A PRELIMINARY INVESTIGATION: PLANT CYANOGENICITY AS A POSSIBLE CO-FACTOR IN A POSSUM SPECIFIC TOXIN

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ABSTRACT

Since the introduction of thirty Australian brushtail possums into New Zealand in 1858 to start a fur trade industry the possum population as grown considerably. New Zealand is now 'home' to approximately 70 million possums which wreak devastation on our native forests and wildlife.

Current effective strategies for the control of possums in uninhabited areas include the use of 1080, brodifacoum, cholecalciferol, cyanide, and trapping or shooting. However these strategies are relatively non-specific in their mode of action and as such cause non-target species, including native wildlife, to die. The use of non-specific toxins and traps is also inappropriate for situations where people, livestock, or pets are present. There is therefore a demand for alternative strategies of possum control that affect only the target species. Methods presently being researched include the development of contraceptive vaccines, possum repellents and possum specific bait stations.

This research investigates the feasibility of developing a cyanogenic bait that is activated by a co-factor within the possum diet. The fast acting hydrogen cyanide poison is present in some plants species in an inactive glycoside form. Upon tissue injury the inactive cyanogenic glycoside is exposed to and hydrolysed by catabolic enzymes within the plant thereby releasing the toxin, hydrogen cyanide, at potentially lethal levels for possums. Some plant varieties within cyanogenic species however, have evolved to be acyanogenic due to the absence of either the cyanogenic substrate, the enzyme, or both. The occurrence of these acyanogenic plants which contain either the substrate or the appropriate enzymes are the target of this research. It is these plants that may provide the necessary co-factor for a cyanogenic possum bait to become lethal.

Preliminary analyses involved measuring and maximising the cyanide release from plant species known to be highly cyanogenic. Clover leaves (*Trifolium repens*), cherries (*Prunus avium*), and almonds (*Prunus amygdalus*) were the plant tissues analysed to determine whether levels of cyanide toxic to possums could be liberated. All three plant varieties underwent *in vitro* analyses in which they were exposed to surplus substrate and/or enzymes at varied temperatures and acidities. The maximum cyanide release

was determined for each plant variety and in the case of almonds (*Prunus amygdalus*) a further *in vivo* study was performed.

Although the clover, cherries and almonds all liberated cyanide after addition of either cyanogenic substrate or enzymes, the almonds were the only plant tissue to liberate sufficient levels of cyanide from the *in vitro* analyses to be considered toxic to possums. The almonds were found to contain high levels of active β-glucosidase enzymes which when incubated with the cyanogenic substrate, amygdalin, released high levels of cyanide. The *in vivo* analyses of almond macerates administered with amygdalin however were inconclusive in showing almonds as an effective co-factor for the hydrolysis of amygdalin. Nevertheless, two possums did die from cyanide poisoning after the administration of amygdalin with and without added enzymes. A third possum displayed signs of severe cyanide poisoning after it was gavaged with amygdalin and β-glucosidase enzymes but it later made a full recovery. As a result of the limitation imposed by the small size of the *in vivo* sample group further experimental trials are recommended to possibly obtain a more accurate set of results.

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ABBREVIATIONS AND TERMINOLOGY

AH	Amygdalin Hydrolase
DOC	Department of Conservation
Dyspnoea	Difficulty in, or laboured, breathing
EU	Enzyme Unit (1 linamarase EU hydrolyses 1 μmol of linamarin per minute at 30°C in phosphate buffer)
Gavage	Introduction of fluid into the stomach by an oesophageal tube passed orally
HCN	Hydrogen Cyanide
HNL	Hydroxynitrile Lyase'
ID	Inner Diameter
i/m	Intra muscular
KCN	Potassium Cyanide
LD	Lethal Dose
LD_{50}	Lethal Dose for 50 % of the Test Animals
LD_{90}	Lethal Dose for 90 % of the Test Animals
MAF	Ministry of Agriculture and Fisheries
MN	Mandelonitrile
MNL	Mandelonitrile Lyase
NaCN	Sodium Cyanide
ND	Not Determined
PH	Prunasin Hydrolase
SAPU	Small Animal Production Unit
Tachycardia	Excessively rapid action of the heart
TCA Cycle	Tricarboxylic Acid Cycle

CHAPTER ONE

INTRODUCTION

1.1 The Possum Peril

Trichosurus vulpecula, the common brushtail possum, is responsible for widespread destruction to New Zealand's indigenous and exotic forests, the spread of bovine tuberculosis, and the disappearance of food for many unique native snails, birds and insects. The harmful effects of the estimated 70 million possums that inhabit New Zealand are of wide concern. This concern, as to the effect that possums have on New Zealand's forests and economy, has prompted research into new methods for controlling possum populations.

In 1858 the first thirty possums were successfully introduced to New Zealand from Australia to start a fur trade industry. Unlike Australia, New Zealand has few natural possum predators or competitors for food and nesting sites, hence the escalation in possum numbers to approximately 70 million in the mid 1990's (Glasgow, 1990). It is now estimated that 92% of New Zealand's land area is colonised by the Australian brushtail possum (Cuddihy, 1993).

