PERSISTENCE AND VERTICAL MOVEMENT OF 2,4-DICHLOROPHENOXYACETIC ACID IN TWO SUBARCTIC SOILS

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A

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Abstract

The persistence of 2,4-dichlorophenoxyacetic acid (2,4-D) was studied in soils from two sub-arctic regions of Alaska. Study sites were established in Delta Junction, where the climate is cold and dry, and Valdez, a more temperate and humid climate. Soil samples were collected from three depth ranges (0 – 7.6 cm, 10 – 30 cm, and 36 – 60 cm) for roughly 360 days following the application of 2,4-D iso-octyl ester at both study sites. 2,4-D was extracted from the soil samples and analyzed using a gas chromatograph and mass selection detector (GC/MSD). Both study sites saw vertical movement of the herbicide, with 2,4-D concentrations detected to a depth of 60 cm in the soil column. The half-life calculated for the Delta Junction site during the growing season was comparable to those observed in studies conducted in temperate climates. However, 2,4-D exhibited longer persistence times at both study sites than the persistence observed in studies conducted in temperate climates, with 2,4-D concentrations present in surface soils one year after application.

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Chapter 1 Introduction

Vegetation management along rights-of-way is a critical and integral part of highway maintenance programs. It helps to ensure the safety of drivers and the integrity of the pavement surface.

Tall, broad-leaf plant species along roadways pose a safety hazard to drivers by interfering with their line of sight, reducing drainage from pavement surfaces, attracting large wildlife, and covering highway signs. In addition, extensive root systems can damage roadway surfaces, regardless of the surface composition. While mechanical methods such as mowers and hydro-axes are commonly used to control vegetation along highway rights-of-way, several states, including Washington and California, utilize herbicides for the same purpose (WS DOT, 2005; Frisbie, 2008).

The Alaska Department of Transportation and Public Facilities (ADOT&PF) is currently evaluating strategies to better manage vegetation along highway rights-of-way. After using mechanical brush cutting methods for 20 years, the ADOT&PF decided to scientifically test combinations of chemical and mechanical vegetation control methods. These tests were aimed at determining the effectiveness and environmental impacts of each method. Results will be used to create policies and conditions for ADOT&PF's Integrated Vegetation Management (IVM) program and a Best Management Plan (BMP) for the control of vegetation along highway right-of-ways.

1.1 Project Scope

ADOT&PF is considering three herbicides, one of which is the phenoxy herbicide 2,4-dicholorophenoxyacetic acid (2,4-D), and which is discussed in this thesis. Few, if any, studies have investigated the persistence and vertical movement of 2,4-D in sub-arctic soils. While studies in temperate climates have shown that 2,4-D quickly dissipates from the soil (Crespín *et al.*, 2001; Meru *et al.*, 1990; Wilson *et al.*, 1997), it is commonly believed that 2,4-D persistence is greater in the sub-arctic environment, where soils are frozen four to six months each year. This research will determine what concentrations of 2,4-D are present in the soil profile over time after a typical broadcast spray application to highway rights-of-way in two sub-arctic climatic zones in Alaska. In addition, this research will test the hypothesis

that the overall persistence of 2,4-D in sub-arctic regions is greater than the persistence observed in more temperate climates.

1.2 Methodology Overview

A 2,4-D iso-octyl ester formulation was chosen and applied at the rate of 2.2 kg acid equivalents per hectare (kg a.e./ha) to two pre-selected plots at two study sites: one in Delta Junction and the other in Valdez, Alaska. During each sample collection event, soil samples were collected following random sampling methodology. These samples were then analyzed using a gas chromatograph/mass selection detector (GC/MSD) at the University of Alaska-Fairbanks Water and Environmental Research Center (WERC) laboratory. Due to the rapid hydrolysis of the 2,4-D iso-octyl ester formulation to the acid form after application noted in literature, only the 2,4-D acid was extracted and analyzed in the laboratory (Stewart & Gaul, 1977). All references to 2,4-D in this document refer to the acid formulation unless otherwise noted.

1.3 Overview of Study Results

The first results drawn from this study indicate that the half-life of 2,4-D in sub-arctic soils is comparable to other more temperate locations. Secondly, persistence of 2,4-D at both study sites was determined to be longer than what is typically found in more temperate regions, with measurable amounts still found in the soil one year after application. The comparable half-life can probably be attributed to the warmer soil temperatures recorded during the first 30 days following the application, which were similar to soil temperatures encountered in many temperate climates. However, the growing season in Alaska, where soil temperatures are above $0^{\circ}C$ for only five or six months each year, is far shorter than in temperate climates. This likely caused the longer 2,4-D persistence at the two study sites because the soils froze before the herbicide had completely dissipated from the soil. Very little of the overall mass of the 2,4-D initially applied was found in the soil at either study site. Due to this, uptake and metabolism in susceptible and non-susceptible plant species, as well as volatilization, are thought to be the primary 2,4-D dissipation mechanisms in this research.

The main conclusion drawn from these results is this: the extremely low 2,4-D concentrations in the soil profile suggest that 2,4-D will most likely have minimal impact on ground-water sources when applied to Alaska's sub-arctic, vegetated soils. It is likely that 2,4-D can be applied successfully to Alaska highway rights-of-way, despite the longer persistence than observed in temperate climates. However, additional research should be conducted to address other areas of 2,4-D behavior in sub-arctic climates before the Alaska DOT&PF begins using 2,4-D iso-octyl ester in their vegetation management program.

A recommendation from this study is that additional research should be conducted on the frequency of herbicide re-application. This further research should include the results from studies that address the overall effectiveness of the herbicide in target species reduction. Additional research should also address the breakdown of 2,4-D in sub-arctic soils, the amount leaching out of the soil profile; and 2,4-D uptake, metabolism, and release mechanisms in surface litter and common Alaskan plant species.

Chapter 2 2,4-D Properties and Dissipation Mechanisms

2.1 Background and Basic Properties

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been used extensively throughout the United States and the world since its development during World War II. The herbicide is used to control broad-leaf plant species and has a wide range of applications, which includes maintenance of personal lawns, agricultural lands, pastures; and highway, railroad, and power line rights-of-way. 2,4-D is generally applied to foliage or soil as either a liquid or granular product (Walters, n.d.).

2,4-D effectively kills target plant species by mimicking the natural auxin growth hormone 3-indoleacetic acid (The Royal Society of Chemistry Information Services [RSC], 1998). Auxins are plant growth regulating hormones that stimulate both RNA and protein synthesis (RSC, 1998; Walters, n.d.). Because 2,4-D is able to mimic the auxin hormone, it is able to produce uncontrolled and disorganized plant growth leading to the eventual death of the affected plant (Tu *et al.*, 2001). Plant death generally occurs 3 to 5 weeks after 2,4-D is applied to foliage or soil (Tu *et al.*, 2001).

While the parent acid is the active compound, most commercially available granular or liquid products are sold as either an ester or amine salt formulation. Formulations are selected based on field conditions such as air temperature at the time of application and the surrounding vegetation (Hart, 2001). The amine salts are considered non-volatile, while the ester formulations are classified as being either low- or high-volatility, depending on their chemical structure (Tu *et al.*, 2001). Several commercially available 2,4-D formulations on the market today also contain other active ingredients like picloram, triclopyr, and MCPA (Tu *et al.*, 2001; Hart, 2001).

Concerns over the toxicity of 2,4-D have persisted since the Vietnam War, when it was a component of the now infamous Agent Orange, which was a combination of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Tu *et al.*, 2001). Agent Orange's toxicity came from dioxin contamination, which was a manufacturing by-product of 2,4-D and 2,4,5-T. Today, 2,4-D manufacturing methods no longer produce dioxin as a by-product and 2,4-D is still approved for use in the United States (Tu *et al.*, 2001). The toxicity of 2,4-D varies depending on the organism. It is considered mildly toxic to mammals, with median lethal

dose (LD_{50}) values ranging from 100 mg/kg for dogs to 1600 mg/kg for rabbits (Walters, n.d.; Montgomery, 2000). The EPA does not consider 2,4-D to be a human carcinogen (2,4-D RED Facts, 2005). The median lethal concentration (LC_{50}) for fish varies widely and is dependent on its formulation, with ester formulations being highly toxic to most fish (Tu *et al.*, 2001). The LC_{50} for cutthroat trout is between 24.5 and 175 mg/L, while the LC_{50} for bluegill sunfish is 263 mg/L (Tu *et al.*, 2001; Montgomery, 2000).

Protecting ground-water resources is an important consideration when applying herbicides because herbicide contamination can affect human health if present at levels above the Environmental Protection Agency's (EPA) maximum contaminant levels (MCL) (Lists of Contaminants & their MCL's, 2008). The current MCL for 2,4-D is 0.07 mg/L (EPA, 2008). As part of the National Water-Quality Assessment (NAWQD) Program, the United States Geological Survey (USGS) sampled more than 3,000 ground-water wells across the U.S. between 1992 and 1996 (Kolpin *et al.*, 2000). In their study, Kolpin *et al.* (2000) sampled 2,306 wells for 2,4-D. Of those 2,306 wells, only 0.43% had detectable amounts of 2,4-D, with the highest detectable concentration being 0.00054 mg/L, well below the EPA MCL (Kolpin *et al.*, 2000).

The molecular formula of 2,4-D is $C_8H_6Cl_2O_3$. Table 2.1 lists some of the important chemical properties for 2,4-D. Under typical conditions, the herbicide has an intermediate potential for mobility in comparison to other herbicides (RSC, 1998; Tu *et al.*, 2001).

Table 2.1. Basic Properties of 2,4-D Acid.

Vapor Pressure[a]	1×10^{-5} Pa @ 20°C
Acid Dissociation Constant, pK_a[a]	2.73
Soil Adsorption Coefficient K_{oc}[b]	0.145 - 875
Water Solubility[a]	890 ppm
Molecular Weight[a]	221.03

[a]RSC, 1998; [b]Bekbölet *et al.*, 1999

Once a herbicide is applied, its attenuation from soil can occur by several different mechanisms: application losses, volatilization, photodegradation, metabolism in plants, metabolism by soil microorganisms, and dilution. The relevance of each mechanism to the overall attenuation will depend on such factors as the type of herbicide, soil type, vegetation

type, and climatic conditions. Each of these loss mechanisms will be discussed in the following sections.

2.2 Application Drift Losses and Volatilization

2,4-D is prone to spray drift losses during application. Spray drift can be divided into two categories: droplet drift and vapor drift. Droplet drift is not dependent on the herbicide formulation; rather it is caused by low output nozzles or applications under high wind conditions. Vapor drift, however, is dependent on the herbicide formulation and is caused by volatilization, generally from foliage or soil surfaces (Piper, 1997). Drift loss becomes problematic when sensitive vegetation, especially cash crops, is in close proximity to the treated area.

Spray drift can be minimized if the application occurs under optimal conditions such as low wind speeds. A study conducted by Grover *et al.* (1985) in Saskatchewan illustrates that point. 2,4-D iso-octyl ester was applied to wheat fields under wind speed conditions of 2.6 m/s. Air samples were collected at six heights during the application at a temporary location downwind of the treated area. The resulting concentrations, the sum of droplet and vapor drift during the application process, represented less than 0.2% of the total amount applied (Grover *et al.*, 1985).

In another study, slight spray drift was observed downwind of two Ontario power line rights-of-way after the areas were treated with 2,4-D tri-isopropaolamine salt (Meru *et al.*, 1990). Meru *et al.* (1990) placed Petri dishes in or near brush at intervals of 0.1, 2, 4, 6, and 10 meters downwind of both application areas. Immediately following the herbicide application, the Petri dishes were collected and analyzed in the laboratory. One site had trace amounts of 2,4-D tri-isopropaolamine salt present 2 meters downwind of the application area, while the other had trace amounts present at 10 meters downwind (Meru *et al.*, 1990).

Herbicide volatility is variable among the different classes and types of herbicides. The 2,4-D iso-octyl ester formulation used in this study is considered to be a low volatile herbicide. Gile (1983) investigated volatile losses of 2,4-D esters (iso-octyl ester and butyl ester) in controlled laboratory studies and found that the majority of herbicide loss through drift and volatilization mechanisms occurred in the first two to four days after the application.

Studies show that 2,4-D esters can volatilize for several days after an application. Grover *et al.* (1985) collected air samples at two-hour intervals for six days following a herbicide application from several heights above the crop canopy at a location near the center of the treated area. The highest vapor densities occurred between 2 ½ and 6 ½ hours after the spray. Diurnal patterns were observed for the first two days, with the minimum vapor densities occurring in the early morning when the surface temperatures were lowest. The diurnal fluxes appeared to be from the crop canopy, which intercepted 52% of the applied herbicide, because 2,4-D ester was still present in the soil even after little to no additional ester concentrations were detected in the air or canopy. Distinct concentration gradients were also observed for the first four days, with the highest concentrations being recorded at the lowest height. By the fifth day, the cumulative losses of 2,4-D iso-octyl ester through volatilization were 20.8% of the total amount applied (Grover *et al.*, 1985).

2.3 Vegetation and Surface Litter Interception

A substantial amount of applied herbicide is likely intercepted by vegetation. When 2,4-D butoxy-propyl ester was applied to two study sites in California, the results showed that 20.7% of the amount applied was intercepted by chamise brush and another 25.2% was intercepted by grass and forbs (Radosevich & Winterlin, 1977). Only 0.1% of the applied amount was accounted for in the soil immediately following application. After thirty days, 5.6% of the total 2,4-D applied was detected in the chamise, 2.0% remained in the grass and forbs, and 0.07% was present in the soil.

Due to a lack of precipitation at the site within the first thirty days following the 2,4-D application, Radosevich and Winterlin (1977) hypothesized that the decrease in concentration may have been caused by volatility losses. After a year, 2,4-D was no longer present in the grass and only 0.01% of the original amount applied remained in the brush (Radosevich & Winterlin, 1977).

