SCOPING RISK FROM NATURAL TOXINS IN NEW ZEALAND CROP PLANTS

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by

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SUMMARY

Crop plants contain many hundreds of chemicals, some of which have beneficial nutritional consequences for humans if consumed, while others may result in adverse health consequences for humans. Some plant chemicals may be both beneficial and harmful, depending on the dose consumed and the context of the consumption event. Chemicals naturally present in plants or produced by the plant in response to environmental factors that can elicit adverse health effects in humans or animals are often referred to as natural plant toxins.

This report is a qualitative assessment of the risks associated with natural toxins in crop plants available in New Zealand, based on currently available data. It was agreed that the summaries for each toxin would answer a series of questions within the framework of a qualitative risk assessment, relating to the evidence for the human toxicity of the toxins and the evidence for exposure to the toxin by the New Zealand population.

While the assessment highlighted considerable uncertainties and data gaps the following general conclusions were reached:

- There are little or no human data supporting the toxicity of caffeic acid, glucosinolates, saponins and quercetin. There is some evidence to suggest beneficial effects from these compounds at dietary levels of exposure, while adverse effects may occur at levels of exposure well above normal dietary levels.
- There are no human data to support the toxicity of proteinase and amylase inhibitors. Forms of foods causing problems in animals (raw soy meal) are not relevant to human diets and enzymes will often be inactivated by normal food processing. These compounds are also being investigated for potentially beneficial therapeutic purposes.
- Potato glycoalkaloids and cucurbitacins may cause adverse health effects in humans under certain environmental circumstances. These circumstances are understood for potatoes (physical injury, plant stress, exposure to light), but not for cucurbits. The extreme bitterness of cucurbitacin-containing foods is usually considered to be a barrier to widespread or prolonged incidents of intoxication.
- Plant products containing cyanogenic glycosides are capable of causing serious cases of cyanide poisoning. However, the main cyanogens-containing foods (e.g. cassava) are not normal components of the New Zealand diet, although they may be increasing in importance.
- Xenoestrogens have been implicated in a range of cancers and non-cancer conditions relating to development of the reproductive organs. However, correlative epidemiological studies have generally focused on synthetic xenoestrogens, rather than phytoestrogens. Some phytoestrogens have also been promoted as having positive health effects. Evidence is currently insufficient to establish the human toxicity of phytoestrogens.

Based on these conclusions, potato glycoalkaloids and cyanogenic glycosides appear to be the toxins associated with cultivated plants consumed in New Zealand with the greatest potential to cause adverse health effects.

1 INTRODUCTION

Crop plants contain many hundreds of chemicals, some of which have beneficial nutritional consequences for humans if consumed, while others may result in adverse health consequences for humans. Some plant chemicals may be both beneficial and harmful, depending on the dose consumed and the context of the consumption event. Chemicals naturally present in plants or produced by the plant in response to environmental factors that can elicit adverse health effects in humans or animals are often referred to as natural plant toxins.

Some food preparation practices (e.g. soaking and cooking of certain pulses) serve to mitigate the impact of such natural plant toxins, while plant breeding has generally aimed to reduce levels of toxins in crop plants.

1.1 Scope of the Current Project

This project aimed to qualitatively assess the risks associated with natural toxins in crop plants available in New Zealand. It was agreed that the summaries for each toxin will answer a series of questions within the framework of a qualitative risk assessment.

Hazard evaluation:

- What evidence is there that the toxic substance causes harm and what evidence is there that it has caused harm to humans?

Hazard characterization:

- What level of hazard exposure would cause concern with respect to public health (dose-response)? Are there existing exposure benchmark doses, such as Acceptable Daily Intakes (ADIs) or Tolerable Daily Intakes (TDIs)?

In most cases the chemicals considered in the current project will not have been assigned ADIs or TDIs. In some cases No Observed Adverse Effects Levels (NOAELs) may be available from laboratory animal studies.

In the absence of derived benchmark levels of exposure a threshold of toxicological concern (TTC) approach may be taken. This approach considers structural aspects of the compound and assigns it to a class with a TTC – a level of exposure that represents a virtually safe dose based on consolidated toxicological data from compounds that are structurally analogous (Barlow *et al.*, 2001; Kroes *et al.*, 2004; Kroes *et al.*, 2005). However, the TTC approach was developed for the consideration of chemicals that are present at low levels in the diet. Natural toxins are normal components of foods and will usually result in exposure at levels above that for the least toxic TTC category (1800 μg/day) (Barlow, 2005).

It is proposed that ADI/TDI be used if available. In the absence of ADI/TDIs, if NOAELs are available from suitably conducted studies than these may be used to suggest a suitable TDI. In the absence of any suitable toxicological data and if the toxin is a trace component of the food, it is proposed that the TTC approach be adopted. As many of the toxins considered in this report are significant components of the crop food, the TTC approach will not be applicable in those cases.

Exposure assessment:

- Is there evidence for the occurrence of the toxic substance in New Zealand consumed crop foods?
- What levels of toxin have been reported in New Zealand or overseas?
- What is the consumption of relevant crops by New Zealanders?
- In the absence of New Zealand data, are there relevant international data?

Risk characterization:

- Is there evidence to suggest that exposure in New Zealand may approach critical dose levels?

1.1.1 Types of plant toxins included in the study

An initial decision needed to be made as to what compounds were to be included in the study.

It was agreed that this project only consider toxic compounds that are generated by an edible crop plant. Mycotoxins are produced by fungi that are able to infect many crop plants, but are products of the fungus rather than the plant. Mycotoxins of potential significance to New Zealand have been considered elsewhere (Cressey and Thomson, 2006).

In discussions of plant toxins a number of different groups are apparent, including:

- Compounds that directly elicit a recognised toxicological response (e.g. organ toxicity, genotoxicity, reproductive toxicity, etc.);
- Compounds that may induce a state of nutrient deficiency (antinutrients, e.g. phytate);
- Compounds that interfere with the normal operation of physiological enzymes (enzyme inhibitors, e.g. soybean protease inhibitor). These may also be antinutritional in outcome;
- Compounds that disrupt the endocrine (hormonal) system; and
- Compounds that elicit immunological responses (allergens).

It was agreed that the current study should not include consideration of antinutrient compounds. These compounds generally have no intrinsic toxicity and are a cause for concern due to their ability to interfere with absorption of dietary nutrients.

Glucosinolates are goitrogenic substances, that is they are able to induce a state equivalent to that of iodine deficiency. While these compounds do seem to influence the availability of iodine to the thyroid gland, they also appear to have an iodine-independent mode of thyroid toxicity and will be included in this study.

While enzyme inhibitors may act to reduce the availability of nutrients they may also allow the build-up of toxic substances and it was agreed that these compounds be included in the current study.

The science relating to compounds that disrupt the endocrine system is developing and complex. Risks associated with plant estrogen-mimicking compounds in New Zealand have been considered previously (Thomson, 2005). It was agreed that this topic not be revisited in the current project, but that the conclusions of the previous project be used to rank issues associated with estrogenic plant compounds along side other toxic plant compounds considered in the current project. Similarly, the issue of toxic cucurbitacins, that may be

present in cucurbits such as zucchinis and cucumbers, has been reviewed previously (Cressey, 2003) and will only be included in the ranking phase of the current exercise.

Allergic reactions have been reported to over 150 different food commodities (Hefle *et al.*, 1996). The allergenic proteins in foods represent a risk to those with the relevant allergy, but represent no risk to the balance of the population. Allergenic proteins were not included in the current project.

Sources of information used to identify plant toxins for inclusion in this project were:

D'Mello JPF, Duffus CM, Duffus JH. (1991) Toxic substances in crop plants. Cambridge: The Royal Society of Chemistry.

Watson DH. (1987) Natural toxicants in food. Progress and prospects. New York: VCH.

Shaw I. (2005) Is it safe to eat? Berlin: Springer-Verlag.

USFDA. Foodborne pathogenic microorganisms and natural toxins handbook ("Bad bug book"). http://www.cfsan.fda.gov/~mow/intro.html

Extoxnet (Department of Food Science and Toxicology, University of Idaho). Natural toxins if foods. http://extoxnet.orst.edu/faqs/natural/page1.htm

Cornell University. Poisonous plants informational database. Toxic agents in plants. http://www.ansci.cornell.edu/plants/toxicagents/index.html

Thomson B. (2002) Natural plant toxins factsheet. ESR Client Report FW0217. Christchurch: ESR.

1.1.2 Plants and toxins included in the current study

The current study would ideally focus on food-toxin combinations that represent the greatest risk to public health. Risk may relate to a toxin present in a commonly consumed food or a toxin distributed across a range of lesser-consumed foods. To cover both of these possibilities the study will approach the topic from two separate direction:

- Food-centric. What toxins are present in our most commonly consumed foods? It was proposed that the 20 most commonly consumed plant foods in New Zealand, on a 'weight consumption per capita' basis be included in the current project.
- Toxin-centric. What are the most widely distributed toxins in the New Zealand food supply?

Based on the criteria outlined above, foods included on the basis of level of consumption were:

- Wheat, potatoes, apples, oranges, tomatoes, grapes, bananas, barley, carrots, onions, pumpkin, pears, peas, cabbage, rice, coffee, lemons/limes, sweetcorn, peaches, and cauliflower.

Toxins considered due their widespread occurrence in the food supply and inherent toxicity were:

- saponins, caffeic acid, cyanoglycosides, quercetin, glucosinolates

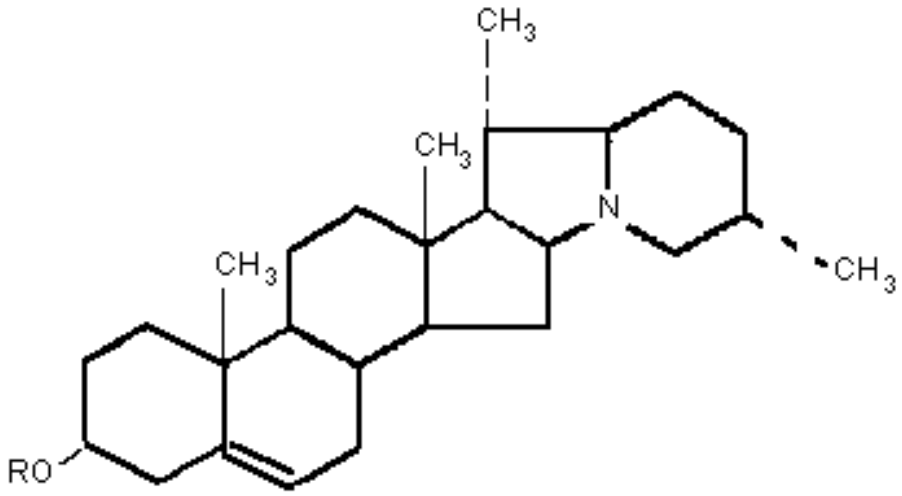
Amylase inhibitors, protease inhibitors and glycoalkaloids were also included due to their presence in highly consumed foods (e.g. wheat, potatoes).

2 GLYCOALKALOIDS

2.1 Structure and Nomenclature

Steroidal glycoalkaloids are produced in plants of the *Solanaceae* family, which includes commonly consumed crops such as potatoes and tomatoes. These compounds are produced biosynthetically from cholesterol (Kuiper-Goodman and Nawrot, 1993). While a number of *Solanaceae* species produce toxic glycoalkaloids, the most important from a public health aspect are those produced by the potato (*Solanum tuberosum*) and, to a much lesser extent, the tomato (*Solanum lycopersicum*). Figure 1 shows the chemical structure of the major glycoalkaloids present in potatoes.

Figure 1: Structure of the major glycoalkaloids from potatoes



R = H	Solanidine
R = D-glucose – D-galactose(*) – L-rhamose	α-Solanine
R = L-rhamose – D-galactose (*) – L-rhamose	α-Chaconine
* Point of attachment	

Reproduced from Kuiper-Goodman and Nawrot (1993)

Alpha-solanine and α-chaconine both have a common aglycone, solanidine. These two glycoalkaloids account for approximately 95% of the glycoalkaloid content of potatoes. Tubers usually contain more α-chaconine than α-solanine.

The major glycoalkaloid present in tomatoes, α-tomatine, has a four carbohydrate moiety (D-xylose – D-glucose (D-galactose(*)) – D-glucose) instead of the three carbohydrate moiety in α-solanine and α-chaconine, and a slightly different ring structure at the distal end (Morris and Lee, 1984).

While the initial glycoalkaloid content of potato tubers appears to be genetically determined, with significant differences between different potato cultivars, levels may increase in response to exposure of the tuber to light or a variety of environmental stresses, such as insect or mechanical damage, or drought stress (Bejarano *et al.*, 2000; Morris and Lee, 1984). Exposure to light can also stimulate chlorophyll synthesis, resulting in 'greening' of the tuber. While greening is often associated with high glycoalkaloid levels, production of glycoalkaloids and chlorophyll occurs by independent processes (Smith *et al.*, 1996)

Table 1 summarises typical glycoalkaloid levels for various parts of the potato plant.

Table 1: Typical glycoalkaloid content of various parts of the potato plant

Plant part	Typical glycoalkaloid content (mg/kg, fresh weight)	Reference
Flowers	2150-5000	(Smith *et al.*, 1996)
Leaves	230-1450	(Friedman and Dao, 1992; Smith *et al.*, 1996)
Stems	23-33	(Smith *et al.*, 1996)
Berries	180-1350	(Coxon, 1981; Friedman and Dao, 1992)
Roots	180-850	(Friedman and Dao, 1992; Smith *et al.*, 1996)
Bitter-tasting tuber	250-800	(Smith *et al.*, 1996)
Normal tuber* - Skin (2-3% of tuber) - Peel (10-12% of tuber) - Flesh - Cortex - Pith	10-150 300-640 150-1070 12-100 125 Not detectable	(Smith *et al.*, 1996)
Sprouts	2000-9970	(Friedman and Dao, 1992; Smith *et al.*, 1996)

* A potato tuber is essentially a plant stem, with analogous parts. The pith is the central axis of the tuber, while the cortex is the outer fleshy part of the tuber, just beneath the skin. The flesh refers to the starchy body of the tuber, including the cortex, the vascular ring, the perimedullary zone and the pith. The skin is the dermal layer of the tuber, while the peel is a pragmatic description of the portion of the potato removed during the food preparation step of peeling (Rastovski *et al.*, 1987).

Glycoalkaloids are concentrated in the skin of the potato tuber. Glycoalkaloid levels are generally higher in the non-tuber components of the plant, with the flowers and sprouts containing particularly high levels.

2.2 Evidence of Toxicity

Potato glycoalkaloid poisoning has been documented for approximately 2000 human cases, including 30 deaths, with case reports from 1865 to as recently as 1983 (Kuiper-Goodman and Nawrot, 1993; Morris and Lee, 1984). Outbreaks of potato glycoalkaloid poisoning have been reported from Germany, Scotland, England and Canada. It has been suggested that many cases of glycoalkaloid poisoning may go undiagnosed due to the similarity of

symptoms to bacterial food poisoning (Smith *et al.*, 1996). Symptoms typically include gastrointestinal (vomiting, diarrhea and severe abdominal pain) and neurological (drowsiness, apathy, confusion, weakness and vision disturbance) aspects and, in severe cases, may progress to unconsciousness and death (Kuiper-Goodman and Nawrot, 1993).

2.2.1 Adverse health effects in New Zealand

The notifiable disease database maintained by ESR (Episurv) contains one outbreak since 1995 that was ascribed to 'solanine poisoning' and involved two people who consumed potato fritters prepared from green potatoes. The glycoalkaloid content was not reported and only gastrointestinal symptoms were recorded.