1.1.1 Deforestation

Browsing by uncontrolled possum populations is a particular problem in native bush and shrub land areas in New Zealand where these marsupials first deplete the area of the most preferred species followed by less palatable species. The possums' systematic stripping of one tree of a particular species before moving onto another of the same species does not allow the tree time to regenerate, thus leading to a progressively degraded forest (Seitzer, 1992). Possums generally target the softer new growth on trees. After an initial browsing, a healthy tree will produce replacement growth. This inevitably attracts possums back and again the tree is heavily browsed of new leaves. The less palatable older leaves which by now are near the end of their average two year life span die thus leaving the tree with no leaves. Therefore a two year period of

persistent defoliation by possums will cause the tree to die (Edwards, 1990). This situation is evident in the dieback of native mistletoe (*Tupeia antarctica, Peraxilla tetrapetalla* and *Ileostylus micranthus*), fuchsia (*Fuchsia excorticata*), titoki (*Alectryon excelsus*), kamahi (*Weinmannia racemosa*), pohutukawa and northern rata (*Metrosideros tomentosa* and *robusta*) which are all edible plant species highly favoured among possums (personal communication with DOC, Palmerston North).

The damage caused by wild possums is not only restricted to native fauna, but also affects native wildlife, insects and introduced plants. Possums deprive birds of berries, nectar and insects, compete for nest sites in hollow trees, and on occasion they will devour eggs and young birds (Seitzer, 1992). By stripping tree foliage, possums considerably reduce the numbers of insects living in the canopy along with a vast amount of organisms inhabiting leaf litter on the forest floor. Commercial pine plantations, orchards, wind breaks and erosion control plantings also undergo heavy browsing. In pine plantations possums not only eat young shoots and catkins, but also cause damage to the tops of the trees as they jump from plant to plant, so browsing is not the only peril to the growing pines (Seitzer, 1992).

1.1.2 Bovine Tuberculosis

Aside from weather extremes New Zealand's farming industry has the added dilemma of the spread of bovine tuberculosis (TB) by the brushtail possum. Bovine TB is a bacterial disease carried by cattle, and to a lesser extent sheep, which is able to infect humans who consume contaminated meat or dairy products. Possums, with an immune system of low resistance, act as an ideal vector for this very easily spread disease. Marsupials acquiring TB suffer extensive lesions in their lungs, gut and lymph nodes. When infected possums graze on farmland these bacteria-filled lesions release bacteria onto pasture through respiration, droppings, urine and weeping wounds thereby providing the contact between possums and livestock (Kelly, 1990).

1.2 The Research Aim

The overall aim of this research is to develop a species-specific cyanogenic toxin for possum control which utilises naturally occurring cyanogenic glycosides or their

catabolic enzymes within the possum diet. The specific scientific aim is to provide preliminary information as to whether cyanogenic plant material will release enough cyanide at the required rate to cause possums to die. This will be achieved by testing the cyanogenicity of selected plant materials (clover, almonds and cherries) *in vitro* and where possible *in vivo* in possums.

This research was planned with the view that future work in this area may lead to the development of a cyanogenic possum bait that is activated by an enzyme or substrate present in the possum diet therefore conferring species specificity. The bait should present greater protection for plant species heavily browsed by possums and therefore increase the chance of survival for the targeted vegetation.

1.3 Cyanogenic Plant Distribution

The ability to make cyanogenic glycosides which liberate hydrogen cyanide on hydrolysis is widespread among plants. At least 2050 species from 110 plant families are able to do this. Although the number of cyanogenic plant species is large, the number of known cyanogenic glycosides and lipids is relatively small, with only 27 having been identified so far, 23 of which are cyanogenic glycosides (Conn, 1980). The remaining few are cyanogenic lipids or pseudocyanogenic glycosides.

Due to the difficulty in isolating and characterising cyanogenic precursors in the field, very few species have had the structures of their cyanogenic glycosides determined. However, the simple qualitative picrate test has enabled many species having a cyanogenic potential to be identified. Plant families noted for being cyanogenic include the Rosaceae (150), Leguminosae (125), Gramineae (100), Araceae (50), Compositae (50), Euphorbiaceae (50) and Passifloraceae (30) (Gibbs, 1974). Because the cyanogenic material belonging to a genus or family usually consists of the same compound or group of compounds, discovery of new cyanogenic glycosides or lipids would be most likely to occur in unstudied families and genera.

Prunasin and amygdalin are two common cyanogenic glycosides generally associated with the Rosaceae family, while Dhurrin is the glycoside commonly associated with

grasses (the gramineae). Linamarin and lotaustralin are present in numerous and unrelated families and coexist with their hydrolytic enzyme, linamarase (**Figure 1.3.1**).