Surface litter concentrations accounted for 54% of the applied herbicide in the study conducted by Radosevich and Winterlin (1977). After thirty days, only 13.7% of the parent acid applied was recovered from the soil, grass and forbs, chamise, and surface litter, with the surface litter accounting for 6% of the amount recovered. This result indicates that the

herbicide dissipates rapidly in a field environment. Throughout the 360 day study, the surface litter always had a far greater percentage of the original herbicide than the soil (Radosevich & Winterlin, 1977), suggesting that surface litter is a major receptor for 2,4-D. This is possibly due to processes such as adsorption, which make the herbicide unavailable for transport through the soil column.

A laboratory study conducted by Norris and Greiner (1967) indicates that rapid degradation occurs in surface litter and demonstrates that the type of litter plays a limited role in the rate of degradation. Using growth chambers, various 2,4-D formulations were applied to forest litter collected in Oregon. When alder, ceanothus, vine maple, big leaf maple, and Douglas fir litter were treated with 2,4-D triethanol amine salt, the percent recoveries for the litter ranged from 60% for alder litter to 75% for Douglas fir litter after fifteen days. When 2,4-D acid, 2,4-D iso-octyl ester, and 2,4-D triethanol amine salt formulations were applied to alder litter, 45% of the 2,4-D acid and 60 to 65% of the 2,4-D iso-octyl ester and triethanol amine salt formulations were recovered after fifteen days (Norris & Greiner, 1967). It is likely that greater variations would exist under field conditions due to individual microenvironments.

2.4 Metabolism of 2,4-D

Metabolism, or biotransformation, of organic herbicides in plants is recognized as a significant process that influences the activity, selectivity and, more importantly to this study, the ultimate fate of 2,4-D following introduction into the environment (Hatzios, 1991). In general, plant species tolerant to auxin herbicides (monocotyledons) such as grasses can detoxify a given concentration of herbicide fast enough to avoid an accumulation to phytotoxic levels in the tissue, while auxin herbicide-susceptible plants (dicotyledons) such as broad-leafed species cannot achieve sufficient detoxification (Devine *et al.*, 1993). Owing to the conversion of the herbicide to other products, plant metabolism into water-soluble conjugates and insoluble "terminal" residuals that remain in the plant during its life, plant metabolism is a mode of attenuation for these herbicides. Hatzios (1991), however, notes that the identity and subsequent fate of bound residuals are poorly understood.

Detoxification of toxic substances is commonly conceptualized as a three-phase process. This concept was first presented by Williams (1959a and 1959b) and further addressed by Parker and Williams (1969). Hatzios (1991) summarizes this concept in the context of herbicide toxicity to plants (phytotoxicity). The primary (Phase I) reactions that take place during the detoxification process are oxidation, reduction, and hydrolyse. The result from this Phase I step is metabolites with reduced or modified phytotoxicity, increased polarity, and a possible predisposition of the parent molecules to further metabolism in Phase II.

Conjugation of xenobiotics (synthetic reactions) occurs in Phase II, resulting in the formation of metabolites with greatly reduced or no phytotoxicity. In metabolism, conjugation is a biochemical process in which a substance is bound to a sugar or amino acid, thereby deactivating its biological activity (Devine *et al.*, 1993). The compounds are also characterized by a higher water solubility and are typically less mobile in the plant. Herbicide conjugates formed in Phase II are converted in Phase III to secondary conjugates or insoluble bound residuals such as lignin biopolymers, which are essentially non-phytotoxic.

Hydrolysis is the first important step in converting the ester formulation of each herbicide to the acid formulation. This step can take place relatively quickly and corresponds to Phase I in the metabolism processes discussed above. Though it is applied as an ester or amine salt, the active compound is hydrolyzed to the parent acid prior to degradation. The hydrolysis of the 2,4-D ester formulation is considered to be a short process, with the ester being completely hydrolyzed to the parent acid a day or two after application. There are, however, a few studies that suggest the hydrolysis process may take longer (Stewart & Gaul, 1977; Grover *et al.*, 1985). High application rates, as shown in a study in Nova Scotia, can increase the time required for complete hydrolysis of the ester (Stewart & Gaul, 1977). In their study, Stewart and Gaul (1977) observed complete hydrolysis of 2,4-D iso-octyl ester one day after it was applied to the soil at a rate of 7.8 kg/ha, while complete hydrolysis occurred two to fourteen days after the soil was treated with 15.7 kg/ha. The time required for complete hydrolysis increased to fourteen to twenty-seven days when applied at the high rate of 31.4 kg/ha. Moreover, Grover *et al.* (1985) found that 2,4-D iso-octyl ester applied to a wheat field in Saskatchewan at a rate of 0.5 kg /ha did not completely hydrolyze to the parent acid until nearly thirty-four days after treatment (DAT), though the acid form was

detected immediately after spraying. In their study, Grover *et al.* (1985) found that roughly 33% of the ester had been hydrolyzed to the acid one DAT. That amount increased to over 70% five DAT, and to roughly 85% nineteen DAT (Grover *et al.*, 1985). While it took roughly thirty-four days for the active compound to completely hydrolize, the results from Grover *et al.* (1985) indicate that the majority was hydrolyzed in the first week. Field conditions such as humidity, soil temperature, soil moisture content, soil type, and air temperature likely contribute to the time necessary for the complete hydrolysis of the active compound to the parent acid. Once in the acid form, 2,4-D follows a known metabolic pathway (Hatzios and Penner, 1982). The resulting residuals formed through these metabolism processes are typically insoluble with limited mobility in the plant.

Metabolism of 2,4-D in plants has been well studied by several researchers (Feung *et al.*, 1971; Feung *et al.*, 1972; Feung *et al.*, 1973; Feung *et al.*, 1974; Loos, 1975; Feung *et al.*, 1976; Feung *et al.*, 1978; Pilmoor & Gaunt,1981). In general, 2,4-D is metabolized in the plant by side chain hydroxylation of the ring structure, conjugation of 2,4-D with plant constituents (sugar and amino acid conjugation), formulation of metabolites, ring cleavage, or by side chain lengthening. Plants resistant to 2,4-D convert the herbicide into inactive, nonphytotoxic carbohydrate conjugates. Susceptible plants convert 2,4-D into amino acid conjugates, which obstruct normal nucleic acid metabolism and protein synthesis, resulting in death of the plant.

Results from two studies performed by Scheel and Sandermann (1981) indicate that there is a tendency toward more metabolism of 2,4-D via hydroxylation in tolerant species and more conjugation to amino acids in susceptible species. From these results, it appears that metabolism in tolerant grasses is primarily to irreversible detoxification products, while that in susceptible species is primarily to reversible conjugates. Further, Davidonis *et al.* (1980) found evidence for the conversion of 2,4-D amino acid conjugates to free 2,4-D in soybean root callus, possibly showing the reversibility of the amino acid conjugates.

2.5 Metabolism by Soil Microorganisms

Dissipation of 2,4-D in the soil occurs primarily through microbial degradation (Walters, n.d.; Tu *et al.*, 2001). Degradation rates can vary widely and depend on variables

such as temperature, soil moisture, and the amount of 2,4-D unavailable due to adsorption by soil particles and uptake by non-susceptible plant species. Both adsorption and uptake can be reversed, which causes the herbicide to be released into the environment where it is available for chemical and microbial degradation (Tu *et al.*, 2001).

Aromatic and heterocyclic ring cleavage of most herbicides is typically stable in plants, resulting in the lack of complete breakdown of the herbicide to CO_2. In contrast, metabolism of herbicides by soil microorganisms commonly includes oxidative, reductive, or hydrolytic cleavage of aromatic and heterocyclic rings (Hatzios, 1991). Pathways for microbial metabolism of 2,4-D have been well studied (Loos *et al.*, 1967a; Loos *et al.*, 1967b; Loos *et al.*, 1967c; Bollage *et al.*, 1967; Helling *et al.*, 1968; Bollage *et al.*, 1968a; Bollage *et al.*, 1968b; Tiedje *et al.*, 1969; Duxbury *et al.*, 1970; Loos, 1971). The two most studied strains of bacteria responsible for the metabolism of 2,4-D are the *Anthrobacter* and *Pseudomonas* species. Loos (1971) summarizes the pathways that have been identified for the metabolism of 2,4-D by each species. Two different alternative basic reaction sequences between the *Anthrobacter* and *Pseudomonas* species were found from the 2,4-D acid to catechol; and thereafter a single basic reaction sequence producing two different end products: a muconic acid for the *Pseudomonas* sequence and a succinic acid in the *Anthrobacter* sequence.

Han and New (1994) isolated eleven bacteria and seventy-two actinomycete that were capable of degrading 2,4-D. Of the seventy-two actincomycete (filamentous) isolates, none could degrade 2,4-D in the absence of an alternative carbon source. In addition, ninety-one fungi capable of degrading 2,4-D were isolated in the soil samples; however, only one of these isolates was able to degrade the herbicide in the absence of an alternative carbon source. This result emphasizes the necessity of soil organic matter in the degradation of 2,4-D by fungi and filamentous microorganisms. These researchers also found that the degradation of 2,4-D was highly dependent upon soil moisture content, with lower moisture contents resulting in negligible degradation rates (Han and New, 1994). Parker and Doxtader (1983) and Bhanumurthy *et al.* (1989) show similar results. In the study conducted by Han and New (1994), bacteria were found to be the most important 2,4-D degrading organisms in relatively moist soils with water potentials above -1.4 MPa. Fungi became an important degrader as the moisture content dropped below the wilting point. The study, which lasted fifty days, also

found that if the water potential dropped to –5.5 MPa, degradation no longer occurred within the soil.

One of the most common and abundant degradation products of 2,4-D is the phenol metabolite 2,4-dichlorophenol (2,4-DCP). Crespín *et al.* (2001) investigated the metabolism of 2,4-D in a field study and found rapid production and disappearance of 2,4-DCP, with the presence of 2,4-DCP being detected one day after application. Rapid loss of 2,4-DCP was attributed to further metabolism and leaching.

2.6 Photodegradation

Among the physical factors influencing the fate of applied herbicides in nature, sunlight, particularly the ultraviolet fraction, appears to greatly influence degradation. After application, photodecomposition will predominantly take place as the droplets dry on the surface of the leaves. Crosby and Tutass (1966) found rapid photodecomposition of 2,4-D in the presence of water and sunlight. Decomposition in this study resulted in the formation of several intermediate compounds, with the final compounds being humic acids.

2.7 Surface Runoff

Provided adequate rainfall, surface runoff can occur for a few days following the application of 2,4-D. When 2,4-D ico-octyl ester was applied at two application rates to both fallow and winter wheat crops situated on south-facing slopes with gradients between 16% and 17% in Pullman, Washington, simulated rainfall of 16 mm over a three-hour period one day after application was enough to cause herbicide runoff (Wilson & Cheng, 1976). Wilson and Cheng (1976) found that the runoff from the plots treated with the higher application rate contained higher concentrations of the herbicide. Since no substantial differences were observed between the fallow and winter wheat plots treated with the same application rate, it can be assumed that the vegetation at a site plays a limited role in the amount of herbicide transported off the site by runoff (Wilson & Cheng, 1976).

A Canadian study along power line rights-of-way also showed runoff from the treated area (Meru *et al.*, 1990). In this study, two sites, with slopes of 16% to 18%, were

treated with 4.8 kg/ha of 2,4-D tri-isopropanolamine salt. Trenches were installed 3, 10, 20, and 30 meters downslope to collect runoff. Both sites experienced runoff within the first week after application, with one site receiving a higher amount of rain than the other (28.2 mm versus 9.7 mm). The site with greater rainfall saw runoff with trace amounts (0.1 to 0.5 μg/L) of 2,4-D in the 3 and 10 meter trenches at four DAT, while the other site had runoff with trace amounts detected in the 1 meter trench at one DAT and in the 3, 10, and 20 meter trenches at three DAT (Meru *et al.*, 1990). Meru *et al.* (1990) found detectable levels of 2,4-D in eleven of the fifty-seven trench water samples for the forty-eight week duration of the project.

2.8 Soil Surface Layer Dissipation

Concentrations of 2,4-D in the surface layer appear to decrease rapidly after application. A field study conducted in Spain found that concentrations in the first 10 cm of the soil profile followed a first-order decay equation (Crespín *et al.*, 2001). Twenty days after the application of 2,4-D amine salt in this study, 2,4-D was no longer present in the surface soil. Acid degradation metabolites, which were present in the soil the day after the application, were present in the surface layer for nearly thirty-five days after treatment (Crespín *et al.*, 2001). The metabolites were never detected in the subsurface.

Other studies confirm the rapid removal of 2,4-D from the surface layer (Wilson & Cheng, 1976; Grover *et al.*, 1985; Stewart & Gaul, 1977). In Pullman, Washington, 2,4-D iso-octyl ester and dimethyl amine salt formulations were applied to winter wheat at two application rates (Wilson & Cheng, 1976). The researchers found that the recovery rates for 2,4-D were greatest in the samples collected from the amine salt treated plots for both application rates. After forty-five days, both amine salt and ester treated plots in this study had significantly reduced concentrations in the surface. By six months, the surface concentrations in all plots accounted for 4% of the applied amount.

In Saskatchewan, 2,4-D iso-octyl ester was applied to a wheat field at a rate of 4.5 kg/ha (Grover *et al.*, 1985). After thirty-four days, the concentration in the surface layer was roughly 2% of the concentration measured one day after the application (Grover *et al.*, 1985). Stewart and Gaul (1977) found that surface sample concentrations in plots treated with 7.8

kg/ha of iso-octyl ester twenty-eight days after treatment were equal to 24% of the concentration detected in samples collected on the first day. After seventy days, the surface concentrations were 1.6% of the original concentration detected in soil, with no detectable levels after one year (Stewart & Gaul, 1977).

Soil moisture appears to affect the biological degradation rate of 2,4-D in surface soils. Wilson *et al.* (1997) found that the half-life for 2,4-D was about five days when the soil moisture was roughly equal to the field capacity. While other factors like soil temperature, soil organic matter, and the rate of application were also evaluated, their research indicated that the soil moisture content was the most important factor influencing 2,4-D dissipation (Wilson *et al.*, 1997).

