PHYTOREMEDIATION OF PERCHLORATE BY TOBACCO PLANTS

by

SARAH ELIZABETH SUNDBERG

(Under the Direction of Jeff Fisher)

ABSTRACT

Perchlorate (ClO_4^-) is an inorganic contaminant found in soil, groundwater, surface water, and irrigation water used for crop production. Previous field and laboratory studies have shown that perchlorate is taken up by plant roots from perchlorate-contaminated soil and water and is accumulated in plant tissues. This research determined the uptake, translocation, and accumulation of perchlorate in tobacco plants. Four hydroponics growth studies were completed under greenhouse conditions. The depletion of perchlorate in the hydroponics nutrient solution and the accumulation of perchlorate in the plant tissues were determined at two-day intervals using ion chromatography. Results suggest that tobacco plants are potential plants for the phytoremediation of perchlorate. A five-compartment plant kinetic model was developed to describe the distribution of perchlorate in tobacco plants. There was good agreement between model predictions and measured concentrations in the plant. The model, once adequately validated, can be applied to other dicot terrestrial plants and inorganic chemicals currently used for both phytoremediation and ecological risk assessment.

INDEX WORDS: Perchlorate, Phytoremediation, Tobacco plants, Plant kinetic model

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SARAH ELIZABETH SUNDBERG

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SARAH ELIZABETH SUNDBERG

Major Professor: Jeff Fisher

Committee: Marsha Black
Hugh Earl

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2003

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INTRODUCTION AND LITERATURE REVIEW

Origins of Perchlorate

Perchlorate (ClO_4^-) is an inorganic contaminant in soils, groundwater, surface waters, and irrigation waters used for crop production. Perchlorate is found in the environment as part of certain geologic deposits, such as the Chilean nitrate deposits, and as a contaminant resulting from the use of solid salts of ammonium, potassium, or sodium perchlorate in industrial and commercial applications[1]. Large-scale production of perchlorate-containing chemicals began in the 1940s. Perchlorate metal salts can undergo mild to explosive reactions when in the presence of oxidizable substances. This characteristic has led to the use of perchlorate salts as oxidizers in a number of commercial applications such as solid propellants for rockets, missiles, fireworks, flares, explosives, and pyrotechnic formulations[2]. Most of the environmental contamination is due to ammonium perchlorate, which is produced almost exclusively as an oxidizer. Since the ammonium ion is a reducing agent, ammonium perchlorate can undergo intramolecular redox reactions that lead to the release of gaseous products. Through such reactions, ammonium perchlorate can act as thrust boosters in various commercial applications[3].

Other industrial applications of perchlorate salts include their use as additives in lubricating oils, in tanning and finishing leathers, as a mordant for fabrics and dyes, in electroplating, aluminum refining, rubber manufacturing, and in the production of paints

and enamels[4]. In addition to large-scale commercial and industrial uses, perchlorate salts are often used on a small scale in the laboratory. Perchlorate can be used to adjust ionic strength, as noncomplexing counterions, or for various applications requiring a strong acid[5].

Chemistry of Perchlorate

The perchlorate anion consists of one chlorine atom surrounded by four oxygen atoms arranged in a nearly perfect tetrahedral structure (ClO_4^-). The average chlorine-to-oxygen bond distance is 1.42 Å units and the oxygen-to-oxygen bond distance is 2.43 Å units[3]. The tetrahedrally-oriented oxygen atoms block the central chlorine atom from any incoming reducing agents. Thus, the activation energy required for the reduction of perchlorate to take place using common reducing agents (such as thiosulfate, sulfite, or ferrous ions) is too high for any reaction to be observed. In the environment, the water solubility of perchlorate salts differ, ranging from a solubility of 0.01 mol L^{-1} for potassium perchlorate to 8.5 mol L^{-1} for sodium perchlorate. Perchlorate adsorbs weakly to most soil minerals, is exceedingly mobile in aqueous systems, and can persist for many decades under typical groundwater and surface water conditions[6].

Human Health Risks

The major route of human exposure to perchlorate is oral uptake. Due to its high charge, perchlorate does not pass readily through skin, and exposure via inhalation is expected to be negligible because the vapor pressure of perchlorate salts is very low at room temperature. Once ingested, perchlorate is readily absorbed by the intestinal tract.

Since the activation energy required for the reduction of perchlorate is so high, perchlorate is not expected to act as an oxidizer under human physiological conditions. Studies of perchlorate absorption, distribution, metabolism, and elimination show that perchlorate is excreted from the body virtually unchanged after absorption[7].

Perchlorate has an ionic radius and charge similar to that of iodide. This allows perchlorate to compete with the uptake of iodide into the thyroid, an essential element for proper thyroid function. Public health concerns for perchlorate are based on its ability to cause an iodide deficiency, which can lead to hypothyroidism. Iodide competition occurs primarily at the sodium-iodide symporter, or NIS, causing a reduction in the thyroid hormones triiodothyronine (T3) and thyroxine (T4). This mode of action results in disturbances of the hypothalamic-pituitary-thyroid axis, giving rise to concerns about the potential for perchlorate to cause developmental, reproductive, neurodevelopmental, immunotoxic, and even carcinogenic effects in humans. There is also concern for toxic effects on various aquatic and terrestrial animals, since studies have confirmed that the competition of iodide uptake by perchlorate has essentially the same sensitivity across many species[8].

Perchlorate in the Environment

Perchlorate began receiving attention from the scientific community in 1997, shortly after development of low-level analytical methods. Since 1997, perchlorate has been discovered at various manufacturing sites and in drinking water and well-water supplies in 14 states. Perchlorate has been found in California water supplies in the range of 18 to 280 parts per billion (ppb or $\mu g\ L^{-1}$), potentially affecting the drinking water supplies of

at least 12 million people[6]. The highest level of perchlorate reported in any public water supply well was 800 ppb in an inactive well in California. Contaminated groundwater locations in California have been associated with facilities which have manufactured, tested, or disposed of solid rocket fuels and propellants for the Department of Defense or the National Aeronautics and Space Administration. Perchlorate has also been detected at low levels (5-9 ppb) in the Colorado River[9] and up to 17 ppb in a Lake Mead inlet, as a result of release from two ammonium perchlorate-manufacturing facilities in Nevada[6]. This water is used for drinking, irrigation, and recreation for millions of people in Nevada, California, and Arizona, and by Native American tribes.

A spring associated with the Las Vegas Wash site had perchlorate concentrations of 1.0 ppb to 130 parts per million (ppm or mg L^{-1}) in surface waters[9]. In Karnack, Texas, a pond that receives water from the Longhorn Army Ammunition Plant contained perchlorate concentrations ranging from 3.5 to 3.8 ppm. On Long Island, New York, perchlorate was found in a number of water supply wells, including several wells down gradient from a fireworks facility. The distribution of perchlorate in New York has been speculated to be a result of low levels of perchlorate contained in fertilizer imported from Chili[1]. Agricultural chemicals have also been considered as a potential source of perchlorate contamination in Nebraska at a shallow well near a fertilizer facility[10].

In addition to water supplies, on-site soils may be contaminated by spills or the disposal of perchlorate solutions, resulting in perchlorate concentrations of less than 1 to 1,470 ppm (mg kg^{-1})[9]. The primary source of perchlorate contamination is the process used to remove or recover propellant from the solid rocket motors. This process results in the generation of large quantities of ammonium perchlorate-concentrated wastewater,

which has been historically disposed of by simply pouring it on the ground. In addition, because of its limited shelf life, perchlorate must be washed out of the United States' missile and rocket inventory and replaced with a fresh supply. Consequently, large volumes of perchlorate have been disposed of in various states since the 1950s[2].

The environmental risks posed by perchlorate are currently being assessed by the United States Environmental Protection Agency, which has placed perchlorate on the Contaminant Candidate List[11] and the Unregulated Contaminant Monitoring Rule[12]. The Contaminant Candidate List (CCL) is the source of priority contaminants for research, guidance and development, regulatory determinations, and monitoring by the states, consisting of 50 chemicals and 10 microbiological contaminants. Under the Unregulated Contaminant Monitoring Rule (UCMR), all large public water systems and a representative sample of small water systems are required to monitor for perchlorate.

Phytoremediation

Phytoremediation is a technology that employs plants to clean contaminated sites. Applications of phytoremediation can be classified based on the contaminant fate, including degradation, extraction, containment, or a combination of these methods. Applications can be further classified on the mechanisms involved, such as extraction from water or soil, concentration of contaminants in plant tissues, degradation by biotic or abiotic processes, volatilization from plants to air, and control of runoff or erosion. Phytoextraction, more specifically, is the uptake of contaminants by plant roots and translocation within the plants. The process involves planting a crop of a species that is known to accumulate contaminants in roots, stems, and leaves of plants, and then

harvesting the plants to remove the contaminants from the site. This process yields a much smaller mass to be disposed of when compared to excavation and land filling, and may also be amendable to contaminant recovery and recycling[13].

Although the emerging technology of phytoremediation is still fairly new, early research has sparked interest in contaminated site owners and citizen groups as an economic and ecologically-sound technology to remediate hazardous sites. There are two considerations influencing the economics of phytoremediation: the potential for application and the cost comparison to conventional methods. Because phytoremediation is an emerging technology, standard cost information is not available. Kidney[14] has estimated the domestic market for phytoremediation at $2 to $3 million for organic contaminant removal from groundwater, and $1 to $2 million for removal of heavy metals from soils. This estimate also projects the cost to increase to $20 to $45 million for removal of organics and $40 to $80 million for removal of heavy metals by the year 2005. However, Glass[15] estimated that the total phytoremediation application system costs would be 50% to 80% lower than alternative cleanup methods. For example, costs for using phytoextraction for the remediation of one acre of 20-inch thick sandy loam were estimated to be $60,000 to $100,000, compared to a minimum of $400,000 for the conventional excavation and storage of the soil[16].

Phytoremediation offers significant ecological promise by providing opportunities to improve the ecological health of contaminated sites. Many contaminated sites support neglected ecosystems, primarily due to the low level of human and animal activity on the site. Phytoremediation could provide revegetation to contaminated sites as well as allow native animals and plants to recolonize the surrounding area. The EPA's Office of

Research and Development (ORD) and the Office of Solid Waste and Emergency Response (OSWER) have several programs that are currently investigating the efficacy, risk, and cost of phytoremediation[13].

There are a few limitations to remediation systems using plants. The primary limitation is root contact. Phytoremediation requires that contaminants be within the root zone of the plant. Therefore, either the plant roots must extend deep enough to reach the contaminants, or the contaminants must be moved in the soil to be within the range of roots. This can be accomplished using agricultural equipment, such as deep plowing, to bring the contaminant within 8-10 inches of the surface[13].

Another limitation to phytoremediation is growth rate of the plants involved. If plant growth rates are small, the process of phytoremediation may take several years, compared to more traditional cleanup technologies such as excavation, disposal, or incineration that may only take weeks or months. Phytoremediation may not be the most effective technology for sites that pose acute risks to humans or the ecosystem and need immediate clean up[13].

The contaminant concentrations in soil and water may also be a limiting factor of phytoremediation. High concentrations of certain contaminants may inhibit plant growth, and most plants do not accumulate significant levels of contaminants in plant tissues. Sites with widespread, medium to low concentrations within the root zone are the best areas for phytoremediation processes. In addition, if herbivorous organisms ingest significant amounts of the phytoremediating plants, there is a concern that the contaminant may cause toxic effects in the organisms. Appropriate exposure control

methods therefore should be implemented, such as perimeter fencing or overhead netting to limit the availability of these plants for consumption[13].

Plant physiology, agronomy, microbiology, hydrogeology, and engineering are all combined to select the proper plant to remove contaminants from sites. The goal of this plant selection process is to choose a plant species with appropriate growth characteristics under the site conditions that meet the objectives of phytoremediation. There are several general criteria that are important in plant selection: disease and insect resistance, climate and stress tolerance, chemical tolerance, cultural requirements, toxic characteristics, growth rate or biomass production, and establishment rate[13].

The efficacy of plants to take up and/or degrade contaminants should be demonstrated under greenhouse conditions prior to use in the field. Also, the contaminant of interest should not cause phytotoxicity in the plants. The success of phytoremediation can be increased by screening plants for useful degradation enzymes and for phytotoxicity to particular contaminants[8].

Potential phytoremediators should possess a fibrous root system that will provide maximum contact with the soil. A fibrous root system has numerous fine roots spread throughout the growth media, providing high surface areas for absorption of water and contaminants. Fescue is a good example of a plant with this fibrous root system. A tap root system, such as alfalfa, is dominated by one central taproot. Although a taproot does not provide as much surface area as fibrous roots, it may have the ability to grow deeper into the root zone[17]. Root depth can vary greatly among different types and species of plants, depending on several local conditions such as soil fertility, soil water content, depth to water, and soil density or structure. Typical plants with fibrous root systems

reach a depth of 6 to 9 inches. However, the roots of legumes, such as alfalfa, can grow up to 30 feet deep in soil given the proper conditions, and certain grasses with fibrous root systems can typically extent 8-10 feet deep. Phreatophytic shrubs, which are deep-rooted plants that obtain water from the water table, have roots that can extend to about 20 feet[13].

