Aluminium Smelting: The Toxicity, Scale And Longevity Of Fluoride Pollution In The Environment

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For My Parents

With Much Love and Thanks

In Loving Memory of Auntie Val
The Brougham's Original Zoologist

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The thesis was written in the style of a series of papers for publication. Therefore, due their nature, there is an element of repetition between chapters in order for them to form discrete entries of work.

Abstract

The release of fluoride from the production of aluminium, and the subsequent impacts of deposition in the environment, is well documented (Weinstein and Davison, 2004). However, changes are occurring within the industry, driven by technological advances and growth in global manufacture, which have the potential to alter the impact of the aluminium industry on the environment. There needs to be a better understanding of the toxicity of Sodium hexafluoroaluminate (cryolite, Na_3AlF_6), the impact of modern operating procedures on the release of particulates in to the environment, and finally, an investigation of the longevity of fluoride concentrations in vegetation from industrial pollution once emissions cease (EU, 2008).

The toxicity of cryolite exposure to 1st and 3rd instar Diamondback moth larvae (*Plutella xylostella (L), Lepidoptera: Plutellidae*) was studied through a series of bioassays. There were inter-stage variations in both the lethal and sub-lethal responses to cryolite, whereby low mortality corresponded with high sub-lethal effects and vice versa. A dose-related response threshold was observed in both instars indicating that the physical nature of cryolite plays a role in the toxic mode of action.

The comparative toxicity of cryolite from the pot-rooms of Lynemouth smelter and pesticide Kryocide© (Cerexagri, Inc) was investigated along with the role of particle size and the purity of the cryolite particulate in the mode of action. Although both physical and chemical factors played a role in toxicity, these factors formed a hierarchy and the mode of action had a significantly over-riding chemical grounding.

Dust samples were collected from the vicinity of Lynemouth smelter for a period of 10 months in order to quantify the impact of modern aluminium production on the environment. Although the volumes of dust collected at any one site were lower than the European Community Short Term Ambient Guideline Concentration of Particulate Matter, dust deposition corresponded with aluminium production rates at the smelter showing that, despite Better Available Technology at the smelter, modern aluminium production still impacts on the environment.

When Anglesey Aluminium Metals Ltd ended its operations in September 2009, it created a unique opportunity to assess the long-term impacts of fluoride pollution on the environment. Samples of soil, leaves and lichens were collected to monitor the changes in fluoride content over a 15 month period. The results showed that fluoride contamination of vegetation from industrial processes had a short-term impact once emissions ceased and that mineral-based soils had limited potential as contaminating sources for plant uptake.

The mode of action of cryolite toxicity and the impact of waste disposal and fugitive release of cryolite in to the environment are discussed.

Thesis Contents

List of Tables

List of Figures

List of Acronyms and Abbreviations

AAM……Anglesey Aluminium Metals Ltd

BAT……Better Available Technology

DDT……Dichlorodiphenyltrichloroethane

EA…….....Environment Agency

EPA…….....Environment Protection Agency

EU……..European Union

LD……Laser Diffraction

MSDS……Material Safety Data Sheet

NE_{dep}……...No Effect Deposition

PPM……...Parts Per Million

SEM……Scanning Electron Microscopy

SLW……...……Specific Leaf Weight

SPL……...……...Spent Pot-Linings

UK……United Kingdom

US……...……United States

USEPA……..United States Environment Protection Agency

XRD……...…….X Ray Diffraction

Chapter 1

General Introduction

1.1 General Introduction

1.1.1 Cryolite; a contemporary global issue

Invariably, industrial expansion brings with it concerns over the resulting cost to the environment. In particular, here we focus on cryolite; Sodium hexafluoroaluminate (Na_3AlF_6), a fluoride compound which plays a significant role within the process of aluminium production, but poses a very real concern as the industry expands.

1.1.2 Fluoride

Fluorine, a highly electronegative chemical, is amongst the top thirteen most abundant elements found within igneous and sedimentary rock within the Earth's crust (Kierdorf *et al*, 1995) and is the most reactive element within the periodic table (Gillespie *et al*, 1989; cited in Camargo, 2003; Weinstein & Davison, 2004). The instability of fluorine means that it is present within the natural environment as organic and inorganic fluoride compounds, including free fluoride ions entering the biosphere through natural processes such as volcanism and rock weathering; (Kierdorf, *et al*, 1995; Camargo, 2003).

Around the world, inorganic fluorides are the most abundant of the fluorine compounds (Gillespie *et al*, 1989; cited in Camargo, 2003) and the most significant of these are fluorapatite ($Ca_5 (PO_4)^3F$), fluorite (CaF_2) and cryolite (sodium hexafluoroaluminate; NA_3AlF_6) which are found within the earth's crust (Camargo, 2003).

1.1.3 Fluoride uptake by animals

The pathways that contribute to an animal's whole-body fluoride concentration consist of; 1. the presence of food residue in the digestive tract, 2. the fluoride absorbed through the digestive and respiratory surfaces and stored in the body and 3. both the wet and dry deposition of fluoride on the outer body surfaces (Davies *et al*, 1996).

Ingestion is the greatest contributing pathway to fluoride uptake (Weinstein & Davison, 2004), through either the consumption of food materials or, as in the case of most marine invertebrates, through direct uptake from the surrounding water medium (Neuhold & Sigler, 1960; Camargo *et al*, 2003).

Both gases and particulates are deposited on the outer surfaces of soils and living organisms such as plants and invertebrates. Surface deposition of fluoride is less toxic than that which is ingested although little information is yet known about the capacity of animal surfaces to retain gas and particulate fluorides (Davies *et al*, 1996). Gaseous fluorides are, however, taken up by plants through the stomatal cells and, predominantly, through direct penetration of the cuticle (Ares *et al*, 1980).

The concentration of fluoride deposited on vegetation is dynamic and vegetation fluoride levels show cyclical patterns (Davison, 1987). Fluoride deposition fluctuates at a rate that suggests that export away from vegetation is at a rate comparable to deposition (Davison, 1987) It enters the food change as deposition on prey items, preening and pollen collecting in bees all leads to the ingestion of fluoride (Davison, 1987).

1.1.4 Cryolite

Cryolite is a naturally occurring inorganic fluorine-compound which forms a white, black, purple or violet coloured solid (Weinstein & Davison, 2004; EPA, 1996). It has a limited natural distribution and, as a consequence of intensive mining in the early twentieth century in Western Greenland, the US, Canada and Russia (Ullmann, 1985; cited in EU Risk Assessment, 2008), the natural resources are all but exhausted. Composed of 12.95% aluminium, 54.29% fluorine and 32.8% sodium, (Ullmann, 1985; cited in EU Risk Assessment, 2008), synthetic cryolite production now supplies industry requirements (EPA, 1996).

Physical properties such as melting point and solubility vary between natural and synthetic forms but the solubility of cryolite is typically 0.042 g/cm^3, and can range between 400-1200 ppm (parts per million). Despite its low solubility (Inchem, 2006), in the presence of sufficient amounts of water, it is broken down in to its constituent elements; Sodium, Aluminium and Fluorine, to near background levels (EPA, 1996).

1.1.5 Uses of cryolite

1.1.5.1 Organic pesticides

Cryolite has been used as an organic insecticide since 1957 and has since been re-registered for use (EPA, 1996). Produced in the form of a dust or wettable powder, it is applied to crops as a liquid spray or dust and appeals as an insecticide due to its low solubility which prevents dilution on the plant (EPA, 1996). The last decade has seen resurgence in applications of cryolite in the USA by commercial growers (Weinstein & Davison, 2004) predominantly targeting leaf-eating invertebrate pests of grape, citrus crops and potatoes (EPA, 1996). Despite such crops often receiving multiple

applications, little research has been conducted into the fundamental mechanisms involved or the sensitivity of different species (Weinstein & Davison, 2004). Described purely as a stomach poison by manufacturers, the importance of digestion in its insecticidal action seems evident as extreme tolerance is demonstrated if it is not absorbed via the gut (Huang, 1995). It has been suggested that ingested cryolite interacts with the micro-flora in the gut, but this interaction depends on the presence of chemical agents such as calcium which form complexes with the fluoride in cryolite (Weinstein & Davison, 2004).

Due to its use as an inorganic pesticide in the US, there is relevance in gathering information on the toxicity of cryolite to invertebrates. Huang (1995) found, through varying the concentration of cryolite deposited on vegetation, that after a period of 48 hours, the LC_{50} for the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) larvae was 15ppm for the first instar and 18ppm for third instar. This study highlighted the fact that sensitivity of individuals to cryolite is not always constant, but can vary with stage.

Investigating the toxicity of cryolite to the Honey bee (*Apis mellifera* L.), an important non-target beneficial invertebrate, the LD_{50} was shown to be greater than 217µg of cryolite per bee, resulting in cryolite being classified as practically non-toxic in the US (Atkins, 1975; cited in EPA, 1996).

1.1.5.2 The aluminium industry

Cryolite is the main constituent of the bath required for the electrolytic reduction of alumina to aluminium in the primary aluminium industry. The commercial production of aluminium was first achieved in 1882 by Charles Hall and separately in France by Paul Louis Toussaint Heroult (Weinstein & Davison, 2004). The process involves

dissolving alumina (Al_2O_2) in molten cryolite at 1000˚C (the electrolytic bath). This production takes place in large electrolyte cells which have a direct input current of 280,000A and 5V. Carbon blocks placed the refractory insulation lining the cells form the cathode. A carbon anode is immersed in the molten cryolite to complete the electrolytic circuit (Weinstein & Davison, 2004).

During the process, Al_2O_3 is broken down to Al^{3+} ions which are attracted to the negative cathode. The negative oxygen ions combine with carbon to produce CO_2 which is then attracted to the positive anode (Alcan, 2001). Moisture within the alumina leads to the formation of hydrogen fluoride which forms half of the gas emissions from the electrolyte cell. A series of volatile and unstable vaporising species exist above the liquid cryolite, and cooling this vapour produces meta-stable particulate matter including particulate cryolite.

The bath components vary within and between sites depending on the quality of the aluminium produced, the process parameters and the substances added to the bath. However, the typical bath comprises of 81.5 – 87% cryolite and the remainder is made up of alumina in solution, aluminium fluoride and calcium fluoride (Alcan, 2001).

1.1.6 China and the aluminium industry

In the twenty-first century, China has demonstrated a significant upturn in its economy through industrial growth and a boom in construction. As a result, China has been the 2nd largest economy behind the United States since 2004 (Alcan Inc, 2004a), spreading global shockwaves throughout the industrial sector as China's demand for natural resources increases (Gee *et al*, 2007). One beneficiary has been the aluminium industry.

A multibillion pound industry: despite the economic downturn, there was a 25% rise in demand for aluminium between 2009 and 2010 alone signalling a rapid price increase of almost 13% between September and November 2010 (Bloomberg.com, 2010). Direct foreign investment into China to the value of $150bn US dollars in 2002 (Bergsdal *et al,* 2004), coupled with sharp falls in the import duties imposed by the World Trade Organisation (WTO), have led to a greater economic consumption amongst China's population due to increases in domestic income (Alcan Inc, 2004b).

In 2008, China was the largest producer of primary aluminium in the world (Bloomberg.com, 2008) and accounted for 40% of the net increase in world production between 1995 and 2002. However, pressure to reduce energy consumption from the international community has forced many smelters in China to reduce their production capacity, significantly impacting the market and further driving up the price of aluminium (Bloomberg.com, 2010)

1.1.7 Global expansion and increased production

Spurred on by China's demand and the increasing value of aluminium, the industry is now experiencing a rapid global expansion. Not only have new smelters been opened throughout the world, in order to reap the benefits of China's economy, technology is being developed and improved in order to reach maximum output capacity. In 1975, when Alcan Europe Inc was opened in Lynemouth, United Kingdom, the smelter had an output of, on average, 80,000 tonnes of aluminium per annum. In 2008, the same smelter was striving to raise production from 160,000 tonnes to 180,000 tonnes per annum to compete within the market (Richard Anderson, Rio Tinto Alcan, personal correspondence, 2010). This smelter, however, is not equipped with the most recent

technological advances and has a maximum output capacity at almost 50% below that which is achievable in many of the newly established smelters. Smelters using new-cell technology can expect to produce a minimum of 350,000 tonnes of aluminium per annum (Richard Anderson, Rio Tinto Alcan, personal correspondence).

One such example is the establishment of Qatarlum; a smelter representing an equal alliance between Qatar Petroleum (QP) and the Norwegian company Norsk Hydro. After a year of operation in 2010, production levels were predicted to reach 585,000 tonnes per annum. However, the new technology used in the smelter suggests that production may eventually reach as high as 1.2 million tonnes per annum (aminfo.com, 2008).

The manufacture of 1 tonne of Aluminium produces between 8 and 20kg of excess cryolite (Alcan, 2001), and it is this excess which has very much become a contemporary concern for industry leaders.

Pot linings, containing cryolite, are renewed every six to eight years and, along with excess cryolite, are typically disposed of in landfill sites or dumping pits along the coast, located behind walls of large rocks to prevent the tides from entering the pits. Once full, the pits are filled with chalk-rich sand, soil and covered with grass (Gislason, 1998). The manufacture and supply of synthetic cryolite has increased in recent years in order to satisfy the world's demand for aluminium. Currently, with fluctuations in aluminium production, a large proportion of excess cryolite is stockpiled in large designated disposal units on-site to be traded between smelters around the world (Richard Anderson, Rio Tinto Alcan, personnel correspondence,

2010). However, critically, there is concern within the industry that the price of aluminium will eventually peak and then fall with economic decline, resulting in falling rates of production subsequently leaving behind a surplus of cryolite to dispose of (Richard Anderson, Rio Tinto Alcan, personnel correspondence, 2010). The number of available disposal sites, particularly in the UK where space is limited, raises concerns about the sustainability of current disposal methods and the potential to release contaminants into the surrounding environment (Gislason, 1998).

1.1.8 Environmental concentrations

The use of fluoride-containing raw materials within any industry has the potential to release gaseous and/or particulate fluoride into the environment with the effect of causing a surge in the background levels in the surrounding areas (Haidouti, 1991). In areas of the world free from fluoride pollution, natural or otherwise, terrestrial fluoride background levels are only just detectable, measuring around 0.1µg m^{-3} air or less (Davison, 1987). In the fallout area of a point source, industrial fluoride particulates may constitute up to half of the total atmospheric fluoride load, (Wright, 1978), and in rural areas of the US, concentrations range from as high as 0.3 µg m^{-3}, the lowest concentration to cause visible damage in vegetation (Davison, 1987), to 0.9µg m^{-3} (Hocking *et al*, 1980). However, this value has been known to soar, in cases of accidental fugitive release, to as much as 50 times this value for short periods of time (Davison, 1987). Typically, in industrial areas, changes in concentration are relative to proximity to local industrial releases.

1.1.9 The concentration gradient

The resulting concentration of fluoride in the emission area of a polluting source is typically a function of distance from the source combined with the local physical-

chemical parameters (Mirlean *et al*, 2006), as fluoride emissions precipitate to the ground- surface (Mirlean *et al*, 2007), and fluoride-rich soils remain localised to the source (Hocking *et al*, 1980). Strong evidence from an abundance of studies in this area have shown that the fall-out of fluoride particulates and gases is greatest in the immediate 2km radius of the source, decreasing in concentration with increasing distance away from the smelter (Bowen, 1988; cited in Madden *et al*, 1997; Haidouti, 1991; Hocking *et al*, 1980; Mirlean *et al*, 2006; Horntvedt, 1983; Mirlean *et al*, 2007; Wright *et al*, 1978). There is faster precipitation of soluble fluoride gases and particulates decreasing rapidly after a radius of 2 km, whereby smaller dust particles made up of less soluble fluoride compounds are carried greater distances of up to 20 km (Mirlean *et al*, 2007).

However, this fall-out area is vulnerable to the effects of weather conditions; wind speed and direction, rain and ground and surface waters, which affect the radial uniformity of the deposit; deposition and vegetation-loading can be reduced to within a few hundred metres from the point of source (Davison *et al* 1976, 1979; cited in Port *et al*, 1997). Similarly, distances of deposition can be increased as wind direction has been found to correlate with higher concentrations of fluoride; however it does not affect the total distance from the source which remains within an approximate 20 km radius of the smelter (Haidouti, 1991).

1.1.10 The introduction of Better Available Technology (BAT)

The Directive of Integrated Pollution, Prevention and Control (IPPC; 96/61/EC), is the regulatory body of the chemical industry, primary aluminium and recycling, glass, ceramics and foundries (EU, Risk Assessment, 2008). Under this directive both particulate and gaseous fluorides are covered. In recent years, a growing public and

government awareness of the nature of fluoride has lead to increasingly stringent containment measures to reduce losses of fluoride to the surrounding environment. Aluminium smelters and the ceramics industry are the largest producers of fugitive releases of hydrogen fluoride of all the heavy industries (Franzaring *et al*, 2006). Gaseous and particulate fluorides emanate from pot roof vents, therefore, electrolytic pots are now tightly hooded and gases are allowed to collect and are drawn off during the reduction process in to the exhaust ducts. The relining of pots, the tapping of aluminium and excess cryolite, the process of adding constituents to the bath and the removal of spent anodes also have considerable volumes of uncontrolled release (Weinstein & Davison, 2004; EU Risk Assessment, 2008). To tackle this problem, the EU now requires fixture of effective distillation columns such as scrubbing systems to reduce atmospheric emissions (Franzaring *et al*, 2006). Scrubbing systems fall into two categories: wet or dry scrubbers. A property of fluoride gases is that they can be chemi-absorbed in to particulates which see them change species once released into the environment (Davies *et al*, 1992). Dry scrubbing systems utilise this property. With an efficiency of 94.65%, the particulates are extracted and transported to the scrubbing system for treatment to remove fluoride from the airborne emissions (Alcan, 2001). Waste fluoride is absorbed into alumina particulate which is recycled back to the cells to be reduced (Wright, 1978). Similarly, environmental monitoring programmes are mandatory records of the accumulation and deposition of fluoride on vegetation, cattle bones and the airborne concentrations in the field in the proximity of smelters.

In recent years, the introduction of these BAT (Better Available Technologies), have resulted in a significant reduction in fluoride emissions in Europe (Franzaring *et al*, 2006). These changes have been witnessed at smelter level with records showing a

dramatic reduction in maximum fluoride output (Hocking *et al*, 1980). However, the use of wet scrubber systems brings with it additional concerns over the welfare of coastal marine communities. Particulate waste from the cells is collected in a mist eliminator grid and treated with sea water (Wright, 1978). The fluoride-containing effluent is predominantly discharged into seas or rivers systems (Wright & Davison, 1975) which is a particular concern as the aluminium industry is primarily a coastal-based industry.

1.1.11 The test species; Plutella xylostella

The Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most economically important pest species of the Brassicae around the world due to its destructive behaviour far exceeding many other pests (Guillox *et* al, 2003; Garcia Campos *et al*, 2006; Sarfraz *et al*, 2005; Talekar, 1996).

1.1.11.1 Target crops

Around the world, both large and small scale production of Brassicae plants occurs, particularly in tropical and temperate climates where any threat to crops poses a substantial economic as well as dietary risk to populations who rely on their production for both income and nutrition. The cultivation of brassicae plants in Taiwan alone uses approximately 50,000ha of land which constitutes 1/3 of all the agricultural land used in the production of crops (Talekar, 1996).

1.1.11.2 Distribution and migration

Due to the synchrony between the distribution of principal foods and the Diamondback moth, the evolution of this species originally was deemed to have

occurred in Europe, in correspondence with the evolution of Brassicae plants. Evidence shows that the origin of parasitoids and hyperparasitoids of the Diamondback moth however is specific to areas of Southern Africa and popular belief is turning to the possibility that this particular species of moth also occurred in Southern Africa from where dispersal on a global scale occurred (Kfir, 1998).

The Diamondback moth is considered to be the most widespread of all lepidopteran pests in the world (Talekar & Shelton, 1993). An important behavioural strategy that contributes to the distribution of the Diamondback moth is their ability to migrate. Migration, stimulated by the detection of a deteriorating habitat, acts as an adaptive mechanism which improves the chances of survival and reproduction (Garcia Campos *et al*, 2006).

Although tropical populations exhibit continuous breeding, in cooler climates the lifecycle is interrupted by the winter temperatures, inducing migration on a huge scale. The passive wind-borne migration of adult moths is so effective that flight can be sustained for periods of several days and, for many populations, transoceanic migration occurs on an annual basis (Talekar & Shelton, 1993).

Combinations of chemical compounds located on the surface of the leaf act as feeding and oviposition stimulants and inhibitors, and aid host-plant recognition (Gupta & Thorsteinson, 1960). In the absence of more favourable food sources, the Diamondback moth can survive on cruciferous plants often regarded as weed species which helps maintain populations in habitats where cultivation is absent (Talekar & Shelton, 1993).

1.1.11.3 Presence in the UK

One example of migration is the high altitude flight of Diamondback moth into the UK (Chapman *et al*, 2002), when warm air currents and high wind speeds optimise flight efficiency which peaks at 23ºC (Shirai, 1991). Overwintering in southern Mediterranean climates (Honda, 1992), Diamondback moth numbers in the UK fluctuate annually. *Plutella xylostella* occur in southern areas of the UK in early May and by mid summer the Diamondback moth is the most abundant nocturnal species detected entering the country (Chapman *et al*, 2002).

1.1.11.4 Insecticide resistance

Originally regarded as a relatively minor pest species, the Diamondback moth developed into a serious problem for the crop production industry with the introduction (and overuse) of broad-spectrum synthetic pesticides in the 1950s. Within any given population there is genetic plasticity capable of modulating life history traits in response to environmental conditions (Garcia Campos *et al*, 2004; 2006). The Diamondback moth quickly developed resistance to a range of pesticide active ingredients (Talekar, 1996), which, when combined with the removal of natural enemies from the field, boosted population numbers. It was reported as the first crop pest species to develop resistance to an insecticide (Talekar & Shelton, 1993), when in 1953 resistance to DDT was discovered (Ankersmit, 1953; cited in Talekar & Shelton, 1993).

The annual cost of losses to crops, research and the application of control measures for this pest species is estimated to be approximately $1 billion US (Talekar, 1992).

The damage to crops and the lack of any suitable control measures have left many areas incapable of continuing crop production.

1.1.12 Aims

Focusing on concerns over the sustainability of synthetic cryolite-disposal held by the aluminium industry, the primary aim of the thesis was to investigate the possible impacts of industrial cryolite on terrestrial invertebrates. Using a series of bioassays, in *Chapters 2* and *3,* the lethal and sub-lethal toxicity were investigated and work was conducted to ascertain which physical properties of cryolite affect its toxicity in the hope of shedding light on the mechanisms involved. Pesticide products containing cryolite as an active ingredient are not registered for use in the UK and so comparative bioassays were also performed between industrial cryolite and Kryocide©, a pesticide used in the US, in order to evaluate how the two compounds differ in their physical properties and toxicity.

Also, with the introduction of BAT to the industry, a number of investigations were conducted to ascertain the current impact of aluminium production on the surrounding environment in terms of both dust deposition, as investigated in *Chapter 4,* and the half life of fluoride in the vicinity of an aluminium smelter after site closure, which is the focus of *Chapter 5*. Ultimately, the thesis aims to define the impact of modern aluminium industry operations on the natural environment.

This series of studies form a four year investigation sponsored by Rio Tinto Alcan, conducted at the University of Newcastle upon Tyne and, in part, represent

collaborative research with Anglesey Aluminium Metals Ltd (AAM) and Emeritus Professor Alan Davison.

Chapter 2

An Investigation of the Lethal and Sub-Lethal Toxicity of Sodium Hexafluoroaluminate (Na_3AlF_6, cryolite)

2.1 Abstract

Understanding and evaluating the lethal and sub-lethal toxicity of chemicals is essential for assessing environmental hazards and making sound management decisions. Fatality alone has been described as an inadequate index of toxicity as death obscures the sub-lethal effects which could reveal the mode of action of the chemical which in turn could lead to a greater understanding of the environmental impacts of release (Sperling, 1976). The recent publication of the European Union risk assessment report (2008), regarding Trisodium hexafluoroaluminate (Na_3AlF_6, cryolite), highlighted a significant absence of information considering either the lethal or sub-lethal effects of exposure on terrestrial invertebrates and therefore a series of studies were devised to provide such data.

Firstly, a series of LD_{50} bioassays were designed and used to assess the comparative toxicity and the effect of exposure time of cryolite between instars of lepidopteran pest *Plutella xylostella*. Further analyses assessed the effects of cryolite on weight, long-term survival and the impacts of nutrition and mouthpart size on toxicity. Industrial cryolite was found to be significantly more toxic to 3rd than 1st instar larvae and toxicity increased significantly with time. Where mortality was low, sub-lethal effects of cryolite exposure were found to be prevalent leading to reduced long-term survival.

The present study suggests that the physical properties of cryolite such as particle size and solubility play a significant function in toxicity and also highlights the importance of considering the sub-lethal effects of exposure, outside the boundaries of bioassays, in order to fully assess the toxic potential of compounds.

2.2 Introduction

2.2.1 The need for toxicity data

Natural resources of Sodium hexafluoroaluminate (Na_3AlF_6, cryolite) have long since been exhausted by the heavy industries where cryolite is used for a wide range of functions including as a flux in the production of aluminium. With the price of aluminium soaring in recent years, metal production has markedly increased and with it the purchase of synthetic cryolite. There are industrial concerns that after the "boom" in the market has reached its peak, the disposal of the excess cryolite, of which little is lost during the smelting process, will be a considerable environmental concern for the industry, particularly in countries such as Britain where landfill, or dumping pit capacity is limited.

With the recent publication of the European Union risk assessment report (2008), regarding cryolite, a significant absence of information considering either the lethal or sub-lethal effects of exposure on terrestrial invertebrates was highlighted. Current concerns within the industry have emphasised the importance of acquiring such information (Richard Anderson, Alcan, personal correspondence, 2008).

2.2.2 Lethal toxicity

The possible chronic effects of exposure were not considered in early studies of human exposure which focused on low concentrations of cryolite (Marcovitch et al 1939, cited in Evans & Phillips, 1939). The emergence of apparent chronic toxicity resulted from exposure of factory workers in cryolite factories (Roholm, 1937) over extended periods of time.

The United States and the European Union differ greatly in their classification of cryolite toxicity. The US classifies cryolite as a category 3 chemical (the second lowest classification) for acute dermal LD_{50} (Hansen et al, 1981, cited in EPA, 1996), and category 4 for acute oral exposure, acute inhalation, and skin irritation (Hazleton Laboratories, America, Inc., 1983, cited in EPA, 1996). Annex 1 of the European Economic Union Council (EEC) directive 67/1548 classifies cryolite with a risk of T: R20/22-N; R48/23/25-Xn; R51-53, which translates to: "toxic: serious danger to health by prolonged exposure through inhalation and if swallowed… may cause long-term adverse effects in the environment" (Environmental Agency, 2008).

The importance of ingestion in invertebrates is evident, as with other fluoride compounds, as extreme tolerance is demonstrated if not absorbed through the gut (Huang et al, 1995). Theories concerning the mode of action include the possibility that cryolite acts an inhibitor to iron, calcium and magnesium-containing enzyme systems (Ware, 1986; cited in Huang et al 1995), and that interaction with micro-flora in the gut in the presence of chemical agents forms complexes, enhancing toxicity (Weinstein & Davison, 2004). Evans & Phillips (1939) concluded that the NaF portion of the cryolite molecule is responsible for its toxic properties in both vertebrates and invertebrates.

2.2.3 Sub-lethal toxicity

Understanding and evaluating the lethal and sub-lethal toxicity of chemicals is essential for assessing environmental hazards and making sound management decisions. The comparison of equitoxic doses has long been a popular method of comparing the toxicity of compounds. Measuring the quantal mortality response of a

species to a compound's toxicity, the objective of an LD_{50} assay is to estimate the magnitude of the dose required to result in a 50% mortality of the population (Busvine, 1971). It is often assumed to define the interaction between the test substance and the biological characteristics of the organism, but in fact the death of the organism is the secondary response to relatively minor initial physical and anatomical responses (Busvine, 1971). Fatality alone has been described as an inadequate index of toxicity as death obscures the sub-lethal effects which could reveal the mechanisms behind the toxic action (Sperling, 1976). By reducing the occurrence of death through short term exposures or low-dose-long term exposure, the probability of sub-lethal responses increases.

2.2.4 Aims

Primarily, the aim of the investigation was to provide information lacking from the EU risk assessment, (2008), regarding the toxicity of cryolite in insects, the mode of action and the sub-lethal effects of exposure.

The toxicity of industrial cryolite, typically emitted from the potrooms of aluminium smelters, was investigated with the Diamondback moth, *Plutella xylostella*, the most destructive pest of brassicas in the world. This species is easily reared in laboratory conditions and produces abundant quantities of larvae suitable for multiple bioassays.

The LD_{50} of industrial cryolite, the dose-mortality relationships and the sub-lethal effects: weight change and mean survival time after 48 hour exposure, were investigated. During the investigations it was noted that the concentration of agar solution used to create a suspension of the particulate for application on to the leaf

discs and also the mouthpart size of the larvae could be affecting the response to cryolite dose. Additional experiments were therefore conducted to investigate these possibilities (Appendix 2 and 3).

2.3 Methods

2.3.1 Estimation of the LD_{50} of particulate industrial cryolite for 1st and 3rd instar Plutella xylostella.

Chinese cabbage plants; *Brassica pekinensis Lour*, were used to maintain a culture of *P. xylostella* and leaves were used in the LD_{50} assays. The plants and insects were housed throughout the investigation in a growth room at a temperature of 20ºC (±2) and with a 16:8 L:D photoperiod. To obtain 1st and 3rd instar larvae, four new *B. pekinensis* plants were added to the culture for 24 hours to stimulate oviposition. These plants were then removed and maintained in a separate cage. This was done six and ten days prior to the start of the 1st and 3rd instar assays, respectively. To ensure that all individuals assigned to the tests were of the required stage, the larvae were selected according to the criteria of length (1.7mm for 1st instar larvae and 7.0 mm for 3rd instar larvae) and the colour of the head (black for 1st instar larvae and brown for 3rd instar larvae) (Capinera, 2000).

In order to emulate cryolite fall-out from a smelter, particulate cryolite used throughout the study was sourced from the pot-rooms of Rio Tinto Alcan Primary Metal Europe, Lynemouth Smelter, Northumberland.

The cryolite was suspended in agar solution to ensure accurate application of doses on to the leaf discs (Appendix 1). For the initial bioassays of both instars, a 0.1% agar solution maintained the test material in suspension. In the second bioassays, as dose increased significantly, a 1% agar solution was required. To control for fluoride from other sources, the water used throughout all of the studies, including that used in the

agar solutions and the water used in the test Petri dishes to maintain moisture levels, was distilled water for analytical purposes (Sigma Aldrich).

The range of 5 concentrations used in the range-finding tests of the LD_{50} assays were based on those used by Huang et al, (1995), in their investigation of pesticide cryolite LD_{50} for 3rd instar Colorado Potato Beetles namely: 6.2375, 12.475, 24.95, 49.9 and 99.8 µg/cm^2 (0.795, 1.096, 1.397, 1.698, 1.999 log µg/cm^2 respectively). The concentrations were produced using a dilution series and the effectiveness of the suspension was tested by measuring the weight of cryolite in a 40µl application administered to weighed glass cover-slips and left for 12 hours to air dry before reweighing (n = 20) (Appendix 1). All solutions and the original agar solution (the control) were stored at 5°C for 24 hours before use.

Discs of *B. pekinensis* (78.38 mm^2) were cut and 8 discs were placed around the perimeter of a filter paper in a 10cm diameter Petri dish. Doses of cryolite in agar solution were applied in 40µl volumes to the surface of each disc and spread across the surface to achieve maximum coverage. Each treatment group consisted of 9 replicate dishes with 8 leaf discs per dish. Distilled water was added to maintain leaf disc turgor, and the dishes were left for approximately 12 hours to allow the cryolite solutions to air dry on to the surface of the discs. Excess water was then removed from the dishes using a pipette, leaving the filter paper within the dishes damp to maintain the turgor of the discs and humidity within the Petri dishes. Six 1st or 3rd instar larvae were selected at random and added to each of the dishes and stored in the growth room.

Dishes were examined for mortality after 24 and 48 hours. A larva was considered dead if it did not move when gently prodded with a fine paintbrush. At 24 hours, the surviving larvae were transferred to freshly-treated dishes and maintained as before. At 48 hours, final counts of surviving larvae were made for each Petri dish.

