GLUCOSINOLATES - A LITERATURE REVIEW

Ian I. Onaga[1]

ABSTRACT

Glucosinolates are syntesised in dicotyledonous plants, especially the order of Capparales; families Resedaceae, Capparidaceae, Caricaceae, Euphorbiaceae, Gyrostemonaceae, Limnanthaceae, Moringgaceae, Salvadorraceae, Toviraceae, Tropaeolaceae and Cruciferae plants' genera Thilasp, Cochlearia, Sisymbrium, Sinapis, Raphanus, Descurainia, Stanleya and Brassica, which includes kale, rape, turnips and Swedes are fed to livestock. Roots and leaves of forage Brassica crops are rich in nutrients for finishing lambs and cattle. Glucosinolates and S-methyl cysteine sulphoxide (SMCO) concentrate in seeds and vegetative tissues. Effect of SMCO is aggravated by the presence of nitriles that deplete glutathione leading SMCO to cause haemolytic anaemia in ruminants. Toxic effects of glucosinolates on the production performance of animals include changes in productive characteristics such as growth rate, egg and milk production, general animal performance including reproduction and weight performance. Adaptive function of glucosinolates associate with protection against herbivore and also influences the degree of herbivory of phytophagous insects. Hhydrolysed products may have a role in pathogen resistance, especially anti-fungal and anti-microbial properties and may act as phago-stimulants in which certain Cruciferae could be stimulate and attract certain species of insects. Breakdown of glucosinolate following their absorption from the digestive tract of animal species need more research work to improve the isolation techniques and isotope labelling. Administration of pure compounds in laboratory animals has proved useful and clarified excretory routes of mercapturic acid derivatives of isothiocyanates in the urine.

Keywords: *Capparales, Cruciferae plants, Brassica, glucosinolates, S-methyl cysteine sulphoxide (SMCO), toxins, allyl isothiocyante, allyl cyanide, thioglucosidas, mercapturic acd, hydorlysis, metabolites, detoxification, in-vitro, in-vivo, zenobiotic, high performance liquid chromatography (HPLC) and enzyme-linked-immunosorbent assay (ELISA)*

INTRODUCTION

Glucosinolates occur in dicotyledonous plants and exist as thioglucosides or sulfur containing glycosides (Duncan and Milne 1989; Tiedink *et al* 1991; Clarke and Clarke 1975; Palmieri *et al* 1986). They are found among the order of Capparales, in the families of Resedaceae, Capparidaceae, Caricaceae, Euphorbiaceae, Gyrostemonaceae, Limnanthaceae, Moringgaceae, Salvadorraceae, Toviraceae, Tropaeolaceae and Cruciferae (Heaney and Fenwick 1980b; Larsen 1981). Cruciferae plants of the genera *Thilasp, Cochlearia, Sisymbrium, Sinapis, Raphanus, Descurainia, Stanleya and Brassica* have been found to contain glucosinolate toxins (Smith and Dacombe 1987). The genus *Brassica* includes kale, rape, turnips and Swedes, which are fed to livestock with potentially harmful consequences (Clarke and Clarke 1975). Forage *Brassica* crops, particularly the roots and leaves are valued as a rich source of nutrients for finishing lambs and cattle at a time of year when pasture is declining or unavailable (Duncan and Milne 1989; Tiedink *et al* 1991). Glucosinolates are well concentrated particularly in the seeds and the vegetative tissues (Heaney and Fenwick 1980a). Some of the R-groups are alkyl, alkenyl, aryl, indole groups, methyl, thiol and hydroxyl groups (Rodman 1978).

BIOSYNTHESIS AND DISTRIBUTION OF GLUCOSINOLATES

Glucosinolates are synthesised in the *Brassica* plants from amino acid precursors. For example, indole glucosinolates are produced from tryptophan while benzyl glucosinolate and hydroxybenzyl glucosinolate are produced from phenylalanine and tyrosine respectively (Underhill 1980). The distribution of amino transferase within the plant was found to be highly correlated with prop-2-enyl glucosinolate concentration in different parts of *Brassica carinata* (Duncan and Milne 1989) and in *Brassica juncea* glycosylation and sulphation steps in glucosinolate bioactivity. The activity of these

[1]Animal & Plant Technical Advisory Services, Department of Agriculture and Livestock, PO Box 2141, BOROKO

enzymes correlates well with glucosinolate concentrations in the glucosinolate biosynthesis (Duncan and Milne 1989) and localised in the vacuoles of cells (Grob and Matile 1980a)

The toxins identified in *Brassica* species include the glucosinolates and S-methyl cysteine sulphoxide (SMCO), which are hydrolysed to dimethyl disulphide (Smith 1974). Smith and Szabo *et al.* (1977) noted that the effect of SMCO is aggravated by the presence of nitriles that deplete glutathione leading SMCO to cause haemolytic anaemia in ruminants.

The toxic effects of glucosinolates on the production performance of animals have been noted in the context of the feeding of glucosinolate-containing rapeseed to farm animals. The effects include changes in productive characteristics such as growth rate, egg and milk production (Bell *et al* 1971; Lo and Bell 1972; Wight *et al* 1987), general animal performance including reproduction and weight performance.

The concentrations of glucosinolates vary within the family Cruciferae plants, and ihe genus *Brassica*. The highest glucosinolate concentrations tend to occur in rapidly growing young *Brassica* plant parts, such as shoot, root tips and seeds (Palmieri *et al* 1986). This may be associated with a defence mechanism against damage by herbivores (Klingauf *et al.* 1972; Nault and Styer 1972; Greenhalgh and Mitchal 1976; Hardman and Ellis 1978). It is known that the toxic effects of glucosinolates include goiter, damage to liver and kidney tissues, and the toxic compounds responsible for these effects could be due to 5-vinyl-2-oxazolidinethione (5-OZT) (Elfvinging 1980) and other breakdown products.

