Evaluation of the effects of atrazine exposure on *Xenopus laevis* in South Africa

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LIST OF ABBREVIATIONS

AchE	-	acetylcholinesterase
ACTH	-	adrenocorticotropic hormone or adrenocorticotrpin
ATP	-	adenosine triphosphate
BCF	-	bioconcentration factors
CSIR	-	Council for Scientific and Industrial Research
CWQG	-	Canadian Water Quality Guidelines
DAC	-	diaminochlorotriazine
DDT	-	dichlorodiphenyltrichloroethane
DEA	-	deethylatrazine
DEHA	-	desethylhydroxyatrazine
DIA	-	deisopropylatrazine
DIHA	-	desisopropylhydroxyatrazine
DO	-	dissolved oxygen
EC_{50}	-	concentration producing 50% maximal response
FETAX	-	frog embryo teratogenicity assay - *Xenopus*
HA	-	hydroxyatrazine
K_d	-	soil-water partition coefficient
K_{oc}	-	organic carbon partition coefficient
K_{ow}	-	octanol/ water partition coefficient
LC_{50}	-	concentration producing 50% mortality
LE	-	Long-Evans hooded
LOD	-	level of detection
LH	-	luteinizing hormone
NADPH	-	nicotinamide adenine dinucleotide phosphate
NF	-	Nieuwkoop & Faber
NHL	-	non-Hodgkin's lymphoma
NOEL	-	no-observed effect concentration
ppm	-	parts per million
SABS	-	South African Bureau of Standards

SD	-	Sprague-Dawley rat
SVL	-	snout-vent length
USEPA	-	United States environmental protection agency
UV-B	-	ultraviolet B
2,4-D	-	2,4-dichlorophenoxyactetic acid
* Corn	-	*Zea mays*

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CHAPTER 1

CHAPTER 1

INTRODUCTION AND LITERATURE OVERVIEW

"If you could tomorrow make water clean in the world, you would have done in one swoop, the best thing that you could have done for improving human health by improving environmental quality."

William C. Clark, speech, Racine, Wisconsin, 1988

During the last decade the loss of biodiversity has been a high-interest topic and the theme of many symposia and workshops. Amphibian declines have emerged as a key example of the global biodiversity crisis. Few other animal groups, if any, received as much attention as amphibians did over the past few years. According to Davidson *et al.* (2001), six primary hypotheses have been proposed to explain amphibian declines. Habitat destruction, increased UV-B radiation, climate change, introduction of exotic predators, disease, and agrochemicals. In this study, we are studying the endocrine effect, if any, that a widely used herbicide, atrazine, has on the African clawed frog (*Xenopus laevis)* under field conditions in South Africa.

In spite of the fact that, every year many thousands of new chemicals are synthesized, there is an enormous lack of knowledge on their environmental properties such as distribution pathways in various compartments of bio-availability, toxicity, bio-degradation, etc. Pesticide use has declined drastically over the past 20 years and when used correctly, they have enormous benefits in increasing crop yield (Zgajnar Gotvajn *et al.* 2001). On the other hand, there may be misuse and especially overuse of xenobiotics, like pesticides, resulting in an increasing concentration in surface and underground waters (Richardson 1996).

To understand the mechanisms through which xenobiotics may modulate endocrine function, it is best to understand the basics through which endocrine disrupters function.

Endocrinology is the study of tissues that secrete chemical messengers called hormones into the blood and the effect these "messengers" have on specific target tissues. Endocrine control represents only one mode of chemical signaling. Many chemical messengers are released into the local extracellular environment of an organism and affects neighboring cells (paracrine control) or the emitting cell (autocrine control). Nerve cells release chemical messengers (hormones or cytokines) into the blood called neuroendocrine control or into the extracellular fluid at specialized points of communication with other nerve cells or non-neuronal cells called synaptic neurotransmission (Kendall *et al.* 1997). Environmental chemicals affect endocrine function through effects on hormone biosynthesis, transport, activity and metabolism in animals (Van der Kraak *et al.* 1998). For example, the exposure of single fish species, White sucker (*Catostomus commersoni*) to bleached kraft pulp mill effluent, leads to significant reductions in plasma sex steroid hormone levels because of the modulation of the endocrine function that causes interference with the biosynthesis or degradation of steroid hormones in the fish (McMaster *et al.* 1992; Van der Kraak *et al.* 1992).

The substance addressed in this report is atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-*s*-triazine) (Fig. 1.1). It has been suggested by Colborn *et al.* (1993) that this widely used chemical is an endocrine disrupter.

Herbicidal *s*-triazines have been on the market for more than 47 years; simazine was introduced in 1956 and atrazine was introduced in 1957 (Worthing 1991). Since that time it has become commonplace in agricultural and forestry practices (Graymore *et al.* 2001). Atrazine an organochlorine compound sold as the active ingredient in a number of wettable, flowable powder, and water-dispersible

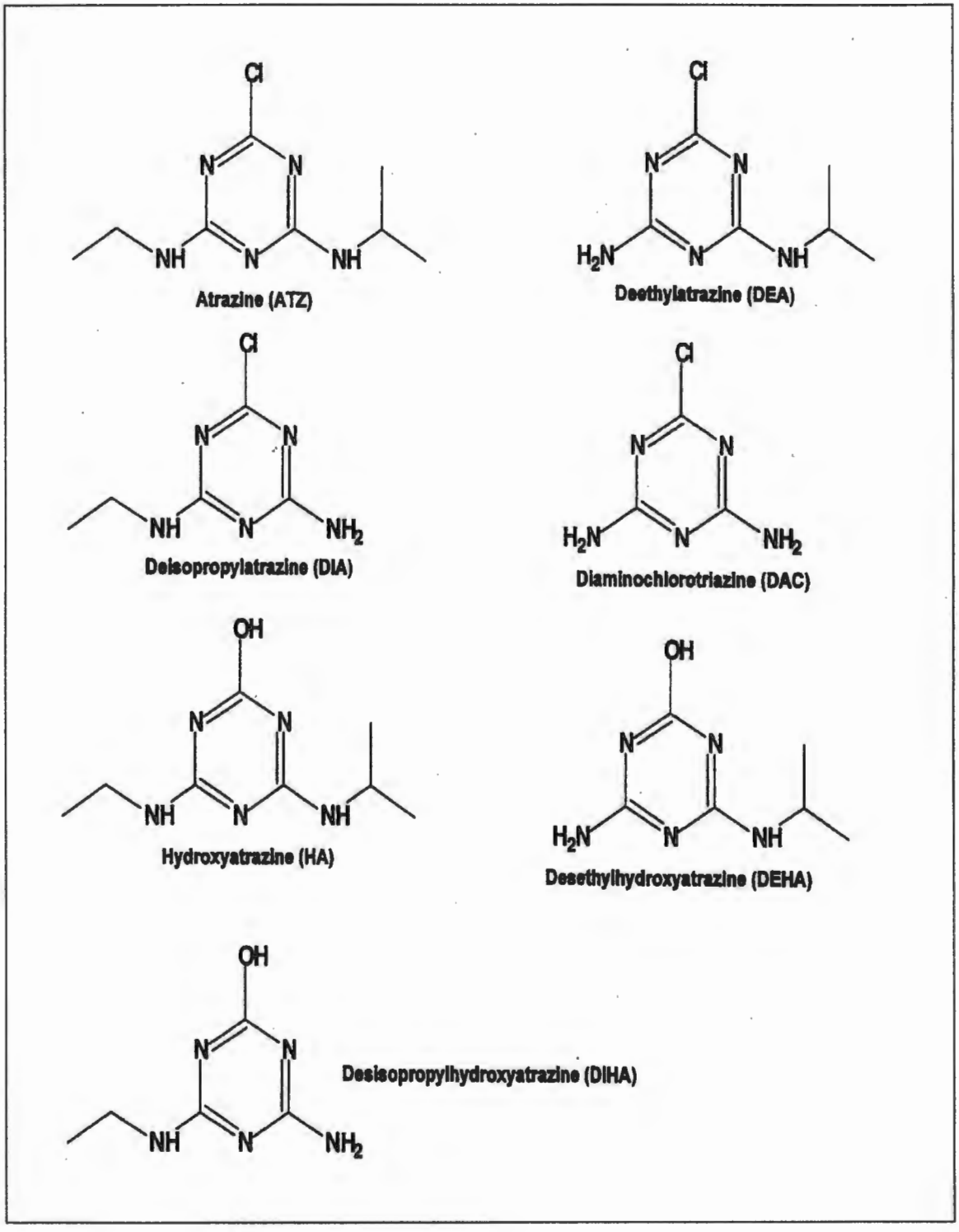

Figure 1.1: The chemical structures of atrazine and its degradation products (Solomon *et al.* 1996).

granular formulations under a variety of trade names as a broadleaf herbicide (Meister *et al.* 1994).

According to amounts applied, atrazine is the predominant herbicide used in the United States and probably the world for the last four decades (Hopenhayn-Rich *et al.* 2002). In 1995, no less than 68 – 73 million pounds (30.8 – 33 million kilograms) of atrazine was applied worldwide (Short *et al.* 1999). Atrazine is used widely as herbicide for broadleaf and grass weed control in selected vegetables, corn, sorghum, sugarcane, fruit and citrus production. Atrazine is usually applied pre-emergence as a water-dispersed spray or in liquid fertilizer, although pre-plant and post-emergent applications are occasionally used.

Herbicidal mechanism of atrazine

Atrazine is a photosynthesis inhibitor (Moreland 1980). According to Hull (1967), exposure to atrazine occurs, primarily through the leaves and roots of the plants and across cell surfaces of unicellular plants. Light energy oxidizes chlorophyll when photosynthesis takes place; oxygen, as a by-product, is produced when these chlorophyll molecules obtain replacement electrons from the cleavage of water (Woolhouse 1981). Like most of the herbicides known to be photosynthetic inhibitors (e.g., triazines, ureas, and uracils), atrazine competes with plastoquinone II and blocks the transport of electrons from Photosystem II; that utilizes water as an electron donor (Hull 1967; Forney *et al* 1981) (Fig. 1.2). Electron flow mediated by light can occur only until all of the chlorophyll molecules in Photosystem II are oxidized, if replacement electrons from water are absent. After this, photophosphorylation (ATP production), reduction of Photosystem I chlorophyll molecules, cyclic photophosphorylation, production of NADPH, and eventually the reduction (fixation) of carbon dioxide in the dark reactions all cease. An inhibition of carbohydrate synthesis, a reduction in the carbon pool, is caused by the blockage as well as a build-up of CO_2 within the

cell (Shabana 1987). Damage to the chlorophyll molecules is caused by extended exposure to light in the presence of blockers of photosynthesis, such as atrazine. This damage does not occur in the absence of light. Atrazine is much more toxic to plants than to animals due to the fact that this photosynthetic metabolic pathway is only found in plants and not in animals.

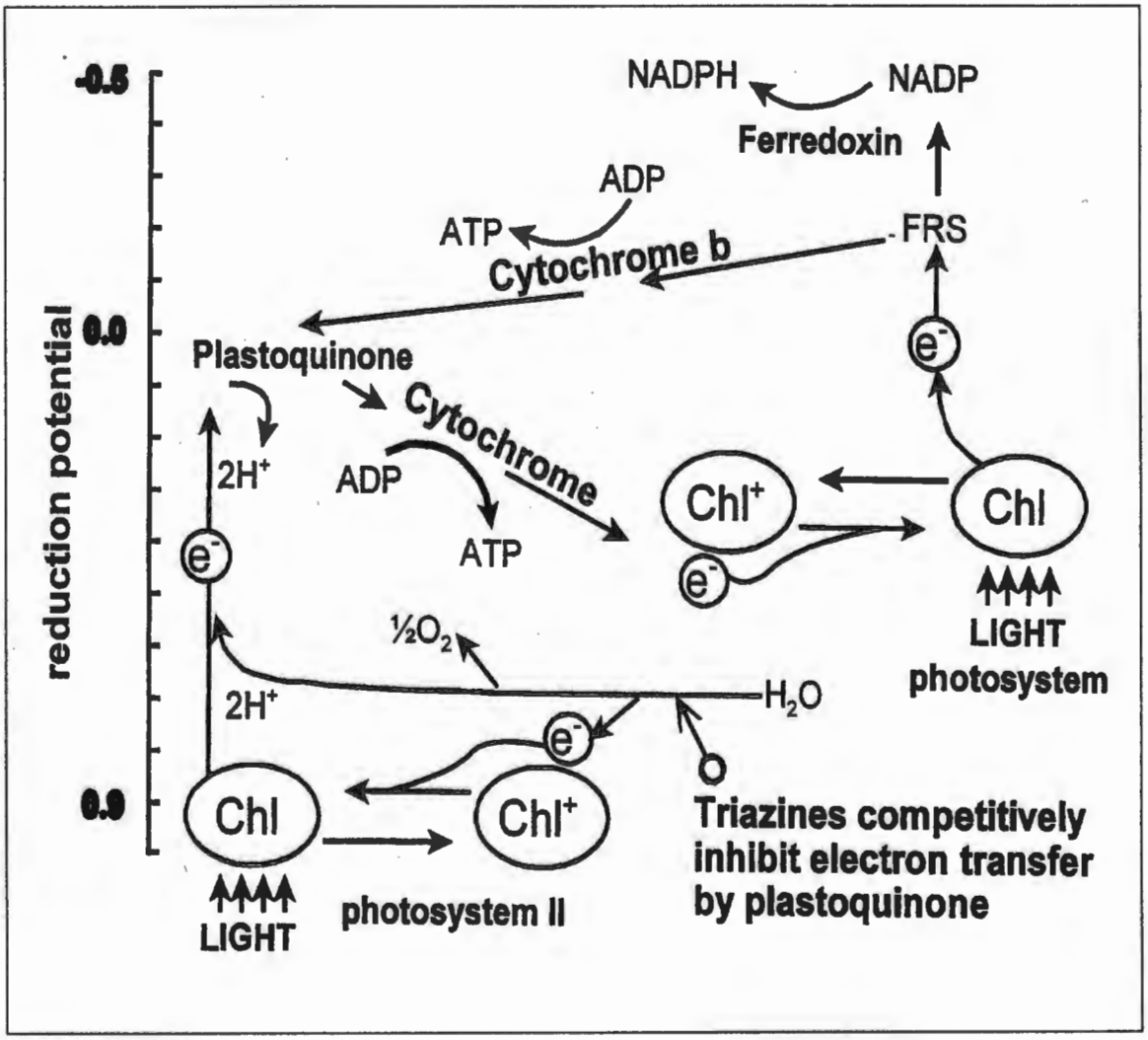

Figure 1.2: The mode of action of atrazine on photosynthesis in the plant (Solomon *et al.* 1996).

Physical and chemical properties of Atrazine

"The pollution of surface and ground waters by chemicals is governed by the physicochemical characteristics of the compounds, by the properties on the medium in which they are applied and by other external factors, such as the local rainfall and wind regimens or the topology of the specific area" (Martinez *et al.* 2000). Among the most important physicochemical properties of agrochemicals are their solubility in water, their capacity to be retained by the organic part of soil (characterized by the K_{oc} coefficient, which is closely related to the octanol-water partition coefficient K_{ow}) and their degradation rate, which is related to their molecular structure and which determines their persistence in soils (Canter *et al.* 1987).

Atrazine is a weakly basic compound with a pK_a of 1.68 (Gunther *et al.* 1970) and water solubility of 33 mg/L at 22°C and a pH of 7 (Novartis 2000). According to a Novartis (2000) study, atrazine has a low vapor pressure, 2.89×10^{-7} mm Hg at 25°C, and atrazine has almost no tendency to volatilize because of it's low Henry's law constant, 2.48×10^{-9} atm m^3 mol^{-1} (Capel *et al.* 2001), implying that volatilization from water and soil surface is negligible (Novartis 2000). The octanol-water partition coefficient (log K_{ow}) of atrazine is 2.68 at a temperature of 25°C (Carpenter 1986). Atrazine's moderate water solubility and relatively low K_d (0.19 to 2.46) and K_{oc} (25.3 to 155) facilitate movement of the chemical (s-triazine ring) primarily in the dissolved state from treated soil to surface or subsurface waters during rain events (Novartis 2000). Based on this physicochemical property, atrazine is not expected to adsorb strongly to sediments and may partition only moderately from the water column (Giddings *et al.* 2000).

There is a continuum in the movement of water, solids and solutes (e.g. atrazine) from terrestrial environments, such as agricultural fields, through a fresh water surface system and eventually into the marine environment. The water usually

moves through a combination of streams, small rivers, and larger integrated rivers, ultimately ending in the marine environment (Capel *et al.* 2001). This continuum can be divided into two parts – soil and stream – determined from the relative abundance of solid particles and water. Thus in agricultural context, the process that connects these two integrated parts of the continuum is termed "field runoff "(Capel *et al.* 2001). The occurrence of soil particles and different kinds of agricultural chemicals in field runoff have been a major concern to the agricultural and environmental communities for decades; especially the last few years. This is one of the predominant pathways through which agrochemicals move from their point of use to the broader environment where unintentional adverse effects may occur.

Through numerous field and laboratory studies, the important factors that govern the extent of particles and chemicals in runoff have been identified (Weber *et al.* 1980). For pesticides, these factors include the attributes of the weather, soil, pesticide properties, and agricultural management practices. It is the specific spatial and temporal combination of these factors that determines the total amount of soil particles, water, and chemicals (pesticides) transported in runoff (Capel *et al.* 2001).

Persistence in a natural environment

The exact relationship between environmental factors and herbicide persistence in soil are not well quantified, because the rate at which herbicides degrade is influenced by several soil as well as weather factors. Soil is a complex medium in which chemical, physical, and biological factors such as pH, texture, organic matter, and microorganisms are important variables (Reinhardt *et al.* 1993). According to Graymore *et al.* (2001), there are five processes that determine the rate of atrazine degradation within soils: hydrolysis, adsorption, volatilization, photo degradation, and microbial degradation. All of these factors may interact to

determine the degradation rate (Walker 1989) as well as the specific compound leaching rate (Weber *et al.* 1980) and hence it's persistence in a natural environment.

The fate of atrazine is influenced by the *s*-triazine ring, which makes this herbicide resistant to microbial attack (Howard 1991), thus making biodegradation due to microbial attack less important than chemical degradation in the environment. The chemical degradation of atrazine occurs at carbon 2, by *N*-dealkylation at carbon 4, and by splitting the triazine ring (Knuesli *et al.* 1969).

Walker *et al.* (1981) found that the half-life of atrazine in several soil types, varied from about one month in warm, wet soil (25°C; field capacity soil water) to about a year in cool, dry soil (5°C; 25% of water at field capacity). Atrazine is also more persistent in cool, dry areas than in warm, moist areas. According to Li *et al.* (1972), the environmental half-life of atrazine has been reported to be 244 days at 25°C and a pH of 4; however, the addition of 2% humic-acid decreased the half-life to 1.73 days. With the addition of 5 mg/L of fulvic acid (a chemical naturally occurring in surface waters), the half-live of atrazine has been reported to be 34.8, 174, 398, and 742 days measured at pH's of 2.9, 4.5, 6.0, and 7.0 respectively (Khan 1978). Aqueous photolysis conducted by Spare (1988) under natural sunlight in the laboratory, indicated a half-life of 355 days for atrazine. Photolysis of atrazine does not occur in water at wavelengths greater than 300 nm (Pape *et al.* 1970). The highest reported concentrations of atrazine are associated with the first rainfall after application (Thurman *et al.* 1992; Hall *et al.* 1993). The concentrations of atrazine in rivers and pond water varies seasonally with the highest concentrations found in the spring and summer months after application (Solomon *et al.* 1996).

A long-term buildup of atrazine in ground water would be expected in areas with continuous corn production under irrigation with yearly applications of atrazine;

however, this build-up has not occurred due to dilution, dispersion, and chemical hydrolysis (Canter *et al.* 1987).

Transformation products of Atrazine

Atrazine may degrade into a number of metabolites, each of varying persistence and toxicity. The most common metabolites of atrazine are deethylatrazine (DEA); hydroxyatrazine (HA); deisopropylatrazine (DIA); diaminochlorotriazine (DAC); and two dealkylated hydroxytriazines, desethylhydroxyatrazine (DEHA) and desisopropylhydroxyatrazine (DIHA) (Fig. 1.1) (Solomon *et al.* 1996). The formation of these transformation products of atrazine has been detected in laboratory studies, and some data are also available for the transformation products detected in the aquatic environment (Giddings *et al.* 2000). In aqueous photolysis studies using natural light, the maximum degradation concentration observed (as percentage of initial atrazine concentrations) were 2.8% for DEA, 2.6% for HA, 1.2% for DIA, 0.9% for DAC, 0.4% for DIHA, and 1.2% for DEHA (Novartis 2000). DIA and DEA are formed through biodegradation, whilst HA and DEHA can be formed by either chemical reactions in soil or biodegradation (Graymore *et al.* 2001). Measurements of persistence of atrazine transformation products are limited. The soil half-lives for DEA, DIA, DAC, and HA have been reported to be 26, 17, 19, and 121 days, respectively (Winkelmann *et al.* 1991), however, aqueous half-lives of atrazine degradates and metabolites are not available. DEA and DIA are phytotoxic. DEA is of most concern and presently is considered almost as toxic as atrazine itself (Winkelmann *et al.* 1991). Bioconcentration data for atrazine transformation products in the natural environment are also limited, because studies reporting biological concentrations focus on metabolite production, rather than the uptake of these degradates from environmental media in biota upon exposure to atrazine (Solomon *et al.* 1996).

Bioconcentration and bioaccumulation of Atrazine

Based on uptake data and measures of bioconcentration factors (BCF), atrazine is not expected to significantly bioconcentrate in aquatic organisms and food chain biomagnification is negligible (Giddings *et al.* 2000). Atrazine's relatively low octanol-water partition coefficient (log K_{ow} = 2.68 at 25°C), relatively great water solubility, and susceptibility to biological metabolism and rapid elimination combine to produce low BCF's in most tested species (Giddings *et al.* 2000). "Consequently, exposure via the food chain is of much lesser importance than via the water column" (Solomon *et al.* 2002).

The following section will give an overview of the effects of triazine on various taxa, with an emphasis on amphibians.

Reported effects and deformities in mammals

In plants, the principal mode of action of atrazine is to inhibit photosynthesis (Gysin *et al.* 1960). "The adverse health effects of these compounds in mammalian species have not been well characterized" (Cooper *et al.* 1999). Atrazine causes numerous endocrine alterations in adult vertebrates, although the mechanisms underlying these alterations are incompletely understood (Crain *et al.* 1999). Atrazine has been tested in mammals for acute, chronic, developmental, and reproductive toxicity as well as potential mutagenic effects (Kendall *et al.* 1997). According to Wetzel *et al.* (1994), atrazine is not a direct-acting carcinogen or a mutagen (Brusick 1994) and lacks in vivo estrogenic activity in mammals (Simpkins 1997).

In studies where Sprague-Dawley (SD) rats have been used, it was reported that dietary exposure to atrazine (400 mg/kg diet) or the related triazine, simazine, led to an earlier onset and greater incidence of mammary tumors (Stevens *et al.*

1994). In a previous study, it has been reported that exposure to atrazine by gavage (75 – 300 mg/kg/day) led to a rapid disruption of ovarian cycling in SD and Long-Evans hooded (LE) females (Cooper *et al.* 1996). "At the highest dose tested, atrazine induced anestrus as indicated by prolonged vaginal diestrus, atrophied ovaries, and basal concentrations of serum gonadotropins and ovarian steroids" (Cooper *et al.* 1996). SD rats were treated for 28 days with atrazine by gavage; at doses of 800 ppm and 4000 mg/kg; atrazine attenuated or blocked estrogen-induced hypersecretion of luteinizing hormone (LH). This effective blockage of LH secretion, at doses that have been demonstrated to induce a continuous estrous state in SD rats, support the theory that the effect of atrazine on LH secretion is an initiation event in the eventual manifestation of tumorogenic effects seen in female SD rats (Morseth 1996; Eldridge *et al.* 1996). In pigs, dietary exposure to atrazine with concentrations of 2 mg/kg/day caused elevated circulating estradiol-17ß prior to expected onset of estrus (Gojmerac *et al.* 1996). In the pigs used in this experiment, estrus never occurred, and histopathological examination revealed the persistence of the corpora lutea.

Atrazine has been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC 1999). This being said, the USEPA (2001) found that a wide margin of safety exists between doses and concentrations of atrazine that causes effects in mammalian toxicology studies in environmental relevant concentrations Patlak (1996) suggested that many chlorinated pesticides, including atrazine, trigger breast cancer development in humans, by affecting the metabolism of estradiol. A recent study in Kentucky suggests a modest association between triazine exposure and an increased risk of breast cancer (Kettles *et al.* 1997).