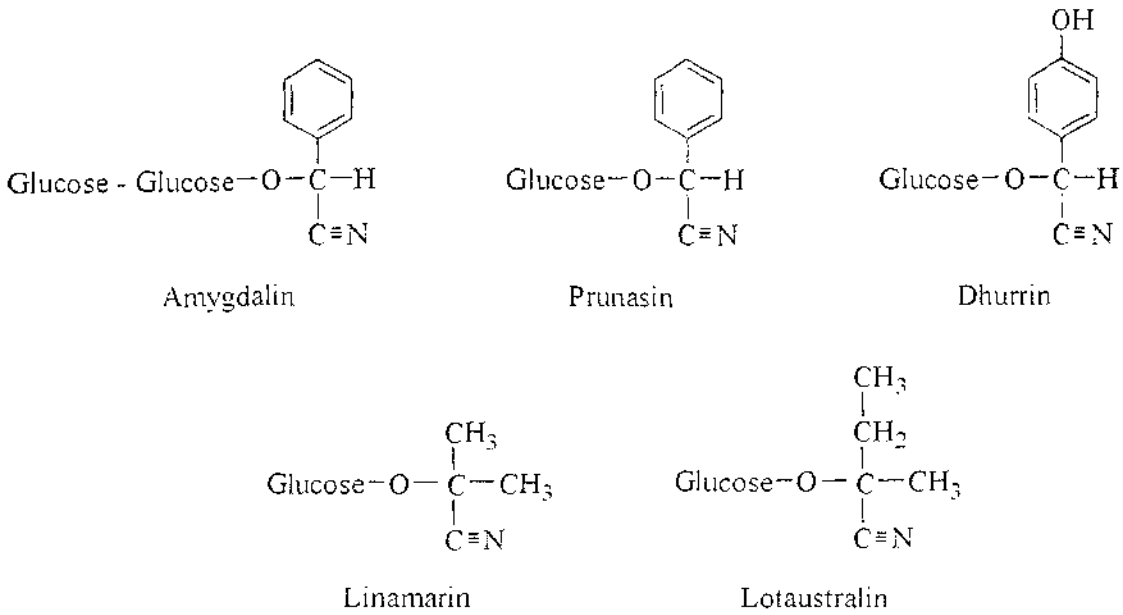

Figure 1.3.1 The Known Cyanogenic Glycosides
(adapted from Table 1., Eyjolfsson, 1970)

A combination of the linamarase and almond emulsin enzymes is capable of cleaving many different cyanogenic glycosides. This ability of a few enzymes to hydrolyse many different glycosides is thought to be an advantage in this research. It may mean that a variety of plant species in the possum diet should have the ability to release cyanide from a synthetic cyanogenic bait.

White clover (*Trifolium repens*), sweet almonds (*Prunus amygdalus*) and sweet black cherry (*Prunus avium*) were the three plant species examined in this research. They were chosen because they are species known to have cyanogenic properties.

1.4 Cyanogenesis

The process by which hydrogen cyanide (HCN) is released from plants containing cyanogenic glycosides or cyanogenic lipids is known as cyanogenesis. Under normal growth conditions the tissues of a cyanogenic plant do not contain detectable HCN and are therefore not toxic (Conn, 1979). The toxicity of the cyanogenic plant is directly

attributable to the liberation of HCN which is effected by mild chemical hydrolysis, or by the action of catabolic enzymes present in the plant which react when the plant tissue is damaged. Damage resulting in HCN liberation may be as a result of maceration, wilting, frosting or stunting of the plant (DOC, 1997).

It has been suggested that the cyanogenic glycosides and the hydrolytic enzymes capable of liberating the cyanide are located in separate compartments, "*The lack of large scale hydrolysis of cyanogenic glycosides in cyanogenic plant tissue until that tissue is crushed or otherwise disrupted is usually attributed to physical separation of the glycoside from its catabolic enzymes*," (Conn, 1980). Conn describes three models proposed to explain the lack of hydrolysis in intact plants. They include the occurrence of glycoside and catabolic enzyme:

- in separate cells or tissues
- in different subcellular compartments within the same cell
- within the same compartment but with inhibitors present to prevent cyanogenesis while the plant remains undisturbed.

All three models give valid explanations for the release of HCN only upon tissue disruption.

1.4.1 Chemical Hydrolysis

Summarised in **Figure 1.4.1.1** are the reactions of several chemical hydrolysing agents on cyanogenic plant materials where the $RR^1C(CN)$-O- moiety is known as an aglycone.

