed the high dose of yellow canary seed groats had statistically significantly higher creatinine levels than rats in the control group; however all were within the normal range.

Electrolytes (Na^+, Cl^- and K^+) and calcium and phosphorus were all within the normal physiological ranges (both genders, all diets, all treatment periods). No differences among groups were observed in glucose levels.

Cholesterol and triglyceride levels were slightly increased in male rats of several groups including the control animals, in the satellite and main study as compared to normal ranges. No effect specific to consumption of canary seed was observed.

Hepatocellular/hepatobiliary panel (total bilirubin, ALP, ALT, AST, GGT and serum bile acids) were all mostly within the normal physiological ranges for both genders, all four test diet groups and all treatment periods. The exceptions were occasional increase in total bilirubin, which was slightly increased in some rats of all groups, including controls. These increases were small, there was no dose-dependency and control animals were also affected, thus these findings were not toxicologically significant.

Histologically, many rats (all groups including the control and both genders) were found to have periportal lipidosis ("fatty liver"). This finding can explain increased cholesterol, triglycerides, total bilirubin and ALT levels. This finding was not unusual for animals fed *ad libitum* for 3 months, during which they were minimally exposed to any stressors (handling, blood collection, etc.) (Medinsky *et al.*, 1986). These slight

increases were obviously diet-related but the controls were equally or more affected, and thus this finding was not necessarily specific for canary seed.

Table 11-7 Serum chemistry for male Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

Parameter	Unit	Group Means ± S.D. (n = 20) Group 1 Control Diet (0%)	Group 2 Low Dose Yellow (2.5%)	Group 3 Mid Dose Yellow (5%)	Group 4 High Dose Yellow (10%)	Group 5 High Dose Brown (10%)	Normal Ranges
A/G	-	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.7 -1.6
ALB	g / L	32 ± 2	32 ± 3	32 ± 3	31 ± 2	33 ± 3	23 -43
GLOB	g / L	28 ± 2	28 ± 1	29 ± 1	29 ± 1	29 ± 2	22 -36
ALP	u / L	80 ± 26	71 ± 13	81 ± 23	75 ± 13	73 ± 17	47 -426
Bil(T)	umol / L	4.4 ± 1.6	3.9 ± 1.5	4.8 ± 2.1	5.8 ± 1.5 *	5.1 ± 1.0	1.7 – 5.7
BUN	mmol / L	3.9 ± 0.6	4.2 ± 0.7	4.4 ± 0.6	4.1 ± 0.7	4.1 ± 0.9	3.0 -8.4
Ca	mmol / L	2.61 ± 0.08	2.60 ± 0.08	2.63 ± 0.08	2.58 ± 0.09	2.63 ± 0.08	2.24 -3.00
Cl	mmol / L	102 ± 3	103 ± 3	102 ± 2	103 ± 1	103 ± 2	90 -116
Creatinine	umol / L	28 ± 2	30 ± 3	30 ± 5	36 ± 4 *	37 ± 5 *	24 -66
Glucose	mmol / L	12.6 ± 3.0	11.6 ± 2.0	12.5 ± 2.9	11.8 ± 3.5	13.1 ± 2.5	0.8 -11.2
P	mmol / L	1.94 ± 0.17	1.90 ± 0.16	2.03 ± 0.15	2.08 ± 0.22	2.04 ± 0.16	1.83 -3.94
K	mmol / L	4.7 ± 0.2	4.8 ± 0.2	4.9 ± 0.3	5.0 ± 0.3 *	5.0 ± 0.3 *	3.7 -7.0
Protein (T)	g / L	60 ± 2	61 ± 3	61 ± 4	60 ± 3	62 ± 4	47 -75
AST	u / L	101 ± 77	84 ± 19	118 ± 112	87 ± 19	85 ± 15	42 -149
ALT	u / L	62 ± 70	42 ± 9	51 ± 30	42 ± 18	45 ± 12	26 -71
Na	mmol / L	140 ± 3	142 ± 3	143 ± 2	143 ± 2 *	144 ± 2 *	136 -152
Triglycerides	mmol / L	1.69 ± 1.01	1.87 ± 1.42	2.22 ± 1.58	1.47 ± 0.71	2.32 ± 1.24	0.10 -1.55
CK	u / L	320 ± 143	315 ± 149	317 ± 148	394 ± 154	290 ± 91	228 -529
Cholesterol	mmol / L	3.04 ± 0.74	2.88 ± 0.56	3.26 ± 0.90	2.94 ± 0.67	3.70 ± 1.05 *	1.00 -3.00
GGT	u / L	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	4 to 6
Bile Acids	umol/L	5.5 ± 3.2	7.1 ± 4.3	21.7 ± 38.3	8.0 ± 4.7	7.7 ± 5.2	0 -24

[1] Magnuson *et al.*, 2014; * Statistically significant difference from Control Group ($p < 0.05$).

Table 11-8 Serum chemistry for female Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

		Group Means ± S.D. (n = 20)					
Parameters	**Unit**	**Group 1 Control Diet (0%)**	**Group 2 Low Dose Yellow (2.5%)**	**Group 3 Mid Dose Yellow (5%)**	**Group 4 High Dose Yellow (10%)**	**Group 5 High Dose Brown (10%)**	**Normal Ranges**
A/G	-	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.8 -1.8
ALB	g / L	41 ± 4	43 ± 5	43 ± 5	44 ± 4	43 ± 4	25 -49
GLOB	g / L	28 ± 1	27 ± 1	27 ± 1	28 ± 1	28 ± 1	22 -34
ALP	u / L	98 ± 63	52 ± 18 *	52 ± 28 *	46 ± 12 *	47 ± 15 *	29 -309
Bil(T)	umol / L	3.5 ± 2.3	3.3 ± 1.9	3.0 ± 1.4	4.7 ± 1.4 *	4.7 ± 1.3 *	1.7 -5.9
BUN	mmol / L	4.5 ± 0.8	4.2 ± 0.5	4.5 ± 0.8	3.9 ± 0.7	4.1 ± 0.7	3.2 -8.0
Ca	mmol / L	2.70 ± 0.10	2.74 ± 0.08	2.78 ± 0.13	2.74 ± 0.07	2.73 ± 0.09	2.31 -3.03
Cl	mmol / L	99 ± 2	100 ± 1	101 ± 3 *	101 ± 2 *	103 ± 2 *	93 -117
Creatinine	umol / L	29 ± 3	29 ± 2	30 ± 4	31 ± 3	32 ± 4 *	23 -66
Glucose	mmol / L	9.9 ± 2.3	11.3 ± 2.8	10.7 ± 2.4	11.2 ± 2.0	10.8 ± 2.9	1.2 -11.4
P	mmol / L	1.79 ± 0.22	1.84 ± 0.18	1.75 ± 0.21	1.82 ± 0.13	1.76 ± 0.21	1.50 -3.47
K	mmol / L	4.4 ± 0.4	4.5 ± 0.2	4.5 ± 0.3	4.7 ± 0.3	4.7 ± 0.4 *	3.6 -6.5
Protein (T)	g / L	69 ± 5	70 ± 5	70 ± 5	72 ± 4	71 ± 4	50 -79
AST	u / L	85 ± 26	78 ± 18	73 ± 18	72 ± 17	82 ± 14	48 -134
ALT	u / L	39 ± 7	36 ± 6	35 ± 5	31 ± 5 *	32 ± 4 *	22 -66
Na	mmol / L	143 ± 2	144 ± 2	146 ± 4 *	144 ± 1	144 ± 2	138 -181
Triglycerides	mmol / L	1.23 ± 0.72	1.48 ± 1.32	1.18 ± 0.59	1.65 ± 1.33	1.47 ± 1.17	0.10 -1.25
CK	u / L	389 ± 226	355 ± 150	313 ± 140	302 ± 137	369 ± 128	158 -556
Cholesterol	mmol / L	2.91 ± 0.79	3.03 ± 0.49	2.52 ± 0.50	2.94 ± 0.58	3.05 ± 0.71	0.94 -3.26
GGT	u / L	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	3 to 8
Bile Acids	umol/L	12.8 ± 4.8	15.6 ± 8.7	18.3 ± 9.0	18.3 ± 8.7	13.1 ± 10.9	0 -24

[1] Magnuson *et al.*, 2014 * Statistically significant difference from Control Group ($p < 0.05$).

000145

Organ Weights

Organ weights were expressed in absolute terms, and as a percent (%) of final body weight and as % of brain weight (Tables 11-9 and 11-10). Statistical differences were observed in some cases, but as will be discussed below, these changes were not considered to be indicative of a toxicological response to canary seed. Statistical differences included lower liver weight of male animals fed the high dose (10%) yellow canary seed when expressed in absolute terms and as a % of brain weight (Table 11-9). There was no statistical difference when expressed as % of body weight. The liver weights of females fed the low dose yellow canary seed and 10% brown canary seed were lower than controls when expressed as a % of body weight only (Table 11-10). The lower liver weights in rats fed the canary seed diets may have been the result of the lower incidence and severity of fatty liver (hepatic lipidosis), which was the most frequent observation during histological evaluations of tissues.

The thymus weight of male rats fed the 10% brown canary seed diet was higher than the controls when expressed in absolute terms, as a % of body weight and as a % of brain weight (Table 11-9). No effect was observed in female rats (Table 11-10) or males fed 10% yellow canary seed (Table 11-9).

The pancreas weight of female rats fed 5% yellow canary seed was higher than the controls when expressed in absolute terms, as a % of body weight and as a % of brain weight, but this was not observed in female rats fed 10% canary seed (Table 11-10) or in male rats (Table 11-9).

The spleen weights of female rats fed 2.5% and 10% yellow canary seed and 10% brown canary seed were lower than the controls when expressed in absolute terms and as % of body weight (Table 11-10). No effect was observed in male rats (Table 11-9). All the above-mentioned organ changes were not considered to be biologically relevant as there was no dose-response relationship, and absolute weights of most of the organs in question were within the normal historical ranges (age and gender matched). The exception is that the weight of the thymus exceeded the normal ranges for all groups including the rats in the control diet groups (normal ranges: 0.28-0.42 g.). Furthermore,

there were no clinical pathology and histopathological findings, which would indicate abnormal findings in any organs in which statistical differences were found, thus these differences were not considered toxicologically significant.

Table 11-9. Summary of absolute and relative organ weights for male rats fed AIN-76 diets containing varying concentrations of glabrous yellow and brown canary seed groats in the 90-day study (Phase 2) [1]

Parameter	Group Means ± S.D. (n = 20)				
	AIN-76 control	Low 2.5% Yellow canary seed groat	Mid 5% Yellow canary seed groat	High 10% Yellow canary seed groat	High 10% Brown canary seed groat
Stomach (Absolute)	2.55 ± 0.34	2.45 ± 0.34	2.67 ± 0.60	2.46 ± 0.28	2.67 ± 0.30
Stomach (% Body Weight)	0.39 ± 0.05	0.38 ± 0.03	0.41 ± 0.14	0.41 ± 0.05	0.40 ± 0.04
Stomach (% Brain Weight)	111.43 ± 15.03	109.49 ± 16.26	118.55 ± 31.58	107.97 ± 11.34	119.12 ± 14.86
Pancreas (Absolute)	0.982 ± 0.313	1.045 ± 0.286	1.036 ± 0.251	1.095 ± 0.279	1.057 ± 0.210
Pancreas (% Body Weight)	0.149 ± 0.049	0.163 ± 0.041	0.159 ± 0.042	0.180 ± 0.044	0.158 ± 0.039
Pancreas (% Brain Weight)	43.014 ± 14.171	46.338 ± 11.757	45.798 ± 10.962	48.197 ± 12.627	47.205 ± 9.832
Spleen (Absolute)	1.079 ± 0.138	1.014 ± 0.187	1.064 ± 0.139	0.959 ± 0.141	0.994 ± 0.144
Spleen (% Body Weight)	0.164 ± 0.019	0.158 ± 0.020	0.163 ± 0.028	0.157 ± 0.015	0.147 ± 0.016
Spleen (% Brain Weight)	47.258 ± 6.371	45.133 ± 7.934	47.150 ± 7.524	42.097 ± 5.204	44.401 ± 6.902
Liver (Absolute)	18.66 ± 2.96	17.24 ± 2.92	17.95 ± 2.61	**15.99 ± 2.35***	19.25 ± 3.68
Liver (% Body Weight)	2.83 ± 0.37	2.68 ± 0.23	2.72 ± 0.27	2.63 ± 0.28	2.83 ± 0.36
Liver (% Brain Weight)	817.37 ± 133.04	766.70 ± 119.54	793.27 ± 120.00	**702.33 ± 91.36***	859.31 ± 168.96
Adrenal Glands (Absolute)	0.085 ± 0.016	0.097 ± 0.026	0.096 ± 0.023	0.086 ± 0.013	0.084 ± 0.011
Adrenal Glands (% Body Weight)	0.013 ± 0.002	0.015 ± 0.004	0.015 ± 0.004	0.014 ± 0.003	0.013 ± 0.002
Adrenal Glands (% Brain Weight)	3.736 ± 0.653	4.355 ± 1.313	4.213 ± 0.966	3.785 ± 0.669	3.760 ± 0.491
Testes (Absolute)	3.83 ± 0.27	3.72 ± 0.45	3.74 ± 0.20	3.83 ± 0.29	3.83 ± 0.50
Testes (% Body Weight)	0.58 ± 0.05	0.59 ± 0.08	0.57 ± 0.07	0.63 ± 0.05	0.57 ± 0.09

Testes (% Brain Weight)	167.73 ±12.56	165.49 ±18.53	165.19 ±12.02	168.90 ±14.51	170.75 ± 20.20
Kidneys (Absolute)	3.78 ± 0.31	3.64 ± 0.61	3.81 ± 0.32	3.70 ± 0.36	3.79 ± 0.45
Kidneys (% Body Weight)	0.58 ± 0.05	0.57 ± 0.06	0.58 ± 0.07	0.61 ± 0.05	0.56 ± 0.06
Kidneys (% Brain Weight)	165.37 ± 11.83	162.10 ± 25.18	168.21 ± 15.01	162.93 ± 14.89	169.06 ± 18.54
Prostate (Absolute)	1.889 ± 0.464	1.794 ± 0.414	1.851 ± 0.624	1.798 ± 0.384	1.737 ± 0.561
Prostate (% Body Weight)	0.286 ± 0.063	0.280 ± 0.058	0.280 ± 0.083	0.297 ± 0.065	0.256 ± 0.072
Prostate (% Brain Weight)	82.603 ± 19.771	79.893 ± 18.102	81.744 ± 27.893	79.133 ± 16.998	77.635 ± 25.126
Lungs and Trachea (Absolute)	2.14 ± 0.25	2.14 ± 0.32	2.04 ± 0.17	1.97 ± 0.19	2.10 ± 0.20
Lungs and Trachea (% Body Weight)	0.33 ± 0.04	0.34 ± 0.04	0.31 ± 0.05	0.33 ± 0.03	0.31 ± 0.02
Lungs and Trachea (% Brain Weight)	93.52 ± 9.82	95.58 ± 14.80	90.33 ± 9.73	86.78 ± 7.43	93.76 ± 9.05
Heart (Absolute)	1.71 ± 0.16	1.69 ± 0.23	1.74 ± 0.11	1.65 ± 0.12	1.73 ± 0.21
Heart (% Body Weight)	0.26 ± 0.024	0.26 ± 0.03	0.27 ± 0.03	0.27 ± 0.02	0.26 ± 0.02
Heart (% Brain Weight)	74.91 ± 6.89	75.31 ± 11.66	76.95 ± 6.61	72.58 ± 4.40	77.15 ± 9.29
Thyroid and Parathyroids (Absolute)	0.036 ± 0.010	0.033 ± 0.009	0.034 ± 0.009	0.033 ± 0.008	0.041 ± 0.008
Thyroid and Parathyroids (% Body Weight)	0.006 ± 0.001	0.005 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Thyroid and Parathyroids (% Brain Weight)	1.581 ± 0.446	1.445 ± 0.384	1.498 ± 0.395	1.468 ± 0.312	1.832 ± 0.345
Thymus (Absolute)	0.581 ± 0.099	0.635 ± 0.135	0.562 ± 0.114	0.516 ± 0.121	**0.704 ± 0.193***
Thymus (% Body	0.088 ± 0.014	0.099 ± 0.016	0.085 ± 0.015	0.085 ± 0.017	**0.104 ± 0.026***

Weight)					
Thymus (% Brain Weight)	25.393 ± 4.225	28.235 ± 5.717	24.790 ± 4.718	22.603 ± 4.945	**31.443 ± 8.766***
Brain (Absolute)	2.29 ± 0.10	2.25 ± 0.10	2.27 ± 0.12	2.28 ± 0.12	2.24 ± 0.09
Brain (% Body Weight)	0.35 ± 0.03	0.36 ± 0.04	0.35 ± 0.03	**0.38 ± 0.03***	0.33 ± 0.03
Epididymis (Absolute)	1.65 ± 0.19	1.64 ± 0.22	1.73 ± 0.21	1.72 ± 0.20	1.70 ± 0.20
Epididymis (% Body Weight)	0.25 ± 0.03	0.26 ± 0.04	0.26 ± 0.04	**0.28 ± 0.04***	0.25 ± 0.04
Epididymis (% Brain Weight)	71.99 ± 8.22	73.27 ± 11.59	76.45 ± 8.49	75.74 ± 9.62	75.91 ± 10.25
Pituitary Gland (Absolute)	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.014 ± 0.002
Pituitary Gland (% Body Weight)	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
Pituitary Gland (% Brain Weight)	0.575 ±0.078	0.564± 0.081	0.561 ± 0.113	0.587 ± 0.089	0.602 ± 0.085

[1] *Magnuson et al., 2014*
*Statistically significant difference from AIN-76 Control Group ($p < 0.05$).

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Table 11-10. Summary of absolute and relative organ weights for female rats fed AIN-76 diets containing varying concentrations of glabrous yellow and brown canary seed groats in the 90-day study (Phase 2)[1]

Parameter	Group Means ± S.D. (n = 20)				
	AIN-76 control	Low 2.5% Yellow canary seed groat	Mid 5% Yellow canary seed groat	High 10% Yellow canary seed groat	High 10% Brown canary seed groat
Stomach (Absolute)	1.76 ± 0.26	1.84 ± 0.25	1.69 ± 0.19	1.74 ± 0.25	1.73 ± 0.31
Stomach (% Body Weight)	0.49 ± 0.06	0.51 ± 0.05	0.49 ± 0.08	0.50 ± 0.07	0.48 ± 0.05
Stomach (% Brain Weight)	86.43 ± 12.66	92.06 ± 12.30	82.68 ± 10.04	83.98 ± 10.68	86.27 ± 15.97
Pancreas (Absolute)	0.618 ± 0.131	0.679 ± 0.155	**0.763 ± 0.189***	0.677 ± 0.117	0.730 ± 0.177
Pancreas (% Body Weight)	0.173 ± 0.037	0.188 ± 0.039	**0.226 ± 0.070***	0.194 ± 0.036	0.205 ± 0.047
Pancreas (% Brain Weight)	30.418 ± 6.596	34.045 ± 7.827	**37.479 ± 9.785***	32.884 ± 5.784	36.263 ± 8.020
Spleen (Absolute)	0.683 ± 0.143	**0.579 ± 0.110***	0.629 ± 0.096	**0.577 ± 0.075***	**0.594 ± 0.111***
Spleen (% Body Weight)	0.189 ± 0.029	**0.160 ± 0.027***	0.184 ± 0.031	**0.165 ± 0.022***	**0.166 ± 0.023***
Spleen (% Brain Weight)	33.548 ± 6.904	**28.974 ± 5.207***	30.760 ± 4.722	**27.985 ± 3.597***	29.749 ± 6.193
Liver (Absolute)	10.55 ± 2.49	9.61 ± 1.23	9.18 ± 1.20	9.43 ± 1.30	9.31 ± 1.65
Liver (% Body Weight)	2.91 ± 0.37	**2.65 ± 0.24***	2.67 ± 0.34	2.69 ± 0.28	**2.59 ± 0.29***
Liver (% Brain Weight)	517.86 ± 117.73	482.24 ± 64.73	448.87 ± 59.40	457.62 ± 65.89	465.50 ± 90.36
Adrenal Glands (Absolute)	0.096 ± 0.025	0.086 ± 0.020	0.088 ± 0.015	0.091 ± 0.020	0.094 ± 0.019
Adrenal Glands (% Body Weight)	0.027± 0.009	0.024 ± 0.006	0.026 ± 0.005	0.026 ± 0.007	0.027 ± 0.006
Adrenal Glands (% Brain Weight)	4.728 ± 1.249	4.319 ± 1.062	4.301 ± 0.769	4.447 ± 1.035	4.699 ± 0.984
Kidneys (Absolute)	2.43 ± 0.41	2.33 ± 0.27	2.35 ± 0.29	2.35 ± 0.32	2.41 ± 0.29
Kidneys (% Body Weight)	0.68 ± 0.05	0.64 ± 0.07	0.68 ± 0.07	0.67 ± 0.09	0.68 ± 0.07
Kidneys (% Brain Weight)	119.69 ± 20.05	116.73 ± 15.15	115.19 ± 15.99	114.16 ± 15.76	120.31 ± 14.68
Ovaries (Absolute)	0.195 ± 0.039	0.167 ± 0.034	0.198 ± 0.052	0.187 ± 0.049	0.179 ± 0.035
Ovaries (% Body Weight)	0.055 ± 0.011	0.046 ± 0.009	0.057 ± 0.013	0.053 ± 0.014	0.050 ± 0.008
Ovaries (% Brain Weight)	9.582 ± 1.981	8.374 ± 1.596	9.686 ± 2.588	9.059 ± 2.355	8.972 ± 1.837
Uterus (Absolute)	0.736 ± 0.158	0.733 ± 0.188	0.733 ± 0.169	0.711 ± 0.154	0.715 ± 0.203

Uterus (% Body Weight)	0.206 ± 0.047	0.204 ± 0.055	0.213 ± 0.045	0.205 ± 0.049	0.200 ± 0.052
Uterus (% Brain Weight)	36.110 ± 7.477	36.642 ± 8.925	36.005 ± 9.038	34.510 ± 7.742	35.570 ± 9.658
Lungs and Trachea (Absolute)	1.57 ± 0.22	1.57 ± 0.24	1.50 ± 0.23	1.50 ± 0.16	1.50 ± 0.20
Lungs and Trachea (% Body Weight)	0.44 ± 0.05	0.43 ± 0.05	0.44 ± 0.06	0.43 ± 0.05	0.42 ± 0.05
Lungs and Trachea (% Brain Weight)	76.97 ± 9.85	78.47 ± 11.71	73.63 ± 11.393	72.50 ± 6.90	75.02 ± 10.66
Heart (Absolute)	1.10 ± 0.13	1.08 ± 0.09	1.11 ± 0.12	1.10 ± 0.11	1.09 ± 0.13
Heart (% Body Weight)	0.31 ± 0.03	0.30 ± 0.30	0.32 ± 0.03	0.32 ± 0.03	0.31 ± 0.03
Heart (% Brain Weight)	54.22 ± 6.17	54.11 ± 4.60	54.52 ± 5.94	53.55 ± 5.46	54.60 ± 6.41
Thyroid and Parathyroids (Absolute)	0.024 ± 0.005	0.021 ± 0.004	0.022 ± 0.004	0.027 ± 0.005	0.026 ± 0.006
Thyroid and Parathyroids (% Body Weight)	0.007 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	**0.008 ± 0.001***	0.007 ± 0.001
Thyroid and Parathyroids (% Brain Weight)	1.179 ± 0.223	1.042 ± 0.192	1.077 ± 0.210	1.321 ± 0.280	1.277 ± 0.330
Thymus (Absolute)	0.419 ± 0.147	0.464 ± 0.112	0.385 ± 0.087	0.439 ± 0.090	0.422 ± 0.140
Thymus (% Body Weight)	0.114 ± 0.031	0.128 ± 0.025	0.111 ± 0.019	0.125 ± 0.019	0.122 ± 0.029
Thymus (% Brain Weight)	20.592 ± 7.323	23.271 ± 5.670	18.853 ± 4.285	21.289 ± 4.214	22.120 ± 7.207
Brain (Absolute)	2.04 ± 0.08	2.00 ± 0.08	2.05 ± 0.09	2.06 ± 0.09	2.01 ± 0.10
Brain (% Body Weight)	0.57 ± 0.07	0.56 ± 0.06	0.60 ± 0.06	0.59 ± 0.06	0.57 ± 0.07
Pituitary Gland (Absolute)	0.016 ± 0.004	0.015 ± 0.004	0.017 ± 0.006	0.018 ± 0.004	0.017 ± 0.004
Pituitary Gland (% Body Weight)	0.005 ± 0.001	0.004 ± 0.001	0.005 ± 0.002	0.005 ± 0.001	0.005 ± 0.001
Pituitary Gland (% Brain Weight)	0.801 ± 0.182	0.775 ± 0.198	0.837 ± 0.275	0.888 ± 0.195	0.854 ± 0.188

[1] Magnuson *et al.*, 2014
*Statistically significant difference from AIN-76 Control Group ($p < 0.05$).

All urinalysis data were unremarkable, within normal ranges and no significant differences between groups were observed. Gross necropsy also revealed no findings of toxicological significance (Magnuson *et al.*, 2014).

Histopathology

Histopathology revealed no findings of toxicological significance, although it should be noted that hepatic periportal lipidosis was noted in most rats of all groups. This is a common finding in well-fed laboratory rats (Medinsky *et al.*, 1986). In controls, the incidence and severity of this finding was slightly greater in females than in males. In both groups of females treated with either 10% of yellow or brown canary seed, and in males treated with 10% yellow canary seed (at the end of the study), there were decreases in incidence and severity in hepatic lipidosis as compared to the corresponding controls (Magnuson *et al.*, 2014).. This may be an indication of some protective properties of canary seed on the liver lipidosis. Further corroboration of possible protective effect of canary seed on lipid metabolism comes from an increase in the incidence and severity of liver lipidosis, i.e. returned to the control levels, in 30-day recovery animals consuming the control diet during this period.

Mineralization in the renal cortico-medullary region was commonly seen in females, but the test article had no apparent effect upon this condition. The occurrence of retinal thinning or degeneration was seen in some males or females of most diet groups, including controls. In addition, some control and treated rats had a variety of degenerative or inflammatory lesions that are commonly seen in laboratory rats and were in no way related to the test article administration.

No significant histopathological findings were noted for the testes, epididymis, prostate and seminal vesicles in male rats or the uterus, ovaries and mammary glands in female rats of the satellite, main and recovery groups for the 4 canary seed diet treatments compared to the control diet (Magnuson *et al.*, 2014).

Summary Phase 2 Rodent Study

In conclusion, analysis of all generated data including clinical observations, clinical pathology, gross necropsy and histopathology revealed no toxicity in rats that

consumed, *ad libitum,* glabrous yellow canary seed groats incorporated into diets at concentration levels of 2.5%, 5% and 10% or glabrous brown canary seed groats incorporated into diets at concentration levels of 10% for a 90-day period (Magnuson *et al.*, 2014).

Also, no toxicity was observed during the subsequent 30-day recovery period. Hepatic periportal lipidosis (and increased cholesterol, triglycerides and in some cases ALT levels) was the only finding that was feeding related (but not related to either yellow or brown canary seed), since there was no dose-response relationship and control rats were equally or more affected than the rats fed canary seed diets.

The above feeding regimen corresponded to average dose levels (gender combined) of 1.30, 2.54 and 5.15 g of yellow canary seed groats or 5.23 g of brown canary seed groats per kg per day, for the four dose levels, respectively.

Under the conditions of this study, a NOEL (No Observed Effect Level) for canary seed groats in rats was considered to be the highest concentration tested at 10% in the diet or 5.15 to 5.23 g/kg body weight per day for 90 days (Magnuson *et al.*, 2014).

11.2 Swine

Two studies evaluating canary seed as a feed for growing swine have been reported (Thacker, 2003; Qiao and Thacker, 2004) and discussed in Section 9.2.3. As the pig is considered to have very similar digestive system to man, these studies are particularly helpful in assessing the nutritional properties of canary seed as a human food; however, the studies did not report toxicological endpoints.

In the study evaluating the growth of grower-finishing pigs fed graded levels of canary seed Thacker concluded that canary seed could be included at levels as high as 57% of the total diet (75% of the cereal portion) without adversely affecting grower pig growth and feed intake or altering carcass characteristics. In addition the author indicated the canary seed diets were palatable, and nutrients were efficiently utilized and any anti-nutritional factors present in canary seed were not at high enough levels to negatively affect pig performance (Thacker, 2003).

11.3 Birds-poultry

Several studies have been conducted on the safety of canary seed as feed for broiler chickens. Newkirk *et al* (2011) studied the toxicological effects on poultry consuming pubescent and glabrous canary seed finding no significant toxicological effects when compared to consumption of a control commercial diet and/or wheat diet.

11.4 Toxicological Considerations Summary

The dietary consumption of canary seed has been investigated in birds, chickens, mice and rats fed pubescent brown and glabrous brown and yellow canary seed that were hulled or dehulled (groats).

Early studies conducted in mice (Bhatt *et al.*, 1984) focused on the carcinogenic and cancer-promoting potential of the silica fibers present on the surface of pubescent canary seed. No evidence of carcinogenicity due to consumption of pubescent canary seed for 18 months was observed in mice that were not initially treated with a skin cancer carcinogen (Bhatt *et al.*, 1984). Chronic irritation from dermal contact with silica fibers on the surface of pubescent canary seed promoted development of skin tumors in mice treated with the carcinogen. The selective breeding of the glabrous canary seed resulted in elimination of the surface silica fibers.

Subsequent toxicology studies conducted in rats demonstrated that brown glabrous canary seed fed in hulled or dehulled (groats) form at a level of 50% of the diet was similar to a diet containing 50% wheat in supporting growth during a 90-day study. No toxicologically significant effects were reported in evaluations of hematology, clinical chemistry, urinalysis, bone marrow assessments, functional observational batteries, ophthalmological evaluations and limited histological assessments. Increased body weights in male rats fed dehulled groats affected relative organ weights, but these were not considered toxicologically significant (Magnuson *et al.*, 2014).

A second 90-day rat study, conducted under GLP, assessed the growth and toxicological effects of the addition of yellow and brown glabrous canary seed groats to the AIN-76 diet at levels up to 10% of the diet. Male rats fed the diet containing 10% yellow canary seed groats consumed statistically significantly less food towards the end of the study, and had significantly lower body weights. No evidence of a dose-response

of these effects was observed in males fed diets with 2.5% or 5% yellow canary seed groats and no similar effects were observed in female rats. Furthermore, no toxicological adverse effects were observed in hematology, clinical chemistry, urinalysis, bone marrow assessments, functional observational batteries, ophthalmological evaluations or histological assessments (Magnuson *et al.*, 2014). The incidence and severity of hepatic lipidosis in the male rats fed 10% yellow canary seed was lower than observed in male rats fed the control diet. Liver lipidosis is a common finding in laboratory rats that are fed *ad libitum*, and tend to become obese (Medinsky et al., 1986). Reduced hepatic lipidosis was also observed in female rats fed diets containing 10% brown or yellow canary seed, as compared to controls. Therefore, the reduced body weight observed in male rats fed 10% yellow canary seed groats was not considered an adverse toxicological effect. No Observed Adverse Effect Levels in this pivotal toxicology study were 5.15 g/kg/d for yellow canary seed groats and 5.23 g/kg/d for brown canary seed groats, which were the highest tested doses.

These studies, in combination with analytical and nutritional data presented in this dossier demonstrating that the levels of nutrients, antinutrients, alkaloids, heavy metals, and mycotoxins are within the acceptable ranges observed in other grains, which support the safety of consumption of yellow and brown canary seed groats as a food cereal grain.

12.0 ALLERGENICITY CONSIDERATIONS

12.1 IgE-Mediated Allergy

Canary seed is not listed as a priority food allergen in North America, Europe, or any other region or country (FARRP, 2013). Cross-reactivities may, however, exist between proteins found in canary seed and major food allergens if there are structural or sequence homologies between the canary seed proteins and other major allergenic proteins. Since canary seed is a grain with comparatively high protein content, the potential for canary seed to sensitize susceptible individuals should also be assessed.

12.1.1. Pollen Allergy

Reports of the pollen from perennial pubescent canarygrass (e.g. *Phalaris aquatica*, *Phalaris arundinacea*) as a major environmental allergen and incidents of allergic reactions to pubescent canary seed on inhalation during handling have been cited in the literature. Using IgE antibodies from sera of 24 grass-pollen-allergic subjects, Suphioglu *et al.* (1993) identified seventeen allergenic fractions of canarygrass (*Phalaris aquatica*) pollen, ranging in molecular mass from 14 to 100 kDa. A 34-kDa protein fraction was found to have the highest frequency of IgE binding (77%) and was tentatively designated as Pha a I. Microsequencing of the N-terminus of this protein showed amino acid sequence homology with Lol p I from rye-grass pollen.

In other studies, significant amino acid sequence homology has been found between the *P. aquatica* allergenic proteins and other allergens from velvet grass, timothy grass and Kentucky bluegrass pollen (Suphioglu and Singh, 1995). Since canarygrass is a member of the *Pooideae* subfamily and is genetically related to other grass species, the possibility of cross-reactive pollen allergens among these various grass species is not surprising. However, pollen allergens are primarily an environmental and occupational issue and do not represent a food safety concern.

Apart from the above described studies, there are no reported studies on the allergenicity of annual canarygrass, particularly the newly developed glabrous yellow and brown *Phalaris canariensis* varieties. Discussions with canary seed producers indicate their preference of working with glabrous (hairless) *P. canariensis* varieties,

versus pubescent (hairy) *P. canariensis* varieties as the glabrous varieties are "itchless" and easier to harvest and manage.

12.1.2. IgE-Mediated Food Allergy

Assessment of the allergenic potential of canary seed is difficult because canary seed has not been a component of the human diet. The pubescent varieties have not been widely consumed and the glabrous varieties are not yet widely produced for human consumption. Not surprisingly, documented cases of food allergy due to canary seed do not exist. Almost no clinical literature exists with respect to the possible presence of ingestion allergens in canary seed, either pubescent or glabrous varieties. Baldo *et al.* (1980), using radioallergosorbent testing (RAST) of sera from subjects orally sensitized to wheat and rye flour, found significant IgE binding with seed extracts of 12 cereals including wheat, durum wheat, triticale, cereal rye, barley, rye grass, oats, canary seed (pubescent *P. canariensis*), rice, maize, sorghum and Johnson grass. However, IgE binding alone is insufficient to prove that allergic reactions would occur if these grains were ingested. To document allergenicity, an oral challenge with the grains or a demonstration of mediator release from activated basophils would be needed. Furthermore, plant sources often have cross-reactive carbohydrate determinants (CCD) on various glycoproteins that bind avidly to IgE but have limited, if any, clinical significance (Chunsheng *et al.*, 2008; van Ree, 2002). While the existence of CCDs was not known at the time of the Baldo *et al.* (1980) study, the role of CCDs in the observed IgE binding could have been significant.

In the absence of any history of ingestion of canary seed, the assessment of the allergenic potential of canary seed could be based upon several factors in a manner consistent with the evaluation of recombinant proteins in genetically modified foods – sequence homology of proteins to known allergens and the digestive stability of proteins to pepsin. However, this approach is difficult for a novel food such as canary seed because it likely contains dozens to hundreds of proteins unlike genetically modified foods that contain only one or a few novel proteins. Furthermore, few proteins in the proteome of canary seed have been purified or sequenced so this approach is essentially unworkable for canary seed.

The potential allergenicity of canary seed can be evaluated to some extent based upon its genetic relationships. Canary seed is part of the *Pooideae* subfamily that also contains wheat, durum wheat, spelt, rye, barley, triticale, and oats. Wheat is a commonly allergenic food. Allergies to other *Pooideae* grains including barley, rye, and oats have been documented but these foods are not commonly allergenic. Canary seed is mostly closely related to oats and oat allergy is rather rarely encountered (Inou *et al.*, 2013). Furthermore, cross-reactive allergy is not known to occur between wheat and other grains in the *Pooidèae* subfamily. This observation casts doubt on the significance of the Baldo *et al.* (1980) study indicating cross-reactive IgE binding.

Boye *et al.* (2013) used SDS-PAGE to separate canary seed proteins. The brown and yellow canary seed cultivars showed similar electrophoretic profiles with the presence of protein bands ranging in molecular mass from ~ 10,000 to 100,000 Da. The most prominent band had a molecular mass of ~ 20,000 – 25,000 Da. To assess the presence of proteins in canary seed that might cross-react with wheat allergens, the reactivities of protein components separated by SDS-PAGE were analyzed by immunoblotting, using pooled sera from 10 wheat allergic individuals. The wheat-allergic sera were obtained from a serum bank and can only be characterized as wheat-sensitized (having IgE that binds to wheat proteins) because the serum donors were not clinically evaluated for wheat allergy by oral challenge or mediator release assays. The immunoblot of the three canary seed protein extracts revealed strong binding of the wheat sera to many of the canary seed proteins. Non-specific binding was suspected and then confirmed; but even exchanging the bovine serum albumin for non-fat dry milk still resulted in some binding of canary seed proteins to wheat sera. The three canary seed composites showed similar antibody-binding patterns.

Gliadin, a component of the gluten complex, is one of the known wheat allergens. To determine if binding would be observed with gluten-specific antibodies, the blots were also probed with polyclonal rabbit IgG anti-gluten antibodies raised specifically against wheat gluten protein (immunogen). In addition, blots were also probed with pooled sera of 7 individuals allergic to sesame seed as well as with anti-β-lactoglobulin antibody tested as negative controls. No binding was observed in any of the three

immunoblots of canary seed suggesting the absence of gluten specific proteins in the three canary seed samples.

To verify if binding would occur with other cereals and pseudo-cereals, the SDS-PAGE and blotting were performed on oat, millet, teff, quinoa, sorghum and buckwheat. Canada Western Red Spring (CWRS) wheat was used as the positive control. The SDS-PAGE results showed major differences in the electrophoretic profiles of the non-wheat cereals. This was expected as the cereals belong to different plant families. As was observed for the canary seeds groats, the pooled wheat sera recognized practically all the different polypeptide bands from the various non-wheat that were clearly visible in the SDS-PAGE profile as well as some that were not previously evident when bovine serum albumin was used as the blocking agent. Blocking with the non-fat dry milk instead of the bovine serum albumin revealed a different pattern with only a few bands recognized. The western blotting was repeated using rabbit polyclonal gluten antibodies with non-fat dry milk as blocking agent. The immunoblot revealed strong binding to many of the wheat proteins and some proteins in oat, millet, quinoa, teff, and to a lower extent with sorghum and buckwheat proteins, which could be either due to cross-reactivities or cross-contamination of the grains with gluten proteins.

To confirm the identity of the predominant protein components recognized by antibodies in the wheat sera, electrophoresis of wheat and non-wheat cereals and pseudo-cereals including glabrous canary seeds was conducted again and the bands showing antibody-antigen binding during immunoblotting were excised and further analyzed by LC/ESI-MS/MS. Because very few proteins from canary seed have been sequenced, none of the IgE-binding proteins from canary seed were identified as belonging to *P. canariensis*. The tryptic peptides identified from the IgE-binding proteins of canary seed did show some homology to sequenced proteins from rice, oats, barley, sorghum, and corn. The only protein with any homology to a wheat protein showed some homology to granule-bound starch synthase I. That protein is not a known wheat allergen (see Boye *et al*, 2013 manuscript for more detail and figures, Appendix 6).

The results obtained by Boye *et al.* (2013) cannot be reliably used to exclude the possibility of some cross-reactivity with canary seed among wheat-allergic individuals. However, the IgE binding observed with canary seed and other non-wheat grains under

some immuoblotting conditions could have been due to CCDs; this possibility was not evaluated by Boye *et al.* (2013).

The safety of glabrous canary seed from an allergy perspective was further assessed by analyzing for the presence of cross-reactivities using commercially available ELISA kits for major allergenic plant foods including gluten, soy, peanuts, tree nuts, sesame and mustard (Boye *et al.*, 2013). In general, analytical tests to determine the amount of the allergenic food residue that might be present in some other food are typically conducted using commercial Enzyme Linked Immuno Sorbent Assays (ELISA). With the exception of gluten, these ELISA kits detect source-specific proteins and are not specific for allergenic proteins from these foods.

All 18 glabrous canary seed composites (6 composite samples of brown canary seed (CDC Maria) and 12 composite samples of yellow canary seed (C05041 & C05091) from the Phase 2 study were tested as per the instructions of the ELISA kits.

Due to reported variability in ELISA results from different test kits, at least two to three commercial test kits from different companies were used for each targeted allergen (when available) and extractions were done in triplicate for each kits and each extract was analyzed in triplicate. As a measure of security, the proposed amounts indicated on the kit instruction were tripled in some instances and the extractions were repeated. When cross-contamination was suspected, samples were visually cleaned and the extractions were repeated. (For methodology details, see Appendix 6: Boye *et al*, 2013).

ELISA results of the canary seed groats for the different allergen kits tested are provided in Table 12-1. All the results were below the Limit of Detection (LOD) and Limit of Quantification (LOQ).

Table 12-1 ELISA results of glabrous brown and yellow canary seed[1]

Allergen	Company	Test kit	ELISA results		
			Glabrous Canary Seed		
			Brown	Yellow	
			CDC Maria	C05041	C05091
Almond	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Gluten	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Hazelnut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Mustard	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
	Sedium R&D	ELISA	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
Peanut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Sesame	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
Soy	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
Walnut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD

[1]Boye et al., 2013

LOD: Limit of detection; LOQ: Limit of quantification.

Overall, these results demonstrate that no proteins from almond, hazelnut, peanut, sesame, soy, walnut, mustard or gluten are present in the canary seed

samples. Furthermore, no protein epitopes capable of reacting with the polyclonal or monoclonal antibodies used in these ELISA kits are present in the canary seed samples. However, these results cannot be used to convincingly demonstrate that cross-reactivity would not occur between canary seed and these commonly allergenic foods as claimed by Boye *et al.* (2013). Evidence of cross-reactivity could only be determined by oral challenges or assays for mediator release from activated basophils. However, based upon the divergent genetic relationships between canary seeds and these other foods, with the exception of wheat gluten, the likelihood of cross-reactivity seems remote.

12.1.3. Gluten

Boye *et al.* (2013) evaluated the possible presence of gluten and gluten-related peptides and proteins using several different approaches. First, as noted above, ELISA kit assays capable of detecting gliadin, the alcohol-soluble fraction of the gluten complex (Mendez *et al.* 2005; Skerritt and Hill, 1991) were conducted on yellow and brown glabrous canary seed. As noted in Table 12-1, three gluten ELISAs were used. Two of these ELISAs use the R5 monoclonal antibody (Mendez et al., 2005) while the third uses the so-called Skerritt antisera (Skerritt and Hill, 1991). The R5 antibody is highly specific for the QQPFP and closely related epitopes found in gliadin. The R5 antibody reacts with prolamins from wheat, barley, rye and related grains but not with oats. The Skerritt antisera are polyclonal and recognize the omega-gliadin fraction of the gluten complex. The Skerritt antisera are highly reactive to wheat and rye prolamins but much less reactive to barley prolamins.

Details of the methodologies used, results obtained, additional tables and figures referred to in the following discussion can be found in Appendix 6 (Boye *et al*, 2013).

As noted in Table 12-1, Boye *et al.* (2013) found no evidence of protein epitopes from canary seed that were reactive with either the R5 or Skerritt antibodies. The absence of reactive proteins in both ELISAs suggests that pure canary seed would not elicit adverse reactions among celiac sufferers. However, the possible presence of reactive prolamin epitopes that would not be recognized by either of these two antibodies cannot be entirely excluded.

Consequently, further evidence of gluten-specific protein fragments was sought by mass spectrometry (MS). Mass spectrometry was used to identify any protein/peptide fragments with homology to known celiac-related gluten sequences of gluten-containing cereals (wheat, barley and rye) (Camafeita *et al.* 1997; Mendez *et al.* 2000). A number of proteins identified from the MASCOT database showed the three glabrous canary samples were mostly homologous with rice, oats, corn, carrot, tomato, radish, beet, and chickpea proteins. No celiac related gluten fragments from wheat, rye, barley or their derivatives were noted in any of the tested glabrous canary samples (Boye *et al*, 2013)

For the glabrous brown canary seed (CDC Maria) three hits were obtained indicating the likely presence of protein disulfide-isomerase (wheat), Em protein H5 (wheat) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) or proteins having similar homology. One hit suggesting the likely presence of cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) or a similar protein was found for CDC 5041. Protein disulfide-isomerase, with a molecular mass of 56,533 Da, is an enzyme in the endoplasmic reticulum in eukaryotes that catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold (Wilkinson and Gilbert, 2004). Em protein H5 (molecular mass, 10,060 Da) is a member of the small hydrophilic plant seed protein family. Cytosolic glyceraldehyde-3-phosphate dehydrogenase (molecular mass, 33,236 Da) belongs to the glyceraldehyde-3-phosphate dehydrogenase family. The amino acid sequences of these three proteins can be found in the reference Boye *et al.*, 2013.