Considering all the general plant characteristics, the optimum plant for the phytoextraction process would: 1) tolerate, translocate, and accumulate high concentrations of environmental contaminants in the stems and leaves, 2) exhibit rapid growth rate, and 3) not be favored for consumption by animals, which would decrease risks to the ecosystem[13].

Nicotiana tabacum

Tobacco belongs to the Solanaceae family and to the genus *Nicotiana*, as established by Linnaeus in 1753[18]. This particular species arose from the hybridizations of two species, *N. sylvestris* and *N. tomentosiformis*[19]. Although there are at least 63 species of *Nicotiana*, *N. tabacum* is the most common species in commercial production, normally grown as an annual crop.

The tobacco plant is a very leafy crop with an extensive fibrous root system. Mature tobacco plants have single leaf areas ranging from 0.09 to 0.14 m^2, and in some cases a single cigar tobacco plant can produce as much as 2.3 m^2 of total leaf area[18]. The tobacco plant also has a very high growth rate. Vickery *et al*.[20] conducted a detailed growth study on the growth rate of tobacco plants from seedling to the ripening of seed, using Connecticut shade-grown tobacco. The growth of the plant can be divided into

three stages. The first stage ranges from 21 to 28 days after planting. During this stage, the seedling establishes itself in the soil or other growth media and increases very little in weight. The second stage occurs during the 35th to 75th day after planting, and is characterized by rapid plant growth. It is during this stage that organic and inorganic substances can very efficiently accumulate in the plant. The last stage, the reproductive period, stretches from day 61 until harvesting. Plants in this stage lose both fresh (wet) weight and dry weight in the leaves while maintaining a fairly constant stem weight.

N. tabacum appears to have strong potential to effectively phytoremediate perchlorate from contaminated soil and water. These plants are characterized by large leaf area and rapid plant growth supported by a fibrous root system. Due to the nicotine content of tobacco, animals tend to avoid consuming the plant, which would decrease the risks to the surrounding ecosystem. Tobacco is an annual crop that can be harvested without difficulty to remove perchlorate contaminated plant material.

Plant Physiology and Modeling

Understanding the potential for the uptake, translocation, and accumulation of toxic chemicals by plants is an important health and ecological issue. Predictive tools are needed to understand and interpret the behavior of chemicals in the plants[21]. It is impractical to test the efficiency of all plant species under greenhouse conditions to phytoremediate chemical contaminants. Mathematical models, which combine the physiochemical properties of a chemical with the anatomical and physiological properties of plants, can be used as a predictive tool to determine the efficiency of a plant to phytoremediate contaminants.

A plant model defines the plant as a set of compartments and is comprised of a set of equations, one for each plant compartment. Formulation of the model is based on identifying appropriate compartments and determining their storage and xylem flow characteristics.

Solution (H_2O) uptake from soil or other growth media by plant roots is driven by mass flow with the transpiration stream[22]. Water evaporation and transpiration from the stoma of the leaf mesophyll cells generates the large negative pressure gradients in the apoplastic water. These negative pressures are transmitted to the xylem, resulting in the passive uptake of water through the roots. Water and solutes enter the root most readily in the apical part of the root via root hairs, which are microscopic extensions of the root epidermal cells[23]. The root hairs greatly increase the surface area of the root, thus providing greater capacity for absorption of water and nutrients. Driven by an energy potential gradient caused by leaf transpiration, water flows through the epidermis and the intercellular spaces of the cortex, and flows to the endodermis and Casparian strip[21]. From the epidermis to the endodermis of the root, there are three pathways though which water can flow[23]. The apoplast pathway is a continuous system of cells and intercellular air spaces in plant tissues. A small amount of water moves through this pathway by diffusion through the cell wall without crossing any membranes. The cellular pathway is made up of two components, the transmembrane pathway and symplast pathway. The transmembrane pathway is the route in which water that enters the cell on one side, exits the cell on the other side, and moves on to the next cell in the series, crossing at least two membranes for each cell in its path. Of the three pathways, the symplast pathway moves

the largest volume of water in the plant. In this pathway, water travels from cell to cell through the entire network of cell cytoplasm interconnected by plasmodesmata[23].

Once water and nutrients have passed through the endodermal cells, collectively known as the root symplast, water enters the root xylem by diffusion. Xylem is the tissue that transports water and minerals from the root system to the aerial portions of the plant, constituting the longest part of the water transport pathway. For example, in a plant one meter tall, more than 99.5% of the water transport pathway through the plant is within in xylem[23]. Water travels through the root xylem and connects to the stem xylem. Once in the stem xylem, water and solutes can pass through the tracheids and sieve cells into the stem pith and stem cortex, together forming stem storage[23].

The stem xylem connects to the leaf xylem. Tracheary elements, which are the conducting cells in the xylem that includes both vessel elements and tracheids, enable the transport of large volumes of water with great efficiency. Water is brought to the leaves via the xylem of the leaf vascular bundle, which branches into a very fine and intricate network of veins throughout the leaf. Dicots, such as tobacco plants, are characterized by the net venation patterns in the leaf, as opposed to the parallel venation pattern in monocots[24]. From the leaf xylem, water is drawn into the mesophyll cells via the cell walls of the leaf. Unlike water, many solutes don't evaporate during stomatal activity of leaves, rather they are subject to metabolism, deposition, or further translocation[21]. Within the mature plant cells of the leaf, central vacuoles occupy 80-90% of the total volume of the cell, and can accumulate water and other dissolved inorganic ions[23].

Previous Research

Perchlorate as an environmental contaminant has been thoroughly reviewed and assessed by the United States Environmental Protection Agency in the Perchlorate Toxicological Review and Risk Characterization document.[8] This document extensively reviews perchlorate occurrences, sources, toxicokinetics, human health effects, animal and ecotoxicology, dose-response assessments, and ecological risk assessments. In addition to reviewing current research findings, this document also identifies important perchlorate issues that warrant further investigation, including the accumulation of perchlorate in terrestrial vascular plants. Due to the lack of evidence of perchlorate phytodegradation, additional plant uptake studies with mass balances would be beneficial in assessing the possible degradation of perchlorate in plant systems.

Since perchlorate is nonvolatile and highly soluble in water, it cannot be removed from water by conventional filtration, sedimentation, or air stripping. Bioremediation processes may provide the best available technology in treating perchlorate-contaminated water and soil. Most attention to date has been focused on developing an anaerobic biochemical reduction process[6]. There are several microorganisms that contain a reductase that allows them to lower the activation energy required for the reduction of perchlorate[25]. In the laboratory, *Staphylococcus epidermidis* has been shown to reduce perchlorate in the absence of nitrate. Nitrate-adapted *Bacillus cereus* also has the ability to reduce perchlorate[26]. Unfortunately, both of these organisms are pathogenic[5]. Rikken *et al.*[27] reported that perchlorate and chlorate can be reduced to chloride by *Proteobacteria* with acetate as a reductant at neutral pH. In California, a pilot-scale bioreactor has been constructed for the Baldwin Park Operable Unit that uses microbes

derived from the food-processing industry to reduce perchlorate. Over a period of several months, perchlorate and nitrate were reduced to undetectable levels[5].

Several studies have suggested that phytoremediation is an effective process for the degradation or extraction of environmental contaminants. Piechalak *et al*.[28] determined that legumes could phytoremediate lead from lead-contaminated nutrient solution. In this study, lead accumulated in the roots, stems, and leaves of three plant species of the Fabacea family. Palmroth *et al*.[29] reported that poplar, pine, and a legume mixture phytoremediated diesel fuel from soil and suggested that these plants may serve as a viable, low-cost remedial technology for diesel-contaminated soils in subarctic regions. The phytoremediation of 1,4-dioxane from a contaminated sandy soil by poplar cuttings was investigated by Ouyang[30]. The 1,4-dioxane primarily accumulated in leaves and stems of poplar cuttings. Schnabel *et al*.[31] reported that edible garden plants, such as carrots, spinach, and tomatoes, phytoremediated and transformed trichloroethylene when irrigated with synthetic groundwater containing the contaminant. Selenium and arsenic have been successfully phytoremediated by various wetland species[32,33].

The possibility of successful phytoremediation of perchlorate-contaminated soil or water depends on the availability of plant varieties with high rates of accumulation and tolerance for perchlorate[34]. Von Burg[35] reported that in most terrestrial plant species, perchlorate soil concentrations in the range of 40 to 80 ppm were toxic to plants. It was also shown that potassium perchlorate concentrations as low as 0.55 ppm in soil inhibited growth in ryegrass and cotton. Soybeans were reported to show toxic symptoms when nurtured with water containing 2.5 to 5 ppm potassium perchlorate[35].

Nzengung *et al.*[36] investigated the phytoremediation of perchlorate from soil and water by several plant species. These authors investigated the use of Willow and eastern cottonwood tree cuttings and *Eucalyptus cineria* plants to decontaminate perchlorate-contaminated water grown in sand and hydroponics growth media. Willow trees decontaminated hydroponics solution amended with 10, 20, and 100 ppm perchlorate to below the analytical detection limit of 0.2 ppm within 70 days of exposure. Mass balance results of this study yielded an average perchlorate recovery of 89%. The 11% of unaccounted perchlorate was assumed to be phytodegraded to chloride. However, possible degradation products of perchlorate such as chlorate, chlorite, hypochlorite, and dichlorooxides were not detected in the media or plant extracts. Eastern cottonwoods, which were the least effective in removing perchlorate from the solution, and *E. cineria* did not thrive well in the hydroponics system.

Previous field and laboratory studies have shown that *Nicotiana tabacum* is fairly tolerant of perchlorate and will accumulate perchlorate in the tissues[37]. Concentrations of perchlorate in tobacco plant tissues have been measured from a 1999 crop grown in soil fertilized with Chilean saltpeter as the perchlorate source. The perchlorate concentration in the leaf lamina was 96.0 ± 0.6 ppm dry weight (14.6 ± 0.1 ppm fresh weight). These perchlorate concentrations are far greater than those known to produce toxic effects in plants as previously mentioned.

A few mathematical models have been developed to describe the fate of chemicals in plants. Boersma *et al.*[21,38], Lindstrom *et al.*[39], and Trapp *et al.*[22] have developed mathematical models for the uptake and translocation of organic chemicals. These authors validated the models with experiments studying the uptake of bromacil by

soybean plants. A fugacity model was developed by Hung and Mackay[40] to predict the uptake and kinetics of organic chemicals in herbaceous agricultural plants. Brennan and Shelley[41] used a mechanistic system dynamics modeling approach to simulate extraction and translocation of lead by a maize plant. The model was developed primarily to test phytoextraction management scenarios, two of which were tested in the studies. More recently, Zhang *et al.*[42] produced a model to describe the transport of methyl *tert*-butyl ether (MTBE) through alfalfa plants. These authors used the model to estimate the diffusion coefficient of MTBE from the root system into the stem.

The first objective of this research was to determine the uptake, translocation, and accumulation of perchlorate in tobacco plants to assess the potential for tobacco to phytoremediate perchlorate. Tobacco plants were chosen for the study because of their known tolerance and accumulation of perchlorate.

The second objective was to develop a simple plant kinetic model for the distribution of perchlorate in tobacco plants, incorporating plant growth. For the application of fate models for phytoremediation purposes, plant growth is an important process to include as the plants used for phytoremediation would likely be in their rapid growth phase. No models were found in the literature for the uptake of inorganic chemicals, including perchlorate, in plants. Unlike organic chemicals that have been previously modeled, perchlorate does not appear to degrade in plant tissues and is not expected to be lost to the atmosphere through transpiration. These factors introduce simplifying assumptions that reduce the number of model parameters and compartments compared to previously developed plant chemical fate models.

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CHAPTER 2

ACCUMULATION OF PERCHLORATE IN TOBACCO PLANTS: POTENTIAL FOR PHYTOREMEDIATION[1]

[1] S. E. Sundberg, J. J. Ellington, J. J. Evans, and J. W. Fisher. Submitted to *Journal* of *Environmental Monitoring*, 1/14/03.

Abstract

Previous studies have shown that tobacco plants are tolerant of perchlorate and will accumulate perchlorate in the plant tissues. This research determined the uptake, translocation, and accumulation of perchlorate in tobacco plants from water. Three hydroponics growth studies were completed under typical greenhouse conditions. In the first study, tobacco plants were grown for 13 days in a hydroponics nutrient solution amended with 25 ppm and 75 ppm perchlorate. The depletion of perchlorate in the hydroponics nutrient solution and the accumulation of perchlorate in the plant parts were determined at two-day intervals. Ion chromatography (IC) was used for the quantitative analysis of perchlorate in the roots, stems, and leaves of the plant, and in the hydroponics growth media. Perchlorate primarily accumulated in the leaves of tobacco plants, yielding a substantial storage capacity for perchlorate. Mass balance results show that perchlorate degradation was negligible in the plants. A second 14-day study was completed to study the uptake of parts-per-billion levels (10 ppb and 100 ppb) of perchlorate, commonly found in the environment. A third 14-day study was completed to determine if tobacco plants were tolerant of 100 ppm perchlorate derived from two perchlorate salts, sodium perchlorate and ammonium perchlorate. The depletion of perchlorate from the hydroponics nutrient solution was determined at the end of the two 14-day studies. No toxicity was observed during the growth studies at any perchlorate concentration used. Results suggest that tobacco plants are potential plants for the phytoremediation of perchlorate.