In a case-control study by Donna *et al.* (1989), women with previous exposure to triazines showed a two- to threefold risk of epithelial ovarian cancer as compared to the unexposed group of women. Chronically exposed workers in chemical plants have revealed no increased incidence of benign or malignant disease

attributable to atrazine. Some case-control studies showed a slight increase of non-Hodgkin's lymphoma (NHL) incidences, while others were negative (Loosli 1995). "Weighted evidence supports no causal association of malignant changes in farming populations with atrazine" (Loosli 1995).

A feeding study on ruminants, under controlled conditions (Johnson *et al.* 1972) showed that triazine doses that were fatal when administered in capsules or as drench are tolerated without adverse reaction if they are adsorbed on fodder. These results contradict reports that attributed losses of ruminants, and occasionally horses, to grazing on pasture strips exposed to triazine spray, for example, through weed control along roadsides or drift from treated fields (Loosli 1995). Experimental toxicity trials showed cattle and sheep to be less tolerant than rodents to oral intake of triazines and other herbicides (Milhaud *et al.* 1977). One dose of 500-mg/kg simazine had a lethal effect in sheep (Hapke 1986).

Reported effects and deformities in non-mammalian vertebrates

Effects of atrazine in Fish

Fish are susceptible to pesticides dissolved in water and can be exposed to these chemicals through skin and gills and by contaminated food (Bisson *et al.* 2002). Exposure in dams and small ephemeral ponds that are situated near agricultural lands where atrazine is used probably represents the highest exposure scenario for this substance in aquatic organisms (Solomon *et al.* 2003).

Endocrine-modulating effects of atrazine in largemouth bass (*Micropterus salmoides*) have been reported (Gross *et al.* 1997; Shrestha *et al.* 1997). These studies involved both in vivo and in vitro treatments. In vitro exposure of bass ovarian tissues to atrazine (10 µg/L) increased secretion of estradiol and suggested an endocrine-modulating effect of atrazine in bass (Shrestha *et al.*

1997). In a single species test, Brook trout (*Salvelinus fontinalis*), exposed to 120 µg/L of atrazine resulted in a reduced growth rate (Dewey 1986); however, the involvement of the endocrine system in this process is unknown.

Fish gills are efficient indicators of water quality, due to their large surface area, external location, and their role in a number of essential life functions (Alazemi *et al.* 1996). Alazemi *et al.* (1996) found gill damage (collapse of secondary lamellae) in freshwater fish *Gnathonemus pertersii* at a concentration of 500 µg/L atrazine; at higher concentrations, of around about 5000 µg/L, deep pits in the gills were observed. According to Alazemi *et al.* (1996) the cause of damage most likely resulted from atrazine denaturing the epithelium of the gill filaments; thus disrupting the ion regulatory function of the gills. Similar effects on the gills of the carp (*Cyprinus carpio*), was reported by Alazemi *et al.* (1996). In a study conducted by Sanderson *et al.* (2001) the effect of the chloro-s-triazine herbicides on vitellogenin production in male carp hepatocytes led to the conclusion that none of the triazine herbicides or their metabolites induced vitellogenin production in male carp hepatocytes; nor did they antagonize the induction of vitellogenin by 100 nM (EC_{50}) 17ß-estradiol. "These findings together with other reports indicate that the estrogenic effects associated with the triazine herbicides in vivo are not estrogen receptor-mediated, but may be explained partly by their ability to induce aromatase in vitro." (Sanderson *et al.* 2001).

The effects of several agro-pesticides including atrazine on the adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) in vitro, have been studied by Bisson *et al.* (2002). A concentration response of ACTH-stimulated cortisol secretion was only observed at atrazine concentrations > 5 µM, a concentration equivalent to 1075 µg/L, also very unlikely to occur; while other metals and pesticides were much more potent than atrazine (Bisson *et al.* 2002).

Howe *et al.* (1998) found that the 96-hour LC 50_s for Channel Catfish (*Ictalurus punctatus*) were 23.8 mg/L for atrazine, 16.7 mg/L for alachlor, and 7.5 mg/L for

a 50:50 mixture of the two. The 96-hour LC 50_s for Rainbow trout (*O. mykiss*) were 20.5 mg/L for atrazine, 9.1 mg/L for alachlor and 6.5 mg/L for a 50:50 mixture of the two.

In a recent laboratory study conducted by Spanó *et al.* (2002), goldfish (*Carasius auratus*) were exposed to atrazine at concentrations of 100 and 1000 µg/L respectively for three weeks. This study showed no consistent effects on vitellogenesis, plasma concentrations of steroids or concentrations of LH in the gonad or pituitary.

Grobler-van Heerden *et al.* (1991), also remarked that during the exposure of *Tilapia sparrmanii* to atrazine, changes in behavior, e.g. decrease in activity, color changes, and a type of "coughing" were observed. The experiment where *T. sparrmanii* were exposed to different sub-lethal atrazine concentrations (active atrazine administered between 0.81 and 16.2 mg/L), indicated that atrazine definitely bio-concentrates in the blood of this fish species. Furthermore, the bio-concentration of atrazine in the blood increased with an increased exposure concentration.

Prasad *et al.* (1991) showed that atrazine at a concentration of 1.1 mg/L decreased oxygen consumption in the Tilapia (*T. mossambica*) in a time dependent manner: from 12% decrease after 7 days to 46% decrease after 90 days.

According to a study conducted by Allran *et al.* (2001), a dramatic inhibition in feeding behavior occurred in frogs exposed to 20 mg/L of atrazine; these findings support other studies in which atrazine decreased food consumption. The Bluegill (*Leponis machiochirus*) exposed to 500 µg/L in flow-through tests for 28 days were lethargic, ate poorly, and swam erratically (Macek *et al.* 1976).

Effects of atrazine in Reptiles

Reports by Gross *et al.* (1995) and Crain *et al.* (1997) suggested that endocrine-modulating effects of atrazine occur in freshwater turtles (Red eared slider, *Trachemys scriptaelegans*) and alligators (*Alligator mississippiensis*) respectively. Because sex differentiation in both of these species is temperature dependent, sex differentiation is easily manipulated in vitro. Red ear slider eggs were incubated at male production temperatures and atrazine was applied to the surface in an oil suspension. The atrazine applied in an ethanol/mineral oil suspension decreased the percentage of male offspring and altered the concentration of associated gonadal/chorioallantoic steroids (Gross *et al.* 1995).

According to Crain *et al.* (1997) atrazine treatment of alligator eggs did not alter sex ratio of offspring or the concentrations of gonadal/chorioallantoic steroids at hatching. Embryonic alligators exposed to high dosage (14 mg/kg) of atrazine caused increased aromatase activity in the gonad/adrenal complex that was characteristic of neither males nor females, thus atrazine exposure may induce endocrine alterations in embryonic alligators (Crain *et al.* 1997). The results of a study conducted by Crain *et al.* (1999), suggested that embryonic exposure to 2,4-D or atrazine did not cause significant alterations in gonadal structure or hepatic steroidogenic enzyme activity of hatchling American alligators. The absence of any noticeable effect may be due to the early developmental stage at which the alligators were exposed to the environmental chemicals.

Effects of atrazine in Amphibians

Living amphibians include three primary groups, frogs and toads (Anura), salamanders (Caudata), and caecilians (Gymnophiona), that are comprised of 4750 described species with perhaps another 500 extant species that have not been described (Sparling *et al.* 2000). Many amphibian species, especially frogs,

complete their life cycle in temporary breeding sites or shallow ponds adjacent to agricultural fields that receive pesticide application. Furthermore, the timing of the application of these agrochemicals (spring and early summer) coincides with amphibian breeding and metamorphoses (Howe *et al.* 1998). Amphibians may be especially sensitive to agrochemicals or environmental contaminants because they have a permeable, exposed epidermis (not covered by tough scales, hair, or feathers), gills and eggs that readily absorb substances (not covered by hard or leathery or calcalous shell) from the environment (Bantle *et al.* 1992). Amphibian larvae are primary consumers that feed on phytoplankton and periphyton, whereas adults act as higher-level carnivores; mainly feeding on insects and other invertebrates, which may expose them to the risk of biomagnification of persistent environmental contaminants (Howe *et al.* 1998). Ouellet *et al.* (1997) described an increase frequency of deformity among frogs, *Rana clamitans* and *R. pipiens*, living in shallow ponds exposed to agricultural pesticide runoff. Of the 853 metamorphosing anurans examined in 14 agricultural habitats, 106 (12 %) had severe degrees of ectromelia and ectrodactyly, compared to only two (0.7 %) of 271 in the 12 control sites. However they did not report concentrations of pesticides in the ponds.

According to Foote (1964), the developmental basis for inter-sexuality in amphibians involves a sexual bi-potentiality of the gonocytes and gonaducts. Early amphibian tadpoles have differentiated gonads that consist of an outer cortex and an inner medulla. These two layers originate from the coelomic epithelium at the medial aspect of the mesonephric kidney in the developing embryo and support the primordial germ cells (George *et al.* 1994). An indifferent or bisexual state occurs before genetic factors induce sex-determining antigens, peptides and hormones that differentiate the cortex into ovarian tissue or medulla into testicular tissue (Noble 1931).

A number of different environmental factors have been reported to influence sexual determination in amphibians. These factors include pH of aquatic

medium, temperature, and the presence of chemicals that might pose an insult to the gonads (Reeder *et al.* 1998). The undifferentiated gonads of amphibians are highly sensitive to steroidal compounds, and treatment of males with estrogens during embryonic development can lead to sex reversal or the formation of an ovotestis (George *et al.* 1994). A study conducted by Reeder *et al.* (1998), on cricket frogs (*Acris crepitans*), suggested that the overall prevalence of inter-sexuality from frogs collected between 1993 and 1995 was 2.6 %. "This may be consistent with the natural prevalence of inter-sexuality or may represent a prevalence altered by hormonally active environmental contaminants. Our 1994 data suggested a relationship between environmental contamination with atrazine and the prevalence of inter-sexuality in cricket frogs" (Reeder *et al.* 1998).

Allran *et al.* (2001) found no difference in hatchability of leopard frog (*R. pipiens*), wood frog (*R. sylvatica*), or American toad (*Bufo americanus*) embryos or mortality of 96-hour post-hatch larvae among atrazine treatments and controls. Atrazine also had no effect on swimming speed of *R. pipiens*. A study conducted by Birge *et al.* (1980), found that the 96-hour LC 50 for atrazine on 4 day post-hatching *R. pipiens* larvae was 7.68 mg/L; this is far less than was reported by Howe *et al.* (1998) on either early- (47.6 mg/L) or late-stage (14.5 mg/L) of *R. pipiens.* Howe *et al.* (1998) also observed abdominal edema in both *R. pipiens* and *B. americanus* larvae after 6 to 24 hour exposures to atrazine ranging from 2.8 to 23 mg/L.

The gray tree frog (*Hyla versicolor*), metamorphosis, in pools exposed to atrazine at 200 and 2000 µg/L by Diana *et al.* (2000), were 5% shorter and had a 10% lower body mass ($p < 0.001$) than those in microcosms exposed to atrazine at 0 or 20 µg/L. Larval period duration was 5% longer in the 2000 µg/L group than in the 200 µg/L group, but did not differ significantly among any other groups. This study does not contradict the assertion made by Solomon *et al.* (1996) that 20 µg/L is the no-observed-adverse-effect-level (NOEL) for atrazine in aquatic

communities. By contrast, adverse effects were seen in the microcosms exposed to atrazine at 200 and 2000 µg/L concentrations. Therefore, the no-observed-adverse-effect-level for atrazine in the experimental system used by Diana *et al.* (2000) was between 20 and 200 µg/L.

The biology of *Xenopus laevis* and effects of atrazine in *X. laevis*

One of the most widespread anuran species in the African sub-continent is the African Clawed Frog *Xenopus laevis*. It has been known to science for the past 200 years and was first described by Daudin in 1803 (Passmore *et al.* 1995). *Xenopus* belongs to a unique family of frogs, the Pipidae. The generic name *Xenopus* is derived from the Greek words "xenos", meaning strange or unusual, and "pous", for foot, while the specific species name *laevis* means smooth or slippery in Latin (Du Preez 1996). *X. laevis* has a wide distribution area within the boundaries of South Africa, occurring from the Western Cape Province northwards, excluding the extreme north of the Northern Cape Province, northern Kwazulu-Natal and the Eastern Mpumalanga Province. It occupies any permanent body of water such as ponds (farm ponds), streams, dams, rivers and water holes (Weldon 1999).

Tavera-Mendoza *et al.* (2002) conducted a study in which they exposed *X. laevis* tadpoles to atrazine at 21 µg/L for 48-hours during gonadal differentiation (stage 56), which occurs during early metamorphosis, resulting in a decrease in testicular volume. The total testicular volume decreased from 0.026 ± 0.003 mm^3 in controls to 0.01 ± 0.001 mm^3 in atrazine exposed tadpoles; this represents a 57% decrease in testicular volume. Furthermore, testicular resorption in 70% of male tadpoles, as well as aplasia was observed in developing larvae exposed to atrazine. "Atrazine may act as an endocrine disrupter at this sensitive stage in the developmental process and may subsequently significantly reduce the reproductive capacity of the organism (in this case *X. laevis*), for life – but no

evidence was found of chromophores, indicating that the pituitary was actively secreting hormones." (Tavera-Mendoza *et al.* 2002).

Hayes *et al.* (2002b) found that there was no effect on mortality, time to metamorphosis, length, or weight at metamorphosis at concentrations of up to 200 µg/L atrazine. There also was a reduction in male metamorph laryngeal muscle size at concentrations > 1 µg/L as well as testicular abnormalities at a concentration as low as 0.1 µg/L. Hayes *et al.* (2002b) suggests that this may be a result of the disruption of steroidegenesis through an increase in the activity of aromatase ($P450_{ARO}$) during early larval development. Cross-sectional measurements of the laryngeal dilator muscle in the Carr *et al.* (2003) study, revealed significant sex differences in both the reference and atrazine treatment groups, with males having a dilator muscle that was 20 to 25 % larger than those of the females. The results of Carr *et al.* (2003) differ from those presented by Hayes *et al.* (2002b) with respect to laryngeal muscle size and gonadal abnormalities. The effects of atrazine on gonadal abnormalities observed at a concentration of 25 µg/L in the study conducted by Carr *et al.* (2003) were only statistically significant, compared to the 0.1 µg/L found by Hayes *et al.* (2002b). That is more than 250-fold greater than the smallest effective dose of atrazine reported to induced gonadal abnormalities in *X. laevis* when ethanol was used as a vehicle for delivering atrazine in Holfretter's medium, rather than the usual FETAX medium (Solomon *et al.* 2002).

Work done by Tavera-Mendoza, Hayes, and other workers on *X. laevis* was conducted under laboratory conditions. The question still remained as to what the situation was in natural populations of *X. laevis* exposed to atrazine.

Due to the geographical distribution of natural *X. laevis* communities in the wild (endemic to Africa), South Africa is a suitable location in which to investigate the effect that atrazine has on *X. laevis* in its natural environment, as well as in semi-natural microcosm systems.

In designing the present study the following were hypothesized:

- Hypothesis 1 states that the concentrations of atrazine observed in natural corn growing areas where *X. laevis* breeds, will not have an effect on *X. laevis* populations.
- Hypothesis 2 states that gonadal anomalies might be expected on *X. laevis* frogs at atrazine concentrations of at least 25 µg/L in semi-natural microcosms.

Study Objectives

The main objectives of this study include the following:

- To determine whether there are any differences in the sex ratios, age, and size classes of *X. laevis* in the reference and exposed sites selected in the Potchefstroom and Viljoenskroon area, South Africa.
- To characterize the exposure of populations of *X. laevis* larvae, metamorphs and adults to atrazine and related triazines in surface waters in reference (no corn production or atrazine/triazine use) and exposed habitats in the proximity of corn production and atrazine/triazine use in the Potchefstroom and Viljoenskroon area of South Africa.
- To determine if exposure to triazines used in corn production increases the incidence or extent of morphological changes in the gonads or intersex gonads in the male and female metamorphs and adults of *X. laevis*.
- To determine whether there are changes in the morphology of the male larynx in metamorphs and adults of *X. laevis* in response to exposures to triazines used in corn production.
- To determine if there are any changes in plasma steroid concentrations of adult *X. laevis* in response to exposures to triazines used in corn production.
- To determine if other frogs present at the sites show any morphological or other changes in the gonads.

- To assess feasibility of determining if *X. laevis* in the exposed sites have been subjected to selection pressure due to atrazine.
- To determine if different concentrations of atrazine (0, 1, 10, 25 µg/L) have any effect on the development of *X. laevis* tadpoles in a semi-natural microcosm study.

This study was designed using a phased approach. During phase one, a feasibility study for site characterization and assessment of histological profile of frogs was conducted. Phase one is represented by Chapter 2 in this document. In phase two, surface water analysis for atrazine and triazine residues were conducted at the specific localities that were identified during phase one. Phase two is represented by Chapter 3 in this document. During phase three, *X. laevis* frogs were collected at each of the reference and experimental sites as indicated. These frogs were then used for histological and hormone analysis, to determine if the atrazine levels found in their nature environment had any affect on them. Although this phase forms an integral and important part of the study at large, it was not included in this thesis as the candidate was not involved with the further processing and analyzing of the data. This phase is represented in Appendix 3. Phase four was conducted by using different concentration levels of atrazine in semi-natural microcosms to determine if atrazine had any affect on *X. laevis* frogs. Chapter 4 represents this part of the study.

The **Introduction and Literature overview** (Chapter 1) is followed by Chapter 2 that deals with the **Feasibility Study for Site Characterization and Assessment of Histological Profile of Frogs**; Chapter 3, **Surface water analysis for Atrazine and Triazine residues**; Chapter 4, **Gonadal Response of *Xenopus laevis* Larvae Exposed to Atrazine Microcosms.** The implications from Chapter 2 to 4 are considered in the **General Discussion** (Chapter 5). The

thesis is concluded with a **Summary** (Chapter 6), after which the **References** follow. Posters and published papers that have been presented as well as data sheets have been added as **Appendices**.

CHAPTER 2

CHAPTER 2

FEASIBILITY STUDY: SITE SELECTION AND *Xenopus laevis* POPULATION PROFILE

2.1 Introduction

The impact of herbicides on aquatic life has raised much concern because of the environmental and health hazards of such chemicals. The latest trend in agriculture has been to avoid persistent pesticides that may produce detrimental ecological effects. For many years long-lived, highly chlorinated pesticides were used in agriculture and human health protection, but they were very persistent in the ecosystem (Klaassen *et al.* 1979). Many also exhibit the potential of biological accumulation and magnification with a chain of undesirable effects. Newer pesticides that have replaced the older ones are less persistent; and apparently less toxic to non-target organisms. However, they may eventually be transported into aquatic ecosystems. There is little information on the distribution and persistence of most agrochemicals in aquatic ecosystems under field conditions.

Atrazine is one of the most heavily used herbicides, and shows good results in controlling target broad leaf weeds in agriculture (Shabana 1987). It is an extremely valuable herbicide because of its remarkable selectivity for several crop plants, including corn (*Zea mays*), sorghum (*Sorghum bicolor*), and sugarcane (*Saccharum officinarum*), which are resistant because they are able to convert the herbicide to non-toxic metabolites very rapidly (Forney *et al.* 1981). Corn is a staple food in Africa and is grown on very large scale in South Africa. It is an important export product of South Africa, not only to other African countries, but to other world countries as well. The majority of corn is grown in the Free State, North West and Gauteng Provinces of South Africa. Atrazine, in various

combinations with other herbicides, has been used as an herbicide in South Africa since 1962. Compared to other herbicides, atrazine is very affordable and remains a very popular choice among farmers. The use of atrazine, and its movement (volatilization, leaching, runoff, etc.) from their target area, may lead to potential contamination of any water-body in the catchment area. Therefore, it is important to assess the adverse impact of any chemical (Gotvajn *et al.* 2001), in this case atrazine, on non-target organisms in the aquatic environment.

The African Clawed frog (*X. laevis*) has been used as a laboratory test animal since the 1930's. The reason why this species is being used is because of its resistance against disease and ease of maintaining large numbers in the laboratory.

X. laevis is endemic to Africa where 17 species are currently known (Tinsley *et al.* 1996). The African Clawed Frog, *X. laevis* is a true aquatic frog found throughout Southern Africa. This species of frog is fully aquatic (all life stages are aquatic), occupy almost every kind of permanent water, including pools, dams, rivers, streams, and waterholes (Du Preez 1996). The study area for this study was selected in the center of the corn production area namely the Viljoenskroon – Potchefstroom area.

2.2 Materials and Methods

2.2.1 Experimental and Reference Sites

2.2.1.1 Selection of sites

The experimental field exposed sites were selected based on the presence of *X. laevis*, relative proximity to areas of corn production as well as previous and planned use of atrazine and terbuthylazine. The selection of reference sites has been selected on the presence of *X. laevis*, lack of corn production and absence of measurable amounts of atrazine and terbuthylazine in the water. Eight reference sites and eight experimental sites were initially identified and evaluated. At each site a 2-L water sample was taken to be analyzed for atrazine concentration. These analyses were conducted at the Potchefstroom University, School of Environmental Sciences and Development by Mr. Peet Jansen Van Rensburg. Aliquot samples were shipped to Dr. Robert Yokley at Syngenta for confirmation.

2.2.2 Biological Sampling and Histological Profile

2.2.2.1 Collecting and transporting of frogs

The method for collecting post-metamorphic *X. laevis* is based on the aquatic nature and feeding behavior of the frog. Frogs were collected in 25-L bucket traps, baited with raw liver (100 g) and raw soup bones (3 bones). *X. laevis* rely strongly on their olfactory sense for locating food. Eight to ten traps (Fig. 2.1) were set per locality in shallow water. Traps were placed upside down with 50 – 100 mm of the trap protruding above the water surface, allowing frogs to breathe through air holes drilled into the bottom of each bucket. Traps were covered with

vegetation to prevent overheating of the traps. The traps were inspected after 48 hours and frogs removed.

The captured frogs were then placed in labeled buckets containing water from the specific locality and transported to the laboratory at the Potchefstroom University. Buckets with frogs were kept in the shade at all times.

Figure 2.1: Baited *Xenopus* trap.

2.2.2.2 Weighing and measuring of frogs

Frogs are often weighed in a plastic bag hanging from a spring balance. This method is not suitable for *X. laevis*, as the frogs tend to jump around in the plastic bag. The frogs become stressed, secreting a milky substance from the skin known as xenoxins (Kreil 1996). Frogs were weighed in an empty 600 ml plastic bottle on an electronic Sartorius BP210S scale (0.01 g accuracy) (Fig. 2.2).

The snout-vent length of the frogs were measured by means of a high quality Teflon Vernier Caliper (accuracy 0.1mm).

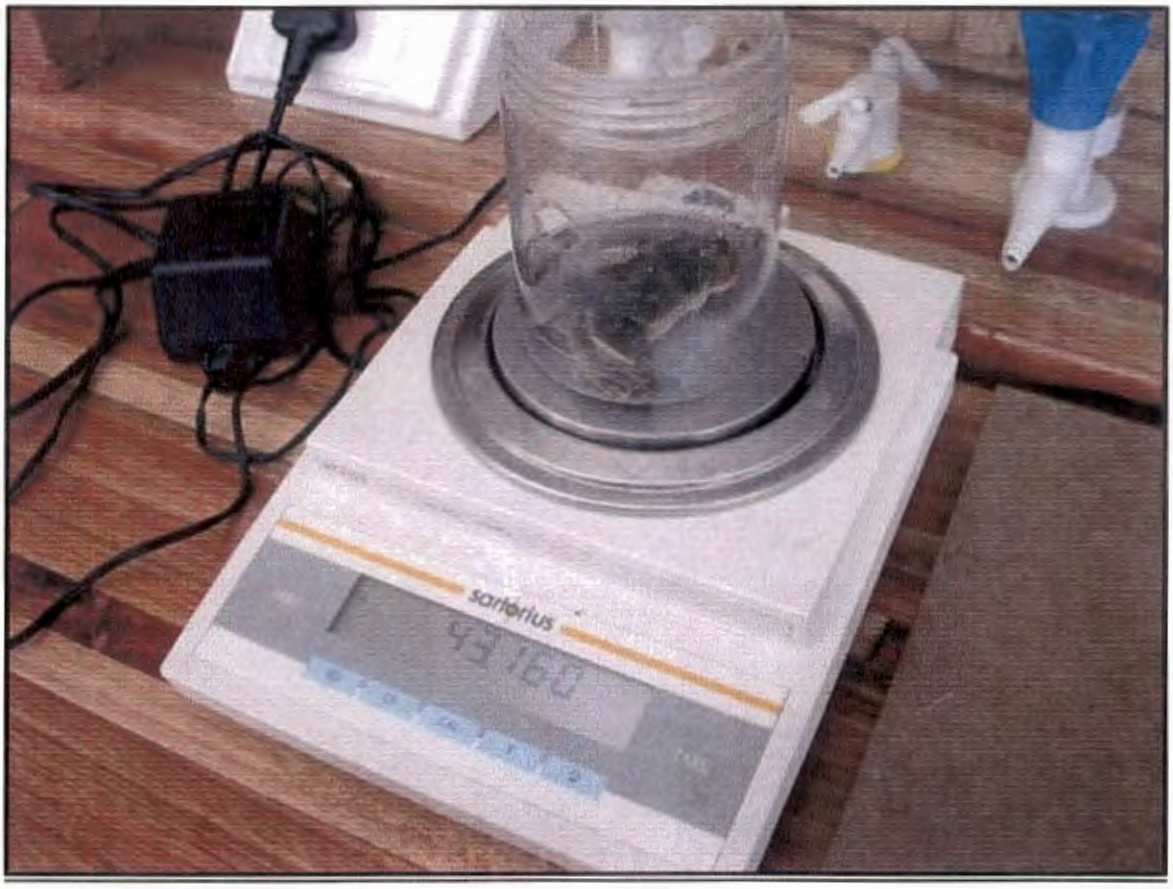

Figure 2.2: Weighing of frogs on the Sartorius BP210S scale.