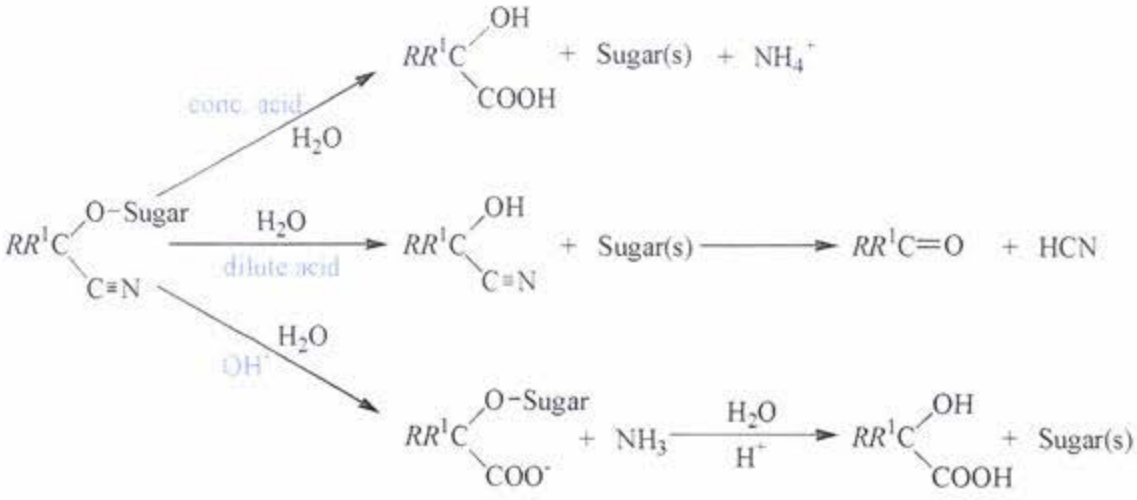

Figure 1.4.1.1 Chemical degradation of cyanogenic glycosides

(Eyjolfsson, 1970)

At elevated temperatures, dilute acid will cleave the β-glycosidic bond between the sugar and aglycone (Conn, 1978) (**Figure 1.4.1.2**). The released aglycone intermediate may then dissociate either spontaneously, or enzymatically. Non-enzymatic dissociation of the aglycone proceeds at a negligible rate below pH 5.5, but with increasing alkalinity it increases its rate of dissociation.

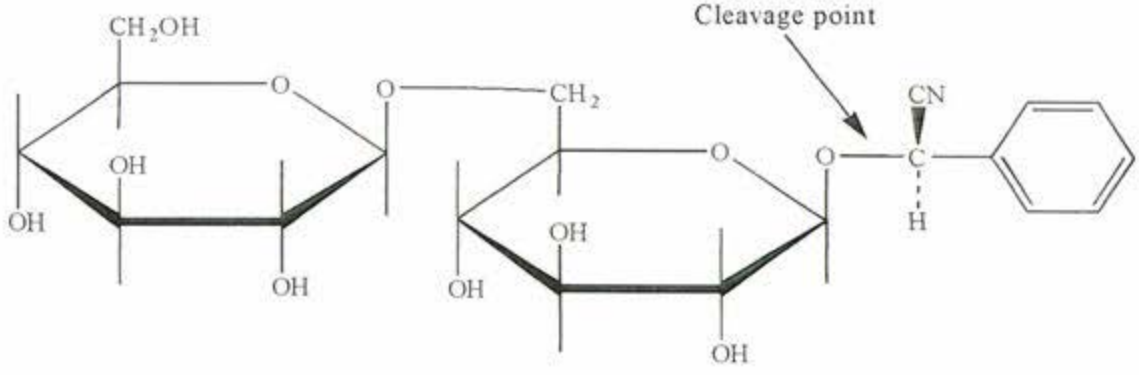

Figure 1.4.1.2 Cleavage point for the β-glycosidic bond of Amygdalin

Concentrated acid hydrolyses the cyanogenic glycoside to its corresponding 2-hydroxy acid, sugar and ammonium ion. Like concentrated acid, mild alkaline conditions also result in the production of the corresponding 2-hydroxy acid, sugar and ammonia (**Figure 1.4.1.1**).

1.4.2 Enzymatic Hydrolysis

Cyanogenic glycosides generally undergo sequential hydrolysis to liberate cyanide. The initial enzymatic reaction in plants involves hydrolysis by β-glucosidases of the β-glycosidic bond joining the aglycone to the sugar group. The sugar moiety of most cyanogenic glycosides is a monosaccharide therefore only the β-glucosidase specific to that particular sugar-aglycone bond is required. In the case of amydalin (**Figure 1.3.1**), vicianin and lucumin (**Figure 1.4.2.1**) however, there is a disaccharide attached to the aglycone (Conn, 1980). These disaccharides require the action of two β-glucosidases to produce the intermediate aglycone. More than one type of cyanogenic glycoside may be present in any one species of cyanogenic plant therefore there is often a requirement for more than one type of β-glucosidase to be present within the plant.

Figure 1.4.2.1 Structure of cyanogenic glycosides vicianin and lucumin
(Conn, 1978)

Upon tissue injury in plants, vacuolar acids and cytoplasmic components mix generating a slightly acidic plant macerate. It is in these slightly acidic conditions (pH 4.0 to 6.2), that the β-glucosidases of most cyanogenic plants have optimal activity for the hydrolytic cleavage of the β-glucosidic bond (Poulton, 1990). The aglycone produced is however relatively stable under these pH conditions and therefore has a negligible rate of non-enzymatic decomposition.

