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Atrazine Contamination in a Rural Source-Water Supply: Spa Lake, Lewisburg, Kentucky

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ATRAZINE CONTAMINATION IN A RURAL SOURCE-WATER SUPPLY:

SPA LAKE, LEWISBURG, KENTUCKY

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Kathryn Jean Seadler

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ATRAZINE CONTAMINATION IN A RURAL SOURCE WATER SUPPLY:

SPA LAKE, LEWISBURG, KENTUCKY

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Abstract

In 1998, Western Kentucky University (WKU) worked in collaboration with the Environmental Protection Agency (EPA), Drinking Water Protection Division, to investigate methods to improve source-water quality for rural-water supply systems (RWSS). Through partial funding from the EPA, WKU developed a Technical Assistance Center for Water Quality (TACWQ), which focused resources and expertise toward assisting RWSS in achieving and maintaining capacity development goals and protecting public health. The TACWQ established the Source Water Protection Initiative (SWPI) to assist RWSS in acquiring and monitoring the technical, financial and managerial capacity needed to provide safe drinking water and achieve the public health protection goals of the EPA Safe Drinking Water Act (Technical Assistance Center, July 1998). The SWPI also provided technical assistance toward identifying and reducing source water impacts throughout Kentucky.

Monthly sampling during 2000 from seven watersheds in western and south central Kentucky showed that levels of several pesticides and herbicides were elevated

above Maximum Contaminant Levels (MCLs) in their source waters. Of the MCL exceedences, three commonly used pesticides (atrazine, alachlor, and simazine) were repeatedly being detected at several sites. Of the three compounds, atrazine, a triazine-class herbicide widely used in Kentucky to control broad leaf and grassy weeds in row crops such as corn, drew the most interest. Atrazine has been classified as a spring use only, "Restricted Use Pesticide due to its potential for groundwater contamination." (EXTOXNET, 1996) It is regulated as a compound with class III (slight) toxicity. In 1994, EPA took atrazine under special review to evaluate the ecological and biological effects it may cause. EPA later deemed atrazine not to significantly increase the risk of cancer in humans and went as far as lifting its use restrictions. Independent researchers still dispute EPA claims.

The exceedences of the MCLs by many compounds in source water do not immediately result in violations. The source water must go through treatment processes. Water-supply operators must strive to meet National Primary Drinking Water Standards (EPA, 1999) prior to going to the consumer. However, the fundamental concept driving the SWPI is that the technical and financial challenges faced by RWSS are proportional to the quality of their source water. At several sites, even treated water that was distributed to customers exceeded federally mandated MCLs. Levels of atrazine in finished water reached 17 parts per billion (ppb) in Lewisburg, Kentucky. The MCL for atrazine is currently 3.0 ppb.

The research presented here is the result of a twenty-two month study to investigate the complex interaction of pesticide application occurrence and the concentration of atrazine detected in the raw and finished water supply. Soil composition plays a large role in the behavior of atrazine absorption. Burkart and others (1999) reported that soil composition accounted for over 33% of the variance of atrazine concentration observed in their study. In the Spa Lake, Lewisburg, Kentucky, watershed it is highly suspect that the variability of atrazine concentrations depends not only on the soil composition but also on application schedules, moisture conditions, rainfall amounts and patterns, chemical mobility of the compounds, geology, and hydrogeologic flow paths.

Chapter 1: Introduction

Drinking water is of vital importance to every human on earth. Some take in for granted. Some struggle to obtain it every day. Having safe water to drink is a luxury that most Westerners assume they are receiving from their local water supplier; but, is the water coming from their faucets really as safe as they might believe?

In 1996, the United States Environmental Protection Agency (EPA) developed the National Drinking Water Regulation, also known as the Safe Drinking Water Act (42 CFR 300g-1) to establish baseline requirements for public water systems to ensure the minimum quality of water which they distributed to consumers (EPA, 1996). The regulation specified contaminants which may have *any* adverse effect on the health of persons, and specified for each contaminant either a maximum contaminant level (MCL), if it is economically and technologically feasible to ascertain the level of such contaminant, or treatment techniques known which may lead to a reduction in the level of such contaminant sufficient to satisfy the requirements of the title (EPA, 1996).

Achieving the MCLs as set for in the Safe Drinking Water Act are certainly a challenge for most water supply systems; however, limited resources often place the regulation out of reach for thousands of rural water supply systems (RWSS). A RWSS is most defined by EPA to provide service to less than 2500 inhabitants, but may service as many as 10,000 if the area is still rural in character (EPA 2004). Non-attainment of the goals set forth in the Safe Drinking Water Act not only places additional financial

burdens on RWSS by way of levies and fines it also exposes consumers to drinking water which would be considered unsafe by public health experts.

In 1998, under a grant funded by EPA (Grant #X826659-01-0), the Technical Assistance Center for Water Quality (TACWQ) was established at Western Kentucky University (WKU) to address the issue of capacity development for RWSS. The TACWQ established programs such as the Source Water Protection Initiative (SWPI) to assist RWSS in acquiring and monitoring the technical, financial and managerial capacity needed to provide safe drinking water and achieve the public health protection goals of the EPA Safe Drinking Water Act (TACWQ, 1998).

From data collected by the SWPI, results from seven demonstration watersheds (Figure 1) showed a number of water quality parameters exceeding the MCLs for untreated *raw* source water. MCL is the highest concentration of a contaminant that is allowed in treated drinking water as set forth by EPA in the National Primary Drinking Water Standards (EPA, 1999). The exceedences of the MCLs in source (raw) water do not result in violations; however, MCL exceedences in the finished water, the water that services the community, can trigger significant fines and violations. With the goals of meeting Drinking Water Standards prior to being used by the consumer and providing safe drinking water, raw source water undergoes various treatment processes. The fundamental concept driving the SWPI is that the technical and financial challenges faced by RWSS are proportional to the quality of their source water (TACWQ, 2000).

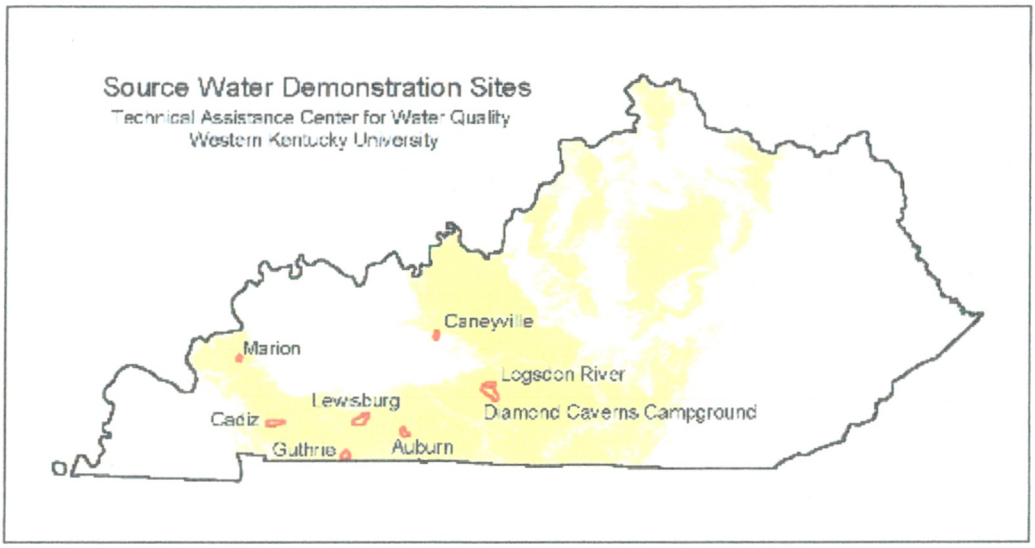


Figure: 1 ▪ Source-Water Protection Initiative Demonstration Watersheds.
Source: Technical Assistance Center for Water Quality. 2000.

Theoretically, the cleaner the source water with which a system can start, the cheaper and easier it will be to treat, resulting in higher water quality and lower consumer costs.

Of the MCL exceedences in raw water, three commonly used pesticides (atrazine, alachlor, and simazine) have been detected repeatedly at several sites. Of these three compounds, atrazine drew the most interest due to its widespread use and highly elevated concentrations. Concurrent to the research conducted by SWPI, Syngenta Corporation, the leading manufacturer of pesticide products containing atrazine, began sampling raw and finished water from the Lewisburg, Kentucky, Water Plant in 1999 under a good stewardship program in collaboration with the Kentucky Department of Agriculture, Division of Pesticides. Syngenta's test results routinely found levels of atrazine exceeding the MCL, peaking as high as 0.017 mg/L. The current MCL for atrazine is

0.003 mg/L (EPA, 1999). Significant concern of the high levels of atrazine resulted in adding Lewisburg as one of the TACWQ's demonstration watersheds.

It is apparent that the variability of atrazine concentrations in source water at Lewisburg depends on a combination of application schedules, moisture conditions within the watershed, rainfall amounts and patterns, chemical mobility of the compounds, geology, soil composition, and hydrogeologic flow paths. The research problem addressed in this work is how and to what degree did these factors influence the concentration of atrazine in the finished and raw water available at the Lewisburg water treatment facility during the 2000 and 2001 agricultural growing seasons.

Chapter 2: Atrazine

I. The Chemical Product and Usage

Atrazine [2-chloro- 4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine] (CAS# 1912-24-9) is a triazine-class herbicide widely used in Kentucky to control annual broadleaf weeds and certain annual grassy weeds in row crops such as field corn, sorghum, and Christmas trees (EXTOXNET, 1996). Atrazine can be used with other crops such as popcorn, sweet corn, grain sorghum (milo), and forage sorghum (sorghum-sudan hybrids) (KDA, 2003). Although not as widely employed, atrazine can also "be used as a nonselective herbicide on non-cropped industrial lands and on fallow lands" (EXTOXNET, 1996).

EXTOXNET (1996) listed that atrazine products may be found on the open market under a variety of trade names, such as: Aatrez, Aktikon, Alazine, Atred, Atranez, Atrataf, Atratol, Azinotox, Crisazina, Farmco Atrazine, G-30027, Gesaprim, Giffex 4L, Malermais, Primatol, Simazat, and Zeapos. The Guidelines for Atrazine Use and Application for Groundwater and Surface Water Protection Best Management Practices (2003) produced by the Kentucky Department of Agriculture (KDA) also identified atrazine and atrazine products on the market under a variety of names, including: A Atrex 4L 90DF, Atrazine 4L 90DF, Axiom AT, Basis Gold, Bicep II, Bicep II Magnum, Buctril+Atrazine, Bullet, Degree Xtra, Field Master, FulTime, Guardsman, Guardsman Max, Harness Xtra, Keystone 5.25L, Laddok, Lariat, LeadOff, Liberty ATZ, Lumax

3.9EC, Marksman, Ready Master ATZ, Simazat 4L 90DF, Sterling Plus 3.2S, Stratos 3.2S, just to name a few of the 130 products available (Blumenstyk, 2003).

In February 2003, the KDA identified atrazine as the most used pesticide in the state of Kentucky. Its superior effectiveness and lower cost when compared to other herbicides made it the applicant of choice across the state.

Atrazine is most often sprayed on the land as a water-based liquid fertilizer, or as an impregnated dry-bulk fertilizer to the soil either as a pre-plant applicant on the soil surface, pre-plant applicant incorporated into the soil, or as a pre-emergence treatment (KDA, 2003). It is labeled as a "spring-use only" product and is not approved for fall application. It may be applied up to 45 days before planting; however, this does not negate its fall application prohibition. Atrazine is less often, but may also be applied after "crop emergence as an early post emergence treatment before corn exceeds 12 inches in height" (KDA, 2003).

II. Mode of Action

Regardless of the application form, atrazine successfully kills most target plants (weeds) by inhibiting the electron-transport chain in their chloroplasts (Assay Designs, 2003). The misconception is untrue that a target plant starves to death when treated with a specific herbicide. Food reserves remain high within the plant despite the onset of chlorosis (yellowing of plant leaves due to lack of chlorophyll) and/or necrosis (dying of

the leaves) (HyperDictionary, 2003), both of which shortly follow herbicide application, regardless of whether employed as a pre- or post-emergent control.

Inhibition of the electron-transport chain is a precise and effective means to destroy a plant. During photosynthesis, light energy is converted into chemical energy in the chloroplasts of a plant through a chemical reaction by two processes: Photosystem I (PI) and Photosystem II (PII). The electron-transport chain involves a series of electron carriers that transfer electrons from a donor carrier, which becomes oxidized, to a receptor carrier, which becomes reduced (Mauseth, 2003). Herbicides can affect the electron-transport chain in two ways. They can inhibit electron transfer or intercept the electrons along the transport chain. Triazine class herbicides, such as atrazine, act on the quinone acceptor complex where it competes with the plastoquinone (PQ) for the quinone B (Q_B) binding site (Taiz and Zeiger, 1998) (see Figure 2). Atrazine displaces the oxidized form of the plastoquinone and inhabits the specific binding site for the quinone acceptor (Taiz and Zeiger, 1998). The binding site is one of five niches on the Q_B protein and is thought to lay on the D1 herbicide-binding proteins (Taiz and Zeiger, 1998; Griffin, 2003). Atrazine is a general purpose herbicide which interferes with the Q_B electron acceptor in the P680 electron-transport chain (Bourgeois, 2003). When atrazine is present, it is unable to accept electrons from the quinone A site, thus electron transport to the plastoquinone pool is inhibited (Taiz and Zeiger, 1998, Griffin, 2003). This causes a backlog of electrons leading to the cessation of adenosine triphosphate (ATP) production (Bourgeois, 2003), which gives the plant energy and is essential for plant survival.

The following diagram demonstrates where the inhibition of the electron-transport chain takes place, between the Q_B and PQ pool (Ashton and Crafts, 1981).

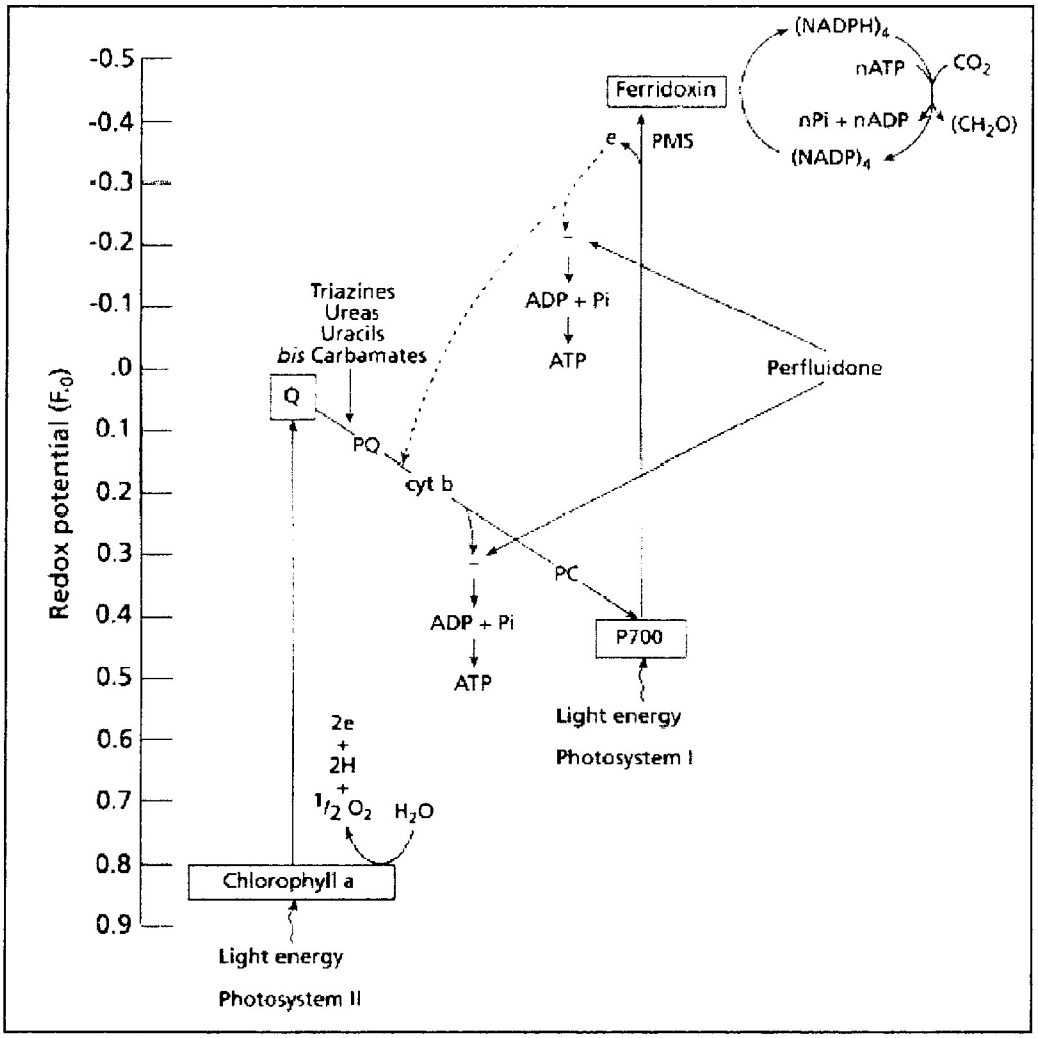


Figure: 2 ▪ The electron-transport chain in photosynthesis indicting various sites of action of herbicide inhibition within the chain. Source: F.M. Ashton and A.S. Crafts. 1981.

Photosynthesis inhibitors were developed beginning in the early 1950s, although their modes of action were not identified until 1961. Atrazine is a heterocyclic ring structure containing three nitrogen atoms alternating with three carbon atoms in a symmetrical pattern (Griffin, 2003). The heterocyclic ring structure is why it is included in the symmetrical-triazine, or s-triazine, class of herbicides (See Figure 3).

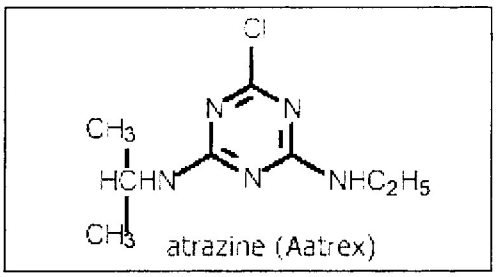


Figure: 3 ▪ Base molecular structure of atrazine, an s-triazine class herbicide (Griffin, 2003).

Research has shown that some plant genome structures are atrazine resistant. To date, there are at least 60 known weed species resistant to atrazine (Griffin, 2003). Erickson and others demonstrated in 1989 that the chloroplast resistance trait can be contributed to a single amino acid substitution in the D1 polypeptide (Erickson *et al.*, 1989). The mutation renders the QB protein invulnerable to atrazine. This resistance has also been proven to be a recessive maternally-inherited trait (Erickson *et al.*, 1989). Sometimes natural evolution of resistance to a product can be manipulated to provide a favorable outcome. With the knowledge that Erickson and others contributed to the field of understanding, researchers have been able to implant the atrazine resistant mutation into certain crop plants, such as corn -- thus allowing fields of the crop to be sprayed with atrazine to eliminate the weeds without killing the crop (Bourgeois, 2003).

It can be agreed upon that atrazine is an effective herbicide, sold at a low cost to the consumers, that has been genetically engineered to selectively control target weeds while not killing the main crop. However, there are also problems associated with the use of the chemical.

III. History and Development

The triazine family was discovered in 1952 by J.R. Geigy in Basel, Switerland, and was introduced onto the market in 1956 by Geigy Colour Company Ltd. Geigy Colour merged with Ciba to form Ciba-Geigy Ltd in 1970. Two decades later in 1992, Ciba-Geigy was renamed Ciba to coordinate with the introduction of a new logo. Four years later in 1996, Sandoz, a pharmaceuticals and textiles development company, and Ciba integrated to form Novartis, which was one of the largest corporate mergers in Swiss history. Novartis Seeds, a division of Novartis, once again merged with AstraZeneca's agribusiness operations to form Syngenta, the first global group focusing exclusively on agribusiness (Syngenta, 2003).

This company, now called Syngenta, came under pressure from the US EPA in the early 1990s to begin monitoring Community Water Systems (CWSs) for elevated pesticide concentrations, including atrazine, as part of an effort of identify, monitor and remediate ecologically vulnerable watersheds. Atrazine is regulated as a compound with class III (slight) toxicity, but since 1994, atrazine has been the subject of a special review by EPA which required Syngenta to monitor 40 indicator watersheds that are representative of areas that may be vulnerable to elevated levels of atrazine contamination (Deegan, 2003). In this program, Syngenta began sampling raw and finished water from the Lewisburg (Kentucky) Water Plant in 1999 under a good stewardship program with the Kentucky Department of Agriculture, Division of Pesticides. Syngenta's test results routinely found levels of atrazine exceeding the MCL,

which prompted EPA to take further action to mitigate the exposure risk in the Spa Lake watershed by implementing a voluntary program that reduced the maximum allowable dry bulk application poundage of atrazine from 2 lb per acre to 1.5 lb per acre for the 2000 and 2001 growing seasons (Givens, 2001). To further benefit the voluntary program, the state mandated a no-till best management practice that was also implemented during the 2000 growing season for the watershed (Givens, 2001).

Under the special review, EPA was required to proceed with a re-registration process for atrazine which included fully evaluating the ecological and biological effects of the herbicide.

IV. Controversy

Concern about the safety of atrazine elevated just as Spa Lake was deemed a watershed of concern. In early 1999, the Natural Resources Defense Council (NRDC) and a coalition of farm and other environmental groups sued EPA, alleging the agency had not evaluated the risk of pesticide usage, especially atrazine, to children as mandated by the 1996 Food Quality Protection Act. The two sides filed a consent decree in 2001 which stated that EPA would review the safety of atrazine by the beginning of 2003. EPA failed to meet this deadline. A second court order required an independent Scientific Advisory Panel to fully review all data linking atrazine to a number of cancers, including prostate cancer and non-Hodgkin's lymphoma (Olson *et al.*, 2003). In the summer of 2003, EPA initiated the advisory panel to evaluate *only* the link between

prostate cancer data and atrazine. In late August 2003, the advisory panel criticized EPA for narrowing its review to only prostate cancer, and further demanded the agency to review data involving atrazine and *all* types of cancers (Olson *et al.*, 2003).

The conflict heightened in October 2003 when EPA handed down its decision to approve the unrestricted use of atrazine and concluded that it is unlikely to cause cancer in humans (Olson *et al.*, 2003). NRDC insisted that the EPA's decision reflected current political, not scientific, influences. Ironically, the same month that the United States maintained that atrazine was safe to use, the 15-nation European Union issued an 18 month ban on the product due to atrazine's health and environmental risks (Olson *et al.*, 2003).

How atrazine affects the environment and the health of wildlife that it encounters is another topic of debate. Tyrone B. Hayes was one of several researchers hired by Ecorisk, Inc., a consulting firm for Syngenta, to investigate the ecological effects of atrazine. Hayes was a developmental endocrinologist in the University of California at Berkeley's department of integrative biology when he was asked to research the effects of atrazine on amphibian development. His research showed that atrazine concentrations as low as one part per billion would inhibit the growth of the larynxes of male African clawed frogs (Blumenstyk, 2003). Syngenta was not enthusiastically receptive to Hayes' findings and disputed his work. Other researchers hired by Ecorisk were unable to reproduce Hayes' results (Blumenstyk, 2003), thus shredding some doubt on the validity of his findings. Due to confidentially agreements, Hayes could not publish his findings

without Syngenta's approval. Publication was exactly what Hayes needed so that his peers, independent of his research or Syngenta, could critically assess his data for accuracy and consistency. On the contrary, Syngenta worked hard to discredit Hayes' research and bury his findings according to one article in the Chronicle of Higher Education (Blumenstyk, 2003).

After separating from the Ecorisk/Syngenta research team, Hayes conducted new studies that looked not only at the larynxes, but also the sex organs of frogs that had been treated with atrazine. In a second, self-funded study, Hayes found atrazine was affecting the sex organs of male frogs at levels as low as 0.1 parts per billion - a concentration a tenth of that affecting the larynxes. Hayes' findings caused a fair amount of concern when he published his independent research in mid-April, 2002. His plight was featured in the Chronicle of Higher Education (Blumenstyk, 2003), as a testament of how research sponsored by big companies doesn't always have a happy ending. Hayes was quoted in the Chronicle, "The testes [of the male test frogs] essentially start changing," because atrazine triggers production of estrogen. "They grow ovaries and eggs." (Blumenstyk, 2003). His research was very disturbing to citizen watch groups like NRDC, as well as the general scientific community interested in atrazine. Recall that the Commonwealth of Kentucky and federal MCL for atrazine in treated drinking water is 0.003 mg/L (or 3.0 parts per billion (ppb)). Hayes' research was in California in a controlled laboratory, but it is plausible to envision similar situations in natural waters anywhere atrazine has been widely used.

Another study by an independent research group indicated an increased rate of cancer among actively working employees at one Syngenta manufacturing facility in Louisiana (Gable, 2002). There have been numerous studies on the evidence of breast cancers due to atrazine exposure primarily in rats and mice (Chapin *et al.*, 1996, Innes *et al.*, Stevens *et al.*, 1994, Wetzel *et al.*, 1994) and humans (Blair *et al.*, 1993, Kettles *et al.*, 1997, Wiklund and Dich, 1994). There is also a concern that estrogen-like chemicals, such as atrazine, may be involved in testicular as well as prostate cancers (Buranatrevedh and Deodutta, 2001). Sharpe and Skakkebaek (1993) described the correlation between the increased use of estrogen-like chemicals and the significant decrease in male sperm counts in the Western world over the past 50 years.

Exposure by the handling of atrazine has been the subject of several environmental health studies. Hoar and others (1993) analyzed three case studies comparing the role of atrazine exposure of farmers to the development of non-Hodgkin's lymphoma (NHL). They concluded that there was little to no increased risk of developing NHL in relation to handling atrazine. Health officials continue to debate this conclusion.

Absorption through the skin is not the only pathway that atrazine can enter into the body. Little is known about the risk of inhalation of atrazine as a fine mist or evaporation product. The most common and most readily absorbing pathway is by ingestion. Upon entering the gastrointestinal tract of rat specimens, studies have shown that only 20% of the product was excreted within a 72 hour period (Stevens and Sumner,

1991). The remaining 80% entered into the specimens' bloodstream by way of absorption across the lining of the gastrointestinal tract. Following an additional 72 hour waiting period, Stevens and Sumner (1991) found as much as 15% of the product remained in the rats' body tissues, including the liver, kidneys, and lungs.

V. Mobility and Transport

With the uncertain knowledge of the safety of atrazine to humans, atrazine has been classified as a Restricted Use Pesticide (RUP) due to its potential for groundwater contamination (Ware, 1986). Atrazine's mobility is gained primarily through rainwater runoff, but may also occur by leaching of the soil. Research by Buchanan and Hiltbold (1973) found that atrazine had an average half-life of 20 days in sandy loams, and its persistence and carryover to future crops in the season was minimal. More recent documentation indicated that atrazine is highly persistent in soils, with a half-life of 60 to > 100 days, and it may even "persist for longer than 1 year under dry or cold conditions" (EXTOXNET, 1996).

