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Determination of Residual Hexazinone in Maine's Soil and Water

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DETERMINATION OF RESIDUAL HEXAZINONE

IN MAINE'S SOIL AND WATER

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An Abstract of the Thesis Presented
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Hexazinone, a systemic herbicide registered for use on wild blueberries in 1983 is credited with increasing Maine's wild blueberry crop by three-fold over a 10 year period, while also increasing overall fruit quality. Unfortunately, the high water solubility of hexazinone gives it a high leaching potential. This solubility factor is exacerbated by the sandy soils where wild blueberries are commonly propagated.

In 1991 a routine screen for pesticides used in blueberry agriculture revealed traces of hexazinone in water samples from property formerly used for blueberry production. This discovery has led to the development of solid phase extraction (SPE) and direct-injection high performance liquid chromatographic (HPLC) methods capable of detecting hexazinone in ground water at limits of quantitation (LOQ) of 0.1 and 0.33 μg/L, respectively. These techniques were proven rapid, accurate and inexpensive.

The HPLC method was used to monitor seven test wells in and near actively managed blueberry agricultural areas. Over a ten-year period, five of these sites showed decreasing hexazinone levels, while two of the wells exhibited large fluctuations in herbicide concentration. The decreased leaching of hexazinone at some sites was

attributed to lower application rates, better management techniques and the development of slow-release formulations, such as impregnated diammonium (DAP) and granulated Pronone.

In 1994, 1998 and 1999 private wells in seven Maine counties, determined to have high potential of hexazinone contamination from blueberry cultivation practices were randomly sampled for hexazinone analysis. Most wells were sampled in the spring, fall and in two separate years. Approximately 61% of the total samples tested positive for the herbicide at levels ranging for 0.1 to 6 μg/L. Levels of hexazinone generally fluctuated little between spring and fall. Concentrations were the same (27%) or lower (66%) in 1998 and 1999 as compared to initial values determined in 1994.

HPLC and Enzyme immuno assay EIA methods were developed to measure the hexazinone content of soil. LOQ's for these techniques were 25 and 50 ng/g for HPLC and EIA, respectively. These methods were used to ascertain the effect of hexazinone formulation type on leaching potential through the soil profile. Granulated Pronone was the most highly retained by soil.

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LIST OF ABBREVIATIONS

ASE	Accelerated solvent extraction
A_p	Plow layer
CE	Capillary electrophoresis
DAP	Diammonium phosphate
EA	Ethyl acetate
EIA	Enzyme immuno assay
ECD	Electron capture detector
ELISA	Enzyme linked immuno sorbent assay
Ft	Feet
GC	Gas Chromatography
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
IC_{50}	Concentration that causes 50% EIA inhibition
K′	Reverse phase HPLC capacity factor
K_{oc}	Sorption Coefficient
Kow	Octanol/water partition coefficient
LH	Litter (organic debris) soil horizon
LLD	Lowest limit of detection
LOD	Limit of detection
LOQ	Limit of quantitation
MDL	Minimum limit of detection
MECl	Methylene chloride or dichloromethane
MEOH	Methanol
mg/L	Milligrams per liter
MSD	Mass spectral detector
NPD	Nitrogen-phosphorous detector
OM	Organic matter
PDA	Photo diode array detector
PPB	Parts per billion
PPM	Parts per million
Pronone 10G	Hexazinone in granular formulation
SIM	Single ion monitoring
SPE	Solid phase extraction
$T_{1/2}$	Half-life
UV	Ultra Violet
µg/L	Micrograms per liter
Velpar L	Hexazinone in liquid formulation
WSSA	Weed Science Society of America

INTRODUCTION

Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4 (1H,3H)-dione] is a pre-emergence, systemic herbicide used primarily for weed control in wild blueberry, forestry, Christmas trees, sugarcane, pineapple, pastures, range land and a number of right-of-ways. It is also registered for use in palm oil, rubber and tea production in a number of foreign countries. Hexazinone is marketed under the trade names Pronone and Velpar and is available in liquid, wetable powder and pelletized formulations. In the late 1970's workers spraying railroad right-of-ways noted that wild blueberries were unaffected by hexazinone treatment. This discovery led to the 1983 registration of the herbicide for use on wild blueberries. The effect of Velpar on Maine's blueberry crop was almost immediate. Along with increased irrigation and the use of honeybees for pollination, hexazinone is credited with expanding wild blueberry production in Maine by threefold, and simultaneously improving fruit quality (Yarborough & Bhowmik, 1989). Thanks in part to hexazinone, Maine now produces 22% of the North American blueberry crop (Holbein, 1995).

In 1991, a routine laboratory screen for pesticide residues showed traces of hexazinone in both surface and groundwater on property formerly used for blueberry production (unpublished data). Subsequent work, performed for the Maine Salmon Commission, found levels ranging to 4 μg/L in several of Maine's eastern watersheds (Evers, 1993). Publicity of these findings, the discovery of traces of the herbicide in dozens of private wells, and public wells in the towns of Gouldsboro (Clancy, 1991) and Franklin (Graettinger, 1994; Bradbury, 1994) have caused a number of concerns by the populations residing near areas used for blueberry production. These worries have led to

the sampling and analysis of hundreds of ground and surface waters as well dozens of soils in Maine over the past decade to study Velpar content, metabolism and movement.

This anxiety by the general public, combined with an overall misunderstanding of the toxicity issues, has led to hexazinone work, which was recently published by a University of Maine graduate student. Najwer-Coyle (1998) weighed the perceived social and economic costs associated with Velpar use, with its agricultural economic benefits. Conceding that at outright ban of the herbicide is unlikely, the author concludes by suggesting several economic incentives aimed at reducing the use of hexazinone in blueberry agriculture.

This thesis will explore the chemical properties, metabolism and toxicity, as well as the fate and transport of hexazinone in the environment, as discussed in the literature. Also discussed are the development of new methods of analysis for the herbicide, data from eight years of groundwater monitoring programs and a study of hexazinone movement through a typical soil profile used for wild blueberry production.

LITERATURE REVIEW

Chemistry

Hexazinone (CAS # 51235-04-02) is a systemic, non-selective herbicide belonging to the triazine family of agrochemicals (figure 1). It works by binding a protein of the photosystem II complex, which in turn blocks the photosynthetic electron-transport chain. This results in a chain reaction of triplet-state chlorophyll reacting with oxygen (O_2) to form singlet oxygen (O). Chlorophyll and O strip hydrogen (H^+) from unsaturated lipids in both the cell and the organelle membranes, to produce free radicals. These lipid radicals attack and oxidize other lipids and proteins, causing the cell and organelle membrane to leak. The leakage of the cellular contents leads to cell death and eventually, the death of the plant. Velpar has a molecular weight of 252.32, a melting point of 115 – 117° C, a vapor pressure of 0.03 Pa at 25° C, and decomposes upon boiling (Royal Society of Chemistry, 1987). The moderately polar structure of hexazinone (fig.1) makes it relatively soluble in water (33,000 mg/l at 25° C).

Toxicity

Hexazinone exhibits low toxicity to birds and mammals. The LD_{50} for oral ingestion is 1690, 860 and 2,258 mg/kg for rats, male guinea pigs and bobwhite quail, respectively (USDA, 1994). Chronic effects are also low. The offspring of female rats fed diets of 150 mg/kg were normal over 2 generations (USDA, 1994). The same publication reported that the Ames test and other assays on living animals showed no changes in chromosomal structure. The USDA publication also noted no carcinogenic effects on rats, mice and dogs fed up to 500 mg/kg during a 1 – 2 year study.

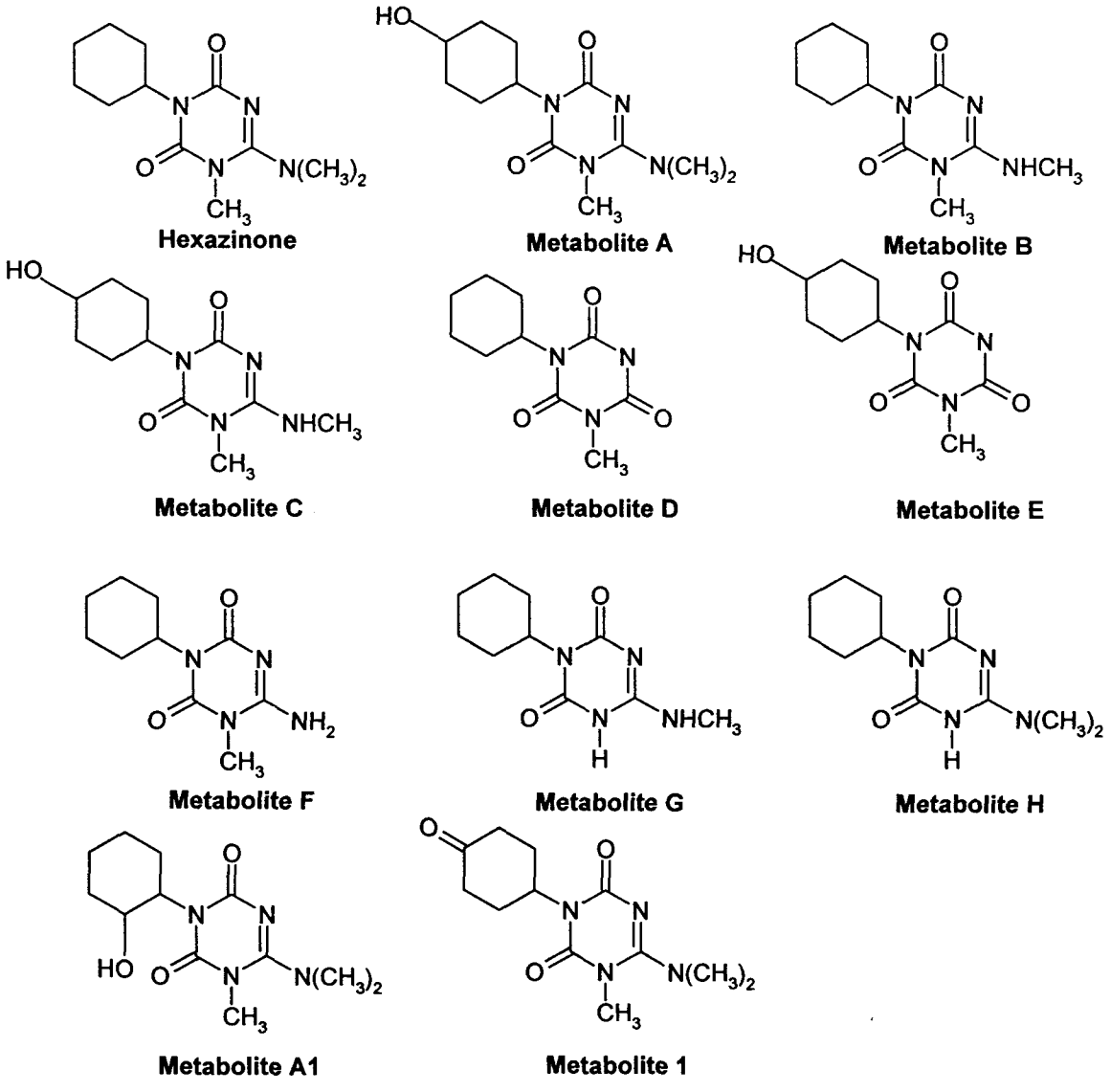

Figure 1. Structures of Hexazinone and its Metabolites.

Hexazinone is quickly excreted by animal systems. Dairy cows and lactating goats given small doses of hexazinone over 30 days, showed no residues of the parent compound in any tissues and had only minute traces of metabolites in their milk (FDA, 1986). There is little chance that the herbicide bioaccumulates in the tissues of any mammal, including humans.

Because blueberry production is most intensive in coastal sections of Downeast Maine, there is great concern over the agrochemical contamination of sensitive watersheds in this region. There is concerted effort by the Federal government to restore populations of the endangered Atlantic salmon to several rivers in the area. Traces of hexazinone found in these streams and rivers have led to re-visitation of the literature in order to ascertain any detrimental effects to native salmon.

There is little reported evidence of the direct toxicity of Velpar to fish. Studies by Rhodes (1980b) and by Mayack et al. (1982) showed no mortality or other effects on bluegill sunfish when they were exposed to levels of up to 1 mg/l of hexazinone for 4 weeks. EXTOXNET (1996) lists the LC_{50} for rainbow trout and bluegill sunfish at 320 and 370 mg/l, respectively. The herbicide was found to be slightly toxic to Pacific salmonids, with an LC_{50} ranging from 236-317 mg/l for chinook, sockeye, chum, rainbow, coho and pink salmon (Wan et al. 1988). Similar work by Kennedy, Jr. (1984) resulted in about 30% less toxicity to similar juvenile populations of salmon.

The toxicological effect of hexazinone on aquatic environment could ultimately disrupt the food chain for salmon populations. Several studies have been conducted to identify negative impacts that the compound might have on other plants or animals found in lake, stream and river habitats. Examination of lakes in boreal forests of Ontario, Canada

revealed a depression of phytoplankton at hexazinone concentrations as low as 0.01 mg/l. These workers also noted that chronic exposure to levels of 0.1 mg/l caused irreversible damage to the plankton (Thompson et al., 1993a). A more extensive study in the same geographical region noted similar declines in zooplankton numbers and concluded that the population change was a result of food resources lost with the suppression of phytoplankton (Thompson et al., 1993b).

Velpar has been shown to have no effect on aquatic insects. Work by Kreutzweiser et al. (1992) and by Schneider et al. (1995) in artificial stream channels to which hexazinone was added, resulted in no adverse impact on insect populations. Earlier studies by Mayack et al. (1992) concluded with similar findings.

The impact of Velpar on periphyton communities may be more serious. Peterson et al. (1997) found a decline in green algae and diatoms exposed to low levels of hexazinone. These researchers speculated that because the herbicide had little effect on cyanobacteria, the organisms could multiply in the absence of competition, and change the aquatic environment. Such changes, the researchers surmised, could lead to contamination of drinking water by algal toxins. Other research supports this theory. Schneider et al. (1995) noted that chronic exposure to hexazinone could have a significant impact on the productivity and recovery of algae populations. Work by Lavy et al. (1989) however, suggests that chronic exposure levels of the herbicide are well below the 0.01 – 0.6 mg/l concentrations required for such detrimental effects.

Fate and Transport

Following the movement and degradation of pesticides after application to agricultural environments is a relatively new field of science, an area that has been given serious

thought only for the past two decades. Commonly described as the study of Fate and Transport, scientists now routinely follow pesticide movement and metabolism in the environment in order to minimize the negative effects on non-target organisms.

Figure 2 depicts a flow diagram for the major routes of travel for pesticides applied on croplands. These processes can be quite complex and are dependent on chemical properties as well as environmental conditions and management practices. Agrochemicals can be adsorbed in the plant canopy either by direct contact with the foliage or by transport through root systems. Some of the applied material can be vaporized into the atmosphere, depending on vapor pressure, wind conditions and spray droplet size. Photolysis may occur if the formulation remains on the surface and is not incorporated into the soil. Pesticides can move laterally with water flow across soil surface, vertically, through the root and vadose zones, or by interflow mechanism, a combination of lateral and vertical flows. Transport of these chemicals across soil surfaces may occur as a solute or bound to a soil particle. Depending on soil type and chemical properties of the compound, much of the pesticide may be bound to the soil in the root zone, where it may be available to attack a target organism or be permanently bound. In this zone, the agrochemical may also be metabolized to more or less toxic compounds via chemical or microbial oxidation. The parent and/or metabolites may also move into the ground water or saturated zone. Table 1 lists the major chemical properties and ecological conditions that affect the movement and degradation of pesticides in the environment. The potential for a pesticide to leach into the ground water is controlled largely by solubility and persistence of the analyte. These two parameters are by and

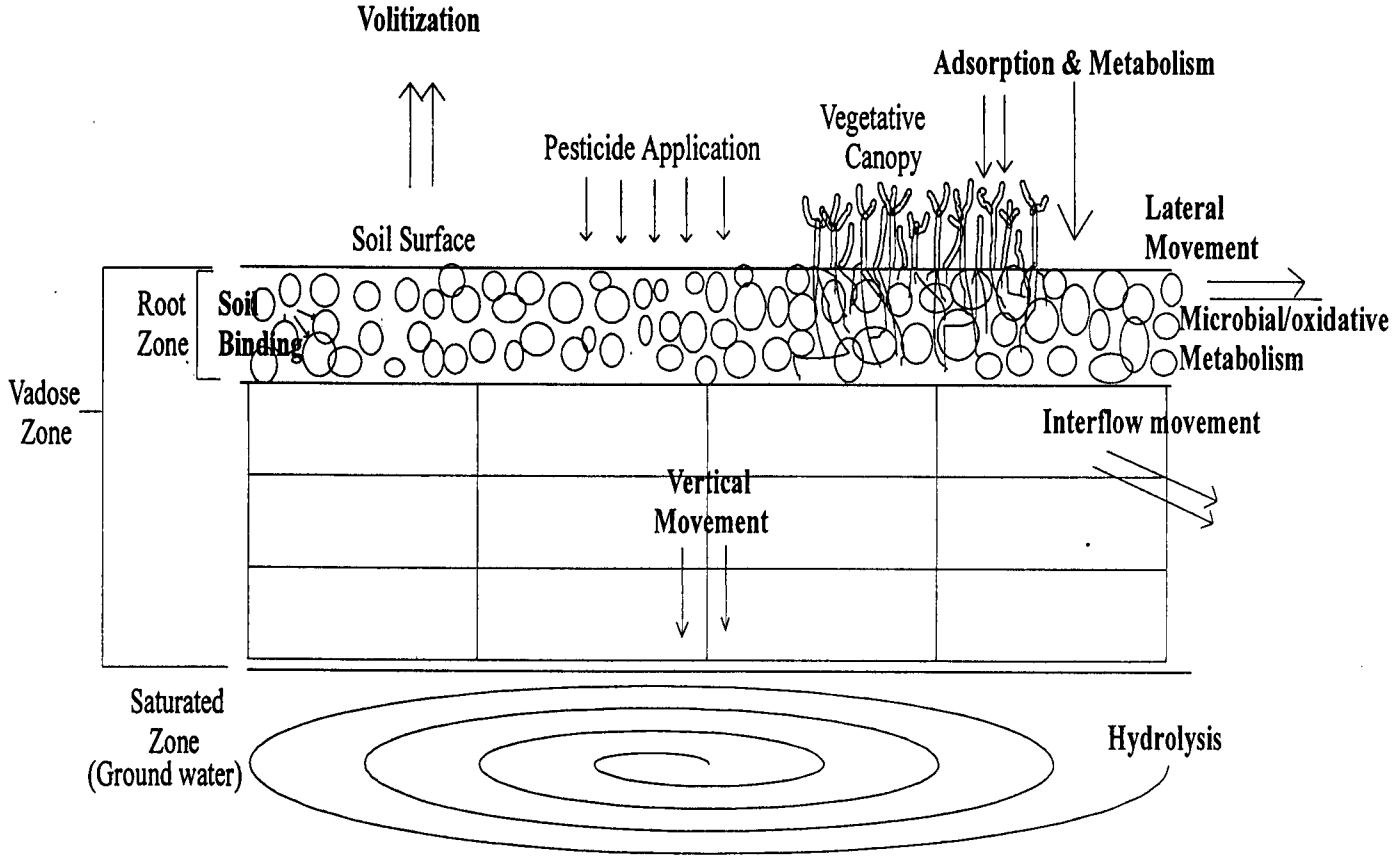

Figure 2. Fate and Transport of Pesticides in the Environment

large, attributes of the chemical properties of the compound. Environmental conditions where the pesticide is utilized vary to a great degree, making the fate of the substance less predictable.

Persistence

Velpar is metabolized into a number of different compounds in the environment, including the metabolites 1, A, A1, B, C, D, E, F, G, H (figure 1). Mechanisms for this degradation, including plant, animal, photolysis, chemical hydrolysis, and microbiological have been the focus of several studies.

Table 1. Effect of Chemical Properties and Environmental Conditions on the Fate and Transport of Pesticides (modified from Probasco and Maughan, 1999)

Chemical Properties	**Environmental Conditions**
Melting point	Ambient temperature range
Boiling point	Vegetative canopy
Density	Rainfall
Vapor Pressure	amount
Dissociation constants	timing
Diffusion coefficients	Soil
Water solubility	texture (% sand, silt & clay)
Partition coefficients	structure (aggregation)
	organic matter (type and content)
	pH
	Exposure to sunlight (photolysis)

Rhodes and Jewell (1980) found that hexazinone-fed rats excreted metabolites A, C, D, and E in both feces and urine. A and C were the prevalent compounds, with very little parent compound remaining. A similar study by Rhodes (1980a) found that bluegill

sunfish exposed to 0.01 – 1.0 mg/l (ppm) in water, resulted in accumulation of ^{14}C labeled parent compound in both liver and flesh, with traces of metabolite A.

Rhodes (1980a) found no chemical hydrolysis of hexazinone in water after 8 weeks at pH ranging from 5 –9 and temperatures of 15, 25 and 37 °C. He found photodegradation was a minimal 10% after 5 weeks of exposure to artificial sunlight. As part of the same study, Rhodes did find that the addition of a photoinitiator (anthaquinone) to distilled water, increased the rate of degradation by three to seven times. The major metabolites, B, H and A, were produced via demethylation.

Hexazinone is absorbed through the root system and the foliage of plants. In non-susceptible species the herbicide is metabolized to less toxic compounds, such as A, D and E. Target plants lack the detoxifying mechanisms and retain the parent compound and the phytotoxic metabolite B (Sidhu and Feng, 1993, Michael et al., 1999).

The chief pathway for Velpar metabolism is microbial and occurs almost exclusively in the soil environment, under aerobic conditions (Rhodes, 1980a, Jensen and Kimball, 1987). Rhodes (1980a) found no hexazinone degradation in soils kept under anaerobic conditions for 60 days, while soils maintained in an aerobic environment lost 45-75 % of the parent compound. Ahrens (1994) lists a $T_{1/2}$ of 90 days for the herbicide, while the DuPont fact sheet (1999) gives a value of 175 days. It can be surmised that the preferred degradation pathway in soils depends on the environmental conditions (temperature, light, moisture, pH) and the predominant micro flora (Van Es, 1990). Test plots in Mississippi, Delaware and Illinois treated with hexazinone each yielded C as the predominant metabolite, with significant levels of A, B and G also reported at each site (Rhodes, 1980b). Rhodes noted that the degradative pathways involved both

demethylation and hydroxylation of the # 4 position on the cyclohexyl ring. Workers in the colder climate of Nova Scotia, found compound B to be the major metabolite in soil (Jensen and Kimball, 1987). The same researchers showed metabolite D was the most abundant product in soils studies in the warm, moist greenhouse environment. Additional studies, which focused on the movement of hexazinone through the soil profile found the presence of metabolites A and B, but did not screen for other metabolites (Neary et al, 1983; Roy et al, 1989).

Solubility

The greater the water solubility of a contaminant, the larger the potential it has to leach into ground water systems. Pesticides with solubilies above 30 mg/l are considered to have high leaching potential if corresponding soil sorption and degradation rates are low (van Es, 1990). A solubility factor of 33,000 mg/l and a relatively long half-life of up to 175 days put Velpar into the category of potential leachers. Table 2 compares the water solubility and $T_{1/2}$ of hexazinone with some other widely used herbicides.

Soil Sorption

A pesticide's potential for adsorption to the soil is defined by its adsorption coefficient (K_{oc}). This coefficient is expressed as:

$$K_{oc} = \frac{\text{concentration adsorbed / concentration dissolved}}{\text{\% organic carbon in soil}}$$

Agrochemicals with low K_{oc} values (<500) have a greater tendency to remain in solution, rather than adsorb to soil particles (van Es, 1990). Hexazinone, with a K_{oc} of 40, is a likely candidate for leaching quickly through the soil profile (table 2).

Table 2. Half-life, Solubility and Sorption Coefficients for Some Commonly Used Herbicides

Herbicide	$T_{1/2}$ (days)	Solubility (mg/L)	K_{oc} (m^3/kg)
Alachlor	200	242	30
Atrazine	160	33	71
Cyanazine	183	171	15
2,4-D	8	620	20
Diuron	98	42	480
Glyphosate	1	12,000	52
Hexazinone	**90-175**	**33,000**	**40**
Imazapyr	510	15,000	100
Sulfometuron	30	10	171
Trichlopyr	55	440	35

Assignment of a K_{oc} value to a pesticide is made with the assumption that pesticide sorption by soils is due entirely to the organic matter (OM) fraction of the soil. This over-simplification is designed to overlook the many variables of soil systems, in order to compare sorption potentials between pesticides, themselves. Likewise, sorption potentials do not take into account the many forms that the OM component may take, including plant debris, lignin, cellulose, hemicellulose, and countless structures of humic acid.

These OM concentrations are almost always present (at significant levels) only in the top six inches of the soil profile. When located on undisturbed soils (i.e., forest soils), OM is usually referred to as the LH horizon, because much of the material is present as leaf and twig litter. Soils that have had mechanical manipulation (plowing or cultivation) usually have an A_p horizon, known as the plow layer. This zone is a mixture of mineral and organic material.

The K_{oc} for a pesticide is an estimate and can be calculated using a number of different methods including molecular properties (water solubility, Kow, k′), topocological indices and linear solvation energy relationships (Gramatica, et al, 2000). Dontati et al. (1994) used k′ (RP-HPLC) and soil sorption isotherm models to determine the K_{oc} for hexazinone and four other triazine and triazine metabolites. Their work determined a K_{oc} of 55 (+/-14) and 98 (+/-102) for the k′ and isotherm models, respectively. Obviously, there is a great deal of inherent variability in the process of determining K_{oc} values. Nonetheless, the K_{oc} of a non-ionic pesticide remains a good general predictor of leaching potential in the soil environment.

It is well known that most non-ionic pesticides bind more strongly to the organic fraction than to the sand, silt and clay components of the soil horizon (table 3). A study of the polarographic reduction and adsorption on lignin by Privman et al. (1994) indicated a poor binding potential of hexazinone to the soil organic fraction, in addition to rapid de-sorption. The researchers noted however, that like many other herbicides, at least 40% of the hexazinone is irreversibly bound and is biologically unavailable. Because hexazinone is poorly retained by the mineral soil fragments, several studies have been conducted that focus on the OM binding potential. Working with undisturbed forest soils in western Canada, Feng et al. (1992) found that hexazinone metabolized or leached from the soil surface within one year of application. They did note however, that the majority of the parent compound and its metabolites were found in the LH zone (top six inches) as compared to the A, B and C horizons. The LH zone was determined to contain 11 – 50% OM. Felding (1992) established that the herbicide moved quickly through the

A_p horizon which contained < 2% OM. This research corroborated similar findings by Zandvoort (1989).

Table 3. Binding Potential of Non-Ionic Pesticides to Soil Components

Soil Fraction	Pesticide Binding Potential
Organic Matter (OM)	Very High
Clay	Medium – High (depending on clay type)
Silt	Low - Medium
Sand	Very Low

Soil Structure

In soil systems, it can be assumed that solutes move through the soil profile at a rate no greater than the solvent front, which in most cases is water. The velocity of water flow varies greatly and is dependent on the soil particle size and shape, as well as the aggregate structures of the soil horizons. For example, water moves relatively quickly through sandy soils, because the relatively large particle size of sand results in bigger spaces between particles. Conversely, soils containing large amounts of clay, retard water flow, due to the very small spaces between clay particles.

The percentages, types and sizes of sand, silt, clay and OM also play a large role in determining soil structure. Soil that crumbles easily when handled is labeled as friable, where as soils that are sticky or very easily molded in the hands are known as non-friable or poorly structured. Friable, or well-structured soil systems have a much greater propensity for water movement than do poorly structured soils, such as clayey tills. The

compact nature of tills can actually make them as impenetrable to water as solid rock. An example of just how dramatic an impact soil particle size and structure have on ground water movement, is illustrated in table 4.

Most of the hexazinone use in Maine occurs in the eastern coastal sections where dozens of indigenous blueberry clones thrive in harsh growing conditions (figure 3). The soil textures in this region consist largely of gravelly sandy loam (Yarborough and Jenkins, 1993), which can promote rapid percolation of water through their profiles. In some areas, the ground water is relatively shallow and resurfaces in close proximity to blueberry fields. The combination of rapid water movement and low soil OM, as well as the low K_{oc} and high solubility of hexazinone, make the herbicide a prime candidate for ground water contamination.

Table 4. Variability in Estimated Permeability of Typical Geological Materials (Illinois State Geological Survey, 1990)

Geological Material	Flow Rate
Clean sand and gravel	100 ft/year
Fine sand and silty sand	100 ft/year – 1 ft/year
Silt	10 ft/year – 1 ft/10years
Gravelly till	1 ft/year – 1 ft/100years
Clayey tills (>25% clay)	1 ft/100years – 1 ft/10,000years
Sandstone	10 ft/year
Fractured rock	10 ft/year
Shale	1ft/100years – 1 ft/1,000,000years
Dense unfractured limestone	1ft/1000years – 1ft/1,000,000years

Stone et al. (1993) created similar "worst-case" conditions in a blueberry field located in eastern Canada. In a study that incorporated a sandy soil with low pH and OM, the

workers found that leachate collected as deep as 150 cm reached a maximum concentration of hexazinone at 80 days (table 5). The researchers also observed that the mulch placed on the soil surface retarded leaching of the herbicide (table 6). Additionally, they noted that OM type and soil pH had little effect on vertical movement of Velpar. They surmised that the OM fraction acted as a "sink", slowly releasing the hexazinone to the lower horizons during precipitation events. In a similar experiment performed on an acidic sandy loam in Downeast Maine, Yarborough and Jenkins (1993) concluded that the mulching layer had no effect on the vertical movement of hexazinone.