2.9 Movement in the Soil Profile

Rainfall and irrigation appear to play a dominant role in the vertical transport of 2,4-D in soil. In several studies, simulated irrigation caused 2,4-D to move to lower layers within the soil profile. In the study conducted by Crespín *et al.* (2001), during the first eight days following the application, 2,4-D was not detected deeper than 10 cm in a soil profile that was composed of primarily clay with some sand and silt. The site was irrigated with 17 mm of water 8 days after the application, causing the herbicide to leach into the soil profile, where it was detected to a depth of 30 cm the following day. After the site was irrigated a second time, the herbicide was detected at a depth of 30 cm to 40 cm (Crespín *et al.*, 2001). Concentrations in the subsurface were always lower than those observed at the surface, with concentration decreasing with increasing depth (Crespín *et al.*, 2001).

While the results were not as immediate as those found by Crespin *et al.* (2001), a study conducted in Washington found that 16 mm of simulated rainfall was enough to cause 2,4-D to leach into a silt loam soil profile (Wilson & Cheng, 1976). In Wilson and Cheng's study, simulated rainfall was applied to the treated fields one day following the herbicide application. Two days after the simulated rainfall, 2,4-D was present at a depth of 24 cm. After four days, the herbicide had leached to a depth of 40 cm (Wilson & Cheng, 1976). For the most part, 2,4-D concentrations decreased as the depth increased. The surface soil samples always had the highest concentrations of 2,4-D, though as the DAT increased, the

subsurface soil samples often saw an increase in concentration with increasing depth (Wilson & Cheng, 1976).

2.10 Overall Persistence Studies

Since the development of 2,4-D in the 1940's, multiple studies have been conducted to determine its persistence in the natural environment. Various field studies have shown the herbicide to persist in soil for periods ranging from thirty-four to three hundred and sixty days, depending on factors such as climate, soil type, and organic matter (Wilson & Cheng, 1976; Wilson *et al.*, 1997; Crespín *et al.*, 2001; Meru *et al.*, 1990; Radosevich & Winterlin, 1977). In the study conducted by Wilson and Cheng (1976), concentrations of 2,4-D in the upper 24 cm of the soil column in plots treated with two different application rates were nearly identical at forty-three days after treatment with 2,4-D iso-octyl ester. The study demonstrated that application rates play a minimal role in the overall dissipation of the herbicide.

Both rainfall/irrigation and moisture content seem to have a profound effect on the persistence of 2,4-D. Two studies, one conducted in Spain (Crespín *et al.*, 2001) and the other in California (Radosevich & Winterlin, 1977), had similar air temperature ranges (16 °C to 34°C at the Spain site and 15 °C to 33°C at the California site), but had different climates and vegetation. The Spanish study had 51 mm of irrigation water added during the first month, while the California study had 9 mm or less of rain within the first month. Forty-two days following the application, the herbicide was not detectable in the Spanish soils, but persisted for over three hundred and sixty days in the California soils. The results of these studies suggest that soil moisture has a role in the rate at which 2,4-D dissipates.

2.11 Herbicide Persistence in Cold Soils

Research conducted in sub-arctic climates (Torstensson & Stark, 1982; Conn & Cameron, 1988) demonstrates that herbicides do have the potential to carry over into the next growing season after application. In Sweden, Torstensson and Stark (1982) applied 2,4-D buthoxiethyl ester at a rate of 2.0 kg a.e./ha to eight plots in three regions of the country.

Only the upper 5 cm of the soil was sampled. Three out of the eight sample locations, which were all located in the same region, had measurable concentrations (> 0.05 μg/sample) of 2,4-D three hundred days following the application (Torstensson & Stark, 1982).

Torstensson and Stark (1982) also investigated triclopyr persistence in the same eight soils in Sweden. Triclopyr euthyleneglycolbuthylether ester was applied to the soil at a rate of 2.2 kg a.e./ha. Unlike 2,4-D, measureable concentrations were detected in all soils three hundred days following the application (Torstensson & Stark, 1982).

Conn and Cameron (1988) investigated the persistence of the mobile herbicide, metribuzin, in soils near Delta Junction, Alaska. Metribuzin was detectable in the soil three years after the application, with 13% of the applied herbicide measured in the soil after one year and 2% after three years. The rates of metribuzin degradation in the growing season were similar to those seen in the contiguous states, but the authors hypothesized that the carry over to future growing seasons was due to the extended periods of cold weather during the winter (Conn & Cameron, 1988).

In a more recent study, Newton *et al.* (2008) applied the herbicides hexazinone, imazapyr, triclopyr, and glyphosate to plots at two different study sites in Alaska. One study site was located along the Gulf of Alaska at Windy Bay and the other was in interior Alaska in Fairbanks. At both study sites, all four herbicides persisted for more than 300 days following application. However, the researchers found that most dissipation occurred during the summer months. Only hexazinone was found to be mobile; vertical movement of the herbicide was detected at the Windy Bay site, where precipitation was high. Samples from the Fairbanks glyphosate plots were analyzed for the metabolite aminomethylphosphoric acid (AMPA). The presence of AMPA at the sites led the researchers to determine that biological degradation of the herbicides was occurring (Newton *et al.*, 2008).

Chapter 3 Methodology

3.1 Field Experimental Design

Two field studies were conducted during the summers of 2006, 2007, and 2008 to determine the persistence and vertical movement of 2,4-D in Alaska. Both sites contained four plots that were treated with Agrisolutions 2,4-D LV6 (2,4-D iso-octyl ester) was selected for both study sites, which was applied consistent with the product label directions. The application rate used during the studies was equal to 2.2 kg a.e./ha of 2,4-D iso-octyl ester. In both locations, application was via a truck equipped with a side delivery broadcast spray nozzle.

The two study sites were selected because they represent different but common sub-arctic climatic zones in Alaska. The first site, established in July 2006, was located approximately 32 km southeast of Delta Junction on the southeast side of Sawmill Creek Road. Delta Junction, located in interior Alaska, is relatively cold and dry with an average annual precipitation of 30.3 cm and an average temperature of –1.9°C. Temperatures vary greatly, with average low temperatures of –23°C in the winter and average high temperatures of 22°C in the summer. The second site, established in July 2007, was located 6 km east of Valdez on the south side of the Richardson Highway. Valdez, located on the south-central coast of Alaska, is humid, with a more temperate climate. Valdez has an average annual precipitation of 171.2 cm and an average temperature of 3.5°C. Temperatures have a far smaller range in Valdez, with average low temperatures of –9°C in the winter and average high temperatures reaching 17°C in the summer. Climate data was obtained from The Alaska Climate Research Center.

Two plots at the Delta Junction site were selected for sample collection and analysis. Each plot was 60.7 m long and 4.6 m wide and consisted of a diverse mix of densely vegetated areas and bare ground with low grasses and small diameter gravel (<1.3 cm). A photo of the site is included in Appendix A. Vegetation at the site included Balsam Poplar (*Populus balsimifera*), Paper Birch (*Betula papyrifera*), Felt-leaf Willow (*Salix alaxensis*), Little-tree Willow (*Salix arbusculoides*), Quaking Aspen (*Populus tremuloides*), Fireweed (*Epilobium angustifolium*), Horsetail (*Equisetum arvense*), Golden Rod (*Solidago sp.*), Yarrow (*Achillea millëfolium*), and Lowbush Cranberry (*Vaccimium vitus*). A complete list

of the vegetation found at the Delta Junction site is listed in Appendix A. The vegetation was not cut prior to the broadcast spray application. Only 8.6 cm of precipitation fell between July 6, 2006 and September 30, 2006. Rainfall data was recorded at the Delta Junction 20 SE weather station and was obtained from the Alaska Climate Research Center. Daylight hours for the 2006 sampling season ranged from twenty-one hours and three minutes on June 21st to ten hours and forty-seven minutes on October 6th. Daylight hours were obtained from the U.S. Naval Observatory.

Two plots at the Valdez site were selected for sample collection and analysis. Each plot was 30.5 m long and 4.6 m wide and was densely vegetated with continuous ground coverage. Vegetation at the site included Alder (*Alnus sp.*), Poplar (*Populus balsamifera*), Salmonberry (*Rubus spectabilis*), Willow sp. (*Salicaceae*), Fireweed (*Epilobium angustifolium*), Yarrow (*Achillea millefolium*), and Cockleburr (*Ranunculus*). A complete list of the vegetation present at the Valdez site can be found in Appendix B. Large pebbles (>15 cm diameter) were found throughout the site; these prevented the collection of some subsurface samples. A photo of the site is included in Appendix B. The vegetation was not cut prior to the broadcast spray application. The total precipitation was 33.7 cm during the period between July 16, 2007 and September 30, 2007. Rainfall data was recorded at the Valdez WSO weather station and was obtained from the Alaska Climate Research Center. Daylight hours for the 2007 sampling season ranged from nineteen hours and twenty minutes on June 21st to thirteen hours and seven minutes on September 13th. Daylight hours were obtained from the U.S. Naval Observatory.

Sample collection methodology was the same at both sites. During each sample event, four locations were randomly selected from individual plots. Three samples were collected at each location, each representing a different depth in the soil column. The samples at the top of the soil column were labeled as surface samples (S) and represented the first 7.6 cm of soil. Samples collected in the range of 10 cm to 30 cm below the surface were labeled as root zone samples (R) and samples collected from 36 cm up to a depth of 60 cm below the surface were designated as below root samples (BR). Pebbles and rocks often prevented BR samples from being collected at a depth of 60 cm. Therefore, the majority of BR samples were representative of the shallower depth range of 36 cm to 45 cm. Sample collection at Delta Junction began on July 7, 2006, one day after 2,4-D was applied, and ended on July 3,

2007. Sample collection at Valdez began a few hours after application on July 16, 2007 and ended on July 18, 2008.

The soil at the Delta Junction site was a silt loam with a hydraulic conductivity of 2 x 10^{-5} cm/s. The pH was 4.5, 5.5, and 5.8 for the surface, root zone, and below root zone depths, respectively. The soil at the Valdez site was a silty sand with a hydraulic conductivity of 2.5 x 10^{-4} cm/s and pH values of 4.9, 5.9, and 6.0 for the surface, root zone, and below root zone depths, respectively. A discontinuous clay layer was observed in the field at the below root zone depth. Hydraulic conductivity measurements were conducted using ASTM D5084: Standard Test Method for Measurement of Hydraulic Conductivity of Saturated Porous Materials Using a Flexible Wall Permeameter. Soil pH measurements were conducted using EPA Method 9045D: Soil and Waste pH.

Samples were collected using stainless steel trowels, stainless steel soil probes, steel hand-powered soil augers, and 4 oz. amber glass jars. Using a stainless steel trowel, vegetation and surface litter was removed and an 8 cm by 8 cm by 7.6 cm cube was removed from the ground. Soil was then removed from the cube and placed in a labeled surface sample amber glass jar. Root zone samples were collected using a stainless steel soil probe that was inserted into the soil column at the same location where the surface sample had been removed. Soil was then removed from the soil probe, excluding the top inch that was discarded to prevent cross-contamination, and placed in a labeled root zone sample jar. Soil augers were used to remove the root zone portion of the soil column. Once this was removed, a soil probe was used to collect the below root zone samples. Again, the top inch was removed and the rest of the soil core was placed into a labeled below root zone amber glass jar. The three samples from each location were placed in a cooler with ice to prevent further biological and photodegradation and for transport to the UAF lab. During a sample event, a total of eight samples were collected from each depth at the study sites, excluding any site condition that prevented sample collection, such as large pebbles in the subsurface.

In the field, several steps were taken to prevent cross contamination. All equipment was washed and sterilized prior to collecting each sample. After a sample was collected, the equipment would be rinsed with water and any remaining soil would be removed with a brush. The equipment was then rinsed with acetone and de-ionized (DI) water. Each piece was then hand dried with a clean paper towel and placed in a clean garbage bag to prevent

contamination from vegetation treated with 2,4-D at the study site. All samples were collected using nitrile gloves that were replaced after each sample was collected.

3.2 Laboratory Extraction Methodology

Samples were stored in a dark cold room set at a temperature of 4°C for a maximum of fourteen days to minimize any additional 2,4-D biological and photodegradation. Once each sample was removed from the cold room, five to thirty grams of soil, depending on the date of sample collection, were placed into a labeled 300 mL bottle. Samples collected later in the study were analyzed using greater amounts of soil to ensure that even low 2,4-D concentrations were detected. Exact weight measurements were recorded to the nearest hundredth in a laboratory notebook. A modified version of a method developed for the extraction of triclopyr by Tsukioka *et al.* (1986) was used to extract the 2,4-D acid (analyte) from the soil.

Dichloroacetic acid (DCAA) was used as the surrogate standard and each weighed soil sample received 25 µL of a 100 ppm DCAA solution. The DCAA was used to determine the percent recovery for each sample. DI water and a 33% potassium hydroxide (KOH) solution, used to preserve the samples and to maintain the analyte in a water-soluble form, were added based on the values shown in the Appendix A. Samples were then covered and shaken for at least two minutes. Since samples were stabilized after the addition of KOH, which prevented 2,4-D degradation, they could be returned to the cold room for a few more weeks before continuing with the analysis.

Samples were divided into labeled centrifuge tubes. Each 300 mL bottle was rinsed with 25 mL of DI water to ensure that all soil particles where the analyte may still have been present were recovered from the bottles. The water was then added to the corresponding centrifuge tube. The 300 mL bottles were rinsed an additional time and the water added to the centrifuge tubes. The centrifuge tubes were placed into a centrifuge and rotated at 3,500 RPM for two minutes. This separated the solids from the water, which then contained the extractable compounds. The water was returned to the appropriate 300 mL bottles, while the centrifuge tubes containing the soil were discarded.

A 20 mL volume of diethyl ether (ether), which was used to extract any ether soluble compounds that were not being analyzed in this study, was added to the 300 mL bottles. The water and ether solution was shaken by hand and vented occasionally (to prevent the build-up in pressure from volatilizing ether) for two minutes. The mixture was placed into new centrifuge tubes, placed in the centrifuge, and rotated at 3,500 RPM for two minutes to separate the water from the ether. The samples were removed from the centrifuge, and the upper ether phase was carefully removed from the tubes with a 1,000 µL pipette and discarded. At that point, the lower water phase was returned to the 300 ml bottles.