All over the world man has been consuming significant amounts of glucosinolates, by eating large amount of cabbage, broccoli, cauliflower, brussels sprout, mustard or horseradish on regular basis (Albert 1987; Tiedink *et al.* 1991). Once these Crucifers are consumed, the glucosinolates are hydrolysed enzymatically, during the preparation for the table or within the stomach after ingestion. There is not much information on the effects of food processing on glucosinolates content and the breakdown products arising following cooking. However, Slominski and Campbell investigated that heat treatment including steaming and cooking resulted in substantial decomposition of indole glucosinolates with thiocyanate ion and indoleacetonitriles accounting for 50% and 30% respectively. Autolysis of indole glucosinolates in raw *Brassica* vegetables resulted in the production of little or no indoleacetonitrils but produced substantial thiocyanate ion and related compounds. The anti-carcinogenic properties of some indoles and isothiocyanates and other glucosinolates derived compounds reacting with nitriles are among the potential positive effects of glucosinolates (Duncan and Milne 1989). In the areas where *Brassica* plants contribute heavily to the cattle fodder, Heaney and Fenwick had reported that the ionic metabolite of thiocyanate ion can be transferred to humans through milk. They also considered that this may be partly responsible for the development of goiter carcinogen when human beings consume raw cabbage to prevent cancer development (Albert 1987).

FUNCTIONS OF GLUCOSINOLATES IN *BRASSICA* PLANTS AND METABOLIC FATE

The function of glucosinolates in *Brassica* plant metabolism is not entirely clear. Their rapid turnover within the plant tissues with the associated metabolic costs indicates an adaptive function, to protect against herbivore damage. This seems analogous to other secondary compounds in other plants (Klingauf *et al.* 1972; Nault and Styer 1972; Hardman and Ellis 1978). An important area of research is the study of the mechanism of protection against insect herbivory, in which the glucosinolate content of various plant species has been shown to affect larval development and pupation. This also influences the degree of herbivory of phytophagous insects (Klingauf *et al.* 1972). Therefore glucosinolate breakdown products may have a role in pathogen resistance, especially those that have been shown to have anti-fungal and anti-microbial properties (Duncan and Milne 1989). Glucosinolates may also act as phago-stimulants in which the certain Cruciferae could be stimulatory and attractive to certain species of insects (Nielsen *et al.* 1979).

The fate of specific glucosinolate breakdown products, following their absorption from the digestive tract of animal species has been less researched, particularly their degradation process. This may be attributed to analytical problems, particularly in determining the hydrolysed products in the digestive fluid. However, the possibility would be that, the isolation techniques and isotope labelling could be improved, thus enabling analysis to be more specific. The digestive fate of glucosinolates in poultry fed rapeseed have been researched extensively, especially the production of 5-OZT and nitrile hydrolysis from progoitrin (Smith and Campbell 1976). The recent studies of glucosinolate recovery in the faeces and urine samples of hens were in the range of 15 – 50 %,

while the un-recovered fraction may have undergone hydrolysis in the digestive tract (Slominski *et al.* 1987; Slominski and Campbell 1988). In hens, caecectomy and addition of antibiotics in the diets increased the hydrolysis by the hind-gut micro-organisms (Freig *et al.* 1987; Slominski and Campbell 1988). About 1 – 2% recovery of intact glucosinolates were seen in the faeces from rats following rapeseed feeding (Marangos and Hill 1974). Low concentrations of 5-OZT were determined in the gut contents of rats and there has been lots of research carried out on 5-OZT with its effect by various authors (Langer and Michajlovskij 1969; Peltola and Krusius 1971; Elfving 1980).

The detection of other hydrolytic products of glucosinolates in the digestive fluids of rats has been difficult (Lo and Hill 1971; VanEtten and Daxenbichler 1977). Administration of pure compounds in laboratory animals has proved useful and clarified excretory routes of mercapturic acid derivatives of isothiocyanates in the urine. However species differences exist in excreting the mercapturic acid (Brusewitz *et al.* 1977; Gorler *et al.* 1982).

GLUCOSINOLATE HYDROLYSIS, METABOLITES AND FACTORS AFFECTING HYDROLYSIS

Glucosinolates are normally associated in plants with the enzyme thioglucosidase (myrosinase). Hydrolysis takes place under the action of myrosinase (Heaney and Fenwick 1980b; Palmieri *et al.* 1986), which catalyses the cleavage of the thioglucoside bond of glucosinolates (Heaney and Fenwick 1980a; Duncan and Milne 1989; Tiedink *et al.* 1991). Once the cellular structure in the *Brassica* plant is disrupted, glucosinolates are broken down by myrosinase to various metabolites including free glucose (Heaney and Fenwick 1980a; Duncan and Milne 1989) and an aglucone intermediate. This is then degraded spontaneously to one of a number of toxic metabolites. The common metabolites normally produced from enzymic hydrolysis are the volatile isothiocyanates, thiocyanate ion and nitriles (Duncan and Milne 1989; Tiedink *et al.* 1991; Duncan and Milne 1992a) while the others are sulphate, hydroxynitriles and hydroxyepithionitriles However different metabolites are formed depending on the conditions during the hydrolysis. Likewise figure IIa and IIb may be seen as possible routes for cysteine conjugate of benzyl isothiocyanate and hydrolysis to form allyl mercaturic acids (AMA) via aglucone.

The production of metabolites following glucosinolates hydrolysis is influenced by factors such as the presence of various protein cofactors, temperature, metallic ion concentrations and pH (Duncan and Milne 1989; Duncan and Milne 1993). These conditions may interact at complex ways to form toxic products such as aglucone, arising from hydrolysis. An aglucone is rearranged to form cyanoepithioalkane by protein co-factor at the expense of aliphatic nitrile. Temperature has an indirect effect associated with the denaturing of certain heat-labile factors involved in glucosinolates hydrolysis, such as epithiospecifier protein (Tookey 1973), however, Gil and MacLeod (1980) have identified that temperature has little effect on the proportions of glucosinolate hydrolysis. The presence of other compounds such as ferrous ions (Fe^{++} and Fe^{+++}) and copper ion (cu_2^{+}) also influences glucosinolate breakdown and consequently may alter their toxicity. Thiol compounds such as cysteine and glutathione increase the action of ferrous ions to favour nitrile production. Also ferrous ions and pH interactions increase the glucosinolate hydrolysis; low and high pH favour nitrile and isothiocyanate production respectively (VanEtten *et al.* 1966; Tookey and Wolff 1970; Uda *et al.* 1986). Glucosinolate hydrolysis is a complex process with the range of products formed depending on the factors discussed above. This of course, is influenced by the conditions in the digestive tract producing toxic products when plants are ingested by the animals. This can be seen with pH, which is low in the monogastric stomach, but higher in rumen of ruminants and this will influence glusosinolate hydrolysis (Duncan and Milne 1989). There has been some evidence that addition of mercaptoethanol to the hydrolysis medium influences glucosinolate hydrolysis; sinigrin is hydrolysed to epithioalkanes and allyl cyanide at the expense of isothiocyanate production. The mechanism is that the mercaptoethanol activates epithiospecifier protein (Duncan and Milne 1989).