In order for atrazine to persist in the soil column it must first be incorporated into the soil medium. Soil composition plays a large role in the behavior of atrazine absorption. Burkart and others (1999) evaluated the concentration of atrazine in groundwater with respect to the overlying soil characteristics. They reported that soil composition accounted for over 33% of the variance of atrazine concentration observed. Although atrazine has historically been considered **not** to strongly adsorb to soil particles

(EXTOXNET, 1996), the case is not always true. Soils with microscopic sized particles, such as those with high clay and humus content, have a better adsorption capacity for binding cations and pesticides. Those microscopic particles, called soil colloids, have extremely large surface areas that are negatively charged. Soil colloids have the ability to trade cations in the soil with other cations introduced into the soil. This swapping of cations is called the cation exchange capacity (CEC) of the soil. The CEC of soil material is directly related to the adsorption and mobility of some pesticides (Jourdan, 1992). Clay minerals exhibit various CECs throughout the year. The CEC is a function of pH and can change seasonally due to the fluctuation of carbon dioxide and water retained in the soil.

The negatively charged soil colloids bind strongly with positively changed pesticides, thus retaining them in the soil for long periods of time. Conversely, negatively charged pesticides are not readily adsorbed onto the soil colloids and should, theoretically, be easily removed from the soil by rain runoff or atmospheric evaporation. Atrazine is a negatively charged pesticide which, when not incorporated into the soil column or taken up by plants, does wash off the soil and crops, thus entering rivers, streams and Community Water Systems (CWSs) in a liquid medium. In theory, atrazine should not be retained in the soil; however, at lower soil pHs, research has shown that some anionic pesticides like atrazine can become cations and result in strong ionic bonds with the soil colloids (Jourdan, 1992). In this circumstance, atrazine adsorbed to soil particles may wash into a water system and be retained by the sediment until water conditions shift, dismembering the ionic bond or degradation occurs. Retention refers to

the mechanisms and forces involved in holding the pesticide to the soil matrix. Jourdan (1992) examined the strength and nature of these factors, such as London-Van Waals forces, hydrogen bonding, protonation, cation and water bridging, cation and anion exchange, covalent bonding, and physical trapping. He determined that under field conditions, wetting and drying of the soil has a significant influence on retention, and that some weakly bonded pesticides can strengthen their bonds to the soil colloids over time.

VI. Fate

Atrazine that doesn't wash off, get adsorbed by plants, or volatilize, remains in the soil. Following incorporation into the soil column, atrazine persists until some form of degradation occurs. Degradation can occur by chemical hydrolysis, atrazine chlorohydrolases, dealkylation and/or photodegradation (EPA, 2001). Chemical degradation is widely believed to play a much greater environmental role than biodregradation (Putters, 2001).

Chemical hydrolysis is an important and specific type of chemical degradation. It is the conversion of organic wastes to more benign compounds through substitution by hydroxide ions (PPL, 2004). Hydrolysis is most effective at extreme pHs and less effective at near neutral pHs (EXTOXNET, 1996). Atrazine can be hydrolyzed at higher temperatures via the action of alkali materials and mineral acids; however, the reaction slows dramatically as the temperature elevates if the pH is neutral (Putters, 2001). Alkaline hydrolysis occurs twice as fast as that of an acidic environment; and, likewise,

reported that chemical hydrolysis is most likely the principle pathway of detoxification in the soil (EXTOXNET, 1996, Schlater, 1994). Since karst environments, such as the area of Spa Lake, usually maintain a near neutral pH, hydrolysis is believed to be retarded in the study area.

Dechlorination must initially occur to begin the chemical hydrolysis process. Following removal of the chlorine (Cl) from the atrazine compound, a hydroxyl group (OH) fills the available position, forming hydroxyatrazine (Figure 4).

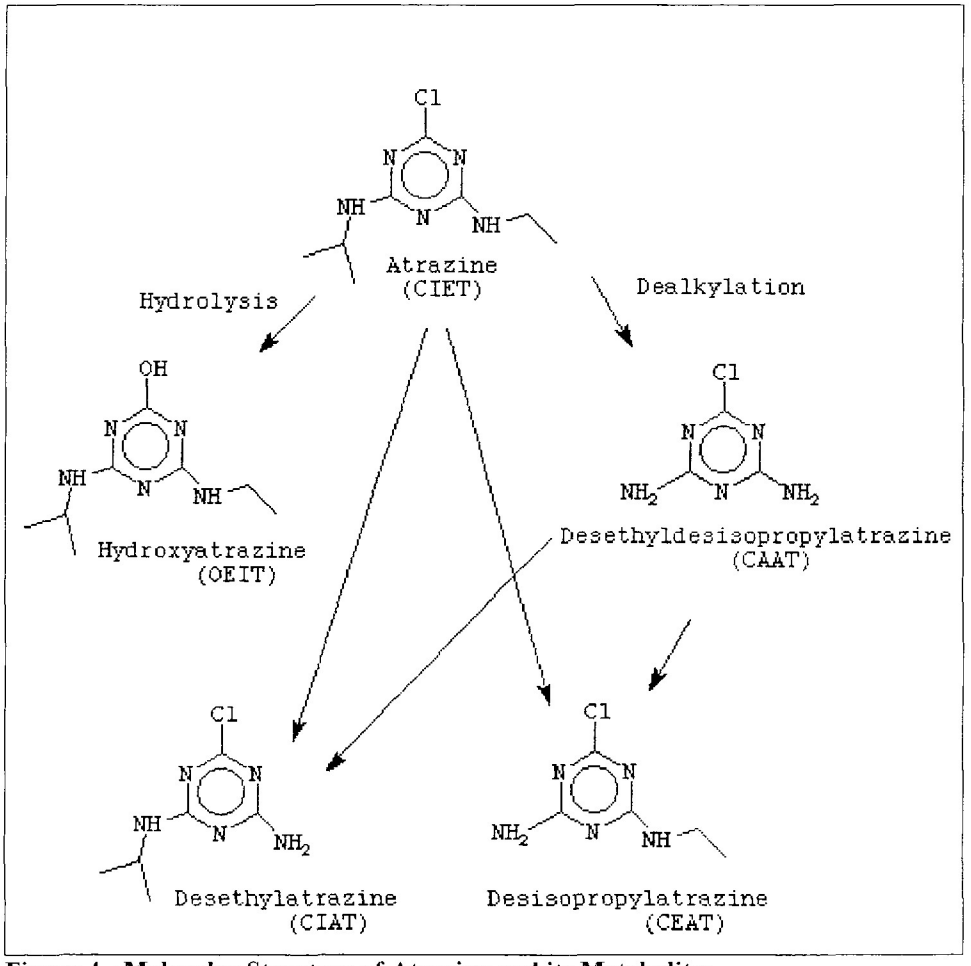


Figure 4 • Molecular Structure of Atrazine and its Metabolites.
Source: Bioremediation of the Herbicide Atrazine. 1994.

It was once believed that chemical hydrolysis was the only means of hydrolysis degradation. However, in a study conducted by the Department of Biochemistry and Institute for Advanced Studies in Biological Process Technology and the Department of Soil Science at the University of Minnesota, biological hydrolysis was documented (Schlater, 1994). Following the formation of hydroxyatrazine, microbial degradation occurs several times to form metabolites. Bacterial degradation of atrazine is termed atrazine chlorohydrolase. This type of degradation results in two compounds: 4-(ethylamino)-2-hydroxy-6-(isopropylamino)-1,3,5-triazine and chloride (Un. College London, 2003). These metabolites are themselves degraded by chlorohydrolase. The final products of chemical hydrolysis are carbon dioxide and ammonia (Schlater, 1994).

In the field, there may be added value to increased rates of atrazine degradation. The University of Minnesota study showed that the addition of an organic material, such as humus, can act as a catalyst, as well as mediate the hydrolysis process by bacterial enzymes (Schlater, 1994). Although it sounds contradictory, humus helps to retain atrazine in the soil column, as well as degrade it to more benign compounds.

A third type of degradation is dealkylation, which, by definition, is the removal of alkyl groups from a compound (Medical Dictionary Search Engine, 2003). Putters (2001) identified dealkylation occurring at the C-4 and C-6 position of the atrazine molecule (Figure 5).

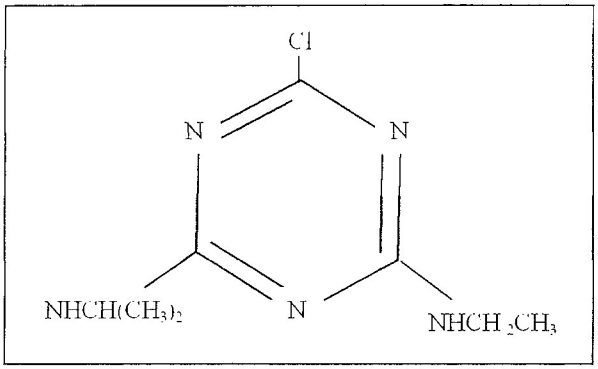


Figure 5 ▪ Molecular Structure of Atrazine.
Design based on model by Putters. 2001.

Through dealkylation, microorganisms assist in removing the alkyl groups from atrazine, thus, converting it into one of its byproducts, such as desethylatrazine, desisopropylatrazine, desisopropyl-desethyl-atrazine, 2-chloro-4-hydroxy-6-amino-1,3,5-triazine (EPA, 2001), 4,5-bis(alkylamino)-1,3,5-triazin-2-ol (via hydrolysis), deethylated hydroxyatrazine (Cornell, 2001), and 2,4-dihydroxy-6(N-ethyl)-amino-1,2,5-triazine (Putters, 2001).

Dealkylation is the most widely studied biodegradation method described in literature (Schlater, 1994). Schlater's 1994 study examined bioremediation opportunities available for atrazine. She reported that no one organism could completely degrade atrazine, but that biodegradation required a whole host of organisms to fully achieve benign results. Through dealkylation, atrazine is initially degraded through the process of oxidative N-dealkylation, forming three atrazine metabolites: deisopropylatrazine (CEAT), deethylatrazine (CIAT), and desethyldesisopropylatrazine (CAAT) (Schlater, 1994), as shown above in Figure 4. For ease of discussion, Schlater abbreviated the

sometimes cumbersome names of metabolites based on their substituents on the s-triazine ring. Those abbreviations have been adopted here (Table 1).

Table 1 ▪ Abbreviations for metabolites based on the substituents on the triazine ring.

Abbreviation	Substituent
A	amino
C	chloro
E	ethylamine
I	isopropylamino
O	hydroxy
T	triazine ring

Source: Bioremediation of the Herbicide Atrazine. Schalter. 1994.

Figure 6 below demonstrates the circuitous route each intermediate reaction travels before forming a common intermediate product, OOOT (Schlater, 1994), commonly known as cyanuric acid (Putters, 2001). Cyanuric acid is degraded through cyanuric aminohydrolase to biuret, which is further degraded by microbial degradation to urea and urease (Putters, 2001). Urea is the final metabolite to break down through the dealkylation process. The final products are carbon dioxide, ammonia and hydrogen (Putters, 2001; Schlater, 1994) – the same final products as chemical hydrolysis and atrazine chlorohydrolase.

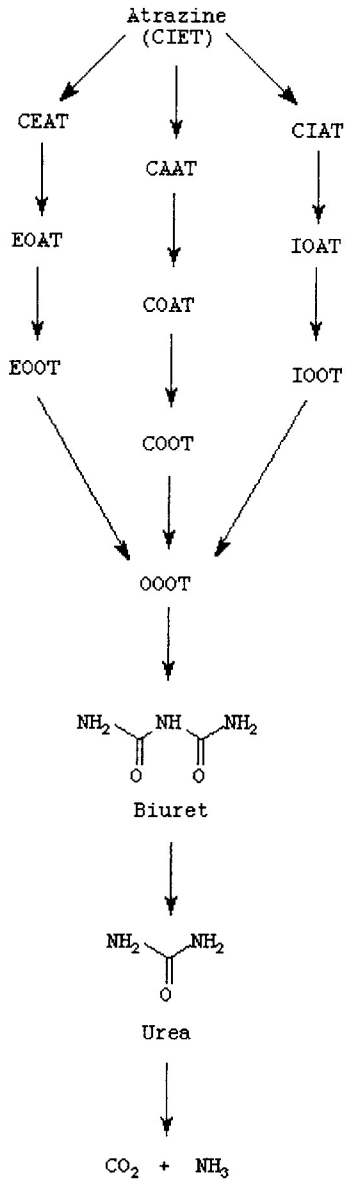


Figure 6 ▪ Atrazine Metabolic Pathways in Microorganisms.
Source: Bioremediation of the Herbicide Atrazine. Schalter. 1994.

Evidence suggests that prolonged persistence of atrazine in the soil and its accumulative effect over many years has the potential to injure sensitive crops that may be used in crop rotation systems, as is common in south central Kentucky (Ashton and Monaco, 1991; Blumhorst and Weber, 1992).

Chapter 3: Site Description

Kentucky characteristically has geological and physiographic diversity. The landscape is commonly shaped by the underlain rock composition and the weathering processes on the surface. The state is divided into five physiographic regions based on surface topography and geology (Figure 7).

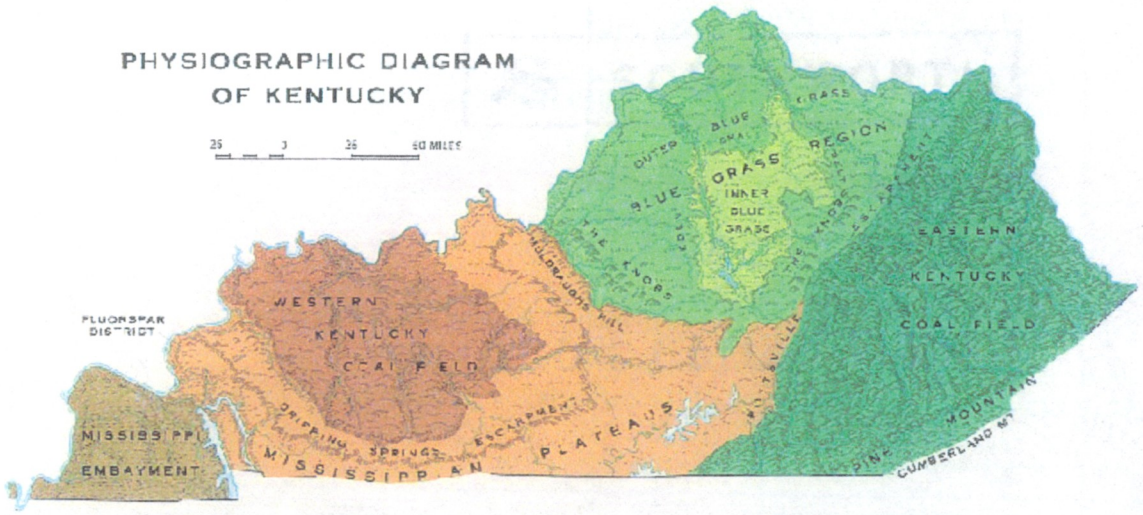


Figure 7 ▪ Physiographic Diagram of Kentucky Source: Kentucky Geologic Survey. 2002.

The physiographic subdivisions of Kentucky generally mimic the geologic map patterns due to the surface topography reflecting geologic controls in each region or subdivision. The state is varied geologically with Tertiary sediments in the Mississippi Embayment region to the west, Upper Paleozoic carbonates and clastics in the south and central regions, Ordovician carbonates and clastics in the Blue Grass region, and Upper Paleozoic carbonates and clastics in the Eastern Kentucky coal fields (Figure 8).

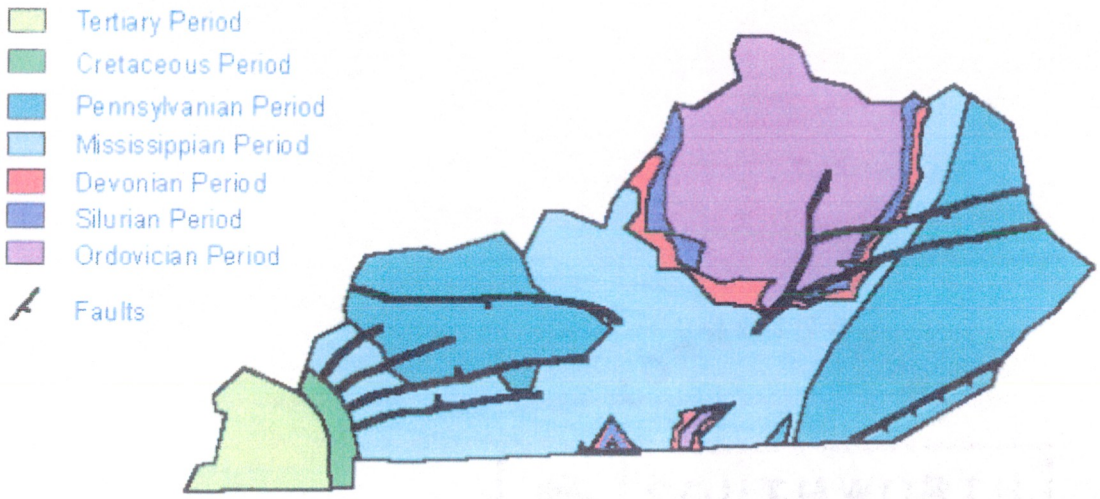


Figure 8 ▪ Geologic Map of Kentucky. Source: Kentucky Geologic Survey. 2002.

As can be noted on the geologic map, much of Kentucky in underlain by rocks of the Mississippian System, which has an age range of 360 to 320 million years (Ma) before the present (Levin, 1994). South central Kentucky is located on thick beds of soluble limestone interlayered with chert and shale and often capped by insoluble quartzose sandstone or conglomerate.

The boundary surrounding the Western Kentucky Coal Fields is known as the Dripping Springs Escarpment (Figure 7). This area is famous for its karst terrain and home to Mammoth Cave National Park, the world's longest cave system. Karst refers to a type of landscape that is generally underlain by carbonate rock, usually limestone or dolostone, where the topography is largely controlled by the dissolution of the rock, and may be characterized by sinkholes, sinking streams, closed depressions, subterranean drainage, caves, springs and swallets, and/or the absence of surface streams (Speleogenesis Info, 2004). Specific morphologic and hydrologic features of soluble

rock are coupled in the term karst. Some morphologic features that may indicate karst include karren, dolinas (sinkholes), caves, caverns. Hydrological features of karst encompass basins of closed drainage, lost rivers/streams, submarine springs, underground streams and incongruity of surface and underground hydrologic divides (Speleogenesis Info, 2004). The term karst is internationally used. It originated from the German form of the Slavic word kras or krs, meaning a bleak waterless place (Speleogenesis Info, 2004). The name was adopted from a district east of Trieste, Germany, for having such a terrain (Speleogenesis Info, 2004).

Wray (2003) examined differences between karst and pseudo-karst and noted that various scientific arguments insist that underlying rock must be water soluble in order to be considered true karst. On the other hand, karst terrain is known to be present on non-carbonate landforms. Less common, karst can occur in volcanic areas, where deserted lava tubes transform into dazzling cave passages. Karst can also develop in artic regions and other landscapes where non-carbonate material are present. These types of landforms are often referred to as pseudo-karst.

The distinguishing characteristic of karst is water soluble rock that dissolves into an underground drainage network of conduits, rock fractures, and bedding-plane joints that redirect and capture the surface water. Jennings (1985) wrote that,

> "... karst is a terrain with distinctive landforms and drainage arising from greater rock solubility in natural waters than elsewhere... Solution is not

always the most prevalent process in karst, nor is it necessarily the dominant one, but it does play a more important role here than in other kinds of landscape."

Figure 9 offers a detailed cross section of a conceptual model of a typical karst landscape.

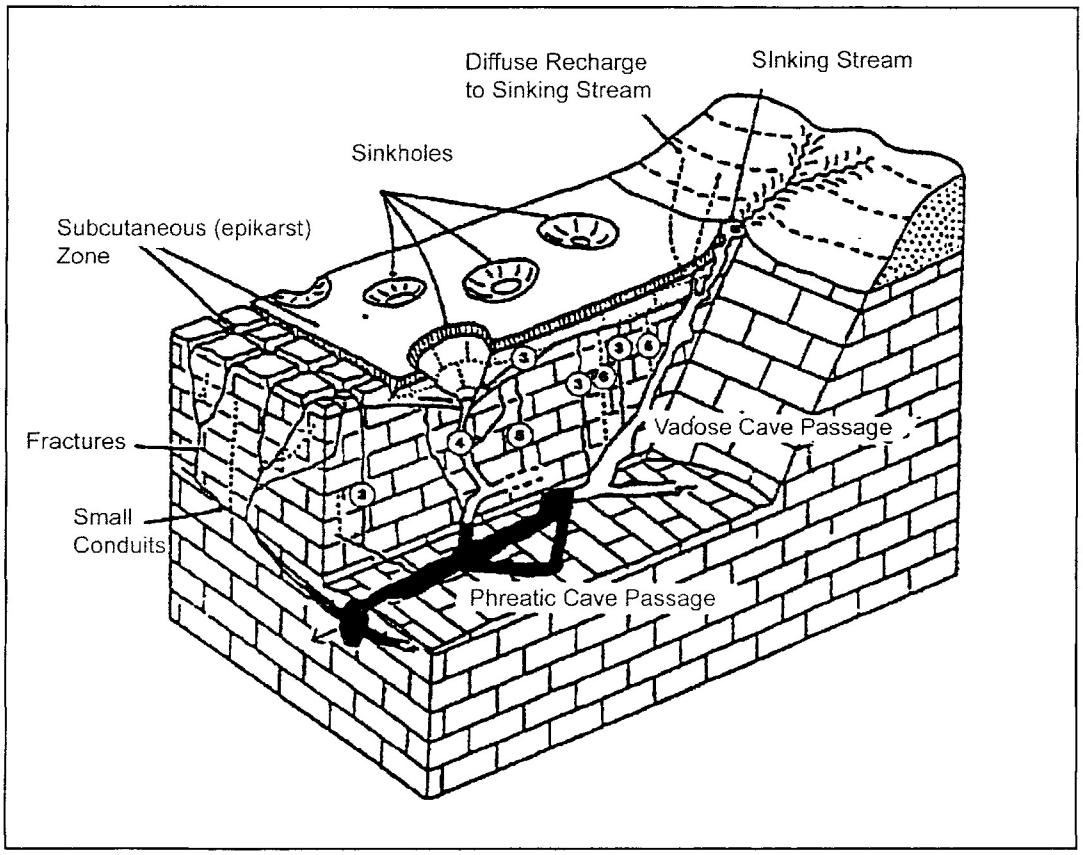


Figure 9 ▪ Conceptual model of a karst landscape. Adapted from Gunn. 1985.

Typically, karst features are formed by dissolution of minerals present in the limestone. Commonly the mineral is calcite or calcium carbonate ($CaCO_3$), better known as limestone. The dissolution process is a series of chemical reactions initiated by contact

of water (H_2O) and carbon dioxide (CO_2) in the atmosphere which creates carbonic acid (H_2CO_3). Carbonic acid dissociates into hydrogen (H^+) and bicarbonate ions (HCO_3), as demonstrated in equations 1 and 2.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \qquad \text{(carbonic acid)} \qquad (1)$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3 \qquad \text{(bicarbonate ion)} \qquad (2)$$

$$HCO_3 \leftrightarrow H^+ + CO_3^{2-}. \qquad \text{(carbonate ion)} \qquad (3)$$

Equations 1-3 ▪ Dissociation of carbon dioxide and water. Boggs. 1995.

This dissociation process leaves free hydrogen ions in solution, thus lowering the pH. When $CaCO_3$ comes in contact with H_2CO_3, it rapidly dissolves into HCO_3 and calcium (Ca^{2+}) ions. The more acidic a solution, the greater the ability to dissolve carbonate material ($CaCO_3$), such as calcite and aragonite crystals. The HCO_3 ions continue to dissolve as previously described above in equation 3. The carbonate/ water/carbon dioxide reaction is summarized as follows:

$$CO_2 + H_2O + \underset{\text{(calcite or aragonite)}}{CaCO_3} \leftrightarrow Ca^{2+} + 2HCO_3 . \qquad (4)$$

Equation 4 ▪ Dissociation of carbon dioxide, water, and calcium carbonate. Boggs. 1995.

Equation 4 represents the most important chemical reaction responsible for the dissolution process and resulting karst landscape in south central Kentucky. The resulting sinkholes and caves offer an open pathway for liquid media to transfer rapidly and relatively unimpeded downstream to the local water table. Without the natural

seepage and filtration opportunities offered by other soil and rock medium, multiple impurities remain in the water and directly enter the open waterways and/or aquifers used for drinking-water supplies.

South central Kentucky is known for its rich agriculture, including beef and dairy cattle operations and fertile crop land. Corn crop production averages 378 hectares per year (Givens, 2000). From such widespread dependence on the land, water-quality issues associated with agriculture usage arise, including elevated levels of pesticides not only in raw water sources but also in finished drinking water as well.

The subject site for this research was Spa Lake, located east, southeast of Lewisburg in south central Kentucky. Spa Lake is a 97.1 hectare lake, created by the damming of a stream valley. This reservoir served as the source-water supply for northern Logan and Todd Counties until 2003. The topographic watershed boundary is depicted in Figure 10.

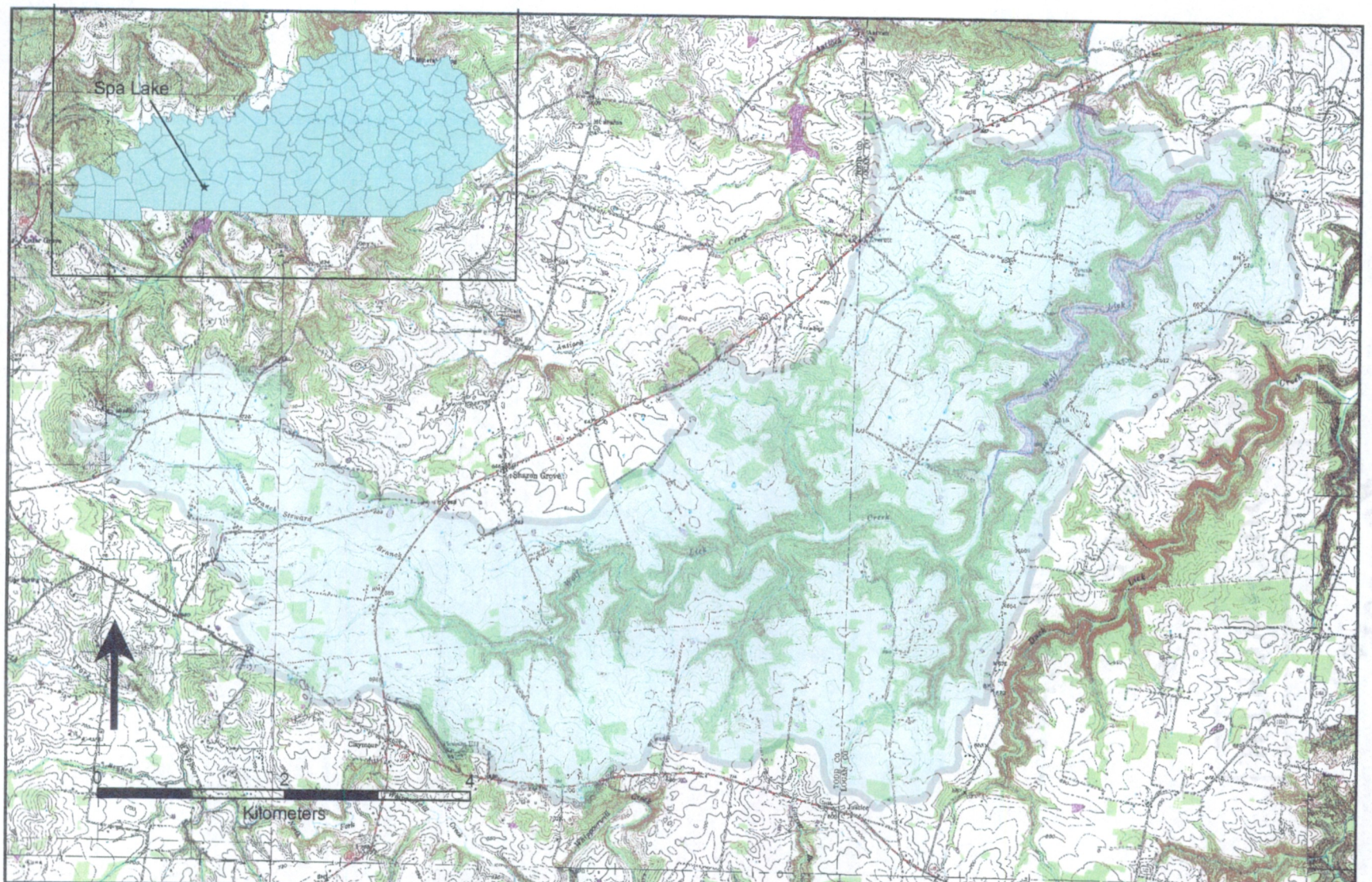


Figure 10 ▪ Topographic map of Spa Lake depicting topographic watershed boundary.
Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

The watershed consists of 42.81 km^2 of various pasture and cropland, and rural communities. Detailed landuse analysis indicated that 18.69 % of the land within the watershed was used for corn-crop production from 1999 to 2001 (TACWQ, 2002). This percentage equates to a total of 8.00 km^2 of potential atrazine coverage that could enter into the local water source, Spa Lake.