A one-part sulfuric acid to three-parts water mixture (1+3 H_2SO_4), which converted the analyte to an ether soluble form, was added to the 300 mL bottles. A 20 mL volume of ether, used to remove the analyte from the water solution, was then added to each 300 mL bottle. Each bottle was capped and shaken for at least two minutes and occasionally vented. The contents of the 300 mL bottles were then poured into centrifuge tubes. The tubes were placed in the centrifuge and rotated at 3,500 RPM. The volume 1+3 H_2SO_4 added can be found in Appendix A.

The samples were removed from the centrifuge and the upper ether phase was removed from the centrifuge tubes using a 1 mL pipette and placed in labeled 40 mL amber glass volatile organic analysis (VOA) vials. The water remaining in the centrifuge tubes was returned to the 300 mL bottles and an additional 10 mL of ether was added to the bottles to ensure that all of the analyte was transferred to the ether. The bottles were shaken by hand for two minutes, with occasional venting. The contents of the 300 mL bottles were then poured into the corresponding centrifuge tubes, after which they were placed in the centrifuge and rotated at 3,500 RPM. The upper ether phase was removed from the tubes and placed into the labeled VOA vials. An additional 10 mL of ether was added to the 300 mL bottles and the process repeated.

Approximately 4 to 5 g of Na_2SO_4, which absorbed any water remaining in the sample, was added to each VOA vial. The contents of the VOA vials were poured into TurboVap tubes, with care given to prevent the Na_2SO_4 from being poured into the tubes with the ether. The Na_2SO_4 in the VOA vials was rinsed with 5 mL of ether to ensure that all the analyte was transferred to the ether. The additional ether was then poured into the TurboVap

tubes. This was repeated once. The TurboVap tubes were then placed into the TurboVap and the samples were evaporated down to 1 mL using nitrogen.

The remaining ether was removed from the TurboVap tubes and placed into 16 mm test tubes using a 500 µL pipette. The residue in the TurboVap tubes was rinsed with 1 mL of ether and transferred to the test tubes, and the step was repeated. The ether in the test tubes was allowed to evaporate, uncapped, overnight in a fume hood.

Once the ether had evaporated, the samples were esterified by adding 0.5 mL of boron trifluoride. Caps were placed on each sample and they were shaken prior to being placed in a block heater. The samples were heated at 80°C for one hour. The heat was then shut off and the samples were allowed to cool for one hour.

Each 16 mm test tube was rinsed with a 10% sodium chloride (NaCl) solution, which forced the analyte into the organic phase, making it soluble in hexane, and poured into a 250 mL separatory funnel. Each separatory funnel had 15 mL of hexane added before being shaken by hand for two minutes and vented occasionally (to prevent the build-up of pressure from volatilizing hexane). The lower water phase, along with a small portion of the upper hexane phase, was removed from the separatory funnel and placed into a clean centrifuge tube. The hexane that remained in the separatory funnel was placed into a labeled VOA vial. The contents of the centrifuge tubes were then poured back into the corresponding separatory funnel and an additional 10 mL of hexane was added. The separatory funnels were again shaken by hand for two minutes and were vented occasionally. Again, the lower water phase was discarded and the upper hexane phase was placed into the corresponding VOA vials.

Approximately 4 to 5 g of Na_2SO_4 were added to the VOA vials and absorbed any water still remaining in the sample. The hexane in the VOA vials was transferred to TurboVap tubes with caution used to ensure that the Na_2SO_4 remained in the VOA vials. The Na_2SO_4 in the VOA vials was rinsed with 5 mL of hexane, which was then poured into the tubes. This step was repeated once. The TurboVap tubes were placed into the TurboVap and the samples were evaporated to 1 mL using nitrogen. The 1 mL of hexane was removed from each TurboVap tube using a 1,000 µL pipette and placed into a labeled gas chromatography (GC) vial. 1,4-dichlorobenzene (1,4-DCB) was used as the internal standard and 25 µL of 1,4-DCB was added to each GC vial. The 1,4-DCB was used to quantify the amount of 2,4-D in the samples. Each GC vial was brought to a volume of 2 mL with hexane.

All laboratory equipment was thoroughly washed and cleaned before use to reduce the risk of cross-contamination. Nitrile gloves were worn for each step, except for the addition of boron trifluoride, which required the use of neoprene gloves.

3.3 Gas Chromatography Analysis

Samples were analyzed using an Agilent Technologies 6890N Network GC System, 7683 Series Injector, and 5973 Network Mass Selective Detector (GC/MSD). Two GC/MSD methods were created to analyze the samples; these were named HERB and HERBREV1. GC/MSD method HERB was originally used to analyze the samples and HERBREV1 was later created to decrease the soil matrix interference with the analyte, which caused peak collusion. GC/MSD method details are shown in Table 3.1. The HERB method was used to analyze the Delta Junctions samples collected between July 7, 2006 and August 22, 2006, while the HERBREV1 method was used to analyze the Delta Junction samples collected between October 6, 2006 and July 3, 2007 and all Valdez samples collected during the study period.

Table 3.1 GC Method Information.

	HERB	HERBREV1
Column	HP-5MS, 30m x 0.25mm, 0.25µm film	HP-5MS, 30m x 0.25mm, 0.25µm film
Oven	1 minute (min) at 60°C, then to 255°C at 10°C/min, then to 290°C at 20°C/min, then to 325°C at 25°/min and hold for16.40 min.	1 minute (min) at 60°C, then to 200°C at 2°C/min, then to 325°C at 25°C/min and hold for 81 min.
Injector	2.0 L	2.0 L
Front Inlet	Initial temp. at 280°C, pressure 2.47 psi	Initial temp. at 280°C, pressure 2.47 psi

By utilizing a GC/MSD system, a mass chromatogram was produced for each sample that allowed the 2,4-D concentrations to be quantified. Mass chromatograms are plots where the x-axis represents the retention time of a compound and the y-axis is the abundance or intensity of the signal as the compound moves from the GC column into the mass selection detector. Standard solutions were created using known quantities of 2,4-D, DCAA, and 1,4-DCB and run through the GC/MSD. The mass chromatograms created from the standard

solutions were used to determine the retention time of each compound and to select five to six ions from each compound, which were then used to quantify the concentrations of the compounds in each field sample. Each ion was identified by its mass to charge ratio (m/z). A selected ion monitoring (SIM) program was created in both of the GC methods so that only the ions used to quantify the 2,4-D, DCAA, and 1,4-DCB concentrations were being evaluated by the GC. Once a mass chromatogram was created for a sample, extracted ion chromatograms were constructed by specifying an m/z value and a time period. The extracted ion chromatograms, which utilized the same axis as the mass chromatograms, would display a peak that represented the specified ion.

Several samples were loaded into an automated GC sampler at one time, with multiple standard solutions comprised of known 2,4-D, DCAA, and 1,4-DCB concentrations also included in the run. To determine the concentration of 2,4-D, DCAA, and 1,4-DCB in each sample and standard solution, extracted ion chromatograms were created for each compound, and the area under each ion peak present at the specified retention times was analyzed using the Agilent ChemStation software. The ratio of 2,4-D abundance to 1,4-DCB abundance (analyte/1,4-DCB) was calculated using the standard solutions. The concentration of 2,4-D was known in the standard solutions and a simple linear relationship was used to determine the 2,4-D concentrations in the samples using the analyte/1,4-DCB ratio found in the samples, the analyte/1,4-DCB ratio, and the known 2,4-D concentration in the standard solutions.

Recovery rates were determined using a method similar to that used to determine sample 2,4-D concentrations. The ratio of DCAA abundance to 1,4-DCB abundance (DCAA/1,4-DCB) was determined for each sample and standard solution. The DCAA/1,4-DCB ratio of the sample was divided by the DCAA/1,4-DCB ratio of the standard and multiplied by 100% to determine the percent recovery. Acceptable recovery rates for soil extractions range from 70% to 130%.

Recovery rates were determined to be 70.22% ± 11.53% for the Delta Junction samples and 124.5% ± 22.3% for the Valdez samples. Recovery rates are not based on all samples due to soil matrix interference with the DCAA. In several samples, the soil matrix interference with the DCAA caused peak collusion with unidentified soil compounds that had the same ions and a similar retention time, making it impossible to analyze areas under the

extracted ion chromatogram peaks and determine recovery rates. While slight variations in soil composition and organic matter existed between samples, the overall recovery rate for each sample location is representative because sample extraction methods were not altered during the study period. The high recovery rates for Valdez may have been caused by laboratory error, but the concentrations still show the overall trends. Sample concentrations have not been adjusted to reflect the recovery rates.

An attempt was made to analyze the 2,4-D metabolites 2,4-dichlorophenol, 2-chlorophenol, and 4-chlorophenol. Metabolite standards, as well as the surrogate standard 2,4-dibromophenol and internal standard 2,5-dibromotoluene, were obtained from Absolute Standards, Inc. The metabolite standards, surrogate standard, and internal standard were analyzed on the GC/MSD to determine their retention times. For the Delta Junction samples collected between the 7^{th} and 9^{th} sampling events, as well as the Valdez samples collected between the 1^{st} and 5^{th} sampling events, 50 µL of a 40 ppm 2,4-Dibromophenol were added to each sample at the same time as the DCAA solution was added. This was eventually used to determine the viability of a simple ether extraction for the 2,4-D metabolites. Rather than discard the ether initially used to extract the compounds not being analyzed in the study, the ether was placed in a labeled VOA vial for each sample. The 300 mL bottle containing the sample was rinsed a second time with ether. The contents of the bottle were again divided into centrifuge tubes and rotated on the centrifuge for 2 minutes at 3,500 RPM. Using a 1,000 µL pipette, the upper ether phase was removed from the centrifuge tubes and added to the labeled VOA vials. Approximately 4 to 5 g of Na_2SO_4 were added to the VOA vials to remove any water in the ether. The samples were then reduced to 1 mL using the TurboVap. An amount of 5 mL of hexane was added to the TurboVap tubes and the samples were reduced to 1 mL again. The hexane was transferred from the TurboVap tubes and placed in labeled GC vials. Each GC vial received 50 µL of a 40 ppm 2,5-diboromotoluene solution and was then brought to 2 mL with hexane. No detectable levels of any of the metabolites were ever observed. It is likely that the simple ether extraction was unsuccessful, possibly because the metabolites were not in an ether soluble form or the concentrations were too low.

Chapter 4 Results and Discussion

4.1 Delta Junction Results and Discussion

No distinct 2,4-D concentration differences were observed between the two plots at the Delta Junction study site, so the data results from the two plots were combined (Table 4.1). All data, separated by plot, can be found in Appendix A. The results reflect only 2,4-D acid (2,4-D) concentrations and do not include 2,4-D iso-octyl ester concentrations, which were not extracted from the soil samples and analyzed. It can be assumed, based on previous studies, that most, if not all, of the iso-octyl ester would have been hydrolyzed within the first week. Stewart and Gaul (1977) saw complete hydrolysis of the iso-octyl ester one day after applying 2,4-D at a rate of 7.8 kg/ha in Nova Scotia, well above the 2.2 kg a.e./ha application rate at the Delta Junction study site. Grover *et al.* (1985) found that 70% of the 2,4-D iso-octyl ester that was applied at a rate of 0.5 kg/ha had hydrolyzed after five days at a study site in Saskatchewan. While it is impossible to know how quickly the iso-octyl ester was hydrolyzed at the Delta Junction site, the Stewart and Gaul (1977) and Grover *et al.* (1985) studies indicate that the vast majority would have been hydrolyzed in the first few days and the overall data trends throughout the study would have remained the same.

As with any field study, there is a large amount of variability among samples as indicated by the range of concentration values, which generally decreases over time, and is shown in Figure 4.1 and Table 4.1. While every effort was made to ensure a uniform spray, site conditions such as the vegetation density and height and the spray truck configuration caused large application variations at both Delta Junction plots. Vegetation often intercepted the spray, which was applied using a truck with a side-mounted nozzle that was driven parallel to the plot length, causing the application to be non-uniform across the width of the plot. Even though concentrations in the vegetation were not measured in Delta Junction, Radosevich and Winterlin (1977) demonstrated that vegetation could intercept a substantial amount of herbicide during an application. The researchers found that nearly 46% of the 2,4-D was intercepted by grass, forbs, and chamise brush immediately following an application in California (Radosevich & Winterlin, 1977). If surface litter is included, then nearly 100% of the 2,4-D was intercepted and did not reach the soil surface immediately following the application (Radosevich & Winterlin, 1977). Thus, the application methods in Delta

Junction, along with the study conducted by Radosevich & Winterlin (1977), suggest that the soil surface in portions of each plot initially may have received no 2,4-D due to vegetation interception.

Drift losses, which transport a herbicide to other regions of the site or to locations that are off-site, can also account for some of the variability at a study site. Grover *et al.* (1985) found that droplet and vapor drift losses accounted for less than 0.2% of the total amount of 2,4-D iso-octyl ester applied. Applications of 2,4-D tri-isopropaolamine salt in Ontario caused trace amounts to be detected up to 10 meters from the study area (Meru *et al.*, 1990). While drift losses were not measured at the Delta Junction site, these studies suggest that it is unlikely drift losses accounted for the variability observed in the sample concentrations, especially considering that the application was conducted under low wind conditions.

While some areas in the study plots may have received no 2,4-D, it is impossible to determine if samples with non-detectable levels of 2,4-D never actually received 2,4-D, or if the 2,4-D concentrations were below the GC/MSD detection limits. Therefore, median concentrations presented in the figures and tables are based on all samples, including those without detectable concentrations, which were assigned a value of zero.