Thiocyanate ion is a known myrosinase induced hydrolysed product of indole glucosinolates and may catalyze the nitrosation reaction (Fenwick *et al.* 1983). Indolyl glucosinolates yield indole compounds, such as indole-3-carbinol, indole-3-acetonitrile, di-indolylmethane and ascorbigen (Tiedink *et al.* 1991). Other compounds such as cyanoepithioalkanes and organic thiocyanate are produced from aliphatic nitriles. Also progoitrin, with its beta-position side chain hydroxy group forms less volatile oxazolidine-2-thiones and epithionitriles (Gil and MacLead 1980; Elfving 1980; Hassan *et al.* 1988; Tiedink *et al.* 1991).

Tiedink *et al.* (1991) investigated the association of sinigrin and some of the glucosinolates in forming N-nitroso compounds following nitrosation. The results showed positive response of hydrolysis occurring in the presence of myrosinase and acidic conditions. However the authors were not convinced of their work, because there was no correlation with the previous work of formation of direct mutagenic N-nitroso compounds in vegetable extracts. Thus, the chemical natures of the precursor of N-nitroso compounds in cruciferous vegetables and biologically active compounds needed further investigation. The activity of various enzymes is affected by the glucosinolate metabolites such as benzyl isothiocyanate, indole-3-carbinol and indole acetonitrile and they may then protect against potential carcinogens (Albert 1987; Duncan and Milne 1989).

In addition to enzymic breakdown, Tiedink *et al.* (1991) reported that glucosinolates undergo chemical hydrolysis although the reaction was found to be very slow. The low pH in the stomach could be responsible for the chemical hydrolysis. The ability of intact and hydrolysed glucosinolates, particularly glucobrassicin and 4-hydroxybrassicin to form N-nitroso compounds showed positive results.

ABSORPTION, STORAGE AND EXCRETION OF GLUCOSINOLATE PRODUCTS

There is no available information regarding the detection of the hydrolytic products of glucosinolates in the blood and tissues of ruminants and monogastrics fed with rapeseed. The detection of glucosinolates in their original form in the gut is also unknown, although post-absorption of the metabolic process of transforming xenobiotics of foreign compounds into excretable compounds is known (Kamrin 1988). Studies performed on laboratory animals, such as rats have proved useful in identifying the excretory routes of the metabolic products. For example, aliphatic isothiocyanates induce glutathione conjugation in the liver as the major excretory route. However, dogs have been found to excrete hippuric acid derivatives while guinea pigs and rabbits excrete cyclic mercapturic acid derivatives, and mice excrete other metabolites as well as mercapturic acid derivatives (Brusewitz *et al.* 1977; Gorler *et al.* 1982). The metabolic fate of glucosinolate-derived nitriles needs further research, though similar aliphatic nitriles have indicated of their likely mode of catabolism. Administration of nitriles increases the urinary thiocyanate ion excretion, due to free cyanide release via hydrolysis in the tissue and then the conventional thiocyanate (SCN) excretion. Early experiments conducted on nitrile toxicity in rodents suggested the compounds are potentially toxic (VanEtten *et al.* 1969; Nishie and Daxenbichler 1980) following the administration of acrylonitrile to rats and observed the excretion of urinary mercapturic acid derivatives. Other experiments suggest that a number of proposed catabolic routes for nitriles can be either direct or indirect conjugation to an epoxide intermediate.

GLUCOSINOLATE TOXICITY AND ITS EFFECTS

Glucosinolate toxicity may occur mostly on a chronic basis while the animals are grazing in paddocks continuously over along period of time. Chronic toxicity effects may occur if the animals graze on the *Brassica* forages or ingest feed such as rapeseed that contains glucosinolates (Kamrin 1988). However, little is known about the digestive fate of glucosinolates in ruminants *in-vivo* (Duncan and Milne 1992a).

Glucosinolate hydrolysis is considered important in identifying the effects of its toxicity. The rapeseed, which is a protein-rich meal contain glucosinolate and is normally fed to farm animals (Palmieri *et al.* 1986). The main production performance problem associated with feeding of rapeseed meal is the presence of glucosinolates, which not pleasant enough to taste due to the toxic effects. These toxic effects have been considered a danger to farm animals and the plant breeders have made lots of progress in breeding programmes to reduce the levels of glucosinolates and erucic acid, which reduces the oil quality in rapeseed (English *et al.* 1988). The effects of rapeseed meal when fed to farm animals show that the productive characteristics affect the growth, egg and milk production (McDonald *et al.* 1992).

The simplest techniques of assessing the toxicity of glucosinolates are by correlating with the amount of glucosinolates present in the diet. But the hydrolysis of glucosinolate is important in altering their toxicity so the processing of diet in various ways to favour the toxic metabolites may influence the resulting toxicity. For example, controlling the hydrolysis of glucosinolates in rapeseed meal fed to rats and chicken may influence the toxicity. Rapeseed meal, which also is rich in nitrile products and 5-vinyl-2-oxazolidinethione (5-OZT) (Duncan and Milne 1989; McDonald *et al.* 1992) causes reduction in live-weight gain. Canola seed which is high in fat and protein, but low in glucosinolates depressed the feed intake and weight gain in pigs and feed containing Canola seed was not utilized efficiently (Cromwell *et al.* 1989). Underlying effects

were kidney enlargement and increase in thyroid weight (Dierschke 1980; Albert 1987). This may have resulted from the presence of 5-vinyl oxazolidinethione (Palmieri *et al* 1986; Duncan and Milne 1989; Duncan and Milne 1992a) and following the hydrolysis of 2-hydroxy-3-butenyl glucosinolate. This may also cause liver damage. Liver haemorrhage may also relate to the presence of glucosinolates in the diet but there seems to be no correlation with the particular metabolites. The cellular damage occurs in the liver and the kidney tissues of rats following ingestion of nitriles possibly as a result of free cyanide release in liver and brain tissue (Nishie and Daxenbichler 1980; Willhite and Smith 1981). The presence of aliphatic nitriles as toxins may inhibit cytochrome oxidase activity and may form thiocyanate ions (Ahmad and Farooqui 1982). Thiocyanate ions may cause a direct goitrogenic effect (McDonald *et al.* 1992; Duncan and Milne 1992a). The metabolites formed from hydrolysis of glucobrassicin can inhibit the neoplastic effects of carcinogens (Tiedink *et al.* 1991).