Gluten epitopes provoking celiac disease typically originate from the gliadin and glutenin fractions and contain high amounts of glutamine and proline amino acid residues and the signature amino acid motif "QP" (Osman *et al.*, 2000 Qiao *et al.*, 2005). The amino acid sequences of the three canary seed protein hits (i.e., protein disulfide-isomerase (wheat), Em protein H5 (wheat) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) did not show any "QP" amino acid motif suggesting little likelihood of them containing a celiac provoking epitope. Overall, the mass spectrometry results of glabrous canary seed proteins suggest either cross contact or homology between canary seed proteins and some rice, oats, corn, carrot, tomato,

radish, beet, and chickpea proteins. Note that none of these three canary seed proteins with some homology to wheat were identified as likely triggers of celiac disease or as IgE-binding proteins using sera from wheat-allergic subjects. These findings suggest canary seed could be gluten-free.

13.0 MICROBIOLOGICAL CONSIDERATIONS

Cereal grains and flours are considered raw agricultural commodities, which undergo minimal processing prior to incorporation into a myriad of food products.

Cereals can contain between 10^2 to 10^9 CFU (colony forming units) of aerobic bacteria per gram, up to 10^6 yeasts and molds. *Salmonella* spp, *Bacillus* spp and *Escherichia* species may also be detected in low numbers (CIGI, 2006; ICMSF, 2005)

13.1 Mycotoxins

Mycotoxins are the most important of the microbial health hazards in cereals and cereal products. Cereal crops harbor many of the most important mycotoxins. The principal mycotoxigenic fungi associated with wheat, barley, and other small grain crops are *Fusarium* species, which produce a range of trichothecene toxins. The most important tricothecenes are deoxynivalenol (DON) and nivalenol (NIV), and the estrogenic toxin, zearalenone (ICMSF, 2005).

Canaryseed, similar to other common cereals and forage grasses, is susceptible to Fusarium Head Blight (FHB). The most common mycotoxin found in grain affected by FHB is deoxynivalenol (DON), also known as vomitoxin. In Saskatchewan, durum wheat, spring wheat and barley are most affected by this disease. The Canadian Grain Commission routinely analyzes grain shipments for *Fusarium* trichothecenes (DON). (Tittlemier et al., 2013). For many countries, the existing maximum limits for DON in cereal grains range from 1.0 to 2.0 mg/kg (ppm) (Tittlemier *et al.*, 2013)

Aflatoxins and vomitoxins (deoxynivalenol-DON) in glabrous canary seed (CDC Maria), pubescent canary seed (Keet) and CWRS wheat (Katepwa) in Phase 1 grown at ten locations in Saskatchewan were analyzed by the Grain Research Laboratory, Canadian Grain Commission (CGC) (Winnipeg, MB). The three crops were found to be free from vomitoxin (within the limit of ELISA technique which was 0.5 ppm). The canary seed and wheat grain were also found to have less than 5 ppb aflatoxin. The CGC issued certificates of analyses for vomitoxin and aflatoxin, which can be found in Appendix 8.

In Phase 2, the brown and yellow canary seed groats were analyzed for the presence of vomitoxin (DON), zearalenone, total fumonisins and Ochratoxin A. As shown in Table 13-1, vomitoxin at the limit of detection (LOD) of 0.1ppm and ochchratoxin A (LOD 0.96ppm) were not detected in any canary seed samples. This low level of ochratoxin is typical of many Canadian grains (<1ppb) and below the limit of other countries (3-50 ppb) (Canadian Grain Commission, 2013).

Total fumonisins with values greater than the 0.13 ppm limit of detection were detected in 8 yellow canary seed samples (0.14 ppm to 0.24 ppm) and in 2 brown canary seed samples (0.13 and 0.20ppm). Eight samples were below the detection level. These levels are below the guidance levels recommended by the US FDA for maize and maize products (2-4 ppm) (FDA, 2001).

Zearalenone was detected in 13 of the 18 glabrous canary seed samples ranging from 13.6 ppb to 40.3 ppb (ug/kg). Five (5) samples presented below the 10.5 ppb limit of detection. The levels detected were less than the maximum limit set by the European Union of 100 ug/kg for unprocessed cereals (EFSA, 2001).

Table 13-1 Mycotoxin levels in glabrous brown and yellow canary seed [1]

Mycotoxin	Limit of Detection	Glabrous Canary Seed	
		Brown	Yellow
		(range)	(range)
Fumonisins (total)	0.13ppm	< 0.13 to 0.20	<0.13 to 0.24
Ochratoxin A	0.96ppb	< 0.96	< 0.96
Vomitoxin	0.1 ppm	< 0.1	<0.1
Zearalenone	10.5 ppb	< 10.5 to 40.3	<10.5 to 33.8

[1] Phase 2 CDCS study

13.2 Microflora

Due to the excessive handling of the small plot samples, the 18 samples of glabrous canary seed used for nutritional and chemical analyses were not analyzed for their microbial profile. Instead, glabrous brown and yellow canary seed grown under field conditions and dehulled under commercial conditions were tested for aerobic plate count, yeasts and molds, and coliforms. The effect of processing on the microbial load was also evaluated.

Tables 13-2 and 13-3 represent the microbial counts of hulled brown and yellow canary seed, whole yellow and brown groats and yellow and brown whole grain flours subjected to various processing conditions-no processing, heat treated at 240°F for 8 minutes; roasted (without prior tempering) at 350°F for 8 minutes and roasted after tempering to 14% moisture at 350°F for 8 minutes.

Results indicate that the microbiological profile of hulled canary seed and canary seed groats falls within the microbiological counts for wheat and other small cereal grains (ICMS, 2005; CIGI, 2006). Raw canary seed, with or without hulls, had approximately 2×10^5 to 1×10^6 cfu/g (total plate count) and 600 to 1500 cfu/g yeasts and mold present on the samples tested. Coliforms (20-80 cfu/g) were detected in the raw flour samples, but not in the whole grain or any of the processed canary seed products. Heat treating at a low temperature (240°F) and roasting (350°F) reduced the microbial load by 2 and 4 to 5 logs respectively.

While cereal grains and their milled products contain bacteria, molds and yeasts due to contamination with soil, feces, insects and other contaminants, they have traditionally been considered low food safety risk commodities due to a low water activity and subsequent heat processing steps when incorporating grains into baked goods and other foods. However, recent food borne outbreaks implicating *Escherichia coli* in raw cookie doughs is changing the way industry views the safety of cereal grains and milled products. A number of control strategies (heat, ozone and irradiation) are being investigated to reduce the incidence of potential pathogens in wheat flours while maintaining the functional and nutritional qualities of grain and milled products (Rose *et al*, 2012). In the meantime, maintaining good agricultural practices and good manufacturing practices throughout the grain supply chain should maintain the microbial

integrity of any processed grain ingredient, including canary seed (Akins-Lewenthal, 2012).

Table 13-2 Microbial analysis of yellow canary seed groats and milled products subjected to different processing conditions*

Canary seed samples	Total Plate Count (CFU/G)[7]	Coliforms Count (CFU/G)	Yeast & Molds Count (CFU/G)
Yellow canary seeds *with hulls*, raw	2.6×10^5	ND[8]	650
Yellow canary seed *groats*, raw	2.4×10^5	ND	420
Whole yellow canary seed *flour*, raw	1.0×10^5	80	100
Yellow canary seed *groats*, without tempering, heat treated[1]	7000	ND	30
Yellow canary seed *groats*, tempered to 14% moisture, heat treated[1]	6200	ND	30
Yellow canary seed *flour*, without tempering, heat treated[1]	4200	ND	910
Yellow canary seed *flour*, tempered to 14% moisture, heat treated[1]	1300	ND	290
Yellow canary seed *flour*, without tempering, roasted[2]	300	ND	110
Yellow canary seed *flour*, tempered to 14% moisture, roasted[3]	100	ND	40

[1,4] Heat Treated = 240°F for 8 minutes ; [2,5] Roasted canary seed without tempering at 350°F for 8 minutes; [3,6] Roasted canary seed with tempered to 14% moisture at 350°F for 10 minutes; [7]CFU, colony forming units
[8]ND-not detected
*Phase 2 CDCS study, unpublished

Table 13-3 Microbial analysis of brown canary seed groats and milled products subjected to different processing conditions

Canary seed Samples	Total Plate Count (CFU/G)	Coliforms Count (CFU/G)	Yeast & Molds Count (CFU/G)
Brown canary seeds *with hulls*, raw	1.01×10^6	ND	800
Brown canary seed *groats*, raw	1.8×10^5	ND	1500
Whole brown canary seed *flour*, raw	1.0×10^5	20	1000
Brown canary seed *groats*, without tempering, heat treated[4]	1000	ND	10
Brown canary seed *groats*, tempered to 14% moisture, heat treated[4]	2000	ND	10
Brown canary seed *flour*, without tempering, heat treated[4]	600	ND	120
Brown canary seed *flour*, tempered to 14% moisture, heat treated[4]	1600	ND	20
Brown canary seed *flour*, without tempering, roasted[5]	110	ND	ND
Brown canary seed *flour*, tempered to 14% moisture, roasted[6]	200	ND	ND

[1,4] Heat Treated = 240°F for 8 minutes ; [2,5] Roasted canary seed without tempering at 350°F for 8 minutes; [3,6] Roasted canary seed with tempered to 14% moisture at 350°F for 10 minutes

*Phase 2 CDCS study, unpublished

14.0 DIETARY EXPOSURE ASSESSMENT

14.1 Potential Forms of Canary Seed Whole Grain

It is proposed glabrous brown and yellow coloured canary seed (*Phalaris canariensis*) be introduced to the US population as a cereal grain in whole groat and milled forms (e.g. flour or flakes) similar to how other cereal grains such as wheat, barley, oats, triticale, rye, buckwheat, ancient grains, millet, and sorghum and pseudo cereals such as buckwheat, amaranth and quinoa are offered. Whole canary seed groats may also be used to replace or complement the use of seeds in food products similar to the use of sesame seed, sunflowers seeds, poppy seed, pumpkin seed and flaxseed as a topping or ingredient in crackers, breads, rolls, buns, cereal/nutrition bars and snaps etc. Canary seed groats could also be used to replace sesame seeds (a food allergen) in some foods (i.e. sesame snaps) to provide alternatives to consumers.

As discussed in Section 5.0 *Manufacturing Methods* product development trials illustrated that canary seed groats or milled products (e.g. flours) at levels up to 25% in most product formulations could be used to replace and/or complement whole grains, refined grains or seed ingredients currently used in food products without greatly affecting functional or sensory characteristics. Levels up to 50% could be used in a standard sugar cookie recipe where canary seed flour could be the sole flour used.

14.2 Estimated Daily Intake of Canary Seed by the U.S. Population from Proposed Food-Uses

Intertek Cantox (Mississauga, ON, Canada) completed the assessment of the potential intake of canary seed by the United States (U.S.) population. The full report is provided in Appendix 9. Canary seed is proposed for use as a grain in the U.S. in baked goods and baking mixes, breakfast cereals, grain products and pastas, and snack foods. Based on product development trials, it is expected that canary seed will primarily be used in whole grain food products. However, in order to estimate the highest possible daily intake of canary seed, both whole grain and refined grain food products in each food category were included, and the highest use levels applied to all

products in that category. Thus, the resulting estimates are unrealistic, but represent a "worst-case" intake scenario, or highest possible intakes for canary seed.

Estimates for the intake of canary seed were based on the proposed food-uses and use-levels for canary seeds in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Canary seed is not intended for use in infant foods. Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of canary seed and the percentage of consumers was determined. Similar calculations were used to estimate the total intake of canary seed resulting from all proposed food-uses of canary seed combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Children, ages >2 to 11;

Female teenagers, ages 12 to 19;

Male teenagers, ages 12 to 19;

Female adults, ages 20 and up;

Male adults, ages 20 and up; and

Total population (all age and gender groups combined).

Intake estimates for infants, ages 0 to 2, were not included, as canary seed is not intended for use in infant foods.

14.2.1 FOOD CONSUMPTION SURVEY DATA

14.2.1.1 Survey Description

NHANES for the years 2009-2010 are available for public use. NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is recognized and used as a nationally representative sample of the U.S. population. It is well-established that the length of a dietary survey affects the estimated consumption of individual users and that

short-term surveys, such as a 1-day dietary survey, may overestimate consumption compared to surveys conducted over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2009-2010, 13,272 individuals were selected for the sample, 10,537 were interviewed (79.4%), and 10,253 were sampled (77.3%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2009-2010 data to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2011; USDA, 2012).

14.2.1.2 Statistical Methods

Statistical analysis and data management were conducted in Creme software (www.cremeglobal.com) (Creme, 2013). Creme Food 3.0 is a probabilistic modeling software tool that uses high-performance computing to allow accurate estimate of

exposure to contaminants, food additives, flavorings, nutrients, food packaging migratory compounds, novel foods, pesticide residues, and microbial contaminants. The main input components are concentration (use level) data and food consumption data. Data sets are combined using the Creme Food 3.0 model to provide accurate and efficient exposure assessments.

For the deterministic assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of canary seed by the U.S. population using Creme software. Estimates for the daily intake of canary seed represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of canary seed averaged over all individuals surveyed, regardless of whether they consumed food products potentially containing canary seed, and therefore includes individuals with "zero" intakes (*i.e.* those who reported no intake of food products potentially containing canary seed during the 2 survey days). All-user intake refers to the estimated intake of canary seed by those individuals who reported consuming food products containing canary seed, hence the "all-user" designation. Individuals were considered 'users' if they consumed 1 or more food products containing canary seed on either Day 1 or Day 2 of the survey.

Mean or percentile intake estimates based on small sample sizes may be less statistically reliable than estimates based on adequate sample sizes (LSRO, 1995). Therefore, for the estimated intakes of canary seed from proposed uses presented herein, values were considered statistically unreliable if the sample included less than 30 respondents. These values were not considered when assessing the relative contribution of specific food-uses to total canary seed consumption and are marked with an asterisk in Appendices A and B of the Intertek Cantox report (Appendix 9).

14.2.2 FOOD USAGE DATA

The individual proposed food-uses and use-levels for canary seed employed in the current intake analysis are summarized in Table 14-1. Canary seed can be added to food in several different forms including the dehulled milled grain, dehulled whole grain flour, or dehulled whole canary seeds. Canary seed is not intended for use in infant foods. The use-levels provided in Table 14-1 represent the total use of the canary seed in all forms within a given food-use in order to reflect the possible inclusion of multiple canary seed-based ingredients.

Food codes representative of each proposed food-use were chosen from the NHANES 2009-2010 (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2013). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C of the Intertek Cantox report (Appendix 9). A given food code may not be associated with both surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

Table 14-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Canary seed in the U.S. (2009-2010 NHANES Data)

Food Category	Proposed Food-Uses	Maximum Proposed Use Level (%)
Baked Goods and Baking Mixes	Bagels	25
	Biscuits	20
	Breads and Rolls	25
	Cakes	20
	Cookies	50
	Cornbread, Corn Muffins, and Tortillas	25
	Crackers	26
	Croissants and Pastries	25
	Doughnuts	25
	Flours and Brans (pre-packaged)	100
	Muffins	20
	Pancakes and Waffles	25
	Pies	10
Breakfast Cereals	Instant and Regular Hot Cereals	15
	Ready to Eat Breakfast Cereals	15
Grain Products and Pastas	Energy, Meal Replacement, and Fortified Bars	25
	Granola and Cereal Bars	25
	Macaroni and Noodle Products	15
	Pasta, Rice and Other Grains	15
Snack Foods	Savory Snacks	25
	Seed-based snacks	40

14.2.3 FOOD SURVEY RESULTS

Estimates for the total daily intakes of canary seed from proposed food-uses are provided in Tables 14.2 and 14.3. Estimates for the daily intake of canary seed from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-6 and B-1 to B-6 of Appendices A and B, respectively of the Intertek Cantox report (Appendix 9).

14.2.3.1 Estimated Daily Intake of Canary seed from All Proposed Food-Uses

Table 14.2 summarizes the estimated total intake of canary seed (g/person/day) from all proposed food-uses in the U.S. population group. Table 14.3 presents this data on a per kilogram body weight basis (g/kg body weight/day). The percentage of users was high among all age groups evaluated in the current intake assessment; greater than 98.7% of the individual population groups comprised users of those food products

in which canary seed is currently proposed for use. (Table 14.2). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

Consumption of proposed food-uses by the total U.S. population resulted in an estimated mean and 90th percentile all-user intakes of canary seed of 47 g/person/day (0.8 g/kg body weight/day) and 85 g/person/day (1.7 g/kg body weight/day), respectively. Within the individual population groups, male adults were determined to have the greatest estimated mean and 90th percentile all-user intakes of canary seed on an absolute basis, at 55 and 100 g/person/day, respectively (Table 14.2).

Table 14.2 Summary of the Estimated Daily Intake of Canary seed from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Children	>2 to 11	46	79	99.9	1,427	46	80
Female Teenagers	12 to 19	46	83	99.4	515	46	83
Male Teenagers	12 to 19	52	96	98.7	560	53	97
Female Adults	20 and up	41	75	99.2	2,627	42	75
Male Adults	20 and up	54	100	99.2	2,368	55	100
Total Population	All Ages	46	84	98.2	7,497	47	85

On a body weight basis, children were the population group identified as having the highest mean and 90th percentile all-user intakes at 1.8 and 3.2 g/kg body weight/day, respectively (Table 14.3). Female and male adults were identified as having the lowest mean all-user intakes of 0.6 g/kg body weight/day, for both population groups, and female adults were determined to have the lowest 95th percentile all-user intakes of 1.1 g/kg body weight/day.

Table 14.3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Canary seed from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/kg bw/day)		All-Users Consumption (g/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Children	>2 to 11	1.8	3.2	99.9	1,427	1.8	3.2
Female Teenagers	12 to 19	0.8	1.4	99.4	515	0.8	1.4
Male Teenagers	12 to 19	0.8	1.6	98.7	560	0.8	1.6
Female Adults	20 and up	0.6	1.1	99.2	2,627	0.6	1.1
Male Adults	20 and up	0.6	1.2	99.2	2,368	0.6	1.2
Total Population	All Ages	0.8	1.7	98.2	7,497	0.8	1.7

14.2.3.2 Estimated Daily Intake of Canary seed from Individual Proposed Food-Uses in the US

In terms of contribution to total mean intake of canary seed, breads and rolls and pasta, rice and other grains were the 2 main sources of intake across all population groups on both an absolute and on a g/kg body weight basis. Breads and rolls contributed 21.9% to total mean intakes or 12.7 to 24.3% among the individual population groups whereas pasta, rice and other grains contributed 20.9% to total mean intakes or 18.7 to 22.8% among the individual population groups. Energy, meal replacement, and fortified bars and seed-based snacks individually contributed ≤0.3% to total mean estimates for canary seed intakes across all population groups (see Tables A-1 to A-6 and/or B-1 to B-6 of the Intertek Cantox report (Appendix 9) for further details). It should be noted that there were no users identified in flours and brans (pre-packaged); thus, there was no intake of canary seed from this category. However, the food codes in this food category are only representative of flour and brans that would have been used by respondents in home baking. Any flours or brans based on canary seed included in prepared foods would have been captured in other food-use categories.

14.3 Summary of Total Daily Intakes

Consumption data and information pertaining to the individual proposed food-uses of canary seed were used to estimate the all-person and all-user intakes of canary seed for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean and 90th percentile intakes of canary seed by the total U.S. population from all proposed food-uses were determined to be 47 g/person/day (0.8 g/kg body weight/day) and 85 g/person/day (1.7 g/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of canary seed by the U.S. population from all proposed food-uses in the U.S., as observed in male adults were estimated to be 55 g/person/day (0.6 g/kg body weight/day) and 100 g/person/day (1.2 g/kg body weight/day), respectively.

15.0 SUMMARY AND CONCLUSIONS

The data and information contained in this report support the safety of annual canary seed (*Phalaris canariensis* L.) as a food cereal grain for human consumption. Glabrous canary seed groats are proposed for use as an ingredient in breads, flours, breakfast cereals, and pastas, as well as baked goods (e.g. biscuits, crackers, cookies, granola bars, nutrition bars, energy bars) and baking mixes (e.g. cakes).

Canary seed provides a source of protein, carbohydrate, essential fatty acids, dietary fiber, minerals and vitamins, as well as phytochemicals. The US Dietary Guidelines for Americans recommend 5-8 servings of grains per day, with at least half of these grains being whole grains. There is an opportunity for glabrous canary seed to be consumed as a whole grain/whole groat in the diet and contribute to dietary eating habits. Canary seed would ideally, as a new whole grain food introduction, be consumed with the other available whole grain diet choices.

The safety assessment process for novel foods, such as canary seed, differs from the conventional approach used in the assessment of an individual food chemical, which leads to the establishment of an Acceptable Daily Intake based on the identification of a no-effect level many times higher than anticipated human exposure (ILSI, 2002). For novel foods, it is recognized that it is not be possible or appropriate to feed a whole food at high levels in the diet, due to major alterations in the nutritional composition of the diet. Instead, the compositional, nutritional and toxicological characteristics and safety assessment of the novel food should be evaluated in the light of anticipated human exposure pattern in the context of normal expectations of food consumption (ILSI, 2003; Health Canada, 2006).

An ILSI expert panel (2003) on the safety assessment of novel foods concluded, "the evaluation should be based on knowledge of the characteristics of the novel food in question using comparisons with conventional foods where appropriate. Critical examination showing the estimated intake of the novel food to be below the level indicated as without toxic or nutritional hazard by the totality of the information available will allow a presumption of reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption."

Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains. *Phalaris canariensis* has a nutritional and compositional profile similar to other commonly consumed cereal grains being mainly comprised of protein (19-23%), starch (53-61%), fat (5.5-8%), dietary fiber (6-8%) and ash (1.9-2.4%). Similar to other cereals the proteins in canary seed are deficient in lysine but rich in cysteine, tryptophan, phenylalanine and arginine. Canary seed contains levels of trace minerals and B vitamins comparable to other cereal grains. As in other cereal grains and legumes, phenolic acids, phytate, trypsin inhibitors and amylase inhibitors are found in the grain. Phytate is present at about twice the level found in wheat, but at similar levels to other cereals, pulses and commonly consumed nuts and seeds. Growth and nutritional studies in swine and rodents confirmed the analytical results, demonstrating growth and food consumption rates comparable to other grains.

Levels of alkaloids, heavy metals, mycotoxins and microbial contamination in canary seed were similar or lower than reported in other cereal grains, and are not of toxicological concern. No evidence of allergenic potential of glabrous brown or yellow canary seed groats was identified from detailed assessments.

Feeding glabrous brown or yellow coloured canary seed groats to rats for 90 days in detailed toxicological studies resulted in no adverse toxicological findings that could be attributed to consumption of glabrous canary seed groats. In the first 90-day study, no adverse effects were observed in rats consuming diets containing 50% brown glabrous canary seed, resulting in NOAELs ranging from 33 to 37 g/kg/d for males and 38 to 42 g/kg/d for females. In the second 90-day study, the observed NOAEL of yellow and brown glabrous canary seed groats were at the highest doses tested, which ranged from 5.1 to 5.7 g/kg/d (Magnuson *et al.,* 2014).

Current consumption levels of whole grains and seeds by the US population, and optimistic projections for the replacement of currently-used grains and seeds with canary seed ingredients in various food products were used to calculate the highest likely consumption levels of canary seed. The average and 90th percentile dietary exposure calculations, using these conservative assumptions, were 0.8 and 1.7 g/kg/d

respectively, for the total population. Not surprisingly, the subgroup with the highest consumption based on body weight was children, with average and 90th percentiles estimated as 1.8 and 3.2 g/kg/d, respectively. The average intakes of grains for children aged 2 to 11 in 2003-2004 was reported to be 6.83 oz per day, or 193.6 g per day (Lin 2011). Based on this average, the daily intake of other grains by a 3 year old child (average weight 14 kg) would be approximately 13.7 g/kg/d.

Thus the highest anticipated exposure levels for canary seed, based on the proposed intended uses and use levels, are well below the levels shown to be safe by both animal safety studies and current levels of consumption of other cereal grains, which are compositionally very similar to canary seed. Safety studies, including both compositional and animal feeding studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake (Health Canada, 2006).

On the basis of the novel food safety assessment guidelines, it is clear that the estimated intakes of canary seed, even for the highest users, are below the level shown to have no adverse effects or nutritional hazards, based on the animal safety studies and nutritional composition comparisons.

The entirety of the available scientific data and studies summarized in this dossier support the conclusion that glabrous brown and yellow coloured canary seed groats and milled products are nutritious and safe to consume for the US population. While two colors of canary seed are available, there is no significant nutritional or safety related differences between canary seed of different colors. Glabrous canary seed groats and milled products would not be expected to cause adverse effects in humans under the conditions of intended use in foods.

Based upon the entirety of the available scientific data and summarized in this dossier, it is concluded that glabrous canary seed groats would be generally recognized as safe for consumption in their intended uses in food.

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APPENDICES: AVAILABLE UPON REQUEST

Submission End

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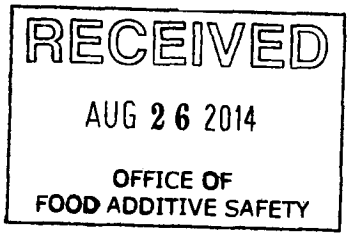

August 18, 2014

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
United States

Attn: Mr. R. Bonnette

Mr. Bonnette,

Please accept this revised version of the GRAS Dossier for file # GRN529. The "commercial confidential" statement in the header has been removed.

This package contains one original version and 2 copies of the following dossier.

1) Dossier: "Documentation supporting the Generally Recognized as Safe (GRAS) status of glabrous annual canary seed (*Phalaris canariensis* L) as a food cereal grain"

Respectfully,

(b) (6)

C.A. Patterson, PhD, PAg
On behalf of the Canaryseed Development Commission of Saskatchewan

Canaryseed
Development
Commission of
Saskatchewan

Documentation Supporting the Generally Recognized as Safe (GRAS) Status of Glabrous Annual Canary Seed (*Phalaris canariensis* L.) as a Food Cereal Grain

March 17, 2014

Prepared for:

Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street Saskatoon, SK S7H 5H9

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March 2014

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE STATUS OF GLABROUS ANNUAL CANARY SEED (PHALARIS CANARIENSIS L.) AS A FOOD CEREAL GRAIN

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Section 3.0 Background Information

Section 7.0 Intended Use

Section 8.0 History of Use

Section 9.0 Nutritional Considerations

Section 10.0 Chemical Considerations

Section 11.0 Toxicological Considerations

LIST OF FIGURES

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GLABROUS ANNUAL CANARY SEED (*PHALARIS CANARIENSIS* L.) AS A FOOD CEREAL GRAIN

EXECUTIVE SUMMARY

The Canaryseed Development Commission of Saskatchewan (CDCS), on behalf of producers of canary seed in Canada, plans to introduce glabrous (hairless) hull varieties of brown and yellow coloured canary seed (*Phalaris canariensis* L.) as a new cereal food grain to be used as an ingredient in food products in the United States.

Canary seed provides a source of protein, carbohydrate, essential fatty acids, dietary fiber, minerals and vitamins, as well as phytochemicals. The US Dietary Guidelines for Americans recommend 5-8 servings of grains per day, with at least half of these grains being whole grains. There is an opportunity for glabrous canary seed to be consumed as a whole grain in the diet and contribute to dietary eating habits. Canary seed would ideally, as a new whole grain food introduction, be consumed with the other available whole grain diet choices.

The purpose of this dossier is to outline information respecting the development of glabrous canaryseed, details of potential manufacturing and processing methods, its intended use and directions for preparation, evidence of traditional use, data to establish glabrous canaryseed is safe for human consumption and estimations of its level of consumption by consumers.

Glabrous canary seed can be considered a novel food crop as its history as a human cereal grain has not been well documented. Glabrous canary seed has been produced by selective breeding techniques.

A major obstacle in developing annual canary seed as a food grain for human consumption was the presence of small silicified hairs (trichomes) or spicules covering the hull surface of commercial cultivars. Due to the increasing importance of canary seed production in Western Canada, a mutation breeding program was initiated at the University of Saskatchewan, Canada, in the 1990s to eliminate hull pubescence (hairy) in canary seed. The objectives in developing glabrous, annual canary seed cultivars were three fold:

a) To reduce the skin irritation encountered by farmers during the harvest process,
b) To eliminate any potential health concerns associated with the *Phalaris* silica trichomes due to their irritative properties (Rabovsky, 1995),
c) To develop cultivars suitable for human consumption (glabrous and yellow coloured grain).

The data and information contained in this report support the safety of consumption of annual canary seed (*Phalaris canariensis* L.) as a human food cereal grain. Glabrous canary seed groats (*i.e.* hull-free grain) are proposed for use as an ingredient in breads, flours, breakfast cereals, and pastas, as well as baked goods (e.g. biscuits, crackers, cookies, granola bars, nutrition bars, energy bars) and baking mixes (e.g. cakes).

Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains. *Phalaris canariensis* has a nutritional and compositional profile similar to other commonly consumed cereal grains being mainly comprised of protein (19-23%), starch (53-61%), fat (5.5-8%), dietary fiber (6-10%) and ash (1.9-2.4%). Similar to other cereals, the proteins in canary seed are deficient in lysine but rich in cysteine, tryptophan, phenylalanine and arginine. Canary seed contains levels of trace minerals and B vitamins comparable to other cereal grains. As in other cereal grains and legumes, phenolic acids, phytate, trypsin inhibitors and amylase inhibitors are found in the grain. Phytate is present at about twice the level found in Western Red Spring wheat, but at similar levels to other cereals, pulses and commonly consumed nuts and seeds. Growth and nutritional studies in swine and rodents confirmed the analytical results, demonstrating growth and food consumption rates comparable to other grains.

Levels of alkaloids, heavy metals, mycotoxins and microbial contamination in canary seed were similar or lower than reported in other cereal grains, and are not of toxicological concern. No evidence of allergenic potential of glabrous brown or yellow canary seed groats was identified from detailed assessments. Feeding glabrous brown or yellow coloured canary seed groats to rats for 90 days in detailed toxicological studies resulted in no adverse toxicological findings that could be attributed to

consumption of glabrous canary seed groats. In the pivotal 90-day study, no adverse effects were observed with the highest doses tested of yellow and brown glabrous canary seed groats, which ranged from 5.1 to 5.7 g/kg/d.

Estimates for the intake of canary seed were based on the proposed food-uses and use-levels for canary seeds in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Optimistic projections for the replacement of currently-used grains and seeds with canary seed products in various food products were used to calculate the highest likely consumption levels of canary seed. Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of canary seed and the percentage of consumers were determined. On an all-user basis, the mean and 90th percentile intakes of canary seed by the total U.S. population from all proposed food-uses were determined to be 0.8 g/kg body weight/day and 1.7 g/kg body weight/day, respectively. Thus the anticipated exposure levels for canary seed, based on the proposed intended uses and use levels, are far below the observed NOAEL of 5.1 to 5.7 g/kg/d in the 90-day rat study.

The entirety of the available scientific data and studies summarized in this dossier support the conclusion that glabrous brown and yellow coloured canary seed groats and milled products are nutritious and safe to consume for the American population. While two colors of canary seed are available, there is no significant nutritional or safety related differences between canary seed of different colors. Glabrous canary seed groats and milled products would not be expected to cause adverse effects in humans under the conditions of intended use in foods.

Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 (Jones & Engelson, 2010) similar to other food cereal grains and pseudocereals consumed by humans.

Based upon the entirety of the available scientific data and summarized in this dossier, it is concluded that glabrous canary seed groats are safe for consumption in its intended use in food.

1.0 Common Name

Annual canary seed (*Phalaris canariensis* L) is commonly known as canary seed or annual canarygrass in North America and "alpiste" in European and South American countries. Dehulled glabrous brown and yellow coloured canary seed grain (also known as groats) and its milled products will be sold as food ingredients.

In the US and Canada, the common name for annual canary seed will be "canary seed".

2.0 Principal Place of Business

Canaryseed Development Commission of Saskatchewan
Bay 6A-3602 Taylor Street Saskatoon, SK Canada S7H 5H9
Executive Director: Kevin Hursh

Description of the Novel Food

3.0 Background Information

The Canaryseed Development Commission of Saskatchewan (CDCS), on behalf of producers of canary seed in Canada, wishes to introduce glabrous (hairless) hull varieties of brown and yellow coloured canary seed (*Phalaris canariensis* L.) as a new cereal food grain to be used as an ingredient in food products in the US.

Glabrous canary seed can be considered a novel food crop as its history of use in human foods has not been well documented and has been developed by selective breeding techniques. Canary seed was recognized by the American Association of Cereal Chemists International (AACCI) as a whole grain in 2006 (Jones & Engelson, 2010) similar to other food cereal grains and pseudocereals consumed by humans. Glabrous canary seed cultivars have the potential to be used as a whole groat (dehulled cereal grain) or as milled grain products in food products similar to the use of other cereal grains.

The gathering of information for the safety assessment of glabrous canary seed has proceeded in two discrete timeframes in the past fifteen years. The initial project

(Phase 1) (1992-2002) involved the development of glabrous canary seed and the identification of both brown and yellow coloured groats amongst the glabrous varieties. In Phase 1, the nutritional and chemical characteristics of glabrous, brown coloured canary seed groats (*P. canariensis*, CDC Maria) were compared to its pubescent (hairy) parent *P. canariensis,* cultivar "Keet" (also a brown coloured groat) and to a Western Red Spring (CHRS) common hard wheat (*Triticum aestivum* subsp. Vulagare[Vill. Host] Mackey), cultivar "Katepwa". The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals, as well as a 90-day rodent trial and two poultry feeding trials.

With the establishment of the Canaryseed Development Commission of Saskatchewan in 2006, the collection of levy funds and the securing of additional funding, the novel food project for glabrous canary seed was once again initiated in 2008. This second project (called Phase 2, 2008-2014) involved a comprehensive comparison of two glabrous yellow coloured cultivars (designated C05041 and C05091) to the glabrous brown coloured cultivar CDC Maria, which had been studied in the Phase 1 project. Nutritional, chemical, additional rodent feeding toxicology studies, and allergenicity studies were conducted. Comprehensive searches of the literature were conducted by C.A. Patterson and B. Magnuson from the initiation of the project through February 2014 for the preparation of the dossier and summation of all available information related to the safety of the consumption of canary seed. Other data were provided by the CDCS.

The purpose of this dossier is to outline information respecting the development of glabrous canaryseed, details of potential manufacturing and processing methods, its intended use and directions for preparation, its history of use, data to establish glabrous canaryseed is safe for human consumption and estimations of its level of consumption by consumers.

3.1 Current production and use of *P. canariensis*

Annual canary seed (*Phalaris canariensis* L), also known as annual canarygrass, is the only annual species of the genus *Phalaris* that has gained commercial importance as a specialty grain crop. Argentina, Morocco and Australia have been the traditional

world producers of annual canary seed as a source of birdfeed but Canada is now the world's largest producer and exporter of annual canary seed with Saskatchewan accounting for about 69% of the tonnage (ca. 125,000 tonnes) of the world canary seed exports in 2011.

Canary seed is primarily used in the birdfeed market as it is a major component in feed mixtures for pet and wild birds. However, Canadian producers are investigating other market opportunities for the glabrous canary seed to mitigate the risk of selling into one market.

Six annual canary seed cultivars are currently registered in Canada–Keet, Elias and Cantate have pubescent (hairy) hulls and CDC Maria, CDC Togo, and CDC Bastia have glabrous (hairless) hulls. All have brown coloured grain kernels. The glabrous cultivars were developed by the University of Saskatchewan in the 1990s. The Food Production and Inspection Branch, Seed Division, Variety Registration Office, Agriculture and Agri-Food Canada issued registration NO 4607 to CDC Maria on 12 June 1997, registration NO 5834 to CDC Togo on 10 June 2004 and registration NO 6259 to CDC Bastia on April 13, 2007.This is not intended to be an exhaustive list of food grade canary seed as addressed by this GRAS determination. Development of new glabrous cultivars is an ongoing process and new cultivars are appearing in Canadian production (Hucl, 2013).

3.2 Projected Uses

The introduction of glabrous canary seed into the human food market will require significant effort from the CDCS and a commercial champion to introduce this specialty crop to the food industry and gain acceptance by consumers. Thus, projecting a realistic dietary exposure to glabrous canary seed is based upon the following factors which will influence its market penetration:

1. Canary seed production volumes: In the last 3 crop years (2009, 2010, 2011) approximately 30-50% of the canary seed produced in Canada was of the glabrous hull brown seeded variety, an average of 74,000 tonnes of glabrous canary seed being grown each year. All of the current pubescent and glabrous canaryseed production goes to the birdfeed market. However, glabrous brown

canary seed could enter the human food market as soon as regulatory approval is gained.

2. Production of glabrous .yellow coloured canary seed: Yellow canary seed varieties are not yet in commercial production, nor registered as a new canary seed variety. Thus it will be at least 1 to 2 years beyond regulatory approval before sufficient glabrous yellow coloured canary seed is available for commercial use as a food ingredient.

3.3 Definitions used in this Dossier

To aid the reader, the following explanations of terminology used in this dossier and accompanying reference literature are provided in Table 3-1.

Table 3-1 Terminology and definitions used in dossier and literature[1,2]

Term	Description	Also known as	In Dossier
Phalaris canariensis	Annual canarygrass	Canary seed Canarygrass Alpiste	Canary seed Annual canarygrass
Glumes	External covering of a cereal grain containing the lemma and palea. Glumes retained after harvesting	Husk Hull Covered grain	Hull or Hulled
Caryopsis	Parts of the cereal grain comprised of pericarp (bran) , endosperm and germ	Grain, Seed Kernel	Grain
Pubescent	Glume (lemma and palea) are covered with silicified trichomes (hairs)	Hairy	Pubescent Hairy
Glabrous	No silicified trichomes (hairs) on the glumes or palea	Hairless	Glabrous Hairless
Dehulling	The process of removing the glumes (outside covering or hull) of the cereal	Dehulling	Dehulling
Dehulled canary seed	Removal of the glumes of canary seed	Grain, kernel, groat	Groat
Whole grain	Whole grains or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same rich balance of nutrients that are found in the original grain seed.		Whole grain canary seed
Conditioning	Water addition under specific conditions to optimize grain for further processing (e.g. grinding and milling)	Tempering	Tempering
Milling	Grain is mechanically processed under controlled conditions of breaking, reduction and separation resulting in separation of various grain components	Milling	Milled fractions to make whole grain flours, flakes, refined flours, brans etc

[1] Serna-Saldivar, 2012; [2] Jones & Engleson, 2010

4.0 CANARY SEED DEVELOPMENT INFORMATION

4.1 History of Organism

Note: The following information has been extracted from the publications by Putnam et al, (1996) and Abdel-Aal and Hucl (2005), which provide a comprehensive description of the history, genetics and breeding, agronomic characteristics, composition and physical properties and processing and utilization of pubescent (hairy) annual canary seed. Glabrous varieties were not commercially available until 1998.

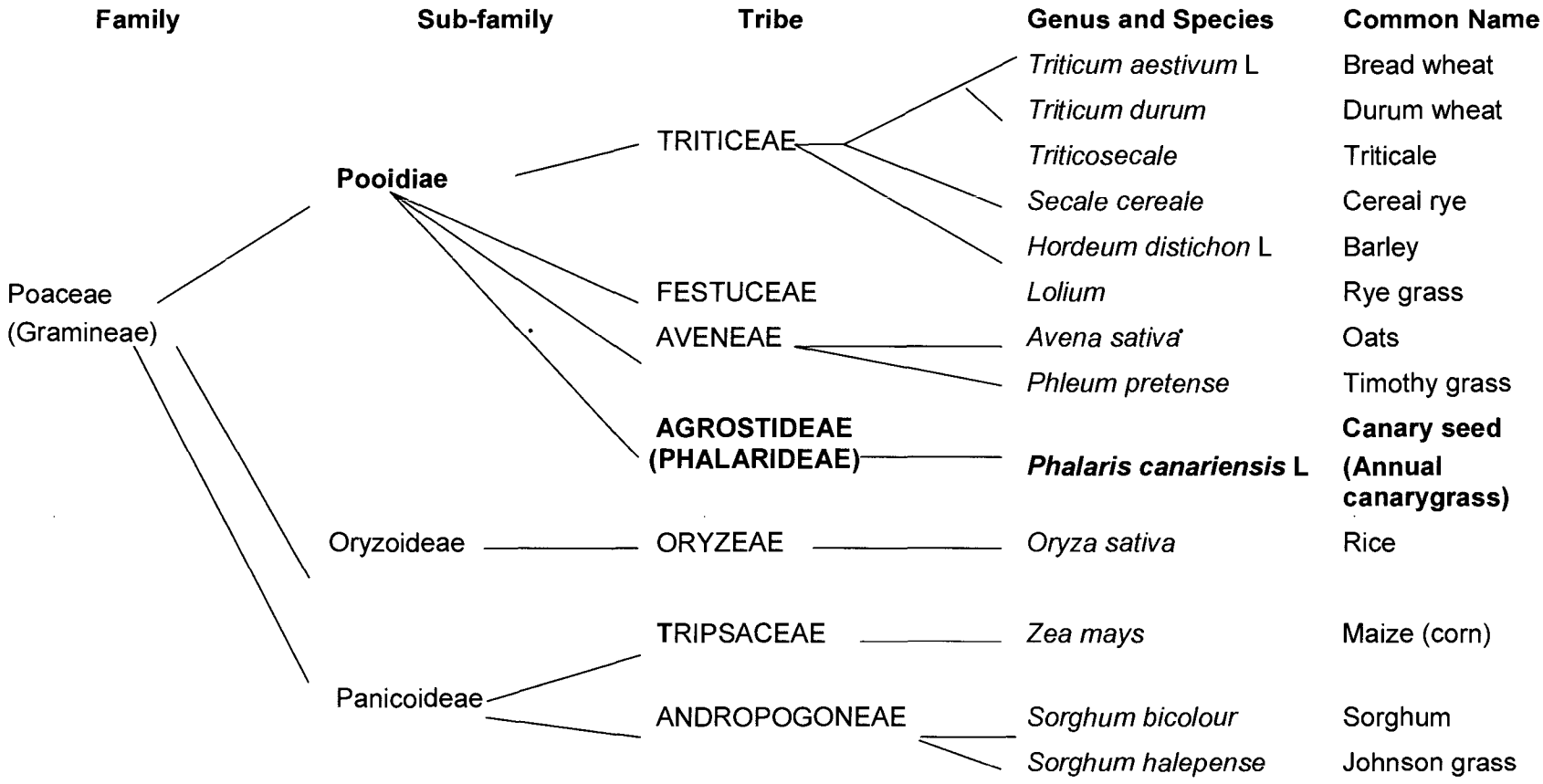
Note: Both "annual canary seed" and "annual canarygrass" are used in many publications referring to Phalaris canariensis.

Annual canarygrass (*Phalaris canariensis*) is a crop belonging to the Poacea (Gramineae) family, Pooidiea subfamily and tribe Agrostideae. This places annual canarygrass in the same subfamily but different tribe as wheat (*Triticum aestivum* L), barley (*Hordeum vulgare* L) and rye (*Secale cereale* L) (all belonging to the Triticea tribe) or oat (Aveneae tribe). Thus, annual canarygrass is somewhat genetically related but completely reproductively isolated from these common cereal crops (Figure 4-1).

Annual canarygrass is of Mediterranean origin. Weedy species of *Phalaris* (e.g., *P. minor*) are found around the Mediterranean basin and farther east. The *P. minor* species (littleseed canarygrass) is a problem weed in wheat fields in Pakistan and India and in Mediterranean climates, including California. Littleseed canarygrass biotypes have developed resistance to a number of herbicides making this species a more problematic weed. Short-spiked canarygrass (*P. brachystachys*) is another problem weed in cereal crops in the Mediterranean basin. Paradoxagrass (*P. paradoxa*) is a major weed in winter wheat production in Australia.

Canarygrass was first domesticated in the Mediterranean region. However, no evidence currently exists to indicate specifically where this domestication took place. A number of seventeenth- and eighteenth century references allude to canary seed or to a morphologically similar species originating in the Canary Islands, in Spain, or in both areas, and being used to feed birds. Canarygrass was assumed to originate in the Canary Islands but it is not clear whether the crop is named after the islands or after the birds (*Serinus canarius*) that originated there. In any case, the grain was fed to canaries and the spread of the two outward from Spain to countries such as Belgium was linked.