A second bioassay was conducted for each instar to confirm the estimated LD_{50} value made by probit analysis. The range of cryolite doses for the second set of bioassays was adjusted to incorporate the predicted LD_{50}, and designed to overlap with the initial dose range to add surety to the initial set of results. The range of doses for 1st instar larvae was 99.8, 199.6, 399.2, 798.4 and 1000µg/cm^2 (1.999, 2.300, 2.601, 2.902 and 3 log µg/cm^2 respectively) with a control of 1% agar solution, and the dose range for 3rd instar larvae was 24.95, 49.9, 99.8, 199.6, 399.2µg/cm^2 (1.397, 1.698, 1.999, 2.300 and 2.601 log µg/cm^2 respectively) with a 1% agar solution acting as the control. The same procedures were followed as in the first set of bioassays.

2.3.1.1 Data analysis

The larval mortalities recorded at 24 and 48 hours were adjusted to correct for control mortality using the Abbott (1925) formula and the LD_{50} values for each instar were calculated using probit analysis (in Excel) based on Busvine (1971). Mortality data were tested for normality using Anderson Darling analysis. In all cases, data were not normally distributed and, as no transformation produced normal data, further analysis was non-parametric. Kruskall Wallis One Way ANOVA (SPSS 15.0) was used to analyse the equality of the mortality response between treatment groups. The data used did not account for differences in control mortality generated through variations in the agar treatments and so did not allow for comparison of data between 1st and

2nd assays within instar groups. Pairwise Mann Whitney U (SPSS 15.0) was used to compare each pair of treatments to identify significant differences.

For all other analyses, data were expressed as corrected percentage mortality (Abbott, 1925), to control for the differences in agar solution used in the controls between assays. Binary Logistic Regression (Minitab 15) was used to examine the effect of exposure time and instar on the relationship between mortality and cryolite treatment.

2.3.2 The effect of cryolite on weight change in Plutella xylostella larvae

Prior to the start of all the LD_{50} bioassays, the groups of 6 larvae were weighed, using a Mettler MT5 microbalance. At the end of the 48 hour bioassay, the surviving larvae were re-weighed to obtain the mean weight of survivors.

2.3.2.1 Data analysis

The mean weight change per individual per treatment was then calculated and the final mean weight of each control group was subtracted from this to allow comparison between assays and instars. Regression analysis (Microsoft Excel) was used to analyse the relationship between weight change and cryolite dose for each instar.

2.3.3 The effect of 48 hour exposure of 1st and 3rd instar larvae to doses of cryolite on long term survival

At the end of the 48 hour exposure of larvae to doses of cryolite, the surviving 1st instar larvae from the second assay and the 3rd instar larvae from both the first and second assays were removed from the cryolite-treated dishes to fresh Petri dishes containing untreated leaf discs, according to instar, assay and treatment group.

Petri dishes were maintained in the culture room and leaf discs were replaced every 2-3 days as required. Mortality counts of larvae, pupae and moths were made every 24 hours until all of the moths had ecloded and no live pupae or larvae remained. Individuals were deemed to be dead if they did not move when touched with a fine paintbrush.

2.3.3.1 Data Analysis

Mortality per day was analysed according to instar, bioassay and treatment with Kaplin Meier Survival Analysis (SPSS 15.0). The equality of survival distributions for the different doses of cryolite was analysed before pairwise analysis was conducted to identify which doses caused a significant effect on survival.

In order to make a comparison between instars, Kaplin Meier Survival Analysis, a statistical tool used to estimate the survival function from life-time data, was used on 1st and 3rd instar, 2nd bioassay data using the results from doses common to both instars, namely: 99.8, 199.6, 399.2 μ/cm^2 (1.999, 2.300, 2.601 log $\mu g/cm^2$ respectively) and the controls which were 1% agar solutions in both cases.

2.4 Results

2.4.1 Estimation of the LD_{50} for industrial cryolite particulate in 1st and 3rd instar P. xylostella.

When investigating the toxicity of industrial cryolite in 1st instar *Plutella xylostella*, probit analysis of the results of the first assay estimated the LD_{50}, at 48 hours, to be 2.905 log μg/cm^2. Analysis of the results of the second assay estimated the LD_{50} as 1.207 log μg/cm^2. The second estimate fell within the range of doses tested in the first bioassay and, as mortality did not reach 50% within this range of doses, it was concluded that industrial cryolite did not cause 50% mortality in 1st instar larvae. Figure 2.1 shows that the mean percentage mortality peaked at dose 2.300 log μg/cm^2, and did not exceed 23.08%. At 48 hours, the LD_{50} of industrial cryolite for 3rd instar larvae was 2.258 log μg/cm^2.

2.4.2 The effect of cryolite dose on mortality in 1st instar P. xylostella

2.4.2.1 Assay 2

There was a highly significant difference (Kruskall Wallis, $X^2 = 21.154$, df = 5, $P < 0.001$), in percentage mortality between treatment groups although these results must be treated with caution due to a possible Type 1 error caused by repeated analyses. Doses 2.902 and 3 log μg/cm^2 yielded significantly lower percentage mortality than doses 1.999 (Mann Whitney U, $P < 0.001$, 0.007 respectively), 2.300 (Mann Whitney U, $P < 0.009$, 0.029 respectively) and 2.601 log μg/cm^2 (Mann Whitney U, $P < 0.003$, 0.009 respectively) (Figure 2.1). Most important to note was that the percentage mortality response to the two highest doses did not significantly differ to that of the control treatment (Mann Whitney U, $P = 0.159$, $P = 0.331$, respectively). There was no significant difference in the pattern of mortality over time between the three

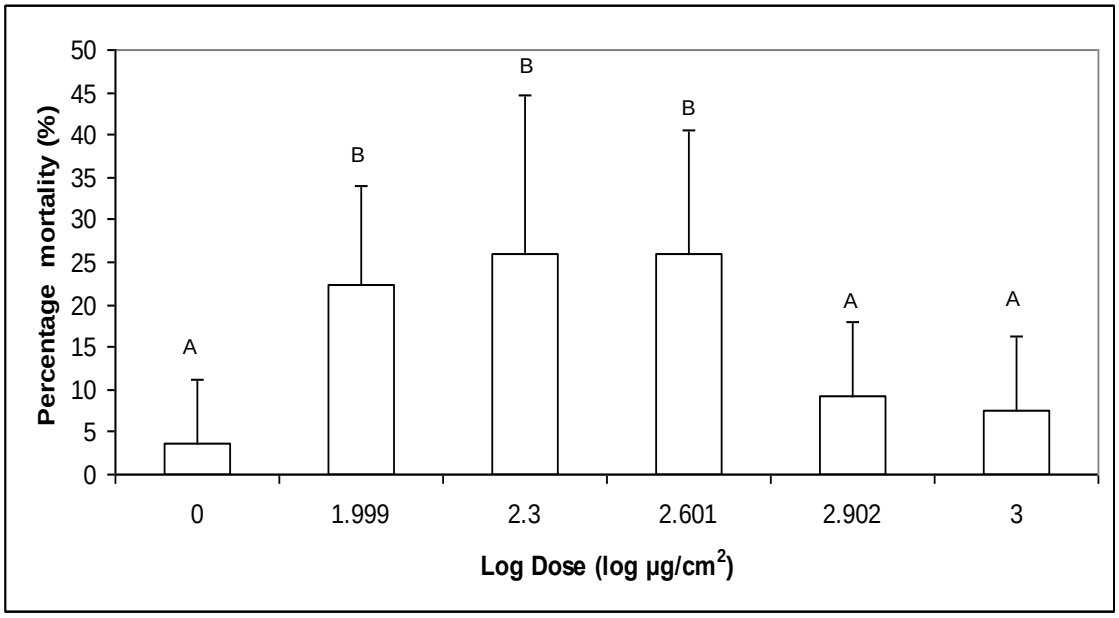

Figure 2.1. The mortality (%) of 1st instar P.xylostella exposed for 48 hours to doses of cryolite in assay 2. N = 52. Error bars indicate standard deviation. Treatments with a different letter are significantly different at P < 0.05.

treatments and therefore at the highest doses tested, cryolite had no significant lethal effect.

2.4.3 The effect of length of exposure on toxicity

2.4.3.1 1st instar: Assay 1 and 2

The length of time that larvae were exposed to doses of cryolite significantly affected the interaction between dose and mortality (Binary logistic regression, $P < 0.05$, df = 2, Z = -2.29, G = 74.439). At the two highest doses of cryolite, there was no significant effect of time on mortality. However, in the remaining doses tested, mortality at each dose significantly increased with time (Binary logistic regression, $P < 0.001$, df = 2, Z = 7.29, G = 68.889) (Figure 2.2).

2.4.3.2 3rd instar: Assay 1 and 2

The percentage mortality at each dose significantly increased as exposure time was extended from 24 to 48 hours (Binary logistic regression, $P < 0.001$, Z = 14.26, G = 387.492) (Fig 2.3). The relationship between dose and mortality also changed significantly as time increased, resulting in greater mortality with increasing dose ($P < 0.001$, Z = 3.85, G = 402.167).

2.4.4 The difference between 1st and 3rd instars

After 24 hours exposure of instars to doses of cryolite, there was no significant difference between the intercepts of the relationships between mortality response and dose ($P = 0.815$, Z = -0.19, G = 6.373) (Figure 2.4), although there was a significant difference ($P < 0.05$, Z = -3.10, G = 16.699) between the slopes of the mortality responses.

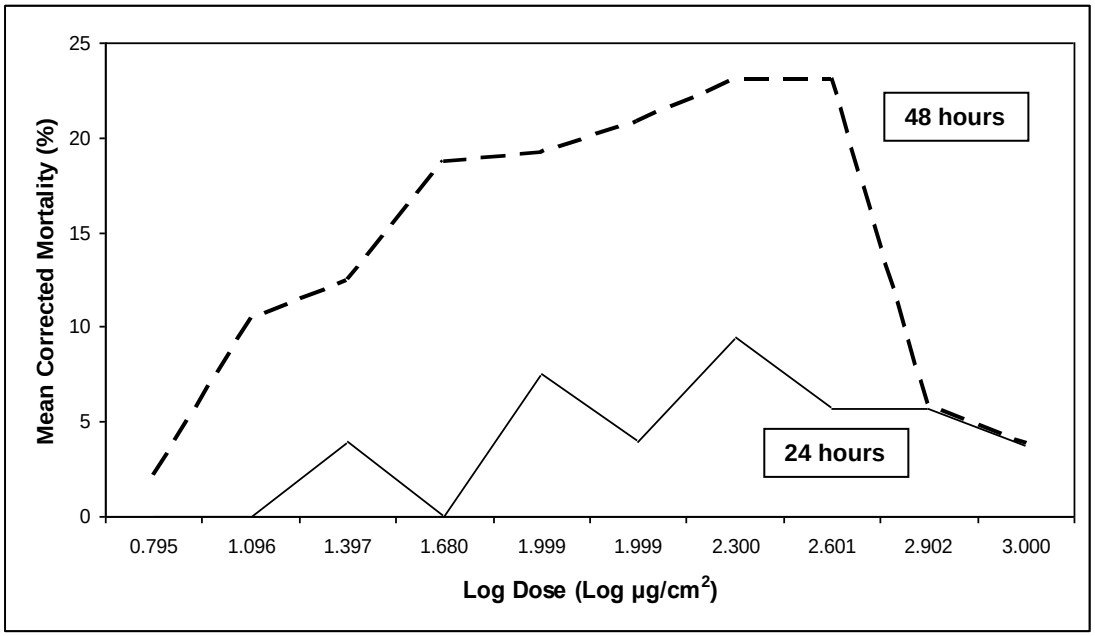

Figure 2.2. The effect of exposure time on the relationship between cryolite concentration (log µg/cm^2), and mean corrected mortality in 1st instar Plutella xylostella.

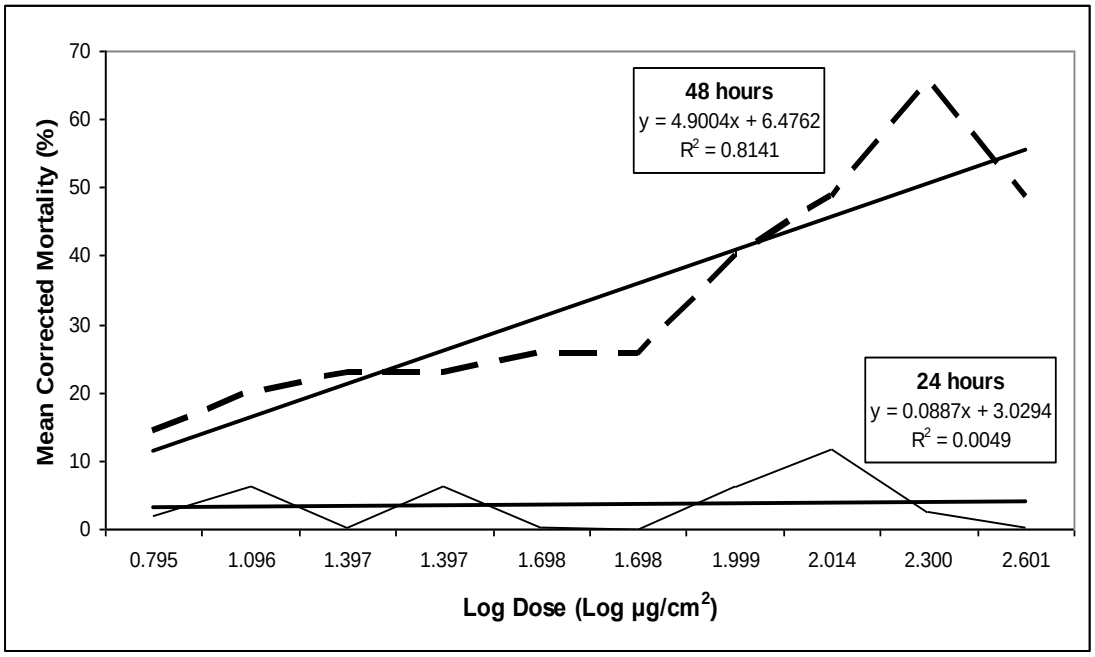

Figure 2.3. The effect of exposure time on the relationship between cryolite concentration (log µg/cm^2), and mean corrected mortality in 3rd instar Plutella xylostella. Mortality corrected with Abbott formula (1925). Trend lines represent linear regression fit.

At 48 hours of exposure, 3rd instar larvae showed significantly greater mortality ($P < 0.001$, $Z = 8.45$, $G = 172.205$) (Fig. 2.4), to doses of cryolite than 1st instar larvae although there was no significant difference ($P = 0.153$, $Z = 1.43$, $G = 174.226$) between the mortality-dose relationships between instars.

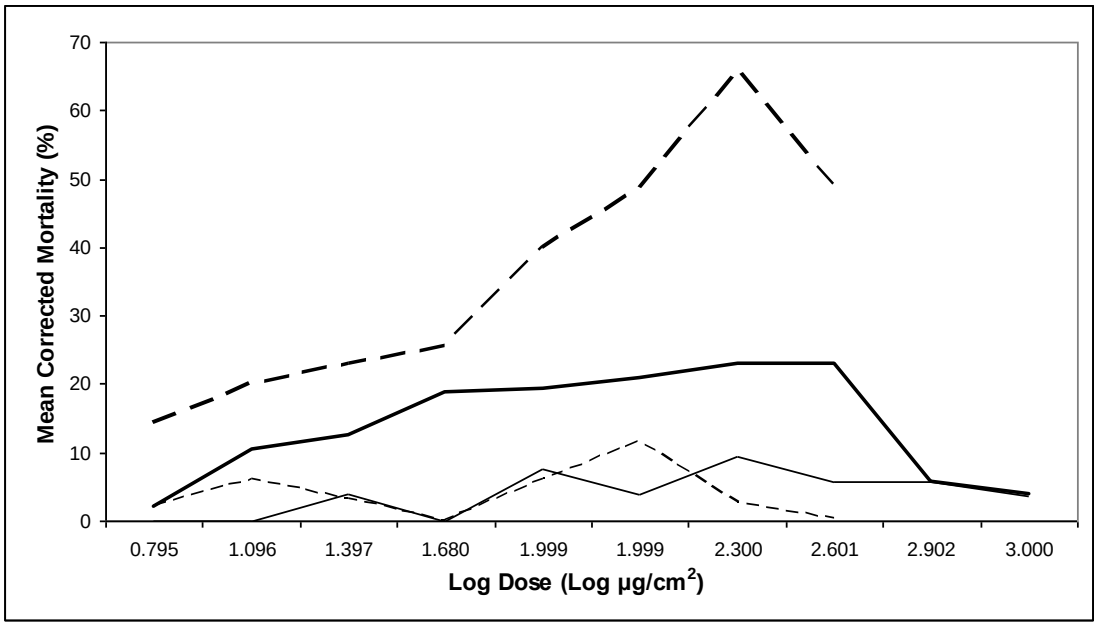

Figure 2.4. The effect of instar on the relationship between cryolite concentration (log µg/cm^2), and mean corrected mortality in Plutella xylostella larvae at 24 and 48 hours of exposure. Mortality corrected with Abbott formula (1925). Full lines refer to 1st instar and dashed lines refer to 3rd instar mortality response. The fine lines are mortality responses at 24 hours of exposure and thicker lines are mortality responses at 48 hours.

2.4.5 The effect of cryolite on weight change in Plutella xylostella larvae

2.4.5.1 1st instar

Doses of cryolite in the range of 0.795 log µg/cm^2 to 3 log µg/cm^2 had a significant negative relationship (y = -228.36x +327.17, R^2 = 0.8232, $P < 0.002$) (Figure 2.5), with weight loss in 1st instar larvae. In all but two treatment groups there was a mean loss in weight after 48 hours of feeding on the treated foliage. Despite the unexpected low corrected mortality results observed in treatments 2.901 and 3 log µg/cm^2 in the mortality tests, it was found that there was no significant corresponding anomalous effect on weight loss at these doses.

2.4.5.2 3rd instar

Weight change remained relatively constant between treatment groups and cryolite had no significant effect on weight change in 3rd instar larvae (y= -334.13x + 4.2366, R^2 = 0.1908, $P = 0.207$) (Figure 2.6).

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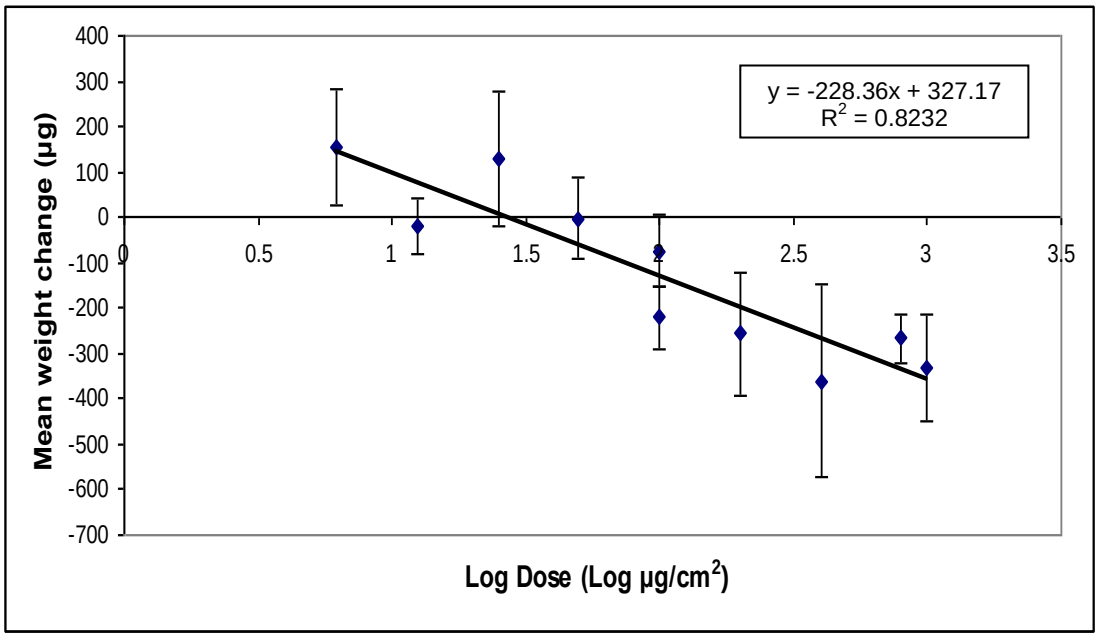

Figure 2.5. The relationship between cryolite concentration (log µg/cm^2), and weight change (µg), in 1st instar Plutella xylostella after 48 hours exposure in both assays 1and 2. Data are displayed as mean corrected weight change. Lines represent linear regression fit. Bars represent standard deviation.

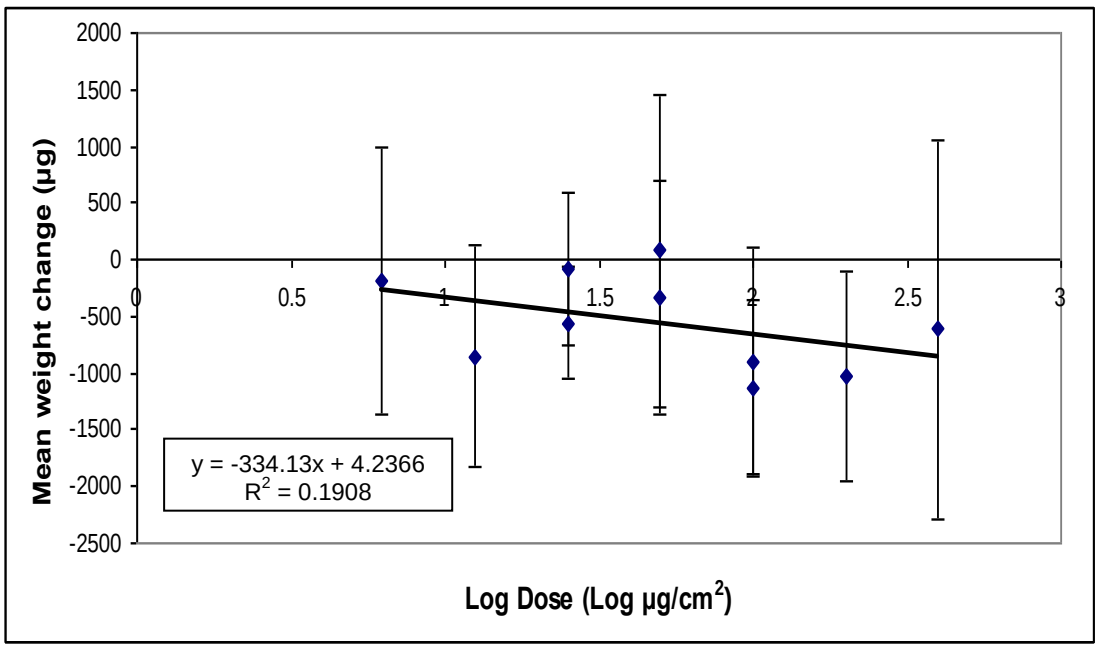

Figure 2.6. The relationship between cryolite concentration (log µg/cm^2), and weight change (µg), in 3rd instar Plutella xylostella after 48 hours exposure in both assays 1and 2. Data are displayed as mean corrected weight change. Lines represent linear regression fit. Bars represent standard deviation.

2.4.6 The effect of 48 hour exposure to cryolite on 1st and 3rd instar larvae long- term survival.

2.4.6.1 1st instar

The dose of cryolite that 1st instar larvae were exposed to had a significant effect on their subsequent survival in the days following the assay (Kaplin Meier, Log rank (Mantel-Cox) chi square = 50.478, df = 5, $P < 0.001$) (Table 2.1).

Pairwise analysis found that mean survival time was significantly greater at dose 3 log µg/cm^2 and the control (Table 2.1, Fig. 2.7) than in the other treatment groups. The control group was the only group to have any censored events (translating as the number of larvae developing into pupae) at 13.5%, and the mean survival length was greater, showing that exposure to cryolite at all doses was detrimental in terms of both survival and development. Exposure of larvae to the lowest dose in this concentration range (1.999 log µg/cm^2) had the greatest negative effect on mean survival.

2.4.6.2 3rd instar: Assay 1

Cryolite dose had no significant effect on the mean survival time of surviving 3rd instar larvae after 48 hour exposure (Kaplin Meier, Log Rank (Mantel - Cox) chi square = 10.169, df = 5, P = 0.071), although this could be an artefact of the small sample sizes caused by the high mortality during the 48 hour period.

2.4.6.3 3rd instar: Assay 2

The doses of cryolite that 3rd instar larva were exposed to in assay 2 had no significant effect on the subsequent mean survival in the days following the assay (Log Rank (Mantel – Cox) Chi square = 9.744, df = 5, $P = 0.083$).

Table 2.1. Pairwise comparisons performed in Kaplin Meier Survival Analysis of survival length based on the dose 1st instar individuals were exposed to in the 2nd assay. Boxed bold values represent significant P values.

Log Dose (log µg/cm^2)	0	1.999	2.3	2.601	2.902	3
0	*	**0.000**	**0.000**	**0.000**	**0.000**	**0.025**
1.999	*	*	**0.048**	**0.025**	**0.005**	**0.000**
2.3	*	*	*	0.874	0.574	**0.007**
2.601	*	*	*	*	0.741	**0.008**
2.902	*	*	*	*	*	**0.014**
3	*	*	*	*	*	*

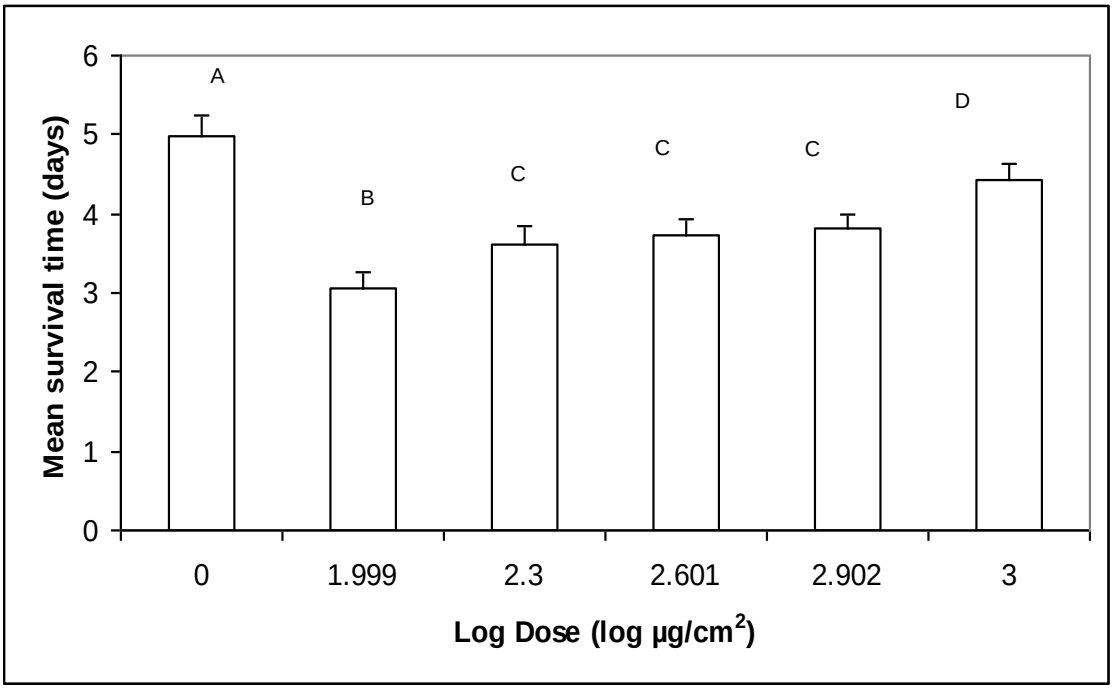

Figure 2.7. The mean survival time (days) of 1st instar P. xylostella larvae after 48 hours of exposure to doses of cryolite. Mean survival time (days) of larvae is plotted against log dose. Error bars indicate standard error. Treatments with different letters are significantly different from each other at P < 0.05.

2.4.6.4 The difference between 1st and 3rd instars

When the data from both instars were analysed by Kaplin Meier Survival Analysis, instar significantly affected the relationship between cryolite dose and the subsequent mean survival, (Log Rank (Mantel = Cox) chi square = 44.151, df = 7, $P < 0.001$).

Dose-pairs between instars were compared (Table 2.2, Figure 2.8). At both the control dose and dose 1.999 log µg/cm^2 mean survival time was significantly greater in 3rd than 1st instar groups. However, in the two highest concentrations; 2.300 and 2.601 µg/cm^2, there was no significant difference in the mean survival time between instars. Due to the smaller survival groups in 3rd instar treatment groups, the standard deviations were much larger.

Table 2.2. Pairwise comparisons between instars performed in Kaplin Meier Survival Analysis of mean survival time of larvae exposed to equal doses of cryolite. Boxed bold values represent significant P values.

Log Dose (log µg/cm^2)	1st instar control	1st instar 1.999	1st instar 2.3	1st instar 2.601	3rd instar control	3rd instar 1.999	3rd instar 2.3	3rd instar 2.601
1st instar control	*	<0.05	<0.05	<0.05	**0.038**	0.362	0.207	0.902
1st instar 1.999	*	*	0.048	0.025	<0.05	**0.002**	0.253	0.013
1st instar 2.3	*	*	*	0.874	<0.05	0.150	0.447	0.037
1st instar 2.601	*	*	*	*	<0.05	0.193	0.516	0.054
3rd instar control	*	*	*	*	*	0.169	0.130	0.327
3rd instar 1.999	*	*	*	*	*	*	0.752	0.557
3rd instar 2.3	*	*	*	*	*	*	*	0.433
3rd instar 2.601	*	*	*	*	*	*	*	*

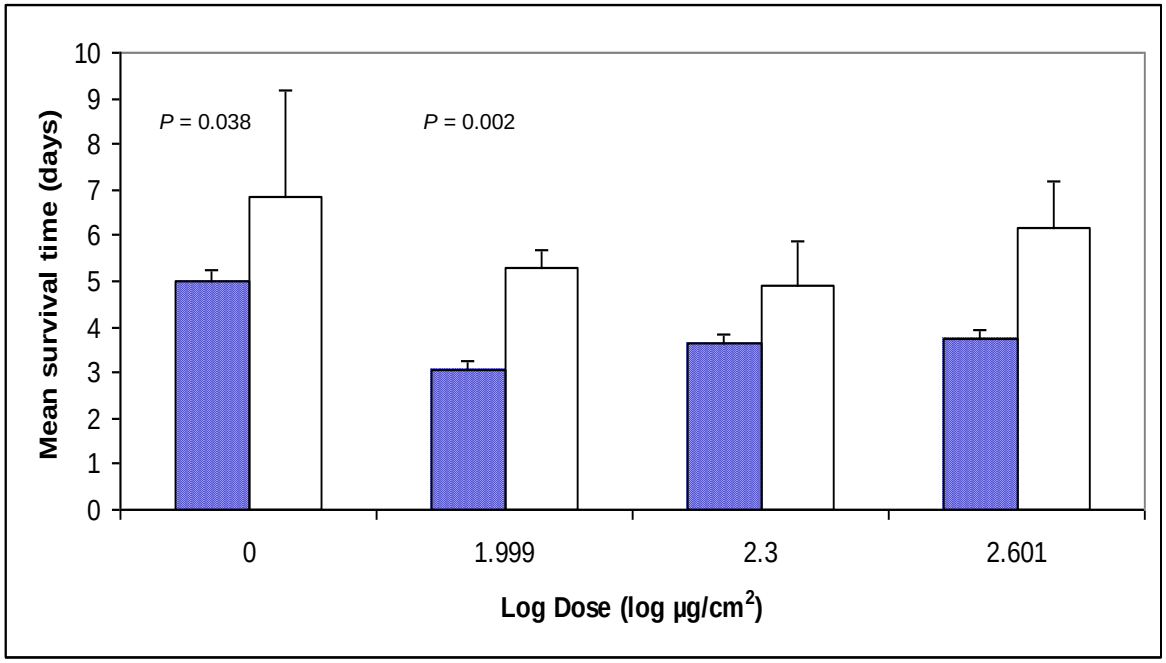

Figure 2.8. The mean survival time (days) of 1st and 3rd instar P.xyostella larvae after 48 hour exposure to doses of cryolite. Shaded bars refer to 1st instar larvae and clear bars refer to 3rd instar larvae. Sample sizes are as follows: control: 1st instar n= 52, 3rd instar n = 35, 1.999 log µg/cm^2:1st instar n =42, 3rd instar n = 18 , 2.300 log µg/cm^2: 1st instar n = 40, 3rd instar n =12, 2.601 log µg/cm^2: 1st instar n = 40, 3rd instar n = 18. Error bars indicate standard error. P values above treatments refer to significantly different instar responses at P < 0.05.