2.2.2 Mechanism of toxicity

The potato glycoalkaloids (α-solanine and α-chaconine) have been reported to have two distinct toxic mechanisms, consistent with the symptoms observed in glycoalkaloid poisoning (Morris and Lee, 1984). These are a membrane disrupting activity, similar that to that of saponins, that causes observed gastrointestinal symptoms due to disruption of gastrointestinal tract membranes, and a cholinesterase inhibiting mechanism, causing the observed neurological symptoms due to build up of the neurotransmitter, acetylcholine (Morris and Lee, 1984).

2.2.3 Toxic and tolerable exposure levels

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered the information available on α-solanine and α-chaconine in 1993 and concluded that the available data "did not permit the determination of a safe level of intake" (Kuiper-Goodman and Nawrot, 1993).

Analysis of data from outbreaks of potato glycoalkaloid poisoning and limited human laboratory studies suggest that doses greater than 2 mg/kg body weight are toxic, while doses in the range 3-6 mg/kg body weight have been lethal in some instances (Morris and Lee, 1984). In one study, an ascending dose study in human volunteers, one of two subjects receiving the highest dose (1.25 mg/kg body weight) experienced symptoms of nausea and vomiting approximately four hours post-administration (Mensinga *et al.*, 2005).

2.2.4 Tolerable concentrations in foods

While having no formal regulatory standing levels of glycoalkaloids up to 200 mg/kg in potatoes has been generally accepted as safe, although a lower limit of 60-70 mg/kg has been suggested (Smith *et al.*, 1996).

2.3 Evidence of Exposure

2.3.1 Glycoalkaloids in New Zealand foods

There is little recent information on the glycoalkaloid content of New Zealand foods. Table 2 summarises available information.

Table 2: Glycoalkaloid content of New Zealand foods

Food	Experimental conditions	Mean (range) TGA concentration (mg/kg)		Reference
		Unexposed	Exposed to light	
Mature potato tubers of cultivars*: Sebago Whitu Ilam Hardy Rua Majestic Glen Ilam Arran Consul	Tubers protected from light (unexposed) and exposed to daylight (mean 510 lux) for 22 days	 5 7 16 18 21 26 65	 25 30 82 90 105 135 100	(Patchett *et al.*, 1977)
Immature potato tubers of cultivars*: Sebago Whitu Ilam Hardy Ilam Hardy Majestic Glen Ilam Arran Consul	Tubers protected from light (unexposed) and exposed to daylight (mean 510 lux) for 18 days	 14 19 29 57 1 76 122	 66 93 191 208 251 475 370	(Patchett *et al.*, 1977)
Mean of 11 cultivars at: 0 months 2 months 4 months	At 0, 2 and 4 months of storage tubers were analysed directly (unexposed) and after exposure to fluorescent light (2905 lux, 12 hours/day for 7 days)	 40 (18-62) 58 (30-140) 55 (33-92)	 112 (46-352) 85 (45-164) 61 (35-121)	(Butcher, 1978)
Mean of four cultivars at: 0 months 2 months 4 months 6 months	At 0, 2, 4 and 6 months of storage tubers were analysed directly (unexposed) and after exposure to fluorescent light (2905 lux, 12 hours/day for 7 days)	 56 (43-74) 59 (44-87) 62 (45-89) 64 (48-92)	 101 (84-128) 103 (85-135) 105 (85-151) 105 (86-149)	(Lammerink, 1985)

TGA = Total glycoalkaloids

* Maturity of potatoes is an imprecise term, but is usually defined in terms of the degree of suberisation or 'skin set', during which the skin of the potato becomes thicker and tougher. Skin set is judged by physical appearance and resistance to abrasion i.e. the skin will not scuff when rubbed. Chemically, this is the point at which the sugar content of the tuber is no longer decreasing (Rastovski *et al.*, 1987).

The glycoalkaloid content of potato tubers grown in New Zealand is generally well below the suggested safe limit of 200 mg/kg when the tubers are protected from exposure to light. However, tubers exposed to light occasionally accumulate levels of glycoalkaloids in excess

of this limit. Given that these New Zealand data are not particularly recent, it is uncertain whether this is a fair representation of the current New Zealand situation.

Non-commercial, early introduced potatoes (10 varieties) have also been analysed and were found to contain a mean level of glycoalkaloids of 76.5 mg/kg (range 38.7 – 142.6 mg/kg) (Savage *et al.*, 2000). Analyses were carried out on freshly harvested, freeze-dried tubers. This type of potato is believed to have been introduced by the French explorer D'Urville and was widely cultivated by Maori, but are not grown commercially due to their poor yield, irregular shaped tubers, deep eyes and unusual colours. The potatoes analysed varied in colour from white fleshed to purple, with most having purple or pink skin colour. The highest glycoalkaloid levels were found in purple-fleshed cultivars.

2.3.2 Potato consumption in New Zealand

According to analysis of data from the 1997 National Nutrition Survey (Russell *et al.*, 1999) Potatoes are consumed by approximately 68% of adult (15 years and over) New Zealanders on any given day (ANZFA, 2001). The mean level of consumption across the whole population is approximately 118 g/day.

Information from Food Balance Sheets suggests a higher level of consumption of approximately 207 g/person/day (http://faostat.fao.org). Similar or higher levels of consumption are reported for a number of countries in Western Europe (Belgium, Denmark , France, Germany, Netherlands, Portugal, Spain), Eastern Europe (Belarus, Hungary, Slovakia, Ukraine), Britain and Ireland, Canada, Greece, Lebanon, Malawi, Peru, the Russian Federation, Rwanda, the Balkans (Bosnia and Herzegovina, Croatia, Romania), the Baltic (Estonia, Finland, Latvia, Lithuania) and Central Asia (Armenia, Azerbaijan, Kazakhstan, Kyrgyzstan).

2.3.3 Glycoalkaloids in foods overseas

2.3.3.1 Australia

The 1992 Australian Market Basket Survey considered potato glycoalkaloids in boiled potatoes and potato crisps (Stenhouse, 1992). The mean level of α-solanine in boiled potatoes was 28 mg/kg, while the mean level of α-chaconine was 25 mg/kg (total 53 mg/kg). For potato crisps, the mean contents of α-solanine and α-chaconine respectively were 29 and 42 mg/kg (total 71 mg/kg).

A study in the Australian Capital Territory determined the total glycoalkaloid content of potatoes classified as good (no obvious damage, blemishes or greening), bad (obvious damage or blemishes) or green (some greening on the surface) (Rigg, 1997). The mean concentration of α-solanine was 14.5 mg/kg (range 'Not detected' to 51 mg/kg), while the mean concentration for α-chaconine was 28.7 mg/kg (range 'Not detected' to 64 mg/kg). There was little difference between the three groups of potatoes with mean total glycoalkaloid contents of 42.1, 43.2 and 44.1 mg/kg for good, bad and green potatoes respectively. Maximum total glycoalkaloid levels were 83, 103 and 91 mg/kg respectively.

2.3.3.2 Other

Table 3 summarises literature information the glycoalkaloid content of potatoes and potato products from a range of countries.

Table 3: Glycoalkaloid content of potatoes and potato products

Country	Food item	Mean (range) glycoalkaloid content (mg/kg, fresh weight)#	Reference
Brazil	Whole potato tubers (cv Monaliza):		(Machado *et al.*, 2007)
	Small tubers:		
	0 days storage	51.4	
	14 days, darkness, room temperature	60.8	
	14 days, darkness, refrigerated	81.8	
	14 days, indirect sunlight	92.5	
	14 days, fluorescent light	107.9	
	Medium tubers:		
	0 days storage	51.5	
	14 days, darkness, room temperature	57.0	
	14 days, darkness, refrigerated	65.0	
	14 days, indirect sunlight	58.9	
	14 days, fluorescent light	103.2	
Italy	cv Agata	10.4	(Finotti *et al.*, 2006)
	cv Primura	18.4	
	cv Arinda	20.0	
	cv Merit	29.0	
	cv Marabel	42.2	
	cv Jelli	35.4	
	cv Frinka	38.1	
	cv Sponta	51.0	
	cv Agria	40.7	
Poland	cv Aster		(Peksa *et al.*, 2002)
	-unpeeled, early harvest	174	
	-unpeeled, mid harvest	136	
	-unpeeled, late harvest	102	
	-peeled, early harvest	45	
	-peeled, mid harvest	65	
	-peeled, late harvest	37	
	cv Mila		
	-unpeeled, early harvest	110	
	-unpeeled, mid harvest	91	
	-unpeeled, late harvest	79	
	-peeled, early harvest	74	
	-peeled, mid harvest	62	
	-peeled, late harvest	41	
	cv Bryza		
	-unpeeled, early harvest	81	
	-unpeeled, mid harvest	56	
	-unpeeled, late harvest	53	
	-peeled, early harvest	46	

Country	Food item	Mean (range) glycoalkaloid content (mg/kg, fresh weight)#	Reference
	-peeled, mid harvest	40	
	-peeled, late harvest	31	
Poland	cv Karlena		(Peksa *et al.*, 2006)
	-unpeeled tuber	129*	
	-potato after peeling	101*	
	-potato after slicing	82*	
	-slices after washing	53*	
	-slices after blanching	38*	
	-chips	24*	
	cv Saturna		
	-unpeeled tuber	165*	
	-potato after peeling	118*	
	-potato after slicing	85*	
	-slices after washing	49*	
	-slices after blanching	37*	
	-chips	23*	
Portugal	Potato tubers-		(Abreu *et al.*, 2007)
	cv Santé		
	-conventional	37.3	
	-integrated crop management	44.2	
	-organic	38.4	
	cv Raja		
	-conventional	79.5	
	-integrated crop management	59.6	
	-organic	44.6	
Sweden	Commercial main crop tubers:		(Hellenas *et al.*, 1995)
	cv Magnum Bonum	254 (61-665)§	
	cv British Queen	223 (154-315)	
	cv Grata	129 (75-224)	
	cv Sabina	110 (58-183)	
	cv Bellona	104 (42-206)	
	cv King Edward VII	93 (69-171)	
	cv Mandel	86 (55-107)	
	cv Bintje	73 (35-112)	
	cv Provita	67 (42-100)	
UK	Whole tubers		(Davies and Blincow, 1984)
	-foreign earlies	125 (60-210)	
	-UK earlies	112 (65-220)	
	-main crop	105 (60-195)	
	Potato powder	88 (39-135)	
	Crisps	83 (32-184)	
	Chips	36 (19-58)	
	Oven chips	47 (27-86)	
	Canned potatoes	55 (29-99)	
USA	Whole tubers (cv White Rose):		(Dao and Friedman, 1994)
	0 days storage	12.4	
	5 days storage, dark	18.3	

Country	Food item	Mean (range) glycoalkaloid content (mg/kg, fresh weight)#	Reference
	13 days storage, dark 20 days storage, dark 5 days storage, light 13 days storage, light 20 days storage, light	30.9 38.9 31.3 32.1 29.3	
USA	Raw potatoes Baked potatoes Boiled potatoes Microwaved potatoes Raw peel Fried peel	103-161 99-113 100-115 124-133 678-763 567-594	(Bushway and Ponnampalam, 1981)
USA	Commercial potato products: Chips Frozen fries, potato balls, mashed potatoes and fried potatoes Dehydrated potato Canned potatoes Fried peels	 27-162 2-123 15-75 0.9-2.5 1390-1450	(Bushway and Ponnampalam, 1981)

cv = cultivar

* Expressed on a dry matter basis

Where results were reported for α-solanine and α-chaconine, the sum of these two compounds was taken as total glycoalkaloid

§ cv Magnum Bonum was subsequently withdrawn from use in Sweden due to its high glycoalkaloid content

There is evidence to suggest that our current commercial potato cultivars have been bred for reduced glycoalkaloid content. Analysis of four wild potato species in Mexico (*S. polytrichon, S. stoloniferum, S. ehrenbergii, S. cardiophyllum*) revealed considerably higher glycoalkaloid levels (1080 – 5540 mg/kg) than in a *S. tuberosum* cultivar grown in the same locale (236 mg/kg) (Sotelo *et al.*, 1998).

The information in Table 3 suggests that the glycoalkaloid content of potato tubers may be influenced by harvest date, exposure to light, storage temperature and growing management system (conventional, organic, integrated). On the basis of studies summarised here, the glycoalkaloid content of potato tubers is increased by early harvest date, exposure of the tubers to light and storage at sub-ambient temperatures. The single study on crop management systems demonstrated equivocal results for one cultivar and lower glycoalkaloid content in organically grown potatoes for a second cultivar.

While increased production of glycoalkaloids in potatoes stored at refrigeration temperatures seems counter-intuitive, an earlier study observed the same phenomenon (Griffiths *et al.*, 1997). It appears likely that the increased rate of production of glycoalkaloids at refrigeration temperatures was due in part to the incomplete dormancy of freshly harvested potatoes and their subsequent reaction to low temperature stress.

2.3.4 Influence of food processing on glycoalkaloid content of foods

Although commercial potato processing has been shown to decrease glycoalkaloid concentrations, as compared to whole raw potatoes, there is mixed evidence for normal

household cooking procedures. While baking, boiling and microwaving have been shown to have little impact on glycoalkaloid content in one study (Bushway and Ponnampalam, 1981), the successive steps of peeling, slicing, washing, blanching and chip making were shown to result in 80-86% reduction of glycoalkaloid content of potatoes processed into chips (Peksa *et al.*, 2006).

2.4 Summary

All potatoes contain at least some glycoalkaloids and there is evidence to suggest that the presence of these toxic chemicals is important for our taste perceptions of the food and for protection of the growing tuber from a range of environmental challenges. While levels of glycoalkaloids in sound, well-stored commercial cultivars of *S. tuberosum* are unlikely to cause adverse health effects, damage to the tuber or inappropriate storage conditions (exposure to light, storage at refrigeration temperatures) can lead to elevation of the glycoalkaloid content and an increased risk of adverse human health outcomes. While very few incidents of potato glycoalkaloid poisoning have been documented in New Zealand, it is likely that less severe cases resulting in only gastrointestinal symptoms would go undiagnosed.

3 CAFFEIC ACID

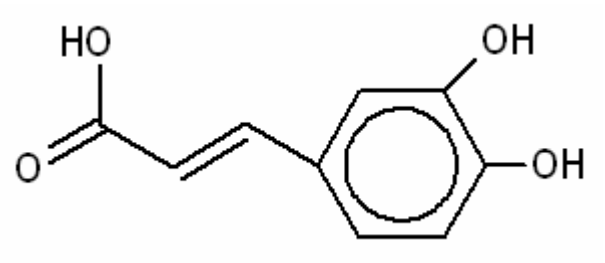
3.1 Structure and Nomenclature

Caffeic acid is a carboxylic acid found in many fruits and vegetables. It may be present as free caffeic acid or in conjugated forms such as chlorogenic acid (IARC, 1993). While little free caffeic acid is found in foods, the conjugates are metabolised to caffeic acid following ingestion (IARC, 1993).