Figure 4-1 Relationship between common cereals and grasses and *Phalaris canariensis



*Adapted from Baldo *et al*, 1980; Jones *et al*, 1995

A mid-1700 dictionary indicates that *alpiste* is a Basque word suggesting annual canarygrass has a long history on the Iberian Peninsula.

Annual canarygrass is sometimes confused with reed canarygrass (*Phalaris arundinacea*), which is a commonly grown perennial forage grass and weed species. Although heads of both plants are panicles, annual canarygrass heads are spike-like and resemble club wheat. The seed of annual canarygrass is larger than reed canarygrass but smaller than wheat (Figure 4-2). The genus also includes Littleseed canary seed (*Phalaris minor* Retz.), a weedy grass also originating in the Mediterranean and which can be found in barley, wheat and seedling alfalfa fields or as a weed on marginal lands, particularly in the western United States. Of the annual species of this genus, *P. canariensis* is the only one that is grown as a grain crop, fitting best as a wheat replacement in a crop rotation.

Although the genus *Phalaris* traces its origins to the Mediterranean basin, the 15 species that make up the genus can be found over a wide range of latitudes. Annual canarygrass is grown in many areas of the world including Argentina, Australia, Netherlands, Hungary, North Africa, the Middle East, the United States and Canada. North American production is primarily in Saskatchewan, Manitoba and Alberta with small acreage in the Red River Valley of North Dakota and Minnesota.

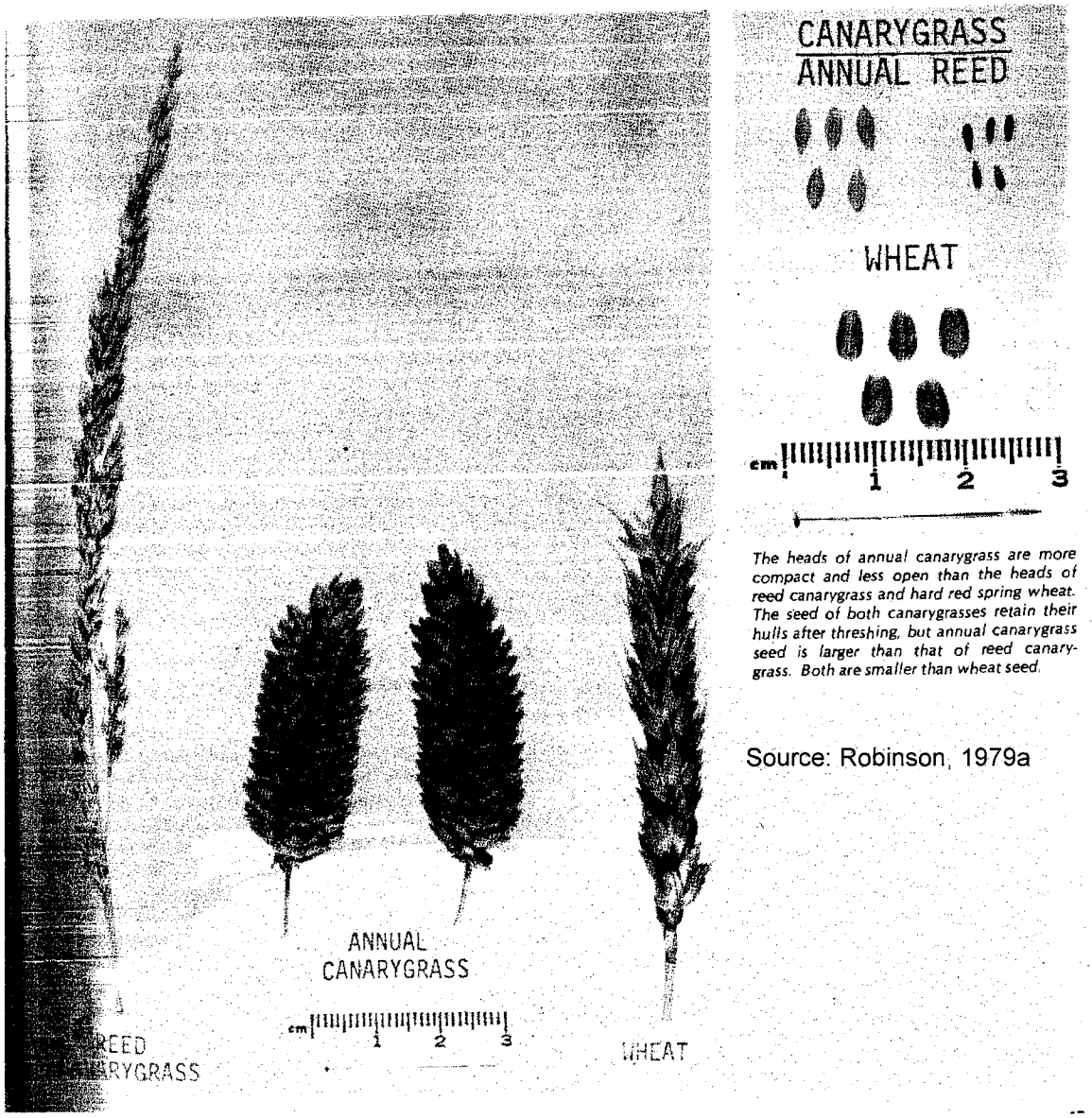
Annual canarygrass is a diploid with ($2n$ = 12), whereas most other *Phalaris* species (annual and perennial) have a basic chromosome number of x = 7. The only other species with $2n$ = 12 are the weedy annual *P. brachystachys* and the perennial *P. truncata* (Anderson, 1961). Based on isozyme and morphological analyses, *P. canariensis* and *P. brachystachys* are closely related (Matus & Hucl, 1999; Matus-Cádiz & Hucl, 2002). Taking into account the chromosome number homology between the two species, one can infer that *P. brachystachys* is probably the ancestral species from which annual canarygrass is derived.

The growth and development of annual canarygrass is quite similar to that of wheat (*Triticum aestivum* L) or oat (*Avena sativa*). It can be grown as either a spring-sown crop in regions with severe winter climates or as a winter-sown crop in Mediterranean climates. Generally, annual canarygrass required about 100-110 days to reach maturity, and is considered a cool-season crop preferring cool, moist conditions.

Even though it is less tolerant of heat and drought than hard red spring wheat, it has been grown successfully for several decades in semi-arid western Saskatchewan, one of the driest regions in Canada. It is frost tolerant and more tolerant of salinity and excess soil moisture than is wheat. Annual canarygrass is best adapted to heavy, moisture retentive soils due to its shallow rooting habit.

Canary seed produces small, elliptical grains with lengths and widths of approximately 4.0-5.1 and 1.5-2.0 mm, respectively (Abdel-Aal et al., 1997). The glabrous grain weighs approximately 7 mg, with an average test weight of 70 kg/hL (Hucl, 2009).

Figure 4-2 Comparison of the panicles and seed size of *P. canariensis*, *P. arundinacea* and hard red spring wheat



The heads of annual canarygrass are more compact and less open than the heads of reed canarygrass and hard red spring wheat. The seed of both canarygrasses retain their hulls after threshing, but annual canarygrass seed is larger than that of reed canarygrass. Both are smaller than wheat seed.

Source: Robinson, 1979a

Reed Canarygrass | Annual Canarygrass | Wheat

4.2 Description of the Genetic Modification

4.2.1 Purpose of the Genetic Modification

Investigations in the 1970s first identified annual canary seed as a potential food grain crop (Robinson, 1978; 1979a,b). However, the presence of small silicified hairs (trichomes) or spicules covering the hull surface of commercial cultivars potentially prevented the use of canary seed as a food grain for human consumption..

Due to the increasing importance of canary seed production in Western Canada, a mutation breeding program was initiated at the University of Saskatchewan in the 1990s to eliminate hull pubescence (hairiness) and brown seed colour in canary seed. The rationale for this project was that exposure to trichomes from different *Phalaris* grass varieties had been proposed as a contributing factor to the high incidence of esophogeal cancer in certain geographical locations (O'Neill *et al.*, 1980). However, a mouse study found no evidence of damage due to consumption of trichomes from *Phalaris canariensis*, although dermal exposure promoted skin cancer in mice exposed to an initiating carcinogen (Bhatt *et al.*, 1984). The relationship between biogenic amorphous silicas in the trichomes and adverse health effects is not clear (Rabovsky, 1995). Thus, the absence of trichomes on glabrous canary seed eliminates concern associated with potential adverse health effects due to exposure. The selection for yellow coloured grain was to improve consumer appeal and acceptability of food products containing canary seed.

The objectives in developing glabrous, annual canary seed cultivars were three fold:

a) To reduce the skin irritation encountered by farmers during the harvest process,
b) To eliminate any potential health concerns associated with the *Phalaris* trichomes,
c) To develop cultivars suitable for human consumption (glabrous and yellow seed).

4.2.2 Pedigree and Breeding Method for the Glabrous Trait

Approximately 625,000 seeds of certified *P. canariensis* Keet (pubescent hull) were subjected to a 2-hour pretreatment soak in water prior to treatment with 1mM sodium azide for 12 hours (Faue *et al*, 1989). Seeds were subsequently flushed with

water and allowed to dry. (Note: Sodium azide is a commonly used agent for grain mutagenesis (Castillo *et al.*, 2001)).

Figure 4-3 provides a schematic of the breeding method for the glabrous and yellow seeded traits. The mutant (M) 1 and M2 populations were grown under field conditions and advanced as bulk samples. Ten kilograms of seed were harvested from the M1 plot. In the M2 and M3, a population size of approximately 80,000 plants in each generation was maintained.

Approximately 15,000 panicles were harvested from the M3 population growing under field conditions. Using a dissecting microscope, a single M3 glabrous panicle, possessing glabrous glumes and hulls, was identified from the M3 population. Ten M4 glabrous plants and their M5 progeny were grown in the greenhouse.

CDC Maria traces its origins to a single putative M4 seed. CDC Maria was selected based on agronomic field evaluation beginning in the M6 (Hucl *et al.*, 2001)

4.2.3 Performance

Since a registration test for annual canary seed did not exist, CDC Maria was evaluated during the years 1992-1996 in the University of Saskatchewan spring cereal testing system and Regional Variety Testing (RVT) system. Yield trials consisted of randomized complete block designs with three replications (Hucl, 2009).

CDC Maria is adapted to the traditional canary seed-growing region of Saskatchewan, the Brown, Dark Brown and Black soil zones.

4.2.4 Yellow Seeded Trait

The mutant populations of the above treated pubescent Keet seeds were also screened for the glabrous yellow seeded phenotype. Yellow-seeded line CY184 was selected from the same sodium azide-treated bulk population of Keet seed as was CDC Maria. CY184 was identified by de-hulling 3 million M4 seeds and subsequently sorting the dehulled seed using a color-sorter (Figure 4-3).

The CY184 breeding line is a pubescent, yellow-seeded line tracing its origin to a single putative M4 seed that breeds true in subsequent generations.

A CDC Maria - CY184 cross yielded brown, glabrous CC9007 (registered as CDC Bastia) and its sister line, glabrous yellow CC9005.

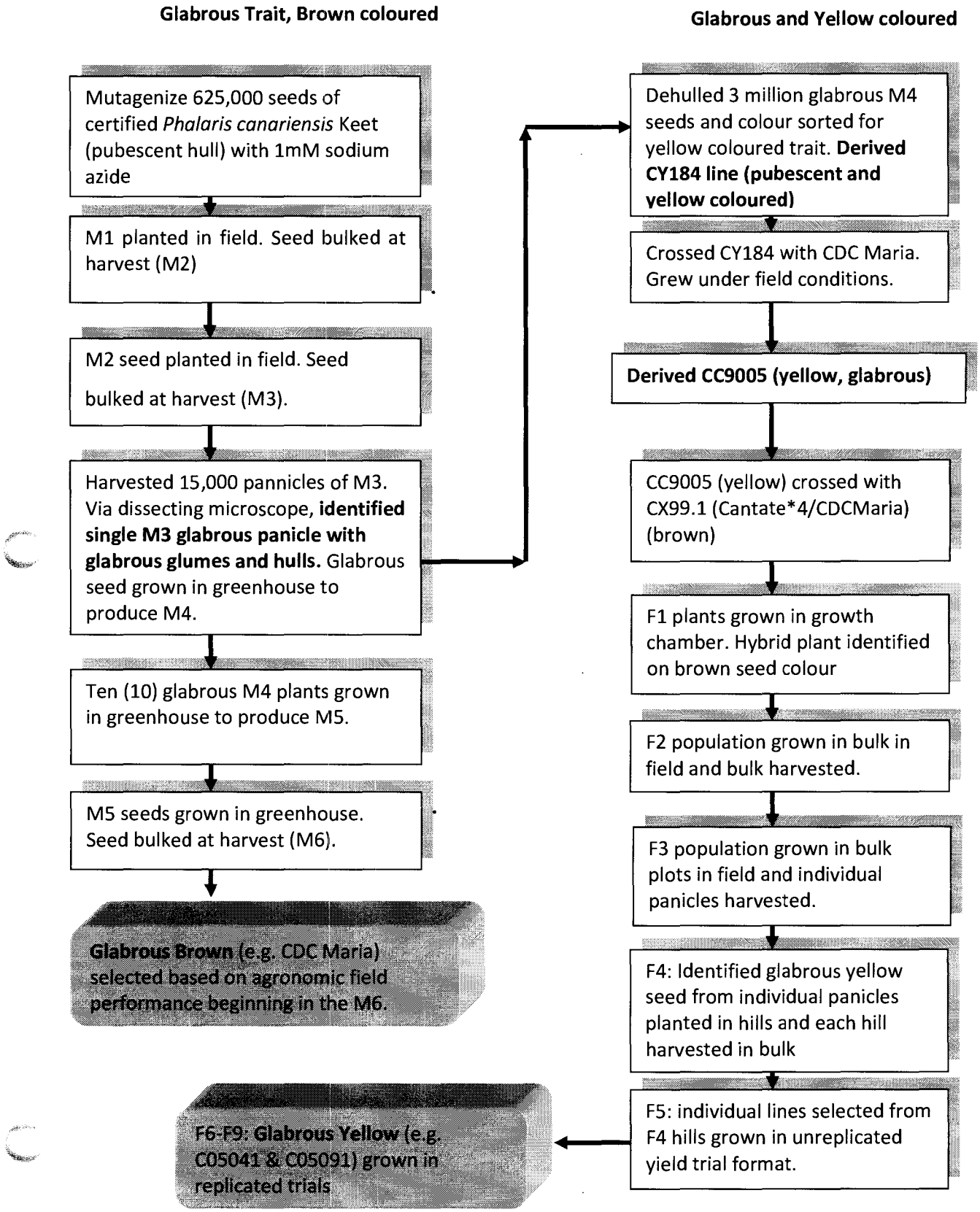
The yellow seeded hairless varieties used for this novel food petition were derived as follows (Figure 4-3). In 2000, CC9005 was crossed with CX99.1 (Cantate*4/CDC Maria) using the approach method in a field crossing nursery. The cross CX99.1 represented the third backcross of CDC Maria to Cantate in which the glabrous trait was selected. Cantate is a pubescent hull cultivar, registered in Canada.

Putative F1 plants were grown in a growth chamber and hybrid plants identified on the basis of brown seed colour. The F2 population was grown in a bulk plot in the field and bulk harvested. F2 families derived from each F1 plant were screened for segregation of hull pubescence and seed colour. The F3 population was grown in bulk plots in the field and individual panicles were harvested. Yellow seed from individual panicles were planted in hills and each hill harvested in bulk. Individual lines from the F4 hills were grown in an unreplicated yield trial format (F5). F6 to F9 generations of C05041 and C05091 were grown in replicated trials at five to six sites in Saskatchewan in the years 2006 to 2012.

The two glabrous, yellow seeded lines (C05041 & C05091) used for this novel food petition have the pedigree of CC9005//Cantate*4//CDC Maria.

The glabrous trait in canary seed is controlled by a single gene (Matus-Cadiz *et al.*, 2003) with the glabrous phenotype being recessive to the pubescent condition. The yellow seed colour is also recessive to the wild-type brown colour.

Figure 4-3 Breeding Program for Glabrous and Yellow Seeded Trait in *Phalaris canariensis*



5.0 METHOD OF MANUFACTURE

Annual canary seed will be processed using common cereal processing methods, the first two steps being harvesting and milling.

Annual canary seed is harvested after complete maturity is reached. Direct harvesting is used as canary seed is resistant to shattering. Once harvested, canary seed is stored in bins due to its low angle of repose (it flows quite easily) and to prevent rodent infestation. Canary seed is safe for storage at 12% seed moisture.

To avoid cross contamination of glabrous cultivars with pubescent cultivars, producers follow the quality management systems designed by the Canadian Seed Growers Association (CSGA) to ensure quality, identify preservation and traceability. Producers already provide documentation showing the canary seed variety. Documentation identifying varietal purity and guaranteeing a glabrous seed source will be critical to the quality chain.

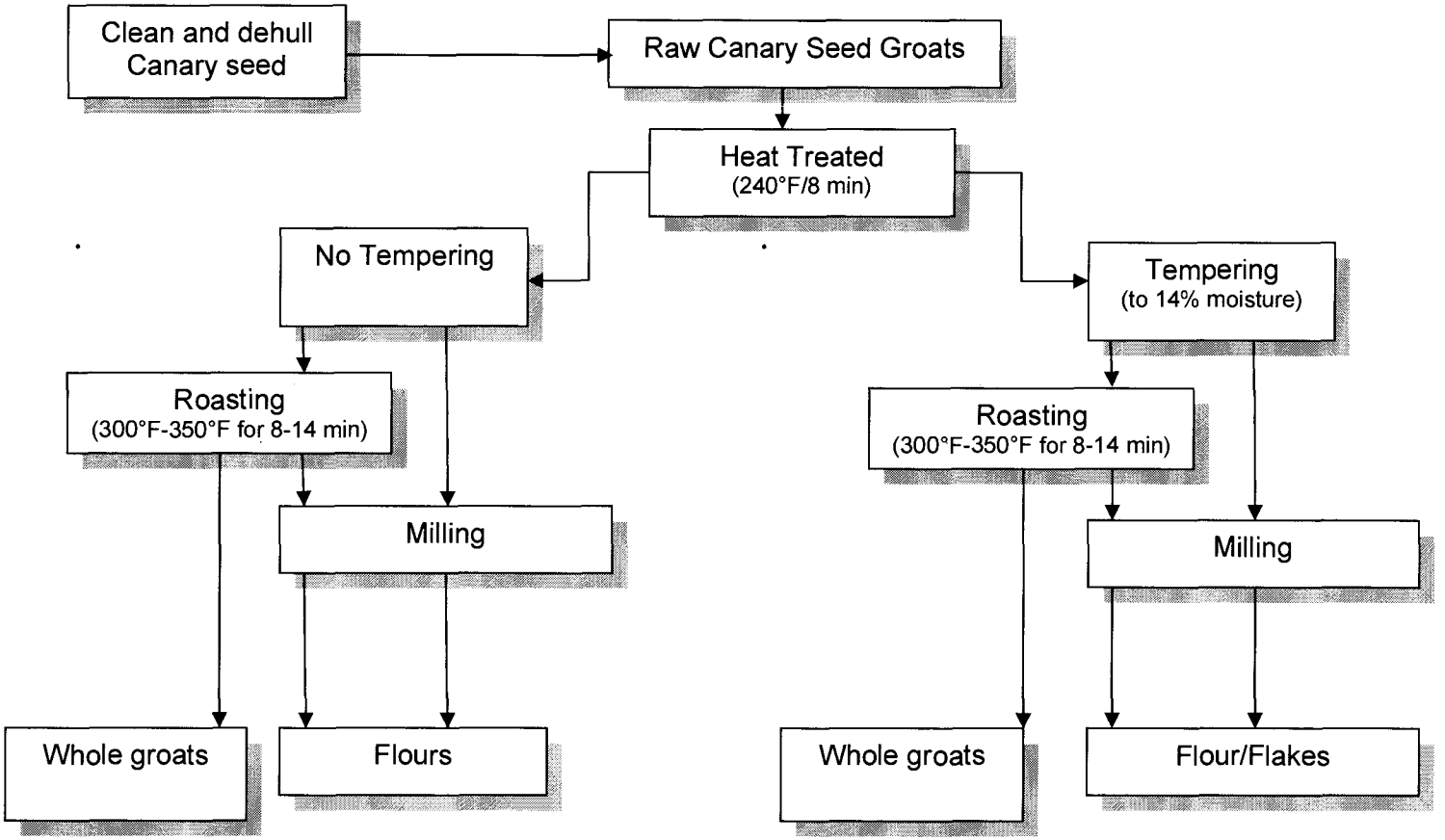
Canary seed processing involves the removal of debris and extraneous material from the harvested crop, removal of hulls, optional tempering of the groat to adjust moisture levels, and grinding and milling of the groats into whole meal flour, milled products or other forms (e.g. flakes). Canary seed groat products will then be sold as food ingredients.

Harvested glabrous canary seed destined as a food ingredient will be cleaned twice prior to dehulling. Dehulling is achieved via cone dehullers or plate dehullers that remove the glumes from the kernels via forced air and screen separation. Canary seed can be dehulled to >99% purity. Once dehulled the canary seed groats are then packaged into 50lb plastic or paper bags that are labeled, palletized and shrink-wrapped. Packaged dehulled canary seed is stored in forced air ventilated rodent-proof 40 foot containers until needed for shipment.

Currently there is no commercial manufacture of canary seed as a food ingredient or its incorporation into manufactured foods in Canada. There are a few canary seed producers/processors with the ability to dehull glabrous canary seed but they are awaiting novel food approval before targeting this niche market.

Processing methods and food products outlined in this submission are based on prototype products developed by the University of Saskatchewan and various Food Technology Centres in Canada. To facilitate processing, glabrous whole canary seed groats can be tempered to 14 % moisture. To enhance sensory properties and prolong shelf life, it can be roasted at 300°F to 350°F for 8-14 minutes and milled to produce whole grain flours or flakes and bran and white flour fractions (Abdel-Aal *et al*, 2010) that can be used directly in standard baking formulations (Figure 5-1). With increasing consumer interest in whole grain flours, the primary focus of product development has been on products containing roasted or unroasted whole groats or milled whole grain canary seed products.

Figure 5-1: Prototype processing methods for glabrous canary seed ingredients[1]



[1]Saskatchewan Food Industry Development Centre, Saskatoon, SK

6.0 DETAILS OF MAJOR CHANGE

The major change with glabrous annual *Phalaris canariensis* is the complete absence of trichomes (silicified hairs) from the glumes (palea and lemma) of canary seed and the selection of yellow coloured seeds in addition to the conventional brown coloured seeds. The presence and absence of hairs on the canaryseed glumes is illustrated in Figures 6-1a, b, respectively. Figure 6-2 shows the variation in canary seed groat colour.

Details relating to how this major change was achieved are outlined in *Section 4.2 Description of Genetic Modification*

Figure 6-1a Pubescent (hairy) hulled *Phalaris canariensis* (Keet)

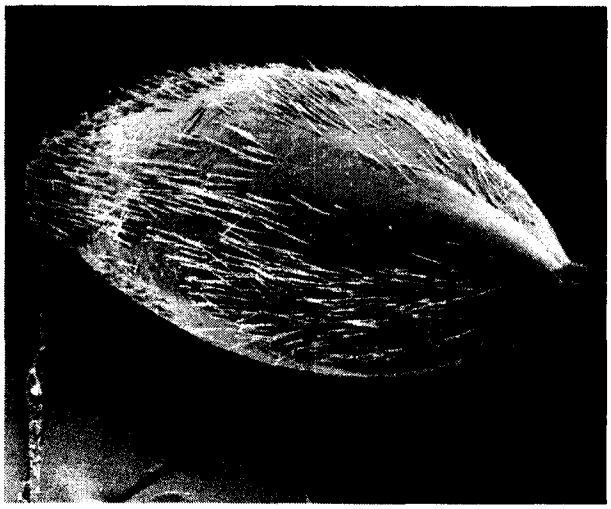
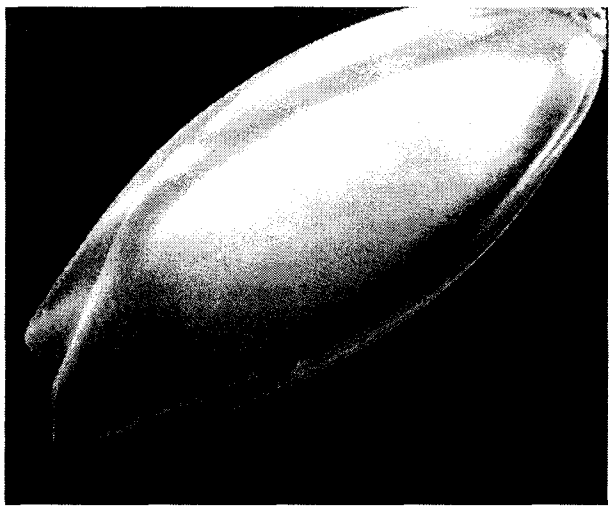
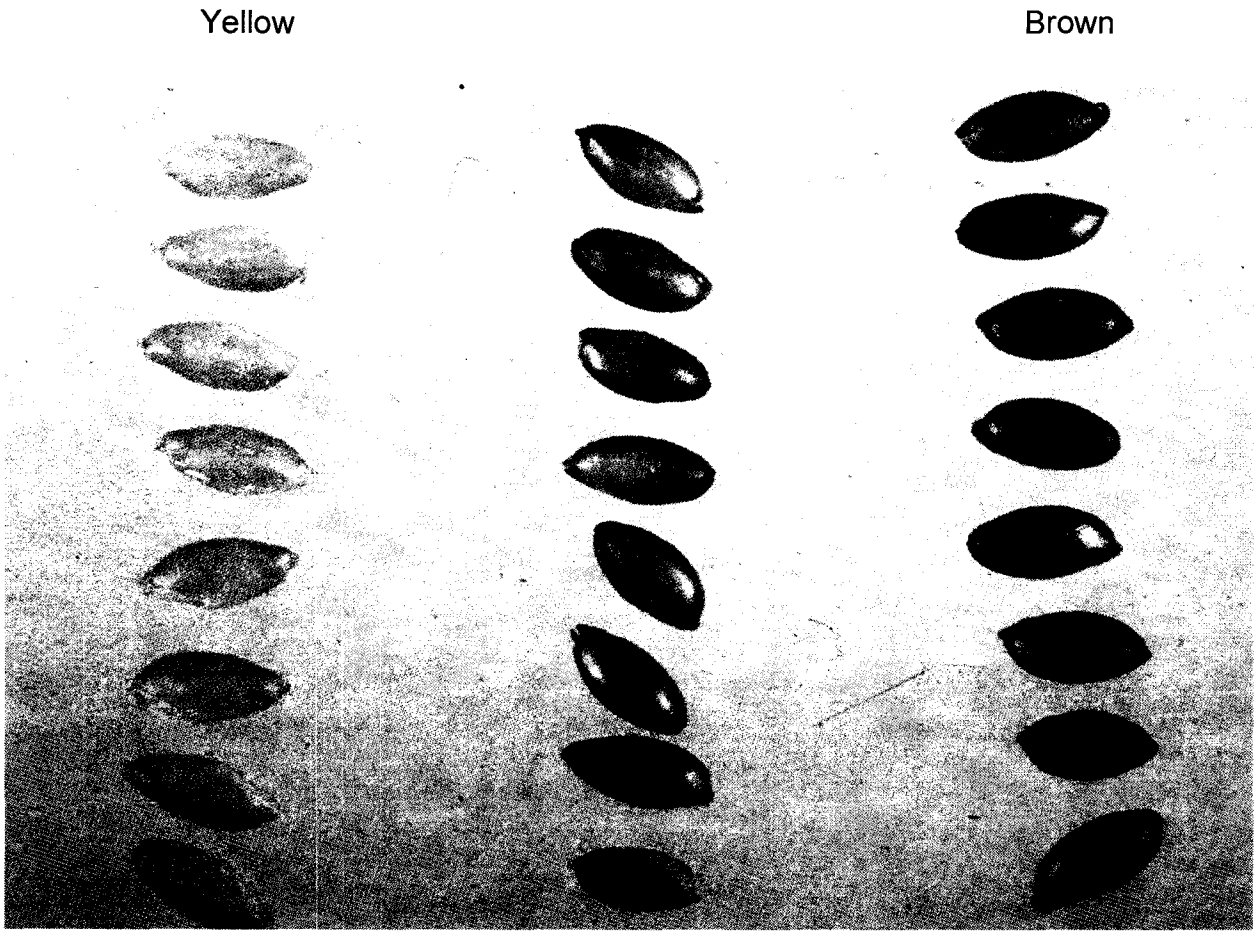


Figure 6-1b Glabrous (hairless) hulled *Phalaris canariensis* (CDC Maria)



(Photos courtesy of P. Hucl, University of Saskatchewan)

Figure 6-2 Canary seed groat colour



(Photo courtesy of P. Hucl, University of Saskatchewan)

7.0 Intended Use and Directions for Preparation

Canary seed groats (dehulled grain) either as a whole groat, whole meal, whole grain flour or a milled product are ideally suited for the bakery, cereal, pasta, snack and nutritional bar market. The grain could also be used as a low fat substitute for sesame seed (a common food allergen) in bread and snack foods or in combination with other seeds as toppings or ingredients.

Canary seed groat products are intended for use as an ingredient in various baked goods, breads, cereals and pasta products. The intended foods and use levels are presented in Table 7-1.

Table 7-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Canary seed in the U.S. (2009-2010 NHANES Data)

Food Category	Proposed Food-Uses	Maximum Proposed Use Level (%)
Baked Goods and Baking Mixes	Bagels	25
	Biscuits	20
	Breads and Rolls	25
	Cakes	20
	Cookies	50
	Cornbread, Corn Muffins, and Tortillas	25
	Crackers	26
	Croissants and Pastries	25
	Doughnuts	25
	Flours and Brans (pre-packaged)	100
	Muffins	20
	Pancakes and Waffles	25
	Pies	10
Breakfast Cereals	Instant and Regular Hot Cereals	15
	Ready to Eat Breakfast Cereals	15
Grain Products and Pastas	Energy, Meal Replacement, and Fortified Bars	25
	Granola and Cereal Bars	25
	Macaroni and Noodle Products	15
	Pasta, Rice and Other Grains	15
Snack Foods	Savory Snacks	25
	Seed-based snacks	40

Intended use and use levels identified above were based upon product prototypes developed at the University of Saskatchewan, the Canadian International Grains Institute, Manitoba Food Processing Development Centre, Guelph Food

Development Centre and the Saskatchewan Food Industry Development Centre using brown and yellow canary seed groats and flours.

Table 7-2 Prototype products from whole canary seed groats or whole grain flour

Centre	Prototype Products
Canadian International Grains Institute (Winnipeg, MB)	Pan bread, pasta, muffins, crackers, cereal bars, tortillas, snaps Topping for: bread and buns, crackers
Manitoba Food Development Centre (Portage La Prairie, MB)	Nutrition bars
Guelph Food Technology Centre (Guelph, ON)	Muffins
Saskatchewan Food Industry Development Centre	Pan bread and cookies
University of Saskatchewan	Pan breads

In all foods tested, the canary seed whole grain flour or whole groat was used to replace and/or complement other ingredients, whether it was refined wheat flour in breads, crackers, pasta, tortillas, muffins, or cookies, quick cooking oats (nutrition bars) or sesame seeds (sesame seed snaps). In the test conditions, up to 50% of refined wheat flour or whole wheat flour was substituted with canary seed whole grain flour in baked good formulations. A 25-35% substitution level produced acceptable food products. One hundred per cent of conventional seed toppings or sesame seed used for bread toppings, crackers, snaps and cereal and fruit bars were substituted with whole roasted canary seed (brown or yellow) groats illustrating the potential to use whole canary seed groats as alternatives to seeds or nuts. Snaps contained 100% substitution for sesame seeds.

Whole grain canary seed flour can also be sold as a stand-alone flour product in the retail market.

All products with the exception of muffins were tested using standard commercial formulations and were prepared in pilot plants. Muffins were tested using a standard household size recipe.

The Technology Centres found that dehulled Canadian glabrous brown and yellow canary seed groats could be processed into flour or roasted as a whole groat to

produce a wide variety of bakery, pasta and snack based products. Few adjustments were required to product formulations or processing conditions when canary seed was used. The flavor of the canary seed was found to be neutral in that it did not contribute nor detract from the flavor of the other ingredients in the formulation. Canary seed did not appear to negatively affect the texture when used as either a flour or whole seed. While food products containing yellow canary seed were more visually appealing than products made with brown canary seed, all products were considered to be acceptable.

All Centres provided the CDCS with prototype formulas and processing methods. Formulations and photographs of these products can be found in Appendix 1.

It is anticipated that canary seed in its whole groat form or as whole grain flour or milled product will first be sold as a food ingredient to secondary processors, with direct sales to consumers being the responsibility of a food processor. The CDCS will endeavor to provide future processors with as much processing information as possible and foresees the development of future recipe books as part of its marketing plan for food grade glabrous canary seed.

8.0 History of Use

Annual canary seed may have been originally used as a human food, although its historical uses are somewhat obscure. It is unclear when it was first used as birdseed, but Linnaeus's original typification and the scientific name *Phalaris canariensis* implies that its use for caged birds was well established in the 16th century. (Anderson, 1961; Baldini and Jarvis, 1991).

A comprehensive literature search in AGRICOLA, PubMed and CABI databases for evidence of human use of *Phalaris canariensis* indicated that canary seed (or alpiste) was recognized as a food in Europe as far back as the late 1500's particularly in those countries bordering the Mediterranean Sea as well as in South America and Mexico. A summary of the literature search is outlined in Table 8-1.

From a North American context, *Phalaris canariensis* appears to have been introduced to this continent in the mid- to late 1800's (Usher, 1974) with the Canadian Ministry of Agriculture growing the annual *Phalaris canariensis* at its Indian Head (SK) Experimental Farm in the late 1890s (MacKay, 1892). The reason for growing was not reported. Pubescent (hairy) canary seed was commercially grown as a grain crop in the northern Great Plains in the Red River valley of North Dakota and Minnesota starting after World War II while commercial production of pubescent canary seed in Canada began in the 1960s in Manitoba and 1971 in Saskatchewan. The primary market has been for use as bird feed.

The seeds of *Phalaris canariensis* are also listed as a food used by the indigenous population of Canada but no further explanations of use were given (Kuhnlein and Turner, 1991).

Other references identify its use as a grain for bread and cereals (Hedrick, 1919; Prance and Nesbitt, 2005) as well as a base for whiskey manufacture (Halliday, 1992). However, no data could be found describing human consumption levels or frequency of consumption for these applications.

Internet searches have shown that ground hulled canary seed is being sold as a beverage powder called "Canary Seed Milk" in the retail markets of Mexico and southern United States, but this appears to be as a traditional medicine rather than as a

food (Estrada-Salas *et al.,* 2014). Whole hulled seed is being sold as a tea (Alpiste) in the food markets of Spain. No data could be found regarding consumption levels.

In 2006, the American Association of Cereal Chemists (AACC) International Whole Grain Working Group Task Force on *Defining Whole Grains in Food* submitted a letter to the United States Food and Drug Administration (FDA) in response to the FDA's announcement in the Federal Register (V71 (33), Feb. 17, 2006) on Whole Grains Label Statements: Availability (AACCI, 2006). This letter (referred to as Docket No. 2006D-0066) included canary seed in its list of edible whole grains. Unfortunately, AACC International used the wrong species name in the whole grains list (*Phalaris arundinacea* rather than P. *canariensis*). An erratum to this Docket now correctly identifying the species name of *Phalaris* as "*canariensis*" was filed with the FDA in June 2011 (AACCI, 2011). A copy of the AACCI Docket response and erratum letter can be found in Appendix 2a & 2b and at the FDA Internet site:

http://www.regulations.gov/#!documentDetail;D=FDA-2006-D-0298-0027.

Links to the appropriate documents can also be found on the AACC International website:

Whole Grain Response:

http://www.aaccnet.org/initiatives/definitions/Pages/WholeGrain.aspx.

The letter itself is located at:

http://www.aaccnet.org/initiatives/definitions/Documents/WholeGrains/WGWGErrataCanarySeedtoFDA.pdf

Table 8-1 References describing the use of canary seed as a food*

Author	Description of Food Use of *Phalaris canariensis*
Jones & Engleson (2010)	The American Association of Cereal Chemists International (AACCI) whole grain working group task force listed canary seed as a true cereal as it fits with the definition of a whole grain.
Prance &Nesbitt (2005)	The author indicated that canaryseed was used as one of many cereals to make a local dish known as "gofio" in the Canary Islands. No other information is given in the artilce.
Halliday (1992)	Halliday noted that canarygrass (alpiste) was used as an ingredient in the making of whiskey. No other details given.
Kuhnlein & Turner (1991)	Authors listed the seed and root of *Phalaris canariensis* as an edible plant food for Canadian Indigenous people (Ch. 5)
Usher (1974)	Usher prepared a dictionary of plants used by man. Indicated canary seed was sometimes used for human consumption in the Mediterranean area.
Hedrick (1919)	In this treatise on edible plants, the author notes that "In Italy, the seeds are ground into a meal and made into cakes and puddings and in the Canary Islands, they are used in the same manner and also made into groats for porridge". No additional information given regarding consumption levels, or frequency of consumption
Piper (1916)	Piper provided background on the historical cultivation and use of annual canarygrass in the Mediterranean region. Refers to canary seed being used as a human food but no further details are given.
Ward (1911)	The Grocer's Encyclopedia: Identified uses for canary seed: as a flour in the manufacture of fine cotton goods and silk stuffs, and as a food in the Canary Islands, Italy and North Africa

*Note: all references, excluding Jones & Engleson, refer to the consumption of hairy varieties of *Phalaris canariensis.*

SAFETY ASSESSMENT

9.0 NUTRITIONAL CONSIDERATIONS

9.1 Compositional Analysis of Canary Seed Groats

Section 3 (Background Information) described the two research programs (Phase 1 and Phase 2) completed to support the safety assessment of glabrous canary seed. In Phase 1 (1992-2002), the nutritional and chemical characteristics of glabrous, brown coloured canary seed groats "CDC Maria" were compared to its pubescent brown coloured parent "Keet" and to Canada Western Red Spring (CWRS) common wheat "Katepwa". The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals.

Phase 2 (2008-2014) involved a comprehensive comparison of two glabrous yellow coloured cultivars (designated C05041 and C05091) to the brown coloured cultivar CDC Maria, which had been studied in the Phase 1 project.

Analytical results from Phase 1 and Phase 2 will be presented simultaneously to permit comparisons between the glabrous brown (CDC Maria) and yellow varieties (C05041 and C05091), the pubescent parent (Keet) and the CWHS wheat. Comparisons to compositional values of commonly consumed cereal grains will also be made.

9.1.1 Methods

9.1.1.1 Source of Grain Materials for Composition and Safety Assessment

The University of Saskatchewan (UofS) Crop Development Centre (CDC) was responsible for growing the pubescent and glabrous *Phalaris canariensis* and wheat used to gather information for the composition and safety assessment.

Phase 1 (1992-2002)

The glabrous canary seed (*P. canariensis* L.), cultivar CDC Maria and the pubescent cultivar Keet were grown in three-replicate randomized complete block experiments in Saskatoon, Saskatchewan in 1996-1998. The CWRS common wheat

Katepwa was grown in plots adjacent to the canary seed field trials. Two replicates from each variety of canary seed and wheat were analyzed separately. The analytical results are expressed as means of two replicates. For heavy metal and mycotoxin testing, the same randomized design was used to obtain samples of the glabrous and pubescent brown canary seed and CWRS wheat from ten sites in Saskatchewan, Canada in 1998.

The hulls of the canary seed grains were removed on an abrasive dehuller followed by air aspiration to produce hull-free grains called groats.

Phase 2 (2008-2014)

Three varieties of glabrous canary seed (brown coloured CDC Maria, and two yellow coloured varieties, C05041 and C05091) were grown at 5 sites throughout the province of Saskatchewan. At each of the five sites, a randomized block design was utilized and three replicate plots of each variety were planted in each of two years (2007 and 2008), providing the project with thirty (30) samples of each of the three varieties for a total of ninety (90) samples for initial analysis. In 2008, the three varieties were also grown in larger plots at the UofS Kernan Farm to provide sufficient grain (~500 kg grain harvested) for food product development, and the rodent toxicology trials and poultry feeding trials.

Statistical analysis of the proximate composition data for the ninety samples indicated there was no statistical difference in proximate composition analysis amongst the 3 replicate blocks of each cultivar at each site location, so hand-harvested grain from the 3 replicate blocks of a single cultivar were combined for further detailed chemical analysis. Three of the five sites produced sufficient quantities of canary seed (6 composite samples for each cultivar for a total of 18 composite samples) to continue in-depth compositional analysis for nutrients, antinutritional factors, inorganic chemicals and mycotoxins.

9.1.1.2 Analytical Methods for Chemical and Nutritional Composition

Tables 9-1 and 9-2 provide a listing of methods used to determine the compositional, nutritional and chemical characteristics of canary seed. Copies of the relevant methods for each analysis can be found in Appendix 3.

The majority of analyses conducted during Phase 1 were performed in-house at the UofS, while analyses for Phase 2 were primarily outsourced to accredited commercial laboratories (POS Biosciences (SK), Silliker Canada Ltd (ON), ALS Laboratory Group (SK), University of Guelph Laboratory Services (ON), Intertek-Sunwest Laboratoratories (SK) and Labs-Mart (AB)) and research laboratories (Agriculture and Agri-Food Canada and University of Manitoba) across Canada. Where necessary, additional methodology details are provided in the body of this dossier.

Table 9-1 Summary of Methodologies Used for Analyses in Phase 1 (1992-2002)

Component	Description	Method	Laboratory	Reference
Proximate Analysis	Moisture	AACC 44-15A	University of Saskatchewan (UofS)	AACC, 1998
	Crude protein	AACC 46-11A		AACC, 1998
	Crude fat	AACC 30-20		AACC, 1998
	Total ash	AACC 08-03		AACC, 1998
Carbohydrate	Starch	AACC76-13		AACC, 1998
	Soluble, insoluble and total dietary fiber	Enzymatic gravimetric procedure, AACC 32-21	UofS	AACC, 1998
	Soluble sugars	Sugar derivatives by gas chromatography		Abdel-Aal *et al.*,1997b
Lipids	Total and purified		UofS	Fölch *et al.*, 1957.
	Fatty acid composition	FAME-GC		Abdel-Aal *et al.*,1997b
Proteins	Fractionation into albumin, globulin, prolamin, glutelin	Successive extraction method based upon Osborne	UofS	Sosulski & Bakal, 1969
	Amino acid composition	Reversed-phased HPLC		Abdel-Aal *et al.*,1997b
	Tryptophan	Spectrometric method		Concon, 1975
	Protein digestibility	Multienzyme technique		Pedersen & Eggum, 1983
Vitamins	Thiamine	AOAC, thiamine 942.23	FDC Northwest Laboratories	AOAC, 1995
	Riboflavin	AOAC, 981.15		AOAC, 1995

	Niacin	AOAC 975.41		AOAC, 1995
Minerals	Major and trace minerals	AOAC 985.01, Inductively coupled argon plasma	FDC Northwest Laboratories	AOAC, 1995
Heavy Metals	Silver, arsenic, bismuth, cadmium, mercury, molybdenum, lead, antimony, tellurium and tungsten	Inductively coupled plasma-atomic emission spectrometry (ICPES)	Saskatchewan Research Council (SRC, Saskatoon, Canada)	Internal method
Mycotoxins	Aflatoxin, vomitoxin	ELISA	Grain Research Laboratory, Winnipeg, MB	
Alkaloids	Phenol, indole and beta-carbolines	GLC/HPLC	UofS	Duynisveld *et al.*, 1990
	Dhurrin	GLC/HPLC		Gorz *et al.*, 1986.
Phenolics	Total	Prussian blue spectrophotometric method	UofS	Price & Butler, 1977
	Condensed tannins	Vanillin assay		Price *et al.*, 1978
	Phenolic acids	Reversed phase-HPLC		Hatcher and Kruger, 1997
Phytate		Anion exchange method, AOAC 32.5.18	UofS	AOAC, 1995
Enzyme Inhibitors	Trypsin inhibitor activity	Spectrophotometric method	UofS	Kakade *et al.* 1974
	Amylase inhibitor activity			Mulimani & Supriya, 1993.