2.5 Discussion

2.5.1 Estimation of the LD_{50} for industrial cryolite particulate in 1st and 3rd instar P. xylostella

The literature considering the toxicity of cryolite as an active ingredient in pesticide products documents interstage variation where predominantly larvae are more susceptible than imagines (Zehnder, 1986; Ferro *et al*, 1993, and Huang *et al*, 1995). In the case of the Colorado Potato Beetle (*Leptinotarsa decemlineata* (Say)), Huang *et al*, (1995) found that the 48 hour LD_{50} for 1st instar larvae was 38.8µg/cm^2 in comparison to 3rd instar LD_{50} of 50.4µg/cm^2. The results of the present study add support to the evidence that the toxicity of cryolite is not uniform between insect life stages.

2.5.2 The effect of cryolite dose on mortality in 1st and 3rd instar P. xylostella

For both instars, the highest mortality occurred at the intermediate dose, 2.3 log µg/cm^2 indicating an apparent response threshold whilst, interestingly, cryolite seemingly had no significant lethal effect at the higher doses in 1st instar larvae.

Evans & Phillips, (1939), found that at high concentrations of fluorine intake, the solubility of the compound played a significant role in its toxicity. Marcovitch *et al*. (1936) (cited in Evans & Phillips, 1939), found that cryolite-rich diets administered to groups of rats over a 29 hour period, led to negligible evidence of toxicity. This is attributed to the insoluble nature of the cryolite compound, which in large doses, remains unabsorbed in the gut, passes through the animals system and is eliminated without lethal effects. Evans & Phillips, (1939), showed that reducing the concentration of cryolite and administering the dose in small quantities every day did

not over-load the animal's system, allowing enough of the compound to be absorbed to produce definite toxic effects. This could possibly explain the lack of toxic effect at doses 2.901 and 3 log µg/cm^2 in 1st instar *P. xylostella* as well as the decline in mortality at dose 2.601 log µg/cm^2 observed in 3rd instar larvae. This decline in mortality was not to the extent of that observed in 1st instars which could be due to the increased capacity of the gut, which is expected due to the significant differences in larval size (Appendix 2).

2.5.3 The effect of length of exposure on toxicity

The toxicity of a substance is a function of exposure which in turn is a function of dose and time (Rozman & Doull, 2000). The present study was unable to confirm the estimation of the LD_{50} value made by probit analysis in 1st instar larvae. Similarly, analysis of the toxicity of a pesticide cryolite product on the Spotted Lady Beetle (*Coleomegilla maculato* (Degeer)*)* (Lucas *et al*., 2004), did not produce an LD_{50} value for either 3rd or 1st instar larvae. Lucas *et al*, (2004) concluded that, for this species, the toxicity of cryolite was low and found that toxic symptoms took a long time to manifest. The initial 48 hour time period was extended to 6 days before a lethal dose was recorded.

Connel & Yu, (2008) found that the internal critical concentration required to produce a lethal effect in organisms exposed to organic pollutants fell in a predictable manner as exposure-time increased. Similarly, Kreutzweiser *et al*, (1994) found that the toxicity of the herbicide Tricloyr ester to species of fish increased as exposure time increased. The absorption-excretion and injury-recovery ratios and the rate of adaptation, result in the reaction of the organism to the toxicant. If the rates of

absorption and injury exceed that of excretion and recovery the increasing exposure time will result in an increase in toxicity (Rozman & Doull, 2000). For both 1st and 3rd instar *Plutella xylostella*, the increasing length of time that groups of larvae were exposed to industrial-cryolite-treated leaf discs significantly increased both the rate at which mortality increased with dose and the magnitude of the mortality response, with the exception of the top two doses when administered to 1st instar larvae. Adhering to the model of toxicity explained by Rozman & Doull (2000), it would seem that within the present study, the absorption of cryolite, and the accumulated injury resulting from this, exceeded both excretion and the rate of recovery eventually resulting in greater mortality within the second 24 hour period.

2.5.4 The difference between 1st and 3rd instars

After 24 hours exposure to doses of cryolite, there was no significant difference between instar mortality responses in either the magnitude of the response or the rate of increase with increasing dose. However, it was found that increasing the length of exposure differentiated the instar response. The rate at which mortality increased with dose did not significantly differ between instars, however, in 3rd instar larvae, the magnitude of the mortality response was significantly greater than in corresponding 1st instar groups after 48 hours exposure. The reason for these differences are currently unknown, but possible explanations could result from an internal difference in the physiology between instars or differences in feeding behaviour and quantity as explained in the following paragraphs:

1. 1st instar larval mouthparts are significantly smaller than those of 3rd instar larvae (Appendix 2). The maximum particle size of cryolite administered to the leaf discs

was approximately 100µm in diameter which was greater than the mean mandible length of 1st instar larvae. Although further investigation is required in to the relationship between mandible length and larval feeding ability, it would be logical to assume that the significant difference in mean mandible length indicates a significant difference in the range of particle sizes these instars are able to consume. Consequently, toxicity in 1st instar larvae may be reduced below that of 3rd instar larvae due to an inability to feed on the toxicant. Refining the particle size further using a sieve and repeating the assays could ascertain the influence of particle size on feeding ability.

2. Similarly, 1st instar larvae often feed beneath the cuticle of the leaf surface, observed in some instances within this study, which reduces larval contact with the cryolite particulate, again resulting in less consumption of cryolite.

3. The cryolite was not administered in conjunction with a wetting agent which would have spread the particulate across the surface of the leaf. Therefore, some areas of the leaf discs were left free of cryolite. The variability of toxicity within any sample group could be due to the different concentrations of toxicant that individuals come into contact with (Singh & Marwaha, 2000) and a smaller body size would indicate a relatively smaller appetite allowing 1st instar larvae to possibly sustain themselves on the areas of leaf disc left free of cryolite. Testing the efficacy of cryolite in conjunction with a wetting agent on the same species of diamondback moth used in this study Rao *et al* (2000), found that the insecticidal property of cryolite was greatly increased when used in conjunction with a wetting agent such as Tween 20 at 0.05%. They concluded that efficacy was increased because the wetting agent spread the

cryolite across the surface of the crop removing any dilution of cryolite in the diet contributed to by untreated areas of the crop surface.

2.5.5 The impact of cryolite exposure on weight change

Port *et al*, (1998), found that a diet containing HF and AlF_3 had no effect on pupal weight of *Pieris brassicae* and concluded that the fluoride pollution around a contaminating source was not sufficient to interrupt chewing herbivore growth. Although most doses of cryolite resulted in a loss in weight below the control group, there was no significant effect of cryolite dose on weight change in 3rd instar *Plutella xylostella* larvae in this present study.

Similarly, Postma *et al*, (1994), found that there were positive consequences of exposure to cadmium for populations of Diptera. Cadmium exposure led to a reduction in population size which in turn increased the food availability for the surviving individuals increasing fitness. As already suggested, the lack of uniform application of cryolite across the surface of the leaf may have given the opportunity for individuals to feed on untreated areas of leaf when competition was reduced through mortality. In this case, small surviving individuals within a treatment may have gained weight due to greater food availability, through reduced competition, therefore raising the mean weight change. The lack of a significant relationship between weight change and dose in 3rd instar larvae may therefore be a consequence of high mortality.

For 1st instar larvae, there was a significant decline in weight with increasing dose during the 48 hour period of exposure. It is unclear whether or not the weight loss

observed in 1st instar was a direct effect of the toxic action of consuming cryolite or an indirect effect on feeding behaviour. Ingestion of cryolite may be blocking cellular processes, leading to increasing weight loss as dose increases. However, even if toxicity on a cellular level is contributing to weight loss, it does not explain weight loss observed in the two highest doses where cryolite effectively had no effect on mortality. As dose increased, the thickness of the cryolite on the leaf surface increased to the extent that at dose 3 log $\mu g/cm^2$ there was a visible crust on the surface of the disc. This may physically prevent larvae from feeding. The presence of cryolite may also act as a repellent as dose increases, again, causing starvation and ultimately weight loss. These results confirm that weight loss is a sub-lethal response to cryolite exposure at the particle size tested.

This study may be criticised on the grounds that analysis was based on results from treatments of variable sample size. The experimental design, unfortunately, dictated that the equation for mean weight change relied upon the surviving larvae from the 48 hour assay. Results may have been an artefact of the mortality caused at the higher doses. In some cases the corrected mortality was so great that as many as five out of six larvae in a treatment dish died during the assay, and the mean weight loss was dependent on the size of the remaining larvae which may not have been representative of the sample. Larger individuals within a dish may have survived whose weight loss was not significant enough to fall below the initial mean weight, thereby representing a mean weight gain where in fact one did not exist. This is analogous of a type 2 error. This is a valid consideration, but was an unavoidable function of the design which could only have been alleviated by reducing the doses of cryolite to the extent that mortality was eradicated. However, at such doses where mortality does not result,

sub-lethal effects may also have been undetectable. What must be considered is that for each treatment there were nine replicates and therefore the chance of an error is small.

In order to investigate this further, future work would have to include a starvation-control treatment group to provide a control for any starvation that is occurring. Also, instead of monitoring the weight change of larvae in groups of six as assigned to the treatment dishes, larvae would be weighed, treated with cryolite for 48 hours and then reweighed separately in order to track individual weight change and remove the possible type 2 error observed with 3rd instar larvae.

2.5.6 The effect of particulate cryolite dose on the mean survival time of larvae after 48 hours exposure

Exposure to cryolite, at all doses, significantly reduced mean survival time of those 1st instar larvae that survived the 48 hour assay. Interestingly, however, it was found that mean survival time increased with increasing dose and therefore the greatest negative effect on survival time was the lowest dose tested: 1.999 log μg/cm^2. A delay in implementing this investigation meant that the surviving larvae from the first assay, and therefore those tested with doses lower than dose 1.999 log μg/cm^2, were not analysed for the impact on mean survival time. This is readily accepted as a flaw in this investigation where it would be useful in order to ascertain whether or not lower doses of cryolite continue to yield greater adverse impacts on survival time.

Both low and intermediate doses of cryolite absorbed in the gut of surviving individuals may not have been adequate to induce mortality in surviving individuals,

but may have been sufficient to cause injury which after exposure shortened the survival time of the individual. However, as concentrations increased, cryolite may have been readily expelled from the organism's system, reducing the weight of the individual but also reducing the significant long term effects on survival (Evans & Phillips, 1939)

In 3rd instar larvae the impact of cryolite exposure on mean survival time was variable between doses and even suggested beneficial properties for survival for those larvae exposed to the lowest dose. With little information available regarding the mechanism behind the toxic action of cryolite, the possible beneficial properties of this compound are unclear especially as both mortality and weight loss occurred above the control level at this dose. This result was not verified with an investigation of assay 1 for 1st instar larvae.

As with the investigation into the effects of cryolite on weight change, an unavoidable function of the experimental design was the variability in sample sizes between treatments groups which have affected the range of results increasing standard deviation and effectively reducing the reliability of the trends observed. This was due to the effectiveness of industrial cryolite as a toxicant to 3rd instar larvae within the 48 hour exposure bioassay.

The investigations undertaken in this chapter highlight several characteristics of cryolite toxicity, but most importantly, contradict claims of a linear relationship between dose and mortality made by the EU Risk Assessment (2008) and indicate that the physical properties of cryolite are an important function of toxicity. Also,

importantly, where mortality is low, injury occurs which affects larval survival further down the line. This shows that the impact of cryolite is greater than that indicated by the LD_{50} and further emphasises the importance of considering sub-lethal injury when assessing the toxicity of compounds.

2.6 Appendix 1

2.6.1 Assessment of the quality and reliability of cryolite-agar solutions prior to bioassays.

The effectiveness of the agar suspensions used to apply cryolite to leaf discs was tested by measuring the weight of cryolite in a 40µl application administered to weighed glass coverslips. Coverslips were left for 12 hours to air dry before reweighing (n = 20). The mean actual weight of each cryolite concentration was then plotted in Excel against mean expected weight and analysed with regression. These data can be seen in figures 2.9 to 2.11.

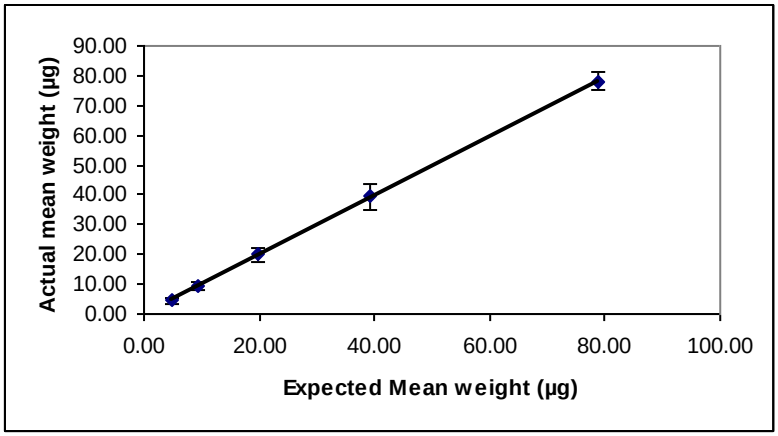

Figure 2.9. Mean weight of cryolite in 40µl application of 5 concentrations of cryolite suspended in 0.1% agar solution. Data is plotted against the expected weight (y = 0.9914x + 0.1216, R^2 = 0.99, P = 1). The cryolite-agar concentrations were used in assay 1 for both 1st and 3rd instar analysis. Bars around the data points represent standard deviation.

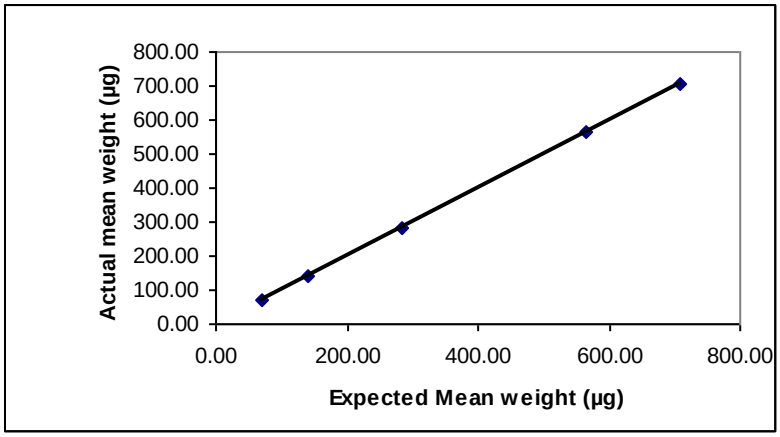

Figure 2.10. Mean weight of cryolite in 40µl application of 5 concentrations of cryolite suspended in 1% agar solution. Data is plotted against the expected weight (y = 0.9999x – 0.2971, R^2 = 1, P = 1). Cryolite-agar concentrations were used in assay 2 for 1st instar analysis. Standard error bars are absent from the figure as values were too low for bars to be visible.

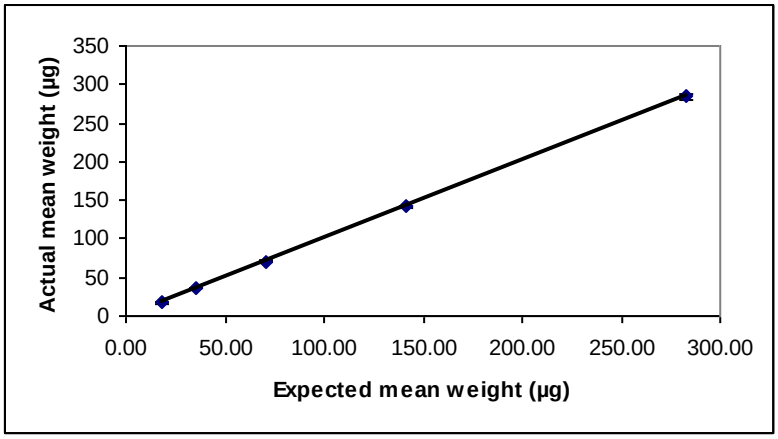

Figure 2.11. Mean weight of cryolite in 40µl application of 5 concentrations of cryolite suspended in 1% agar solution. Data is plotted against the expected weight (R^2 = 1, P = 1). Cryolite-agar concentrations were used in assay 2 for 3rd instar analysis. Standard error bars are absent from the figure as values were too low for bars to be visible.

2.7 Appendix 2

2.7.1 Methods employed to measure mandible size in 1st and 3rd instar Plutella xylostella larvae.

2.7.1.1 Mouthpart size in 1st and 3rd instar larvae

First and 3rd instar larvae were cultured and collected from the plant material as already described. Approximately 50 1st instar larvae and 30 3rd instar larvae were collected and separated by instar into sample pots. Samples were then processed ready for electron microscopy.

Five SEM images of the mouthparts of different individuals from each instar were taken and the lengths of the mandibles were measured. Using the scale bar accompanying the image, the measurements (mm) were converted to actual length (µm) (examples in Figures 2.13 and 2.14). After testing for normality the data were analysed by ANOVA to compare mean mandible length between instars.

In order to fix the samples, they were covered with 2% gluteraldehyde in Sorensons Phosphate Buffer and stored at 5°C for 24 hours. Samples were then rinsed with several changes of Sorensons Phosphate Buffer before dehydration. Initial dehydration was carried out by submerging samples in 25%, 50% and then 75% ethanol for 30 minutes each. Samples were then submerged in 100% ethanol for two hours. Final dehydration was carried out using carbon dioxide in a Samdri 780 critical point dryer whereby, under high pressure, the ethanol in the cells was replaced with liquid carbon dioxide. Slowly reducing the pressure down to atmospheric pressure caused the carbon dioxide to become gaseous leading to immediate dehydration. Through this method, shrinkage of the samples is minimised to <20%.

Both instars produced copious amounts of silk when submerged in the gluteraldehyde solution and so, after dehydration, several individuals of each instar were carefully separated under a stereo microscope using tweezers and a mounted needle. Both the separated larvae and the remaining bound group of larvae were mounted onto adhesive carbon discs mounted on to aluminium stubs, positioning the larvae to maximise the view of the mouthparts.

The mounted samples were left to dry overnight and then coated with the standard 15nm coating of gold using a Polaron SEM Coating Unit. Specimens were examined using a Stereoscan S40 Electron Microscope. Help with the processing and the supply of the equipment were provided by the EM Research Services, Newcastle University, Newcastle upon Tyne.

2.7.2 Results

2.7.2.1 Mouthpart size in 1st and 3rd instar larvae

Of the five individuals analysed for each instar, the mean mandible length was 61.34669µm (SD = 5.612463µm) and 107.3468µm (SD = 24.84756µm) for 1st and 3rd instar larvae respectively. There was a significant difference in mean mandible length (µm), between 1st and 3rd instars (ANOVA, $F = 16.304$, $P < 0.01$) (Figure 2.12).

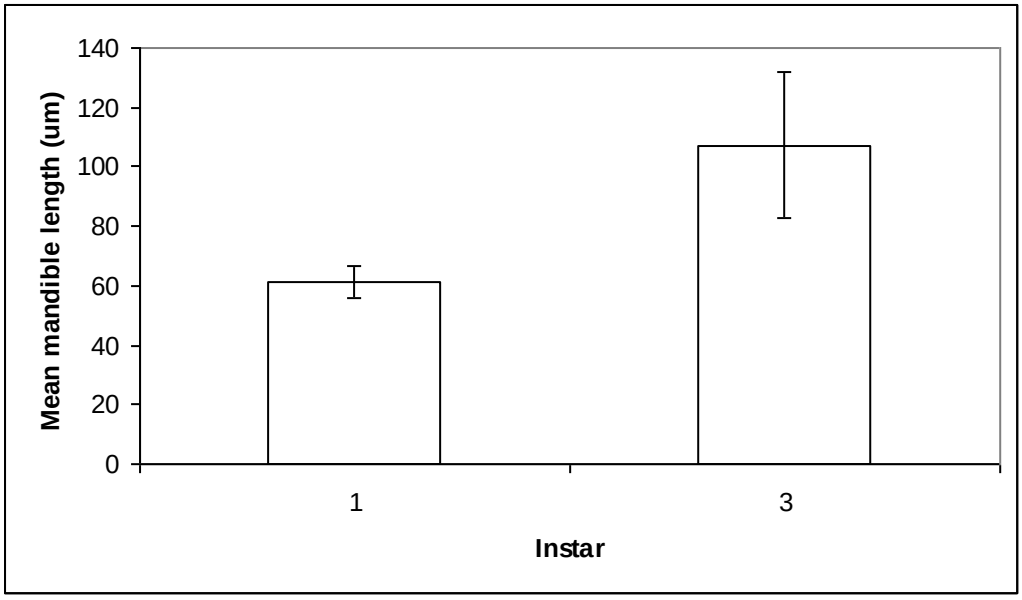

Figure 2.12. The mean mandible length 1st and 3rd instar Plutella xylostella larvae examined using Scanning Electron Microscope images. In both cases the sample size n=5. The error bars represent standard deviation.

Figure 2.13. Scanning Electron Microscope image of 1st instar Plutella xylostella larvae. Scale bar at the top represents 100µm length. The white line demonstrates the measurement used to determine mandible length.

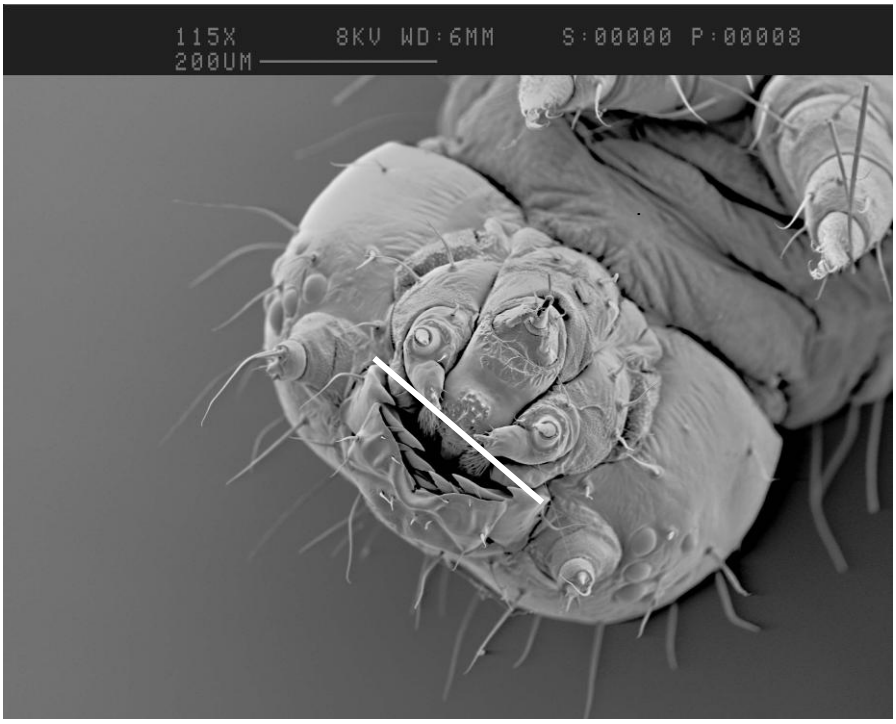

Figure 2.14. Scanning Electron Microscope image of 3rd instar Plutella xylostella larvae. Scale bar at the top represents 200µm length. The white line demonstrates the measurement used to determine mandible length.

2.8 Appendix 3

2.8.1 The effect of nutrition on the lethal and sub-lethal effects of cryolite exposure in P. xylostella.

2.8.1.1 The effect of agar on the lethal effects of cryolite in 1st and 3rd instar larvae

The impact of agar concentration on mortality was investigated using cryolite concentrations that were used in both assays. Mean mortality (not adjusted for control mortality) was plotted against dose. Instars were analysed separately. For instar 1 there were just 2 data points: control and 1.999 log µg/cm^2. For 3rd instar larvae the groups compared were the control, 1.397, 1.698 and 1999 log µg/cm^2. Mann Whitney U analysis (Minitab 15) was used to compare pairwise differences of mortality between agar concentrations.

2.8.1.2 The effect of agar concentration on weight change after 48 hour exposure to doses of cryolite

The data in assays 1 and 2 for 1st and 3rd instars were used to investigate the impact of agar concentration on weight change. Mean weight change (not adjusted for the control) was plotted against dose. Instars were analysed separately and the impact of agar was investigated using treatment groups that were used across both assays as above. Mann Whitney U pairwise comparison tests were performed (Minitab 15) to ascertain any significant effects of agar concentration on weight change.

2.8.2 Results

2.8.2.1 The effect of agar on the lethal effects of cryolite in 1st and 3rd instar larvae

When plotting percentage mortality against log dose, (Fig. 2.15), it was found that increasing the agar solution concentration 10-fold from 0.1 to a 1% solution caused a

decrease in overall mortality of between 7 and 8% in 1st instar larvae. This however, could only be confirmed from the results of the concentrations that overlapped in both assays which effectively was only the control and the dose of 1.999 log µg/cm^2.

Despite this observation, there was no significant effect of agar concentration on percentage mortality in either the control treatment (Mann Whitney U, $P = 0.340$) or dose 1.999 log µg/cm^2, (Mann Whitney U, $P = 0.258$).

The agar concentration applied with the treatments of cryolite had no significant effect on mortality in 3rd instar larvae. Percentage mortality for control groups, dose 1.397 log µg/cm^2 and 1.698 log µg/cm^2 resulted in equal mean percentage mortality between assays despite the concentration of agar. Only at dose 1.999 log µg/cm^2 did the mean percentage mortality increase by 18.6% when the agar concentration was increased ten-fold. However, there was significant overlapping of standard deviation between the two assays and therefore at no doses of cryolite treatment was there a significant effect of agar concentration on mean percentage mortality.

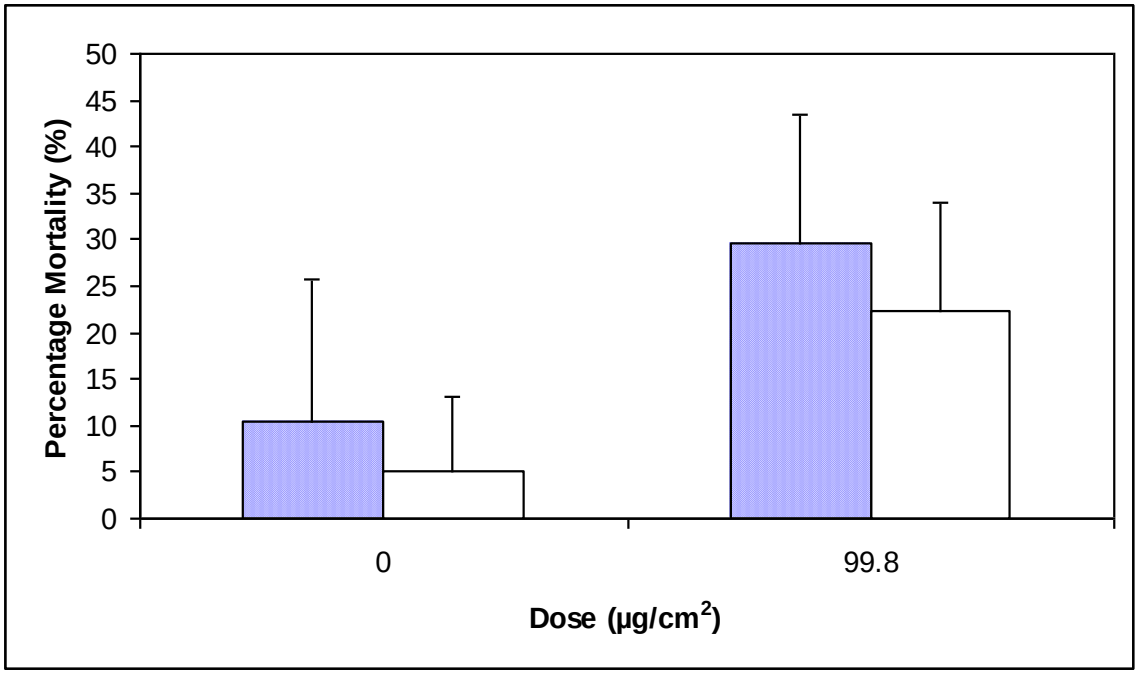

Figure 2.15. The effect of the agar concentrations 0.1 and 1% solutions, on percentage mortality in 1st instar larvae exposed to doses 0 and 99.8µg/cm^2 of cryolite for 48 hours. Shaded bars represent 0.1% agar concentration treatments and clear bars represent 1% agar solution treatments. Error bars represent standard deviation.

2.8.3 The effect of agar concentration on weight change in 1st and 3rd instar P.xylostella larvae after 48 hour exposure to doses of cryolite

When 1st instar larvae were exposed to a ten-fold increase in agar concentration mean weight increased in both the control group and at dose 1.999 log µg/cm^2 (Figure 2.18). There was almost a two-fold mean weight increase caused by 1% agar solution compared to 0.1% agar solution in treatment group 1.999µg/cm^2 and more than two-fold increase in mean weight increase in the control group when exposed to 1% agar solution. The effect in the control treatments was highly significant (Mann Whitney U, $P < 0.001$), and there was also a significant effect of agar concentration on weight change within the treatment group 1.999 log µg/cm^2 (Mann Whitney U, $P < 0.001$).

Increasing the agar solution from 0.1 to a 1% solution caused a reduction in mean weight gain in all 4 treatments (Figure 2.19). However, the effect was only significant within the control treatment (Mann Whitney U, $P < 0.005$).

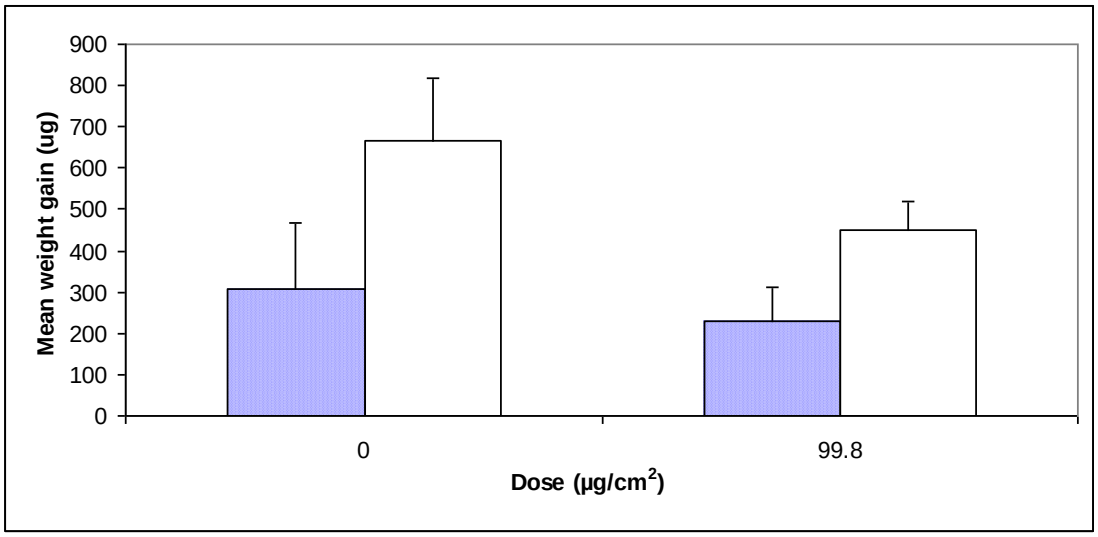

Figure 2.18. The effect of the agar concentrations 0.1 and 1% solutions on weight change in 1st instar larvae exposed to doses 0 and 99.8µg/cm^2 of cryolite for 48 hours. Shaded bars refer to 0.1% agar solution and clear bars refer to 1% agar solution. Error bars demonstrate standard deviation.