Introduction

Perchlorate (ClO_4^-) is an inorganic contaminant in soils, groundwater, surface waters, and irrigation waters used for crop production. Perchlorate is found in the environment as part of certain geologic deposits, such as the Chilean nitrate deposits, and as a contaminant resulting from the use of solid salts of ammonium, potassium, or sodium perchlorate in industrial and commercial applications[1]. Perchlorate metal salts can undergo mild to explosive reactions when in the presence of oxidizable substances. This characteristic has led to the use of perchlorate salts as oxidizers in a number of commercial applications such as solid propellants for rockets, missiles, fireworks, explosives, and pyrotechnic formulations[2].

In the environment, the water solubility of perchlorate salts differ, ranging from a solubility of 0.01 mol L^{-1} for potassium perchlorate to 8.5 mol L^{-1} for sodium perchlorate. The perchlorate anion is very unreactive and persists for many decades under typical groundwater and surface water conditions[3]. The environmental risks posed by perchlorate are currently being assessed by the United States Environmental Protection Agency, which has placed perchlorate on the Contaminant Candidate List[4] and the Unregulated Contaminant Monitoring Rule[5].

Since 1997, perchlorate has been discovered at various manufacturing sites and in drinking water and well-water supplies in California, Utah, Nevada, and Texas. Perchlorate has been found in California water supplies in the range of 18 to 280 ppb (μg L^{-1}), potentially affecting the drinking water supplies of at least 12 million people[3]. The groundwater locations in California have been associated with twelve facilities, which have manufactured or tested solid rocket fuels for the Department of Defense or the

National Aeronautics and Space Administration. Perchlorate has also been detected at low levels (5-9 ppb) in the Colorado River[6] and up to 17 ppb in Lake Mead, as a result of release from two ammonium perchlorate-manufacturing facilities in Nevada[3].

A previous report by Ellington *et al.*[7] indicated that the perchlorate in a fertilizer that contained the anion as a natural component was taken up by the roots and accumulated in the leaves of tobacco plants. Samples of chewing tobaccos, cigars, and cigarettes were purchased at retail stores in Athens, Georgia, in November and December 1999, which represented products from seven tobacco companies. Perchlorate was found in all but one of the products, with the highest perchlorate concentration of 149.3 ± 3.0 ppm (mg kg^{-1}) dry weight being found in a chewing tobacco. Although the leaf curing practices for these products were unknown, it can be assumed that the perchlorate contained in the products originated in the soil or fertilizer, was translocated during the plant growing process, and persisted in the plant leaves over several years of curing and aging processes. This raises questions regarding the presence of perchlorate in other food crops and products being consumed by human and animal populations that were exposed to perchlorate contamination.

Perchlorate has an ionic radius and charge similar to that of iodide. This allows perchlorate to compete with the uptake of iodide into the thyroid, an essential element for proper thyroid function. Public health concerns for perchlorate are based on its ability to cause an iodide deficiency, which can lead to hypothyroidism. Thyroid hormones are very important for normal growth and development in humans[8].

A number of approaches have been investigated for the removal of perchlorate contamination from water including reverse osmosis, anion exchange, adsorption by

activated carbon, and phytoremediation[9]. Phytoremediation employs plants to remove contaminants from polluted soils in a cost-effective and ecologically sound manner. The possibility of successful phytoremediation of perchlorate-contaminated soil depends on the availability of plant varieties with high rates of accumulation and tolerance for perchlorate[10]. Von Burg[11] reported that in terrestrial species, perchlorate soil concentrations in the range of 40 to 80 ppm were toxic to plants. It was also shown that potassium perchlorate concentrations as low as 0.55 ppm in soil inhibited growth in ryegrass and cotton. Soybeans were reported to show toxic symptoms when nurtured with water containing 2.5 to 5 ppm (mg L^{-1}) potassium perchlorate[11].

Field and laboratory studies have shown that perchlorate is transported from contaminated soils through the root system and is accumulated in the tissues and organs of vascular plants. Concentrations of perchlorate in tobacco plant tissues and soils have been measured from a 1999 crop grown in soil fertilized with Chilean saltpeter[7]. The perchlorate concentration in the leaf lamina was 96.0 ± 0.6 ppm dry weight (14.6 ± 0.1 ppm fresh weight). Perchlorate concentration in a composite soil sample collected from the field at the end of the growing season was 0.34 ± <0.01 ppm dry weight. This research has shown that tobacco plants are fairly tolerant of perchlorate, will accumulate perchlorate in the leaves of the plant, and may be an effective plant for the phytoremediation of perchlorate.

In this study, tobacco plants were grown in perchlorate amended hydroponics nutrient solutions where applied perchlorate concentrations were known and uptake levels could be monitored. Perchlorate concentrations in roots, stems, and leaves of tobacco plants were measured by ion chromatography (IC).

Experimental Procedures

Six flats of 250 *Nicotiana tabacum* var. K326 seedlings per flat were obtained from the Coastal Plains Experimental Station in Tifton, Georgia, in mid-April 2002. The six-week old seedlings were placed in a greenhouse at the EPA Ecosystem Research Division in Athens, Georgia. They were watered once or twice daily, depending on sun and heat exposure, with a hydroponics solution made by adding 47.85 g of Peter's 5-11-26 Hydrosol (Hummerts, St. Louis, MO, Cat. #07-5570), 31.65 g of calcium nitrate 15.5-0-0 (Hummerts, St. Louis, MO, Cat. #07-0355), and 50 L of water. All water used throughout the study was 18 MΩ cm water. When the seedlings reached 8-10 cm in height, or approximately 10 weeks of age, 200 seedlings of equal height and stem width were removed from the flats and the soil was gently and thoroughly rinsed from the roots with water. The seedlings were placed into white plastic 32 oz. Sweetheart deli/food containers (Thornton Bros., Athens, GA, Cat. #GW33TH) containing approximately 850 mL of the hydroponics solution. A 0.75-inch hole was drilled into each container snap-cap lid, through which the stems and roots were placed. Each container and lid was wrapped in aluminum foil to prevent light from penetrating through the white plastic. Small pieces of foil were wrapped around the base of the stems to help provide support.

The growth containers were refilled with the hydroponics solution on a daily basis for four weeks while the tobacco plants acclimated to the solution. The hydroponics solutions used for the uptake studies were prepared as previously described but in addition, perchlorate was added to give the desired concentrations at the start of the studies.

A. Phytoremediation Study

After 4 weeks of growing in the hydroponics solution, 49 plants of equal height and stem width were chosen for the phytoremediation study. The plants were transferred to foil-covered plastic containers containing 800 mL of hydroponics solutions with known concentrations of perchlorate. All perchlorate-amended nutrient solutions were made up in single batch lots and perchlorate concentrations were verified using ion chromatography prior to dispensing into the plastic containers. Twenty-one plants were placed into 25 ppm and 21 plants were placed into 75 ppm perchlorate solutions. Seven control plants remained in perchlorate-free hydroponics solution.

Seven harvest days were designated at 1, 3, 5, 7, 9, 11, and 13 days after treatment with perchlorate. Three plants per concentration and one control were harvested each harvest day, as described later in this section. During this 13-day period, measured volumes of perchlorate-free nutrient solution were added to each container to replace the volume of nutrient solution taken up by the plant.

B. Uptake of Low Concentrations (ppb) of Perchlorate

In another study, the ability of the tobacco plant to phytoremediate perchlorate at very low concentrations (parts-per-billion) was studied. Six plants of equal height and stem width were transferred to foil-covered white plastic containers with 800 mL of water fortified with 10 ppb perchlorate and 100 ppb perchlorate (three plants each). The ionic matrix of the nutrient solution required a 1/1000 dilution before IC analysis. Therefore, these plants were grown in water without the nutrient solution, unlike studies A and C, which allowed the detection of low concentrations of perchlorate at the end of the growth study. Two plants grown in water without treatment served as controls, and the tobacco

plants were replenished with measured volumes of perchlorate-free water when needed. At the end of the 14-day growth period, the plant roots were rinsed into the plastic containers and the solutions were brought up to the initial 800 mL volume. Aliquots of the solutions were analyzed to determine the amounts of perchlorate left in the solutions.

C. Uptake of High Concentrations (ppm) of Perchlorate

In the preliminary study at this laboratory, tobacco plants showed signs of toxicity with 100 ppm perchlorate within 12 days of exposure (data not shown). To verify this observation, six plants of equal height and stem width were transferred to foil-covered white plastic containers containing 850 mL of solution with 100 ppm perchlorate derived from sodium perchlorate and ammonium perchlorate (three plants each). Two plants were maintained as controls. The plants were grown for 14 days, and the nutrient solutions were replenished as needed with measured volumes of perchlorate-free solution. At the end of the 14-day growth period, the solutions were brought up to the initial 850 mL volume. Aliquots of the solutions were analyzed to determine the amounts of perchlorate left in the solutions. These plants were not harvested, however visual signs of toxicity were noted.

D. Plant Extraction Methods

A method for the analysis of perchlorate in plants was developed, based on dry and wet weight, by Ellington and Evans[12]. In this method, freeze-dried and ground samples were added to water and heated to precipitate proteins. The aqueous phase was centrifuged, exposed to alumina, and filtered through a cartridge filled with divinylbenzene to yield a water-clear extract for IC analysis. This method was chosen because it allows the quantitative analysis of perchlorate with little to no plant matrix interference.

At 12:00 noon on the designated harvest days, the tobacco plants were brought into the lab for harvesting. The leaves were removed from the stem at the base of the petioles using a razor blade. Each leaf was placed on a clean glass plate and individually cut down the center of the midrib, then sliced into half-inch square pieces. The cut leaf material, including the midrib, was placed into a pre-weighed plastic bag, and the total leaf fresh weights were obtained using a Mettler AE240 balance. The stem and roots of the plant were removed from the container and the roots were placed over a 1 L graduated cylinder to drain. The remaining hydroponics solution was also poured into the 1 L cylinder and the volume was recorded to determine the amount of solution that was transpired the previous day. The root mass was rinsed with water into the graduated cylinder until the volume was brought up to the original starting volume of 800 mL for parts A and B, and 850 mL for part C. Samples of the hydroponics solutions were taken and stored at 4^{o}C until analysis. As a precautionary measure, the roots were further rinsed with four 1 L portions of water each to ensure removal of any residual perchlorate. These rinses were discarded since they contained only 1-5 ppb perchlorate. The stem and roots were dried using paper towels, and the roots were sectioned off from the stem at the start of the first lateral root. The stem was cut with a razor blade into 2 mm cross sections, placed into a pre-weighed plastic bag, and the total stem fresh weights were obtained. The roots were cut into 1 cm pieces using scissors, placed into a pre-weighed glass bottle, and the total root fresh weights were obtained. All plant material was freeze-dried for 48-72 hours and the weight recorded. This harvesting procedure was repeated for each plant, starting with the controls and ending with the highest perchlorate concentration.

The freeze-dried samples were ground through a 30-mesh screen in a Wiley Mill and stored in air-tight containers in a freezer until used. Approximately 100 mg of each freeze-dried sample was weighed in duplicate into aluminum weigh boats, heated at 105^{o}C for 24 hours, and re-weighed to determine the dry weights of the plant samples based on the original wet weight.

Duplicate 100-mg samples of each freeze-dried sample were weighed into 40-mL glass vials. Five milliliters of water were pipetted into each vial. The vials were capped tightly and placed in a boiling water bath for 15 minutes, shaken every 5 minutes, to precipitate the proteins and to saturate the dry material. The cooled aqueous extracts were filtered through one layer of Kimwipes to remove the majority of the fibrous material and precipitated proteins. One milliliter of each filtered extract was placed on 500 mg of water-washed and dried DD-6 alumina (Alcoa, Port Allen, LA) for approximately 18 hours. The treated extract was then diluted with 9 mL water to give a 1/10 dilution, and filtered through a 0.45 μM Pall Gelman Acrodisc ion membrane syringe filter (Fisher Scientific, Fairlawn, NJ, Gelman part #4485). An aliquot of the 1/10 dilution was further diluted 1/100, to give a final dilution of 1/1000.

The 1/1000 dilutions were filtered through activated On-Guard RP cartridges (Dionex, Sunnyvale, CA, part #39595) with a 0.2 μM Pall Gelman Acrodisc ion membrane syringe filter (Fisher Scientific, Fairlawn, NJ, Gelman part #4483) attached in tandem. All On-Guard RP cartridges were activated with 5 mL of methanol and 10 mL of water. Three 2-mL fractions of each sample were collected into IC Autosampler sample vials (National Scientific, Lawrenceville, GA, cat. #C4011-1) after discarding the

first 15 drops (0.75 mL). The samples were stored at 4^{o}C until their analysis using the ion chromatograph.

Data obtained from ion chromatography was analyzed using linear regression analysis. Correlation coefficients and linear equations were determined for the rate of solution uptake in the controls, 25 ppm, and 75 ppm perchlorate amended solutions, and for the wet and dry weights of the controls, 25 ppm, and 75 ppm perchlorate amended tobacco plants.