Glucosinolates toxicity may involve glucosinolate extracts from glucosinolate free diets, which are fed to rats had problem of productive characteristics. Rats dosed with butenyl cynide prepared from rapeseed showed depressed live-weight gain (Sirvastava *et al.* 1975). Allyl isothiocynate (AITIC) and other isothiocynates are well documented electrophilic compounds, which are very reactive and this may underlie toxic nature due to molecular polarity. However, this evidence is based on a limited number of experiments. It was found that AITIC dosing increase plasma phospholid concentrations in rats (Muztar *et al.* 1979a) and this work was confirmed by Idris and Ahmad (1975), also both authors have found to reduced the plasma glucose and uric concentrations (Idris and Ahmad 1975; Muztar *et al.* 1979b). AITC dosed rats appear with hypothyroidism during carbohydrate metabolism, following the altered activity of liver succinic dehydrogenase and kidney xanthine oxidase (Ahmad *et al.* 1967). Rats dosed with also inactivated the antidiuretic hormone and addition of AITC on to the sulphydry group of tyrosine may reduce thyroid hormone synthesis. Phenyl isothiocyanate also affects the presence of iodine and plasma thyroxin (T_4). Other AITC reactions, which are important biologically, are unclear (Duncan and Milne 1989). In an *in-vitro* experiment, proton-potassium adenosine triphosphatase has been seen to be inhibited by the presence of AITC (Takeguchi *et al.* 1983). Similarly a number of *in-vitro* and *in-vivo* experiments demonstrated the effects on thyroid function (Muztar *et al.* 1979b). These effects showed that there were potential toxic actions of isothiocyanate as a result of sulphydryl groups. Benzyl isothiocyanate cleaved the disulphide bonds of important proteins, which led to toxic actions (Tang 1974).

In-vitro and *in-situ* trials carried out with sheep in Russia and had found that neutral detergent fibre disappeared much faster with high glucosinolate than with low glucosinolate containing substrates. The authors argued that the high glucosinolates in the forage did not interrupt the fermentation and was infact more degradable than low glucosinolate diet. In a growth trial, fresh forage with low and high glucosinolates fed to lambs, and it was found that there was an improved growth performance in high glucosinolate fed lambs than low glucosinolate fed lambs. This experiment suggested that ruminal digestive function may tolerate a high level of dietary glucosinolate (Pearce *et al.* 1989). However this level of glucosinolate may exist where digestive function is compromised without noticeable toxicity symptoms in the animals. Duncan and Milne (1992a), reported the growth of sheep fed with *Brassica* crops was low, though the crops were highly digestible. But the *Brassica* forage seed had an adverse effect on the animals and resulted in low voluntary forage intake, due to the presence of glucosinolate formaing toxic compounds (Duncan and Milne 1992a). Voluntary food intake (VFI) was depressed by the administration of allyl cyanide (ACN), but the blood gluthathione concentration and plasma urea were not affected, while the plasma creatinine varied in the concentration depending on the treatment (Duncan and Milne 1992a). The treatment also affected the concentration of plasma gamma-glutamyltrans-peptdase. The kidney cytochrome oxidase activity, a terminal enzyme involved in the electron transport chain, was not affected (Duncan and Milne 1992a).

In a subsequent experiment Duncan and Milne (1992a) investigated the rumen microbial degradation of ACN in sheep fed with chopped cabbage, *Brassica oleraccea va capitata* and dried grass pellets. Rumen fluid analysis suggested that in animals fed with grass pellets, the ACN was stable, in contrast to rapid decline in ACN concentration in animals fed with cabbage.

Indolyl glucosinolates may inhibit the neoplastic effects of carcinogens. Besides the anti-carcinogenic effects of indole-3-acetonitile, the compound is reported to be a precursor of N-nitroso compound, which have been identified from nitrile treated Chinese cabbage. Although indole-3-acetonitrile is reported to be anti-carcinogenic, the nitrosation seemed to initiate and promote tumour in rats, however the effects of such compounds in

man are not known (Tiedink *et al.* 1991). These authors also reported that indole compounds are mutagenic to bacteria, after the nitrite treatment and its rapid reaction endogenously.

The plants mentioned in the introduction can cause haematuria in dairy cattle, particularly by the poisonous AITC (Albert 1987), though not many animal deaths have been reported. Death may occur due to exhaustion, resulting from intensive irritation of the alimentary tract (Clarke and Clarke 1975).

Brassica species such as *Sisymbrium irio* present in United Kingdom (UK) do not seem to poison the chickens, pigs, cattle, sheep and horses (Clarke and Clarke 1975). However, cattle in Argentina have died of gastroenteritis after the consumption of *Sisymbrium irio*. In France and Western Australia, fatal poisoning in lambs and cattle have occurred because animals were allowed to grazed on the field covered with wild radish, in which the flowers were in advanced stage. In America, the adverse effect of ingesting *Descurainia pinata* had caused blindness and paralysis of tongue (Clarke and Clarke 1975). Also the inclusion of rapeseed meal in the diet of growing chickens and pigs have reduced or inhibited the growth rate and increased the weight of liver, kidney and thyroid gland (Wight *et al.* 1987).

The thyroid gland problem can be caused by the thiocyanate ion, which is a goitrogen causing hypothyroidism. Hypothyroidism occurs as a result of hyperplasia, prevents the thyroid gland from taking up the iodide ion and inhibits the iodination of tyrosine (McDonald *et al.* 1992), the precursor of thyroxin, to produce thyroid hormones. Most goitrogenic effects have been experimented on rabbits, but goitre is noticeable in human as "big neck" (Dierschke 1980; Robinson 1980; Albert 1987). The goitrogenic effect in lambs can be prevented by intramuscular injection of iodine (Clarke and Clarke 1975). Supplying of adequate iodine to animal diet which are exposed to some form of iodine deficiency may also prevent goitrogenic effect (McDonald *et al.* 1992). Despite the discovery of goitrogenic activity in cabbage and other plants of the *Brassica* family, the chemical agents responsible for this biological effect have not been pinpointed whether the presence of such an effects exists. It has been proved on rats and guinea pigs that cabbage consumption has a marked goitrogenic potency, through significant increase in the thyroid weight (Langer and Stolc 1965). However the total iodine content in the thyroid was not significantly altered, but the serum protein-bound iodine (PBI) was significantly depressed (Langer and Stolc 1965). Different authors have reported the effect on iodine uptake by the thyroid hormone (Langer and Michajlovskij 1969; Marangos and Hill 1974; Akiba and Matsumoto 1976; Elfving 1980). Attempts also have been made to counter-act the growth depressing effect by processing and the extraction of dietary supplements to reduce the glucosinolate levels.