Table 5. Concentration of Herbicides in Soil Water 80 –130 days – Post Treatment (modified from Stone et al., 1993)

Soil Depth (cm)	Sulfometuron (µg/L)	Tebuthiuron (µg/L)	Hexazinone (µg/L)
10	0.5	42.7	113.1
20	0.4	44.0	126.3
40	0.0	24.1	52.8
150	0.0	9.0	62.0

Table 6. Effect of Litter Type on Herbicide Movement (modified from Stone et al., 1993)

Humus	Tebuthiuron (µg/L)	Hexazinone (µg/L)
Control (no humus)	12.6	77.8
Pine	4.1	29.8
Hardwood	0.6	29.1

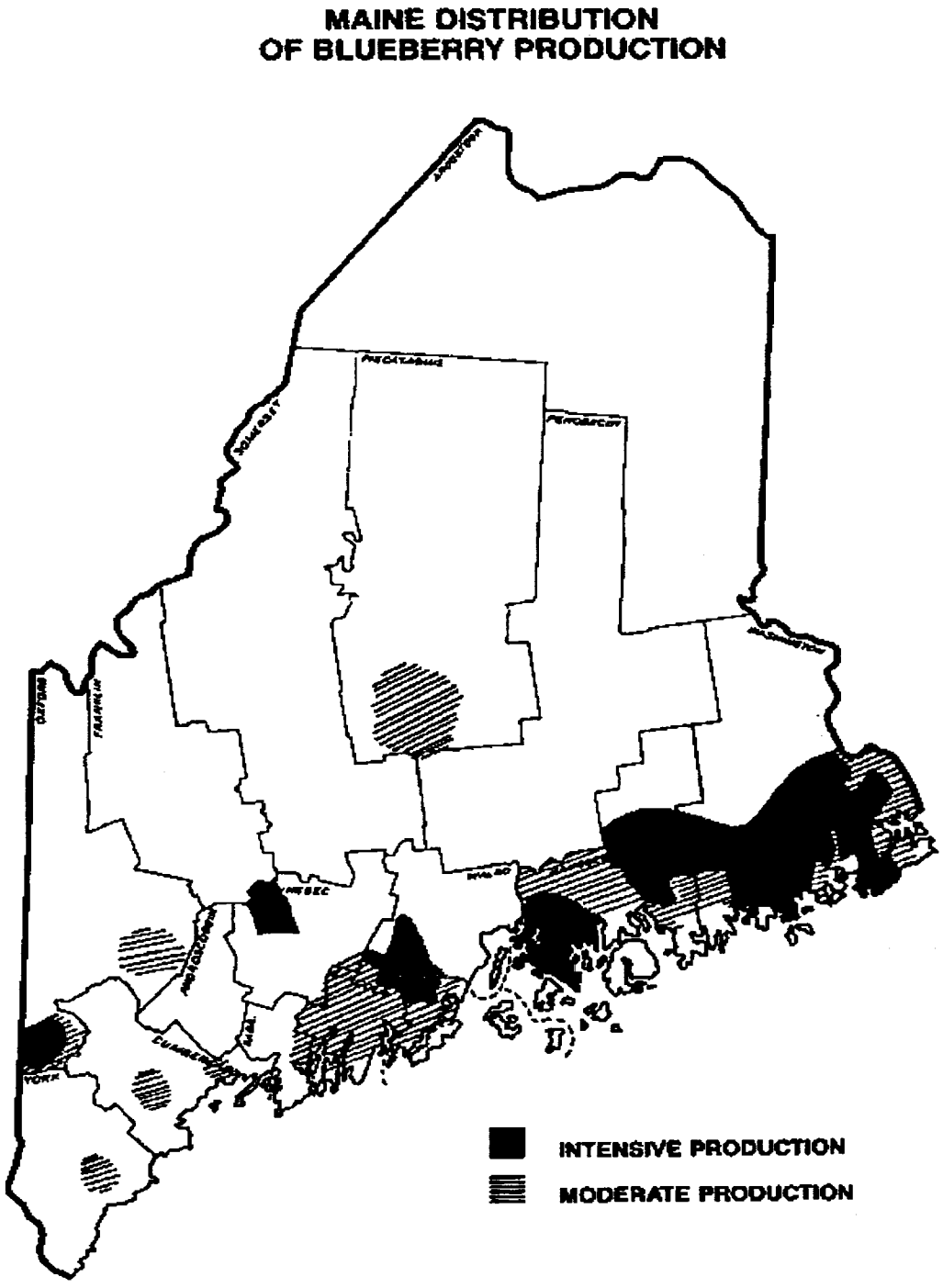

Figure 3. Areas of Blueberry Production in Maine (Yarborough, 1995)

Earlier work with forest soils showed virtually no movement through sandy or clay soils, with 88-98 % of the Velpar retained in the top organic horizons (Ray et al., 1989). Conversely, Allender (1991) noted both lateral and vertical movement of the herbicide on four sites, ranging from sandy loam to clay in texture. Lavy et al. (1989) found perpendicular movement of the chemical when used on a well drained silt loam, even on slopes as steep as 40 %. Application of Velpar on a sandy loam up to two meters thick, in the Upper Piedmont region of Georgia resulted in dry period pulses of up to 44 ug/l in local streams (Neary et al. 1983). This is indicative of rapid vertical transport.

Methods of Analysis

Analysis of hexazinone and some of its metabolites in soil and water has been accomplished by using several techniques, including gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and enzyme immuno sorbent assay (EIA or ELISA). These analytical systems can be assembled using a variety of separation implements (columns) and an array of detection devices. Each analytical technique has inherent advantages and disadvantages, which include such issues as cost, ease of use, sensitivity, specificity and sample matrix effects.

The following sections represent a review of extraction and clean-up approaches for hexazinone in water and soil matrices, as well as separation and detection methods for the parent compound and several metabolites.

Extraction Techniques

Water

Until the mid 1980's most methodologies for the extraction of residual pesticides from water matrices involved the use of liquid-liquid partitioning. The benefits of this procedure are two-fold, combining concentration and clean-up steps. Table 7 lists several solvents that analysts have employed for Velpar extraction, including chloroform (Bouchard et al., 1983; Solomon et al., 1988 and Lavy et al., 1989), ethyl acetate (Feng and Feng, 1988), acetone/methylene chloride (Wan et al., 1988) and methylene chloride (Miles et al., 1990). Partitioning into these types of organic solvents is expensive, time-consuming, potentially hazardous and generates large volumes of toxic waste. For these reasons, this extraction technique is no longer as widely accepted.

Solid phase extraction (SPE) has gained broad acceptance for the concentration and clean up of a wide range of agrochemicals in water samples. Disposable, non-polar C-18 SPE cartridges and extraction disks are offered by a number of vendors and work well for removing Velpar from water (Perkins and Bushway, 1999; Baranowski and Pieszko, 2000). Cartridges packed with a newer graphitized carbon material were used by Kubilius and Bushway (1998) to successfully extract the parent herbicide, as well as metabolites A, B, C, D and E from ground water. Hennion (2000) has described various interactions, including hydrophobic, electronic and ion exchange properties of graphitic carbon surfaces as explanations for the superior ability of this phase for trapping water-soluble analytes from aqueous sources. Baranowski and Pieszko (2000) found that sulfonic SPE cartridges worked as well as C-18 SPE, for the removal of residual

Table 7. Methods for Hexazinone Analysis in Water

Analyte matrix	Separation/Detection	Extraction	Clean-up	LOQ	Metabolites	Notes	Reference
Water	HPLC – 254nm (C8 column)	liquid/liquid (chloroform)	reconstitute - methanol	1.0 ug/l	none	Confirmation GC/MS	Bouchard et al., 1983
Water	GC/NPD (packed column)	liquid/liquid (ethyl acetate)	none	not listed	A,B		Feng & Feng, 1988
Water	GC/NPD (packed column)	liquid/liquid (chloroform/water)	reconstituted in ethyl acetate	not listed	none		Solomon et al., 1988
Water	GC/NPD	liquid/liquid (95%MECl 5% acetone)	none	not listed	none		Wan et al., 1988
Water	HPLC – 254nm	liquid/liquid (chloroform)	none	20 ug/l	none		Lavy et al., 1989
Water	GC/NPD (capillary HP-5)	liquid/liquid (methylenechloride)	reconstitute in acetone	0.3 ug/l	none		Miles et al., 1990
Water	EIA	none	none	0.13 ug/l	A, A1, 1, B, C	Not specific for met. Cross-reactive	Bushway & Ferguson, 1996
Water	EIA	none	none	0.10 ug/l	A, A1, 1, B, C	Not specific for met. Cross-reactive	Bushway et al., 1996
Water	CE/UV – 247nm	SPE (graphitized carbon)	none	0.5 ug/l	A, B, C, D, E		Kubilius & Bushway, 1998
Water	HPLC/DAD – 247nm (C8 column)	SPE (tC18)	none	0.2 ug/l	none	Potential for metabolite B	Perkins & Bushway, 1999

Table 7. Cont.

Analyte matrix	Separation/Detection	Extraction	Clean-up	LOQ	Metabolites	Notes	Reference
Water	HPLC/UV – 247nm (C8 column)	none	none	0.33 ug/l	none	Potential for metabolite B	Perkins & Bushway, 1999
Water	HPLC/UV – 254nm (C8 column)	SPE (C18)	none	0.30 ug/l	none	Multi-pesticide method	Baranowski & Pieszko, 2000
Water	HPLC/UV – 254nm (C8 column)	SPE (sulfonic)	none	0.30 ug/l	none	Multi-pesticide method	Baranowski & Pieszko, 2000

hexazinone from water. While there is no published record for the use of copolymer (styrene-divinylbenzene) for Velpar extraction, it has been used successfully for a wide range of other herbicides. The use of this SPE material for binding the polar atrazine metabolites deethylatrazine, deisopropylatrazine and didealkylatrazine (Tanabe et al., 2000) shows promise for extracting hexazinone metabolites of similar polarity from water samples. Other polymeric SPE compounds, which have been used to successfully bind pesticides with higher polarities, include divinylbenzene-N-vinyl pyrollidine (Potter et al., 2000) and ethylvinylbenzene-divinylbenzene (Tolosa et al., 1999). Hennion and Pichon (1994) found that the polymeric sorbents had 20 to 40 times more retentive capacity than C-18 for removing polar aromatic compounds from water. The authors of Solid Phase Extraction, Principles and Practice (Thurman et al., 1998) list several reasons for these phenomena, including higher surface areas than C-18 phases, as well as the strong interaction between the sorbent and the π bonds of the solute.

Soil

For several reasons the extraction of hexazinone from soil is far more challenging than working with water. The binding potential of the herbicide to soil particles can be strong, depending on the soil type. For example, organic and clay fractions tend to bind compounds more tightly than sand and silt particles. Breaking the soil-hexazinone bond is essential for efficient extraction. Additionally, soils tend to exhibit more complex matrices than do water samples. In order to break the soil-Velpar attraction many of these matrices are co-extracted with the target analyte(s) and need to be removed from the extract, prior to sample analysis. Such sample clean up can be costly, time consuming and often results in smaller sample sizes and lowered detection limits.

Finally, because of its particle size distribution and different mineral make-up, it is more difficult to collect homogeneous soil samples than water samples. Therefore, lack of a carefully planned sampling protocol can easily result in reproducibility problems and data error.

Over the past two decades, a number of solvent systems have been employed to extract hexazinone and its metabolites from soil. In order to report residue levels in a consistent manner (dry weight basis), most soil samples are dried and weighed before analysis proceeds. This drying can take place at room temperature or in a drying oven. Because drying can further bind the target analyte, water is often employed in extraction solvents in the theory that it will re-hydrate the soil and increase extraction efficiency.

Table 8 lists extraction solvents, which have been successfully exploited for hexazinone extraction. Holt (1981), Roy (1981), Bouchard and Lavy (1983), and Solomon et al. (1988) all used mixtures of acetone:water (4:1) as an extractant. Perez et al. (1998) and Zhu et al. used the same solvent system in a 9:1 ratio. Other popular water-solvent mixtures include methanol:water at 50:1 (Feng, 1992), 2:1 (Allender, 1991; Lyndon et al., 1991) and 4:1 (Fischer and Michael, 1995; Bushway et al., 1997) proportions and 4:1 acetonitrile:water (Baranowski and Pieszko, 2000). All of these solvent systems should also co-extract the more polar hexazinone metabolites, although only a few of these mixtures were used for this purpose.

Only three non-aqueous extracting schemes were found in the literature. One involves an eighteen-hour soxhlet extraction with acetone (USEPA, 1996). This is a general procedure used for the removal of a broad spectrum of pesticides in soil. Another process uses chloroform and is also broad spectrum in nature (Baranowski and Pieszko, 2000).

Finally, although the authors made no note of soil water content, Subtrova et al. (1990) used 100% methanol as a soil extractant.

Most soil extraction methods require further clean up before analysis of the sample extract can be completed. Until recently, the most common way to accomplish this was with various liquid-liquid partitioning solvents, including chloroform, ethyl acetate or dichloromethane. In fact, some of these protocols were quite arduous, involving up to eight partitioning and drying steps (Holt, 1981). Although the resulting preparation was quite clean, it could take an entire day to prepare two samples.

Nearly all of the sample clean up methodology developed during the past ten years for hexazinone extraction has involved the use of SPE cartridges. This technology has greatly increased sample throughput and has greatly reduced the costs associated with toxic solvent use and disposal. Although florisil packing material has been used extensively to prepare extracts in non-aqueous diluents, the most commonly used SPE phase for hexazinone in a solvent-water mixture is probably C-18. Fischer and Michael (1996) found that this material worked well for hexazinone residues in soil, as well as more complex plant materials. Baranowski and Pieszko (2000) developed a multi-pesticide residue method for soil using a similar C-18 cartridge and found that a sulfonic SPE phase worked equally well. Finally, Feng (1992) developed his own mixed function SPE, using sodium sulfate, aluminum oxide and florisil. This micro column was inexpensive and it retained metabolites A and B quite well. Other extraction-clean up methods that have been used for residual hexazinone include gel permeation chromatography (GPC) and accelerated solvent extraction (ASE). GPC is a size-exclusion technique, which is very useful for the separation of the humic fractions

Table 8. Methods for Hexazinone Analysis in Soil

Analyte matrix	Separation/Detection	Extraction	Clean-up	LOQ	Metabolites	Notes	Reference
Soil	GC/NPD (packed column)	80:20 (acetone:water)	extensive	40 ug/kg	A, B, C, D, E	derivitized (TFA)	Holt, 1981
Soil	GC/NPD (packed column)	80:20 (acetone:water)	extensive	not listed	A, B	no derivitization	Roy et al., 1981
Soil	HPLC/UV – 254nm C8 (column)	1:4 (acetone:water)	dichloromethane reconstitute - water	10 ug/kg	none	several soil types	Bouchard & Lavy, 1983
Soil	GC/NPD (packed column)	80:20 (methanol:water)	chloroform reconsitute – ethyl acetate	10 ug/kg	A, B, C	metabolites difficult	Jensen & Kimball, 1987
Soil	GC/NPD	4:1 (acetone:water)	Multiple liquid/liquid partitioning	30 ug/kg	A ,B	no derivitization	Feng & Feng, 1988
Soil/ sediment	GC/NPD (packed column)	80:20 (acetone:water)	chloroform reconsitute – ethyl acetate	not listed	none		Solomn et al., 1988
Soil	HPLC/UV – 254nm C8 (column)	1:4 (acetone:water)	dichloromethane reconstitute - water	50 ug/kg	none		Lavy et al., 1989
Soil	GC/NPD (capillary column-HP5)	4:1 (ethyl acetate: methanol)	Reconstitute - toluene	20 ug/kg	none		Miles et al., 1990
Soil	HPLC/UV – 254nm C18 (column)	methanol	dichloromethane reconstitute - methanol	10 ug/kg	none		Subrtova et al., 1990

Table 8. Cont.

Analyte matrix	Separation/Detection	Extraction	Clean-up	LOQ	Metabolites	Notes	Reference
Soil	HPLC/UV – 254nm C18 (column)	2:1 (methanol:water)	dichloromethane reconstitute - methanol	not listed	none		Allender, 1991
Soil	HPLC/UV – 254nm C18 (column)	2:1 (methanol:water)	GPC	5 ug/kg	none		Lyndon et. al, 1991
Soil	GC/NPD (capillary column DB17)	200 + 4 (methanol+water)	micro-column (Nasulfate/AlO_3/florisil)	12.5 ug/kg	A, B	DB-17 gives good metab. separation	Feng, 1992
Soil	HPLC – MS (thermospray) C18 (column)	4:1 (methanol:water)	SPE (C18)	5 ug/kg	A,B,C,D,E,G	also for vegetation	Fischer & Michael, 1995
Soil	GC/MS (capillary column)	acetone (soxhlet – 18 hours)		not listed	none	long extraction time	USEPA, 1996
Soil	EIA	80:20 (methanol:water)	none – interferences diluted	50 ug/kg	A,A1,1,B,C	not specific for met. cross-reactive	Bushway et al., 1997
Soil	GC/NPD GC/MS confirmation	90:10 (acetone:water) ultrasonic extr.	Reconstitute in ethyl acetate	10 ug/kg	none	Soil packed in column – low solvent volumes	Perez et al., 1998
Soil	HPLC/UV – 254nm C18 & C8 (columns)	chloroform	SPE (C18)	1.4 ug/kg	none	Multi-pesticide method	Baranowski & Pieszko, 2000

Table 8. Cont.

Analyte matrix	Separation/Detection	Extraction	Clean-up	LOQ	Metabolites	Notes	Reference
Soil	HPLC/UV – 254nm C18 & C8 (columns)	9:1 (acetonitrile:water)	SPE (sulfonic)	1.4 ug/kg	none	Multi-pesticide method	Baranowski & Pieszko, 2000
Soil	GC/MS (HP5 column)	ACE water/acetone	none	2.5 ug/kg	none	novel extraction	Zhu et al., 2000

(found in soils containing significant OM) from a variety of pesticides (Lyndon et al., 1991). ASE is a new technology that utilizes high pressures and temperatures to reduce sample preparation time while simultaneously increasing extraction efficiency. It has found a great deal of use for the extraction of pesticides from soil, including hexazinone (Zhu et al., 2000). The disadvantages of ASE are the initial capital expense ($ 2,000-50,000) and the increased likelihood of interfering co-extractants from the complex matrices commonly associated with soil.

Detection Methods

The number of steps required for extract clean up depends largely on the instrumentation used for detection. Some detection methods are very analyte-specific or detect only certain classes of compounds. Examples of such methodologies include enzyme immunoassay (EIA), gas chromatography (GC) with nitrogen-phosphorous detection (NPD). Less analyte specific instrumentation includes high performance liquid chromatography (HPLC) with ultra violet (UV) or photodiode array (PDA) detection. GC or HPLC separation with mass spectral detection (MSD) can vary in sensitivity and specificity, depending on the mode of operation (single ion monitoring vs. total ion scanning) and the ionization properties of the analyte.

The majority of the earliest pesticide residue methods were accomplished using GCs equipped with packed columns and NPD or electron capture detection (ECD). Both of these detection systems are quite sensitive. Since hexazinone and it's accompanying metabolites contain significant percentages of nitrogen, many researchers have relied on packed columns and NPD to establish residual levels of this herbicide in a number of different matrices, including water and soil (Holt, 1981; Roy et al., 1981; Jensen and

Kimbal, 1987; Feng and Feng, 1988; Solomon et al., 1988; Wan et al., 1988). The development of the capillary fused silica column in the late 1980's led to better chromatographic resolution and allowed better and faster separations, as well as lower detection levels for hexazinone (Miles et al., 1990; Feng, 1992).

The introduction of relatively inexpensive, bench-top MS detection has enabled the chromatographer to simultaneously determine and confirm residual hexazinone. Single ion monitoring (SIM) permits investigators to collect data from only the predominant hexazinone ions, resulting in greater sensitivity and selectivity of the method (USEPA, 1996; Perez et al., 1998; Zhu et al., 2000). Quadrupole and ion trap detectors are the most common MSDs available in pesticide residue laboratories. Each has certain advantages over the other. The quadrupole instrument is generally both more quantitative and more forgiving of complex sample extracts than is the ion trap apparatus, which provides more accurate information of actual mass of the target analyte.

HPLC separation with UV and PDA detection has been used extensively for the isolation of hexazinone from both water and soil extracts. The parent compound exhibits excellent absorption at 254 nm, which worked well for older fixed wavelength UV detectors (Bouchard et al., 1983; Lavy et al., 1989). Other workers using a 254 nm wavelength as well as reverse-phase (RP) C-8 or C-8 Columns are listed in Tables 7 and 8. Using a PDA detector, Bushway et al. (1996) monitored hexazinone at its UV max of 247 nm. Using this system, Perkins and Bushway (1999) were able to establish a limit of quantitation (LOQ) of 0.2 μg/L, and used the herbicides unique UV spectrum for confirmation.

Only one HPLC-MSD method was found in the literature. Fischer and Michael (1995) used a thermospray device to achieve a LOQ of 5 µg/kg in soil and were able to detect metabolites A, B, C, D, E and G.

CE is another newer technology that has found use in pesticide residue analysis. Kubilius and Bushway (1998) developed a CE-PDA method for hexazinone and several metabolites in water that was sensitive to 0.5 µg/L. CE allows charges to be applied to target compounds, which is particularly useful for separating polar compounds, such as hydroxylated pesticide metabolites. The improvement of CE interfaces for MS detectors will greatly enhance the sensitivity of CE systems and may make such instruments invaluable for pesticide residual analysis.

EIA kits for pesticide analysis were developed by a small Maine company in the late 1980's, as spin-offs from clinical formats. While these kits retail for up to $ 600 for approximately 100 assays, they are relatively inexpensive, when compared to the capital necessary for more traditional HPLC and GC systems. EIA is also easy to use, with little training required. Bushway et al. (1996 and 1997) published three papers, which describe EIA applications for residual hexazinone in water and soil matrices. This methodology has the advantage/disadvantage that it does not differentiate between parent and metabolite compounds (table 9). This lack of differentiation between hexazinone metabolites can be considered a benefit in light of the EPA's directive to consider residual parent and corresponding metabolites as one value, while at the same time; this causes confusion, due to the different cross-reactivity concentrations. While the cross-reactivity may have a minor effect on quantitative accuracy, EIA remains an invaluable tool for inexpensively screening large numbers of environmental samples.

Table 9. Cross-Reactivity of Metabolites in the Hexazinone Plate and Tube EIA (modified from Bushway et al., 1996)

Compound	Plate EIA IC_{50}[b] (ppb)	Plate EIA LLD[c] (ppb)	Tube EIA IC_{50}[b] (ppb)	Tube EIA LLD[c] (ppb)
Hexazinone	1.0	0.10	1.0	0.10
Metabolite A	2.8	0.22	3.8	0.22
Metabolite A1	7.0	0.44	8.0	0.44
Metabolite 1	2.8	0.22	2.3	0.22
Metabolite B	4.2	1.1	5.4	1.1
Metabolite C	8.0	1.1	11.0	1.1
Metabolite D	*	*	*	*
Metabolite E	*	*	*	*

* No cross-reactivity at 1 ppm.

[b] Concentration that causes 50% inhibition.

[c] Lowest limit of detection at % B_0 of less than 90.

DETERMINATION OF HEXAZINONE IN GROUND WATER BY DIRECT-INJECTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Hexazinone has been detected at levels ranging from 0.2 to 50 μg/L in many ground water samples from eastern Maine over the past decade. A rapid and inexpensive direct-injection high-performance liquid chromatographic (HPLC) method has been developed to monitor contamination levels of the herbicide. The method is sensitive (limit of quantitation = 0.33 μg/L) and is linear to 33.0 μg/L (R^2 = 0.9995). Direct injection results from 50 field samples compared well (R^2 = 0.98) with an HPLC method using solid-phase extraction for concentration and cleanup. The technique is very reproducible (coefficients of variation of 0-8.4% within day and 3.0-13.2% between day) and eliminates loss of analyte because of fewer steps in the procedure.

Introduction

Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione); trade name of Velpar; E.I. Dupont de Nemours & Co., Wilmington, DE] is a selective herbicide used primarily in forestry, but has also been effective in alfalfa, pineapple and wild blueberry agriculture. Hexazinone has been credited with dramatically increasing the yield of the blueberry crop in Maine, while also increasing the size and quality of the berries (Yarborough and Bhowmik, 1989). Unfortunately, the thin, low base, sandy soils (Stone et al., 1993) often associated with blueberry agriculture, coupled with the high solubility of hexazinone (33,000 mg/L) have led to the contamination of local ground water supplies (Bushway et al., 1996).

Ground water from susceptible areas in Maine has been monitored routinely for hexazinone since 1990, when residues first appeared. Using a solid phase extraction technique (SPE) our laboratory assays 150-200 samples per year for research, private and regulatory interests. A large percentage of these samples have been positive for the herbicide, with concentrations as high as 50 μg/L.

There are several published methods describing techniques for the determination of hexazinone and its metabolites in various matrices, including capillary electrophoresis (CE; Kubilius and Bushway, 1998), high-performance liquid chromatography (HPLC; Bouchard and Lavy, 1983, Lyndon et al., 1991), gas chromatography with nitrogen-phosphorous thermionic detection (GC-NPD; Holt, 1981, Solomon et al., 1990, Feng, 1990), and GC with mass spectrometry (MS; Fischer and Michael, 1995). Although these procedures provide detailed information for metabolite and parent residues, they are time consuming and expensive. The increased demand in Maine for testing of ground

water for parent hexazinone has led to the development of a faster and less expensive direct injection technique described in this paper.

Experimental

Liquid Chromatographic System

(a) *Pump.*-HP 1050 gradient (Hewlett Packard, Inc., Wilmington, DE).

(b) *Detector.*-Hitachi Model L205, variable wavelength (Hitachi Instruments, San Jose, CA).

(c) *Integrator.*-Model 3376 (Hewlett Packard, Inc.).

(d) *Injector.*-Model EQ6 fitted with a 500 µL loop and a 2 mL glass barrel syringe (Valco Instruments, Houston TX).

(e) *Column.*-Zorbax C8, 5µm, 250 x 4.6 mm (Phenomenex, Inc., Torrance, CA).

Reagents

(a) *Solvents.*- Acetonitrile, methanol and water were all HPLC grade (VWR Scientific, Bridgeport, NJ).

(b) *LC elution solvent.*-Water:acetonitrile:methanol (60:25:15, v/v/v).

(c) *Hexazinone standard.*-Analytical grade (Environmental Protection Agency, Research Triangle Park, NC).

(d) *Hexazinone Metabolites* -A, A1, B, C, D and E.(E.I. Dupont de Nemours and Co., Wilmington, DE).

LC Method

(a) *Standard preparation.*-Stock solutions of hexazinone and each metabolite were prepared by dissolving a known weight of each compound in 25 mL of acetonitrile. Standards are stable for several months when stored at –20 °C. A standard curve consisting of 0.33, 0.66, 1.32, 3.3, 6.66 and 32.8 µg/L hexazinone was prepared daily in HPLC grade water.

(b) *Analysis.*-The LC mobile phase consisted of water-acetonitrile-methanol (60 + 25

+15, v/v/v). Assay conditions were as follows: temperature, ambient; flow rate, 1.7 mL/min.; UV detection wavelength, 247 nm.

(c) *Direct injection reproducibility study.*-Seven ground water samples known to contain varying levels of hexazinone residues were collected from the Pineo Ridge area of Cherryfield, ME. The water was collected in methanol rinsed, clear, 1 L jars and stored at 5 ° C. No preservatives were added and no pH adjustments were made, since hexazinone is stable for at least 4 weeks under these conditions. Samples were allowed to warm to room temperature before injecting into the HPLC system. The injector and syringe were flushed several times with HPLC grade water before injecting 500 μL of the sample or standard. Hexazinone concentration was calculated by comparing peak heights of samples to standards. Each sample was injected 6 times within 1 day and 1 time each day over 6 days to determine method reproducibility.

(d) *Correlation of direct injection with SPE-LC method.*-A total of 50 ground water samples collected from various locations in eastern Maine were assayed by the LC direct-injection and by an internally validated LC method that used SPE for sample preparation.

Results and Discussion

The current federal and state of Maine drinking water guidelines for hexazinone are 200 and 210 μg/L, respectively. The HPLC method described is sensitive to 0.33 μg/L of hexazinone (signal to noise, 3:1) and linear to at least 33 μg/L. A clean ground water sample (Figure 4) shows a chromatogram with no interfering peaks at the elution time of hexazinone.

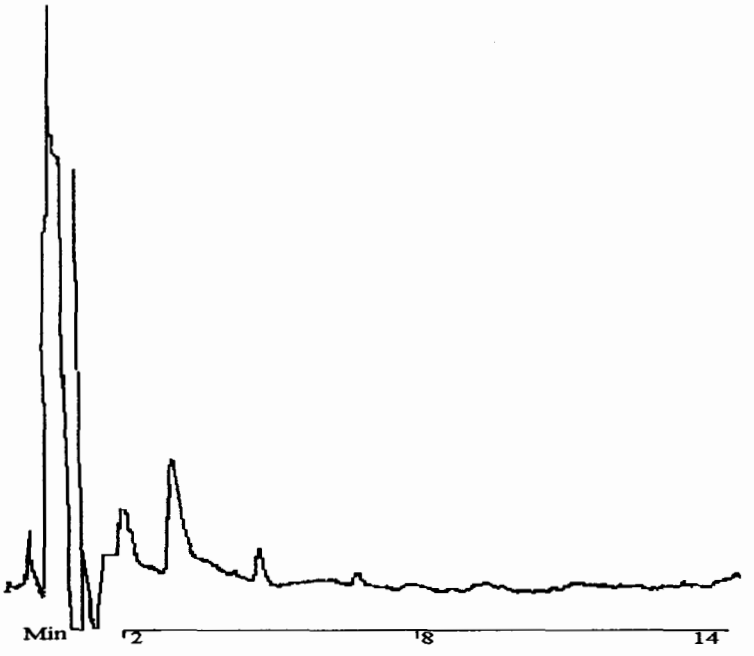

Figure 4. Chromatogram of Clean Groundwater Sample (blank).

The chromatogram in Figure 5 depicts a spring water sample with a hexazinone peak at 7.9 minutes.

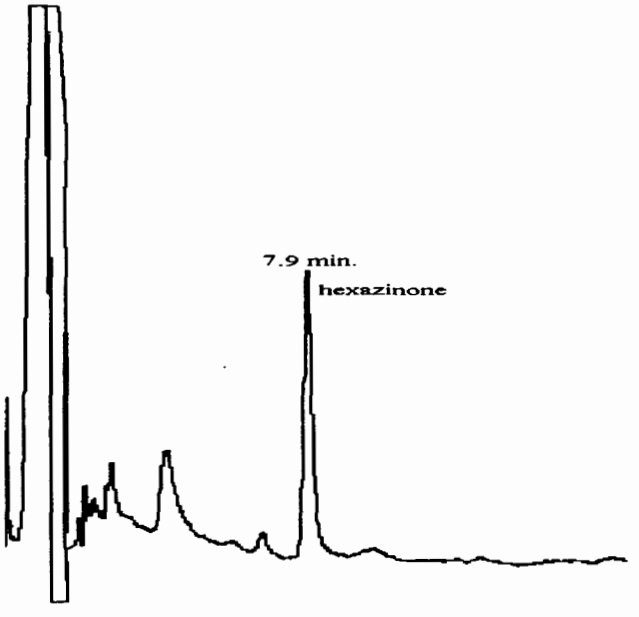

Figure 5. Chromatogram of Spring Water Sample Containing 6.6 µg/L Hexazinone

Hexazinone metabolites A, A1, B, C, D, G were injected into the HPLC system and found not to co-elute with the parent compound These metabolites are more polar and elute earlier than does the parent compound. Most are also relatively unstable in aqueous environments and don't often appear in ground water samples. Neary et al. (1983) found only traces of metabolites A and B in surface runoff, after treating the top soil with hexazinone. Recent work by Kubilius and Bushway (1998) found B to be the only metabolite to contaminate ground water consistently, at measurable levels. With use of the direct-injection method, metabolite B eluted at 6.5 min. and was not strongly absorbed at 247 nm. The λ_{max} for metabolite B is 230 nm. At 247 nm the LOQ for this compound is 10 μg/L, which is too high to determine using this method.