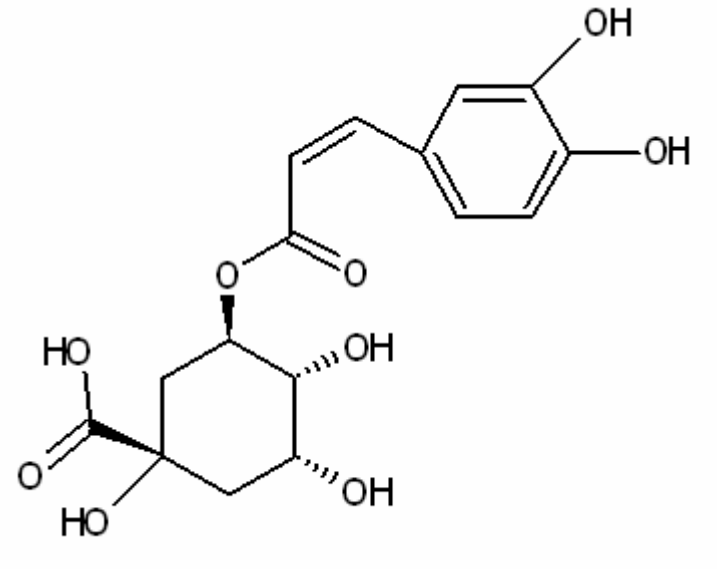
Caffeic acid and its conjugates are constituent of plants from the families Umbelliferae, Cruciferae, Cucurbitaceae, Polygonaceae, Compositae, Labiatae, Solanaceae, leguminosae, Saxifragaceae, Caprifoliaceae, Theaceae and Valerianaceae (IARC, 1993).

The structures of caffeic acid and its major conjugate, chlorogenic acid, are shown in Figure 2. While the name 'caffeic acid' is similar to 'caffeine', there is no structural relationship between these compounds.

Figure 2: Chemical structure of caffeic acid and chlorogenic acid



Caffeic acid



Chlorogenic acid

Biosynthesis of caffeic acid, chlorogenic acid and isochlorogenic acid in plant material has been reported to increase following infection or stress (e.g. physical injury, drought, etc.) in

potatoes and sweet potatoes (IARC, 1993). While not specifically reported for other fruits and vegetables, it is likely that caffeic acid concentration will increase in non-tuber species in response to infection or stress.

3.2 Evidence of Toxicity

No data were found on the toxicity of caffeic acid in humans. The International Agency for Cancer Research (IARC) classified caffeic acid as possibly carcinogenic to humans (group 2B), but concluded that there was sufficient evidence for its carcinogenicity in animals (IARC, 1993).

Concerns regarding caffeic acid have come from studies in rodents, where an increase in the incidence of kidney and forestomach hyperplasia and tumours was observed at high caffeic acid dose rates (Hagiwara *et al.*, 1991; Hirose *et al.*, 1990; Hirose *et al.*, 1998; Kagawa *et al.*, 1993). However, the carcinogenicity of caffeic acid appears to be extremely dose dependent and appears to be carcinogenic at a dose level of 2% of the diet and tumour promoting at dose levels in the range 0.5-1.0% of the diet. At lower dose levels (0.05-0.5%) caffeic acid has been shown to have anticarcinogenic activity (Tanaka *et al.*, 1993; Wattenberg *et al.*, 1980).

3.2.1 Mechanism of toxicity

Caffeic acid is generally considered to be non-genotoxic and it has been suggested that its ability to cause tumours is due to its cytotoxicity and its ability to induce cell proliferation (Kagawa *et al.*, 1993). Kagawa *et al.* (1993) compared the action of genotoxic and non-genotoxic (including caffeic acid) carcinogens and demonstrated that the non-genotoxic carcinogens tended to produce simple or papillary hyperplasia (SPH) and mild basal cell hyperplasia (BCH), rather than the atypical hyperplasia (AH) produced by genotoxic carcinogens. The SPHs caused by caffeic acid were reversible, gradually resolving after cessation of administration. The AHs caused by genotoxic carcinogens tended to progress to papillomas and carcinomas after cessation of administration.

The observation that caffeic acid appears to be anticarcinogenic at low doses and carcinogenic at high doses is supported by a study of the dose-response relationship with respect to cell proliferation (Lutz, 1997). A J-shaped curve was observed indicating suppression of cell proliferation at low doses and induction of cell proliferation at high doses.

3.2.2 Toxic and tolerable exposure levels

No tolerable intake level has been established for caffeic acid. The lack of evidence of adverse health effects in humans due to caffeic acid exposure does not allow establishment of a toxic exposure level. Toxic exposure levels in experimental animals seem to be of the order of 0.5-2% (2% in the diet equates to an exposure level of approximately 1400 mg/kg body weight/day) of the diet.

Given that caffeic acid is a relatively common chemical in foods the Threshold of Toxicological Concern (TTC) approach is not appropriate for this compound (see section 1.1).

3.2.3 Tolerable concentrations in foods

No tolerable concentrations for caffeic acid in foods have been established.

3.3 Evidence of Exposure

3.3.1 Caffeic acid in New Zealand foods

The New Zealand Food Composition Database contains no information on the caffeic acid content of New Zealand foods and contains only two entries for chlorogenic acid (in coffee, based on overseas data).

3.3.2 Caffeic acid in foods overseas

The assessment of caffeic acid carried out by IARC in 1993 included a significant amount of information on the caffeic acid content of foods, mainly based on the work of Herrmann and co-workers (Herrmann, 1989; IARC, 1993). Caffeic acid is generally determined after hydrolysis and the analysis will measure free caffeic acid as well as conjugated forms. Where data was presented for the individual conjugates these have been converted to caffeic acid equivalents, based on relative molecular weights. This information is summarized in Table 4.

Table 4: Caffeic acid content of foods

Food/Plant	Plant part	Caffeic acid concentration (mg/kg, fresh weight)
Vegetables		
Bean, broad	Hulls Unripe fruit	12-14 <0.5-9
Beetroot	Whole vegetable Outside Heart	5-17 5 4
Beet, sugar	Whole vegetable	3-4
Broccoli	Florets	8-10
Brussels sprouts		34-50
Cabbage, Chinese	Outer leaves	11-52
Cabbage, red	Outer leaves Head	6-24 12-17
Cabbage, savoy	Outer leaves Head	9-36 4-7
Cabbage, white	Outer leaves Head	<0.5-62 <0.5-12
Carrot	Whole vegetable Rind Central cylinder	18-96 27-141 8-73
Cauliflower	Leaves Florets	9-90 1-6
Celery	Whole vegetable Outside Heart	89-104 87-122 84-109

Food/Plant	Plant part	Caffeic acid concentration (mg/kg, fresh weight)
	Root	168
Chives	Green leaves	<0.5
Courgette/zucchini	Whole vegetable	10
Eggplant/aubergine	Ripe vegetable	360-436
Fennel	Tuber	100
Garlic	Dry bulb skin Fleshy tissue of bulb	<20 7-14
Horseradish	Whole vegetable Peel Heart	10-11 14 4
Kale	Stalk and midrib Leaf and stalk Leaf and blade	9-13 77-92 51-305
Kohlrabi	Leaves Tuber	15-113 <0.5-5
Lettuce	Leaves	767-1440
Onion	Green leaves	<0.5-19
Parsley	Whole plant Leaf	6 <0.5
Pea	Unripe seeds Hulls	<0.5-1 <0.5
Pepper, sweet (capsicum)	Green capsicum Red capsicum	3-7 4-10
Potato	Peel Whole tuber	163-205 3-33
Radish	Whole vegetable Peel Heart Leaves	13-17 47-52 3-9 376-417
Radish, black	Whole vegetable Peel Heart Leaves	5-8 6-7 7 156-247
Rhubarb	Leaves	6-16
Salsify	Whole vegetable Outside Heart	49-212 106 62
Tomato	Unripe green Ripe red Peel Pulp Seeds	13-79 32-97 97 41 119
Fruit		
Apple	Fruit	32-196
Apricots	Fruit	79
Blueberry	Fruit	83-588

Food/Plant	Plant part	Caffeic acid concentration (mg/kg, fresh weight)
Cherries	Fruit	
-sweet		145
-sour		138
Currant	Black	14-93
	Red	8-16
	White	10-21
Gooseberry	Green	24-32
	Red	29-32
	Yellow	23-27
Grapefruit	Fruit	11-40
	Peel	14-51
Lemon	Fruit	13-27
	Peel	16-35
Orange	Fruit	19-50
	Peel	12-36
Pears	Fruit	33-142
Plums	Fruit	316
Peaches	Fruit	138
Strawberry	Fruit	<0.5-14
Sweet melon	Fruit	3
	Peel	<0.5
Watermelon	Peel or fruit	<0.5

Table adapted from IARC (1993) and Herrmann (1989)

Coffee beans are one of the richest dietary sources of chlorogenic acid, with green coffee beans containing 6-10% chlorogenic acid, on a dry weight basis (Clifford, 1999). A 200 ml cup of roasted and ground coffee may contain 20-675 mg of chlorogenic acid. Soluble coffee powder provides approximately 70 to 220 mg of chlorogenic acid per 200 ml cup.

3.3.3 Influence of food processing on caffeic acid content of foods

Storage of shredded carrot in air results in an initial increase in levels of free caffeic acid and caffeic acid esters (Babic *et al.*, 1993). Generally levels decrease again after approximately three days storage, although cultivar differences were observed. In storage experiments with shredded iceberg lettuce, following washing with chlorinated, ozonated or tap water, levels of caffeic acid derivatives were generally unchanged or decreased during 5-8 days of storage (Baur *et al.*, 2004).

Boiling of freshly cut sweet potato (*Ipomoea batatas* L.) in water results in rapid decreases in the levels of caffeic acid derivatives (Takenaka *et al.*, 2006). This was largely, but not completely due to elution of these phenolic compounds into the water. The authors suggested that the additional decrease in caffeic acid derivatives was due to the action of the enzyme polyphenol oxidase (PPO).

Processing of nine types of berry fruit into jam did not have a significant impact on the caffeic acid content, measured after acid hydrolysis (Amakura *et al.*, 2000).

Roasting of coffee beans results in a loss of chlorogenic acid of about 8-10% for every 1% loss of dry matter (Clifford, 1999)

3.3.4 Estimates of dietary exposure to caffeic acid

The caffeic acid exposure of New Zealanders has been estimated to be in the range 0.7-0.9 mg/kg body weight/day, based on New Zealand food consumption data and literature data on the caffeic acid content of foods (Thomson, 1996). A similar exposure level of 1 mg/kg body weight/day has been reported overseas, although the derivation of this values is unclear (Lutz and Schlatter, 1992). These estimates do not include the contribution due to consumption of coffee and related beverages.

Phenolic acid intake was estimated for a group of Bavarian adults, aged 19-49 years, with a average for caffeic acid of 206 mg/day (Radtke *et al.*, 1998). This would equate to an intake of 2.9 mg/kg body weight/day for a 70 kg adult. Coffee contributed 92% of the total caffeic acid intake.

An estimate of caffeic acid exposure for the US population was made of 0.02-0.2 mg/kg body weight/day from food alone, plus 0.9-9 mg/kg body weight/day from coffee consumption (NRC, 1996).

3.4 Summary

Caffeic acid, mainly in the form of its quinic acid conjugate chlorogenic acid, is present in a wide range of fruits, vegetable, spices and stimulants. Typical levels of dietary exposure are likely to be in the range 0.1-1 mg/kg body weight/day from food alone, or up to 10 mg/kg body weight/day including coffee consumption.

Caffeic acid has been shown to cause forestomach tumours in experimental animals. However, the carcinogenicity of caffeic acid appear to be extremely dose-dependent, with dose levels of 2% of the diet (~1400 mg/kg body weight/ day) being carcinogenic, while doses in the range of 0.5-1.0% of the diet (350-700 mg/kg body weight/day) are tumour promoting. Lower doses appear to exert an anticarcinogenic effect.

These data suggest that human dietary levels of caffeic acid may be more likely to exert a protective effect, rather than a carcinogenic effect, although it is uncertain whether rodent data are directly applicable to humans.

4 PROTEINASE INHIBITORS

Plant foods are an important source of dietary protein. In order for the human body to digest this protein, enzymes are produced that breakdown proteins. Some plant species produce proteins that are able to inhibit the action of these proteolytic enzymes.

The mechanism by which these inhibitors work is that after the enzyme cleaves (hydrolyses) the appropriate bond the inhibitor does not leave the active site due to the presence of another bond which keeps the two fragments in close association. Thus an enzyme-inhibitor complex is formed (Norton, 1991).

4.1 Structure and Nomenclature

The inhibitors have been classified according to type and family from which they were originally isolated (Norton, 1991):

- Soybean trypsin inhibitor (Kunitz) family (occur in soyabean and some other legumes)
- Soyabean proteinase inhibitor (Bowman-Birk) family (occur in soyabean, lima bean, other beans, peanuts, cowpea, wheat germ, rice bran)
- Potato I inhibitor family (may be up to 15-25% of soluble protein in potato tubers)
- Potato II inhibitor family
- Barley trypsin inhibitor family (occurs in barley and corn)
- Squash inhibitor family (occur in squash, summer squash, zucchini, cucumber, bitter gourd)

While all of these inhibitors are proteins with unique structures, some common elements exist (Norton, 1991). At the active site of the inhibitor (the peptide bond that will be cleaved by the proteinase) there will be a characteristic amino acid, recognised by the proteinase. In trypsin inhibitors this will be lysine or arginine; in chymotrypsin inhibitors it will be tyrosine, phenylalanine, tryptophan, leucine or methionine, and in elastase inhibitors either alanine or serine (Norton, 1991). Table 5 summarises key details of the known proteinase inhibitors relevant to crop plants.

Table 5: Plant proteinase inhibitors; families, plant species, proteinases inhibited and active site amino acid structure

Inhibitor family	Plant species	Designation	Enzymes inhibited	Active site sequence
Kunitz	Soybean (*Glycine max*)	STI	T	Arg-Ile
	Winged bean (*Psophocarpus tetragonolobus*)	WBI	T	Arg-Ile
Bowman-Birk	Soybean (*Glycine max*)	BBI	T C	Lys-Ser Leu-Ser
	Lima bean (*Phaseolus lunatus*)	LBI	T C	Lys-Ser Leu-Ser
	Common bean (*Phaseolus vulgaris*)	GBI	E T	Ala-Ser Arg-Ser
	Peanut (*Arachis hypogaea*)	GI	T,C T	Arg-Arg Arg-Ser

Inhibitor family	Plant species	Designation	Enzymes inhibited	Active site sequence
	Cowpea (*Vigna unguiculata*)	CPI	T C	Lys-Ser Phe-Ser
	Wheat (*Triticum aestivum*)	WGTI	T	Arg-Ser
	Rice (*Oryza sativa*)	RBTI	T T	Lys-Pro Lys-Met
Potato Inhibitor I	Potato (*Solanum tuberosum*)	PI-I	C	Leu-Asp
Potato Inhibitor II	Potato (*Solanum tuberosum*)	PI-II	T	Lys-Ser
Barley Trypsin Inhibitor	Barley (*Hordeum vulgare*)	BTI	T	Arg-Leu
Squash Trypsin Inhibitor	Winter squash/pumpkin (*Cucurbita maxima*)	CMTI	T	Arg-Ile
	Filed pumpkin/zucchini (*Cucurbita pepo*)	CPTI	T	Lys-Ile
	Cucumber (*Cucumis sativus*)	CSTI	T	Arg-Ile

Adapted from Norton (1991)
T = trypsin, C = chymotrypsin, E = elastase
Ala = alanine, Arg = arginine, Asp = asparagine, Ile = Isoleucine, Leu = leucine, Lys = lysine, Phe = phenylalanine, Pro = praline, Ser = serine

4.2 Evidence of Toxicity

There is little information available on adverse health effects in humans due to ingestion of proteinase inhibitors. An outbreak of gastrointestinal illness associated with consumption of a soy protein extender was investigated (Gunn *et al.*, 1980). The investigators reported symptoms including nausea, abdominal cramps, diarrhea, headache, vomiting and difficulty breathing in 508 cases, with symptoms usually occurring within one hour of consumption. Investigations by the manufacturer demonstrated that an increase in the heating regime eliminated the problem. While proteinase inhibitors were not established as the cause of this outbreak, it has been cited as evidence of the potential for inadequately processed soybean products to find their way onto the market (Liener, 1994).