The use of rapeseed meals, *Brassica napus* for pigs and poultry are restricted in the diet because of the presence of thiocyanate and 5-vinyloxazlidine-2-thione (McDonald *et al.* 1992). In Canada, careful selection of *Brassica campestris* has resulted in obtaining low contents of glucosinolates and erurcic acid, which causes heart lesions in experimental animals. The meals such as Canola produced from this careful selection technique are being used widely (English *et al.* 1988; McDonald *et al.* 1992). The toxicity of glucosinolates can be denatured by cooking or heating, which greatly reduces or inactivates the goitrogenic potency of the plant (Slominski and Campbell 1989; Dietz *et al.* 1991; McDonald *et al.* 1992). However, in autolysis of raw *Brassica* vegetables substantial quantities of thiocyanate ion and related compounds (indolemethanols) can be experienced. It may be said that the anticarcinogenic properties of *Brassica* vegetables depend on the method of preparation (Slominski and Campbell 1989).

Rats dosed with glucosinolates, such as allyl isothiocyanate have been noted to develop vesication and slow healing of ulcers, and the vapours are harmfull to lungs. Ingestion of large doses causes severe gastrointestinal inflamation and my result in circulatory collapse and death (Ahmad *et al.* 1967).

In stomach intubation of 1-cyano-3,4-epithiobutane (CEB), rats showed symptoms of strong vocalization (make noise) to touch, lost right reflex action, and muscle tone. Administration of CEB and 4-pentenenitrile by stomach intubation showed a significant retardation in weight gain (Dietz *et al.* 1991). These symptoms were further examined on histopathological assessment with a mild to moderate periacinar necrosis and congestion in liver. The kidney had tubular degeneration from terminal hypoxia, a result of CEB administration. This investigation confirmed the acute toxicity of epithionitriles. LD50 of 3-hydroxy-4-pentenenitrile administered by stomach intubation lead to a loss of right reflex and intermittent drooling seizers followed by death (Nishie and Daxenbichler 1980).

DETOXIFICATION

The importance of understanding the metabolic fate and potential excretary routes of glucosinolates when ingested through different diets would allow the understanding of the potential of the toxicity in the animal species. However, research in this area has been rather limited and the knowledge of the digestive fate of glucosinolates is very poor. The systemic fate of glucosinolate hydrolysis products show that the chemical nature had helped to provide metabolic routes and detoxification processes.

The metabolic fate of glucosinolate hydrolytic products following absorption are well known, however attempt to detect hydrolytic products of glucosinolates in the blood and tissues of animals fed on rapeseed have been fruitless.

Different species of animals have excreted different levels of mercapturic acids in urine based on the effective detoxification system in an animal (Gorler *et al.* 1982). Glutathione conjugation of isothiocyanates especially conjucgation of benzyl isothiocyante (Bruggemann *et al.* 1986) have been studied *in vitro* experiments and *in vivo* with rats fed on Brussels sprouts (Godlewski *et al* 1985) and cabbage (Stoewsand *et al.* 1986). While the metabloic fate of glucosinolates derived nitriles has the chemical nature of aliphatic nitriles such as acrylonitrile in catabolism had been known due to the nature of compound in the manufacture of plastics (Szabo *et al.* 1977; Langvardt *et al* 1980) and metabolism of similar unsaturated nitriles such as allyal cyanide (Willhite and Smith 1981). The nitrile metabolism experiments in urinary thiocyanate ion excretion has been continuously been studied, especially in the areas of conventional excretion of cyanide as SCN.

The complexity of xenobiotic metabolism has been highlighted following the consumption of the certain cruciferous vegetables, especially the beneficial effect of inhibiting the tumour formation (Wattenberg 1977). Similar situation of interactions in the metabolism of other xenobiotics may exist and have significant relationship to the overall effects of the anti-nutritive factors of the Cruciferae.

ANALYTICAL METHODS

A wide range of analytical methods are being applied to analyse glucosinolate metabolites as a means of screeing glucosinolates hydrolysis. These analytical procedures can quantify both the individual glucosinolates and the total concentration of glucosinolates. The determination of individual glucosinolate is exclusively performed by chromatographic methods and lots of improvements have been made to obtain high resolution and quantification over the years. Early methods included paper chromatography, which identified the thiourea derivatives of isothiocyanates in the chemo-taxonomic studies, but linked into quantitative analysis (Larsen 1981). The quantitative analysis of glucosinolates is performed by using gas liquid chromatography (GLC). Originally this method was used for separating hydrolysis products, after autolysis with exogenous myrosinase (Daxenbichler *et al.* 1970; Daxenbichler *et al.* 1977; Macleod *et al.* 1978). But now, this method is identified to suit the volatile nature of glucosinolate metabolites with high resolution during separation of indicidual glucosinolates (Grob Jr. and Matile 1980b).

The recent and current analyses are concentrated on the identification of parent glucosinolates, either by GLC or high performance liquid chromatography (HPLC). The GLC methods or techniques of glucosinolate separation are based on the derivatization with trimethsilane (TMS) to increase the volatile condition of the metabolites (Thies 1976), and detection level (Heaney and Fenwick 1980a; Heaney and Fenwick 1982). HPLC method is now increasingly used to identify the parent glucosinolates due to the method's simplicity and flexibility (Minchington *et al.* 1982; Spinks *et al.* 1984).

The total glucosinolates analysis is based on the detection of glucose level after the enzymatic breakdown by myrosinase (Joseffeson and Appelquvist 1968). This analytical method is suited to vegetative and seed material, it involves the retention of glucosinolates on an ion exchange resin, followed by washing and on-column hydrolysis (Heaney and Fenwick 1981, Heaney *et al.* 1988, VanEtten *et al.* 1974; VanEtten and Daxenbichler 1977). Another analytical method recently reported is the enzyme-linked-immunosorbent assay (ELISA), which screens a large number of samples very quickly for sinigrin levels, but is not appropriate for the determination of other glucosinolates or their metabolites (Hassan *et al.* 1988).

CONCLUSION

The physiological effects in animals consuming Cruciferae plants containing glucosinlates are influenced by many factors. These give rise to constraint effects on biosynthesis in the plant and to the efficiency of glucosinolate catabolism in the metabolic system of an animal. The studies have centred mainly on the aspects of glucosinolate

chemistry, particularly the enzymic hydrolysis and give rise to the identification of metabolites. Studies have not established the gross effect of glucosinolates in animals. The adaptative significance of the structural diversity of glucosinolates and their relative effects of different glucosinolates are not known at the moment.