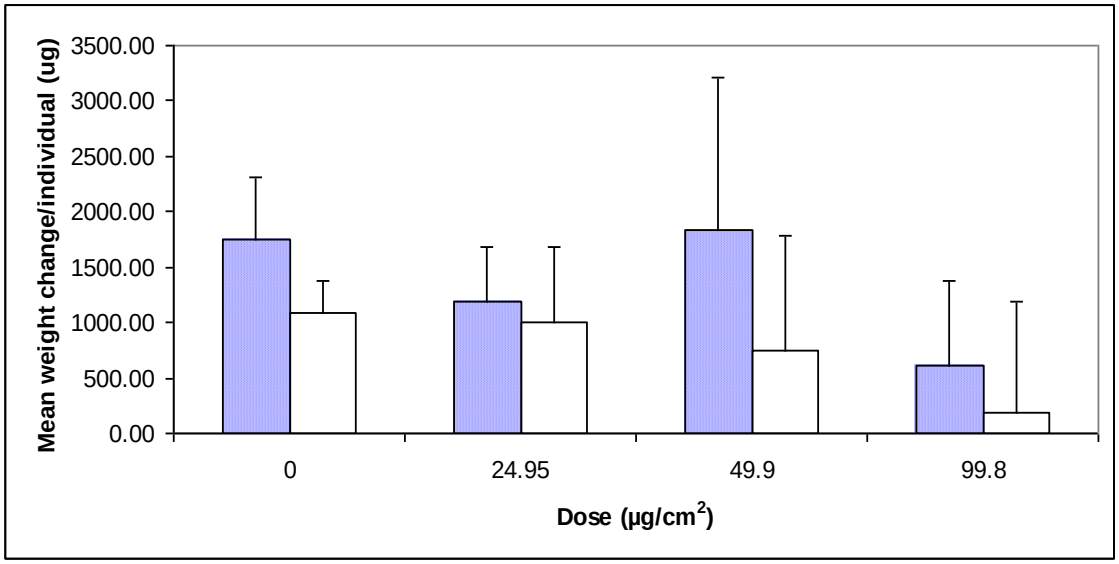

Figure 2.19. The effect of the agar concentrations 0.1 and 1% solutions on weight change in 3rd instar larvae exposed to doses 0, 24.95, 49.9 and 99.8µg/cm^2 of cryolite for 48 hours. Shaded bars refer to 0.1% agar solution and clear bars refer to 1% agar solution. Error bars demonstrate standard deviation.

2.8.4 Discussion

2.8.4.1 The impact of agar concentration on the lethal effects of cryolite

Increasing food availability has been shown to reduce the toxic affect of substances either through behavioural or intracellular processes (Postma *et al*, 1994: Barry *et al*, 1995).

Agar is the commercial name of a desiccated gelatinous extract obtained from specific species of red seaweed of the class Rhodophyta (Nilson *et al*, 1940). Composed of polysaccharides: agarose and agaropectin, the nutritional benefits of agar still remain unclear with the bulk of research originating from the mid 1930s and 40s.

Of the limited data available to assess the impacts of agar solution on the toxicity of cryolite in 1st instar larvae, at both treatment levels (both the control and doses 1.999 log $\mu g/cm^2$), increasing levels of agar was seen to reduce mortality. As the reduction in mortality in the cryolite treatment did not exceed that of the control, the results indicate that agar reduces "natural" mortality and does not impact on the lethal effects of cryolite in 1st instar larvae.

An obvious hypothesis for these results would suggest that increasing agar concentration 10-fold increases nutritional intake which in turn improves the health of the insect, reducing mortality that would occur naturally despite the toxic action of the cryolite.

Each treatment that was assessed represented 9 replicates each consisting of 6 larvae. However, as this assessment was based on only two treatment groups, the results

cannot be considered conclusive. The limits of the study presented here are acknowledged, but emphasis must be made that the study is merely investigative. The implications made are interesting yet further work is required to examine this relationship further. Therefore in order to confirm these observations, the full range of cryolite treatments ranging from the control to dose 3 log µg/cm^2 should be analysed through bioassays, each with a 0.1% and a 1% solution group and set of replicates.

Additionally, these results require verification as they conflict with those observed in the 3rd instar larval results where increasing agar concentration was seen to have no significant impact on either "natural" mortality or that which was caused by the ingestion of cryolite. This implies a difference in the reaction to nutrition between stage groups.

On the basis of the results presented in this investigation, agar appears to have no significant effect on the lethal effects of industrial cryolite in either 1st or 3rd instar larvae.

2.8.4.2 Impact of agar on the effects of cryolite on weight loss effects

For 1st instar larvae, increasing the concentration of the agar solution caused a significant increase in mean weight within both the control group and in the presence of cryolite in the treatment group 1.999 log µg/cm^2. These results agree with those observed by Nilson *et al*, (1940), who found that adding an agar solution to feed significantly increased weight gain in rats believed to be caused by beneficial effect of agar on the functioning of the intestinal tract. The mean weight increase observed in the cryolite treatment group did not exceed that observed in the control, and therefore

it is assumed that increasing agar solution did not impact on the sub lethal effect of cryolite toxicity on weight change.

The impact of increasing agar solution on weight change in 3rd instar larvae was variable and did not provide a linear relationship with dose. However, at none of the cryolite treatments was there a significant weight loss with increasing agar concentration. The results showed that in 3rd instar larvae, weight loss was a natural factor of increasing agar solution as there was a significant weight loss with increasing agar solution within the control group. Nilson *et al*, (1940) found that the digestibility of food decreased progressively with increasing concentrations of agar resulting in weight loss although this does not explain the differences between instars observed in the present study.

The 1st instar larvae are very small measuring approximately 1.7mm in length. At this stage, larvae feed both at the edges of the leaf and mine beneath the leaf surface to feed on the leaf tissue. There is the possibility that individual larvae may not have come into contact with the layer of agar situated on the leaf surface to the extent of that of 3rd instar larvae who consume all layers of the leaf. Therefore, they may have benefitted from the protection from desiccation afforded to the leaf by the presence of agar, resulting in weight gain. As 3rd instar larvae consume the whole of the leaf, they may have suffered the adverse affects caused by the agar solution. These theories are speculative and further work is required. Examination of the leaves under a microscope after the assay as well as analysis of the area consumed using a leaf-area meter will establish whether or not 1st instar larvae are mining under the leaf surface and avoiding consumption of the agar solution. Results for 1st instar larvae depended

on only two treatment groups due to the small overlap of doses between assay 1 and assay 2. The number of treatment groups tested for the effects of agar on weight change in 1st instar larvae should be increased in order to confirm the observations from this investigation.

Chapter 3

The Comparative Toxicity of Industrial and Pesticide-cryolite, the Mode of Action and the Implications of Environmental Release

3.1 Abstract

Cryolite, (Sodium hexafluoroaluminate, Na_3AlF_6), is one of the most commercially exploited fluoride-containing minerals in the world. It has been used as a pesticide in the US since the late 1930s and is also the main constituent of the electrolytic bath required in the reduction of alumina to aluminium in the primary aluminium industry. Due to the efficacy of cryolite as a pesticide, aluminium industry leaders have raised concerns over the consequences of such releases in to the surrounding environment and the subsequent monetary back-lash of such effects. Described simply as a 'stomach poison or disrupter' by pesticide manufacturers, little is known about the mode of action of cryolite or the physical and chemical differences between pesticide cryolite and that which is emitted from industrial processes. A series of LD_{50} bioassays were performed to evaluate the comparative toxicity of industrial cryolite and the pesticide product Kryocide© (Cerexagri, Inc) and, by manipulation of the compounds, the role that particle size and compound purity play in toxicity was examined. Industrial cryolite was shown to have a significantly different physical and chemical composition to that of the pesticide which, subsequently, results in the pesticide compound being significantly more toxic to *Plutella xylostella* larvae. This study found that although both factors play a significant role in toxicity, ultimately, it is the chemical composition of the compound which most significantly affects toxicity.

3.2 Introduction

Cryolite (Sodium hexafluoroaluminate), is one of the most commercially exploited fluoride-containing minerals in the world. A halide mineral of fluorine, cryolite is composed of aluminium, fluorine and sodium ions and is the main constituent of the electrolytic bath required in the reduction of alumina to aluminium in the primary aluminium industry. Cryolite is also used in an array of other industries including brick and ceramic works and has been applied as an agricultural insecticide in the US since the early 1930s.

Cryolite was used as a pesticide in the US, along with a range of other fluoride-containing pesticides, in response to a ban by the UK on imported crops with high arsenical residues (DeLong, 1934). Fluoride was considered an effective replacement for arsenicals as it caused high acute toxicity and caused little damage to plants and human health.

Presently, there are four insecticidal products on the market, produced by leading manufacturers Ceraxagri, Inc. and Gowan Co., whose products range from 96% cryolite down to 20% in baiting products. These pesticides are applied to many fruit, vegetable and ornamental crops and are predominantly used against the pests of grapes, potatoes, citrus and brassica plants which include Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), blue-green citrus root weevil (*Pachnaeus litus* (Germar)), grape berry moth (*Endopiza viteana* (Clemens)) and the Diamond-back moth (*Plutella xylostella* (L)) (EPA, 1996). Popular due to its efficacy, low solubility, its status as an organic pesticide, and the inability of insects to develop resistance, cryolite was replaced for a period of time by more specialised products. However, in

recent years, it has reappeared on the market, particularly in the vineyards of California, US, and has been described as "…unsurpassed in terms of efficiency and cost/benefit ratio..." (Wahlstrom *et al*. 1996).

Surprisingly, the abundant use of cryolite and other fluorine insecticides in the US has stimulated very little research into the mechanisms behind the toxicity. Cryolite has been described as a stomach poison by the US Environmental Protection Agency (EPA, 1996) and they refer to work conducted by Corbett, *et al*, (1974), who state that cryolite complexes with metal-containing enzymes in the stomach. Ware, (1986; cited by Huang *et al*, 1995) outlines these systems as those involving calcium, magnesium and iron.

Particle size is one important characteristic to consider when investigating the toxicity of a substance. In the 1940s, it was found that toxicity generally increased with declining particle size (Smith and Goodhue, 1942). The inverse exponential relationship between surface area and particle size determines the potential number of reactive groups on the surface of the material which theoretically should determine the reactivity and therefore the toxicity of the substance (Nell *et al*, 2006). Manufacturers claim that cryolite acts as a stomach disrupter and causes abrasion to the foregut equivalent to consuming glass (Gowan, private correspondence). Again, particle size is an important factor to consider when investigating mechanical damage to tissues. The results of research conducted by Shelton *et al*, (2007), found that mortality of chewing herbivores increased with increasing particle size and concluded that this was due to abrasion in the gut.

The manufacture of 1 tonne of aluminium produces between 8 and 20kg of excess cryolite of which the typical fugitive release amounts to approximately 3.36g/second (Alcan, 2001). Despite both fugitive and controlled emissions from aluminium smelters, of which two are currently operating in Britain, cryolite is not included in the review programme for the BPD (Biocidal Products Directive) for use in biocidal products and therefore cannot legally be used as an insecticide in the EU (HSE, private correspondence).

3.2.1 Aims

The function of both particle size and sample purity in toxicity were examined in an attempt to gain insight in to the mechanism behind cryolite toxicity and to evaluate the comparative toxicity of industrial cryolite and Kryocide© (Cerexagri, Inc), a cryolite insecticide.

3.3 Methods

The LD_{50} bioassay methods used in this chapter broadly followed those outlined in *Chapter 2*. Third instar *Plutella xylostella* (L.) (Lepidoptera: *Plutellidae*) larvae were used in each of the three toxicity investigations to allow comparison with the LD_{50} value of industrial cryolite highlighted in the previous chapter. For each investigation the initial ranging bioassays employed nine replicates of each concentration of cryolite in the range of 0.795 – 1.999 log µg/cm^2, (Huang *et al,* 1995) and a control of 1% agar solution. Each replicate used six 3rd instar larvae. All of the bioassays described were conducted using a 1% agar solution as the medium of the suspended treatments. Nine replicate dishes treated with dose 2.258 (log µg/cm^2) of industrial cryolite were included in each bioassay to ensure the quality of the bioassay was equal to that conducted in *Chapter 2*, and therefore that a comparison was viable.

3.3.1 The comparative toxicity of pesticide and industrial cryolite

The LD_{50} of Kryocide was determined in a 48 hour ranging bioassay (n = 9), to allow a comparison with the toxicity of industrial cryolite assessed in *Chapter 2*.

3.3.2 The role of particle size in the toxicity of cryolite

Scanning Electron Microscope images of industrial cryolite and Kryocide were produced and studied to establish whether or not there was a difference in particle size between the two materials (Appendix 1).

Samples of both Kryocide and industrial cryolite were analysed by Intertek (Redcar, UK) using a Coulter LS230 Laser Diffraction Particle Size Analyser fitted with the Variable Fluids Module in order to determine the particle size distribution.

Industrial cryolite was then sieved through a 38µm Endecott sieve to reduce the particle sizes within the material and the LD_{50} was investigated in a ranging bioassay (n=9). In the second bioassay, the concentrations of sieved material ranged from 1.999 to 3 log µg/cm^2, as described in *Chapter 2*.

3.3.3 The role of impurities in the toxicity of cryolite

In collaboration with the CEAM Advanced Materials Division, Newcastle University, the mineral phases of both Kryocide and industrial cryolite were assessed from qualitative X Ray Diffraction (XRD). Quantities of each material were hand-pressed in to sample holders and processed in a Phillips X'pert XRD Analyser to produce phase diagrams describing the x ray patterns of the crystal phases in the samples. The International Centre for Diffraction Data (ICDD) was used to reference the peaks of the phase diagrams and determine the substances within each material.

A sample of 99.98% pure cryolite (Sigma Aldrich) was sieved through a 250 mesh Endecott sieve to mirror the particle size range of the industrial cryolite tested in *Chapter 2*. The LD_{50} was determined in a ranging bioassay (dose range 0.795 - 1.999 log µg/cm^2, n=9).

3.3.4 Data analysis

When comparing LD_{50} values, treatments were deemed to be significantly different when the 95% confidence limits did not overlap (Robertson & Preisler, 1992). Because of the nature of the particle size distribution data obtained through laser diffraction, formal statistics could not be performed to analyse the significance of the difference between particle size ranges between compounds.

3.4 Results

3.4.1 The comparative toxicity of pesticide and industrial cryolite

At 48 hours, industrial cryolite, with a particle diameter less than 38µm, had an LD_{50} of 2.964 log µg/cm^2, whereas, Kryocide© had an LD_{50} of 1.970 log µg/cm^2 and an LD_{50} of 1.859 log µg/cm^2 for cryolite (99.98% cryolite content) was determined (Table 3.1). When comparing the overlap of the 95% confidence intervals of each of the LD_{50} vales it was found that there were significant differences in toxicity between all 3 treatments and between the LD_{50} of industrial cryolite found in *Chapter 2* (Table 3.1).

3.4.2 The role of particle size in the toxicity of cryolite

Laser diffraction analysis of a sample of Kryocide and a sample of industrial cryolite, sieved through the 250 mesh sieve, showed the particle size ranges of the two compounds differed and that Kryocide had a higher proportion of smaller particles than industrial cryolite, as can be seen in Table 3.2 and Figure 3.1. At the smaller range of particle sizes, industrial cryolite did not elicit a response threshold for mortality. Although mortality plateaued at the intermediate doses, it was seen to significantly increase at dose 3 log µg/cm^2 (Fig 3.2).

3.3.3 The role of impurities in the toxicity of cryolite

X Ray Diffraction (XRD) found that there were stark differences in the composition of the industrial cryolite and Kryocide (Fig. 3.3 and 3.4). The Material Safety Data Sheet (MSDS) for electrolytic bath (Alcan, 2006) states that the standard composition of industrial cryolite sourced from Alcan contains between 75 and 85% cryolite. Electrolytic bath typically contains 5-7% calcium fluoride, 5-7% aluminium fluoride

and 2-8% alumina (Chanania and Eby, 2000). XRD showed that industrial cryolite also contained quantities of chiolite, sodium calcium aluminium fluoride and fluorite (Fig. 3.3). In contrast to this, XRD showed Kryocide to only contain cryolite (Fig. 3.4), which the manufacturer reports comprises 94-96% of the material volume (Ceraxagri Inc, 2006). The other components were in such small quantities that they were not recognised using XRD, however a similar product manufactured by Gowan Inc. contains 1% Chiolite, 0.3% sulphur and 0.2% silica (Gowan Company, personal. correspondence, 2009.).

Table 3.1. The LD_{50} and 95% confidence limits of four cryolite compounds to 3rd instar Plutella xylostella at 48 hours of exposure

Compound	Maximum particle size (µm)	Cryolite concentration (%)	LD_{50} (log µg/cm^2)	95% CL ± (log)
Industrial cryolite (*Chapter 2*)	256.9	75-85	2.258	0.023
Kryocide	101.1	94-96	1.97	0.032
Industrial cryolite	38	75-85	2.964	0.018
Cryolite (Sigma Aldrich)	256.9	99.98	1.859	0.015

Table 3.2. The particle size distributions of industrial cryolite passed through a 250 mesh sieve, and Kryocide, as analysed by laser diffraction.

Compound	10% <µm	50% <µm	90% <µm	Minimum Particle size (µm)	Maximum Particle size (µm)	Mean Particle size (µm)
Industrial cryolite	11.9	41	106	0.04	101.1	50.4
Kryocide©	7.1	21.4	47.5	0.04	256.9	24.6

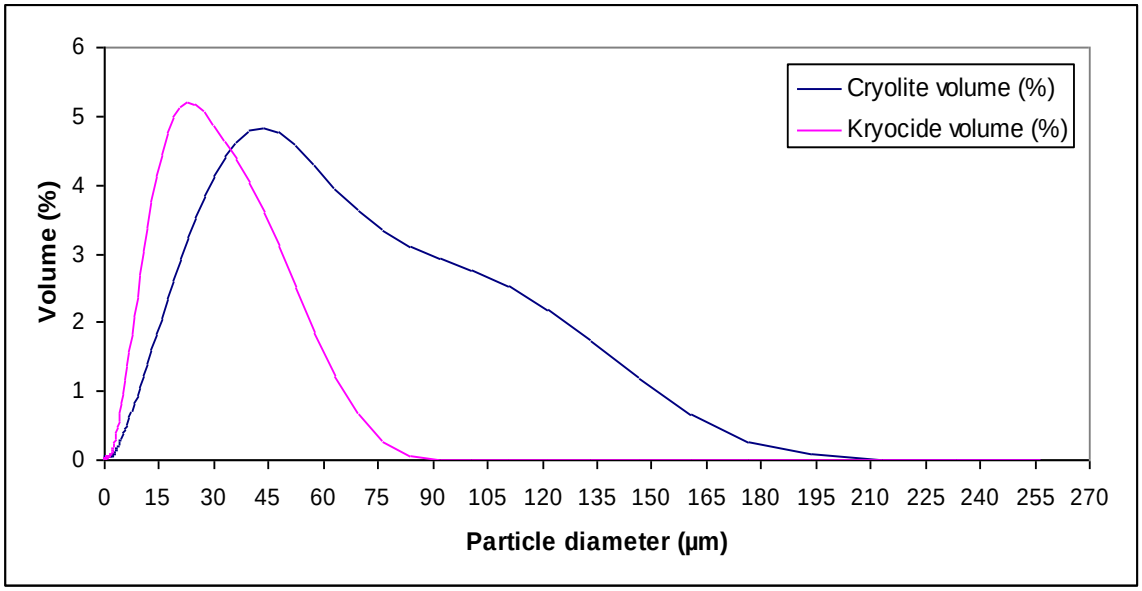

Fig.3.1. Particle size distribution frequencies of industrial cryolite and Kryocide as analysed by laser diffraction.

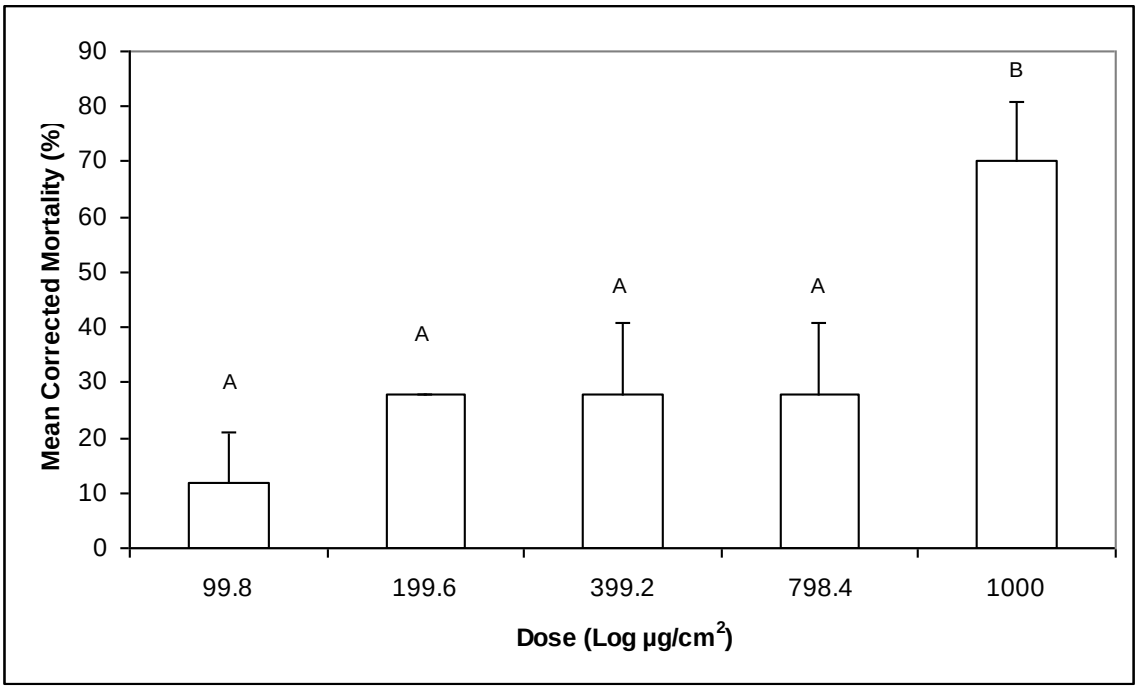

Fig. 3.2. The mean corrected mortality (%) of 3rd instar P.xylostella larvae exposed for 48 hours to doses of industrial cryolite, with a particle diameter less than 38µm.. N = 52. Error bars indicate standard deviation. Treatments with a different letter are significantly different at P < 0.05.

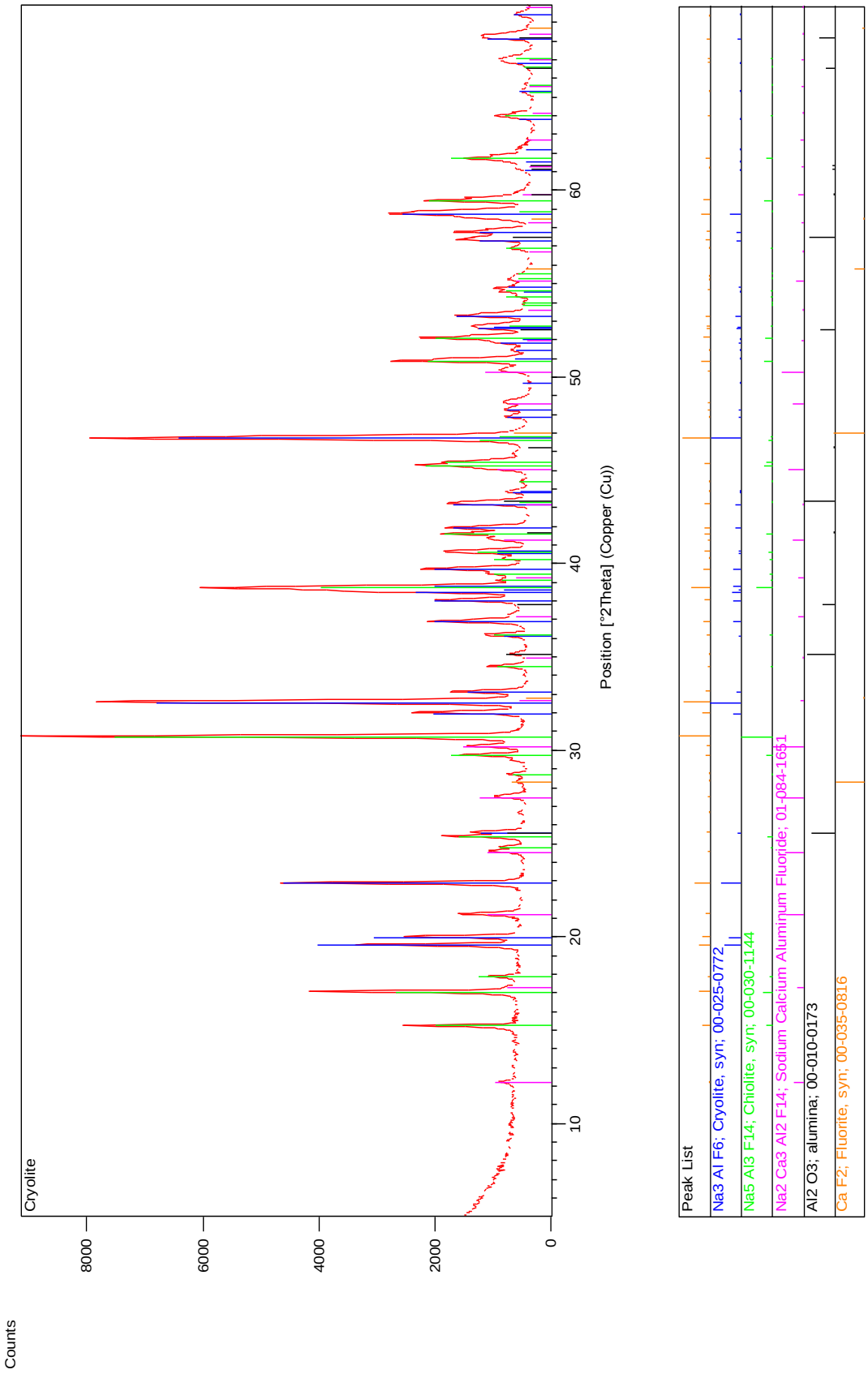

Fig. 3.3. Phase diagram of industrial cryolite produced by X Ray Diffraction. The Peak List links the crystal phase peaks with the minerals in the compound.

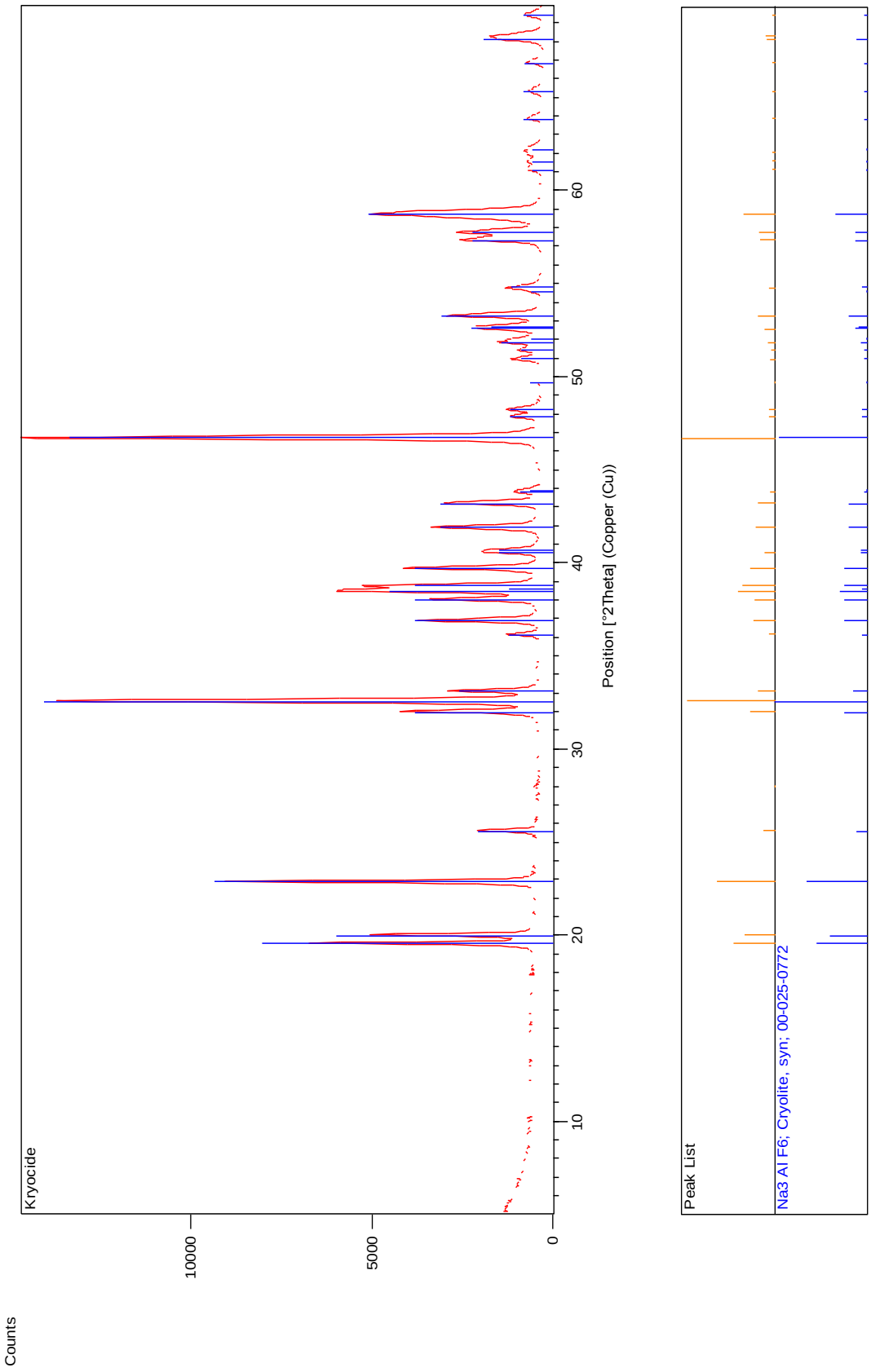

Fig. 3.4. Phase diagram of Kryocide produced by X Ray Diffraction. The Peak List links the crystal phase peaks with the minerals present in the compound.

3.5 Discussion

Particle-characteristics that determine tissue injury are particle size, chemical composition, surface structure, solubility, shape and aggregation (Nel *et al*, 2006). The results of this study show that when studying the comparative mortality caused by industrial cryolite and Kryocide, both particle size and the chemical composition of the sample are important considerations.

Particle size was shown to have a significant positive association with mortality which corresponds with those observations noted by Shelton et al, (2007), when investigating the effect of cryolite crystal size on mortality in termites. Shelton *et al.* (2007) suggest that the mortality caused by cryolite results from physical abrasion of the crop and foregut of the insect which increases with particle size, leading to tissue damage, desiccation and mortality. However, despite the greater toxicity of larger particles, when particles are small, the lack a response threshold indicates that the solubility of the compound increases as the surface area of the particles increase, reducing the potential to over-load the digestive system and preventing expulsion of cryolite from the gut. Although Shelton *et al*, (2007) do not suggest physical abrasion as an exclusive mechanism, they do imply a predominantly benign process involving limited interaction at the cellular, sub-cellular and protein levels. The present study found that the chemical composition of the compound similarly played a significant role in toxicity as the results of the Kryocide bioassay showed that industrial cryolite was significantly less damaging, regardless of the particle size ranges tested.

XRD revealed that industrial cryolite contained several impurities, typical of electrolytic bath from an aluminium smelter. Molten electrolytic bath consists

predominantly of cryolite and chiolite which form the electrolytic pot lining. However, fluorspar (calcium fluoride) and aluminium fluoride are added to the bath to reduce the melting point from 1009ºC to 920-980ºC. Dissolved alumina is the raw material from which aluminium is derived and which also aids the reduction of the melting temperature. Calcium oxide is a naturally-occurring impurity present in alumina. In the weeks of first operation of a newly lined electrolytic pot, sodium carbonate is added to the bath to replace the sodium lost from the system through preferential absorption in to the lining. Aluminium fluoride is also added to the system to match the added sodium content and restore the optimum ratio. Therefore, the compounds recorded in the XRD of the industrial cryolite sample were consistent with production.

An important finding of this study was that there is a hierarchy of factors which contribute towards cryolite toxicity. The concentration of cryolite within the sample had greater influence than particle size in determining its damaging effect. When the compounds tested had equal chemical composition, it was the compound containing the larger particle sizes which caused greatest mortality, and when both compounds had equal particle size ranges, it was the compound with the greatest quantity of cryolite that was more toxic. However, when both chemical composition and particle size varied between test compounds, it was the compound with the greatest proportion of cryolite which was most toxic. This of course is subject to what the contaminants in the sample are and we must assume that the hazard-potential of the contaminants in industrial cryolite were relatively benign.

The mode of action of cryolite has never been clearly established and there is a considerable lack of evidence to support theories surrounding this issue. Interestingly, the results from this study serve to cast doubt on the claims made by cryolite insecticide manufacturers that their products act as stomach disrupters resulting in severe abrasion, equivalent to the insect consuming glass. Although physical abrasion may be a contributing factor to mortality, as indicated when particle size was altered in the bioassay, the results of this study lend support to those theories of chemical rather than physical action. Evans *et al* (1939) suggested that the NaF portion of the cryolite molecule is responsible for its toxicity, whereas other theories consider that the lethal and sub-lethal effects may be due to the cryolite inhibiting the iron, calcium and magnesium-containing enzyme systems (Ware, 1986: cited in Huang *et al*. 1995).