Wolf Lick Creek is the primary source of water contributing to Spa Lake. The catchment area has moderately developed karst landforms with only initial ponor and doline dissolution features. Wolf Lick Creek has deeply incised the relatively gently graded upland fields and pastures. The upstream, western branches of Wolf Lick Creek, known as Steward Branch, had intermittent flow throughout the study period. Much of the upland drainage area is underlain by the Golconda Formation, which is part of the Chester Series within the Mississippian System. The Haney Limestone, Big Clifty Sandstone, and Beech Creek Limestone Members comprised the Golconda Formation, respectively trending eastward and descending stratigraphically (Figures 11 and 12).

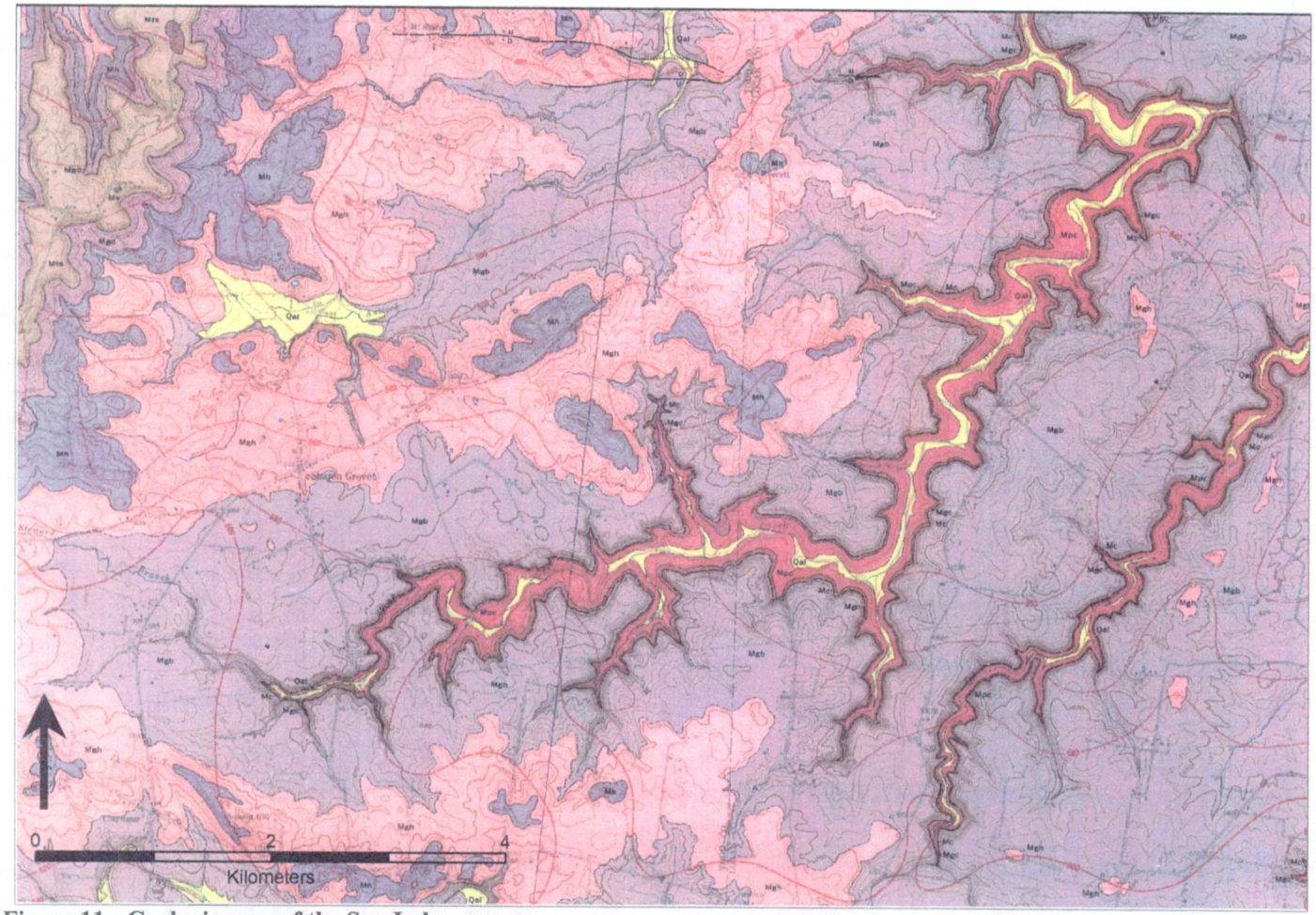


Figure 11 ▪ Geologic map of the Spa Lake area.
Based on the Ulrich 7.5 Minute US Geologic Map, Sharon Grove Quadrangle. Logan and Todd Counties, Kentucky. 1966.

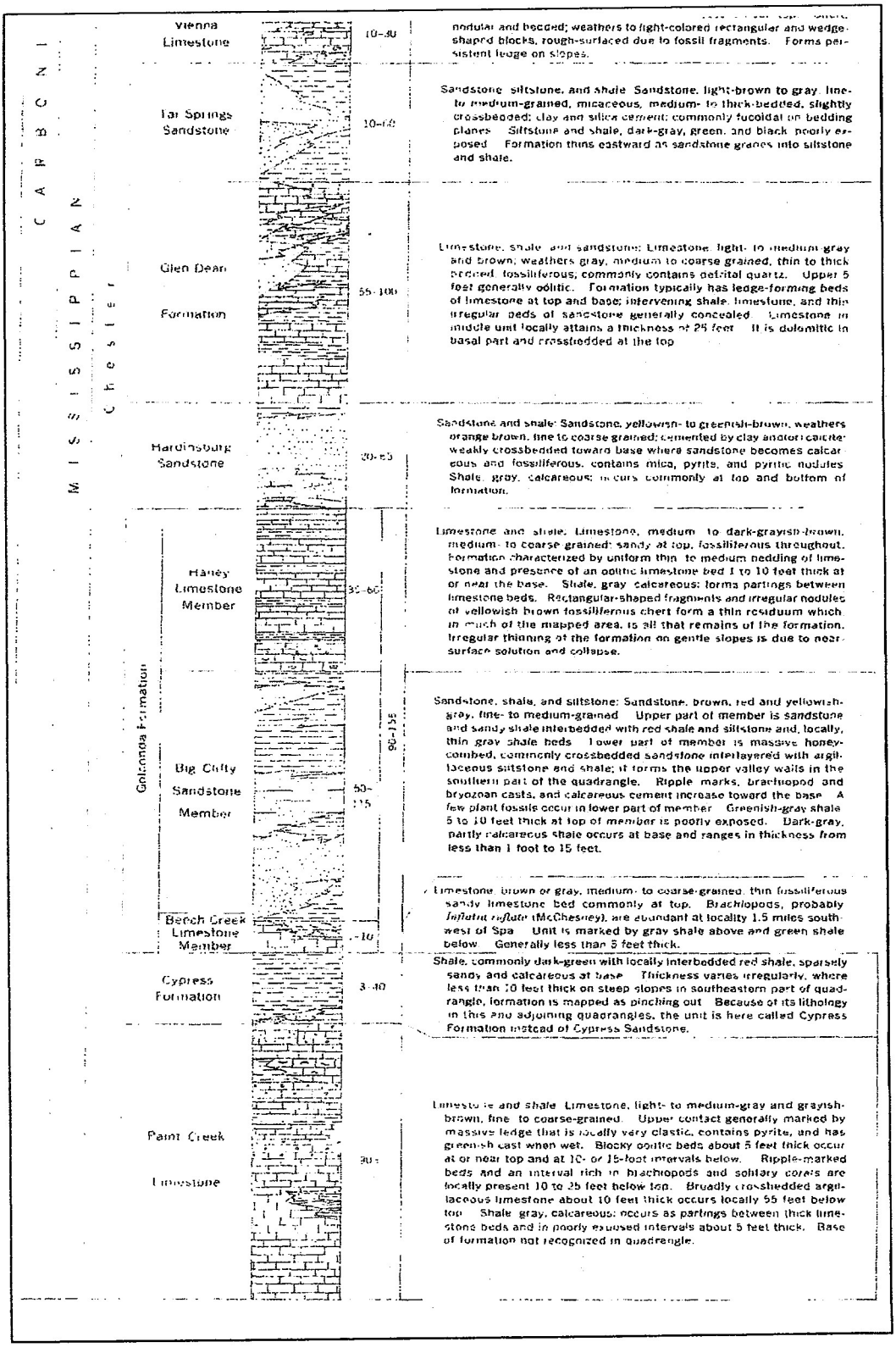


Figure 12 ▪ Stratigraphic column. Source: US Geologic Survey, Ulrich. 1966.

The Haney Limestone Member is a thin to medium, uniformly bedded, medium- to dark-grayish-brown, medium- to coarse-grained limestone. Gray calcareous shale is interbedded throughout the limestone beds and, rectangular-shaped fragments and nodules of fossiliferous chert form a residual layer near the bottom of the formation. A small number of shallow sinkholes was observed in the field and are discernable in the upstream stretches of Wolf Lick Creek on both the Sharon Grove, KY 7.5 minute topographic and geologic quadrangles (Figures 10 and 11 above). The few sinkholes observable in the Haney Limestone and the overlying Hardinsburg Sandstone are attributed to the near-surface solution and collapse of Haney (Ulrich, 1966).

As the creek meanders east-northeast, Steward Branch flows intermittently across the Big Clifty Sandstone Member, which can range from 15- to 30-meters thick. The Big Clifty is actually a combination of crossbedded sandstone and calcareous shale interbedded with argillaceous siltstone and shale. Well preserved in some areas where ripple marks, brachiopods, and bryozoan casts are are present in the exposure near the base of the formation. The Big Clifty Sandstone Member forms the uppermost walls of the deep valley that Wolf Lick Creek has cut through the area.

The Beech Creek Limestone is the lowest and thinnest member of the Golconda Formation. This thin, fossiliferous sandy limestone bed is little exposed and typically ranges from 0.3 to 3.0 meters in thickness, and is bound by gray shale above and green shale below. A rich bed of brachiopods, thought to be *Inflatia inflate*, is identified on the

7.5 minute Sharon Grove Geologic Quadrangle (Ulrich, 1966). This outcrop of fossils is located on the northern shore of Spa Lake, just north of the island.

The Beech Creek Limestone is rarely seen on the steeply graded slopes bordering Wolf Lick Creek and Spa Lake. Only one outcrop was identified during field research for this project.

Just below the Beech Creek Limestone, the green shale mentioned above signifies the beginning of the Cypress Formation. The green shale indicative of the Cypress is locally interbedded with red shale and is mapped as pinching out in the southern portion of the 7.5 minute Sharon Grove Geologic Quadrangle, which includes the southern tributaries of Spa Lake (Ulrich, 1966). The Cypress Formation surrounding the lake is roughly 6 to 12 meters thick. In other areas, the Cypress Formation is also known as the Cypress Sandstone; however, its lithology in the Spa Lake area prohibits such description.

The Cypress Formation becomes sandy and calcareous at its base as it grades into the Paint Creek Limestone, which creates the base of the Wolf Lick Creek and Spa Lake. The Paint Creek is a light- to medium-gray and grayish-brown limestone with gray calcareous shale partings poorly exposed in 1.5 meter intervals (Ulrich, 1966). The upper contact was well identified in the field based upon the geologic quadrangle description of a "massive ledge that is locally very clastic, contains pyrite, and has (a) greenish cast when wet" (Ulrich, 1966). This formation has blocky oölitic beds in 1.5 meter intervals

down to 4.5 meters, then is characterized by ripple marked beds and an outcrop abundant with brachiopods and solitary rugose corals down to roughly 7.6 meters below the top of the bed (Ulrich, 1966).

Field observations reveal numerous seeps, springs, and sinks along the Wolf Lick Creek bed where the Paint Creek Limestone Formation is exposed, as well as in the alluvium fill material that covers much of the lower creek and lake floor. A large karst spring is identified as a major contributor to Spa Lake. It is significant because it means that the watershed area calculated for Spa Lake based on topographic boundaries may have been underestimated, since the spring itself may drain areas outside of Spa Lake's topographic drainage basin, as is common in karst-flow systems. This unnamed spring (sampling site #13) empties into Wolf Lick Creek upstream of the traversable lake (see Figure 13). It is accessible by foot at the bottom of a steep ravine. The first visit to the spring in May 2001 revealed that the feature was indeed a karst spring, flowing 566 liters per second, which equates to 58.6 million liters per day. The calculated flow rate was believed to represent a high flow volume as roughly 5 centimeter of rainfall had occurred during the preceding 24 hours. Additional flow measurements during summer 2001 indicated an average base flow volume of 192.6 liters per second, or 16.6 million liters per day.

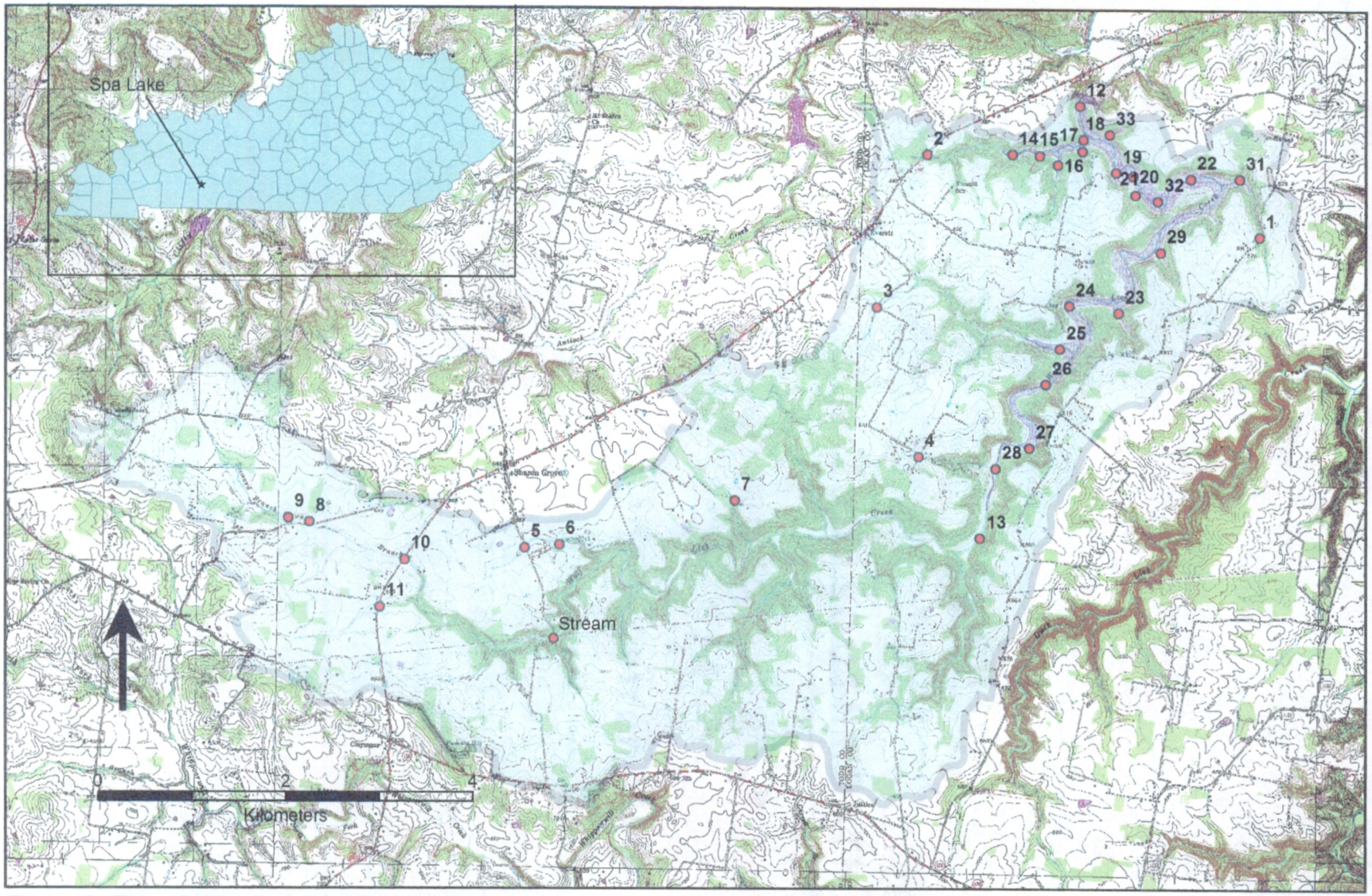


Figure 13 ▪ Water Quality Sampling Site Locations.
Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

The topographic boundary for the watershed lies just east of the spring location, sampling site #13.. It was hypothesized that the spring was capturing flow from either further upstream of Wolf Lick Creek, still within the topographic watershed boundary, or Duck Lick Creek to the southeast, outside the topographic watershed boundary. The two streams run on approximately parallel paths beginning near the Logan and Todd County line. Duck Lick Creek has very similar morphology to Wolf Lick Creek. Both streams originate on the Big Clifty Sandstone and incise deep, steep walled valleys. At the valley bottoms, both Wolf Lick and Duck Lick Creeks are in contact with the Paint Creek limestone. Wolf Lick Creek appears to flow continuously, except for perhaps in very dry conditions in the far upstream reaches. Duck Lick Creek sinks shortly after coming into contact with the Paint Creek Limestone in all but flood conditions, and resurges a short distance downstream from the sink point. Numerous vortices, boils, seeps, and springs were found along both streams. Investigation of tributary streams feeding Wolf Lick Creek found that they also sink near the contact of the Big Clifty Sandstone and the Paint Creek Limestone. None of these tributaries resurged before their confluence with Wolf Lick Creek.

To better understand which areas were contributing atrazine to the Spa Lake system, eleven tributaries (#1-11) were identified for monthly sample collection (Figure 13 and Table 2). One tributary (#1) was located on the south side of the lake. The subbasin contributing to sampling site #1 consisted of 0.78 km^2, of which 0.09 km^2 was noted as corn crop fields in 1999-2001, as identified for aerial photographs and landuse analysis (Table 2).

Ten tributaries (#2-11) drain land primarily from the upland, sandstone capped, crop fields north of Spa Lake. Sample site #2 is located on the far most northwestern tributary of Spa Lake where it crosses under State Highway 106. This tributary is situated at the down-slope edge of a row-crop field. Sample sites #3 and #4 are located just south of the community of Everett. Each tributary drains approximately 0.72 and 0.24 km^2 of land, which includes 0.13 and 0.14 km^2 of corn crop. Sample sites #5 and #6 are located due south of the road intersection at Sharon Grove. Sample Site #5 and #6, as well as sampling sites #1-4, are on the upland sandstones of the Big Clifty; however, sample site labeled "Stream" dropped to base level of the stratigraphic column to a dry creek bed covered with small boulder and large gravel size rocks. Sample sites #7, #10, and #11 are positioned on the Big Clifty Sandstone member, while sample sites #8 and #9 are only slightly higher in elevation and are located at the base of the Haney Limestone. Sample site #8 is just down steam of a six inch pipe which drained a tiled row crop field. Representatives from the Kentucky Department of Agriculture, Division of Pesticides indicated that such a pipe was in violation of Kentucky's Best Management Plan (BMP) for pesticide use (Collins, 2000; Ragan, 2000). Sampling sites #7-10 are all located at the edge of row-crop fields. Sampling site #11 was surrounded by a cattle farm. Sample sites with their associated subbasin area and percentage of corn crop are summarized in Table 2.

Table 2 ▪ Sub-basin Land-use Summary

Sample Site	Area of Subbasin (km^2)	% of Total Watershed	Area of Corn Crop in Subbasin (km^2)	% of Corn Crop within Subbasin
1	0.78	1.81	0.09	11.44 %
2	0.68	1.59	0.15	21.79 %
3	0.72	1.67	0.13	17.38 %
4	0.24	0.57	0.14	59.14 %
5	0.97	1.77	0.97	100.00 %
6	1.16	1.22	1.04	89.33 %
Stream	10.58	24.72	4.39	41.44 %
7	0.80	1.86	0.00	0.00 %
8	3.05	7.12	1.29	42.41 %
9	2.72	6.36	1.21	44.31 %
10	5.01	11.71	2.25	44.84 %
11	1.36	3.19	0.57	41.87 %

Sampling site #12 is located at the boat ramp for Spa Lake. The boat ramp is positioned just south of the manmade dam containing Spa Lake. This is also where the water intake cistern was located which supplied the Lewisburg Water Plant. Samples were obtained from shore.

As mentioned above, sample site #13 was a large karst spring located on the south side of Wolf lick Creek, just north, northwest of an intermittent tributary branching to the south. Sites #14-33 are various locations within the lake accessible by boat. See Figure 14 for subbasin landuse map. The landuse identified as corn crop within each subbasin (5.12 km^2) in 1999, 2000, or 2001 is outlined on Figure 14. Compare Figure 14 to Figure 15 which illustrates all the identified corn crop landuse for the same years, which totaled 8.00 km^2.

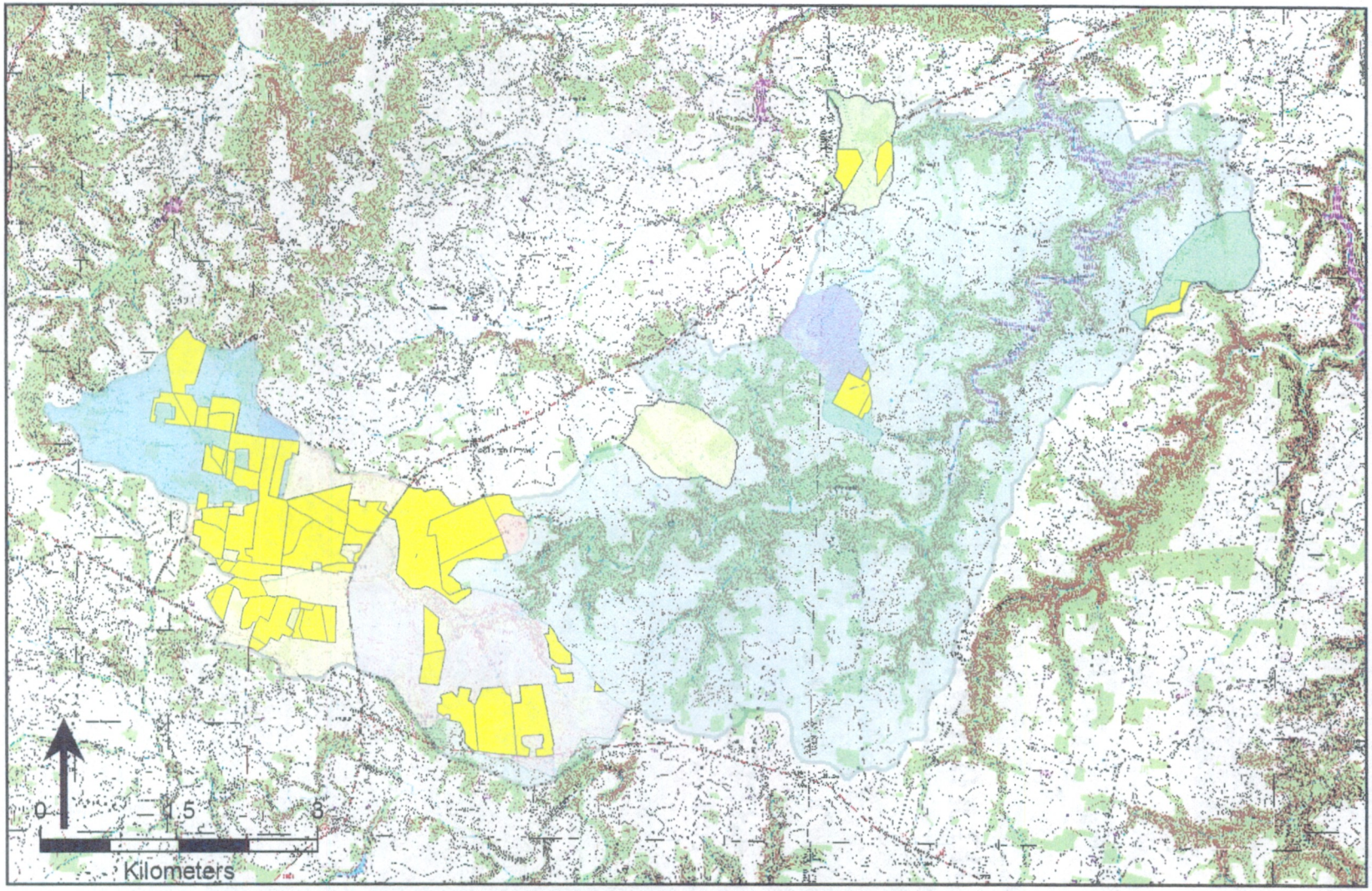


Figure 14 ▪ Corn-Crop Landuse outlined within Subbasins of Spa Lake Watershed.
Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