E. Instrumentation

Ion chromatography was performed on a Dionex DX-500 system (Sunnyvale, CA), equipped with a GP50 gradient pump, LC30 chromatography oven, AS3500 autosampler, and ED electrochemical detector. The ED40 was equipped with a conductivity cell and a DS3 detection stabilizer maintained at 30^{o}C within the LC30 oven. The conductivity cell was mounted at the end of the Anion Micro Membrane Suppressor III (2 mm) running in the displacement chemical regeneration (DCR) mode, which allowed detection of anions in the 0-10 $\mu S\ cm^{-1}$ conductivity range. Anions were separated on an IonPac AS16 analytical Microbore separation column (2 x 250mm) in tandem with an IonPac AG16 guard column (2 x 50 mm). Samples and standards were run in the isocratic mode (0.38 mL/min) using 50 mM NaOH as eluent. Elution time of perchlorate on a clean column varied from 9 to 11 minutes. The elution time of perchlorate in samples was dependent on the concentration on non-perchlorate ions and elution time decreased directly with progressive column degradation. A 1,000 μL injection loop was used.

The ion chromatograph was calibrated by injections of calibration standards prepared by diluting a 1,000 ppm stock solution of perchlorate. This stock solution was

prepared by weighing 1.231 g of sodium perchlorate into a 1 L volumetric flask. The flask was brought to volume with water. Eight dilutions of the stock dilution were made to span the range of 5-200 ppb. The calibration standards were 5, 10, 20, 50, 75, 100, 150, and 200 ppb. Injections of these standards in duplicate were used to calibrate the IC. The equation for the linear regression line was used in the IC analysis of sample extracts to calculate the quantity of perchlorate. Calibration check standards were injected prior to and among the analysis of samples, along with water blanks, and system performance was accepted if the concentration estimated from the regression equation was within 10% of the true value. The correlation coefficient for regression of the averaged values was always greater than 0.999. A typical regression equation: Area(ClO_4^- peak) = 4909.1 (mg ClO_4^-) + (-3953.9) where area is the area of the perchlorate peak in the IC chromatogram of the sample and -3953.9 is the y-intercept of the calibration regression line.

Results and Discussion

The uptake, translocation, and accumulation of perchlorate in tobacco plants were determined in this study. Volumes of nutrient solution taken up by individual plants were recorded daily during the 13-day growth study (Figure 2.1). After 1 day of treatment, the plants (controls, 25 ppm, and 75 ppm perchlorate amended plants) had taken up an average of 148 ± 31 mL and after 13 days of treatment, 3714 ± 310 mL were translocated throughout the plant. In general, total plant fresh weights (Figure 2.2) doubled from an average of 90.8 ± 8.4 g after 1 day to 208.6 ± 11.1 g after 13 days. Total plant dry weights (Figure 2.3) increased from an average of 11.0 ± 1.9 g after 1 day to 26.4 ± 1.1 g

after 13 days, a 2.5 fold increase. The study was terminated after 13 days of treatment when the plastic containers could no longer support the rapidly growing tobacco plants.

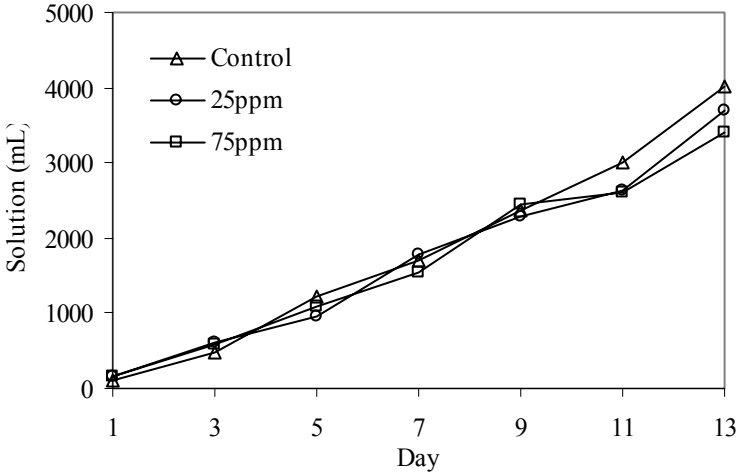

Figure 2.1: Cumulative uptake of nutrient solution by tobacco plants containing 0 ppm (n =1), 25 ppm (n =2), and 75 ppm (n =2) perchlorate. Linear regression analysis results including correlation coefficients (r^2) and linear equations for the treatments were: Control r^2 = .986, y = 0.6451x-0.7113; 25ppm r^2 = .980, y = 0.5722x-0.5576; 75ppm r^2 = .976, y = 0.5396x-0.4706.

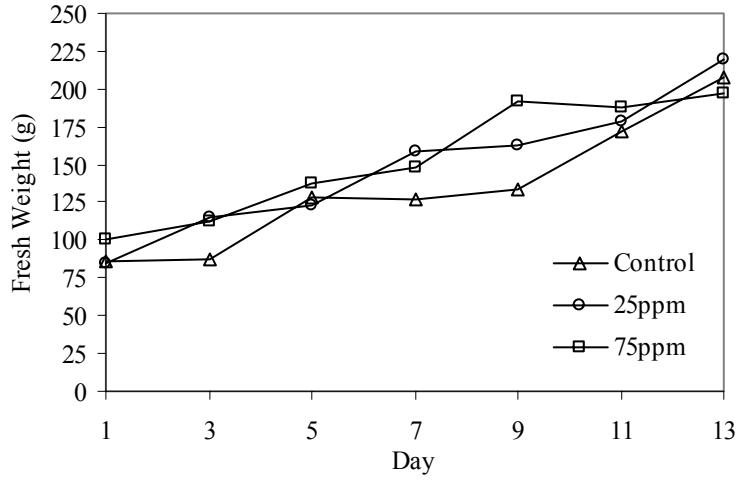

Figure 2.2: Total fresh weights (leaves, stems, roots) of tobacco plants containing 0 ppm (n =1), 25 ppm (n =2), and 75 ppm (n =2) perchlorate. Linear regression analysis results

including correlation coefficients (r^2) and linear equations for the treatments were: Control r^2 = .909, y = 0.19.083x+58.726; 25ppm r^2 = .962, y = 20.313x+67.789; 75ppm r^2 = .940, y = 17.767x+82.744.

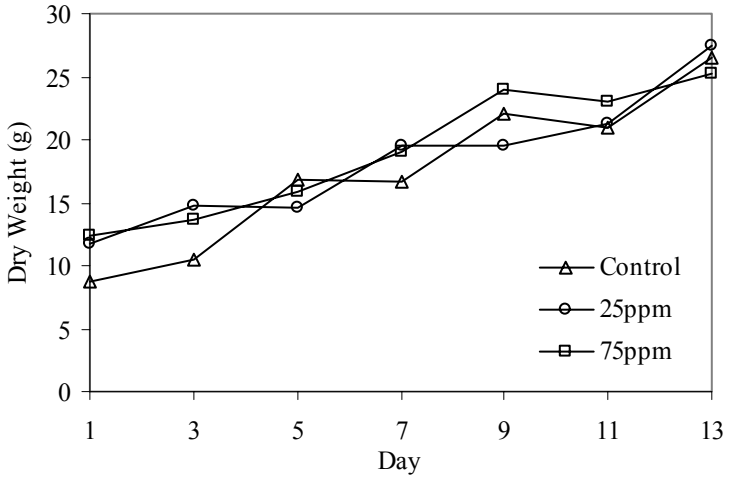

Figure 2.3: Total dry weights (leaves, stems, roots) of tobacco plants containing 0 ppm (n =1), 25 ppm (n =2), and 75 ppm (n =2) perchlorate. Linear regression analysis results including correlation coefficients (r^2) and linear equations for the treatments were: Control r^2 = .935, y = 2.8281x+6.139; 25ppm r^2 = .919, y = 2.3267x+9.1233; 75ppm r^2 = .945, y = 2.3684x+9.5173.

Plant variability in size and health was observed throughout the growth studies. Due to this plant variation, the averages of two of the three plants in each concentration and harvest group were used in the final results. Determination of which two plants were to be used was based on solution uptake by the roots, fresh and dry weights, and the overall appearance and health of the plants. Plant variability may be due to physiological variations or to the lack of aeration of the nutrient solution over the 13 days.

The results of the phytoremediation study showed that perchlorate was taken up by the root system, traveled up the stem via the xylem, and probably accumulated in the

vacuoles of the various cell types in the leaves. As shown in Table 2.1, the highest perchlorate content found in the roots was after 1 day, but then decreased as perchlorate

Table 2.1: Perchlorate content in tobacco plants and solution.

	mg ClO_4^-			
Plant	**Leaf**	**Stem**	**Root**	**Solution**
25ppm[a]				
Day 1	3.1 ± 0.3[b]	0.5 ± 0.1	1.1 ± 0.2	16.7 ± 0.4
Day 3	10.4 ± 1.0	0.8 ± < 0.1	0.8 ± < 0.1	9.8 ± 0.5
Day 5	13.6 ± 0.9	0.7 ± < 0.1	0.7 ± 0.1	4.9 ± 1.4
Day 7	16.3 ± 0.9	0.7 ± 0.1	0.3 ± < 0.1	2.3 ± 0.9
Day 9	18.3 ± 1.0	0.6 ± < 0.1	0.3 ± 0.1	1.1 ± 0.1
Day 11	18.0 ± 0.4	0.6 ± < 0.1	0.2 ± < 0.1	0.9 ± 0.1
Day 13	18.3 ± 1.5	0.7 ± 0.1	0.27 ± < 0.1	0.4 ± 0.5
75ppm[c]				
Day 1	8.2 ± 0.3	1.4 ± 0.1	2.7 ± 0.2	47.8 ± 1.8
Day 3	23.9 ± 0.5	1.9 ± 0.2	2.4 ± 0.2	31.3 ± 1.1
Day 5	37.9 ± 1.6	1.9 ± 0.3	1.4 ± 0.5	16.4 ± 0.3
Day 7	40.8 ± 1.01	1.8 ± 0.1	1.0 ± < 0.1	15.0 ± 3.5
Day 9	48.6 ± 1.0	1.7 ± < 0.1	0.7 ± 0.1	4.5 ± 0.2
Day 11	53.0 ± 1.9	1.6 ± < 0.1	0.7 ± 0.1	3.7 ± 1.0
Day 13	54.4 ± 2.8	1.2 ± 0.1	0.3 ± < 0.1	2.5 ± 1.4

a Actual initial ClO_4^- concentration was 25.2 ppm (20.1 mg).
b Milligrams ClO_4^- ± standard deviation, n=2.
c Actual initial ClO_4^- concentration was 75.4 ppm (60.3 mg).

was taken up into the stems and leaves. The perchlorate content in the stem remained fairly constant and the perchlorate accumulated in the leaves over the 13-day growth period. The perchlorate content in the nutrient solution decreased over time, and in some instances dropped below the method detection limit of 0.5 ppb of the IC. Figures 2.4 and

2.5 clearly show these relationships for the 25 ppm and 75 ppm perchlorate amended plants.

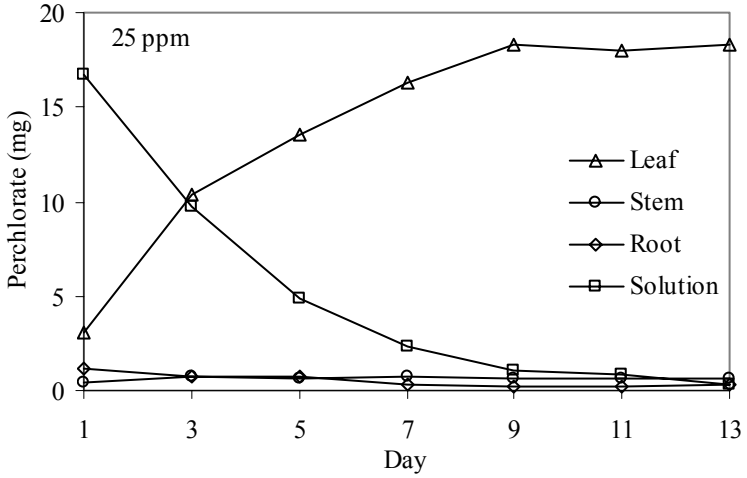

Figure 2.4: The fate of perchlorate in 25 ppm (n =2) perchlorate amended tobacco plants and depletion of perchlorate from the nutrient solutions.

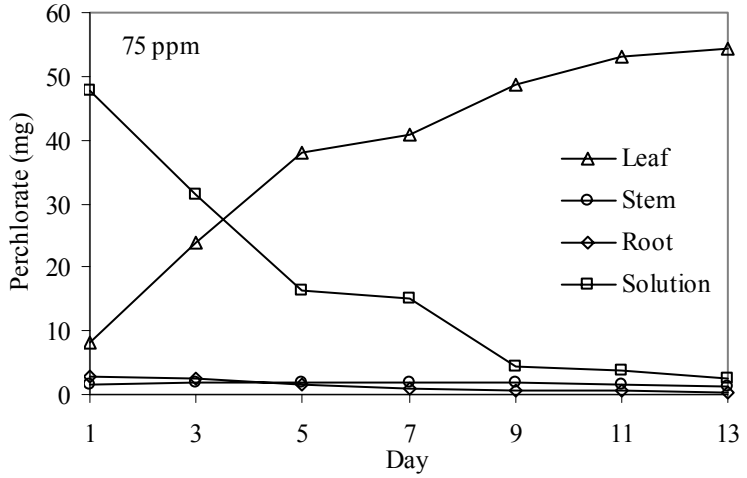

Figure 2.5: The fate of perchlorate in 75 ppm (n =2) perchlorate amended tobacco plants and depletion of perchlorate from the nutrient solutions.