Although the aglycones (α-hydroxynitriles or cyanohydrins) will dissociate non-enzymatically due to their instability (particularly at alkaline pH), the presence of plant hydroxynitrile lyases will catalyse the more rapid dissociation of the aglycone to HCN and its product ketone, or aldehyde (**Figure 1.4.2.2**).

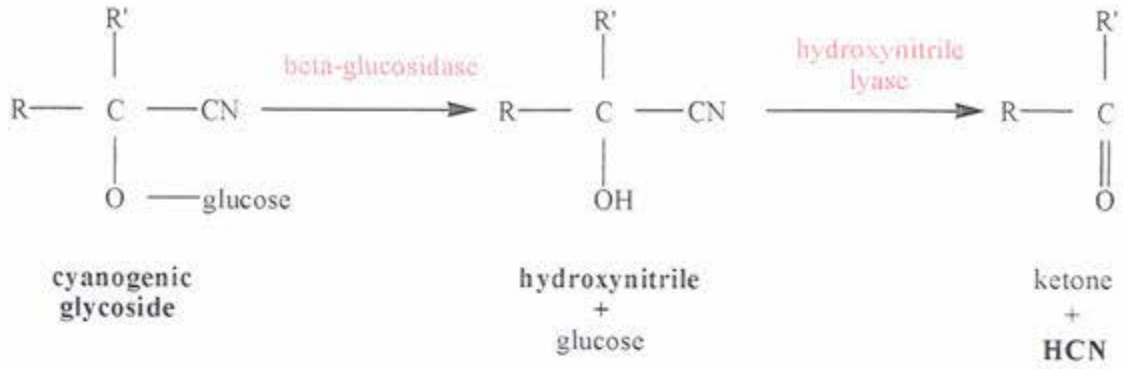

Figure 1.4.2.2 The hydrolytic release of HCN from cyanogenic glycosides

A study by Selmar *et al.* in 1989 used the hydroxynitrile lyase (HNL) of *Hevea brasiliensis* on the cyanogenic glycoside, linamarin, and the aglycone, mandelonitrile. The study showed that addition of HNL increased the rate of HCN liberation up to 20-fold therefore demonstrating the significance of HNL for rapid cyanogenesis. Its physiological importance is shown by the fact that only plants possessing high hydroxynitrile lyase activity are able to liberate HCN efficiently.

Because cyanide is not a cumulative poison (refer to section 1.5) it is the activity of the cyanide-liberating enzymes upon an appropriately toxic quantity of substrate within the cyanogenic plant that is the major determining factor in the toxicity of the plant.

1.5 Cyanide Toxicology and Detoxification

Hydrogen cyanide is a respiratory poison that has a capacity to form a reversible complex with the terminal oxidase of the mitochondrial electron transport pathway. Cyanide is not a cumulative poison and as such it is only toxic when the rate of absorption exceeds that of detoxification and elimination. The danger of cyanide exposure derives from its rapid absorption which overwhelms the natural defensive detoxification mechanisms of the body.

In cases of acute cyanide poisoning, the inhibition of cytochrome aa_3, the terminal oxidase, results in death. With a sub-lethal cyanide intake, HCN removal can be achieved by elimination or by detoxification (**Figure 1.5.1**).