Table 9-2 Summary of Methodologies Used for Analyses in Phase 2 (2008-2014) [1]

Component	Description	Method	Laboratory	Reference
Proximate Analysis	Moisture	AOCS Ba2a--38 (meal)	POS	AOCS 2009
	Crude protein	AOCS Ba 4e-93	Biosciences	AOCS 2009
	Crude fat	Swedish tube (internal method)		
	Total ash	AOAC Bc 5-49		AOAC 2003
Carbohydrate	Starch	AACC 76-13		AACC 2003
	Crude fiber	AOCS Ba 6-84		AOCS 2009
	Soluble and insoluble	AACC 32-21	POS	AACC 2003
	Total dietary fiber	AACC 32-05		AACC 2000
	Acid detergent and lignins	AOAC 973.18		AOAC 2003
	Neutral	AACC 32-20 (Modified)		AACC 2003
	Soluble sugars	AOAC 980.13	Sunwest Food Laboratories (Saskatoon)	AOAC 2003
Lipids	Fatty acid composition	AOAC 969.33 prep, AOAC 996.06 quant. modified		AOAC 2003
	Unsaponifiable matter	AOCS Ca 6a-40	POS	AOAC 2003
Proteins	Amino acid composition	Reversed-phased HPLC Waters Pico-Tag Method and Internal Method	POS	Internal Method
	Protein dispersibility index	AOCS Ba 10a-65		AOCS 2009
Vitamins	Thiamine (B1)	AOAC 942.23	Silliker Canada Co.	AOAC 2003
	Pyridoxine (B6)	AOAC 961.15 (USFDA 400)		AOAC 2003
	Riboflavin	AOAC 981.15		AOAC 2003
	Niacin	AOAC 975.41 (USFDA 340)		AOAC 2003
	Folic Acid	AACC 86-47.01	Labs-Mart (Edmonton, AB)	AACC 2013
Minerals	Microelement panel (Al, As, B, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se, tin, titanium, zinc)	Toxi-024- Metals in biological materials by ICP-OES	University of Guelph Laboratory Services	Internal method provided
	Macro element panel (Ca, Mg, P, K, Na, S, Fe)	Metals in biological metals by ICP-OES (Toxi-024)	University of Guelph Laboratory Services	Internal method provided
Heavy Metals	Arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr),	ICPMS Analysis of Metals in Foods (Toxi-064)	University of Guelph Laboratory Services	Internal method provided

	copper (Cu), iron (Fe), lead (Pb), manganese (Mn), molybdenum, (Mo) nickel (Ni), zinc (Zn),			
	Silver (Ag), arsenic (As), bismuth (Bi), cadmium (Cd), mercury (Hg), molybdenum (Mo), lead (Pb), antimony (Sb), tellurium (Te)and tungsten (w)	Metals in environmental matrices by Inductively Coupled Plasma Mass spectrometry (ICP/MS)	ALS Laboratory Group (Edmonton, AB)	Internal method provided
Mycotoxins	Vomitoxin	Vomitoxin ELISA IMC-411	University of Guelph Laboratory Services	Method provided
	Ochratoxin A	RIDASCREEN®FAST Ochratoxin A Test	Intertek-Sunwest (SK)	Internal method
	Fumonisins (total)	AOAC – 2001.06 RIDASCREEN® FAST Fumonisin: Total Fumonisin in Corn		,AOAC, 19th edition 2012
	Zearalenone	AOAC 994.01 RIDASCREEN®FAST Zearalenone Enzyme Immunoassay for Quantitative Determination of Zearalenone		AOAC,19th edition, 2012
Alkaloids	Phenol, indole and beta-carbolines Dhurrin	GLC/HPLC, UPLC – internal method developed by AAFC GLC/HPLC	Agriculture and Agri-Food Canada, Saskatoon, SK	Duynisveld *et al.*, 1990, Muir *et al*, 1992 Gorz *et al.*, 1986.
Phenolics	Total Condensed tannins Phenolic acid composition	Folin-Ciocalteau Vanillin assay Reversed phase-HPLC	University of Manitoba	Li *et al.*, 2010 Price *et al.*, 1977 Li *et al*, 2011
Phytate	Phytic acid determination	Anion exchange method	University of Manitoba	Latta & Eskin 1980
Enzyme Inhibitors	Trypsin inhibitor activity Amylase inhibitor activity	Spectrophotometric method	University of Manitoba	Kakade *et al.*, 1974 Deshpande *et al*, 1982.
Phytosterols	Sterols and tocopherols	Capillary gas chromatography	POS	Slover *et al.*, 1983

[1] Methods for proximate analysis were used on the 90 samples and on the 18 composite samples.

9.1.1.3 Statistical Analysis

Phase 1

All analyses were carried out using at least two separate determinations for each sample. Analysis of variance was performed to determine significant differences between cultivars for nutrients, minerals, and vitamins using Minitab Software (version 12, Minitab Inc., State College, PA, USA). Differences were examined using the least significant difference (LSD) method and were considered to be significant when $p < 0.05$.

Phase 2

All analyses were carried out using at least two separate determinations for each sample. For the individual 90 samples, analysis of variance was carried out to assess the variation amongst the canary seed samples to determine the amount of variability between cultivars for protein, oil, ash, moisture and carbohydrate and to determine whether test plots of a specific variety from one site could be combined. In this study, varieties were nested in subsamples, subsamples in blocks, blocks in locations, and locations in years.

The variance components analysis was performed to assess the variation within each level of the dataset for the ninety samples to determine 1) the amount of between-site variation, and 2) whether further statistical analysis should be conducted on individual subsamples or averaged subsamples.

The subsample displayed little variation, and implied strong consistencies within the laboratory analyses. Little variation attributable to the experimental blocks indicated consistent environments within each field site and thus enabled composite samples to be prepared from the replicate plots.

Mixed effects models (Hurlbert, 1984) were used to assess how the varieties differed from each other with year, location and block specified as random effects. These models were fit using the "lme" function in the "nlme" library in the R package. (Crawley, 2007).

Orthogonal contrasts were used to assess whether there was a difference between varieties. Contrasts were only performed on models after the initial mixed model indicated significant differences.

9.1.2 Nutrient Composition of Raw Canary Seed Groats

Hand-harvested samples from each of the test plots were dehulled and hand cleaned. The hulls of the canary seed grains were removed on an abrasive dehuller followed by air aspiration to produce hull-free grains called groats.

9.1.2.1 Chemical Composition

For the purposes of this dossier, chemical and nutrient values for the two glabrous yellow cultivars (C05041 & C05091) analyzed in Phase 2 have been combined to provide the mean and range of values for yellow canary seed. Similarly, values for the glabrous brown variety (CDC Maria) include results from Phase 1 and Phase 2. Nutrient values for pubescent brown canary seed (Keet) and the CWRS wheat (Katepwa) are from the Phase 1 study only.

Microstructure analysis of canary seed illustrated that canary seed is a true cereal similar to wheat, oats, barley and rice containing three main components: bran, the germ and the starchy endosperm (Abdel-Aal *et al.*, 2011a).

Glabrous brown and yellow canary seed cultivars have a proximate composition profile similar to the pubescent parent, Keet (Table 9-3). Glabrous varieties were slightly lower in crude fat content and higher in protein content but had similar ash content to the pubescent cultivar. All canaryseed varieties (glabrous or pubescent) were higher in ash, crude fat and protein than the Canadian Western Red Spring (CWRS) wheat (Table 9-3). Robinson (1978) reported that canary seed caryopses were much higher in nitrogen, ash, oil, phosphorous and potassium but lower in fiber than other grain crops. The nitrogen-to-protein conversion factor used for canary seed protein was 5.7 as recommended for cereals by Sosulksi & Imafidon (1990).

For comparative purposes, the chemical composition of glabrous canary seed groats (dehulled canary seed) is compared to commonly consumed cereal grains such as wheat, barley, oats and rye and, in some instances, to other specialty whole grains (e.g. sorghum, millet), pseudocereals (e.g. amaranth, quinoa and buckwheat) and brown rice (Jones & Engleson, 2010).

Table 9-3 Comparison of proximate chemical composition (% dry basis) of glabrous brown and yellow coloured canary seed groats to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed[1, 2]								Pubescent Canary Seed[1]			Wheat[1]	
	Brown				Yellow				Brown			CHRS	
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range	Mean	SD
			Min	Max			Min	Max					
Ash	2.4	±0.2	2.1	2.6	2.2	±0.2	1.9	2.4	2.1	±0.1	2.0-2.1	1.7	±0.1
Crude Fat	6.2.	±0.3	5.5	6.6	6.2	±0.2	5.8	6.4	. 8.7	±0.3	8.4-8.9	2.3	±0.1
Protein (Nx5.7)	21.8	±0.7	20.8	23.1	21.0	±1.0	19.3	22.8	18.7	±2.7	15.6-20.3	15.0	±2.0
Carbohydrate (by difference)	69.3	±0.7	68.4	70.4	70.6	±0.9	69.3	72.1	70.5	NR	NR	65.7	NR

[1] Abdel-Aal *et al.*, 1997b
[2] Phase 2, CDCS study
NR: not reported

Protein concentrations for glabrous canary seed ranged from 19.3 % to 23.1 %. These protein values are higher than those found in wheat (10-16%) (OECD, 2004), barley (7.6-14.4%) (OECD, 2003) and oats (13.8 – 22.5 %) (McMullen, 2000). The protein level for glabrous canary seed is also higher than protein levels in other specialty cereals such as millet (8.8% db (N x 6.25), sorghum (12.1 % db (N x 6.25) (Ragaee *et al.*, 2006),amaranth (16.8% N x 5.85) (Bejosana & Corke, 1998), buckwheat (12.5% N x 5.7), brown rice (7.9% N x 6.25) (Rosell & Marco, 2008) and quinoa (14.5 %, N x 5.96) (Alvarez-Jubete *et al.*, 2010). Glabrous canary seed has a higher content of crude fat (~6%) compared to wheat and barley (2.31%), millet (4.22%), rye (2.53%) and sorghum (3.32%) (Chung & Ohm, 2000). The content of crude fat in canary seed is very similar to oats (3.1-11.6%), quinoa (5.01-5.95 %) and amaranth (6.56-10.3%) and higher than buckwheat (2.4-2.8%) (Schoenlechner *et al.*, 2008) and rice (2.9%) (Rosell & Marco, 2008). The ash content in canary seed groats ranged from 1.94 to 2.6% across all varieties and sites examined. This range is comparable to the range of ash content found in other common cereals such as wheat (1.17-2.96%) (OECD, 2004), barley (2.0-5.0%) (OECD, 2003) and field maize (1.1-3.9%) (OECD, 2002) and pseudocereals such quinoa (2.4-3.3%)(Schoenlechner *et al.*, 2008). Canary seed has a mineral content lower than amaranth (3.25%) but higher than buckwheat (1.37-1.67%) (Schoenlechner *et al.*, 2008) and rice (1.5%) (Rosell & Marco, 2008).

As discussed in Methods (Section 9.1.1.3), statistical analysis of the proximate composition (protein, ash, crude fat) on the ninety individual samples grown in Phase 2 indicated that glabrous canary seed from replicate plots at one location could be combined to provide an adequate volume of grain for more detailed compositional and nutritional analysis. Three of the five test sites produced sufficient quantities of grain to produce 6 composites of each variety (18 samples) for further in-depth analysis.

9.1.2.2 Protein and Amino Acid Composition

The protein content in the canary seed groats was higher than that reported in the literature for barley, oat or wheat (Gutierrez-Alamo *et al.*, 2008; Quinde *et al*, 2004).

Glabrous canary seed has an amino acid profile similar to that of its pubescent parent (Table 9-4); the notable difference being the lower lysine range of the pubescent

cultivar (1.1-1.4 g AA /100g protein) compared to the glabrous varieties (1.4-2.6 g amino acid (AA) /100g protein). The lysine content in canary seed is slightly lower than that found in wheat, barley and oats, but is comparable to maize (Table 9-5).

Compared to other cereals, canary seed proteins have higher contents of tryptophan, phenylalanine, and cysteine, the methionine-sparing amino acid (Table 9-5). Tryptophan is nutritionally important as it is a precursor for important metabolites such as serotonin and nicotinamide (WHO, 2007). Its content is low in cereals, especially maize. The range of tryptophan in glabrous canary seed (2.7 -3.1 g AA/100g protein) is twice as high as that found in many cereals and pseudocereals. Comai *et al* (2007) reported tryptophan levels (all as g AA/100g protein) in spelt, 1.17; wheat, 1.16; quinoa, 1.14; sorghum, 1.1; oat, 0.97; pearl millet, 0.97; barley, 0.96; rye 0.82 and maize, 0.49. The phenylalanine content in glabrous canary seed ranged from 6.2 to 6.7 g AA/100g protein, higher than reported for wheat (3.5-5.4 g AA/100g), barley (4.2-5.4 g AA/100g) and oats (5.3 g AA/100g). Canary seed groats had cysteine levels ranging from 2.4 to 3.4 g/100g higher than wheat, oats, and barley (Table 9-5).

While the range of total essential amino acids in canary seed protein is higher than those of wheat, the higher canary seed amino acid values are comparable to those of oats, barley and maize (Table 9-5). The values of the non-essential amino acids in canary seed were comparable to wheat, oats, barley and corn.

Table 9-4 Comparison of protein (%), non-protein nitrogenous material (%) and amino acid profile (gAA/100g protein) of glabrous brown and yellow canary seed compared to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed								Pubescent Canary Seed[1]			CWRS[1]
	Brown[1,2]				Yellow[2]				Brown			Wheat
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range	Mean
			Min	Max			Min	Max				
Protein (N x 5.7) (%)	21.8	±0.8	20.8	23.06	21.0	±0.2	1.9	2.4	18.7	±2.7	15.6-20.3	15.0
Non-protein nitrogen (%)	0.8	±0.1	0.7	0.90	0.8	±0.1	0.7	0.9				
Amino Acid Profile												
Alanine	4.5	±0.1	4.5	4.6	4.5	±0.1	4.4	4.6	4.1	±0.1	4.1-4.2	3.0
Arginine	6.5	±0.2	6.3	6.8	6.6	±0.2	6.3	6.9	6.9	±0.1	6.8-7.0	5.1
Aspartic acid	4.4	±0.2	4.1	4.7	4.5	±0.1	4.2	4.7	4.6	±0.1	4.5-4.6	4.4
Cystine	2.5	±0.1	2.2	3.4	2.5	±0.1	2.4	2.6	3.3	±0.1	3.2-3.3	2.3
Glutamic acid	26.1	±0.6	25.2	26.7	26.5	±0.4	25.6	27.0	30.6	±0.2	30.4-30.7	33.0
Glycine	3.1	±0.1	3.0	3.2	3.1	±0.1	2.9	3.2	3.0	±0.1	3.0-3.1	3.8
Histidine	1.7	±0.1	1.6	1.9	1.7	±0.1	1.6	1.8	1.8	±0.1	1.7-1.9	2.1
Isoleucine	3.9	±0.1	3.4	4.1	3.9	±0.1	3.8	4.1	3.5	±0.1	3.5-3.6	2.8
Leucine	7.6	±0.2	7.1	7.8	7.6	±0.2	7.4	7.8	7.0	±0.1	7.0-7.1	5.3
Lysine	2.6	±0.2	1.4	2.8	2.5	±0.1	2.5	2.6	1.4	±0.2	1.1-1.4	1.9
Methionine	1.9	±0.2	1.4	2.2	1.9	±0.2	1.7	2.2	1.4	±0.1	1.3-1.5	1.4
Phenylalanine	6.5	±0.1	6.3	6.7	6.5	±0.1	6.2	6.6	6.7	±0.4	6.4-7.1	5.4
Proline	6.2	±0.1	6.1	6.3	6.3	±0.1	6.1	6.4	5.4	±0.1	5.3-5.4	8.6
Serine	4.5	±0.1	4.5	4.5	4.5	±0.1	4.3	4.9	4.2	±0.1	4.1-4.2	4.3
Threonine	2.7	±0.1	2.7	2.8	2.8	±0.2	2.5	2.9	2.7	±0.1	2.7-2.8	2.8
Tryptophan	2.8	±0.1	2.7	2.9	2.9	±0.2	2.7	3.1	2.8	±0.3	2.6-3.1	1.2
Tyrosine	3.6	±0.1	3.3	3.8	3.6	±0.2	3.4	3.8	3.2	±0.1	3.2-3.3	3.5
Valine	4.8	±0.1	4.7	4.9	4.8	±0.1	4.7	4.9	4.6	±0.2	4.5-4.8	3.8
Total A. A.	95.9	±1.2	94.5	97.6	96.6	±1.2	94.9	97.5	97.2	±0.3	97.0-97.5	94.7

[1] Values from Abdel-Aal *et al.*, 1997b
[2] Values from Phase 2, CDCS study

Table 9-5 Comparison of amino acid composition of glabrous canary seed to four common cereal grains

Amino Acid	Canary Seed[a] (g/100g protein)	Wheat[b] (% total protein)	Barley[c] (g/100 g protein)	Maize[d] (g/16gN)	Oats[d] (g/16g N)
Essential AA					
Methionine	1.4-2.2	1.3-1.7	1.4-3.2	1.8	2.5
Cysteine	2.2-3.4	1.7-2.7	1.0-1.8	1.1	1.6
Lysine	1.4-2.8	2.2-3.0	3.1-4.2	2.6	4.2
Tryptophan	2.7-3.1	1.0-2.7	1.5[d]	0.7	1.3
Isoleucine	3.4-4.1	3.0-4.3	3.1-3.9	3.7	3.9
Histidine	1.6-1.9	2.0-2.8	1.9-3.3	2.8	2.2
Valine	4.7-4.9	4.4-4.8	3.9-5.3	5.3	5.3
Leucine	7.1-7.8	5.0-7.3	5.4-7.1	13.6	7.4
Phenylanlanine	6.3-6.7	3.5-5.4	4.2-5.4	5.1	5.3
Tyrosine	3.4-3.8	1.8-3.7	1.9-2.8	4.4	3.3
Threonine	2.7-2.9	2.4-3.2	3.0-3.7	3.6	3.3
Total essential AA	**36.95-43.75**	**26.3-41.6**	**30.4-42.19**	**44.7**	**40.3**
Non-essential AA					
Alanine	4.4-4.6	3.4-3.7	4.4-4.6	7.9	5.0
Arginine	6.3-6.9	4.0-5.7	4.2-6.2	3.8	6.9
Aspartic acid	4.1-4.7	4.8-5.6	6.8-7.4	6.3	8.9
Glutamic acid	25.2-26.9	29.9-34.8	21.9-26.1	18.9	23.9
Glycine	2.9-3.2	3.8-6.1	4.2-5.1	3.4	4.9
Proline	6.1-6.4	9.8-11.6	11.4-12.4	8.3	4.7
Serine	4.3-4.7	4.3-5.7	3.7-5.4	4.8	4.2

[a] Data range canary seed analysis (Phase 1 and Phase 2, yellow and brown glabrous canary seed))
[b] From OECD, 2004
[c] From OECD, 2003, except for tryptophan (Lookhart & Bean, 2000 Table 2)
[d] From Lookhart and Bean, 2000 Table 2

9.1.2.3 Fatty Acid Profile

Glabrous and pubescent canaryseed groats contain approximately 3 to 4 times the amount of crude fat than the CWRS wheat. Crude fat levels in the parent pubescent canaryseed ranged from 8.4-8.9%, the glabrous brown ranged from 5.5-6.6%; and the glabrous yellow ranged from 5.8-6.4%. The CWRS wheat in the study contained 2.3% crude fat. Glabrous canary seed has a higher content of crude fat (~6%) compared to wheat and barley (2.3%), millet (4.2%), rye (2.5%) and sorghum (3.3%)(Chung & Ohm,

2000). The content of crude fat in canary seed is within the range of crude fat in oats (3.1-11.6%).

Like other cereal grains, the predominant fatty acids in glabrous brown and yellow canary seed are palmitic (range: 11.2-12.3%), oleic (range: 26.7-33.6%) and linoleic acids (range: 48.2-54.9%)(Table 9-6). These values are comparable to that of the pubescent canary seed parent Keet (10.7%, 29.8% and 55.4%, respectively) (Table 9-6 and Abdel-Aal *et al.*, 1997b) and consistent with fatty acid values (palmitic, 12%; oleic, 32%; and linoleic, 54%) in other tested pubescent canary seed cultivars (Malik & Williams, 1966).

As a relative percentage of fatty acids, palmitic acid was present in lower levels (11.0-13.3%) in canary seed than found in the CWRS wheat (~16%, Table 9-6), other wheat varieties (17-24%), barley (19-28%) and rye (12-19%)(Chung & Ohm, 2000). Canary seed contained a relatively higher level of oleic acid (28.7-35.5%) than these cereal grains [wheat (8-21%), barley (9-17%) and rye (12-17%)] with a very similar relative level to oats (22-39%) (Youngs and Püskülcü, 1976) and buckwheat (37%) (Taira *et al*, 1986). Linoleic acid is the major fatty acid in canary seed oil, constituting about 55% of the total fatty acids compared to 61% in wheat oil.

Canary seed contains approximately 85% unsaturated fatty acids, of which approximately 32% is monounsaturated and 55% are polyunsaturated fatty acids (Table 9-7). Canary seed has a higher unsaturated to saturated fat ratio (~85:13) than wheat, barley and oats (all about 75:25) but contains a lower percentage of polyunsaturated fatty acids (~55%) than wheat (~66%) and barley (~60%) but more than oats (~48%). Canary seed has been found to exhibit antioxidant properties for fats and oils primarily due to the presence of caffeic acid esters and phytosterols (Takagi & Iida, 1980). Canary seed groats contain about 2% omega-3 fatty acids (Table 9-7), similar to other cereal grains.

Table 9-6 Comparison of fatty acid composition (% total fatty acids) of brown glabrous and yellow canary seed to pubescent brown canaryseed and CWRS wheat

Fatty Acid		Glabrous Canary Seed								Pubescent Canary Seed[1]		Wheat[1]
		Brown[1,2]				Yellow[2]				Brown		CWRS
		Mean	SD	Range		Mean	SD	Range		Mean	SD	Mean
				Min	Max			Min	Max			
Crude Fat (%)		6.2	±0.3	5.5	6.6	6.2	±0.2	5.8	6.4	8.7	±0.3	2.3±0.1
Monounsaturated FA												
Hexadecenoic	C16:1	0.2	±0.0	0.2	0.2	0.1	±0.0	0.1	0.2	nr		nr
Oleic	C18:1	30.9	±2.1	28.7	33.6	29.9	±1.8	26.7	32.4	29.5	±0.8	16.6
Octadecenoic	C18:1	0.7	±0.0	0.7	0.8	0.6	±0.1	0.5	0.8	nr		nr
Eicosenoic	C20:1	1.0	±0.1	0.9	1.1	0.9	±0.2	0.1	1.1	nr		nr
Erucic	C22:1	0.1	±0.0	0.1	0.1	0.1	±0.0	0.1	0.1	0.1	±0.1	0.0
Polyunsaturated FA												
Linoleic	C18:2	51.1	±2.1	48.2	53.2	52.2	±1.8	49.8	54.9	55.4	±1.0	61.2
Linolenic	C18:3	2.2	±0.3	1.9	2.6	1.9	±0.5	0.0	2.4	2.7	±0.2	4.6
Saturated FA												
Myristic	C14	0.2	±0.0	0.2	0.2	0.2	±0.01	0.2	0.2	0.2	±0.1	0.2
Palmitic	C16	11.9	±0.2	11.8	12.3	11.6	±0.3	11.2	12.1	10.7	±0.3	15.8
Stearic	C18	1.3	±0.1	0.9	1.4	1.4	±0.1	1.3	1.5	1.0	±0.1	0.8
Arachidic	C20	0.1	±0.0	0.1	0.1	0.2	±0.0	0.0	0.2	0.1	±0.1	0.0
Behenic	C22	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.1	0.1	±0.1	0.2
Others		0.1	±0.0	0.0	0.2	0.1	±0.0	0.0	0.1	0.9	0.1	0.7

*nr: Not reported
[1]Abdel-Aal *et al.*, 1997
[2]Values from Phase 2, CDCS study

Table 9-7 Comparison of the Fatty Acid Profile (% of total fatty acids) in glabrous brown and yellow canary seed groats

	Brown[1,2]				Yellow[2]			
	Mean	STDEV	Range		Mean	STDEV	Range	
			Min	Max			Min	Max
Saturates	13.7	±0.3	13.5	14.1	13.5	±0.4	13.0	14.1
Monounsaturates	32.9	±2.1	30.6	35.6	31.9	±1.7	29.5	34.3
Polyunsaturates	53.3	±2.3	50.2	55.8	54.6	±2.0	51.6	57.4
Omega 3	2.2	±0.3	1.9	2.6	2.0	±0.2	1.8	2.4
Omega 6	51.1	±2.1	48.2	53.2	52.5	±1.8	49.8	55.0
Omega 9	32.1	±2.1	29.7	34.8	31.0	±1.7	28.7	33.6

[1]Abdel-Aal *et al.*, 1997
[2]Values from Phase 2, CDCS study

9.1.2.3.1 Tocopherol and Phytosterol Composition

Tocol derivatives (tocopherols and tocotrienols) are responsible for the vitamin E activity in plant tissues and various combinations of all eight tocol derivatives are found among the cereal grains (Chung & Ohm, 2000).

Wheat has 4 major tocol derivatives (α-tocopherol, α-tocotrienol, β-tocopherol and β-tocotrienol) present and barley has all eight naturally occurring tocopherols. Oats contain six of the tocopherols derivatives (α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol, δ-tocopherol and trace of Δ-trienol) (Chung & Ohm, 2000).

In the Phase 2 study, α-tocopherol and δ-tocopherol were detected in both brown and yellow glabrous canary seed (Table 9-8). Phytosterols were not determined in the Phase 1 study. The total tocopherol range in canary seed (1.8-3.4 mg/100g) is somewhat less than the total tocopherol content reported in wheat (4.9-5.8 mg/100g), barley (4.22-8.0 mg/100g), but similar to the levels found in oats (1.3-3.0 mg/100g) (Peterson *et al.*, 2007).

Cereals are recognized as significant plant sterol sources. The most abundant sterols in plant sources, including oilseeds and fresh vegetables, are sitosterol, campesterol, stigmasterol, Δ5-avenasterol and Δ7-avenosterol where sitosterol is the predominant sterol (Piironen *et al.*, 2002). The total phytosterol contents of bread wheat grains have been reported to range from 0.67-0.96 mg/g (db) with the differences being attributed to genetic variation, environmental factors and analytical methods (Pirronen *et al.*, 2009). β-sitosterol comprises about 60% of the total sterols in barley and in wheat, 41-53% of the total sterols. Campesterol is the next most abundant sterol found in barley (OECD, 2003) and wheat (OECD, 2004). Canary seed groats have the same sterol profile as other common cereals with β-sitosterol as the primary sterol comprising about 41.5 to 43% of the total sterols in canary seed, followed by campersterol, stigmasterol and cholesterol. However, the range of total sterols (0.44-0.50 mg/g dm) is similar to oats (0.35-0.49 mg/g dm) (Maata *et al.*, 1999) but less than found in wheat (0.67-0.96 mg/g dm) (Piironen *et al.*, 2009) and barley (0.89-1.1 mg/g dm) (Andersson *et al.*, 2008).

Table 9-8 Comparison of the tocopherol (mg/100g) and sterol (mg/g) content of glabrous brown and yellow canary seed groats[1]

	Brown[1]				Yellow[1]			
	Mean	SD	Range		Mean	SD	Range	
			Min	Max			Min	Max
Tocopherols (mg/100g)								
α-tocopherol	2.2	±0.3	1.8	2.8	1.9	±0.2	1.6	2.4
δ tocopherol	0.6	±0.2	0.3	1.0	0.5	±0.2	0.1	0.8
Total Tocopherols	2.8	±0.5	2.3	3.4	2.2	±0.3	1.8	2.8
Sterols (mg/g)								
β-sitosterol	0.20	±0.01	0.18	0.21	0.20	±0.01	0.19	0.21
Campesterol	0.11	±0.01	0.10	0.12	0.11	±0.00	0.11	0.12
Stigmasterol	0.01	±0.00	0.00	0.01	0.01	±0.00	0.01	0.01
Cholesterol	0.001	±0.00	0.001	0.001	0.00	±0.00	0.000	0.00
Other Sterols	0.15	±0.01	0.14	0.16	0.14	±0.01	0.12	0.15
Total Sterols	0.47	±0.03	0.44	0.50	0.45	±0.01	0.43	0.48
Unsaponifiable Matter (%)	1.71	±0.10	1.55	1.88	1.64	±0.17	1.43	1.94

[1] Phase 2, CDCS Study

9.1.2.4 Carbohydrate Fraction

Cereal grains are considered an important source of starch (40-90% of their dry weight) as are pulses (30-70%) and tubers (65-85%) (Shelton & Lee, 2000). Glabrous canary seed contains about 55-59% starch (db) (Table 9-9). The pubescent parent canary seed, Keet, has been reported to contain 54-65% starch (Abdel-Aal *et al*, 1997a). The starch content in glabrous canary seed is less than that reported in wheat (63-72%), corn (65-78%) and sorghum (60-77%) but is within the range reported for oats (43-61%) and barley (57.6-59.5) (Shelton & Lee, 2000).

Abdel-Aal and co-workers (1997a) studied starch extracted from pubescent canary seed and found that more than 95% of the polygonal shaped canary seed starch granules were an average size of 2.0µm. Previous studies on pubescent canary seed starch have reported granule size ranges of 2.5-5.0 µm (Goering & Schuh, 1967). The granule size of amaranth (1-3 µm) (Capriles *et al.*, 2008) and quinoa starch (0.6 to 2.0 µm) (Lorenz, 1990; Lindeboom *et al.*, 2005) are comparable to canary seed. Wheat starch granules range from 1-40 µm and, like barley and rye starches, have a bimodal size distribution containing large lenticular granules (25-40µm) and small spherical granules (1-10µm) (Shelton & Lee, 2000).

The amylose content (16.2-19.5%) in canary seed starch was less than in wheat (22.7%) and corn (24.5%) but fit within the range for that found in eight quinoa lines (3-20%) (Lindeboom *et al.*, 2005). Canary seed starch has A-type starch crystals, characteristics of most cereal starches with a high degree of crystallinity (Abdel-Aal *et al*, 1997a).

Cereals contain small amounts of free sugars: wheat (1-2%), barley (2-3%), corn (1-3%), oats (1-2%) and rye (~3%)(Shelton & Lee, 2000). The free sugars vary among cereal grains with sucrose, glucose, and fructose being predominant. Other sugars have been reported in cereals including raffinose, stachyose, and arabinose. Glabrous canary seed cultivars contained 0.6 to 1.1% soluble sugars, while the pubescent cultivar contained 1.7% and the CWRS wheat control contained 2.9% soluble sugars (Abdel-Aal *et al*, 2011a). Individual free sugars were measured in the pubescent parent canaryseed cultivar with that cultivar containing about 0.8% sucrose, 0.1%fructose, and 0.1%

glucose (Abdel-Aal *et al.*, 1997b), similar to that found in the glabrous cultivars. Sucrose was the predominant sugar in glabrous and pubescent canary seed (Table 9-9). Arabinose was also detected but not maltose.

9.1.2.4.1 Dietary fiber

There is quite a wide range in the dietary fiber content of cereals, ranging from 9.3% (db) in millet (Pönte *et al.*, 2000) to 25% (db) in rye (Gebruers *et al.*, 2008). Durum wheat, spring wheat and winter wheat all differ in the ranges of dietary fiber content. The European HEALTHGRAIN diversity screen determined that winter wheat ranged from 11.5-18.3% (db), spring wheat 12.1-17.5% (db) and durum wheat 10.7 to 15.5% (db). The diversity screen also found that dietary fiber levels in barley, rye and oat samples were higher than in wheat, with values (db) from 15.0 to 23.7% in barley, 20.4 to 25.2% in rye and 10.6 to 23.4% in oats (Gebruers *et al.*, 2008.) The majority of dietary fiber in cereals is composed of insoluble dietary fiber ranging from 1.87 % in soft wheat to ~22% in barley. Barley and rye have been reported to have the highest levels of soluble fiber, 2.56% and 3.7% respectively (Ragaee *et al*, 2006) although high levels (4.1-4.9%) have also been reported in oats (Manthey *et al.*, 1999). In comparison with these cereals, canary seed groats contain less total dietary fiber (range 5.9 to10.2%) with the majority being insoluble and less than 1% being soluble (Table 9-10). The dietary fiber content in the pubescent canary seed ranged from 5.5-8.3%, comprised of about 1% soluble fiber and the remaining insoluble fiber (Abdel-Aal *et al.,* 1997b).

Canary seed has a dietary fiber content similar to buckwheat (~7% db) (Wijngaard & Arendt, 2006), lower than quinoa (12.88% db)) and amaranth (11.14%db) (Schoenlechner *et al.,* 2008) and higher than brown rice (3.5-4.6% db) (Rosell & Marco, 2008).

Table 9-9 Comparison of the starch (%db) and sugars (% db) content of glabrous brown and yellow canary seed groats to pubescent brown canary seed groats

	Glabrous Canary Seed								Pubescent Canary Seed[1]	
	Brown[1,2]				Yellow[2]				Brown	
	Mean	SD	Range		Mean	SD	Range		Mean	SD
			Min	Max			Min	Max		
Total Starch	56.1	±1.1	54.2	57.6	57.1	±2.7	53.0	61.2	60.0	±2.6
Arabinose	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.2	tr	
Fructose	0.1	±0.0	0.0	0.1	0.1	±0.0	0.0	0.2	0.1	±0.0
Glucose	0.2	±0.1	0.1	0.3	0.1	±0.0	0.0	0.1	0.1	±0.0
Maltose	Nd	-	nd	nd	nd	-	nd	nd	nd	Nd
Sucrose	0.6	±0.1	0.5	0.7	0.6	±0.1	0.4	0.8	0.8	±0.1
Unknown									0.79	±0.1
Total Sugars	0.9	±0.1	0.8	1.1	0.8	±0.2	0.6	1.1	1.75	±0.1

[1]Abdel-Aal *et al*, 1997
[2] Phase 2 CDCS study
nd-not detected ; tr: trace

Table 9-10 Comparison of the dietary fiber content (% db) of glabrous brown and yellow coloured canary seed groats to pubescent brown canary seed groats and CWRS wheat

	Glabrous Canary Seed[1]								Pubescent Canary Seed[2]		
	Brown				Yellow				Brown		
	Mean	SD	Range		Mean	SD	Range		Mean	SD	Range
			Min	Max			Min	Max			
Lignins (%)	0.6	±0.3	0.3	1.0	0.6	±0.2	0.3	0.9	ND		
Soluble Fiber (%)	0.3	±0.2	0.1	0.7	0.4	±0.3	0.1	1.1	0.9	±0.1	0.8-0.9
Insoluble Fiber (%)	8.1	±0.9	7.1	9.1	8.1	±1.1	5.5	10.0	5.1	±0.5	4.7-5.6
Total Dietary Fiber (%)	8.4	±0.9	5.9	9.3	8.6	±1.2	6.0	10.2	6.6	±1.0	5.5-8.3

[1]Phase 2, CDCS study
[2]Abdel-Aal *et al.*, 1997
ND-not determined

9.1.2.5 Micronutrient composition

9.1.2.5.1 Vitamins

Levels of the B vitamins thiamine, riboflavin and niacin were measured in glabrous and pubescent canary seed cultivars and the CWRS wheat in Phase 1. These three B vitamins plus pyridoxine and folate was measured in the canary seed cultivars in the Phase 2 study. Thiamine content in canary seed (0.7 mg/100g db) was almost twice that measured in the CWRS wheat (0.4 mg/100 g (db)) with riboflavin levels being very similar (0.1-0.2 mg/100 g (db)). However, the niacin content in canary seed (ca.1.0 mg/100 g db) was significantly less than the niacin measured in CWRS wheat (7.3 mg/100 mg db) (Table 9-11). Measured levels of pyridoxine in glabrous canaryseed from Phase 2 were approximately 0.2 mg/100 g (db).

The thiamine content range reported here for glabrous canaryseed was comparable to the ranges reported in wheat, barley, oats and maize (Table 9-12). Riboflavin values for glabrous canary seed were similar to reported values for wheat and oats and higher than reported values for barley and field maize. Pyridoxine content in canary seed (0.2 mg/100g (db)) was less than reported levels in wheat, barley and maize, but similar to oat. However, canary seed contains less niacin than reported for wheat, barley and field maize, and is more similar to oat (Table 9-12).

Total folate content in glabrous canary seed ranged from 0.07-to 0.12 mg/100g (db) for yellow and brown coloured varieties; higher than the folate values reported for wheat (0.02-0.09 mg/100g db), barley (0.019-0.03 mg/100g db), maize (0.017-0.045 mg/100g db) and oats (0.06-0.07 mg/100g db) (Bock, 2000; OECD, 2002, 2003, 2004) (Table 9-12). Folate content in canary seed was comparable to those values reported for the pseudocereals amaranth (0.05-0.73 mg/100g db) and quinoa (0.13 mg/100g db), and higher than buckwheat (0.02 mg/100) (Schoenlechner *et al.,* 2010) and rice flour (0.006 mg/100 g) (Yazynina *et al.,* 2008).

Table 9-11 Comparisons of B vitamins (mg/100g db) content of glabrous brown and yellow canary seed groats compared to pubescent canary seed groats and CWRS wheat

Vitamin	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[2]	Wheat[2]
	Brown				Yellow				Brown	CWRS
	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Thiamine (B_1)	0.7	±0.1	0.6	0.9	0.7	±0.1	0.6	0.8	0.8	0.4
Riboflavin (B_2)	0.1	±0.0	0.1	0.2	0.1	±0.0	0.1	0.1	0.2	0.2
Niacin (B_3)	1.3	±0.1	0.7	1.3	1.1	±0.2	1.0	1.4	0.9	7.3
Pyridoxine (B_6)	0.2	±0.0	0.2	0.2	0.2	±0.0	0.2	0.2	ND	ND
Folic Acid (B_9)	0.09	0.01	0.07	0.12	0.08	0.01	0.07	0.10	ND	ND

[1]Phase 2, CDCS study
[2]Abdel-Aal et al., 2011a
ND-not determined

Table 9-12 Comparison of B Vitamin contents (mg/100g) in four cereal grains

Vitamin	Wheat [a]	Barley[b]	Field Maize[c]	Oats[d]
Thiamine (B_1)	0.13-0.99	0.12-1.6	0.23-0.86	0.77
Riboflavin (B_2)	0.06-0.31	0.08-0.07	0.025-.056	0.18
Niacin (B3)	2.20-11.10	4.6-14.7	0.93-7.0	1.8
Pyridoxine (B_6)	0.09-0.79	0.27-1.15	0.46-0.96	0.13
Folic acid (B_9)	0.02-0.09	0.019-0.03	0.017-0.045	0.06-0.07

[a] OECD, 2004, wheat
[b] OECD, 2003, barley
[c] OECD, 2002, maize
[d] Bock, 2000 (pg 482, Table 5)

9.1.2.5.2 Mineral Content

Cereals make up a significant dietary source of minerals and trace elements with cereals and cereal products in a typical Western diet contributing about 50% of the dietary manganese and iron, about 30% of copper and magnesium and about 20% of the zinc and phosphorous (Piironen *et al.*, 2009).

There are substantial differences in micronutrient concentrations in various grains depending upon type of grain, genotype, growing conditions and fertilizer application (Zhao *et al.*, 2009). In wheat, iron, zinc, copper and manganese contents are low. For many minerals (e.g. calcium, magnesium, copper, iron and selenium) the range in contents can be up to 10 fold (Piironen *et al.*, 2009). It appears soil type can cause more variation than the genotype or species. Table 9-13 provides examples of the micronutrient variation in four cereal grains–wheat, barley, field maize and oats.

Glabrous and pubescent canary seed cultivars had similar levels of major and trace minerals and all canary seed cultivars had significantly higher levels of phosphorous, sulphur, magnesium, calcium, iron, manganese and zinc than the CWRS wheat (Table 9-13). However, the values obtained for P, S, Mg, Ca, Fe, Mn and Zn are comparable to those reported in the literature for these nutrients in a number of wheat varieties (as given in Table 9-14). Glabrous canary seed exceeded oat and barley in phosphorous, magnesium and iron content.

Table 9-13 Comparison of the major mineral (mg/100g db) and trace mineral (mg/kg db) contents of glabrous brown and yellow canary seed to pubescent brown canaryseed and CWRS wheat

	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[1]	Wheat[1]
	Brown				Yellow				Brown	CWRS
	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Major Minerals (mg/100g)										
Phosphorous	645	±49	577	710	611	±38	540	660	590	430
Potassium	401	±33	349	443	370	±25	318	407	340	355
Sulfur	270	±23	242	305	270	±19	241	297	300	200
Magnesium	217	±10	200	233	210	±8	196	220	195	155
Calcium	32	±3	27	40	32	±5	24	41	40	20
Sodium	<MDL	-	<MDL	<MDL	<MDL	-	<MDL	<MDL	10	10
Trace Minerals (mg/kg)										
Iron	82	±16	64	110	77	±4	66	81	55	42
Manganese	51	±6	42	63	57	±6	48	68	71	59
Zinc	34	±2	30	39	30	±3	23	34	35	25
Copper	7	±1	5	22	6	±1	5	8	24	28
Nickel	3	±1	2	4	3	±1	2	3	3	0.3
Selenium	2	±1	2	4	2	±1	1	3	3	2

<MDL: less than method detection limit of 20 ppm

[1] Abdel-Aal *et al*, 2011a

[2] Phase 2, CDCS study

Table 9-14 Comparison of mineral content in four grain cereals

Mineral	Wheat[a]	Barley (whole grain)[b]	Field Maize[c]	Oats[b]
		Major Minerals (mg/100g, dry basis)		
Phosphorous	220-910	470	234-750	340
Potassium	280-730	630	320-720	460
Magnesium	20-220	140	82-1000	140
Calcium	10-80	90	3-100	95
Sodium	4.6	11.8	0-150	8.6
		Trace Minerals (mg/kg, dry basis)		
Iron	16-163	60	1.0-100	70
Manganese	10-90	18	NR	50
Copper	1.0-14.0	9	0.9-10	40
Selenium	0.4[d]	NR	0.01-1.0	NR
Zinc	15-102	40	12-30	39

*NR Information not reported in reference
[a]Piironen *et al.*, 2009
[b]Bock, 2000
[c]OECD, 2002
[d]Gawalko, 2002

9.1.2.6 Anti-nutrient Composition

Phytate, phenols, tannins, trypsin inhibitor, amylase inhibitor, glucosides and alkaloids may all be present in common cereal crops. The anti-nutrient composition of pubescent and glabrous canary seed cultivars compared to the CWRS wheat was evaluated with the following anti-nutrients being measured—phytate, total phenols, condensed tannins, trypsin inhibitor and amylase inhibitor (Table 9-15). Alkaloids are discussed in *Section 10, Chemical Considerations*.

Table 9-15 Comparison of anti-nutrient factor content in glabrous brown and yellow canary seed to pubescent brown canary seed and CWRS wheat

	Glabrous Canary Seed[1,2]								Pubescent Canary Seed[1]	Wheat[1]
	Brown				Yellow				Brown	CWRS
	Mean	SD	Range		Mean	SD	Range		Mean	Mean
			Min	Max			Min	Max		
Phytate (mg/g)	18.7	±3.4	14.1	22.3	18.2	±3.3	13.8	23.2	17.5	10.7
Total Phenols (mg/g)	1.79	±0.14	0.87	1.85	1.97	±0.07	1.89	2.09	0.83	0.81
Trypsin Inhibitor (TIU/mg)	0.50	±0.15	0.34	0.64	0.71	±0.10	0.60	0.90	0.51	0.47
Amylase Inhibitor (AIU/mg)	6.24	±2.45	2.8	10.06	5.56	±1.21	4.17	8.33	2.84	2.66
Condensed tannins	ND				ND				ND	ND

[1]Abdel-Aal *et al.*, 2011b; Li *et al*, 2010
[2] Phase 2, CDCS study
ND: not detected; TIU: Trypsin Inhibitor Units, AIU: Amylase Inhibitor units.

9.1.2.6.1 Phytate

Phytate (phytic acid and its salts) is found in the cotyledon of legumes and oilseeds or in the bran of cereal grains (Reddy & Sathe, 2002). It is considered an anti-nutrient due to its role in chelating mineral elements such as calcium and zinc in the human body. On the other hand, phytate is reported to have some potential beneficial effects such as its ability to lower blood glucose and its role in reducing plasma cholesterol and triacylglycerols, and cancer risk (Jenab & Thompson, 2002; Schlemmer *et al.*, 2009; Kumar *et al.*, 2010).

Glabrous and pubescent canary seed cultivars were found to contain about two times the phytate content of the control CWRS wheat. Phytate values for canary seed ranged from 13.8 to 23.2 mg/g (db) while the phytate content in the CWRS wheat was 10.7 mg/g (Table 9-15).