This review is mainly on the potential effect of different glucosinolates, especially on the limited number of breakdown products in number of animal species which have been studied. Because of the importance of the physiological effects of glucosinolates in the animal, it is a significant area of research for the agriculture and livestock sector.

REFERENCES

AHMAD, K.; RAHMAN, F. M. M.; RAHMAN, A. AND BEGUM, R. (1967). Biochemical effect of allylisothiocyanate. Pharmacological and Toxicological effects of food components-Effects of pharmacologiques et toxicologiques des composants alimentaris – Pharmakologische und toxkologische effekte von nahrungsbestandteilen. Proceedings of the 7th international congress on nutrition, Vol. 5, p. 815 – 819.

AHMED, P. AND FAROOQUI, M. Y. H. (1982). Comparative toxicities of aliphatic nitriles. *Toxicological letters*, **12**, 157 – 163.

AKIBA, Y. AND MATSUMOTO, T. (1976). Antithyroid activity of goitrin in chicks. *Poultry science*, **55**, 716 – 719.

ALBERT, A. (1987). Xenobiosis: Food, drugs and poisons in the human beings. Chapman and Hall, London, p. 66.

BELL, J. M.; YOUNG, C. G. AND DOWNEY, R. K. (1971). A nutritional comparison of various rapeseed and mustard seed solvent-extracted meals of different glucosinolate composition. *Canadian journal of animal science*, **51**, 259 – 269.

BRUGGEMANN, I. M.; TEMMINK, J. H. M. AND VAN BLADEREN, P. J. (1986), **83**, 349.

BRUSEWITZ, G.; CAMERON, B. D.; CHASSEAUD, L. F.; GORLER, K.; HAWKINS, D. R.; KOCH, H. AND MENWICK, W. H. (1977). The metabolism of benzyl isothiocyanate and its cysteine conjugate. *Biochemical journal*, **162**, 99 – 107.

CLARKE, E. G. C. AND CLARKE, M. L. (1975). Veterinary toxicology. 1st edition, Willlams and Bailliere Tindall, London, p. 294 – 298.

CROMWELL, G. T.; STAHLY, T. S. AND RANDOLPH, J. H. (1989). Raw, full-fat canola seed as a pro protein and energy source for growing-finishing swine. American dairy science association and American society of animal science combined annual meeting: Teaming up for animal agriculture. *Journal of animal science*, **67**, supplement No. 2. 39 – 40.

DAXENBICHLER, M. E.; SPENCER, G. F.; KLEIMAN, R.; VANETTEN, C. H. AND WOLFF, I. A. (1970). Gas-liquid chromatographic determination of products from the progoitrins in crambe and rapeseed meals. *Analytical biochemistry*, **38**, 373 – 382.

DAXENBICHLER, M. E. AND VANETTEN, C. H. (1977). Glucosinolates and derived products in Cruciferous vegetables: Gas-liquid chromatographic determination of the aglucone derivatives from cabbage. *Journal of association of official analytical chemistry*, **60**, 950 – 953.

DIERSCHKE, D. J. (1980). Basic controlling mechanisms: nervous, endocrine, and neuroendocrine. In: *Animal agriculture*: The biology, husbandry, and use of domestic animals. Ed. Cole, H. H. and Garret, W. N., 2nd edition, W. H. Freeman and Company, New York, P. 370.

DIETZ, H. M.; PANIGRAHI, S. AND HARRIS, R. V. (1991). Toxicity of hydrolysis products from 3-butenyl glucosinolate in rats. *Journal of agriculture and food chemistry*, **39**, 311 – 315.

DUNCAN, A. J. AND MILNE, J. A. (1989). Anti-nutritional factor, potentially toxic substances in plants. *Aspect of applied biology*, **19**, 75 – 92.

DUNCAN, A. J. AND MILNE, J. A. (1992a). Effect of long-term intra-ruminal infusion of the glucosinolate metabolite allyl cyanide on the voluntary food intake and metabolism of lambs. *Journal of the science of food and agriculture*, **58**, 9 – 144.

DUNCAN, A. J. AND MILNE, J. A. (1993). Effects of oral administration of *Brassica* secondary metabolites, allyl cyanide, allyl isothiocyanates and dimethyl disulphide, on the voluntary food intake and metabolism of sheep. *British journal of nutrition*, **70**, 631 – 645.

ELFVING, S. (1980). Studies on the naturally occurring goitrogen 5-vinyl-2-thiooxazolidone, metabolism and antithyroid effect in the rat. *Annals of clinical research*, **12**, supplement 28, p. 9 – 47.

ENGLISH, P. R.; FOWLER, V. R.; BAXTER, S. AND SMITH, B. (1988). The growing and finishing pig: Improving efficiency, Farming Press, Ipswich, p. 227 – 228.

FENWICK, G. R,; HEANEY, R. K. AND MULLIN, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. CRC. *Critical review of food science and mutation*, **18**, 123 – 201.

FREIG, A. A. H.; CAMPBELL, L. D. AND STANGER, N. E. (1987). Fate of ingested glucosinolates in poultry. *Nutritional reports information*, **36**, 1337 – 1345.

GIL, V. AND MACLEOD, A. J. (1980). Glucosinolates of *Lepidum stivum* and 'garden cress'. *Journal of the science of agriculture*, **31**, 739 – 741.

GODLEWSKI, C. E.; BOYD, J. N.; SHARMAN, W. K.; ADERSON, J. L. AND STOEWSAND, G. S. (1985). *Cancer Letters*, **28**, 151.

GORLER, K.; KRUMBIEGEL, G.; MENNICKE, W. H. AND SIEHL, H. V. (1982). The metabolism of benzyl isothiocyanate and its cysteine conjugate in guinea pigs and rabbits. *Xenobiotica*, **12**, 535 – 542.

GREENHALGH, J. R. AND MITCHELL, N. D. (1976). The involvement of flavour volatiles in the resistance to downey mildew of wild and cultivated forms of *Brassica oleraceae*. *New phytologist*, **77**, 391 – 398.

GROB, K. AND MATILE, P. H. (1980a). Vacuolar location of glucosinolates in horseradish root cells. *Plant Science Letters*, **14**, 327 – 335.

GROB, JR. K. AND MATILE, P. H. (1980b). Capillary GC of glucosinolate-derived horseradish constituents. *Phytochemistry*, **19**, 1789 – 1793.