There is equivocal evidence on the ability of plant proteinase inhibitors to inhibit the activity of human trypsin and chymotrypsin (Norton, 1991), although an in-depth review concluded that legume proteinase inhibitors can inactivate human trypsin and chymotrypsin and these inhibitors are not affected by pepsin or pH during passage through the stomach (Weder, 1986).

The primary effect of proteinase inhibitors in test animals is to depress growth rate. The mechanism of action is not apparently the expected inhibition of protein digestion. Instead the enzymatic inhibition stimulates the pancreas to excessively secrete more enzyme which leads to pancreatic enlargement and increased demand for amino acids (methionine stress caused by the increased production of methionine rich digestive enzymes) (Bender, 1987). Damage to the pancreas, including pancreatic cancer, is another result.

In recent years, most of the literature on proteinase inhibitors has focused on positive aspects of their impact on human health and in particular their ability to prevent cancer (Kennedy, 1998; Qi *et al.*, 2005).

4.2.1 Mechanism of toxicity

Trypsin in the intestinal lumen is considered to inhibit pancreatic secretion by inhibiting release of the stimulant hormone cholecystokinin (McGuinness *et al.*, 1984). Inhibition of trypsin removes this 'negative feedback' control and allows uncontrolled release of cholecystokinin, which has been shown to stimulate pancreatic hypertrophy and hyperplasia in rats (McGuinness *et al.*, 1984). Feeding of raw soy flour has been demonstrated to result in elevation of circulating cholecystokinin levels. Similar feedback control of pancreatic secretion in humans has been demonstrated, with administration of Bowman-Birk inhibitor resulting in a two to threefold increase in output of pancreatic enzymes (Liener *et al.*, 1988).

It should be noted that studies showing an influence of soy flour feeding on pancreatic problems in laboratory animals was the result of feeding raw soy flour at levels of 5-100% of the diet. Neither the form nor the quantity of this food are relevant to normal human food consumption.

No studies linking human proteinase inhibitor exposure to pancreatic cancer were found.

4.2.2 Toxic and tolerable exposure levels

No tolerable intake level has been established for proteinase inhibitors. The lack of evidence of adverse health effects in humans due to proteinase inhibitor exposure does not allow establishment of a toxic exposure level.

Given that proteinase inhibitors are relatively common chemicals in foods the Threshold of Toxicological Concern (TTC) approach is unlikely to be appropriate for this compound (see section 1.1).

4.2.3 Tolerable concentrations in foods

No tolerable concentrations for proteinase inhibitors in foods have been established.

4.3 Evidence of Exposure

4.3.1 Proteinase inhibitors in New Zealand foods

The trypsin inhibitor activity of a number of New Zealand-grown pea (*Pisum sativum* L.) cultivars was examined (Morrison *et al.*, 2007). Activities ranged from 0.33 trypsin inhibitor units (TIU)/mg of dry matter for the wrinkled-seeded freezer cultivar, Bolero to 0.75 TIU/mg for the green variety, Prussian Blue. Soaking and boiling of peas reduced the activity by an average of 78%. An earlier study found higher trypsin inhibitor levels in New Zealand field peas (0.52-2.44 TIU/mg), with only 11% of inhibitor activity being destroyed by heating in an autoclave (James *et al.*, 2005).

Trypsin inhibitors have been isolated from New Zealand-grown kumara (*Ipomoea batatis*), although the concentrations present were not reported (Scott and Symes, 1996).

4.3.2 Proteinase inhibitors in foods overseas

Consideration of data on the proteinase inhibitor content of foods is complicated by the different methods of analysis used to measure them and different modes of presenting results and units of measurement employed. While some studies apparently present results in the same units (Doell *et al.*, 1981; Rackis *et al.*, 1986), between study comparisons should be made with caution.

The proteinase inhibitor activity of a range of soy products and soy containing foods was examined in the United States (DiPietro and Liener, 1989). Total inhibitory activity was expressed as 'inhibitor units per mg'. A wide range of inhibitor activities were found in soy flours (10.8-286.3 IU/mg), soy concentrates (<5-74.2 IU/mg) and soy isolates (4.9-46.3 IU/mg). Amongst soy foods, the highest inhibitor levels were found in dehydrated soy milk (118.6 IU/mg), textured soy protein (14.3 IU/mg) and a wheat/soy pancake mix (13.0 IU/mg). Other soy containing foods all contained less than 5 IU/mg.

Rackis and co-workers, from the US Department of Agriculture (USDA), have reviewed a large amount of information on the proteinase inhibitor content of foods (Rackis *et al.*, 1986). Studies by their research group and others demonstrated trypsin inhibitor activity (TIA) in raw defatted soy flour in the range 23-42 mg/g. This decreased to 5-8 mg/g after toasting. Soy protein concentrates exhibited a wide range of trypsin inhibitor activities (4-29 mg/g), with approximately 80-90% of inhibitor activity destroyed by heating. Foods made from soy (tofu, soy sauce) generally exhibited even lower trypsin inhibitor activities (0.1-0.7 mg/g).

The TIA content of a range of legumes has been measured, relative to soybean (Rackis *et al.*, 1986). All had significantly lower TIA contents than soybeans, with highest levels in French bean (*Phaseolus vulgaris*; 80% of soybean), cow pea (*Vigna catjung*; 79% of soybean) and lima bean (*Phaseolus lunatus*; 77% of soybean). Other commonly consumed legumes included garden pea (*Pisum sativum*; 5-25% of soybean) and lentils (*Lens esculenta*; 25% of soybean).

A broad survey of trypsin inhibitor activity in British foods was carried out (Doell *et al.*, 1981). Results were expressed in terms of mg of pure trypsin inhibited per gram of food (parts per thousand). The highest trypsin inhibitor levels were seen in raw sweetbreads (pancreas or thymus gland of young lambs or calves; 23.3 mg/g) and raw soybean (18.7 mg/g). Most other foods (dairy, meat products, fish, fats and oils, vegetables, fruits, nuts, cereals and beverages) contained less than 1 mg/g of trypsin inhibitor activity. Levels of 3-7 mg/g were found in hen's eggs, with boiling reducing, but not eliminating, the trypsin inhibitor activity. Other foods containing greater than 1 mg/g of trypsin inhibitor activity were potato powder (6.2-8.6 as powder and 1.0-1.5 reconstituted), potatoes, new fresh (1.3 mg/g), fried pig (1.3 mg/g) and sheep (2.8 mg/g) livers and miso (4.1 mg/g).

The levels of trypsin, chymotrypsin and carboxypeptidase inhibitors in potatoes was examined during storage for up to 20 days, either in the dark or exposed to light (Dao and Friedman, 1994). Results were expressed in 'units/g'. Only minor changes in inhibitor

activities were observed during the 20 day period and no significant effect due to storage conditions was observed.

4.3.3 Influence of food processing on proteinase inhibitor activity content of foods

In general, plant protein foods from legumes and oilseeds require moist heat treatment to increase their nutritional value. Heating inactivates proteinase inhibitors and phytohemagglutinins (lectins that agglutinate mammalian red blood cells) and converts the protein into a more digestible form (Rackis *et al.*, 1986).

The trypsin inhibitor activity of raw soy meal is almost completely destroyed by treatment with live steam at atmospheric pressure for 15 minutes or longer (Rackis *et al.*, 1986). However, even after prolonged heating some residual TIA remains. Extrusion cooking, roasting, infra-red cooking, dielectric heating and microwave cooking can be equally effective in inactivating TIA, as long as attention is paid to temperature, time and moisture conditions (Rackis *et al.*, 1986).

Proteinase inhibitors in other legumes show similar heat stability to soybean inhibitors, while cereal trypsin inhibitors appear to be more stable with heating for one hour at 100ºC only reducing the inhibitor activity of wheat, rye and triticale by 38-71%, 36% and 7% respectively (Rackis *et al.*, 1986).

Activity of potato inhibitors I and II was completely destroyed by boiling for 30 minutes or microwave cooking for 3-5 minutes (Huang *et al.*, 1981). Inhibitor II activity was destroyed by baking at 190ºC for one hour, but significant inhibitor I activity remained in some parts of the potato after baking for 80 minutes.

4.3.4 Estimates of dietary exposure to proteinase inhibitors

Two studies were found that estimated the total dietary exposure to proteinase inhibitors (Billings *et al.*, 1990; Doell *et al.*, 1981).

Billings *et al*. (1990) analysed duplicate dietary samples from 31 free-living subjects in the United States for soluble trypsin and chymotrypsin inhibitory activity. The median estimated daily intake of trypsin inhibitor activity was 4.6 mg/day (range 0-127.9 mg/day), while the median estimated intake of chymotrypsin inhibitor activity was 1.6 mg/day (range 0-31.1 mg/day).

Doell *et al*. (1981) estimated an average intake of trypsin inhibitor activity for the British population of 295 mg/person/day, with the major contributors being eggs (93.6 mg/day), milk and milk products (56.8 mg/day), potatoes (42.5 mg/day) and other vegetables (37.8 mg/day).

The significant differences between the estimates of trypsin inhibitor activity intake in these two studies is likely to be methodological as much as actual.

4.4 Summary

While there is a plausible mechanism for promotion of pancreatic cancer by plant proteinase inhibitors, there is no evidence that this occurs. The proteinase inhibiting activity of plant foods will be severely reduced by normal food processing practices.

Differences in methodologies for determining and expressing the proteinase inhibitor content of foods make it difficult to establish a likely consensus level of exposure.

5 AMYLASE INHIBITORS

Alpha-amylase is one of the most important carbohydrate degrading enzymes in plants and animals. Proteinaceous inhibitors of α-amylase are produced by cereals, legumes, potatoes, peanuts and some fruit (Buonocore and Silano, 1986). Most amylase inhibitors present in plants show activity against animal amylases (including insect amylases), but are inactive against bacterial, fungal and plant enzymes (Buonocore and Silano, 1986).

Unlike proteinase inhibitors there is no single consistent mode of interaction between the inhibitor and the enzyme substrate (Svensson *et al.*, 2004). Interactions may be via direct hydrogen bonds, through hydrogen bonds via a water network or via a fully hydrated calcium ion (Svensson *et al.*, 2004).

5.1 Structure and Nomenclature

Amylase inhibitors from plants have been classified into six groups on the basis of sequence homology and three-dimensional structure. Details of these inhibitor families are included in Table 6.

Table 6: Plant amylase inhibitors; families, plant species, proteinases inhibited and active site amino acid structure

Inhibitor family	Plant species	Designation	Enzymes inhibited	Size (amino acid residues)
CM-proteins	Barley (*Hordeum vulgare*) Wheat (*Triticum aestivum*) Rye (*Secale cereale*) Millet (*Eleusine coracana*)	BMAI-I WMAI-I, WRP25, WRP26 RATI	Mammalian, insect, bacterial	124-160
Kunitz-type	Barley (*Hordeum vulgare*) Wheat (*Triticum aestivum*) Rice (*Oryza sativa*)	BASI WASI RASI	Cereal, insect	176-181
Thaumatin-like	Maize (*Zea mays*)		Insect	173-225
Legume lectin-like	Common bean (*Phaseolus vulgaris*)	αAI1, αAI2	Mammalian, insect, (fungal)	240-250
γ-Thionin-like	Sorghum (*Sorghum bicolor*)	SIα1, SIα2, SIα3	Insect, (mammalian)	47-48
Knottin-like	Amaranth (*Amaranthus hydrochondriacus*)	AAI	Insect	32

Adapted from (Svensson et al., 2004)

5.2 Evidence of Toxicity

There is little information available on adverse health effects in humans due to ingestion of amylase inhibitors.

Considerable interest has focused on the potential for amylase inhibitors to decrease starch digestion in humans. This has the potential to act as a management tool for conditions such as diabetes mellitus and obesity. A purified amylase inhibitor from white bean (*Phaseolus vulgaris*) has been shown to inhibit more than 99.9% of intraluminal amylase activity and decreased the intraluminal digestion of starch (Layer *et al.*, 1985). Acute and subchronic toxicity studies carried out on this product in rats did not demonstrate any toxicological concerns (Chokshi, 2007; Harikumar *et al.*, 2005).

A purified wheat amylase inhibitor (WAI) has also been trialled for treatment of diabetes and obesity (Lankisch *et al.*, 1998). However, although WAI delayed carbohydrate absorption and reduced peak post-prandial glucose concentration, overall carbohydrate absorption was only minimally decreased.

5.2.1 Toxic and tolerable exposure levels

No tolerable intake level has been established for amylase inhibitors. The lack of evidence of adverse health effects in humans due to amylase inhibitor exposure does not allow establishment of a toxic exposure level.

Given that amylase inhibitors are relatively common chemicals in foods the Threshold of Toxicological Concern (TTC) approach is unlikely to be appropriate for this compound (see section 1.1).

5.2.2 Tolerable concentrations in foods

No tolerable concentrations for amylase inhibitors in foods have been established.

5.3 Evidence of Exposure

5.3.1 Amylase inhibitors in New Zealand foods

No information was found on the amylase inhibiting activity content of New Zealand foods.

5.3.2 Amylase inhibitors in foods overseas

Australian barley cultivars were found to contain levels of barley amylase/subtilisin inhibitor (BASI) at concentrations in the range 136-232 mg/kg using an ELISA technique (Jarrett *et al.*, 1997). Analysis of 12 Australian wheat cultivars by the same method found much lower inhibitor levels (mean = 3.2 mg/kg), although this may have been a consequence of the monoclonal antibody used in the assay having a higher affinity for barley inhibitor than wheat inhibitor.

Analysis of a range of cereals for α-amylase inhibitor activity against human salivary α-amylases found the highest activity in white flour (590 units/g), followed by whole wheat

flour (351 units/g) and whole rye flour (186 units/g) (Granum, 1979). Oat and barley flour did not contain detectable activity. Red beans had low inhibitory activity (41 units/g), while split peas, brown rice, potato, carrot and swede contained no detectable inhibitor activity (Granum, 1979).

McCue and co-workers examined the ability of extracts from a range of plant foods to inhibit the activity of porcine pancreatic α-amylase (McCue *et al.*, 2005). Highest amylase inhibiting activities were found in ginger, coccinia (scarlet gourds), mustard, cinnamon, turmeric, eggplant, red grape, green pepper, broccoli sprouts and string beans. The study did not consider more usual sources of amylase inhibitors.

5.3.3 Influence of food processing on amylase inhibitor activity of foods

Amylase inhibitors in wheat flour are modified by the heat associated with bread baking (Xu *et al.*, 1999).

Breadbaking reduced the α-amylase inhibitor activity of white bread and whole rye bread by 80-90%, while no activity remained in whole wheat bread (Granum, 1979). Spaghetti with an initial activity of 248 units/g, retained less than 2% of its inhibitory activity after boiling for 15 minutes (Granum, 1979).

Soaking of chickpeas in a solution of sodium bicarbonate, followed by pressure cooking for 30 minutes reduced the amylase inhibiting activity by approximately 40% (Saxena *et al.*, 2003).

Soaking of kidney and faba beans reduced their α-amylase inhibitor activities by approximately 10-15%, while germination for up to 72 hours reduced the activity by 30-40% (Alonso *et al.*, 2000). Extrusion at 152-156°C destroyed all remaining α-amylase inhibitory activity. Similar results were demonstrated with pea seeds (*Pisum sativum*) (Alonso *et al.*, 1998).

5.3.4 Estimates of dietary exposure to amylase inhibitors

No estimates of dietary exposure to amylase inhibitors were found.