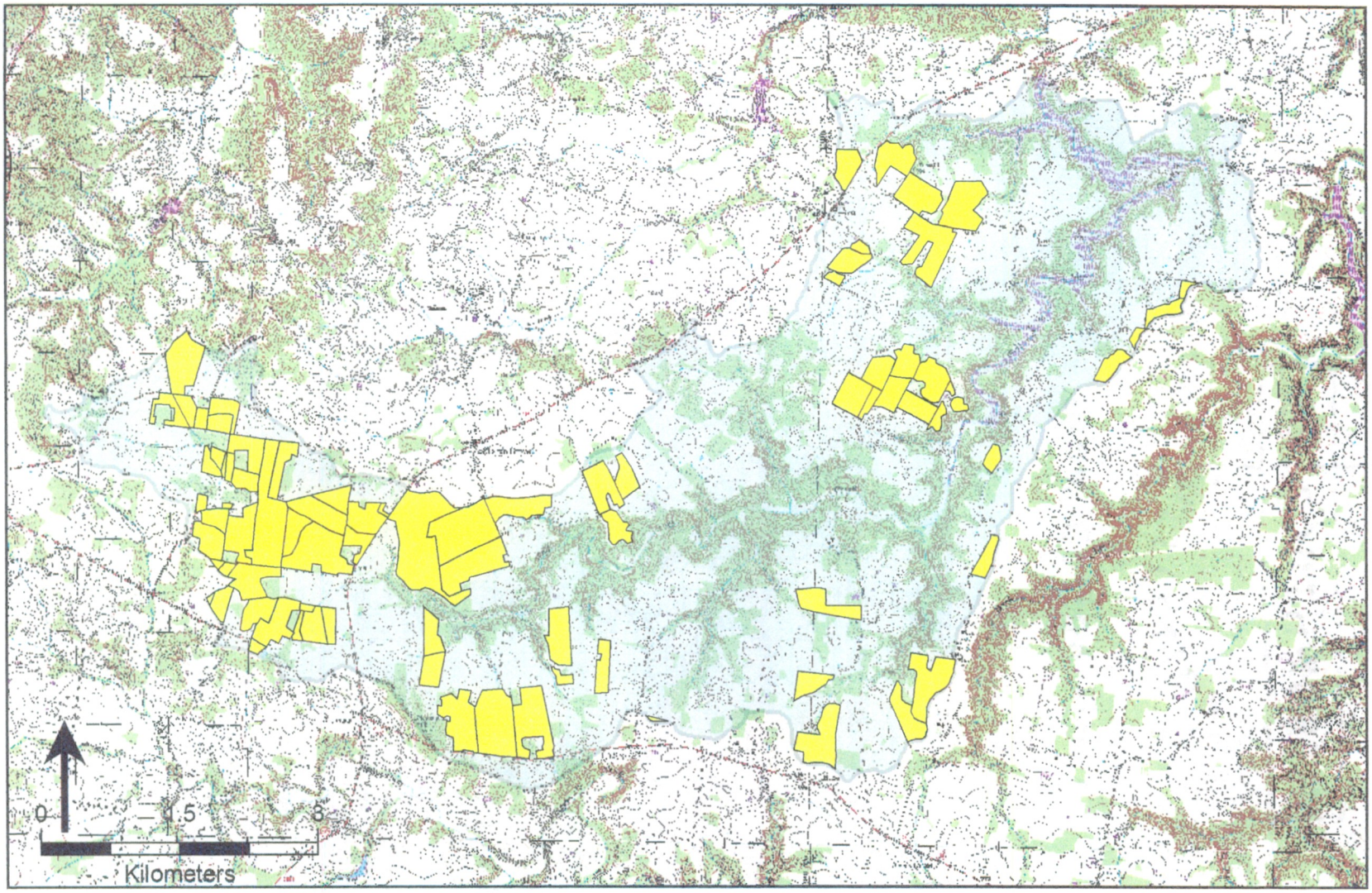


Figure 15 ▪ Corn-Crop Landuse Outlined for 1999 to 2001.
Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

The soil overlying most of the sandstone capped upland area was classified as the Zanesville-Frondorf-Talbott association. Broadly, this association is typified by gently sloping to steep, deep and moderately deep soils on the uplands. It is generally well drained to moderately well drained soils that have a loamy or clayey subsoil (USDA, 1975). The soils of the research area were illustrated on map 13 of the Logan County, Kentucky Soil Survey (USDA, 1975). The Zanesville silt loams ranged from 2-6 % slope (ZaB) on ridgetops, to 6-12 % slope (ZaC) below the ridge tops on upper sides of the slopes. The Zanesville silt loam has only a moderate natural fertility, generally low organic-matter content, and usually easy to till. Due to a moderate erosion hazard, soil-conservation practices are suggested. On the lower graded slopes the root zone is moderately deep; however, below the ridge tops, the soil layer is usually only 12-15 cm deep (USDA, 1975).

Sadler silt loams (SaB) were also identified on the upland where the grade was 2-6 % slope. The Sadler silt loams are also moderately well drained on board ridge tops. They formed primarily in loess mantles and in the underlying residuum derived from sandstone, siltstone, and shale (USDA, 1975). The surface layer (0-18 cm in depth) of brown silt loam gives way to a yellowish-brown silty loam, which extends to 122 cm in depth. The lower part of this loam creates a compact fragipan of mottled shades of brown and gray light silty clay (USDA, 1975).

Moving down the slope of the subject area, the Frondorf silt loam can be found on slopes of 6-12% (FrC) and 12-20% (FrD). On very steep slopes of 12-50 %, the Frondorf

stony complex (FrS) can be found. These areas are covered with moderately deep, well-drained soil on the tops and sides of ridges. Similar to the Sadler silt loams, the Frondorf silt loams and stony complexes formed in thin mantles of loess and in material weathered from sandstone, siltstone, and shale (USDA, 1975).

In the tributaries of Wolf Lick Creek, the Wellston silt loam can be seen. It usually grades from 6 to 12% slope (WeC). In the creek itself, the Cuba silt loam (Cu) has been documented (USDA, 1975).

Chapter 4: Methodology

I. Fieldwork Methodology

Water-sample collection began at Lewisburg with bi-weekly sampling of the source and finished water from February to April, 2001. Sampling increased to weekly collection in May, June, and July, due to these months historically being associated with the highest pesticide levels following spring application. Collection returned to a bi-weekly schedule in August 2001 continuing through April 2002. Weekly sample collection resumed in May and continued through December 2002, with the exception of September 2002. No samples were collected that month due to personnel changes. These weekly/biweekly samples were collected by personnel at the Lewisburg Water Plant. Each collector was trained on proper collection and handling techniques and documentation of physical parameters, as well as supplied with nitrile gloves and sampling containers. The sampling technique was as follows:

- Open the finished water tap 100% and allow to flush for a minimum of five minutes.
- While wearing nitrile gloves label and collect a finished water sample in supplied 40 mL glass amber, polyurethane lined capped VOA vial, ensuring zero head space in container.
- Don a new pair of nitrile gloves and repeat for raw water sample.

The samples were packed for transport in a small cooler and stored in a refrigerator at 4.0° C or less. A representative from the Kentucky Department of Agriculture, Division of Pesticides, transported the samples from the Lewisburg Water Plant to Western Kentucky University for analysis.

Fieldwork also included sampling each of the eleven tributaries on a monthly basis from March through August in both 2001 and 2002. Each site was measured for water quality parameters including pH, temperature, and conductivity using an Oakton Con 300 series multi- parameter meter. Discharge was measured using a Marsh McBerney FlowMate 2000 flow-velocity meter using standard methods for stream flow measurements.

Water samples from the lake (#14-33) were collected from May to July 2001. A 3.6 meter John-boat equipped with a 40 horsepower electric trolling motor was utilized to collect water samples from the lake to be analyzed for various water-quality parameters. The same instruments described above were used to monitor water-quality parameters of the lake. All samples were placed on ice immediately following collection and stored in a cooler at a temperature of 4.0° C or less until analysis was performed. All samples were analyzed within two weeks of collection, per analytical method requirements, EPA Method 4670.

In an attempt to determine the source of the large spring (sample site #13) and whether or not there was indeed inter-basin exchange between Duck Lick and Wolf Lick

Creeks, several dye traces were preformed using field proven groundwater dye tracing techniques. Backgrounds levels for natural fluorescence and/or synthetic dyes were established by placing activated, coconut charcoal, carbon dye receptor packets at 10 locations on Wolf Lick and Duck Lick Creeks. The dye receptors were collected for analysis and a second set of background receptors were secured approximately three weeks later. Several of the original receptors were destroyed or not recovered. It is believed they were either washed away by heavy precipitation or were scavenged by wildlife. One expert in the field of groundwater dye tracing stated that the thieves were most likely packrats, and that turtles were mostly likely to blame for eating the activated charcoal (Crawford, 2001). Homemade wire cages were constructed to help protect the remaining charcoal packets. Dye receptors were also placed 3-4 meters inside the mouth of the spring, allowing for little chance of mixing with the creek water.

Three dyes were chosen to perform a simultaneous dye trace: Fluorescein (Acid Yellow 73), Eosine (Acid Red 87), Sulphorhodamine B (Acid Red 52). Figure 16 shows the locations of the dye receptors.

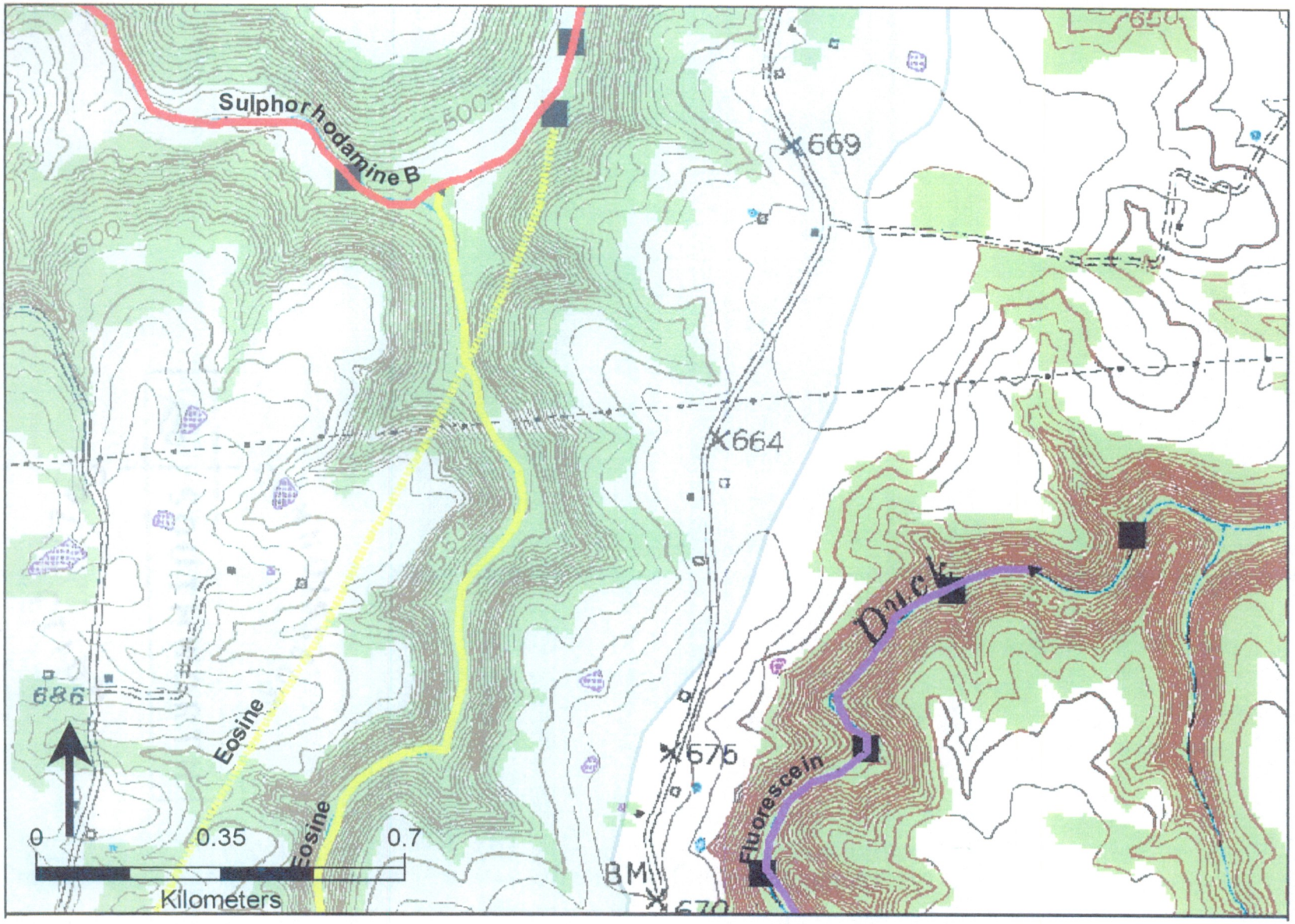


Figure 16 ▪ Dye Receptor Locations. Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

II. Chemical Analysis

To define the presence of atrazine in surface and subsurface tributaries of Spa Lake, EPA Method 4670, Triazine Herbicides as Atrazine in Water by Quantitative Immunoassay, was chosen for its time and cost effectiveness and its demonstrated usefulness as a quantitative screening tool for atrazine and related compounds. As described in EPA report SW-846 (1998), method 4670 explains the analytical procedures used for the determination of atrazine in water using competitive immunoassay technique. This method applies the principles of enzyme linked immunosorbent assay (ELISA) employing antibody molecules that bind to the target analyte (atrazine) and other related compounds. Commercially available test kits (Atrazine RaPID Assay®) purchased from Strategic Diagnostics, Inc. were employed, as well as an RPA-1 spectrophotometer.

All samples, plus the standards and controls in the test kit, were brought to room temperature prior to analysis. Test tubes coated with antibodies specific to atrazine were labeled and placed in the upper portion of the magnetic test tube rack. The magnetic base was set aside. Two hundred microliters (μL) of standard, control or sample, followed by 250 μL of enzyme conjugate, also called enzyme labeled atrazine, were added to each tube. Next, 500 μL of paramagnetic particles attached with antibodies specific to atrazine were pipetted into each tube. Both the atrazine (which may be in the sample) and the enzyme labeled atrazine (enzyme conjugate) compete for antibody binding sites

on the magnetic particles. All titrations were performed using an Ependorf Research 0-1000 μL adjustable pipetter and disposable tips.

The following schematics illustrate how the antibody coating on the prepared test tubes compete either for atrazine or enzyme conjugate. This is why the procedure requires the sample or standard to be added to the test tubes first (step 1).

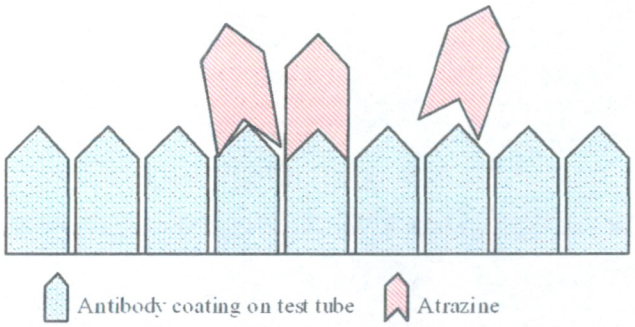


Figure 17 ▪ Schematic of ELISA of atrazine, Step 1.

The antibody sites which are not filled by sample (or standard) are occupied by the enzyme conjugate (step 2).

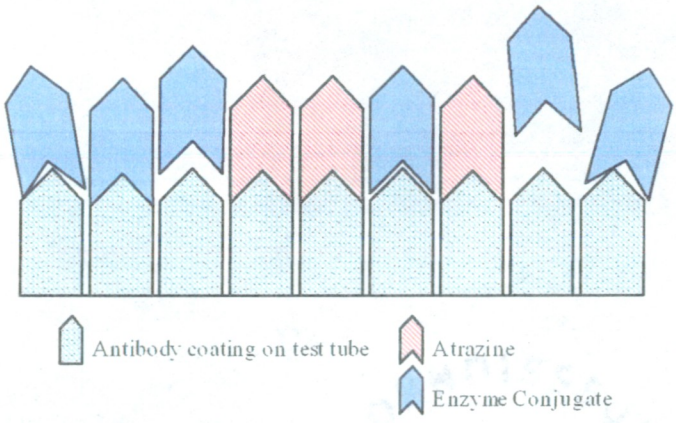


Figure 18 ▪ Schematic of ELISA of atrazine, Step 2.

A magnetic particle antibody was then added that capped the sequence of molecules to be retained in the test tubes. Following an incubation period of 30 minutes, a magnetic field was applied and unbound conjugate and sample analyte were removed by duplicate washing with 1 mL of organic-free reagent water. Atrazine and bound labeled atrazine analog that remained attached to the antibodies on the magnetic particles were held in the tube in proportion to their original concentrations (Strategic Diagnostics, 1999).

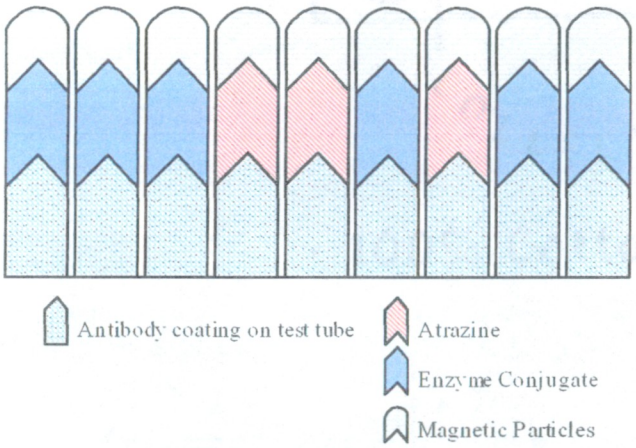


Figure 19 ▪ Schematic of ELISA of atrazine, Step 3.

The presence of atrazine was determined by adding an enzyme substrate (hydrogen peroxide) and chromogen (3,3',5,5' – tetramenthylbenzidine). The enzyme labeled atrazine analog bound to the atrazine antibody catalyzed the conversion of the substrate/chromogen mixture to a blue colored product.

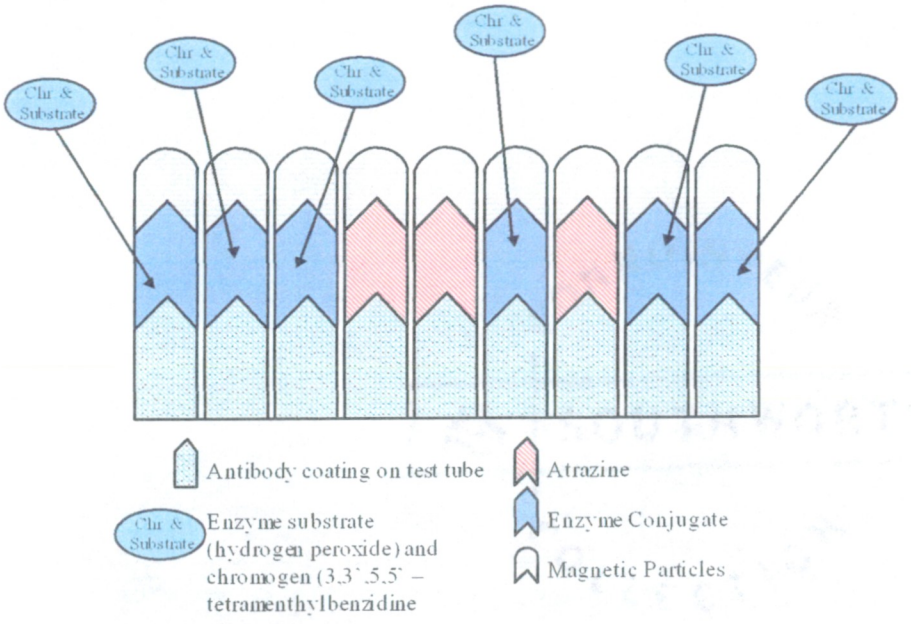


Figure 20 ▪ Schematic of ELISA of atrazine, Step 4.

Following an additional 20-minute incubation period the reaction was stopped by the addition of 500 µL of 2 M sulfuric acid stopping solution, turning the liquid yellow.

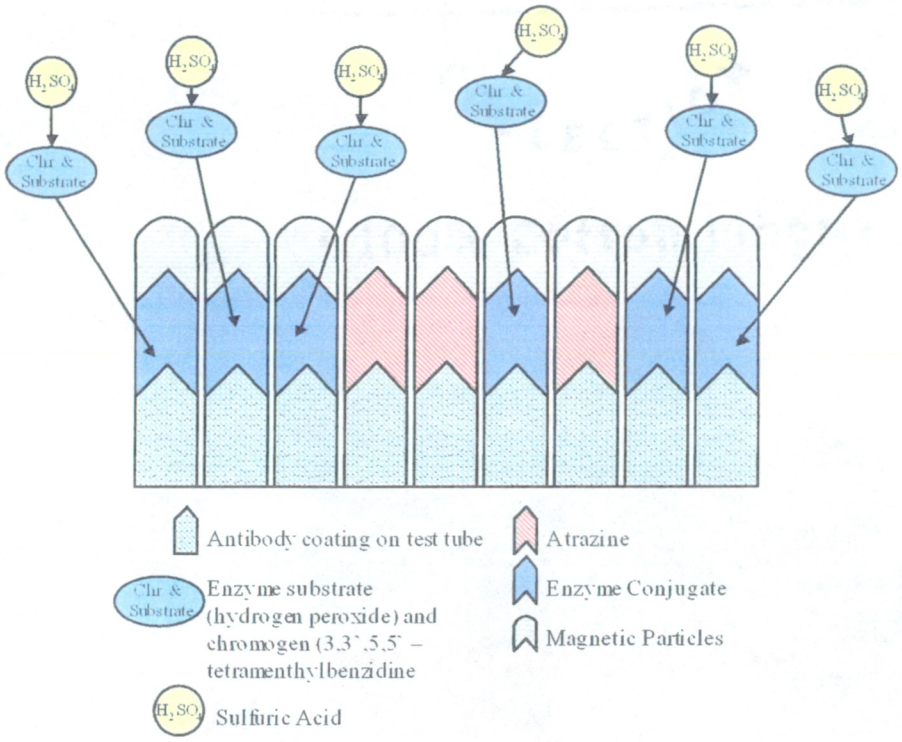


Figure 21 ▪ Schematic of ELISA of atrazine, Step 5.

Since the labeled atrazine (enzyme conjugate) was in competition with the unlabeled atrazine (sample) for the antibody sites, the color intensity that developed was ***inversely*** proportional to the concentration of atrazine in the sample (Strategic Diagnostics, 1999). The absorbance of the solution was measured by photometric interpretation using a RPA-I photometer at 450 nm.

A disadvantage to using the ELISA method for analysis, although it is a quantitative method, is that it is considered a screening tool, not a certifiable quantitative number, as is EPA Method 525.2. ELISA methods have some interference products which can skew its results. The RaPID Assay® kits employed in this project could not differentiate 100% between atrazine and other related compounds. The Table 3 lists

compounds that may have contributed to higher results due to cross-reactivity. The method detection limit (MDL) is the lowest concentration that can be detected by the assay. The limit of quantification (LOQ) is the lowest concentration of a compound that can be quantified by the assay. The IC05 is the concentration required to inhibit 50% of the color produced by the negative control (Strategic Diagnostics, 1999). The percent reactivity (% Rec) summarizes the cross-reactivity percent of other triazine compounds related to atrazine (USEPA, 2000).

Table 3 ▪ Immunoassay Cross-reactivity Compounds

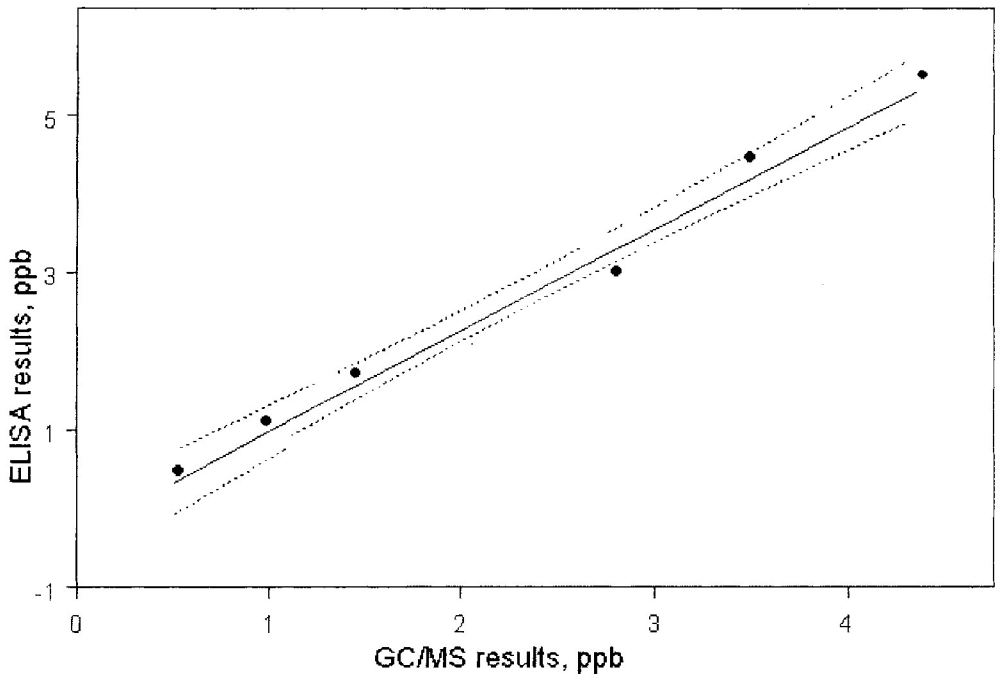
Compound	MDL (ppb)	LOQ (ppb)	IC 50 (ppb)	% Rec.
Atrazine	0.046	0.1	0.72	100
Propazine	0.033	0.1	0.74	97
Ametryn	0.053	0.05	0.39	185
Prometryn	0.054	0.09	0.64	113
Prometon	0.056	0.31	2.22	32
Desethyl Atrazine	0.062	0.45	3.21	22
Terbutryn	0.090	0.76	5.50	13
Terbutylazine	0.310	2.15	15.5	5
Simazine	0.340	0.68	4.90	15
Desisoprpyl Atraizne	0.800	30.1	217	0.3
Cyanazine	1.0	>10000	>10000	<0.1
6-Hydroxy Atrazine	1.1	20.6	148	0.5

Source: Strategic Diagnostics, 1999

Despite the cross-reactivity of the above listed compounds, several compounds do not interfere with the ELISA method up to 1000 ppb, such as aldicarb, aldicard sulfoxide, aldicard sulfone, alachlor, benomyl, butachlor, butylate, captan, dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl. Other compounds have been found to have no significant effect on the test kits up to 100 ppm, for example: humic acid, iron, sulfide, and sulfite; while more common compounds, such as copper,

nickel, sulfate, magnesium, calcium, nitrate, and thiosulfate were found to have no significant effect up to 250 ppm. Sodium chloride showed the least cross-reactivity in conjunction with atrazine without effecting the results up to a concentration of 0.65 M (Strategic Diagnostics, 1999).

For quality-control purposes, a spilt sample was analyzed by both ELISA, method 4670, and gas chromatography/mass spectroscopy (GC/MS), EPA Method 508.1. The ELISA literature reported an expected correlation with GC/MS of r= 0.943; however, this project produced a *r* value of 0.9899 (Graph 1).



Graph 1 ▪ ELISA vs. GC/MS Results Comparison.

III. Statistical Analysis

In an effort to see a broader picture of the true nature of occurrence of atrazine in the raw drinking water supplied by Spa Lake, data collected in this project was paired with earlier data collected by Syngenta. The publicly supplied data were generated by an independent laboratory hired by Syngenta to screen for atrazine by the ELISA method described above.