HARDMAN, J. A. AND ELLIS, P. R. (1978). Host plant factors influencing the susceptibility of Cruciferous crops to cabbage root attack. *Entomology: Experimental applied*, **24**, 193.

HASSAN, F.; ROTHNIE, N. E.; YEUNG, S. P. AND PALMER, M. V. (1988). Enzyme-linked immunosorbent assays for alkenyl glucosinolates. *Journal of the agricultural and food chemistry*, **36**, 398 – 403.

HEANEY, R. K. AND FENWICK, G. R. (1980a). The analysis of glucosinolates in *Brassica* species using gas chromatography. Direct determination of the thiocyanate ion precursors, glucobrassicin and neo glucobrassicin. *Journal of the science of food and agriculture*, **31**, 593 – 597.

HEANEY, R. K. AND FENWICK, G. R. (1980b). The glucosinolate content of *Brassica* vegetables. A chemotaxonomic approach to cultivar identification. *Journal of the science of food and agriculture*, **31**, 794 – 801.

Heaney, R. K. and Fenwick, G. R. (1981). A micro-column method for the rapid determination of total glucosinolate content of Cruciferous material. Zeitschrift fur Pflanzenzuechtung/ Journal of plant breeding, **87**, 89 – 95.

HEANEY, R. K. AND FENWICK, G. R. (1982). The quantitative analysis of indole glucosinolates by gas chromatography - the importance of the derivatisation conditions. *Journal of the science of food and agriculture*, **33**, 68 – 70.

HEANEY, R. K.; SPINKS, E. A. AND FENWICK, G. R. (1988). Improved method for the determination of the total glucosinolate content of rapeseed by determination of anzymatically released glucose. *Analyst*, **113**, 1515 – 1518.

IDRIS, R. AND AHMAD, K. (1975). The effect of allylisothiocyanate and other antithyroid compounds on blood coagulation in rats. *Biochemical pharmacology*, **24**, 2003 – 2005.

JOSEFFSON, E. AND APPELQVIST, L. A. (1968). Glucosinolates in seed of rape and turnip rape as affected by variety and environment. *Journal of the science of food and agriculture*, **19**, 564 – 570.

KAMRIN, M. A. (1988). Toxicology: A primer on toxicology principles and applications. Lewis Publishers, Inc., Michigan.

KLINGAUF, F.; SENGONCA, C. AND BENNEWITZ, H. (1972). Effect of sinigrin on sucrose uptake by some polyphagous and oligophagous aphids (aphidae). *Oecologia*, **9**, 53 – 57.

LANGER, P. AND STOLC, V. (1965). Goitrogenic activity of allyl isothiocyanate – a widespread

natural mustard oil. *Endocrinology*, **76**, 151 – 155.

LANGER, P. AND MICHAJLOVSKIJ, N. (1969). Studies on the antithyroid activity of naturally occurring L-vinyl-2-thiooxazolidone and its urinary metabolite in rats. *Acta Endocrinological*, **62**, 21 – 30.

LANGVARDT, P. W.; PUTZIG, C. L.; BRAUM, H. AND YOUNG, J. D. (1980). *Journal of Toxicological Environment and Health*, **6**, 273.

LARSEN, P. O. (1981). Glucosinolates. In: *The biochemistry of plants*. Vol. 7. Lee P. A., Ed. Stumph Publication Academic Press.

LO, M. T. AND HILL, D. C. (1971). Toxicity of a glucosinolate concentrate prepared from rapeseed meal. *Canadian journal of animal science*, **51**, 187 – 192.

LO, M. T. AND BELL, J. M. (1972). Effects of various dietary glucosinolates on growth, feed intake and thyroid functions in rats. *Canadian journal of animal science*, **52**, 295 – 302.

MACLEOD, H. A.; BENNS, G.; LEWIS, D. AND LAWRENCE, J. F. (1978). Detection of goitrin and its heptafluorobutyryl derivative by gas-liquid chromatography with electrolytic conductivity and sulfur detectors. *Journal of chromatography*, **157**, 285 – 290.

MARANGOS, A. AND HILL, R. (1974). The hydrolysis and absorption of thioglucosides of rapeseed meal. *Nutritional society proceedings*, 33, supplement 1, p. 90A.

MCDONALD, C. H.; EDWARDS, R. A. AND GREENHALGH, J. F. G. (1992). Animal nutrition 4th edition, Longman Scientific and Technical, New York.

MENNICKE, W. H.; KRAL, T.; KRUMBIEGEL, C. AND RILTMAN, N. (1987). Determination of N-acetyl-s-(N-allylthiocarbamoyl)-L-cysteine. *Journal of chromatography*, **414**, 19 – 24.

MICHINGTON, I.; SANG, J.; BURKE, D. AND TRUSCOTT, R. J. W. (1982). Separation of desulphoglucosinolates by reversed-phase high performance liquid chromatography. *Journal of chromatography*, **247**, 141 – 148.

MUZTAR, A. J.; AHMAD, P.; HUQUE, T. AND SLINGER, S. J. (1979a). A study of the chemical binding of allyl isothiocyanate with thyroxin and of the effect of allyl isothiocyanate on lipid metabolism in the rat. *Canadian journal of physiology and pharmacology*, **57**, 385 – 389.

MUZTAR, A. J.; HUQUE, T.; AHMAD, P. AND SLINGER, S. J. (1979b). Effect of allyl isothiocyanate on plasma and urine concentrations of some biochemical entities in the rat. *Canadian journal of physiology and pharmacology*, **57**, 504 – 509.

NAULT, L. R. AND STYER, W. E. (1972). Effects of sinigrin on host selection by aphid. *Entomology: Experimental and applied*, **15**, 423 – 439.

NIELSEN, J. K.; DALGAARD, L.; LARSEN, L. M. AND SORENSEN, H. (1979). Hosts plants selection of the horse-radish flea beetle *Phyllotreta armoraciae* (Coleoptra: Chrysomelidae): feeding response to glucosinolates from several Crucifers. *Entomologia experimentalis et applicata*, **25**, 227 – 239.

NISHIE, K. AND DAXENBICHLER, M. E. (1980). Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin U found in Cruciferae. *Food cosmetic and toxicology*, **18**, 159 – 172.

PALMIERI, S.; IORI, R. AND LEONI, O. (1986). Myrosinase from *Sinpis alba* L.: A new method of purification for glucosinolate analysis. *Journal of the agriculture and the food chemistry*, **34**, 138 – 140.