As Table 4.1 shows, the first notable result is the relatively low concentrations measured in the surface and subsurface soils at the Delta Junction site (note that concentration values are presented in units of micrograms per kilogram). Since 2,4-D iso-octyl ester was applied once during the study period, it was expected that the concentration of 2,4-D would decrease over time. The median surface concentration values show a general downward trend over time (Figure 4.1 and Figure 4.2), though a slight increase in median concentration is noted at five DAT, at 47 DAT, and again at 288 DAT. The highest median surface concentration occurred during the second sample event at five DAT. However, the highest observed surface concentration during the study period occurred one DAT and is shown in Table 4.1. Hydrolysis of the 2,4-D ester to the acid form, which Grover *et al.* (1985) found to take several days, may explain why the highest median concentration of 2,4-D acid occurred five days after the application. It is also likely that vegetation interception played a primary role in the surface concentrations in soils collected during the week following the 2,4-D application. Areas with little to no vegetation had a direct application of 2,4-D to the soil

surface, while areas with dense vegetation had the application intercepted by foliage and surface litter, thus delaying the movement of 2,4-D to the soil surface.

Table 4.1 Sample Event Data for Delta Junction, Alaska. The soil temperatures listed are a 24-hour average based on temperatures taken at two-hour intervals. Soil moisture contents were determined on a mass basis. Root zone and below root zone samples were not collected at 288 DAT because those soil layers were still frozen at that time. Concentrations do not account for recovery rates.

Sample Date	DAT	No. of Samples	Median Conc. (µg/kg)	Low Conc. (µg/kg)	High Conc. (µg/kg)	Soil Temp. (°C)	Soil Moisture Content (%)
Surface							
7/7/06	1	8	23.2	0.0	252.6	NC	32.9
7/11/06	5	7	33.4	44.1	139.6	NC	28.5
7/17/06	11	7	10.4	30.4	91.1	17.6	32.5
8/2/06	27	8	7.5	9.5	31.5	16.0	30.3
8/22/06	47	8	19.7	30.0	90.8	9.5	33.2
10/6/06	92	6	15.0	8.6	29.7	3.3	36.2
4/20/07	288	8	28.2	38.1	128.7	1.3	43.9
5/18/07	316	7	8.2	10.9	32.2	7.8	37.1
7/3/07	362	7	3.1	1.9	6.8	15.4	32.7
Root Zone							
7/7/06	1	8	1.0	0.0	9.2	NC	34.8
7/11/06	5	6	1.5	0.0	11.5	NC	26.0
7/17/06	11	8	4.4	0.0	6.8	14.7	31.4
8/2/06	27	7	5.1	4.1	10.4	14.2	32.0
8/22/06	47	8	3.5	2.7	7.9	10.0	30.6
10/6/06	92	6	2.1	0.0	2.6	4.3	29.9
4/20/07	288	N/A	N/A	N/A	N/A	-0.4	N/A
5/18/07	316	5	0.6	0.4	0.7	5.4	34.6
7/3/07	362	8	0.0	0.0	0.0	14.0	29.3
Below Root Zone							
7/7/06	1	8	2.1	0.0	3.6	NC	25.0
7/11/06	5	7	0.5	0.0	1.2	NC	18.8
7/17/06	11	7	0.0	0.0	4.7	13.0	23.8
8/2/06	27	8	3.2	1.2	3.9	12.3	22.8
8/22/06	47	7	2.6	1.6	5.2	10.5	20.0
10/6/06	92	6	1.2	0.0	1.7	5.5	21.9
4/20/07	288	N/A	N/A	N/A	N/A	-1.2	N/A
5/18/07	316	8	0.2	0.0	0.5	2.4	20.8
7/3/07	362	8	0.0	0.0	0.0	11.7	20.7

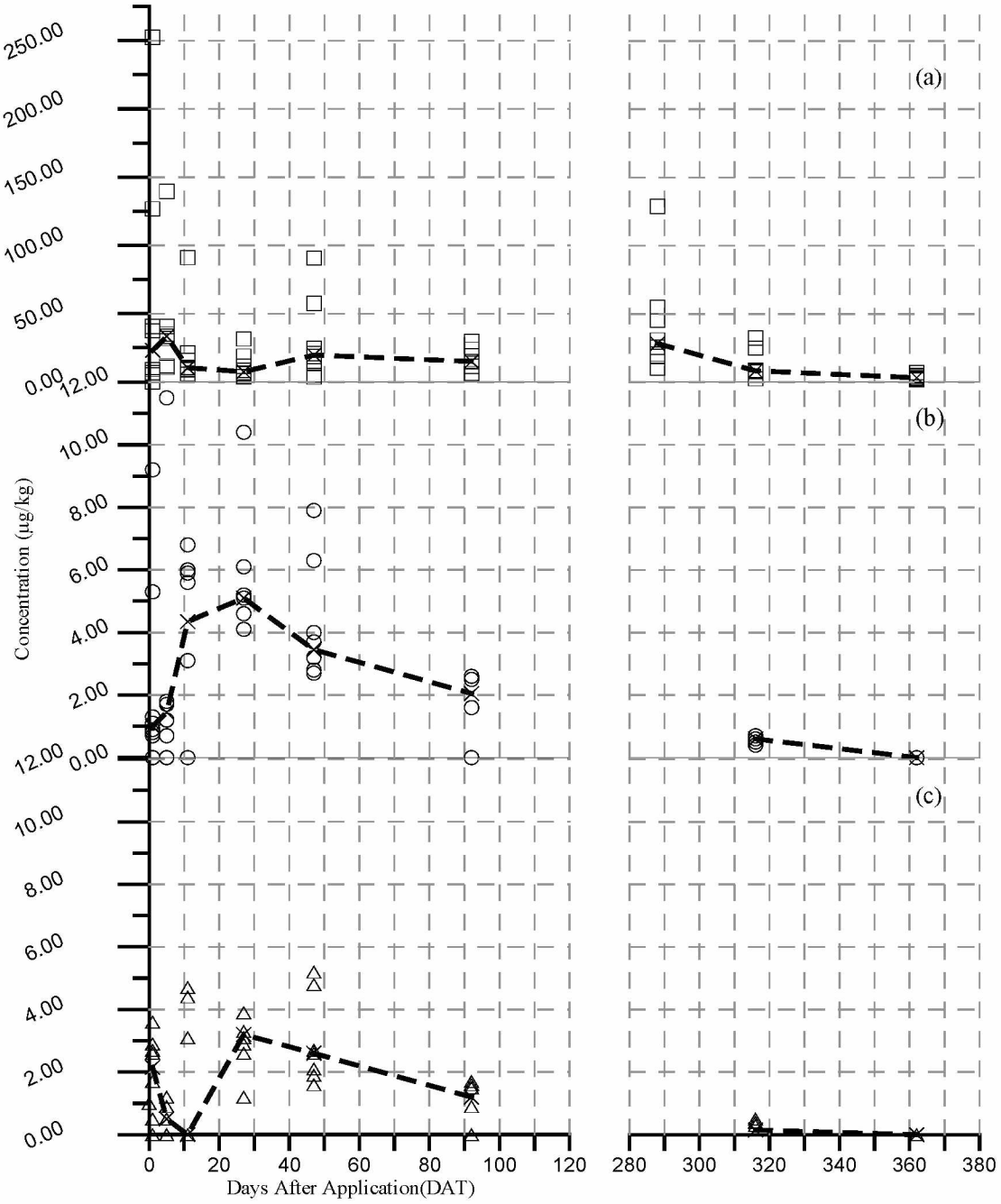

Figure 4.1 Delta Junction 2,4-D Concentrations. 2,4-D concentrations measured in surface (a, 0-7.6 cm), root zone (b, 10-30 cm), and below root zone (c, 36-60 cm) soil at the Delta Junction site. The trend line in each plot represents the median sample concentration. Herbicide was applied to the site on July 6, 2006. The soil froze approximately 120 days after application and thawed approximately 286 days after application. The break in the ordinate axis represents this frozen period. Note the concentration scale difference between (a) and both (b) and (c). Concentrations do not account for recovery rates.

The increase in concentration forty-seven DAT closely followed a rain event of 11.2 mm and is shown in Figure 4.2. Other rain events occurred prior to day forty-seven, but none generated more than 5 mm of precipitation and did not result in increased surface soil concentrations. Other studies have not reported a similar phenomenon following a rain event (Crespín *et al.*, 2001; Wilson & Cheng, 1976), though triclopyr (a synthetic auxin herbicide like 2,4-D) concentration increases have been observed after spring breakup (Torstensson & Stark, 1982). Based on the research conducted in Delta Junction and on current literature, it is impossible to know if the same release mechanisms are involved after a rain event and spring breakup. The rain event may have caused surface wash-off from the vegetation and contributed to the concentration increase, though the majority of the herbicide was likely removed from the foliage surface prior to day forty-seven through the processes of plant uptake, volatilization, and photodegradation. Other processes such as reverse conjugation in the roots of susceptible species from amino acids to the free acid form may have contributed to the increase, allowing the free acid to be transported from the roots to the soil by the precipitation (Davidonis *et al.*, 1980). The increase in soil moisture following the rain event may have caused additional hydrolysis of the ester to the acid form, which then would cause a release of additional 2,4-D to the soil surface. Grover *et al.* (1985) found that the hydrolysis process appeared to be positively affected by increased moisture contents. The increase was not observed in the root zone or below root zone samples, though an increase could have occurred prior to sample collection on day ninety-two as the herbicide leached through the soil profile (Figure 4.2).

Another increase in the median surface concentration occurred 288 DAT, right after the surface soils thawed during spring breakup. However, it should be noted that random sampling conducted at the site may have contributed to the observed concentration increase if locations with elevated 2,4-D concentrations prior to freezing were sampled. It is also possible that the increased surface concentrations after spring breakup were caused by a release mechanism similar to the one that caused the concentration increase at day forty-seven, such as the reverse conjugation of the amino acids to free 2,4-D in the roots of susceptible plants (Davidonis *et al.*, 1980). If the concentration increase is valid, then the Delta Junction results would confirm the results found by Torstensson and Stark (1982), who also observed increased concentrations of the herbicide triclopyr in soils following spring

breakup in Sweden. As suggested by a study conducted by Iwata and Hirota (2005), the freeze-thaw cycle may have contributed to the increase. Iwata and Hirota (2005) found that as the freezing front advanced in a soil column, upward pore water flow was induced as the water moved to areas of lower water potential. The results of the Iwata and Hirota (2005) study suggest that as the freezing front advanced in Delta Junction, pore water with dissolved 2,4-D may have flowed from the root zone and below root zone depths to the surface, and increased the concentration of 2,4-D when the surface layer froze. The surface samples collected immediately following spring breakup were collected when the root and below root zone depths were still frozen, which means that any additional 2,4-D in the surface was unable to leach through the soil column before the sample event at day 288.

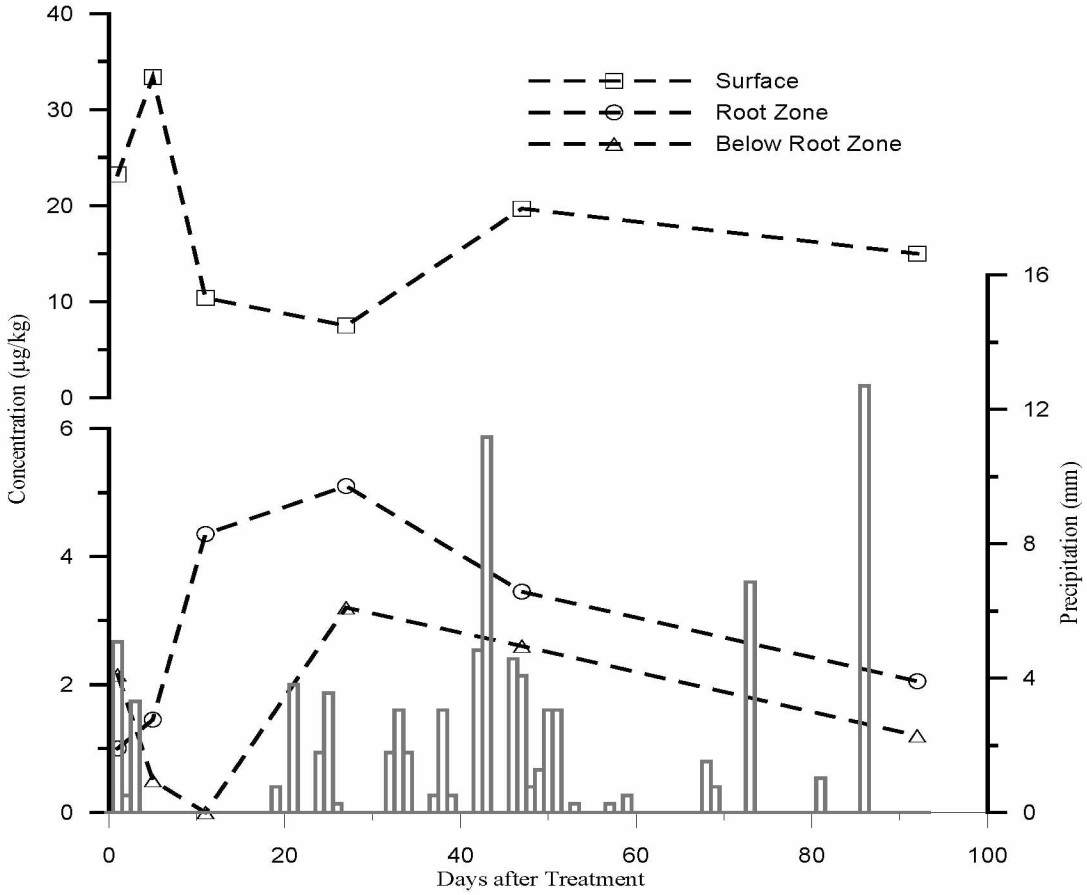

Figure 4.2 Median Delta Junction Surface Concentrations versus Precipitation. Precipitation amounts were measured at the Delta Junction 20 SE weather station for the first 92 days after treatment. Concentrations do not account for recovery rates.

An increase in concentration was not observed in subsurface soils following spring breakup. It is possible that a concentration increase occurred as the thaw front moved through the soil column, but root zone and below root zone samples were collected over a week after the ground thawed, allowing time for any increased 2,4-D to leach through the soil column.