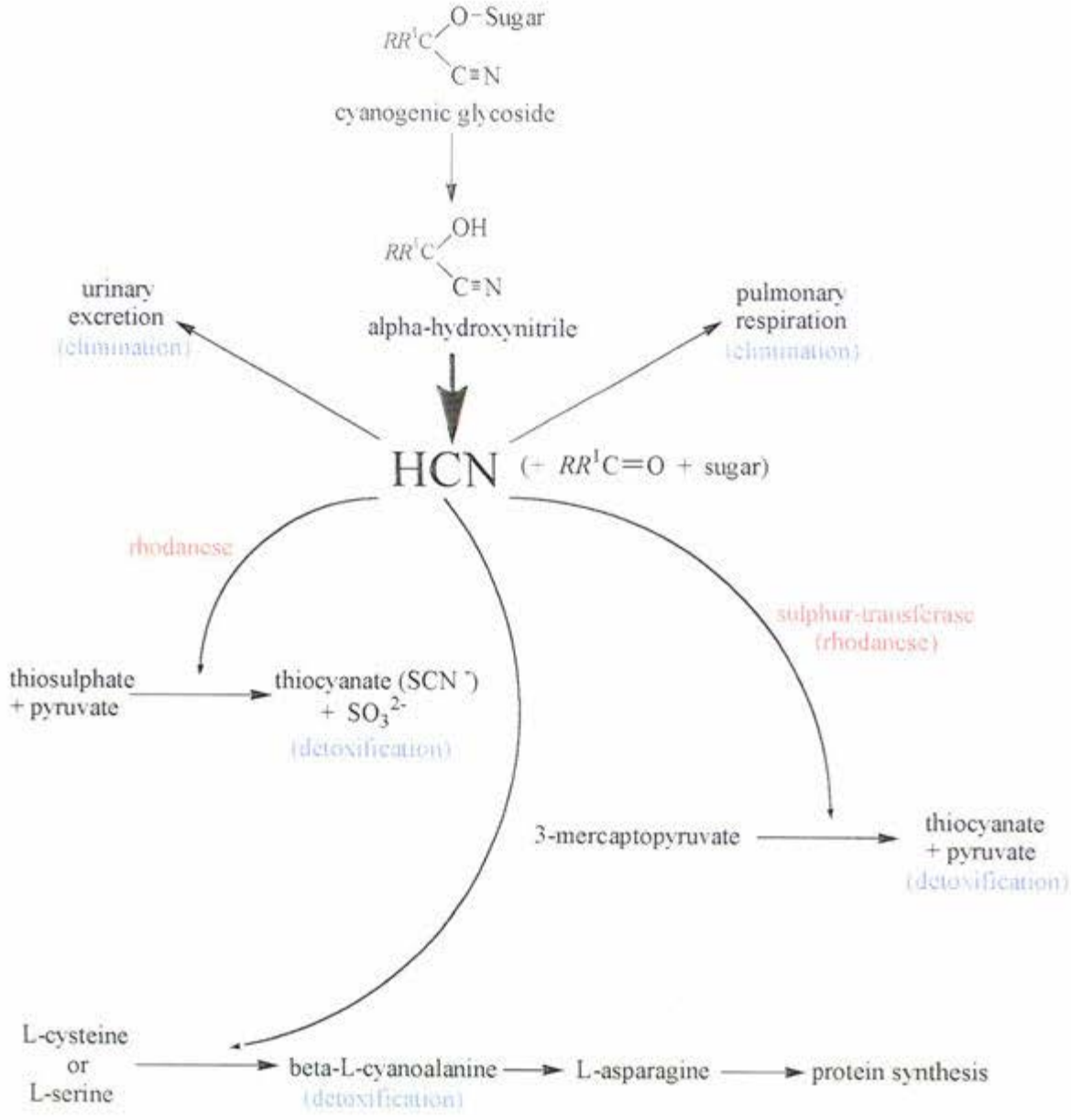

Figure 1.5.1 The metabolic disposal of inorganic cyanide

(adapted from Montgomery, 1969)

A small amount of cyanide can be eliminated by pulmonary respiration or by excretion in urine. Further removal of cyanide is achieved by its incorporation into vitamin B_{12} or cysteine, or by its oxidation to formate and carbon dioxide (Salkowski and Penney, 1994).

The body's principle metabolic pathway for detoxification is by reaction with thiosulphate to form thiocyanate (SCN) and sulphite, the reaction being catalysed by

rhodanese (sulphur-transferase) (**Figure 1.5.1**). The SCN produced, although less toxic than cyanide, is still a harmful substance that inhibits the organic binding of iodine in tissues of the thyroid gland (Greer *et al*, 1966). The rhodanese enzyme is widespread in living tissues, reaching its highest concentrations in the liver, kidney, thyroid, adrenal, and pancreas. The thiocyanate produced is excreted in the urine. The presence of sulphur in the blood facilitates cyanide detoxification.

The dose of cyanide, along with the speed of ingestion, is of importance in ensuring that the amount of cyanide taken is fatal. To be most effective, the cyanide release from cyanogenic glycosides needs to occur between mastication of the plant material and its subsequent delivery to the stomach. This is because the acidity of the monogastric stomach is not favourable for the reaction of the HNL catalysing the formation of HCN (and aldehyde or ketone) from the intermediate aglycone. The pH of the duodenum is more favourable for the HNL's which have a slightly alkaline pH optimum (Conn,1979). Further factors affecting the toxicity of cyanogenic plant material to animals includes the age of the plant material (younger growth is generally more cyanogenic), degree of mastication, rate of digestion, size and type of animal and its ability to detoxify cyanide.

As with the present use of cyanide paste, this project aims to produce a product that would release a high dose of cyanide rapidly, to ensure a quick and humane death. The LD_{50} for hydrogen cyanide in possums is approximately 11 mg/kg. With the average possum weighing around 2.5 kg, approximately 28 mg of cyanide is required to kill 50 percent of the possums.

1.6 Signs of Cyanide Poisoning

The effect of cyanide poisoning in animals is dependent upon the dose taken. Literature on the sub-lethal consumption of cyanide in possums is rare. However studies on beagles have recorded a period of dyspnoea and tachycardia occurring in the animals before complete recovery (Salkowski and Penney, 1994). A recent publication by Gregory *et al*, (1998) describes the death process from lethal potassium cyanide (KCN) ingestion in possums. Signs that were noted in the trial included a short period (approximately one minute) of impaired balance and co-ordination about 2¼ minutes

after cyanide consumption, followed by a phase where the possums were prostrate and experienced short periods of dyspnoea or hyperpnoea. Convulsions presented on average three minutes and 40 seconds into the experiment and lasted in general 19 seconds for 73% of the test animals. Limb movements and spasms were also observed. In the final and longest phase of cyanide poisoning the possums were relatively inactive in a prostrate position. Respiration ceased on average 14 minutes and 8 seconds after cyanide (19 mg/kg) consumption.