PEARCE, P. E.; HUNT, C. W.; LANCASTER, L. L.; AULD, D. L.; KIVIOJA, D. A. AND MILLER, J. C. (1989). Effects of high versus low glucosinolate rapeseed forage on digestion and fermentation characteristics. American dairy science association and American society of animal science combined annual meeting: Teaming up for animal agriculture. *Journal of animal science*, **67**, supplement 2, p. 190.

PELTOLA, P. AND KRUSIUS, F-E. (1971). The effects of small doses of 1-5-vinylthiooxazolidone on the human thyroid function during long term treatment. In: *Further advances in thyroid research*. Ed. Fellinger and Hofer, Viena medical academy, Viena. pp. 149 – 153.

ROBINSON, D. W. (1980). General nutritional considerations. In: *Animal agriculture*: The biology, husbandry, and use of domestic animals.

Ed. Cole, H. H. and Garrett, W. N. 2nd edition, W. H. Freeman and Company, New York, p. 512.

RODMAN, J. E. (1974). Systematic and evolution of the genus *cakile* (Cruciferae) contribution. *Gray Herb, Harvard university*, **205**, 3 – 146.

RODMAN, J. E. (1978). Glucosinolates, methods of analysis and some chemosystematic problems. *Phytochemical bulletin*, **11**, 6 – 32.

SLOMINSKI, B. A.; CAMPBELL, L. D. AND STANGER, N. E. (1987). Influence of hydrolysis in the metabolism of nitriles to cyanide *in vivo*. *Drug, metabolism and disposition*, **10**, 495 – 498.

SLOMINSKI, B. A. AND CAMPBELL, L. D. (1988). Extent of hydrolysis in the intestinal tract and potential absorption of intact glucosinolates in laying hens. *Journal of the science of food and agriculture*, **42**, 305 – 314.

SMITH, R. H. (1974). Kale poisoning. *Report of the Rowett Research Institute*, **30**, 112 – 131.

SMITH, T. K. AND CAMPBELL, L. D. (1976). Rapeseed meal glucosinolates; metabolism and effect on performance in laying hens. *Poultry science*, **55**, 861 – 867.

SPINKS, E. A.; SONES, K. AND FENWICK, G. R. (1984). The quantitative analysis of glucosinolates in Cruciferous vegetables, oilseeds and forage crops using high performance liquid chromatography. *Fette seifen anstrichmittel*, **86**, 228 – 231.

SRIVASTAVA, V. K.; PHILBRICK, D. J. AND HILL, D. C. (1975). Response of rats and chicks to rapeseed meal subjected to different enzymatic treatments. *Canadian journal of animal science*, **55**, 331 – 335.

STOEWSAND, G. S.; ANDERSON, J. L. AND LISK, D. J. (1986). *Proceedings of Society of Experimental Biological Medicine* **182**, 95. ???

SZABO, S.; VAILEY, K. A.; BOOR, P. J. AND JAEGER, R. J. (1977). Acrylonitrile and tissue glutathione: different effects of acute and chronic interaction. *Biochemistry and biophysical research communication*, **79**, 32 – 37.

TANG, C. S. (1974). Benzyl isothiocyanate as a naturally occurring papain inhibitor. *Journal of food science*, **39**, 94 – 96.

TAKEGUCHI, N.; NISHURA, Y.; WATANBE, T.; MORI, Y. AND MORII, M. (1983). A pungent ingredient of mustard, allyl isothiocyanate, inhibits (H^++K^+)-ATPase. *Biochemical and biophysical research communications*, **112**, 464 – 468.

Thies, W. (1976). Quantitative gas liquid chromatography of glucosinolates on a microlitre scale. *Fette seifen anstrrichmittel*, **78**, 231 – 234.

TIEDINK, H. G. M.; MALINGRE, C. E.; VAN BROEKHOVEN, L. W.; JONGEN, W. M. F.; LEWIS, J. AND FENWICK, R. (1991). Role of glucosinolates in the formation of N-nitroso compounds. *Journal of the agriculture and the food chemistry*, **39**, 922 – 926.

TOOKEY, H. L. AND WOLFF, I. A. (1970). Effect of organic reducing agents and ferrous ion on thioglucosidase activity of crambe abyssinica seed. *Canadian journal of chemistry*, **48**, 1024 – 1028.

TOOKEY, H. L. (1973). Crambe thioglucosidase glucohydrolase. Separation of a protein required for epthiobutane formation. Canadian journal of chemistry, **51**, 1654 – 1660.

UDA, Y.; KURATA, T. AND ARAKAWA, N. (1986). Effects of pH and ferrous ion on the degradation of glucosinolates by myrosinase. *Agricultural and biological chemistry*, **50**, 2735 – 2740.

VANETTEN, C. H.; DAXENBICHLER, M. E.; PETERS, J. E. AND TOOKEY, H. L. (1966). Variation in enzymatic degradation products from the major thioglucosidases in crambe abyssinica ana *Brassica napus* seed meals. *Journal of agricultural and food chemistry*, **14**, 426 – 430.

VANETTEN, C. H.; GAHNE, W. E.; ROBBINS, D. J.; BOOTH, A. N.; DAXENBICHLER, M. E. AND WOLFF, I. A. (1969). Biological evaluation of crambe seed meals and derived products by rat feeding. *Cereal chemistry*, **46**, 145 – 155.

VANETTEN, C. H.; MCGRAW, C. E. AND DAXENBICHLER, M. E. (1974). Glucosinolate determination in Cruciferous seeds and meals by measurement of enzymatically released glucose. *Journal of agricultural and food chemistry*, **22**, 483 – 487.

VANETTEN, C. H. AND DAXENBICHLER, M. E. (1977). Glucosinolates and derived products in

Cruciferous vegetables: Total glucosinolates by retention on anion exchange resin and enzymic hydrolysis to measure released glucose. *Journal of association of official analytical chemistry*, **60**, 946 – 949.

WATTENBERG, L. W. (1977). *Journal of National Cancer Institute*, 58, 395.

WIGHT, P. A. L.; SCOUGALL, R. K.; SHANNON, D. W. F. AND WELLS, J. W. (1987). Role of glucosinolates in the causation of liver haemorrhages in laying hens fed with water extracted or heat-treated rapeseed cakes. *Research in veterinary science*, **43**, 313 – 319.

WILLHITE, C. C. AND SMITH, R. P. (1981). The role of cyanide liberation in the acute toxicity of aliphatic nitriles. *Toxicology and applied pharmacology*, **59**, 589 – 602.