The repeatability of the method was assessed by conducting intra- (Table 10) and interday (Table 11) injections. Statistical analysis showed acceptable repeatability, with coefficient of variation levels ranging from 0 to 8.4% for within-day injections and 3.0 to 13.2% for between day injections.

Table 10. Direct Injection Reproducibility Within Day Analysis

Sample	Rep-1 (μg/L)	Rep-2 (μg/L)	Rep-3 (μg/L)	Rep-4 (μg/L)	Rep-5 (μg/L)	Rep-6 (μg/L)	%CV
PR-spring	0.292	0.292	0.292	0.292	0.292	0.292	0
Y-29	0.510	0.510	0.437	0.437	0.510	0.437	8.4
Y-13	1.729	1.670	1.729	1.729	1.789	1.789	2.6
Y-4	2.270	2.270	2.385	2.385	2.385	2.385	4.0
spring-5	6.321	6.757	6.691	6.757	6.691	6.560	2.5
spring-6	9.840	9.971	9.906	9.840	10.034	9.709	1.2
spring-8	4.890	4.592	4.330	4.592	4.330	4.461	4.6

Table 11. Direct Injection Reproducibility Between Day Analysis

Sample	Day-1 (µg/L)	Day-2 (µg/L)	Day-3 (µg/L)	Day-4 (µg/L)	Day-5 (µg/L)	Day-6 (µg/L)	%CV
PR-spring	0.292	0.358	0.394	0.394	0.292	0.358	13.2
Y-13	1.729	1.729	1.640	1.662	1.749	1.749	3.8
Y-29	0.510	0.477	0.525	0.590	0.656	0.552	11.5
Y-4	2.207	2.207	2.362	2.211	2.385	1.895	7.9
spring-5	6.321	6.321	6.560	6.191	6.691	6.268	3.0
spring-6	9.060	9.060	9.840	9.287	10.04	9.330	4.3
spring-8	4.890	4.890	4.592	4.128	4.330	3.936	8.9

To test the accuracy of the direct injection method, 50 ground water samples with various levels of hexazinone contamination (0.3 – 10 µg/L) were compared with an HPLC-photodiode array (PDA) method, which used a SPE concentration and cleanup step. The SPE method was previously validated by using HPLC-MS and CE-PDA (Kubilius and Bushway, 1998) and was sensitive to 0.05 µg/L. The correlation of the two methods showed excellent agreement throughout the concentration range, with R^2 = 0.98 (Figure 6).

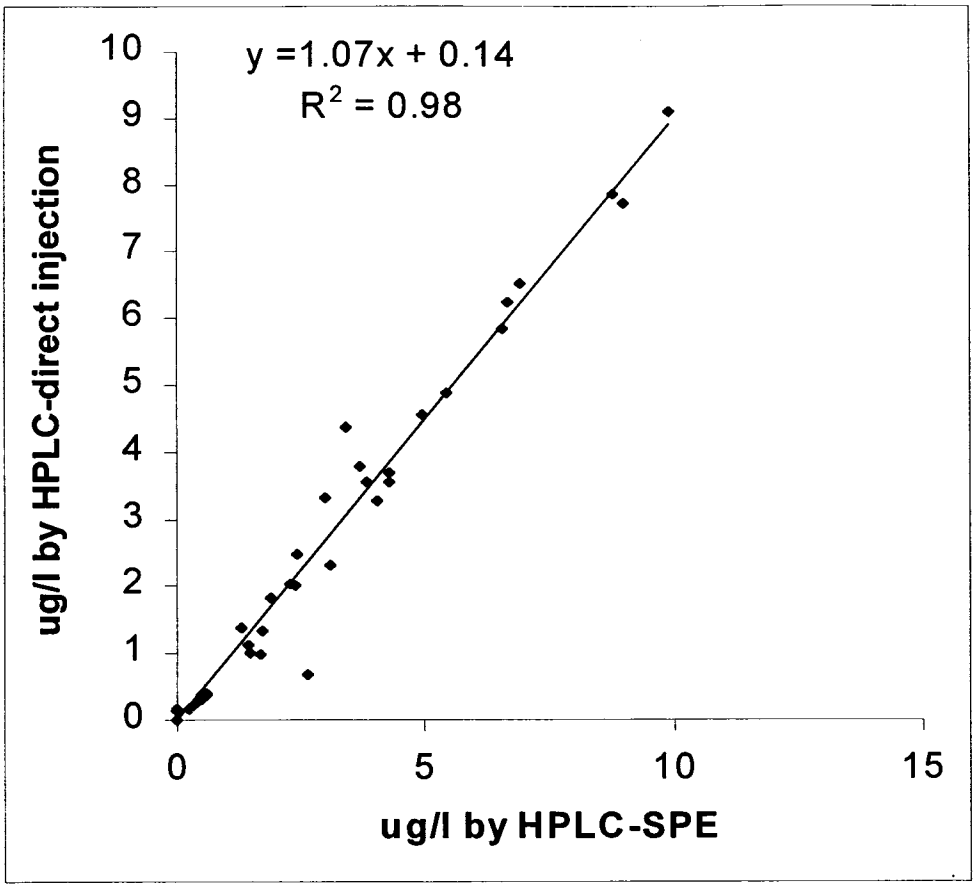

Figure 6. Correlation of Hexazinone by LC-SPE to LC-Direct Injection

Conclusion

This is a sensitive, rapid, reliable and inexpensive method for the analysis of hexazinone residues in groundwater. System automation could be easily accomplished by the addition of an inline filter and auto sampler. Metabolite B, which is often found when the parent herbicide is present, could be detected simultaneously by using a sufficiently sensitive photo diode array detector.

ANALYSIS OF HEXAZINONE IN MAINE'S GROUND WATER

Introduction

Ever since the 1991 discovery of residual hexazinone in Maine's surface and ground water, a number of government agency and special interest groups have taken an interest in determining the extent of the contamination. These groups include the Maine Board of Pesticides Control (MBPC), the Maine Sea Run Salmon Commission (MSRSC), the Department of Marine Resources (DMR), the Maine Organic Farmers and Growers Association (MOFGA), the Maine Blueberry Commission (MBBC), as well as a number of private citizens whose drinking water is threatened by contamination with the herbicide. Although reasons for concern vary from such issues as the effect on clams (DMR) and effect on endangered sea run salmon (MSRSC) to exposure to humans, these organizations have collected hundreds of environmental samples in attempts to ascertain both the concentration and the mobility of hexazinone.

Because of human exposure concerns via drinking water; two of these agencies have assumed the responsibility for monitoring hexazinone in ground water. The MBBC became involved in long-term water sampling after a monitoring well in a commercial blueberry field repeatedly yielded Velpar concentrations in the 30 µg/L range. The MBPC began to participate in hexazinone analysis of drinking water as part of its mandate to evaluate and control pesticide use, misuse and pollution of the environment.

Data for this chapter is divided into two sections. Part 1 involves long-term, analysis of water at regular intervals, from seven wells known to contain detectable levels of hexazinone. These wells include monitoring sites installed in blueberry fields between

1986 and 1991 by the Maine Department of Conservation, in addition to wells used for potable water by the general public. Part 2 includes nearly a decade of random sampling from privately owned wells located near blueberry growing areas. The MBPC sampling occurred statewide, with a majority of the work occurring in Washington County, which is considered the heart of Maine's blueberry agriculture.

Materials and Methods

Part I - Long-Term Monitoring of Contaminated Wells

Site Selection

Seven sites in eastern Maine were chosen to monitor ground water for residual hexazinone. These areas are representative of intensive blueberry agriculture and are located in several counties (figure 7). All of the wells had tested positive for hexazinone in the past. The soils on these sites are all sandy loams or loamy sands and vary in depth. Table 12 lists the depths of all wells except 23 and 31, for which there is no available data. Wells 9, 11 and 12 are test wells, which are located in blueberry fields. Figure 8 (well 12) illustrates the constructive design of these test wells. These sites were selected to represent worst-case scenarios of hexazinone movement into the ground water. The other locations have drilled wells, which provide potable water for general human consumption. Well 13 was chosen because of its proximity to an elementary school. Wells 23, 31 and 32 were selected due to their location in a different part of the state.

As shown in table 12, three types of hexazinone formulations were used, including Velpar L (liquid), Velpar impregnated on DAP (diammonium phosphate) and Pronone

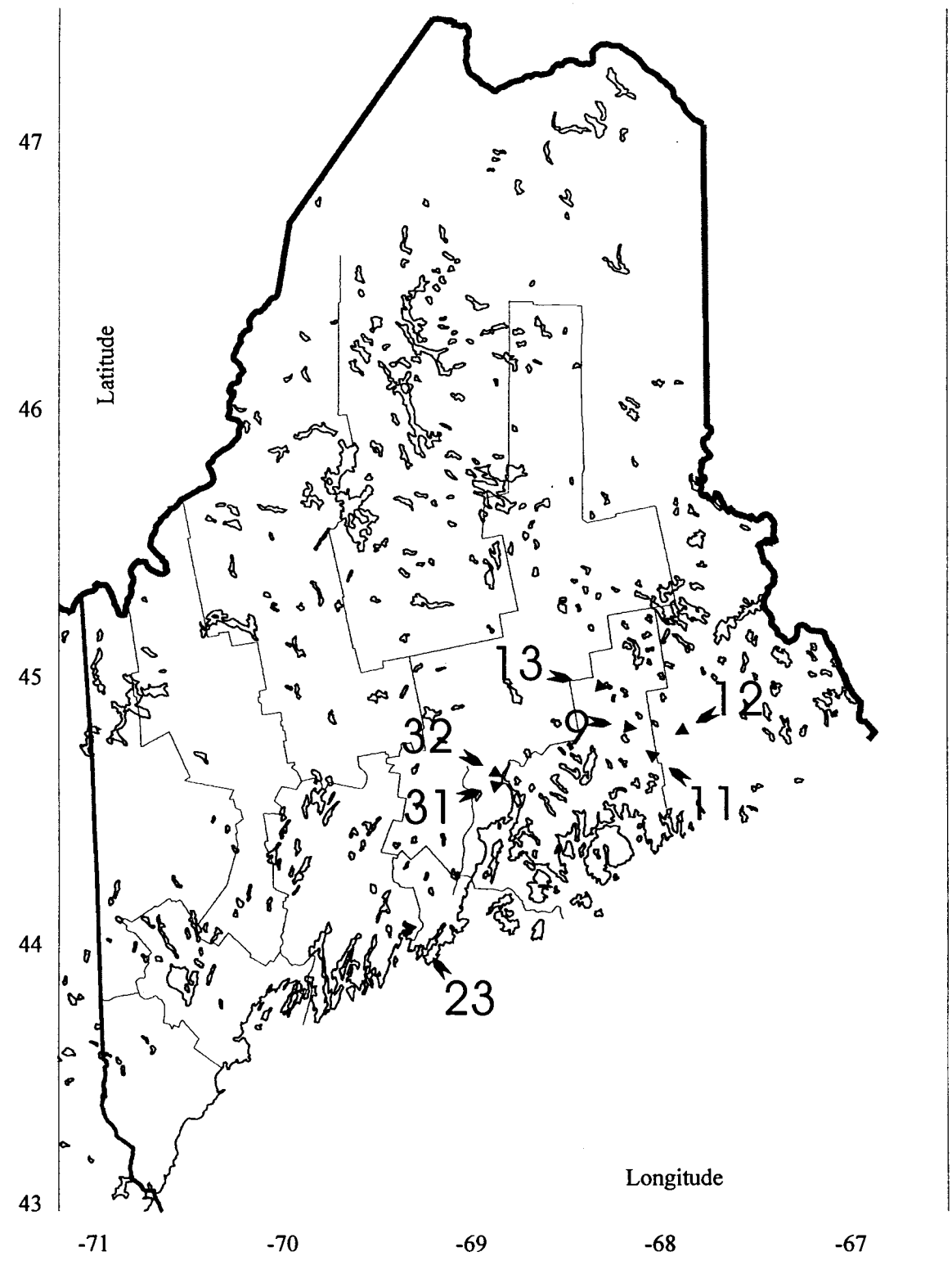

Figure 7. Location of Time-Series Wells Sampled for Residual Hexazinone

Table 12. Description of time-series wells sampled for residual hexazinone

Well No.	County	Town	Description	Depth (ft)	Treatment	Notes
9	Washington	T 22	Test well in field	23	No hexazinone after 1993	Originally showed 30 ug/L
11	Washington	Deblois On Deblois Plain	Test well in field	35	Velpar L	Near small irrigation pond
12	Washington	Columbia On Pineo Ridge	Test well in field	25	Pronone 10G	1993 treatment Terbacil no Velpar
13	Hancock	Aurora	Drilled-potable	100	Velpar impreganated DAP	School water supply (500 ft from field
23	Lincoln	Waldoboro	Drilled-potable	unknown	Pronone 10G	No longer used for drinking as of 2000
31	Waldo	Stockton Springs	Drilled-potable	unknown	Pronone 10G	Downgrade from well 32
32	Waldo	Stockton Springs	Drilled-potable	245	Pronone 10G	Near Velpar loading zone

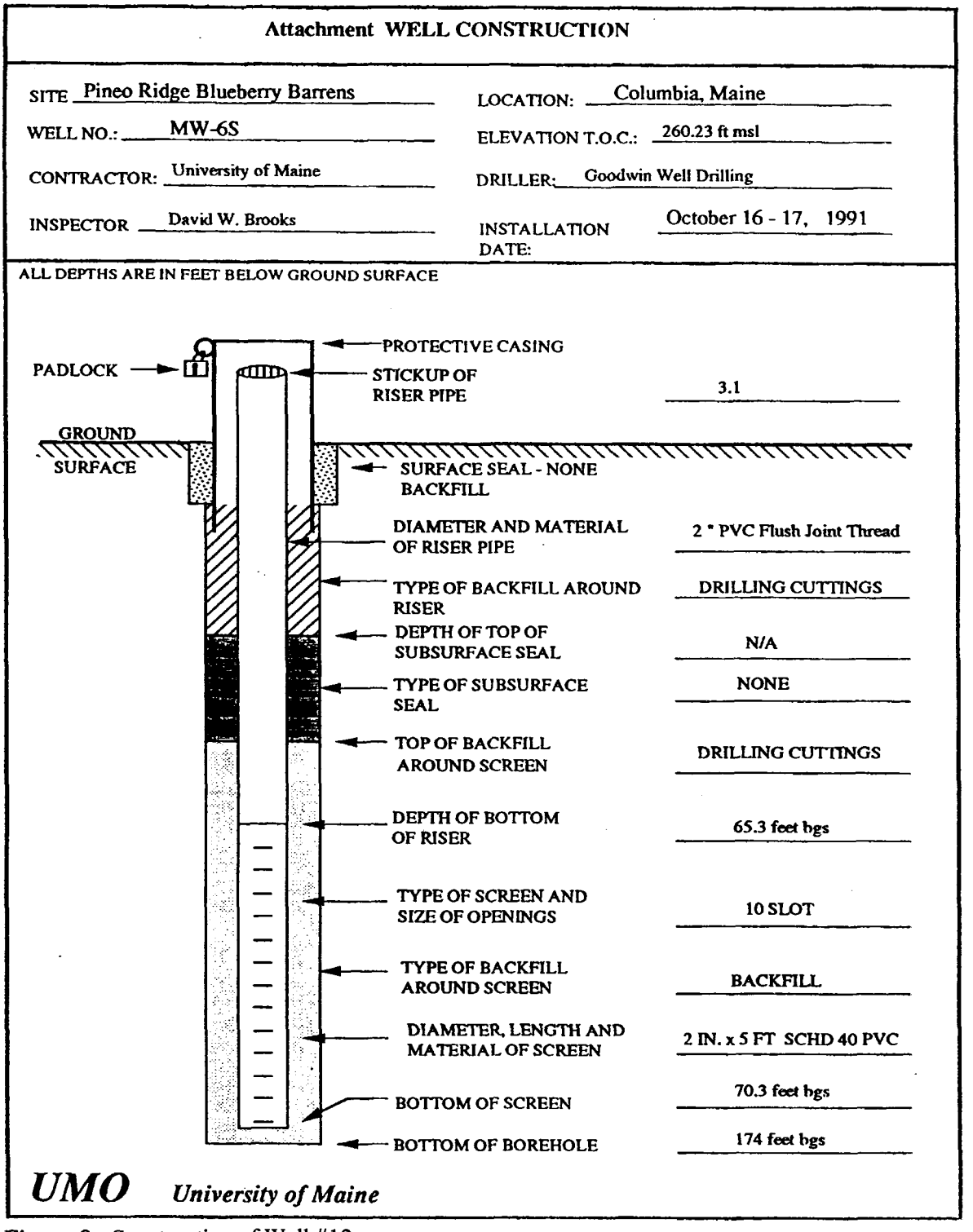

Figure 8. Construction of Well #12

10G (granular). The one exception to this formulation use was the field where well 9 was located. This site has received no hexazinone treatment after 1993.

Sample Collection

Whenever possible the wells were sampled monthly, from early May to October, during the free-flow period for ground water. In 1997 this work actually began in April. The study spanned as many as 10 (well 9) and as few as 6 years (well 23).

Sample collection in the test wells was accomplished by using one of two pumping systems. The first system consisted of up to 50 feet of ½ inch polypropylene tubing fitted with a stainless steel ball valve footer (Cole Parmer, Vernon Hills, IL). This system required a vigorous up and down "pumping" motion to bring water through the tubing. The second arrangement (figure 9) utilized an electric Redi-Flow 2 pump (Grundfos Pumps, Clovis, CA) coupled with a rented 5000-watt generator and ½ inch polypropylene tubing.

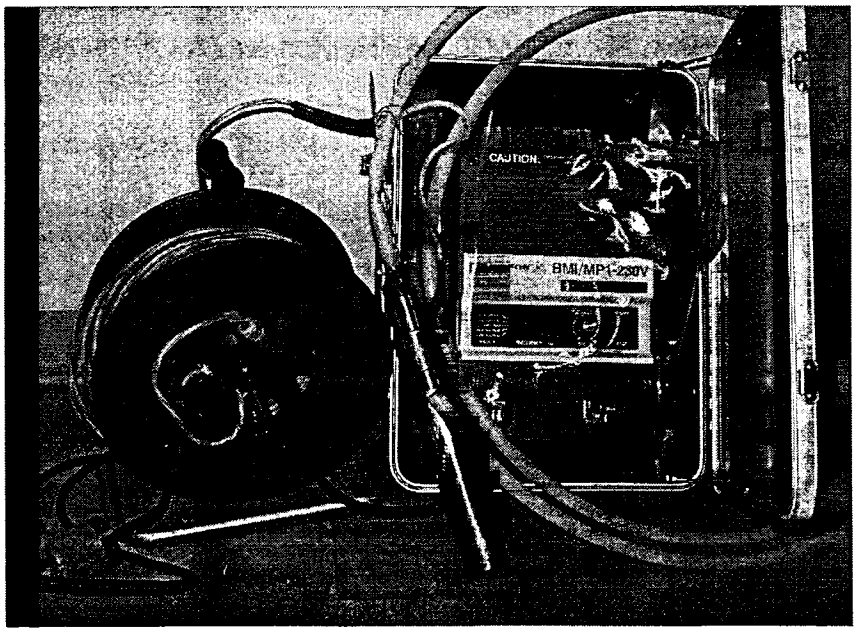

Figure 9. Grundfos Redi-Flow 2 Pumping System

Samples from wells 13, 23, 31 and 32 were collected from commercial and residential sources. The pumping system of each location was purged for several minutes to ensure that the well and not the plumbing was being sampled.

Water samples were collected in 500 ml canning jars purchased from a local department store. All wells were sampled over a 1 – 2 day period and stored over ice until they could be transported to the University of Maine for laboratory analysis. Samples were extracted within 3 days of sampling and extracts were stored at –20° C, until they were analyzed by HPLC for hexazinone content.

Part II - Monitoring of Randomly Selected Wells

Site Selection

Ground water sources for the determination of hexazinone contamination were identified by the MBPC through a process of stratified-random selection. After deciding how many sites were to be sampled, individual 7.5-minute topographic maps containing information pertaining to pesticide/commodity use were randomly selected. In this case, the pesticide was hexazinone and the commodity was wild blueberries. Field inspection staff provided this information. To further randomize the sampling program, each 7.5-minute topographic map was then overlaid with a 10 x 10 numbered grid. A random number list for each map then directed the sampler to subsections of the 7.5-minute topographic map, in search of a candidate sampling site. If there was more than one candidate site within the subsection, then the sampler assigned a number to each site (working south to north and /or east to west). Using a random number table the sampling site was then chosen. These additional steps were used to minimize sampler bias when searching for candidate sites. Within the gridded subsections, the sampler chose a well

with three criteria. First, the well location had to be within 1/4 mile of an actively managed blueberry field for which hexazinone was used. Also, the well was required to be downgrade of the blueberry field. Finally, it had to be a private domestic source, currently used for drinking water. Wells from the selected residences were sampled in 1994, 1998 and 1999. Wells that tested positive for hexazinone were assayed in subsequent years. Some wells were sampled each of as many as three years, while others were sampled only once.

Sample Collection

Samples were collected in duplicate 1 L non-actinic residue-free bottles (Fisher Scientific, Pittsburgh, PA). Before collection, the water at each site was allowed to run for 5 minutes, to purge the plumbing. Samples were stored in coolers, over ice and transported to the University within 2 days of collection. Samples were stored at 5° C for no longer than 2 days before extraction. Sample extracts were stored at –20° C until they could be analyzed by HPLC.

Sample Analysis

All samples for both the Part I and Part II studies were analyzed using the SPE and HPLC procedure developed by Perkins and Bushway (1999), listed below.

Extraction

All water samples were extracted using tC-18 SPE cartridges (Waters Assoc., Milbridge, MA) and a 12 port Vac-Elute system (J.T. Baker, Phillipsburg, NJ). The extraction cartridges were prepared by treating with 5 mL methanol, followed by 5 mL of deionized water. Samples were passed through the cartridges at a rate of 10 mL per minute. Care was taken to ensure that the cartridges did not dry out during sample

extraction. Five hundred mL sample volumes were used for part I, while 1000 mL volumes were used for part II. After the entire volume of sample passed through, the SPE cartridges were dried under vacuum for 20 minutes, to remove all traces of moisture. The dried cartridges were eluted with 4 mL of 90:10 (methyl-tert-butyl ether:ethyl acetate) and collected in a 7 mL sample vial. Sample eluates were brought to dryness under a stream of nitrogen and re-constituted in 40:40:20 (acetonitrile:water:methanol) using a sonicating water bath. Re-suspended samples were filtered with 0.45 μm PTFE discs (Fisher Scientific, Pittsburgh, PA) before injecting to the HPLC system.

HPLC Analysis

The HPLC system consisted of a Hewlett Packard model 1050 isocratic pump, auto sampler and diode array detector. The analytical column was a Zorbax C-8, 5 μm, 250 x 4.6 mm. The mobile phase was a mixture of 40:40:20 (acetonitrile:water:methanol) and the flow rate was set at 1.0 mL per minute. The signal was monitored at 247 nm and the UV spectra was collected from 190 to 450 nm. Data was collected using HP Chemstation (version A03.01) software.

Hexazinone analytical standard was obtained from the EPA repository (Fort Meade, MD). A stock solution of the standard was prepared by dissolving 25 mg in 25 mL of acetonitrile. The stock solution was stable for at least six months, when stored at –20° C. A working solution of 776 ng/mL was prepared, weekly by diluting an appropriate aliquot of stock solution in 25 mL of the mobile phase.

Fifty μL of standard and each sample were injected into the HPLC system. Quantification of hexazinone was accomplished by comparing the peak area response for the samples with peak area of the standard, using the following equation:

$$\frac{\text{Sample Area (MAU)}}{\text{Standard Area (MAU)}} \times \text{Standard concentration (ng/ml)} \times \frac{\text{Final Sample Volume (ml)}}{\text{Original Sample Volume (ml)}}$$

Confirmation for water samples showing positive response for hexazinone was accomplished by comparing the sample UV spectra with the standard UV spectra.

Results and Discussion

Part I - Long-Term Monitoring of Contaminated Wells

Chromatograms for the hexazinone standard and an extract from well 9 are illustrated in figures 10 and 11. The target analyte elutes at 5.4 minutes and is resolved from any interfering peaks. The spectra from the standard and from well 9 are superimposed in figure 12. This spectrum is unique to hexazinone, which aids in the confirmation of positive samples, and also provides valuable peak purity information. Ground water extracts tend to be very clean, and interfering compounds (peaks) i.e., humic acid fractions are generally not a problem.

Results for the monthly analysis of Well 9 for residual hexazinone from 1992 to 2001 are listed in table 13. Also included in this table is a column containing the mean hexazinone concentration for each sampling year. Monthly residues for each year are also shown in figure 13. This graph illustrates the low variability of hexazinone levels between months, within the same year. It should be noted that although there were a number of months that this well was not sampled, the hexazinone levels have declined steadily over the years. The field in which this test well is located has not been treated with Velpar after 1993, because of concern over high (29 μg/L) concentrations of the herbicide. The shallow depth of the well, the sand-gravel soil structure and the poor vegetative cover, all have contributed to this unusually high hexazinone level. The mean

concentrations for each year are plotted in figure 14, which shows a progressive decline in Velpar concentrations since use of the herbicide was halted on this field.