5.4 Summary

While amylase inhibitors theoretically have the potential to inhibit human digestive enzymes, there is no evidence of this occurring except in cases where therapeutic preparations enriched in amylase inhibitors have been used. In these cases the inhibition of intraluminal amylases was seen as a beneficial tool for the management of diabetes and obesity, rather than as a toxicological risk.

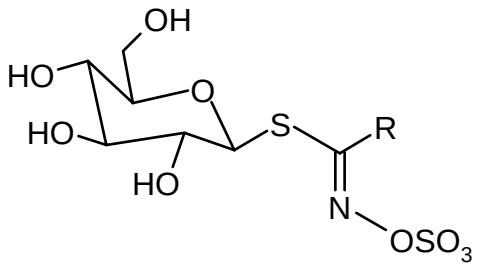
Exposure to amylase inhibitors will be frequent as the sources of well characterized inhibitors (wheat, rice, maize) are significant foods in our diet.

6 GLUCOSINOLATES

6.1 Structure and Nomenclature

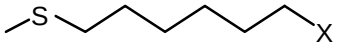
Glucosinolates, and their breakdown products isothiocyanates, were known as "mustard oils" in the 17th century because of their association with the sharp taste of mustard seeds. The two glucosinolates, sinigrin and sinalbin, were isolated from black (*Brassica nigra)* and white (*Sinapis alba*) mustard seeds in the early 1830s, with the general structure of glucosinolates being elucidated in 1956 (Fahey *et al.*, 2001). Glucosinolates include a wide range of different chemicals (at least 120), comprising a glucosinolate moiety and one of a variety of side chains. The side chain may be a straight or branched carbon chain, a substituted carbon chain, an aromatic or substituted aromatic chain or a heterocyclic moiety (Figure 3). The largest single group (one-third of all glucosinolates) contains a sulphur atom.

Figure 3: **Glucosinolate general structure and examples**

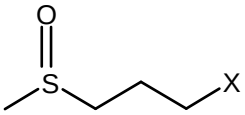


glucosinolate moiety = X

R= *sulphur containing*
eg

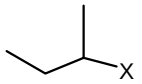


7-(Methylthio)heptyl



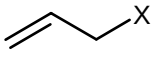
glucoraphanin

R= *branched aliphatic*
eg



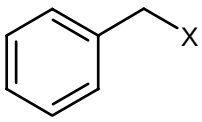
glucocochlearin

R= *aliphatic olefin*
eg



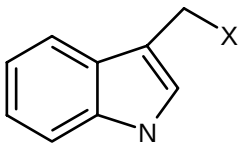
sinigrin

R= *aryl aromatic*
eg



gluconasturtiin

R= *indole aromatic*
eg



glucobrassicin

Adapted from Fahey et al. (2001)

6.2 Evidence of Toxicity

The relatively nonreactive glucosinolates are converted to thiocyanates, isothiocyanates, nitriles, epithionitriles, oxazolidine-2-thiones, or indolyl compounds by the enzyme myrosinase (found in plants and gut microflora).

No data were found on the toxicity of glucosinolates, or their breakdown products, in humans.

Initially glucosinolates were studied because of potential anti-nutritional and toxic effects observed in animal studies, that showed high glucosinolate diets affected the morphology and function of various cells and organs. Goitrogenic effects of induced iodine deficiency, enlarged thyroids, reduced levels of circulating thyroid hormones and non goitrogenic effects including abnormalities in the liver, kidney and suprarenal gland, and detrimental effects on growth and reproductive performance were observed in various species (Heaney and Fenwick, 1995; Tripathi and Mishra, 2007). Plant breeding or processing was subsequently utilised to remove glucosinolates from animal feeds (Fahey *et al.*, 2001; Holst and Williamson, 2004). For example, the oilseed crop "Canola" was developed from rapeseed (*Brassica napus*) in the 1970s by traditional plant breeding techniques, to produce an oilseed crop with low erucic acid and low glucosinolate content.

However, neither liver nor thyroid toxicities were associated with *Brassica* or glucosinolate ingestion in two human clinical trials over period of four weeks (Heaney and Fenwick, 1995; Shapiro *et al.*, 2006). In one study, volunteers consumed cooked Brussels sprouts with a progoitrin (2-hydroxybut-3-enyl glucosinolate) content equivalent to 40 mg goitrin per day for four weeks (Heaney and Fenwick, 1995). In the more recent study, doses were either 25 or 100 μmol of glucoraphanin (4-methylsulphinylbutyl glucosinolate) equivalent to approximately 11 or 46 mg per person per day for seven days (Shapiro *et al.*, 2006).

Untreated juice of cruciferous vegetables induced DNA damage in both bacterial and mammalian cells *in vitro* with glucosinolates and particularly isothiocyanates implicated as causative agents (Kassie *et al.*, 1996). Genotoxic, and probably carcinogenic effects have also been evidenced in animal studies (Fahey *et al.*, 2001; Kassie *et al.*, 1996; Stoner *et al.*, 2002), although a further study reported a much reduced carcinogenic effect under *in vivo* compared with *in vitro* test conditions (Kassie *et al.*, 1999). The potential carcinogenic effects contrast with demonstrated anticarcinogenic or chemoprotective properties of glucosinolates that are the focus of numerous studies (Fahey *et al.*, 2001; Holst and Williamson, 2004; McNaughton and Marks, 2003) and US patent (Fahey and Talalay, 2001).

6.2.1 Adverse health effects in New Zealand

There are no known reports of adverse human health effects associated with glucosinolates in New Zealand.

6.2.2 Mechanism of toxicity

The glucosinolates comprise a wide range of compounds that do not all elicit the same effects (Heaney and Fenwick, 1995; Holst and Williamson, 2004; Tripathi and Mishra, 2007). Glucosinolate effects may be mediated by different mechanisms, including estrogenic,

goitrogenic and carcinogenic mechanisms (Fahey *et al.*, 2001; Heaney and Fenwick, 1995; Holst and Williamson, 2004; Mawson *et al.*, 1994). Holst and Williamson (2004) proposed that the effect, and the mechanism, is also likely to be dose dependent. At low concentrations glucosinolates products are likely to activate mitogen-activated protein kinases that lead to gene expression of survival and defensive genes, as observed in beneficial effects. Increasing the dose will additionally activate the caspase pathway, leading to apoptosis, and potential cell toxicity. Further increasing the dose may account for non-specific, necrotic cell death and genotoxic effects (Holst and Williamson, 2004). This view is supported by Ju and co-workers who found that *Brassica* vegetable extracts exerted anti-estrogenic effects at low doses, whereas at higher doses they can act as estrogen mimics (Ju *et al.*, 2000).

6.2.3 Toxic and tolerable exposure levels

The lack of evidence of adverse health effects in humans due to glucosinolates exposure does not allow establishment of a toxic exposure level.

Given that glucosinolates are relatively common chemicals in foods the Threshold of Toxicological Concern (TTC) approach is unlikely to be appropriate for this compound (see section 1.1).

6.2.4 Tolerable concentrations in foods

Tolerance levels for total glucosinolates in animal feeds have recently been published and vary for different animals: ruminants (1.5-4.22 µmol/g diet), pig (0.78 µmol/g diet), rabbits (7.0 µmol/g diet), poultry (5.4 µmol/g diet) and fish (3.6 µmol/g diet) (Tripathi and Mishra, 2007). Expressed on a weight basis, assuming a molecular weight of 460, the tolerance concentrations range from 400 – 3,200 mg/kg diet, with pigs the least tolerant animal and rabbits the most tolerant.

6.3 Evidence of Exposure

6.3.1 Glucosinolates in New Zealand foods

A single paper reporting the level of allyl isothiocyanate (AITC) in New Zealand grown wasabi was found, with levels in the rhizome in the range 1564-3366 mg/kg on a fresh weight basis (Sultana *et al.*, 2002).

6.3.2 Glucosinolates in foods overseas

Glucosinolates are prevalent in 15 botanical families of which the *Brassicaceae* is the most important dietary source for humans (Holst and Williamson, 2004). Concentrations of individual glucosinolates vary with plant species and growing conditions. McNaughton and Marks (2003) have evaluated the literature and compiled a database of total glucosinolate content of cruciferous vegetables from eighteen published studies (Table 7). Papers that measured only a specific glucosinolate compound and did not report total glucosinolates were excluded. Total glucosinolate content was based on one of two analytical methodologies (a glucose release method or intact glucosinolates by HPLC or GC). Data from different studies were aggregated to derive a median and range of concentrations

Table 7: Total glucosinolate content (mg/100g fresh weight) of edible cruciferous vegetables

Food	Processing	No. samples	Mean (Range)
Broccoli	Raw	2	40.0 (19.3-127.5)
		2	127.5
		6	86.0
		NR	19.3
		NR	61.1
		25	62.3
	Cooked	NR	37.2
Broccoli (frozen)	Raw	NR	50.7
	Cooked	NR	20.7
Brussels sprouts	Raw	2	445.5 (80.1-445.5)
		6	252.7
		40	126.6
		109	200.2
		NR	80.1
		44	226.2
		10	292.0
		2	247.0
	Boiled	2	148.0
		44	123.7
Brussels sprouts, frozen	Raw	NR	90.5
	Cooked	NR	61.3
Cabbage, unspecified	Raw	2	58.9 (42.7-108.9)
		43	108.9
		2	42.7
	Boiled	43	78.6
Cabbage, Chinese, pak-choi	Raw	3	53.4 (17.3-54.8)
		NR	17.3
		2	57.2
		68	54.8
Cabbage, Chinese, pe-tsai	Raw	19	19.8 (8.9-54.1)
		56	21.3
		2	8.9
		18	54.1
Cabbage, red	Raw	8	76.6 (26.5-76.6)
		1	18.8
		17	26.5
		2	64.2
		NR	66.9
	Cooked	NR	54.8
Cabbage, savoy	Raw	4	77.0 (59.5-209.0)
		17	59.5
		11	209.0
Cabbage, white	Raw	67	51.1 (8.4-90.0)
		32	90.0
		17	37.7
		2	22.9
		NR	8.4

Food	Processing	No. samples	Mean (Range)
Cauliflower	Raw	5	43.2 (11.7-78.6)
		2	11.7
		NR	36.5
		44	62.0
		27	78.6
	Cooked	44	42.0
Cauliflower, frozen	Raw	NR	40.5
	Cooked	NR	27.9
Coleslaw	Raw	NR	42.2
Collards	Raw	5	200.7
Cress	Raw	NR	389.5
		NR	658.2
Horseradish	Raw	NR	160.1
Kale, unspecified	Raw	1	317.1 (6.7-317.1)
		5	100.7
		NR	6.7
Kale, Chinese	Raw	2	62.2
		24	80.4
Kale, curly	Raw	NR	89.4
	Cooked	NR	69.1
Kohlrabi	Raw	2	52.4 (19.7-109.3)
		NR	19.1
		1	39.3
		N	109.3
	Cooked	NR	73.4
Mustard greens	Raw	28	118.1 (118.1-544.5)
		2	544.5
		2	281.5
Radish, unspecified	Raw	NR	12.5
		2	172.4
Radish, black	Raw	NR	92.8
		1	123.4
Radish, European	Raw	6	44.8
Radish, white	Raw	NR	71.0
		7	76.8
Radish, red	Raw	36	67.6
Radish, Asian	Raw	4	138.0
		15	108.8
Swede	Raw	33	92.0
Turnip	Raw	9	93.0 (20.4-140.5)
		60	140.
		NR	20.4
Turnip-swede	Raw	44	56.0
	Cooked	44	29.1
Watercress	Raw	NR	95.0

Adapted from (McNaughton and Marks, 2003)

6.3.3 Influence of storage, processing and cooking on glucosinolate content of foods

The relatively non-reactive glucosinolates are converted to isothiocyanates by the enzyme myrosinase (found in plants and gut microflora). In plant tissue, myrosinase is present in separate compartments from glucosinolates and is released as a result of processes such as cutting, cooking or freezing (Fahey *et al.*, 2001).

No significant decrease in glucosinolate content of broccoli, Brussels sprouts, cauliflower nor green cabbage was found when stored at ambient temperature for 7 days, but losses ranging from 11 to 27% were observed when stored in a domestic refrigerator for 7 days and greater losses of 33% were seen after freezing and thawing (Song and Thornalley, 2007).

Verkerk *et al.* found an increase of some glucosinolates, especially indole glucosinolates after chopping (Verkerk *et al.*, 1997). For example, levels of glucobrassicin (indol-3-ylmethyl glucosinolate) increased four-fold in cut cabbage. Whilst an increase in isothiocyanates is expected due to release from sequestered or conjugated forms by myrosinases, an increase in glucosinolate is unexpected. It was suggested that the glucosinolates may be formed as part of the plant's defence mechanism in response to injury (in this case, chopping). In contrast, Song and Thornalley (2007) reported that up to 75% of the glucosinolate content was lost after vegetables were finely diced or shredded with much less loss if the vegetables were only coarsely shredded. Thirty to fifty percent of the loss was accounted for by conversion to isothiocyanates.

Glucosinolates and some of their breakdown products are water-soluble and may therefore be lost into the cooking water of boiled vegetables. The losses can be more than 50%, but differ between species, and between cultivars within a species (Fahey *et al.*, 2001; McNaughton and Marks, 2003; Song and Thornalley, 2007). Song and Thornalley (2007) reported a loss of glucosinolate content on boiling of 58-77% of baseline values after 30 minutes boiling. However, steaming, microwave and stir-fry cooking gave no significant loss of total glucosinolate content. McNaughton and Marks (2003) report an average loss during cooking of about 36%.

6.3.4 Estimates of dietary exposure to glucosinolates

An estimate of exposure to total glucosinolates was made by combining concentration data from Table 7 with New Zealand consumption data for average serving sizes across all respondents 15 years and over, in the 1997-98 National Nutrition Survey (NNS) (Russell *et al.*, 1999). For those foods that are generally cooked (broccoli, cabbage, cauliflower, swede and turnip) data for cooked foods was used, or in the case of swede and turnip an adjustment for a conservative estimate of mean loss of 36% on cooking, was assumed (McNaughton and Marks, 2003). The contributing values and exposure estimate are shown in Table 8.

Exposure to total glucosinolates across the adult population is estimated to be 17 mg/day from an average consumption of 31 g of cruciferous vegetables per person per day. However, these average values may be exceeded by subjects who like cruciferous vegetables and consume larger portions. For example, the average daily consumption of turnips across the population was 0.3 g/day yet the maximum serving size was 359 g. The maximum serving size of watercress was 1450 g across the population, while the average daily consumption was only 1.8 g. For the very worst case of the same consumer eating the

maximum serving size for each cruciferous vegetable, the estimated exposure to total glucosinolates is 3 g/day.

Table 8: Estimated average exposure to total glucosinolates from cruciferous vegetables (mg/day)

Food	Total glucosinolate concentration (mg/100g)	Daily average consumption (g/day)	Exposure (mg/day)
Broccoli, cooked	37.2	6.0	2.2
Brussels sprouts	136.0	1.1	1.5
Cabbage	66.7	8.2	5.5
Cauliflower	42.0	7.5	3.1
Coleslaw	42.2	4.7	2.0
Radish	83.2	0.1	0.1
Swede	58.9	1.7	1.0
Turnip	61.4	0.3	0.2
Watercress	95.0	1.8	1.7
Total		**31.4**	**17.3**

An average exposure of 17 mg/day is lower, but in the same order of magnitude as the 46 mg/day and 36 mg/day estimated for a German population in the winter and summer respectively based on average consumption of 54 g/day of cruciferous vegetables (Holst and Williamson, 2004) and the average UK intake of 29 mg/day from cooked Brussels sprouts, cabbage, cauliflower and turnips/Swedes (Sones *et al.*, 1984). Sones and co-workers (1984) also estimated that a high consumer might consume in excess of 300 mg/day.