2.2.2.3 Mark and recapture

The methodology of mark and recapture is based on the basic principle that by repeatedly catching, marking and releasing individuals from a specific locality, the population size can be estimated through the integration of data. The mark and recapture technique has been in use since the 1920's (Woodbury 1956). Herpetologists routinely use this technique to study amphibian species. Estimates of population size in the experimental and reference sites were determined using a modified version of the Jolly-Seber Stochastic Method (Donnely *et al.* 1994).

First the number of marked individuals at risk on day *i* (M*i*) was estimated using the equation:

$$M_i = m_i + \frac{z_i r_i}{y_i}$$

Where: m_i = the number of marked animals caught on day *i*.

r_i = the number of marked animals released on day *i*.

y_i = the number of animals marked and released on day *i* and caught after day *i*.

z_i = the number of animals marked before day *i* that are not caught on day *i* but are caught after day *i*.

Population size (N_i) was estimated as follows:

$$N_i = \frac{M_i (n_i + 1)}{(m_i + 1)}$$

Where: n_i = the number of animals caught on day *i*.

The estimations of survival rate ($\varnothing_i$) and gains (g_i) are given by the equations:

$$\varnothing_i = \frac{M_i + 1}{(M_i - m_i + r_i)} \qquad \text{and} \qquad g_i = N_{i+1} - \varnothing_i N_i$$

Standard error for estimate population size was calculated as follows:

$$SE_{Ni} = \{N_i(N_i - n_i)[\frac{M_i - m_i + r_i}{M_i} \frac{1}{(y_i - r_i)} \frac{1}{+ m_i} \frac{1}{- n_i}]\}^{\frac{1}{2}}$$

A total of a hundred frogs were caught at every site and, by means of the mark and recapture method, the population size of every site was determined.

In addition to estimate population size, information concerning community structure (sex ratio and body measurements) was gathered during the mark and recapture procedure.

2.2.2.4 Farming history in the vicinity of selected sites

All farmers in the catchment area of sites were visited and interviewed to establish the history of pesticide use over the past few years, as well as an indication of crops planted and tillage practices used. This estimation helped to determine in more detail the farming history of the catchment area of each experimental site.

2.2.2.5 Cryo-branding of frogs

A set of branding irons made from 2 mm bronze wire was used for numbering the frogs. The branding irons were cooled in liquid nitrogen. A number corresponding to the site was branded onto the ventral surface posterior to the sternum (Daugherty 1976) (Fig. 2.3). Young frogs are difficult to handle and were

temporarily anaesthetized in MS-222 (3–amino benzoic acid ethyl ester) before branding.

Figure 2.3: A female *X. laevis* captured twice in site number 2. Note that the second "2" was branded upside down to indicate the second capture event.

2.2.2.6 Sexing of frogs in reference and experimental sites

Females were identified through distinct swollen labial folds on either side of the vent. Males were identified through dark colouration on the palms of the forelimbs, which can be observed during the mating season and by the lack of swollen labia folds (Weldon 1999). The use of morphological characteristics to

distinguish sexes is not reliable for individuals smaller than 30 mm and was therefore not attempted.

2.2.2.7 Toe clipping of frogs

The last two digits of the longest toe on one foot were removed for skeletochronology in order to establish the age of frogs. The frog was placed on its ventral side and kept secure with the hand. The left foot was stretched out and with a firm action of a disinfected scalpel the toe was cut between phalanges two and three. The toe was then placed in labeled vial containing Bouin's fixative (Hemelaar *et al.* 1980). The frog was then placed back in water directly after toe clipping. After the toes had been clipped, frogs were observed for 24 hours to ensure that no infection occurred, whereafter frogs were released at the site where they were captured.

2.2.2.8 Skeletochronology

When frogs hibernate in winter, bone cells that are formed are more compact. Over years this creates a set of growth rings similar to those of a tree. By sectioning laterally through a toe it is possible to establish the age of the frog without killing the specimen. This technique is referred to as skeletochronology. Toes fixed in Bouin's fixative were rinsed in water, transferred to 70 % ethyl alcohol and dehydrated in an alcohol series, decalcified in Perrenyies solution, cleared in xylene and embedded in wax using a Slee Embedding Center. Histological sectioning were cut at 6 µm, stained with Heamatoxalin (Gill's Heamatoxalin), counterstained with Eosin and permanently mounted using DPX mounting medium (Humason 1978). Slides were examined under a compound microscope; the first ring around the bone cavity is the endostyle and was not counted. Starting from the endostyle, the growth rings were counted revealing the age of the specific specimen.

2.3 RESULTS

2.3.1 Physical Properties of Reference and Experimental sites

Potential reference and experimental sites were identified on 1:50 000 topographic maps. Eight potential reference and eight potential experimental sites were identified.

2.3.1.1 Water analysis

Atrazine concentrations measured for various sites by Potchefstroom University and Syngenta are given in Tables 2.1 and 2.2. Unfortunately some of the water samples shipped to Syngenta for atrazine analysis broke and could not be analyzed. Results of analyses that were conducted are given in Tables 2.1 and 2.2.

Table 2.1: Atrazine analyses conducted on water samples collected at the reference sites.

Site	Potch Value (µg/L)	Syngenta Value (µg/L)
R1	<0.10	Broken
R2	0.15	0.13
R3	<0.10	<0.10
R4	0.11	Not analyzed
R5	<0.10	Not analyzed
R6	<0.10	Not analyzed
R7	<0.10	Not analyzed
R8	<0.10	Not analyzed

Table 2.2: Atrazine analyses conducted on water samples collected at the experimental sites.

Site	Potch Value (µg/L)	Syngenta Value (µg/L)
E1	1.23	0.96
E2	<0.10	<0.10
E3	0.32	0.19
E4	0.12	Broken
E5	<0.10	<0.10
E6	0.68	1.1
E7	0.51	Not analyzed
E8	0.84	Not analyzed

After the *X. laevis* population size and specific atrazine concentrations of the evaluated site were determined, three reference sites (R1, R3, and R6) (Fig. 2.4) and five experimental sites (E1, E3, E4, E6, and E8) (Fig. 2.5) were selected that fitted the profile.

2.3.2 Physical Properties of the Selected Reference and Experimental sites

Physical properties of the reference, as well as the experimental sites are presented in Tables 2.3 to 2.10. This includes more detailed descriptions of properties, including; grid reference, surface area, watershed area, deepest point, depth at reference point, Secci depth and an indication of the aquatic and surrounding vegetation.

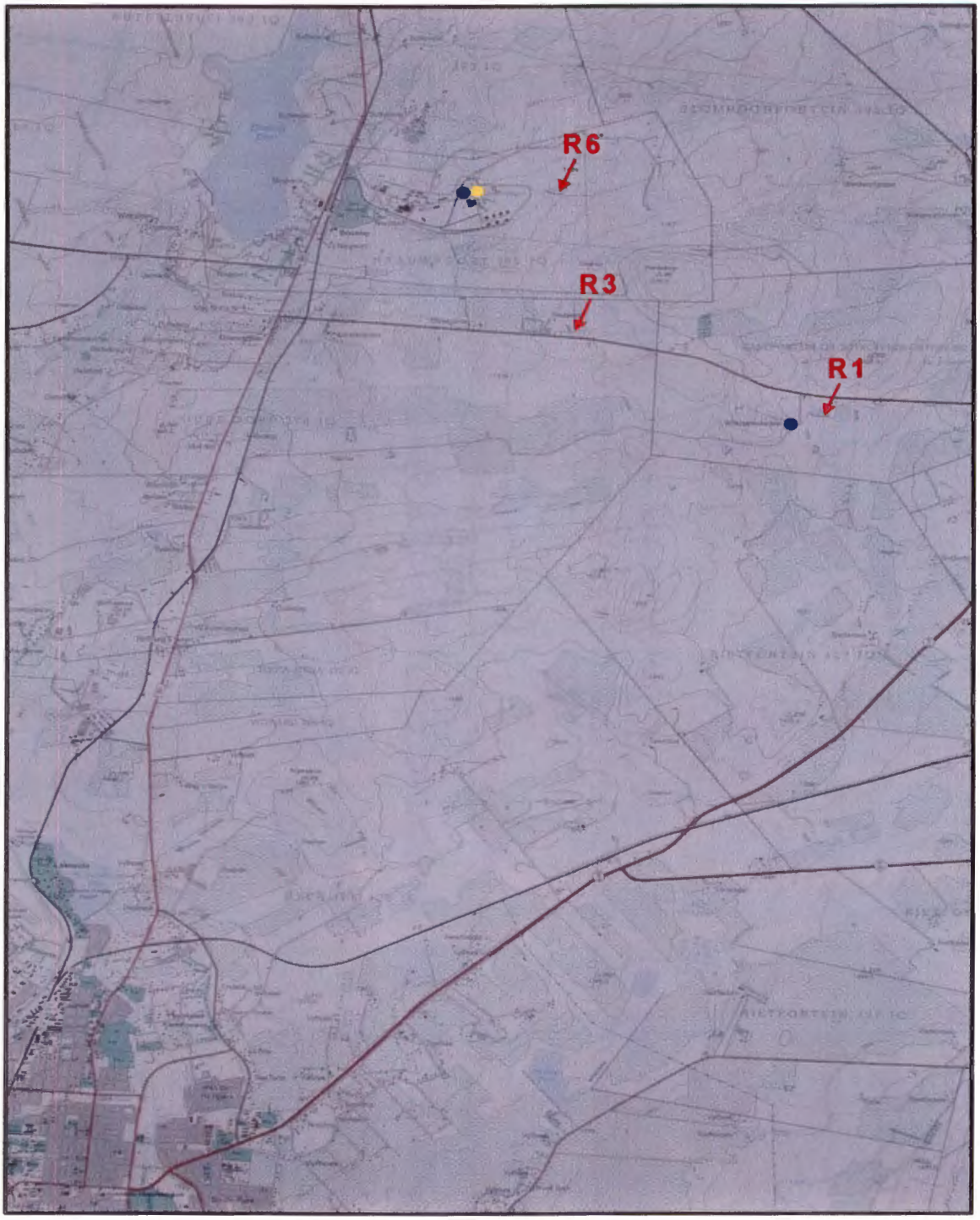

Figure 2.4: Map of the Potchefstroom area showing sites evaluated as reference sites. Blue dots indicate stations where rainfall data were obtained while the yellow dot indicates the position of the Agricultural Research Council weather station where detailed weather data capture was taken on a daily basis.

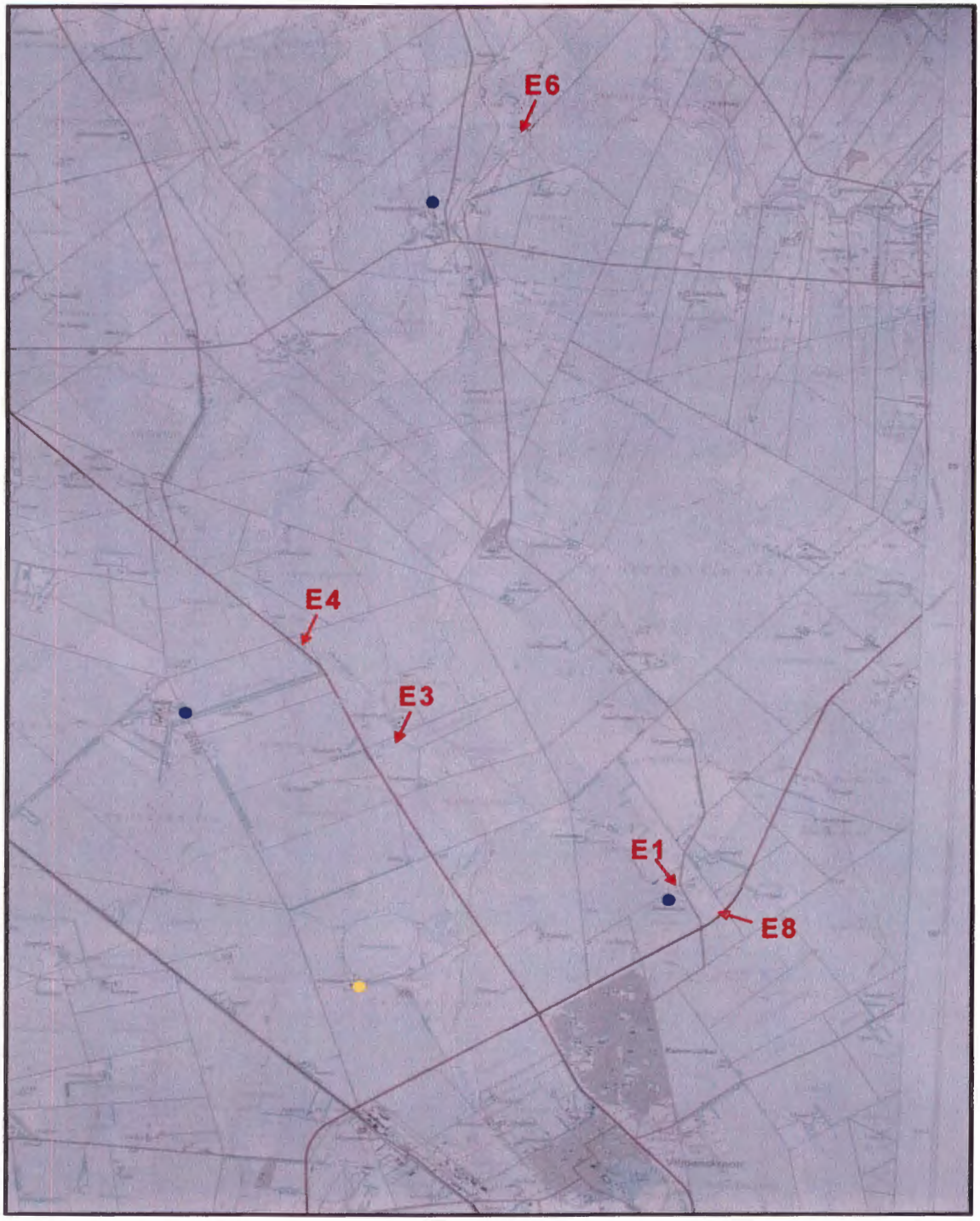

Figure 2.5: Map of the Viljoenskroon area showing sites evaluated as experimental sites. Blue dots indicate stations where rainfall data were obtained while the yellow dot indicates the position of the Agricultural Research Council weather station where detailed weather data capture was taken on a daily basis.

Table 2.3: Physical properties of reference site R1.

R1	
	
Grid reference	26°35'40"S 27°11'47"E
Surface area (Sept 2001)	20,500 m^2
Watershed area	244 ha
Deepest point	261 cm
Source of water	Rainfall + fountain feeding into dam
Reference point	27.5 cm
Secci depth	11.5 cm
Aquatic vegetation	*Paspalum* sp. Along ± 10% periphery. *Juncus excertus*. Isolated patches along periphery. ± 4% of periphery. *Aponogeton junceus*. Isolated patches along periphery. ± 1% of periphery.
Surrounding vegetation	Wooded thorn-field. Natural vegetation. No crops in catchment. Cattle farm.

Table 2.4: Physical properties of reference site R3.

R3	
	
Grid reference	26°35'01"S 27°10'09"E
Surface area (Sept 2001)	2,000 m^2
Watershed area	170 ha
Deepest point	38.5 cm
Source of water	Rainfall + fountain feeding into dam
Reference point	20 cm
Secci depth	32 cm
Aquatic vegetation	*Juncus* sp. Cover about 90% of water body. *Paspalum* sp. Along periphery.
Surrounding vegetation	Wooded thorn-field. Natural vegetation. No crops in catchment. Cattle farm.

Table 2.5: Physical properties of reference site R6.

R6	
	
Grid reference	26°33'41"S 27°09'35"E
Surface area (Sept 2001)	14860 m^2
Watershed area	480 ha
Deepest point	104 cm
Source of water	Rainfall
Reference point	9.5 cm
Secci depth	6.5 cm
Aquatic vegetation	*Juncus* sp. Cover about 90% of water body. *Paspalum* sp. Along periphery.
Surrounding vegetation	Wooded thorn-field and open grassland. Natural vegetation. No crops in catchment. Security buffer zone outside explosive factory.

Table 2.6: Physical properties of experimental site E1.

E1	
	
Grid reference	27°09'42"S 26°57'42"E
Surface area (Sept 2001)	7,406 m^2
Watershed area	1990 ha
Deepest point	118 cm
Source of water	Rainfall
Reference point	26.5 cm
Secci depth	Light penetrates to bottom.
Aquatic vegetation	*Juncus* sp. Isolated patches along periphery. ± 4% of periphery *Potamogeton pusillus.* Cover bottom of dam. *Chara* sp. Abundant floating in water.
Surrounding vegetation	10-50 m natural field to cornfields.

Table 2.7: Physical properties of experimental site E3.

E3	
	
Grid reference	27°08'06"S 26°54'45"E
Surface area (Sept 2001)	46,076 m^2
Watershed area	1 046 ha
Deepest point	133 cm
Source of water	Rainfall
Reference point	26.5 cm
Secci depth	Light penetrates to bottom.
Aquatic vegetation	*Juncus* sp. Thick stands in flooded area and along 90% of periphery. *Potamogeton capensis*. Cover bottom of dam. *Typha capensis*. Isolated patches along 4% of periphery.
Surrounding vegetation	30-100 m natural field to cornfields.

Table 2.8: Physical properties of experimental site E4.

E4	
	
Grid reference	27°06'45"S 26°53'35"E
Surface area (Sept 2001)	2,400 m^2
Watershed area	448 ha
Deepest point	44 cm
Source of water	Rainfall
Reference point	23 cm
Secci depth	Light penetrates to bottom.
Aquatic vegetation	*Persicaria salicifolium.* Isolated patches along ± 8% of periphery. *Chara* sp. Floating in water. *Lagarosiphon muscoides.* Floating in water.
Surrounding vegetation	80 m natural field to cornfields.

Table 2.9: Physical properties of experimental site E6.

E6	
	
Grid reference	27°01'49"S 26°55'35"E
Surface area (Sept 2001)	68,722 m^2
Watershed area	515 ha
Deepest point	370 cm
Source of water	Rainfall + fountain feeding into dam
Reference point	39.5 cm
Secci depth	207 cm
Aquatic vegetation	*Juncus* excertus. Along 80% of periphery. *Persicaria salicifolium*. Along ± 5% of periphery. *Potamogeton pusillus*. Covers most of bottom of dam. Even in deep waters. *Kniphofia ensifolia* Isolated patches. *Ceratophylum demersum* dense areas floating in shallow water.
Surrounding vegetation	30-50 m natural field to cornfields. *Eucalyptus* and willow trees along dam

Table 2.10: Physical properties of experimental site E8.

E8	
	
Grid reference	27°09'49"S 26°58'11"E
Surface area (Sept 2001)	2,400 m^2
Watershed area	1 100 ha
Deepest point	175 cm
Source of water	Rainfall
Reference point	33 cm
Secci depth	Light penetrates to bottom.
Aquatic vegetation	*Juncus* sp. Thick stands along periphery. *Eleocharus palustris*. Short grass covering bottom of dam. *Chara* sp. Floating in parts of dam. Filament algae in some areas of dam.
Surrounding vegetation	± 100 m natural field to cornfields.

2.3.3 Farming History in the Vicinity of Selected Sites

2.3.3.1 Crops planted over the last several years

The overview of the survey conducted to establish crops planted, pesticide usage and tillage practices revealed the following; No crops were planted and thus no herbicides applied in the catchment area of reference sites. Crops planted from 1996 up to 2002 include corn, wheat, sunflower, groundnuts and sorghum (Table 2.11). Corn was the most dominant crop, with ± 80% of the surface planted, followed by wheat, and sunflowers. During the season 2001 - 2002, the major crop planted in the catchment area of site E1 was corn (95.3% in 2001, and 99% in 2002); at site E3, corn (96% in 2001, and 88% in 2002); at site E4, corn (88% in 2001, and 73% in 2002); at site E6, corn (60% in 2001, as well as in 2002); and site E8, corn (95.3% in 2001, and 99% in 2002).

This shows that there is a definite pattern in crop selection at every experimental site catchment area over the past five years. From this pattern it is likely that the agro-chemicals used during the year at every site will be the same.

Table 2.11: Estimated percentage of each crop planted in the agricultural area of each experimental site.

E1					
Year	**Corn**	**Wheat**	**Sunflower**	**Groundnuts**	**Sorghum**
1996	98	0	0	2	0
1997	96	0	0	2	2
1998	98	0	0	2	0
1999	96	0	0	2	2
2000	97	0	1	2	0
2001	95.3	0.2	0.5	2	2
2002	99	0.5	0	0.5	0

Table 2.11: (continue).

E3					
Year	**Corn**	**Wheat**	**Sunflower**	**Groundnuts**	**Sorghum**
1996	96	0	1	3	0
1997	96	0	1	3	0
1998	96	0	1	3	0
1999	96	0	1	3	0
2000	96	0	1	3	0
2001	96	0	1	3	0
2002	88	10	2	0	0

E4					
Year	**Corn**	**Wheat**	**Sunflower**	**Groundnuts**	**Sorghum**
1996	98	0	1	1	0
1997	98	0	1	1	0
1998	98	0	1	1	0
1999	98	0	1	1	0
2000	95	3	1	1	0
2001	88	10	1	1	0
2002	73	25	2	0	0

E6					
Year	**Corn**	**Wheat**	**Sunflower**	**Groundnuts**	**Sorghum**
1996	100	0	0	0	0
1997	95	0	5	0	0
1998	80	0	20	0	0
1999	65	0	35	0	0
2000	58	0	40	2	0
2001	60	0	30	10	0
2002	60	5	25	10	0

Table 2.11: (continue).

E8					
Year	**Corn**	**Wheat**	**Sunflower**	**Groundnuts**	**Sorghum**
1996	98	0	0	2	0
1997	96	0	0	2	2
1998	98	0	0	2	0
1999	96	0	0	2	2
2000	97	0	1	2	0
2001	95.3	0.2	0.5	2	2
2002	99	0.5	0	0.5	0

2.3.4 Tillage Practices

Farmers do not plough in the Viljoenskroon area, but practice mutch tillage whereby plant residues are left on the surface. Farmers rip deep (700 – 900 mm) in previous plant rows (ridge tillage). This practice is followed as this area is known for sand-storms early in season that damage the young plants.

Tillage practices on the cornfields annually follow the following program:

- September – October — Rip deep. Ridge tillage.
- 1 November – 4 December — Plant corn. Apply herbicides and insecticides.
- 30 December – January — Weeding.
- January — Apply herbicides and insecticides.
- June — Harvest crop.
- August — Plough or let cattle pick up leftovers.

2.3.5 Sampling of *X. laevis* Populations

2.3.5.1 Number, sex ratio and population estimates of *X. laevis* collected

The biological sampling of the frogs was conducted by means of baited traps, as discussed in Materials and Methods. The number and sex ratio of the *X. laevis* collected (Table 2.12) ranged from as low as 22 males collected up to 61 females caught at once, at one specific site. All of this chapter's raw data are given in Appendix 4.

Table 2.12: Number and sex ratio of *X. laevis* collected. (J=Juvenile, M=Male, F=Female).