Using S-Plus statistical analysis computer software, data were tabulated for the 1999 and 2000 growing seasons (supplied by Syngenta), and the 2001 and 2002 growing seasons (generated by the research described herein). A growing season is defined by the initial preparation and treating of the crop fields, including application of pre-emergent pesticides such as atrazine, through the growing period, harvest, and winter until early spring of the following year. Following conversations with the local producers and personnel with the Natural Resource Conservation Service (NRCS), March 15th of each year was assigned the first day of the growing season. The independent variable for each data set was time elapsed since application (TESA), and the dependent variable was the raw drinking water value for atrazine.

To evaluate the data sets qualitatively, the data were smoothed using a smoothing spline technique. Smoothing spline interpolation is designed to smooth data sets which are mildly contaminated with isolated errors. A smoothing spline is a locally weighted

average of the y values based on the relative locations of the x values (UCAR/CGD, 2004).

After the data were smoothed, the points were evaluated relative to the TESA and the amount of rainfall the area received in the seven days prior to collection of the sample (P1W). It was hypothesized that the amount of atrazine in the raw drinking water sample was partially controlled by the quantity rainfall early in the season as well as in the prior one week, in addition to the TESA.

Chapter 5: Results

I. Fieldwork Results

Hydrologic inventorying and placement of background dye receptors for Wolf Lick and Duck Lick Creeks began on December 20, 2001 and continued through February 18, 2002. On December 20, 2001 the inventory began by walking from the head waters of Duck Lick Creek downstream recording all significant hydrologic features. A handheld geographic positioning system (GPS) was utilized to determine exact location of features observed. A plunge pool was identified at N 36°89.795' W 87°2.056' ± 10 m that was formed as the creek turns sharply around a bend. A black pipe was observed exiting from the hillside into the plunge pool. A local farmer stated that the pipe had previously been used to pump water up on to the flat lands for the livestock. A spring was located about 10 m upstream. The specific conductance of water coming directly out of the spring was 313 microSiemens (μS) and the temperature was 12.5° C. The conductivity above the spring was 197.6 μS and the temperature was 9.2° C. The conductivity below the spring was 280 μS and the temperature was 11.3° C. Three dye receptors in cages were placed around this feature, one upstream and two downstream.

Continuing downstream on Duck Lick Creek, at N 36°53.946' W 87°1.803' ± 8.5 m the stream split around a debris pile and sank. On the right side there was a depression where it was clear that the water from Duck Lick Creek sank. The left side appeared to take on any overflow.

The creek bed remained dry for several hundred meters downstream until the water resurged at N 36°53.981' W 87°1.683' ± 6.5 m in the form of three seeps and a small spring with a moderate output of water. The specific conductance at this location was measured to be 270 μS and the temperature 11.5° C. A caged dye receptor was placed at this location. No other significant hydrologic features were noted along Duck Lick Creek.

The hydrologic inventory continued on January 28, 2002, along Wolf Lick Creek. The conductivity reading at the mouth of the spring was 371 and the temperature was 9.0 °C. The conductivity reading upstream of the spring was 380 and the temperature was 6.8 °C. The conductivity reading downstream from the spring was 372 and the temperature was 9.2 °C.

The original background receptors were collected on January 28, 2002. The second set of background receptors was collected February 18th. Dye injection locations were chosen based on the following fours scenarios.

Scenario #1: The spring (sampling site #13) was fed by Duck Lick.

Scenario #2: The spring was fed by Wolf Lick (i.e. it was a subsurface meander cutoff).

Scenario #3: The spring was fed by the tributary just slightly upstream from it.

Scenario #4; The spring was fed from diffuse recharge within the topographic watershed boundary.

Dye was injected on February 21, 2002 at the head waters of Duck Lick Creek and Wolf Lick Creek, as well as a major tributary to Wolf Lick Creek. Dye receptors were collected on March 4 and March 29, 2002. Flourescein was detected at all four receptors in Duck Lick. It was not detected at any locations in Wolf Lick, including the spring (sample site #13) (Figure 22).

Eosine (Acid Red 87) was recovered at the spring, as well as up and down stream of the spring (Figure 22).

Sulphorhodamine B (Acid Red 52) was recovered at the spring, but neither upstream nor downstream of it (Figure 22).

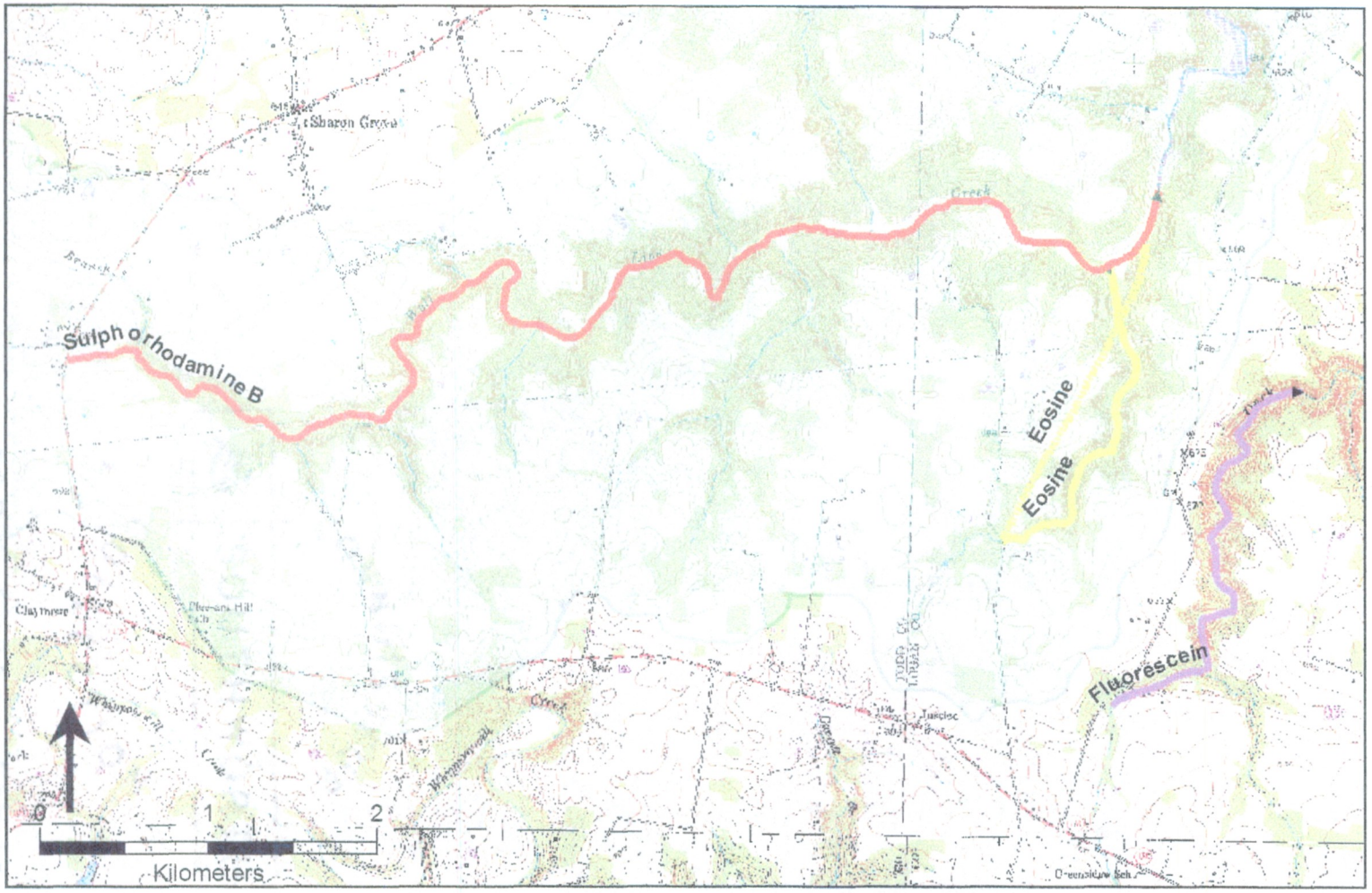


Figure 22 ▪ Dye Trace.
Based on the 7.5 Minute US Topographic Map, Sharon Grove Topographic Quadrangle. 1951, photorevised 1983.

II. Analytical Results

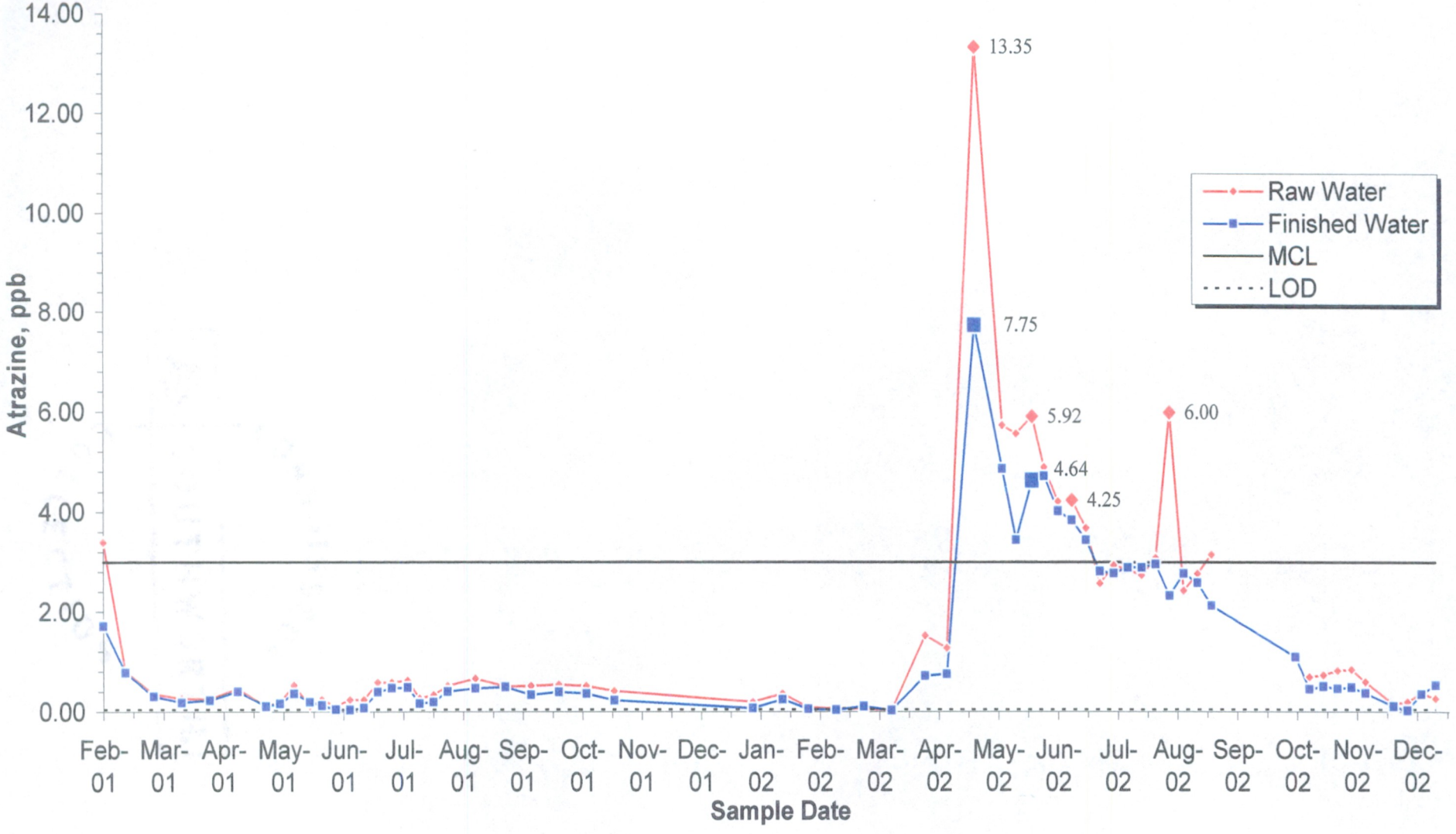
Data collected from the Lewisburg Water Treatment Plant for atrazine in the raw and finished water from February 15, 2001 through December 16, 2002, are summarized in Table 4 and Graph 2. Recall the MCL for atrazine is 3.00 µg/L, also known as parts per billion (ppb). Values exceeding the MCL are shown in bold.

Table 4 ▪ Raw & Finished Atrazine Results

Sample Date	Raw Water Atrazine, ppb	Finished Water Atrazine, ppb
2/15/2001	**3.40**	1.72
2/26/2001	0.81	0.79
3/12/2001	0.36	0.31
3/26/2001	0.27	0.19
4/9/2001	0.26	0.23
4/23/2001	0.47	0.41
5/7/2001	0.11	0.11
5/14/2001	0.19	0.17
5/21/2001	0.53	0.37
5/29/2001	0.12	0.20
6/4/2001	0.25	0.13
6/11/2001	0.11	0.05
6/18/2001	0.24	0.04
6/25/2001	0.24	0.08
7/2/2001	0.59	0.40
7/9/2001	0.59	0.48
7/17/2001	0.63	0.49
7/23/2001	0.27	0.17
7/30/2001	0.34	0.20
8/6/2001	0.52	0.41
8/20/2001	0.67	0.48
9/4/2001	0.51	0.50
9/17/2001	0.52	0.34
10/1/2001	0.55	0.40
10/15/2001	0.52	0.37
10/29/2001	0.42	0.23
1/7/2002	0.20	0.08

Sample Date	Raw Water Atrazine, ppb	Finished Water Atrazine, ppb
1/22/2002	0.37	0.25
2/4/2002	0.09	0.06
2/18/2002	0.07	0.04
3/4/2002	0.07	0.11
3/18/2002	0.03	0.03
4/4/2002	1.53	0.72
4/15/2002	1.28	0.76
4/29/2002	**13.35**	**7.75**
5/13/2002	**5.74**	**4.87**
5/20/2002	**5.57**	**3.45**
5/28/2002	**5.92**	**4.64**
6/3/2002	**4.90**	**4.73**
6/10/2002	**4.22**	**4.03**
6/17/2002	**4.25**	**3.85**
6/24/2002	**3.69**	**3.45**
7/1/2002	2.58	2.82
7/8/2002	2.94	2.78
7/15/2002	2.85	2.90
7/22/2002	2.74	2.90
7/29/2002	**3.09**	2.96
8/5/2002	**6.00**	2.33
8/12/2002	2.43	2.77
8/19/2002	2.77	2.59
8/26/2002	**3.16**	2.13
10/7/2002	Broken bottle	1.10
10/14/2002	0.70	0.46
10/21/2002	0.74	0.51
10/28/2002	0.83	0.47
11/4/2002	0.85	0.49
11/11/2002	0.60	0.38
11/25/2002	0.16	0.11
12/2/2002	0.20	0.03
12/9/2002	0.38	0.36
12/16/2002	0.27	0.54
Average	**2.49**	**1.93**

Graph 2▪ Raw and Finished Atrazine Levels



Data collected within the Spa Lake Watershed, both tributaries and lake samples, from March 9, 2001, through August 23, 2002, are summarized in Tables 5, 6, and 7. The absence of data indicates no water was flowing for a particular location at the time of collection and is notated as NF (no flow) in the data tables. Bold values indicate values over the MCL. If a sample was not collected for a particular date/sample locations, it is noted as NS (no sample). Graphs 3 -14 illustrate atrazine levels found at sampling site locations #1-10, 12, and 13 over the same period of time. A graph for sample site location #11 is absent because it only had flowing water once, thus was only sampled once, during the course of the study period. These graphs clearly demonstrate the effect that drought conditions had on the sampling schedule. Graphs 15 and 16 illustrate atrazine levels of sample site location #13-33 on June 15 and July 24, 2002, respectively.

Table 5 ▪ Sample Sites LE01-LE11

Date	LE01	LE02	LE03	LE04	LE05	LE06	Stream	LE07	LE08	LE08Tile	LE09	LE10	LE11
3/9/2001	0.03	0.01	0.03	0.03	0.18	0.09	NF	NF	NF	NF	NF	NF	NF
4/12/2001	0.01	0.25	0.01	0.03	NF	NF	NF	0.04	0.36	NF	0.85	1.93	0.07
5/22/2001	**23.40**	NF	0.29	**73.00**	NF	**131.80**	NF	0.42	**14.10**	NF	0.62	**24.30**	NF
6/24/2001	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
7/31/2001	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
8/31/2001	0.17	0.13	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
3/21/2002	0.02	0.00	0.00	0.04	NF	0.09	**4.01**	0.01	**5.48**	0.12	NF	**4.64**	NF
4/29/2002	0.03	0.03	NF	0.00	NF	0.06	**11.25**	0.03	**3.89**	0.02	**4.67**	**11.65**	NF
5/31/2002	0.04	0.15	0.03	0.15	0.43	**3.82**	NF	0.10	**7.65**	**11.16**	**6.21**	**6.12**	NF
7/17/2002	0.24	0.15	0.14	NF	NF	0.34	NF	0.14	0.83	0.78	2.30	1.50	NF
8/14/2002	0.08	0.05	0.02	0.02	0.00	0.04	0.31	0.01	NF	NF	0.35	0.16	NF
Average	**2.67**	**0.10**	**0.07**	**10.47**	**0.20**	**19.46**	**5.19**	**0.11**	**5.39**	**3.02**	**2.50**	**7.19**	**0.07**

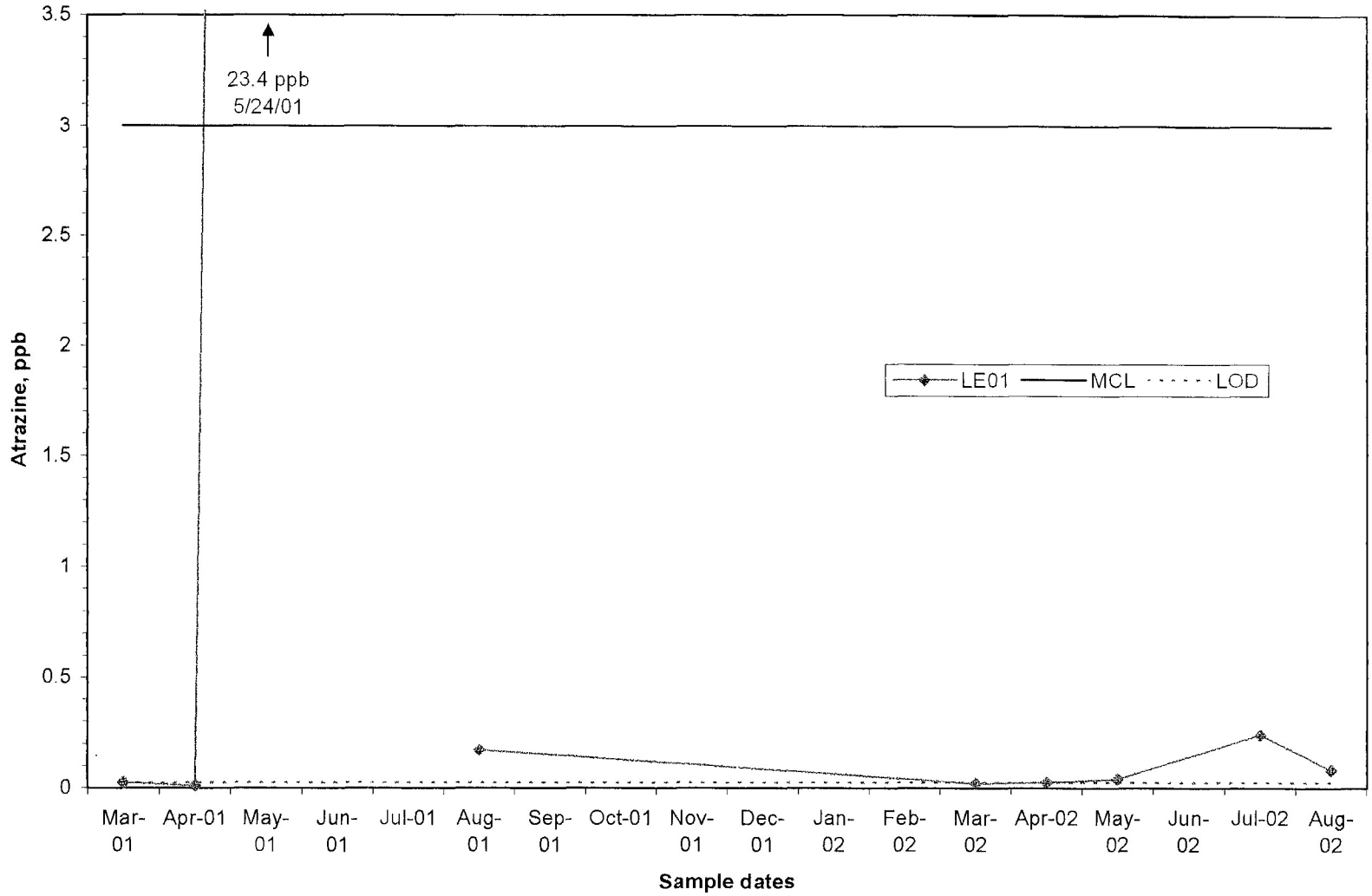
Table 6 ▪ Sample Sites LE12-LE21

Date	LE 12	LE13	LE13D	LE13U	LE14	LE15	LE16	LE17	LE18	LE19	LE20	LE21
3/9/2001	NF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4/12/2001	NF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5/22/2001	0.40	**12.40**	NS	NS	0.32	1.20	0.94	0.64	0.65	0.57	0.75	0.80
6/24/2001	0.49	0.18	NS	NS	0.24	0.19	0.61	0.35	0.54	0.20	0.25	0.67
7/31/2001	0.71	0.65	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
8/31/2001	0.00	NF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3/29/2002		0.66	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4/29/2002	**15.35**	**4.43**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5/31/2002	**7.38**	2.93	**2.89**	**3.75**	NS	NS	NS	NS	NS	NS	NS	NS
6/15/2002	**10.00**	0.46	0.51	0.23	**8.40**	**9.10**	**4.75**	**9.40**	**8.40**	**9.80**	**8.80**	**3.06**
7/17/2002	**4.16**	1.31	1.27	1.81	NS	NS	NS	NS	NS	NS	NS	NS
7/24/2002	**3.50**	NS	NS	NS	**3.75**	**3.83**	**3.54**	**3.54**	**3.82**	**3.51**	**3.91**	**3.62**
8/14/2002	**3.43**	0.38	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average	**0.00**	**2.60**	**1.56**	**1.93**	**3.18**	**3.58**	**2.46**	**3.48**	**3.35**	**3.52**	**3.43**	**2.04**

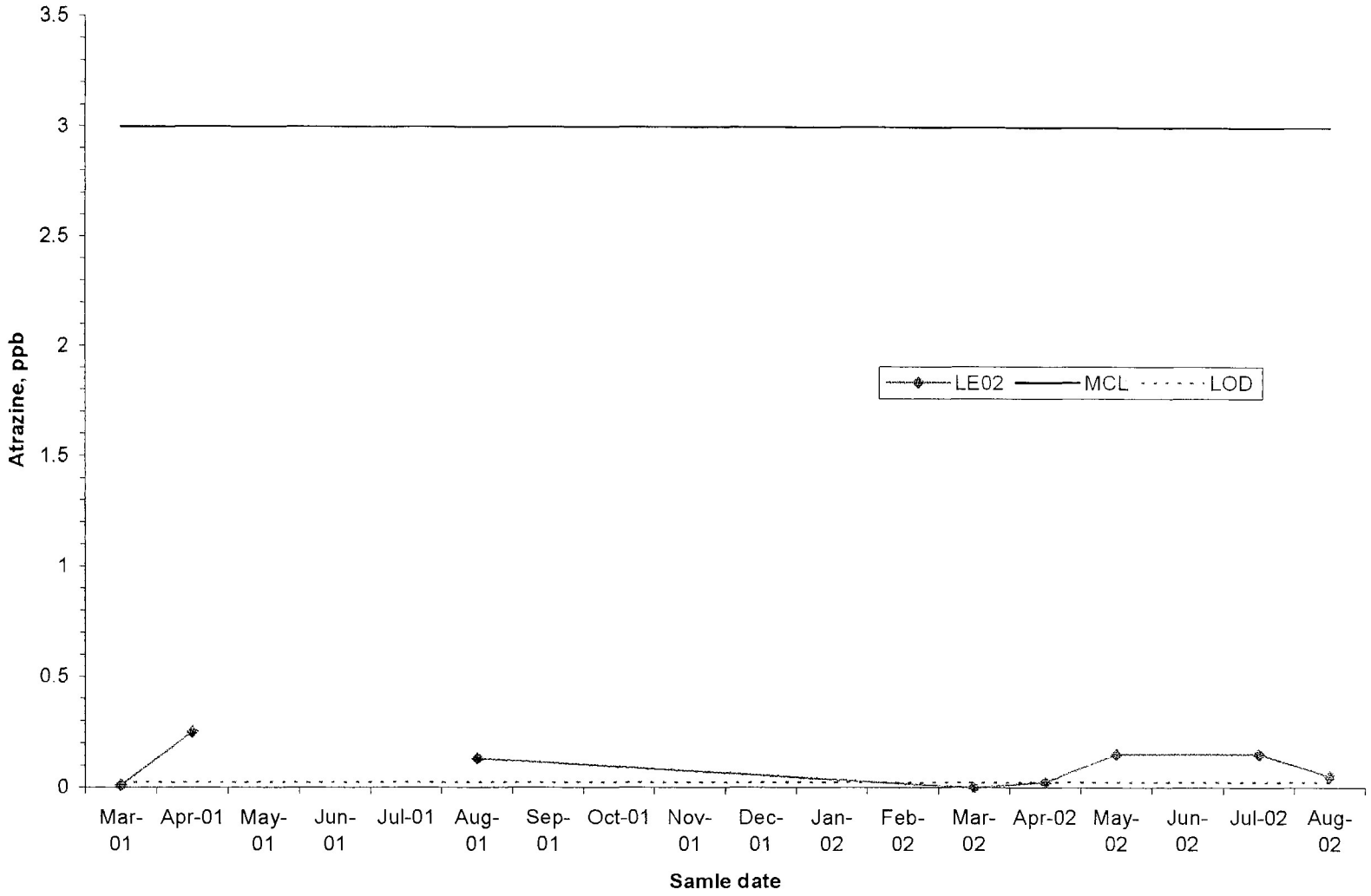
Table 7 ▪ Sample Sites LE22-LE33

Date	LE22	LE23	LE24	LE 25	LE26	LE27	LE28	LE29	LE30	LE31	LE32	LE33
3/9/2001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4/12/2001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5/22/2001	0.63	0.49	1.33	**4.35**	**3.34**	**4.56**	**9.90**	1.24	0.76	0.65	0.58	0.72
6/24/2001	0.63	1.35	1.17	0.91	0.73	0.70	0.52	1.18	0.72	0.42	0.38	1.16
7/31/2001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
8/31/2001	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3/21/2002	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4/29/2002	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5/31/2002	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
6/15/2002	**10.60**	**8.80**	**1.80**	**7.60**	**8.20**	**3.22**	NS	**9.50**	**10.30**	**10.60**	2.64	**11.40**
7/17/2002	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
7/24/2002	**3.96**	**3.74**	**3.35**	**3.95**	**3.74**	**3.62**	**3.63**	**3.98**	**4.71**	**4.07**	**4.18**	**4.38**
8/14/2002	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average	**3.96**	**3.60**	**1.91**	**4.20**	**4.00**	**3.03**	**4.68**	**3.98**	**4.12**	**3.94**	**1.95**	**4.42**