6.4 Summary

The relatively non-reactive glucosinolates are converted to a range of breakdown products through hydrolysis by the enzyme myrosinase, resulting in adverse effects in rats, fish, pigs, poultry, ruminants, rabbits and sheep. This has resulted in the setting of maximum tolerance levels for glucosinolates in animal feed. However, there is no evidence of adverse effects in humans and there is evidence to suggest beneficial health effects due to glucosinolate exposure at dietary levels.

Glucosinolates occur in a wide variety of plants with cruciferous vegetables (broccoli, Brussels sprouts, cabbage, cauliflower, radish, horseradish, swede, turnip and watercress) the most important dietary source for humans. Concentration of individual glucosinolates varies with plant species and growing conditions. Glucosinolate content decreases with refrigeration, freezing and fine shredding. Glucosinolates and some of their breakdown products are water-soluble and are progressively lost with increased time of boiling. However, steaming, microwave and stir-fry cooking give no significant loss of total glucosinolate content.

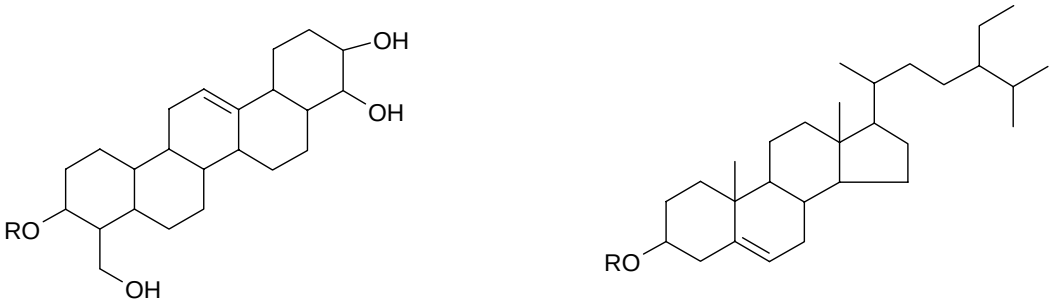
An average New Zealand adult is estimated to be exposed to around 17 mg per day total glucosinolates although an extreme consumer may be exposed to considerably higher levels of glucosinolate. This level of exposure is similar to reported levels from European countries. Exposure up to 50 mg per day has not shown adverse effects for humans (Shapiro *et al.*, 2006).

7 SAPONINS

7.1 Structure and Nomenclature

Saponins, named because they form stable, soap-like foams in aqueous solutions, comprise a diverse and chemically complex group of compounds that occur naturally in over 100 plant families (Fenwick *et al.*, 1991), most probably to protect the plant from potential pathogens. All saponins contain either a triterpenoid or a steroidal aglycone backbone linked to one or more sugar chains. Variations in functional groups bound to the aglycone backbone and the position, number and nature of the sugar groups gives rise to a wide range of different saponins. The naming of saponins is not particularly systematic and many saponins are named to reflect their botanical origin, hence for example, the soyasaponins (from soya), phaseollosides A-E (from *Phaseolus vulgaris*), asparasaponins I and II and officinalisnins I and II (from *Asparagus officinalis*) and glycyrrhizin (from liquorice, *Glycyrrhiza gabra*) (Fenwick *et al.*, 1991). Examples of the chemical structure of a triterpenoid and a steroidal saponin are shown in Figure 4.

Figure 4: Chemical structures of the aglycone skeletons of a triterpenoid (soyasaponin A) and steroidal (sitosterol) saponin found in beans and onions respectively



Soyasaponin A

Sitosterol

R = a sugar moiety

7.2 Evidence of Toxicity

A diverse range of biological effects have been reported in association with saponins. These are mostly beneficial but also potentially harmful and including membrane permeabilising (haemolytic), immunostimulating, cholesterol lowering, anti-carcinogenic, antioxidant, antiviral, antibiotic and antifungal have been ascribed to saponins (Francis *et al.*, 2002; Sparg *et al.*, 2004). Saponins are extremely toxic to cold-blooded animals but their toxicity to animals and humans is poorly characterised. Haemolytic saponins have high mammalian toxicity when administered intravenously, with minimum lethal doses as low as 40 mg/kg body weight being reported for rabbits (Price *et al.*, 1987).

Based on limited evidence, there is an understanding of low oral toxicity to humans (Price *et al.*, 1987; Sparg *et al.*, 2004). It has been suggested that, although haemolytic saponins are able to increase the permeability of the gut, the high surface area of the gut compared to the concentration of saponins in the diet mediate against toxic effects (Price *et al.*, 1987).

Price and colleagues (1987) cite three reports from the 1960s of patients who developed hypertension as a result of eating large quantities of licorice-based confectionary.

7.2.1 Adverse health effects in New Zealand

There are no known reports of adverse health effects due to saponin exposure in New Zealand or overseas

7.2.2 Mechanism of toxicity

Two reviews cover the current knowledge of the mechanisms of the biological action of saponins (Francis *et al.*, 2002; Sparg *et al.*, 2004) but toxicity is not ascribed to any of these effects. It has been hypothesised that deleterious effects observed in cold-blooded animals are due to the ability of haemolytic saponins to increase membrane permeability.

7.2.3 Toxic and tolerable exposure levels

Neither toxic nor tolerable exposure levels have been set for saponins in food.

Under the Threshold of Toxicological Concern (TTC) approach saponins would be considered to be structural class I (Barlow, 2005; Munro *et al.*, 1996), with an associated TTC of 1800 μg/day (0.03 mg/kg body weight/day for a 60 kg adult). However, given that saponins are relatively common chemicals in foods the TTC approach is unlikely to be appropriate for this compound.

7.2.4 Tolerable concentrations in foods

No limits have been set for tolerable concentrations of saponins in foods.

7.3 Evidence of Exposure

Saponins occur in many plant species including wild plants and cultivated crops, with the triterpenoid saponins predominant in cultivated crops and steroid saponins common in plants used as herbs for their health-promoting properties (Francis *et al.*, 2002). Triterpenoid saponins have been detected in many legumes including soyabeans, beans, peas and also in alliums (garlic, onion and leeks), tea, spinach, sugar beet, licorice, sunflower, horse chestnut, and ginseng, although not necessarily detected in the edible part of the plant. Steroid saponins are found in oats, capsicum pepper and tomato seeds, alliums, asparagus, yam, fenugreek, yucca and ginseng (Francis *et al.*, 2002; Price *et al.*, 1987).

7.3.1 Saponins in New Zealand foods

No data was found for saponin levels in New Zealand foods.

7.3.2 Saponins in foods overseas

A summary of total saponin concentration data from the compilation of Rao and Gurfinkel, for foods that might be consumed as part of a New Zealand diet, is shown in Table 9 (Rao

and Gurfinkel, 2000). The triterpenoid saponins of legumes have been the most widely studies, but there is limited data for saponin content of the widely consumed alliums. While saponins are present in tea leaves, there are no data on the saponin content of tea as consumed. Variability of concentration ranges for a particular food is a combination of natural variation and differences in analytical methodologies. For this reason, the year of publication is included.

Table 9: Concentration of total saponins in plant foods

Food	Total saponins (g/kg)[1]	Year of publication
Asparagus	1.3[2]	1983
	0.14-0.80[3]	2006
Beans, unspecified	0.245	1986
Broad bean	3.1[2]	1983
	3.5	1998
Butter bean	1.0	1986
Canned baked beans	1.1[2]	1983
Cauliflower	24	1988
Chickpea	2.3	1986
	50[2]	1983
	16	1988
	1.5	1987
	56	1998
	0.71, 0.76	1996
Garlic	1.1[2]	1983
	2.9	1998
	930	1978
Green bean	13	1998
	1.0[2]	1983
Haricot bean	16[2]	1983
	3	1987
	19	1998
	4.1	1986
Kidney bean	14[2]	1983
	2	1987
	1.7	1998
	16	1998
	3.5	1986
Leek	1.0	1978
Lentil	3.2-4.0[2]	1983
	3.7-4.6	1998
	1.1	1986
	0.7,1.1	1996
Lima beans	1.1	1998
Mung bean	0.5	1986
	5.1[2]	1983
Navy beans	4-18[2]	1983
	4.5-21	1998
Oats	0.9[2]	1983
	1.0	1998

Food	Total saponins (g/kg)[1]	Year of publication
	0.4	1993
Onion	0.2	1978
Pea	1.8	1985
	1.5	1997
	2.4,2.5	1994
	11	1998
	1.1, 1.2	1994
	0.6	1998
	1.8	1986
	2.5[2]	1983
Peanut	16	1969
	5.8[2]	1983
	1.0	1998
	6.3	1998
	<0.1	1986
	6.3	1992
Scarlet runner bean	1.8	1998
	3.4	1986
Silverbeet	5.0[2]	1983
Soybean	39[2]	1983
	6.5	1986
	3.5	1985
	2.4	1998
	43	1998
	0.9-5.3	1995
Soybean- flour	4.7-5.3	1995
Soybean- protein isolate	8.1	1995
Soymilk	2.6-3.9	1995
Soy -Tofu	3.0-3.3	1995
Soy - Miso	1.5	1995
Spinach	5.5[2]	1983
	23.5	1988
	47	1998

Adapted from Rao and Gurfinkel (2000)

1 Dry or fresh weight basis not specified

2 Concentrations in foods as consumed (Fenwick and Oakenfull, 1983)

3 (Schwarzbach *et al.*, 2006)

In addition to the compilation of data on total saponins, there are limited concentration data for specific saponins, in particular glycyrrhizin in beverages and licorice (Fenwick *et al.*, 1991). US, UK and Belgian confectionery contained 0.4-7.9, 0.1-1.7 and >2.2 mg/g glycyrrhizin respectively.

7.3.3 Influence of storage, processing and cooking on saponin content of foods

The saponin content of asparagus spears did not change with storage at -18ºC for 20 weeks (Schwarzbach *et al.*, 2006). However, studies have consistently shown that soaking, cooking and canning reduces the saponin content of food (refs. in Rao and Gurfinkel, 2000). While

most of the losses are likely the result of leaching of water-soluble saponins, chemical alteration may also occur.

7.3.4 Estimates of dietary exposure to saponins

Information on saponin intake from a human diet is extremely limited. Ridout and co-workers estimated daily intakes of saponins from legumes as ranging from 15 mg for a typical Western diet to over 200 mg/day for a vegetarian diet (Ridout *et al.*, 1988). Total dietary saponin intake from all foods would be higher but no estimates have been found.

Mean intakes of glycyrrhizin, from licorice, of approximately 2-10 mg/day have been calculated for US, Belgium, UK and Danish consumers of licorice-containing beverages, herbal products and confectionery (Fenwick *et al.,* 1991).

7.4 Summary

Saponins are a diverse group of secondary plant metabolites, found in a variety of vegetables, predominantly legumes and most likely with a natural role to protect the plant from potential pathogens. Saponins are associated with a range of biological effects, mostly positive, and there is an understanding of low oral toxicity to humans although this is not well defined.

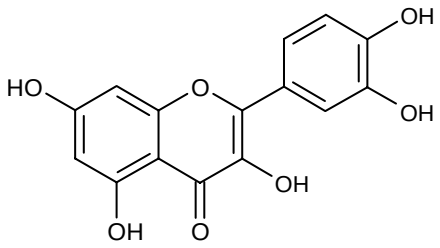
Humans are potentially exposed to saponins from the consumption of legumes including soyabeans, beans, peas and also alliums (garlic, onion and leeks), asparagus, ginseng, horse chestnut, licorice, oats, spinach, sugar beet, sunflower, tea and yams. Different saponins probably account for different effects and therefore information on exposure to individual saponins is necessary to define toxic doses for humans. Lack of availability of individual compounds has limited research in this area.

8 QUERCETIN

8.1 Structure and Nomenclature

Quercetin is one of the major flavonoids found in fruits and vegetables (IEH, 2000; Pillow *et al.*, 1999).

Figure 5: **Chemical structure of quercetin**



8.2 Evidence of Toxicity

There are no data available on the toxicity of quercetin to humans.

Quercetin has been reported to be carcinogenic in rats, but not mice, studies with a dose rate of 10.1 mg/kg body weight/day inducing tumors in half of the test animals (Gold *et al.*, 2006). The International Agency for Research on Cancer, in 1987 and 1999, concluded that no evaluation could be made of the carcinogenicity of quercetin to humans on the basis of limited data from animal studies and no data for humans (IARC, 1987; 1999). IARC concluded that there was limited evidence for the carcinogenicity of quercetin in animals, due to increased incidence of intestinal and bladder tumours in one study and an increased incidence of renal tubule neoplasms in another (IARC, 1987). Conversely, quercetin has also been reported to inhibit carcinogenesis in both rats and mice (NRC, 1996).

Quercetin is weakly estrogenic in *in vitro* cell based assays (Thomson, 2005).

8.2.1 Adverse health effects in New Zealand

There are no known reports of adverse health effects from quercetin in New Zealand

8.2.2 Mechanism of toxicity

Quercetin caused a concentration dependent increase in DNA damage, and perturbation of the oxidative state of liver nuclei of rats *in vitro* by increasing lipid peroxidation and decreasing glutathione content and glutathione S-transferase activity (IARC, 1999).

At a cellular level, quercetin inhibited certain biochemical pathways relating to energy production, markedly inhibited growth of several cell lines and caused altered liver function in rodent and human microsomes (cell fragments), including the inhibition of detoxification pathways (IARC, 1999).

Evaluation of a two-year rat study concluded that the increased incidence of renal tumours in male rats receiving medium and high doses of quercetin (1.0 and 4.0% of the diet) was due to exacerbation of spontaneous chronic progressive nephropathy (CPN) (Hard *et al.*, 2007). This mechanism was not believed to have any relevance for extrapolation to humans.

8.2.3 Toxic and tolerable exposure levels

No tolerable exposure limits for quercetin in the diet have been established.

Under the Threshold of Toxicological Concern (TTC) approach quercetin would be considered to be structural class I (Barlow, 2005; Munro *et al.*, 1996), with an associated TTC of 1800 μg/day (0.03 mg/kg body weight/day for a 60 kg adult). However, given that quercetin is a relatively common chemical in foods the TTC approach is unlikely to be appropriate for this compound.

8.2.4 Tolerable concentrations in foods

No tolerable concentrations for quercetin in foods have been established.

8.3 Evidence of Exposure

8.3.1 Quercetin in New Zealand foods

Limited data on the levels of quercetin in New Zealand apples and manuka honey have been published (McGhie *et al.*, 2005; Yao *et al.*, 2003). McGhie and colleagues reported the total concentration of quercetin plus quercetin glycosides in 10 apple varieties to range from 119 to 177 mg/kg. This value is high compared with a mean value of 36 mg/kg found in apples in The Netherlands (Hertog *et al.*, 1992). Yao and co-workers (2003) reported a mean concentration of 4.3 mg/kg of quercetin in two New Zealand manuka honeys (Yao *et al.*, 2003).

8.3.2 Quercetin in foods overseas

Hertog and co-workers (1992) reported seasonal variability of quercetin concentrations in leafy vegetables such as lettuce with higher concentrations in summer produce. In addition, plants grown in glasshouse conditions had lower flavonoid content.

Compilations of literature values for the concentration of five flavonoids, including quercetin, in human foods have been published (IEH, 2000; Pillow *et al.*, 1999) with a summary of concentration data for quercetin shown in Table 10.