After the 13-day phytoremediation growth study in the perchlorate-amended nutrient solution, 95.4% of the total perchlorate applied was taken up by the 25 ppm perchlorate amended plants, and 92.7% of the total perchlorate applied was in the 75 ppm perchlorate amended plants. Most of the remaining perchlorate was accounted for in the amended nutrient solution. The highest perchlorate concentration in the plants was found on day 11 in the leaf, where 0.37% of the dry matter was perchlorate (Table 2.2). This suggests that tobacco plants are effective plants for the phytoremediation of perchlorate from the environment. Table 2.2 shows that perchlorate concentrations peaked after 9 days in the leaf, 3 days in the stem, and 1 day in the roots. After 9 days, very little additional perchlorate was accumulated in the leaves, which can be attributed to the near-depletion of perchlorate in the nutrient solution. However, the plants continued to grow which added to the total mass of the plant. This resulted in the dilution of perchlorate on a dry or wet weight basis within the plants. Perchlorate recoveries ranged from 97.4% to 107.6% in the 25 ppm perchlorate amended plants and nutrient solutions, and from 92.0% to 99.8% in the 75 ppm plants and nutrient solutions. These high recoveries suggest that the perchlorate is being stored in the plant and that the degradation of perchlorate is negligible, if degradation occurs. The lack of degradation can be attributed to the energy required to degrade the perchlorate anion and/or the lack of the proper degradation enzyme(s) in plant tissues. Assuming there is no degradation of perchlorate, the recovery errors may be the result of methodology.

Table 2.3 provides the results of the phytoremediation study at very low and high concentrations of perchlorate. Tobacco plants were shown to be very effective in phytoremediating parts-per-billion concentrations of perchlorate, which are commonly

found in the environment. Two of the three 10 ppb perchlorate amended plants' water concentrations were below the method detection limit of the IC, indicating that at least 95% of the perchlorate was taken up by the plants. The third plant exposed to 10 ppb had to be discarded due to disease. Two of the three 100 ppb perchlorate amended plants' water concentrations were below the detection limit as well. The third plant's water had 0.0021 mg perchlorate (2.7% of the total perchlorate applied) remaining after the 14 days,

Table 2.2: Perchlorate concentration in tobacco plants and solution.

	ppm ClO_4^- (Dry and Fresh Weight Basis)						ppm ClO_4^- Solution
	Leaf		Stem		Root		
Plant	Dry	Fresh	Dry	Fresh	Dry	Fresh	
25ppm[a]							
Day 1	368.3 ±14.2[b]	53.3 ± 1.9	226.6 ± 34.2	30.0 ± 0.7	770.1 ± 9.4	55.7 ± 3.3	20.9 ± 0.5
Day 3	1000.3 ± 26.6	143.8 ± 10.1	287.2 ± 12.6	35.3 ± 2.3	458.1 ± 70.5	35.4 ± 6.6	12.2 ± 0.6
Day 5	1401.3 ± 108.8	188.3 ± 3.5	220.6 ± 21.1	25.4 ± 3.5	374.9 ± 85.1	28.1 ± 4.8	6.1 ± 1.7
Day 7	1268.7 ± 72.0	175.8 ± 5.6	160.9 ± 11.5	19.3 ± 1.6	153.0 ± 32.9	12.4 ± 3.5	2.9 ± 1.1
Day 9	1469.4 ± 38.8	187.87 ± 1.2	140.4 ± 36.9	16.4 ± 3.0	103.7 ± 5.8	9.6 ± 0.8	1.4 ± 0.1
Day 11	1336.2 ± 20.5	149.3 ± 23.9	107.8 ± 6.4	13.0 ± 0.2	85.6 ± 3.2	8.8 ± 0.6	1.1 ± 0.2
Day 13	1122.1 ± 7.7	143.3 ± 4.4	88.2 ± 2.0	11.5 ± 0.2	76.0 ± 8.1	6.5 ± 1.3	0.5 ± 0.7
75ppm[c]							
Day 1	957.9 ± 98.5	134.3 ± 3.6	627.7 ± 84.1	75.6 ± 6.9	1870.0 ± 100.3	134.1 ± 8.7	59.8 ± 2.2
Day 3	2536.4 ± 178.5	354.3 ± 41.2	783.0 ± 1.6	89.6 ± 4.7	1319.4 ± 55.4	102.9 ± 7.2	39.2 ± 1.3
Day 5	3567.3 ± 168.0	452.3 ± 1.4	616.7 ± 137.2	70.0 ± 9.7	686.1 ± 153.8	52.5 ± 6.8	20.5 ± 0.3
Day 7	3207.0 ± 131.2	457.8 ± 4.9	428.5 ± 23.1	50.4 ± 0.2	481.2 ± 14.0	42.9 ± 0.1	18.7 ± 4.3
Day 9	3181.8 ± 384.1	428.0 ± 76.9	304.9 ± 60.3	34.9 ± 5.4	242.4 ± 29.8	25.2 ± 2.2	5.6 ± 0.2
Day 11	3700.9 ± 33.9	472.9 ± 5.8	260.2 ± 46.2	32.1 ± 5.4	238.0 ± 39.2	24.1 ± 3.2	4.6 ± 1.2
Day 13	3559.0 ± 419.6	469.3 ± 53.1	173.4 ± 31.4	22.7 ± 4.7	114.1 ± 29.5	12.0 ± 3.0	3.1 ± 1.7

a Actual initial ClO_4^- concentration was 25.2 ppm (20.1 mg).
b Dry and fresh weights ± standard deviation, n=2.
c Actual initial ClO_4^- concentration was 75.4 ppm (60.3 mg).

indicating that 97.3% of the total perchlorate applied was taken up by the plant. Chlorosis was evident in these water-grown plants after approximately 6 days due to the lack of nutrients during the 14 day growth study. However, this did not affect perchlorate uptake by the plants.

Table 2.3: Perchlorate remaining in solution after 14 days (n =1).

Plant	mg ClO_4^-	ppm ClO_4^-
10ppb[a] Plant 1	ND[b]	ND
10ppb Plant 2	ND	ND
100ppb[c] Plant 1	ND	ND
100ppb Plant 2	0.002	0.0027
100ppb Plant 3	ND	ND
100ppm[d] $NaClO_4$ Plant 1	$1.6 \pm < 0.1$[e]	$1.9 \pm < 0.1$
100ppm $NaClO_4$ Plant 2	8.9 ± 0.2	10.4 ± 0.3
100ppm[f] NH_4ClO_4 Plant 1	$1.1 \pm < 0.1$	$1.2 \pm < 0.1$
100ppm NH_4ClO_4 Plant 2	$2.0 \pm < 0.1$	$2.3 \pm < 0.1$

a Actual initial ClO_4^- concentration was 9.7 ppb (7.8µg)
b Non detectable
c Actual initial ClO_4^- concentration was 97.7 ppb (78.2 µg)
d Actual initial ClO_4^- concentration was 102.2 ppm (86.9 mg)
e Milligrams ClO_4^- ± standard deviation
f Actual initial ClO_4^- concentration was 101.4 ppm (86.2 mg)

The sodium and ammonium salts of perchlorate were used to determine whether any toxicity was due to the perchlorate or excess sodium ions. Table 2.3 shows that three out of the four plants exposed to 100 ppm perchlorate had less than 2% of the total perchlorate applied remaining after the 14 days. The fourth plant had approximately 10% of the perchlorate remaining in the solution, most likely due to physiological variations of the plant. No signs of toxicity were observed during the study for either sodium or ammonium perchlorate plants. Although the preliminary study had suggested that an

initial concentration of 100 ppm perchlorate might be toxic to the plants, the age at which the plants were exposed must be considered. The plants in the preliminary study were exposed at 10 weeks of age, as opposed to this study's plants being exposed at 14 weeks of age. This implies that the toxicity of perchlorate may be related to the stage of maturity and size of the tobacco plants at the time of exposure to perchlorate.

Based on these results, the tobacco plant appears to be a potential candidate for the phytoremediation of perchlorate. The tobacco roots are fibrous with large surface areas for the uptake of perchlorate, the observed storage capacity (0.37%) of perchlorate in the leaves is substantial, and the plants are fairly tolerant of perchlorate at initial concentrations up to 100 ppm. This storage capacity may be greater since the upper limits of amended perchlorate were reached in this study.

There are a few limitations to the use of tobacco for phytoremediation. Tobacco plants would be limited to phytoremediating perchlorate contaminated water within the rhizosphere, from sources such as irrigation or surface water, where growth conditions are appropriate for tobacco plants. Since tobacco roots don't extend beyond the rhizosphere, these plants would be ineffective in phytoremediating perchlorate contaminated groundwater. Also, since the accumulated perchlorate is not degraded in the tobacco plant, the plants would have to be removed after remediation of the perchlorate. Incineration may be an effective method to dispose of perchlorate contaminated plants since perchlorate decomposes around 300^{o}C [3].

A Plant Kinetic (PK) model has been developed based on the results reported here to describe and predict the uptake, translocation, and accumulation of perchlorate in tobacco plants[13].

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CHAPTER 3

A MODEL FOR THE UPTAKE, TRANSLOCATION, AND ACCUMULATION OF PERCHLORATE IN TOBACCO PLANTS[2]

[2] S. E. Sundberg, J. W. Fisher, J. J. Ellington, J. J. Evans, and D. A. Keys. Submitted to *Journal of Environmental Monitoring*, 1/14/03.

Abstract

A simple mathematical model was developed to describe the uptake, translocation, and accumulation of perchlorate in tobacco plants under rapid growth conditions. The fate of inorganic chemicals such as perchlorate has not been modeled previously. The Plant Kinetic (PK) model defined a plant as a set of compartments, described by mass balance differential equations and plant-specific physiological parameters. The tobacco plant was divided into five compartments: solution, roots, stem, stem storage, and leaves. Transport of perchlorate from the hydroponics solution into the root tissue, through the stems, and to the leaves was modeled by mass flow with the transpiration stream. The translocation of perchlorate from the root tissue into the xylem was described as a diffusion-limited process. Transport of perchlorate to and from a stem storage compartment, described as first-order processes, were necessary to predict perchlorate content in the stem. Growth dilution was described by incorporating measured tobacco growth curves. Hydroponic solution utilization data and plant growth data were used to predict xylem flow rates and growth curves as functions of time. Root diffusion and stem bidirectional transport rates were optimized to fit plant kinetic data at 25 ppm and 75 ppm solution perchlorate concentrations. Data obtained from a separate study with multiple solution perchlorate concentrations were used to validate predicted root, stem, and leaf concentrations. There was good agreement between model predictions and measured concentrations in the plant. The PK model, once adequately validated, can be applied to other terrestrial plants and nondegradable inorganic chemicals currently used for both phytoremediation and ecological risk assessment applications.

Introduction

Perchlorate is an inorganic contaminant in soils, groundwater, surface waters, and irrigation waters used for crop production resulting from the use of solid salts of ammonium, potassium, or sodium perchlorate in industrial and commercial applications. Perchlorate is also found in the environment as part of certain geologic deposits, such as the Chilean nitrate deposits[1]. In the environment, perchlorate salts are very soluble and are weakly sorbed to soil minerals. Consequently, perchlorate is exceedingly mobile in aqueous systems and is persistent for many decades under typical groundwater and surface water conditions[2].

Since 1997, perchlorate has been reported at various manufacturing sites and in drinking water and well water supplies in California, Utah, Nevada, and Texas. Perchlorate has been found in California water supplies in the range of 18 to 280 ppb (μg L^{-1}), potentially affecting the drinking water supplies of at least 12 million people in the United States[2]. The groundwater locations in California have been associated with facilities, which manufactured, tested, or disposed of solid rocket fuels and propellants for the Department of Defense or the National Aeronautics and Space Administration. In addition to water supplies, soils may be contaminated by spills or the disposal of perchlorate solutions. Perchlorate concentrations from less than 1 ppm (mg kg^{-1}) to 1,470 ppm have been reported in soils[3]. Contamination occurs as a result of flushing rockets, improper disposal of rocket fuel, explosives, or dumping of manufacturing wastes. The environmental risks posed by perchlorate are currently being assessed by the United States Environmental Protection Agency, which has placed perchlorate on the Contaminant Candidate List[4] and the Unregulated Contaminant Monitoring Rule[5].

The possibility of successful phytoremediation of perchlorate-contaminated soil depends on the availability of plant varieties with high rates of accumulation and tolerance for perchlorate[6]. Von Burg[7] reported that in terrestrial species, perchlorate soil concentrations in the range of 40 to 80 ppm were toxic to plants. It was also shown that potassium perchlorate concentrations as low as 0.55 ppm in soil inhibited growth in ryegrass and cotton. Soybeans were reported to show toxic effects when irrigated with water containing 2.5 to 5 ppm (mg L^{-1}) potassium perchlorate[7].