The content of phytate in cereals as reported by Anjum *et al.* (2002) and Hidvegi & Lasztity (2002) were 2.4-10.5 mg/g in wheat, 8.5-11.8 mg/g in barley and 9.0-14.2 g/mg in oat. As Table 9-16 illustrates, phytate levels in canary seed are within the range

found in common foods including whole grains, pulses, seeds and nuts. The amount of phytate can vary from 0.6 to 22 mg/g in cereals and 0.8 mg to 60 mg/g in cereal milled fractions and protein products (Reddy & Sathe, 2002). For instance, values reported for triticale are 5.0-18.9 mg/g; corn, 7.5-22 mg/g; wheat bran 25-58 mg/g; beans, 8.9-27 mg/g; and soybean 10-22.2 mg/g.

Other reported phytate values for common foods include edible nuts such as peanuts 1.7-44 mg /g (Schlemmer *et al.*, 2009); almonds 21.1 mg/g; cashews, 12.3 mg/g; pistachios, 28.4 mg/g and filberts 23.4 mg/g (Harland *et al*, 2004)].

Environmental fluctuations, growing location, soil type, fertilizer applications and year of growth influences the phytate content of seeds and grains (Reddy & Sathe, 2002).

Table 9-16 Phytate content of cereals, pulses and edible nuts			
Grain/Pulse/Edible Nuts	**Fraction**	**Phytate (mg/g)**	**Reference**
Canary seed	Whole groat	14.1-23.2	Abdel-Aal *et al.*, 2011b
Common Wheat	Whole grain	22	Harland & Oberleas, 1986
	Flour	3-8.24	Bos *et al.*, 1991, Tangkongchitr *et al.*, 1981
	Bran	25-58	Graf & Eaton, 1993
Durum wheat	Whole grain	9.8-14.3	Tabekhia & Donnelly, 1982
	Flour	4.5-7.2	
	Bran	23.3-43.2	
	Semolina	1.6-3.4	
Barley	Whole grain	9.8-11.5	Bos *et al.*, 1991
	Flour	6.93-8.45	Graf & Eaton, 1993
Oat		7.8-13.3	Miller *et al.*, 1980
Rice	Uncooked, ground	2.46-2.92	Harland & Oberleas, 1986
	Unpolished, cooked	12.7-21.6	Kumar *et al.*, 2010
Brown rice	Unpolished, uncooked	13.2	Moongngarm & Saetung, 2010
Sorghum	Pearled grain	2-13	Cilliers & Van Niekirk, 1986
	Whole grain flour	10.12	Garcia-Estepa et al., 1999
Rye		5.35-5.65	Kikunaga *et al.*, 1985.
Amaranth		5.2-6.1	Lorenz & Wright, 1984
		10.6-15.1	Kumar *et al.*, 2010
Buckwheat	Whole grain	9.2-16.2	Kumar *et al*, 2010
Pearl millet		1.79-3.06	Simwemba *et al.*, 1984. Kumar & Chauhan, 1993
Quinoa	Raw	10.5-13.5	Vega-Galvez et al., 2010
Soya	Dehulled	11.5	Thompson & Erdman, 1982
	Defatted	18.2	
Lentils	Whole	2.7-10.5	Reddy & Sathe, 2002
Peas	Whole	2.2-12.2	
Kidney beans	Whole	8.9-15.7	
Chickpeas	Whole	2.8-12.6	
Sesame seed	Roasted	39.3-57.2	Kumar *et al*, 2010
Peanut	Flour	15.6-19.4	Harland & Oberleas, 1986
	Whole	1.7-44	Schlemmer *et al*, 2009
Almonds	Oil roasted, blanched,	21.1	Harland *et al*, 2004
Cashews	Dry roasted	12.29	
Filberts	Shelled, dried	23.4	
Pistachios	Whole	28.35	

9.1.2.6.2 Total phenolics

Phenolic compounds are present in a variety of chemical forms in plants. The antinutritional properties of phenolics refer to their astringency and role in reducing the availability of certain minerals and amino acids. Conversely, phenolic compounds have antioxidant activity, which controls the oxidation of lipids (Naczk & Shahidi, 2006).

In Phase 1, canary seed groats (derived from the brown pubescent or glabrous cultivars) were found to have a total phenolic content (TPC) similar to that of the CWRS wheat, averaging 0.84 mg/g (Table 9-15).

In Phase 2, glabrous brown canary seed has significantly less total phenolic content (1.79 mg/g) than the glabrous yellow cultivar (1.95 mg/g) (Table 9-15) and the TPC levels in both brown and yellow cultivars in the Phase 2 study were about two times higher than those TPC values found in the Phase 1 for pubescent and glabrous cultivars tested. Variation in phenolic content is to be expected due to methodology (Zhou & Yu, 2004) and due to genotype and environmental effects (Moore *et al.*, 2006). A recent study on wheat phenolics showed that six Canadian wheat varieties grown in western Canada had mean total phenolics content ranging from 1.7-1.9 mg/g (db) (Mpofu *et al.*, 2006), higher than the CWRS wheat tested in Phase 1, but comparable to the TPC in the glabrous canary seed tested in Phase 2.

There is also a wide variation in total phenolics content amongst various grains. Whole grain rye ranges from 0.65-3.0 mg/g dm (Bondia-Pons *et al.*, 2009), barley ranges from 0.25-0.67 mg/g (db) (Andersson *et al.*, 2008), with millet containing 0.38 mg/g (dm), and sorghum 0.41 mg/g (dm) (Ragaee *et al.*, 2006). The HEALTHGRAIN study found differences between conventional wheats [spring (0.61mg/g); winter (0.66 mg/g db), durum (0.69 mg/g db)] and ancient wheats [spelt (0.58 mg/g db), einkorn (0.62 mg/g db) and emmer (0.78 mg/g db)] (Li *et al.*, 2008). TPC for wheat whole meal and wheat bran and flour ranged from 0.77 to 1.29 mg/g and 2.28 to 3.44 mg/g respectively. Thus the phenolic content of canary seed is within the range found in other food cereals.

The predominant phenolic acids in glabrous canary seed are ferulic, caffeic, sinapic and p-coumaric (Abdel-Aal *et al.*, 2011b; Li *et al*, 2010). Ferulic acid is the predominant phenolic acid in wheat and barley as well (Naczk & Shahidi, 2006).

Glabrous brown and yellow canary seed groats exhibit the same flavonoid profiles, being rich in flavonoid glycosides. High concentrations of O-pentosyl vitexin and O-pentosyl isovitexin were detected (Li *et al.*, 2011).

9.1.2.6.3 Condensed Tannins

Condensed tannins (proanthocyanidins) were not detected in glabrous canary seed as confirmed by analysis (Abdel-Aal *et al.*, 2011b; Li *et al*, 2011).

9.1.2.6.4 Other Phytochemicals

The carotenoid content of glabrous brown and yellow canary seed were determined in a project separate from the safety assessment but summary data are presented here to show the increased interest in investigating the attributes of canary seed as a new cereal food. Total carotenoid content in the whole meal canary seed ranged from 7.57 to 10.03 mg/kg with a mean of 9.21 mg/kg in the brown canary seed and ranged from 8.73 to 10.02 mg/kg with a mean of 9.34 mg/kg in the yellow varieties Li & Beta (2012). The major carotenoids detected in the glabrous brown and yellow varieties were β-carotene, lutein and zeaxanthin. On average, canary seed wholemeal contained 4946, 2316 and 530 µg/kg of β-carotene, lutein and zeaxanthin, respectively, in the brown cultivar, and 4974, 2238 and 440 µg/kg, respectively in the yellow cultivars. Li & Beta (2012) indicated that the total carotenoid content of glabrous canary seed was similar to the intermediate group of durum wheat (9.7-11.0 mg/kg), but that canary seed contained much higher levels of β-carotene compared to wheat (30-100 µg/kg), rice (66-150 µg/kg), and corn (49.2-458 µg/kg).

9.1.2.6.5 Enzyme Inhibitors

Trypsin and amylase inhibitors are found in raw cereal grains and legumes. These enzyme inhibitors have nutritional implications in human diet, but are typically not considered a problem because they are destroyed during the application of heat used in most cooking techniques.

Low levels of trypsin inhibitor were detected in pubescent (0.5 TIU/mg) and glabrous canary seed cultivars (0.51-0.71 TIU/mg) and the CWRS wheat (0.47 TIU/mg) (Table 9-15; Abdel-Aal *et al.*, 2011b) compared to 30.26 TIU/mg in soybean, a rich source of trypsin inhibitor (data not shown). Soybean was included as a sample check due to its high trypsin inhibitor activity.

In Phase 1, amylase inhibitor activity was measured in canary seed and wheat grains using soluble starch as a substrate and pure α-amylase with and without inhibitor extracts (Mulimani and Supriya, 1993). Canary groat and common wheat had similar α-amylase activities with means ranging from 2.66 AU/mg for wheat to 2.8 AIU/mg for canary seed (Table 9-15; Abdel-Aal *et al.*, 2011b).

In Phase 2, a slightly different method was used to determine alpha-amylase inhibitor activity. The inhibitory activity was measured by the decrease of α-amylase activity from the inhibitors using soluble starch as a substrate and pure α-amylase (from *Bacillus licheniformis*, Sigma) based on method by Deshpande *et al.* (1982). This is likely why slightly higher α-amylase inhibitor activities (5.47 to 6.24 AIU/mg) for the glabrous brown and yellow cultivars are being reported (Table 9-15).

9.1.3 Nutrient Composition of Processed Canary Seed Groats

As discussed in Section 5.0 *Manufacturing,* the Canaryseed Development Commission of Saskatchewan contracted with Food Technology Centres in Canada to optimize the post-harvest processing of dehulled canary seed, determine the nutritional composition and shelf stability of processed canary seed and develop prototype food products with canary seed ingredients.

Nutrient analysis was conducted on brown and yellow canary seed groats subjected to combinations of mild heat, tempering conditions and roasting treatments. Brown and yellow canary seed groats were subjected to the following treatments:

1) Treatment 1: No tempering, heat treatment: 240°F, 8 minutes;
2) Treatment 2: Tempering (to 14% moisture), heat treatment: 240°F, 8 minutes;
3) Treatment 3: No tempering, roasting 350°F, 10 minutes;
4) Treatment 4: Tempering (to 14% moisture), roasting 350°F for 8 minutes.

Nutrient composition results are presented in Table 9-17. The nutritional composition of processed glabrous canary seed was similar to that of the raw groats (as described in the previous section 9.1.2) indicating that processing does not change the nutritional profile of canary seed groats.

Phytate content was measured in the whole meal flours produced from processed canary seed groats. Compositional analyses on raw canary seed groats indicated phytate levels ranged from 14.1 mg/g to 23.2 mg/g (equivalent to 1.4% to 2.3%) (Table 9-15). Phytate levels in whole meal flours produced from treated canary seed groats ranged from 1.8% to 2.7% (Table 9-17), well within the range of phytate values for commonly consumed cereals, pulse and nuts (Table 9-16). Since phytates are heat stable and are not easily removed by cooking, autoclaving, roasting or other conventional heat processing methods (Venkatachalam & Sathe, 2002), a reduction in phytate levels in heat treated canary seed groats was not expected.

Table 9-17 Nutritional composition (per 100 g db) of processed glabrous brown and yellow canary seed groats

	Glabrous Canary Seed[1]							
	Brown				Yellow			
Nutrient	No tempering, heat treated	Tempering, heat treated	No tempering, Roasted	Tempering, Roasted	No tempering, heat treated	Tempering, heat treated	No tempering, Roasted	Tempering, Roasted
Calories, Total (per 100g, db)	431.9	431.5	436.8	438.5	432.2	429.3	434.4	434.7
Protein (g)	22.1	21.1	23.5	22.3	20.7	20.7	21.1	20.2
Carbohydrates (g)	65.9	67.4	64.8	65.4	67.8	68.7	68.0	68.5
Fat (g)	8.9	8.6	9.3	9.8	8.7	8.1	8.7	8.8
Ash (g)	2.8	2.6	2.9	2.8	2.7	2.7	2.7	2.8
Saturated Fatty Acids (g)	1.3	1.4	1.3	1.5	1.2	1.1	1.2	1.2
trans-Fatty Acids (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cholesterol (mg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dietary Fiber (g)	8.1	8.2	8.4	9.2	7.9	8.1	8.5	9.1
Sugars (g)	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.6
Fructose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Galactose/Glucose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sucrose (g)	0.5	0.4	0.6	0.6	0.5	0.4	0.6	0.6
Maltose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactose (g)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin A (IU)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vitamin C (mg)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sodium (mg)	1.1	1.6	1.5	1.6	1.0	1.8	1.4	1.5
Calcium (mg)	38.5	40.4	40.6	39.0	39.6	40.4	40.5	43.2
Iron (mg)	6.4	6.9	7.1	6.5	6.3	6.6	7.5	6.9
Canary Seed Flours Phytate (%)	2.0±0.2	1.9±0.2	2.3±0.2	2.2±0.1	2.1±0.2	1.8±0.2	2.3±0.2	2.7±0.1

1 Phase 2 CDCS study, not published

9.1.3.1 Nutrient Composition of Prototype Food Products

To provide examples of the nutrition composition of potential food products, nutrition fact tables (NFT) were generated for two products a) unbaked nutrition bars containing roasted canary seed groats incorporated at a 5%, 10%, 15%, 20% and 25% inclusion rates (Table 19-18); and b) muffins containing 7% roasted whole grain yellow canary seed flour (replaced 20% of the refined wheat flour in formulation) (Table 19-19).

Results of the canary seed chemical analyses (as shown in Table 9-17) were input into a Genesis® R & D SQL nutrient database to generate Canadian nutritional facts tables. Since there was little difference in the nutritional composition of the brown and yellow canary seed groats, NFTs were only generated for bars containing roasted glabrous brown canary seed groats. The nutritional composition of the bars as shown by the nutrition fact tables in Figure 19-1 was essentially unchanged by increased levels of canary seed. A NFT was generated for a muffin containing roasted yellow canary seed flour (Figure 19-2).

Table 19-18 Formulation (%) for prototype unbaked nutrition bar at differing inclusion levels of brown or yellow, roasted canary seed groats[1].

	Canary Seed Inclusion Level (%)				
Ingredients	**5**	**10**	**15**	**20**	**25**
Brown rice syrup	19.2	19.2	19.2	19.2	19.2
Honey	10.7	10.7	10.7	10.7	10.7
Canola oil	3.8	3.8	3.8	3.8	3.8
Monoglycerides	1.0	1.0	1.0	1.0	1.0
Canary seed	**5.0**	**9.9**	**14.9**	**19.8**	**24.8**
Quick oats	21.9	17.0	12.0	7.1	2.1
Oats #5	18.6	18.6	18.6	18.6	18.6
Rice crisps	4.4	4.4	4.4	4.4	4.4
Cranberries	7.6	7.6	7.6	7.6	7.6
Pecan pieces	7.6	7.6	7.6	7.6	7.6
Cinnamon	0.2	0.2	0.2	0.2	0.2

[1]Phase 2 CDCS study, not published

Figure 9-1 Nutritional fact tables for nutrition bars with differing inclusion level of roasted brown canary seed[1]

5% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	6 %
Saturated / saturés 0.5 g + Trans / trans 0 g	3 %
Omega-6 / oméga-6 0.8 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	1 %
Carbohydrate / Glucides 20 g	7 %
Fibre / Fibres 2 g	8 %
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	4 %

10% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	6 %
Saturated / saturés 0.5 g + Trans / trans 0 g	3 %
Omega-6 / oméga-6 0.9 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	1 %
Carbohydrate / Glucides 20 g	7 %
Fibre / Fibres 2 g	8 %
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

15% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 120	
Fat / Lipides 4 g	6 %
Saturated / saturés 0.5 g + Trans / trans 0 g	3 %
Omega-6 / oméga-6 0.9 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	1 %
Carbohydrate / Glucides 20 g	7 %
Fibre / Fibres 2 g	8 %
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

20% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 130	
Fat / Lipides 4 g	6 %
Saturated / saturés 0.5 g + Trans / trans 0 g	3 %
Omega-6 / oméga-6 1 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	1 %
Carbohydrate / Glucides 20 g	7 %
Fibre / Fibres 2 g	8 %
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

25% level

Nutrition Facts / Valeur nutritive	
Per 1 bar (30 g) / par 1 bar (30 g)	
Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 130	
Fat / Lipides 4 g	6 %
Saturated / saturés 0.5 g + Trans / trans 0 g	3 %
Omega-6 / oméga-6 1 g	
Omega-3 / oméga-3 0.1 g	
Monounsaturated / monoinsaturés 2 g	
Cholesterol / Cholestérol 0 mg	
Sodium / Sodium 15 mg	1 %
Carbohydrate / Glucides 20 g	7 %
Fibre / Fibres 2 g	8 %
Sugars / Sucres 7 g	
Protein / Protéines 2 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	2 %
Iron / Fer	6 %

[1]Phase 2 CDCS Study, not published

Roasted, whole yellow canary seed flour replaced all purpose wheat flour at 10%, 15% and 20% in a muffin formula. The formula and the nutrition facts table for muffins containing ~ 7% roasted ground whole grain yellow canary seed flour (20% replacement of all purpose flour) is presented in Table 19-19 and Figure 19-2, respectively.

Table 9-19 Formulation (%) for prototype muffin containing roasted whole ground yellow canary seed flour at 20% replacement levels of all purpose flour[1]

Ingredient	%
Roasted Canary seed Flour	*7.10*
All purpose flour	*28.38*
2% Milk	*23.08*
Canola Oil	*10.60*
Sugar	*19.07*
Whole Egg	*9.46*
Baking Powder	*1.74*
Salt	*0.57*
TOTAL	*100.00*

[1]Phase 2 CDCS Study, not published

Figure 9-2 Nutritional facts table for prototype muffins containing ~7% roasted whole ground canary seed flour [Phase 2 CDCS Study, not published)

Nutrition Facts
Valeur nutritive
Per 1 muffin (43 g) / pour 1 muffin (43 g)

Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 170	
Fat / Lipides 6 g	**10** %
Saturated / saturés 0.5 g + Trans / trans 0 g	**4** %
Polyunsaturated / polyinsaturés 2 g	
Omega-6 / oméga-6 1.5 g	
Omega-3 / oméga-3 0.5 g	
Monounsaturated / monoinsaturés 3.5 g	
Cholesterol / Cholestérol 20 mg	
Sodium / Sodium 190 mg	**8** %
Carbohydrate / Glucides 23 g	**8** %
Fibre / Fibres 1 g	**3** %
Sugars / Sucres 9 g	
Protein / Protéines 4 g	
Vitamin A / Vitamine A	0 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	6 %
Iron / Fer	8 %

9.1.3.2 Food Grade Specifications

Based upon the data provided in this dossier, the food grade specifications outlined in Tables 9-20 and 9-21 could be used as guidelines for the introduction of glabrous canary seed into the food market. It is expected that the values for proximate analysis may vary from those given in this table due to cultivar and environmental conditions, similar to that experienced in other cereal grains.

Table 9-20 Physical and chemical properties of canary seed

Physical Standard	Whole Groat	Milled
Appearance	Uniform brown or yellow colour	Uniform yellow colour/ uniform brown colour with or without darker flecks
Odour	No off odors	No off odors
Texture	Smooth, free-flowing granulation	Free-flowing powder
Bulk density	c. 65-70 kg/hl	c.41 kg/L (loose)
Particle sizes	Various depending upon size of kernel	Various

Table 9-21 Food Grade Specifications Whole Grain Canary Seed (dry basis)[a]			
Parameter	Unit	Specification Whole Groat	Specification Milled
Proximates			
Protein (N x 5.7)	(%)	18-25	18-25
Carbohydrates	(%)	68-72	68-72
Ash	(%)	1.9-2.6	1.9-2.6
Dietary fiber	(%)	5.9-10.2	5.9-10.2
Total Fat	(%)	5.5-6.4	5.5-6.4
Heavy metals[b]			
Lead	mg/kg	<0.2	<0.2
Cadmium	mg/kg	<0.2	<0.2
Mercury	mg/kg	<0.1	<0.1
Arsenic	mg/kg	<0.2	<0.2
Microbial[c]			
Aerobic plate count	CFU[d]/g	$<10^6$	$<10^6$
Coliforms	CFU/g	$<10^4$	$<10^4$
Yeast/Mold	CFU/g	$<5 \times 10^3$	$<5 \times 10^3$
Pathogens (*E.coli, Salmonella, S. aureus*	Absent	Absent	Absent

[a] Specifications defined based upon data presented in this dossier. Values may vary from year to year depending upon cultivar and environmental conditions
[b] As recommended by Codex Alimentarius, 2007
[c] As identified in ICMSF, 2005
[d] CFU: colony forming units

9.1.4 Nutritional Summary

From a nutritional perspective, glabrous canary seed would provide macro- (protein, starch, fat) and micronutrients (vitamins and minerals) at levels comparable to other cereal grains such as wheat, barley, oats and rye. Dietary fiber levels are similar to millet but lower than some of the other grains. Canary

seed contains approximately 19-22% protein, 5-7% crude fat, 2% ash, 55% starch and 6-10% dietary fiber. Similar to other cereal grains, the proteins in canary seed are deficient in lysine but rich in cystine, tryptophan, and phenylalanine, which could make them good complements to legumes. Canary seed contains the B vitamins, thiamine and riboflavin, at levels comparable to other cereals. Niacin levels are lower than in wheat and barley but similar to oat. Total folate content in canary seed is higher than the common cereals (wheat, barley and oats) and similar to the pseudocereals, amaranth and quinoa. Of the antinutritional compounds present in canary seed, the level of phytate was about two times higher than the tested CWRS wheat but still within the range of phytate content of other commonly consumed foods such as some cereals, pulses and edible nuts.

9.2 Nutritional Bioavailability

Canary seed is being introduced as a new cereal grain. As indicated in Section 8.0 *History of Use*, canary seed has limited history as a human food. Consequently there is limited information about its nutritional bioavailability in the scientific literature.

In vitro protein digestibility was evaluated as part of the Phase 1 study, and in Phase 2 the *in vitro* protein digestibility of thermally treated canary groats was studied. Two 90-day oral toxicity trials, one trial conducted in each Phase, and a 28-day study in Phase 2 evaluated the effect of consuming canary seed on growth of rats.

9.2.1 *In vitro* Protein digestibility

Digestibility of proteins is a factor that impacts nutritional value.

The *in vitro* digestibility of canary seed (84) is comparable with that of other plant protein sources (WHO, 2007) – [e.g. corn (87); wheat (86); oat (86); soy flour (86); and higher than other specialty grains such as millet (79) (WHO, 2007), amaranth (74) (Bejosana & Corke, 1998) and buckwheat (79.9) (Wijngaard & Arendt, 2005) but less than that of casein (95) (WHO, 2007).

Rajamohamed and coworkers (2013) examined the *in vitro* protein digestibility of canary seed under simulated gastrointestinal conditions and evaluated the impact of thermal treatment on protein digestibility. *In vitro* digestibility of yellow and brown canary seed proteins from raw groats, roasted groats (176°C (348.8°F), 12 min) and boiled (98°C (208.8° F), 12 min) flours under gastric, duodenal and sequential gastric–duodenal conditions was evaluated according to the method outlined in Rajamohamed *et al.*, (2013). The results indicated the canary seed proteins were digested more easily under sequential gastric–duodenal conditions than under gastric or duodenal conditions alone. Roasting of canary seeds altered the electrophoretic profile of the proteins and resulted in fainter bands compared to those observed for boiled and raw canary seeds. Thermal processing generally improved canary seed protein digestibility.

9.2.2 Rodents

The effect of consumption of glabrous canary seed on growth of rats was assessed in the Phase 1 90-day oral toxicity study and the Phase 2 28-day and 90-day oral toxicity studies (Magnuson *et al.*, 2014).

The objective of the Phase 1 study was to compare the growth and toxicological effects of glabrous (hairless) hulled and glabrous dehulled brown canary seed (CDC Maria) with the parent pubescent (hairy) hulled canary seed (Keet) and a common grain, Canada Western Red Spring (CWRS) wheat (CDC Teal), at maximal tolerable levels (50%) in the diet.

The objective of Phase 2 was to compare the growth and toxicological effects of the glabrous brown versus glabrous yellow canary seed cultivars. In Phase 2, the test diets included three concentration levels of dehulled yellow groats cultivar (C05041) and one concentration of dehulled glabrous brown (CDC Maria) compared to the AIN-76 rodent reference diet. Relevant nutritional information from the rodent studies is presented in the following summary tables (Phase 1, Table 9-22; Phase 2, Tables 9-25 and 9-26) and discussion.

Toxicological endpoints for these studies will be discussed in *Section 10: Toxicological Considerations*.

Table 9-22 Summary of 90-day rat study (CTR0012) (Phase 1)[1]

Objective: to compare the toxicological and growth effects of glabrous brown canary seed (CDC Maria) and pubescent brown parent (Keet) with that of CWRS (CDC Teal) wheat in rats

- Protocol followed OECD Test Guideline No.408
- 4-week old Sprague-Dawley rats (male and female); n=10/sex/group (total 80 rats)
- 4 diet groups:
  - Diet 1: 50% dehulled glabrous brown canary seed (CDC Maria)
  - Diet 2: 50% hulled glabrous brown canary seed (CDC Maria)
  - Diet 3: 50% hulled pubescent brown canary seed (Keet)
  - Diet 4: 50% CWRS wheat (CDC Teal) (control diet)
- All test diets provided the same amount of apparent metabolizable energy (AME) (3,500 kcal/kg) and crude protein (20%). Crude fat content of the diets varied 9.0%, 10.4%, 10.6% and 9.4% for Diets 1, 2, 3 and 4, respectively.
- Diets also contained corn and soybean meal in varying amounts.
- Water and test diet fed *ad libitum* for 90 days

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- Feed consumption data showed no difference among the various diet regimens
- Male rats had higher weight gain on dehulled glabrous canary seed than on the hulled glabrous canary seed or hulled pubescent Keet, but gain was similar to that of the CWRS wheat diet. There was no difference in weight gain among female rats on the diets.

Conclusions

- Values for feed consumption and body weight gain in rats fed diets containing 50% canary seed were comparable to values when fed a 50% wheat diet.

[1]Magnuson *et al.*, 2014

In Phase 1 a 90-day oral toxicity study was performed on Sprague Dawley rats using glabrous hulled canary seed and dehulled canary seed (groats) (CDC Maria), pubescent hulled canary seed (Keet) and CWRS wheat (CDC Teal) as the test ingredients according to OECD Test Guideline 408 for "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD, 1998). This study consisted of two identical trials staggered 8 days apart to make sample collection within a one-day period more manageable. Only one test ingredient concentration level (50%) was studied (limit test). The nutritional and compositional information on canary seed and its similarity to wheat composition did not show any potential

toxic elements (Section 9.1, Nutrient Composition). Complete Phase 1 study details are available in Appendix 4.

Four groups of animals, each consisting of 10 males and 10 females, were fed diets containing 50% CWRS wheat (control), 50% glabrous brown canary seed groats (CDC Maria); 50% glabrous hulled (CDC Maria) canary seed or 50% pubescent hulled canary seed cultivar (Keet). Diets (Table 9-23) were formulated to contain 3500 kcal/kg AME, 20.00% crude protein, 0.75% calcium, 0.15% sodium, 0.0781% choline, 1.20% lysine, 0.65% methionine and 0.80% threonine to meet or exceed the requirements for rat reproduction (National Research Council, 1995). All diets were provided in mash form.

Table 9-23 Specifications for formulation of rodent test diets[1]

Ingredients (%)	Diet Treatment			
	No. 1 Dehulled Glabrous canary seed	No. 2 Hulled Glabrous canary seed	No. 3 Hulled Pubescent canary seed	No. 4 CWRS wheat
Dehulled glabrous canary seed	50.0			
Hulled glabrous canary seed		50.0		
Hulled pubescent canary seed			50.0	
CWRS wheat				50.0
Corn	29.51	23.63	17.75	19.46
Soybean meal-48	11.22	14.90	21.20	18.41
Canola oil	4.65	6.83	6.73	7.73
Dicalcium phosphate[2]	0.72	0.79	0.83	0.88
Limestone	1.58	1.53	1.47	1.47
Sodium chloride	0.34	0.34	0.34	0.34
Vitamin/mineral premix[3,4]	0.50	0.50	0.50	0.50
Choline Chloride	0.15	0.15	0.15	0.15
DL-Methionine	.034	0.37	0.36	0.36
L-Threonine	0.24	0.25	0.17	0.17
L-Lysine HCl	0.74	0.71	0.48	0.53

[1]Magnuson *et al.*, 2014
[2]Dicalcium phosphate (15% Ca, 21% P)
[3]Supplied per kg diet: vitamin A (retinal acetate + retinyl palmitate), 11000 IU; vitamin D, 2200IU; vitamin E (dl-α-tocopherol acetate), 30 IU; menadione, 2.0 mg; thiamine, 1.5 mg, riboflavin, 6.0 mg; niacin, 60 mg;, pyridoxine, 4.0 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; biotin, 0.15 mg, ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[4]Supplied per kg feed: iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

Final body weight, weight gain and feed consumption are shown in Table 9-24. Total mean feed consumption data showed no difference between the various diet treatments for male or female rats; males consuming on average 1.9 to 2.1 kg and females 1.4 kg over the 90 day trial. Males fed the glabrous canary seed groats had higher final body weights and greater mean body weight change over the 90 days than those fed the glabrous hulled canary seed or the pubescent hulled canary seed, but were not statistically different from rats fed the control CWRS wheat diet. A similar trend was observed for females, but differences were not statistically significant. Higher weight gain in rats fed glabrous dehulled canary seed with similar intake as hulled canary seed is likely due to higher caloric value of feed per gram due to removal of hulls and lower indigestible fiber.

Males consumed 34, 33, 37 and 35 g per kg body weight per day of the CWRS wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively. Females consumed 43, 38, 42 and 42 g per kg body weight per day of the CWRS wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively.

Table 9-24: Summary of body weights, body weight changes and food consumption in the 90-day rat feeding study (Phase 1)[1]

		Body Weight Means[2] ±SD (g)				Body Weight Change (g)[3]	Total Feed Consumed (g)
Test diet	Sex	Day 1	Day 28	Day 56	Day 90		
50% dehulled glabrous canary seed[2]	M	97±5	329± 20	471 ±35	**572[a] ±52**	**475[a] ±51**	1994 ± 138
50% hulled glabrous canary seed[3]	M	98± 6	320± 31	431±29	**514[b] ±42**	**416[b] ± 38**	2058 ± 147
50% hulled pubescent canary seed[4]	M	97±12	302± 27	422± 35	**517[b] ± 55**	**419[b] ± 49**	1973 ± 188
Control - 50% Wheat[5]	M	97±7	316± 13	445± 15	**536[ab]± 21**	**439[ab] ± 20**	1980 ± 100
50% dehulled glabrous canary seed	F	89 ±7	202±13	262± 40	290 ±25	201 ±26	1302 ± 82
50% hulled glabrous canary seed	F	89± 5	196± 8	238 ±16	271± 29	182± 28	1366 ± 117
50% hulled pubescent canary seed	F	90± 5	189± 21	236± 20	267± 29	178± 30	1372 ± 162
Control - 50% Wheat	F	88±9	199± 17	243± 24	265± 27	177± 28	1364 ± 88

[1]Magnuson *et al.*, 2014
[2]n=10
[3]Mean in the same column with different letters are significantly different at P<0.05.

The Phase 2 rat studies evaluated the effects of consumption of glabrous yellow canary seed groats incorporated into diets at concentrations levels of 2.5%, 5% and 10% and of glabrous brown canary seed groats incorporated into diets at a concentration level of 10%. Rats were fed diets *ad libitum* over 2 time periods a) a 28-day period (Table 9-25) and b) a 90-day period followed by a 30-day recovery period (Table 9-26). Test diets were in the form of hard cold-pressed rodent chow pellets. The 28-day trial was used to establish testing parameters for the pivotal 90-day study. The studies were conducted by NucroTechnics and monitored by Cantox Intertek. The study reports for the 28-day and 90-day studies are described in Appendix 5a & 5b, respectively.

The rationale for the Phase 2 28-day and 90-day rodent study design was as follows:

- The objective of the novel food initiative is to obtain regulatory approval for use of glabrous brown and yellow cultivars in human foods.
- Glabrous yellow cultivars had not been evaluated in the Phase 1 rodent trial. There were no significant differences in the nutritional composition between brown and yellow glabrous canary seed, and only minor differences in antinutritional compounds, indicating high nutritional value and low toxicity.
- The Phase 1 90-day rat feeding study had shown no significant differences in growth or adverse toxicological effects in rats fed the glabrous brown cultivar or the pubescent parent brown cultivar as compared to CWRS wheat, when added to the diet at a level of 50%. Only one dose level of canary seed was evaluated.
- A standardized approach to the safety assessment of novel food ingredients is to determine the dose-response of any effects of consumption of the ingredients added to a standardized diet as compared to animals fed the standardized diet.
- Limited histopathology had been conducted in the Phase 1 90-day study, thus the brown cultivar was also included in the Phase 2 study.
- The dehulled form (groat) of glabrous canary seed is to be consumed by the human population, not the hulled form (with hull).
- Dietary levels of 2.5%, 5% and 10% were chosen for several reasons:

- When testing whole foods, using high concentrations presents the potential for inducing nutritional imbalances.
- Toxicology studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake (Health Canada, 2006).
- The high concentration, 10%, was chosen to reflect consumption levels higher than that targeted for the American population. Based upon the potential human consumption values obtained from the human dietary exposure assessment conducted in Phase 2 (Section 14 *Dietary Exposure*) using conservative and optimistic assumptions, the highest users (90^{th} percentile) in the general population were estimated to consume 1.7 g glabrous canary seed per kg body weight (BW) per day (Section 14 *Dietary Exposure*). The results of the Phase 1 90-day feeding study indicated that male and female rats consumed on average 33 g and 38 g glabrous dehulled canary seed/kg/day, respectively, when 50% of the test diet was canary seed. Using female intake (38 g/kg/d), if similar food intake occurs, a 10% concentration would result in consumption of 7.6 g/kg/day, which is about 5-fold the 90^{th} percentile intake expected by the human population. The lower doses were included to assess dose-response of any observed effects.

Table 9-25 Summary of the 28-day study in Sprague Dawley rats with brown and yellow canary seed groats (Phase 2)[1]

Objective: to compare the toxicological and growth effects of dehulled glabrous brown with dehulled glabrous yellow canary seed (groats)

- Protocol followed OECD Test Guideline N0. 408
- 5 groups of male and female Sprague-Dawley rats (25 male and 25 female/test diet), each group consisting of 5 male and 5 female rats (Strain: Crl:CD (SD)BR-Sprague-Dawley)
- 5 diet groups:
  - Diet 1: Control: AIN-76A (0% canary seed)
  - Diet 2: 2.5% dehulled glabrous yellow canary seed (C05041)
  - Diet 3: 5.0% dehulled glabrous yellow canary seed (C05041)
  - Diet 4: 10% dehulled glabrous yellow canary seed (C05041)
  - Diet 5: 10% dehulled glabrous brown (CDC Maria)
- Diets were formulated by Research Diets, Inc (New Jersey, U.S.A) to contain 20% protein and 5% fat and 3.9 kcal/g. Equivalent protein, carbohydrate, fat and fiber levels were achieved by varying levels of casein, corn starch, corn oil and cellulose. The diets were assessed for protein, fat, sugar profile, vitamin A and vitamin D_3 at the beginning and end of the experiment to confirm formulation and stability.
- Water and test diets fed *ad libitum* daily for 28 days.

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- No significant differences in body weight and body weight gains, gender matched, between the control and test diets
- No apparent differences in feed consumption between control and test diets.
- Slightly lower feed consumption was noted for males and females in the latter days of study, but body weights were not affected.

Conclusions:

- Rats fed diets containing 2.5%, 5% and 10% yellow and 10% brown canary seed showed no significant differences in body weight and body weight gains compared to the control diet throughout the study period indicating canary seed was nutritionally adequate.

[1]Magnuson *et al.*, 2014

Table 9-26 Summary of the 90-day study in Sprague Dawley rats with brown and yellow canary seed groats (Phase 2)[1]

Objective: to compare the toxicological and growth effects of dehulled glabrous brown canary seed (CDC Maria) with dehulled glabrous yellow canary seed (C05041) in rats

- Protocol followed OECD Test Guideline NO. 408
- 5 groups of male and female Sprague-Dawley rats (35 male and 35 female/test diet) consisting of 20 M/F in main group, 10M/F in satellite group and 5M/F in recovery group (30 days on control diet).
- 5 diet groups:
  - Diet 1: Control: AIN-76A (0% canary seed)
  - Diet 2: 2.5% dehulled glabrous yellow canary seed (C05041)
  - Diet 3: 5.0% dehulled glabrous yellow canary seed (C05041)
  - Diet 4: 10% dehulled glabrous yellow canary seed (C05041)
  - Diet 5: 10% dehulled glabrous brown (CDC Maria)
- Diets were formulated by Research Diets, Inc (New Jersey, U.S.A) to contain 20% protein and 5% fat and 3.9 kcal/g. Equivalent protein, carbohydrate, fat and fiber levels were achieved by varying levels of casein, corn starch, corn oil and cellulose. Each diet preparation was assayed for protein, fat, sugar profile, vitamin A and vitamin D_3 at the beginning and end of the experiment to confirm formulation.
- Water and test diets fed *ad libitum* daily for 90 days followed by a 30-day recovery period.

Measured endpoints for growth evaluation:

- Body weight and feed consumption

Results:

- No significant differences in body weights and weight gain among diet groups, except for the body weights of male rats fed the 10% yellow canary seed; which were lower than controls at the end of the study.
- Food consumption for this group was also lower in the latter days of study
- Normalization of body weights at Day 91 per total feed consumption showed no differences between treatment groups and the control group.

Conclusions:

- Rats fed diets containing 2.5%, 5% and 10% glabrous brown canary seed showed no differences in body weight and body weight gains compared to the control diet throughout the study period. A reduction in food consumption in males fed and 10% glabrous yellow canary seed in the latter weeks of the study resulted in reduced body weight compared to controls. Overall the study indicated canary seed was nutritionally adequate.

[1]Magnuson *et al.*, 2014

In the 28-day study, there were no statistical differences in body weights and body weight gains, gender matched, among the control and test groups. Food consumption mirrored the body weight gains. There were no appreciable differences in food consumption amongst the groups (see study report, Appendix 5a). Food spillage did not indicate an excessive wastage of food. The feeding regimen of glabrous canary

seed at levels of 2.5%, 5% or 10% a*d libitum* for 28 days corresponded to average dose levels (gender combined) of 1.8, 3.6 and 7.0 g of yellow canary seed groat for the four concentration levels, respectively, and 6.9 g of brown canary seed groat per kg body weight per day.

In the Phase 2 90-day, statistical analysis of body weights and weight gain (ANOVA; p=0.05) showed no differences amongst the groups except the body weights of male animals in the group fed 10% yellow canary seed groat were lower at Day 85 (7% of control) and Day 90 (8% of control) (Table 9-27). Feed consumption mirrored the body weight gains. There were no apparent differences in total feed consumption amongst the groups, although feed consumption was significantly reduced in male animals fed the 10% yellow canary seed groat at during Days 78 to 85 (10% of control) and Days 85 to 90 (11% of control). Normalization of body weights at Day 91 per total feed consumption showed no differences between treatment groups and the control group. Furthermore, as is discussed in toxicological considerations (Section 10), the reduced body weight in males fed 10% yellow canary seed as compared to male rats fed the control diet was not associated with any adverse biochemical or histological effects. In contrast, male rats fed the 10% yellow canary seed diet has lower incidence and severity of liver lipidosis (fatty liver) as compared to male rats fed the control diet. Liver lipidosis is a common finding in laboratory rats that are fed *ad libitum* and tend to become obese. Thus, the slightly lower body weights and food consumption levels in male rats fed the 10% yellow canary seed diet at the end of the study period, are not considered adverse or to have toxicological effects.

Table 9-27 Summary of body weights, body weight changes and food consumption in the 90-day rat study (Phase 2) [1,2]

Group	Sex	Body Weight Means ± S.D. (g)				Mean Body Weight Change (Day 1 to 90) (g)	Mean Total Food Consumption (kg)
		Day 1[3]	Day 29[3]	Day 57[4]	Day 90[4]		
1. Control Diet	M	318 ± 17	492 ± 34	597 ± 49	668 ± 64	350 ± 56	2.4 ± 0.2
	F	220 ± 16	303 ± 26	346 ± 37	366 ± 46	146 ± 38	1.6 ± 0.2
2.Low Dose (2.5% Yellow canary seed)	M	311 ± 23	489 ± 44	596 ± 66	666 ± 84	355 ± 65	2.4 ± 0.3
	F	216 ± 14	297 ± 27	349 ± 28	373 ± 35	155 ± 28	1.6 ± 0.2
3.Mid Dose (5% Yellow canary seed)	M	316 ± 23	498 ± 44	602 ± 58	665 ± 81	352 ± 68	2.4 ± 0.2
	F	216 ± 16	295 ± 28	335 ± 38	351 ± 39	134 ± 32	1.5 ± 0.2
4.High Dose (10% Yellow canary seed)	M	310 ± 23	478 ± 36	560 ± 44	615* ± 56	311 ± 43	2.3 ± 0.2
	F	216 ± 14	296 ± 24	341 ± 31	359 ± 35	141 ± 28	1.6 ± 0.1
5.High Dose (10% Brown canary seed)	M	311 ± 21	491 ± 41	610 ± 47	687 ± 62	376 ± 52	2.5 ± 0.2
	F	216 ± 14	298 ± 30	340 ± 36	363 ± 41	148 ± 34	1.6 ± 0.2

[1] Magnuson *et al.*, 2014
[2] Prestudy body weights were also recorded but were not reported (they are recorded in the raw data)
[3] n = 35
[4] n = 25; as noted in Section 11, a satellite group of 10 rats per group were terminated after 6 weeks on diet.
* Statistically significant from Control Group ($p<0.05$).

Feed consumption in females was similar in the canary seed diet groups when compared to control animals with the exception of female rats in the mid-dose group experiencing statistically significant lower feed consumption during Days 64 to 71 (10% of control) and Days 85 to 90 (15% of control) and female rats in the low-dose group experiencing lower feed consumption (10% of control) during the period from Days 85 to 90. Given the fact that the body weights in these groups were not affected and no adverse effects were observed, this effect was considered to be of no toxicological significance. As observed in male rats, female rats fed the 10% canary seed diets also

displayed reduced incidence of liver lipidosis, further indicating that the lower feed consumption levels at the end of the study period did not adversely affect animal health.

The 90-day feeding regimen corresponded to average dose levels (gender combined) of 1.30, 2.54 and 5.15 g of yellow canary seed groats or 5.23 g of brown canary seed groats per kg per day, for the four dose levels, respectively.

Rodent Trials Summary

In summary, the results of the Phase 1 and Phase 2 rodent trials indicate that rodents fed diets containing 2.5%, 5% and 10% glabrous brown canary seed groats or diets containing 50% glabrous hulled or dehulled brown canary seed or pubescent hulled canary seed showed no differences in body weight and body weight gains compared to the control diets throughout the study periods. The only significant finding was a reduction in body weight of male rats fed 10% glabrous yellow canary seed, which was attributed to a reduction in food consumption during the latter period of the study. Overall, the results of these studies indicate that canary seed is nutritionally adequate.

9.2.3 Swine

Two studies evaluating canary seed as a potential feed for growing-finishing swine have been reported (Thacker, 2003; Qiao and Thacker, 2004). As the pig is considered to have very similar digestive system to man, these studies are particularly helpful in assessing the nutritional properties of canary seed as a human food. The first study, summarized in Table 9-28, evaluated growth of pigs fed graded levels of pubescent canary seed (cv. Elias) in the diet (Thacker, 2003).

Table 9-28 Summary performance of growing-finishing pigs fed diets containing graded levels of hulled pubescent brown canary seed (Thacker, 2003)

Objective: to determine the performance and carcass characteristics of growing-finishing pigs fed diets containing graded levels of pubescent canary seed (cv.Elias)

- Cross bred pigs; each diet fed to groups of 6 or7 gilts and 6 castrates each (n=12 or 13/diet)
- 5 diet groups:
  - Diet 1: 0 % canary seed (cv.Elias); 100% barley in diet,
  - Diet 2: 25% pubescent canary seed (cv.Elias), 75% barley in basal diet
  - Diet 3: 50% pubescent canary seed (cv.Elias), 50% barley in basal diet
  - Diet 4: 75% pubescent canary seed (cv.Elias), 25% barley in basal diet
  - Diet 5: 100% pubescent canary seed (cv.Elias) , 0% barley in basal diet
- Pigs were provided diets ad libitum for 30minutes, twice daily, during the growing period (34.4 to 84 kg) and the finishing period (84-107.8kg) (time not reported)
- Canary seed replaced 25 to 100% of barley ingredient in the basal diet.

Measured endpoints for growth evaluation

- Digestibility for dry matter, crude protein, and gross energy
- Performance parameters including daily weight gain and feed conversion
- Carcass traits including slaughter weight, carcass weight, dressing percentage, carcass value index, lean yield, loin fat and loin lean.