	J/M/F	R1	R3	R6	E1	E3	E4	E6	E8
Number of frogs	**J**	0	3	1	4	0	3	1	6
	M	39	52	41	51	46	22	34	45
	F	61	45	58	45	54	43	65	49

At every site, except sites R3 and E1, there were more females collected during this survey (Table 2.12 & Fig. 2.6). The percentage of males collected ranged from 34% for sites E4 and E6 to 54% for site R3. A chi-square test showed that the proportions of female and male *X. laevis* at each site was not the same ($p = 0.03$), however, a two-sided two-sample t-test (assuming equal variances) does not reject ($p = 0.67$) the null hypothesis that the mean of the percentages of female at reference sites equals the mean of the percentages of males at the experimental sites.

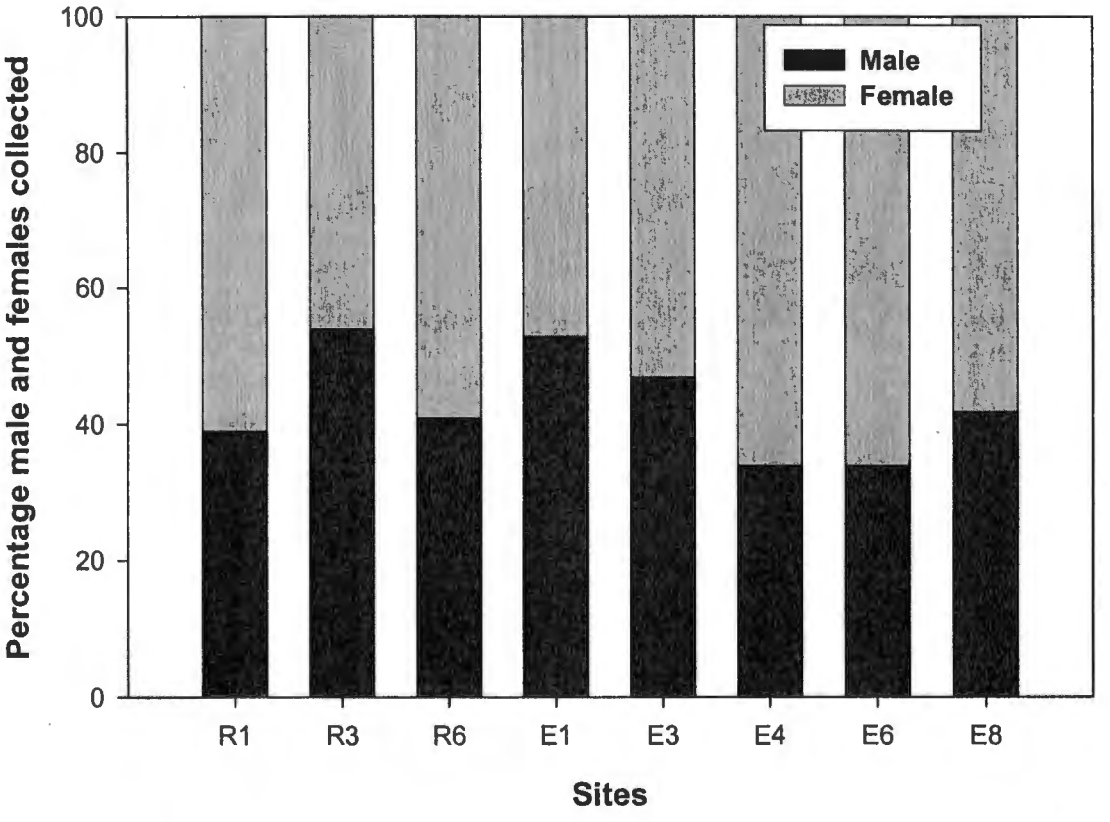

Figure 2.6: Histogram showing the percentage of male and female frogs at each of the chosen sites.

The estimated population size of the reference sites range between 360 (R3) and 950 (R6), while the experimental sites have a range between 216 (E8) and 1218 (E3) (Table 2.13). At all the selected sites, *X. laevis* were breeding and had sustainable populations. Looking at the total number of frogs collected, most frogs were collected from sites R6 (621) and E3 (620), with least collected from sites E4 (84) and E8 (96).

Table 2.13: The results of the mark and recaptured of frogs, with estimated population size.

Site	Collection no.	Number of frogs collected	Recapture 1	Recapture 2	Recapture 3	Recapture 4	Estimated population size
R1	1	86					
	2	115	19				**713**
	3	88	6	2			
R3	1	73					
	2	88	38				**360**
	3	68	3	27			
R6	1	181					
	2	24.5	64				**950**
	3	195	26	61			
E1	1	64					
	2	25	9				**370**
	3	16	3	1			
	4	13	2	0	1	0	

Table 2.13: (continue).

Site	Collection no.	Number of frogs collected	Recapture 1	Recapture 2	Recapture 3	Recapture 4	Estimated population size
E3	1	163					**1218**
	2	235	38				
	3	222	13	59			
	4	9	1	4	1	0	
E4	1	13					**354**
	2	34	1				
	3	14	0	7			
	4	9	1	4	1		
	5	14	1	0	1	0	
E6	1	29					**909**
	2	18	2				
	3	10	0	0			
	4	65	1	0	0		
	5	73	0	1	0	8	

Table 2.13: (continue).

Site	Collection no.	Number of frogs collected	Recapture 1	Recapture 2	Recapture 3	Recapture 4	Estimated population size
E8	1	35					
	2	14	2				
	3	5	0	0			
	4	15	2	0	1		**216**
	5	27	3	1	0	4	

2.3.5.2 Mass of frogs collected

The mass of the juvenile frogs that were collected was never more than 7.3 grams and never less than 2.1 g (Table 2.14 & Fig. 2.7). The mean weight of the males collected ranged between 14.5 and 29.3 g. The females on the other hand, were mutch larger with a maximum mean mass of 45.0 g and a minimum mean mass of 20.8 g. Because the variances of the weights of the frogs differed significantly at the different sites, the null hypothesis that the mean weight at a site was the same for all sites was tested using a Kruskal-Wallis Test (which is the same as a Wilcoxon Rank-Sum test when there are only two groups). The null hypothesis was rejected in females and males at 5% significance level ($p < 0.0001$ and $p < 0.0001$, respectively). The mean of the mean weights of female frogs at reference and experimental sites were significantly different at 5% significance level (using two-sided two-sample t-test assuming equal variances, $p = 0.02$) but those in the males were not ($p = 0.24$). Since the weight of females is dependent on the state of ovarian development (which could not be determined without sacrificing the animals), this result was not surprising.

Table 2.14: Mass of frogs collected at the chosen sites.

		R1	R3	R6	E1	E3	E4	E6	E8
Mass of juveniles	Mean	-	3.8	6.4	4.2	-	4.0	6.8	6.0
	Min	-	3.3	-	2.1	-	3.6	-	3.6
	Max	-	4.7	-	6.3	-	4.3	-	7.3
Mass of males	Mean	18.6	17.3	29.3	14.5	15.4	21.1	18.5	18.1
	Min	7.4	6.9	7.1	5.3	7.3	14.2	9.9	9.2
	Max	41.7	65.2	70.0	49.0	49.1	43.8	35.8	41.7
Mass of females	Mean	45.0	34.9	37.6	20.8	29.6	33.8	28.6	26.5
	Min	7.6	6.1	6.3	7.3	5.7	14.3	9.0	8.4
	Max	204.5	117.8	142.5	124.8	134.0	120	95.6	70.6

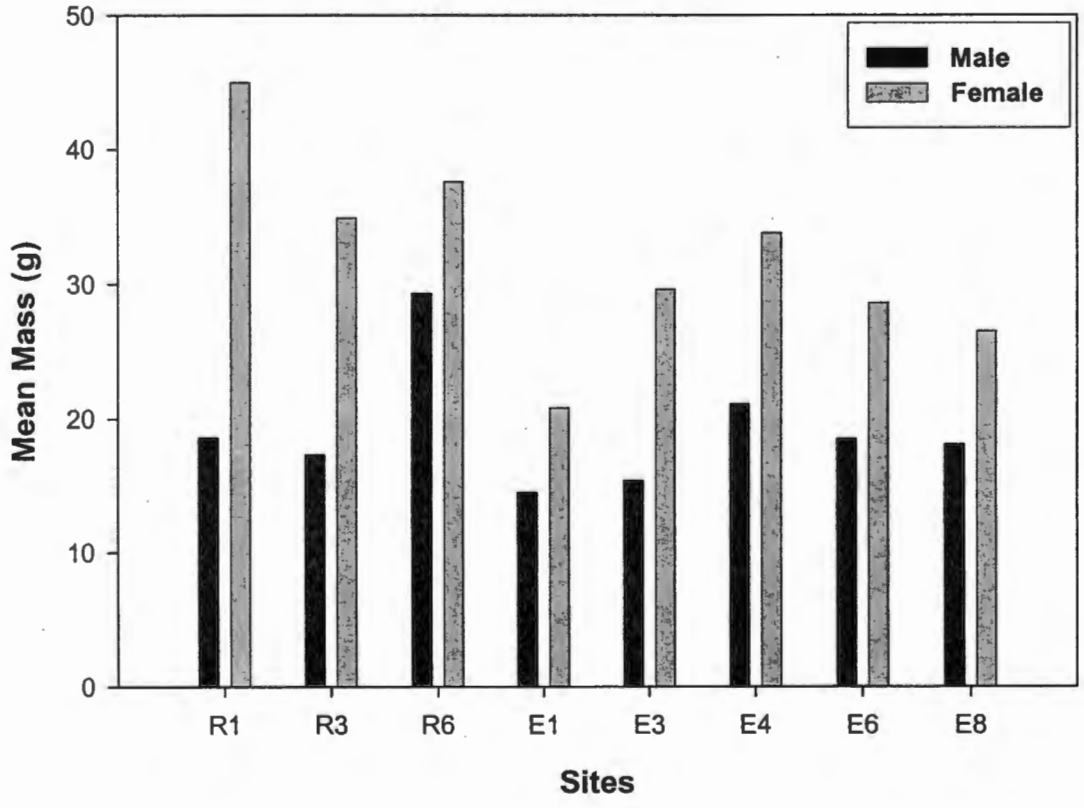

Figure 2.7: Histogram showing the mean average mass of both the male and female *X. laevis* collected at the chosen sites.

2.3.5.3 Snout-vent length of frogs collected

X. laevis males are smaller than females as reflected by measured snout-vent length (Table 2.15). The snout-vent length of the females was greater than that of the males, ranging from a minimum mean of 57.1 mm to a maximum mean of 71.6 mm, comparing this with the minimum mean of 50.3 mm and a maximum mean of 62.8 mm of males. The null hypothesis that the mean snout-vent length for females and males at a site was the same for all sites was rejected using a Kruskal-Wallis Test ($p < 0.0001$ and $p < 0.0001$, respectively). The mean of the mean snout-vent lengths of frogs at reference sites were not significantly different (two-sided two-sample t-test, assuming equal variances, $p = 0.07$ for females and $p = 0.25$ for males).

Table 2.15: Snout-vent length of frogs collected.

		R1	R3	R6	E1	E3	E4	E6	E8
S v L of juveniles	**Mean**	-	32.3	37.5	33.8	-	32.7	37.7	36.3
	Min	-	30.2	-	27.9	-	32.2	-	32.1
	Max	-	35.0	-	40.0	-	33.6	-	40.4
S v L of males	**Mean**	55.7	52.8	62.8	50.3	50.8	57.9	54.3	54.3
	Min	41.5	39.5	38.9	35.6	39.5	50.4	41.1	40.5
	Max	73.1	85.6	84.7	79.6	75.8	75.3	69.9	75.7
S v L of females	**Mean**	71.6	65.8	67.9	57.1	60.6	68.7	63.1	62.0
	Min	33.9	38.8	38.3	39.2	36.4	51.2	42.5	41.4
	Max	125.8	105.2	113.1	105.2	105.1	108.1	99.3	90.0

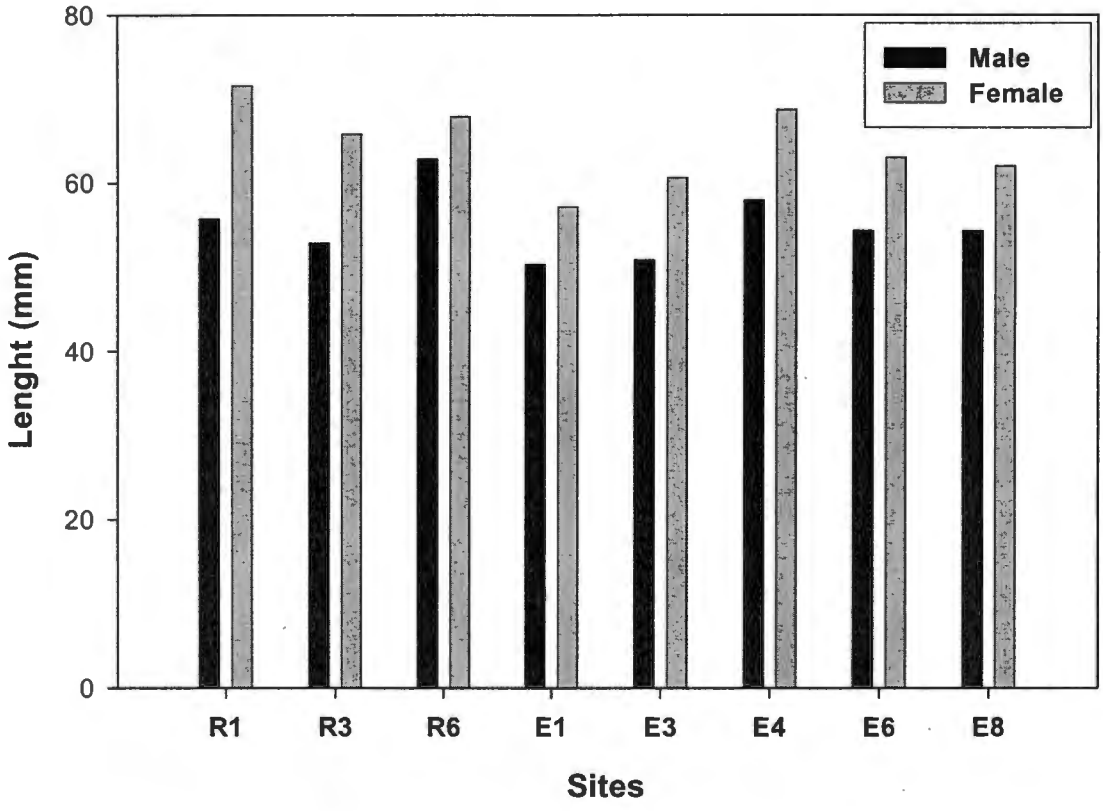

Figure 2.8: Histogram showing the average snout-vent length of both male and female *X. laevis* collected at the chosen sites.

2.3.6 Age profile of Frogs Collected

The interpretation of the skeletochronology sections turned out to be very complicated. While some sections indicated clear growth rings, the majority had very faint or even no rings making the interpretation very difficult. In the end only those in which the growth lines could be determined with certainty were included in the study. Fig. 2.9 and 2.10 shows photomicrographs of histological section through the toes of a one year-old and a six year-old frogs respectively.

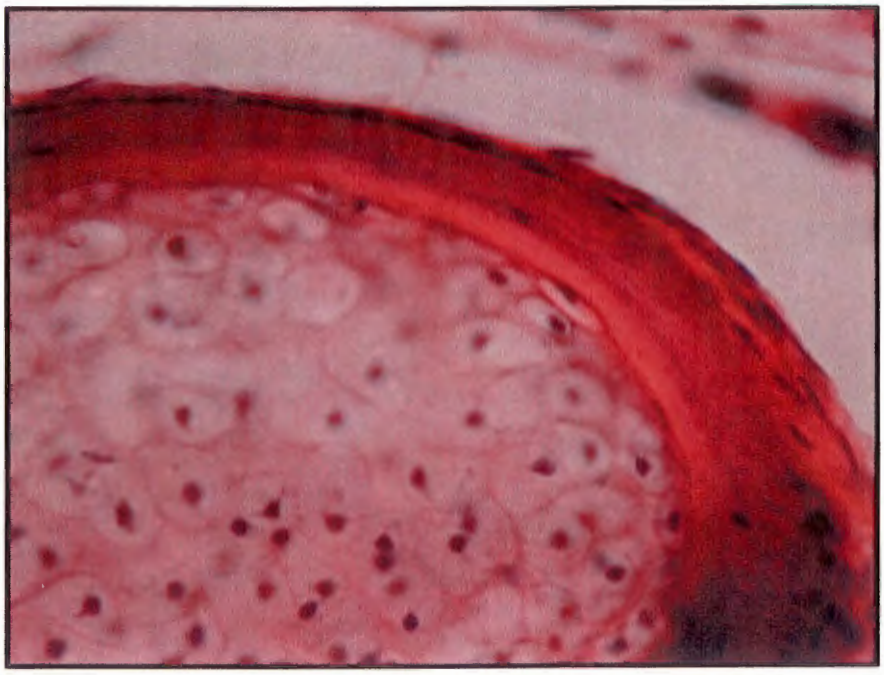

Figure 2.9: Photomicrograph of histological section through the toe of an one year-old frog.

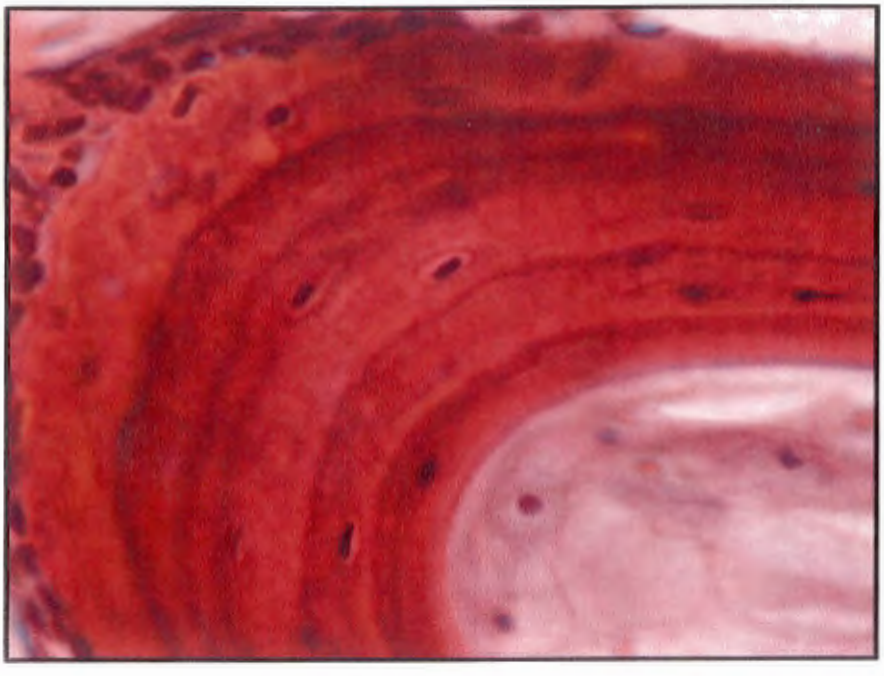

Figure 2.10: Photomicrograph of histological section through the toe of a six year-old frog.

The age structure of the frogs collected ranged from one to eight years, with the most frogs being one or two years old (Table 2.16 & Fig. 2.11). The mean age profile of all the sites ranges from 1.44 years to 2.62 years. A total of 53% of the frogs from reference sites and 38% from experimental sites were between one and two years of age. The age-class data were used to test the null hypothesis that the proportions of frogs in each age group in corn and non-corn growing areas were not statistically different. The difference in age distributions between the reference and experimental sites was tested by pooling the sites in each group and comparing them using a chi-square homogeneity statistic. Due to within-group site differences, however, this statistic would not be expected to follow a chi-square distribution. Consequently a permutation distribution was computed and used to calculate the p-value for this test. This p-value turned out to be $p = 0.7143$. Thus, while there are quite significant age differences among sites, there is no evidence that the group of exposed and the group of reference sites are statistically different. The mean numbers of adult male and female frogs in each age class were not significant between the reference and experimental sites (t-test $p = 0.43 – 0.76$).

Table 2.16: Table giving age structure of frogs collected.

	R1	R3	R6	E1	E3	E4	E6	E8
1 year	9	43	49	34	24	14	21	14
2 years	22	16	14	15	12	11	21	27
3 years	13	3	6	9	4	18	9	6
4 years	10	2	1	6	2	8	4	5
5 years	1	0	1	3	0	1	4	2
6 years	1	0	0	1	0	1	1	1
7 years	0	0	0	0	0	0	0	0
8 years	0	0	0	1	0	0	0	0
Total number	58	64	76	70	42	53	61	56
Mean age	2.62	1.44	1.58	2.07	1.62	2.51	2.20	2.20

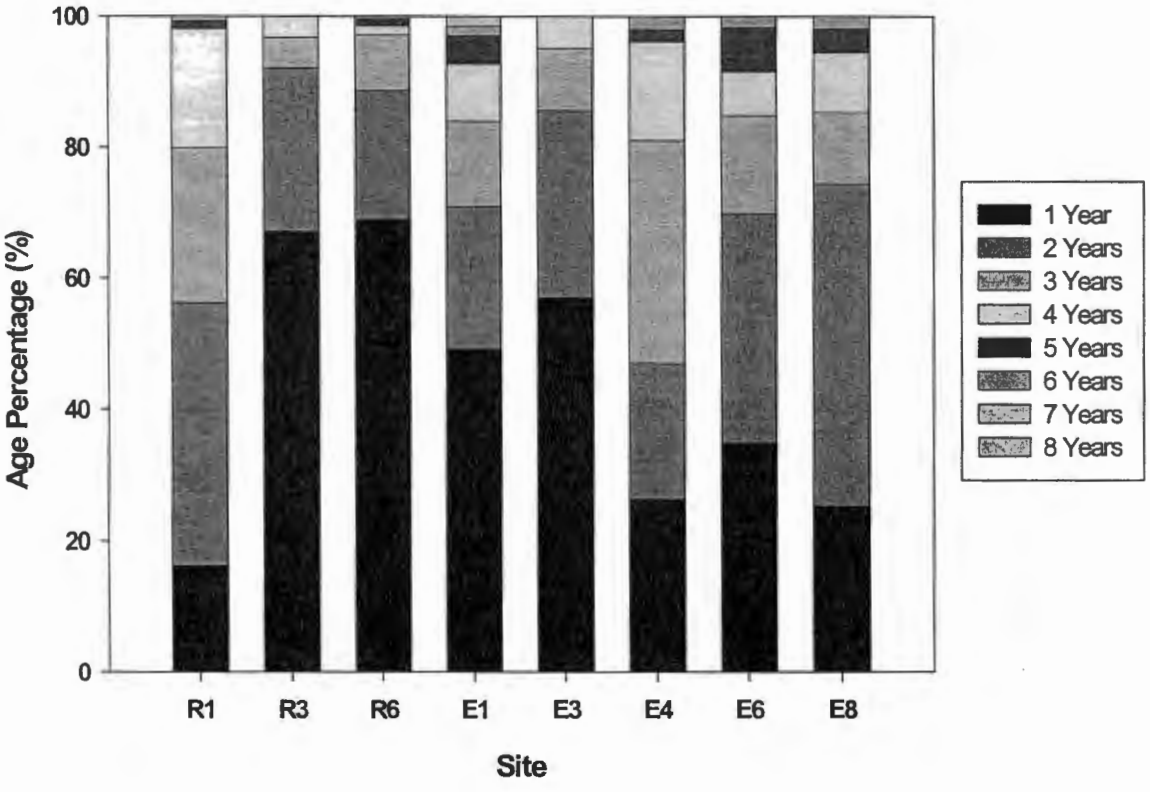

Figure 2.11: Histogram showing the age profile of *X. laevis* collected.

2.4 Discussion

At the beginning of the study, a survey was conducted and all the crops planted in the catchment area were plotted. With the help of the individual farmers the data of crops planted at specific localities were determined for the last five years and it seems like corn was the most dominant crop planted with about 80% of the surface planted with it (Table 2.11). No crops were planted in the vicinity of the reference sites. The habitat that surrounds these sites is mostly grass fields for cattle grassing.

Relatively few juveniles were collected during the present study. *X. laevis* has an extended breeding season from September to April. As a result, juveniles vary in size. For the purpose of this study, only very small individuals, the sex of which could not be determined, were labeled as juveniles. Traps were set at the time of year when the least number of frogs would be expected to enter the traps. This, together with the fact that only three to five trappings were performed, implies that the estimated population size is uncertain and is probably an underestimated of (Table 2.13).