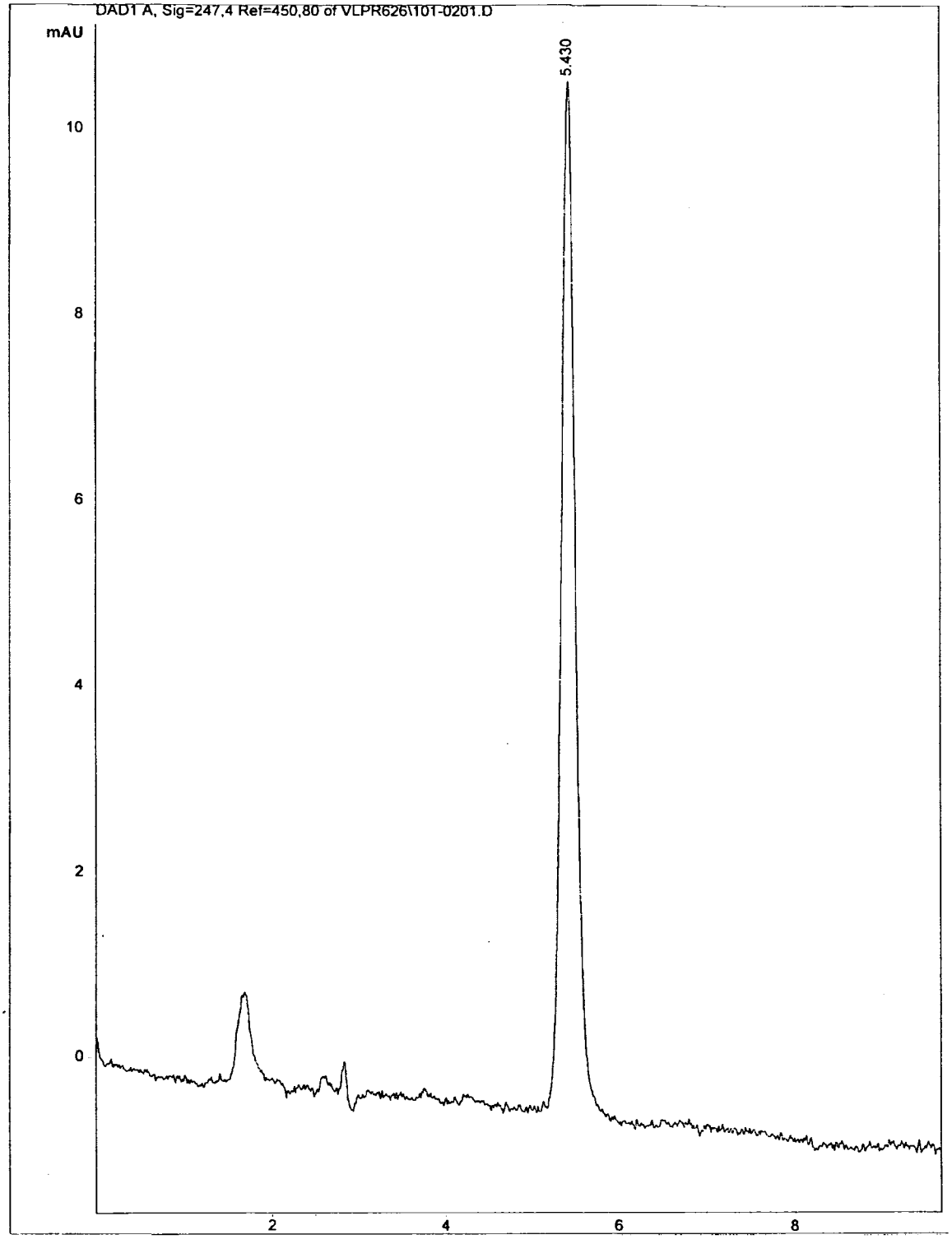

Figure 10. HPLC-DAD Chromatogram of a Hexazinone Standard

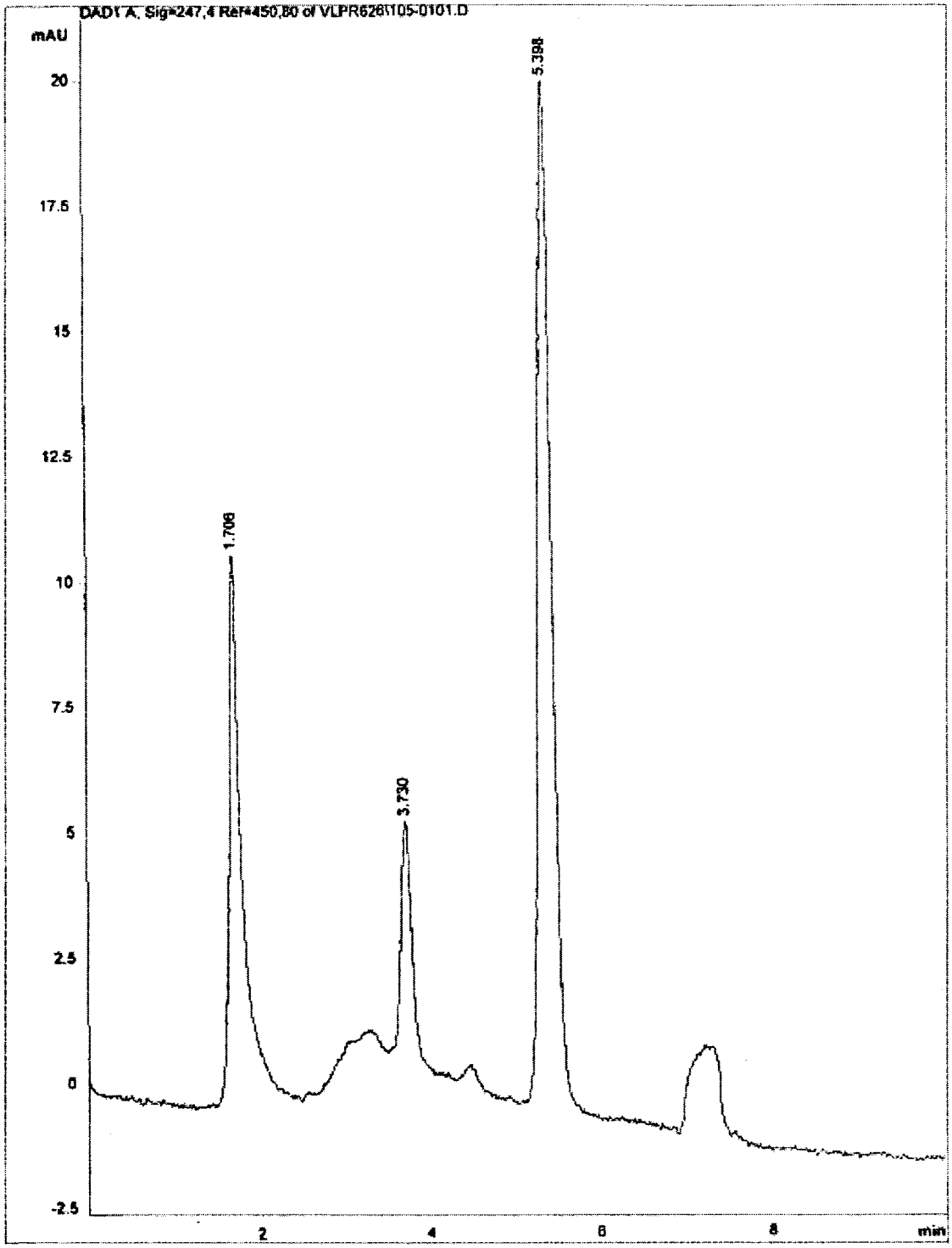

Figure 11. HPLC-DAD Chromatogram of an Extract from Well 9

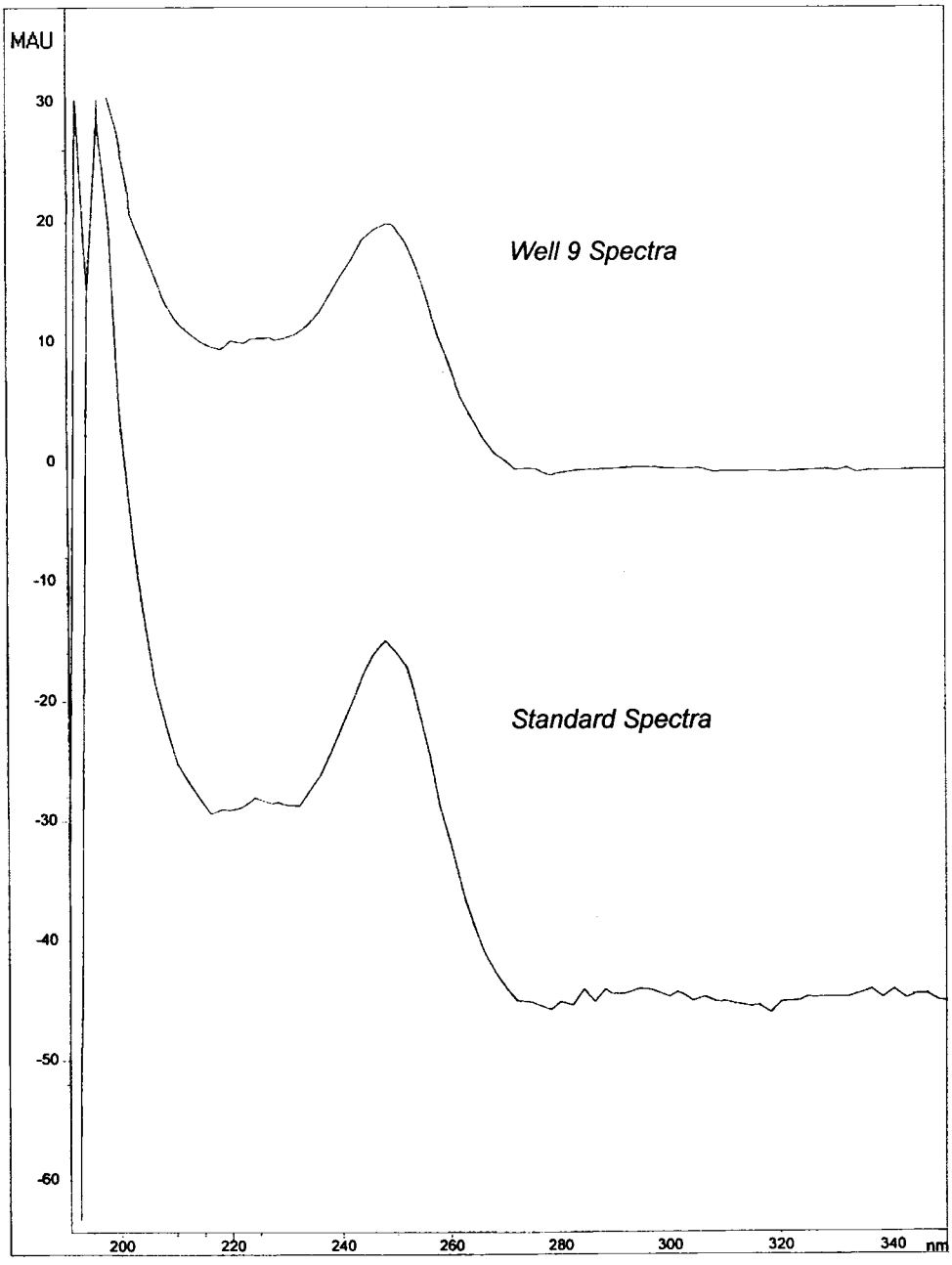

Figure 12. Superimposed Hexazinone Spectra for Standard and Sample Extract

Table 13. Well 9 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean (µg/L)
1992	*	25	22	*	*	*	26.7	24.57
1993	*	*	26.8	18.7	25.1	25.4	29	25.00
1994	*	25.4	19.6	24.9	23.1	23.9	*	23.38
1995	*	17	19	18	20	13	12.1	16.52
1996	*	15.4	13.4	13.9	10.5	11.2	8.9	12.22
1997	9.5	7.8	8.9	*	8.3	9.7	*	8.84
1998	*	9.5	8.5	11.2	10.4	3.8	5.9	8.22
1999	*	8.4	4.5	5.2	4.5	*	4.5	5.42
2000	*	2.7	2.9	2.3	2.5	1.5	*	2.38
2001	*	0.74	0.75	0.74	0.8	*	2.67	1.14

* not sampled or missing data

Note - no hexazinone treatment after 1992

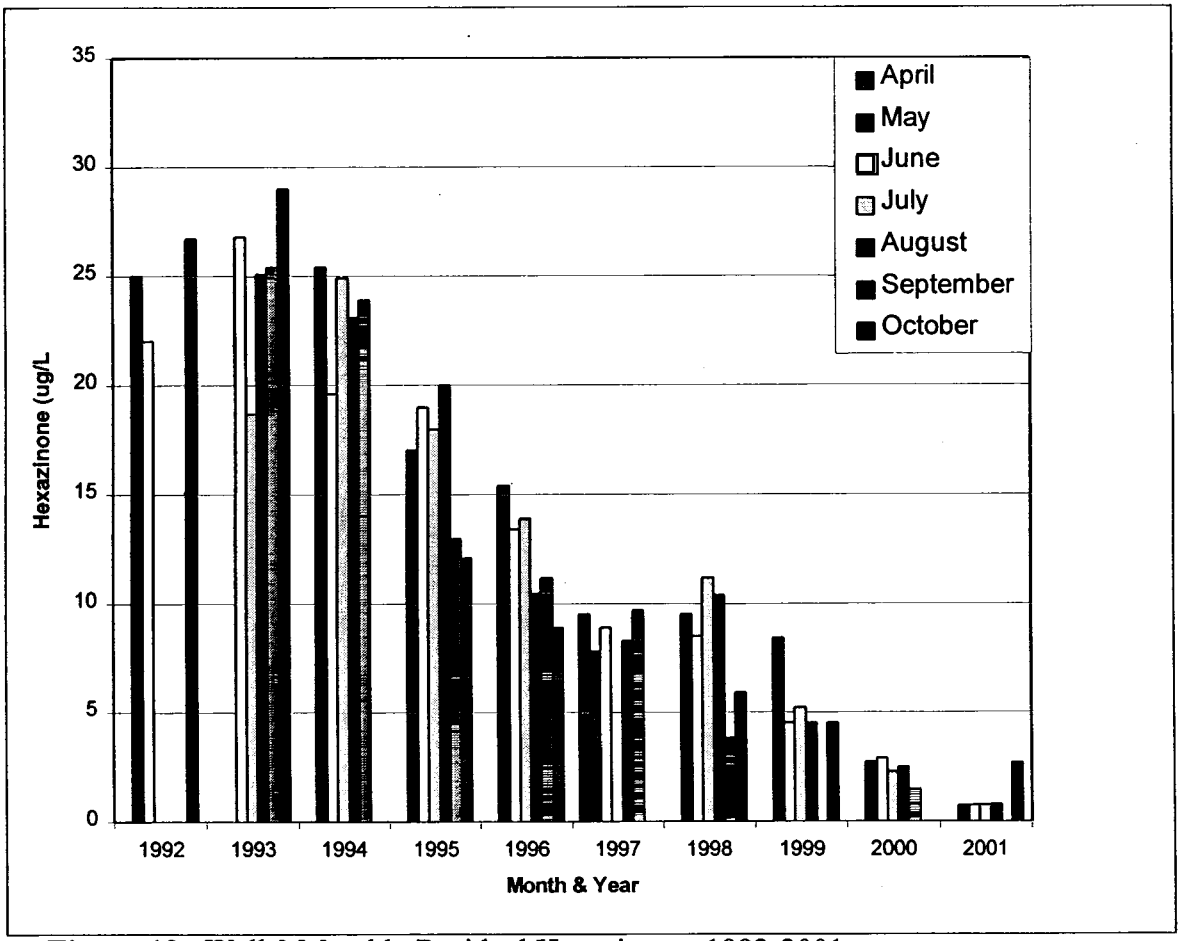

Figure 13. Well 9 Monthly Residual Hexazinone: 1992-2001

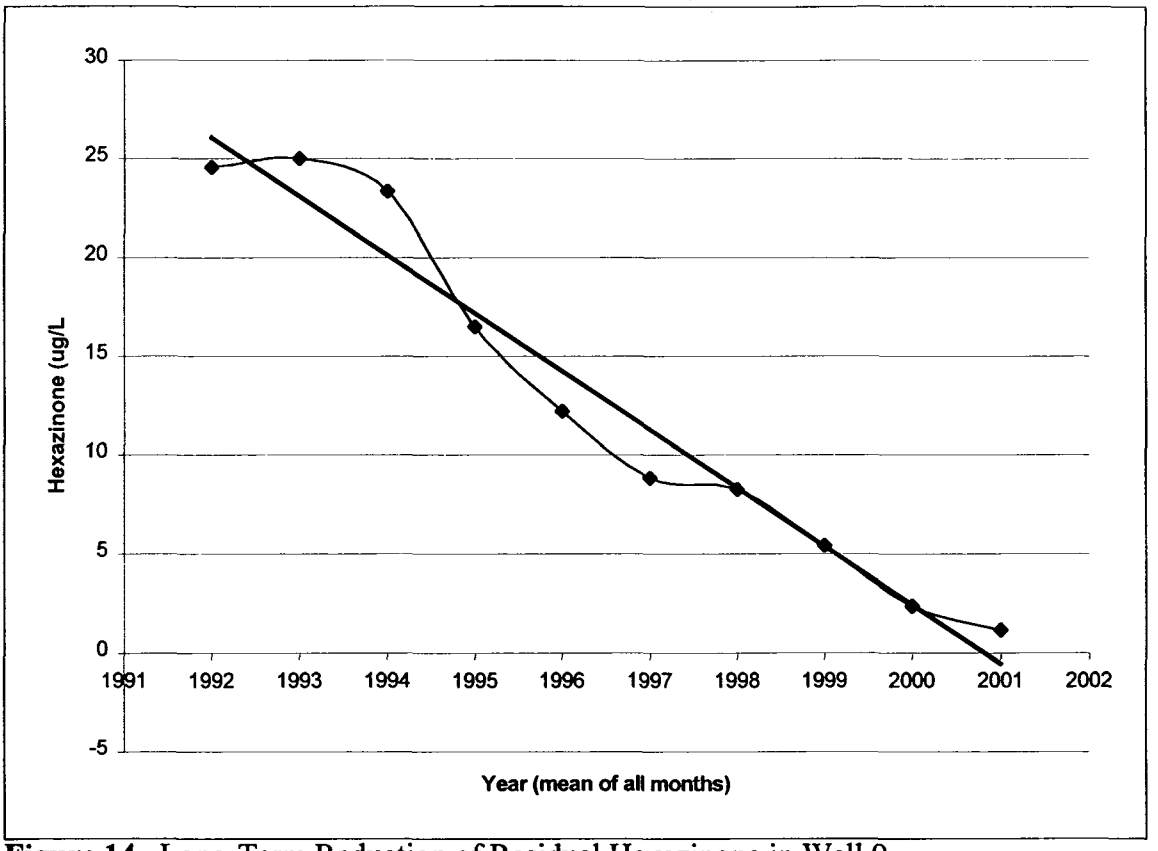

Figure 14. Long-Term Reduction of Residual Hexazinone in Well 9

Site 11 is located on the Deblois Plain, a very flat area covered by hundreds of acres of intensively managed blueberry fields. This test well is also at a relatively shallow depth of 35 ft. The areas surrounding Well 11 have been treated with a liquid formulation (Velpar L) since at least 1992. This is the most water-soluble form of hexazinone, and is therefore expected to move quickly through the soil profile. Table 14 lists the monthly hexazinone levels for the years of 1993 – 2001. These monthly values are graphed in figure 15 and range from a high 11.6 of to a low of 0.31 µg/L. This low value, although included in the reported data, is likely the result of laboratory error(s). Likely errors include improper preparation of the SPE cartridge, or incomplete drying of the cartridge before elution with the MTBE/EA solvent.

Table 14. Well 11 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean (µg/L)
1993	*	9.4	8.2	13.2	7.5	•	11.6	9.98
1994	•	8.9	7.6	4.3	10.5	11.2	*	8.50
1995	*	10	*	•	10.5	8.2	6.9	8.90
1996	•	6.9	7.4	4.2	5.8	5.5	4.3	5.68
1997	6.5	6.2	7.8	*	6.2	9.5	*	7.24
1998	*	8.9	11.5	5.4	5.6	8.2	7.9	7.92
1999	*	5.8	7	4.6	6.3	*	4.8	5.70
2000	*	2.9	3.2	3.4	2.3	2.6	*	2.88
2001	*	2.61	0.31	3.34	5.72	*	2.61	2.92

• not sampled

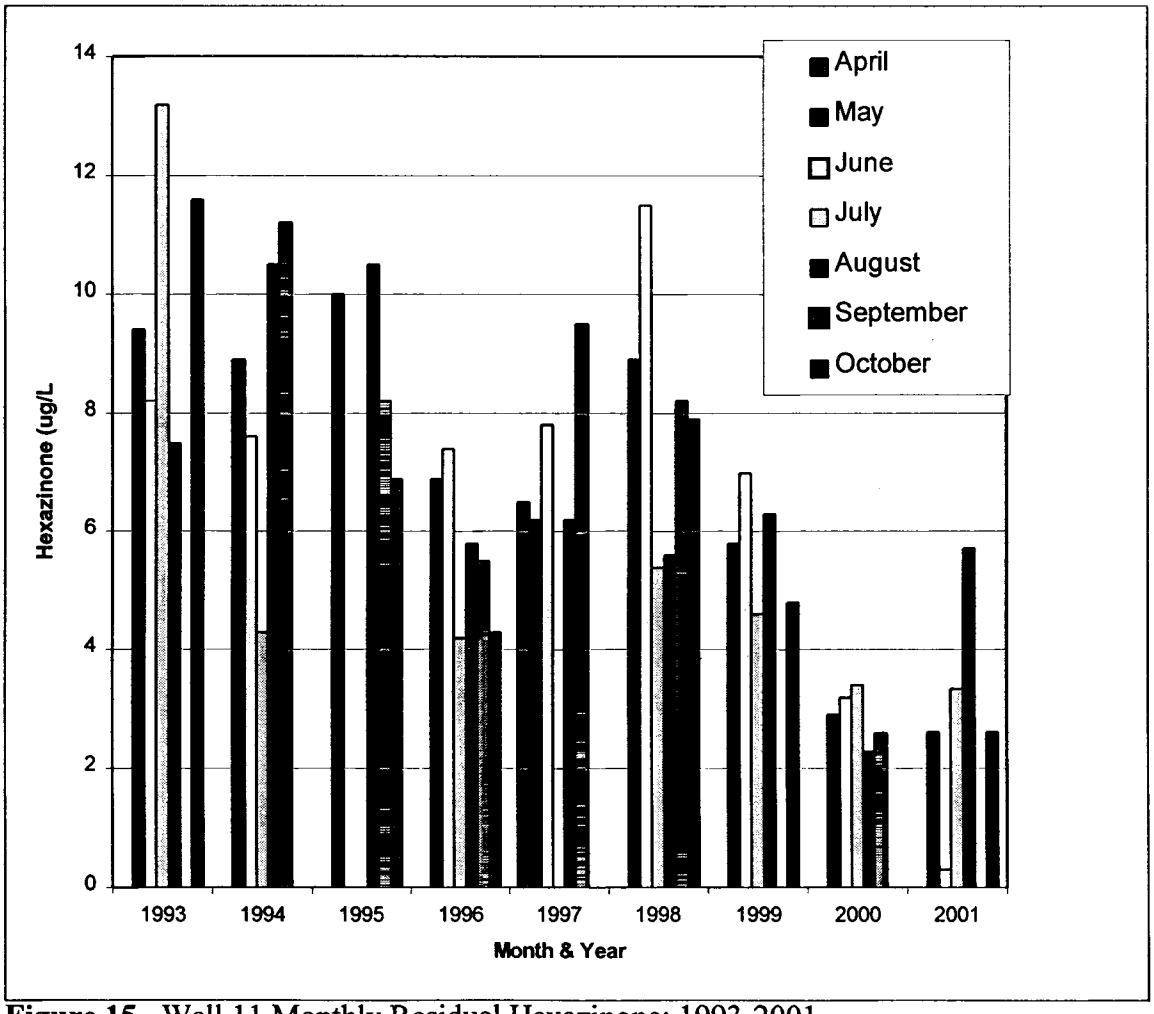

Figure 15. Well 11 Monthly Residual Hexazinone: 1993-2001

The long-term residual trend for hexazinone in Well 11 is downward (figure 16) however, levels did increase slightly in 1997 and 1998. This may be the result of a dry summer in 1996, followed by increased rainfall in the following two years. The falling concentrations of hexazinone in 1999 – 2001 may be a combination of below normal precipitation, coupled with improved management practices of the fields associated with this site.

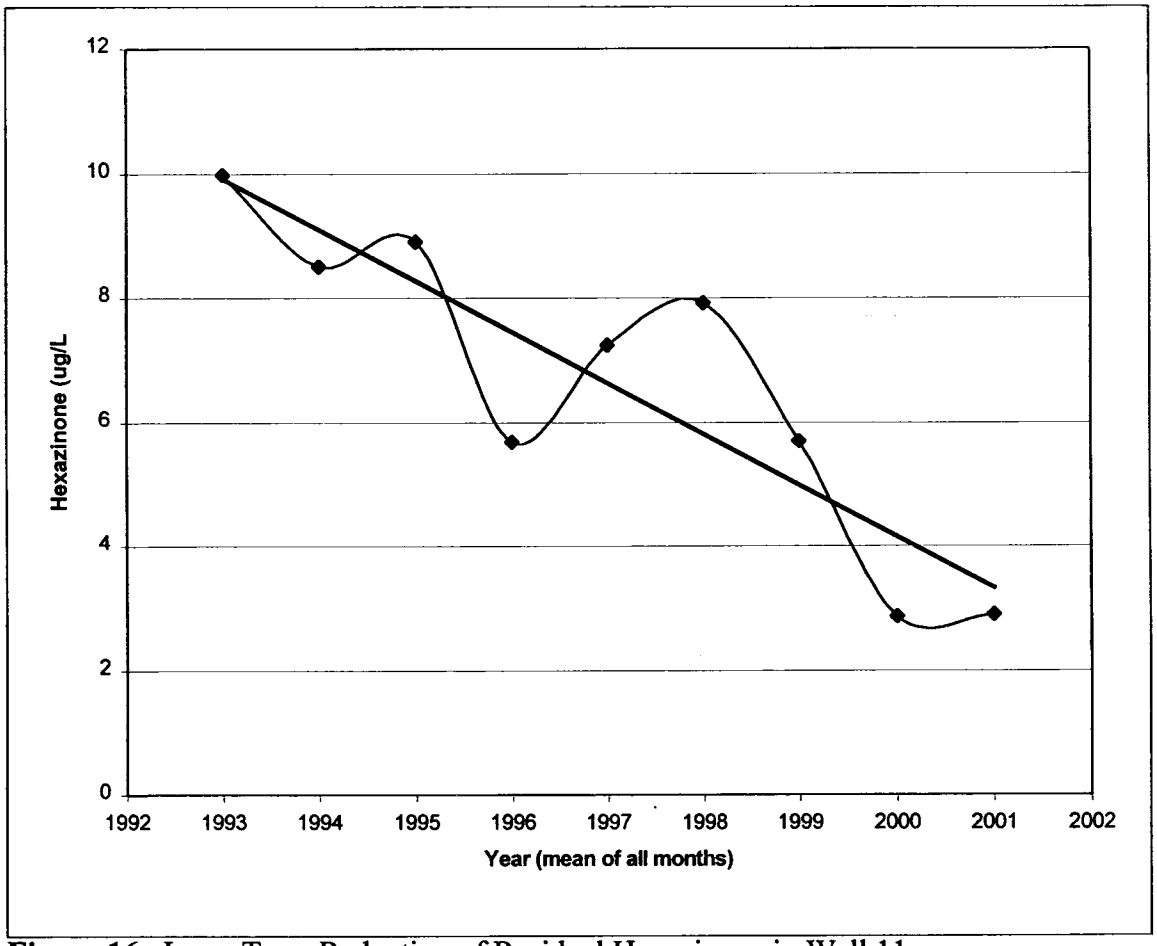

Figure 16. Long-Term Reduction of Residual Hexazinone in Well 11

Test Well 12 is situated on the elevated area of Pineo Ridge. It is shallow (25 ft.) and has been treated with Pronone G (granulated hexazinone) since 1994. Table 15 lists the data collected from this source from 1993 – 2001. Hexazinone levels in 1993 were

consistently lower (1 µg/L) than in any other year (figure 17). This phenomenon can be explained by the fact that the surrounding fields were treated with the terbacil instead of hexazinone in 1993. Data from this site indicates that several forces could influence hexazinone movement into the water table. First, it was observed that within one year after treatment resumed, the residual ground water levels increased to 10 µg/L. This indicates that hexazinone (even in a slow-release granular formulation) can move quickly into the ground water. The data from 1994 shows an almost constant increase in hexazinone concentration as the season progresses. This pattern follows the partitioning of the herbicide through the soil horizon. In subsequent years the hexazinone eventually reaches an equilibrium concentration within the organic horizon and is released, at a relatively constant level into the sandy horizons, where it moves freely with the solvent (water) front. Figure 18 plots the long-term trend for Well 12. There is no pattern followed for hexazinone concentration over time, however, the past two years have shown a downward trend. This may be a result of a recent drought.

Table 15. Well 12 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean
1993	*	*	1	0.9	1.2	*	1.4	1.125
1994	*	1.3	2.2	4.3	10.5	11.2	29.5	9.83
1995	*	4.2		4.3	5.5	3.7	3.3	4.20
1996	*	2.2	4	3.2	1.2	3.3	3.1	2.83
1997	3.2	4.4	3.5	*	3.1	10.2	*	4.88
1998	*	5.4	6.2	6.7	4.8	5	3.3	5.23
1999	*	4.6	6.8	8.5	7.6	*	*	6.88
2000	*	4.8	5.4	4.1	3.8	2.8	*	4.18
2001	*	1.94	3.62	3.51	0.34	*	*	2.35

* not sampled or missing data

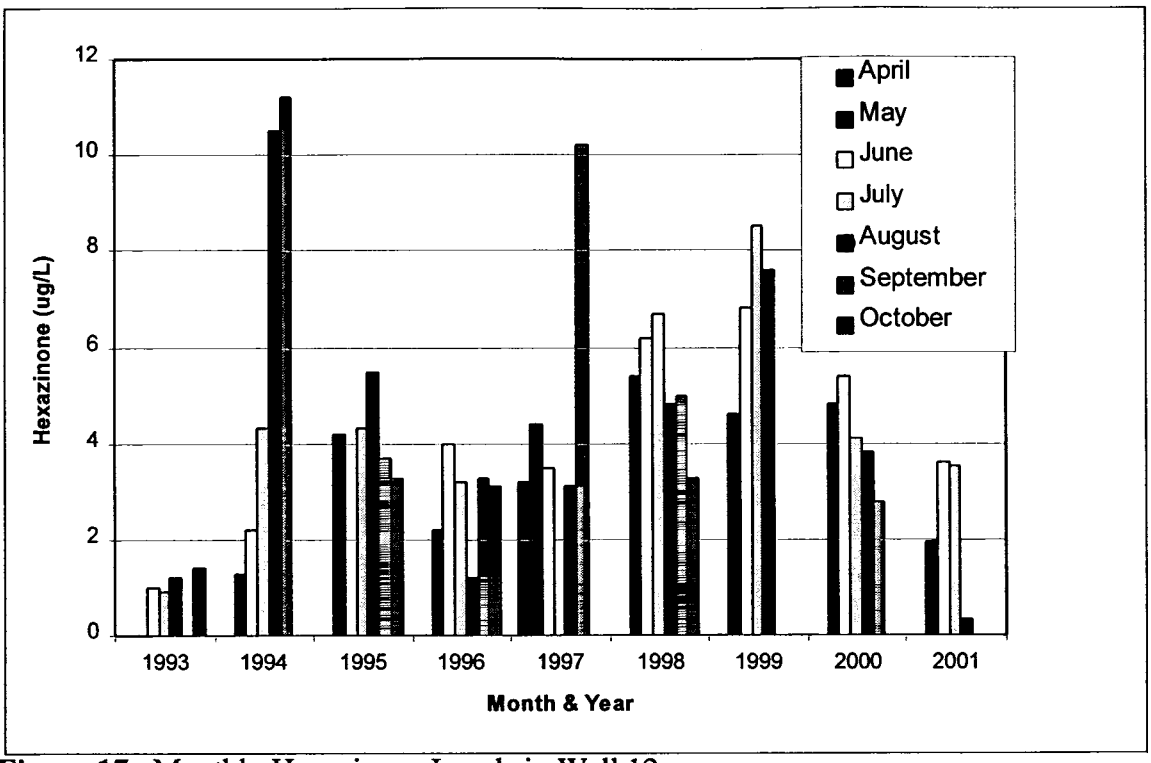

Figure 17. Monthly Hexazinone Levels in Well 12

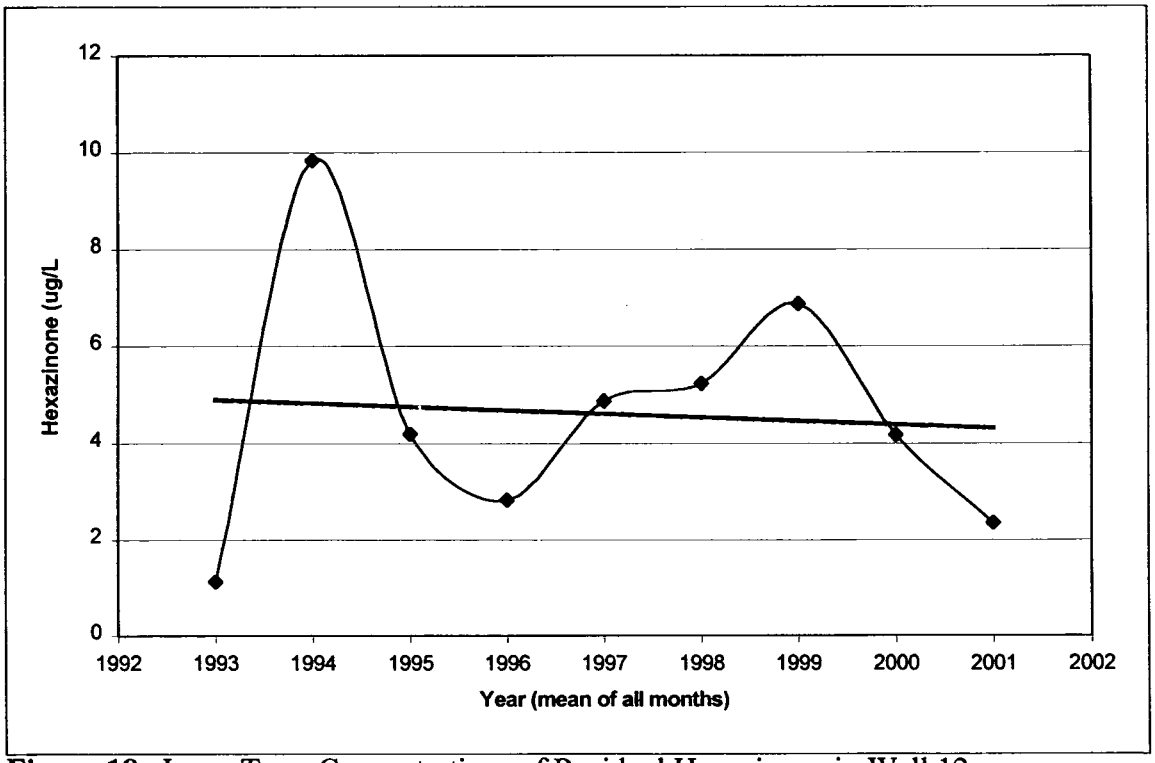

Figure 18. Long-Term Concentrations of Residual Hexazinone in Well 12

Well 13 is the water supply for the Airline Consolidated School and is situated 500 ft. from an actively managed blueberry field. Hexazinone was first detected in this well in 1993 and has been monitored for nine years (table 16). Except for the months of July in 1993 and June of 1998 (figure 19) the Velpar levels at this site were remarkably constant, especially when compared to the test wells 9, 11 and 12. These relatively stable concentrations can probably be explained by the 100 ft. depth of the well and perhaps the geological materials associated with the ground water at this location. The test wells are positioned in shallow rub down/gravel aquifers, which exhibit very localized hydrological features. Surface water percolates very quickly into the saturated zone through these porous soils. Conversely, the ground water tapped by well 13 is a much deeper source and may have over-lying materials that are less permeable, such as silt, clay or fractured bedrock. The movement of water from the surface to the saturated zone may take months or years, damping any high concentration pulses of solubilized hexazinone. The high-level spikes in 1993 and 1998 could have been caused by sudden rain events, with large volumes of water washing over the surface, running down the casing and into the well.