Overall, soil concentrations decreased with increasing depth (Table 4.1, Figure 4.1, and Figure 4.2). While degradation products were not measured in the Delta Junction samples, Crespín *et al.* (2001) measured the concentration of 2,4-D degradation products in samples collected from an area treated with 2,4-D amine salt in Spain. The researchers found degradation products present in the surface soil layer, but not in the subsurface, suggesting that 2,4-D degradation occurs primarily in the organic matter-rich surface layer (Crespín *et al.*, 2001). If 2,4-D concentrations are reduced in the surface through degradation pathways, then there is a reduced amount of 2,4-D available for transport through the subsurface soil layers. Therefore, the reduced subsurface 2,4-D concentrations may be caused by the degradation of 2,4-D in the surface soil layer.

The majority of root zone and below root zone samples exhibited small concentrations of 2,4-D one DAT. Rain events immediately following the application may have contributed to the transport of 2,4-D to the subsurface so soon after the application. While the Delta Junction 20 SE weather station did not record any rain on July 6, 2006, the day of the application, a rain event was observed at the site by the researchers present at the site. Other studies have indicated that small rain events can induce herbicide leaching through a soil profile. Crespín *et al.* (2001) irrigated a site with 17 mm of water eight days following an application of 2,4-D amine salt and found detectable concentrations at a depth of 30 cm the following day. In another study, Wilson and Cheng (1976) applied 16 mm of water to a site treated with 2,4-D iso-octyl ester the previous day and found detectable concentrations at a depth of 24 cm two days later. The small rain event at the Delta Junction study site, while likely smaller than the irrigation events in the Crespín *et al.* (2001) and Wilson and Cheng (1976) studies, may reasonably explain the 2,4-D detected in the root and below root zone samples one day following the application. Secondary porosities in surface soils created by root channels, burrowing animals, and insects can create preferential flow paths for infiltrating water. Such preferential flow paths could be the reason a small rain event was able to cause leaching at the Delta Junction site one DAT. In addition, the rain

event on July 6, 2006 occurred within three hours after the application when little herbicide dissipation had occurred.

The root zone and below root zone samples had similar detectable concentrations, especially when compared to the concentrations detected in the surface samples (Table 4.1). As shown in Figure 4.2, the highest median concentrations in the root zone and below root zone samples occurred twenty-seven DAT. The median concentrations in the root zone increased from day one through day twenty-seven, at which point they decreased throughout the study period and were no longer detected 362 days following the application. The below root zone samples, which exhibited a higher median concentration than the root zone samples one DAT, had decreasing median concentrations through day eleven. The below root zone samples saw an increase in median concentration on day twenty-seven, after which a steady decrease was observed through the rest of the study period, with no detectable concentrations on day 362 (Figure 4.1).

Concentrations of 2,4-D were detected after spring breakup in both the surface and subsurface samples (Table 4.1 and Figure 4.1). These results contradict studies in temperate climates that have shown 2,4-D to rapidly degrade, with 2,4-D rarely persisting over sixty days. Wilson *et al.* (1997) conducted thirteen dissipation studies in a temperate climate using a liquid 2,4-D iso-octyl ester formulation and found that only two of the study sites had 2,4-D persistence greater than sixty days. However, 2,4-D dissipation studies in sub-arctic environments have shown increased persistence. A study conducted in Sweden by Torstensson and Stark (1982) found 2,4-D concentrations present in the soil a year after application in three out of eight study sites. Of the sites where 2,4-D dissipated before the ground froze, only one site had no detectable concentrations after sixty days (Torstensson & Stark, 1982). The results from Delta Junction, compared with those observed in the Torstensson and Stark (1982) and Wilson *et al.* (1987) studies, suggest that 2,4-D applied in sub-arctic climates tends to persist in soils longer than 2,4-D applied in temperate climates.

The increased persistence time is likely due to the colder soil temperatures in sub-arctic climates. Microorganisms have specific temperature ranges in which they thrive, with the majority of microorganisms classified as mesophilic with temperatures ranging from around 15°C to as high as 45°C (Vaccari *et al.*, 2006). Since most microorganisms are

mesophilic, it is widely accepted that microbial degradation slows as soil temperatures decrease.

Veeh *et al.* (1996) found that the maximum degradation rate for 2,4-D decreased with decreasing temperature. While many temperate climates enjoy long periods of warm soil temperatures, the average surface soil temperature dropped below 10°C by August 22, 2006, or day forty-seven, at the Delta Junction study site. This temperature drop likely caused a decrease in the microbial degradation of 2,4-D. Microbial degradation rates probably continued to decrease until the soil froze, at which point the degradation rates were roughly zero. Decreased microbial degradation in the soils are one explanation for the increased persistence of 2,4-D in sub-arctic soils, because the amount of 2,4-D being degraded by the soil microorganisms would decrease with declining temperatures.

Other studies have shown that herbicide dissipation follows first-order decay models (Wilson *et al.*, 1997; Crespín *et al.*, 2001). However, the studies conducted by Wilson *et al.* (1997) and Crespín *et al.* (2001) were conducted in temperate climates where 2,4-D concentrations were rarely detected after forty days and no additional 2,4-D mass was added to the soil. Temperate climates enjoy longer growing periods with warmer soil temperatures than in Alaska. A reduction in soil temperatures will reduce the biological degradation of 2,4-D, thus slowing its dissipation. Johnson *et al.* (1995) found that a 15°C decrease was enough to increase the time required for the disappearance of 50% of the initial soil concentration by up to twenty days. Unlike the studies conducted by Wilson *et al.* and Crespín *et al.*, increases in median 2,4-D concentration in the surface layer at the Delta Junction site were detected as noted previously.

Due to the increased surface concentration and the decrease in soil temperature, only the first thirty days were used for the Delta Junction first-order decay model so that the half-life values observed in temperate climates could be compared to those observed during the height of the growing season in Alaska. Also, using the first thirty days allows for a comparison of the initial dissipation between the Delta Junction study and studies conducted in temperate climates. First order decay is as follows:

$$C(t) = C_0 e^{-kt} \tag{4.1}$$

where C_0 is the concentration at time 0 and k is the rate constant. Estimating k from a best fit of equation 4.1, the half-life can be determined as follows:

$$t_{1/2} = - \ln (1/2) / k \quad (4.2)$$

The half-life for 2,4-D based on the first 30 days following the application was determined to be 13.4 days and the correlation coefficient for the best fit was 0.73. The half-life model is shown in Figure 4.3. Average half-life values for 2,4-D in temperate climates is ten days (Tu *et al.*, 2001), which is within the same order of magnitude as the half-life calculated for Delta Junction based on the thirty days following the 2,4-D iso-octyl ester application.

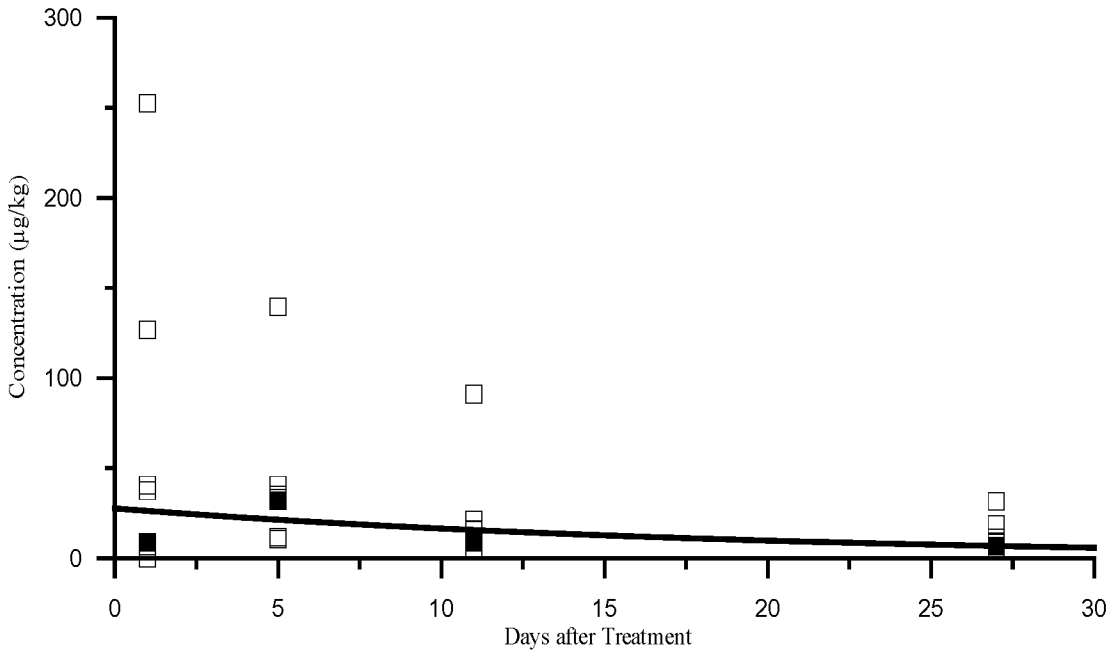

Figure 4.3 First Order Decay Curve for Delta Junction Samples. First order decay is based only on the first 30 days. The correlation coefficient for best fit was calculated as 0.73. Concentrations do not account for recovery rates.

When the mass of 2,4-D measured in the soil is compared with the total mass of 2,4-D applied, the percentage of mass accounted for in the soil can be determined (Figure 4.4). The calculation used to determine the amount of mass in the top 60 cm of soil is simply an integration of the measured concentration in the three sample horizons with depth. The percentage of mass accounted for in the soil one day following the 2,4-D application was determined to be roughly 4.5%, though based on the upper and lower quartiles, it could be as low as 1.5% or as high as 11%. Based on the upper quartiles, the maximum percentage of mass accounted for in the soil column at any given time is 11%, which indicates that very little of the 2,4-D ever reached the soil column. As demonstrated in other field studies,

vegetation and surface litter interception may have prevented the majority of 2,4-D from reaching the soil surface during the study. Radosevich and Winterlin (1977) found that vegetation and surface litter at a site in California intercepted nearly 100% of the applied 2,4-D butoxypropyl ester, with 45% of the applied 2,4-D being intercepted by the vegetation alone. In Saskatchewan, Grover *et al.* (1985) found that wheat intercepted 52% of the applied 2,4-D iso-octyl ester. While the vegetation and ground cover at the site in Delta Junction is invariably different from that encountered at the study sites in California and Saskatchewan, it still indicates that a substantial amount of 2,4-D was likely intercepted in Delta Junction and never reached the soil surface.

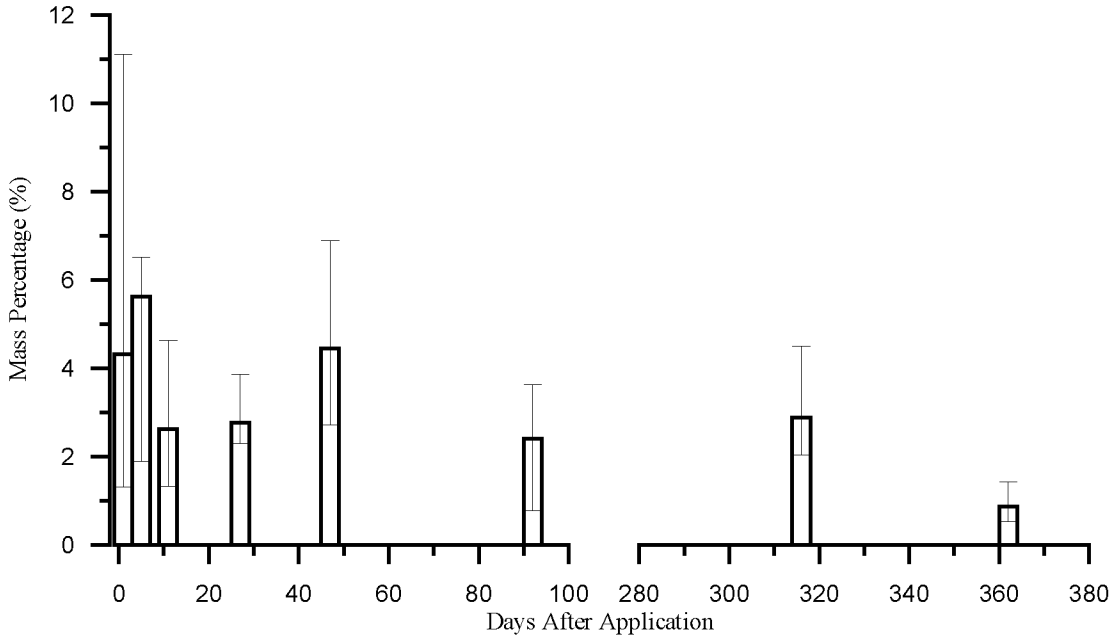

Figure 4.4 Mass of Applied 2,4-D Accounted for in the Delta Junction Soil Profile. Bars represent the ratio of the median value of mass measured in the soil to the total mass applied. Error bars indicate the upper and lower quartile of the measured mass datum set.

Amounts of 2,4-D intercepted by the vegetation at the Delta Junction site were likely reduced through plant uptake and volatility. Herbicide adsorption into plants can be regarded as a three-phase process. Once applied, the herbicide first partitions into the cuticle and then diffuses through the cuticle, and finally partitions out of the cuticle into apoplast, or the epidermal cell well area (Devine *et al.*, 1993). The rate that a herbicide will partition into and distribute throughout the cuticle is controlled by the factors that dictate the rate of diffusion.

These factors include: thickness of the cuticle, herbicide concentration on the leaf surface, magnitude of the molecular diffusion coefficient for the herbicide in these materials, and the tortuosity of the diffusion pathways. Movement of herbicides into plants is relatively rapid. Bucholtz and Hess (1985) measured a rapid penetration of 2,4-D into intact cabbage with only 10% of the applied herbicide mass being recovered at any time after application. If the amount of 2,4-D vegetation penetration was as high in Delta Junction as it was in the Bucholtz and Hess (1985) study, then little of the 2,4-D would have been transported to the soil column.