The male-female ratio, mass and snout-vent-ratio for the experimental and reference sites were very similar. The mean percentage of males and females in the reference and the exposed sites was not different ($p = 0.67$). The age profiles, however, show some differences. Four of the sites (R1, E4, E6 and E8) had relatively small numbers of one-year old frogs. Sites R3 and R6 had large numbers of one-year old frogs, while in both sites E1 and E3 about 50% of the frogs were in the one-year old category. The two-year old category is more homogenous, varying from 11% in site E4 to 27% in site E8. The mean age profile was determined to be between 1.44 years and 2.62 years across the reference and experimental sites respectively. The mean of the mean ages at reference sites was not different from mean of the mean ages at experimental sites ($p = 0.28$). The interpretation of the skeletochronology proved to be a very

difficult process as a large number of frogs had only very faint or no visible growth lines at all in their toes. A possible explanation for this phenomenon could be that the sites used in this study do not dry up completely in the winter or the winters are usually relatively short. The frogs therefore do not hibernate by burrowing into the mud. This does not explain why some frogs had distinct growth lines. There are several possible explanations. If parts of the site dried up, frogs trapped in these sections would hibernate and develop growth rings. Frogs from the parts of the site that did not dry up would not develop growth rings. *X. laevis* have been reported to migrate between ponds, particularly when ponds dry up. Since frogs were branded, it was easy to determine if they migrated between ponds. No migration of frogs between any of the sites was observed. The rainfall for 2001 was above average and none of the sites dried up over the winter period. The possibility of migration, by non-marked specimens cannot be excluded, however, it is considered to be highly unlikely. Another possible explanation for the presence of growth lines only in some frogs could be due to a difference in hibernation behavior. It is possible that some frogs hibernate while others remain active.

The mass as well as the snout-vent length data, are significantly higher for female frogs than for male frogs. The mass of the females was never lower than 5.7 grams and reached a maximum weight of 204.5 grams, while the mass of the males ranged between 5.3 grams and 70.0 grams. There were no differences in the mean weights of the male frogs at the reference and experimental sites ($p = 0.24$), but there were significant differences in the mean weights of males and females across the sites and the mean weights of the females at the experimental and reference sites ($p = 0.02$), this may be due to the different stages of ovarian development. The snout-vent length of the females ranged between 57.1 mm to 71.6 mm, whereas the males' snout-vent length ranged from 50.3 mm to 62.8 mm. There were significant differences in snout-vent length (SVL) in male and female frogs across both the reference and experimental sites,

however, the mean SVL at reference sites was not different from that at the exposed sites in females ($p = 0.07$) and males ($p = 0.24$).

X. laevis populations from both atrazine exposed sites and reference sites appear to do well in the study area. Populations in exposed and reference sites were comparable. No adverse effects of atrazine or other triazines present in the water have been observed. One would expect that if atrazine had a negative effect on *X. laevis*, which would reflect in the population dynamics of the species, as this herbicide has been used in the study area for more than four decades.

* Data were collected by Mr. Ché Weldon.

CHAPTER 3

CHAPTER 3

SURFACE WATER ANALYSIS FOR ATRAZINE AND TRIAZINE RESIDUES

3.1 Introduction

The indirect effects of many pesticides on specific habitat quality have the potential to seriously influence wildlife populations in a number of ways, and more attention should be given to such possibilities (Bunyan *et al.* 1983). Many human activities, including agriculture, contribute simultaneously to habitat changes, and it is difficult to isolate alterations due to pesticides and to identify their particular influence on animal populations. However, research on pesticides has contributed a great deal of new knowledge about the habits, behavior, reproduction, food, and population dynamics of wildlife species (Keith 1991).

The toxicity of accumulated environmental chemicals in amphibians and reptiles is a major concern in the evaluation of chemicals hazards and ecological risk for these types of organisms. For amphibians and reptiles, the toxicity of surface waters, sediments, and soils that are potentially contaminated by different chemicals can be estimated using two approaches: a toxicity test approach or a chemistry-based approach. In the toxicity-based approach, toxicity tests directly measure toxic effects. In the chemistry-based approach, chemical analyses and laboratory-generated water quality criteria are used to determine possible hazards at specific sites (Sparling *et al.* 2000). The latter method was used during this study to determine the potential risks from atrazine in the environment.

It has been noted that amphibians may be at increased risk to the effects of environmental stressors due to their limited home range or their fidelity to site, their ecosystem diversity, their trophic position and their bio-accumulation

potential, and the relative ease with which chemicals are transported across membranes throughout various stages of development (Berrill *et al.* 1993; Diana *et al.* 1998; Matson 1998). As has been mentioned above, amphibians are located in small, permanent or temporary ponds and forest areas, which may be targets of aerial pesticide spray (Berrill *et al.* 1994; Berrill *et al.* 1995), or the sinks for agricultural field or urban runoff (Dial *et al.* 1984; Birdsall *et al.* 1986), industrial chemicals (Bishop *et al.* 1998), nutrients (Berger 1989; Lips 1998) and metals (Hall *et al.* 1984), that place them in a very sensitive environment for eco-toxicological stressors.

Affected communities can be identified by field surveys. These surveys can provide information for assessing adverse ecological effects potentially caused by hazardous waste. However, field surveys alone cannot identify causes of effects (Sparling *et al.* 2000).

This study was specifically conducted in South Africa because *X. laevis* occurs naturally in this part of the world, and South Africa has a high annual usage of atrazine in some agricultural areas. The moderate water solubility of atrazine and small K_d and K_{oc} favor movement of the chemical in the dissolved state from treated soil in surface water during rain events (Solomon *et al.* 1996). Thus the high annual usage combined with the moderate rainfall results in atrazine runoff into the small dams in between sprayed fields were *X. laevis* is found, thus making it the ideal monitoring place for the effects of atrazine on *X. laevis* in its natural environment.

3.2 Materials and Methods

3.2.1 Experimental and Reference Sites

Reference sites situated in the Potchefstroom area (see Fig. 2.4) were chosen on the basis of the combination of lack of corn planting, triazine use in the watershed and lack of measured residues of triazines in water, as well as suitable *X. laevis* populations.

Experimental sites located in the agricultural area of Viljoenskroon (see Fig. 2.5), were selected based on use of atrazine and other triazines in the watershed as well as measurements of exposure, and the population density of *X. laevis*.

Figures 2.4 and 2.5 show the location of the sites, as well as the rainfall and weather stations, from which rainfall and weather data were obtained.

3.2.2 Aerial Photographs

On 31 January 2002, aerial photographs were taken of all the sites from a fixed wing plane using a 35 mm Canon EOS5 SRL camera. Figure 3.1 shows the catchment area of sites E1, E6 and E8. Note the extent of the cornfields. Closer photographs of the sites reveal the physical properties of the sites (Fig. 3.2 & Fig. 3.3).

Figure 3.1: Aerial photographs of the catchment areas of the experimental sites E1, E6, and E8.

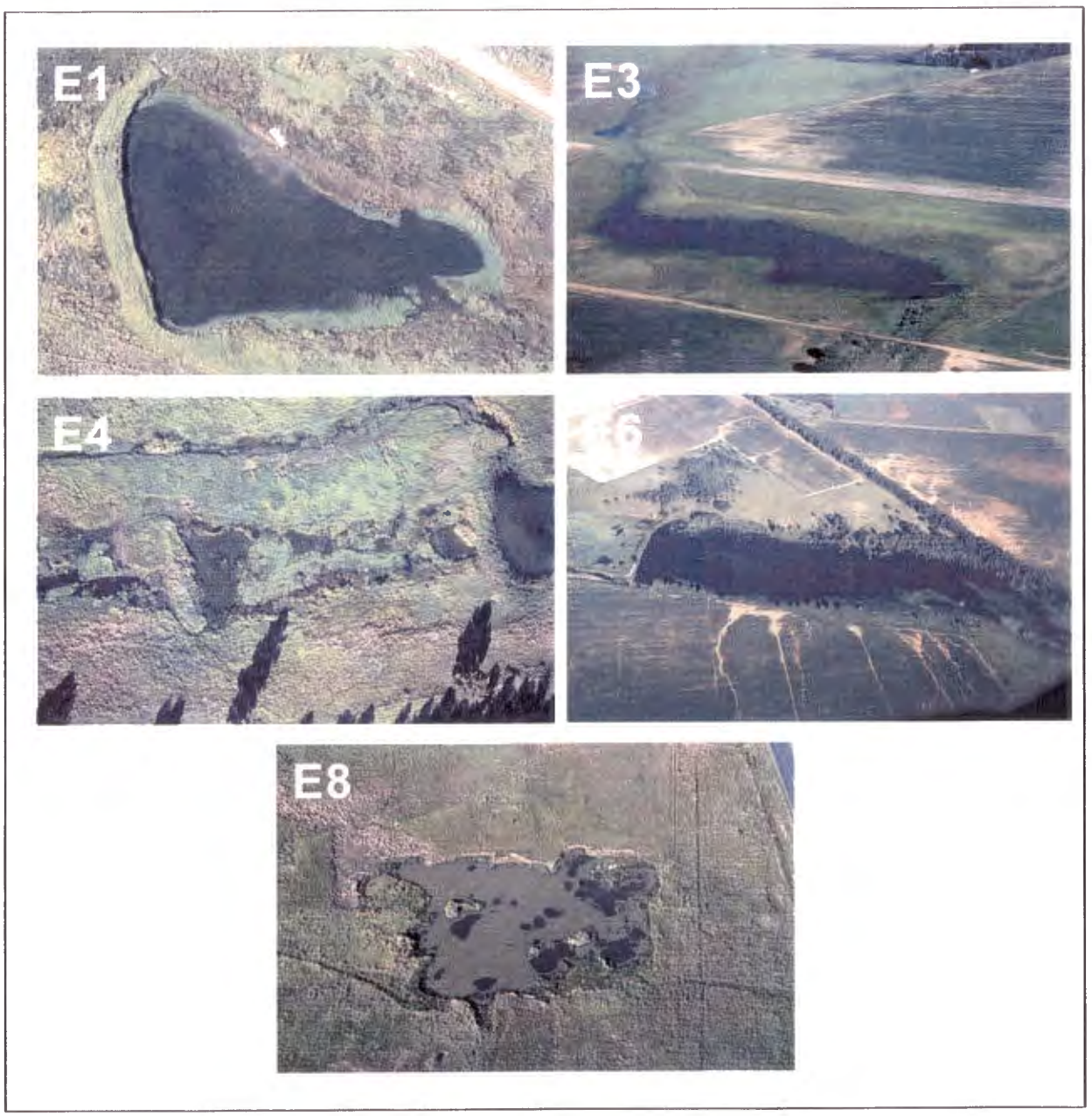

Figure 3.2: Aerial photographs of the experimental sites (E1, E3, E4, E6, & E8) in the Viljoenskroon area.

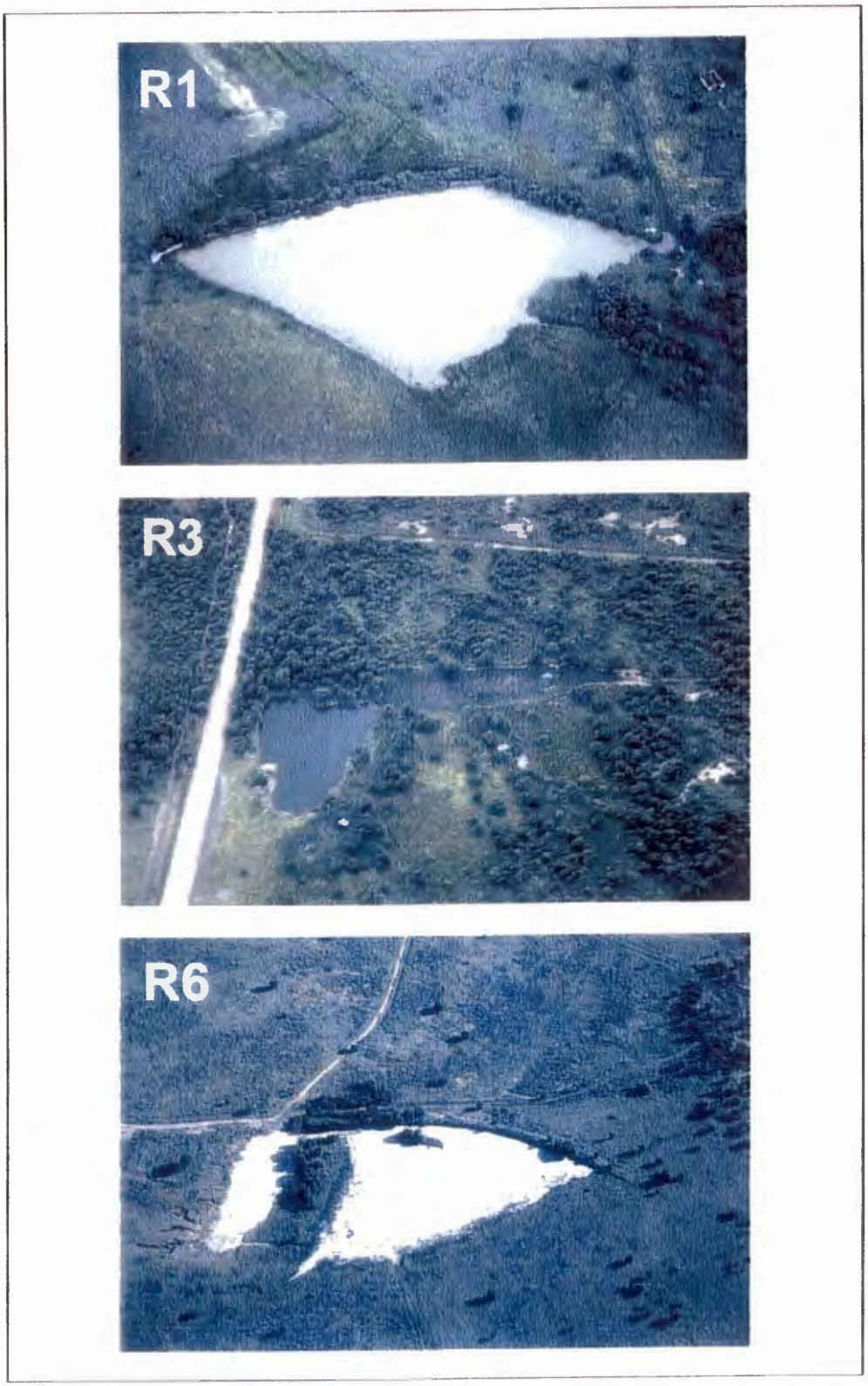

Figure 3.3: Aerial photographs of the reference sites (R1, R3, & R6) in the Potchefstroom area.

3.2.3 Collection of Environmental Samples and Site-Specific Measurements

3.2.3.1 Collection of water and sediment samples

From 5 November 2001 until 18 February 2002, water samples were taken on a weekly basis, and from the 4 March 2002 until 10 June 2002, every 14 days. During each sampling event and at each of the reference and experimental sites, four one-litre samples were taken 100 mm below the surface at fixed points around the each specific locality. These four samples were then pooled in a four-litre glass container, mixed, and a one-litre sub-sample taken. These labelled one-litre samples were then placed in cool boxes with blocks of blue ice to cool the water samples. Samples were transported to Potchefstroom University within five hours of sampling and stored in a cool room at 4°C until shipping to the analytical laboratory later the same day (Fig. 3.4). Chain of custody forms were filled in for all the samples. Sediment samples were collected at the same fixed points where the water samples were collected. Only the top layer of sediment (3 cm) was collected at every locality. This was done once a month, with the first sampling of water. Sediment samples were also transported in cool boxes, stored and shipped as for the water samples (Fig. 3.4).

Figure 3.4: Water and sediment samples in cool boxes for transportation.

3.2.3.2 Site-specific measurements

At the beginning of the study a reference marker (a steel rod) was placed at a specific location in each site. This marker was used as a reference point, at which the depth of the water and other water quality parameters were measured every time water samples were collected at the sites.

A YSI 556 multi probe system data logger was used to measure dissolved oxygen (mg/L), conductivity (µS/cm), pH, and water temperature (ºC). Air temperature was measured in the shade at a height of one meter above the ground using a digital thermometer. All of these measurements were taken at the reference point. Specific weather conditions such as % cloud cover; rainfall and wind were also taken. Data recorded on the data logger were filled in on data

sheets at every site as a back up. Data in the logger were uploaded to a personal computer and transferred to Excel spreadsheets for further analysis.

3.2.3.3 Collection of climatological data

Rainfall figures were obtained from the farmers on whose farms the sites were located (Table 3.1). In addition to the data obtained from the farmers, rainfall figures, minimum and maximum temperatures, wind speed, evaporation and hours of sunlight were obtained from the Agricultural Research Council of South Africa, with weather stations situated at Naschem (see Fig. 2.4) and on the farm Rietpan close to the experimental sites (see Fig. 2.5). In Tables 3.1 and 3.2 the sources as well as their distance from specific sites of the climatological data are indicated.

Table 3.1: Sources of rainfall data.

Rainfall		
Site	**Weather station**	**Distance to site**
R1	Mr. J. Taggart	150 m
R3	Naschem	3000 m
R6	Naschem	1800 m
E1	Mr. J. Bosman	60 m
E3	Mr. S. Meintjies	3500 m
E4	Mr. S. Meintjies	2600 m
E6	Mr. S Botha	2000 m
E8	Mr. J. Bosman	750 m

Table 3.2: Sources of other climatological data.

Other climatological data		
Site	**Weather station**	**Distance to site**
R1	Naschem	6500 m
R3	Naschem	1800 m
R6	Naschem	1800 m
E1	Rietpan	5600 m
E3	Rietpan	4500 m
E4	Rietpan	6700 m
E6	Rietpan	17200 m
E8	Rietpan	6000 m

3.2.4 Chemical Analyses of Water and Sediment Samples

The pesticide and metabolite analyses of the water samples that were collected at the specific sites were conducted by the analytical laboratories of the South African Bureau of Standards (SABS) at the Council for Scientific and Industrial Research (CSIR) and by Mr. Peet Jansen van Rensburg at the Department of Microbiology, School of Environmental Sciences and Development, Potchefstroom University for CHE, Potchefstroom. Elemental scans of both water and sediment samples were conducted at SGS (Midrand, Johannesburg, South Africa). Both the SABS and SGS are GLP accredited analytical laboratories and analyses conducted by Mr. Jansen van Rensburg were conducted in the spirit of GLP. The detection limits for the analytes tested by the different companies are shown in Table 3.3.

Table 3.3: Detection limits of chemical analyses done by Potchefstroom University, SGS, and CSIR (µg/L).

Analytical laboratory	Detection limit (LOD)
Potchefstroom University	0.01 µg/L
SGS	0.01 µg/L
CSIR	0.05 µg/L

3.2.5 Agricultural Activities in the Catchment of Experimental Sites

A survey was conducted throughout the study area of the type of crops planted in the catchment area of all the experimental sites at the beginning of the study. A detailed list of pesticides and the amount of each applied to specific fields were compiled for the complete catchment area. This was done with the help of individual farmers and representatives of chemical companies. Data were recorded on data sheets.

3.3 Results

3.3.1 Climatological Data

Very high rainfalls were recorded for the period between September and December 2001. In both the Potchefstroom and Viljoenskroon areas, rainfall for the month of December 2001 was more than double the ten-year mean. The temperatures for the study period correlated well with the ten-year mean for both the areas (Fig. 3.4 & Fig. 3.5).

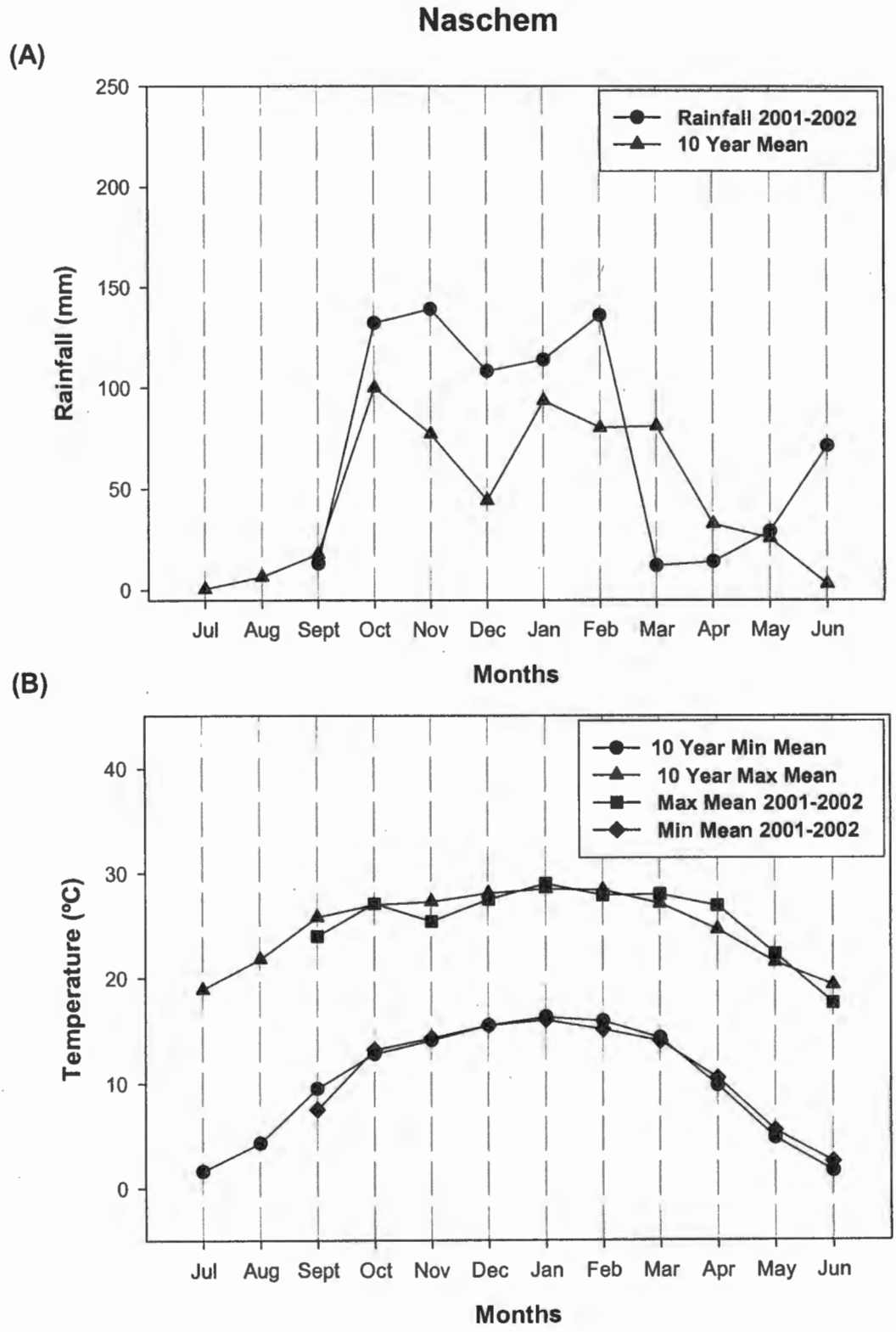

Figure 3.4: Mean rainfall **(A)** and temperature **(B)** data for the Potchefstroom area (Reference Sites) 2001-2002.