Table 16. Well 13 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean
1993	*	3.3	2.3	8.9	1.2	*	2.4	3.62
1994	*	2.4	2.1	2.1	2.1	2.7	*	2.28
1995	*	2.1	*	2.2	2.4	1.9	1.8	2.08
1996	*	2.2	1.8	0.3	0.2	0.6	1.4	1.08
1997	1.6	1.3	2	*	1.6	2.1	*	1.72
1998	*	2.4	6.4	2.4	2	1.8	1.5	2.75
1999	*	2.4	1.9	2.3	1.5	*	2	2.02
2000	*	1.6	1.8		1.7	1.4	*	1.63
2001	*	*	2.3	2.12	nd	*	nd	2.21

* not sampled nd - none detected at method limit

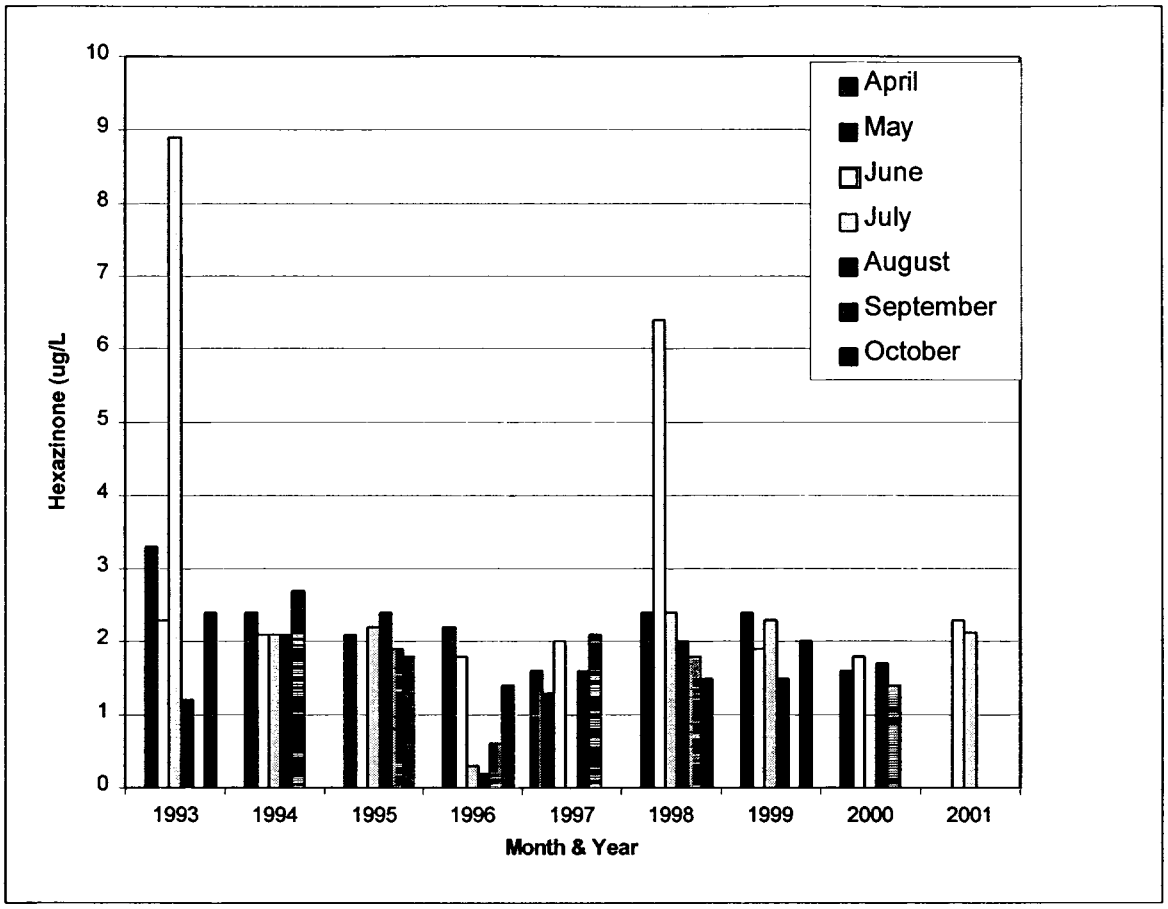

Figure 19. Monthly Hexazinone Levels in Well 13

Figure 20 depicts the average annual hexazinone concentrations from 1993 to 2001. The unusually high fluxes of the herbicide in 1993 and 1998 are reflected by the skewed line graph. A trend-line added to this graphic indicates that residual hexazinone has dropped slightly over the years, after a Velpar impregnated DAP regimen was begun in 1993.

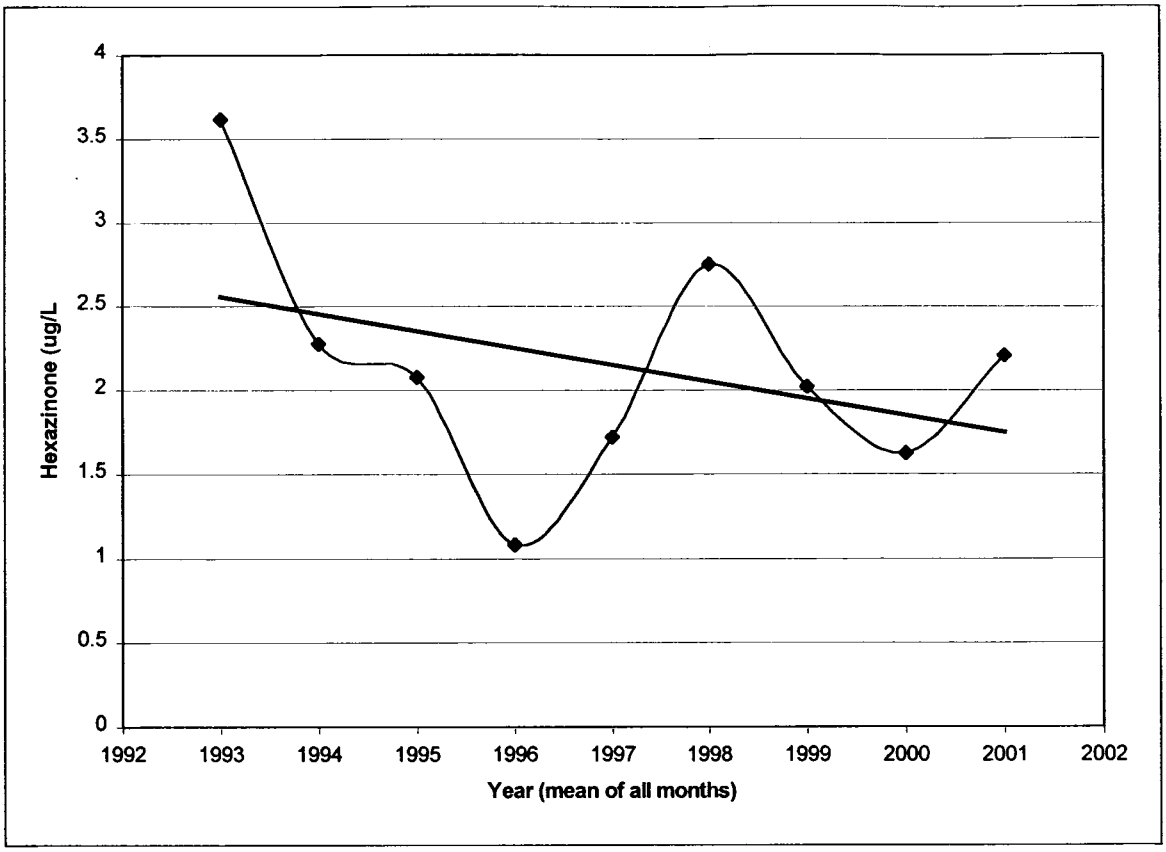

Figure 20. Long-Term Concentrations of Residual Hexazinone in Well 13

Site 23 has a drilled well, which provided potable water (prior to 1999) and is located in the southern costal town of Waldoboro. The monthly data for this site is listed in table 17. Water from the location was monitored because of its proximity to the southern zone of Maine blueberry production. After 1994, this well showed relatively stable hexazinone concentrations (figure 21), with average annual levels ranging from 1.5 to 2.1 µg/L (figure 22). This tendency may be due to a combination of lower use rates and the DAP impregnated formulation. Because residual hexazinone was relatively constant and the well is no longer used, sampling at this site was discontinued in 1999.

Table 17. Well 23 Residual Hexazinone (µg/L)

	Month						
Year	April	May	June	July	August	September	October
1993	*	*	*	*	*	*	*
1994	*	2.2	4.4	2.6	4.8	4.9	*
1995	*	*	*	2	2.6	2	1.5
1996	*	2.3	1.9	2.3	0.3	1.3	1.5
1997	1.8	1.7	2.9	*	1.6	2.3	
1998	*	2.3	1.6	1.7	*	1.3	0.5
1999	*	2		*	*	*	*
2000	*	*	*	*	*	*	*
2001	*	*	*	*	*	*	*

* not sampled

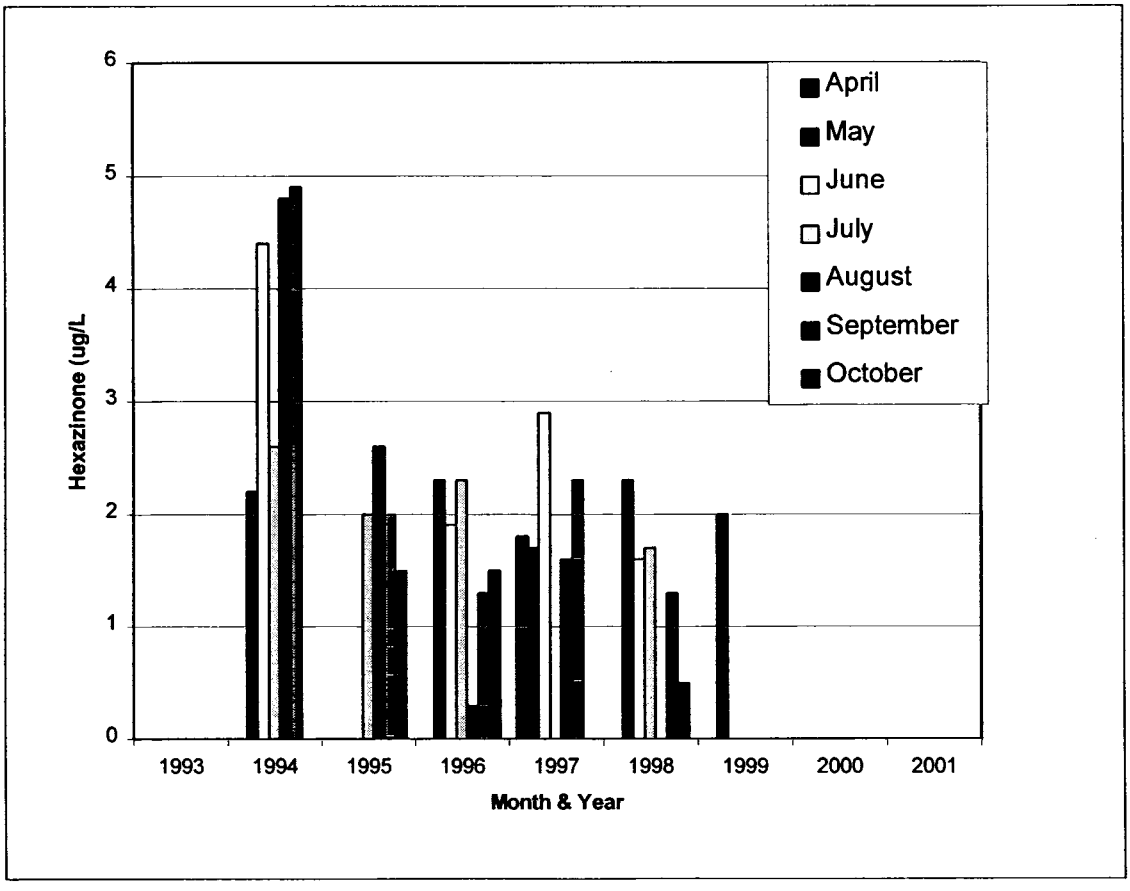

Figure 21. Monthly Hexazinone Levels in Well 23

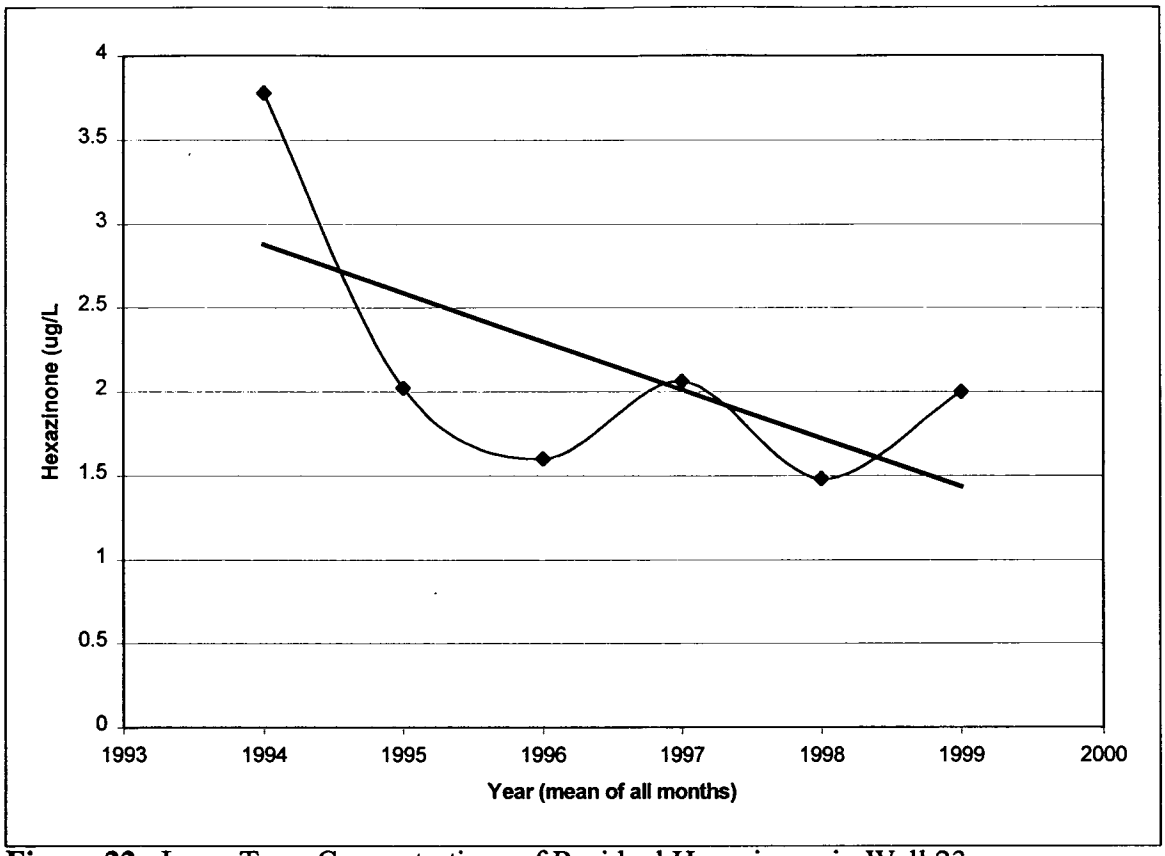

Figure 22. Long-Term Concentrations of Residual Hexazinone in Well 23

Sites 31 and 32 are located in Stockton Springs near a field where Pronone G is used for weed control. Well 32 was drilled to 245 feet. Well 31, downgrade from 32, was also drilled, but its depth is not known. The monthly hexazinone levels for these sites are listed in tables 18 and 19. In 1997, residual hexazinone in Well 32 increased from a relatively stable level of 10 µg/L to 105 µg/L (figure 23).

Theoretically, such a large pulse of hexazinone should not suddenly appear in water collected from a 245 ft. depth. Because this well is located near the staging area for hexazinone application it is quite possible that this site was contaminated by a point source spill.

To test this premise, Yarborough (1997) compared residual Velpar levels with this site with other areas of the field. Yarborough found that concentrations of hexazinone in soil from the staging area were four to ten times higher than soil from other spots in the field. This data combined with the observation that the staging area was also free of vegetation supported the conjecture of an accidental spill. Together with several regulatory agencies, Yarborough surmised that the hexazinone was transported into the groundwater by one of two means. First, a heavy precipitation event or snowmelt could have carried the herbicide down the outside of the well casing, which seems particularly likely since the contamination event seems to have occurred while the ground was still frozen. Another possible infiltration route could be through fractured bedrock. The well is located on land with a shallow soil of 20 to 30 inches, which is classified as Tunbridge/Lyman. Hexazinone could move quickly through this porous earth and rapidly seep through cracks in the underlying bedrock.

Data from the well (figure 24) reveal a steady decrease in residual hexazinone from 1997 through the year 2001, when levels averaged 8.2 μg/L. This decrease supports the argument for a single point source pollution event.

Table 18. Well 32 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean
1993	*	*	*	*	*	*	*	*
1994	*	*	*	*	*	*	*	*
1995	*	*	5.6	4.5	*	4.5	3.6	4.6
1996	*	9.7	7.8	6.5	*	10.7	11.8	9.3
1997	105	54	29.5		26.1	25.5	29.2	44.9
1998	*	46	36	44.6	32.7	12.3	15.4	31.2
1999	*	15.3	14	18.2	13.3	*	16.7	15.5
2000	*	13.6	12.1	12	11.6	9.1	*	11.7
2001	*	1.63	11.6	9.5	8.35	*	10.1	8.2

*not sampled

Table 19. Well 31 Residual Hexazinone (µg/L)

	Month							
Year	April	May	June	July	August	September	October	Mean
1993	*	*	*	*	*	*	*	
1994	*	*	*	*	*	*	*	
1995	*	*	3.9	3.9	4.8	3.3	8.8	4.9
1996	*	3.9	3.9	3.7	11.4	3.8	5.6	5.4
1997	1.9	3.6	6.3	*	5.1	7.1	6.4	5.1
1998	*	2.8	2.4	6	*	5.4	4.3	4.2
1999	*	5.7	5.7	4.9	4.9	*	4.3	5.1
2000	*	3.6	3.2	4.2	4.1	2.9	*	3.6
2001	*	2.7	4.7	5.3	2.7	*	5.2	4.1

* not sampled

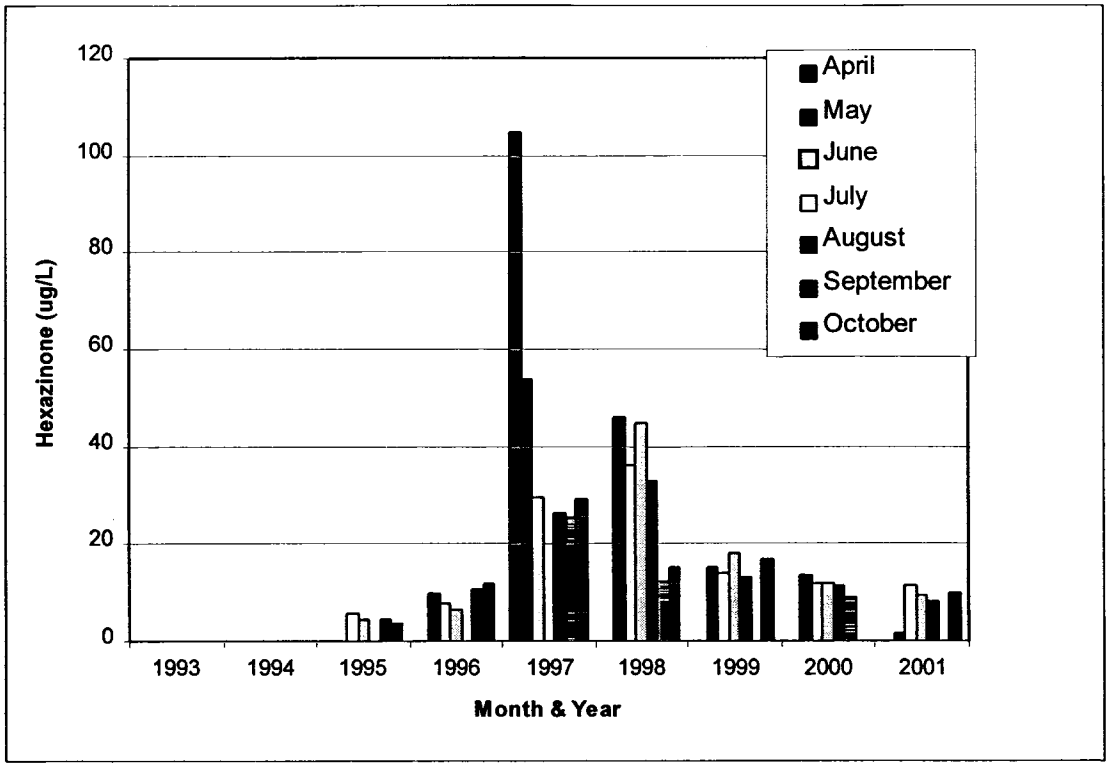

Figure 23. Monthly Hexazinone Levels in Well 32

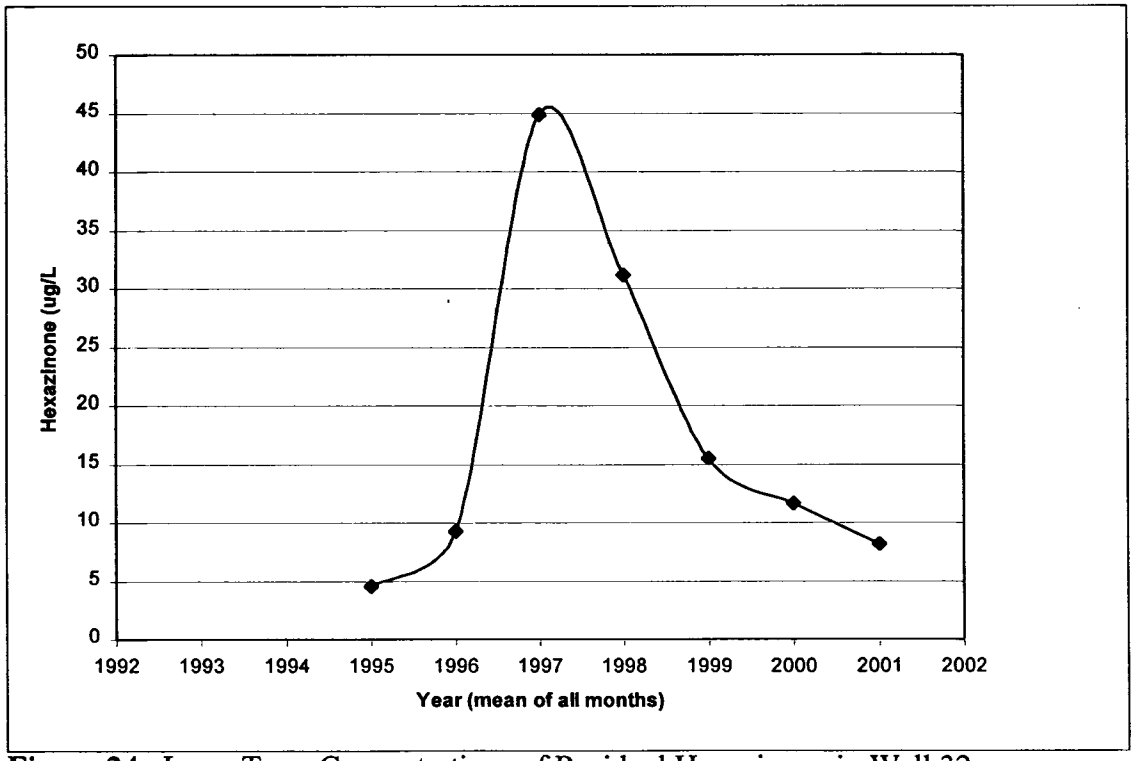

Figure 24. Long-Term Concentrations of Residual Hexazinone in Well 32

Figure 25 shows monthly hexazinone levels for the years 1995 through 2001 for well 31. Spikes of 8.5 µg/L in April, 1995 and 11.5 µg/L in May of 1996 are the only aberrations in what are otherwise relatively stable hexazinone concentrations. It is of interest to note that these two elevated Velpar values occurred before the 1997 pulse in Well 32. One might expect to see elevated levels of the herbicide in Well 31 due to its downgrade position from Well 32. Conversely, figure 26 indicates that a downward trend in hexazinone concentration was observed, supporting the notion that one or both of the following situations could have occurred. First, if the hexazinone contamination at Well 32 occurred because of surface water running down the well casing, the pulse could have been very localized and would have been quite dilute before reaching the water source at Well 31. Also, because the depth of Well 31 is not known, it is quite possible that this well taps a different water source.

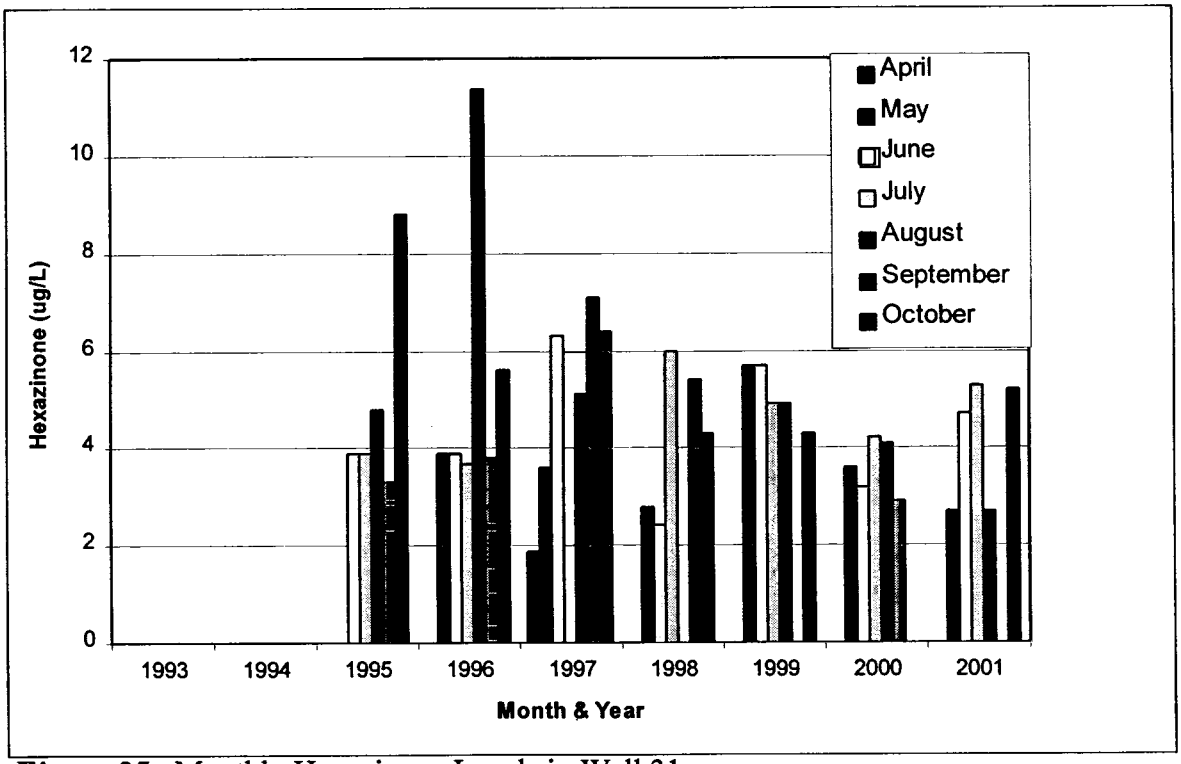

Figure 25. Monthly Hexazinone Levels in Well 31

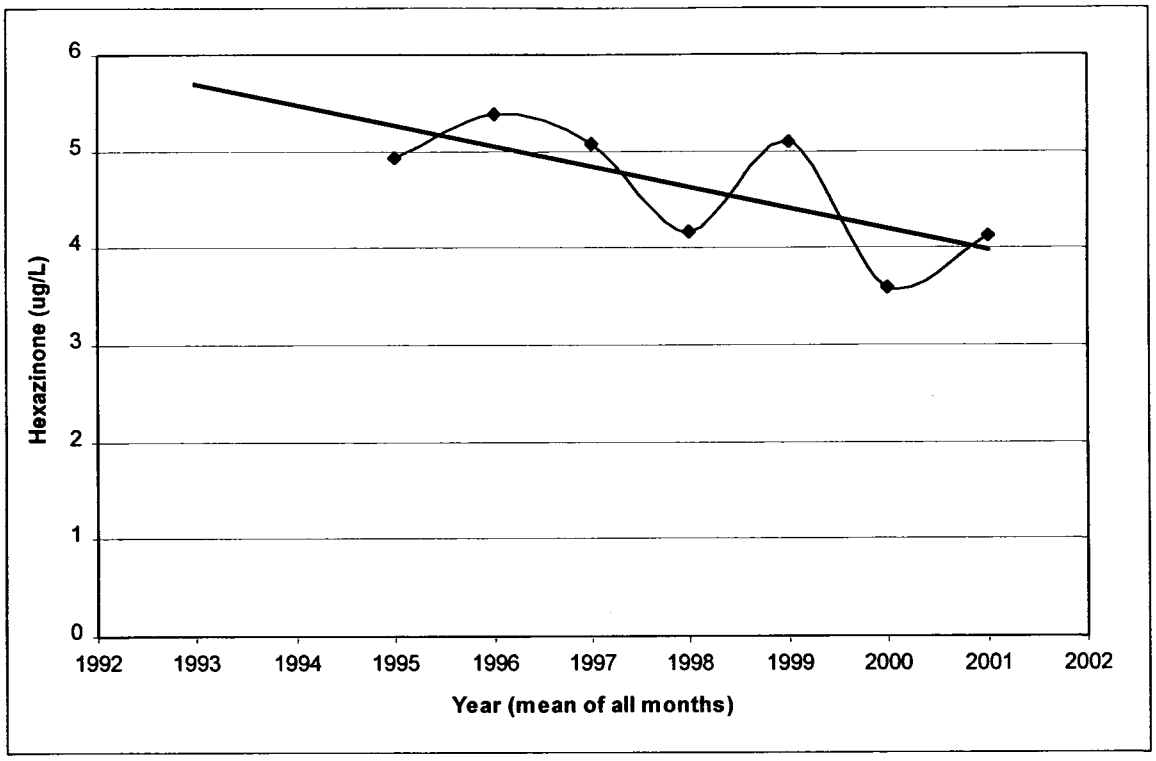

Figure 26. Long-Term Concentrations of Residual Hexazinone in Well 31

Part II - Monitoring of Randomly Selected Wells

Beginning in 1994, a number of private wells were monitored for residual hexazinone by the MWBPC and the University of Maine Chemical Food Safety Laboratory. Samples were collected from 8 of Maine's 16 counties, with the majority coming from the blueberry producing areas in Washington, Waldo, Hancock, Lincoln and Kennebec (figure 27). Almost half the wells were in Washington County due to its high concentration of blueberry agriculture.