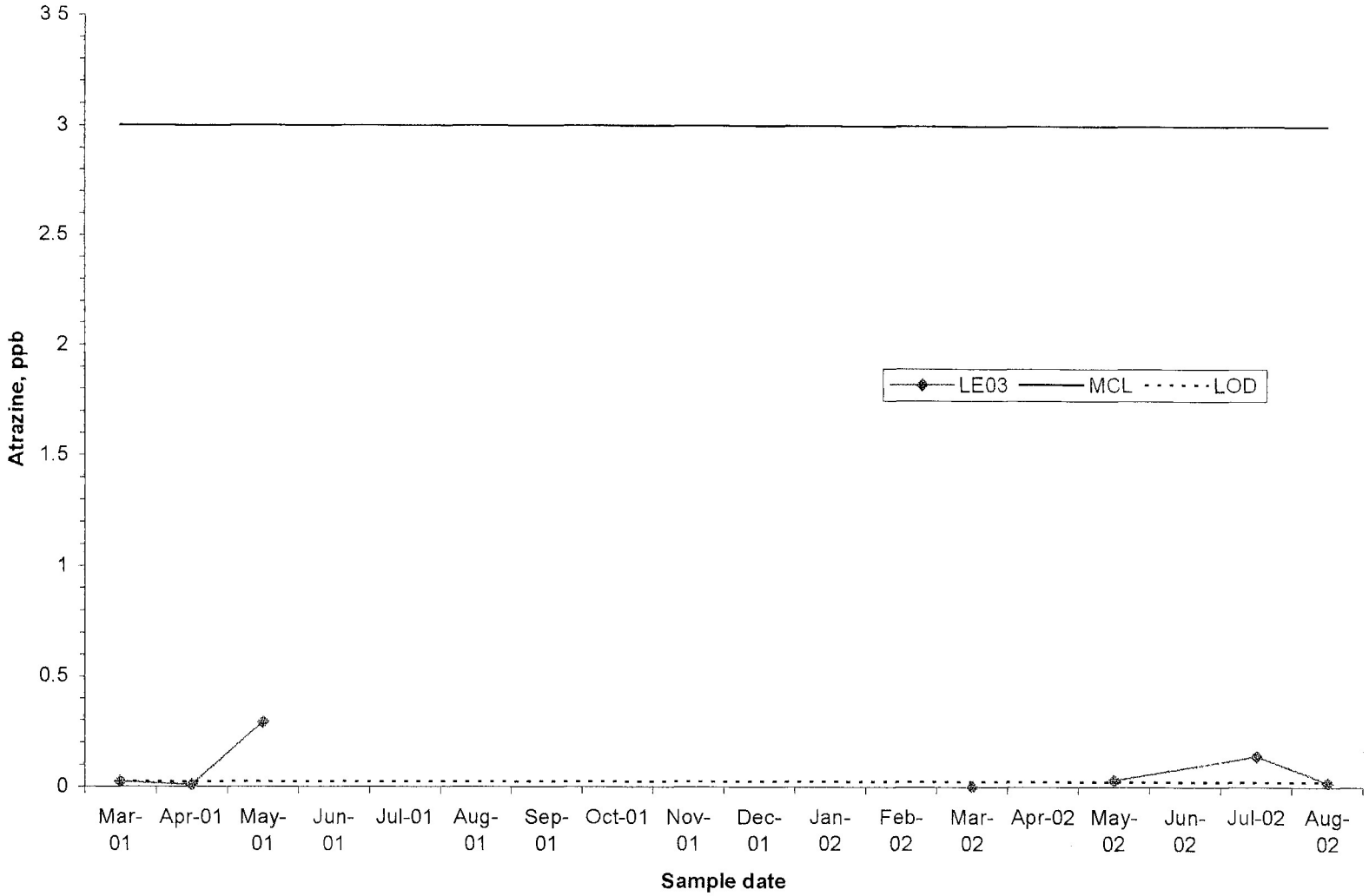
Graph 3 ▪ LE01


3.5
3
2.5
2
1.5
1
0.5
0
Atrazine, ppb
23.4 ppb
5/24/01
LE01
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample dates

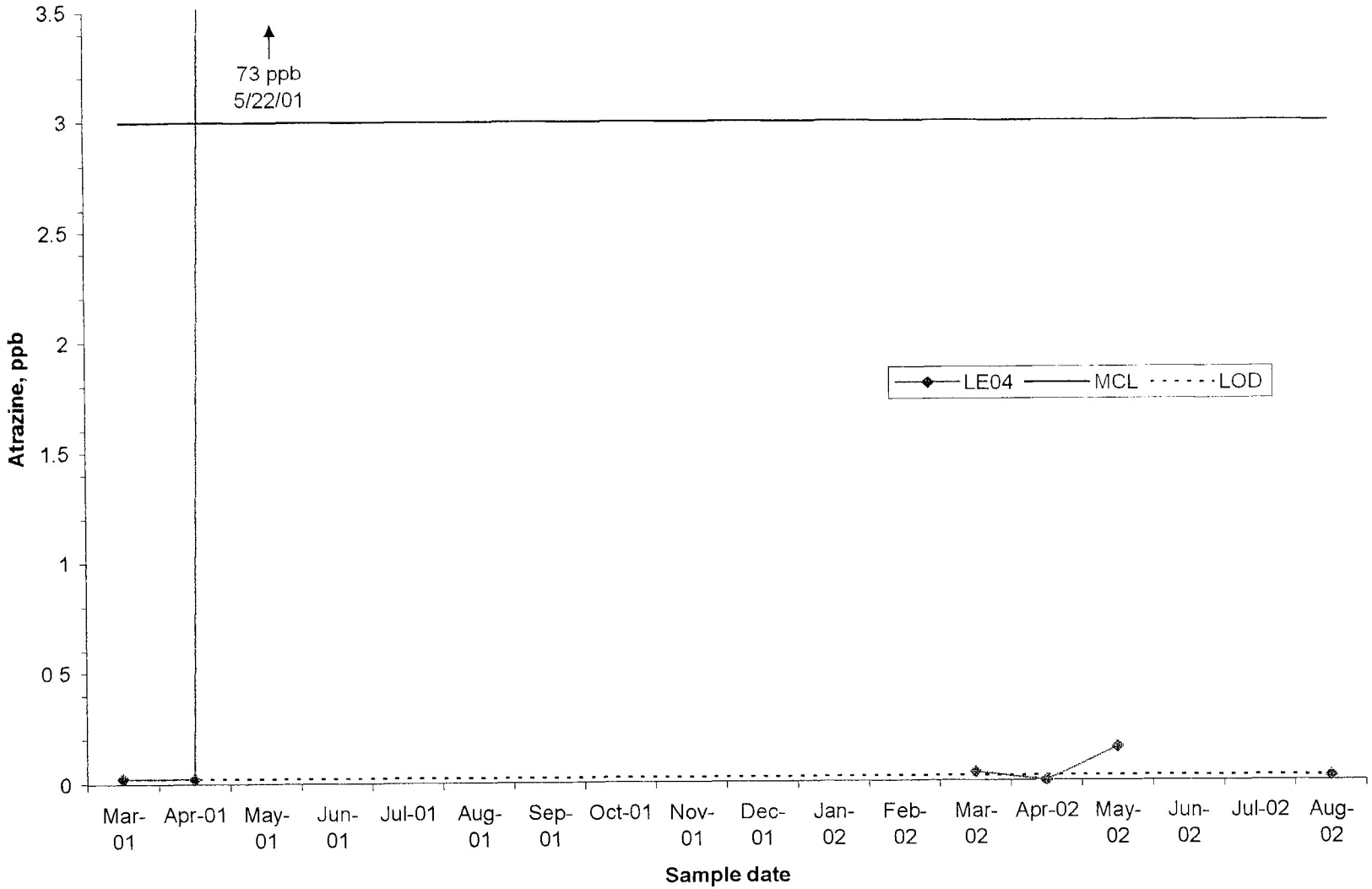
Graph 4 ▪ LE02


3.5
3
2.5
2
1.5
1
0.5
0
Atrazine, ppb
LE02
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Samle date

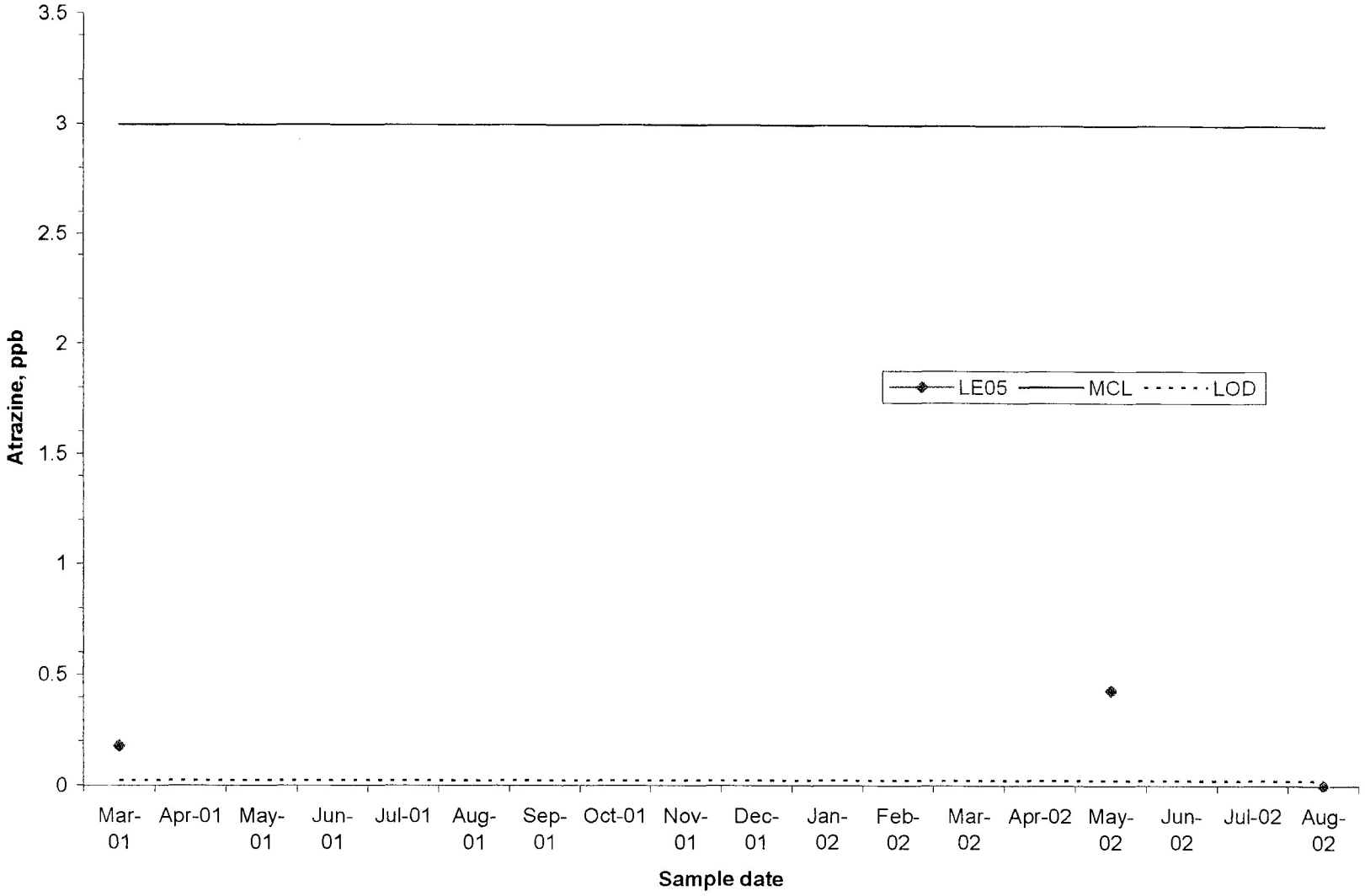
Graph 5 ▪ LE03


3 5
3
2.5
2
1.5
1
0.5
0
Atrazine, ppb
LE03
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample date

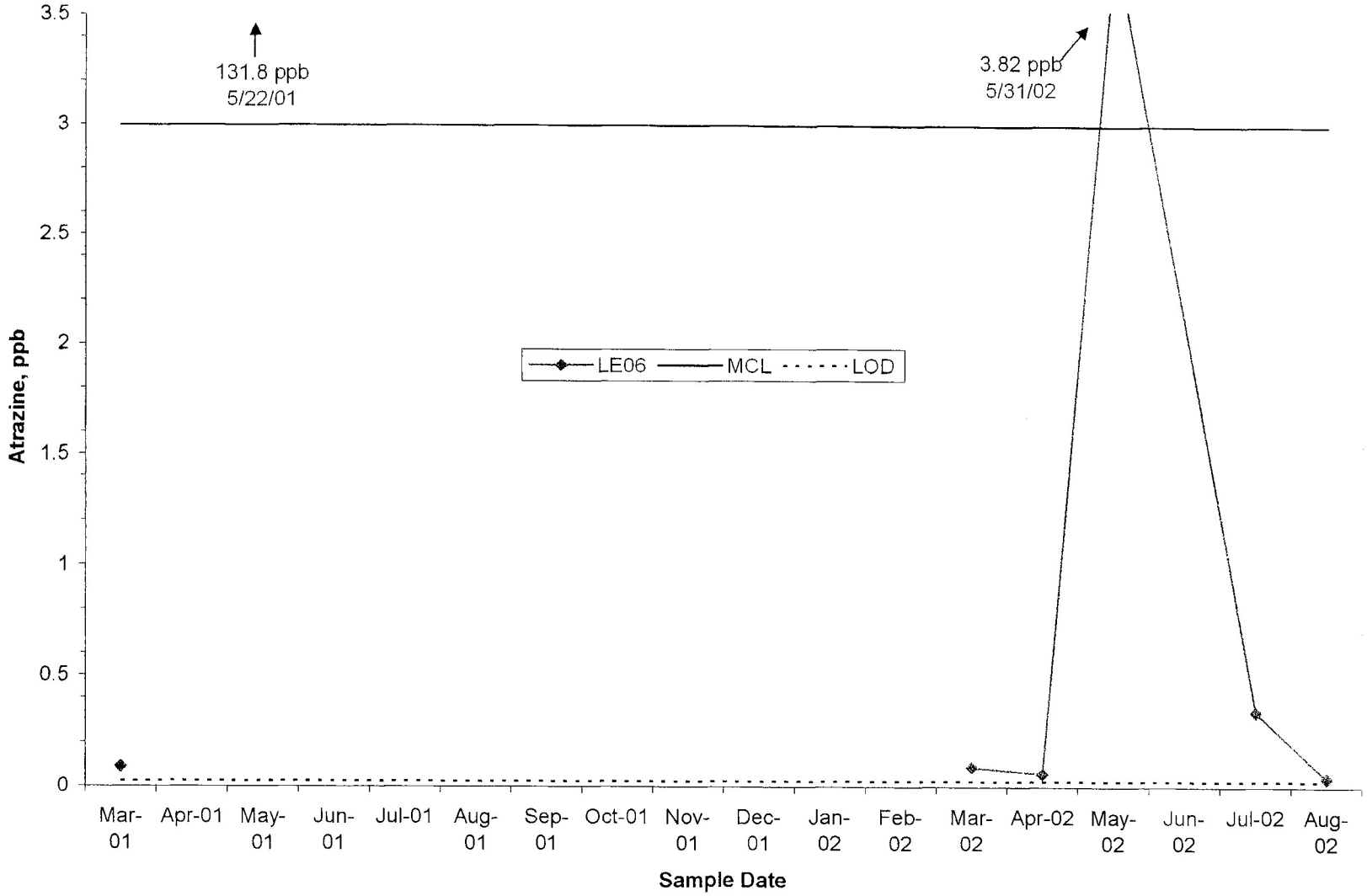
Graph 6 ▪ LE04


3.5
3
2.5
2
1.5
1
0 5
0
73 ppb
5/22/01
Atrazine, ppb
LE04
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample date

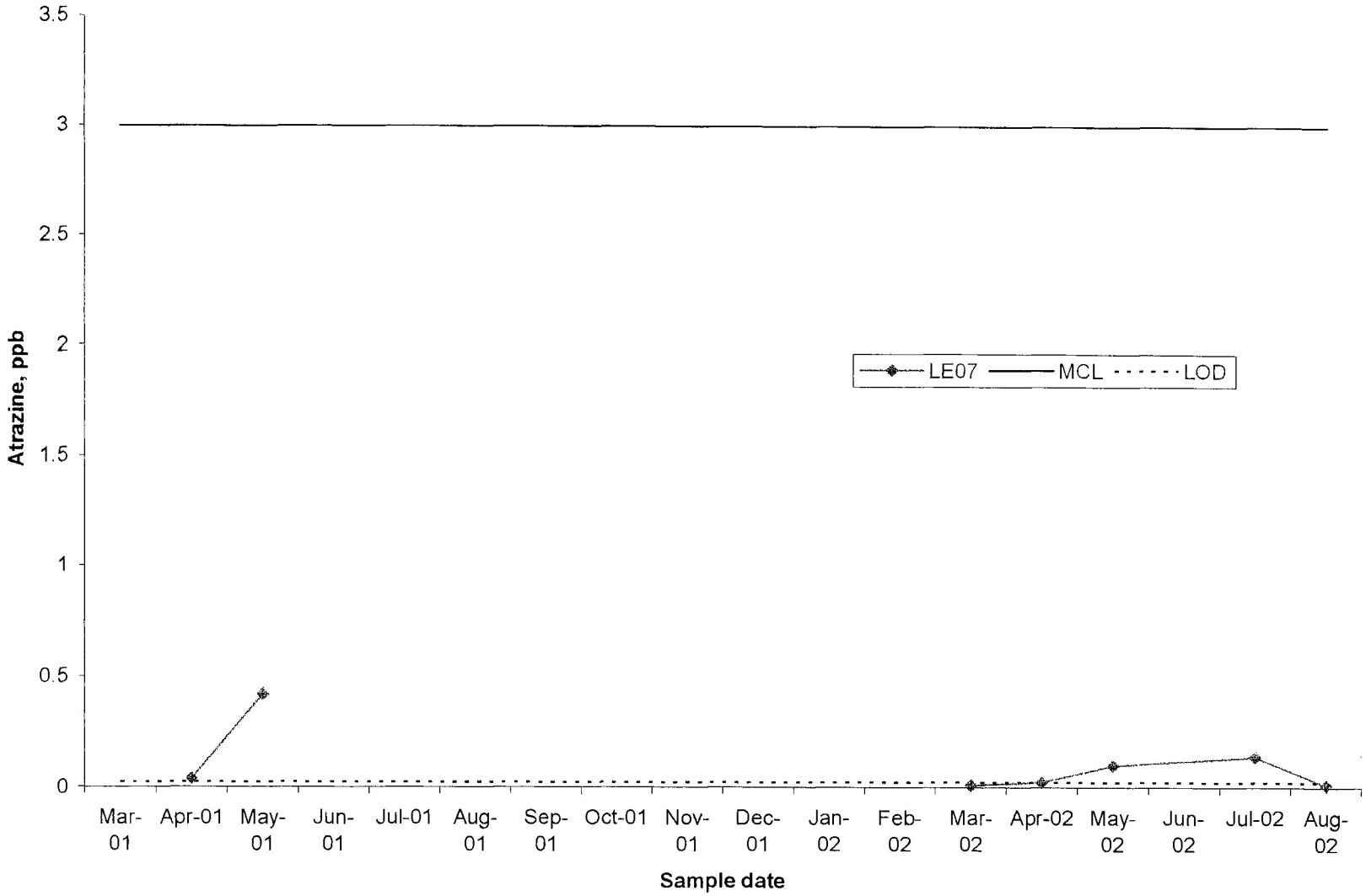
Graph 7 ▪ LE05


3.5
3
2.5
2
1.5
1
0.5
0
Atrazine, ppb
LE05
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample date

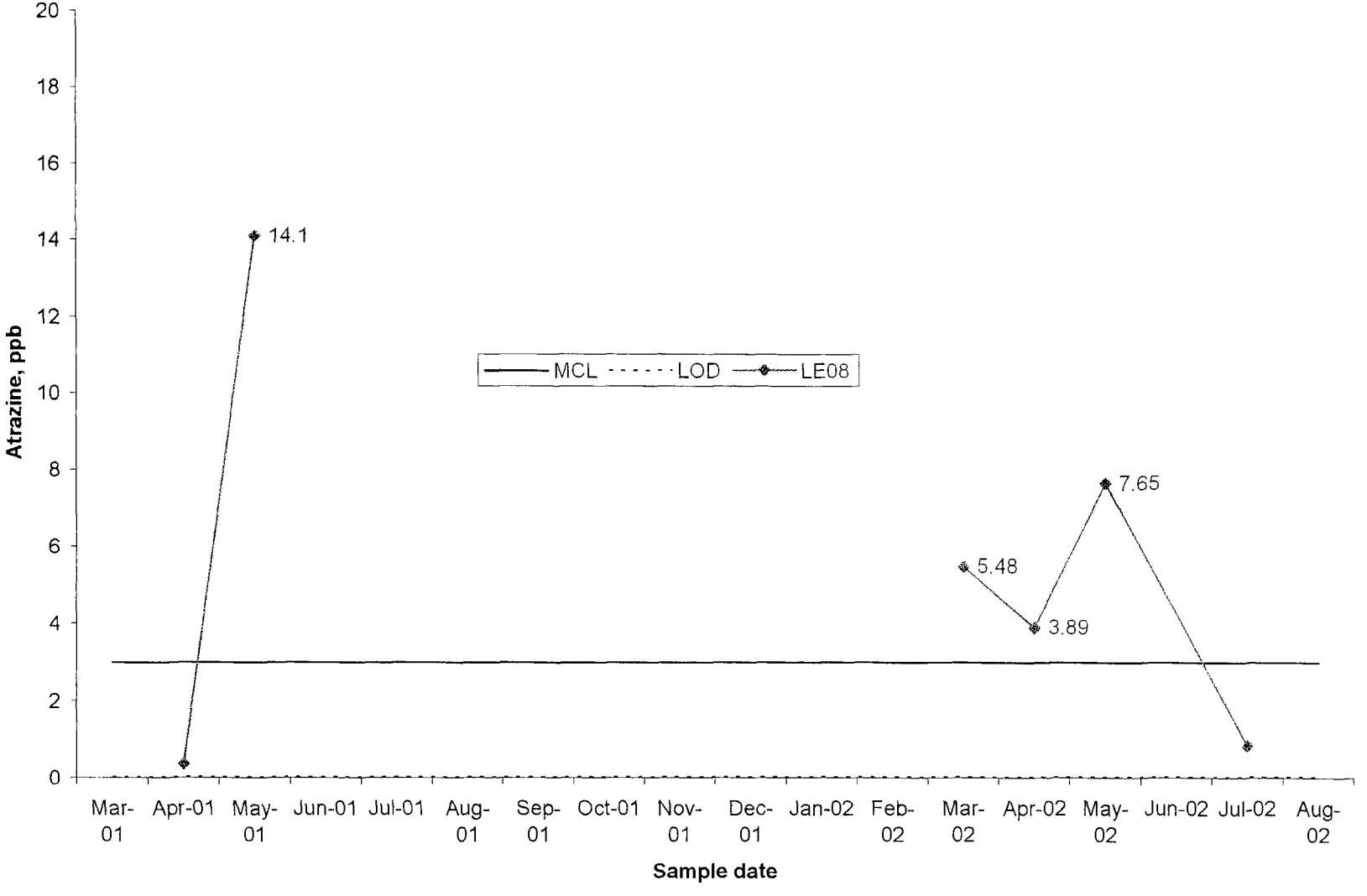
Graph 8 ▪ LE06


131.8 ppb
5/22/01
3.82 ppb
5/31/02
LE06
MCL
LOD
Atrazine, ppb
3.5
3
2.5
2
1.5
1
0.5
0
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample Date

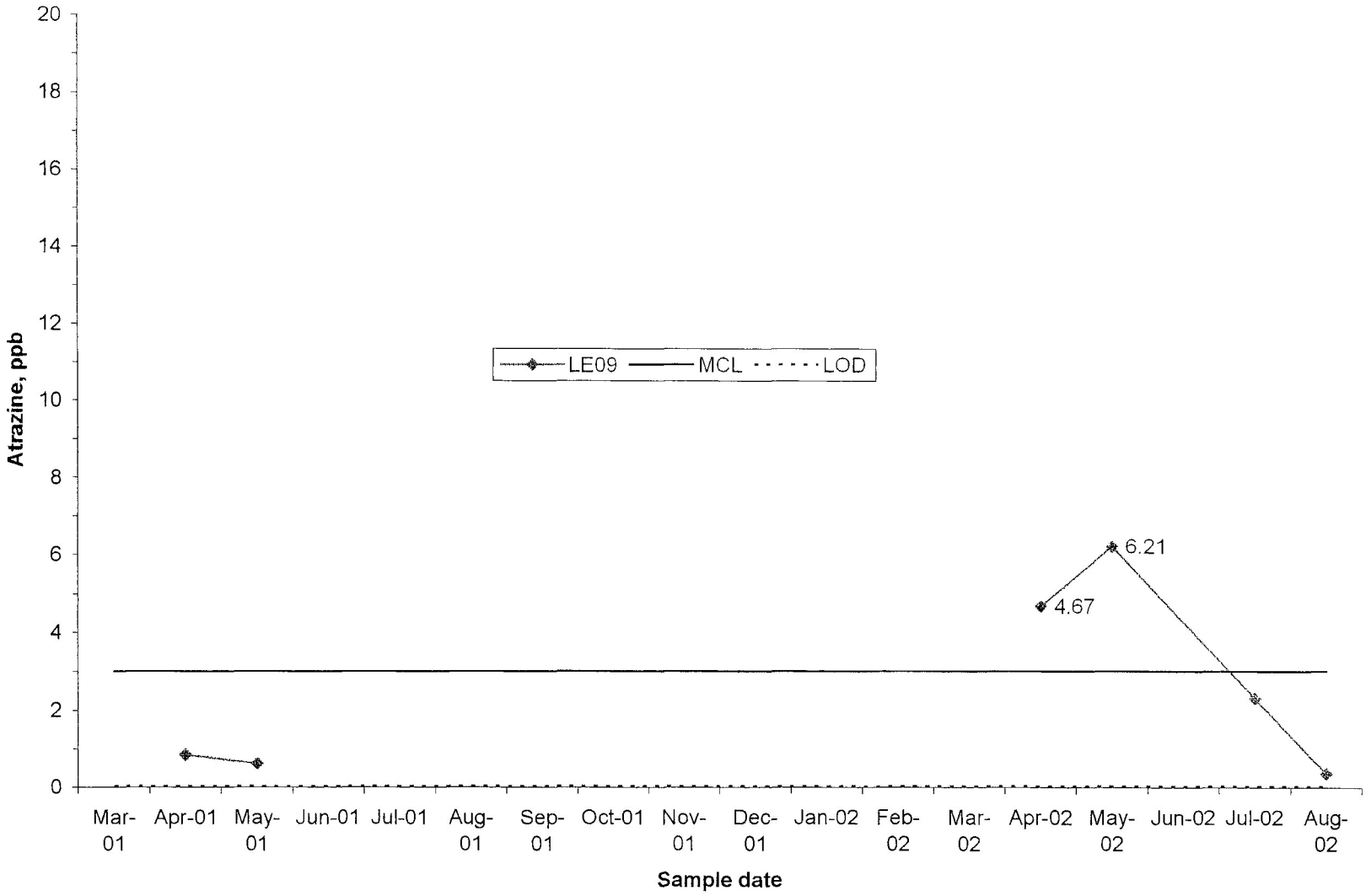
Graph 9 ▪ LE07


3.5
3
2.5
2
1.5
1
0.5
0
Atrazine, ppb
LE07
MCL
LOD
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Nov-01
Dec-01
Jan-02
Feb-02
Mar-02
Apr-02
May-02
Jun-02
Jul-02
Aug-02
Sample date