Results

- Decrease in dry matter digestibility with increasing canary seed level possibly due to higher fiber content of canary seed compared to barley. Increasing crude protein digestibility determined with increasing level of canary seed.
- Gross energy digestibility not affected by level of canary seed.
- Pigs fed diet containing 25% canary seed had highest weight gain; lowest weight gain observed on diets containing 100% canary seed.
- Feed intake and feed conversion not affected by level of canary seed
- Carcass traits not affected by canary seed inclusion in diet.

Conclusions

- Results for growth and feed intake of pigs suggest canary seed could be included up to 57% of the total diet (75% of cereal portion) without adversely affecting grower pig performance or altering carcass characteristics.
- Canary seed is palatable and nutrients can be effectively utilized.
- Canary seed did not appear to have any negative effect on pig performance.

This study (Thacker, 2003) on swine was conducted to determine the performance and carcass characteristics of growing-finishing pigs fed diets containing graded levels of pubescent hulled canary seed, cultivar Elias. Canary seed replaced the barley portion of the barley/soybean meal diet at levels of 25, 50, 75 or 100%. Thacker found that during the grower period, pigs fed the diet containing 25% canary seed had

the highest rates of gain (1.0 kg/day) and pigs fed the 100% canary seed diet had the lowest gain (0.90 kg/day). Pigs fed a diet containing 50% and 75% canary seed showed a daily gain of 0.98 kg/d and 0.97 kg/d, respectively, higher than the control diet where a daily gain of 0.93 kg was noted. In the finishing period, pigs fed the diet containing 50% canary seed had the highest gain (1.07 kg/d) while pigs fed the 100% canary seed diet showed the poorest growth (0.94 kg/d). Weight gains on the control diet (1.0 kg/d); 25% canary seed diet, (1.02 kg/d) and 75% canary seed diet (1.0 kg/d) were comparable. Daily intake and feed conversion during both periods were unaffected by level of canary seed. Canary seed diets were considered to be palatable and the nutrients effectively used. It appeared the canary seed did not contain any antinutritional factors at high enough levels to have a negative impact on pig performance. In general, Thacker found that canary seed could be included up to 57% of the total diet (75% of cereal portion) without adversely affecting grower pig performance or altering carcass characteristics.

The second swine study (Qiao & Thacker, 2004)(Table 9-29) focused on determining if a new method –mobile nylon bag technique (MNBT) – could accurately predict the digestible energy (DE) content of swine feed for use in ration formulation programs. The researchers evaluated 22 traditional (e.g. barley, corn, oats and wheat) and non-traditional feeds (e.g. low viscosity ryes, legumes, oilseeds and canary seed) to determine the potential of the MNBT as a tool to determine DE. Three varieties of canary seed (glabrous hulled CDC Maria, glabrous dehulled CDC Maria and pubescent hulled Keet) were evaluated as part of the study.

Table 9-29 Summary of determination of digestible energy content of traditional and non-traditional swine feeds (Qiao & Thacker, 2004)

Objective: to compare the dry matter and energy digestibility of swine feed ingredients using a mobile nylon bag technique.

- Crossbred pigs with duodenal cannuale were fed on a grower diet. After simulating gastric digestion, nylon bags containing feed samples were inserted into the duodenum. Bags were recovered for analysis of feces content.
- 22 traditional and non-traditional swine feed ingredients tested including 3 canary seed ingredients and the CDC Teal wheat

Canary seed samples tested;

- Dehulled glabrous canary seed (CDC Maria)
- Hulled glabrous canary seed (CDC Maria)
- Hulled pubescent canary seed (Keet)

Measured endpoints:

- Digestibility for dry matter, crude protein and gross energy
- Digestible energy

Results:

- Dry matter digestibility of hulled CDC Maria (75.2%) and Keet (76.3%) were similar to barley (74%) and less than CDC Teal wheat (84.9%)
- Greater dry matter digestibility for dehulled CDC Maria (92.4%) comparable to oat groats (92-94%)
- Similar pattern observed for energy digestibility

Analytical results for canary seed showed that glabrous dehulled CDC Maria had greater % dry matter digestibility, % energy digestibility, gross energy (MJ/kg) and higher digestible energy (MJ/kg) than either the glabrous or pubescent hulled canary seeds products. The glabrous dehulled canary seed had similar dry matter and energy digestibility values to the high fat oat groats; all being higher than the traditional cereal grains (barley, corn, oats and hard red spring wheat). The DE for dehulled CDC Maria was much higher (17.61 MJ/kg) than the traditional cereals (range: 11.25-14.26 MJ/kg) or secondary cereal grains (range: 13.53-16.95 MJ/kg) tested in the study. The hulled glabrous and pubescent cultivars had DE values slightly lower (13.76 and 13.82 respectively) than corn (13.89) and wheat (14.23), but higher than oats (11.25) and barley (12.40). Glabrous dehulled canary seed (CDC Maria) also showed higher digestible energy values than the CWRS wheat (CDC Teal) (DE, 14.62 MM/kg) which in turn was slightly higher than the DE values for hulled glabrous and pubescent cultivars.

These results showed that the digestible energy values for glabrous or pubescent canary seed cultivars were within the reported DE ranges of traditional and secondary cereal grains used as swine feeds.

9.2.4 Nutritional Bioavailability Summary

In general, the results of the animal nutritional studies (rodents & swine) support the conclusion that growth of animals on diets containing hairless canary seed (brown or yellow coloured groats) is as good or as better than growth of animals containing similar amounts of CWRS wheat in the control diets. No adverse effects on growth were noted during the study periods and the presence of the higher phytate levels in canary seed as compared to the CWRS wheat (Section 9.1.2.6.1) did not appear to negatively impact growth characteristics.

10.0 CHEMICAL CONSIDERATIONS

10.1 Alkaloids

10.1.1 Alkaloids in *Phalaris* spp.

Prior to this novel food initiative on glabrous brown and yellow canary seed for human food use, there have been no reports of alkaloids present in the seeds (grain/groats) of any of the *Phalaris* species. Determination of alkaloids in *Phalaris* species has been entirely restricted to analysis of leaf material (Anderton *et al.*, 1999; Duynisveld *et al.,* 1990; Kalén *et al.,* 1992; Majak and Bose, 1977; Majak *et al.*, 1978; Ostrem, 1987; and Zhou *et al*, 2006.)

Alkaloids are nitrogen containing organic compounds that can be potentially toxic to humans. They are found in some families and species of higher plants, particularly in leguminosae, as byproducts of plant metabolism, as a reservoir for protein synthesis or as protective agents (Facchini, 2001).

Alkaloids may occur in the seeds of a number of species of interest for both animal and human consumption. Raw barley seeds, for instance, may contain small amounts of alkaloids. An examination of barley varieties used in the brewing industry for the presence of alkaloids showed that gramine was not detected in the seed of five barley cultivars tested. However, hordenine (0.7 µg/gm) was detected in one of the five cultivars and N-methyltyramine was detected in all five cultivars at levels ranging from 0.3 to 11.4 µg/gm (Poocharoen, 1983). Lupins (*Lupinus* spp.) accumulate significant quantities of quinolizidine alkaloids in their seeds; however in some cultivars of yellow lupin (*Lupinus luteus* L.), the indole alkaloid gramine, is the most abundant alkaloid. Gramine concentrations reported for this cultivar range from 166 to 1894 mg/kg (Jamroz & Kubizna, 2008; Wasilewko & Buraczewska, 1999). The ANZFA report of 2001 (ANZFA, 2001) provides a summary of the alkaloid profile and potential toxicity of the alkaloids in sweet lupins. The mean alkaloid content in sweet lupins is 130-150 mg/kg; however the varieties tested in this report did not contain gramine. The ANZFA report suggests a tolerable level of exposure of lupin alkaloids for humans of 35µg/kg/day.

10.1.2 Alkaloid Results

The analysis of alkaloids in pubescent and glabrous brown canary seed groats were conducted in Phase 1 (Abdel-Aal *et al.*, 2011b). The alkaloids, gramine, nonadecane, tryptamine and norharmane were determined in canary seed groats and CWRS wheat milling fractions by gas liquid chromatography (GLC) as described by Duynisveld and others (1990). HPLC was also used to confirm the alkaloids results as outlined by Muir and colleagues (Muir *et al.*, 1992). Detection of alkaloids was performed at 270 nm and a standard solution of gramine, tryptamine and β-carboline (5 mmol) was used for calibration and identification. No alkaloids were detected in the groats of the glabrous brown cultivar (CDC Maria) or its pubescent parent (Keet).

In Phase 2 of the canary seed project, a new method based upon the method of Muir *et al* (1992) was developed. In this Phase 2 study alkaloids were also evaluated in the grain (seed) of the perennial reed canarygrass (*P. arundinaceae* L) and compared to commercially grown samples of the annual glabrous *P. canariensis* cultivar, CDC Maria. The complete report outlining the methodology used and results of the alkaloid study for Phase 2 can be found in Appendix 7 (Muir *et al.*, 2010)

Seeds from three cultivars of *P. arundinacae* reed canarygrass (forage cultivars known to have significant foliar levels of the alkaloids gramine and hordenine) were used to develop a method for extraction and analysis of alkaloids from the seeds. Spike recovery experiments using gramine were undertaken during method development to ensure that the extraction and analytical process was appropriate to detect low levels of indole alkaloids in *Phalaris* seed samples.

The alkaloid content was evaluated in seeds of three cultivars of *P. arundinacea* reed canarygrass (Vantage, Rise and Rival) obtained from Plant Gene Resources of Canada (Agriculture Agri-Food Canada, Saskatoon) and a commercially available sample of glabrous CDC Maria (Crop Development Centre, University of Saskatchewan) (Table 10-1). Reference materials included gramine, 5-Methoxy-N,N-dimethyltryptamine, tryptamine, O-methylserotonin HCl, and tyramine. At the time of the analysis, no reference standard could be found for hordeine.

The major peak found in the seeds of all three reed canarygrass cultivars was the amine tyramine (15.12-18.96 µg/g) (Figure 10-1). Tyramine was essentially absent

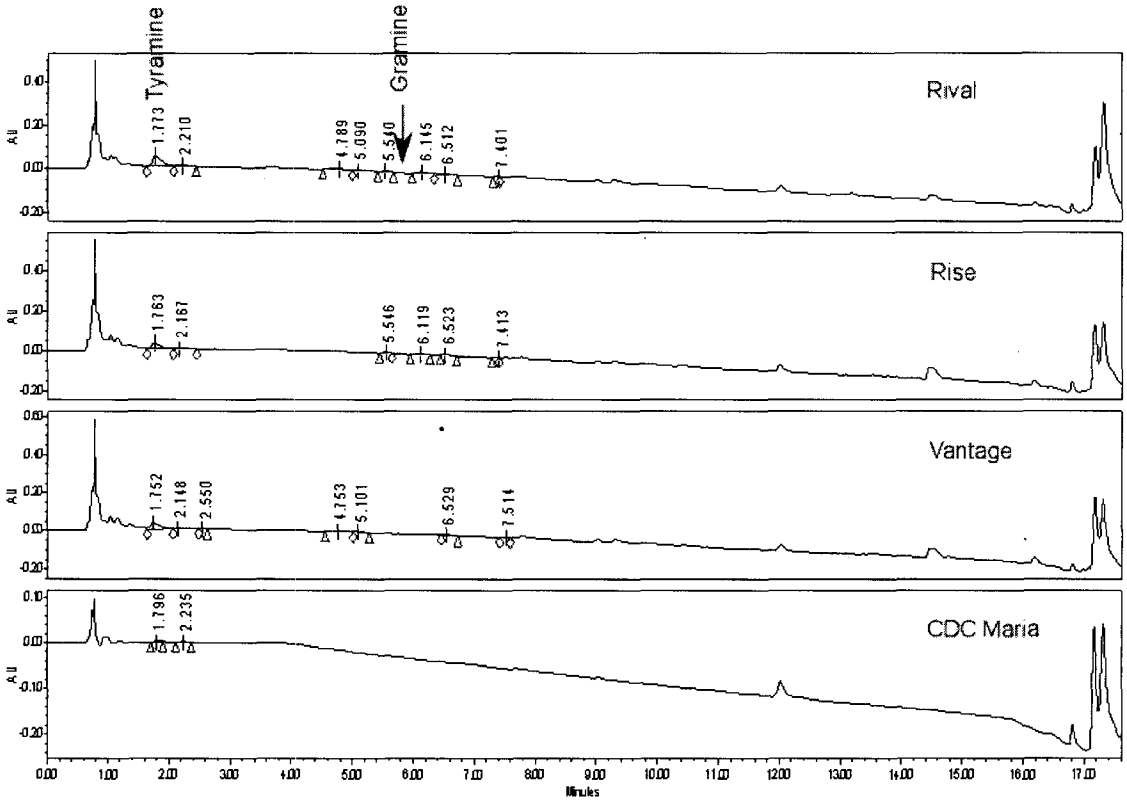
in the groat of commercially grown glabrous canary seed cultivar (CDC Maria). No peaks were found that co-chromatographed with gramine in any sample. A number of minor peaks were observed in the reed canarygrass grain extracts and examination of the UV spectra indicated that the peaks with retention times had UV spectra similar to gramine or related indole or phenylethylamine alkaloids for which reference standards were available. Mass spectral analysis also indicated the presence of nitrogen but the concentration was too low to obtain a positive identification of any compound. Because of the presence of nitrogen and a UV spectrum similar to gramine or tyramine, these peaks were presumed to be alkaloids or amines and the concentration was estimated using the external standard calibration curve for gramine.

Table 10-1. Tyramine and alkaloid-like compounds present in MeOH:NH_4OH (99:1) extracts of reed canarygrass and commercial glabrous canary seed (CDC Maria) seeds.[1]

	Tyramine µg/g sample (n=3)	**Total alkaloid-like compounds (excluding Tyramine)** µ/g sample (n=3)
CDC Maria canary seed	0.77	0.76
Vantage canarygrass	16.56	19.69
Rise canarygrass	15.12	10.53
Rival canarygrass	18.96	21.51

[1]Muir, 2010, report for CDCS, unpublished; Appendix 7

Figure 10-1. UPLC analysis (Symmetry C18 column) of seed extracts of reed canarygrass (*P. arundinacea*) and glabrous canary seed (*P. canariensis*) for the presence of alkaloids and amines. UV = 214 nm. The arrow indicates the retention time for gramine.[1]



[1]Muir, 2010, report for CDCS, unpublished; Appendix 7

Determination of the alkaloid content in glabrous hulled and dehulled canary seed.

The 18 composite samples of glabrous brown (6 composites of CDC Maria) and yellow canary seed (6 composites each of C05041 & C05091) were analyzed both as intact grain (with hulls) and dehulled grain (groats). All samples were extracted in triplicate and each lab sample was analysed in triplicate by Ultra Performance Liquid Chromatography (UPLC) (Table 10-2). Values reported are means of the 6 composite samples. The mean laboratory replicate values for each field replicate are reported in Appendix 7. Gramine was not detected in any of these samples. As reported above, the

major peak in all chromatograms was identified as tyramine and this is reported separately in Table 12-2. The criteria for considering a peak to be alkaloid-like included a UV spectrum similar to one of the reference standards and the presence of molecular or daughter ions indicating the presence of nitrogen in the molecule.

Table 10-2. Tyramine and alkaloid-like compounds present in MeOH:NH_4OH (99:1) extracts of glabrous hulled canary seed and dehulled canary seeds (n=6) [1]

	Tyramine µg/g	STDEV µg/g	Alkaloid-like compounds µg/g	STDEV µg/g
Glabrous Hulled				
Brown CDC Maria	3.50	±1.91	2.26	±0.67
Yellow C05041	21.19	±5.13	7.95	±0.70
Yellow C05091	20.80	±3.63	4.93	±0.96
Glabrous De-Hulled				
Brown CDC Maria	2.83	±0.61	1.23	±0.33
Yellow C05041	23.55	±6.09	5.69	±1.28
Yellow C05091	20.11	±6.56	7.07	±2.12

[1] Muir, 2010, report for CDCS, unpublished; Appendix 7

The commercially grown glabrous brown CDC Maria grain used in the comparison study with reed canarygrass appears to have lower concentrations of tyramine (0.77 µg/g) and alkaloid-like compounds (0.76 µg/g) (Table 12-1) than the glabrous brown CDC Maria grown in the small replicated plots for the Phase 2 study (tyramine, 2.83-3.5 µg/g; alkaloid-like compounds, 1.23-2.36 µg/g) (Table 12-2).

Both yellow coloured cultivars contained more tyramine (20.1-23.6 µg/g) and alkaloid-like compounds (5.7-7.1 µ/g) than the brown cultivar (2.8 µg/g and 1.2 µg/g), respectively (Table 12-2). While the tyramine levels in the glabrous yellow cultivars were similar to that measured in the reed canarygrass (Table 12-1), the alkaloid-like compounds were less.

The levels of both tyramine and alkaloid-like compounds were not significantly different between the hulled and dehulled grain indicating that most if not all of these compounds are residing in the embryo and cotyledon and not in the hull.

Levels of the biogenic amine tyramine detected in the grain of pubescent and glabrous canary seed were significantly below the level considered to have a biological

effect (e.g. >6000µg in two typical food servings sizes) (McCabe-Sellars et al., 2006). In all cases, the concentrations present were too low to allow positive identification of the individual compounds.

At the time of this alkaloid analysis for the novel food initiative, an authentic reference sample for hordeine could not be found. Consequently, the absence or presence of hordeine in the canary seed samples could not be confirmed. However, the researchers observed that of the unknown peaks that had enough absorbance to do a spectral analysis, no match for hordeine could be seen. This suggests that, if hordeine was present, it was below the detection threshold and below any level that could be quantified.

10.1.3 Alkaloid Summary

Gramine was not detected in any of the canary seed samples and the major peak in the chromatogram was identified as the amine tyramine. The glabrous yellow canary seed contained more tyramine and alkaloid-like compounds that the glabrous brown canary seed. However all detected levels of alkaloid-like compounds were too low to allow positive identification of the individual compounds. The concentrations of tyramine observed in both brown and yellow cultivars were also well below any level considered to have a biological effect (e.g. >6000µg) (McCabe-Sellars et al., 2001).

10.2 Heavy metals

Heavy metal concentrations in crops are dependent upon the environment, soil structure and agronomic practices (crop rotation, fertilizer application) as well as natural variation in the uptake and distribution of trace elements among crop species and among cultivars within species (Grant *et al.*, 2008). Heavy metals in plant foods represent a large group of constituents that are either essential or potentially toxic to human health.

In the Phase 1 study, samples of glabrous canary seed (CDC Maria), pubescent canary seed (Keet) and the CRSW wheat (Katepwa) obtained from ten sites in Saskatchewan were ground and wet digested using a mixture of nitric acid and perchloric acid for heavy metal analysis by inductively coupled plasma emission spectrometry (ICPES) at Saskatoon Research Centre (SRC), Saskatoon. In Phase 2, the heavy metal contents in 18 samples of glabrous canary seed (n=6 for each of CDC Maria (brown), C05041& C05091 (yellow)) from three sites in Saskatchewan were measured by inductively coupled plasma mass spectrometry (ICPMS) at ALS Laboratories (Saskatoon, SK).

Ten (10) heavy metals (molybdenum, antimony, tellurium, tungsten, arsenic, bismuth, cadmium, mercury, lead and silver) were measured in glabrous (CDC Maria) and pubescent (Keet) canary seed and compared with wheat as a traditional food in Phase 1 (Table 10-3). These same ten metals plus cobalt were measured in the brown and yellow glabrous varieties (CDC Maria, C05041, and C05091) in Phase 2 (Table 10-4)

The mean molybdenum concentration in glabrous canary seed samples ranged from 0.51 to 0.93 mg/kg, in pubescent canary seed, 0.41 mg/kg and in the CWRS wheat 0.93 mg/kg. However over all sites and for all crops, molybdenum values ranged from 0.10 to 2.40 mg/kg. These values are similar to those found in other cereal crops grown on the Canadian prairies: barley, 0.9 mg/kg; oats, 1.1 mg/kg; wheat, 1.0 mg/kg and rye, 0.6 mg/kg (McCartney *et al.*, 2006)

Three heavy metals with little or unknown effects on humans when ingested were measured in both project phases. This group is considered as neutral metals and

includes antimony, tellurium and tungsten. In Phase 1, there were no significant differences in the level of antimony (Sb), tellurium (Te) or tungsten (W) in hairless and hairy canary seed compared with wheat. In Phase 1, all these metals were present in very low amounts ranging between 0.1 and 0.29 mg/kg (Table 10-3). In Phase 2, the levels of these metals in the glabrous canary seed samples were all below the method detection limit for each metal (Sb, 0.05 mg/kg; Te, 0.50 mg/kg and W, 0.80 mg/kg) (Table 10-4).

The content of five heavy metals with potential toxicity for humans was also measured. Arsenic, bismuth, cadmium, lead and mercury were all detected at low concentrations in the pubescent and glabrous canary seed cultivars and the control wheat. Similar average concentrations of arsenic (0.2 mg/kg), bismuth (0.2 mg/kg), cadmium (0.1 mg/kg) and mercury (0.03 mg/kg) in both types of canary seed as well as wheat were found (Table 10-3) in Phase 1. In Phase 2, all canary seed sample results for bismuth, cadmium, lead, mercury, and silver were below the method detection limit for these metals (Bi, 0.30 mg/kg; Cd, 0.5 mg/kg; Pb, 0.1 mg/kg; Hg, 0.01 mg/kg and Ag, 0.08 mg/kg) (Table 10-4). Arsenic levels ranged from 0.06-0.10 mg/kg for the Phase 2 analysis, less than the 0.2 mg/kg average levels found during Phase 1 analysis.

In Phase 1 there was a slight, but insignificant difference between glabrous and pubescent canary seed in lead level (0.21 and 0.37 mg/kg, respectively) while wheat had only 0.13 mg/kg. In Phase 2, the lead content ranged from below the method detection limit of 0.02 mg/kg (in the yellow cultivars) to 0.059 ppm in the brown canary seed. The levels of lead obtained in both Phase 1 and Phase 2 studies were all within the range of 0.030-0.37 mg/kg reported in the literature for cereal crops (Cubadda *et al.*, 2003). The mean lead content in pubescent (0.43 mg/kg) and glabrous canary seed (0.21 mg/kg) analyzed in Phase 1 was slightly higher than the accepted 0.2 mg/kg (wet weight) for wheat and 0.1 mg/kg (wet weight) for other cereals (Codex, 2007). However, the range of lead in canary seed was wide, ranging from 0.10 to 1.20 mg/kg in pubescent canary seed and 0.1 to 0.7 mg/kg in glabrous canary seed suggesting that growing conditions and/or environmental factors may cause a high degree of fluctuation in lead content. Phase 2 canary seed lead values (<0.02 to 0.059 mg/kg) were all less than the Codex limit. Variations in lead content in Canadian grown barley (0.073-

0.21ppm), oats (0.110 to 0.130ppm) and wheat (0.087 to 0.18ppm) have also been reported (Dudas and Pawluk, 1977). Zook *et al.,* (1970) reported differences in lead content based upon wheat type [hard, 0.50 ppm (mg/kg); soft, 1.0 ppm (mg/kg); and durum (0.42 ppm (mg/kg)].

Reported literature values for arsenic, bismuth and cadmium in wheat were less than 0.05 mg/kg and mercury was less than 0.02 mg/kg on different soil types and under varying growth conditions (Cubadda *et al.*, 2003; Lavado *et al.*, 2001; Yager *et al.*, 2004). The cadmium level in hairless and hairy canary seed in Phase 1 was at the acceptable limit of 0.1 mg/kg set for cereals (other than buckwheat and quinoa) and less than the value set for wheat of 0.2 mg/kg (Codex, 2007), and in Phase 2, all samples results are reported as less than the method detection limit of 0.005 mg/kg (ppm). Cadmium levels in spring wheat, barley, oat and maize generally contain cadmium concentrations below 0.1 mg/kg (Grant et al., 2008). Reported literature values for cadmium levels in crops on the Canadian prairies has ranged from 0.05 mg/kg to 0.23 mg/kg for wheat durum (Clarke *et al*, 2002, Dudas & Pawluk, 1977), 0.30 to 0.12 mg/kg for barley and 0.04 to 0.065 mg/kg for oats (Dudas & Pawluk, 1977) but higher for flaxseed (0.2 to 0.4 mg/kg) (Clarke et al., 2010). Cadmium accumulation in a plant is dependent upon genotype and environment (Clarke *et al,* 2002; Grant *et al*, 1998).

Mercury levels in the Phase 1 canary seed samples and the control wheat were all less than 0.03 mg/kg while mercury levels in the Phase 2 glabrous samples were all less than 0.01 mg/kg. These values are similar to the reported literature values for oat (0.01-0.012 mg/kg), barley (0.006-0.012 mg/kg) and wheat (0.0053-0.01 mg/kg) grains (Dudas & Pawluk, 1977).

Due to the differences in methods and limits of quantification (LOQ) used in the two study phases, the arsenic levels in the Phase 1 canary seed analysis (where the LOQ = 0.2 mg/kg) were higher (0.2 mg/kg) than those values found in the Phase 2 analyses where all samples had arsenic levels less than the limit of quantification (<0.02mg/kg). Phase 2 arsenic results were well below the range (0.06-0.08 mg/kg) found in an extensive evaluation of cereal grains in Europe (EFSA, 2009) and less than some reports of arsenic levels found in wheat (0.17 mg/kg) (Raber *et al*, 2012) or rice

(0.02-0.36 mg/kg) (EFSA, 2007). Silver is reported for glabrous canary seed at levels less than the detectable limit of 0.08 mg/kg.

All heavy metals tested were within regulatory and/or acceptable levels.

Table 10-3. Comparison of heavy metal content (mg/kg) of glabrous and pubescent canary seed groats to CWRS wheat grown at 10 sites in Saskatchewan (Phase 1)[1]

Metal	Glabrous Brown Canary Seed			Pubescent Brown Canaryseed			CWRS Wheat		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Essential Metals									
Molybdenum (Mo)	0.51	0.71	0.10-2.20	0.23	0.41	0.1-1.4	0.64	0.94	0.10-2.40
Neutral Metals									
Antimony (Sb)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Tellurium (Te)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Tungsten(W)	0.2	0	0.2-0.2	0.22	0.04	0.2-0.3	0.2	0	0.2-0.2
Toxic Metals									
Arsenic (As)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Bismuth (Bi)	0.2	0	0.2-0.2	0.2	0	0.2-0.2	0.2	0	0.2-0.2
Cadmium (CD)	0.1	0	0.1-0.1	0.1	0	0.1-0	0.1	0	0.1-0.1
Mercury (Hg)	0.03	0	0.03-0.03	0.03	0	0.03-0.03	0.03	0	0.03-0.03
Lead (Pb)	0.21	0.23	0.10-0.70	0.37	0.42	0.10-1.20	0.13	0.09	0.10-0.40
Silver (Ag)	0.1	0	0.1-0.1	0.1	0	0.1-0	0.1	0	0.1-0.1

[1] Abdel-Aal, 2011b

Table 10-4 Comparison of heavy metal contents (mg/kg) of glabrous brown and yellow canary seed groats (Phase 2)[1]

		Glabrous Canary Seed							
		Brown				Yellow			
	Detection Limit (mg/kg)	Mean	SD	Range		Mean	SD	Range	
				Min	Max			Min	Max
Essential metals									
Molybdenum (Mo)	0.05	0.70	±0.32	0.41	1.15	0.93	±0.33	0.48	1.56
Neutral Metals									
Antimony (Sb)	0.05	<0.05	na	<0.05	<0.05	<0.05	na	<0.05	<0.05
Cobalt (Co)	0.50	<0.50	na	<0.50	<0.50	<0.50	na	<0.50	<0.50
Tellurium (Te)	0.50	<0.50	na	<0.50	<0.50	<0.50	na	<0.50	<0.50
Tungsten (W)	0.80	<0.80	na	<0.08	<0.08	<0.80	na	<0.80	<0.80
Toxic Metals									
Arsenic (As)	0.02	<0.02	na	<0.02	<0.02	<0.02	na	<0.02	<0.02
Bismuth (Bi)	0.30	<0.30	na	<0.30	<0.30	<0.30	na	<0.30	<0.30
Cadmium (Cd)	0.005	<0.005	na	<0.005	<0.005	<0.005	na	<0.005	<0.005
Lead (Pb)	0.02	<0.037	0.004	0.02	0.059	<0.02	na	<0.02	<0.03
Mercury (Hg)	0.01	<0.01	na	<0.01	<0.01	<0.01	na	<0.01	<0.01
Silver (Ag)	0.08	<0.08	na	<0.08	<0.08	<0.08	na	<0.08	<0.08

[1] CDCS Phase 2 study, unpublished

10.3 Pesticides

The following pesticides are registered for use on pubescent and glabrous canary seed *(Phalaris canariensis)* in Canada. Uses and application rates are similar to those of other cereal crops (wheat, barley, oats etc) grown in Canada and the US (CFR, 2013). One potential exception is the use of difenzoquat, which is currently under re-evaluation in both countries.

Table 10-5 Pesticides registered for use on canary seed in Canada[1]

	Product Name	Active Ingredient	Registrant
Herbicides	Avadex – granular formulation	Triallate	Gowan Co.
	Avenge	Difenzoquat	AmVac Crop/Syngenta
	Pardner, Koril, Bromotril, Brotex	Bromoxynil	Bayer
	Buctril M, Logic M, Mextrol 450, Badge	Bromoxynil + MCPA ester	Bayer
	Curtail M	Clopyralid + MCPA amine	NuFarm
	Banvel, Oracle, VMD 480 Dicamba	Dicamba + MCPA amine	BASF
	Target, Sword, Tracker XP	Dicamba +mecoprop+MCPA	Syngenta
	Prestige	Fluroxypyr + clopyralid + MCPA ester	Dow
	Trophy	Fluroxypyr + MCPA ester	NuFarm
Fungicides	Tilt, Bumper, Pivot	Propiconozole	Syngenta
Insecticides	Cygon, Lagon	Dimethoate	IPCO Cheminova UAP
	Malathion	Malathion	ICPO UAP

[1] Saskatchewan Guide to Crop Production 2014.

11.0 Toxicological Considerations

Although limited, there is some evidence of history of use of canary seed as human food in North America. This section describes all safety evaluation studies identified in the literature, as well as the studies conducted to support the GRAS determination.

Background: The gathering of information for the safety assessment of glabrous canary seed has proceeded in two discrete timeframes in the past fifteen years. The initial project (Phase 1) (1992-2002) involved the development of glabrous canary seed and the identification of both brown and yellow coloured groats amongst the glabrous varieties. In Phase 1, the nutritional and chemical characteristics of glabrous brown coloured canary seed groats (*P. canariensis*, CDC Maria) were compared to its pubescent parent *P. canariensis,* cultivar "Keet" (also brown coloured groat) and to a Canada Western Red Spring (CWRS) wheat. The project involved analysis of the nutrient composition, antinutritional components, alkaloids and heavy metals, as well as a 90-day rodent trial.

Phase 2 (2008-2014) involved a comprehensive comparison of two yellow glabrous coloured cultivars (designated C05041 and C05091) to the brown coloured glabrous cultivar CDC Maria, which had been studied in the Phase 1 project. The toxicology studies conducted during Phase 1 and Phase 2, plus those in the published literature are summarized below.

11.1 Rodents

11.1.1 Mice

Bhatt *et al* (1984) investigated the carcinogenic promoting effect of the silica hairs from the pubescent hulls of *Phalaris canariensis.* Swiss mice were orally administered pubescent canary seed in one experiment, and in other experiments, dermal exposures to the silica hairs was undertaken. In all experiments, an initiator-promoter protocol was used. The initiator was 15,16-dihydro-11-methylcyclopental(a)phenanthren-17-one, which initiates skin cancer when injected intramuscularly or by dermal application. The tumor promoter was croton oil applied to

the skin. For oral administration of the pubescent canary seed, seeds were ground to a coarse meal, mixed with 50% by weight egg white, air-dried in a thin layer and broken into fragments. In the oral canary seed experiment, there were 5 treatment groups. Mice (20 male, 10 female) in Group 1 were injected with the initiator, and fed the canary seed mixture fragments in their food hoppers 4 days per week and standard mouse diet for the remaining 3 days per week. Group 2 (10 mice/sex) were fed the same dietary regime as Group 1, but did not receive the initiator. Group 3 (10 mice/sex) were injected with the initiator and fed the standard diet. Group 4 (10 mice/sex) received the initiator, standard diet and croton oil applications. Group 5 (10 mice/sex) received the standard diet and croton oil applications, but no initiator. Tumor incidence was assessed after 78 weeks (18 months). In the absence of the carcinogen initiator (Group 2), mice fed the pubescent canary seed were in normal health and 15% heavier than the control groups fed a standard mouse diet. No tumors were observed in mice in Group 2. Histopathological examination showed neither gross abnormalities in the oesophagus or stomach, or any significant incidence of internal tumours in any of the mice. The authors reported that no toxic effects were observed, and confirmed exposure as silica fibers on the grain hulls were recovered from the gut contents throughout its length and also from washed gut tissues. A promoting effect of dermal exposure to pubescent canary seed was demonstrated in Group 1 "initiated" mice fed canary seed. These mice developed tumors around in the facial trunk and ventral trunk. Most were benign squamous papilloma. The amount of canary seed consumed was not reported. Tumors were also observed in initiated mice fed the standard diet, (Groups 3 and 4). Subsequent experiments confirmed dermal contact of purified *P. canariensis* silica fibers promoted phenanthrene-induced skin tumors (Bhatt *et al*, 1984.)

11.1.2 Rats

The University of Saskatchewan and the Canaryseed Development Commission of Saskatchewan sponsored two 90-day oral sub-chronic rat studies using i) pubescent and glabrous canary seed, and ii) glabrous brown and glabrous yellow coloured canary seed varieties. A 28-day oral rat study was also conducted. The descriptions and results

of these studies have been published (Magnuson *et al.*, 2014), and are described in detail below.

11.1.2.1 90-day rat study on glabrous and pubescent canary seed (Phase 1)

In this Phase 1 90-day rat feeding study, a single concentration (50%) of either glabrous canary seed (CDC Maria) or pubescent canary seed (Keet) as test ingredient in the diet was compared to CWRS wheat (50%) as the control. Diets were formulated according to National Research Council (1995) specifications to ensure nutritional equivalency. The high level of test ingredient was chosen to represent an artificially high dose of canary seed in the human diet. The test ingredient results revealed no significant adverse effects in growth, behavior, hematology, clinical chemistry or gross pathology. Histological assessment consisted of examining 4 animals per sex per group. Thus, this study provides support for the safety of oral consumption of the novel food, glabrous canary seed.

Table 11-1 provides a summary of the objective, protocol, data collected and results for this trial. Full protocol details can be found in Appendix 4 and Magnuson *et al.*, 2014.

Table 11-1 Summary of the 90-day rodent study (CTR0012) (Phase 1) [1]

Objective: to compare the toxicological and growth effects of glabrous canary seed CDC Maria and pubescent canary seed Keet with that of CWRS wheat in rats

- Protocol followed OECD Test Guideline No.408 (repeated dose 90-day toxicity study in rodents)
- 4-week old Sprague-Dawley rats (male and female); n=10/sex/group (total 80 rats)
- 4 diet groups:
  - Diet 1: 50% dehulled glabrous CDC Maria canary seed
  - Diet 2: 50% hulled glabrous CDC Maria canary seed
  - Diet 3: 50% hulled pubescent Keet canary seed
  - Diet 4: 50% CWRS wheat (control diet)
- Diets were formulated with additions of corn, soybean, canola oil, amino acids, vitamins and minerals to meet or exceed minimum nutrient requirements for rats.
- All test diets provided the same amount of apparent metabolizable energy (AME) (3,500 kcal/kg) and crude protein (20%). Crude fat ranged from 9% to 10.5%.
- Water and test diet fed *ad libitum* for 90 days

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational battery, hematology, clinical chemistry, organ weights, urinalysis, and limited histopathology.

Results:
No toxicologically significant effects were observed in rats fed diets containing 50% glabrous hulled canary seed, 50% glabrous dehulled canary seed, or 50% pubescent hulled canary seed as compared to rats fed diets contain 50% CWRS wheat for 90 days.

[1]Magnuson *et al.*, 2014),

Four groups of 20 Sprague-Dawley rats (10 per sex) were fed diets containing 50% CWRS wheat (control), 50% glabrous canary seed groats (dehulled) (CDC Maria), 50% glabrous hulled CDC Maria or 50% pubescent hulled canary seed cultivar Keet. Diets were formulated with additions of corn, soybean, canola oil, amino acids, vitamins and minerals to meet or exceed minimum nutrient requirements for rats. Diets contained 3500 kcal/kg AME, 20% crude protein, 0.75% calcium, 0.15% sodium, 0.078% choline, 1.2% lysine, 0.65% methionine and 0.80% threonine to meet or exceed the requirements for rat reproduction (National Research Council, 1995). The test diet was provided in mash form for 90 days. Other details of the experimental protocol are found in Appendix 4 and Magnuson *et al.*, 2014. The results from this study will be summarized below, but most data are not shown. The study report is provided in Appendix 4.

Final body weight, weight gain and feed consumption are shown in Table 9-22. Males fed the glabrous canary seed groats had a greater mean body weight change over the 90 days than those fed glabrous hulled canary seed, the pubescent hulled canary seed or the control wheat diet. A similar trend was observed for females, but differences were not statistically significant. Higher weight gain in rats fed dehulled glabrous groats with similar food intake as other diets, is likely due to higher nutritional bioavailability of feed per gram due to removal of hulls and lower indigestible fiber. Total mean feed consumption data showed no difference between the various diet groups for male or female rats. Males consumed 34, 33, 37 and 35 g per kg body weight per day of the wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed respectively. Females consumed 43, 38, 42 and 42 g per kg body weight per day of the wheat, dehulled glabrous canary seed, hulled glabrous canary seed and hulled pubescent canary seed, respectively.

Organ weights are shown in Table 11-2 as both absolute and relative to final body weight. No differences in absolute organ weights were observed among diet groups, with the exception of liver weights in male rats. Male rats fed the diet containing dehulled glabrous canary seed had significantly higher liver weights as compared to male rats fed the diets containing hulled glabrous or hulled pubescent canary seed, but were similar to those fed the control wheat diet. As male rats fed the dehulled form of canary seed also had higher body weights than rats fed the hulled form, the difference in absolute liver weight is likely due to higher body weight. This is further illustrated by the lack of significant differences in liver weights relative to body weight among the diet groups.

Compared to male rats fed the hulled canary seed diets and the wheat diet, increased body weights of male rats fed dehulled glabrous canary seed groats diet resulted in slightly reduced testes weights relative to body weight. No other significant differences in organ weights in rats fed canary seed as compared to the wheat control were observed, although some differences were observed among the canary seed diets. Differences were not considered toxicologically significant.

Table 11-2 Organ weights, total (g) and relative (g/100 g BW) in the Phase 1 90-day study with male and female rats fed diets containing 50% various types of canary seed or wheat*

		Dehulled glabrous canary seed groat[1]	Hulled glabrous canary seed[2]	Hulled pubescent canary seed[3]	Wheat (control)[4]
Males					
Heart	(g)	1.44 ± 0.16	1.34 ± 0.12	1.34 ± 0.14	1.38 ± 0.6
	(g/100 g BW)	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.01
Spleen	(g)	0.84 ± 0.16	0.79 ± 0.15	0.84 ±0.11	0.84 ± 0.11
	g/100 g BW)	0.15 ± 0.02	0.15 ± 0.03	0.16 ± 0.02	0.16 ± 0.02
Liver	**(g)**	**22.4 ± 2.4[a]**	**19.4 ± 2.6[b]**	**18.74 ± 2.2[b]**	**20.4 ± 2.8[ab]**
	(g/100 g BW)	3.91 ± 0.12	3.77 ± 0.32	3.62 ± 0.19	3.67 ± 0.23
Adrenals	(g)	0.069 ± 0.026	0.077 ± 0.018	0.067 ± 0.011	0.082 ± 0.036
	(g/100 g BW)	0.12 ± 0.004	0.15 ± 0.003	0.013 ± 0.002	0.015 ± 0.007
Kidneys	(g)	3.52 ± 0.37	3.45 ± 0.52	3.33 ± 0.31	3.47 ± 0.33

	(g/100 g BW)	0.62 ± 0.06	0.67 ± 0.08	0.65 ± 0.035	0.65 ± 0.07
Epididymides	(g)	1.41 ± 0.22	1.45 ± 0.15	1.53 ± 0.15	1.51 ± 0.20
	(g/100 g BW)	0.25 ± 0.05^{b}	0.28 ± 0.04^{ab}	0.30 ± 0.06^{a}	0.28 ± 0.04^{ab}
Testes	(g)	3.22 ± 0.14	3.41 ± 0.30	3.42 ± 0.27	3.57 ± 0.33
	(g/100 g BW)	**0.57± 0.6^{a}**	**0.67 ± 0.10^{b}**	**0.67 ± 0.09^{b}**	**0.67 ± 0.08^{b}**
Brain	(g)	2.22 ± 0.09	2.18 ± 0.08	2.22 ± 0.10	2.24 ± 0.07
	(g/100 g BW)	0.39 ± 0.03^{b}	0.43 ± 0.03^{a}	0.43 ± 0.05^{a}	0.42 ± 0.02^{ab}
Thymus	(g)	0.79 ± 0.22^{a}	0.63 ± 0.11^{ab}	0.56 ± 0.16^{b}	0.65 ± 0.15^{ab}
	(g/100 g BW)	0.14 ± 0.03	0.12 ± 0.02	0.11 ± 0.03	0.12 ± 0.03
Females					
Heart	(g)	0.84 ± 0.07	0.85 ± 0.08	0.84 ± 0.10	0.84 ± 0.09
	(g/100 g BW)	0.29 ± 0.02	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.03
Spleen	(g)	0.49 ± 0.09	0.48 ± 0.07	0.44 ± 0.05	0.47 ± 0.04
	(g/100 g BW)	0.17 ± 0.04	0.18 ± 0.03	0.17 ± 0.02	0.18 ± 0.02
Liver	(g)	10.2 ± 0.88	9.76 ± 1.8	9.82 ± 1.04	9.85 ± 0.91
	(g/100 g BW)	3.55 ± 0.29	3.58 ± 0.35	3.67 ± 0.18	3.67 ± 0.23
Adrenals	(g)	0.075 ± 0.023	0.083 ± 0.022	0.085 ± 0.026	0.077 ± 0.020
	(g/100 g BW)	0.026 ± 0.007	0.031 ± 0.009	0.032 ± 0.010	0.029 ± 0.007
Kidneys	(g)	1.91 ± 0.16	1.87 ± 0.22	1.86 ± 0.16	1.88 ± 0.23
	(g/100 g BW)	0.66 ± 0.78	0.69 ± 0.06	0.70 ± 0.06	0.70 ± 0.06
Ovaries	(g)	0.12 ± 0.04	0.14 ± 0.04	0.12 ± 0.02	0.12 ±0.04
	(g/100 g BW)	0.040 ± 0.015	0.051 ± 0.015	0.046 ± 0.010	0.046 ± 0.019
Uterus	(g)	0.50 ± 0.10	0.58 ± 0.14	0.59 ± 0.17	0.55 ± 0.10
	(g/100 g BW)	0.17 ± 0.03^{b}	0.21 ± 0.05^{ab}	0.22 ± 0.05^{a}	0.21 ± 0.06^{ab}
Brain	(g)	1.98 ± 0.06	1.97 ± 0.08	1.98 ± 0.07	2.01 ± 0.05
	(g/100 g BW)	0.69 ± 0.07	0.73 ± 0.07	0.75 ± 0.09	0.75 ± 0.08
Thymus	(g)	0.47 ± 0.18	0.42 ± 0.12	0.46 ± 0.19	0.40 ± 0.21
	(g/100 g BW)	0.16 ± 0.06	0.15 ± 0.04	0.17 ± 0.07	0.15 ± 0.07

[1] n=10
[2] Glabrous brown canary seed (CDC Maria cultivar) groats
[3] Glabrous brown canary seed (CDC Maria), hulled.
[4] Pubescent hulled brown canary seed (Keet cultivar), hulled.
[5] Canadian Western Red Spring wheat
Means in the same row with different letters are significantly different at P<0.05
*Magnuson *et al.*, 2014

There were no significant differences among rats on the various diets for either daily or monthly FOB. There was no association of ophthalmology lesions with a canary seed diet. There was no hematology, serum chemistry or urinalysis findings considered to be diet-related. There were no significant differences related to diet in terms of prothrombin time and activated partial thromboplastin time (Magnuson *et al.*, 2014; data provided in study report in Appendix 4).