Results for the analysis of hexazinone from these sources are listed in table 20 in a county-by-county format. The limit of quantification for this study was 0.1 µg/L, instead

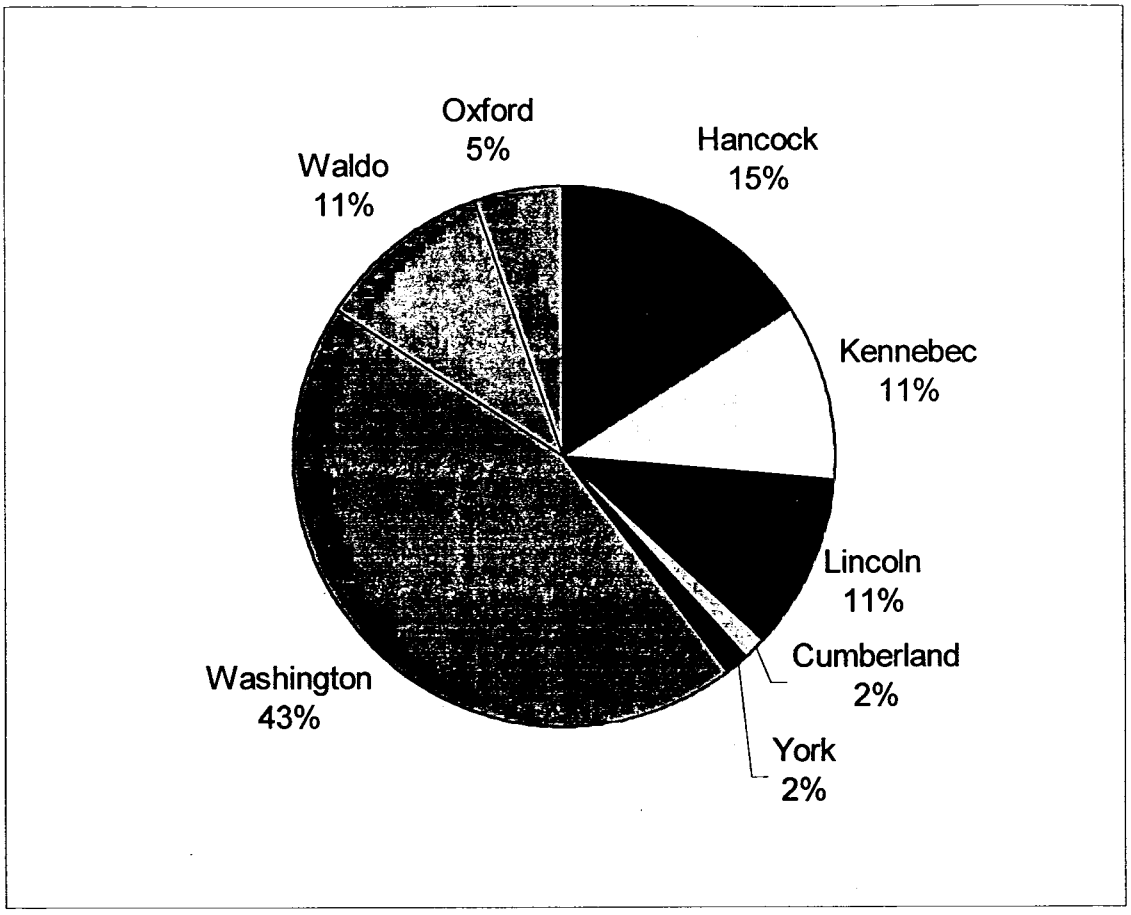

Figure 27. MBPC Private Well Water Samples for Hexazinone – by County

of the 0.2 µg/L listed in the method of Perkins and Bushway (1999). A doubling of sample volume from 0.5 to 1.0 L was responsible for this increase in sensitivity.

Of the eight counties sampled, only York and Cumberland yielded no positive outcomes. Because only one well in each county was tested, this lack of affirmation can be considered insignificant. Under ideal conditions, MBPC inspectors would have located more willing participants from these two counties for this sampling program.

Of the remaining 6 counties, Kennebec had the highest rate of positive results with 86%, where one well contained a concentration of 4.18 µg/L of the herbicide. Seventy-

one percent of the private wells in Lincoln County were positive for hexazinone, with a maximum level of 3.8 μg/L found in the town of Jefferson. Hancock County had a positive response rate of 62% with 4.9 μg/L detected in a Bucksport well. Of the 59 samples taken from Washington County, 59% contained detectable traces, with a high of 5.6 μg/L found in Wesley. Four of the seven wells in Oxford County gave positive results with 6 μg/L quantified in Otisfield. Finally, 50% of Waldo Counties private water sources located near blueberry agriculture showed traces of hexazinone, with the highest concentration detected only 1.2 μg/L.

In addition to the survey of rural inhabitants exposure to hexazinone via drinking water, another goal of this study was to measure seasonal and long-term changes of the herbicide in groundwater sources. To this end, many of the wells in the study were sampled up to 3 times, usually before the spring thaw (prior to surface water infiltration) and again in the late summer or early fall. It was theorized that infiltration of recently applied Velpar would occur during the spring and summer months, raising residual levels by late in the season. Table 21 shows that no real pattern emerged. Levels were higher (by at least 20%) in the February/March period as often as in the months of August and September. This result is not surprising, since little is known about soil types or aquifers associated with each groundwater system. Furthermore, hexazinone is applied biennially, so sampling of these sites over several more years would be needed in order to see any emerging patterns. Finally, little was known about formulation types, application rates or rainfall patterns at any of these of locations. The extent of each of these and other variables is probably quite large.

Table 20. Hexazinone in Private Wells Sampled by the MBPC (1994, 1998 & 1999)

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
05BPCG005	15-Mar-94	2.57	Gouldsboro	Hancock
05BPCG005	13-Sep-94	2.18	Gouldsboro	Hancock
05BPCG005	26-Feb-98	ND	Gouldsboro	Hancock
05BPCG006	5-Apr-94	4.78	Bucksport	Hancock
05BPCG006	1-Sep-94	4.88	Bucksport	Hancock
05BPCG007	1-Sep-94	4.29	Bucksport	Hancock
05BPCG007	4-Mar-98	ND	Bucksport	Hancock
05BPCG008	13-Sep-94	0.17	Prospect Harbor	Hancock
05BPCG008	26-Feb-98	0.34	Prospect Harbor	Hancock
05BPCG009	1-Sep-94	ND	Penobscot	Hancock
05BPCG010	13-Sep-94	3.74	Gouldsboro	Hancock
05BPCG011	1-Mar-99	ND	Penobscot	Hancock
05BPCG013	26-Mar-99	ND	Ellsworth	Hancock
05BPCG014	26-Mar-99	0.23	Lamoine	Hancock
05BPCG015	29-Mar-99	ND	Surry	Hancock
05BPCG016	29-Mar-99	0.22	Surry	Hancock
05BPCG016	18-Jan-00	ND	Surry	Hancock
05BPCG017	29-Mar-99	0.22	Hancock	Hancock
05BPCG020	30-Mar-99	ND	Gouldsboro	Hancock
05BPCG021	30-Mar-99	0.32	Gouldsboro	Hancock
05BPCG022	31-Mar-99	0.21	Franklin	Hancock
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
16	**21**	**13**	**61.9**	**<0.1- 4.88**

Table 20. Cont.

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
06BPCG026	25-Mar-94	0.093	Vienna	Kennebec
06BPCG026	12-Sep-94	0.1	Vienna	Kennebec
06BPCG026	25-Feb-98	0.30	Vienna	Kennebec
06BPCG027	4-Apr-94	ND	Mt. Vernon	Kennebec
06BPCG028	6-Apr-94	0.550	Gardiner	Kennebec
06BPCG028	30-Aug-94	3.65	Gardiner	Kennebec
06BPCG028	25-Feb-98	0.30	Gardiner	Kennebec
06BPCG030	30-Aug-94	4.18	Gardiner	Kennebec
06BPCG030	25-Feb-98	1.16	Gardiner	Kennebec
06BPCG031	30-Aug-94	0.13	Gardiner	Kennebec
06BPCG031	25-Feb-98	ND	Gardiner	Kennebec
06BPCG032	12-Sep-94	0.57	Mt. Vernon	Kennebec
06BPCG033	12-Sep-94	0.67	Mt. Vernon	Kennebec
06BPCG045	11-Mar-99	0.85	Vienna	Kennebec
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
8	**14**	**12**	**85.8**	**<0.1- 4.18**

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
09BPCG018	29-Mar-94	5.97	Otisfield	Oxford
09BPCG018	12-Sep-94	3.14	Otisfield	Oxford
09BPCG018	2-Mar-98	2.15	Otisfield	Oxford
09BPCG020	12-Sep-94	0.34	Otisfield	Oxford
09BPCG020	23-Feb-98	ND	Otisfield	Oxford
09BPCG021	12-Sep-94	ND	Otisfield	Oxford
09BPCG021	2-Mar-98	ND	Otisfield	Oxford
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
4	**7**	**4**	**57**	**<0.1- 5.97**

Table 20. Cont.

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
08BPCG008	4-Apr-94	1.50	Jefferson	Lincoln
08BPCG008	30-Aug-94	3.77	Jefferson	Lincoln
08BPCG008	25-Feb-98	1.66	Jefferson	Lincoln
08BPCG009	4-Apr-94	0.304	Dresden	Lincoln
08BPCG009	30-Aug-94	0.33	Dresden	Lincoln
08BPCG009	24-Feb-98	0.14	Dresden	Lincoln
08BPCG010	30-Aug-94	0.90	Dresden	Lincoln
08BPCG010	26-Feb-98	2.1	Dresden	Lincoln
08BPCG011	30-Aug-94	0.35	Jefferson	Lincoln
08BPCG011	25-Feb-98	0.33	Jefferson	Lincoln
08BPCG012	30-Aug-94	ND	Jefferson	Lincoln
08BPCG012	25-Feb-98	ND	Jefferson	Lincoln
08BPCG013	30-Aug-94	ND	Dresden	Lincoln
08BPCG013	24-Feb-98	ND	Dresden	Lincoln
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
6	**14**	**10**	**71**	**<0.1- 3.77**

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
03BPCG012	4-Apr-94	ND	Brunswick	Cumberland
03BPCG012	26-Feb-98	ND	Brunswick	Cumberland
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
1	**2**	**0**	**0**	**<0.1**

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
16BPCG016	29-Mar-94	ND	Kennebunk	York
16BPCG016	23-Feb-98	ND	Kennebunk	York
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
1	**2**	**0**	**0**	**<0.1**

Table 20. Cont.

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
14BPCG004	25-Mar-94	0.707	Pembroke	Washington
14BPCG004	20-Sep-94	1.29	Pembroke	Washington
14BPCG004	9-Mar-98	0.93	Pembroke	Washington
14BPCG005	17-Mar-94	ND	Cutler	Washington
14BPCG005	25-Feb-98	ND	Cutler	Washington
14BPCG006	17-Mar-94	0.315	Lubec	Washington
14BPCG006	13-Sep-94	1.71	Lubec	Washington
14BPCG006	25-Feb-98	ND	Lubec	Washington
14BPCG007	11-Mar-94	0.844	Wesley	Washington
14BPCG007	19-Sep-94	5.56	Wesley	Washington
14BPCG008	11-Mar-94	0.494	Columbia Falls	Washington
14BPCG008	14-Sep-94	0.23	Columbia Falls	Washington
14BPCG008	23-Feb-98	ND	Columbia Falls	Washington
14BPCG009	11-Mar-94	0.251	Dennysville	Washington
14BPCG009	13-Sep-94	ND	Dennysville	Washington
14BPCG009	3-Mar-98	ND	Dennysville	Washington
14BPCG010	11-Mar-94	0.251	Steuben	Washington
14BPCG010	13-Sep-94	0.27	Steuben	Washington
14BPCG010	26-Feb-98	ND	Steuben	Washington
14BPCG011	20-Apr-94	2.53	Jonesport	Washington
14BPCG011	14-Sep-94	2.21	Jonesport	Washington
14BPCG011	23-Feb-98	0.45	Jonesport	Washington
14BPCG012	14-Sep-94	4.27	Jonesport	Washington
14BPCG012	23-Feb-98	1.52	Jonesport	Washington
14BPCG013	14-Sep-94	0.39	Columbia Falls	Washington
14BPCG013	2-Mar-98	ND	Columbia Falls	Washington
14BPCG014	13-Sep-94	ND	Lubec	Washington
14BPCG014	25-Feb-98	ND	Lubec	Washington
14BPCG015	13-Sep-94	ND	Dennysville	Washington

Table 20. Cont.

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
14BPCG016	14-Sep-94	ND	Columbia Falls	Washington
14BPCG016	23-Feb-98	ND	Columbia Falls	Washington
14BPCG017	14-Sep-94	ND	Columbia Falls	Washington
14BPCG018	14-Sep-94	0.51	Columbia Falls	Washington
14BPCG018	3-Mar-98	ND	Columbia Falls	Washington
14BPCG019	14-Sep-94	3.12	Steuben	Washington
14BPCG020	13-Sep-94	ND	Lubec	Washington
14BPCG020	25-Feb-98	ND	Lubec	Washington
14BPCG021	20-Sep-94	ND	Meddybemps	Washington
14BPCG021	9-Mar-98	ND	Meddybemps	Washington
14BPCG022	13-Sep-94	ND	Dennysville	Washington
14BPCG022	3-Mar-98	ND	Dennysville	Washington
14BPCG023	20-Sep-94	0.27	Meddy Bemps	Washington
14BPCG023	9-Mar-98	0.37	Meddy Bemps	Washington
14BPCG024	14-Sep-94	1.31	Jonesport	Washington
14BPCG024	23-Feb-98	0.57	Jonesport	Washington
14BPCG025	19-Sep-94	0.50	Wesley	Washington
14BPCG026	19-Sep-94	1.03	Wesley	Washington
14BPCG026	25-Feb-98	1.02	Wesley	Washington
14BPCG027	13-Sep-94	0.76	Steuben	Washington
14BPCG027	26-Feb-98	0.57	Steuben	Washington
14BPCG029	30-Mar-99	1.12	Cherryfield	Washington
14BPCG031	31-Mar-99	0.95	Addison	Washington
14BPCG032	31-Mar-99	0.31	Columbia	Washington
14BPCG033	1-Apr-99	0.43	Jonesboro	Washington
14BPCG034	1-Apr-99	ND	Jonesboro	Washington
14BPCG035	1-Apr-99	0.93	Machiasport	Washington
14BPCG036	1-Apr-99	ND	Cutler	Washington

Table 20. Cont.

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
14BPCG038	2-Apr-99	1.3	Alexander	Washington
14BPCG039	2-Apr-99	ND	Alexander	Washington
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
34	**59**	**35**	**59.3**	**<0.1- 5.56**

WELL ID	SAMPLE DATE	HEXAZINONE (ug/L)	CITY/TOWN	COUNTY
15BPCG016	5-Apr-94	0.183	Stockton Springs	Waldo
15BPCG016	14-Sep-94	ND	Stockton Springs	Waldo
15BPCG016	23-Feb-98	ND	Stockton Springs	Waldo
15BPCG018	5-Apr-94	ND	Belfast	Waldo
15BPCG018	23-Feb-98	ND	Belfast	Waldo
15BPCG019	1-Sep-94	0.98	Stockton Springs	Waldo
15BPCG019	2-Mar-98	1.07	Stockton Springs	Waldo
15BPCG020	1-Sep-94	0.117	Stockton Springs	Waldo
15BPCG020	23-Feb-98	ND	Stockton Springs	Waldo
15BPCG021	13-Sep-94	1.05	Stockton Springs	Waldo
15BPCG021	23-Feb-98	1.23	Stockton Springs	Waldo
15BPCG024	25-Feb-99	ND	Belfast	Waldo
15BPCG025	25-Feb-99	ND	Searsport	Waldo
14BPCG028	25-Feb-99	1.21	Stockton Springs	Waldo
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
8	**14**	**7**	**50**	**<0.1 - 1.23**

ALL COUNTIES				
Total Wells	**Total Samples**	**Positive Samples**	**% Positive**	**Range (ug/L)**
78	**133**	**81**	**60.9**	**<0.1 - 5.97**

Table 21. Spring-Fall Fluctuation of Hexazinone Levels in Private Wells

Total Wells	Higher levels in Spring (% of total)	Higher levels in Fall (% of total)	Same levels in Spring and Fall Within 20% (% of total)
16	4 (25)	5 (31)	7 (44)

Following the initial 1994 study, many wells were re-sampled in 1998. Allowing for a 20% margin of error, table 22 indicates that detectable hexazinone concentrations have dropped dramatically over a four to five year period. Of the 29 wells that were re-sampled in 1998, nineteen (two thirds) of them showed significantly reduce levels of the herbicide, while only two of the private water sources were higher. Improvement of these contamination numbers is quite likely a result of better agricultural practices, combined with improved (slow-release) formulations.

Table 22. Comparison of Residual Hexazinone Between 1994 & 1998

Total Wells	Higher levels in 1994 (% of total)	Higher levels in 1998 (% of total)	Same levels in 1994 & 1998 (% of total)
29	19 (66)	2 (7)	8 (27)

Conclusion

None of the hundreds of groundwater samples, including in-field test wells and private wells ever exceeded the 210 µg/L drinking water health advisory level set by the EPA. In fact, with few exceptions detectable concentrations of the herbicide hovered between 0.1 and 6 µg/L. Furthermore, the data presented in this chapter indicates a strong trend of

reduced contamination of groundwater from 1994 to 2001. This is probably due to improved hexazinone formulation, as well as lower usage rates and better agricultural practices.

Monitoring programs for both the MWBC and the MBPC will continue into the foreseeable future. The acquisition of GC-MS technology will allow the screening of samples for common metabolites, including met. B, which often accompanies the parent compound in contaminated ground water.

ANALYSIS OF HEXAZINONE IN SOIL

Introduction

As discussed in preceding sections, the fate and transport of hexazinone is affected by many variables, including the amount of herbicide, formulation, soil type, slope and depth to ground water. Because hexazinone is applied to blueberry fields in April and May when there is little vegetative cover, much of this systemic herbicide is actually applied directly to the soil surface. In order to maximize weed-control effectiveness and to minimize ground water contamination it is important to understand the effect of formulation type on the persistence and mobility of hexazinone. Also, unpublished observations of damage to a large blueberry field in Maine shows that a high residual level of the herbicide, under certain conditions, can damage and even kill wild blueberry plants. To these ends, controlled field studies were performed using a variety of hexazinone formulations. HPLC and EIA methods were developed to assay the soil hexazinone residues for this study.

Materials and Methods

Experimental Design

The study was carried out during the 1995 and 1997 growing seasons. In 1995 Velpar L, Pronone 10G, Pronone 10MG and Velpar/DAP were applied to field plots under controlled conditions. In 1997 the study was repeated with Velpar DF, Pronone MG and Velpar/MAP. In 1997 each plot received one inch of precipitation or irrigation per week, to insure that adequate moisture was moved through the soil profile. Soil

samples were collected periodically and analyzed by HPLC for residual hexazinone. Details for the experimental design for formulation application and sample collection are given in appendices A and B.

Sample Analysis

Soil samples for HPLC and EIA method development were collected from Florida, as well as eastern, western and southern Maine. The newly developed HPLC method was used to study the effect of formulation type on hexazinone movement at Blueberry Hill Farm in Jonesboro, Maine.

Extraction

One gram of air-dried soil was weighed into a 25 mL polypropylene bottle, followed by the addition of 5 small stainless steel ball bearings and 10 mL of 80:20 (methanol:distilled water). Samples were shaken vigorously by hand for 10 minutes. The mixtures were allowed to stand overnight to ensure complete extraction before shaking again for 5 more minutes. One hundred μL and 5 ml aliquots were removed for EIA and HPLC analysis, respectively.

EIA Analysis

The EIA kit (tube format) was purchased from Millipore Corp. (Bedford, MA). The 100 μl extract aliquot was added to 0.9 mL of HPLC grade water so that the sample contained 8% methanol. A 200 μL aliquot of the sample and standards were added to the appropriate EIA tubes, followed by 200 μl of the enzyme conjugate. Each tube was mixed by swirling and then incubated for 20 minutes at room temperature. The tubes were then rinsed 4 times under running tap water and blotted dry with a paper towel. Five hundred μl of K-blue substrate (Elisa Technologies, Lexington, KY) was added to

each tube before a second incubation period of 10 minutes. Three hundred µl of stop solution (1 N HCl) was added to the tubes to stop the reaction and to change the color from blue to yellow. The absorbance of each standard and sample was measured at 450 nm using an Enviroguard (Millipore Corp.) tube reader. Samples outside the standard linearity range were diluted with an appropriate volume of 8% methanol solution.

Control tubes were assayed with each set of tubes to calculate %B values of standards and samples. Standards were run at the beginning and end of each day, with the average of both runs used to plot the standard curve. Plotting % B against the log of hexazinone concentration derived this curve. Hexazinone levels in the soil samples extracts were calculated by extrapolating the values from this curve.

HPLC Analysis

The soil extracts were cleaned-up using activated tC18 Sep-Paks (Waters Associates, Milford, MA). This activation was accomplished by passing 5 mL of HPLC grade methanol through the Sep-Pak, followed by 5 mL of HPLC grade water. One hundred mL HPLC grade water was added to each 5 ml extract before passing the entire mixture through the tC18 cartridge. After drying under vacuum for 20 minutes, the Sep-Paks were eluted with 4 mL of 80:20 (methyl-tert-butyl ether:ethyl acetate). The eluates were evaporated to dryness under a stream of nitrogen and reconstituted with 1.0 mL of the HPLC mobile phase. A 50 µL aliquot was injected into the HPLC system.

The HPLC system consisted of a Hewlett-Packard (Wilmington, DE) 1050 photodiode array detector set to monitor at 247 nm, 1050 isocratic pump, 1050 auto-injector and a Zorbax C18 column (4.6 mm I.D. x 250 mm, 5 µ particle size) from Phenomenex, (Torrance, CA).

The mobile phase was a mixture of 40:40:20 (acetonitrile:water:methanol) with a flow rate of 1.0 mL per minute. Data was collected using HP Chemstation software.

Results and Discussion

The limit of detection for both HPLC and EIA was 25 ng/g (ppb). Typical HPLC generated chromatograms for a hexazinone standard and a soil extract are shown in figures 28 and 29, respectively. The large wide (non-integrated) peak that elutes before hexazinone appears in most of the soil extracts and is probably associated with humic acid fractions found in the upper soil horizons. While humic co-elution was generally not a problem during the study, lower detection limits could be attained by further sample clean up or an adjustment of solvent concentrations in the mobile phase.

The linear range for hexazinone by EIA was from 0.22 to 17.6 ng/g, with an IC_{50} (concentration of hexazinone at a %B value 50) of 3.0 ng/g. The limit of detection (LOD) for EIA was 25 ng/g, while the LOQ was 50 ng/g. The 8% methanol in the standard and sample solutions imparted a slight inhibitory effect on the immunoassay, but an evaporation step was avoided in favor of faster analyses. Dilution of the sample to reduce inhibition by the methanol, made it impossible to attain an LOQ of 25 ng/g.

A correlation study comparing HPLC and EIA methods was completed on the 78 soil samples obtained from treated blueberry fields in Maine and Florida. Results for these analyses are listed in table 23. Figure 31 shows that the agreement between the two techniques was acceptable ($R^2 = 0.9075$). The linear equation of $y = 0.745 x + 206$ indicates a low bias for EIA, but soil type or pH had no effect on this phenomenon.