Using a simulated ecosystem in a lab, Gile (1983) found that 15% of the total 2,4-D iso-octyl ester applied was lost through volatility. The percentage lost through volatility was even greater in a field study conducted in Saskatchewan (Grover *et al.*, 1985). Grover *et al.* (1985) found that cumulative volatility losses, determined over the first five days following an application of 2,4-D iso-octyl ester, accounted for 20.8% of the total amount applied. While volatility losses at the Delta Junction site were not investigated, the studies conducted by Gile (1983) and Grover *et al.* (1985) suggest that volatility losses at the Delta Junction site may have been substantial, reducing the amount of 2,4-D that could reach the soil surface and leach through the soil column.

The overall mass accounted for in the soil varies little over time except for the last sample event, where the percent accounted for in the soil drops to roughly 1%. The percent accounted for in the soil is based on concentrations in the surface, root zone, and below root zone soil layers. While the surface soils experienced a decrease in concentration, the root zone and below root zone soil samples experienced an increase in 2,4-D concentrations (Figures 4.1 and 4.2). Since the percent accounted for in the soil remained fairly constant over the course of the study period, the results may indicate that limited biological and chemical degradation is occurring in the surface soil and that leaching is the primary dissipation path for 2,4-D in the soil. The mass of herbicide measured at any time after application in the surface soils is likely a combination of herbicide sorbed to organic matter in the organically rich surface soils and herbicide that resides in the susceptible plant roots as 2,4-D acid. Sorption of herbicide onto organic matter may result in less herbicide availability for microbial degradation, which may in part explain the fairly constant amount of mass measured in the soil over time. Proving this assertion has been shown to be difficult owing to

multiple breakdown mechanisms that occur in the soil beyond microbial. As discussed, Davidonis *et al.*, (1980) showed the reversibility from amino acid conjugates to free 2,4-D in a susceptible species, providing some evidence for the possibility of measuring 2,4-D in roots in the surface soils at the Delta Junction study site.

Further, the degradation rate of a herbicide will be slowed because of the reduced microbial degradation due to the relatively cold soil temperatures. Soil temperatures measured in Delta Junction during the study period ranged between -12.4°C and 22.4°C for the surface zone, -10.9°C and 16.4°C for the root zone, and –8.8°C and 14.7°C. While the upper values of the soil temperature ranges for the soil layers were at or near the temperature range for mesophilic microorganisms (Vaccari *et al.*, 2006), the median temperatures, measured to be 0.1°C for the surface zone, 0.6°C for the root zone, and 0.7°C for the below root zone, were not. Given that mesophilic microorganisms are the most abundant form of microorganism, the low median soil temperatures may indicate a reduced amount of microorganisms available to degrade to the 2,4-D.

4.2 Valdez Results and Discussion

Results reflect concentration data from the only plot at the Valdez site with detectable concentrations of 2,4-D; these results are shown in Table 4.2. It is possible that the plot with no detectable concentrations was never treated with 2,4-D iso-octyl ester due to difficulties with the spray truck. In addition, photographs from the site do not reveal any signs of plant death, further supporting the notion that the site was never treated. All data, separated by plot, is located in Appendix B. Results only reflect 2,4-D acid concentrations.

Overall trends for the Valdez results were difficult to define because the median values were based on four samples or less (Table 4.2). Several surface samples and a majority of root zone and below root zone samples had no detectable amounts of 2,4-D present. This can be seen in the Appendix B.

The median surface concentrations peak the day after application and then decrease seven DAT. Following the decrease at day seven, the median surface concentration remains constant through the final sample event of 2007, taken on day fifty-nine (Figure 4.5). Surface concentrations collected after spring breakup were relatively high when compared with

concentration values from previous sample events. Most subsurface samples had no detectable concentrations of 2,4-D, with 2,4-D detected in only seven out of twenty-three root zone samples and three out of twenty below root zone samples. One root zone sample had detectable concentrations of 2,4-D after spring breakup, with a high concentration of 2,4-D relative to samples collected during previous sample events. No below root zone samples had detectable amounts of 2,4-D after spring breakup.

Table 4.2 Sample Event Data for Valdez, Alaska. The soil temperatures listed are a twenty-four hour average based on temperatures taken at two-hour intervals. Soil moisture contents were determined on a mass basis. Root zone and below root zone samples were not collected 0.5 DAT because it was unlikely the 2,4-D had leached into the soil at that point. Concentrations do not account for recovery rates.

Surface							
Sample Date	DAT	No. of Samples	Median Conc. (µg/kg)	Low Conc. (µg/kg)	High Conc. (µg/kg)	Soil Temp. (°C)	Soil Moisture Content (%)
7/16/07	0.5	2	5.9	0.0	11.9	13.2	33.0
7/17/07	1	4	19.0	0.0	21.8	11.9	31.6
7/23/07	7	3	7.8	0.0	8.8	12.2	38.2
8/8/07	23	4	7.7	0.0	15.9	12.5	29.0
9/13/07	59	3	8.4	3.8	34.2	9.7	33.4
5/19/08	308	4	62.0	29.9	271.4	NA	NA
7/18/08	368	4	0.0	0.0	42.1	NA	NA
Root Zone							
7/16/07	0.5	N/A	N/A	N/A	N/A	11.3	N/A
7/17/07	1	4	3.3	0.0	37.1	10.8	26.5
7/23/07	7	4	0.0	0.0	8.7	11.2	25.0
8/8/07	23	4	3.3	0.0	23.2	11.8	27.5
9/13/07	59	3	0.0	0.0	2.6	9.7	20.4
5/19/08	308	4	0.0	0.0	55.2	NA	NA
7/18/08	368	4	0.0	0.0	0.0	NA	NA
Below Root Zone							
7/16/07	0.5	3	N/A	N/A	N/A	9.2	N/A
7/17/07	1	3	6.5	0.0	12.9	9.4	25.2
7/23/07	7	3	0.0	0.0	0.0	9.9	19.0
8/8/07	23	3	0.0	0.0	5.1	10.2	16.7
9/13/07	59	3	0.0	0.0	0.0	9.3	23.5
5/19/08	308	4	0.0	0.0	0.0	NA	NA
7/18/08	368	4	0.0	0.0	0.0	NA	NA

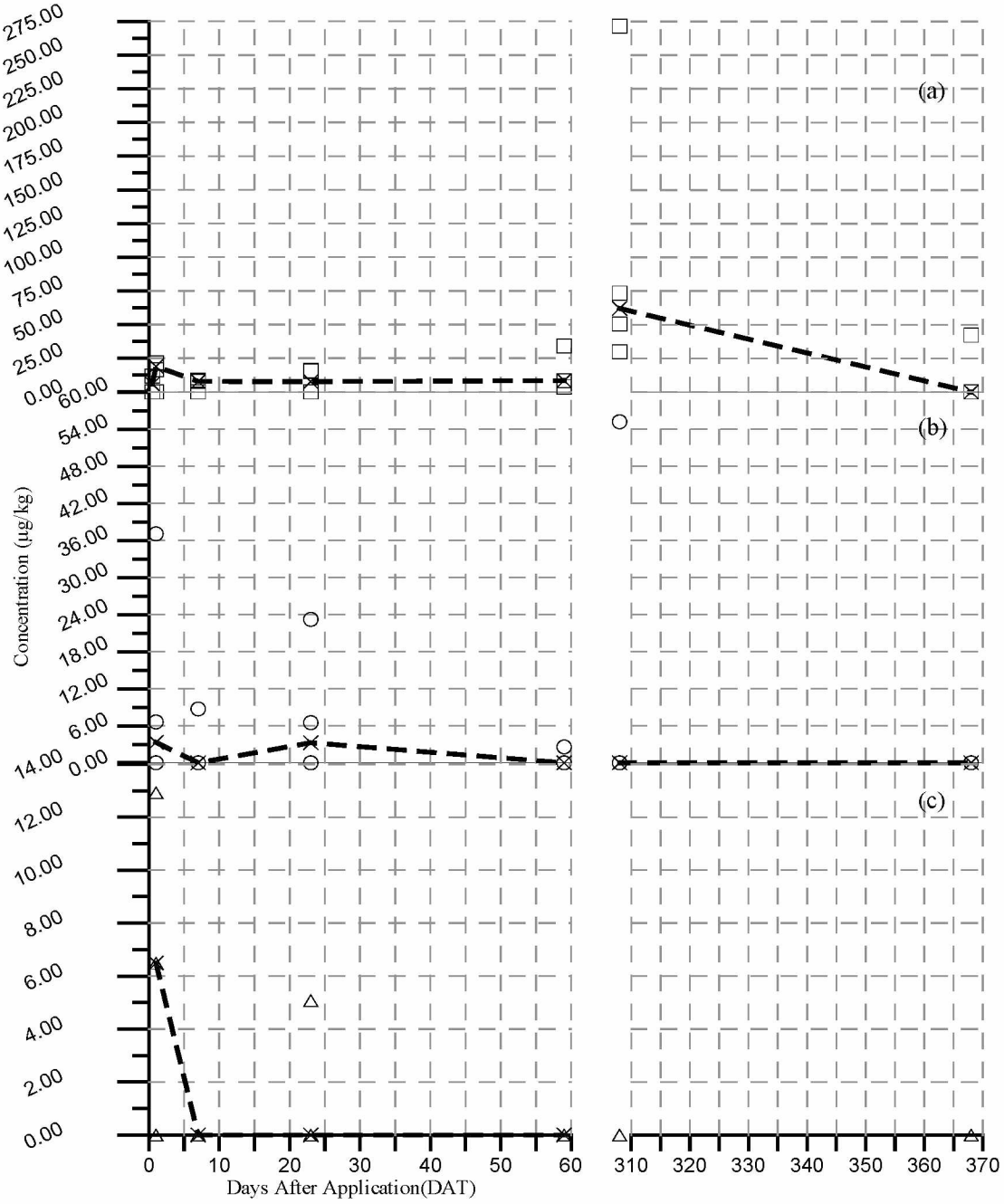

Figure 4.5 Valdez 2,4-D Concentrations. 2,4-D concentration measured in surface (a, 0-7.6 cm), root zone (b, 10-30 cm), and below root zone (c, 36-60 cm) soil at the Valdez site. The trend lines in each plot represents the median sample concentration. Herbicide was applied to the site on July 16, 2007. The soil froze approximately 150 days after application and thawed approximately 270 days after application. The break in the ordinate axis represents this frozen period. Note the concentration scale difference between (a), (b), and (c). Concentrations do not account for recovery rates.

It is likely that vegetation interception, uptake, and metabolism contributed to the low sample concentrations observed in the samples collected during the 2007 season. Research conducted by Radosevich and Winterlin (1977) and Grover *et al.* (1985) demonstrate that a large percentage of applied 2,4-D can be intercepted by vegetation. In their study, Radosevich and Winterlin (1977) found that nearly 46% of the applied 2,4-D butoxy-propyl ester was intercepted by chamise brush, grass, and forbs, while Grover *et al.* (1985) found that 52% of applied 2,4-D iso-octyl ester was intercepted by wheat. Both sites had different vegetation, which invariably impacted the amount of herbicide intercepted. The site in Valdez, which was in a humid climate, was covered in dense, woody vegetation that was seven feet tall in most areas. It is likely that this vegetation intercepted a higher percentage of the applied herbicide than was intercepted in the Radosevich and Winterlin (1977) and Grover *et al.* (1985) studies.

While sampling and laboratory error cannot be ruled out, a change in site variables may have contributed to or caused the unusually high concentrations observed in the samples collected in the spring and summer of 2008. In the spring of 2008, the Alaska DOT&PF accidentally mowed the site before samples were collected on May 19, 2008. It is unknown how mowing may have affected the site without further research, though it may have caused additional 2,4-D to leach into the soil column. As previously mentioned, the low soil concentrations during the 2007 season were partially attributed to vegetation interception and plant uptake. It is likely that some of the 2,4-D acid was not metabolized in the plants prior to mowing based on other studies that have found 2,4-D in vegetation and surface litter a year after 2,4-D applications (Radosevich & Winterlin, 1977). That same vegetation, which was cut into several pieces and left on the ground surface by mowing, was surface litter when samples were collected in 2008. If 2,4-D was still present in the vegetation when the site was mowed, then the cuts in the plant stems may have made it easier for the 2,4-D to be washed out of the vegetation by precipitation and to leach into the soil column.

The cut vegetation may have also contributed to sampling error. As mentioned in Chapter 3, surface litter was removed from sample locations prior to collection. This technique was employed to ensure that measured concentrations were from the soil only. Therefore, if surface litter was collected (samples were collected by others possibly resulting in a deviation from the sampling technique used to obtain the previous samples) with the

surface soil samples during the 2008 season, the concentrations measured in the laboratory would have included 2,4-D concentrations extracted from both the surface litter and soil. This scenario would cause surface soil sample concentrations to be positively skewed.

Only one subsurface sample, collected from the root zone, had detectable levels of 2,4-D during 2008. This may indicate that the 2,4-D had not been transported to the root zone and below root zone levels at the time of the May 2008 sampling event. By the time of the final sampling event 368 DAT, only one surface sample and no subsurface samples had detectable levels of 2,4-D. It is possible that the 2,4-D may have leached out of the sampled depths in the soil profile, which would have led to the non-detectable root zone and below root zone concentrations 368 DAT. However, the root zone and below root zone results from 2008 could potentially validate the idea that the high surface concentrations in 2008 were due to deviation in sampling technique resulting in a sample containing surface litter. As indicated by other researchers, 2,4-D can persist in surface litter a year after application (Radosevich and Winterlin, 1977). If the surface samples contained a high amount of surface litter, then the high surface soil concentrations may have been primarily due to surface litter. The 2,4-D bound in the surface litter would not likely be available for transport, which may explain why 2,4-D was only detected in one subsurface soil sample in 2008.

As hypothesized in the Delta Junction study results, volatility losses may partially account for the low sample concentrations observed in Valdez. While volatility losses were not determined, other studies have shown that volatility losses for 2,4-D iso-octyl ester can be substantial (Gile, 1983; Grover *et al.*, 1985).