Table 10: Concentration of quercetin in foods consumed by humans (mg/kg)

Food	Quercetin	Food	Quercetin	Food	Quercetin
Apple	23-36	Grapefruit juice	4.9	Potatoes	2
Apple juice	2.8	Green beans	29-39	Red cabbage	3.7-4.6
Apricot	25	Green pepper	18	Red currant	13
Broad beans	19-20	Kale	40-110	Red pepper	<1
Broccoli	18	Leek	<1	Red wine	11
Cauliflower, Brussels sprouts	<1	Lettuce	14	Strawberry	8.6
Celery	<1	Onion	347-445	Sweet cherry	15
Cherries	15	Orange juice	3.4-5.7	Tea	18-25
Cranberry juice	172	Pear	4.5-6.4	Tomato	7-8
Endive	<1	Plum	6-9	Tomato juice	13
Grape	12-15				

Adapted from IEH (2000) and Pillow et al. (1999)

8.3.3 Influence of storage, processing and cooking on quercetin content of foods

Quercetin levels in processed beans, red cabbage and kale were approximately 50% lower than in fresh products (Hertog *et al.*, 1992). By contrast, processed cherries had higher quercetin levels than fresh sweet cherries and quercetin levels in applesauce were comparable with those in most varieties of apples. No quercetin was found in processed apricots but 25 mg/kg quercetin was found in fresh apricots. This observation may be due to varietal differences.

Apple juice is predicted to be stable with respect to quercetin levels at ambient or refrigerated storage conditions for up to six months (van der Sluis *et al.*, 2005).

8.3.4 Estimates of dietary exposure to quercetin

Dietary exposures of 6.7-6.9 mg/day for young males, 8.6-8.8 mg/day for adult males over 25 years of age, 9.6-9.8 mg/day for adult females over 25 years of age and 9.4-9.5 mg/day for vegetarian females have been estimated for New Zealand population groups based on New Zealand consumption and overseas concentration data (Thomson, 2005). The major contributing foods were apples, apricots, green beans, onions and broccoli but the relative contributions varied for the different population groups.

The mean daily exposure for the adult Dutch population has been determined to be 16 mg/day (Hertog *et al.*, 1993), similar to mean intake estimates for the United States of 16 mg/day for women from the Nurses' Health Study (71,976 women) and 17 mg/day for men from the Health Professionals Follow-up Study (35,425 men) (Lin *et al.*, 2006). The mean intakes of quercetin from a small study of Japanese consumers was estimated at 21 mg/day (Kita *et al.*, 2004).

8.4 Summary

Quercetin is present in a number of commonly consumed foods, resulting in dietary exposures of between 10 and 20 mg/day with the major contributing foods being apples, apricots, green beans, onions and broccoli.

There is limited evidence that quercetin is carcinogenic to rats, but not mice, and no evidence of toxicity to humans. However, the results from rat studies suggest that tumour formation is via a secondary mechanism (non-genotoxic) with limited relevance to humans and was only noted at high doses levels, well above human dietary levels.

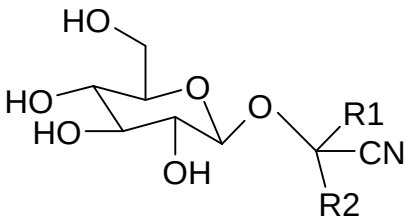
As with other chemicals included in the current assessment, there is contradictory evidence as to whether quercetin is carcinogenic or anti-carcinogenic.

9 CYANOGLYCOSIDES

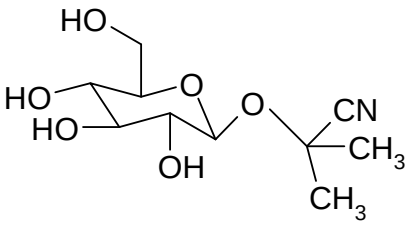
9.1 Structure and Nomenclature

Cyanoglycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. More than 2000 plant species are believed to contain cyanogens, with approximately 300 cyanoglycosides identified (Davis, 1991). With only a few exceptions cyanoglycosides comprise a glucose moiety bound to a cyanhydrin. The basic structure and two exemplar cyanoglycosides are shown in Figure 6.

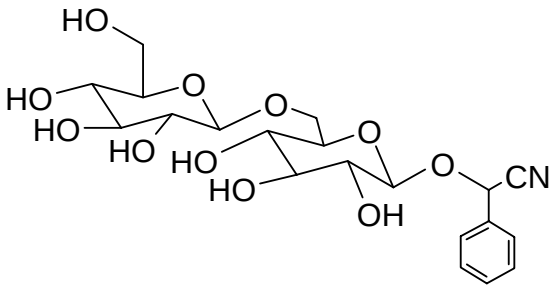
Figure 6: **Cyanoglycoside general structure and examples**



Cyanoglycoside basic structure



linamarin



amygdalin

9.2 Evidence of Toxicity

Potential toxicity of cyanoglycosides arises from enzymatic degradation to produce hydrogen cyanide, resulting in acute cyanide poisoning. Clinical symptoms of acute cyanide poisoning include rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhea, mental confusion, stupor, blue discolouration of the skin due to lack of oxygen, twitching and convulsions (FSANZ, 2004; Speijers, 1993).

Several diseases are associated with chronic dietary intake of cyanoglycosides, although there is some debate over the causal relationships due to confounding nutritional factors (Davis, 1991; FSANZ, 2004; Speijers, 1993). For example, malnourished individuals appear to be more susceptible to the effects of cyanoglycosides.

- Konzo is a motor neuron disease characterized by irreversible weakness in the legs. In severe cases, patients are not able to walk, and speech and arms may be affected. Konzo particularly affects children and women of childbearing age in East Africa in times of food shortage and is associated with a high and sustained intake of cassava

(*Manihot esculenta*) in combination with a low intake of protein (Davis, 1991; FSANZ, 2004).

- Tropical ataxic neuropathy (TAN) describes several neurological symptoms effecting the mouth, eyesight, hearing or gait of mostly older males and females. TAN is attributed to cyanide exposure from the chronic consumption of foods derived from cassava (FSANZ, 2004).

- Goitre and cretinism due to iodine deficiency can be exacerbated by chronic consumption of insufficiently processed cassava. Cyanoglycosides from cassava are detoxified to thiocyanate that competes with iodine in the thyroid, effectively increasing the dietary requirement for iodine.

9.2.1 Adverse health effects in New Zealand

There are two known reports of cyanide poisoning in New Zealand from the consumption of apricot kernels. In one case a woman was admitted to North Shore hospital after consuming 60 ground apricot kernels mixed with orange juice (Atkinson, 2006). In an earlier case, reported by Waikato hospital (Tebbutt, 2001), 30 apricot kernels containing 3 mg cyanide/g kernel caused a significant poisoning.

Effects arising from chronic consumption of cyanogenic foods are not likely to be an issue for the general population in New Zealand since food insecurity and dietary intake of protein is adequate and neither cassava, nor other cyanogenic foods are staples of the general diet. New Zealanders do have a low iodine intake (Vannoort and Thomson, 2005) and therefore goitre or cretinism are possible concerns for individuals who may regularly consume cyanogenic foods.

9.2.2 Toxic and tolerable exposure levels

The acute oral lethal dose of hydrogen cyanide for humans is 0.5-3.5 mg/kg body weight (Speijers, 1993). For an 80 kg person this equates to 40-280 mg HCN.

9.2.3 Tolerable concentrations in foods

At present there is no maximum level of hydrogen cyanide specified in New Zealand foods (FSANZ, 2002). However, Food Standard 1.4.1 specifies limits for total hydrocyanic acid from the addition of flavouring substances in confectionary (25 mg/kg), stone fruit juices (5 mg/kg), marzipan (50 mg/kg) and alcoholic beverages (1 mg/kg per 1% alcohol content).

The United Kingdom Food Standards Agency and the New Zealand Food Safety Authority consider a safe intake of apricot kernels to be one to two kernels a day (NZFSA, 2006; UKFSA, 2006).

The WHO has set a safe level of cyanogens in cassava flour as 10 mg HCN/kg, while an acceptable level in Indonesia is 40 ppm (FSANZ, 2004; Speijers, 1993).

9.3 Evidence of Exposure

9.3.1 Cyanoglycosides in New Zealand foods

No data was found for the level of cyanoglycosides in New Zealand foods.

9.3.2 Cyanoglycoside levels in foods overseas

Humans may be exposed to cyanogenic glycosides from the consumption of cassava root, bamboo shoots, beans, almonds and marzipan, apricot stones, cherry pits, plum stones, peach stones, apple seeds, flax seed meal, sorghum leaf and giant taro leaves (Davis, 1991; Haque and Bradbury, 2002). Data on the concentration of cyanoglycosides in food plants are limited and extremely variable depending on plant variety, age, growing conditions and post harvest changes and the method of analysis used.

Data on the cyanogenic glycoside content of some foods are given in Table 11.

Table 11: Cyanogenic glycoside content of food potentially consumed by humans

Food	Major cyanogenic glycoside present	Cyanogen content (mg HCN/kg)	Reference
Cassava (*Manihot esculenta*) - root	Linamarin	25-27 380 (bitter tubers) 445 (sweet tubers)	(Haque and Bradbury, 2002) (Simeonova and Fishbein, 2004) (Simeonova and Fishbein, 2004)
Sorghum (*Sorghum vulgare*) – leaves	Dhurrin	750-790	(Haque and Bradbury, 2002)
Flax (*Linum usitatissimum*) – seed meal	Linamarin, linustatin, neolinustatin	360-390	(Haque and Bradbury, 2002)
Lima beans (*Phaseolus lunatus*)		2000-3000	(Simeonova and Fishbein, 2004)
Giant taro (*Alocasia macrorrhizos*) – leaves	Triglochinin	29-32	(Haque and Bradbury, 2002)
Bamboo (*Bambusa arundinacea*) – young shoots	Taxiphyllin	1010-1060 7700	(Haque and Bradbury, 2002) (Simeonova and Fishbein, 2004)
Apple (*Malus* spp.) – Seed	Amygdalin	690-790	(Haque and Bradbury, 2002)
Peach (*Prunus persica*) – Kernel	Amygdalin	710-720	(Haque and Bradbury, 2002)
Apricot (*Prunus armeniace*) – Kernel	Amygdalin	785-813 89-2170 2.2 (juice)	(Haque and Bradbury, 2002) (Simeonova and Fishbein, 2004) (Simeonova and Fishbein, 2004)
Plum (*Prunus* spp.) – Kernel	Amygdalin	696-764	(Haque and Bradbury, 2002)
Nectarine (*Prunus persica var nucipersica*) – Kernel	Amygdalin	196-209	(Haque and Bradbury, 2002)
Cherry (*Prunus* spp.)	Amygdalin	4.6 (juice)	(Simeonova and Fishbein, 2004)
Bitter almond (*Prunus dulcis*)	Amygdalin	4700	(Shragg *et al.*, 1982)

While cyanogenic glycosides were determined in giant taro (*Alocasia macrorrhizos*), no information was located on the cyanogens content of common taro (*Colocasia esculenta*), the species eaten in New Zealand. No information could be found on the amygdalin content of pear seeds, however, given the close relationship of pears to apples, it seems reasonable to assume that pear seeds will also contain significant amounts of amygdalin.

Different bamboo species have different levels of cyanide with limited reports citing from 1000 to 8000 mg HCN/kg bamboo shoot (Haque and Bradbury, 2002; Simeonova and Fishbein, 2004). A concentration closer to 1000 mg/kg is considered more likely for the bamboo varieties normally eaten, although concentration decrease rapidly following harvest and the actual concentrations present in bamboo, as consumed, may be substantially lower (Haque and Bradbury, 2002).

Cassava (*Manihot esculenta*), also known as manioc, yucca and tapioca, is the most important cyanogenic food crop for humans, being a major source of dietary energy in many tropical regions (Davis, 1991). There are a number of varieties of cassava that range from low cyanide content, referred to as "sweet cassava" to higher cyanide content, known as "bitter cassava". During periods of drought the cyanide content of both sweet and bitter cassava varieties increases (Bokanga *et al.*, 1994). The major cyanoglycoside found in cassava is linamarin, with lesser amounts of lotaustralin, although cyanoglycoside concentration is usually expressed as HCN equivalents per kg cassava. Cassava is consumed in a number of forms: flour used for cooking; root slices; root chips; baked, fried, steamed or boiled grated root; steamed whole root; and tapioca pearls made as pudding and the cyanogens content decreases markedly with processing (Table 12).

Table 12: Total cyanogens content of cassava foods (mg HCN/kg)

Food type	Country of origin	Number of samples	Total cyanogens content	Reference
Peeled roots	Cameroon	36	197-951	(Agbor-Egbe and Lape Mbome, 2006)
Flour-	Mocambique	144	13 (±19)-76 (±39)	(FSANZ, 2004)
	Indonesia	59	5 (±4), 54 (±51)	(FSANZ, 2004)
	Tanzania	-	131 (±71)	(FSANZ, 2004)
	Central African Republic	-	32	(FSANZ, 2004)
"Baton de manioc" (steam cooked dough)	Cameroon	4	2.5-6.4	(Agbor-Egbe and Lape Mbome, 2006)
"Fufu" (boiled paste)	Cameroon	4	4.8-10.3	(Agbor-Egbe and Lape Mbome, 2006)
"Gari" (boiled grits)	Cameroon	4	1.5-2.8	(Agbor-Egbe and Lape Mbome, 2006)

9.3.3 Influence of storage, processing and cooking on cyanoglycosides content of foods

Cyanogen levels in cassava decrease markedly with soaking, mashing, grinding, sun drying and cooking- that might be steaming, boiling, frying or baking. Agbor-Egbe and Labe Mbome (2006) recently demonstrated reductions of 97-99% for three different cassava based foods across 4 different varieties (Table 13). Processing may include soaking for several days with approximately 10 fold reductions in cyanogens content when soaked for 48 compared with 24 hours.

Table 13: Changes in concentration of cyanogens between raw (peeled roots) and three cassava products ("Fufu", "Baton de manioc" and "Gari" (mg HCN/kg cassava)

	Variety 1	Variety 2	Variety 3	Variety 4
Peeled roots	449	667	951	655
Final product -"Fufu"	13	19	27	19
Peeled roots	511	248	197	511
Final product – "Baton de manioc"	6	3	3	6
Peeled roots	740	428	798	450
Final product - " Gari"	3	1	3	2

Adapted from Agbor-Egbe & Lape Mbome

The cyanide content in bamboo shoots decreases substantially following harvesting (FSANZ, 2004).

9.3.4 Estimates of dietary exposure to cyanoglycosides

Nine respondents in the 1997 New Zealand National Nutrition Survey ate between 40 and 704 g of cooked, boiled or baked cassava (Russell *et al.*, 1999). Cassava consumption is largely confined to the Polynesian populations of south and west Auckland and in the Wellington suburb of Porirua (FSANZ, 2004). The varieties of cassava available in New Zealand from the Pacific Islands have low cyanide contents. The lack of specific concentration data for cassava in New Zealand and the wide range of variability in cyanogen content makes it difficult to estimate dietary exposure for these individuals. Assuming the concentrations of 197-951 mg/kg found in peeled roots (Agbor-Egbe and Labe Mbome, 2006) for a high consumer of cassava (700 g/day) a worst case exposure is 140-670 mg/day. This may be toxic, but is most likely overestimated because it does not allow for reductions with processing. Using concentration values of 13-76 mg/kg for cassava flour (Table 12), exposure to cyanide, for a high consumer of cassava are in the order of 9-53 mg/day, below a lethal dose.

One respondent in the 1997 New Zealand National Nutrition Survey consumed 70 g cooked bamboo shoots (Russell *et al.*, 1999). Assuming a concentration of 1000 mg/kg, this equates to an exposure of 70 mg/day, not allowing for any decrease in cyanogen content with cooking.

No exposure estimates were found in the literature.