Serum chemistry values for rats fed canary seed were not significantly different from rats fed the wheat diet, except for ALT levels, which were significantly lower for both genders when fed the glabrous canary seed groat diet than with the other diets. However, all values were within the normal physiological ranges and were not toxicologically significant. No significant differences between genders or diets were noted in urinalysis data (Magnuson *et al.*, 2014). Data are not shown (study report in Appendix 4).

All rats underwent gross examination and no diet-related lesions were noted. The limitation of this study is that tissues from only 32 of the 80 rats (i.e. 4 out of 10 rats per treatment/sex) were assessed histologically. The few observed lesions did not appear to be associated with any diet and consisted of mostly very mild changes, including mild inflammatory lesions in various tissues. Data are not shown (study report in Appendix 4).

In summary, rats fed a diet containing 50% hulled or dehulled glabrous canary seed, or hulled pubescent canary seed for 90 days had similar or improved growth, hematological and clinical chemistry parameters, as rats fed a diet containing 50% CWRS wheat. No adverse effects were observed. Although the study had limited histology, these findings support the safety of glabrous canary seed as a human food. The NOAEL for glabrous canary seed ranged from 33 to 37 g/kg/d for males and 38 to 42 g/kg/d for females (Magnuson *et al.*, 2014).

11.1.2.2 Rodent studies on yellow and brown glabrous canary seed (Phase 2)

The Phase 2 (2008-2014) rat studies examined the effects of administering yellow or brown glabrous canary seed groats in the diet at concentrations levels of 2.5%, 5% and 10% canary seed groats to rats *ad libitum* over 2 time periods: a) a 28

day period and b) a 90-day period followed by a 30-day recovery period. The rationale for the Phase 2 28-day and 90-day rodent study design was outlined in Section 9.22. The studies were conducted by NucroTechnics and monitored by Cantox Intertek. The objectives, protocols and results of these studies are summarized in Table 11-3 (28-day study) and 11-4 (90-day study).

The experimental protocols and full results including summary tables and raw data are available in the accompanying study reports (28-day study, Appendix 5a; 90-day study, Appendix 5b). Only a few summary tables, when noted, are included in the body of this dossier. These studies have been published (Magnuson *et al.*, 2014).

In establishing whether individual or group values were "normal" or "abnormal", Nucro-Technics' historical data and Charles-River published data for Sprague- Dawley rats were used (Charles River, 1984). Additional references for interpretation of clinical pathology findings were also used (Car, 2006; Clapp, 1982; Levine, 2002; Lewis, 1996; Ramaiah, 2007).

The study was conducted in accordance to the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 407.

11.1.2.2.1 28-Day feeding study on yellow and brown glabrous canary seed in rats

The 28-day study was conducted in accordance to the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 407.

Table 11-3 Twenty-eight (28) day dose range finding study in Sprague Dawley rats fed brown and yellow canary seed groats (Phase 2) [1]

Objective: a) to assess the effects of 3 dose levels of glabrous yellow dehulled canary seed (yellow groats) and 1 dose level of glabrous dehulled brown canary seed (brown groats) and b) to validate the diet preparation process and stability/homogeneity of different components in the diet. Information to be used in the 90 day study.

- 5 groups of male and female Sprague-Dawley rats (5 male and 5 female/test diet)
- 5 diet groups:
  - Diet 1: Control: AIN-76A
  - Diet 2: 2.5% dehulled yellow canary seed
  - Diet 3: 5.0% dehulled yellow canary seed
  - Diet 4: 10% dehulled yellow canary seed
  - Diet 5: 10% dehulled brown canary seed (CDC Maria)
- Diets were formulated to ensure test diets contained similar macro- and micronutrients as the AIN-76A diet. All diets contained 20% protein and 5% fat with total Kcal/g of 3.9.
- Water and test diets fed *ad libitum* daily for 28 days

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational batteries, hematology, clinical chemistry, urinalysis, organ weights and gross necropsy.

Data Type	Results
Mortality	All animals survived to scheduled euthanasia/necropsy date
Hematology	No findings attributable to consumption of canary seed diets
Functional Observational Batteries	Normal.
Organ weights and Growth	No appreciable differences in body weights and body weight gains. No notable changes in absolute organ weights and relative organ weights (to brain/body weights) except for higher relative lung weight (relative to brain weight) in Gr. 3 males. Not considered biologically relevant as there was no dose-response relationship.
Plasma chemistry	No significant findings
Coagulation	No significant findings
Urinalysis	No significant findings
Gross Necropsy	No significant findings
Histopathology	No histopathological assessment carried out

[1] Magnuson *et al.*, 2014

This 28-day rodent study examined the safety (systemic toxicity and target organs for toxicity) of yellow and brown canary seed glabrous groats incorporated into a diet at concentration levels of 2.5%, 5% and 10% and administered to rats *ad libitum* over a 28-day period. This study was initiated to identify the baseline parameters for the pivotal 90-day study.

Five groups of rats were used in the study (1 control, 4 test). Each test and control group consisted of 5 male and 5 female rats (Strain: Crl:CD®(SD)BR-Sprague-Dawley).

Based on the test groups' average body weights and food consumption, male rats consumed 1.7, 3.4 and 6.6 and 6.5 g/kg body weight per day, and females consumed 1.9, 4.0, 7.8 and 7.6 g/kg body weight per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% (glabrous yellow canary seed) or 10% (glabrous brown canary seed), respectively, over a 28-day period. The gender-combined consumption was 1.8, 3.6 and 7.0 g of yellow canary seed groat or 6.9 g of brown canary seed groat per kg body weight per day, for the four dose levels, respectively (Magnuson, *et al.,* 2014).

Various endpoints were monitored as well as body weight assessment, food consumption, clinical pathology, organ weights, and gross pathology. Daily clinical observations and weekly physical examinations showed no diet-related toxicity over a 28-day treatment period in any of the groups of rats.

Animals from all diet groups consumed food and gained body weight over the treatment period. There were no statistical differences in food consumption and body weight gains between the control and test groups of animals.

There were no haematology, serum chemistry or urinalysis findings considered to be diet-related and gross necropsy and organ weights and organ weight ratios were unremarkable (Magnuson, *et al.,* 2014). Full study details are available in Appendix 5a.

In conclusion, this study including clinical observations, clinical pathology and gross necropsy revealed no toxicity in rats that consumed yellow or brown canary seed groats incorporated into diets at concentration levels of 2.5%, 5% or 10% *ad libitum* for a 28-day period. These dose levels were used for the subsequent Phase 2 90-day study.

11.1.2.2.2 90-Day rat feeding study on glabrous yellow and brown canary seed (Phase 2)

This 90-day study was conducted in compliance with the Good Laboratory Practices of the United States Food and Drug Administration (21 CFR Part 58 and

subsequent amendments), and in accordance with the US FDA Center for Food Safety and Applied Nutrition Redbook (2000) and OECD Testing Guidance No. 408 with the exception of the test diet formulation and preparations which were conducted by Research Diets, Inc., New Brunswick, New Jersey, U.S.A. Although the diets were not prepared under strict GLP conditions, the preparation of the diets was designed to be consistent with the requirements of GLP. Full study details are available in Appendix 5b.

Table 11-4 Ninety (90) day safety study in Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats (Phase 2) [1]

Objective: to compare the toxicological and growth effects of dehulled glabrous canary seed (brown groats) with dehulled glabrous yellow canary seed (yellow groats) in rats

- Protocol followed OECD Test Guideline N0. 408
- 5 groups of male and female Sprague-Dawley rats (35 male and 35 female/test diet) consisting of 20 M/F in main group, 10M/F in satellite group and 5M/F in recovery group (30 days on control diet).
- 5 diet groups:
  - Diet 1: Control: AIN-76A
  - Diet 2: 2.5% dehulled yellow canary seed
  - Diet 3: 5.0% dehulled yellow canary seed
  - Diet 4: 10% dehulled yellow canary seed
  - Diet 5: 10% dehulled brown canary seed
- Diets were formulated to ensure test diets contained similar macro- and micronutrients as the AIN-76A diet. All diets contained 20% protein and 5% fat with total Kcal/g of 3.9.
- Water and test diets fed *ad libitum* daily for 90 days followed by a 30 day recovery period on control diet.

Measured endpoints for toxicological evaluation: body weight, food consumption, functional observational batteries, ophthalmology, hematology, bone marrow analysis, coagulation, clinical chemistry, urinalysis, organ weights, gross pathology and complete histology.

Data Type	Results
Bone marrow	No significant findings
Hematology	No findings attributable to consumption of canary seed diets
Functional Observational Batteries	Normal
Ophthalmological Examination	No findings attributable to test article
Organ weights and Growth	No findings attributable to consumption of canary seed diets
Plasma chemistry	No significant findings
Coagulation	No significant findings
Urinalysis	No significant findings
Gross Necropsy	No significant findings
Histopathology	No findings attributable to consumption of canary seed diets

[1] Magnuson *et al.*, 2014

Based on the test groups' average body weights and food consumption, male rats consumed 1.23, 2.45 and 4.92 or 5.03 g/kg per day, and females consumed 1.41, 2.68, 5.53 or 5.57 g/kg per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% yellow canary seeds or 10% brown canary seeds, respectively, over a 90-day period (Magnuson *et al.*, 2014).

Various biomarkers were monitored as well as body weight, feed consumption, ophthalmology, clinical pathology, organ weights, gross pathology and histopathology.

Daily clinical observations and weekly physical examinations showed no test article related toxicity over the 90-day period as well as over the subsequent 30-day recovery period, in any of the diet groups (Data available in Appendix 5b).

Animals from all groups consumed feed and gained body weight over the treatment period. There were no differences in feed consumption and body weight gains between the control and test groups of animals, with the following exceptions: mean weights of male rats treated with 10% yellow canary seed groats were lower at Day 85 (7% of control) and Day 90, (8% of control). This finding was also mirrored with slightly reduced feed consumption in these rats during the same time period: Days 78-90. Normalization of the body weights at day 91 per total feed consumption showed no differences between control and treatment groups. There was no dose-response in reduced body weight or food consumption observed in male rats fed the yellow canary seed groats, and no differences in body weight or food consumption was observed in female rats fed 10% yellow canary seed groats (see Table 9-25, Section 9.2.2). Thus, the differences in feed consumption and body weight were considered to be of no toxicological significance.

Based on the test groups' average body weights and food consumption, male rats consumed 1.23, 2.45 and 4.92 or 5.03 g/kg per day, and females consumed 1.41, 2.68, 5.53 or 5.57 g/kg per day of canary seed groats, in groups offered 2.5%, 5.0%, 10% yellow canary seeds or 10% brown canary seeds, respectively, over a 90-day period.

Ophthalmology

There was no apparent dose-dependency in observations, or findings specific to the test article groups, thus findings were not considered to be diet-related.

Clinical Pathology

There was no hematology, serum chemistry or urinalysis findings considered to be diet-related (Magnuson *et al.*, 2014). It should be however noted that in some rats (across all groups, both genders and including controls) cholesterol and triglyceride levels were increased and in some rats, as well as increases in total bilirubin and ALT. These findings were associated with hepatic lipidosis, which is not uncommon in well-fed obese rats (Medinsky *et al.*, 1986).

Hematology

Summary tables for hematology data are presented in Appendix 5b and in Tables 11-5 and 11-6. There were no hematology findings that were considered to be related to the consumption of the diets (Magnuson *et al.*, 2014).

RBC counts, reticulocytes, hemoglobin, (Hb), Hematocrit (Hct) and RBC indices (MCV, MCH and MCHC) were all within the normal physiological limits throughout the study. WBC counts and differential counts were also all within the normal historical ranges for both genders, for all groups, and test periods, with the following exceptions: large unstained cells (LUC's) (a part of lymphocyte lineage) was marginally increased in male rats fed 5% yellow canary seed groats and the control diet. This finding was not associated with dose-dependent increases and male control rats were affected as well, thus this finding was not considered to be diet-related. Platelet counts were also within the normal historical ranges for all groups, both genders and all time points.

Statistically significant differences were observed in the hematocrit and MCHC values between males fed the control diet and males fed various canary seed diets. In both cases, the values were well within the normal ranges and this effect was considered to be of no biological relevance.

Table 11-5 Hematology data for male Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

		Group Means ± S.D. (n = 20)					
Parameters	**Unit**	**Group 1 Control Diet (0%)**	**Group 2 Low Dose Yellow (2.5%)**	**Group 3 Mid Dose Yellow (5%)**	**Group 4 High Dose Yellow (10%)**	**Group 5 High Dose Brown (10%)**	**Normal Ranges**
RBC	$x10^{12}$ / L	8.49 ± 0.54	8.72 ± 0.42	8.56 ± 0.37	8.59 ± 0.42	8.80 ± 0.43	6.06 -9.46
Hb	g / L	142 ± 9	144 ± 5	142 ± 6	146 ± 6	146 ± 6	120 -181
Hct	%	44.1 ± 2.7	45.0 ± 1.7	45.3 ± 1.6	46.1 ± 1.8 *	46.8 ± 2.1 *	37.3 -50.2
MCV	fL	52.0 ± 2.1	51.6 ± 1.4	52.9 ± 1.6	53.7 ± 2.3	53.3 ± 2.3	47.5 -66.1
MCH	pg	16.7 ± 0.7	16.5 ± 0.5	16.6 ± 0.6	17.0 ± 0.7	16.6 ± 0.7	15.8 -23.1
MCHC	g / L	322 ± 6	319 ± 7	314 ± 8 *	317 ± 7 *	311 ± 5 *	287 -401
Platelets	$x10^9$ / L	905 ± 234	982 ± 123	1040 ± 216	978 ± 205	1022 ± 107	579 -1641
WBC	$x10^9$/ L	8.86 ± 2.48	8.70 ± 2.32	10.54 ± 10.02	7.96 ± 2.53	8.72 ± 2.69	5.00 -15.28
Neutrophils	$x10^9$ / L	1.37 ± 0.52	1.19 ± 0.49	2.16 ± 4.58	1.20 ± 0.36	1.16 ± 0.29	0.05 -2.37
Lymphocytes	$x10^9$ / L	6.99 ± 2.02	7.07 ± 1.93	7.32 ± 3.09	6.33 ± 2.15	7.10 ± 2.40	1.67 -14.00
Monocytes	$x10^9$/ L	0.23 ± 0.13	0.19 ± 0.07	0.27 ± 0.21	0.21 ± 0.09	0.24 ± 0.10	0 -0.46
Eosinophils	$x10^9$ / L	0.12 ± 0.03	0.11 ± 0.05	0.58 ± 2.06	0.11 ± 0.03	0.11 ± 0.03	0 -0.21
Basophils	$x10^9$ / L	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.04	0.02 ± 0.01	0.02 ± 0.01	0 -0.06
LUC	$x10^9$ / L	0.13 ± 0.12	0.11 ± 0.04	0.19 ± 0.33	0.09 ± 0.05	0.10 ± 0.05	0 -0.14
Reticulocytes	$x10^9$ / L	211.6 ± 45.1	203.9 ± 51.0	204.5 ± 37.2	182.0 ± 28.6	202.1 ± 36.4	100 - 400

[1] Magnuson *et al.*, 2014

*Statistically significant difference from Control Group ($p < 0.05$)

Table 11-6 Hematology data for female Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

Parameters	Unit	Group Means ± S.D. (n = 20) Group 1 Control Diet (0%)	Group 2 Low Dose C Yellow (2.5%)	Group 3 Mid Dose Yellow (5%)	Group 4 High Dose Yellow (10%)	Group 5 High Dose Brown (10%)	Normal Ranges
RBC	$x10^{12}$ / L	8.21 ± 0.30	8.12 ± 0.28	8.20 ± 0.41	8.21 ± 0.44	8.11 ± 0.36	6.16 -9.09
Hb	g / L	141 ± 4	141 ± 4	142 ± 5	141 ± 5	140 ± 5	127 -172
Hct	%	43.0 ± 1.3	42.9 ± 1.4	43.4 ± 1.4	43.4 ± 1.9	42.9 ± 1.6	35.3 -47.5
MCV	fL	52.3 ± 1.3	52.8 ± 1.6	53.0 ± 2.0	53.0 ± 1.9	52.9 ± 1.7	47.5 -64.0
MCH	pg	17.2 ± 0.4	17.4 ± 0.5	17.3 ± 0.7	17.3 ± 0.7	17.3 ± 0.5	17.9 -21.6
MCHC	g / L	328 ± 4	329 ± 5	327 ± 5	325 ± 6	327 ± 5	325 -385
Platelets	$x10^{9}$ / L	864 ± 156	878 ± 145	865 ± 113	889 ± 175	942 ± 125	526 -1648
WBC	$x10^{9}$ / L	5.61 ± 1.81	5.48 ± 1.46	5.06 ± 1.38	5.48 ± 0.98	5.43 ± 1.52	4.30 -13.00
Neutrophils	$x10^{9}$ / L	0.86 ± 0.39	0.73 ± 0.29	0.65 ± 0.20	0.80 ± 0.35	0.71 ± 0.27	0.10 -2.67
Lymphocytes	$x10^{9}$ / L	4.39 ± 1.40	4.43 ± 1.16	4.12 ± 1.25	4.37 ± 0.74	4.42 ± 1.31	0.33 -11.60
Monocytes	$x10^{9}$ / L	0.18 ± 0.09	0.15 ± 0.06	0.14 ± 0.05	0.14 ± 0.07	0.15 ± 0.06	0 -0.30
Eosinophils	$x10^{9}$ / L	0.08 ± 0.04	0.08 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.04	0 -0.20
Basophils	$x10^{9}$ / L	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0 -0.04
LUC	$x10^{9}$ / L	0.09 ± 0.06	0.08 ± 0.03	0.08 ± 0.03	0.11 ± 0.13	0.08 ± 0.03	0 -0.11
Reticulocytes	$x10^{9}$ / L	173.1 ± 34.3	164.9 ± 37.3	154.4 ± 27.7	160.6 ± 27.3	193.2 ± 72.5	100 - 400

[1] Magnuson *et al.*, 2014

* Statistically significant difference from Control Group ($p < 0.05$).

Blood Coagulation

There were no coagulation alterations that were attributed to consumption of canary seed. Individual coagulation data can be found in Appendix 5b of this report.

Serum Chemistry

There were no serum chemistry changes that were attributed to the consumption of the test diets. Summary tables for male and female rats from the main 90 day study are presented in Tables 11-7 and 11-8, respectively.

Total protein, albumin, globulin and A/G ratios were not affected by the test diets. BUN levels were not affected by the diets. In the main study, male rats in the groups of high dose yellow canary seed groats and brown canary seed groats, and female rats fed the high dose of yellow canary seed groats had statistically significantly higher creatinine levels than rats in the control group; however all were within the normal range.

Electrolytes (Na^+, Cl^- and K^+) and calcium and phosphorus were all within the normal physiological ranges (both genders, all diets, all treatment periods). No differences among groups were observed in glucose levels.

Cholesterol and triglyceride levels were slightly increased in male rats of several groups including the control animals, in the satellite and main study as compared to normal ranges. No effect specific to consumption of canary seed was observed.

Hepatocellular/hepatobiliary panel (total bilirubin, ALP, ALT, AST, GGT and serum bile acids) were all mostly within the normal physiological ranges for both genders, all four test diet groups and all treatment periods. The exceptions were occasional increase in total bilirubin, which was slightly increased in some rats of all groups, including controls. These increases were small, there was no dose-dependency and control animals were also affected, thus these findings were not toxicologically significant.

Histologically, many rats (all groups including the control and both genders) were found to have periportal lipidosis ("fatty liver"). This finding can explain increased cholesterol, triglycerides, total bilirubin and ALT levels. This finding was not unusual for animals fed *ad libitum* for 3 months, during which they were minimally exposed to any stressors (handling, blood collection, etc.) (Medinsky *et al.*, 1986). These slight

increases were obviously diet-related but the controls were equally or more affected, and thus this finding was not necessarily specific for canary seed.

Table 11-7 Serum chemistry for male Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

Parameter	Unit	Group Means ± S.D. (n = 20)					
		Group 1 Control Diet (0%)	Group 2 Low Dose Yellow (2.5%)	Group 3 Mid Dose Yellow (5%)	Group 4 High Dose Yellow (10%)	Group 5 High Dose Brown (10%)	Normal Ranges
A/G	-	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.7 -1.6
ALB	g / L	32 ± 2	32 ± 3	32 ± 3	31 ± 2	33 ± 3	23 -43
GLOB	g / L	28 ± 2	28 ± 1	29 ± 1	29 ± 1	29 ± 2	22 -36
ALP	u / L	80 ± 26	71 ± 13	81 ± 23	75 ± 13	73 ± 17	47 -426
Bil(T)	umol / L	4.4 ± 1.6	3.9 ± 1.5	4.8 ± 2.1	5.8 ± 1.5 *	5.1 ± 1.0	1.7 – 5.7
BUN	mmol / L	3.9 ± 0.6	4.2 ± 0.7	4.4 ± 0.6	4.1 ± 0.7	4.1 ± 0.9	3.0 -8.4
Ca	mmol / L	2.61 ± 0.08	2.60 ± 0.08	2.63 ± 0.08	2.58 ± 0.09	2.63 ± 0.08	2.24 -3.00
Cl	mmol / L	102 ± 3	103 ± 3	102 ± 2	103 ± 1	103 ± 2	90 -116
Creatinine	umol / L	28 ± 2	30 ± 3	30 ± 5	36 ± 4 *	37 ± 5 *	24 -66
Glucose	mmol / L	12.6 ± 3.0	11.6 ± 2.0	12.5 ± 2.9	11.8 ± 3.5	13.1 ± 2.5	0.8 -11.2
P	mmol / L	1.94 ± 0.17	1.90 ± 0.16	2.03 ± 0.15	2.08 ± 0.22	2.04 ± 0.16	1.83 -3.94
K	mmol / L	4.7 ± 0.2	4.8 ± 0.2	4.9 ± 0.3	5.0 ± 0.3 *	5.0 ± 0.3 *	3.7 -7.0
Protein (T)	g / L	60 ± 2	61 ± 3	61 ± 4	60 ± 3	62 ± 4	47 -75
AST	u / L	101 ± 77	84 ± 19	118 ± 112	87 ± 19	85 ± 15	42 -149
ALT	u / L	62 ± 70	42 ± 9	51 ± 30	42 ± 18	45 ± 12	26 -71
Na	mmol / L	140 ± 3	142 ± 3	143 ± 2	143 ± 2 *	144 ± 2 *	136 -152
Triglycerides	mmol / L	1.69 ± 1.01	1.87 ± 1.42	2.22 ± 1.58	1.47 ± 0.71	2.32 ± 1.24	0.10 -1.55
CK	u / L	320 ± 143	315 ± 149	317 ± 148	394 ± 154	290 ± 91	228 -529
Cholesterol	mmol / L	3.04 ± 0.74	2.88 ± 0.56	3.26 ± 0.90	2.94 ± 0.67	3.70 ± 1.05 *	1.00 -3.00
GGT	u / L	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	4 to 6
Bile Acids	umol/L	5.5 ± 3.2	7.1 ± 4.3	21.7 ± 38.3	8.0 ± 4.7	7.7 ± 5.2	0 -24

[1] Magnuson *et al.*, 2014; * Statistically significant difference from Control Group ($p < 0.05$).

Table 11-8 Serum chemistry for female Sprague Dawley rats fed glabrous brown and glabrous yellow canary seed groats in the 90-day safety study (Phase 2) [1]

		Group Means ± S.D. (n = 20)					
Parameters	**Unit**	**Group 1 Control Diet (0%)**	**Group 2 Low Dose Yellow (2.5%)**	**Group 3 Mid Dose Yellow (5%)**	**Group 4 High Dose Yellow (10%)**	**Group 5 High Dose Brown (10%)**	**Normal Ranges**
A/G	-	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.8 -1.8
ALB	g / L	41 ± 4	43 ± 5	43 ± 5	44 ± 4	43 ± 4	25 -49
GLOB	g / L	28 ± 1	27 ± 1	27 ± 1	28 ± 1	28 ± 1	22 -34
ALP	u / L	98 ± 63	52 ± 18 *	52 ± 28 *	46 ± 12 *	47 ± 15 *	29 -309
Bil(T)	umol / L	3.5 ± 2.3	3.3 ± 1.9	3.0 ± 1.4	4.7 ± 1.4 *	4.7 ± 1.3 *	1.7 -5.9
BUN	mmol / L	4.5 ± 0.8	4.2 ± 0.5	4.5 ± 0.8	3.9 ± 0.7	4.1 ± 0.7	3.2 -8.0
Ca	mmol / L	2.70 ± 0.10	2.74 ± 0.08	2.78 ± 0.13	2.74 ± 0.07	2.73 ± 0.09	2.31 -3.03
Cl	mmol / L	99 ± 2	100 ± 1	101 ± 3 *	101 ± 2 *	103 ± 2 *	93 -117
Creatinine	umol / L	29 ± 3	29 ± 2	30 ± 4	31 ± 3	32 ± 4 *	23 -66
Glucose	mmol / L	9.9 ± 2.3	11.3 ± 2.8	10.7 ± 2.4	11.2 ± 2.0	10.8 ± 2.9	1.2 -11.4
P	mmol / L	1.79 ± 0.22	1.84 ± 0.18	1.75 ± 0.21	1.82 ± 0.13	1.76 ± 0.21	1.50 -3.47
K	mmol / L	4.4 ± 0.4	4.5 ± 0.2	4.5 ± 0.3	4.7 ± 0.3	4.7 ± 0.4 *	3.6 -6.5
Protein (T)	g / L	69 ± 5	70 ± 5	70 ± 5	72 ± 4	71 ± 4	50 -79
AST	u / L	85 ± 26	78 ± 18	73 ± 18	72 ± 17	82 ± 14	48 -134
ALT	u / L	39 ± 7	36 ± 6	35 ± 5	31 ± 5 *	32 ± 4 *	22 -66
Na	mmol / L	143 ± 2	144 ± 2	146 ± 4 *	144 ± 1	144 ± 2	138 -181
Triglycerides	mmol / L	1.23 ± 0.72	1.48 ± 1.32	1.18 ± 0.59	1.65 ± 1.33	1.47 ± 1.17	0.10 -1.25
CK	u / L	389 ± 226	355 ± 150	313 ± 140	302 ± 137	369 ± 128	158 -556
Cholesterol	mmol / L	2.91 ± 0.79	3.03 ± 0.49	2.52 ± 0.50	2.94 ± 0.58	3.05 ± 0.71	0.94 -3.26
GGT	u / L	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	< 5 ± 0	3 to 8
Bile Acids	umol/L	12.8 ± 4.8	15.6 ± 8.7	18.3 ± 9.0	18.3 ± 8.7	13.1 ± 10.9	0 -24

[1] Magnuson *et al.*, 2014 * Statistically significant difference from Control Group ($p < 0.05$).

Organ Weights

Organ weights were expressed in absolute terms, and as a percent (%) of final body weight and as % of brain weight (Tables 11-9 and 11-10). Statistical differences were observed in some cases, but as will be discussed below, these changes were not considered to be indicative of a toxicological response to canary seed. Statistical differences included lower liver weight of male animals fed the high dose (10%) yellow canary seed when expressed in absolute terms and as a % of brain weight (Table 11-9). There was no statistical difference when expressed as % of body weight. The liver weights of females fed the low dose yellow canary seed and 10% brown canary seed were lower than controls when expressed as a % of body weight only (Table 11-10). The lower liver weights in rats fed the canary seed diets may have been the result of the lower incidence and severity of fatty liver (hepatic lipidosis), which was the most frequent observation during histological evaluations of tissues.

The thymus weight of male rats fed the 10% brown canary seed diet was higher than the controls when expressed in absolute terms, as a % of body weight and as a % of brain weight (Table 11-9). No effect was observed in female rats (Table 11-10) or males fed 10% yellow canary seed (Table 11-9).

The pancreas weight of female rats fed 5% yellow canary seed was higher than the controls when expressed in absolute terms, as a % of body weight and as a % of brain weight, but this was not observed in female rats fed 10% canary seed (Table 11-10) or in male rats (Table 11-9).

The spleen weights of female rats fed 2.5% and 10% yellow canary seed and 10% brown canary seed were lower than the controls when expressed in absolute terms and as % of body weight (Table 11-10). No effect was observed in male rats (Table 11-9). All the above-mentioned organ changes were not considered to be biologically relevant as there was no dose-response relationship, and absolute weights of most of the organs in question were within the normal historical ranges (age and gender matched). The exception is that the weight of the thymus exceeded the normal ranges for all groups including the rats in the control diet groups (normal ranges: 0.28-0.42 g.). Furthermore,

there were no clinical pathology and histopathological findings, which would indicate abnormal findings in any organs in which statistical differences were found, thus these differences were not considered toxicologically significant.

Table 11-9. Summary of absolute and relative organ weights for male rats fed AIN-76 diets containing varying concentrations of glabrous yellow and brown canary seed groats in the 90-day study (Phase 2)[1]

Parameter	Group Means ± S.D. (n = 20)				
	AIN-76 control	Low 2.5% Yellow canary seed groat	Mid 5% Yellow canary seed groat	High 10% Yellow canary seed groat	High 10% Brown canary seed groat
Stomach (Absolute)	2.55 ± 0.34	2.45 ± 0.34	2.67 ± 0.60	2.46 ± 0.28	2.67 ± 0.30
Stomach (% Body Weight)	0.39 ± 0.05	0.38 ± 0.03	0.41 ± 0.14	0.41 ± 0.05	0.40 ± 0.04
Stomach (% Brain Weight)	111.43 ± 15.03	109.49 ± 16.26	118.55 ± 31.58	107.97 ± 11.34	119.12 ± 14.86
Pancreas (Absolute)	0.982 ± 0.313	1.045 ± 0.286	1.036 ± 0.251	1.095 ± 0.279	1.057 ± 0.210
Pancreas (% Body Weight)	0.149 ± 0.049	0.163 ± 0.041	0.159 ± 0.042	0.180 ± 0.044	0.158 ± 0.039
Pancreas (% Brain Weight)	43.014 ± 14.171	46.338 ± 11.757	45.798 ± 10.962	48.197 ± 12.627	47.205 ± 9.832
Spleen (Absolute)	1.079 ± 0.138	1.014 ± 0.187	1.064 ± 0.139	0.959 ± 0.141	0.994 ± 0.144
Spleen (% Body Weight)	0.164 ± 0.019	0.158 ± 0.020	0.163 ± 0.028	0.157 ± 0.015	0.147 ± 0.016
Spleen (% Brain Weight)	47.258 ± 6.371	45.133 ± 7.934	47.150 ± 7.524	42.097 ± 5.204	44.401 ± 6.902
Liver (Absolute)	18.66 ± 2.96	17.24 ± 2.92	17.95 ± 2.61	**15.99 ± 2.35***	19.25 ± 3.68
Liver (% Body Weight)	2.83 ± 0.37	2.68 ± 0.23	2.72 ± 0.27	2.63 ± 0.28	2.83 ± 0.36
Liver (% Brain Weight)	817.37 ± 133.04	766.70 ± 119.54	793.27 ± 120.00	**702.33 ± 91.36***	859.31 ± 168.96
Adrenal Glands (Absolute)	0.085 ± 0.016	0.097 ± 0.026	0.096 ± 0.023	0.086 ± 0.013	0.084 ± 0.011
Adrenal Glands (% Body Weight)	0.013 ± 0.002	0.015 ± 0.004	0.015 ± 0.004	0.014 ± 0.003	0.013 ± 0.002
Adrenal Glands (% Brain Weight)	3.736 ± 0.653	4.355 ± 1.313	4.213 ± 0.966	3.785 ± 0.669	3.760 ± 0.491
Testes (Absolute)	3.83 ± 0.27	3.72 ± 0.45	3.74 ± 0.20	3.83 ± 0.29	3.83 ± 0.50
Testes (% Body Weight)	0.58 ± 0.05	0.59 ± 0.08	0.57 ± 0.07	0.63 ± 0.05	0.57 ± 0.09

Testes (% Brain Weight)	167.73 ±12.56	165.49 ±18.53	165.19 ±12.02	168.90 ±14.51	170.75 ± 20.20
Kidneys (Absolute)	3.78 ± 0.31	3.64 ± 0.61	3.81 ± 0.32	3.70 ± 0.36	3.79 ± 0.45
Kidneys (% Body Weight)	0.58 ± 0.05	0.57 ± 0.06	0.58 ± 0.07	0.61 ± 0.05	0.56 ± 0.06
Kidneys (% Brain Weight)	165.37 ± 11.83	162.10 ± 25.18	168.21 ± 15.01	162.93 ± 14.89	169.06 ± 18.54
Prostate (Absolute)	1.889 ± 0.464	1.794 ± 0.414	1.851 ± 0.624	1.798 ± 0.384	1.737 ± 0.561
Prostate (% Body Weight)	0.286 ± 0.063	0.280 ± 0.058	0.280 ± 0.083	0.297 ± 0.065	0.256 ± 0.072
Prostate (% Brain Weight)	82.603 ± 19.771	79.893 ± 18.102	81.744 ± 27.893	79.133 ± 16.998	77.635 ± 25.126
Lungs and Trachea (Absolute)	2.14 ± 0.25	2.14 ± 0.32	2.04 ± 0.17	1.97 ± 0.19	2.10 ± 0.20
Lungs and Trachea (% Body Weight)	0.33 ± 0.04	0.34 ± 0.04	0.31 ± 0.05	0.33 ± 0.03	0.31 ± 0.02
Lungs and Trachea (% Brain Weight)	93.52 ± 9.82	95.58 ± 14.80	90.33 ± 9.73	86.78 ± 7.43	93.76 ± 9.05
Heart (Absolute)	1.71 ± 0.16	1.69 ± 0.23	1.74 ± 0.11	1.65 ± 0.12	1.73 ± 0.21
Heart (% Body Weight)	0.26 ± 0.024	0.26 ± 0.03	0.27 ± 0.03	0.27 ± 0.02	0.26 ± 0.02
Heart (% Brain Weight)	74.91 ± 6.89	75.31 ± 11.66	76.95 ± 6.61	72.58 ± 4.40	77.15 ± 9.29
Thyroid and Parathyroids (Absolute)	0.036 ± 0.010	0.033 ± 0.009	0.034 ± 0.009	0.033 ± 0.008	0.041 ± 0.008
Thyroid and Parathyroids (% Body Weight)	0.006 ± 0.001	0.005 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Thyroid and Parathyroids (% Brain Weight)	1.581 ± 0.446	1.445 ± 0.384	1.498 ± 0.395	1.468 ± 0.312	1.832 ± 0.345
Thymus (Absolute)	0.581 ± 0.099	0.635 ± 0.135	0.562 ± 0.114	0.516 ± 0.121	**0.704 ± 0.193***
Thymus (% Body	0.088 ± 0.014	0.099 ± 0.016	0.085 ± 0.015	0.085 ± 0.017	**0.104 ± 0.026***

Weight)					
Thymus (% Brain Weight)	25.393 ± 4.225	28.235 ± 5.717	24.790 ± 4.718	22.603 ± 4.945	**31.443 ± 8.766***
Brain (Absolute)	2.29 ± 0.10	2.25 ± 0.10	2.27 ± 0.12	2.28 ± 0.12	2.24 ± 0.09
Brain (% Body Weight)	0.35 ± 0.03	0.36 ± 0.04	0.35 ± 0.03	**0.38 ± 0.03***	0.33 ± 0.03
Epididymis (Absolute)	1.65 ± 0.19	1.64 ± 0.22	1.73 ± 0.21	1.72 ± 0.20	1.70 ± 0.20
Epididymis (% Body Weight)	0.25 ± 0.03	0.26 ± 0.04	0.26 ± 0.04	**0.28 ± 0.04***	0.25 ± 0.04
Epididymis (% Brain Weight)	71.99 ± 8.22	73.27 ± 11.59	76.45 ± 8.49	75.74 ± 9.62	75.91 ± 10.25
Pituitary Gland (Absolute)	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.014 ± 0.002
Pituitary Gland (% Body Weight)	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
Pituitary Gland (% Brain Weight)	0.575 ±0.078	0.564± 0.081	0.561 ± 0.113	0.587 ± 0.089	0.602 ± 0.085

[1] *Magnuson et al., 2014*

*Statistically significant difference from AIN-76 Control Group ($p < 0.05$).

Table 11-10. Summary of absolute and relative organ weights for female rats fed AIN-76 diets containing varying concentrations of glabrous yellow and brown canary seed groats in the 90-day study (Phase 2)[1]

Parameter	Group Means ± S.D. (n = 20)				
	AIN-76 control	Low 2.5% Yellow canary seed groat	Mid 5% Yellow canary seed groat	High 10% Yellow canary seed groat	High 10% Brown canary seed groat
Stomach (Absolute)	1.76 ± 0.26	1.84 ± 0.25	1.69 ± 0.19	1.74 ± 0.25	1.73 ± 0.31
Stomach (% Body Weight)	0.49 ± 0.06	0.51 ± 0.05	0.49 ± 0.08	0.50 ± 0.07	0.48 ± 0.05
Stomach (% Brain Weight)	86.43 ± 12.66	92.06 ± 12.30	82.68 ± 10.04	83.98 ± 10.68	86.27 ± 15.97
Pancreas (Absolute)	0.618 ± 0.131	0.679 ± 0.155	**0.763 ± 0.189***	0.677 ± 0.117	0.730 ± 0.177
Pancreas (% Body Weight)	0.173 ± 0.037	0.188 ± 0.039	**0.226 ± 0.070***	0.194 ± 0.036	0.205 ± 0.047
Pancreas (% Brain Weight)	30.418 ± 6.596	34.045 ± 7.827	**37.479 ± 9.785***	32.884 ± 5.784	36.263 ± 8.020
Spleen (Absolute)	0.683 ± 0.143	**0.579 ± 0.110***	0.629 ± 0.096	**0.577 ± 0.075***	**0.594 ± 0.111***
Spleen (% Body Weight)	0.189 ± 0.029	**0.160 ± 0.027***	0.184 ± 0.031	**0.165 ± 0.022***	**0.166 ± 0.023***
Spleen (% Brain Weight)	33.548 ± 6.904	**28.974 ± 5.207***	30.760 ± 4.722	**27.985 ± 3.597***	29.749 ± 6.193
Liver (Absolute)	10.55 ± 2.49	9.61 ± 1.23	9.18 ± 1.20	9.43 ± 1.30	9.31 ± 1.65
Liver (% Body Weight)	2.91 ± 0.37	**2.65 ± 0.24***	2.67 ± 0.34	2.69 ± 0.28	**2.59 ± 0.29***
Liver (% Brain Weight)	517.86 ± 117.73	482.24 ± 64.73	448.87 ± 59.40	457.62 ± 65.89	465.50 ± 90.36
Adrenal Glands (Absolute)	0.096 ± 0.025	0.086 ± 0.020	0.088 ± 0.015	0.091 ± 0.020	0.094 ± 0.019
Adrenal Glands (% Body Weight)	0.027± 0.009	0.024 ± 0.006	0.026 ± 0.005	0.026 ± 0.007	0.027 ± 0.006
Adrenal Glands (% Brain Weight)	4.728 ± 1.249	4.319 ± 1.062	4.301 ± 0.769	4.447 ± 1.035	4.699 ± 0.984
Kidneys (Absolute)	2.43 ± 0.41	2.33 ± 0.27	2.35 ± 0.29	2.35 ± 0.32	2.41 ± 0.29
Kidneys (% Body Weight)	0.68 ± 0.05	0.64 ± 0.07	0.68 ± 0.07	0.67 ± 0.09	0.68 ± 0.07
Kidneys (% Brain Weight)	119.69 ± 20.05	116.73 ± 15.15	115.19 ± 15.99	114.16 ± 15.76	120.31 ± 14.68
Ovaries (Absolute)	0.195 ± 0.039	0.167 ± 0.034	0.198 ± 0.052	0.187 ± 0.049	0.179 ± 0.035
Ovaries (% Body Weight)	0.055 ± 0.011	0.046 ± 0.009	0.057 ± 0.013	0.053 ± 0.014	0.050 ± 0.008
Ovaries (% Brain Weight)	9.582 ± 1.981	8.374 ± 1.596	9.686 ± 2.588	9.059 ± 2.355	8.972 ± 1.837
Uterus (Absolute)	0.736 ± 0.158	0.733 ± 0.188	0.733 ± 0.169	0.711 ± 0.154	0.715 ± 0.203

Uterus (% Body Weight)	0.206 ± 0.047	0.204 ± 0.055	0.213 ± 0.045	0.205 ± 0.049	0.200 ± 0.052
Uterus (% Brain Weight)	36.110 ± 7.477	36.642 ± 8.925	36.005 ± 9.038	34.510 ± 7.742	35.570 ± 9.658
Lungs and Trachea (Absolute)	1.57 ± 0.22	1.57 ± 0.24	1.50 ± 0.23	1.50 ± 0.16	1.50 ± 0.20
Lungs and Trachea (% Body Weight)	0.44 ± 0.05	0.43 ± 0.05	0.44 ± 0.06	0.43 ± 0.05	0.42 ± 0.05
Lungs and Trachea (% Brain Weight)	76.97 ± 9.85	78.47 ± 11.71	73.63 ± 11.393	72.50 ± 6.90	75.02 ± 10.66
Heart (Absolute)	1.10 ± 0.13	1.08 ± 0.09	1.11 ± 0.12	1.10 ± 0.11	1.09 ± 0.13
Heart (% Body Weight)	0.31 ± 0.03	0.30 ± 0.30	0.32 ± 0.03	0.32 ± 0.03	0.31 ± 0.03
Heart (% Brain Weight)	54.22 ± 6.17	54.11 ± 4.60	54.52 ± 5.94	53.55 ± 5.46	54.60 ± 6.41
Thyroid and Parathyroids (Absolute)	0.024 ± 0.005	0.021 ± 0.004	0.022 ± 0.004	0.027 ± 0.005	0.026 ± 0.006
Thyroid and Parathyroids (% Body Weight)	0.007 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	**0.008 ± 0.001***	0.007 ± 0.001
Thyroid and Parathyroids (% Brain Weight)	1.179 ± 0.223	1.042 ± 0.192	1.077 ± 0.210	1.321 ± 0.280	1.277 ± 0.330
Thymus (Absolute)	0.419 ± 0.147	0.464 ± 0.112	0.385 ± 0.087	0.439 ± 0.090	0.422 ± 0.140
Thymus (% Body Weight)	0.114 ± 0.031	0.128 ± 0.025	0.111 ± 0.019	0.125 ± 0.019	0.122 ± 0.029
Thymus (% Brain Weight)	20.592 ± 7.323	23.271 ± 5.670	18.853 ± 4.285	21.289 ± 4.214	22.120 ± 7.207
Brain (Absolute)	2.04 ± 0.08	2.00 ± 0.08	2.05 ± 0.09	2.06 ± 0.09	2.01 ± 0.10
Brain (% Body Weight)	0.57 ± 0.07	0.56 ± 0.06	0.60 ± 0.06	0.59 ± 0.06	0.57 ± 0.07
Pituitary Gland (Absolute)	0.016 ± 0.004	0.015 ± 0.004	0.017 ± 0.006	0.018 ± 0.004	0.017 ± 0.004
Pituitary Gland (% Body Weight)	0.005 ± 0.001	0.004 ± 0.001	0.005 ± 0.002	0.005 ± 0.001	0.005 ± 0.001
Pituitary Gland (% Brain Weight)	0.801 ± 0.182	0.775 ± 0.198	0.837 ± 0.275	0.888 ± 0.195	0.854 ± 0.188

[1] Magnuson *et al.*, 2014
*Statistically significant difference from AIN-76 Control Group ($p < 0.05$).

All urinalysis data were unremarkable, within normal ranges and no significant differences between groups were observed. Gross necropsy also revealed no findings of toxicological significance (Magnuson *et al.*, 2014).

Histopathology

Histopathology revealed no findings of toxicological significance, although it should be noted that hepatic periportal lipidosis was noted in most rats of all groups. This is a common finding in well-fed laboratory rats (Medinsky *et al.*, 1986). In controls, the incidence and severity of this finding was slightly greater in females than in males. In both groups of females treated with either 10% of yellow or brown canary seed, and in males treated with 10% yellow canary seed (at the end of the study), there were decreases in incidence and severity in hepatic lipidosis as compared to the corresponding controls (Magnuson *et al.*, 2014).. This may be an indication of some protective properties of canary seed on the liver lipidosis. Further corroboration of possible protective effect of canary seed on lipid metabolism comes from an increase in the incidence and severity of liver lipidosis, i.e. returned to the control levels, in 30-day recovery animals consuming the control diet during this period.

Mineralization in the renal cortico-medullary region was commonly seen in females, but the test article had no apparent effect upon this condition. The occurrence of retinal thinning or degeneration was seen in some males or females of most diet groups, including controls. In addition, some control and treated rats had a variety of degenerative or inflammatory lesions that are commonly seen in laboratory rats and were in no way related to the test article administration.