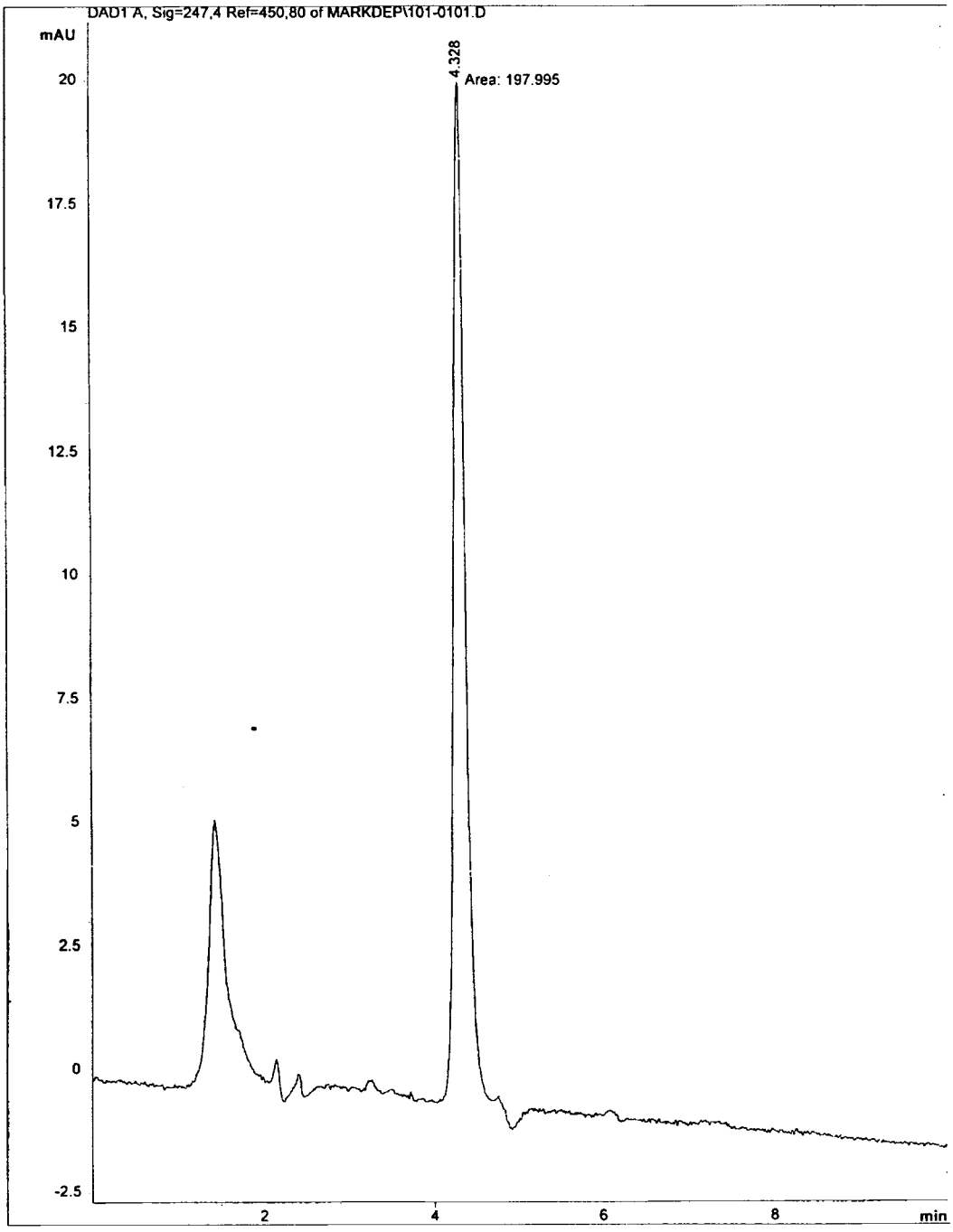

Figure 28. HPLC Chromatogram of Hexazinone Standard for Soil Method

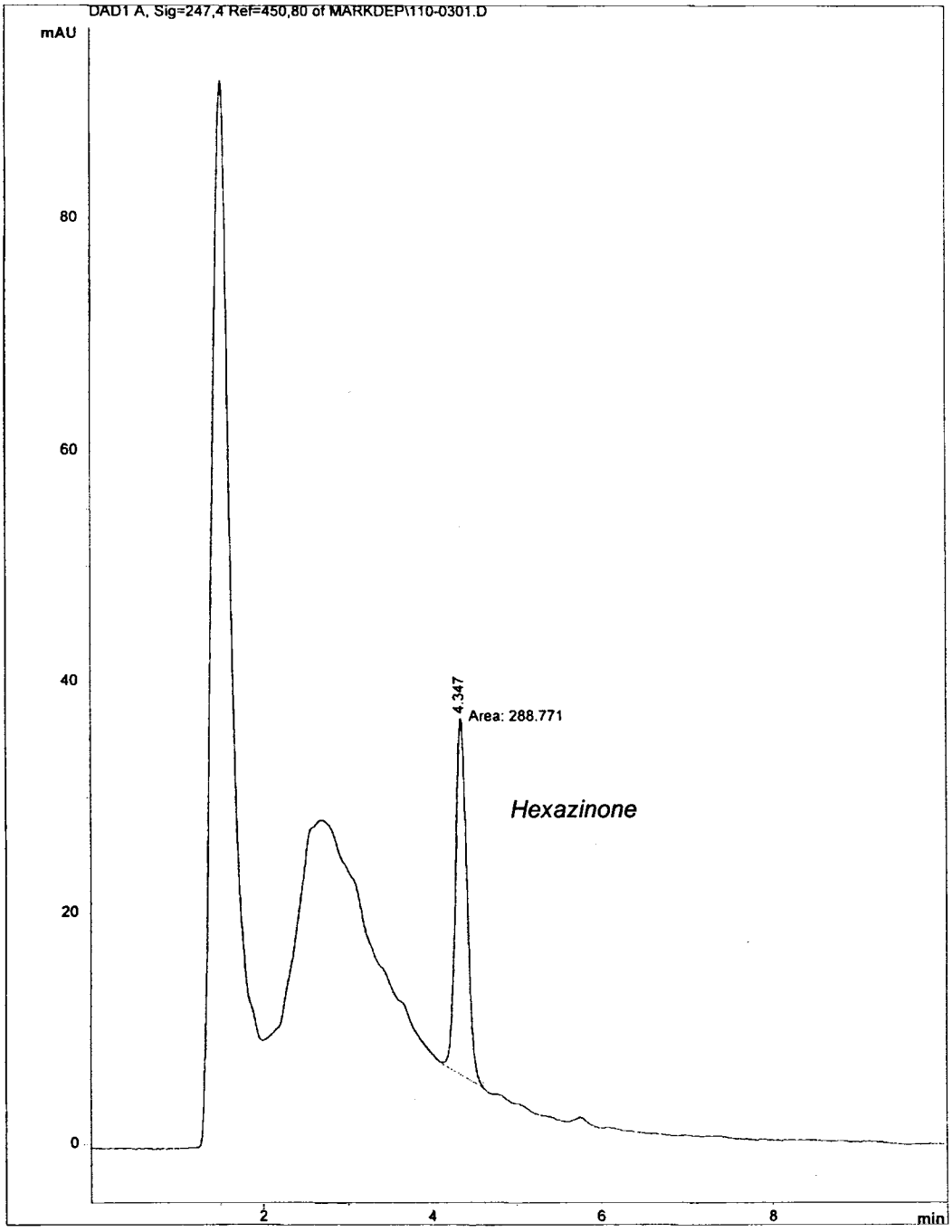

Figure 29. Typical HPLC Chromatogram for Hexazinone in a Soil Extract

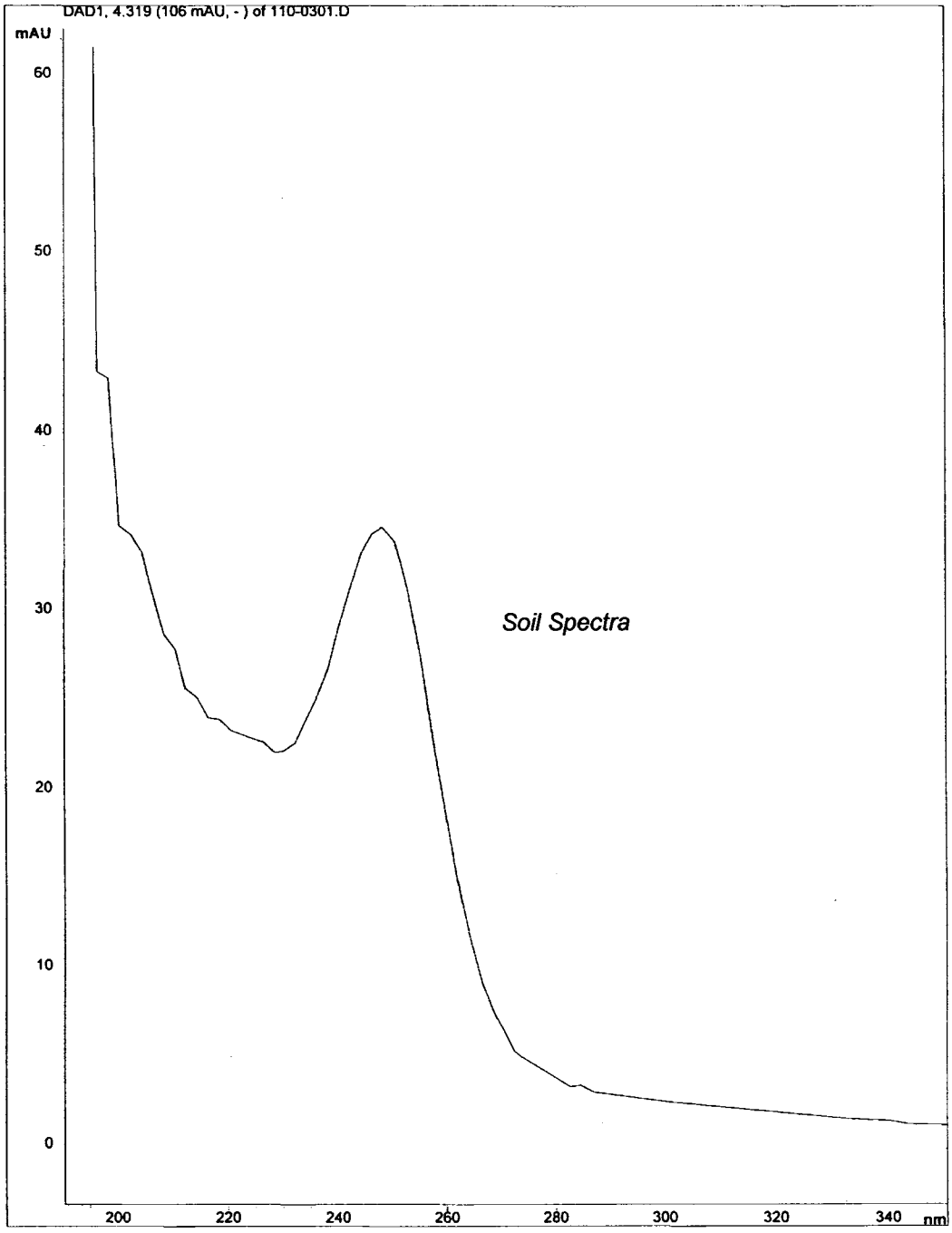

Figure 30. UV Spectra for HPLC Generated Hexazinone Peak from a Soil Extract

Table 23. Comparison of HPLC and EIA Methods for Hexazinone in Soil

Sample	Hexazinone (ug/g)		Sample	Hexazinone (ug/g)		Sample	Hexazinone (ug/g)	
	HPLC	EIA		HPLC	EIA		HPLC	EIA
Soil - 1	143	54	Soil - 27	847	1000	Soil - 53	8521	4800
Soil - 2	1036	1015	Soil - 28	106	96	Soil - 54	14706	9800
Soil - 3	242	230	Soil - 29	1207	980	Soil - 55	4930	4900
Soil - 4	967	900	Soil - 30	15310	10000	Soil - 56	5335	6300
Soil - 5	197	64	Soil - 31	642	540	Soil - 57	909	680
Soil - 6	127	54	Soil - 32	9499	8000	Soil - 58	268	275
Soil - 7	178	74	Soil - 33	14320	15000	Soil - 59	101	95
Soil - 8	184	120	Soil - 34	5272	4300	Soil - 60	979	920
Soil - 9	1270	1600	Soil - 35	1018	1410	Soil - 61	3125	5000
Soil - 10	1560	1900	Soil - 36	181	230	Soil - 62	4531	5400
Soil - 11	660	1000	Soil - 37	301	200	Soil - 63	644	780
Soil - 12	1450	1800	Soil - 38	1104	920	Soil - 64	910	900
Soil - 13	253	110	Soil - 39	2802	1740	Soil - 65	687	540
Soil - 14	1136	1600	Soil - 40	3127	1860	Soil - 66	1056	760
Soil - 15	3370	4000	Soil - 41	251	200	Soil - 67	899	735
Soil - 16	948	1200	Soil - 42	249	245	Soil - 68	5984	5600
Soil - 17	178	190	Soil - 43	180	68	Soil - 69	8679	6000
Soil - 18	216	325	Soil - 44	435	465	Soil - 70	163	65
Soil - 19	181	94	Soil - 45	7797	5000	Soil - 71	929	1320
Soil - 20	867	1450	Soil - 46	8834	5250	Soil - 72	727	460
Soil - 21	850	1000	Soil - 47	1911	1600	Soil - 73	233	112
Soil - 22	119	46	Soil - 48	5503	4650	Soil - 74	438	245
Soil - 23	1270	1250	Soil - 49	8920	6259	Soil - 75	353	200
Soil - 24	200	170	Soil - 50	8293	4300	Soil - 76	395	230
Soil - 25	97	100	Soil - 51	1264	1280	Soil - 77	222	290
Soil - 26	353	320	Soil - 52	556	330	Soil - 78	242	145

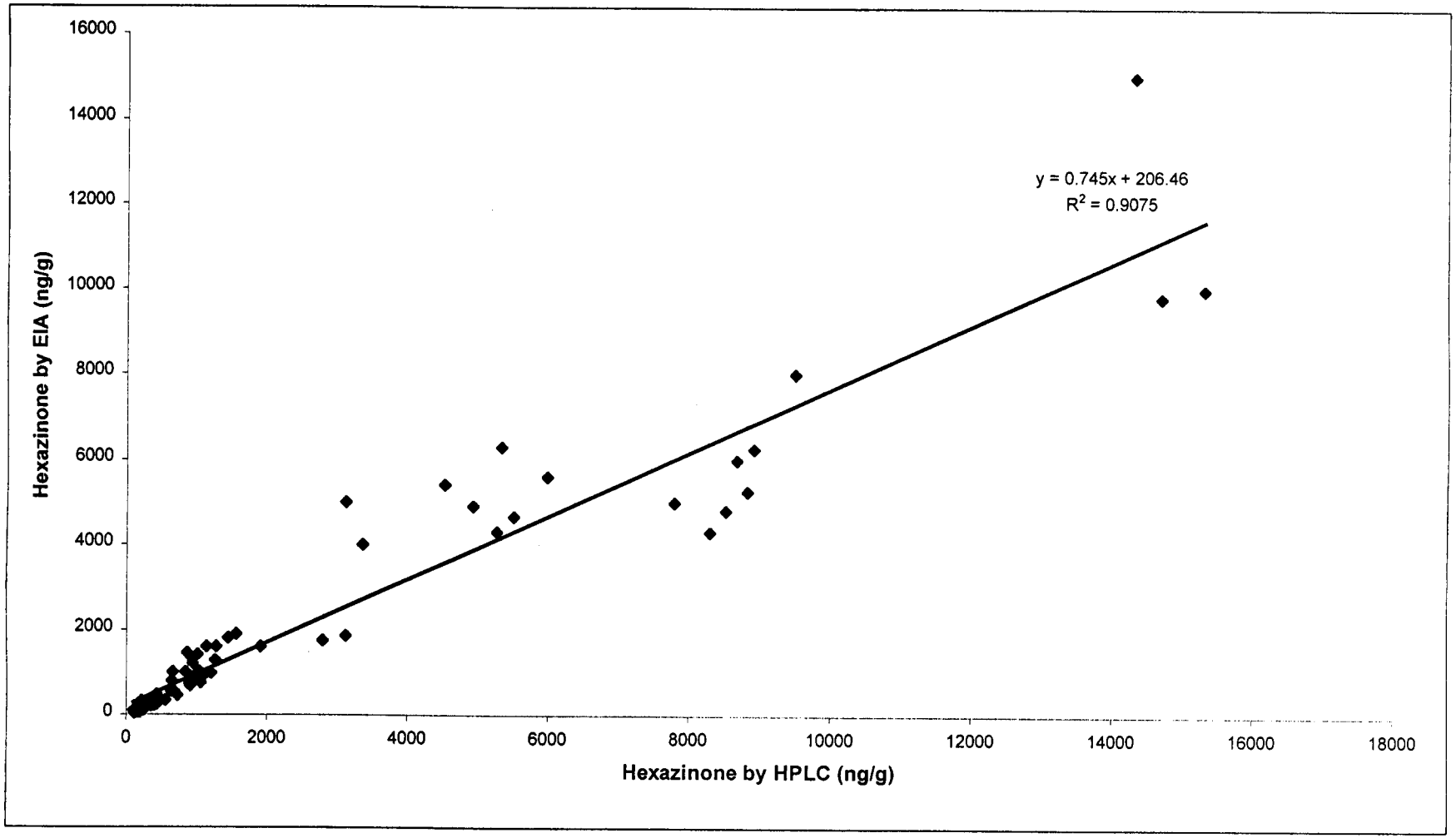

Figure 31. Correlation of HPLC and EIA Methods for Hexazinone in Soil

In fact, comparison of individual soil analyses shows that EIA values are often higher than HPLC levels.

The HPLC procedure was utilized for a two-year study of the effect of formulation on hexazinone mobility in loamy sand soils found in most Maine blueberry soils. In a 1995 evaluation Yarborough et al. found that Velpar/DAP was retained at higher levels in the soil profile than both Pronone and Velpar L (appendix A). The researchers repeated the study in 1997 and concluded that Pronone was least likely to leach into ground water, followed by Velpar DF and Velpar MAP (appendix B).

Conclusion

To maximize the effectiveness of the herbicidal activity and minimize the contamination of ground water supplies, it is important to keep as much of the parent compound as possible in the upper soil horizons. Both the HPLC and EIA methods represent good tools for the analysis of residual hexazinone in soil.

SUMMARY

The methods for the analysis of residual hexazinone in soil and water discussed in the preceding chapters represent relatively inexpensive and efficient techniques when compared to many other published means. Direct-injection of groundwater into the described HPLC system yielded an LOQ of 0.33 μg/L, saving significant time, material and associated solvent disposal costs. The HPLC method developed for soil analysis entails a rapid extraction and clean-up process and provides adequate sensitivity (LOQ = 25 ng/ml). The accompanying EIA technique is a good example of how advances in technology can eliminate the huge capital cost of traditional HPLC and GC purchases. EIA also has the advantage of speed, reduced clean-up, lower use of toxic solvents, while matching the sensitivity and quantitation of traditional instrumentation. The combination of EIA screening with HPLC confirmation provides an efficient and powerful set of tools for the analysis of residual hexazinone in both soil and groundwater.

From the data presented in the second chapter, it is apparent that hexazinone contamination of rural ground water supplies is widespread, with between 50 and 70 percent of wells testing positive for trace levels of the herbicide. However, none of the private wells showed concentrations above 6 μg/L, and a majority of the positive samples were in the 1 μg/L range. This places hexazinone contamination approximately two orders of magnitude lower than the government health advisory of 210 μg/L. Furthermore, the trends from ground water sampling from both test and private wells show decreases in residual hexazinone.

These decreases are likely the result of lower hexazinone application rates to an average of 1 lb/acre, as well as a range of better management practices. Some of these practices include: application of the herbicide only when necessary; avoiding outcrops and ledges; using during the cropping year, when there is more foliage to absorb the herbicide; and using slow release formulations, such as granulated Pronone.

So, there are still unanswered questions. How much more can be done to control hexazinone leaching? How much more should be done? This is generally the point at which the analysist's role ends and the somewhat political duties of the toxicologist, state or federal regulator, grower and homeowner begin.

From the toxicologist's point of view, this is a non-issue. No one is being exposed to Velpar concentrations even approaching the 210 μg/L health advisory. The trends established in this study coupled with improved cropping practices, indicate that this will continue to be the case.

Unfortunately, toxicology is not an exact science. Laboratory and computer modeling cannot take every situation into account. There are often unanswered questions such as: What are the negative synergistic effects on non-target organisms when hexazinone in combined with one or more pesticides? What are the long-term effects of the herbicide on these organisms? How does one accurately translate effects on experimental animals (fish, rats, dogs) to humans?

The duties of government regulators are more complex. These groups must balance the economic impacts on producers, the well being of private citizens, the established law(s) and the out-cry of citizen groups. How does one balance these concerns fairly? Weed control with hexazinone has been credited with increasing Maine's blueberry crop

by three-fold over the last 10 years. This rise has not gone unnoticed, especially in Downeast sections of Maine where per capita income is below average. But, does anyone have the right to contaminate someone else's water supply? What chemical and non-chemical alternatives does the grower have? Terbacil and diruon herbicides exhibit higher toxicities than hexazinone and are just as prone to leaching. Also, what responsibility does the laboratory analyst bear, while continuing to lower detection analytical detection limits to levels which may have no effect on most biological systems?

The solution to these questions is compromise. Hexazinone is a valuable tool to the blueberry industry. The continued monitoring of Maine's ground water coupled with experimentation with new sulfonylurea herbicides, good management practices and the use of slow release hexazinone formulations should result in less residual hexazinone in Maine's ground water. Citizens reluctant to ingest hexazinone can have their water tested for a nominal charge and install inexpensive activated charcoal filtration systems to remove the herbicide from their drinking water.

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APPENDICES

APPENDIX A

WEED CONTROL AND PRUNING - 1997 Blueberry Research Advisory Report

INVESTIGATORS: David E. Yarborough, Associate Professor of Horticulture
Timothy M. Hess, Research Associate
Brian Perkins, Research Scientist

4. TITLE: Effect of hexazinone formulation on movement through the soil profile.

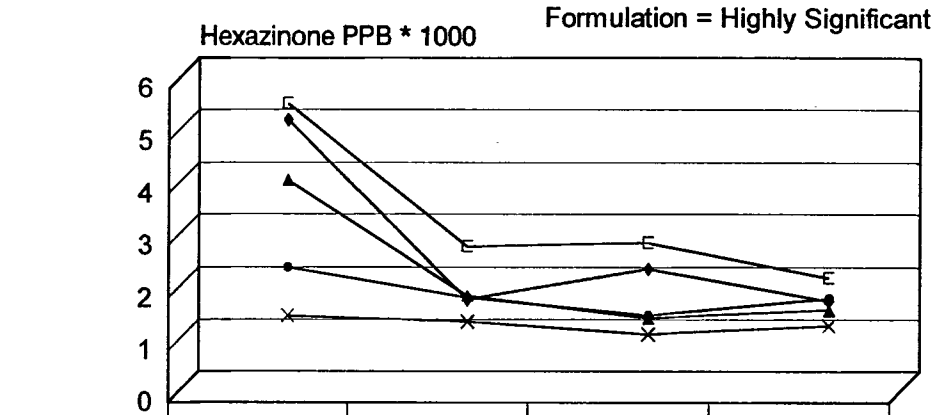
METHODS: A randomized complete block design trial to study the effect of hexazinone formulation on soil movement and weed control was established and treated with one lb ai/a Velpar® L, Pronone® 10G, Pronone® 10MG, Velpar/DAP or left untreated May 25, 1995. Each treatment also received 200 lbs/a diammonium phosphate (DAP). Plot size was 10 X 20 ft with 10 ft alleyways, 3 blocks and 5 treatments for a total of 15 plots. Soil was sampled on 6-25-95, 8-25-95, 11-25-95 and 5-24-96 one, three, six months and one year post treatment, from 0-2", 2-6" and 6-10". Carryover effects to wild blueberries and weeds was evaluated in mid June 1996.

RESULTS: The Velpar/DAP formulation had the highest concentration over time at the 0-2" (0-5 cm) depth and the untreated control had the lowest (Figure 1). One year after application the Velpar/DAP formulation had the highest concentration of hexazinone at the 2-6" (5-15 cm) depth (Figure 2) followed by the Pronone® formulations. A similar fluctuation occurred at the 6-10" (15-25 cm) depth with Velpar/DAP, Pronone® 10G and Pronone® 10MG formulation retained in the soil at higher concentrations (Figure 3). Most of the hexazinone was retained at the 0-2" (0-5 cm) level one year later (Figure 4). Even though the untreated control did not receive any hexazinone treatment in 1995, hexazinone was still detectable from the treatment in May 1993 (Figure 4). Precipitation was well below normal for the summer of 1995 compared to the average (Figure 5).

CONCLUSION: If hexazinone leaching and groundwater is a concern at a particular site, this research indicates the Velpar/DAP formulations of hexazinone is retained in the soil profile the longest and will thus, be least likely to leach into groundwater, followed by Pronone® formulations. Velpar® L was the most likely to leach out of all soil horizons.

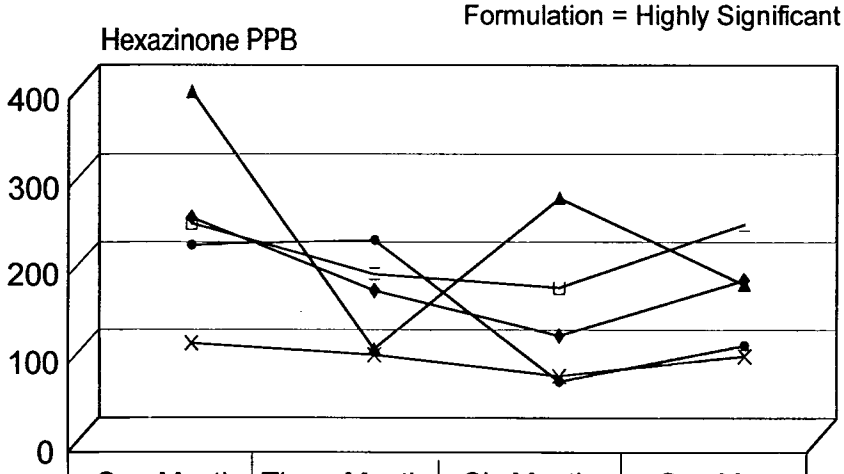
RECOMMENDATIONS: This experiment should be reevaluated with the Velpar® DF formulation with irrigation to insure there is adequate moisture to move the hexazinone through the soil profile.

Figure1. Effect of Velpar Formulation on Hexazinone Movement Through the Soil Profile at 0-2 Inches



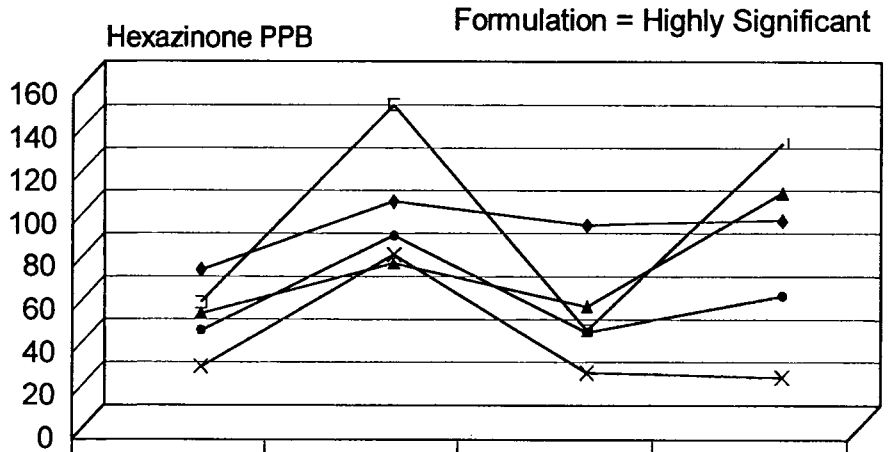
	One Month	Three Months	Six Months	One Year
Velpar L	2	1.423	1.083	1.41
Pronone 10G	4.812	1.383	1.966	1.35
Pronone 10MG	3.656	1.45	1.019	1.173
Velpar/Dap	5.126	2.4	2.466	1.8
Untreated	1.069	0.954	0.716	0.88

Figure 2. Effect of Velpar Formulation on Hexazinone Movement Through the Soil Profile at 2-6 Inches



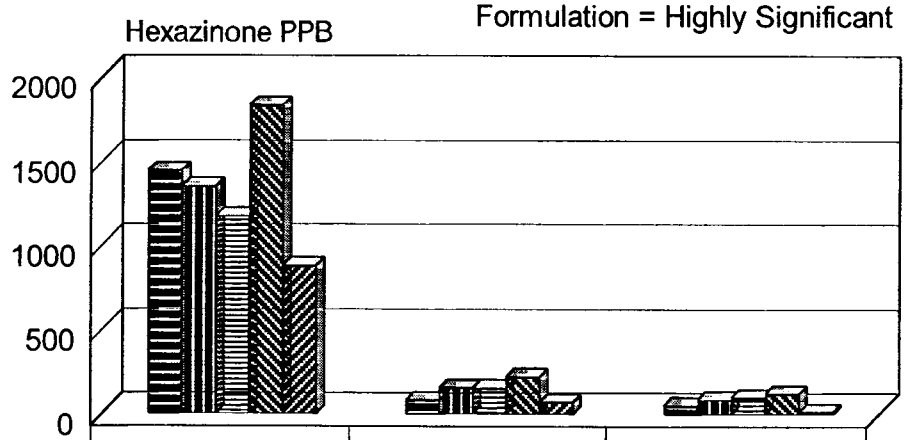
	One Month	Three Months	Six Months	One Year
Velpar L	196	202	41	82
Pronone 10G	228	144	93	156
Pronone 10MG	370	78	250	151
Velpar/Dap	221	163	147	220
Untreated	84	71	47	70

Figure 3. Effect of Velpar Formulation on Hexazinone Movement Through the Soil Profile at 6-10 Inches



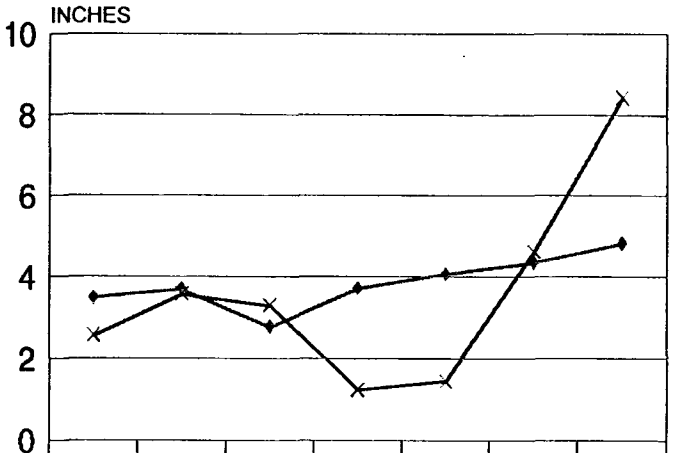
	One Month	Three Months	Six Months	One Year
Velpar L	35	79	34	51
Pronone 10G	63	95	84	86
Pronone 10MG	43	66	46	99
Velpar/Dap	48	140	35	122
Untreated	18	70	15	13

Figure 4. Comparison of Formulation on Hexazinone Movement After One Year



	0-5 cm	5-15 cm	15-25 cm
Velpar L	1443	82	51
Pronone 10G	1350	157	86
Pronone 10MG	1173	152	99
Velpar/DAP	1833	220	122
UTC	880	70	13

Figure 5. Blueberry Hill Farm Precipitation



	May	June	July	Aug	Sep	Oct	Nov
1995	2.6	3.6	3.3	1.2	1.5	4.6	8.4
4 Year Average	3.5	3.7	2.8	3.7	4.1	4.4	4.8

APPENDIX B

PRUNING AND WEED CONTROL - 1998 Wild Blueberry Research Advisory Committee

INVESTIGATORS: David E. Yarborough, Associate Professor of Horticulture
Timothy M. Hess, Research Associate
Brian Perkins, Research Scientist

2. TITLE: Effect of hexazinone formulation on movement through the soil profile.

METHODS: A randomized, complete block design trial to study the effect of hexazinone formulation on soil movement and weed control was established and treated with one lb ai/a Velpar DF®, Pronone MG®, Velpar DF® impregnated on monammonium phosphate (MAP) or left untreated May 22, 1997. Each treatment also received 200 lbs/a MAP. A similar trial was initiated in 1995 during a dry growing season. To analyze the effects of precipitation on hexazinone movement, each plot received a total of 1" of rainfall or irrigation per week from trial initiation until September 1, 1997. Plot size is 10 X 20 ft with 5 ft alleyways and has 3 blocks and 4 treatments for a total of 12 plots. Soil was sampled on 6-23-97, 8-26-97, 11-12-97 for one, three and six months post treatment, from the 0-2", 2-6" and 6-10" soil depths. Soils will be sampled again in May 1998 for the 12 month post treatment. Weed control and injury to wild blueberries will be evaluated in mid June 1998.

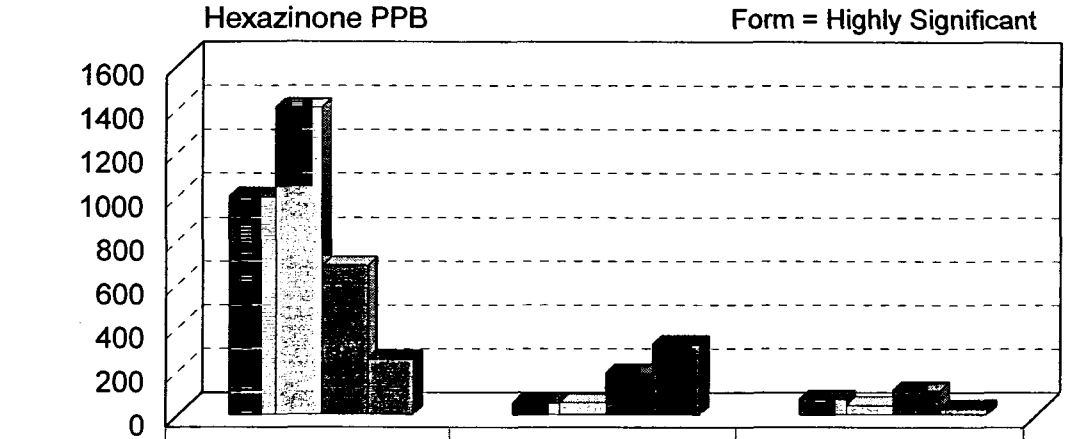
RESULTS: The Pronone® formulation had the highest levels at the 0-2" layer at both 1 and 3 months sample times (Figure 1) followed by the DF formulation and Velpar DF®/MAP. At 2-6", both VEL/MAP and the control, a residual from 2 years prior application, have the highest concentration (Figure 1). Similarly, at 6-10" Velpar DF®/MAP had the highest concentration at both sampling dates.

CONCLUSION: In both 1995 and 1997, high levels of Pronone® were retained at 0-2" after 1 month (Figures 1 and 2) although in 1997 the levels are only 20% of those 1995 and do not increase at the deeper soil levels indicating they have been leached from the root zone or broken down by micro organisms (Figure 2). At 3 months sampling in 1997, all forms of hexazinone are retained at almost the same levels in the first month (Figure 3) where as levels decreased dramatically in 1995 (Figure 4). Overall

trends indicate Velpar DF®/MAP or DAP formulations leach more readily during wet growing seasons with Pronone® being retained the most.

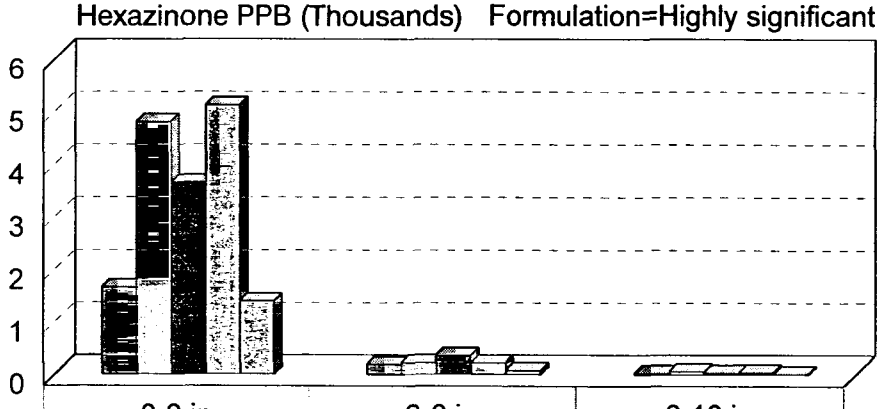
RECOMMENDATIONS: Continue with future sampling date then terminate trial.