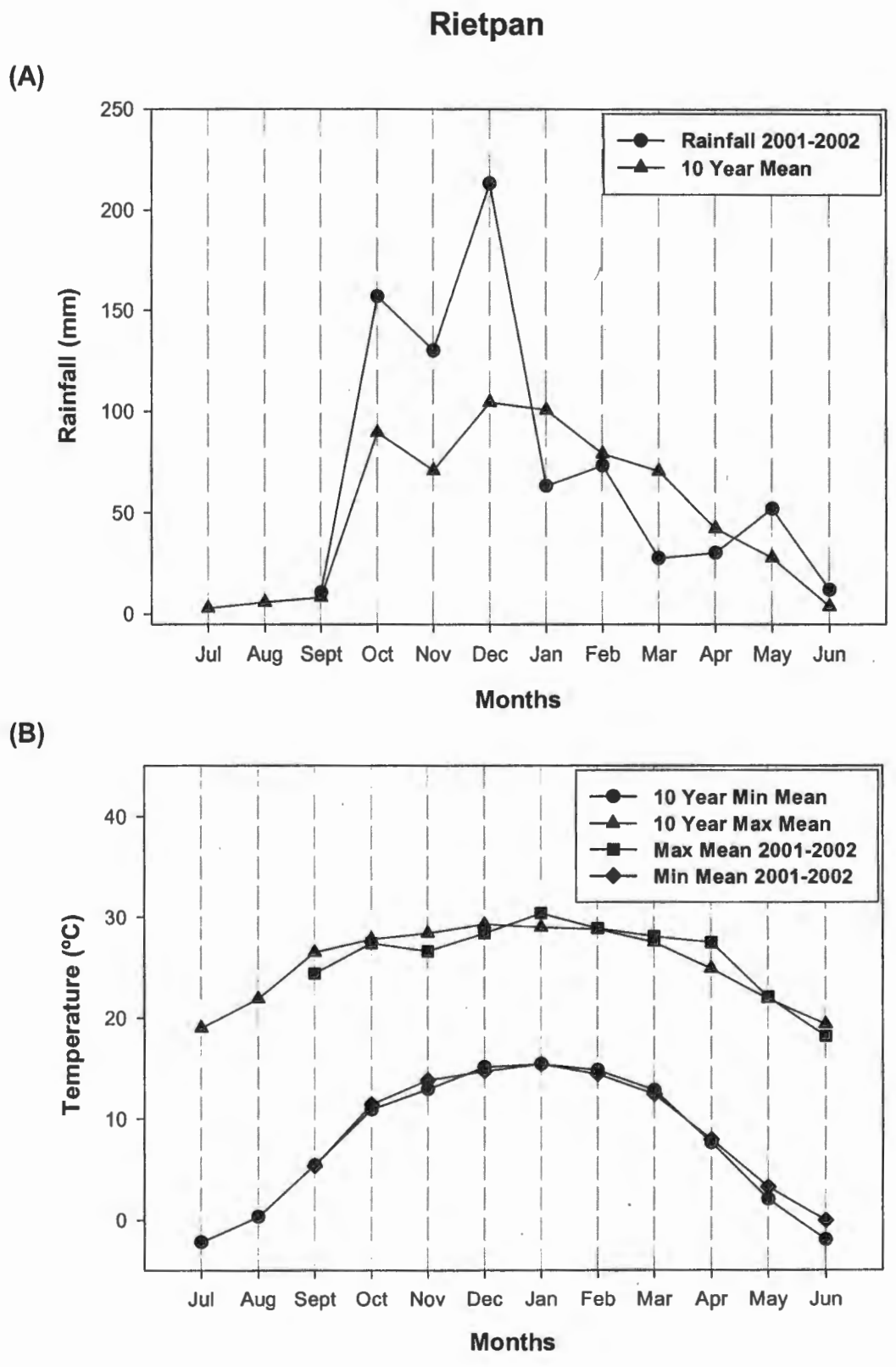

Figure 3.5: Mean rainfall **(A)** and temperature **(B)** data for the Viljoenskroon area (Experimental Sites) 2001-2002.

3.3.2 Agricultural Activities During the Study Period

Due to very high rainfall in November and December 2001, farmers were not able to plant a great deal of sunflower, sorghum, and groundnuts in the catchment area of the experimental sites. As a result, corn became the primary crop in the catchment area (Fig. 3.6). While the regular season for planting corn is from the beginning of November to mid-December, planting was delayed due to excessive rains with some farmers only planted as late as the first week of January 2002. As a result, the application of insecticides and herbicides was delayed as well.

Due to the very high rainfall in the catchment area of the experimental sites, all the dams overflowed and were flushed. All the experimental dams, except dam E6, had stopped overflowing by early January.

The amounts of herbicide active ingredient, converted to g/ha, applied in the catchment area of the experimental sites were calculated. This is presented for atrazine (Fig. 3.7), acetochlor (Fig. 3.8), simazine (Fig. 3.9), terbuthylazine (Fig. 3.10) and S-metolachlor (Fig. 3.11).

The amounts of insecticide active ingredient, converted to g/ha, applied in the catchment area of the experimental sites were also calculated. This is presented for cypermethrin (Fig. 3.12), monochrotophos (Fig. 3.13) and terbufos (Fig. 3.14).

Atrazine, terbuthylazine, acetochlor, cypermetrin and terbufos were applied to most of the corn fields at the recommended rate (Figs. 3.7, 3.10, 3.8, 3.12 & 3.14). On two fields adjacent to sites E1 and E8 and on two fields in the catchment of site E6 atrazine was applied at a very high rate (Fig. 3.7). Simazine was only applied on two fields adjacent to sites E1 and E8 and one field next to site E6 (Fig. 3.9). S-metolachlor and monocrotophos were only applied to 10 and three fields respectively (Figs. 3.11 & 3.13).

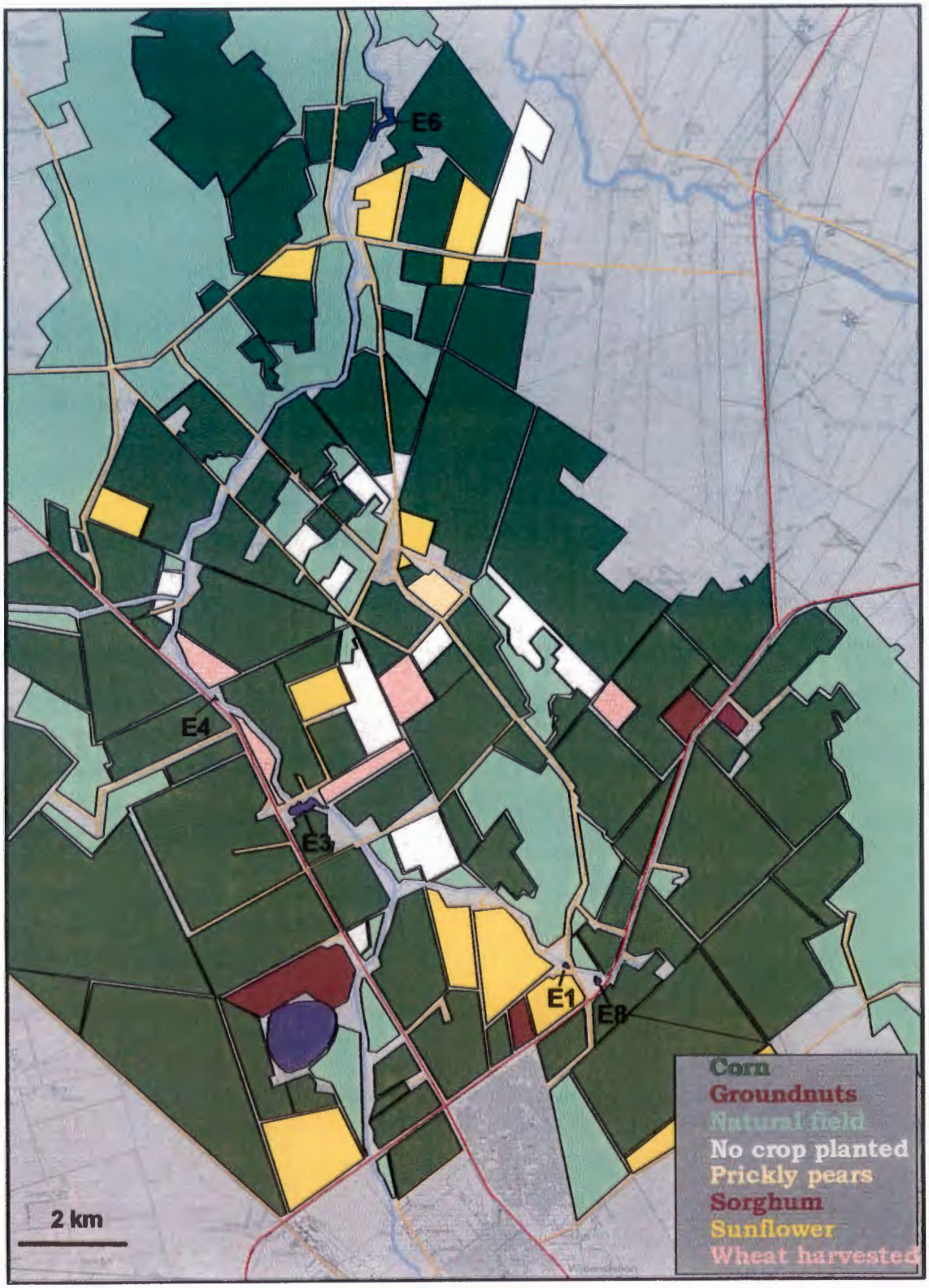

Figure 3.6: Map of the Viljoenskroon area indicating crops planted during the 2001 – 2002 season.

3.3.3 Agrochemicals Applied During the Study Period

Presented in Table 3.4 are all the herbicides used in the catchment area of the experimental sites during the study. Atrazine, terbuthylazine, and acetochlor were the dominant products used. Bullet, Robust, and A-mazing (commercial names), contain both atrazine and acetochlor as active ingredients. According to the formulation of the herbicide, atrazine was applied in different rates on the agricultural fields.

Table 3.5 shows the insecticides used in the catchment area of the experimental sites. The most commonly used insecticide was terbufos. This insecticide is used for the control of nematodes and soil insects. Terbufos is sold under the commercial names of Counter and Terraphos.

Table 3.4: Summary of herbicides applied during the study period.

Herbicides		
Registered trade name	**Active ingredient**	**Rate of application of formulation**
With planting		
Bullet	250 g/l Acetochlor 125 g/l Atrazine 125 g/l Terbuthylazine	2.75 l/ha @ 33% of surface or 2.1 l/ha @ 100% of surface
Dual Gardomil	916 g/l S-metolachlor 102.8 g/l S-metolachlor 248.6 g/l Atrazine 248.6 g/l Terbuthylazine	2.2 l/ha @ 33% of surface
Guardian	800 g/l Acetochlor	0.75 l/ha @ 33% of surface
Harness	900 g/l Acetochlor	0.3 l/ha @ 33% of surface or 1 l/ha @ 100% of surface
Lasso	480 g/l Acetochlor	1.1 l/ha @ 33% of surface
Metagan Gold	916 g/l S-metolachlor	0.8 l/ha @ 40 % of surface
Primagram	229.2 g/l S-metolachlor 371 g/l Atrazine	2 l/ha @ 20% of surface

Herbicides		
Registered trade name	**Active ingredient**	**Rate of application of formulation**
With planting		
Robust	160 g/l Acetochlor 165 g/l Atrazine 165 g/l Simazine	1 l/ha @ 33% of surface or 3 l/ha @ 100% of surface
Suprazine	250 g/l Atrazine 250 g/l Terbuthylazine	1.5 l/ha @ 100% of surface
Zeazine	167 g/l Atrazine 333 g/l Cyanazine	2.3 l/ha @ 33% of surface
3-6 weeks after planting		
A-mazing	250 g/l Acetochlor 125 g/l Atrazine 125 g/l Terbuthylazine	2 - 3 l/ha @ 100% of surface
Frontier	900 g/l Dimethenomid	0.65 l/ha @ 100% of surface
Gesaprim super	300 g/l Atrazine 300 g/l Terbuthylazine	2 l/ha @ 20% of surface
Harness	g/l Acetochlor	0.5–1 l/ha @ 100% of surface
Metagan Gold	916 g/l S-metolachlor	0.6 l/ha 1.5 l/ ha @ 100% of surface
Suprazine	250 g/l Atrazine 250 g/l Terbuthylazine	1.5 - 2 l/ha @ 33% of surface

Table 3.5: Summary of insecticides applied during the planting period.

Insecticides applied with planting				
Registered trade name	**Active ingredient**	**Rate of application of formulation**	**Effective against**	**When applied**
Counter	Terbufos 100 g/kg	4.4 kg/ha	Nematodes and soil insects	With planting
Magnum	Cypermethrin 200 g/l	33 ml/100m in row	Cutworm	With planting
Monostem	Monocrotophos	1.5 l/ha	Stalk borer	30 days after planting
Polytrin	Cypermethrin 200 g/l	33 ml/100m in row	Cutworm	With planting
Terraphos	Terbufos 100 g/kg	4.6 kg/ha	Nematodes and soil insects	With planting

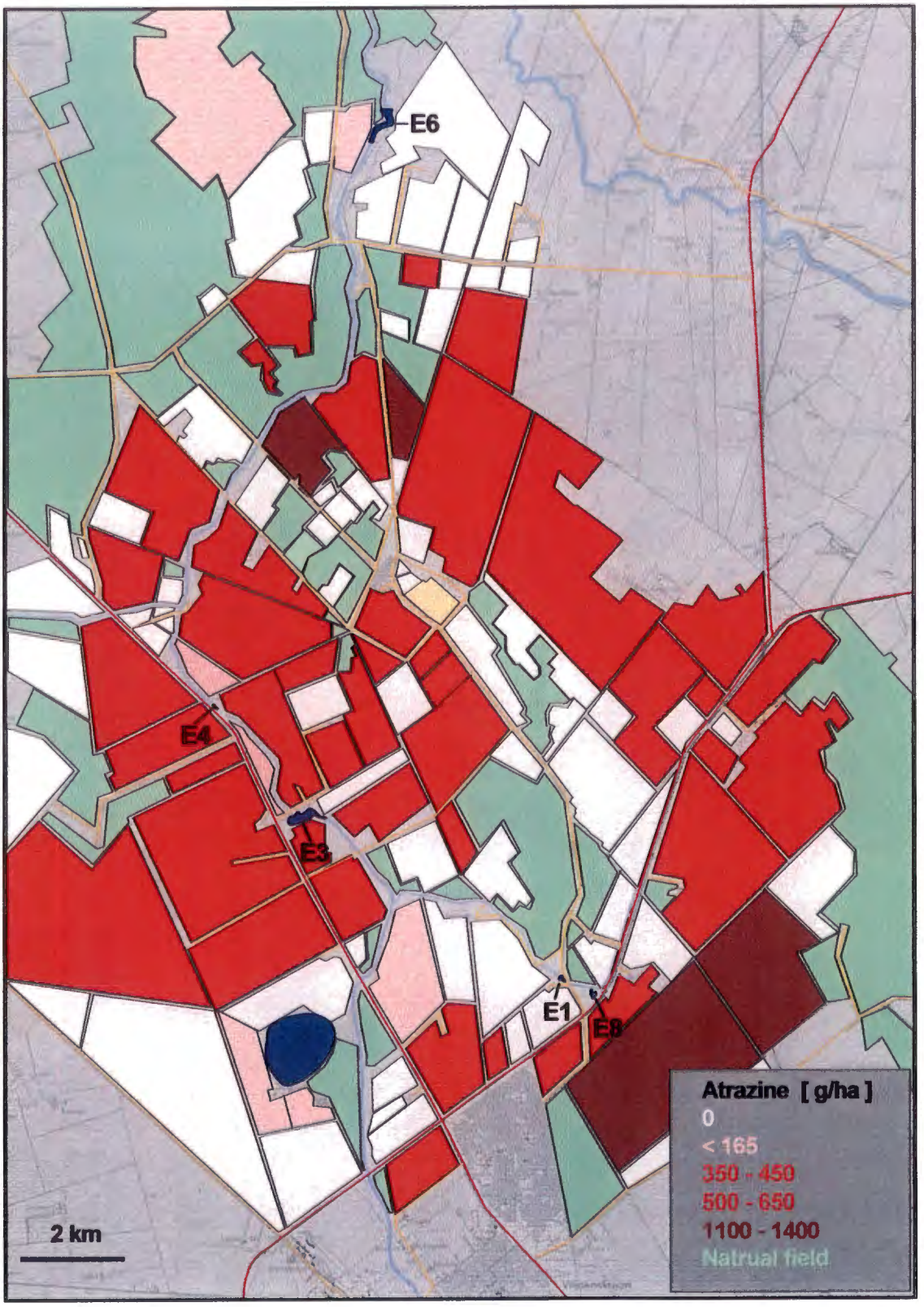

Figure 3.7: Amount of atrazine applied in catchment areas of experimental sites.

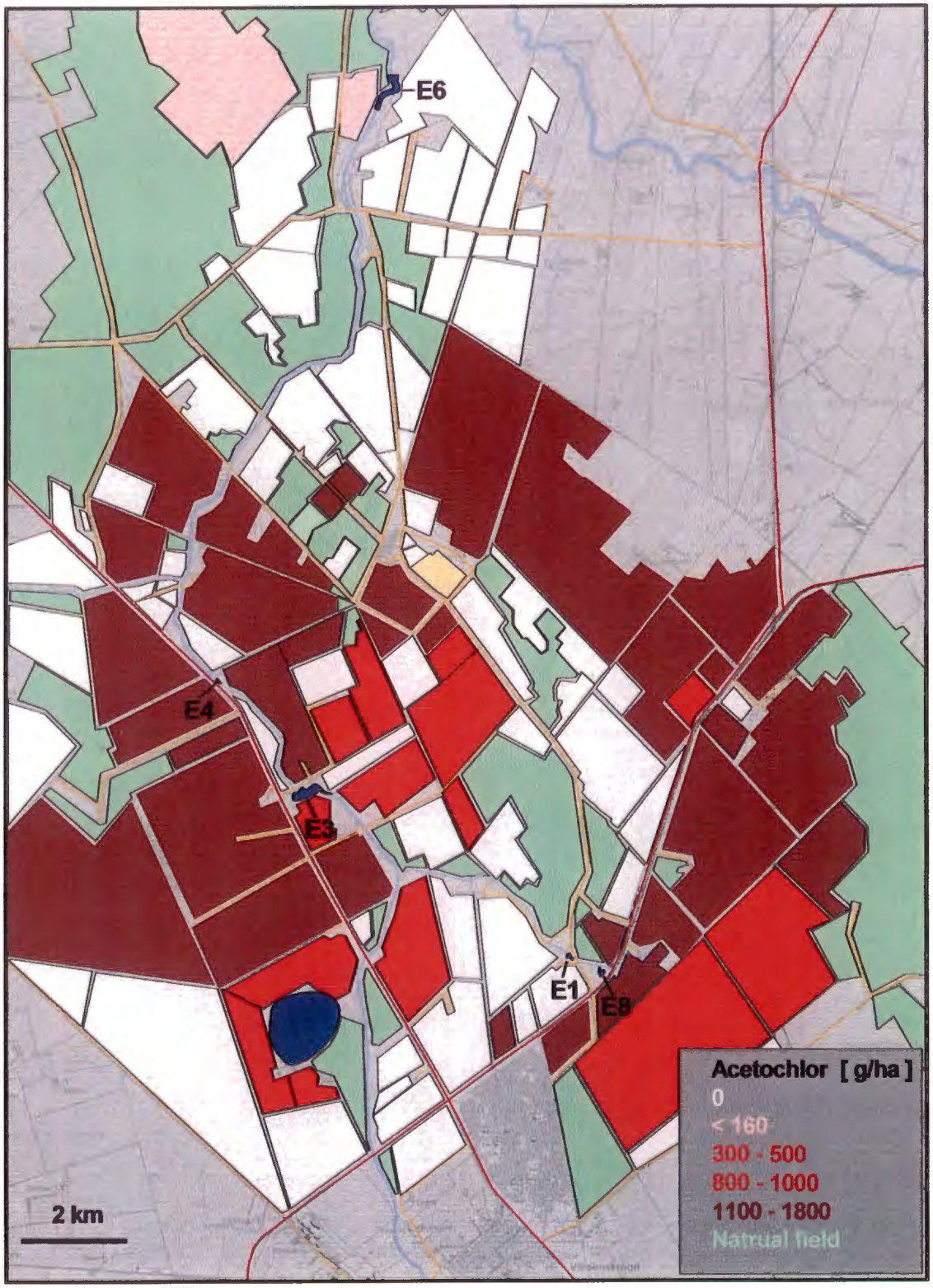

Figure 3.8: Amount of acetochlor applied in catchment areas of experimental sites.

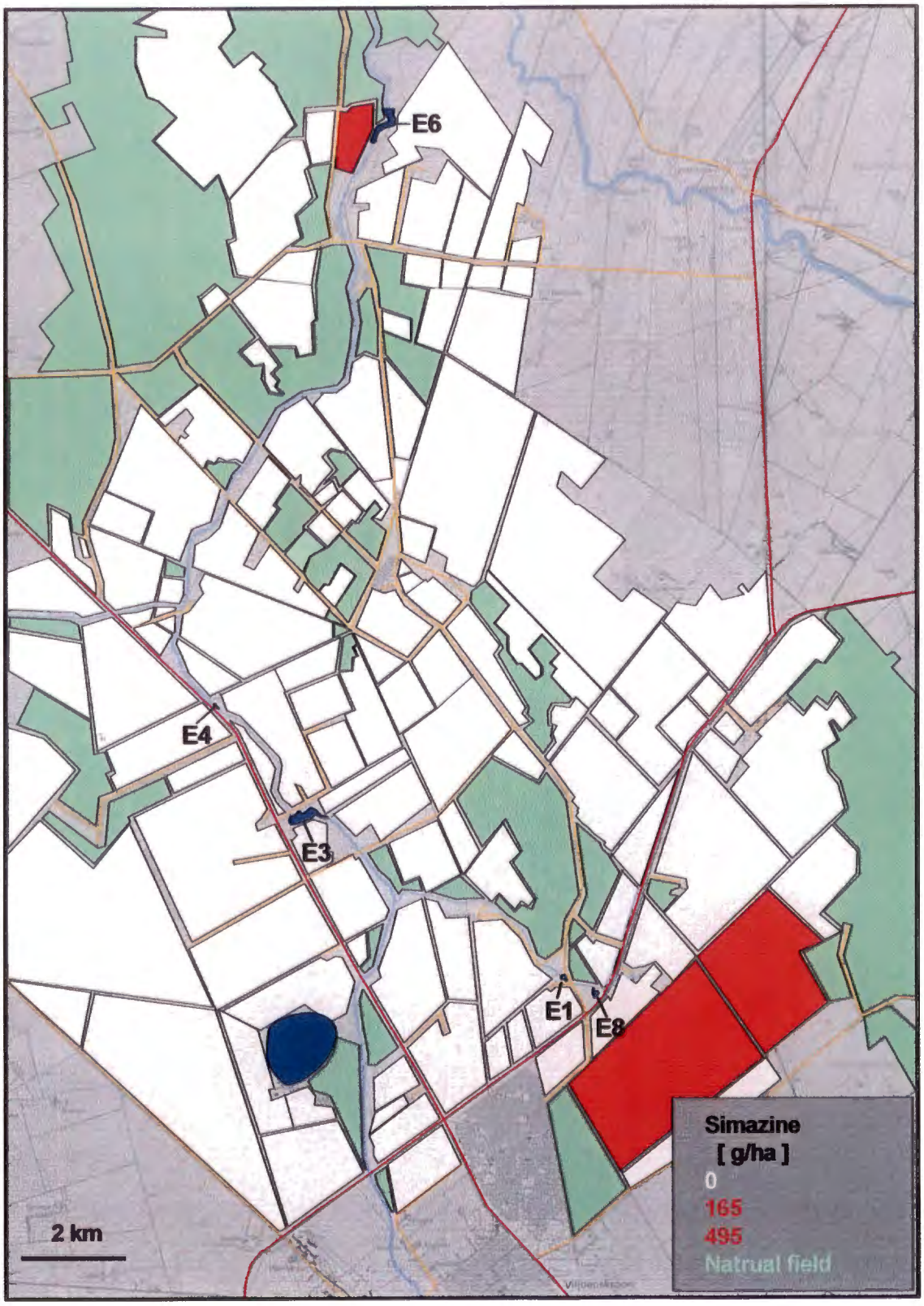

Figure 3.9: Amount of simazine applied in catchment areas of experimental sites.