Ultimately, an important question to address is: are releases of industrial cryolite from aluminium smelters in the UK more potentially damaging than applications of pesticide cryolite? The factors to consider are particle size, purity of sample and concentration. Kryocide particle size ranges from 0.04 to 36.24µm with a mean particle size of 24.6µm. Although not investigated in this study, a compliance check monitoring the emissions of the smelter to air was conducted at Rio Tinto Alcan, Lynemouth in 2007. The report detailed the particle sizing of the final emission after treatment by the bag filters. The dust collector and treatment system associated with cryolite emissions was not included in the survey, but essentially the equipment is the same. The smelter releases particulates in the size range of 0.32 to 11.5µm diameter showing that the particle sizes are smaller than those recorded for the Kryocide sample. We know that pesticide cryolite has a greater purity (%) than industrial cryolite. As for concentrations emitted into the environment, the maximum seasonal

application of cryolite to crops in the US equates to approximately 17.3 g/m^2 (EPA, 1996) which in 2008 was reported be approximately 23 times greater than the deposits of cryolite around the worst polluting smelters in the world (EU, 2008). With recent improvements in Better Available Technologies (BAT) it is estimated that concentrations in the vicinity of smelters are very low. Although only a tentative conclusion, based on the information available, it is likely that the concentrations of industrial cryolite, emitted from any aluminium smelter, are not as damaging as that applied to crops in the US.

3.6 Appendix 1

3.6.1 SEM analysis of particle size

A series of Scanning Electron Microscopy (SEM) images of samples of industrial cryolite and Kryocide (© Ceraxagri) were taken (Fig. 3.5 to 3.8) in order to observe the potential differences in particle sizes, a positive analysis of which would result in a more in-depth assessment of the particle size ranges. Industrial cryolite, sourced from the pot rooms of Lynemouth Aluminium smelter, was sieved through a 250 mesh sieve, as done in *Chapter 2*. The two compounds were mounted onto adhesive carbon discs which in turn were mounted onto aluminium stubs. Both dabbing and blowing techniques were employed in mounting the samples to achieve a variety of dispersal densities across the surface of the discs.

The mounted samples were left to dry overnight and then coated with the standard 15nm coating of gold using a Polaron SEM Coating Unit. Specimens were examined using a Stereoscan S40 Electron Microscope. Help with the processing and supply of the equipment was provided by the EM Research Services, Newcastle University, Newcastle upon Tyne.

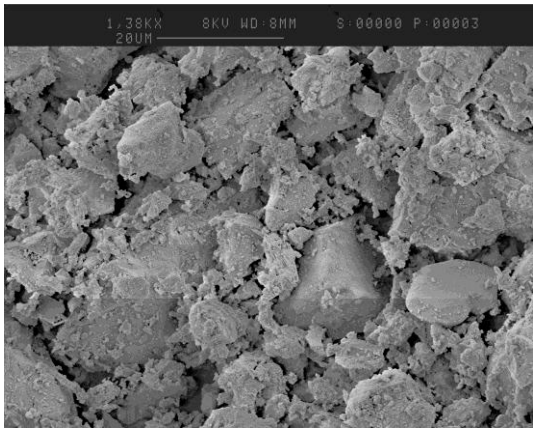

Figure 3.5. Scanning Electron Microscope image of industrial cryolite, applied to the carbon disc by dabbing the particulate. Scale bar at the top represents 20µm length.

Figure 3.6. Scanning Electron Microscope image of Kryocide (© Ceraxagri), applied to the carbon disc by dabbing the particulate. Scale bar at the top represents 20µm length.

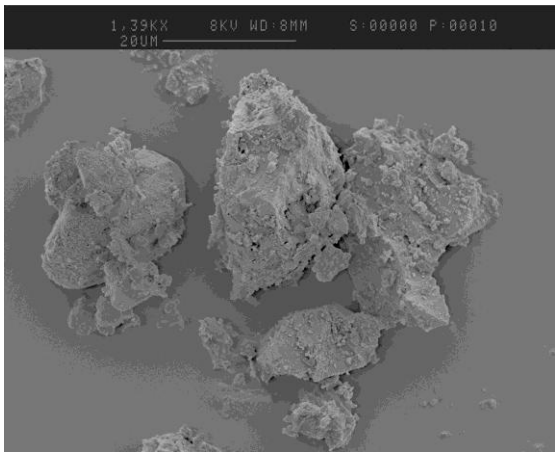

Figure 3.7. Scanning Electron Microscope image of industrial cryolite, applied to the carbon disc by blowing the particulate. Scale bar at the top represents 20µm length.

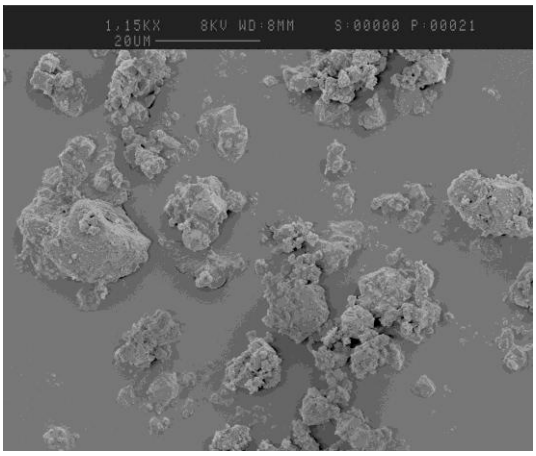

Figure 3.8. Scanning Electron Microscope image of Kryocide (© Ceraxagri), applied to the carbon disc by blowing the particulate. Scale bar at the top represents 20µm length.

Chapter 4

The Impact of Modern Aluminium Smelting Practice on the Emission of Particulates in to the Environment

4.1 Abstract

Aluminium smelting has historically been one of the predominant sources of fluoride pollution of all the heavy industries. Due to the introduction of the Directive of Integrated Pollution, Prevention and Control (IPPC; 96/61/EC), the Part A (1) Environmental Permit operated by smelters has led to the implementation of Better Available Technology (BAT) which has significantly reduced fugitive-fluoride loads (Rio Tinto Alcan, personnel correspondence, 2007) . However, little is known about the volume and environmental toxicity of cryolite (Sodium hexafluoroaluminate, Na_3AlF_6) released in to the environment, a fluoride-containing compound used in the smelting process which is fugitively released in to the environment from chimneys and designated storage units. An analysis of the toxicity of industrial cryolite release can be made once the concentration of cryolite in the environment is quantified. Therefore, the deposition of dust was monitored over a period of ten months, at several locations within the vicinity of a smelter operating BAT, in order to quantify the concentration of airborne cryolite.

The study found that the volume of dust at any one site did not exceed the European Community Short Term Ambient Guideline Concentration of Particulate Matter or the volume required for analysis by X Ray Diffraction and, as such, the deposition of cryolite could not be quantified. A significant relationship was observed between aluminium production at the smelter and deposition at the four sites yielding the

greatest volume of dust which indicates that, despite low dust emissions, the production of aluminium still impacts the environment.

4.2 Introduction

The use of fluoride-containing raw materials within any industry has the potential to release gaseous and/or particulate fluoride in to the environment (Haidouti, 1991). Aluminium smelters and the ceramics industry are the largest producers of fugitive releases of fluoride of all the heavy industries (Weinstein & Davison, 2004; Franzaring *et al*, 2006). Fluoride is released to both air and the surrounding waters through airborne emissions and effluent, causing a surge in the background levels of fluoride in the local area (Buse, 1986).

Historically, industrial fluoride particulates constituted up to half of the total atmospheric fluoride load in the fall out area of a source (Wright & Thompson, 1978), but concentrations have been known to soar in cases of accidental fugitive release to as much as 50 times the normal concentration for short periods of time (Davison, 1987). For over a century there has been a clear understanding of the cause and effect relationship between these heavy industries and subsequent damage that they cause (Wright & Thompson, 1978; Davison, 1987; Davies *et al*, 1998).

However, in accordance with the Directive of Integrated Pollution, Prevention and Control (IPPC; 96/61/EC) (EU Risk Assessment, 2008), in response to growing public and government awareness of the nature of fluoride, increasingly stringent containment measures to reduce losses of fluoride to the surrounding environment have been implemented in aluminium smelters. The implementation of such Better Available Technology (BAT) includes the hooding of electrolytic pots to collect gases and the installation of effective distillation columns in the form of scrubbing systems which have significantly reduced fluoride loads (Franzaring *et al*, 2006; Alcan, 2001).

Cryolite, (Sodium hexafluoroaluminate), is a fluoride-containing compound which forms the main constituent of the electrolytic bath used in the aluminium industry to reduce alumina to aluminium. In 2001, it was calculated that the manufacture of 1 tonne of aluminium produced between 8 and 20kg of excess cryolite of which the typical fugitive release amounted to approximately 3.36g/second (Alcan, 2001).

Cryolite has also been applied as an organic pesticide in the US since the 1930s, targeting the pests of potatoes, grapes, brassica and citrus plants (EPA, 1996). The focus of *Chapter 3* was a comparison of toxicity between Kryocide, a cryolite-pesticide product, and industrial cryolite emitted from an aluminium smelter. The study concluded that, based on the results, the pesticide product is more toxic to the Diamondback moth (*Plutella xylostella*), than the industrial cryolite.

The maximum seasonal application of cryolite to crops in the US equates to approximately 17.3 g/m^2 (EPA, 1996) which in 2008 was reported be approximately 23 times greater than the deposits of cryolite around the worst polluting smelters in the world (EU Risk Assessment, 2008). The aim of the present study was quantify the airborne concentration of cryolite in the vicinity of an aluminium smelter in order to allow a comparison of toxicity between the environmental concentration and the concentration of pesticide cryolite applied to crops in the US.

In light of the introduction of BAT, this study also aimed to investigate the impact of aluminium production on the deposition of particulates, and more specifically cryolite, in the fall out area of the smelter.

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4.3 Methods

4.3.1 Sampling

Dust samples were collected monthly for a period of ten months from seven sites (Table 4.1 and Fig. 4.1) surrounding the aluminium smelter at Lynemouth, Northumberland (NZ 29443 89711), between April 2009 and January 2010. Sites were selected based on pre-existing Stevenson Screen boxes positioned by the smelter for the monitoring of airborne fluoride concentrations. Due to this, all sites were positioned upwind from the emission-source as downwind sampling would require samples taken from positions in the North Sea. A series of Frisbee dust collectors were constructed based on the design of the dry Frisbee dust deposit gauge designed by Ian Hanby (Vallack, 1995). Plastic Frisbees with a surface area of 0.0594m^2 and a 29.5cm diameter were secured to the lids of 4 litre collection bottles. A hole was drilled though both the Frisbee and the bottle lid to allow rain water and dust to run through. Each dust collector was secured to the existing sampling equipment located at 7 sites surrounding the aluminium smelter, in order to add height away from vegetation which could obstruct dust deposition and also to add some protection from theft. A metal ring was placed around the neck of each bottle and bungee cords were used to secure the bottle in place (Fig. 4.2).

4.3.2 Field collection

At monthly intervals, the Frisbees at each site were cleaned into the collection bottles using 100ml distilled water and a rubber pipette. The collection bottles and their contents were then removed and replaced with clean bottles and secured back in to place.

Table 4.1. The National Grid References of the seven dust sampling sites located in the vicinity of Lynemouth Aluminium Smelter.

Site	National Grid Reference
1	NZ 29303 89433
2	NZ 29461 89325
3	NZ 29657 89235
4	NZ 29117 89761
5	NZ 30096 89761
6	NZ 25984 94041
7	NZ 24488 89694

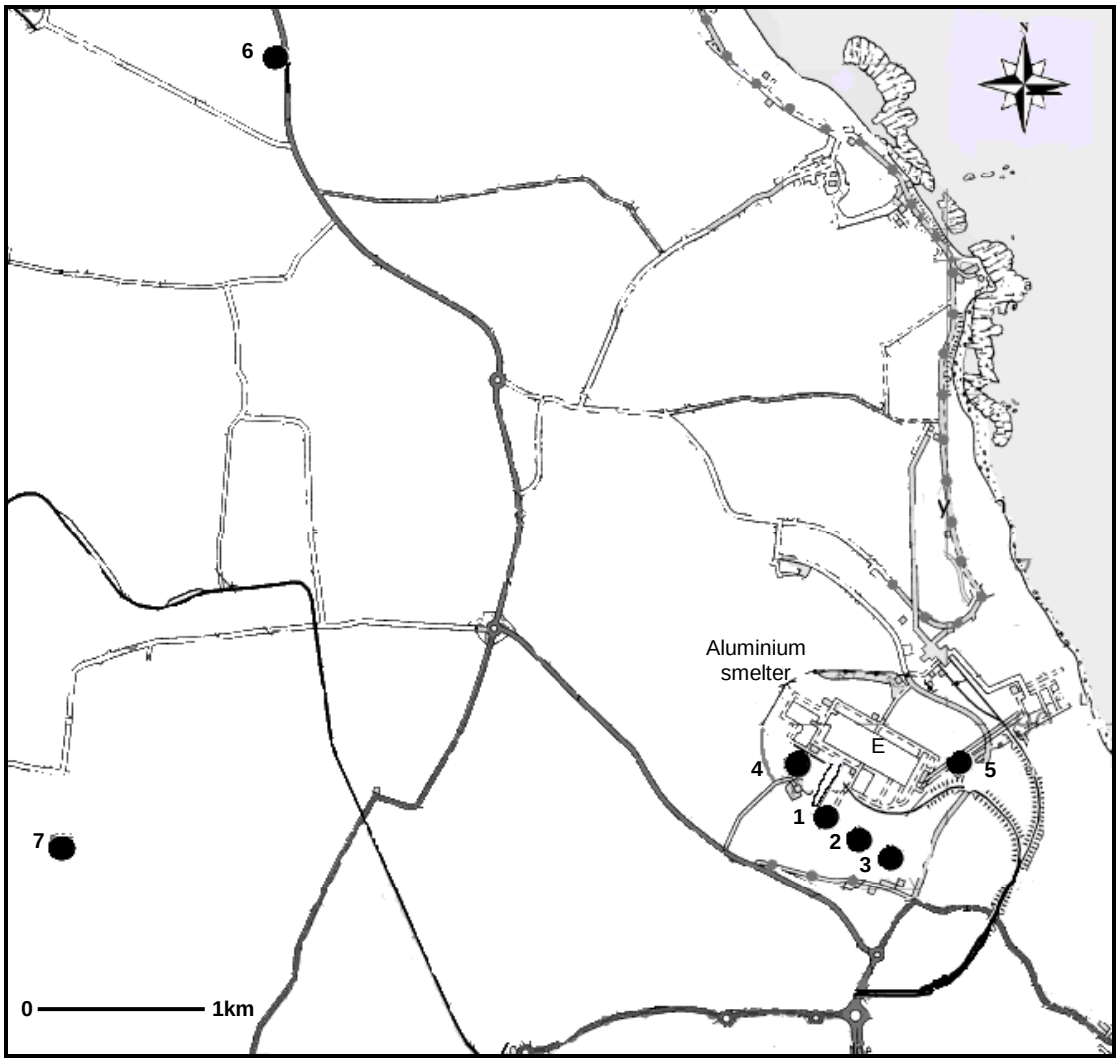


Figure 4.1. The locations of the seven Frisbee dust gauges located around the aluminium smelter in Lynemouth, Northumberland. E indicates the site of the emission source.

Figure 4.2. An example of a Frisbee dust collector positioned in the field and secured at height to ensure unobstructed deposition of dust in to the bottle.

4.3.3 Laboratory treatment

Nine centimetre-diameter Whatman ash-less filter papers were dried in an oven for 1 hour at 80ºC and equilibrated in a desiccator for 2 hours before being weighed on a microbalance. The contents of the collection bottle were filtered through a 20cm diameter funnel leading to a 1mm mesh tea strainer, to remove extraneous material.

Under suction, the contents were passed through a Whatman 3 piece filter, containing the pre-weighed filter paper, leading to a 2 litre Buchner flask. A rubber pipette and a wash bottle containing distilled water were use to dislodge deposits from inside the collection bottle which were passed through the filter. The filter papers were then dried, equilibrated and re-weighed (Vallack, 1995).

4.3.4 The impact of distance from the emission source on dust deposition

Using a Garmin Etrex GPS system, the distances of the 7 sites from the emission source were calculated using Memory Map software and the relationship between dust deposition and distance was examined through regression analysis. Using data provided from the aluminium smelter, the interaction between dust deposition and aluminium production was investigated.

4.3.5 The effect of meteorological factors on dust deposition

Meteorological data recording wind speed, temperature and rainfall were provided by Rio Tinto Alcan for each sampling period and the relationship between dust deposition and the meteorological variables were examined though plots of the data and regression analysis. The affect of wind direction on dust deposition could not be analysed due to a malfunction of the equipment during the sampling period.

4.3.6 Quantitative analysis of environmental concentrations of cryolite around the smelter

A minimum of 12g of dust is required to conduct quantitative X Ray Diffraction (XRD). Over the period of sampling, the total volume of dust recovered from the dust gauges did not exceed 1g at any site and totalled just 5.255g from all sites. Therefore, XRD and the subsequent calculation of environmental concentrations of cryolite could not be made.

4.4 Results

4.4.1 Monthly dust deposition at each site

There was a significant positive exponential relationship, at $P < 0.05$, between dust deposition and month at each of the seven sites when month runs from April to January (Fig. 4.3 to 4.8) although it does not appear to be a close fit. In six of the seven sites, the maximum mean deposition ($g/m^2/day$) was recorded in January 2010, but at site 1 the greatest mean volume of dust ($g/m^2/day$) was recovered in November (Fig. 4.3). The mean deposition of dust fluctuated monthly between sites (Fig. 4.3-4.8) and there was no one site that consistently yielded the greatest or the lowest volume of dust (Fig. 4.42-4.51).

4.4.2 The effect of distance from the emission source on dust deposition and the difference between sites

There was no significant relationship, between distance from the emission source and dust deposition in any month ($P > 0.05$) (Fig. 4.31 – 4.40). Due to evidence that large particulate matter deposits close to the source of emission resulting in greater deposition of dust within 2km of aluminium smelters (Mirlean *et al*. 2007) sites exceeding a distance of 2km from the emission source were removed from the analysis and the linear regression was repeated (Fig. 4.41). There was still no significant linear effect of distance from the emission source and mean dust deposition ($g/m^2/day$) ($R^2 = 0.1735$, $P = 0.28$).

The greatest total deposition of dust was recorded at site two located 519 metres from the emission source, which also yielded the highest minimum monthly deposition and the greatest mean within the 10 month period (Table 4.1 and Fig. 4.52). The greatest maximum monthly deposition of dust was recorded at site six, located 5500 metres

from the emission source (Table 4.1, Fig. 4.42-4.51). The lowest maximum, mean and total volume of deposition was recorded at site one, located 486 metres from the source of emission which was the closest site to the smelter (Table 4.1).

4.4.3 The effect of rainfall on dust deposition

There was no significant linear relationship between total rainfall (mm/month), and dust deposition at any of the seven sampling sites (Fig 4.9 - 4.15). January stood out at six out of the seven sites (excluding site one), as yielding an anomalously high volume of dust deposition with intermediate levels of rainfall. The data from January were removed from the analysis and the mean deposition of dust (g/m^2/day) across all seven sites was plotted against total rainfall (mm) per month (Fig. 4.16). There was no significant relationship ($R^2 = 0.3814$, $P = 0.076$) between the two variables.

4.4.4 The effect of temperature on dust deposition

There was a significant negative relationship between mean temperature/month (ºC), and mean dust deposition (g/m^2/day) at site four ($R^2 = 0.6335$, $P < 0.01$) (Fig. 4.20), and site seven ($R^2 = 0.1295$, $P < 0.05$) (Fig. 4.23). There was no significant relationship between temperature and dust deposition at any of the remaining five sampling sites ($P < 0.05$) (Fig 4.17, 4.18, 4.19, 4.21, 4.22).

4.4.5 The effect of wind speed on dust deposition

There was a significant positive linear relationship between mean wind speed (mph/month) and mean dust deposition (g/m^2/day) at site four ($R^2 = 0.5121$, $P < 0.05$) (Fig. 4.27). No significant relationship was observed between the two variables at any of the remaining six sampling sites (Fig. 4.24 – 4.30).

4.4.6 The impact of rates of aluminium production on dust deposition

There was a significant positive linear relationship between aluminium production (tonnes/day/month) and mean dust deposition (g/m^2/day/month) at site two (R^2 = 0.5994, $P < 0.01$) (Fig. 4.54), site four (R^2 = 0.5622, $P < 0.05$) (Fig. 4.56), site six (R^2 = 0.4366, $P < 0.05$) (Fig, 4.58), and site seven (R^2 = 0.3659, $P < 0.01$) (Fig. 4.59). There was no significant relationship observed between the two variables at sites one, three or five (Fig. 4.53 – 4.59).

Table 4.2. The minimum, maximum, mean and total dust deposition recorded at each sampling site over the sampling period of March to January 2009-2010. Values highlighted in bold refer to the highest or lowest values.

Site	Minimum dust deposition(g/m^2/day)	Maximum monthly dust deposition (g/m^2/day)	Mean dust deposition (g/m^2/day)	Total dust deposition (g)
1	0.007	**0.127**	**0.040**	**0.307**
2	**0.009**	0.369	**0.110**	**0.579**
3	0.005	0.140	0.051	0.450
4	0.004	0.494	0.095	0.422
5	0.008	0.235	0.054	0.391
6	**0.003**	**0.594**	0.103	0.338
7	0.008	0.481	0.093	0.394

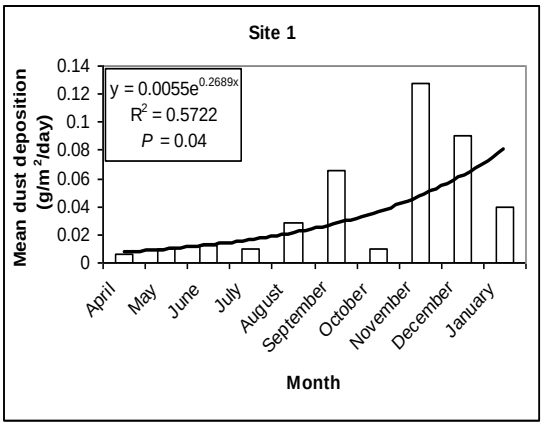


Figure 4.3

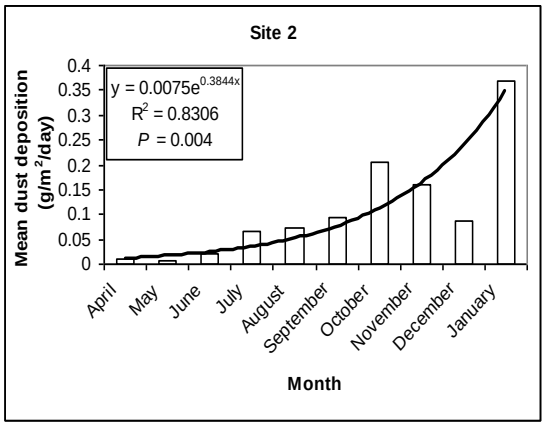


Figure 4.4

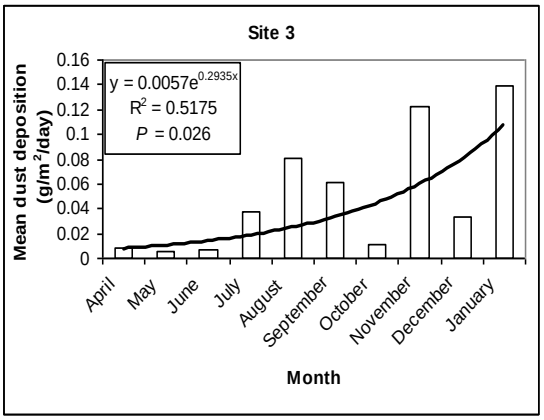


Figure 4.4

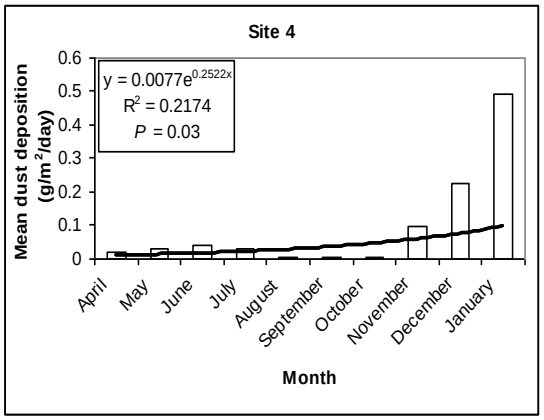


Figure 4.5

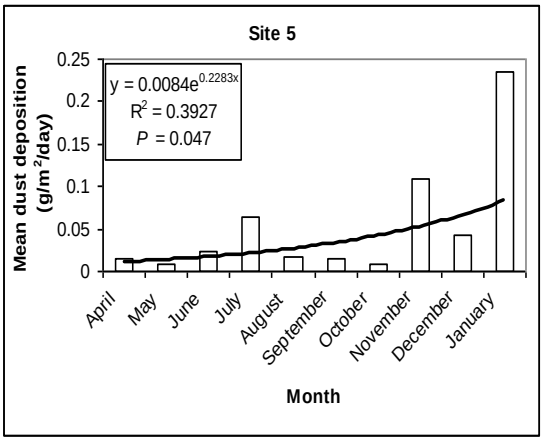


Figure. 4.6

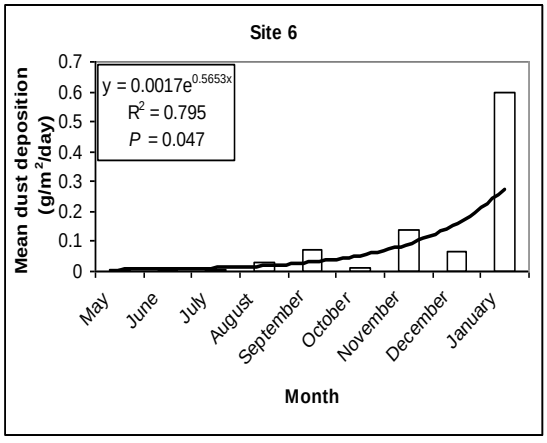


Figure 4.7

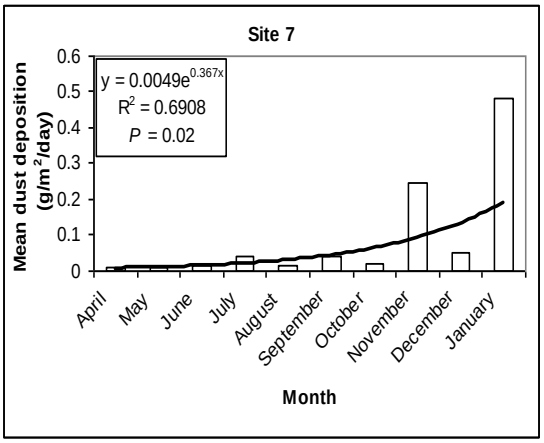


Figure 4.8

Figure 4.3- 4.8. Plots of mean monthly deposition of dust (g/m^2/day) at each of the seven sampling sites. The line shows the exponential trend and the R^2 and P values show the significance of the relationship at $P<0.05$.

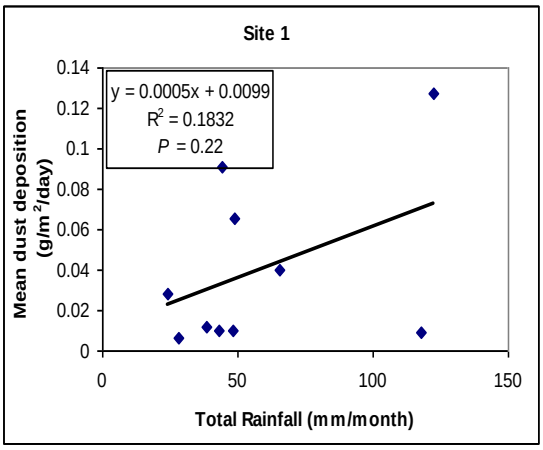


Figure 4.9

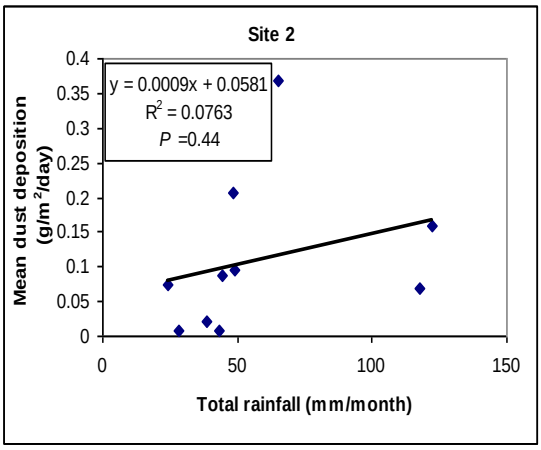


Figure. 4.10

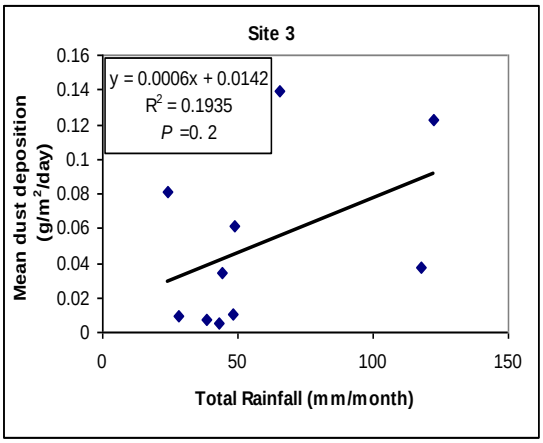
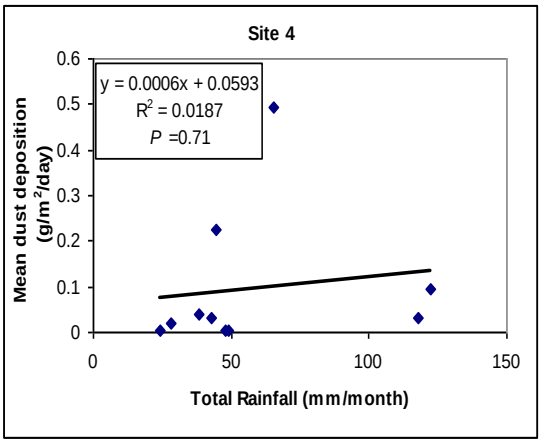


Figure. 4.11



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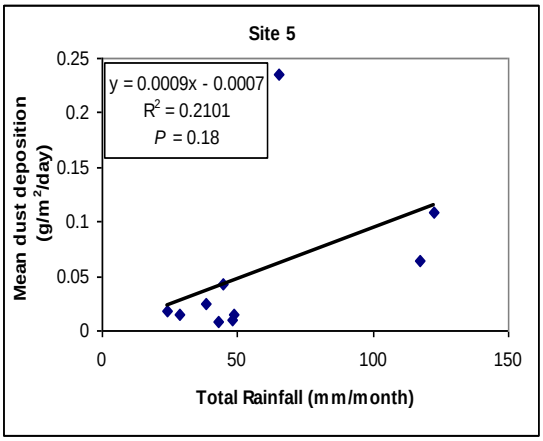

Figure. 4.13

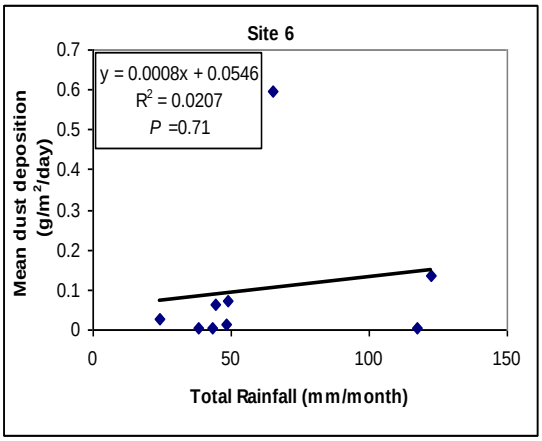

Figure 4.14

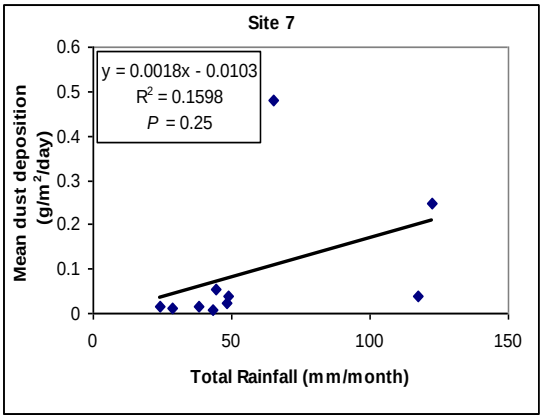

Figure 4.15

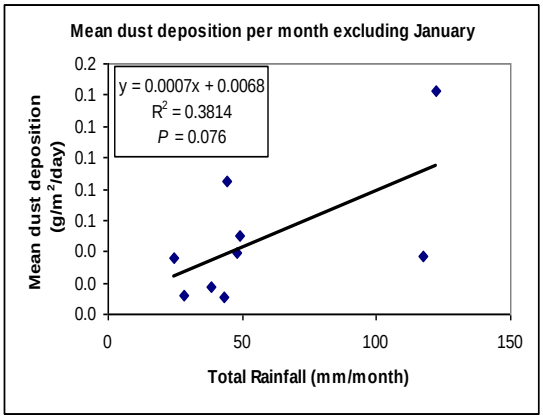

Figure 4.16

Figure 4.9 – 4.15. Plots of mean monthly dust deposition (g/m^2/day) against total monthly rainfall (mm) at each sampling site. The trend lines show the linear relationship between the two variables and the R^2 and P values show the significance of the relationship at $P < 0.05$.