Previous field and laboratory studies have shown that tobacco plants are tolerant of perchlorate and will accumulate perchlorate in the plant tissues[8,9]. Concentrations of perchlorate in tobacco plant tissues were measured from a 1999 crop that was side-dressed with a fertilizer that contained Chilean saltpeter[8]. The perchlorate concentration in the leaf lamina was 96.0 ± 0.6 ppm dry weight (14.6 ± 0.1 ppm fresh weight). In a greenhouse study, leaves of tobacco plants grown in 75 ppm perchlorate amended nutrient solution accumulated up to 3,701 ± 34 ppm perchlorate dry weight (473 ± 6 ppm fresh weight)[9]. The perchlorate concentrations in tobacco plants reported in these studies are greater than those previously shown to produce toxic effects in various plant species. In addition, tobacco plants are characterized by their large leaf area and rapid plant growth supported by a fibrous root system. Mature tobacco plants have leaf areas ranging from 0.09 to 0.14 m^2, however in some cases a single cigar tobacco plant can produce as much as 2.3 m^2 of leaf area[10]. This allows large amounts of perchlorate to be stored in the various leaf tissues. Sundberg *et al.*[9] observed a storage capacity of perchlorate in tobacco leaves of 0.37% dry weight.

Understanding the potential for the uptake, translocation, and accumulation of toxic chemicals by plants is an important public health and ecological issue. Predictive tools are needed to understand and interpret the behavior of chemicals in the plants[11]. It is impractical to test the efficiency of all plant species under greenhouse conditions to phytoremediate chemical contaminants. Mathematical models, which combine the physiochemical properties of a chemical with the anatomical and physiological properties of plants, can be used as a predictive tool to assess the temporal aspects of phytoremediation and the efficiency of plants to phytoremediate environmental contaminants.

The plant uptake, translocation, and accumulation of environmental contaminants are very dependent on the anatomy and physiology of plants. The features that enable plants to accumulate nutrients and water from soil also enable them to accumulate anthropogenic chemicals or substances[11]. With the exception of some hormone-like chemicals, plant uptake and translocation of anthropogenic chemicals appears to be carried out by passive transport (mass flow or diffusion). There is no evidence for active transport of anthropogenic chemicals as they move through the endodermis of the root and into the vascular system of plants[12].

Solution (H_2O) uptake by plant roots is driven by mass flow with the transpiration stream[11]. Water evaporation and transpiration from the cell walls of the leaf mesophyll cells generates the large negative pressure gradients in the apoplastic water. These negative pressures are transmitted to the xylem, resulting in the passive uptake of water through the roots. Water and solutes enter the root most readily in the apical part of the root via root hairs, which are microscopic extensions of the root epidermal cells[13].

Driven by the energy potential gradient caused by leaf transpiration, water flows through the epidermis and the intercellular spaces of the cortex, and flows to the endodermis and Casparian strip[14]. From the epidermis to the endodermis of the root, there are three pathways though which water can flow: the apoplast pathway, symplast pathway, or transmembrane pathway[13]. Of the three pathways, the symplast pathway moves the largest volume of water in the plant. In this pathway, water travels from cell to cell through the entire network of cell cytoplasm interconnected by plasmodesmata.

Once water and nutrients have passed through the endodermal cells, collectively known as the root symplast, they enter the root xylem by diffusion. Xylem is the tissue that transports water and minerals from the root system to the aerial portions of the plant, constituting the longest part of the water transport pathway[13]. Water travels through the root xylem and connects to the stem xylem. Once in the stem xylem, water and solutes can pass through the tracheids and sieve cells into the stem pith and stem cortex, together forming stem storage.

The stem xylem connects to the leaf xylem. Tracheary elements, which are the conducting cells in the xylem that includes both vessel elements and tracheids, enable the transport of large quantities of water with great efficiency. Water is brought to the leaves via the xylem of the leaf vascular bundle, which branches into a very fine and intricate network of veins throughout the leaf. From the leaf xylem, water is drawn into the mesophyll cells via the cell walls of the leaf. Within the mature mesophyll cells of the leaf, central vacuoles, which occupy 80-90% of the total volume of the cell, will accumulate water and other dissolved inorganic ions[13].

A few mathematical models have been developed to describe the fate of chemicals in plants. Boersma *et al.,*[14,15] and Trapp *et al.*[11] developed mathematical models to describe the uptake and translocation of organic chemicals. These authors validated the models with experiments based on uptake of bromacil by soybean plants. Model validations were performed assuming steady-state conditions with respect to plant growth and transpiration rates. A fugacity model was developed by Hung and Mackay[16] to predict the uptake and kinetics of organic chemicals in herbaceous agricultural plants. More recently, Zhang *et al*.[17] constructed a model to describe the transport of methyl *tert*-butyl ether (MTBE) into alfalfa plants. These authors used the model to estimate a diffusion coefficient for the transfer of MTBE from the root system into the stem. No models were found in the literature for uptake of inorganic chemicals including perchlorate, in plants. Unlike organic chemicals that have been previously modeled, perchlorate does not appear to degrade in plant tissues and is not expected to be lost to the atmosphere through transpiration. These factors introduce simplifying assumptions that reduce the number of model parameters and compartments compared to previously developed plant chemical fate models. The objective of this study was to develop a simple PK model for the uptake, translocation, and accumulation of perchlorate in tobacco plants incorporating plant growth. For the application of fate models for phytoremediation purposes, plant growth is an important process to include as the plants used for phytoremediation would likely be in their rapid growth phase.

Experimental Procedures

A. Model Calibration Study

To calibrate the PK model, a 13-day hydroponics uptake study was completed. Tobacco plants were grown in 25 ppm and 75 ppm perchlorate amended nutrient solutions. At two-day intervals groups of plants (including controls) were harvested, and perchlorate concentrations in the solution, roots, stems, and leaves were determined by ion chromatography. The study is described in detail elsewhere[9].

B. Model Structure and Development

The PK model defined the plant as a set of anatomical compartments based on the anatomy and physiology of the plant. The tobacco plant was divided into five compartments: solution, roots, stem, stem storage, and leaves (Figure 3.1). The compartments were described by mass balance differential equations and plant-specific physiological parameters.

The PK model was developed using Advanced Continuous Simulation Language (ACSL) Version 11.8.4 (AEgis Technologies, Huntsville, AL). Refer to the Appendix for ACSL model code and command files. To mathematically describe the processes within a living plant using mass balance differential equations, a few simplifying assumptions were made: (a) movement of water between compartments occurs by mass flow with the transpiration stream and diffusion, (b) phloem transport of perchlorate from the leaves back down to the roots is negligible, (c) no significant degradation of perchlorate occurs in the plant system, and (d) no evaporative loss of perchlorate occurs in the stomatal activity of the plant[9].

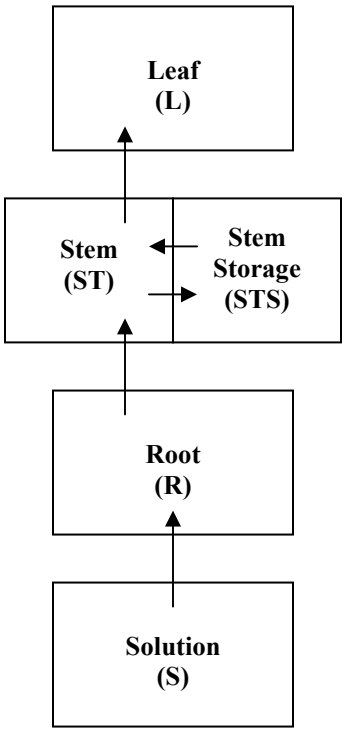

Figure 3.1: Sequence of plant compartments based on plant anatomy and physiology used in the PK model.

The PK model is comprised of a set of equations, one for each plant compartment. The rate of uptake of the hydroponics solution into the plant was estimated by measuring the rate of solution utilization ($R_S(t)$, L day^{-1}) in the 13-day phytoremediation study. The rate of uptake of perchlorate into the root system from the solution and the accumulation of perchlorate in the leaf tissue were described by flow-limited conditions, governed by the transpiration stream. That is, the rate-limiting step in uptake of perchlorate in the root and leaf is the transpiration stream flow rate. Clearance of perchlorate from the root tissue was described as a diffusion-limited process, where diffusion across the cell membranes of the endodermis was the rate-limiting step. In the stem, perchlorate

distribution from the xylem into the stem storage tissue and visa versa was described as a bi-directional first-order diffusional process.

The PK model assumed a constant supply of nutrient solution. The xylem flow rate was assumed to equal measured average daily nutrient solution utilization rates. The xylem flow rate and plant growth curves were determined by linear interpolation of measured solution utilization rates and plant weights at two-day intervals during the growth study (Figures 3.2 and 3.3). Linear interpolations were implemented in ACSL using a TABLE function.

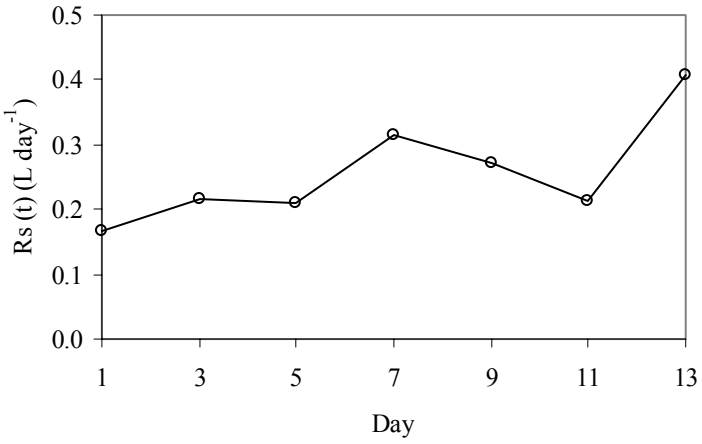

Figure 3.2: Mean nutrient solution utilization rate, Rs(t), of 25 ppm and 75 ppm perchlorate amended plants from the 13-day hydroponics study (n = 4).

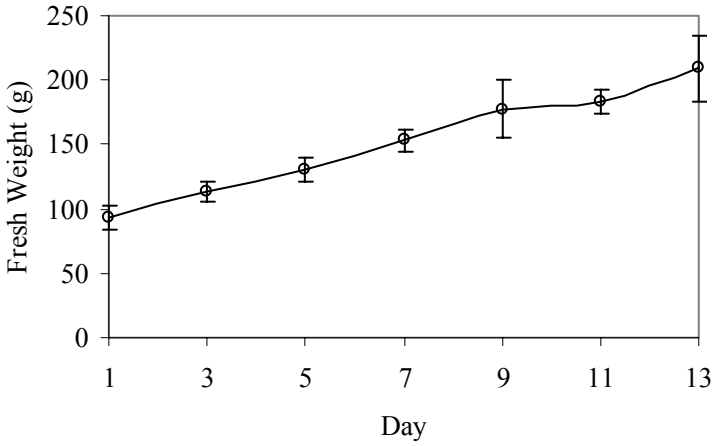

Figure 3.3: Average fresh weights of 25 ppm and 75 ppm perchlorate amended plants from the 13-day hydroponics study (n = 4). Error bars indicate standard deviations.

Descriptions of the six equations yielding the rate of change of perchlorate (mg day^{-1}) for each compartment are as follows. Model terms used in the equations along with their definitions are given in Table 3.1.

Solution Compartment

Mass change = - uptake by root.

$$\frac{dA_S}{dt} = -R_S(t) \times C_S$$

where $C_S = \frac{A_S}{V_S}$.

Root Compartment

Mass change = + uptake from solution

- diffusion across endodermis to stem.

$$\frac{dA_R}{dt} = [R_S(t) \times C_S] - [DF_R(t) \times C_R]$$

where $DF_R(t) = DF \times R_S(t)$ and

$$C_R = \frac{A_R}{M_R(t)} \ .$$

Stem Compartment

Mass change = + diffusion across endodermis from root

- diffusion into stem storage

+ diffusion from stem storage

- mass flow with transpiration stream to leaf.

$$\frac{dA_{ST}}{dt} = [DF_R(t) \times C_R] - [T_{ST} \times A_{ST}] + [T_{STS} \times A_{STS}] - [R_S(t) \times C_{ST}]$$

where $C_{ST} = \frac{A_{ST}}{M_{ST}(t)}$.

Stem Storage Compartment

Mass change = + diffusion to stem storage

- diffusion from stem storage.

$$\frac{dA_{STS}}{dt} = [T_{ST} \times A_{ST}] - [T_{STS} \times A_{STS}] .$$

Leaf Compartment

Mass change = + mass flow with transpiration stream from stem.

$$\frac{dA_L}{dt} = [R_S(t) \times C_{ST}] .$$

Table 3.1: Symbols and subscripts used in the PK model mass equations.