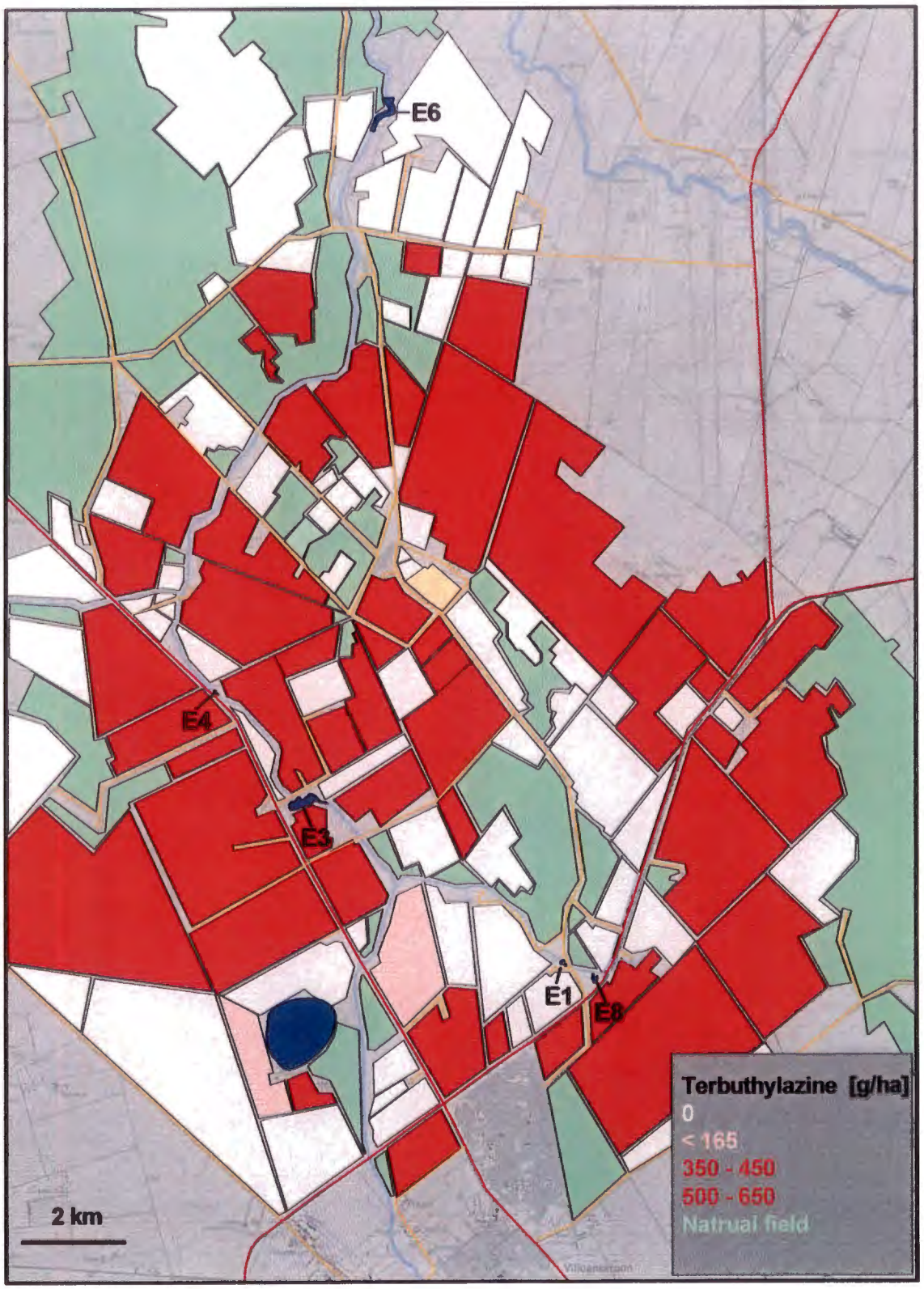

Figure 3.10: Amount of terbuthylazine applied in catchment areas of experimental sites.

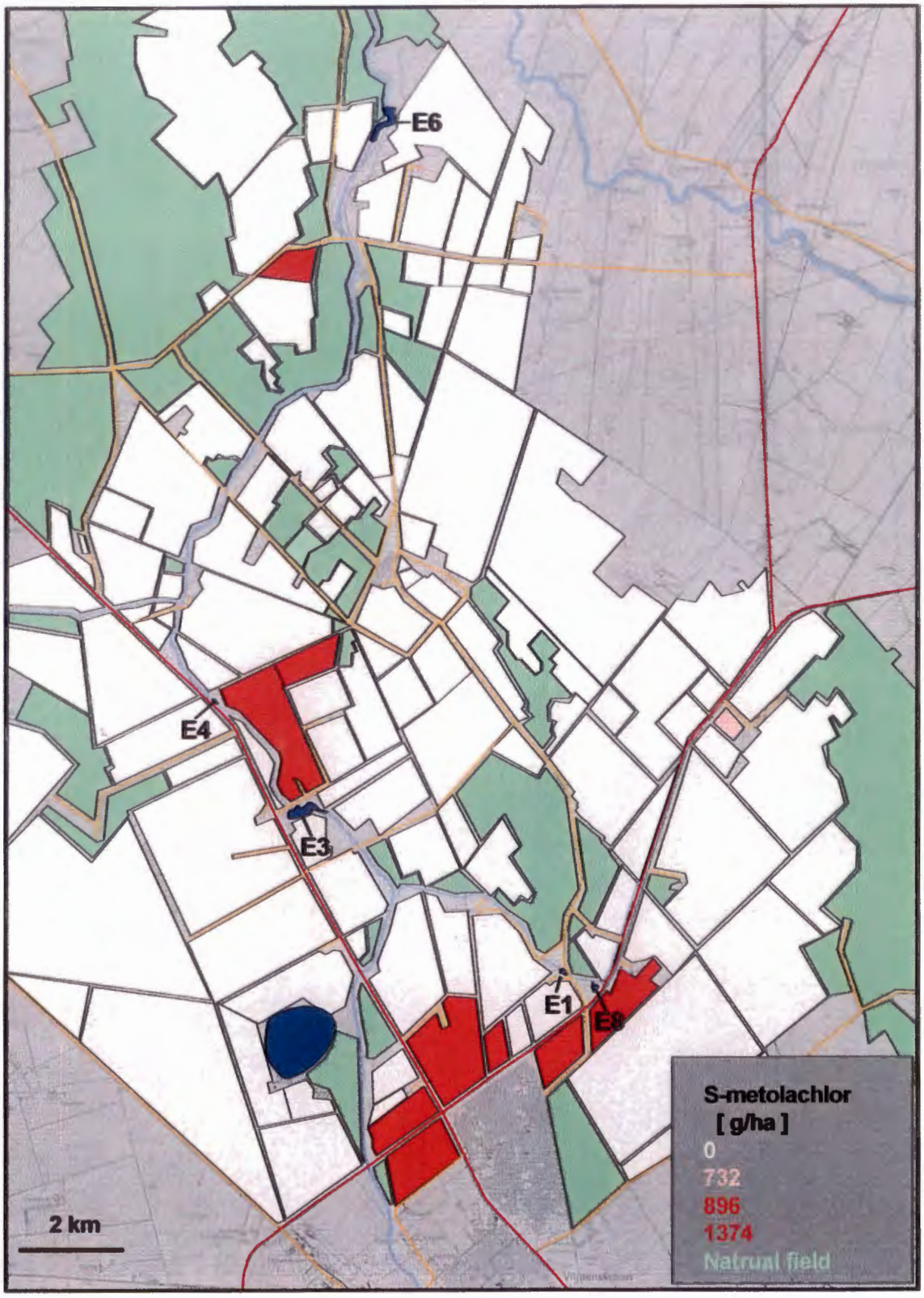

Figure 3.11: Amount of S-metolachlor applied in catchment areas of experimental sites.

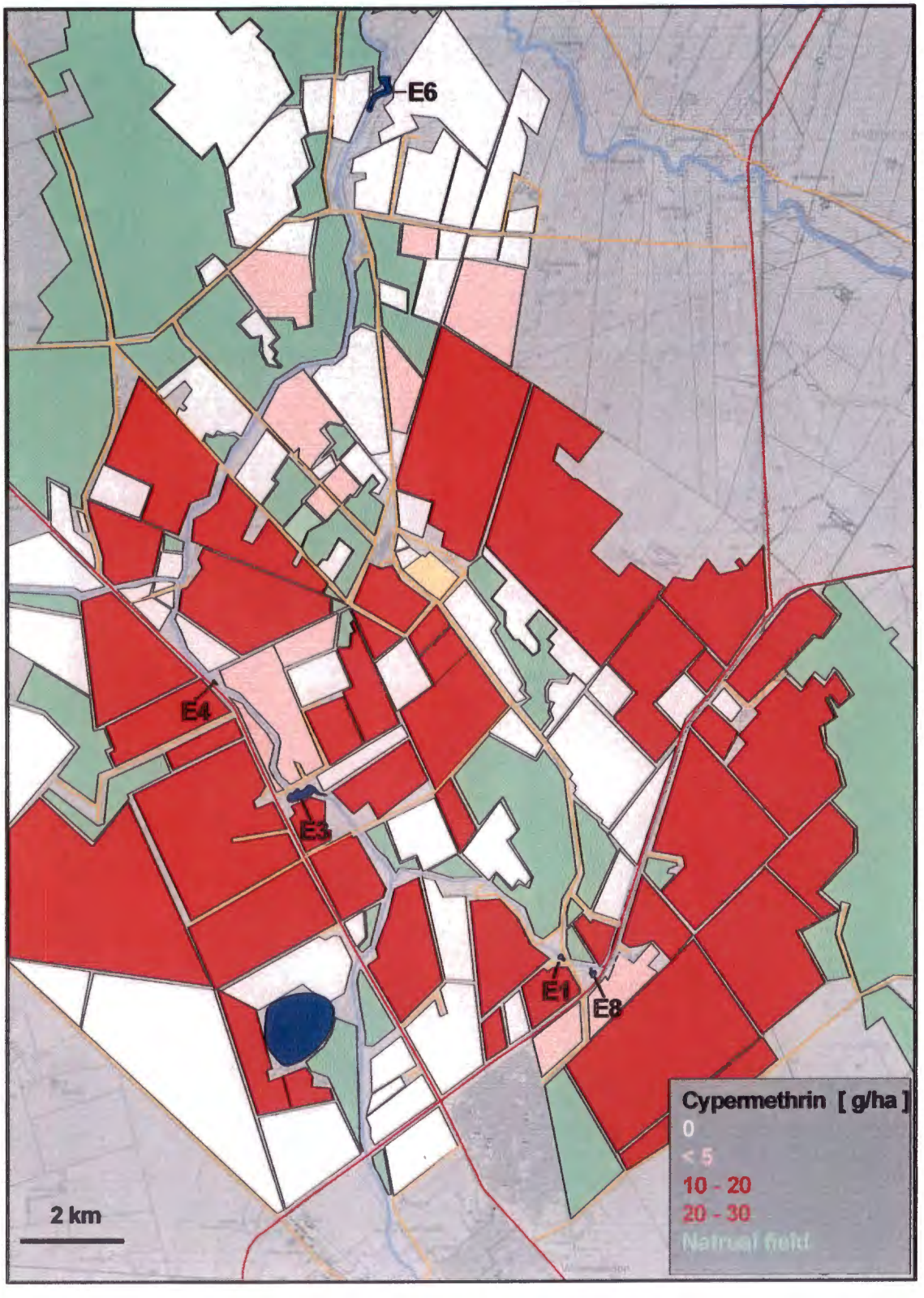

Figure 3.12: Amount of cypermetrin applied in catchment areas of experimental sites.

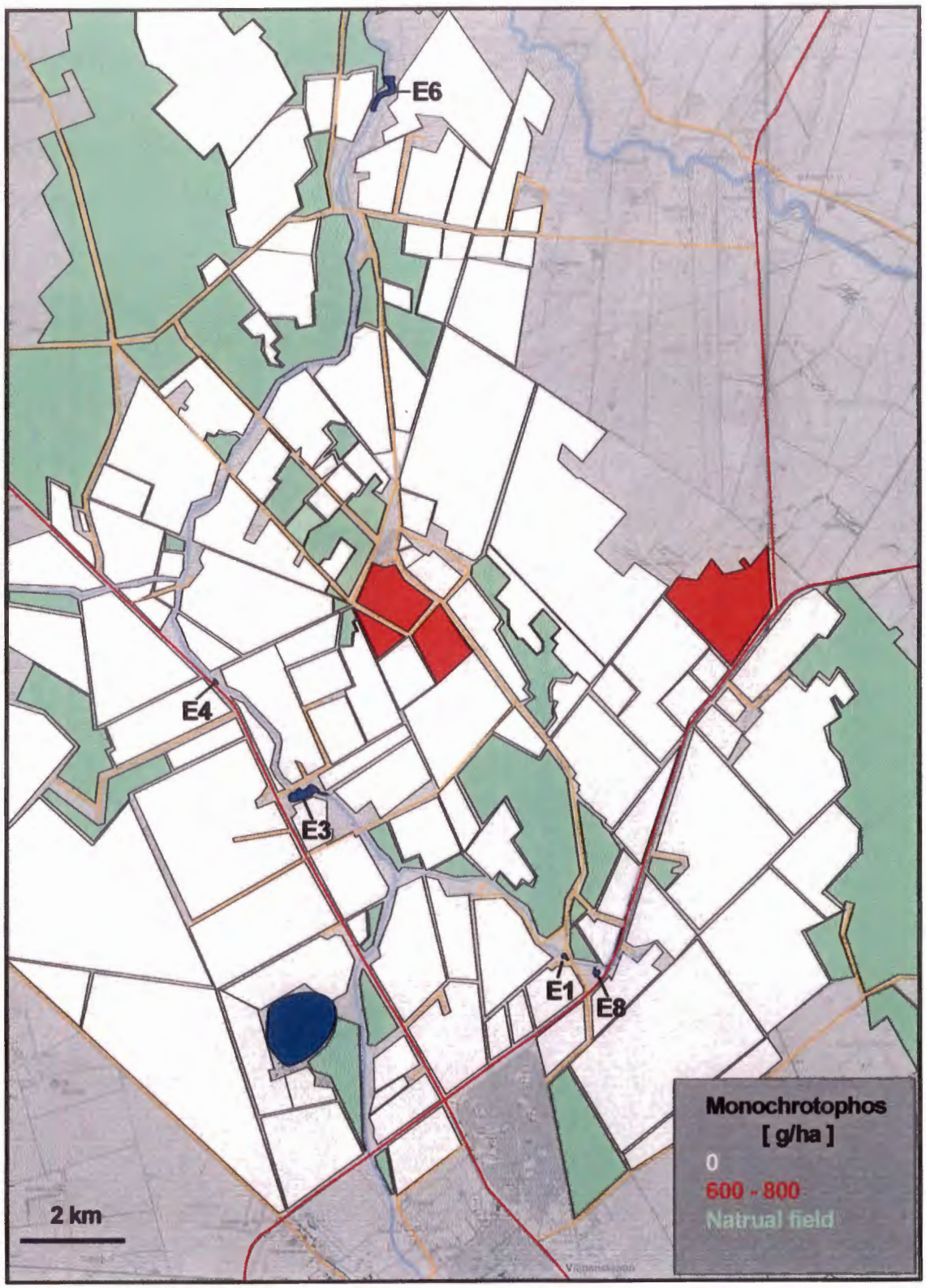

Figure 3.13: Amount of monochrotophos applied in catchment areas of experimental sites.

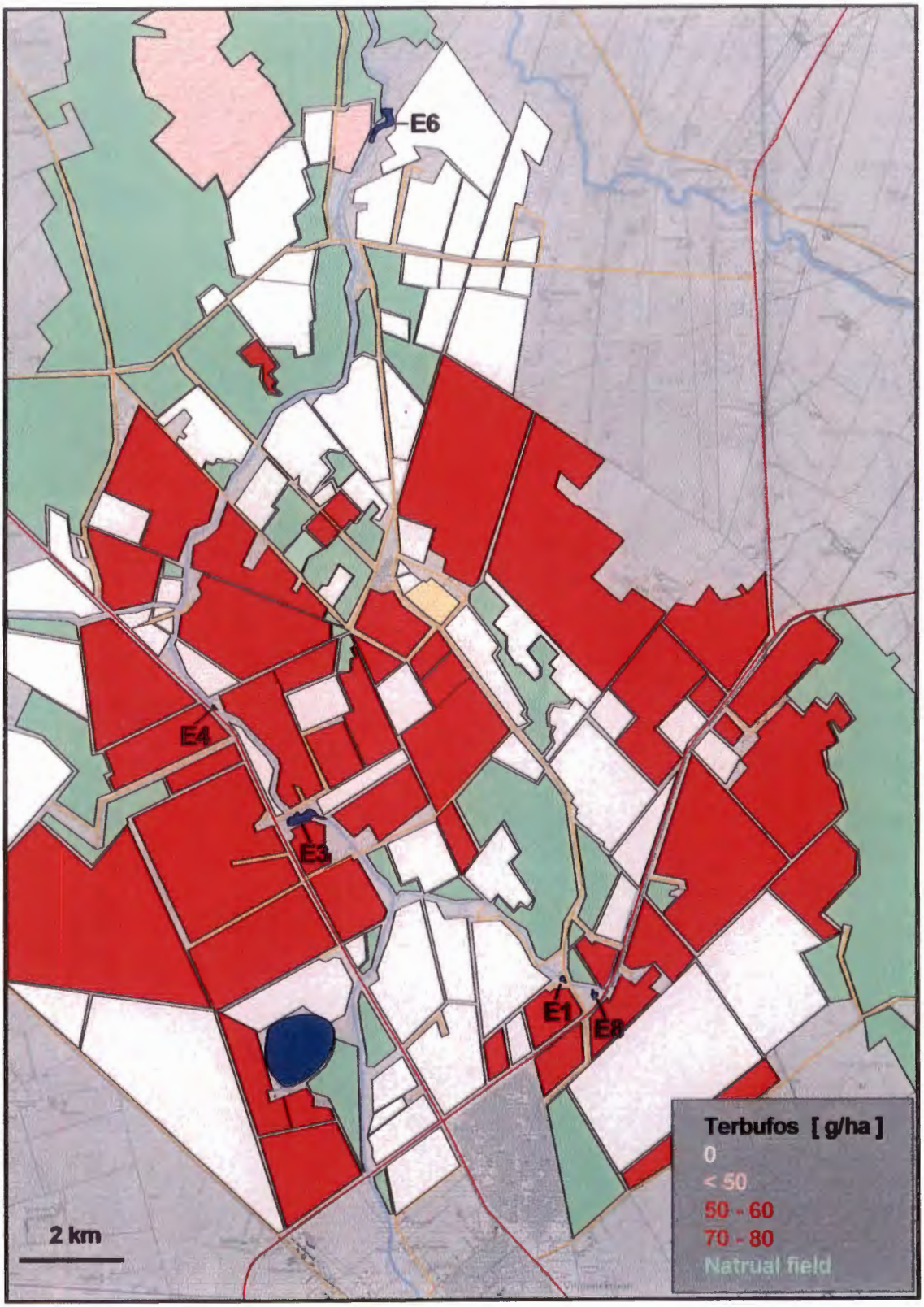

Figure 3.14: A mount of terbufos applied in catchment areas of experimental sites.

3.3.4 Herbicides in Water and Sediment samples

No herbicides were detected in any of the sediment samples analyzed. Concentrations of herbicides found in water from reference sites are presented in Fig. 3.15 – Fig. 3.17 and those found in experimental sites in Fig. 3.18 – Fig. 3.22. A complete Table with all the data is attached as Appendix 5. For the purpose of this report, values lower than the LOD of 0.5 µg/L are presented in the Figures as a value of 0.25 µg/L and values lower than the LOD of 0.1 µg/L are presented as 0.05 µg/L.