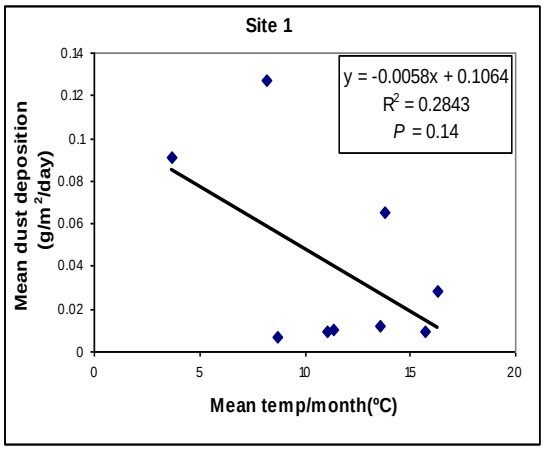

Figure 4.17

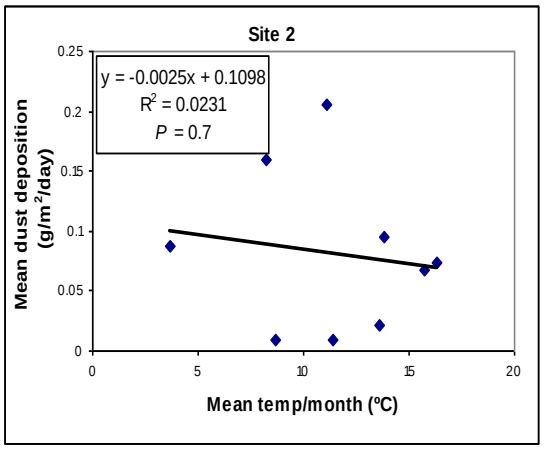

Figure 4.18

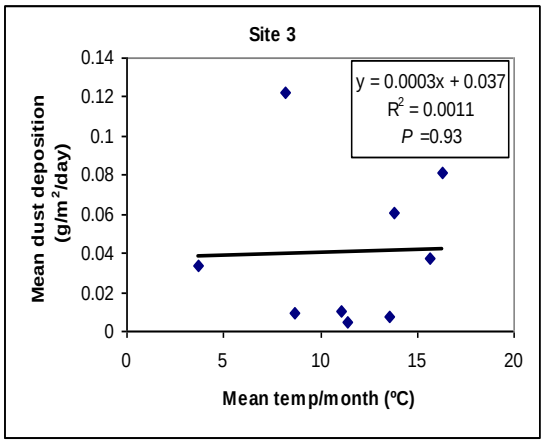

Figure 4.19

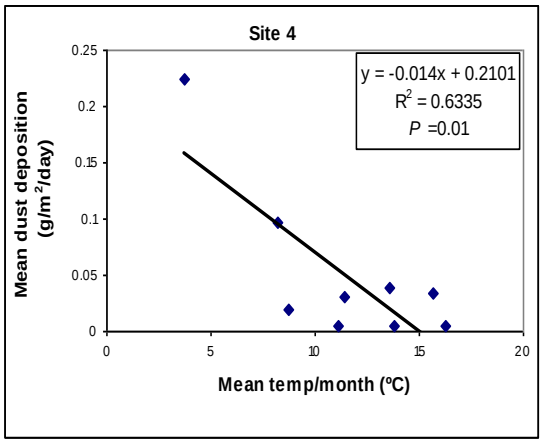

Figure 4.20

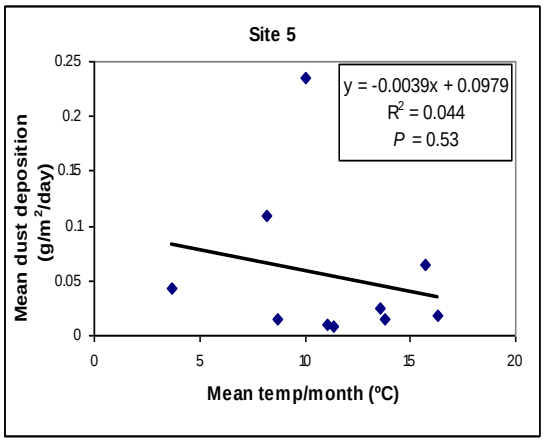

Figure 4.21

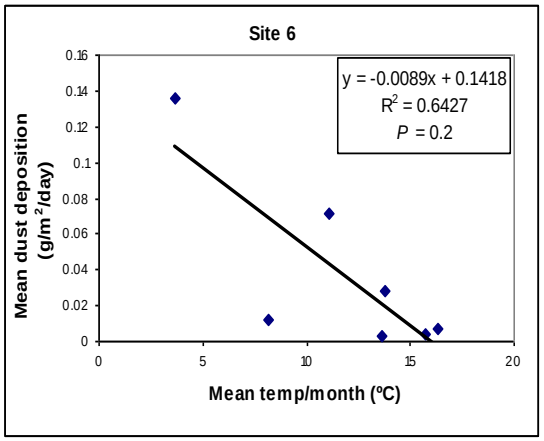

Figure 4.22

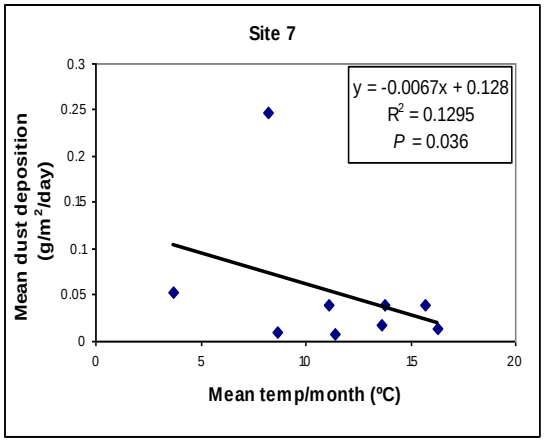

Figure 4.23

Figure 4.17 – 4.23. Plots of mean monthly dust deposition (g/m^2/day) against mean monthly temperature (ºC) at each sampling site. The trend line, R^2 and P values show the significance of the relationship at $P<0.05$.

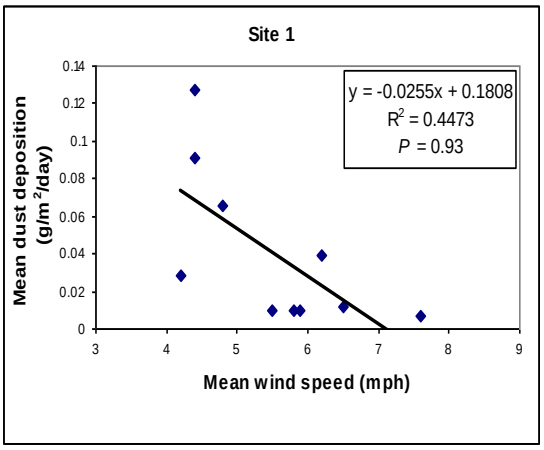

Figure 4.24

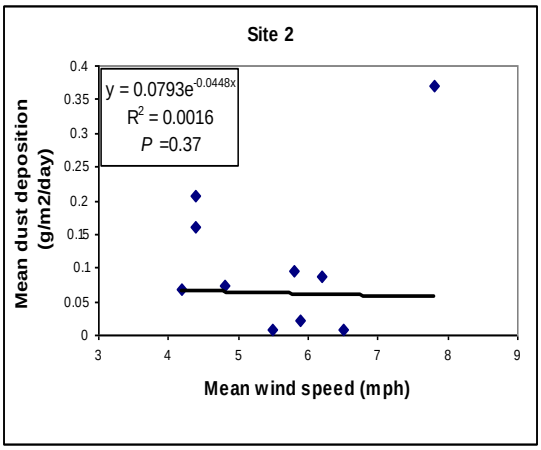

Figure 4.25

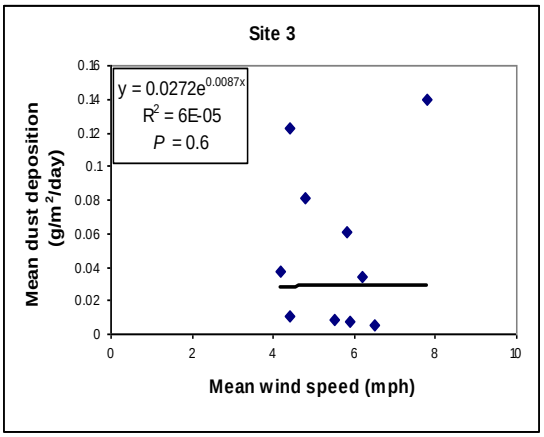

Figure 4.26

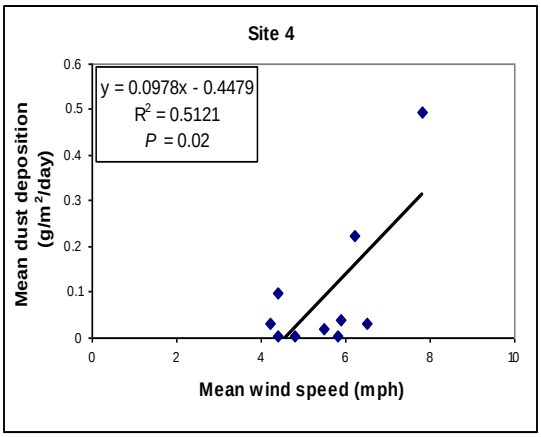

Figure 4.27

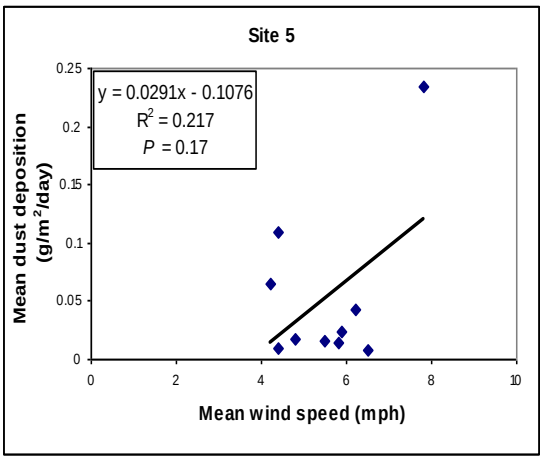

Figure 4.28

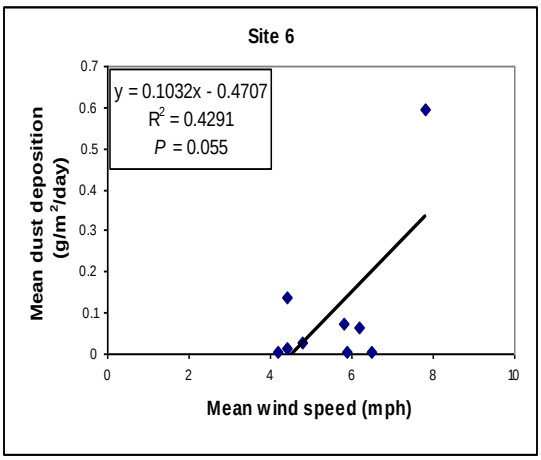

Figure 4.29

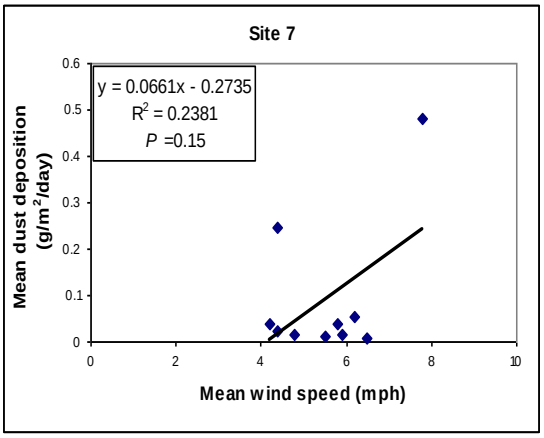

Figure 4.30

Figure 4.24 – 4.30. Plots of mean monthly dust deposition (g/m^2/day) against mean monthly wind speed (mph). The Trend line, R^2 and P values show the significance of the relationship at $P<0.05$.

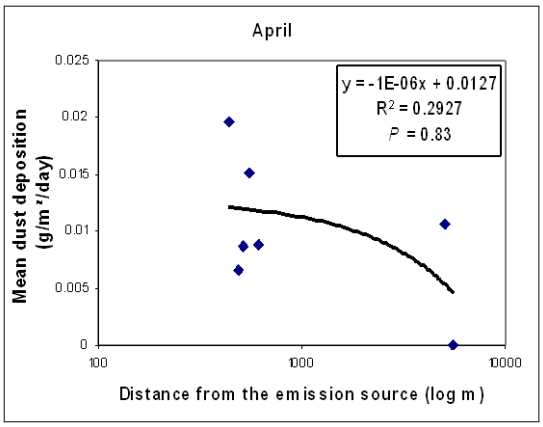

Figure 4.31

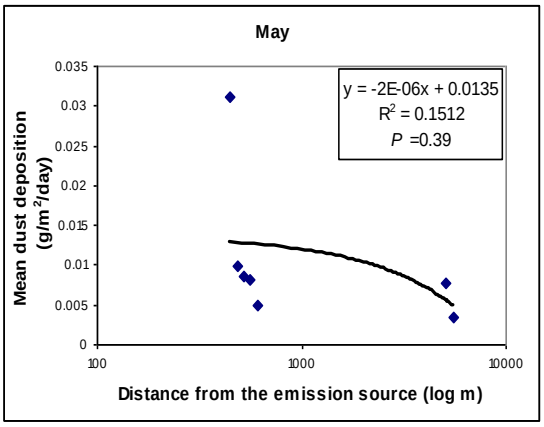

Figure 4.32

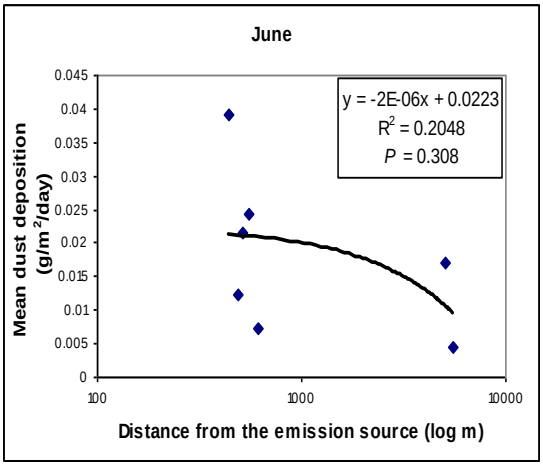

Figure 4.33

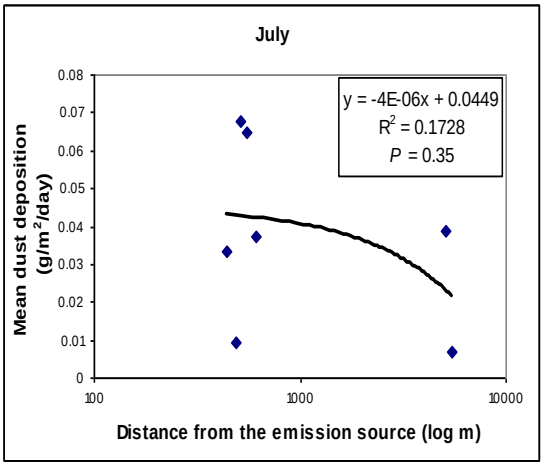

Figure 4.34

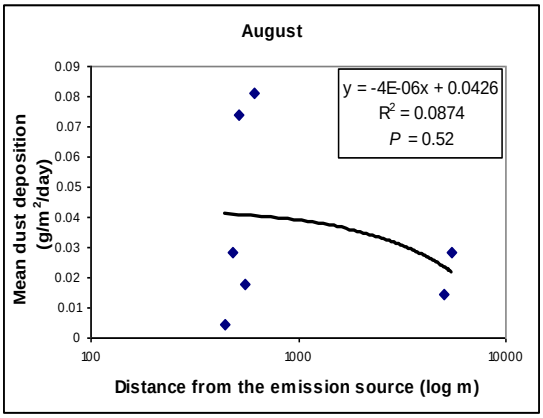

Figure 4.35

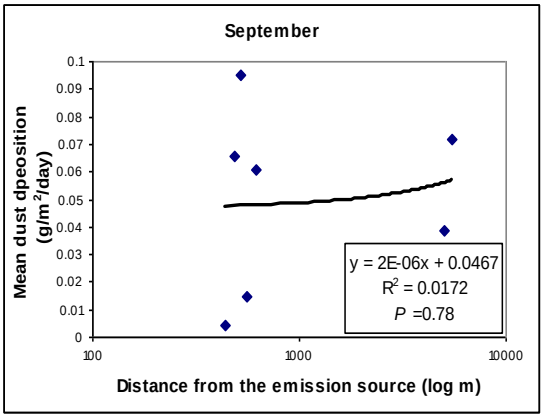

Figure 4.36

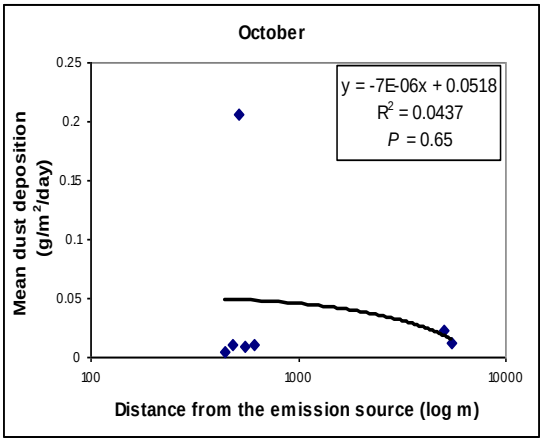

Figure 4.37

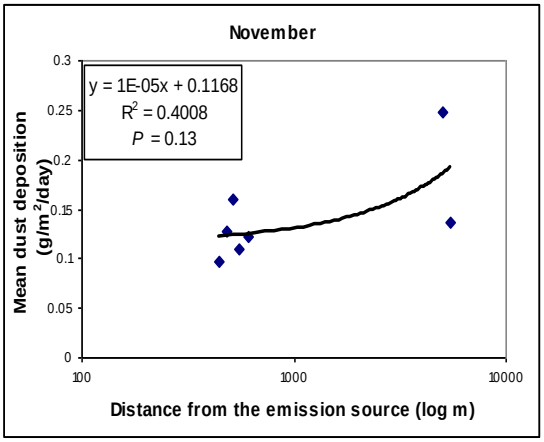

Figure 4.38

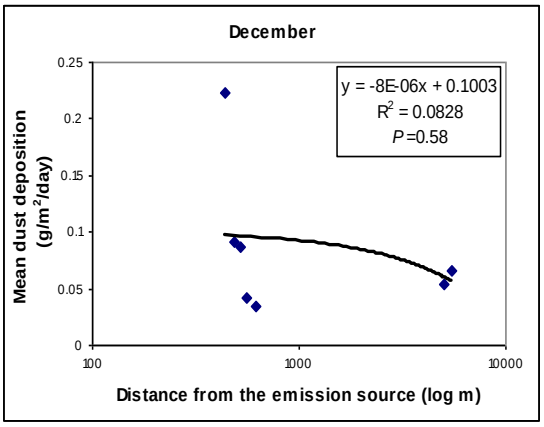

Figure 4.39

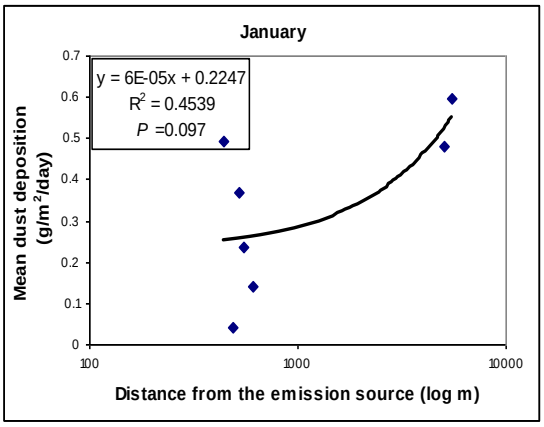

Figure 4.40

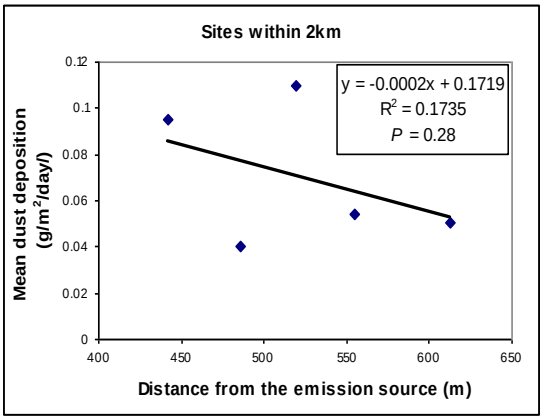

Figure 4.41

Figure 4.31 – 4.40. Log scaled plots of mean dust deposition (g/m^2/day) at each of the seven sampling sites against distance of each site from the emission source (m) at each month. Fig 4.41 shows a plot of mean dust deposition (g/m^2/day) over the 10 month sampling period at each sampling site within 2km of the emission source, against distance (m) from the emission source The trend lines, R^2 and P values show the significance of the relationship

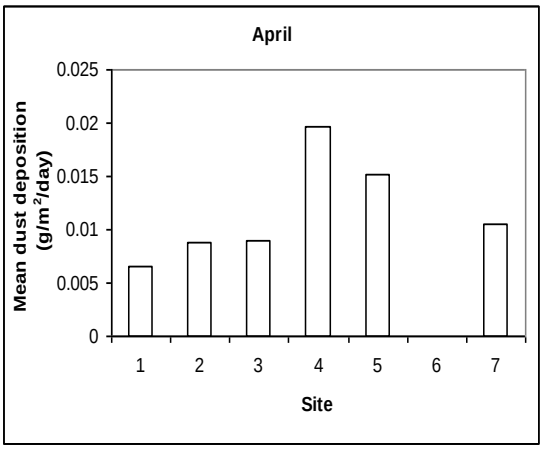

Figure 4.42

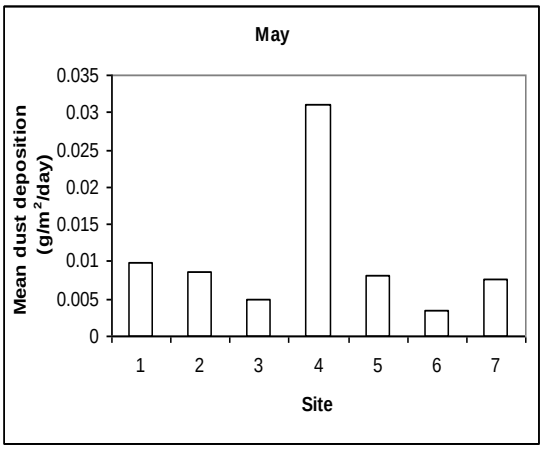

Figure 4.43

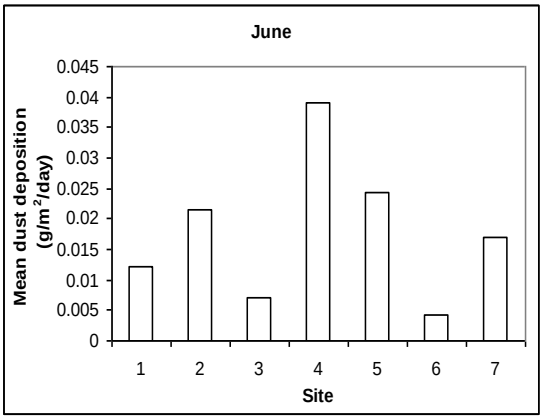

Figure 4.44

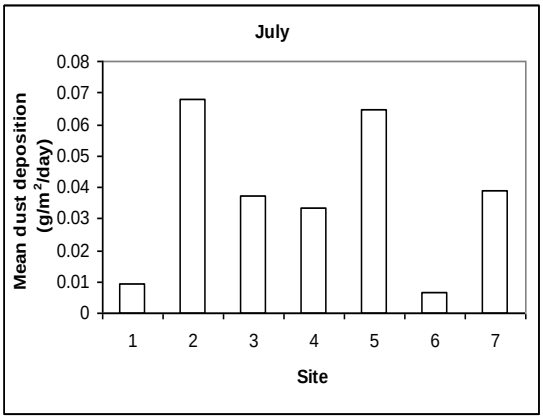

Figure 4.45

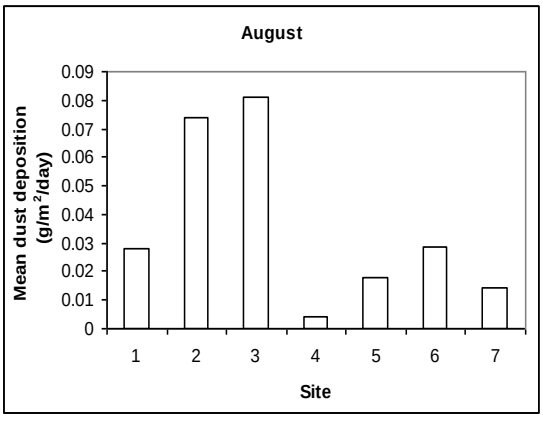

Figure 4.46

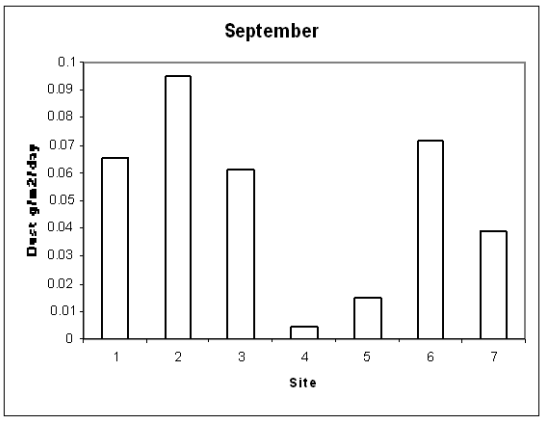

Figure 4.47

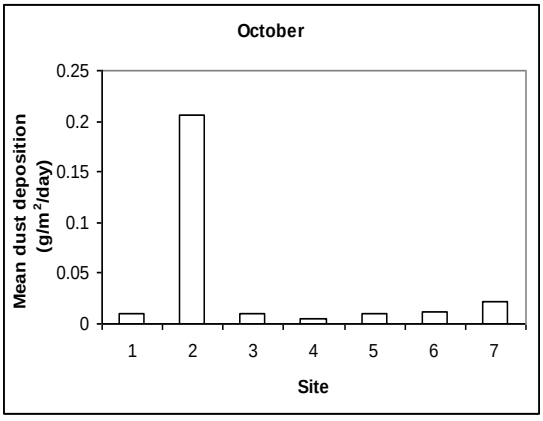

Figure 4.48

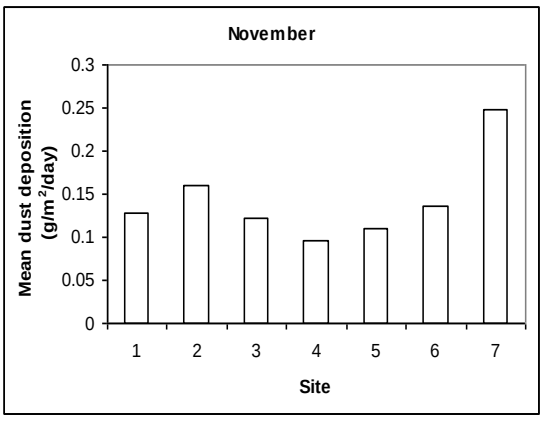

Figure 4.49

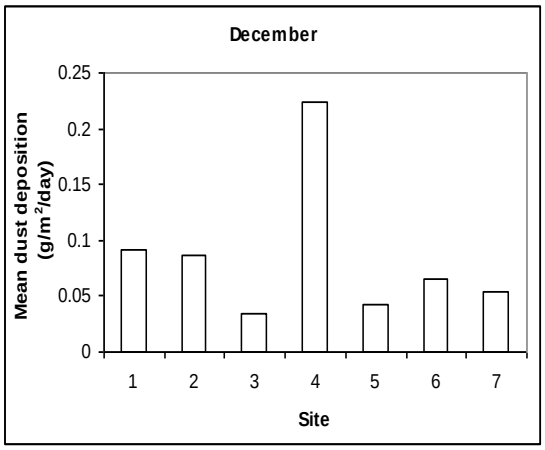

Figure 4.50

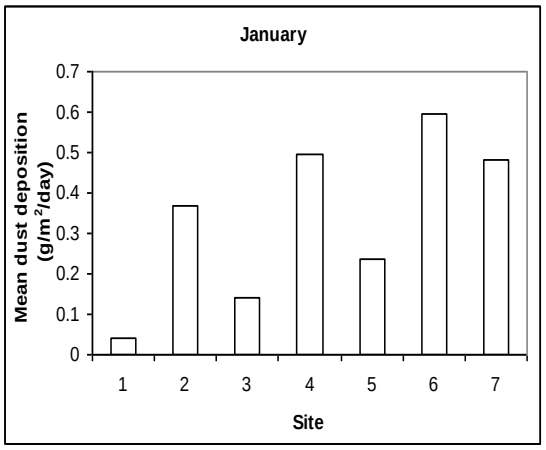

Figure 4.51

Fig

Figure 4.42 – 4.51. Plots of mean monthly dust deposition (g/m^2/day) at each site.