No significant histopathological findings were noted for the testes, epididymis, prostate and seminal vesicles in male rats or the uterus, ovaries and mammary glands in female rats of the satellite, main and recovery groups for the 4 canary seed diet treatments compared to the control diet (Magnuson *et al.*, 2014).

Summary Phase 2 Rodent Study

In conclusion, analysis of all generated data including clinical observations, clinical pathology, gross necropsy and histopathology revealed no toxicity in rats that

consumed, *ad libitum,* glabrous yellow canary seed groats incorporated into diets at concentration levels of 2.5%, 5% and 10% or glabrous brown canary seed groats incorporated into diets at concentration levels of 10% for a 90-day period (Magnuson *et al.*, 2014).

Also, no toxicity was observed during the subsequent 30-day recovery period. Hepatic periportal lipidosis (and increased cholesterol, triglycerides and in some cases ALT levels) was the only finding that was feeding related (but not related to either yellow or brown canary seed), since there was no dose-response relationship and control rats were equally or more affected than the rats fed canary seed diets.

The above feeding regimen corresponded to average dose levels (gender combined) of 1.30, 2.54 and 5.15 g of yellow canary seed groats or 5.23 g of brown canary seed groats per kg per day, for the four dose levels, respectively.

Under the conditions of this study, a NOEL (No Observed Effect Level) for canary seed groats in rats was considered to be the highest concentration tested at 10% in the diet or 5.15 to 5.23 g/kg body weight per day for 90 days (Magnuson *et al.*, 2014).

11.2 Swine

Two studies evaluating canary seed as a feed for growing swine have been reported (Thacker, 2003; Qiao and Thacker, 2004) and discussed in Section 9.2.3. As the pig is considered to have very similar digestive system to man, these studies are particularly helpful in assessing the nutritional properties of canary seed as a human food; however, the studies did not report toxicological endpoints.

In the study evaluating the growth of grower-finishing pigs fed graded levels of canary seed Thacker concluded that canary seed could be included at levels as high as 57% of the total diet (75% of the cereal portion) without adversely affecting grower pig growth and feed intake or altering carcass characteristics. In addition the author indicated the canary seed diets were palatable, and nutrients were efficiently utilized and any anti-nutritional factors present in canary seed were not at high enough levels to negatively affect pig performance (Thacker, 2003).

11.3 Birds-poultry

Several studies have been conducted on the safety of canary seed as feed for broiler chickens. Newkirk *et al* (2011) studied the toxicological effects on poultry consuming pubescent and glabrous canary seed finding no significant toxicological effects when compared to consumption of a control commercial diet and/or wheat diet.

11.4 Toxicological Considerations Summary

The dietary consumption of canary seed has been investigated in birds, chickens, mice and rats fed pubescent brown and glabrous brown and yellow canary seed that were hulled or dehulled (groats).

Early studies conducted in mice (Bhatt *et al.*, 1984) focused on the carcinogenic and cancer-promoting potential of the silica fibers present on the surface of pubescent canary seed. No evidence of carcinogenicity due to consumption of pubescent canary seed for 18 months was observed in mice that were not initially treated with a skin cancer carcinogen (Bhatt *et al.*, 1984). Chronic irritation from dermal contact with silica fibers on the surface of pubescent canary seed promoted development of skin tumors in mice treated with the carcinogen. The selective breeding of the glabrous canary seed resulted in elimination of the surface silica fibers.

Subsequent toxicology studies conducted in rats demonstrated that brown glabrous canary seed fed in hulled or dehulled (groats) form at a level of 50% of the diet was similar to a diet containing 50% wheat in supporting growth during a 90-day study. No toxicologically significant effects were reported in evaluations of hematology, clinical chemistry, urinalysis, bone marrow assessments, functional observational batteries, ophthalmological evaluations and limited histological assessments. Increased body weights in male rats fed dehulled groats affected relative organ weights, but these were not considered toxicologically significant (Magnuson *et al.*, 2014).

A second 90-day rat study, conducted under GLP, assessed the growth and toxicological effects of the addition of yellow and brown glabrous canary seed groats to the AIN-76 diet at levels up to 10% of the diet. Male rats fed the diet containing 10% yellow canary seed groats consumed statistically significantly less food towards the end of the study, and had significantly lower body weights. No evidence of a dose-response

of these effects was observed in males fed diets with 2.5% or 5% yellow canary seed groats and no similar effects were observed in female rats. Furthermore, no toxicological adverse effects were observed in hematology, clinical chemistry, urinalysis, bone marrow assessments, functional observational batteries, ophthalmological evaluations or histological assessments (Magnuson *et al.*, 2014). The incidence and severity of hepatic lipidosis in the male rats fed 10% yellow canary seed was lower than observed in male rats fed the control diet. Liver lipidosis is a common finding in laboratory rats that are fed *ad libitum*, and tend to become obese (Medinsky et al., 1986). Reduced hepatic lipidosis was also observed in female rats fed diets containing 10% brown or yellow canary seed, as compared to controls. Therefore, the reduced body weight observed in male rats fed 10% yellow canary seed groats was not considered an adverse toxicological effect. No Observed Adverse Effect Levels in this pivotal toxicology study were 5.15 g/kg/d for yellow canary seed groats and 5.23 g/kg/d for brown canary seed groats, which were the highest tested doses.

These studies, in combination with analytical and nutritional data presented in this dossier demonstrating that the levels of nutrients, antinutrients, alkaloids, heavy metals, and mycotoxins are within the acceptable ranges observed in other grains, which support the safety of consumption of yellow and brown canary seed groats as a food cereal grain.

12.0 ALLERGENICITY CONSIDERATIONS

12.1 IgE-Mediated Allergy

Canary seed is not listed as a priority food allergen in North America, Europe, or any other region or country (FARRP, 2013). Cross-reactivities may, however, exist between proteins found in canary seed and major food allergens if there are structural or sequence homologies between the canary seed proteins and other major allergenic proteins. Since canary seed is a grain with comparatively high protein content, the potential for canary seed to sensitize susceptible individuals should also be assessed.

12.1.1. Pollen Allergy

Reports of the pollen from perennial pubescent canarygrass (e.g. *Phalaris aquatica*, *Phalaris arundinacea*) as a major environmental allergen and incidents of allergic reactions to pubescent canary seed on inhalation during handling have been cited in the literature. Using IgE antibodies from sera of 24 grass-pollen-allergic subjects, Suphioglu *et al.* (1993) identified seventeen allergenic fractions of canarygrass (*Phalaris aquatica*) pollen, ranging in molecular mass from 14 to 100 kDa. A 34-kDa protein fraction was found to have the highest frequency of IgE binding (77%) and was tentatively designated as Pha a I. Microsequencing of the N-terminus of this protein showed amino acid sequence homology with Lol p I from rye-grass pollen.

In other studies, significant amino acid sequence homology has been found between the *P. aquatica* allergenic proteins and other allergens from velvet grass, timothy grass and Kentucky bluegrass pollen (Suphioglu and Singh, 1995). Since canarygrass is a member of the *Pooideae* subfamily and is genetically related to other grass species, the possibility of cross-reactive pollen allergens among these various grass species is not surprising. However, pollen allergens are primarily an environmental and occupational issue and do not represent a food safety concern.

Apart from the above described studies, there are no reported studies on the allergenicity of annual canarygrass, particularly the newly developed glabrous yellow and brown *Phalaris canariensis* varieties. Discussions with canary seed producers indicate their preference of working with glabrous (hairless) *P. canariensis* varieties,

versus pubescent (hairy) *P. canariensis* varieties as the glabrous varieties are "itchless" and easier to harvest and manage.

12.1.2. IgE-Mediated Food Allergy

Assessment of the allergenic potential of canary seed is difficult because canary seed has not been a component of the human diet. The pubescent varieties have not been widely consumed and the glabrous varieties are not yet widely produced for human consumption. Not surprisingly, documented cases of food allergy due to canary seed do not exist. Almost no clinical literature exists with respect to the possible presence of ingestion allergens in canary seed, either pubescent or glabrous varieties. Baldo *et al.* (1980), using radioallergosorbent testing (RAST) of sera from subjects orally sensitized to wheat and rye flour, found significant IgE binding with seed extracts of 12 cereals including wheat, durum wheat, triticale, cereal rye, barley, rye grass, oats, canary seed (pubescent *P. canariensis*), rice, maize, sorghum and Johnson grass. However, IgE binding alone is insufficient to prove that allergic reactions would occur if these grains were ingested. To document allergenicity, an oral challenge with the grains or a demonstration of mediator release from activated basophils would be needed. Furthermore, plant sources often have cross-reactive carbohydrate determinants (CCD) on various glycoproteins that bind avidly to IgE but have limited, if any, clinical significance (Chunsheng *et al.*, 2008; van Ree, 2002). While the existence of CCDs was not known at the time of the Baldo *et al.* (1980) study, the role of CCDs in the observed IgE binding could have been significant.

In the absence of any history of ingestion of canary seed, the assessment of the allergenic potential of canary seed could be based upon several factors in a manner consistent with the evaluation of recombinant proteins in genetically modified foods – sequence homology of proteins to known allergens and the digestive stability of proteins to pepsin. However, this approach is difficult for a novel food such as canary seed because it likely contains dozens to hundreds of proteins unlike genetically modified foods that contain only one or a few novel proteins. Furthermore, few proteins in the proteome of canary seed have been purified or sequenced so this approach is essentially unworkable for canary seed.

The potential allergenicity of canary seed can be evaluated to some extent based upon its genetic relationships. Canary seed is part of the *Pooideae* subfamily that also contains wheat, durum wheat, spelt, rye, barley, triticale, and oats. Wheat is a commonly allergenic food. Allergies to other *Pooideae* grains including barley, rye, and oats have been documented but these foods are not commonly allergenic. Canary seed is mostly closely related to oats and oat allergy is rather rarely encountered (Inou *et al.*, 2013). Furthermore, cross-reactive allergy is not known to occur between wheat and other grains in the *Pooideae* subfamily. This observation casts doubt on the significance of the Baldo *et al.* (1980) study indicating cross-reactive IgE binding.

Boye *et al.* (2013) used SDS-PAGE to separate canary seed proteins. The brown and yellow canary seed cultivars showed similar electrophoretic profiles with the presence of protein bands ranging in molecular mass from ~ 10,000 to 100,000 Da. The most prominent band had a molecular mass of ~ 20,000 – 25,000 Da. To assess the presence of proteins in canary seed that might cross-react with wheat allergens, the reactivities of protein components separated by SDS-PAGE were analyzed by immunoblotting, using pooled sera from 10 wheat allergic individuals. The wheat-allergic sera were obtained from a serum bank and can only be characterized as wheat-sensitized (having IgE that binds to wheat proteins) because the serum donors were not clinically evaluated for wheat allergy by oral challenge or mediator release assays. The immunoblot of the three canary seed protein extracts revealed strong binding of the wheat sera to many of the canary seed proteins. Non-specific binding was suspected and then confirmed; but even exchanging the bovine serum albumin for non-fat dry milk still resulted in some binding of canary seed proteins to wheat sera. The three canary seed composites showed similar antibody-binding patterns.

Gliadin, a component of the gluten complex, is one of the known wheat allergens. To determine if binding would be observed with gluten-specific antibodies, the blots were also probed with polyclonal rabbit IgG anti-gluten antibodies raised specifically against wheat gluten protein (immunogen). In addition, blots were also probed with pooled sera of 7 individuals allergic to sesame seed as well as with anti-β-lactoglobulin antibody tested as negative controls. No binding was observed in any of the three

immunoblots of canary seed suggesting the absence of gluten specific proteins in the three canary seed samples.

To verify if binding would occur with other cereals and pseudo-cereals, the SDS-PAGE and blotting were performed on oat, millet, teff, quinoa, sorghum and buckwheat. Canada Western Red Spring (CWRS) wheat was used as the positive control. The SDS-PAGE results showed major differences in the electrophoretic profiles of the non-wheat cereals. This was expected as the cereals belong to different plant families. As was observed for the canary seeds groats, the pooled wheat sera recognized practically all the different polypeptide bands from the various non-wheat that were clearly visible in the SDS-PAGE profile as well as some that were not previously evident when bovine serum albumin was used as the blocking agent. Blocking with the non-fat dry milk instead of the bovine serum albumin revealed a different pattern with only a few bands recognized. The western blotting was repeated using rabbit polyclonal gluten antibodies with non-fat dry milk as blocking agent. The immunoblot revealed strong binding to many of the wheat proteins and some proteins in oat, millet, quinoa, teff, and to a lower extent with sorghum and buckwheat proteins, which could be either due to cross-reactivities or cross-contamination of the grains with gluten proteins.

To confirm the identity of the predominant protein components recognized by antibodies in the wheat sera, electrophoresis of wheat and non-wheat cereals and pseudo-cereals including glabrous canary seeds was conducted again and the bands showing antibody-antigen binding during immunoblotting were excised and further analyzed by LC/ESI-MS/MS. Because very few proteins from canary seed have been sequenced, none of the IgE-binding proteins from canary seed were identified as belonging to *P. canariensis*. The tryptic peptides identified from the IgE-binding proteins of canary seed did show some homology to sequenced proteins from rice, oats, barley, sorghum, and corn. The only protein with any homology to a wheat protein showed some homology to granule-bound starch synthase I. That protein is not a known wheat allergen (see Boye *et al*, 2013 manuscript for more detail and figures, Appendix 6).

The results obtained by Boye *et al.* (2013) cannot be reliably used to exclude the possibility of some cross-reactivity with canary seed among wheat-allergic individuals. However, the IgE binding observed with canary seed and other non-wheat grains under

some immuoblotting conditions could have been due to CCDs; this possibility was not evaluated by Boye *et al.* (2013).

The safety of glabrous canary seed from an allergy perspective was further assessed by analyzing for the presence of cross-reactivities using commercially available ELISA kits for major allergenic plant foods including gluten, soy, peanuts, tree nuts, sesame and mustard (Boye *et al.*, 2013). In general, analytical tests to determine the amount of the allergenic food residue that might be present in some other food are typically conducted using commercial Enzyme Linked Immuno Sorbent Assays (ELISA). With the exception of gluten, these ELISA kits detect source-specific proteins and are not specific for allergenic proteins from these foods.

All 18 glabrous canary seed composites (6 composite samples of brown canary seed (CDC Maria) and 12 composite samples of yellow canary seed (C05041 & C05091) from the Phase 2 study were tested as per the instructions of the ELISA kits.

Due to reported variability in ELISA results from different test kits, at least two to three commercial test kits from different companies were used for each targeted allergen (when available) and extractions were done in triplicate for each kits and each extract was analyzed in triplicate. As a measure of security, the proposed amounts indicated on the kit instruction were tripled in some instances and the extractions were repeated. When cross-contamination was suspected, samples were visually cleaned and the extractions were repeated. (For methodology details, see Appendix 6: Boye *et al*, 2013).

ELISA results of the canary seed groats for the different allergen kits tested are provided in Table 12-1. All the results were below the Limit of Detection (LOD) and Limit of Quantification (LOQ).

Table 12-1 ELISA results of glabrous brown and yellow canary seed[1]

Allergen	Company	Test kit	ELISA results		
			Glabrous Canary Seed		
			Brown	Yellow	
			CDC Maria	C05041	C05091
Almond	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Gluten	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Hazelnut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Mustard	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
	Sedium R&D	ELISA	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
Peanut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
	R-Biopharm	Ridascreen	< LOD	< LOD	< LOD
Sesame	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
Soy	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD
	ELISA System	ELISA	< LOQ	< LOQ	< LOQ
	Neogen	Veratox	< LOQ	< LOQ	< LOQ
Walnut	Gen-Probe/Tepnel	Biokit	< LOD	< LOD	< LOD

[1]Boye et al., 2013

LOD: Limit of detection; LOQ: Limit of quantification.

Overall, these results demonstrate that no proteins from almond, hazelnut, peanut, sesame, soy, walnut, mustard or gluten are present in the canary seed

samples. Furthermore, no protein epitopes capable of reacting with the polyclonal or monoclonal antibodies used in these ELISA kits are present in the canary seed samples. However, these results cannot be used to convincingly demonstrate that cross-reactivity would not occur between canary seed and these commonly allergenic foods as claimed by Boye *et al.* (2013). Evidence of cross-reactivity could only be determined by oral challenges or assays for mediator release from activated basophils. However, based upon the divergent genetic relationships between canary seeds and these other foods, with the exception of wheat gluten, the likelihood of cross-reactivity seems remote.

12.1.3. Gluten

Boye *et al.* (2013) evaluated the possible presence of gluten and gluten-related peptides and proteins using several different approaches. First, as noted above, ELISA kit assays capable of detecting gliadin, the alcohol-soluble fraction of the gluten complex (Mendez *et al.* 2005; Skerritt and Hill, 1991) were conducted on yellow and brown glabrous canary seed. As noted in Table 12-1, three gluten ELISAs were used. Two of these ELISAs use the R5 monoclonal antibody (Mendez et al., 2005) while the third uses the so-called Skerritt antisera (Skerritt and Hill, 1991). The R5 antibody is highly specific for the QQPFP and closely related epitopes found in gliadin. The R5 antibody reacts with prolamins from wheat, barley, rye and related grains but not with oats. The Skerritt antisera are polyclonal and recognize the omega-gliadin fraction of the gluten complex. The Skerritt antisera are highly reactive to wheat and rye prolamins but much less reactive to barley prolamins.

Details of the methodologies used, results obtained, additional tables and figures referred to in the following discussion can be found in Appendix 6 (Boye *et al*, 2013).

As noted in Table 12-1, Boye *et al.* (2013) found no evidence of protein epitopes from canary seed that were reactive with either the R5 or Skerritt antibodies. The absence of reactive proteins in both ELISAs suggests that pure canary seed would not elicit adverse reactions among celiac sufferers. However, the possible presence of reactive prolamin epitopes that would not be recognized by either of these two antibodies cannot be entirely excluded.

Consequently, further evidence of gluten-specific protein fragments was sought by mass spectrometry (MS). Mass spectrometry was used to identify any protein/peptide fragments with homology to known celiac-related gluten sequences of gluten-containing cereals (wheat, barley and rye) (Camafeita *et al.* 1997; Mendez *et al.* 2000). A number of proteins identified from the MASCOT database showed the three glabrous canary samples were mostly homologous with rice, oats, corn, carrot, tomato, radish, beet, and chickpea proteins. No celiac related gluten fragments from wheat, rye, barley or their derivatives were noted in any of the tested glabrous canary samples (Boye *et al*, 2013)

For the glabrous brown canary seed (CDC Maria) three hits were obtained indicating the likely presence of protein disulfide-isomerase (wheat), Em protein H5 (wheat) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) or proteins having similar homology. One hit suggesting the likely presence of cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) or a similar protein was found for CDC 5041. Protein disulfide-isomerase, with a molecular mass of 56,533 Da, is an enzyme in the endoplasmic reticulum in eukaryotes that catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold (Wilkinson and Gilbert, 2004). Em protein H5 (molecular mass, 10,060 Da) is a member of the small hydrophilic plant seed protein family. Cytosolic glyceraldehyde-3-phosphate dehydrogenase (molecular mass, 33,236 Da) belongs to the glyceraldehyde-3-phosphate dehydrogenase family. The amino acid sequences of these three proteins can be found in the reference Boye *et al.*, 2013.

Gluten epitopes provoking celiac disease typically originate from the gliadin and glutenin fractions and contain high amounts of glutamine and proline amino acid residues and the signature amino acid motif "QP" (Osman *et al.*, 2000 Qiao *et al.*, 2005). The amino acid sequences of the three canary seed protein hits (i.e., protein disulfide-isomerase (wheat), Em protein H5 (wheat) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (barley) did not show any "QP" amino acid motif suggesting little likelihood of them containing a celiac provoking epitope. Overall, the mass spectrometry results of glabrous canary seed proteins suggest either cross contact or homology between canary seed proteins and some rice, oats, corn, carrot, tomato,

radish, beet, and chickpea proteins. Note that none of these three canary seed proteins with some homology to wheat were identified as likely triggers of celiac disease or as IgE-binding proteins using sera from wheat-allergic subjects. These findings suggest canary seed could be gluten-free.

13.0 MICROBIOLOGICAL CONSIDERATIONS

Cereal grains and flours are considered raw agricultural commodities, which undergo minimal processing prior to incorporation into a myriad of food products.

Cereals can contain between 10^2 to 10^9 CFU (colony forming units) of aerobic bacteria per gram, up to 10^6 yeasts and molds. *Salmonella* spp, *Bacillus* spp and *Escherichia* species may also be detected in low numbers (CIGI, 2006; ICMSF, 2005)

13.1 Mycotoxins

Mycotoxins are the most important of the microbial health hazards in cereals and cereal products. Cereal crops harbor many of the most important mycotoxins. The principal mycotoxigenic fungi associated with wheat, barley, and other small grain crops are *Fusarium* species, which produce a range of trichothecene toxins. The most important tricothecenes are deoxynivalenol (DON) and nivalenol (NIV), and the estrogenic toxin, zearalenone (ICMSF, 2005).

Canaryseed, similar to other common cereals and forage grasses, is susceptible to Fusarium Head Blight (FHB). The most common mycotoxin found in grain affected by FHB is deoxynivalenol (DON), also known as vomitoxin. In Saskatchewan, durum wheat, spring wheat and barley are most affected by this disease. The Canadian Grain Commission routinely analyzes grain shipments for *Fusarium* trichothecenes (DON). (Tittlemier et al., 2013). For many countries, the existing maximum limits for DON in cereal grains range from 1.0 to 2.0 mg/kg (ppm) (Tittlemier *et al.*, 2013)

Aflatoxins and vomitoxins (deoxynivalenol-DON) in glabrous canary seed (CDC Maria), pubescent canary seed (Keet) and CWRS wheat (Katepwa) in Phase 1 grown at ten locations in Saskatchewan were analyzed by the Grain Research Laboratory, Canadian Grain Commission (CGC) (Winnipeg, MB). The three crops were found to be free from vomitoxin (within the limit of ELISA technique which was 0.5 ppm). The canary seed and wheat grain were also found to have less than 5 ppb aflatoxin. The CGC issued certificates of analyses for vomitoxin and aflatoxin, which can be found in Appendix 8.

In Phase 2, the brown and yellow canary seed groats were analyzed for the presence of vomitoxin (DON), zearalenone, total fumonisins and Ochratoxin A. As shown in Table 13-1, vomitoxin at the limit of detection (LOD) of 0.1ppm and ochchratoxin A (LOD 0.96ppm) were not detected in any canary seed samples. This low level of ochratoxin is typical of many Canadian grains (<1ppb) and below the limit of other countries (3-50 ppb) (Canadian Grain Commission, 2013).

Total fumonisins with values greater than the 0.13 ppm limit of detection were detected in 8 yellow canary seed samples (0.14 ppm to 0.24 ppm) and in 2 brown canary seed samples (0.13 and 0.20ppm). Eight samples were below the detection level. These levels are below the guidance levels recommended by the US FDA for maize and maize products (2-4 ppm) (FDA, 2001).

Zearalenone was detected in 13 of the 18 glabrous canary seed samples ranging from 13.6 ppb to 40.3 ppb (ug/kg). Five (5) samples presented below the 10.5 ppb limit of detection. The levels detected were less than the maximum limit set by the European Union of 100 ug/kg for unprocessed cereals (EFSA, 2001).

Table 13-1 Mycotoxin levels in glabrous brown and yellow canary seed [1]

Mycotoxin	Limit of Detection	Glabrous Canary Seed	
		Brown	Yellow
		(range)	(range)
Fumonisins (total)	0.13ppm	< 0.13 to 0.20	<0.13 to 0.24
Ochratoxin A	0.96ppb	< 0.96	< 0.96
Vomitoxin	0.1 ppm	< 0.1	<0.1
Zearalenone	10.5 ppb	< 10.5 to 40.3	<10.5 to 33.8

[1] Phase 2 CDCS study

13.2 Microflora

Due to the excessive handling of the small plot samples, the 18 samples of glabrous canary seed used for nutritional and chemical analyses were not analyzed for their microbial profile. Instead, glabrous brown and yellow canary seed grown under field conditions and dehulled under commercial conditions were tested for aerobic plate count, yeasts and molds, and coliforms. The effect of processing on the microbial load was also evaluated.

Tables 13-2 and 13-3 represent the microbial counts of hulled brown and yellow canary seed, whole yellow and brown groats and yellow and brown whole grain flours subjected to various processing conditions-no processing, heat treated at 240°F for 8 minutes; roasted (without prior tempering) at 350°F for 8 minutes and roasted after tempering to 14% moisture at 350°F for 8 minutes.

Results indicate that the microbiological profile of hulled canary seed and canary seed groats falls within the microbiological counts for wheat and other small cereal grains (ICMS, 2005; CIGI, 2006). Raw canary seed, with or without hulls, had approximately 2×10^5 to 1×10^6 cfu/g (total plate count) and 600 to 1500 cfu/g yeasts and mold present on the samples tested. Coliforms (20-80 cfu/g) were detected in the raw flour samples, but not in the whole grain or any of the processed canary seed products. Heat treating at a low temperature (240°F) and roasting (350°F) reduced the microbial load by 2 and 4 to 5 logs respectively.

While cereal grains and their milled products contain bacteria, molds and yeasts due to contamination with soil, feces, insects and other contaminants, they have traditionally been considered low food safety risk commodities due to a low water activity and subsequent heat processing steps when incorporating grains into baked goods and other foods. However, recent food borne outbreaks implicating *Escherichia coli* in raw cookie doughs is changing the way industry views the safety of cereal grains and milled products. A number of control strategies (heat, ozone and irradiation) are being investigated to reduce the incidence of potential pathogens in wheat flours while maintaining the functional and nutritional qualities of grain and milled products (Rose *et al*, 2012). In the meantime, maintaining good agricultural practices and good manufacturing practices throughout the grain supply chain should maintain the microbial

integrity of any processed grain ingredient, including canary seed (Akins-Lewenthal, 2012).

Table 13-2 Microbial analysis of yellow canary seed groats and milled products subjected to different processing conditions*

Canary seed samples	Total Plate Count (CFU/G)[7]	Coliforms Count (CFU/G)	Yeast & Molds Count (CFU/G)
Yellow canary seeds *with hulls*, raw	2.6×10^5	ND[8]	650
Yellow canary seed *groats*, raw	2.4×10^5	ND	420
Whole yellow canary seed *flour*, raw	1.0×10^5	80	100
Yellow canary seed *groats*, without tempering, heat treated[1]	7000	ND	30
Yellow canary seed *groats*, tempered to 14% moisture, heat treated[1]	6200	ND	30
Yellow canary seed *flour*, without tempering, heat treated[1]	4200	ND	910
Yellow canary seed *flour*, tempered to 14% moisture, heat treated[1]	1300	ND	290
Yellow canary seed *flour*, without tempering, roasted[2]	300	ND	110
Yellow canary seed *flour*, tempered to 14% moisture, roasted[3]	100	ND	40

[1,4] Heat Treated = 240°F for 8 minutes ; [2,5] Roasted canary seed without tempering at 350°F for 8 minutes; [3,6] Roasted canary seed with tempered to 14% moisture at 350°F for 10 minutes; [7]CFU, colony forming units
[8]ND-not detected
*Phase 2 CDCS study, unpublished

Table 13-3 Microbial analysis of brown canary seed groats and milled products subjected to different processing conditions

Canary seed Samples	Total Plate Count (CFU/G)	Coliforms Count (CFU/G)	Yeast & Molds Count (CFU/G)
Brown canary seeds *with hulls*, raw	1.01×10^6	ND	800
Brown canary seed *groats*, raw	1.8×10^5	ND	1500
Whole brown canary seed *flour*, raw	1.0×10^5	20	1000
Brown canary seed *groats*, without tempering, heat treated[4]	1000	ND	10
Brown canary seed *groats*, tempered to 14% moisture, heat treated[4]	2000	ND	10
Brown canary seed *flour*, without tempering, heat treated[4]	600	ND	120
Brown canary seed *flour*, tempered to 14% moisture, heat treated[4]	1600	ND	20
Brown canary seed *flour*, without tempering, roasted[5]	110	ND	ND
Brown canary seed *flour*, tempered to 14% moisture, roasted[6]	200	ND	ND

[1,4] Heat Treated = 240°F for 8 minutes ; [2,5] Roasted canary seed without tempering at 350°F for 8 minutes;
[3,6] Roasted canary seed with tempered to 14% moisture at 350°F for 10 minutes
*Phase 2 CDCS study, unpublished

14.0 DIETARY EXPOSURE ASSESSMENT

14.1 Potential Forms of Canary Seed Whole Grain

It is proposed glabrous brown and yellow coloured canary seed (*Phalaris canariensis*) be introduced to the US population as a cereal grain in whole groat and milled forms (e.g. flour or flakes) similar to how other cereal grains such as wheat, barley, oats, triticale, rye, buckwheat, ancient grains, millet, and sorghum and pseudo cereals such as buckwheat, amaranth and quinoa are offered. Whole canary seed groats may also be used to replace or complement the use of seeds in food products similar to the use of sesame seed, sunflowers seeds, poppy seed, pumpkin seed and flaxseed as a topping or ingredient in crackers, breads, rolls, buns, cereal/nutrition bars and snaps etc. Canary seed groats could also be used to replace sesame seeds (a food allergen) in some foods (i.e. sesame snaps) to provide alternatives to consumers.

As discussed in Section 5.0 *Manufacturing Methods* product development trials illustrated that canary seed groats or milled products (e.g. flours) at levels up to 25% in most product formulations could be used to replace and/or complement whole grains, refined grains or seed ingredients currently used in food products without greatly affecting functional or sensory characteristics. Levels up to 50% could be used in a standard sugar cookie recipe where canary seed flour could be the sole flour used.

14.2 Estimated Daily Intake of Canary Seed by the U.S. Population from Proposed Food-Uses

Intertek Cantox (Mississauga, ON, Canada) completed the assessment of the potential intake of canary seed by the United States (U.S.) population. The full report is provided in Appendix 9. Canary seed is proposed for use as a grain in the U.S. in baked goods and baking mixes, breakfast cereals, grain products and pastas, and snack foods. Based on product development trials, it is expected that canary seed will primarily be used in whole grain food products. However, in order to estimate the highest possible daily intake of canary seed, both whole grain and refined grain food products in each food category were included, and the highest use levels applied to all

products in that category. Thus, the resulting estimates are unrealistic, but represent a "worst-case" intake scenario, or highest possible intakes for canary seed.

Estimates for the intake of canary seed were based on the proposed food-uses and use-levels for canary seeds in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2009-2010 (CDC, 2011; USDA, 2012). Canary seed is not intended for use in infant foods. Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of canary seed and the percentage of consumers was determined. Similar calculations were used to estimate the total intake of canary seed resulting from all proposed food-uses of canary seed combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Children, ages >2 to 11;
Female teenagers, ages 12 to 19;
Male teenagers, ages 12 to 19;
Female adults, ages 20 and up;
Male adults, ages 20 and up; and
Total population (all age and gender groups combined).

Intake estimates for infants, ages 0 to 2, were not included, as canary seed is not intended for use in infant foods.

14.2.1 FOOD CONSUMPTION SURVEY DATA

14.2.1.1 Survey Description

NHANES for the years 2009-2010 are available for public use. NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. Each year about 7,000 people from 15 different locations across the U.S. are interviewed, and approximately 5,000 complete the health examination component of the survey. Any combination of consecutive years of data collection is recognized and used as a nationally representative sample of the U.S. population. It is well-established that the length of a dietary survey affects the estimated consumption of individual users and that

short-term surveys, such as a 1-day dietary survey, may overestimate consumption compared to surveys conducted over longer time periods (Anderson, 1988). Because two 24-hour dietary recalls administered on 2 non-consecutive days are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 15 PSUs are visited per year. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2009-2010, 13,272 individuals were selected for the sample, 10,537 were interviewed (79.4%), and 10,253 were sampled (77.3%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Sample weights were incorporated with NHANES 2009-2010 data to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2011; USDA, 2012).

14.2.1.2 Statistical Methods

Statistical analysis and data management were conducted in Creme software (www.cremeglobal.com) (Creme, 2013). Creme Food 3.0 is a probabilistic modeling software tool that uses high-performance computing to allow accurate estimate of

exposure to contaminants, food additives, flavorings, nutrients, food packaging migratory compounds, novel foods, pesticide residues, and microbial contaminants. The main input components are concentration (use level) data and food consumption data. Data sets are combined using the Creme Food 3.0 model to provide accurate and efficient exposure assessments.

For the deterministic assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of canary seed by the U.S. population using Creme software. Estimates for the daily intake of canary seed represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were generated. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. All-person intake refers to the estimated intake of canary seed averaged over all individuals surveyed, regardless of whether they consumed food products potentially containing canary seed, and therefore includes individuals with "zero" intakes (*i.e.* those who reported no intake of food products potentially containing canary seed during the 2 survey days). All-user intake refers to the estimated intake of canary seed by those individuals who reported consuming food products containing canary seed, hence the "all-user" designation. Individuals were considered 'users' if they consumed 1 or more food products containing canary seed on either Day 1 or Day 2 of the survey.

Mean or percentile intake estimates based on small sample sizes may be less statistically reliable than estimates based on adequate sample sizes (LSRO, 1995). Therefore, for the estimated intakes of canary seed from proposed uses presented herein, values were considered statistically unreliable if the sample included less than 30 respondents. These values were not considered when assessing the relative contribution of specific food-uses to total canary seed consumption and are marked with an asterisk in Appendices A and B of the Intertek Cantox report (Appendix 9).

14.2.2 FOOD USAGE DATA

The individual proposed food-uses and use-levels for canary seed employed in the current intake analysis are summarized in Table 14-1. Canary seed can be added to food in several different forms including the dehulled milled grain, dehulled whole grain flour, or dehulled whole canary seeds. Canary seed is not intended for use in infant foods. The use-levels provided in Table 14-1 represent the total use of the canary seed in all forms within a given food-use in order to reflect the possible inclusion of multiple canary seed-based ingredients.

Food codes representative of each proposed food-use were chosen from the NHANES 2009-2010 (CDC, 2011; USDA, 2012). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2013). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). All food codes included in the current intake assessment are listed in Appendix C of the Intertek Cantox report (Appendix 9). A given food code may not be associated with both surveys; as with each new survey the food code list has been updated to reflect the availability of new foods and the discontinuation of certain obsolete codes.

Table 14-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Canary seed in the U.S. (2009-2010 NHANES Data)

Food Category	Proposed Food-Uses	Maximum Proposed Use Level (%)
Baked Goods and Baking Mixes	Bagels	25
	Biscuits	20
	Breads and Rolls	25
	Cakes	20
	Cookies	50
	Cornbread, Corn Muffins, and Tortillas	25
	Crackers	26
	Croissants and Pastries	25
	Doughnuts	25
	Flours and Brans (pre-packaged)	100
	Muffins	20
	Pancakes and Waffles	25
	Pies	10
Breakfast Cereals	Instant and Regular Hot Cereals	15
	Ready to Eat Breakfast Cereals	15
Grain Products and Pastas	Energy, Meal Replacement, and Fortified Bars	25
	Granola and Cereal Bars	25
	Macaroni and Noodle Products	15
	Pasta, Rice and Other Grains	15
Snack Foods	Savory Snacks	25
	Seed-based snacks	40

14.2.3 FOOD SURVEY RESULTS

Estimates for the total daily intakes of canary seed from proposed food-uses are provided in Tables 14.2 and 14.3. Estimates for the daily intake of canary seed from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-6 and B-1 to B-6 of Appendices A and B, respectively of the Intertek Cantox report (Appendix 9).

14.2.3.1 Estimated Daily Intake of Canary seed from All Proposed Food-Uses

Table 14.2 summarizes the estimated total intake of canary seed (g/person/day) from all proposed food-uses in the U.S. population group. Table 14.3 presents this data on a per kilogram body weight basis (g/kg body weight/day). The percentage of users was high among all age groups evaluated in the current intake assessment; greater than 98.7% of the individual population groups comprised users of those food products

in which canary seed is currently proposed for use. (Table 14.2). Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

Consumption of proposed food-uses by the total U.S. population resulted in an estimated mean and 90th percentile all-user intakes of canary seed of 47 g/person/day (0.8 g/kg body weight/day) and 85 g/person/day (1.7 g/kg body weight/day), respectively. Within the individual population groups, male adults were determined to have the greatest estimated mean and 90th percentile all-user intakes of canary seed on an absolute basis, at 55 and 100 g/person/day, respectively (Table 14.2).

Table 14.2 Summary of the Estimated Daily Intake of Canary seed from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/day)		All-Users Consumption (g/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Children	>2 to 11	46	79	99.9	1,427	46	80
Female Teenagers	12 to 19	46	83	99.4	515	46	83
Male Teenagers	12 to 19	52	96	98.7	560	53	97
Female Adults	20 and up	41	75	99.2	2,627	42	75
Male Adults	20 and up	54	100	99.2	2,368	55	100
Total Population	All Ages	46	84	98.2	7,497	47	85

On a body weight basis, children were the population group identified as having the highest mean and 90th percentile all-user intakes at 1.8 and 3.2 g/kg body weight/day, respectively (Table 14.3). Female and male adults were identified as having the lowest mean all-user intakes of 0.6 g/kg body weight/day, for both population groups, and female adults were determined to have the lowest 95th percentile all-user intakes of 1.1 g/kg body weight/day.

Table 14.3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Canary seed from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (g/kg bw/day)		All-Users Consumption (g/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Children	>2 to 11	1.8	3.2	99.9	1,427	1.8	3.2
Female Teenagers	12 to 19	0.8	1.4	99.4	515	0.8	1.4
Male Teenagers	12 to 19	0.8	1.6	98.7	560	0.8	1.6
Female Adults	20 and up	0.6	1.1	99.2	2,627	0.6	1.1
Male Adults	20 and up	0.6	1.2	99.2	2,368	0.6	1.2
Total Population	All Ages	0.8	1.7	98.2	7,497	0.8	1.7

14.2.3.2 Estimated Daily Intake of Canary seed from Individual Proposed Food-Uses in the US

In terms of contribution to total mean intake of canary seed, breads and rolls and pasta, rice and other grains were the 2 main sources of intake across all population groups on both an absolute and on a g/kg body weight basis. Breads and rolls contributed 21.9% to total mean intakes or 12.7 to 24.3% among the individual population groups whereas pasta, rice and other grains contributed 20.9% to total mean intakes or 18.7 to 22.8% among the individual population groups. Energy, meal replacement, and fortified bars and seed-based snacks individually contributed ≤0.3% to total mean estimates for canary seed intakes across all population groups (see Tables A-1 to A-6 and/or B-1 to B-6 of the Intertek Cantox report (Appendix 9) for further details). It should be noted that there were no users identified in flours and brans (pre-packaged); thus, there was no intake of canary seed from this category. However, the food codes in this food category are only representative of flour and brans that would have been used by respondents in home baking. Any flours or brans based on canary seed included in prepared foods would have been captured in other food-use categories.

14.3 Summary of Total Daily Intakes

Consumption data and information pertaining to the individual proposed food-uses of canary seed were used to estimate the all-person and all-user intakes of canary seed for specific demographic groups and for the total U.S. population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

In summary, on an all-user basis, the mean and 90th percentile intakes of canary seed by the total U.S. population from all proposed food-uses were determined to be 47 g/person/day (0.8 g/kg body weight/day) and 85 g/person/day (1.7 g/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of canary seed by the U.S. population from all proposed food-uses in the U.S., as observed in male adults were estimated to be 55 g/person/day (0.6 g/kg body weight/day) and 100 g/person/day (1.2 g/kg body weight/day), respectively.

15.0 SUMMARY AND CONCLUSIONS

The data and information contained in this report support the safety of annual canary seed (*Phalaris canariensis* L.) as a food cereal grain for human consumption. Glabrous canary seed groats are proposed for use as an ingredient in breads, flours, breakfast cereals, and pastas, as well as baked goods (e.g. biscuits, crackers, cookies, granola bars, nutrition bars, energy bars) and baking mixes (e.g. cakes).

Canary seed provides a source of protein, carbohydrate, essential fatty acids, dietary fiber, minerals and vitamins, as well as phytochemicals. The US Dietary Guidelines for Americans recommend 5-8 servings of grains per day, with at least half of these grains being whole grains. There is an opportunity for glabrous canary seed to be consumed as a whole grain/whole groat in the diet and contribute to dietary eating habits. Canary seed would ideally, as a new whole grain food introduction, be consumed with the other available whole grain diet choices.

The safety assessment process for novel foods, such as canary seed, differs from the conventional approach used in the assessment of an individual food chemical, which leads to the establishment of an Acceptable Daily Intake based on the identification of a no-effect level many times higher than anticipated human exposure (ILSI, 2002). For novel foods, it is recognized that it is not be possible or appropriate to feed a whole food at high levels in the diet, due to major alterations in the nutritional composition of the diet. Instead, the compositional, nutritional and toxicological characteristics and safety assessment of the novel food should be evaluated in the light of anticipated human exposure pattern in the context of normal expectations of food consumption (ILSI, 2003; Health Canada, 2006).

An ILSI expert panel (2003) on the safety assessment of novel foods concluded, "the evaluation should be based on knowledge of the characteristics of the novel food in question using comparisons with conventional foods where appropriate. Critical examination showing the estimated intake of the novel food to be below the level indicated as without toxic or nutritional hazard by the totality of the information available will allow a presumption of reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption."

Detailed analysis of the composition of macronutrients, micronutrients, and antinutritional factors demonstrated that glabrous canary seed is similar to other commonly consumed cereal grains. *Phalaris canariensis* has a nutritional and compositional profile similar to other commonly consumed cereal grains being mainly comprised of protein (19-23%), starch (53-61%), fat (5.5-8%), dietary fiber (6-8%) and ash (1.9-2.4%). Similar to other cereals the proteins in canary seed are deficient in lysine but rich in cysteine, tryptophan, phenylalanine and arginine. Canary seed contains levels of trace minerals and B vitamins comparable to other cereal grains. As in other cereal grains and legumes, phenolic acids, phytate, trypsin inhibitors and amylase inhibitors are found in the grain. Phytate is present at about twice the level found in wheat, but at similar levels to other cereals, pulses and commonly consumed nuts and seeds. Growth and nutritional studies in swine and rodents confirmed the analytical results, demonstrating growth and food consumption rates comparable to other grains.

Levels of alkaloids, heavy metals, mycotoxins and microbial contamination in canary seed were similar or lower than reported in other cereal grains, and are not of toxicological concern. No evidence of allergenic potential of glabrous brown or yellow canary seed groats was identified from detailed assessments.

Feeding glabrous brown or yellow coloured canary seed groats to rats for 90 days in detailed toxicological studies resulted in no adverse toxicological findings that could be attributed to consumption of glabrous canary seed groats. In the first 90-day study, no adverse effects were observed in rats consuming diets containing 50% brown glabrous canary seed, resulting in NOAELs ranging from 33 to 37 g/kg/d for males and 38 to 42 g/kg/d for females. In the second 90-day study, the observed NOAEL of yellow and brown glabrous canary seed groats were at the highest doses tested, which ranged from 5.1 to 5.7 g/kg/d (Magnuson *et al.,* 2014).

Current consumption levels of whole grains and seeds by the US population, and optimistic projections for the replacement of currently-used grains and seeds with canary seed ingredients in various food products were used to calculate the highest likely consumption levels of canary seed. The average and 90th percentile dietary exposure calculations, using these conservative assumptions, were 0.8 and 1.7 g/kg/d

respectively, for the total population. Not surprisingly, the subgroup with the highest consumption based on body weight was children, with average and 90th percentiles estimated as 1.8 and 3.2 g/kg/d, respectively. The average intakes of grains for children aged 2 to 11 in 2003-2004 was reported to be 6.83 oz per day, or 193.6 g per day (Lin 2011). Based on this average, the daily intake of other grains by a 3 year old child (average weight 14 kg) would be approximately 13.7 g/kg/d.

Thus the highest anticipated exposure levels for canary seed, based on the proposed intended uses and use levels, are well below the levels shown to be safe by both animal safety studies and current levels of consumption of other cereal grains, which are compositionally very similar to canary seed. Safety studies, including both compositional and animal feeding studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake (Health Canada, 2006).

On the basis of the novel food safety assessment guidelines, it is clear that the estimated intakes of canary seed, even for the highest users, are below the level shown to have no adverse effects or nutritional hazards, based on the animal safety studies and nutritional composition comparisons.

The entirety of the available scientific data and studies summarized in this dossier support the conclusion that glabrous brown and yellow coloured canary seed groats and milled products are nutritious and safe to consume for the US population. While two colors of canary seed are available, there is no significant nutritional or safety related differences between canary seed of different colors. Glabrous canary seed groats and milled products would not be expected to cause adverse effects in humans under the conditions of intended use in foods.

Based upon the entirety of the available scientific data and summarized in this dossier, it is concluded that glabrous canary seed groats would be generally recognized as safe for consumption in their intended uses in food.

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APPENDICES: AVAILABLE UPON REQUEST