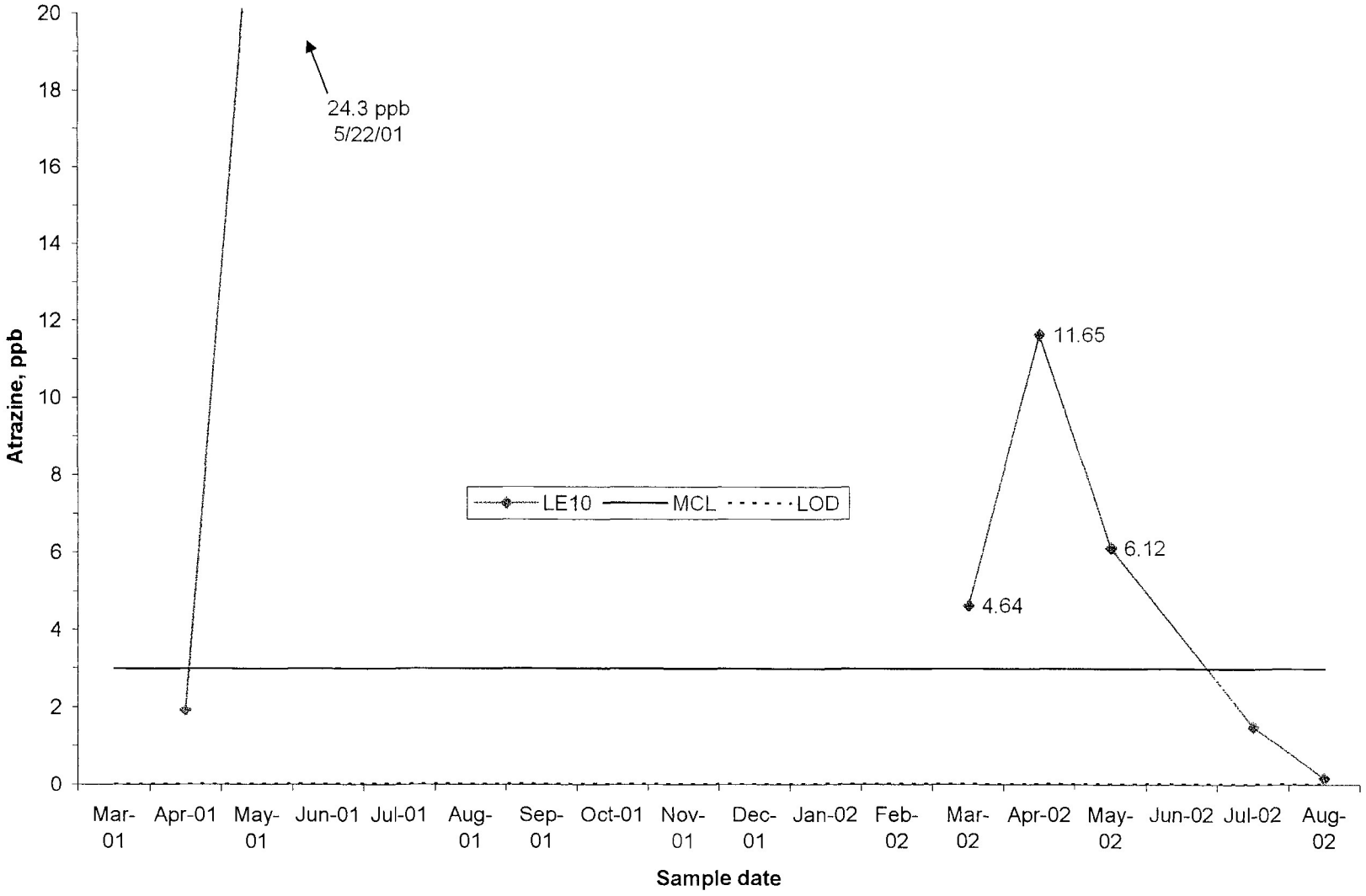
Graph 10 ▪ LE08



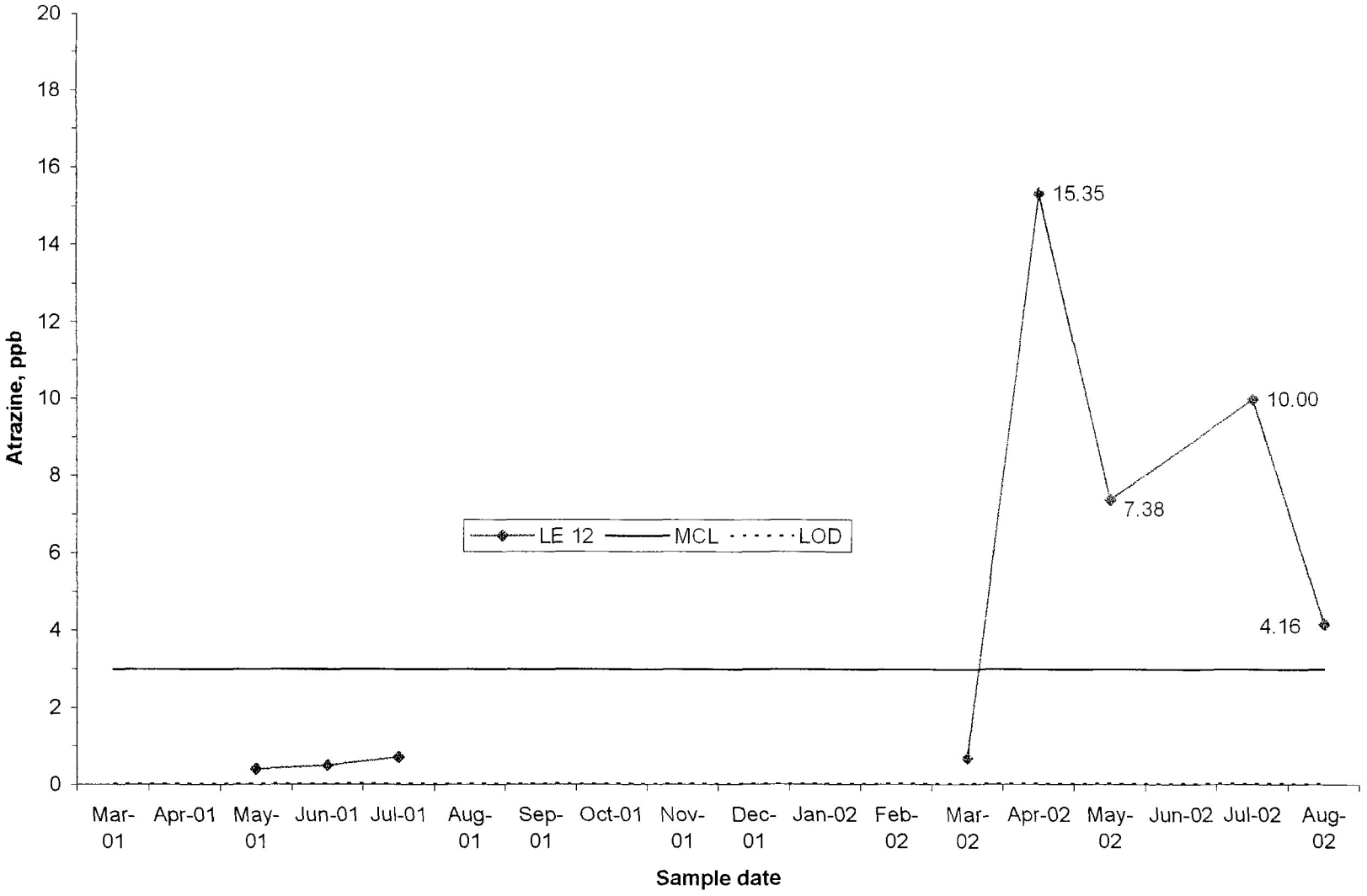
Graph 11 ▪ LE09



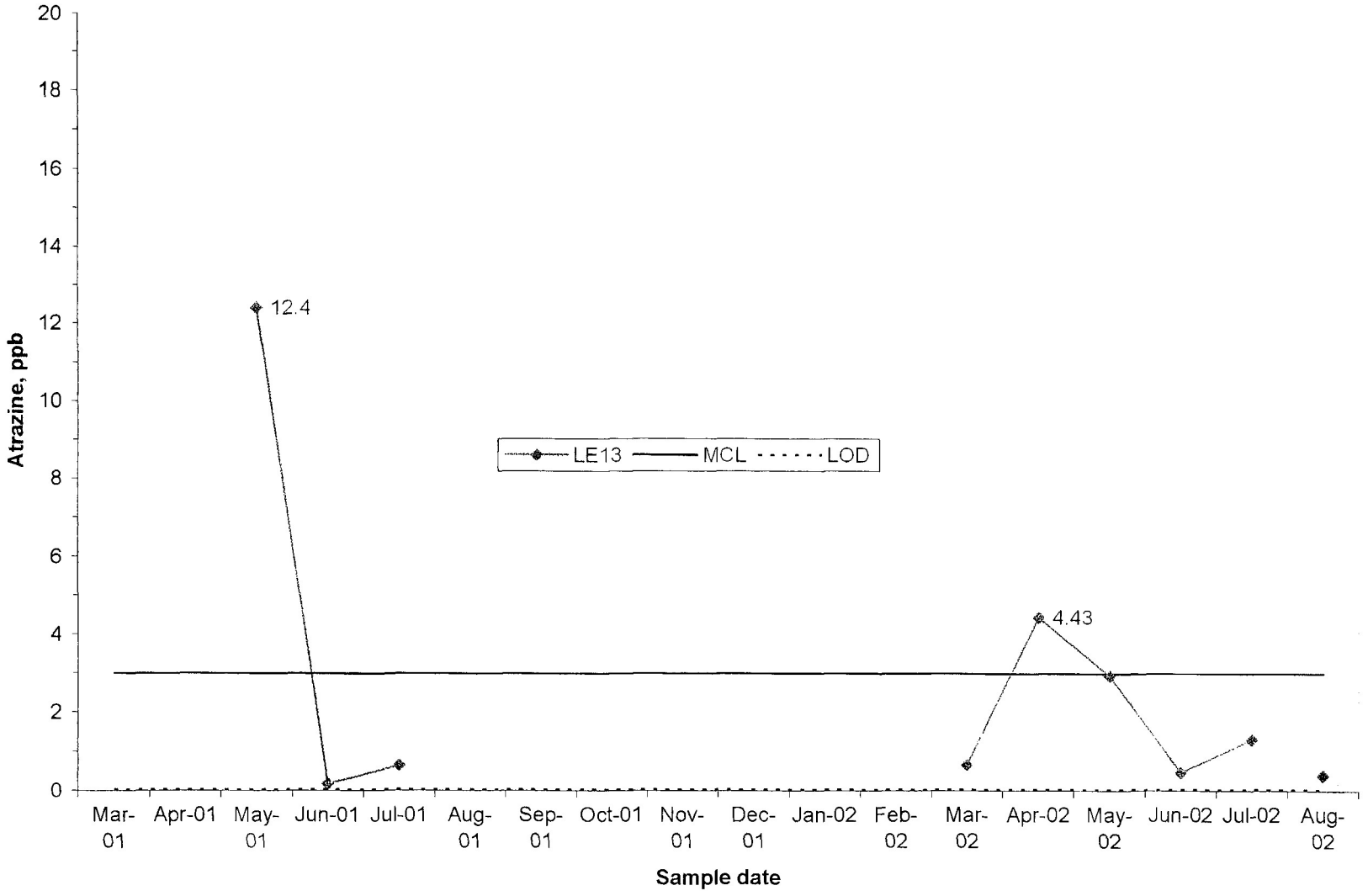
Graph 12 ▪ LE10



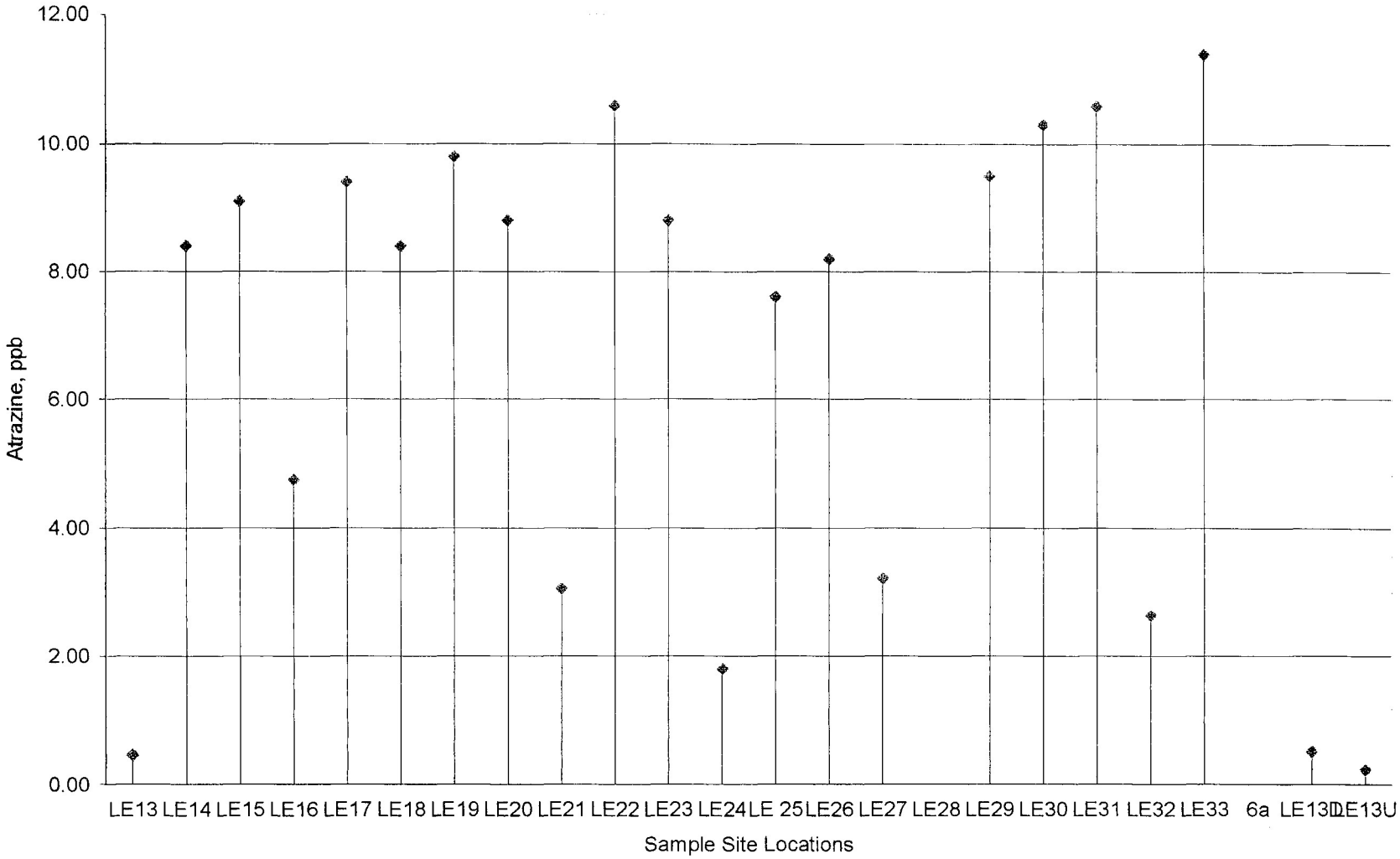
Graph 13 ▪ LE12



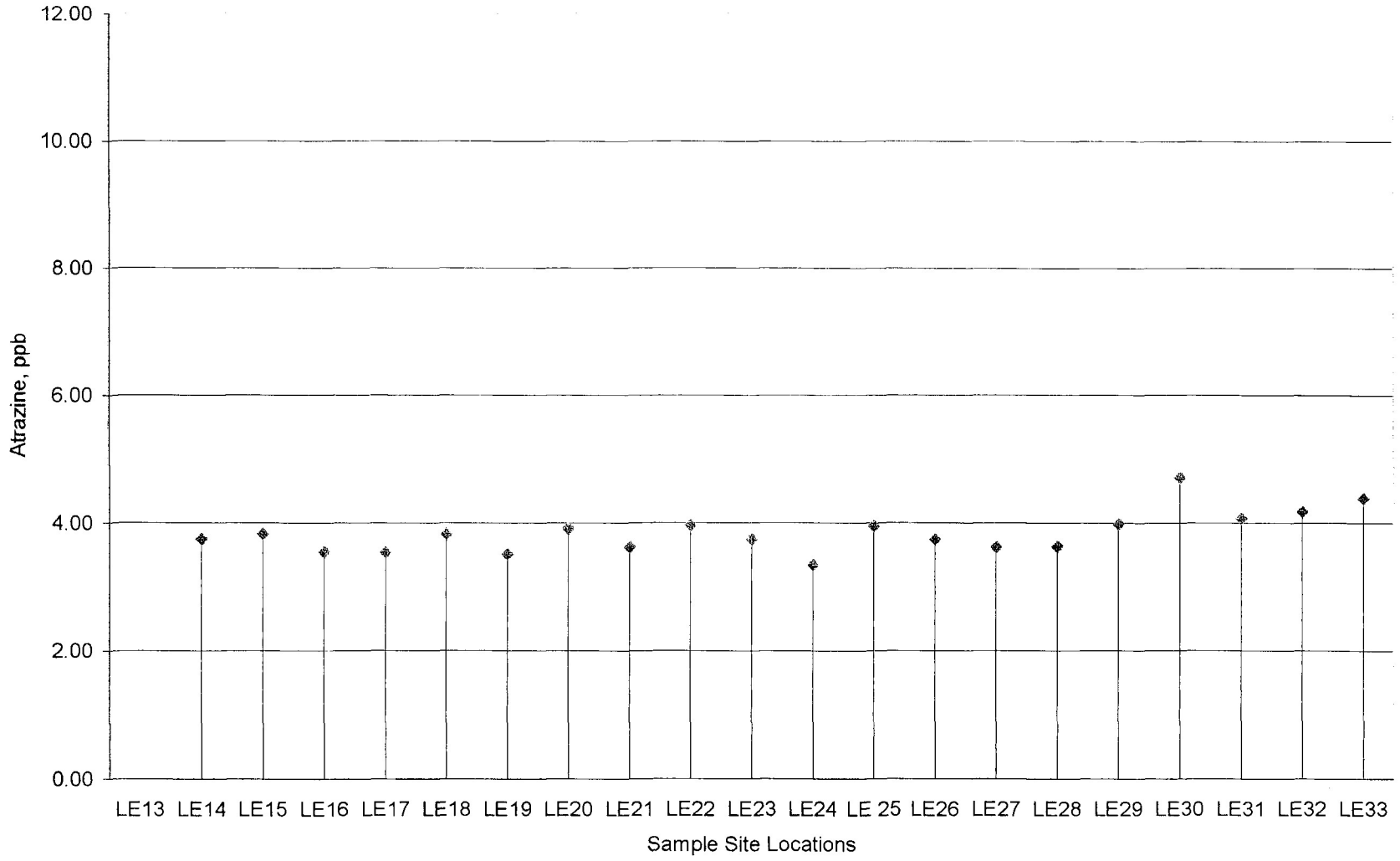
Graph 14 ▪ LE13



Graph 15 ▪ June 15, 2002



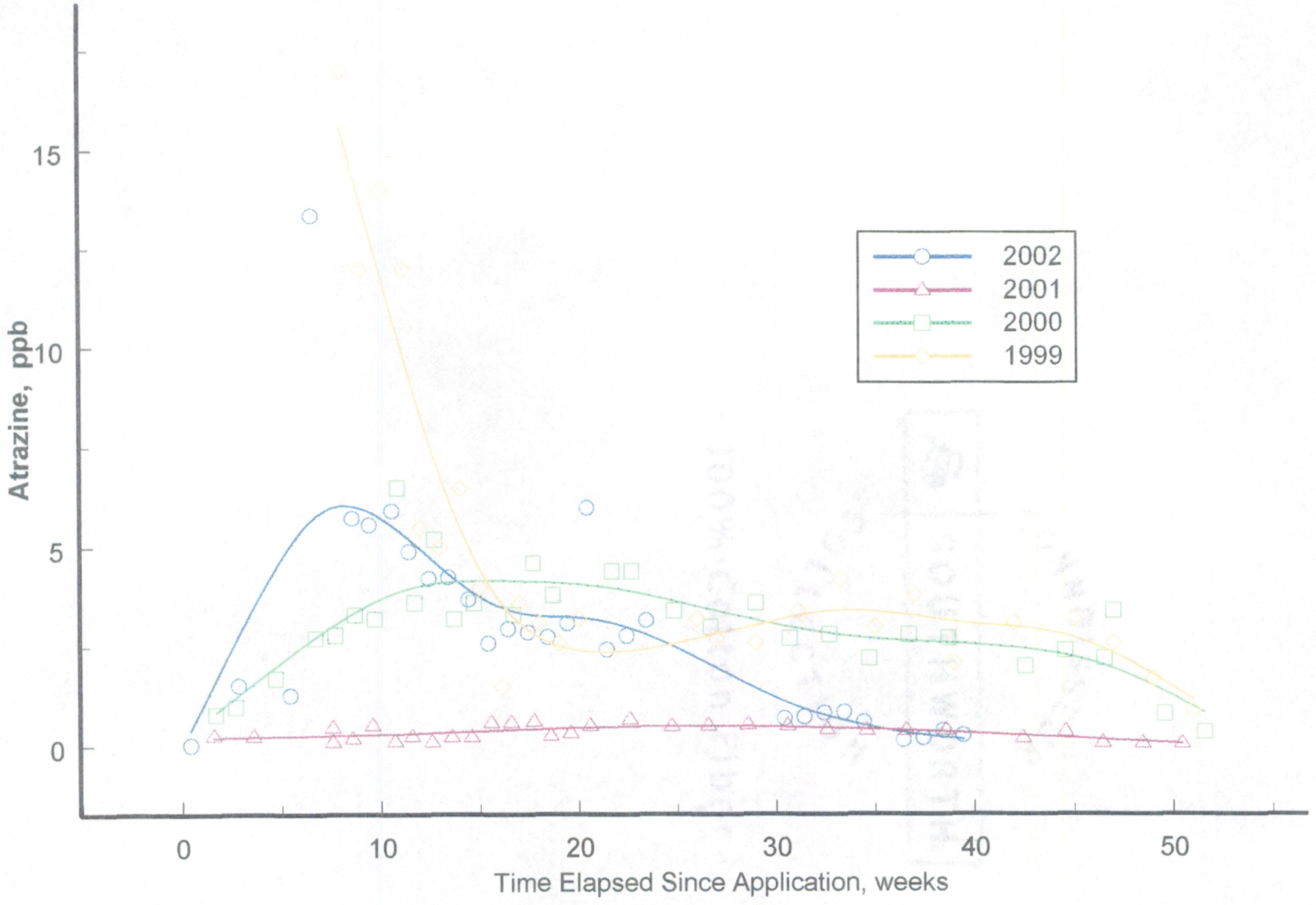
Graph 16 ▪ July 24, 2002



II. Statistical Results

Data publicly provided by Syngenta for the 1999 and 2000 growing seasons and data generated by this research for the 2001 and 2002 are shown in Graph 17. Each year is also illustrated independently in Graphs 18-21, respectively. The graphs are based on Tables 8-11 which detail the date each sample was collected, the time elapsed since application in weeks (TESA), the atrazine value as analyzed by the ELISA method – reported in μg/L (ppb), and the quantity of rainfall in the previous one week (P1W) as recorded at a nearby weather station in Russellville, Kentucky. The data from 1999 were interpolated to have begun low early in the year prior to initiation of Syngenta's voluntary monitoring program.