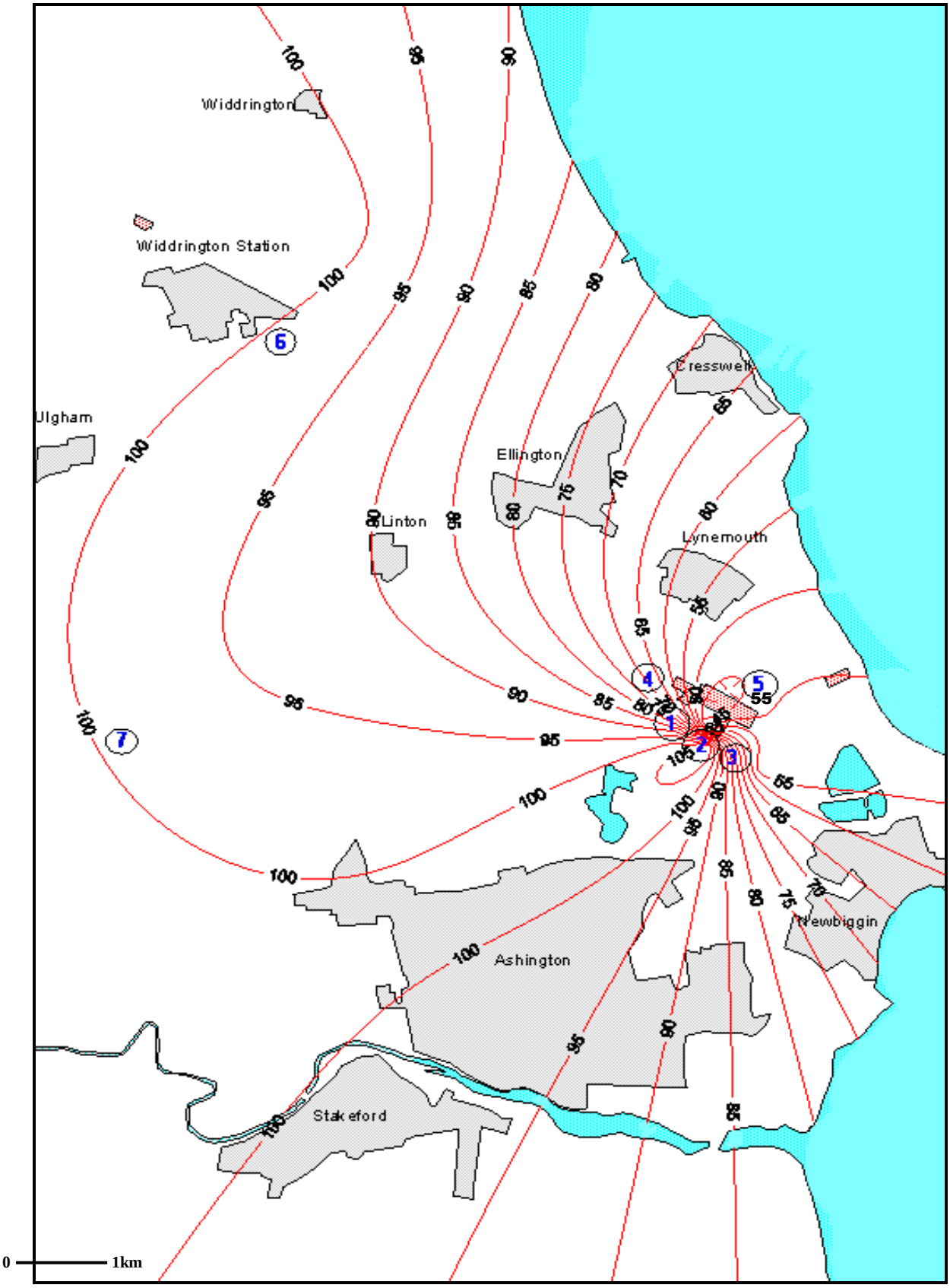

Figure 4.52. A map of the sampling area around the aluminium smelter generated in Surfer version 8 which has mapped contours (red lines) of dust deposition based on data collected at the seven sampling sites. The numbers associated to the contour lines represent the mean deposition of dust (mg/m^2/day) deposited along that contour over the 10 month sampling period. Blue numbers represent the seven sampling sites

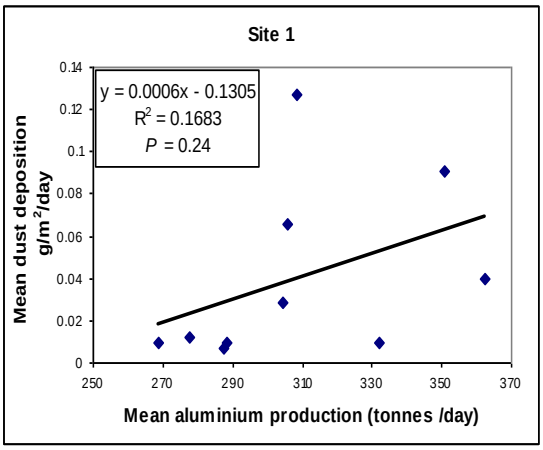

Figure 4.53

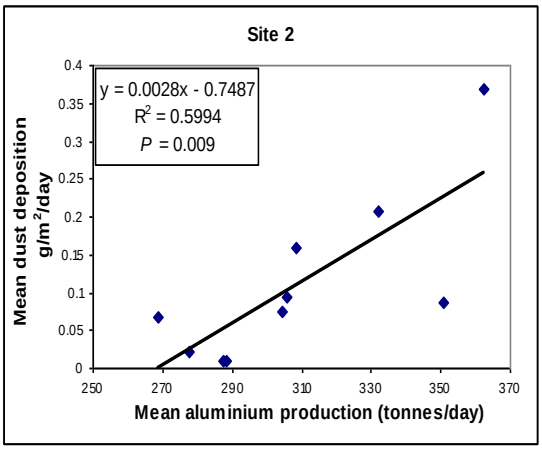

Figure 4.54

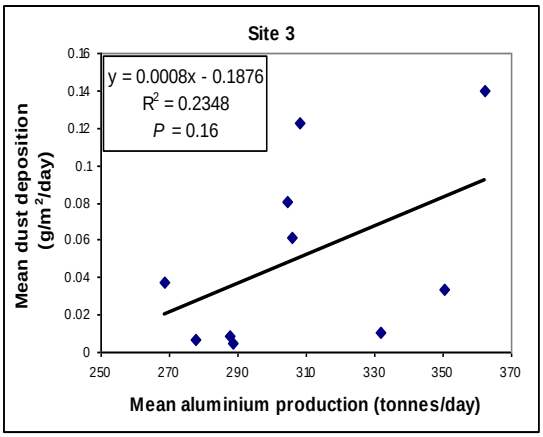

Figure 4.55

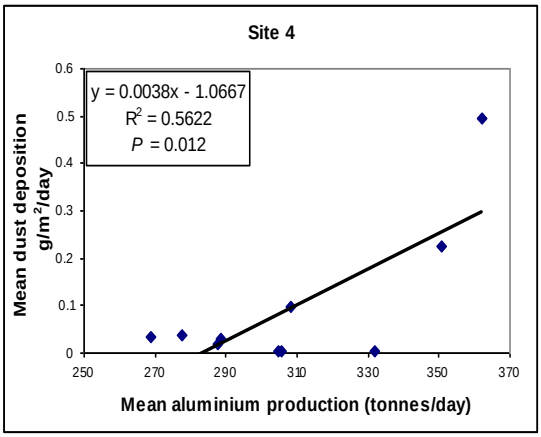

Figure 4.56

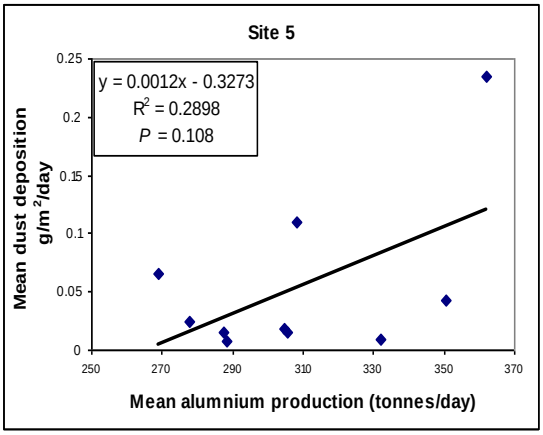

Figure 4.57

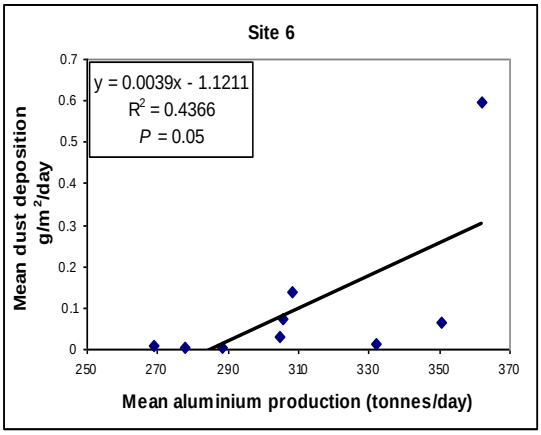

Figure 4.58

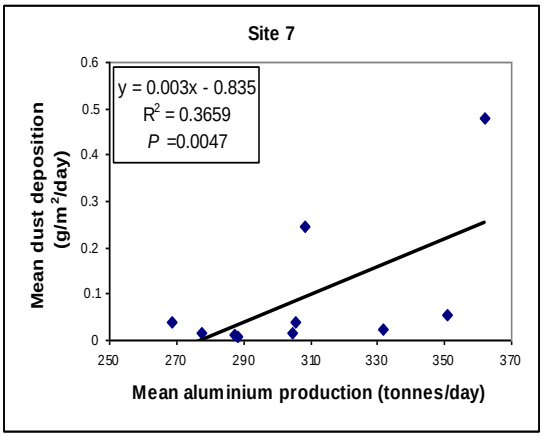

Figure 4.59

Figure 4.53 – 4.59. Plots of mean monthly dust deposition (g/m^2/day) against mean monthly aluminium production (tonnes/day) at each of the seven sampling sites. The trend lines, R^2 and P show the significance of the relationship at $P<0.05$.

4.5 Discussion

An examination of the direct effects of heavy industries on pollution is a complex task as modern day industrial practices are heavily regulated and emissions are much reduced. Meteorological factors, land use, and other aspects of human activity all contribute to this complexity, making it difficult to isolate the effects of just one factor (Weinstein &Davison, 2004). Apart from the significant exponential increase in mean dust deposition between April 2009 and January 2010 at all sites, in the present study, there was no factor that was investigated that significantly affected mean dust deposition consistently across all sites or between months.

Feng *et al*, (2003) found that the highest total deposition of fluoride particulates in Beijing was observed in the summer which was attributed to 75% of the annual precipitation falling during this season. Although a significant exponential relationship was observed between dust deposition and month we must consider that it was not a close fit and no similar relationship was observed between dust deposition and the seasonal variables that accompany changes in month between April and January, namely: precipitation, temperature and wind speed. During January 2010, there was significant snowfall and it was not known what effect this might have on particulate deposition. When the data from January were removed from the analysis and regression analysis was performed, there was still no significant relationship between rainfall and dust deposition. Field investigations remove an element of control from the researcher and can limit the accuracy of collecting samples. Due to the nature of the sampling protocol, which required that the collection bottles be thoroughly cleaned of all dust whilst in the field, variation in the accuracy in this task could have occurred which would explain anomalous results such as the seemingly significant relationship recorded between month and deposition.

The greatest mean deposition of dust was recorded at sites two, four, six and seven located 519m, 442m, 5500m and 5060m, respectively, from the source of emissions. The resulting concentration of fluoride in the area of a polluting source has regularly been described as a function of the distance from the source in conjunction with the local physical-chemical parameters, and there is strong evidence that that the fall-out of particulates and gas emissions is concentrated to the immediate 2km radius of the source, decreasing in concentration with increasing distance (Wright *et al*, 1978; Horntvedt, 1983; Hocking *et al*, 1980; Davison, 1987; Bowen, 1988; Mirlean & Roisenberg, 2007; Franzaring et al, 2006). Both sites six and seven were located beyond the 2km boundary surrounding the aluminium smelter and, as both are located on agricultural land, the high deposition of dust recorded here could be attributed to the disturbance of land at these sites. Without analysis through X Ray Diffraction, to determine the composition of the dusts, this is inconclusive. However, the strong relationship between mean aluminium production at the smelter and dust deposition at each of the four sites suggests that the relatively high levels of deposition at sites six and seven are the result of industrial and not agricultural processes.

Lynemouth smelter is unique in its design in that emissions are released from a height of 80 meters from the discharge stacks which is considered to be high (Richard Anderson, Rio Tinto Alcan, personal communication, 2011). For many years, stack heights have been used to control pollution by elevating the discharge away from the ground and dispersing it further away from the local environment (Hall, 1995). Therefore, it would not be unusual to record the deposition of industrial particulates at distances exceeding 2km, and at greater volumes than those recorded in the local vicinity of the smelter. All four sites are located to the west and north-west of the emission source (Fig. 4.1), upwind from the smelter. Deposition of dust would be

expected to travel downwind towards the east of the emission source. It is hypothesised that a measure of dust deposition at locations exceeding 5km downwind from the smelter would show a greater deposition of dust than that observed at site six and seven due to the effect of wind direction on particulate distribution.

Of all the sites, site four was located closest to the smelter at just 442 metres from the emission source. Due to the design of the chimneys, deposition of particulates at this site would be expected to be relatively low. However, this site yielded the third greatest mean deposition of dust of the seven sites at 0.095 g/m^2/day. Site four is located to the west of the emission source upwind from the emission source (Fig. 4.1) close to the bath plant, the site of the electrolytic bath at the smelter which contains cryolite and other particulates and located close to the designated disposal building housing cryolite for disposal or trade with other aluminium smelters. Leaks from buildings and the manual handling of bath in the vicinity of this site is expected to generate emissions from a low level and may lead to localised deposition of particulate dust (Richard Anderson, personal communication, 2009). The deposition of particulates at site four would also be affected by the relatively low stack height of the chimney (just 35 metres) located close to this sampling point, maintaining localised dispersion of particulates. The location of site four also explains the significant relationship between aluminium production and deposition recorded here as increased aluminium production would increase output from the chimney stack and the manual handling of bath.

The greatest mean deposition of dust over the ten month period was 0.11g/m^2/day recorded at site two located south west and 519 metres from the emission source which is below the European Community Short Term Ambient Guideline

Concentration of Particulate Matter of 0.3g/m^2/day (Hall, 1995). This site is upwind and within 1 km from the emission-source and, therefore, due to the stack height of the smelter, high levels of dust deposition are not expected at this site. However, there was a strong significant relationship between aluminium production and dust deposition indicating that the dust recorded here was probably emitted from the smelter. No similar recording was made at sites one and three, that, despite being located close to site two (Fig. 4.1), yielded the lowest mean deposition of dust over the ten month period (Table 4.1), and had no significant relationship between mean dust deposition and aluminium production (Fig. 4.53 and 4.54).

In accordance with Integrated Pollution Protectional Control (IPPC), heavy industries must employ the latest BAT (Best Available Technologies) in order to comply with their licensing agreements. The aluminium industry uses effective distillation columns such as scrubbing systems to reduce atmospheric emissions as required by the EU (Franzaring *et al*, 2006). The introduction of this technology has resulted in a significant reduction in fluoride emissions in Europe (Franzaring *et al*, 2006) which has been witnessed at smelter level with records showing a dramatic reduction in maximum fluoride output (Hocking *et al*, 1980). However, as discussed, at four of the seven sites, a significant relationship was observed between aluminium production and dust deposition. Therefore, despite employing BAT to reduce emissions, production of aluminium at the smelter does still affect the surrounding environment, although the impact is small in terms of the quantity of dust that is dispersed. The lack of a significant relationship between dust deposition and aluminium production a sites one, three and five requires further investigation in order to find an explanation. This requires an increase in the number of sampling sites, particularly downwind from the emission source, as the volumes of dust that were deposited at each site were low. A

longer period of sampling is also recommended to allow analysis of the dust composition through X Ray Diffraction.

Chapter 5

The Impact of Aluminium Smelter Shut-Down on the Concentration of Fluoride in Vegetation and Soils

5.1 Abstract

Gaseous and particulate forms of fluoride are released into the atmosphere from a large number of sources including volcanoes, aluminium smelters and phosphate fertiliser factories. The deposition of fluoride on vegetation, the uptake by plants and the subsequent impact on grazing herbivores is well-documented but little is known about what happens to the concentrations of fluoride in vegetation and the potential for soils to act as contaminating sources of fluoride once industrial emissions cease. The closure of Anglesey Aluminium Metals Ltd smelter in September 2009 provided a unique opportunity to investigate this by monitoring the fluoride concentrations in a variety of plant species and soil within 1 km of the emission-source for a period of 58 weeks following closure of the smelter.

At the start of the investigation, the highest mean concentrations of fluoride were recorded downwind from the smelter and, at the most contaminated grass site, exceeded the Washington Standard (the recommended maximum monthly concentration of fluoride in forage to minimise the toxic effects in cattle) by almost 26 times. Despite this, the investigation showed that even at the most severely contaminated site it took only the relatively short period of just 91 days for the mean fluoride concentration in grass to recover to the Washington Standard. The results highlighted the limited potential for mineral-based soils to act as contaminating sources for plant uptake as the mean concentration of grass at each site fell to

background concentrations of <10 ppm within the period of sampling and a decline in mean fluoride concentration was observed within each of the materials at each site once emissions had ceased.

5.2 Introduction

Gaseous and particulate forms of fluoride are released into the atmosphere from a large number of sources including volcanoes, aluminium smelters and phosphate fertiliser factories (NAS, 1974; Weinstein & Davison, 2004). The main gaseous form is hydrogen fluoride; it is the most phytotoxic of all air pollutants and, historically, has been responsible for large-scale economic and aesthetic damage (NAS, 1974; Weinstein & Davison, 2004)). The deposition of fluoride on vegetation or uptake by plants can cause fluorosis in herbivores if there is an excess in their diet. All of this is well documented (Weinstein & Davison, 2004), but questions about the long-term fate and cycling of deposited fluoride still arise when a new industrial source of fluoride is planned or when there is a volcanic eruption.

Regulatory bodies and the public are often concerned about the potential that build-up of fluoride in the soil has to increase uptake through the roots and consequently elevate plant fluoride, even when a source ceases emission. In general, the chemistry of soil fluorine and our understanding of the physiology of fluoride uptake suggest that there is little risk from long-term deposition of fluoride on neutral to alkaline mineral soils but uncultivated, acid soils may be a greater risk (Weinstein & Davison, 2004; Davison & Weinstein, 2006). However, there is very little known about fluoride dynamics in organic materials such as humus and peat so the risk to plants rooted in those media cannot be evaluated with any certainty (Davison & Weinstein, 2006). An indication that soil fluoride does not impact on the fluoride content of leaves would be if the concentration in vegetation falls to background levels once emissions cease. Sidhu (1976), for example, reported that the fluoride content of balsam fir and black spruce needles fell from 251 and 370 mg kg^{-1} to 25 and 8 respectively over a period

when a phosphate factory ceased emissions between May and October 1975. Similarly, it was observed that the fluoride content of conifer needles fell each year as emissions from an aluminium smelter at Sunndal, Norway dropped (Anon, 1994). For example, in the 1960s the concentration ranged from about 50-60 mg kg^{-1} but by the 1990s, when emission rates were down by around 88% and close to background levels of 10-12 mg kg^{-1}.

The problem is that very few studies of post-emission fluoride concentrations have been published and although these examples provide useful data there is still a need for more information for a greater range of species and situations. The opportunity to obtain additional information was therefore taken when the Anglesey Aluminium Metals Ltd smelter (Grid Reference: SH 26600 80900) closed in September 2009. Samples of soil, leaves and lichens were collected to follow the changes in fluoride content over a 15 month period.

5.3 Methods

5.3.1 Sampling locations and mapping

Vegetation and soil samples were collected regularly between 30th September 2009 (the day of smelter shutdown) and 6th January 2010 (14 weeks after shutdown). For the duration of this period, the mean temperature was 8.32°C, total precipitation was 475mm and there was a prevailing south westerly wind (D. Perkins, 2010). The sampling period was extended beyond the initial 3 months to incorporate all four seasons in order to avoid seasonal fluctuations of fluoride. A final collection of all sample-types was made on the 10th June 2010 at the start of the following year's growing season (36 weeks after shutdown), and further samples of lichen were collected at the beginning of December 2010 in order to increase the size of the data set and further monitor the concentration of fluoride.

Each site was mapped using a Garmin Etrex H GPS system in combination with Memory Map software (Fig. 5.1). Table 5.1 shows the distance and direction (from the smelter chimney) for each of the sample types and locations.

All sampling locations were within 1km of the smelter chimney and, with one exception, sites were within 1 km of the pot line roof, which are the main sources of fluoride pollution emanating from the smelter (Fig. 5.1). The samples collected at the sites North East from the chimney were downwind from the emission source

5.3.2 Sampling

All of the material sampled was from that year's growth to avoid fluoride sinks and in order to collect material representative of the current fluoride concentrations. In the

initial sampling period, samples of all the materials collected were taken on the first day that the electrolytic pots were shut down. Grass material of mixed sword, representative of forage for cattle, was sampled weekly for eight weeks from four sites situated around the smelter (Fig 5.1). Further samples were then taken after a two week and then a four week period. At each site three sub-samples were collected from an area approximately 5m and cut to avoid fluoride contamination from roots and soil debris.

Three samples of the lichen *Ramalina siliquosa* were collected monthly from a location downwind from the smelter (Fig. 5.1) and three samples of coniferous needles were collected monthly from three trees at locations both upwind (*Pinus contorta*) and downwind (*Picea sitchensis*) from the smelter (Fig. 5.1). Three samples of Sycamore leaf, *Acer pseudoplatanus*, were collected weekly from three trees located downwind from the smelter until leaf fall. A further set of vegetation and soil samples were collected from all locations 254 days after site closure.

Soil material was collected monthly from each of the four grass-collection sites. At each site, three samples of soil were removed from the top 2cm of the soil layer.

5.3.3 Chemical analyses

The fluoride content (PPM) of both plant and soil material was measured using standard operating procedures employed by UK aluminium reduction plants in their routine fluoride monitoring programmes.

Table 5.1. The given name, the distance (metres) and the direction of each site from the source of the fluoride emissions at Anglesey Aluminium.

Material	Site name	Direction from the emission source	Distance from the emission source (metres)
Grass	G1	North west	596
	G2	North east	486
	G3	South west	943
	G4	South-south-east	932
Soil	S1	North west	596
	S2	North east	486
	S3	South west	943
	S4	South south- east	932
Conifer	E1	North-west-west	542
	E2	North east	486
Sycamore	D	North east	486
Lichen	L	North-east-east	486

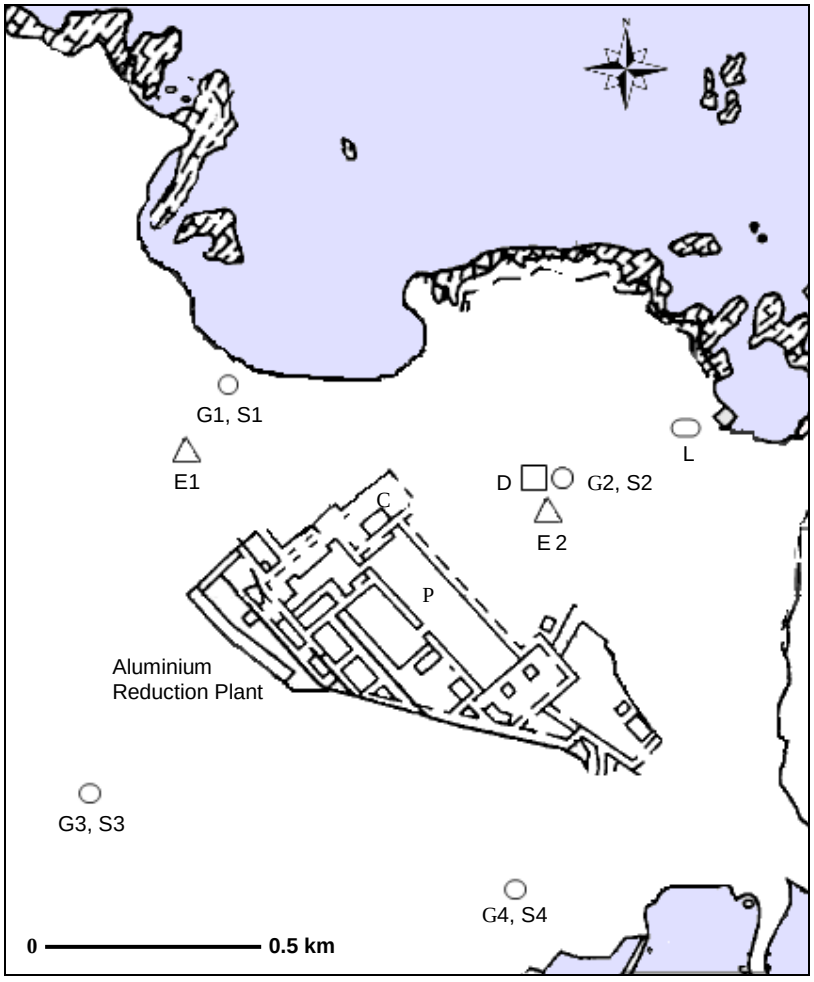

Figure. 5.1. Sampling sites near the Anglesey smelter for grass and soil (○, 1-4); Conifer sampling (Δ, 1-2); Sycamore leaf sampling (⌈); and lichen (⬭),. The chimney (c) and potroom roof (P) are the sources of fluoride emissions and are marked on the smelter site plan.

5.3.3.1 Plant material

Vegetation samples were dried for 24 hours at 70ºC and then ground to a fine powder using a Cyclotec 1093 tissue grinding mill. Between 200-300mg of each sample was added to 2ml 0.25M NH_2SO_4, thoroughly wetted and left for two hours at room temperature to digest.

Six millilitres of trisodium citrate buffer was then added and stirred. The fluoride content of the solution was assessed using an Elit 8221 fluoride-sensitive electrode. The calibration was made using NaF standard solutions and the equipment was calibrated during every set of analyses along with, three standard vegetation samples of 100ppm Assam tea which were included in each batch to ensure the quality of the procedures.

5.3.3.2 Soil material

The samples were air dried and sieved to remove debris. Between 200 and 500mg of sample was measured into a platinum crucible. Two hundred milligrams of anhydrous Analar Na_2CO_3 and drops of nanopure water (Sigma Aldrich) were added and mixed to create slurry and gently heated over a bunsen burner until samples were white and carbon-free.

The heat was increased and the residue was fused until it turned clear. It was then left to cool in a fluoride-free environment. Ten millilitres of 0.25M NH_2SO_4 was added a few drops at a time and allowed to digest for one hour. Six millilitres of trisodium citrate buffer was then added and the concentration of fluoride in the soils was calculated using the same method as used with the vegetation samples.

The mean weight of the organic content of the soil samples was analysed for each site. A sample of each of the soil samples was dried and pre-weighed in to a platinum crucible before adding drops of nanopure water and mixing to create slurry. Crucibles were heated over a bunsen burner for approximately 20 minutes until the water had evaporated and samples had burnt. After cooling, the samples were then reweighed.

5.3.4 Statistical analyses

The mean fluoride concentration (ppm) and standard deviation was calculated for each material on each sampling date (Appendix 1). For each material and site, the mean fluoride concentration (ppm) was plotted against the number of days since smelter closure. A power trend line was fitted to the data and the R^2 calculated in Excel. Where the *P* value was less than 0.05, and therefore significant, the regression equation generated from the line was used to calculate the half life of fluoride at each site, in each sample material. Where the concentration of fluoride in the grass samples exceeded 40ppm at the start of the analyses, the regression equation was also used to calculate the number of days it took for the fluoride concentration (ppm) to fall to the Washington State standard of 40ppm, a standard concentration of fluoride in vegetation regarded as an acceptable level to graze livestock on for a period of one month.

5.4 Results

5.4.1 Results from grass analysis

At the start of the monitoring programme, the mean concentrations of extractable fluoride exceeded background levels of <10ppm (Davison *et al*, 1983) at all four grass sites. With the exception of site G1, concentrations were in excess of the Washington State standard of 40ppm, with higher concentrations recorded at sites situated downwind from the smelter (Fig 5.2). On the day of site closure, the highest mean concentration of fluoride was 1032 ppm at site G2, and the lowest was 27 ppm at site G1 (Appendix 1).

Over the period of 254 days, all grass sites showed a decrease in mean fluoride (Fig 5.2-5.5). Both G1 and G3, located upwind from the emission-source, had starting fluoride concentrations <50 ppm. Between the first day of site closure and day 49, at both sites, mean fluoride fluctuated and then steadily declined in the following days (Fig. 5.3 and 5.5).

At sites G2 and G4, situated downwind from the emission-source, there was a strong negative power relationship between mean fluoride concentrations and time (R^2 = 0.8402 and 0.9206 respectively) (Fig 5.4 and 5.6).

At all four grass sites mean concentrations of fluoride had declined to background levels of below 10 ppm by day 254 of sampling.

The half life of fluoride at site G2 was 11.15 days, and from the power line fitted to the data, it took 90.96 days for the fluoride concentration to decline to the Washington

State standard of 40ppm. At site G4, the half life of fluoride was calculated to be 7.3 days, and it took 21.5 days for the concentration to fall to the Washington standard.

5.4.2 Results from the soil analysis

The mean organic content of the soils at each site was between 5-10 % of the total dry weight (Table 5.3).

All of the soil sites, excluding site S2, had a mean fluoride concentration <328ppm at the start of the monitoring programme (Fig. 5.7), but all exceeded the background concentration of <20 ppm for soil. The lowest mean fluoride concentration at the start of the monitoring programme was 199 ppm, measured at site S1, upwind from the emission-source. Only site S2, located directly downwind from the emission-source, had a mean fluoride concentration exceeding 1000 ppm at 1017ppm (Fig. 5.7).

At each sampling date there had been a decrease in mean soil fluoride concentration at each site (Appendix 1).

The shortest half life of fluoride was recorded at site S2 (Table 5.3), the most polluted soil site, situated downwind from the emission site. At the end of the monitoring period, the lowest mean fluoride concentration was recorded upwind from the emission source at site S1 and the highest mean concentration was recorded at site S2 (Table 5.3).

Table 5.2. The mean organic content (g/g) and standard deviation of the soil samples at each sampling site.

Site	Mean organic content (g/g soil)	Standard deviation
S1	0.05	0.01
S2	0.09	0.06
S3	0.10	0.02
S4	0.08	0.06

Table 5.3. The half life and mean concentration of fluoride at day 254 at each soil sampling site after shutdown. The regression coefficient was obtained from power trend lines fitted to the data

Site	Half life of fluoride (days)	R^2	Mean conc' F at day 254
S1:NW:596	79.98	0.9754	71
S2:NE:486	45.50	0.7983	231
S3:SW:943	260.60	0.6974	124
S4:SSE:932	86.78	0.7271	80

5.4.3 Results from the Evergreen analysis

The mean concentration of fluoride from conifer foliage, on the day of shutdown, was higher at site E2 than upwind from the emission-source at site E1 (Fig 5.8) measuring a mean of just 38ppm at the site upwind from the source compared to 2387.2 ppm downwind.

Downwind from the smelter, mean fluoride was seen to decrease by each sampling date (Appendix 1), starting at 2387.2 ppm on the day of shutdown and falling to 492.6 ppm by day 254. However, upwind from the emission source, at site E1, the mean concentration fluctuated throughout the sampling period, starting at 38.2 ppm and ending at 23.1 ppm on day 254. The half life of fluoride, at site E2, was 37.7 days (R^2 = 0.7735).

5.4.4 Results from deciduous analyses

Of all the materials analysed, the mean concentration of fluoride in the sycamore leaf samples, at site D, was the greatest concentration recorded at the start of the monitoring programme (Fig. 5.9). The sycamore leaf matter had a fluoride concentration of 2681.4 ppm on the day of shutdown and fluctuated within the first 42 days (Appendix 1), peaking at 4821 ppm on day 42. The mean concentration had fallen to 10 ppm by day 254.

5.4.5 Results of lichen analyses

The mean concentration of fluoride in lichen on the day of shutdown, was the lowest concentration analysed of the materials sampled north-east of the emission-source (Fig.5.9). The mean concentration started at 377.2 ppm on the first day of sampling,

fluctuated throughout the sampling period, was recorded as 323 ppm on day 254 and had fallen to a mean of 117 ppm by December 2010.

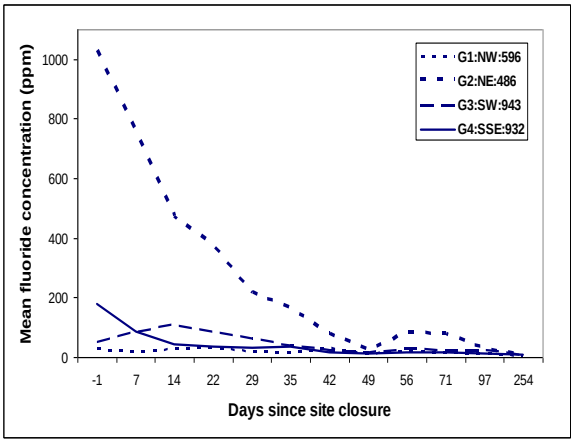

Figure 5.2. Mean fluoride concentrations (ppm) at four grass sites over 254 days following the shutdown of an aluminium plant.

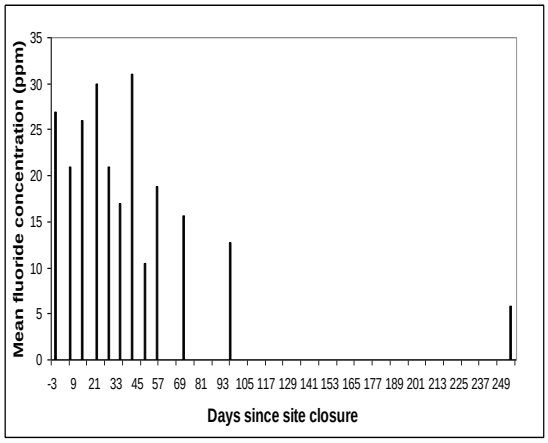

Figure 5.3. Mean fluoride concentration (ppm) in grass samples from site G1 in the days following the shutdown of the aluminium plant.

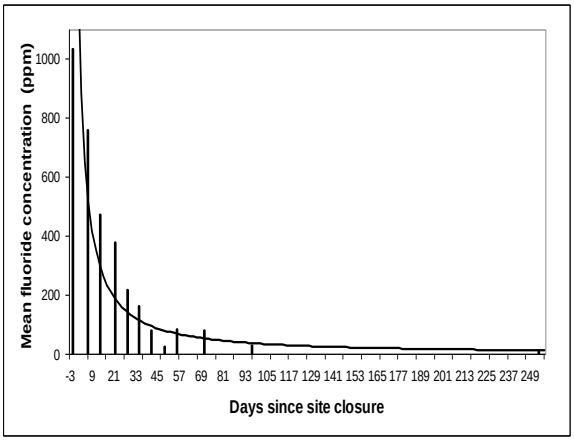

Figure 5.4. Mean fluoride concentration (ppm) in grass samples from site G2 in the days following the shutdown of an aluminium plant.