The salivation, nausea, vomiting and anxiety associated with low doses of cyanide poisoning in humans were not apparent in possums (Gregory *et al*, 1998).

1.7 Possum Control

For nearly five decades *Trichosurus vulpecula* have resisted sporadic pest control measures in New Zealand. A variety of toxins have been used alongside the conventional pest culling methods of shooting and trapping but to no avail as possum numbers are still escalating. An important point in the inability to bring the possum population under control is the lack of a suitably toxic and environmentally friendly species specific bait.

With the present possum toxins, the only realistic strategy for combating the possum onslaught in New Zealand is through sustained control. Irrespective of how successful an initial kill is, it must be followed up with maintenance control measures to prevent a new possum population from establishing itself. Eradication, although the most desirable and cost-effective option in the long term, is restricted to islands and peninsulas where reinvasion from neighbouring areas can be prevented. Successful eradication of *Trichosurus vulpecula* has been achieved for Kapiti island where the benefits to native vegetation and wildlife are now beginning to show (James, 1990).

Although all of the current culling methods are effective in reducing possum numbers, they all have the unfortunate disadvantage of inadvertently killing and maiming native wildlife, farm animals and pets. For the toxins this can be through non-targeted species directly ingesting poisoned bait or by secondary poisoning. The latter occurs when a non-target species consumes a poisoned insect or carcass.

With increasing public concern over the impact of pesticides on the environment there is a real need to develop more environmentally friendly and humane methods of pest control.

1.8 Present Control Methods

The Department of Conservation (DOC) presently use the acute poisons cholecalciferol, cyanide and sodium monofluoracetate (1080), and the anticoagulant brodifacoum in their fight against the possum invasion. These toxins are used along side non-chemical control measures such as shooting and trapping.

1.8.1 Cholecalciferol

Cholecalciferol (vitamin D3) is a naturally synthesised compound in animal skin and is also present in egg yolks, fish liver, fish oils and milk fat. Its action, after its conversion in the body to 25-hydroxycholecalciferol, is to mobilise calcium from bone into the bloodstream. Excessive cholecalciferol levels in the body cause hypercalcaemia and calcification of blood vessels. In possums this is believed to lead to heart failure within 4-7 days of a lethal dose being consumed. Signs of cholecalciferol poisoning in possums include loss of appetite, constipation and lethargy followed by death (Jolly *et al.* 1993, as referenced in DOC, 1997).

Although cholecalciferol is not designed as a species-specific bait it is distributed in specially made bait stations that limit accessibility by non-target species. Cinnamon flavouring and green colouring to deter birds further reduces the risk of non-targeted species consuming the bait. This bait does have a low risk of primary poisoning from loose bait and from secondary poisoning via consumption of poisoned carcasses (DOC, 1997).

1.8.2 Cyanide

Cyanide paste and 1080 are the possum poisons most commonly used in New Zealand. The ability of cyanide to kill quickly via its interference with the mitochondrial electron

transport chain makes this form of pest control ideal for possum skin recoverers as the killed possums are found within a short distance of the bait station.

The conventional cyanide bait does have a high risk of killing non-target species, but careful placement can minimise accidental poisoning of livestock, wildlife and humans. Unfortunately cyanide paste is among the possum baits that can induce possum shyness if a sub-lethal dose is taken.

Feratox , a new cyanide pellet recently released on the market, has been designed to overcome the learned aversion problem. Between 85 and 90 mg of KCN is encapsulated in a hard pellet and is released rapidly into the mouth when the pellet is crushed upon chewing (Feratox, 1997). Possums are rendered unconscious within one minute from when the bait is crushed, with time to death only three minutes. Trials using this bait show it to have an approximate 90% kill rate. Further success in the use of this bait has been due to a specially designed self feeding bait station in which the pellets are housed, which restricts access to the bait to possums.

There is a minimal risk of secondary poisoning with cyanide bait due to its rapid degradation to less toxic substances which is an advantage that cyanide has over 1080.

1.8.3 Sodium Monofluoroacetate (1080)

Since 1955, 1080 has been used extensively as a pest control in New Zealand. It contains a synthetically made and chemically identical monofluoroacetate to that occurring naturally in plants such as gifblaar (*Dichapetalum cymosum*), rat weed (*Palicourea margravii*), ratsbane (*Dichapetalum toxicarium*) and some 40 other plant species in Australia (DOC, 1997).

The metabolic conversion of monofluoroacetate to fluorocitrate in animals results in the inhibition of the energy producing tricarboxylic acid cycle (TCA cycle). Specifically, fluorocitrate interferes with the conversion of citrate to isocitrate with a resultant build up of citrate in the body. These high citrate levels can inhibit the phosphofructokinase enzyme of the glycolytic pathway. As a consequence of the TCA cycle disruption, the animal suffers from energy deprivation prior to death. Signs of poisoning in possums

first begin about 30 minutes after consumption and may include vomiting, cyanosis, tremors, drowsiness, staggering, respiratory and cardiac failure (DOC, 1997). Time to death is generally between 8 and 48 hours from time of ingestion. It therefore has a much longer kill time than that of cyanide.