Figure 1. Comparison of Formulation on Hexazinone Movement After One Month-1997



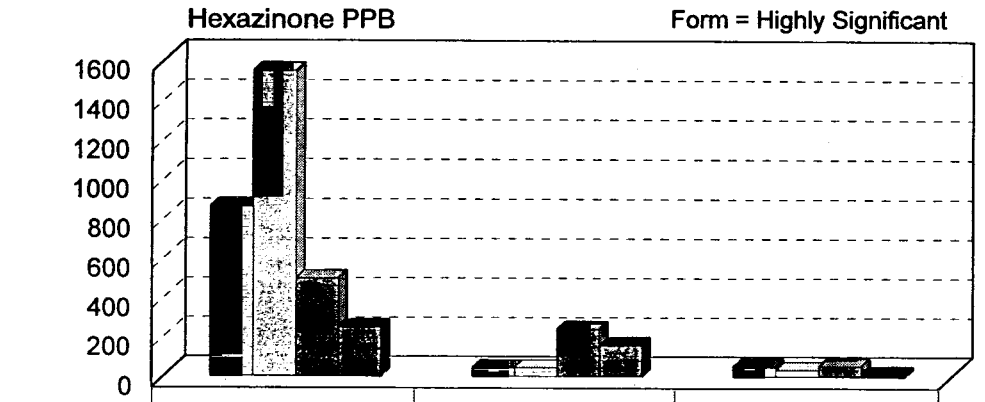
	0-2 in	2-6 in	6-10 in
Velpar DF	994	52	68
Pronone MG	1401	55	43
Velpar/MAP	681	188	109
UTC	254	319	27

Figure 2. Comparison of Formulation on Hexazinone Movement After One Month-1995



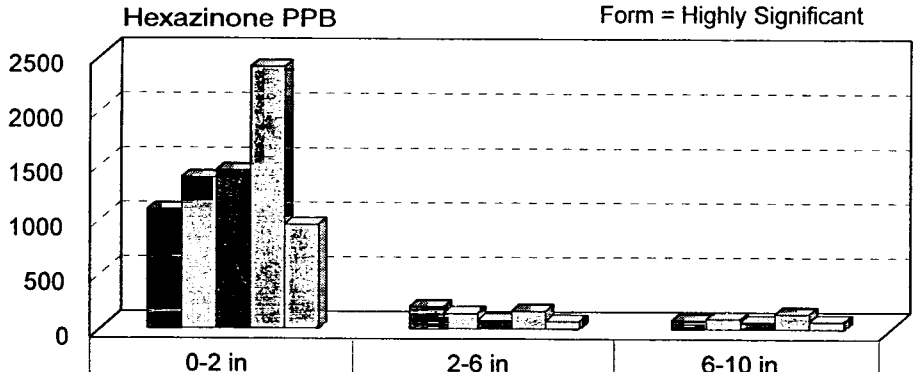
	0-2 in	2-6 in	6-10 in
Velpar L	1.666	0.196	0.035
Pronone 10G	4.812	0.229	0.063
Pronone 10MG	3.656	0.37	0.043
Velpar/DAP	5.127	0.222	0.048
UTC	1.402	0.084	0.018

Figure 3. Comparison of Formulation on Hexazinone Movement After Three Months -1997



	0-2 in	2-6 in	6-10 in
Velpar DF	861	44	50
Pronone MG	1547	46	35
Velpar/MAP	499	240	54
UTC	251	159	13

Figure 4. Comparison of Formulation on Hexazinone Movement After Three Months-1995



	0-2 in	2-6 in	6-10 in
Velpar L	1090	202	79
Pronone 10G	1383	143	95
Pronone 10MG	1450	78	66
Velpar/DAP	2400	163	141
UTC	954	71	70

APPENDIX C

DETERMINATION OF CAPSAICINOIDS IN SALSA BY LIQUID CHROMATOGRAPHY AND ENZYME IMMUNOASSAY

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ABSTRACT

Two simple and rapid methods were developed to monitor pungency of salsa in production. Capsaicin (C) and dihydrocapsaicin (DHC) were quantified in 17 commercially available tomato-based salsas by enzyme immunoassay (EIA) and by high performance liquid chromatography (LC) with fluorescent detection. Samples were extracted with methanol and the extracts were subjected to solid phase extraction (SPE) using polystyrene-divinylbenzene columns. Analysis of the SPE eluates showed good correlation (r^2=0.953) between LC and EIA, with a slightly high bias for EIA. Salsa fortified with C and DHC from 0.118 to 103.2 ug/g resulted in recoveries of 90 - 112% (C) and 76 – 97% (DHC). Limits of detection by LC were 0.1 ug/g for each capsaicinoid and 0.1 ug/g by EIA for total capsaicinoids. The LC on-column response was linear from 0.2 to 100 ng for both C and DHC, while the working range for EIA was 0.1 to 2.0 ppm. Variability in pungency was noted between different salsa brands labeled mild, medium and hot.

INTRODUCTION

Hot sauces and tomato-based salsas containing hot peppers (*Capsicum* fruit) have enjoyed strong gains in consumer acceptance in recent years and now account for an estimated 500 million American dollars in annual sales (1). Consumers can now choose from a wide variety of salsas, which are available in a wide range of pungencies.

The capsaicinoids (vanillyl amide structures with saturated and unsaturated C_9-C_{11} branched fatty acids) are responsible for the pungent or hot sensation associated with salsa (figure1). This burning sensation is commonly measured in Scoville Units (SU), a widely accepted organoleptic test developed by Wilbur Scoville in 1912 (2). Table 1 compares SU values for the capsaicinoids commonly occurring in *Capsicum* fruit. There are three capsaicinoids commonly found in hot peppers, including capsaicin (C) and dihydrocapsaicin (DHC), which account for between 80 and 90+ % of the pungency, while nordihydrocapsaicin (NDHC) is normally present in much lower concentrations (3,4). Traces of homocapsaicin, homodihidrocapsaicin, nornodihydrocapsaicin, as well as other analogues and homologues have also been reported in the literature (5,6,7,8).

Numerous methods have been published describing the identification and quantification of capsaicinoids in hot peppers, oleoresins and hot sauces. The techniques employed include liquid chromatography with ultraviolet and fluorescence detectors (6-10), LC with mass spectral detectors (6), gas chromatography with MS detectors (5), and micellar electrokinetic capillary chromatography with ultraviolet and electrochemical detection (12). Most of these techniques are quite useful for research and quality control functions of expensive ingredients such as oleoresins, but are too costly and time consuming to be used for the analysis of end products, such as salsa.

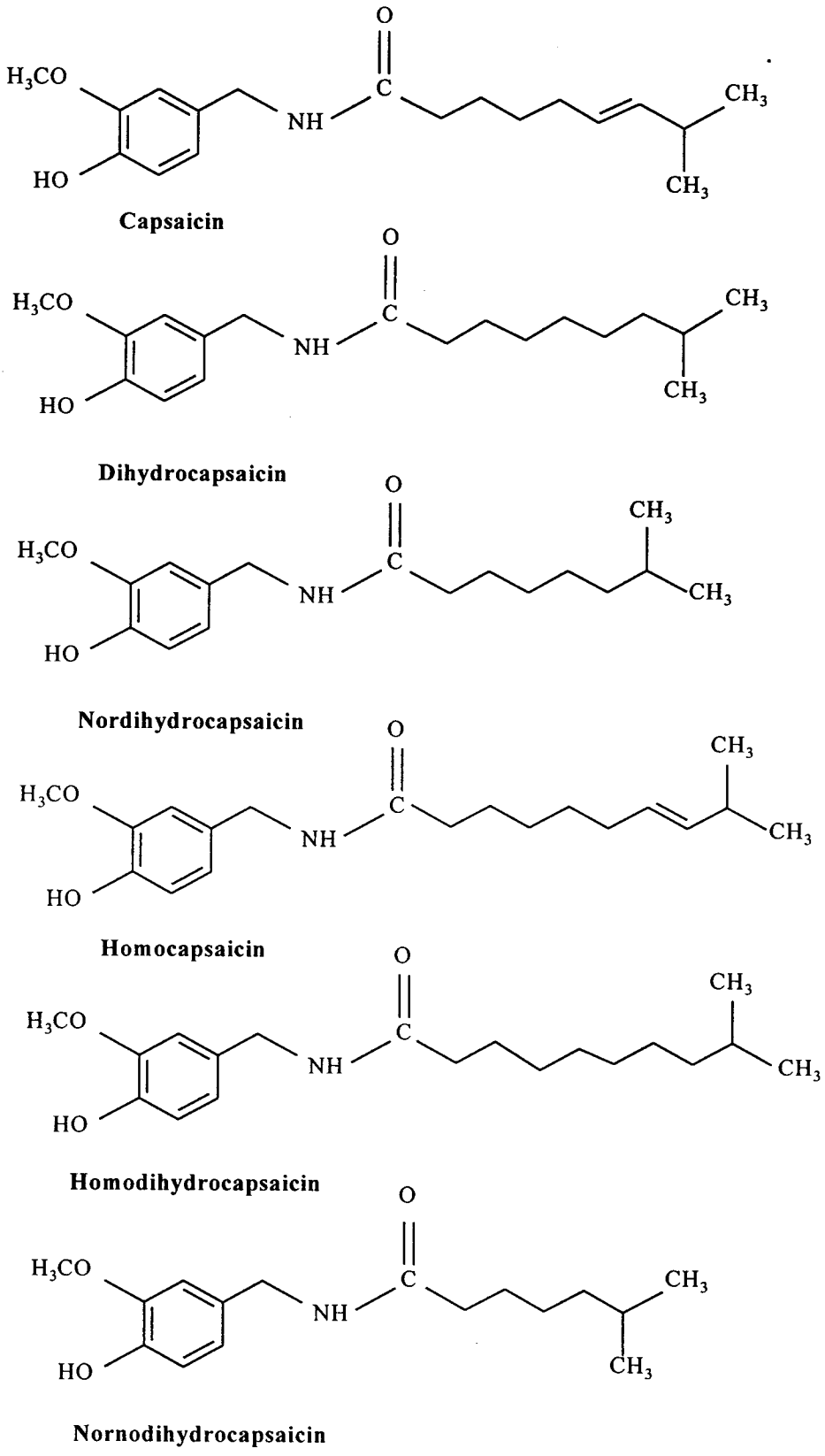

Figure 1. Capsaicinoid Structures

Table 1. Relative Pungencies of Capsaicinoids

Capsaicinoid (ug)	Pungency (SU)
capsaicin	16
dihydrocapsaicin	16
nordihydrocapsaicin	9.1
homocapsaicin	8.6
homodihydrocapsaicin	8.6

We have found no published methods for the analysis of capsaicinoids in salsa. This paper compares a novel and rapid EIA method developed with a commercially available kit with a simple LC assay for the analysis of the capsaicinoids, C and DHC in processed, tomato-based salsa.

EXPERIMENTAL

Apparatus

(a) LC/FLD system.-HP 1100 series (Hewlett Packard, Burlington, MA) equipped with a Prodigy C18, 4.5 x 250 mm column, maintained at ambient temperature (Phenomenex, Inc., Torrance, CA). The mobile phase was a mixture of 55:45 (acetonitrile:water) with an isocratic flow of 1 ml/min. The fluorescence detector was programmed to monitor the signal with an excitation of 280 nm and an emission of 325 nm. Twenty ul of sample was injected into the system. Data was collected and analyzed with HP Chemstation software.

(b) EIA system.-Plate reader (Molecular Devices, Sunnyvale, CA) with absorbance measured at 450 nm. Capsaicin test kit, manufactured by Beacon Analytical Systems (Portland, ME).

(c) Blender.-Waring model 33BL79 (East Windsor, NJ).

(d) Polytron.-Model CH-6010 (Brinkman Instruments, Westbury, NY).

(e) Solid phase 12 position manifold.- (Allied Signal-Burdick & Jackson, Muskegon, MI).

(e) Centrifuge.-Model TJ-6, 15000 x g (Beckman, Palo Alto, CA).

Reagents

(a) Methanol, acetonitrile, and water.-HPLC grade (Fisher Scientific, Fairlawn, NJ).

(b) SPE cartridges.-Waters Corp. Oasis (Milford, MA) 200 mg, 6 ml.

(c) Centrifuge tubes.-Disposable, 50 ml polypropylene (VWR Scientific, Bridgeport, (NJ).

(d) Salsa.-Purchased from local supermarkets.

(e) Standard stock solutions.-Prepare C (97%) and DHC (90%) (Sigma, St. Louis, MO) by weighing 10 mg of each into separate 25 ml volumetric flasks and dilute to volume with acetonitrile.

(f) Intermediate and working solutions.-Dilute 1 ml from stock solutions to 50 ml with acetonitrile for both C and DHC. Dilute intermediate solutions with appropriate volumes of acetonitrile to make 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0 ug/ml working standards.

Extraction

Puree the entire jar of salsa in the blender for 2 min to ensure a homogeneous sample. Weigh a 5 g sub sample into a 50 ml centrifuge tube and add 25 ml methanol. Polytron the mixture for 3 min at medium speed and centrifuge for 10 min at 15,000 x g. Remove a 0.5 ml aliquot for EIA and evaporate it to dryness under a stream of nitrogen. Pipette 10 ml of supernatant from the tube and mix with 100 ml of distilled water. Care must be

taken not to disturb the pellet, for any particles introduced to the clean-up procedure can easily clog the SPE cartridge frit.

Clean-up

Apply the entire diluted sample to the SPE cartridge after activating by successive rinses with 5 ml of methanol and 5 ml of water. Elute the solution at a rate of 5 ml per min. Rinse the cartridge with 5 ml of distilled water. Allow the cartridge to dry under vacuum for 3 min, then elute with acetonitrile, collecting the first 3.0 ml of eluate. Inject 20 ul of the eluate into the LC system.

EIA Procedure

Warm all reagents to room temperature. Reconstitute dried sample into 0.5 ml of 90:10 (water:methanol). Pipette 100 ul of sample or calibrator into each mixing well, followed by 100 ul of enzyme conjugate. Mix contents of each well by gently aspirating a few times with the pipette, then transfer 100 ul of the mixture to the antibody-coated reaction wells. Incubate the plate for 10 min at room temp, then rinse the wells with tap water by filling and decanting. Add 100 ul of substrate to each well and incubate for 10 min. Stop the reaction by adding 100 ul of stop solution and read plate absorbance at 450 nm. Samples with absorbance values exceeding the standard curve must be diluted and re-assayed. Calculate the $\%B_0$ values from the absorbance data. Refer to product insert sheet (provided by manufacturer) for detailed procedure.

LC Recovery Assays

Because all salsa tested contained capsaicinoids, the recovery procedure for LC analysis was estimated by spiking a salsa (mild) sample at six levels of capsaicin (0.14,

3.096, 10.32, 25.8, 57.6 and 103.2 ppm) and dihydrocapsaicin (0.097, 3.48, 10.44, 24.36, 48.72 and 97.44 ppm) after first determining the capsacinoid levels naturally present in the sample. Recovery values were calculated by subtracting the natural from the fortified levels for both capsaicin and dihydrocapsaicin. The fortification-recovery procedure was repeated over a period of six days to determine the ruggedness of the LC method.

RESULTS AND DISCUSSION

Although acetonitrile is often used as an extraction solvent for capsaicinoid analysis due to its efficiency and low co-extractive properties (7,10), a less expensive and less toxic solvent would facilitate use of these methods by the food industry. After analyzing several samples extracted with acetonitrile, methanol, and ethanol by LC, methanol was chosen for use in this study. We noticed no difference in extraction efficiency between the three solvents and although methanol and ethanol extracted more pigment, the chromatograms for all extracts were similar.

The on-column response for C and DHC was linear to 100 ng (R^2=0.990 and R^2=0.998, respectively). Typical chromatograms for standard and sample injections are shown in figure 2, where near-baseline separation was realized for each capsaicinoid, within 13 minutes. There were no interfering peaks observed for any of the salsa samples that we assayed. Although nordihydrocapsaicin was likely present in many of the samples (figure 2b), we were unable to obtain an analytical standard for positive identification. Other researchers, using similar reverse-phase LC conditions to separate capsaicinoids in oleoresin and hot pepper extracts, generated similar chromatograms. All showed NDHC eluting immediately before the C peak (6,9,10,11).

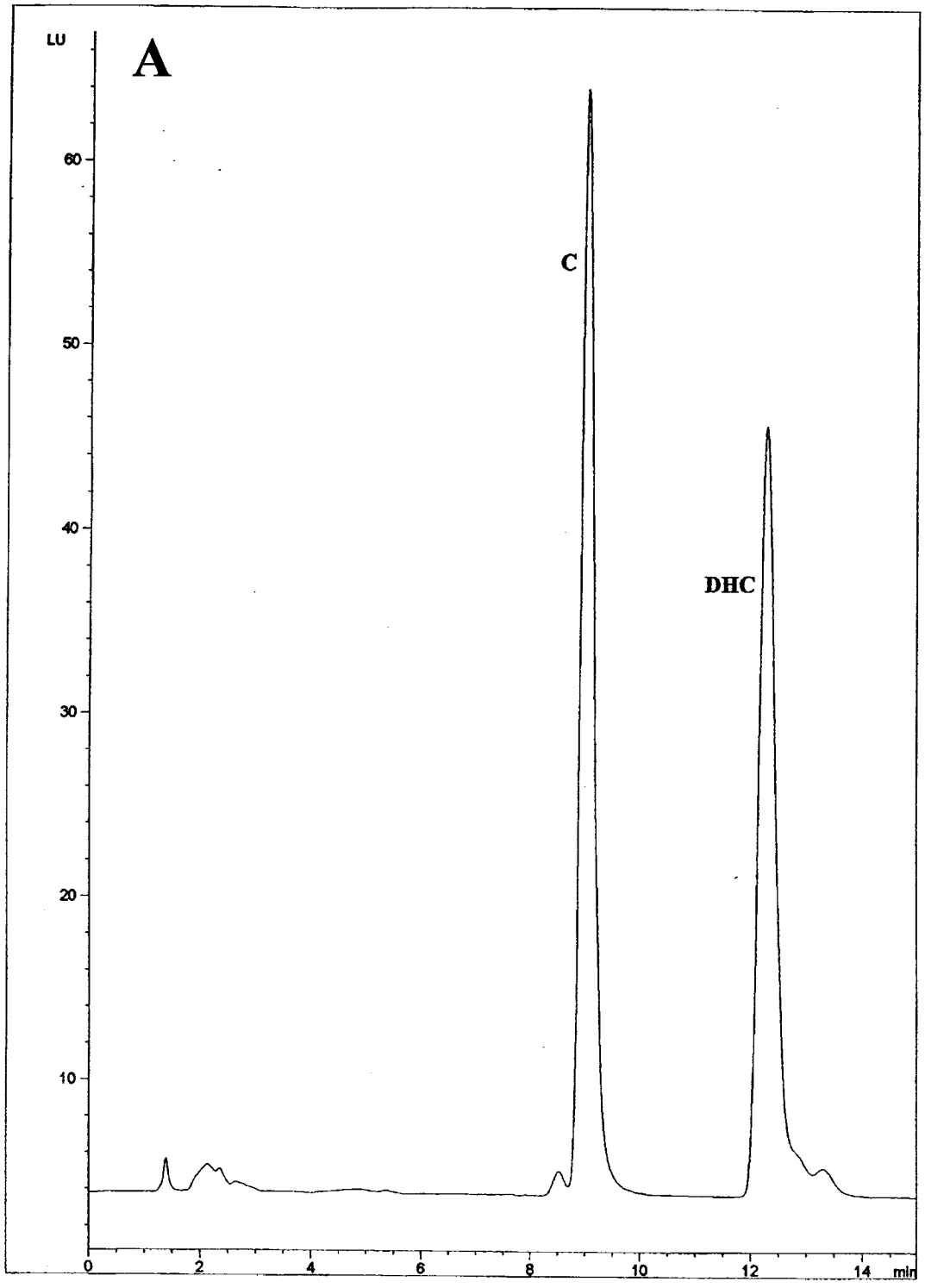

Figure 2a. Chromatogram of C and DHC Mixed Standard

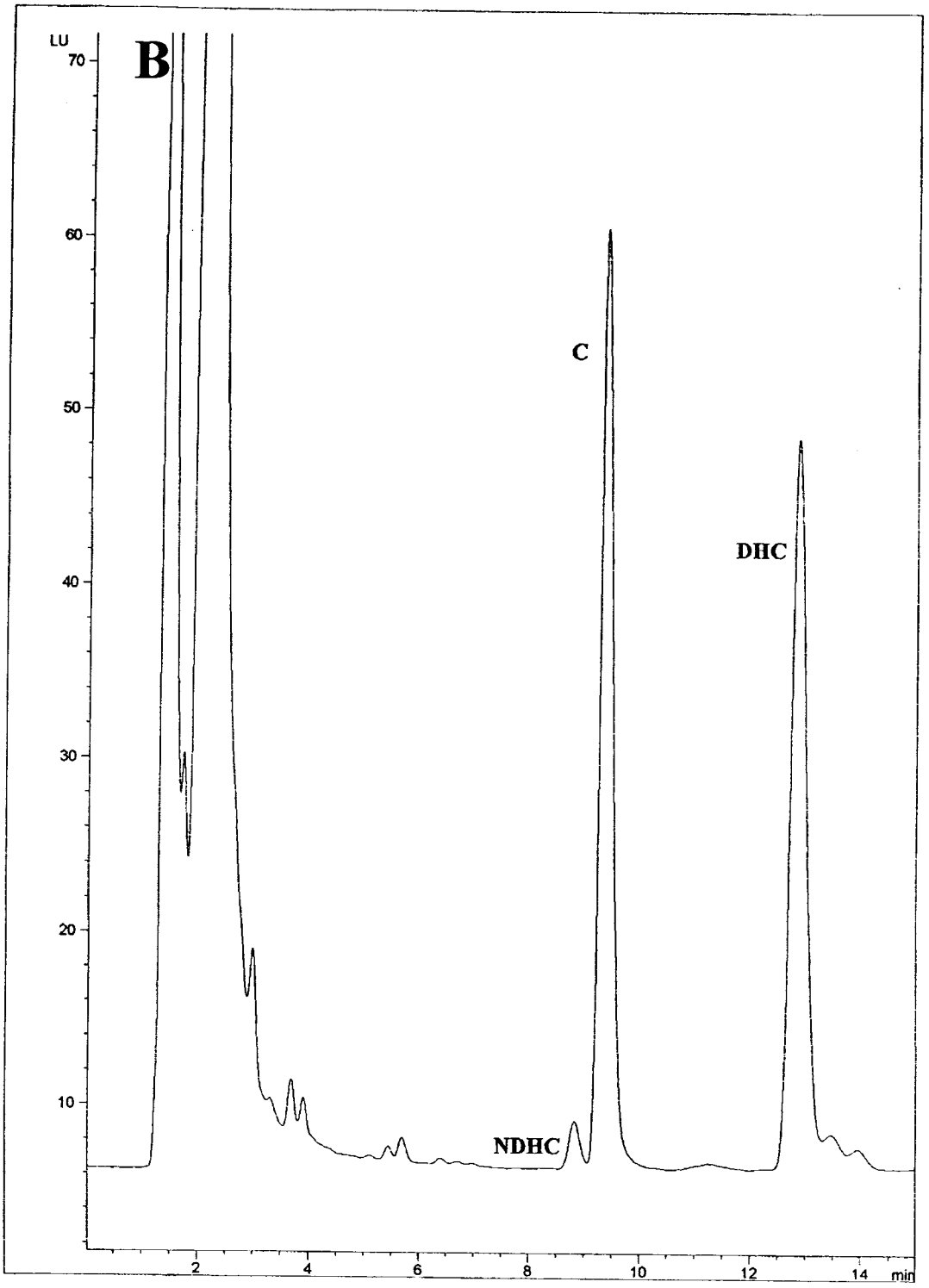

Figure 2b. Chromatogram of Salsa of Medium Pungency

Results for the fortification-recovery study are listed in table 2. Recoveries ranged from 77.15 to 112.5% for both C and DHC for samples fortified from 0.118 to 103.2 ug/g. Relative standard deviations were acceptable for all spiking levels, with exception of the lowest spiking regime, which resulted in RSDs above 20%. This variability is explained by noting that the fortification level (C=0.12 ug/g and DHC=0.118 ug/g) was an order of magnitude lower than the capsaicinoids naturally present in the "mild" salsa (C=1.4 ug/g and DHC=1.7 ug/g). Small variations in recovery of the natural capsaicinoids greatly increased the RSD values of the low spikes.

Table 2. Capsaicin and Dihydrocapsaicin Recovery by LC/FLD

Spike Level (ug/g)		Mean Recov. (ug/g)		Mean Recov. (%)		SD (ug/g) n=6		RSD (%)	
Cap	DHCap	Cap	DHCap	Cap	DHCap	Cap	DHCap	Cap	DHCap
0.120	0.118	0.1350	0.1126	112.5	95.42	0.031	0.032	23.1	28.3
3.096	3.480	3.3085	3.388	106.9	97.36	0.250	0.344	7.54	10.1
10.32	10.44	10.76	10.05	104.3	96.26	0.420	0.872	3.90	8.67
25.80	24.36	24.79	21.68	96.09	89.00	1.88	2.32	7.58	10.7
51.60	48.72	47.62	37.59	92.29	77.15	3.44	4.21	7.24	11.2
103.2	97.44	92.85	74.38	89.97	76.33	3.38	5.26	3.63	7.07

Seventeen salsa samples ranging from "extra mild" to "hot" were assayed by both LC and EIA for C and DHC content. The data generated from these two techniques correlated well, with a value of 0.957 (figure 3). The slight bias toward EIA may be due in part, to the cross-reactivity of NDHC to the antibody. This capsaicinoid was not quantified by LC. Results for both assays are given in table 3. It is of interest to note the great variability in total capsaicinoid content and pungency between brands, with some samples containing 3x the value as others, within the same pungency category.

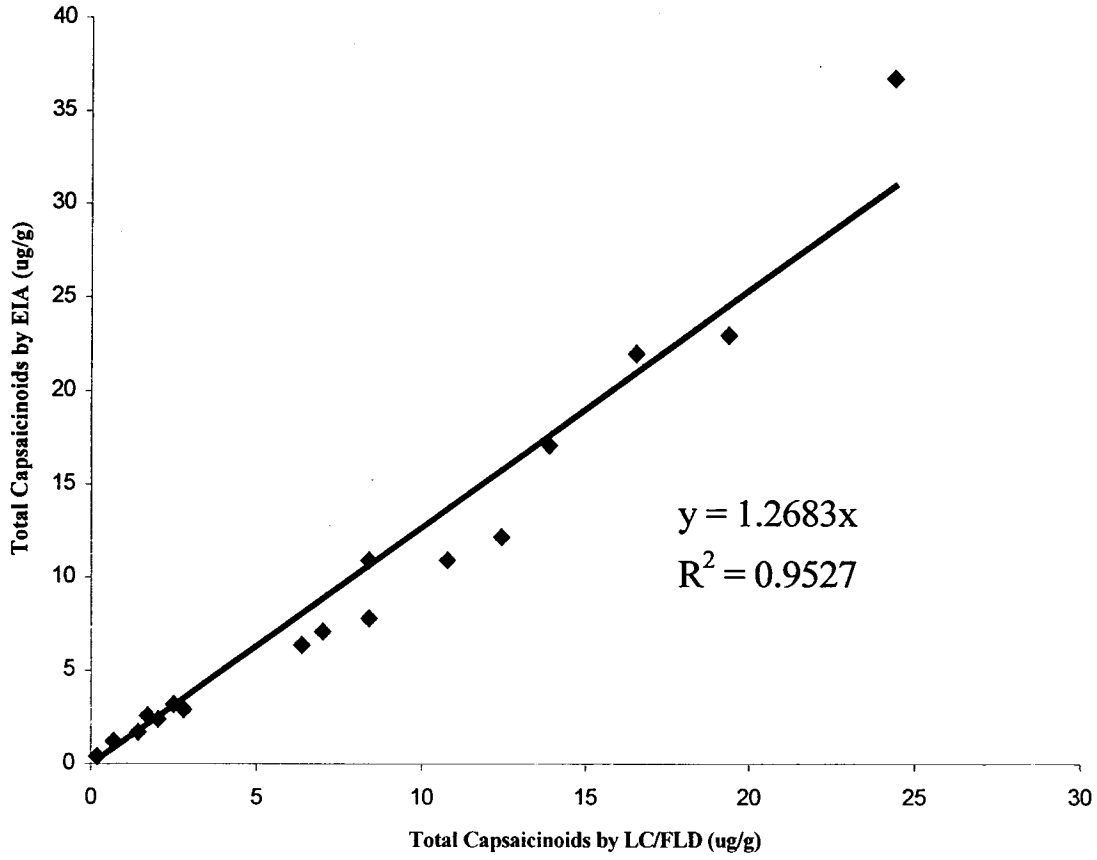

Figure 3. Correlation Between LC-FLD and EIA Techniques for Total Capsaicinoid Analysis

Table 3. Comparison of LC/FLD with EIA for Total Capsaicinoids in Salsa

Salsa	LC/FLD (ug/g)	EIA (ug/g)
A-hot	24.40	36.80
A-medium	2.50	3.20
B-hot	13.90	17.10
B-medium	7.00	7.10
C-medium	8.40	7.80
C-mild	1.70	2.60
D-medium	8.40	10.90
E-mild	2.02	2.41
E-medium	10.79	10.92
E-hot	19.38	22.98
F-extra mild	0.19	0.39
F-mild	1.42	1.70
F-medium	6.37	6.38
F-hot	16.55	22.00
G-mild	0.68	1.22
G-medium	2.80	2.92
G-hot	12.44	12.16

CONCLUSION

Both of the methods described in this paper are rapid and accurate. The LC procedure provides processors who possess basic HPLC equipment the ability to easily monitor salsa production lines for consistent pungency. The EIA technique requires minimal equipment and up to 10 samples per hour can processed by an analyst, with little training.

ACKNOWLEDGMENTS

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BIOGRAPHY OF THE AUTHOR

L. Brian Perkins was born in Lewiston Maine on April 6, 1960. He was raised in Buckfield, Maine and graduated from Buckfield High School in 1978. He attended the University of Maine and in 1984 received a Bachelor's degree in Plant and Soil Sciences. In 1993 Brian completed a Master of Science degree program in Food Science while working full-time for the University of Maine.

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