Symbol	Definition
R(t)	Xylem flow rate (L day $^{-1}$)
T	Transfer parameter (day $^{-1}$)
DF	Diffusion factor (unitless)
DF(t)	Diffusion factor, dependent on time (L day $^{-1}$)
V	Volume (L)
M(t)	Mass (wet weight), dependent on time (kg)
C	Concentration of ClO_4^- (ppm)
A	Amount of ClO_4^- (mg)
Subscript	**Definition**
S	Solution
R	Roots
ST	Stem
STS	Stem Storage
L	Leaf

The root diffusion factor was optimized by maximum likelihood estimation as implemented in ACSL Math Version 11.8.4 (AEgis Technology, Huntsville, AL). The Nelder-Mead algorithm was used for likelihood estimation. The error model was fit to the experimental data. The starting value for the DF parameter was found by visually fitting the model to the data prior to running the optimization. The two perchlorate concentrations (25 ppm and 75 ppm) were first fit separately and then simultaneously to the data for parameter estimation (Table 3.2). There was successful convergence to final parameter estimates.

The diffusion values of the transfer parameters (unitless) between the stem and stem storage compartments were optimized by maximum likelihood estimation as described above (Table 3.2). Proportions of measured total stem weights from experimental data allocated to the stem and stem storage were 0.07 and 0.93, respectively, as determined for a normalized dicot plant by Boersma *et al.*[15]

Table 3.2: Model calibration and plant anatomy parameters.

Parameter	Value	Description	Source
T_{ST}	16.33 ± 2.74 [a]	Transfer parameter from ST to STS	Optimized [b]
T_{STS}	0.31 ± 0.06 [a]	Transfer parameter from STS to ST	Optimized [b]
DF	0.40 ± 0.02 [a]	Diffusion factor	Optimized [b]
P_{ST}	0.07	Proportion of total stem weight allocated to ST	Boersma *et al.*[15]
P_{STS}	0.93	Proportion of total stem weight allocated to STS	Boersma *et al.*[15]

a Values of T_{ST}, T_{STS}, and DF ± standard deviation.
b Parameters were fit to calibration data by maximum likelihood estimation.

D. Model Validation Study

To validate the PK model, a second uptake study was completed. Procedures of this study followed those of the 13-day phytoremediation study (Methods Part A), with the exception that the plants in this study were grown in 850 mL of 10, 50, 75, and 100 ppm perchlorate amended nutrient solution for a two-day period. Three plants per concentration and two controls were harvested two days after treatment began. Harvest procedures, plant extraction methods, and analytical instrumentation are described in detail elsewhere[9].

Results and Discussion

The predicted perchlorate uptake from the hydroponics solution amended with 25 and 75 ppm perchlorate closely followed the measured values from the 13-day phytoremediation study (Figure 3.4). Perchlorate concentrations in the plant roots (Figure 3.5) increased rapidly during the first day of exposure. This can be attributed to the initial entry of perchlorate and nutrient solution into the root cortex, comprised of cells and free space in which water and solutes freely move. The filling of the cortex cells and free space occurs as soon as exposure is initiated due to the plant roots being completely bathed in the

nutrient solution. Such behavior is not expected in soil-grown plants[18]. On the first modeling attempt, the amount of perchlorate in the roots was under-predicted compared to the measured values from the 13-day hydroponics growth study (Figure 3.5). A parameter was added to the root compartment mass balance equation to adjust the perchlorate content in the root tissue entering the root xylem and stem xylem (Table 3.2). To mathematically describe the diffusion of perchlorate from the root tissue, $R_S(t)$ (L day^{-1}) was multiplied by a diffusion factor (DF, unitless). Thus, the diffusion of perchlorate out of the root tissue was assumed to be proportional to the increase in the transpiration rate, reflecting growth of the root system. This approach is mathematically equivalent to the approach taken previously by Trapp *et al.*[11], where DF is equivalent to the TSCF (Transpiration Stream Concentration Factor).

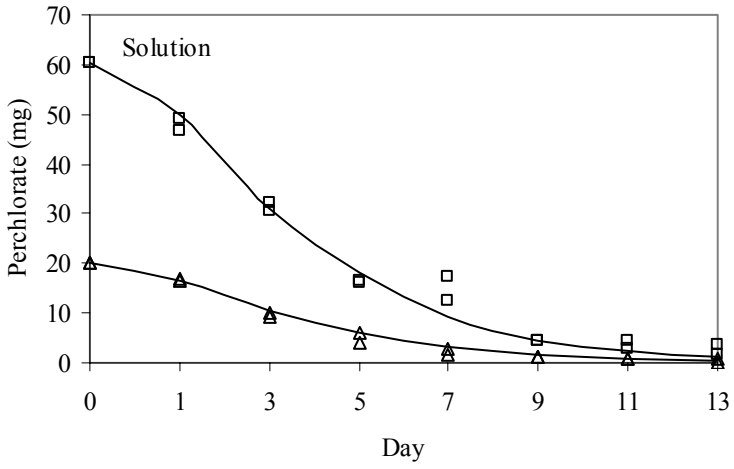

Figure 3.4: Perchlorate content in hydroponics solution exposed to 25 ppm and 75 ppm. Smooth lines follow model simulations, triangles indicate measured values from the 25 ppm growth study, and squares indicate measured values from the 75 ppm growth study.

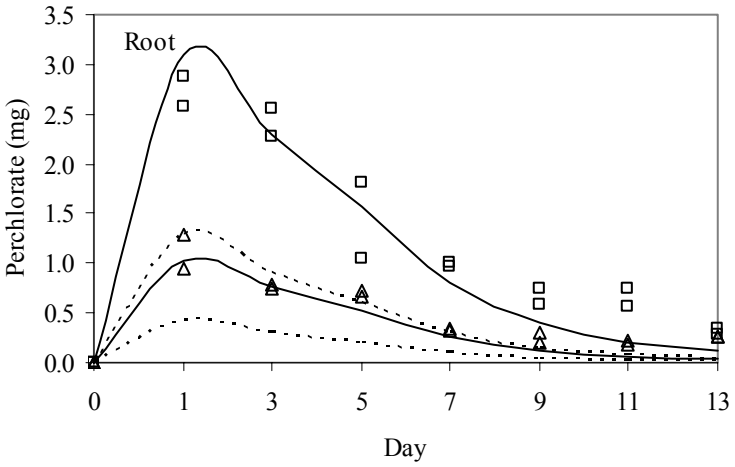

Figure 3.5: Perchlorate content in tobacco roots exposed to 25 ppm and 75 ppm perchlorate. Smooth lines follow model simulations with the diffusion factor included in the model equations, dotted lines follow model simulations without the diffusion factor, triangles indicate measured values from the 25 ppm growth study, and squares indicate measured values from the 75 ppm growth study.

Perchlorate kinetic behavior in the stem (Figure 3.6) is characterized by an initial increase in concentration during the first three days of exposure and retention in the stem. In the 75 ppm perchlorate amended plants, there was a gradual decrease in perchlorate concentration after day 9 as the perchlorate is depleted from the solution and accumulated in the leaves. By day 3, it appears that the stem perchlorate concentration in the 25 ppm perchlorate amended plants reached equilibrium with the concentration in the transpiration stream. On the first modeling attempt, the amount of perchlorate in the stem was under-predicted compared to the measured values from the growth study. Consequently, a stem storage compartment was added to the model to better describe perchlorate retention in the stem. Perchlorate transfer between the stem xylem and stem storage was described as an unsymmetrical bi-directional first-order process to represent diffusion of perchlorate in and out of the stem storage compartment. These first-order

constants allowed for the description of a short retention of perchlorate until the concentrations of the stem xylem and stem storage reached equilibrium, at which time perchlorate was released from storage and further translocated up the stem xylem into the leaves. Peak stem concentration was dependent on perchlorate concentration, and no saturation point was observed.

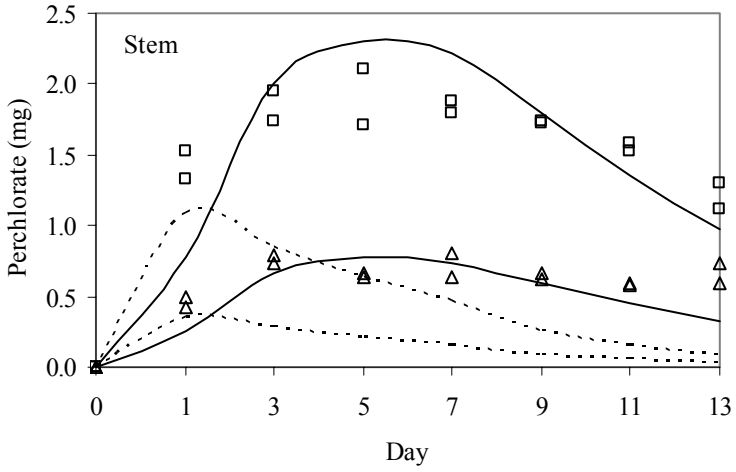

Figure 3.6: Perchlorate content in tobacco stems exposed to 25 ppm and 75 ppm perchlorate. Smooth lines follow model simulations with the stem storage compartment included in the model equations, dotted lines follow model simulations without the stem storage compartment, triangles indicate measured values from the 25 ppm growth study, and squares indicate measured values from the 75 ppm growth study.

There are a few possible explanations for the retention of perchlorate in the stem storage compartment. This compartment is comprised of cortex and pith tissues of the stem, both of which contain plant cells. It is possible that perchlorate in the cortex and pith tissue diffuses through the cell membranes and enters the cell. Within the cell, perchlorate can be drawn into the vacuoles, which, like the vacuoles in the leaf mesophyll cells, are capable of accumulating water and dissolved inorganic ions. The retention

could be due to ionic attraction or interaction of perchlorate with cations within the vacuoles. In addition, a very small fraction of perchlorate may bind to cell proteins.

Perchlorate accumulation in the leaves (Figure 3.7) was modeled without the addition of calibration parameters. Thirteen days after exposure, the model predicts that 96.3% of both the 20.13 mg perchlorate applied in the 25 ppm perchlorate amended plants and of the 60.34 mg perchlorate applied in the 75 ppm perchlorate amended plants will be accumulated in the leaves. In the 13-day phytoremediation study, 90.8% of the 25 ppm and 95.2% of the 75 ppm perchlorate amended plants was accumulated in the leaves 13 days after exposure

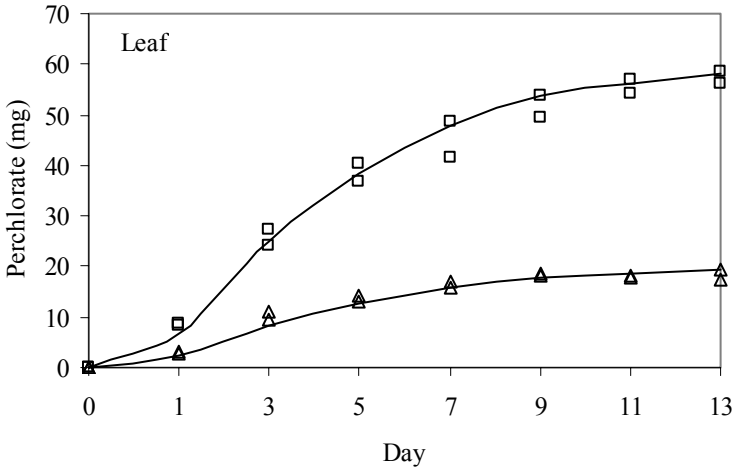

Figure 3.7: Perchlorate content in tobacco leaves exposed to 25 ppm and 75 ppm perchlorate. Smooth lines follow model simulations, triangles indicate measured values from the 25 ppm growth study, and squares indicate measured values from the 75 ppm growth study.

There are a couple limitations of the PK model for the uptake, translocation, and accumulation of perchlorate by tobacco plants. Instantaneous exposure to perchlorate, as observed in the hydroponics growth study, would not occur under typical field conditions

except where a spill of contaminated water would reach the root zone of the plants in a very short period of time. For this type of condition, the model should be run as presented here. However, for field conditions in which there is a low level, chronic exposure to perchlorate, such as plants growing in contaminated soils, the model parameters should be adjusted accordingly. A slower rate of uptake would be expected as roots interface with soil solution, vapor, and solid phases rather than a hydroponics solution. In addition, for the purpose of simplifying the model, the compartments were assumed to be homogenously mixed compartments. In field conditions, there may be significant differences between upper and lower sections of the plant roots, stems, and leaves, within the xylem as well as in storage tissues. Greater variability in the uptake of perchlorate in field conditions would be expected due to varying soil properties, such as pH, organic matter, and moisture content.

The separate 2-day phytoremediation study was used to validate the PK model. Measured and predicted amounts of perchlorate are given in Table 3.3. Across the four concentrations, 10, 50, 75, and 100 ppm perchlorate, all but two of the predicted values were within the calculated 95% confidence intervals of the measured values. In the 75 ppm perchlorate amended plants, perchlorate content in the leaf was under-predicted, and in the 100 ppm perchlorate amended plants, perchlorate content in the root was over-predicted. These results suggests that the PK model is most reliable for perchlorate concentrations less than 75 ppm. The pharmacokinetics of perchlorate in the stem appears to be complex and may be governed by a saturable transport process. Further research is needed to gain a better understanding of perchlorate kinetics in the tobacco plant at high perchlorate concentrations.