Graph 17 ▪ Atrazine Time Series with Spline Smoothing



Graph 18 ▪ 1999 Growing Season

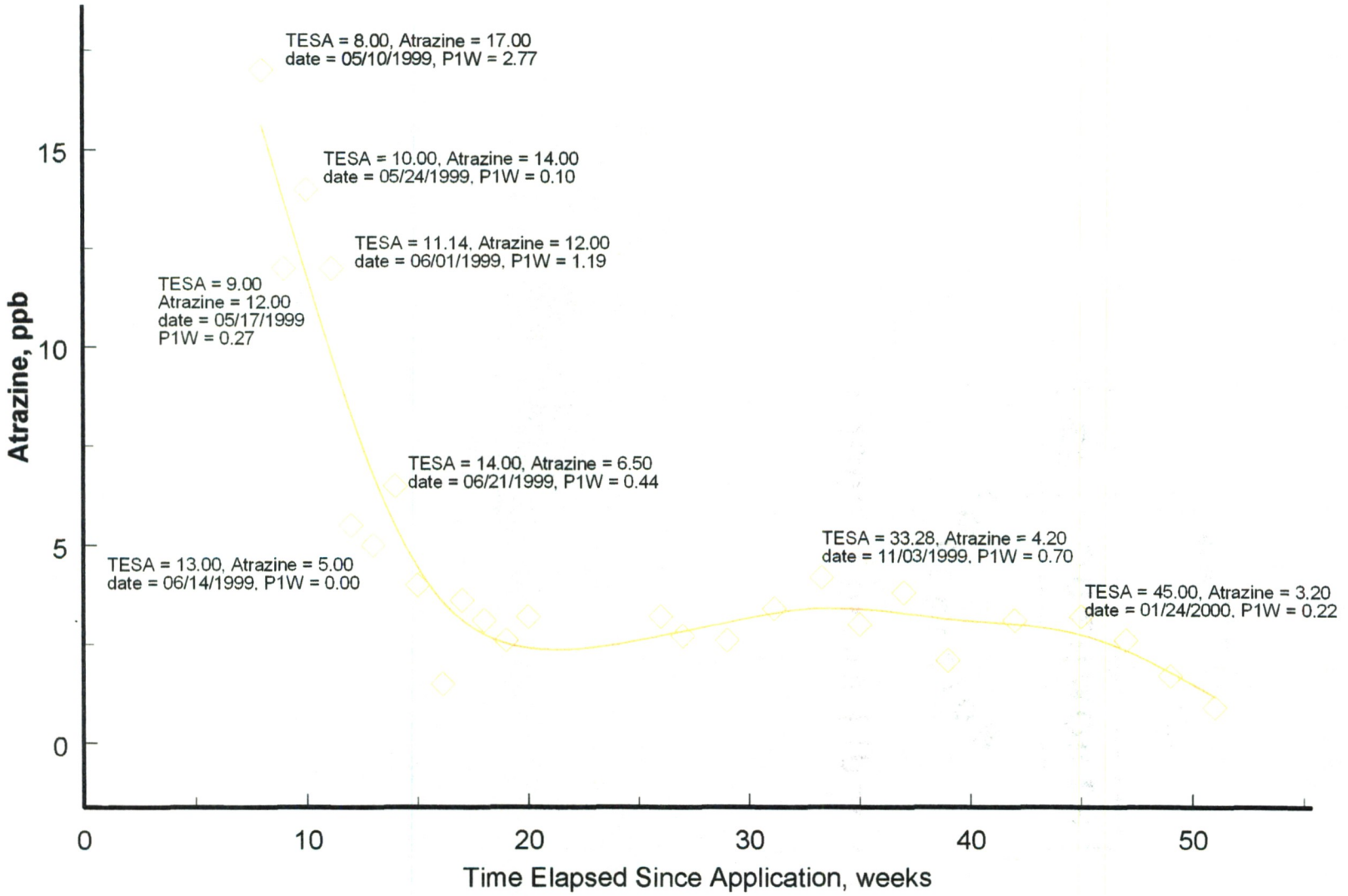


Table 8 ▪ 1999 Growing Season

DATE	TESA, weeks	ATRAZINE, ppb	P1W, inches
5/10/1999	8.000000	17.000000	2.770000
5/17/1999	9.000000	12.000000	0.270000
5/24/1999	10.000000	14.000000	0.100000
6/1/1999	11.140000	12.000000	1.190000
6/7/1999	12.000000	5.500000	1.560000
6/14/1999	13.000000	5.000000	0.000000
6/21/1999	14.000000	6.500000	0.440000
6/28/1999	15.000000	4.000000	2.130000
7/6/1999	16.140000	1.500000	2.040000
7/12/1999	17.000000	3.600000	0.060000
7/19/1999	18.000000	3.100000	0.130000
7/26/1999	19.000000	2.600000	0.220000
8/2/1999	20.000000	3.200000	0.100000
9/13/1999	26.000000	3.200000	0.000000
9/20/1999	27.000000	2.700000	0.370000
10/4/1999	29.000000	2.600000	0.070000
10/19/1999	31.140000	3.400000	0.000000
11/3/1999	33.280000	4.200000	0.700000
11/15/1999	35.000000	3.000000	0.000000
11/29/1999	37.000000	3.800000	0.460000
12/13/1999	39.000000	2.100000	1.070000
1/3/2000	42.000000	3.100000	0.000000
1/24/2000	45.000000	3.200000	0.220000
2/7/2000	47.000000	2.600000	0.000000
2/21/2000	49.000000	1.700000	2.920000
3/6/2000	51.000000	0.900000	0.050000

Graph 19 ▪ 2000 Growing Season

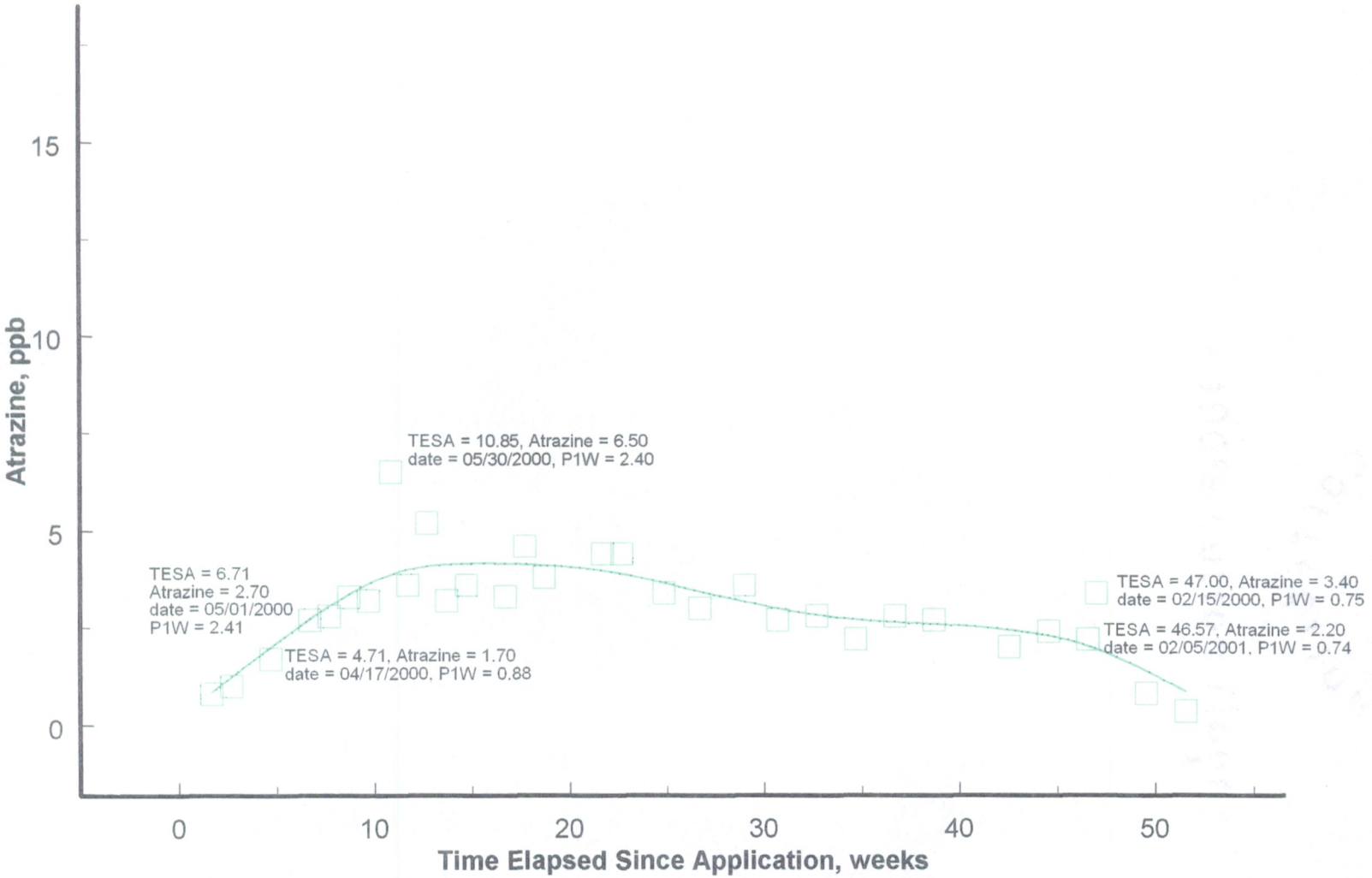


Table 9 ▪ 2000 Growing Season

DATE	TESA, weeks	ATRAZINE, ppb	P1W, inches
3/20/2000	1.710000	0.800000	1.820000
4/3/2000	2.710000	1.000000	0.490000
4/17/2000	4.710000	1.700000	0.880000
5/1/2000	6.710000	2.700000	2.410000
5/8/2000	7.710000	2.800000	0.740000
5/15/2000	8.710000	3.300000	0.560000
5/22/2000	9.710000	3.200000	0.240000
5/30/2000	10.850000	6.500000	2.400000
6/5/2000	11.710000	3.600000	0.000000
6/12/2000	12.710000	5.200000	0.130000
6/19/2000	13.710000	3.200000	0.830000
6/26/2000	14.710000	3.600000	0.600000
7/10/2000	16.710000	3.300000	0.080000
7/17/2000	17.710000	4.600000	0.000000
7/24/2000	18.710000	3.800000	0.440000
8/14/2000	21.710000	4.400000	0.600000
8/21/2000	22.710000	4.400000	0.160000
9/5/2000	24.850000	3.400000	0.000000
9/18/2000	26.710000	3.000000	1.600000
10/4/2000	29.000000	3.600000	0.000000
10/16/2000	30.710000	2.700000	0.000000
10/30/2000	32.710000	2.800000	0.000000
11/13/2000	34.710000	2.200000	2.050000
11/27/2000	36.710000	2.800000	1.150000
12/11/2000	38.710000	2.700000	0.030000
1/8/2001	42.570000	2.000000	0.080000
1/22/2001	44.570000	2.400000	1.140000
2/5/2001	46.570000	2.200000	0.740000
2/15/2001	47.000000	3.400000	0.750000
2/26/2001	49.570000	0.810000	1.860000
3/12/2001	51.570000	0.360000	0.280000

Graph 20 ▪ 2001 Growing Season
Voluntary No-Atrazine Year

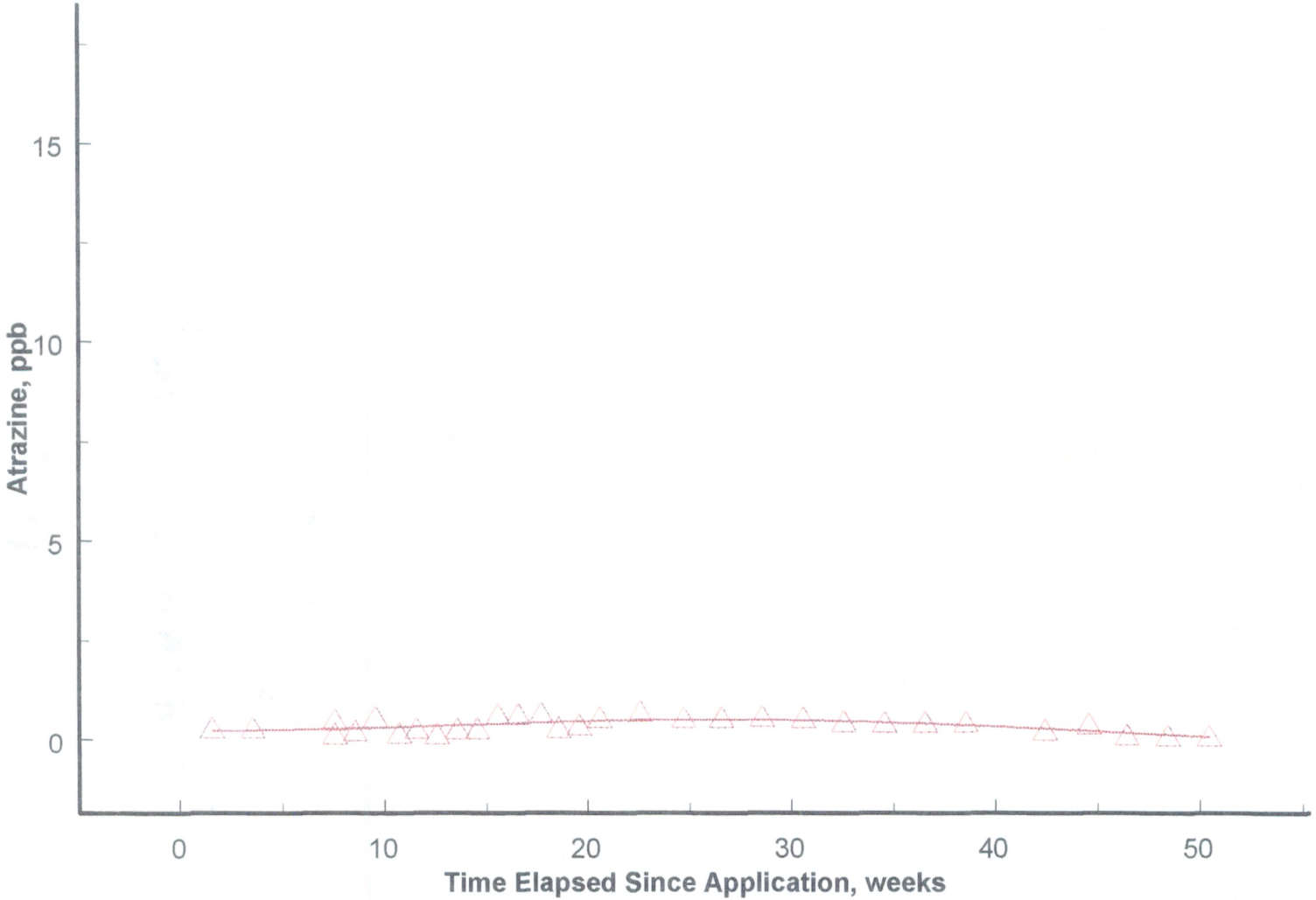


Table 10 ▪ 2001 Growing Season

DATE	TESA, weeks	ATRAZINE, ppb	P1W, inches
3/26/2001	1.570000	0.270000	0.390000
4/9/2001	3.570000	0.260000	0.680000
4/23/2001	7.570000	0.470000	0.100000
5/7/2001	7.570000	0.110000	0.230000
5/14/2001	8.570000	0.190000	1.860000
5/21/2001	9.570000	0.530000	0.180000
5/29/2001	10.710000	0.120000	1.650000
6/4/2001	11.570000	0.250000	1.770000
6/11/2001	12.570000	0.110000	0.770000
6/18/2001	13.570000	0.240000	1.600000
6/25/2001	14.570000	0.240000	0.080000
7/2/2001	15.570000	0.590000	1.330000
7/9/2001	16.570000	0.590000	0.990000
7/17/2001	17.710000	0.630000	0.000000
7/23/2001	18.570000	0.270000	1.310000
7/30/2001	19.570000	0.340000	2.240000
8/6/2001	20.570000	0.520000	0.450000
8/20/2001	22.570000	0.670000	0.170000
9/4/2001	24.710000	0.510000	0.580000
9/17/2001	26.570000	0.520000	0.840000
10/1/2001	28.570000	0.550000	0.560000
10/15/2001	30.570000	0.520000	3.970000
10/29/2001	32.570000	0.420000	0.460000
11/12/2001	34.570000	0.400000	0.000000
11/26/2001	36.570000	0.400000	1.620000
12/10/2001	38.570000	0.400000	2.070000
1/7/2002	42.430000	0.200000	0.160000
1/22/2002	44.570000	0.370000	0.330000
2/4/2002	46.430000	0.090000	0.830000
2/18/2002	48.430000	0.070000	0.070000
3/4/2002	50.430000	0.070000	0.610000

Graph 21 ▪ 2002 Growing Season

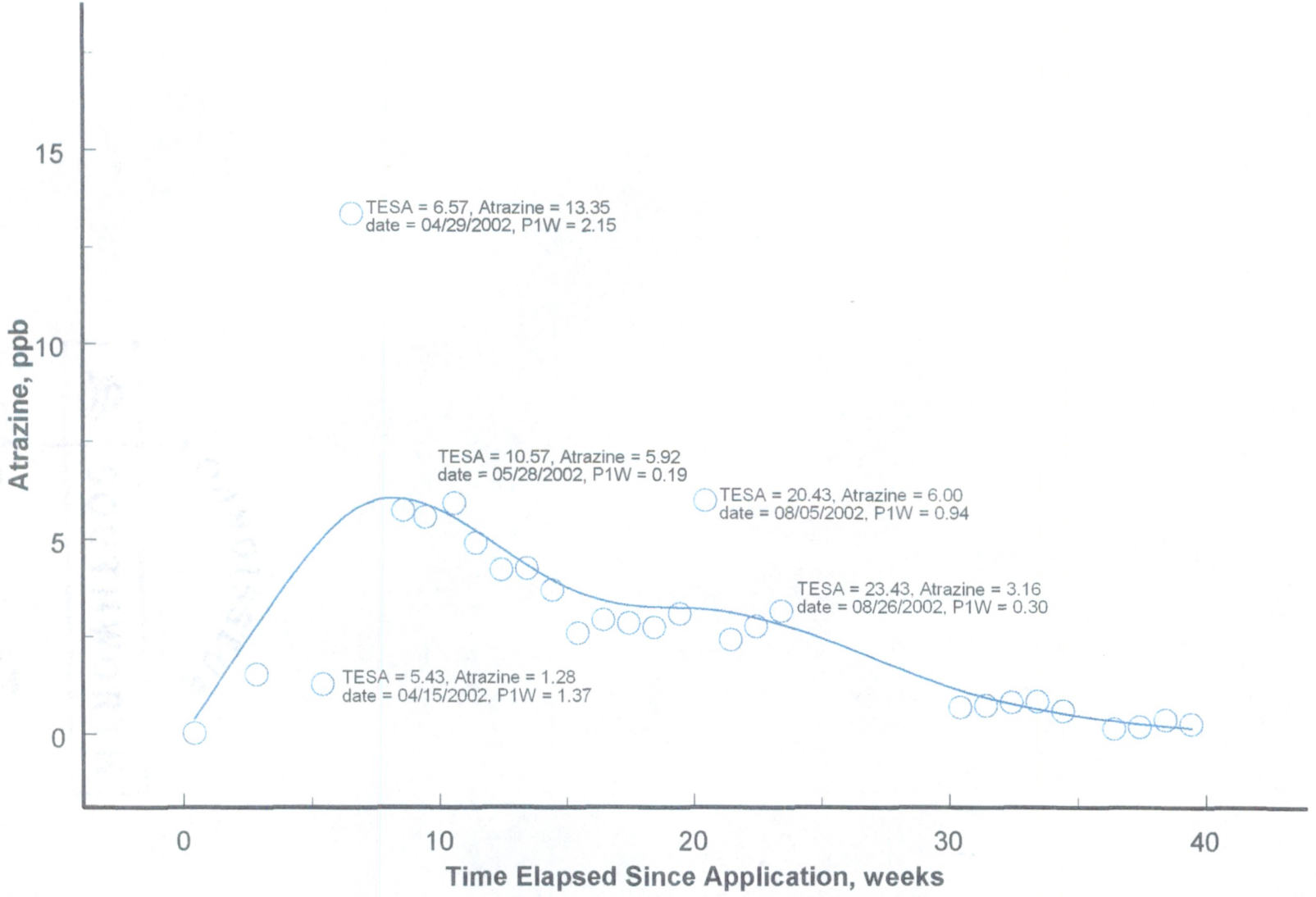


Table 11 ▪ 2002 Growing Season

DATE	TESA, weeks	ATRAZINE, ppb	P1W, inches
3/18/2002	0.430000	0.030000	1.470000
4/4/2002	2.850000	1.530000	1.000000
4/15/2002	5.430000	1.280000	1.370000
4/29/2002	6.570000	13.350000	2.150000
5/13/2002	8.570000	5.740000	0.390000
5/20/2002	9.430000	5.570000	4.600000
5/28/2002	10.570000	5.920000	0.190000
6/3/2002	11.430000	4.900000	0.030000
6/10/2002	12.430000	4.220000	0.640000
6/17/2002	13.430000	4.250000	0.480000
6/24/2002	14.430000	3.690000	0.000000
7/1/2002	15.430000	2.580000	0.240000
7/8/2002	16.430000	2.940000	0.820000
7/15/2002	17.430000	2.850000	2.240000
7/22/2002	18.430000	2.740000	0.000000
7/29/2002	19.430000	3.090000	0.390000
8/5/2002	20.430000	6.000000	0.940000
8/12/2002	21.430000	2.430000	0.000000
8/19/2002	22.430000	2.770000	2.570000
8/26/2002	23.430000	3.160000	0.300000
10/7/2002	29.430000	broken bottle	0.560000
10/14/2002	30.430000	0.700000	3.270000
10/21/2002	31.430000	0.740000	0.460000
10/28/2002	32.430000	0.830000	0.180000
11/4/2002	33.430000	0.850000	1.670000
11/11/2002	34.430000	0.600000	2.080000
11/25/2002	36.430000	0.160000	0.060000
12/2/2002	37.430000	0.200000	0.090000
12/9/2002	38.430000	0.380000	1.290000
12/16/2002	39.430000	0.270000	1.410000

Chapter 6: Discussion

Concurrent periods of severe drought in southern, central, and western Kentucky in 2001, followed by a year of average rainfall in 2002, highlight the effects climatic variability can have on concentrations of atrazine found in a water source. Field work proved to be a valuable tool for observing drought conditions, and thus, lack of flow, in many of the tributaries to Wolf Lick Creek and Spa Lake.

The drought conditions of 2001 undoubtedly reduced the observed amounts of atrazine reaching the water treatment facility; however, other factors also contributed to the minimal amounts of atrazine permeating Spa Lake. In conversations with local producers, a major corn producer in the Sharon Grove area reported that he replaced atrazine with the herbicide Balance. In 1998, EPA concluded that residues of isoxaflutole, the active ingredient in Balance, and its metabolites do not contribute significantly to the aggregate cancer or non-cancer human health risk in drinking water at the present time (Federal Register, 1998). Several producers followed suit by enrolling in a "No Atrazine" program designed by the Kentucky Department of Agriculture, Division of Pesticides to help monetarily compensate farmers for not using atrazine during the 2001 growing season. Checks were issued to the producers in January 2002 following submittal of their regulatory paperwork.

Unfortunately, the Division of Pesticides did not have sufficient funding to support a second year of the No Atrazine program. Many producers had no choice but to resume the use of atrazine due to it's comparably lower cost and known effectiveness. As expected, with the resumption of atrazine use in 2002, elevated levels of the product were observed in April and May 2002.

Usage of atrazine doesn't fully explain its occurrences in a system. Precipitation patterns played a key role in the occurrence of atrazine. As described earlier, atrazine is highly seasonal, and, therefore, the highest concentrations were, as expected, found in the spring of each year. One observation that was made is that if a precipitation event occurs during or shortly after the application period (mid-March to early April) then the source water atrazine concentrations were more likely to exceed EPA drinking water regulations.

Growing season 2001 offers a good example of this theory. As demonstrated in the previous graphs, atrazine concentrations of the raw drinking water in 2001 were well below the MCL of 3.0 ppb. One exception to very little atrazine detected in 2001 occurred on May 22, 2001, when the sampling event immediately followed a significant rain event of over 4 cm. Many tributaries and lake samples dramatically responded to the rain event with significantly higher atrazine values than recorded the remained of the season. Analytical results from May 2001 indicated 12.4 ppb of atrazine in the water flowing from the karst spring (sample site #13), which exceeds EPA's MCL by 4 times. Analytical results from June 2001 indicated 0.18 ppb of atrazine flowing from the same location. Graphs 17 and 20 illustrate the minimal amount of atrazine observed in 2001.

On the other hand, no precipitation lends itself to either no sample being collected from the tributaries and very low concentrations of atrazine moving through the watershed. When growing season 2000 is considered, the severe drought must also be evaluated. Graph 19 indicates that the first significant rain event following application occurred the last week of April. This rain event did not move a considerable amount of atrazine through the system for the May 1st sample date. However, the precipitation in the week preceding the May 30th sample event presumably contributed to the atrazine concentration of 6.5 ppb, twice that of the previous week. The 2000 growing season never demonstrated the characteristic spring spike then gradual decline of atrazine, as evident in the 1999 and 2002 seasons (See Graph 17). A lesser flushing effect of atrazine early in 2000 may have contributed to its data points remaining slightly higher than the 1999 and 2002 observations later in the season.

Another interesting observation showed on Graphs 17 and 18 was that in 1999 elevated atrazine values early in the season, coupled with fair to moderate precipitation events, may have flushed the system of atrazine resulting in the steep, rapid decrease in concentration observations through July 6, 1999. The following months received little precipitation and, likewise, steady values of atrazine were observed. The slight trending upward in October and November was mostly likely due to lake turnover that slightly agitated the lake sediment, thus releasing a nominal amount of adsorbed atrazine into the water system.

Further support of precipitation largely governing observed atrazine concentrations is demonstrated in Graph 21. In addition to the resumption of atrazine use in many parts of the watershed, the entire region received at or near average precipitation amounts during 2002. A significantly wet March - over 16.2 cm of precipitations - suggests that application may have been delayed until early to mid April. Graph 21 and Table 11 illustrate a significant spike in atrazine following significant precipitation events in the preceding week. Again, an early spring flush helped wash the atrazine through the system, thus moderating the observations later in the season. Also, later in the growing season and following emergent of the crops, theatrically, no product was being used, and the atrazine concentrations trended downward.

In addition to analytical data obtained from the samples collected, field data are important to consider. The hydrologic inventory and dye traces revealed valuable pieces of information to the project. Based on the results of the simultaneous dye trace, and the fact that there were moderate-to-high flow conditions/precipitation over the course of the trace, it was determined that there was no inter-basin transfer of water between Duck Lick and Wolf Lick Creeks. The most likely source of water for the spring (sample site #13) is Wolf Lick Creek and its tributaries, supplemented by recharge water from the hill above the spring. This finding suggests that the atrazine found in Spa Lake most likely originated within the Wolf Lick Creek / Spa Lake watershed.

The failure to detect sulphorhodamine B dye either upstream or downstream of the spring is likely due to dilution of the dye. The small amount of dye used in the trace

combined with the relatively high flows during the trace was probably responsible for the dilution of the dye into non-detectable concentrations. Breakdown of the dye in sunlight may have also contributed to the failure to the detect it, but given the short length of the trace, this is not likely to have been a major factor.

The detection of eosine dye in the spring and both upstream and downstream of it indicated two of the hypothetical scenarios were correct. Dye in the spring confirms a direct relationship between the predominately dry tributary directly upstream of the spring and the volume of water exiting the spring. The detection of eosine upstream of the spring indicates some surface flow contributing the Wolf Lick Creek. The over surface flow was presumably from a significant precipitation event during the course of the dye trace.

Chapter 7: Conclusions

In studies such as this one, investigators are often left with new questions rather than clear cut and definitive answers. The factors driving the continued use of atrazine are apparent. Atrazine is a proven, effective, low-cost treatment readily available to producers. Atrazine's ability to inhibit the electron transport chain is highly efficient for row crops in controlling board leaf weeds and grasses. In the long term, its degradation products are harmless elements found in nature.

Yet despite all the advantages atrazine may provide, controversy continues to surround its safety and suspected health risks. Scientists continue to investigate the biological and ecological effects of atrazine. At the time of this writing, USEPA continues to support the use of atrazine, while the European Union upholds its ban on the product. The EU is slated to issue a decision on the use of atrazine in the spring of 2005.

It is painfully obvious from this research that the reduction of atrazine in a watershed significantly reduces the amount of product entering into the source water of a RWSS. A goal of the SWPI was to assist RWSS in acquiring and monitoring the technical capacity needed to provide safe drinking water and to achieve the public health protection goals of the EPA Safe Drinking Water Act. This research reinforces the notion that the cleaner the source water, the cheaper it is to treat and the healthier it is for the consumers.

These problems are by no means restricted to Spa Lake, Lewisburg, Kentucky. Pesticide contamination was evident in four of the seven demonstration watersheds reviewed by the TACWQ. On a broader scale, atrazine is widely used across the United States as well as in other agriculture producing nations. The effectiveness of the product can not be denied; however, concerns associated with atrazine are numerous in the news and scientific literature. The concerns identified at Lewisburg are not isolated circumstances, but are typical of many rural communities across the United States.

As research continues in the area of atrazine contamination is a RWSS, there are several issues that should be investigated further, but are out to the scope of this project. Variation in soil pH is known to influence the CEC of atrazine and should be explored as a cause for fluctuating retention rates versus application amounts. Exact application amounts, time of application and rainfall with 24-48 hour may reveal more information as to the loading characteristics of atrazine.

Although Spa Lake is no longer a drinking water supply facility for northern Logan and Todd counties, the TACWQ is also working with the Kentucky Department of Agriculture, Division of Pesticides, to identify solutions to the lingering problems at Lewisburg. This problem is clearly a concern to the Environmental Protection Agency and the Commonwealth of Kentucky. Through funding grants that helped support this, and other, research, EPA has demonstrated a vested interest in identifying issues that must be addressed so that cleaner water may be provided to all citizens, both rural and urban.

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