Low concentrations of 2,4-D were detected after spring breakup in both the surface and root zone samples. A study conducted by Torstensson and Stark (1982) in Sweden found 2,4-D concentrations present in the soil a year after application in three out of eight study sites. Of all the sites where 2,4-D dissipated before the ground froze, only one site had no detectable concentrations after sixty days (Torstensson & Stark, 1982). Studies in temperate climates have shown 2,4-D to rapidly degrade, with 2,4-D rarely persisting over sixty days. Wilson *et al.* (1997) conducted thirteen dissipation studies using a liquid 2,4-D iso-octyl ester formulation, and found that only two of the study sites saw 2,4-D persistence greater than sixty days. The results from Valdez, combined with those observed in the Delta Junction research and the Torstensson and Stark (1982) study, suggest that herbicides applied in sub-

arctic climates tend to persist in soils longer than the herbicides that are applied in temperate climates.

Chapter 5 Conclusion

The results from both Delta Junction and Valdez found detectable concentrations of 2,4-D following spring breakup the year after application. The 2,4-D remaining in the soils after spring breakup quickly dissipated from subsurface samples, with no concentrations detected by the final sampling event. Concentrations of 2,4-D were still present in some surface samples collected during the final sampling event at both sites. Compared with temperate climates, where 2,4-D persistence is rarely over sixty days, 2,4-D persistence in Delta Junction and Valdez, both sub-arctic regions, was far greater with values over 300 days. However, the half-life for Delta Junction during the growing season, which was calculated using the concentration values for the first thirty days, was 13.4 days and comparable to half-life values for 2,4-D in temperate climates. This indicates that the initial dissipation for 2,4-D in Alaska is comparable to that seen in temperate climates.

The mass of applied 2,4-D accounted for in the soil at Delta Junction was relatively low, with percentages under 12%. Vegetation interception, volatilization, and plant uptake may explain the small amount of 2,4-D reaching the soil column. The large number of Valdez samples with no detectable levels of 2,4-D further supports this theory, especially when the dense, woody vegetation encountered at the site is considered.

Detectable concentrations of 2,4-D were observed in the subsurface one day following applications in both Delta Junction and Valdez. Concentrations were generally small, but still indicated a potential for leaching. Rain events may have contributed to the transport of 2,4-D to surface and subsurface soils.

5.1 Applicability of Results

Based on the small concentrations of 2,4-D in the surface and subsurface samples at both sites, and the results of the groundwater study conducted between 1992 and 1996 (Kolpin *et al.*, 2000), there is a low probability of health and environmental risks when applied correctly in sub-arctic climates. However, it may be wise to conduct a risk assessment to truly determine the health and environmental impacts associated with applying 2,4-D iso-octyl ester to highway rights-of-way in Alaska.

It should be noted, though, that site conditions in the sub-arctic can vary substantially, as indicated by the climatic differences between Delta Junction and Valdez. Conditions that may impact the transport and persistence of 2,4-D in the soil column include soil composition, vegetation density, rainfall amount, and temperature. In addition, application rates will likely affect the transport and persistence of 2,4-D.

5.2 Future Research Needs

The results from the field studies conducted in Delta Junction and Valdez highlighted the need for further investigation into several areas that may aid in the understanding of 2,4-D dissipation and transport mechanisms in sub-arctic climates.

An area that warrants further research is the uptake, metabolism, and release of 2,4-D in common plant species in Alaska. As discussed in Chapter 4, the vegetation at both study sites likely intercepted much of the applied 2,4-D. The time necessary for complete uptake of 2,4-D on a leaf surface is important to understand because any 2,4-D remaining on the plant surface may be available for transport to the soil surface during a rain event. Understanding 2,4-D metabolism and release mechanisms in vegetation is also important, especially when considering the increase in surface concentrations observed after spring breakup in Valdez, where the vegetation was cut during the study.

Another important topic that requires additional investigation is the uptake, metabolism, and release of 2,4-D from surface litter. Both study sites had extensive amounts of surface litter, though its impact on the results was not investigated during the field study. It was unknown how much 2,4-D was absorbed into the surface litter in Delta Junction and Valdez and if the surface litter was able to metabolize the herbicide. In addition, release mechanisms, if any, were not known. As discussed in Chapter 4, an increase in surface concentrations was observed following a rain event. There is the possibility that the increase in 2,4-D may have come from surface litter.

Surface runoff should also be investigated because several Alaskan highways have surface water bodies (marshes, ponds, etc.) that could potentially be contaminated by 2,4-D. Understanding 2,4-D surface runoff is especially critical in areas like Valdez, where rain events are frequent and could increase the chance of off-site movement.

2,4-D persistence was more than a year in both Delta Junction and Valdez. However, the research only focused on the dissipation and persistence of 2,4-D following one application of 2,4-D. If the Alaska DOT&PF were to start applying 2,4-D to highway rights-of-way, then applications would occur every few years. Additional research should be conducted to determine the effects of 2,4-D re-application to soil surfaces in the sub-arctic.

Only 2,4-D was extracted from the soil samples and analyzed. Once 2,4-D is applied, it begins to breakdown, eventually mineralizing to carbon dioxide. Several 2,4-D breakdown products, such as 2,4-dichlorophenol and phenol, are known and have been investigated by other researchers. Due to the persistence of 2,4-D in Alaska, further research should be conducted to determine if 2,4-D completely mineralizes to CO_2 in sub-arctic soils. In addition, the persistence of 2,4-D breakdown products and their potential health and environmental risks should also be investigated.

Detectable concentrations of 2,4-D were found in below root zone samples at the Delta Junction and Valdez study sites. However, it was not known how much 2,4-D leached to a depth below the below root zone sample depth. To ensure that groundwater sources are protected during 2,4-D applications, the amount of 2,4-D leaching out of the below root zone depth should be investigated.

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Appendix A

Delta Junction Site Information

Table A-1: Delta Junction Vegetation List. Site vegetation provided by the USDA Subarctic Agricultural Research Unit located on the University of Alaska – Fairbanks campus.

Common Name	Scientific Name
Paper Birch	*Betula papyrifera*
Cinquefoil	*Dasiphora fruticosa*
Balsam Poplar	*Populus balsimifera*
Quaking Aspen	*Populus tremuloides*
Felt-leaf Willow	*Salix alaxenis*
Little-tree Willow	*Salix arbusculoides*
Bebb's Willow	*Salix bebbiana*
Blueberry	*Vaccinium uliginosum*
Lowbush Cranberry	*Vaccinium vitus*
Yarrow	*Achillea millefolium*
Moonwort	*Botrychium* species
Lambsquarter	*Chenopodium album*
Crepis	*Crepis* species
Fireweed	*Epilobium angustifolium*
Horsetail	*Equisetum arvense*
Strawberry	*Fragaria virginiana*
Bedstraw species	*Gallium* species
Gentian species	*Gentian* species
Grass	Grass like
Mint species	*Labiatae* species
Jacob's Ladder	*Polemonium acutiflorum*
Potentilla	*Potentilla* species
Buttercup species	*Ranunculus* species
Groundsel/Ragwort	*Senecio* species
Golden Rod	*Solidago* species
Dandelion	*Taraxacum officinale*
Clover	*Trifolium repens*
Polemoniium	Unknown Polemonium

Figure A-1 Delta Junction Site Photograph, July 6, 2006. The photograph was taken at the time of application; the herbicide spray stream can be seen.

Table A-2 Laboratory Sample Additions. Amounts of DI water, 33% KOH, and 1+3 H_2SO_4 added to each sample based on soil sample weight. The values shown in the table are valid for samples collected in Delta Junction and Valdez.

Soil Weight (g)	DI Water (mL)	33 % KOH (mL)	1+3 H_2SO_4
5	15	2	4
10	30	4	8
15	45	6	12
20	60	8	16
25	75	10	20
30	90	12	24

Table A-3 Complete Data Set from Delta Junction. Sample locations in each plot were not the same at each sample event. ND = non-detect, NC = not collected or destroyed. Concentrations do not account for recovery rates.

Plot		Plot 1				Plot 2			
Sample Location		1	2	3	4	1	2	3	4
Date	DAT	Surface Concentrations (µg/kg)							
07/07/06	1	252.6	9.0	5.2	ND	37.5	126.8	40.7	6.9
07/11/06	5	40.6	33.4	10.7	31.9	35.0	11.7	139.6	NC
07/17/06	11	91.1	10.4	5.8	15.8	8.9	21.3	9.3	NC
08/02/06	27	6.6	3.8	8.4	4.2	31.5	18.9	5.8	11.4
08/22/06	47	24.7	4.9	20.8	3.5	13.3	90.8	57.6	18.6
10/06/06	92	24.1	14.9	29.7	NC	11.4	15.1	6.2	NC
04/20/07	288	128.7	54.8	18.7	18.9	10.1	45.2	30.8	25.5
05/18/07	316	7.4	8.5	32.2	2.4	24.8	NC	8.2	7.9
07/03/07	362	4.9	3.1	1.6	1.5	4.2	6.8	2.2	NC
		Root Zone Concentrations (µg/kg)							
07/07/06	1	9.2	ND	0.7	0.9	0.8	5.3	1.1	1.3
07/11/06	5	NC	0.7	1.8	1.7	1.2	NC	ND	11.5
07/17/06	11	ND	ND	5.6	5.9	6.0	ND	6.8	3.1
08/02/06	27	6.1	5.2	4.6	4.6	5.1	10.4	NC	4.1
08/22/06	47	4.0	2.7	3.2	3.7	2.8	2.8	7.9	6.3
10/06/06	92	2.6	2.6	2.5	NC	1.6	ND	ND	NC
05/18/07	316	NC	0.6	0.7	0.6	0.5	0.4	NC	NC
07/03/07	362	ND	ND	ND	ND	ND	ND	ND	ND
		Below Root Zone Concentrations (µg/kg)							
07/07/06	1	0.5	ND	3.6	2.7	2.9	2.6	1.7	0.5
07/11/06	5	0.9	1.2	ND	ND	1.2	NC	ND	0.5
07/17/06	11	ND	ND	ND	4.7	4.4	NC	ND	3.1
08/02/06	27	3.9	2.9	3.1	2.6	3.3	1.2	3.3	3.3
08/22/06	47	5.2	2.1	1.9	NC	2.7	1.6	2.6	4.8
10/06/06	92	1.5	1.7	1.6	NC	0.9	ND	ND	NC
05/18/07	316	ND	0.5	0.4	0.5	0.3	ND	ND	ND
07/03/07	362	ND	ND	ND	ND	ND	ND	ND	ND

Appendix B

Valdez Site Information

Table B-1 Valdez Vegetation List. Site vegetation provided by the USDA Subarctic Agricultural Research Unit located on the University of Alaska – Fairbanks campus.

Common Name	Scientific Name
Alder	*Alnus* species
Poplar	*Populus balsamifera*
Currant	*Ribes triste*
Salmonberry	*Rubus spectabilis*
Yarrow	*Achillea millėfolium*
Indian Paintbrush	*Castilleja* species
Delphinium	*Delphinium* species
Fireweed	*Epilobium angustifolium*
Equisetum	*Equisetum arvense*
Fleabane	*Erigeron* species
Bedstraw species	*Gallium* species
Grass species	*Gramineae*
Pushki (Cow parsnip)	*Heracleum lanatum*
Common plantain	*Plantago major*
Fern species	*Pteridophyta*
Pyrola	*Pyrola* species
Cockleburr	*Ranunculus*
Yellow rattlebox	*Rhinanthus minor*
Sitka burnett	*Sanguisorba stipulata*
Watermellonberry	*Streptopus amplexifolius*
Dandelion	*Taraxacum officinale*
Clover species	*Trifolium* species

Figure B-1 Valdez site photograph, July 16, 2007. The photograph was taken at the time of application; the herbicide spray stream can be seen to the left of the truck.

Table B-2 Complete Data Set from Valdez. Sample locations in each plot were not the same at each sample event. ND = non-detect, NC = not collected or destroyed. Concentrations do not account for recovery rates.

Plot		Plot 1				Plot 2			
Sample Location		1	2	3	4	1	2	3	4
Date	DAT	Surface Concentrations (µg/kg)							
07/16/07	0.5	NC	NC	ND	11.9	ND	ND	ND	ND
07/17/07	1	21.4	21.8	16.5	ND	ND	ND	ND	ND
07/23/07	7	8.8	NC	7.8	ND	ND	ND	ND	ND
08/08/07	23	ND	15.9	ND	15.4	ND	ND	ND	ND
09/13/07	59	34.2	NC	8.4	3.8	ND	ND	ND	ND
05/19/08	308	73.4	271.4	29.9	50.5	ND	ND	ND	ND
07/18/08	368	ND	42.1	ND	ND	ND	ND	ND	ND
		Root Zone Concentrations (µg/kg)							
07/16/07	0.5	NC	NC	NC	NC	NC	NC	NC	NC
07/17/07	1	6.6	37.1	ND	ND	ND	ND	ND	ND
07/23/07	7	8.7	ND	ND	ND	ND	ND	ND	ND
08/08/07	23	23.2	ND	ND	6.5	ND	ND	ND	ND
09/13/07	59	ND	NC	2.6	ND	ND	ND	ND	ND
05/19/08	308	ND	55.2	ND	ND	ND	ND	ND	ND
07/18/08	368	ND	ND	ND	ND	ND	ND	ND	ND
		Below Root Zone Concentrations (µg/kg)							
07/16/07	0.5	NC	NC	NC	NC	NC	NC	NC	NC
07/17/07	1	12.9	NC	ND	6.5	ND	ND	ND	ND
07/23/07	7	ND	NC	ND	ND	ND	ND	ND	ND
08/08/07	23	5.0	ND	NC	ND	ND	ND	ND	ND
09/13/07	59	ND	ND	NC	ND	ND	ND	ND	ND
05/19/08	308	ND	ND	ND	ND	ND	ND	ND	ND
07/18/08	368	ND	ND	ND	ND	ND	ND	ND	ND