Table 3.3: Predicted perchlorate content from the PK model and measured perchlorate content after 2 days of treatment in validation study.

	Perchlorate (mg)	
	Predicted	**Measured**[a]
10ppm[b] Leaf	2.24	2.71 (1.99-3.43)
Stem	0.21	0.19 (0.07-0.31)
Root	0.39	0.37 (0.20-0.54)
Solution	5.97	5.88 (5.26-6.50)
50ppm[c] Leaf	10.85	13.27 (6.79-19.75)
Stem	1.03	0.92 (-0.94-2.78)
Root	1.89	1.72 (1.43-2.01)
Solution	28.91	29.31 (23.86-34.76)
75ppm[d] Leaf	16.27	21.32 (16.28-26.36)
Stem	1.55	1.48 (1.19-1.77)
Root	2.83	2.18 (1.13-3.23)
Solution	43.37	42.72 (35.82-49.62)
100ppm[e] Leaf	21.61	27.88 (21.60-34.16)
Stem	2.05	1.92 (1.51-2.33)
Root	3.76	2.76 (1.85-3.67)
Solution	57.59	56.66 (52.36-60.96)

a Measured values from validation study and 95% confidence intervals, n = 3
b Actual initial ClO_4^- concentration was 10.36 ppm (8.81 mg)
c Actual initial ClO_4^- concentration was 50.21 ppm (42.68 mg)
d Actual initial ClO_4^- concentration was 75.32 ppm (64.02 mg)
e Actual initial ClO_4^- concentration was 100.02 ppm (85.02 mg)

The PK model can be applied to other dicot vascular plants that are tolerant of perchlorate by measuring plant fresh weights and nutrient solution utilization rates throughout the period of perchlorate accumulation. The PK model can also be applied to similar nonvolatile, nondegradable inorganic chemicals for prediction of accumulation. For predicting root and stem translocation, calibration parameters should be estimated from plant tissue kinetic data. The PK model could then be used as a screening tool for phytoremediation or environmental risk applications. More specifically, the PK model can aid in field trial design for testing the ability of tobacco to phytoremediate perchlorate.

In conclusion, a simple five compartment mathematical model was developed to describe the fate of perchlorate in tobacco plants under non-steady state plant growth conditions. The PK model adequately predicts perchlorate uptake by roots from the nutrient solution, translocation into the xylem by diffusion across the root endodermis cell membranes, transfer between the stem xylem and stem storage, and accumulation in plant leaves.

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CHAPTER 4

CONCLUSIONS

The phytoremediation study showed that perchlorate was taken up by the root system, traveled up the stem via the xylem, and probably accumulated in the vacuoles of the various cell types in the leaves. Figures 2.4 and 2.5 clearly show the behavior of perchlorate in the plant for the 25 ppm and 75 ppm perchlorate amended plants. After the 13-day hydroponics growth study in the perchlorate-amended nutrient solution, 95.4% of the total perchlorate applied was taken up by the 25 ppm perchlorate amended plants, and 92.7% of the total perchlorate applied was in the 75 ppm perchlorate amended plants. Most of the remaining perchlorate was accounted for in the amended nutrient solution. The highest perchlorate concentration in the plants was found on day 11 in the leaf, where 0.37% of the dry matter was perchlorate (Table 2.2). This suggests that tobacco plants are very effective plants for the phytoremediation of perchlorate from water. Perchlorate recoveries ranged from 97.4% to 107.6% in the 25ppm perchlorate amended plants and nutrient solutions, and from 92.0% to 99.8% in the 75ppm plants and nutrient solutions. These high recoveries suggest that the perchlorate is being stored in the plant and that the degradation of perchlorate is negligible, if degradation occurs. The lack of degradation can be attributed to the energy required to degrade the perchlorate anion and/or the lack of the proper degradation enzyme(s) in plant tissues. Assuming there is no degradation of perchlorate, the recovery errors may be the result of methodology.

Based on these results, the tobacco plant appears to be a good candidate for the phytoremediation of perchlorate. The tobacco roots are fibrous with large surface areas for the uptake of perchlorate, and the storage capacity (0.37%) of perchlorate in the leaves is substantial. However, since the accumulated perchlorate is not degraded in the tobacco plant, the plants would have to be removed after remediation of the perchlorate.

Following the growth study, a five compartment mathematical model was developed to describe the fate of perchlorate in tobacco plants. The model adequately predicts perchlorate uptake by roots from the nutrient solution, translocation up the xylem by mass flow with the transpiration stream, movement between the stem xylem and stem storage, and accumulation in plant leaves (Figures 3.4-3.7). The separate 2-day phytoremediation study was used to validate the PK model. Measured and predicted amounts of perchlorate are given in Table 3.3. Across the four concentrations, 10, 50, 75, and 100 ppm perchlorate, all but two of the predicted values were within the calculated 95% confidence intervals of the measured values. In the 75 ppm perchlorate amended plants, perchlorate content in the leaf was under-predicted, and in the 100 ppm perchlorate amended plants, perchlorate content in the root was over-predicted. These results suggest that the PK model is most reliable for perchlorate concentrations less than 75 ppm.

Since no tobacco plant specific parameters were used in the formulation of the model, the model can be applied to other dicot vascular plants that are tolerant of perchlorate by substituting appropriate plant wet weight growth curves and expected solution uptake curves. The model can also be applied to similar nonvolatile,

nondegradable inorganic chemicals by entering in the appropriate tissue partition coefficients specific to the chemical.

The results of this research suggest that further work is warranted in this area. The PK model developed can be used by scientists to aid in the development of soil-grown tobacco greenhouse studies as well as field studies to further study the potential for these plants to phytoremediate perchlorate from perchlorate contaminated soils. In addition, other plants commonly grown in potential perchlorate contaminated areas should be studied to determine their tolerance of perchlorate and to assess the toxicity of perchlorate across a variety of plant species. Aquatic species may be useful to phytoremediate perchlorate from perchlorate-contaminated waters, such as Lake Mead and the Colorado River. Finally, the ability of perchlorate to be taken up into food crops irrigated with perchlorate contaminated irrigation waters should be assessed to determine the potential of perchlorate to be found in the fruit and vegetable section of local grocery store chains.

APPENDIX

ACSL Model Code (.csl file)

PROGRAM: Perchlorate & Tobacco Uptake Model (PTUM)

'Created 09/19/02 by Sarah Sundberg & Deborah Keys'

'Describes Uptake of Perchlorate in Roots, Stems, Leaf of Tobacco Plant'

INITIAL	$'Start of initial'
ALGORITHM IALG=2	
CINTERVAL CINT=.25	
CONSTANT TSTOP=14	
'Solution Parameters'	
CONSTANT SOLCON=25.16	$'Initial concentration of ClO_4 in solution mg/L'
SAMT=SOLCON*VS	$'Initial amount of ClO_4 in solution mg'
CONSTANT VS=0.8	$'Volume of solution L'
'Plant Parameters'	
CONSTANT DF=0.403	$'Diffusion factor'
CONSTANT TST=16.327	$'Transfer from stem to stem storage'
CONSTANT TSTS=0.314	$'Transfer from stem storage to stem'
CONSTANT PSTS=0.93	$'Proportion of stem storage weight'
PST=1-PSTS	$'Proportion of stem weight'

```
'Data for average rate of uptake of water L/day for days where measured'
TABLE RS, 1, 7/1,3,5,7,9,11,13 &
,0.1655,0.2155,0.209375,0.31575,0.2705,0.212,0.40875/
'Data for root weight (kg) where measured'
TABLE VR, 1, 7/1,3,5,7,9,11,13 &
,0.0201435,0.0226625,0.02601825,0.02551325,0.0262915,0.0251545,0.0306005/
'Data for stem weight (kg) where measured'
TABLE VSTTOTAL, 1, 7/1,3,5,7,9,11,13 &
,0.017144,0.02115925,0.02649,0.037043,0.04012475,0.0453715,0.0558995/
'Data for leaf weight (kg) where measured'
TABLE VL, 1, 7/1,3,5,7,9,11,13 &
,0.05937125,0.07006025,0.07791475,0.0909195,0.1052875,0.11062525,0.122364/
END     $'End of initial'

DYNAMIC
DERIVATIVE TOB
TERMT(T.GE.TSTOP)                $'Condition for termination of run'
RScalc = RS(T)                   $'Calculates rate of solution uptake as function of time'
VRcalc = VR(T)                   $'Calculates weight of root as function of time'
VSTTOTALcalc = VSTTOTAL(T)       $'Calculates weight of stem as function of time'
VLcalc = VL(T)                   $'Calculates weight of leaf as function of time'
VST = VSTTOTAL(T)*PST            $'Stem weight (kg)'
VSTS = VSTTOTAL(T)*PSTS          $'Stem storage weight (kg)'
```

'Perchlorate in solution'

RAS = -RS(T)*CS $'Rate of change of ClO_4 in solution (mg/day)'

AS = INTEG(RAS,SAMT) $'Amount of ClO_4 in solution (mg)'

CS = AS/VS $'Concentration of ClO_4 in solution (mg/L)'

'Perchlorate in roots'

RAR = (RS(T)*CS)-(RS(T)*DF*CR) $'Rate of change of ClO_4 in roots (mg/day)'

AR = INTEG(RAR,0) $'Amount of ClO_4 in root (mg)'

CR = AR/VR(T) $'Concentration of ClO_4 in root (mg/kg)'

'Perchlorate in stem'

RAST = (RS(T)*DF*CR)-(TST*AST)+(TSTS*ASTS)-(RS(T)*CST)

$'Rate of change of ClO_4 in stem (mg/day)'

AST = INTEG(RAST,0) $'Amount of ClO_4 in stem (mg)'

CST = AST/VST $'Concentration of ClO_4 in stem (mg/kg)'

'Perchlorate in stem storage'

RASTS = TST*AST-TSTS*ASTS $'Rate of change in stem storage (mg/day)'

ASTS = INTEG(RASTS,0) $'Amount of ClO_4 in stem storage (mg)'

CSTS = ASTS/VSTS $'Concentration of ClO_4 in stem storage (mg/kg)'

ASTTOTAL = AST+ASTS $'Total amount of ClO_4 in stem (mg)'

CSTTOTAL = ASTTOTAL/VSTTOTAL(T) $'Total concentration in stem (mg/kg)'

'Perchlorate in leaf'

RAL = RS(T)*CST $'Rate of change of ClO_4 in leaf (mg/day)'

AL = INTEG(RAL,0) $'Amount of ClO_4 in leaf (mg)'

CL = AL/VL(T) $'Concentration of ClO_4 in leaf (mg/kg)'

```
END                                  $'End of derivative ONE'


DYNAMIC
'Perchlorate Mass Balance'
TMASS=AS+AR+ASTTOTAL+AL $'Total amount (mg)'
END                                  $'End of dynamic'
TERMINAL
END                                  $'End of terminal'
END                                  $'End of program'
```

ACSL Model Command (.cmd)

data ppm25s & !(25ppm, mg ClO_4)

(t,as)

1 16.96

1 16.44

3 10.09

3 9.44

5 3.95

5 5.86

7 2.89

7 1.67

9 1.18

9 1.04

11 0.76

11 0.96

13 0.00

13 0.75

END

data ppm25r & !(25ppm, mg ClO_4)

(t,ar)

1 0.95

1 1.29

3 0.75

3 0.78

5 0.67

5 0.73

7 0.35

7 0.32

9 0.30

9 0.20

11 0.18

11 0.23

13 0.27

13 0.18

END

data ppm25st & !(25ppm, mg ClO_4)

(t,asttotal)

1 0.43

1 0.49

3 0.73

3 0.79

5 0.66

5 0.64

7 0.64

7 0.81

9 0.62

9 0.67

11 0.60

11 0.58

13 0.73

13 0.60

END

data ppm25l & !(25ppm, mg ClO_4)

(t,al)

1 2.87

1 3.27

3 11.07

3 9.68

5 14.19

5 12.95

7 15.65

7 16.96

9 18.41

9 18.11

11 18.62

11 14.07

13 19.32

13 17.22

END

data ppm75s & !(75ppm, mg ClO_4)

(t,as)

1 46.58

1 49.08

3 30.56

3 32.07

5 16.56

5 16.20

7 12.52

7 17.42

9 4.62

9 4.37

11 3.00

11 4.39

13 1.50

13 3.46

END

data ppm75r & !(75ppm, mg ClO_4)

(t,ar)

1 2.87

1 2.20

3 2.28

3 2.55

5 1.81

5 1.05

7 1.01

7 0.97

9 0.74

9 0.59

11 0.57

11 0.74

13 0.29

13 0.13

END

data ppm75st & !(75ppm, mg ClO4)

(t,asttotal)

1 1.33

1 1.52

3 1.95

3 1.74

5 2.11

5 0.55

7 1.88

7 1.80

9 1.74

9 1.12

11 1.31

11 1.58

13 1.12

13 1.30

END

data ppm75l & !(75ppm, mg ClO_4)

(t,al)

1 8.40

1 8.05

3 24.27

3 23.53

5 36.74

5 38.97

7 41.56

7 40.06

9 47.48

9 47.15

11 54.33

11 51.64

13 56.33

13 52.44

END