9.4 Summary

Cyanogenic glycosides present in human foods have caused adverse health effects, including death, in a number of documented cases overseas. There is some evidence of toxicity in New Zealand relating to the consumption of apricot kernels, leading to advice to limit consumption to one or two kernels per day.

Cyanogenic glycosides occur naturally in cassava, bamboo shoots, beans, and the seeds of many *Prunus* and *Malus* species, with cassava the predominant food source except for those

people who may consume apricot kernels for claimed health benefits. No information is available on the cyanogens content of the taro species consumed in New Zealand. Levels of cyanoglycosides are significantly reduced with processing but no concentration data exists for cassava or bamboo shoots as consumed by a small percentage of New Zealanders. There is evidence that a few individuals consume up to 700 g of cassava per day, with the potential for acute toxicity. A goitrogenic effect of chronic exposure to cyanoglycosides from cassava, is possible given the low iodine status in New Zealand, especially among the subpopulation of cassava consumers from the Pacific Islands.

10 TOXINS IN FOODS COMMONLY CONSUMED IN NEW ZEALAND

The toxins assessed in previous sections were selected through an initial screening exercise, on the basis that they may be present in a range of foods in the New Zealand diet. However, significant exposure to a natural toxin may result from consumption of a single food if that food is consumed in sufficiently large amounts. The following sections consider the 20 most consumed foods in the New Zealand diet, on a 'g/person/day' basis, and summarises information on any toxins that have been reported to occur in the food. Where the toxin has been dealt with in another section of this report the information will not be repeated here. Foods covered and their mean daily levels of consumption are given in

Table 14: Twenty most consumed foods in the New Zealand diet

Food	Mean consumption (g/person/day)	Food	Mean consumption (g/person/day)
Wheat	119.2	Pumpkin	12.4
Potatoes	117.9	Pears	12.0
Apples	58.2	Peas	11.0
Oranges	53.8	Cabbage	10.8
Tomatoes	46.4	Rice	10.2
Grapes	37.7*	Coffee	9.8
Bananas	32.3	Lemons, limes	8.4
Barley	23.6*	Sweetcorn	8.0
Carrots	19.5	Peaches	7.9
Onions	15.5	Cauliflower	7.7

* Estimates for consumption of grapes and barley include the equivalents of these commodities that are consumed in the form of wine and beer

10.1 Wheat (*Triticum aestivum*)

Wheat is consumed principally in the form of baked products, such as bread, biscuits and cakes, and as pasta. Wheat, and particularly the germ of wheat, contains potent amylase inhibitors (see section 5).

Wheat also contains a ribosome-inactivating protein (RIP), tritin (Coleman and Roberts, 1982). RIPs exert their toxicity by inhibiting protein synthesis in cells, by causing damage to the ribosome through an enzymatic (catalytic) mechanism (Stirpe, 2004). Tritin is a Type 1 RIP and, although no toxicological studies on tritin were found, Type 1 RIPs have been demonstrated to be of low mammalian toxicity (Battelli, 2004).

10.2 Potatoes (*Solanum tuberosum*)

The principal toxins associated with potatoes are the glycoalkaloids (see section 2), which may cause illness if levels increase due to exposure to light or other plant stress situations. Potatoes also contain caffeic acid (see section 3) and proteinase inhibitors (see section 4) although the activity of the inhibitors is rapidly destroyed by heating.

10.3 Apples (*Malus domestica*)

Apples contain caffeic acid (see section 3) and quercetin (see section 8). The seeds of the apple contain cyanoglycosides (amygdalin; see section 9), although apple seeds are generally not consumed.

10.4 Oranges (*Citrus sinensis*)

Oranges contain low levels of caffeic acid (see section 3).

10.5 Tomatoes (*Solanum lycopersicum*)

Tomatoes contain caffeic acid (see section 3), saponins (see section 7) and the glycoalkaloid, α-tomatine.

There have been no documented cases of human intoxication due to α-tomatine ingestion. Some studies have indicated that the toxicity of α-tomatine in experimental animals is similar to that of the potato glycoalkaloids (Morris and Lee, 1984), while other studies have show rodent toxicity, as indicated by LD_{50} values, to be 20 times lower than potato glycoalkaloids (Friedman, 2002). Inhibitory activity against bovine and human acetylcholinesterases is also lower for α-tomatine than α-solanine or α-chaconine (Friedman, 2002).

Levels of α-tomatine are high (up to 500 mg/kg) in immature green tomatoes, but are degraded to less than 5 mg/kg during the ripening process (Friedman, 2002).

10.6 Grapes (*Vitis spp.*)

Chlorogenic acid and caffeic acid are found in grapes and in wine produced from grapes (Hennig and Burkhardt, 1960) (see section 3).

10.7 Bananas (*Musa spp.*)

No reports were found of natural toxins occurring in the banana.

10.8 Barley (*Hordeum vulgare*)

Barley is most often consumed, after malting and brewing, as a component of beer. Barley (*Hordeum vulgare*) contains both proteinase (see section 4) and amylase inhibitors (see section 5). However, there is some evidence to suggest that these inhibitors survive the malting and brewing processes (Perrocheau *et al.*, 2005).

Barley contains a Type 1 RIP, however, there is no evidence that this toxin exerts any significant mammalian toxicity (Motto and Lupotto, 2004).

10.9 Carrots (*Daucus carota*)

Carrots contain moderate levels of caffeic acid (see section 3). Carrots may produce intensely bitter isocoumarins when subjected to stress and, particularly when exposed to ethylene (Talcott *et al.*, 2001). Bitterness has been particularly linked to one of these compounds; 6-

methoxy-8-hydroxy-3-methyl-3,4-dihydroisocoumarin, also know as 6-methoxymellein (Talcott *et al.*, 2001). Ethylene may accumulate when carrots are stored in poorly ventilated environments, such as refrigerated storage (Lafuente *et al.*, 1996). There are no verified cases of human intoxication due to carrot isocoumarins and the compounds have generally low toxicity to mammalian cells (Superchi *et al.*, 1993). It is probably that the intense bitterness of the isocoumarins would render carrots inedible at concentrations below those required to elicit toxicological responses in humans.

10.10 Onions (*Allium cepa*)

Onions contain relatively low levels of caffeic acid in the green leaves (see section 3). The bulb contains saponins (see section 7) and quercetin (see section 8).

10.11 Pumpkin (*Cucurbita spp.*)

No references were found to potentially toxic substances produced by the pumpkin. Intensely bitter chemicals, known as cucurbitacins are produced by fruit of cucurbit species. These compounds are able to cause serious gastrointestinal symptoms in humans (Cressey, 2003). The intense bitterness of these chemical has lead to the breeding of cultivars with negligible amounts of cucurbitacin, although occasional incidents associated with zucchini and squash have been reported (Kirschman and Suber, 1989; Rymal *et al.*, 1984). However, no reports were found of this problem in commercial pumpkin cultivars.

10.12 Pears (*Pyrus spp.*)

Pears contain significant amounts of caffeic acid (33-143 mg/kg; see section 3). The seeds of the pear probably contain cyanoglycosides (see section 9), although pear seeds are generally not consumed.

10.13 Peas (*Pisum sativum* L.)

Cultivated peas contain saponins (see section 7) and low levels of proteinase inhibitors (see section 4).

Consumption of grass pea (*Lathyrus sativus*) has caused a neurological condition known as lathyrism or neurolathyrism in humans (Yan *et al.*, 2006). The causative neurotoxin is a small non-protein amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP). ODAP is formed biosynthetically from its precursor, β-(isoazolin-5-on-2-yl)-L-alanine (BIA) (Kuo *et al.*, 1998). However, although BIA is produced by garden pea (*Pisum sativum*) seedlings, it does not appear to undergo metabolism to ODAP, as it does in *Lathyrus sativus* (Kuo *et al.*, 1998).

10.14 Cabbage (*Brassica oleracea*)

Cabbage contains caffeic acid (see section 3) and is a major dietary source of glucosinolates (see section 6).

10.15 Rice (*Oryza sativa*)

Rice contains Bowman-Birk-type proteinase inhibitors (see section 4) and Kunitz-type amylase inhibitors (see section 5).

10.16 Coffee (*Coffea spp.*)

Coffee is a rich source of caffeic acid (see section 3) and, in most cases, exposure to caffeic acid and its precursors will depend on the amount of coffee consumed.

Coffee contains significant levels of the alkaloids caffeine, theophylline and theobromine (Love, 1989). Caffeine levels are typically in the range 1.2-2.2% of green coffee, on a dry weight basis (IARC, 1991). Evidence for the toxicity of caffeine at dietary levels is equivocal. An extensive OECD review of toxicological information concluded that (OECD, 2002):

- Low doses stimulate the central nervous system, while high blood concentrations can result in restlessness, excitement, tremor, tinnitus, headache and insomnia.
- No association between moderate coffee/caffeine consumption and cardiovascular disease was seen in recent studies. Effects on cardiac rhythm are still under debate.
- There is inadequate evidence of carcinogenicity in humans.
- While moderate caffeine intake (equivalent to 3-4 cups/day of coffee) is unlikely to cause spontaneous abortions or reduced birth weights, associations between higher caffeine intakes and these toxicological endpoints cannot be excluded.

10.17 Lemons/limes (*Citrus x limon/ Citrus spp.*)

Both lemons and limes are moderate sources of caffeic acid (see section 3).

10.18 Sweetcorn (Hybridised form of *Zea mays*)

Corn contains amylase inhibitors active against insect amylases (see section 5). Corn also contains a Type 3 RIP (Motto and Lupotto, 2004), but there is no evidence that this possesses significant mammalian toxicity (Stirpe, 2004). The corn enzyme is unusual in being synthesised as a pro-enzyme in the seed, which becomes activated during germination through proteolysis of acidic residues (Krawetz and Boston, 2000). Corn for human consumption is harvested and processed prior to germination.

10.19 Peaches (*Prunus persica*)

Peaches contain significant amounts of caffeic acid (see section 3). The stone of the peach contain cyanoglycosides (see section 9), although peach stones are generally not consumed.

10.20 Cauliflower (*Brassica oleracea*)

Cauliflower contains caffeic acid (see section 3) and glucosinolates (see section 6).

11 RANKING RISKS ASSOCIATED WITH DIETARY EXPOSURE TO NATURAL TOXINS FROM CROPS IN NEW ZEALAND

While it is usually not possible to attribute individual cases of human disease resulting from natural toxins in New Zealand crop plants, information is available from which to consider the relative importance of risks due to various natural toxins in New Zealand. Such a ranking exercise needs to consider:

- The likely adverse health effects due to exposure to each toxin, and the seriousness of those effects.
- The weight of evidence for a causative role of the toxin in the adverse health effect observed.
- The exposure level or dose at which the toxin can exert its toxic effects. A surrogate for this exposure level is the tolerable daily intake or equivalent for each toxin.
- The proximity of New Zealand exposure estimates to tolerable limits and the availability of sufficient data to make those estimates realistic. This would need to include information on imported foods.

In general, this study has encountered difficulty in establishing suitable reference exposure levels to assess the toxicological significance of dietary exposure to many of the natural toxins investigated, while for some of the natural toxins considered no estimate of the likely level of exposure in New Zealand was available. Because of these data deficiencies, natural toxins included in this study have been primarily ranked on the basis of the weight of evidence for a relationship between dietary exposure and a human disease state. A secondary ranking has been applied, based on the seriousness of potential health outcomes, with toxins capable of causing fatalities or chronic disease rated a greater risk than those causing non-fatal transitory disease.

The weight of evidence approach for assessing a causative relationship between human exposure and disease states tends to favour situations of overt acute toxicity and the evidence linking any of the investigated natural toxins to chronic diseases is generally weak. Although this has resulted in toxins linked to chronic disease states being ranked lower than those linked to acute diseases, it should be stressed that the ranking is based on information **currently available** and may change if new information emerges.

Table 15 summarises information reviewed in this risk profile relevant to risk ranking.

While the information in Table 15 contains considerable uncertainties and data gaps the following general comments can be made:

- There are little or no human data supporting the toxicity of caffeic acid, glucosinolates, saponins and quercetin. There is some evidence to suggest beneficial effects from these compounds at dietary levels of exposure, while adverse effects may occur at levels of exposure well above normal dietary levels.
- There are no human data to support the toxicity of proteinase and amylase inhibitors. Forms of foods causing problems in animals (raw soy meal) are not relevant to human diets and enzymes will often be inactivated by normal food processing. These compounds are also being investigated for potentially beneficial therapeutic purposes.
- Potato glycoalkaloids and cucurbitacins may cause adverse health effects in humans under certain environmental circumstances. These circumstances are understood for potatoes (physical injury, plant stress, exposure to light), but not for cucurbits. The

extreme bitterness of cucurbitacin-containing foods is usually considered to be a barrier to widespread or prolonged incidents of intoxication.

- Plant products containing cyanogenic glycosides are capable of causing serious cases of cyanide poisoning. However, the main cyanogen-containing foods are not widely consumed in New Zealand, although their consumption may be increasing.
- Xenoestrogens have been implicated in a range of cancers and non-cancer conditions relating to development of the reproductive organs. However, correlative epidemiological studies have generally focused on synthetic xenoestrogens, rather than phytoestrogens. Some phytoestrogens have also been promoted as having positive health effects. Evidence is currently insufficient to establish the human toxicity of phytoestrogens.
- There are little or no human data supporting the toxicity of additional toxins mentioned under Section 10 (Type 1 and Type 3 RIPs, isocoumarins, caffeine, α-tomatine).

Based on these conclusions, potato glycoalkaloids and cyanogenic glycosides appear to be the toxins associated with cultivated plants consumed in New Zealand with the greatest potential to cause adverse health effects.

Table 15: Risk ranking information for natural crop toxins in the New Zealand food supply (toxins ranked in order of descending assessed risk)

Toxin	Human health effects	Animal health effects	Weight of evidence[#]	Critical exposure limit (mg/kg body weight/day)	New Zealand dietary exposure (mg/kg body weight/day)	Major contributing foods
Glycoalkaloids	Gastrointestinal and neurological symptoms		High	2	<0.2*	Potatoes
Cyanoglycosides	Acute cyanide poisoning, thyroid effects		High		*	Cassava, bamboo, fruit seeds
Cucurbitacins	Gastrointestinal effects		Medium-High			Zucchini, some squash species
Phytoestrogens	Implicated in the aetiology various cancers and conditions of the reproductive organs		Low-medium			Legumes, fruit, vegetables
Saponins	Hypertension	Haemolysis, increased membrane permeability	Low-medium		0.2-2.9	Legumes, alliums, various other plant products
Glucosinolates		Goitrogenic and carcinogenic effects	Low		0.2	Cruciferous vegetables
Caffeic acid		Kidney and forestomach hyperplasia and	Low		0.7-0.9	Coffee, various fruits and vegetables

	Human health effects	Animal health effects	Weight of evidence[#]	Critical exposure limit (mg/kg body weight/day)	New Zealand dietary exposure (mg/kg body weight/day)	Major contributing foods
		tumours				
Quercetin		Intestinal, bladder and renal tumours	Low		0.1-0.16	Various fruits and vegetables
Proteinase inhibitors		Depressed growth rate, pancreatic damage, including pancreatic cancer	Low			Potatoes, other vegetables
Amylase inhibitors			Low			Cereals

The weight of evidence assessment is subjective, but is based on the strength and consistency of associations between toxin exposure and specified human disease. The consistency between human and animal disease is also considered. A definitive cause and effect relationship between the toxin and human disease (e.g. cyanide poisoning) is classified as a high weight of evidence, while failure to identify a human disease state (e.g. amylase inhibitors) is classified as a low weight of evidence.

* Adverse health effects are acute and the normal level of exposure has little significance.

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