Unlike cyanide paste, the bait is extremely safe to handle and at the spread rate of 5 kg a hectare, any poison entering the waterways would be sufficiently diluted so as not to harm humans or animals drinking it (51,000 litres would contain a fatal dose for a human). Sub-lethal doses tend not to result in learned aversion as cyanide does (Agricultural Pest Destruction Council, 1971). A major disadvantage of the bait is that it is not species specific.

1.8.4 Brodifacoum

Brodifacoum is an anticoagulant and as such disrupts the normal synthesis of vitamin K-dependent clotting factors in the liver. Poisoning commonly results in anaemia, weakness and haemorrhaging from the orifices. The effects of this bait may take several weeks to develop and time to death varies among possums according to the major haemorrhage site (DOC, 1997).

The potency and persistence of this second generation anticoagulant results in a high risk for primary and secondary poisoning in both targeted and non-targeted species.

1.8.5 Phosphorus

Phosphorus is no longer used by DOC, but regional councils use it in areas where 1080 is a risk to domestic animals. Phosphorus does not have a high risk of secondary poisoning as the toxin is not absorbed into muscle tissue like 1080. Death from phosphorus poisoning can occur by either cardiac failure or at a later stage by hepatic failure.

There are three phases in the action of phosphorus. The first is the acute gastrointestinal, abdominal and circulatory phase where vomiting, diarrhoea, shock, cyanosis and coma may occur. This is followed by a dormant phase whereby the

possum stabilises or may even recover slightly. In the third and final stage liver failure appears. Time to death may vary from 1 day to several weeks (DOC, 1997).

1.8.6 Shooting

Shooting, other than the new Feratox bait, is at present the only other pest control method that is species specific. This form of control is, however, time consuming and restricted to easily accessible parts of the New Zealand native bush. Many areas are not accessible by road or foot, so this method can really only be effective in reducing possum numbers New Zealand-wide if used in conjunction with an aerial bait.

1.8.7 Trapping

Like shooting, trapping is restricted to those areas in New Zealand that are accessible by foot. Many trapping devices, in particular the Lane's Ace trap (more commonly known as a gin trap), are controversial because of the inhumane way in which the animal suffers and often mutilates itself. An animal caught in the gin trap is held until the hunter kills it which legally has to be within 24 hours from the time of capture. Kill traps in which the animal dies instantly, although more humane, are impractical to use in many areas because of their large size and weight (Allen, 1989).

1.8.8 Bio-dynamic Repellents

A bio-dynamic technique using burnt possum pelts and testes was tested as a form of possum repellent by Forest Research scientists. This trial was unsuccessful however, with the material having no effect on the test possums (Atkinson, 1991).

1.9 Future Control Methods

The main requirements of any new pest control are that it be effective, humane in use, safe for non-target species, environmentally friendly and safe to handle.

Biological control using contraceptive vaccines to cause sterility in female possums is a definite possibility for a species-specific control. This form of pest control is, however,

reliant on natural mortality to reduce possum numbers, and as such will take a long time period to produce a noticeable decline in the population.

Research currently undertaken at Lincoln University, New Zealand, includes an immunocontraception method which will cause the female's immune system to attack the sperm. Reproductive physiologist, Dr Janine Duckworth, is working at reducing the reproductive rate of possums by injecting a vaccine into female possums that produces antibodies against one or more proteins in possum sperm. Preliminary studies have shown the vaccine to cause 80 per cent of the sample group of female possums to cease reproducing. Further research to find a way of spreading the biological control agent is presently being investigated. The present method of subcutaneous injection is not a practical method for spreading this or any form of similar pest control. Identification of a possum-specific pathogen or parasite would be the most effective mode of spreading a vaccine such as this (Gee, 1995).

The use of repellents to suppress possum browsing is another area in which there is current interest. Although this method does not set out to kill possums, the ability to repel possums from targeted areas would be of great benefit in forestry and related industries where possums cause a lot of seedling damage each year. Orchards, commercial and private, could also benefit from this sort of pest control. The ability to use repellents to shepherd possums to more accessible areas for shooting or trapping would also be of benefit. Research into the use of sulphur-rich formulations have had positive repellency results with rabbits and hares being repelled for up to two months and possums refusing to eat treated apples (Woolhouse and Morgan, 1995). Further studies include the use of biological fluids from carnivorous predators of herbivores.

Development of a cyanogenic bait that is activated by an enzyme or substrate common to the possum diet is another new pest control concept for the fight against possums. The ability to produce a bait that is non-toxic unless coupled with particular plant species would be a novel way to produce a species specific toxin. This preliminary study investigates whether such a toxin is feasible.