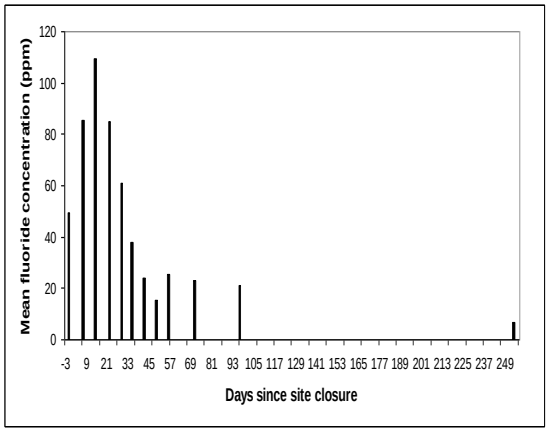

Figure 5.5. Mean fluoride concentration (ppm) in grass samples from site G3 in the days following the shutdown of an aluminium plant.

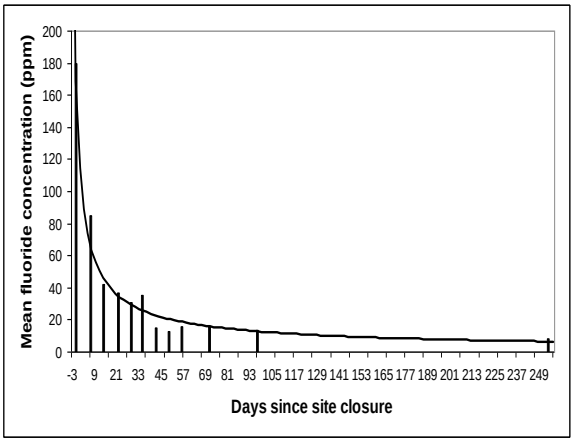

Figure 5.6. Mean fluoride concentration (ppm) in grass samples from site G4 in the days following the shutdown of an aluminium plant.

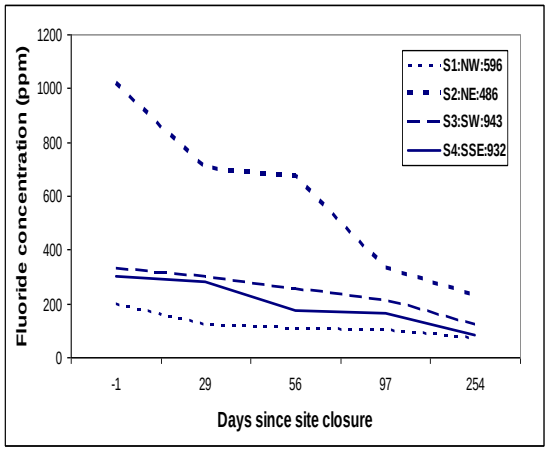

Figure 5.7. Mean fluoride concentration (ppm) at 4 soil sampling sites over 254 days following the shutdown of an aluminium plant.

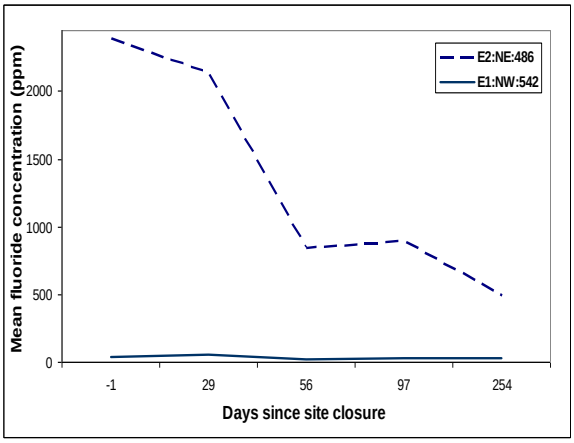

Figure 5.8. Mean fluoride concentration (ppm) of evergreen samples collected at locations upwind and downwind from the emission-source over a period of 254 days following the shutdown of an aluminium plant.

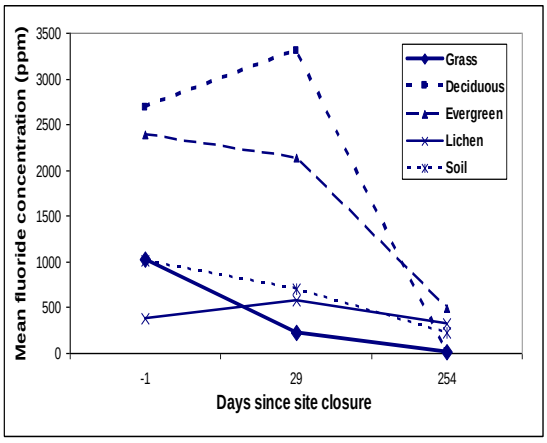

Figure 5.9. Mean fluoride concentration (ppm) of vegetation and soil samples collected downwind from the emission source over a period of 254 days following the shutdown of an aluminium plant.

5.5 Discussion

There is a significant amount of published research which highlights both inter and intraspecies variation in the uptake, accumulation and sensitivity of plants to fluoride (Murray, 1981; Vike & Habjorg, 1995; Wullf & Karenlampi, 1995; Ruan & Wong, 2000; Weinstein & Davison, 2004). These differences are demonstrated by so-called fluoride-accumulators such as tea (*Camellia sinensis*) and camellia (*Camellia japonica*) (Weinstein & Davison, 2004). However, the results of the present study not only highlight differences in species' accumulation-capacity, but also variations in their rate of loss. Both the sycamore trees and lichen leaf matter were sampled from the same location, downwind from the source, and yet showed dramatically different rates of fluoride decline.

The uptake of fluoride by leaves is determined by the boundary layer, the nature of the leaf surface and the nature of the stomatal apertures. A combination of a dense canopy and large leaves, such as Sycamore used in the present study, would be expected to have a reduced rate of gas conductance in comparison to an open canopy and needle-like leaves such as conifers (Weinstein & Davison, 2004). However, the reverse was observed in the present study and a higher mean concentration was recorded in the sycamore leaf matter than the coniferous at the start of the monitoring programme. This could be a function of the season in which the monitoring began. Sycamore leaves accumulate fluoride during the growing season over the summer period and concentrations reach their highest during the autumn season when dead patches act as sinks for fluoride, raising concentrations further (Vike & Hablong, 1995; Davison, 2010). When determining the concentration of fluoride in receptors, estimates of fluoride deposition are typically based on the weight of the leaf (Specific

Leaf Weight (SLW)) or soil. The weight of leaves and soils is however not constant and changes with temperature and light, and in leaves, ageing and senescence. Davison & Blakemore (1976) demonstrated this effect when they recorded a steady increase in fluoride concentration over a two month period primarily due to a decrease in SLW by senescence. In the present study these factors did not counter-balance the effect of ceasing the emissions at the smelter and, of all the species sampled, the sycamore material showed the greatest overall reduction in mean fluoride concentration over the monitoring period and had reduced to background levels 256 days after emissions had ceased. This could be a function of their deciduous nature, which, unlike the conifers and lichens sampled, allows the trees to shed these so called fluoride-sinks.

Historically, lichens in the vicinity of Anglesey smelter contained mean concentrations of <10µg (F^-) g^{-1} before emissions from the smelter commenced in 1970 (Perkins & Millar, 1987), but by 1977 there was an 88% loss in foliose lichen coverage and, after 10 years of production, those located within 1 km of the smelter contained in excess of 400µg (F-) g-1 dry weight (Perkins & Millar, 1987).

Lichens are recognized as highly sensitive to air pollution (Gries, 1996) and are successfully used as biomonitors (Weinstein & Davison, 2004). Lichens are long-lived perennials which, when in the vicinity of a source, are exposed to fluoride all year round with no deciduous parts with which to shed fluoride loads. Lichens have a large number of ion-exchange sites controlled through physicochemical processes with limited biological control. Consequently, there is little discrimination between the uptake of beneficial nutritious elements required for growth and harmful, toxic

elements such as fluoride which explains their capacity to accumulate deposited elements in high concentrations (Weinstein & Davison, 2004).

Despite this unregulated method of gaseous uptake, inexplicably, lichens had the lowest mean concentration of fluoride downwind from the source at the start of the monitoring programme. However, lichens also underwent the lowest proportional decline in mean concentration within the monitoring period and mean concentrations exceeded background concentrations by almost 12 times 15 months after the smelter had closed. This emphasises the sensitivity of this group and the long-lasting impacts of exposure to fluoride.

Once production of aluminium had ceased at the smelter, the site underwent a period of clean-up whereby excess alumina was removed from the electrolytic pots in preparation of the pots being dismantled the following year. This period of clean-up ran from day 5 to day 81 of the monitoring programme and therefore spanned a number of days on which vegetation and soil samples were collected. It was reported at the time that localised dust emissions resulting from the clean-up were minimal and emissions were kept under good control although some alumina may have been released in to the environment (Anglesey Aluminium Metals Ltd, personal correspondence, 2011). Despite the apparent minimal impact of these operations on the surrounding environment, the results of the present study report fluctuations in mean fluoride concentrations in the sycamore material and both the upwind conifer and grass samples during this period of clean-up. Murray, (1984), recorded fluctuations in the concentration of fluoride in Shiraz Grape despite exposure to a continuous concentration of 0.27 $\mu g\ m^{-3}$ fluoride, however typically, fluoride in

vegetation fluctuates in parallel with atmospheric fluoride concentrations (Davison, 1987). The correspondence between the fluctuations recorded in the present study and the period of clean-up could be attributed to fugitive release of fluoride during this time.

However, fluctuations were not recorded in several sample-types collected downwind from the source during this same period. These samples were the soils at all sites and the grass at the two most polluted sites downwind from the source. This result is not a function of distance and direction from the source or significantly high mean concentrations of fluoride within the testing material as fluctuations were recorded in the lichens, coniferous and sycamore material collected at the same location downwind from the source which all showed significantly high concentrations at the start of the monitoring period. The reason for these observations is unclear and raises significant questions about the dynamics of fluoride loss from soils and grasses and the seemingly negligible effect that small fluctuations in ambient fluoride concentrations have when the concentrations in the material are significantly high. Almost 40 years of operation at Anglesey Aluminium Metals Ltd resulted in significant accumulation of fluoride in sensitive plant species and soils within 1 km of the source. Most notable were the concentrations in those plants and soils located downwind which, in certain cases, had concentrations several hundred times greater than the background concentrations. Despite this long and consistent history of exposure, all of the plants and soils sampled showed a decrease in mean fluoride content within 10 months of site closure, with some materials, such as the grass, showing a decline within 7 days, and both grass and sycamore declining to background concentrations by the end of the monitoring period.

Davison (1979) showed that the rate of concentration change was a function of the mean; the greater the mean, the greater the daily increase or decrease in plant fluoride. This corresponds with the results of the present study which found that the greatest decline in concentration between monitoring days was recorded at the most contaminated site downwind from the source. This was true for all the environmental receptors sampled where there was a comparable upwind site.

The results of this study are of significant importance as they: 1. highlight the limited effect of soil-fluoride on plant uptake; 2. demonstrate the short term effects of heavy industries on forage; and 3. demonstrate interspecies differences in accumulation-capacity and rate of loss. According to the US Department of Agriculture (1971) "Airborne fluorides (F) have caused more worldwide damage to domestic animals than any other air pollutant" (cited in Prival & Fisher, 1972). Fluoride has a strong affinity with calcium with which it binds (Weinstein & Davison, 2004) and, consequently, the impact of accumulated fluoride on the bones and teeth of grazing livestock is well documented. These include dental lesions, brittle and chalky teeth (Shupe, 1969), localized enlargements of bones (Hobbes, 1962; Shupe, *et.al*, 1963), and mineralisation of the ligaments and tendons around the joints which leads to lameness (Suttie, 1971; Shupe, 1969). The concentration of fluoride in plants has long been used by many countries as a regulatory tool and forms an important basis for assessing the impact of heavy industries on crops. Monitoring plant fluoride concentrations assesses the potential for fluorosis in grazing livestock which was particularly important across Europe in 2010 when it was used to assess the impact of the eruption of the Icelandic volcano, Eyjafjallajökull, on fluoride concentrations in grazing forage; an important factor for informing outdoor grazing policies. In

response to public and governmental concerns, there are now standard maxima for fluoride deposition on forage, the most widely adopted being the Washington Standard which stipulates a maximum annual exposure of 40 ppm in order to minimise the effects of ingestion (Suttie, 1977). An exceedance of the Washington Standard was recorded at 3 of the four grass sites at the start of the monitoring period with one site recording more than 25 times the recommended concentration.

In the vicinity of long-established sources, the potential for a significant build-up of fluoride in soils raises the important question of the retention-capacity of soils and the potential for soils to act as a source of fluoride for plant uptake when emissions cease. This is an important issue to address as it reveals the scale and longevity of fluoride contamination and the magnitude of the impact of fluoride-producing industries on the long-term quality of forage. Research into soil and plant uptake of fluoride was stimulated in the 1930s and 40s by concern over the exposure of livestock to potentially toxic levels of the contaminant via the application of high fluoride-containing phosphate fertilisers to the forage. As such, a lot is known about the mineralogy of soil fluoride and the processes of uptake, retention and loss of fluoride in vegetation (Weinstein & Davison, 2006).

The background concentrations of soils in uncontaminated locations are thought to be less than 15ppm. (Thompson, Sidhu & Roberts, 1979; Omueti & Jones, 1980). The mineral horizons of soils, however, affect the concentration of fluoride which can range from under 100 to several thousand mg kg^{-1} dry wt. (NAS, 1971; Kabata-Pendias & Pendias, 2001), where deposits of minerals such as fluorspar occur (Cooke *et al*, 1976; Geeson *et al*, 1998). Generally, the upper organic horizon of the soils has

less sorption capacity than the mineral soil which increases with depth (Arnesen, *et al* 1995) as fluoride is lost from the upper horizons through leaching into the deeper soils base (Robinson & Edgington, 1946; Omueti & Jones, 1980; Loganathan *et al*, 2001). As the soil at the site had a low organic content, fluoride would be retained within the mineral horizons at the surface of the soil and leaching would be slow. The high mineral content also indicates a high sorption capacity of fluoride at the soil surface and the potential to adsorb fluoride remaining in the atmosphere into the upper horizon of the soil profile would be high. This would explain why concentrations did not decline to background levels within the period of monitoring.

Mineral soils have little discernible effect on root uptake of plants (Weinstein & Davison, 2004; Weinstein & Davison, 2006). A negligible impact of soil-fluoride on the concentration of fluorides in plants would result in a fall in plant fluoride concentrations to background levels once emissions cease, which, despite the retention-capacity of the soils, resulted at each sampling site by the end of the monitoring period. These results corresponded with those recorded by Braen & Weinstein (1985) who found that contaminated soils were not an important source of fluoride for plants.

When Anglesey Aluminium Metals Ltd ended its operations in September 2009, it created a unique opportunity to assess the long-term impacts of fluoride pollution on the environment. This body of research provides significantly important information for the aluminium industry as concerns are regularly raised by both the regulatory authorities and the public over the extent of the impact of their operations on the environment. When considering the period of operation at the smelter, the relatively

short period of time that it took for the forage to recover to the Washington Standard at the most severely contaminated sites should be viewed as an extremely encouraging indicator of the short-term impact of industrial fluoride on forage and the limited potential for mineral-based soils to act as contaminating sources.

5.6 Appendix 1

Table 5.4. The mean concentration of fluoride (ppm) in grass samples and the standard deviation at each site collected on each date of collection.

Sample	Grass 5		Grass 7		Grass 10		Grass 11	
Date	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation
30/09/2009	26.97	9.23	1032.14	154.69	49.68	3.25	179.84	15.17
07/10/2009	21.00	1.00	759.67	109.20	85.33	20.50	85.33	20.50
14/10/2009	26.33	2.08	473.33	67.60	109.67	2.08	42.00	6.56
22/10/2009	30.33	4.73	376.00	65.64	85.00	16.64	36.67	5.77
29/10/2009	21.15	5.84	218.02	24.14	60.63	8.11	31.00	6.05
05/11/2009	16.72	3.77	160.77	35.72	37.79	1.12	35.00	10.21
12/11/2009	30.70	8.06	76.69	38.67	23.55	9.92	15.00	0.41
19/11/2009	10.46	1.46	23.49	3.37	15.22	2.24	12.89	1.62
26/11/2009	18.85	1.55	81.03	24.56	25.61	6.39	16.11	1.35
11/12/2009	15.59	3.89	77.43	26.32	22.98	2.49	15.89	4.56
06/01/2010	12.74	2.61	27.90	15.31	23.06	8.50	13.30	3.90
10/06/2010	5.80	0.70	7.64	0.45	6.61	1.35	8.54	1.00

Table 5.5. The mean concentration of fluoride (ppm) in soil samples and the standard deviation of each sample on each date of collection.

Sample	Soil 5		Soil 7		Soil 10		Soil 11	
Date	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation
30/09/2009	198.61	61.43	1017.25	48.85	327.8	26.87	297.66	15.05
29/10/2009	124.33	15.48	702.36	26.54	298.86	27.93	278.93	15.27
26/11/2009	106.63	12.19	669.41	58.5	254.09	8.33	174.17	48.34
06/01/2010	101.72	1.92	331.85	152.45	214.47	27.54	161.27	13.24
10/06/2010	70.56	20.53	230.54	50.31	124.3	15.8	79.93	16.88

Table 5.6. The mean concentration of fluoride (ppm) in coniferous foliage and the standard deviation of each sample at each collection date

Sample	Downwind Evergreen		Upwind Evergreen	
Date	Mean fluoride concentration (ppm)	Standard deviation	Mean fluoride concentration (ppm)	Standard deviation
30/09/2009	2387.23	846.19	38.22	13.37
29/10/2009	2134.75	828.57	51.83	23.94
26/11/2009	839.02	116.89	18.38	1.11
06/01/2010	893.59	156.59	29.10	10.07
10/06/2010	492.61	38.92	23.12	5.89

Table 5.7. The mean concentration of fluoride (ppm) in Lichen and the standard deviation of each sample at each collection date.

Sample	Lichen	
Date	Mean fluoride concentration (ppm)	Standard deviation
30/09/2009	377.17	99.91
29/10/2009	571.22	147.34
26/11/2009	219.87	67.94
06/01/2010	208.85	23.41
10/06/2010	323.03	63.05
04/12/2010	117.21	21.83

Chapter 6

General Discussion

6.1 General Discussion

The research presented in this thesis was initially stimulated by concerns raised by aluminium industry leaders. Rises in aluminium prices are leading to the global expansion of the industry, increasing production to meet the demands of the market. The subsequent parallel rise in the manufacture of synthetic Sodium hexafluoroaluminate (cryolite, Na_3AlF_6) is raising concerns over the environmental impact of managing the waste disposal of these increasing loads when the market takes an inevitable down-turn.

Throughout the process of developing this body of work, the research has progressed and expanded. A combination of EU restrictions on the application of cryolite-based compounds in the field, the release of the European Union Risk Assessment Report of Sodium hexafluoroaluminate in 2008, highlighting significant gaps in ecotoxicological research, and opportunities that arose through the closure of Anglesey Aluminium Metals Ltd in 2009, transferred the focus of the thesis to investigations into the mode of action and toxicity of cryolite and other environmental issues related to aluminium production.

Ultimately, the thesis aims can be split into two sections; firstly, an investigation into the toxic mode of action of cryolite as a bi-product of aluminium smelting and an active ingredient in pesticides in the US, and secondly, an assessment of the impact of modern aluminium production practices on the environment in terms of the toxicity to

insect populations, the deposition of dust in the environment and the longevity of fluoride contamination in forage and vegetation.

6.1.1 The mode of action

Historically, cryolite has been described as a stomach poison. The most descriptive assessment, upheld by the United States Environment Protection Agency (USEPA,1996), states that fluoride ions released in to the gut form toxic complexes with metal-containing enzymes in the stomach (Corbett, *et al*, 1974; cited in EPA, 1996). This is in agreement with the manufacturers of cryolite-based pesticides in the US who also claim it acts as an abrasive with the consistency of "glass" (Gowan Company, personal correspondence, 2008). This thesis has shown that the mode of action of cryolite, when ingested, has the potential to be significantly more complicated than reported in the early studies of the 1970s, 80s and 90s.

There are strong effects of several factors which operate on the toxicity of cryolite which sheds further light on its mode of action as an active ingredient in pesticides in the US. Firstly, it is evident that the toxicity of cryolite has some physical grounding of which solubility and particle size have significant impacts. A dose-related response threshold recorded in this thesis contradicts previous beliefs that mortality simply increased with dose (EU Risk Assessment, 2008). The low solubility of cryolite means that large particles administered in high doses are not significantly toxic to insects and small mammals (Marcovitch *et al*, 1936, cited in Evan & Phillips, 1937). The negative relationship between particle size and surface area ratio would suggest that the solubility and, therefore, the toxicity of the particle would increase as particle size decreases. However, the LD_{50} results suggest that the reverse is true and that

toxicity increases with particle size which in turn indicates that larger particle sizes cause abrasion to the crop and foregut of the insect leading to desiccation and mortality as suggested by Shelton *et al*, (2007). It is important to consider, however, the lack of a mortality response threshold when the particle sizes of industrial cryolite are below 38µm. Despite the results of the LD_{50} assays, at high doses, smaller particles are in fact potentially more toxic than large particles. Therefore, despite the greater toxicity of larger particles, greatly contributed to by abrasion of the gut, when particles are small, the solubility of the compound potentially increases, reducing the potential to over-load the digestive system, preventing expulsion of cryolite from the gut.

An attempt to create a hierarchy of factors revealed that chemical composition plays a significantly more dominant role than physical nature in driving mortality responses to cryolite, adding weight to the theories that the release of fluoride ions are released which disrupt the chemical equilibrium of the gut of the insect (Corbett, *et al*, 1974; cited in EPA, 1996; Ware, 1986; cited by Huang *et al*, 1995). The studies also revealed the significance of exposure time, the life stage of the insect, the insect species and the dose that insects are exposed to.

6.1.2 The environmental impact of modern aluminium production and proposals for future work

Within the EU there are currently three major pathways of cryolite release into the environment. These are releases through emissions and effluent from heavy industrial processes, releases from dumping pits for spent potlinings and fugitive release from

designated disposal buildings where pot-linings are stored above ground in preparation for disposal.

The EU Risk Assessment Report (2008) for Sodium hexafluoroaluminate concluded that there is a real need for further information regarding waste management and the subsequent emissions of cryolite, particularly from aluminium smelters which generate the largest quantities of waste of all the industries. The hope is that the findings of this thesis go some way to providing some of the missing data.

6.1.2.1 The environmental impact of emissions

Both the physical and chemical form of the particulates and the concentration of emissions from smelters are not conducive of high toxicity in the natural environment. The results from this thesis indicates that particles emitted in the EU from smelters employing Better Available Technology (BAT) such as dry scrubbing and fabric filtration systems are small in the range of 0.32 to 11.5µm diameter and comprise a mixture of several impurities such as chiolite and alumina: constituents of the electrolytic bath in the Hall-Heroult process. Also, using the Rio Tinto Alcan Lynemouth Aluminium Smelter as a case study, the volume of particulate deposition, at its greatest, was around a third of the recommended standard volume set by the European Community Short Term Ambient Guideline (Hall, 1995).

Due to the 80m stacks employed at Lynemouth Aluminium smelter, which elevates the discharge of pollution away from the local environment (Hall, 1995), this plant does not represent a significant source of fluoride pollution for the immediate 1 km radius. Anglesey Aluminium Metals Ltd (AAM) emits fluoride from a stack height of

122 meters, dispersing pollution away from local area, but, significantly, also from the pot-line roof at just 14 metres high also disperses gaseous and particulates into the immediate 1km vicinity of the smelter. This provided an opportunity to assess the longevity of significant fluoride pollution well in excess of background and recommended standard concentrations.

Concentrations of fluoride in vegetation, which represent a significant risk to health, have a very short half life, often falling to background concentrations in a relatively short period of time. Significantly, contaminated soils, of the type analysed, do not act as effective sources of contamination for forage and so, despite the high retention capacity of some mineral-rich soils, the risk posed to foraging insects and mammals is limited by the capacity of vegetation to retain fluoride, which this research shows is limited, and the pH of the soils, which in agricultural cultivated land typical of that surrounding aluminium smelters tends to be alkaline and, therefore, poses a limited threat.

The findings lead to two significant conclusions. Firstly, that emissions of particulate cryolite from aluminium smelters employing BAT would have no significant impact on insect populations located in the fall-out zone and, secondly, that, encouragingly, even when the concentration of both gaseous and particulate fluorides are considered high, with the potential to have significant toxic impacts for grazing herbivores, these environmental conditions are reversible and can be considered short-term when emissions cease.

6.1.2.2 The impact of landfilling/dumping pits

The linings of electrolytic pots consist of carbon which, at high temperatures of 940ºC, absorbs cryolite, alumina and other chemicals present in the electrolytic bath. At such high temperatures the potential for the lining to crack increases requiring replacement approximately every three to seven years. By this time, through absorption of compounds in the electrolytic bath, the linings have a mass of 35 to 55 tonnes and approximately 7.5-22% of this mass comprises fluoride which is predominantly cryolite (Kumar *et al*, 1992). Cryolite is also absorbed on to the spent anodes which, in smelters employing pre-bake technology, are cleaned every month further adding to the volume of cryolite for disposal. The disposal of spent pot-linings (SPL) and anode coal scrap is the greatest volume of solid waste that smelters are responsible for and causes great concern within the industry regarding the impact on their environmental profile (Spigel & Pelis, 1990).

Landfill sites and dumping pits, designated for this disposal, are typically located close to coast lines behind walls constructed with large concrete boulders. Once full, the pits are covered with chalk-rich sand, soil and grass (Gislason, 1998). Sea water penetrates these barriers and, as approximately 30% of the fluoride associated with these linings is water-leachable, the impact of the leaching of these compounds is well documented, typically recording no impact on the diversity and abundance of intertidal communities (Ingolfsson, 1990; Svavarsson, 1990). In terms of cryolite, this may be due to its behaviour in water as it is broken down to its constituent elements of sodium, aluminium and fluoride to near background levels when in sufficient quantities of water (EPA, 1996).

Continuous leaching of cryolite from these pits onto land would, presumably, be a slow process, prolonging the exposure time of cryolite in the soil. The toxicity of cryolite increases with time which potentially could mean that the slow leaching of cryolite onto land from dumping pits would cause significant mortality in the surrounding terrestrial invertebrate communities. However, although there is little, if any, research investigating the impact of these dumping pits on terrestrial invertebrate communities, the EU Risk Assessment (2008) concluded that it was likely that when cryolite enters the soil matrix and comes into contact with water, it is again broken down to its constituent elements and invertebrates would not be exposed to dissolved cryolite. A tentative NE_{dep} (No Effect Deposition) of 1.7 µg/cm^2 for short term exposure in soils was made (EU, 2008), but slow leaching through the soil column, such as from dumping pits, was deemed to have a negligible effect on invertebrate populations due to the dissolution of cryolite during that time and no limit to the NE_{dep} was derived.

6.1.2.3 The environmental impact of stockpiling pot-linings

The major concern of industrial leaders is the environmental impact of stockpiling synthetic cryolite for disposal or for dispensation to third parties for use. As with dumping pits, the designated disposal units contain concentrated volumes of cryolite which are typically above ground within large storage units which have the potential to release particulate matter into the environment when the doors of the unit are opened. Therefore, these units pose a greater potential for release in to the wider environment than covered dumping pits.

The difference between stack emissions and those from stockpiled pot-linings is the particle size of cryolite deposited on the local vegetation. Particle sizes from stacks are attenuated by filtration and scrubbing systems whereas stockpiled cryolite comes in a diverse range of sizes from large boulders to fine powder. In agreement with the findings of Shelton *et al*, (2007), the findings of this thesis emphasise the important role that increasing particle size plays in toxicity.

The volume of dust discharged in to the surroundings of the disposal units at Rio Tinto Alcan, Lynemouth, was significantly greater than that deposited at some of the other sites monitored. However, this site did not yield the greatest concentration of dust during this sampling period indicating that emissions from the stacks are greater than that from the disposal units. Also, the volumes that were collected at this site were again much lower than those recommended by the European Community Short Term Ambient Guideline (Hall, 1995).

Although further work is recommended below, the results of this thesis indicate the discharge rates from disposal units are less than those from stacks, thereby posing a lower threat to the environment.

6.1.2.4 Conclusions

The above evaluation of these results indicates that the discharge of cryolite and other fluorides in to the environment from modern-day smelting is limited due to the installation of BAT such as scrubbing systems. In cases such as Anglesey Aluminium Metals where, in the present thesis, high concentrations of fluoride were recorded, the effects were short lived once emissions cease and there are no long-term effects in

forage. However, this research also served to highlight the impacts of exposure to low doses of cryolite and the long-term effects of this exposure on *P. xylostella* such as changes to weight and the shortening of survival rates. Therefore, an important conclusion to take from this work is that at a time where significant emphasis is being placed on sustaining the world's biodiversity and minimising human impact on the environment, any release of fluoride into the environment should be minimised in order to avoid detrimental effects on insect communities and grazing livestock.

6.1.3 Recommendations for future research

Where appropriate, considerations of future work have been discussed in the relevant chapters. Recommendations for further research, based on the findings of the current thesis, are discussed below.

The investigations into the toxicity of cryolite in *Chapter 2* could be developed by extending the testing period of the bioassay for 1st instar larvae with the aim of attaining an LD_{50} value of toxicity. The effects of body size on the efficacy of cryolite could be explored further by running a series of bioassays employing different ranges of particle sizes and monitoring the effect on the area of leaf consumed by each instar. Research into the sub-lethal effects of cryolite exposure on weight change could profit from future research by way of a starvation treatment group. This would explore the hypotheses that larvae survive exposure to cryolite by not feeding on the contaminated foliage during the bioassay resulting in survival but also weight loss. As suggested in *Chapter 2,* in order to avoid a type 2 error the weight of individuals should be monitored individually rather than in treatment groups.

Regarding the mode of action of cryolite, further in-depth investigations need to be made through physiological, cellular and biochemical approaches in order to aid a better understanding by pesticide manufacturers of how both mechanical and chemical processes contribute to toxicity. Even if concentrations of cryolite emitted from industrial processes in the EU are below that which is lethal, by pinpointing the mode of action, a better understanding can be gained of how these processes might be affecting insect populations on a sub-lethal level.

Although there were several important outcomes from the results of *Chapter 4* and several inferences can be made about the impact of aluminium smelting on the environment and surrounding insect communities, there is a lack of information on the actual concentration of industrial cryolite particulate in the vicinity of aluminium smelters and the toxicity of these concentrations. This needs to be explored further through extended periods of monitoring the volume of dust deposition in the vicinity of a smelter and subsequently analysing the particulate through quantitative XRD. Similarly, by increasing the sampling period, analysis of the composition of the deposition collected at sites exceeding 5 km from the source will highlight whether the higher volumes of dust are due to agricultural activity surrounding these sites or the 80m stacks at the smelter depositing dust at greater distances than expected away from the source.

Regarding the long-term impacts of industrial emissions on vegetation, although a decline in the mean concentration of fluoride in lichens was observed, the concentrations were 10 times the background concentrations at the end of the monitoring period. Again, extended monitoring periods are the next step in fully

understanding the scale of the impact and further investigations are required into interspecies differences in fluoride retention capacities to explain the differences observed in *Chapter 5*.

The EU Risk Assessment Report for cryolite (2008) calls for more explicit descriptions of the specific waste fractions of designated disposal stores in order to assess the potential releases of particulate cryolite in to the environment. Detailed analyses of the gaseous and particulate soil and vegetation fluoride concentrations and the diversity of invertebrate communities in the vicinity of these stores are recommended to confirm their impact

As discussed, a significant amount of research focuses on the impact of dumping pits on intertidal communities due to the likelihood of sea water penetrating the barriers constructed around the perimeter. It is likely that a large proportion of the 30% leachable fluoride from these pits is lost to the sea and therefore the effect on the fluoride concentration of soils and plants in the vicinity is expected to be minimal. An investigation into the concentration of fluoride in the soils and vegetation surrounding dumping pits and the subsequent impact on invertebrate populations is recommended to confirm this.

Finally, it is the hope that the studies presented in this thesis address some of the concerns raised by industry leaders regarding the scale and longevity of the impact of their operations on the environment, and that, these studies form a firm basis on which to further research the toxicity of cryolite and the fate of fluorides generated and released into the environment by the aluminium industry.

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