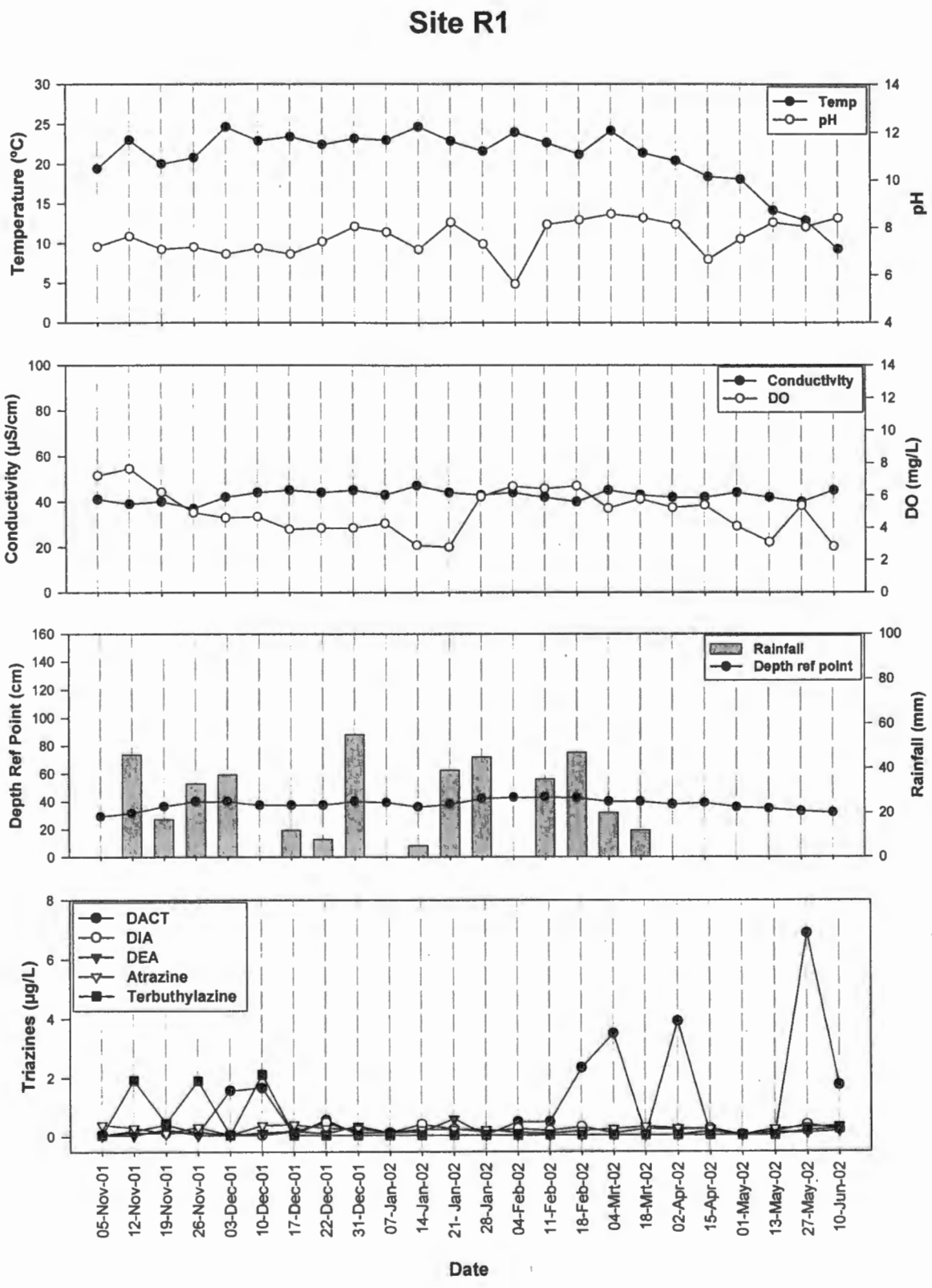

Figure 3.15: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

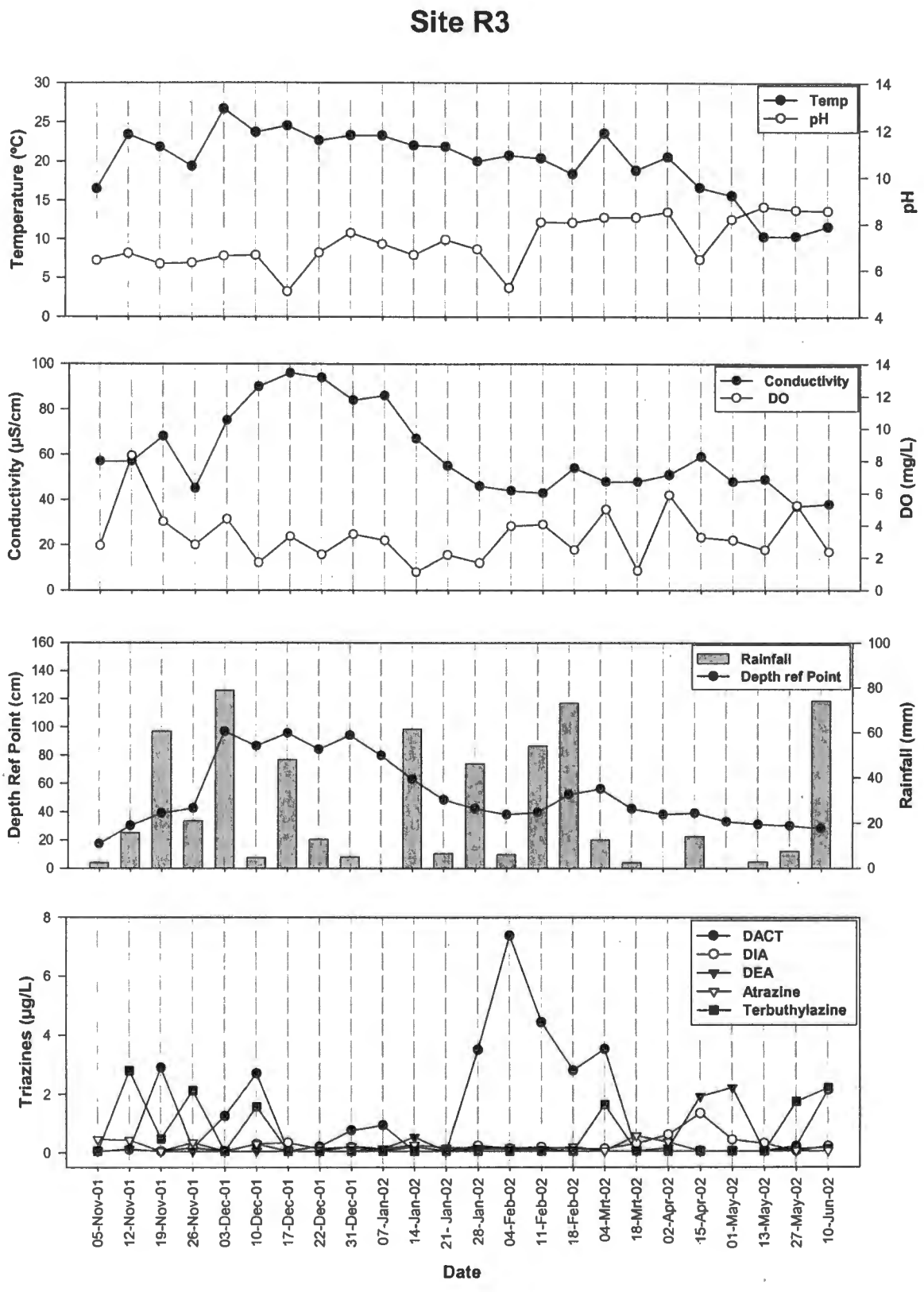

Figure 3.16: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

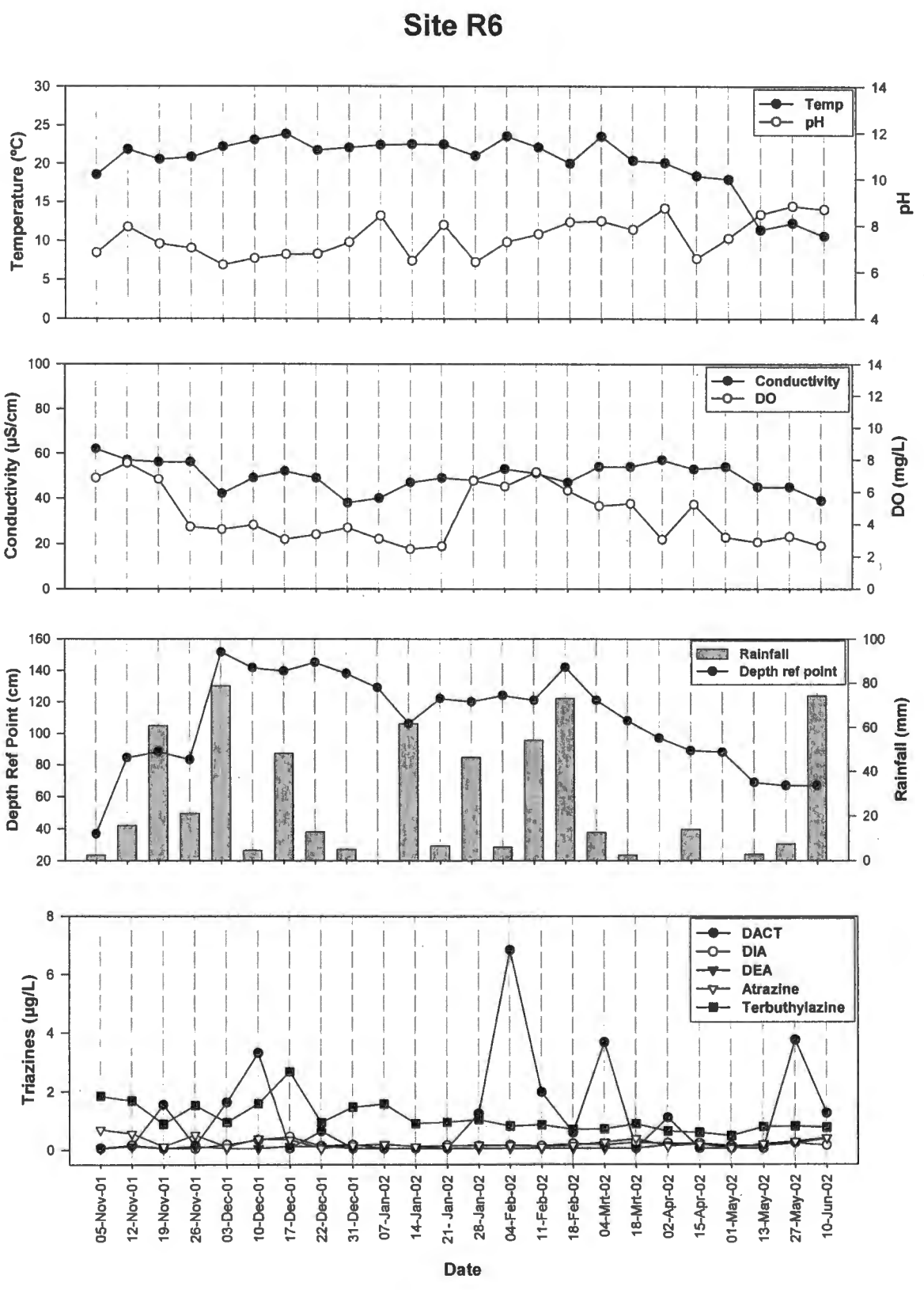

Figure 3.17: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

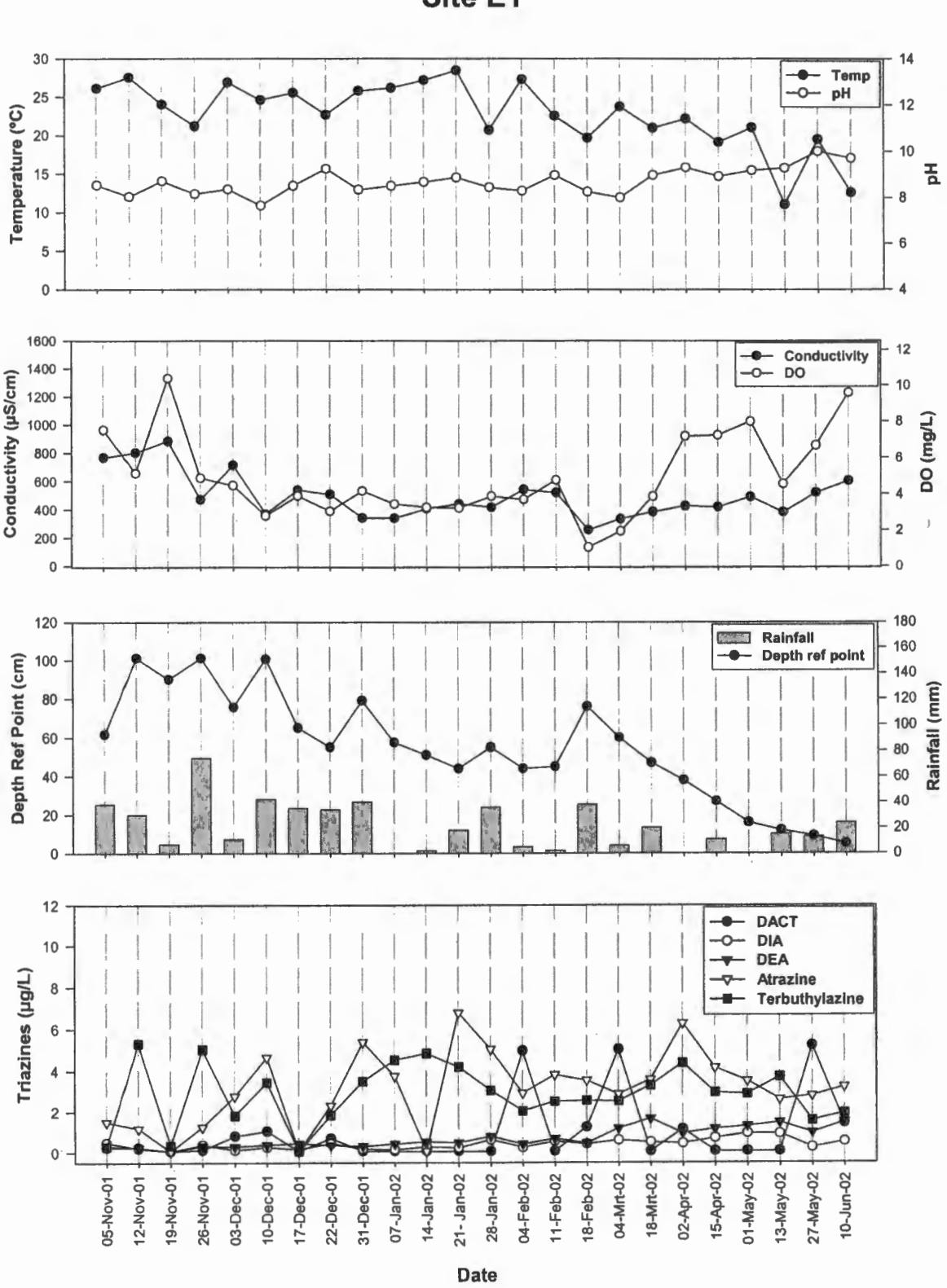

Figure 3.18: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

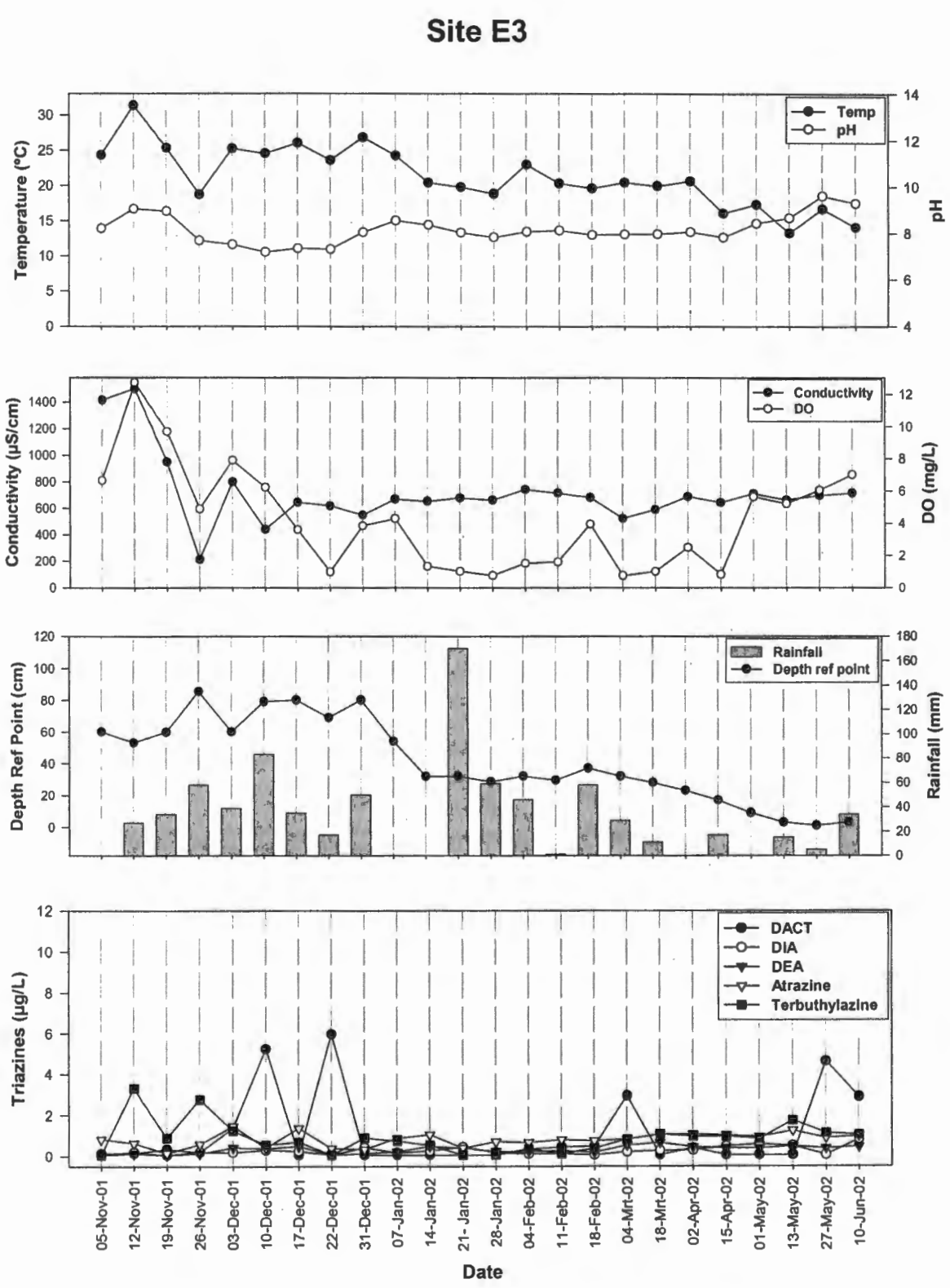

Figure 3.19: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

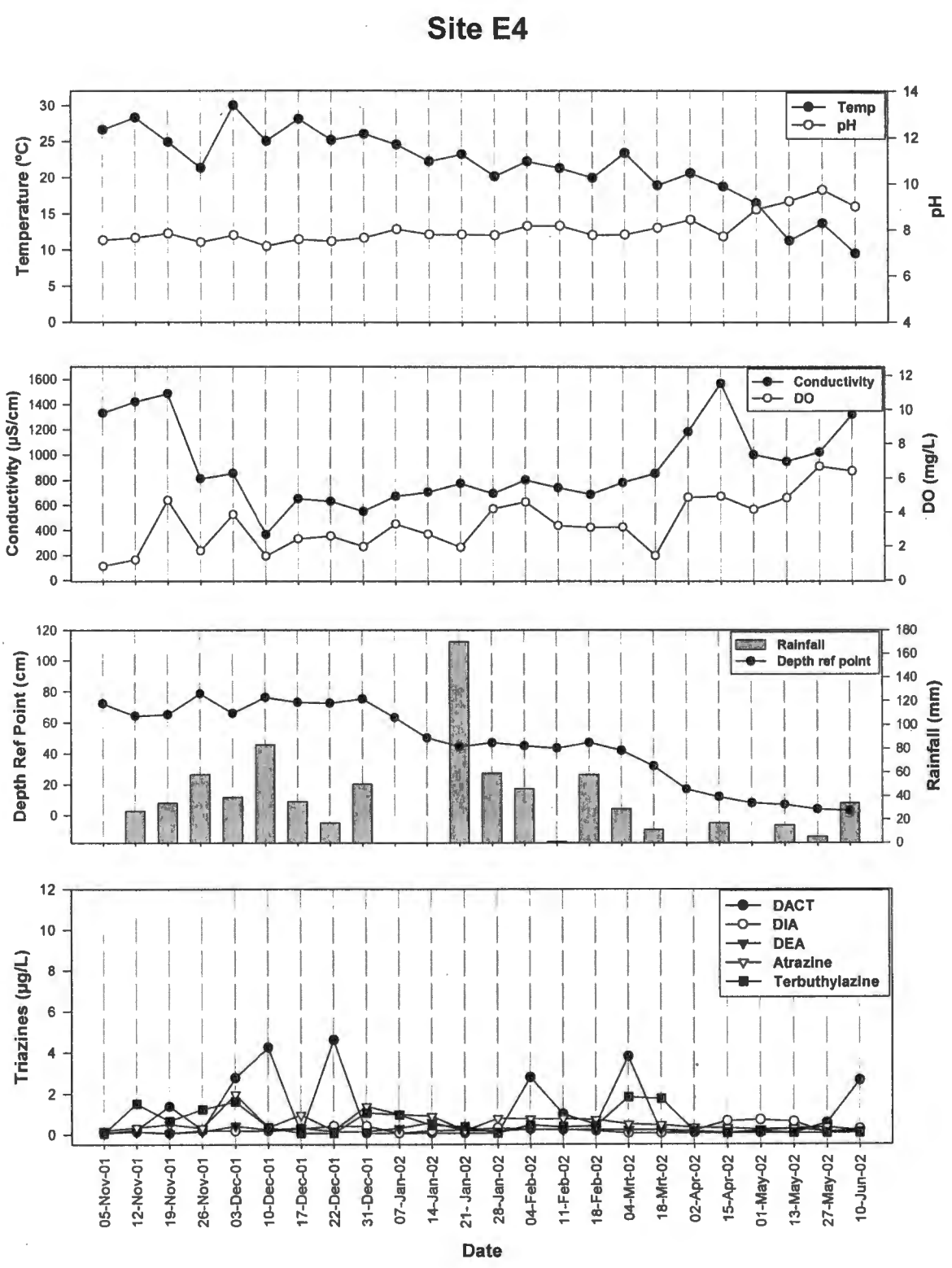

Figure 3.20: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

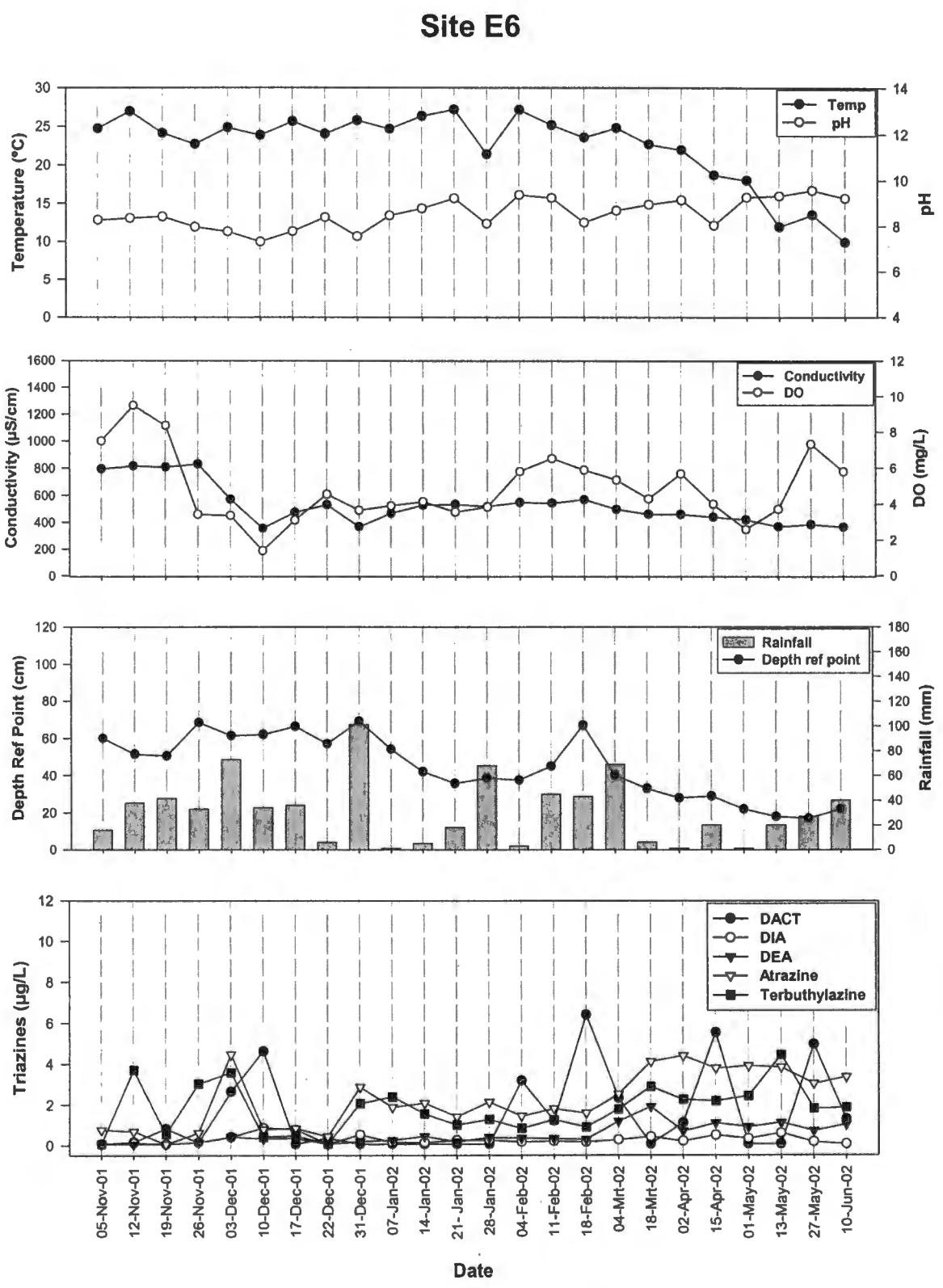

Figure 3.21: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

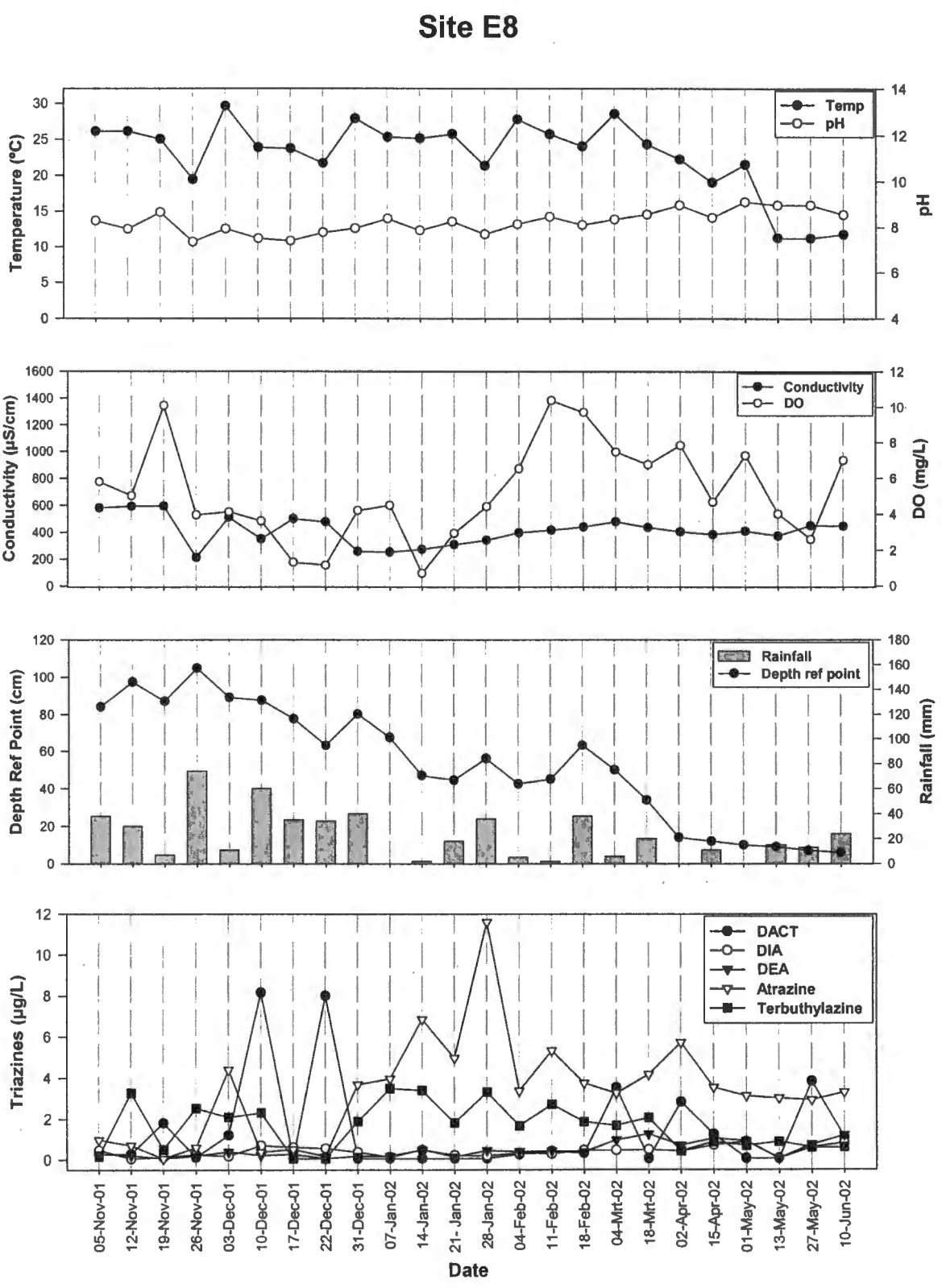

Figure 3.22: Water temperature, pH, conductivity, dissolved oxygen, water depth, rainfall at reference point and chemical residues detected.

3.3.5 Elemental Determinations Conducted on Water and Sediment Samples

A summary of results of the elemental determinations conducted on water samples are presented in Table 3.6 and those on sediment samples in Table 3.7. For comparison, water quality guideline concentrations are shown as well. No analyses were performed for the month of April 2002, for May 2002; nitrate and nitrite were analyzed and, for June 2002, only nitrate.

Table 3.6: Concentrations of elements and ions in water at reference and experimental sites.

Elements and ions	Units:	R1	R3	R6	E1	E3	E4	E6	E8	Guideline**
Aluminum as Al	mg/l	6.0	2.5	33.8	2.1	0.6	0.6	0.1	0.1	0.005-0.1
Antimony as Sb	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Arsenic as As	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.005
Barium as Ba	mg/l	0.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	Not available
Beryllium as Be	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Bismuth as Bi	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Boron as B	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Cadmium as Cd	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.000017
Calcium as Ca	mg/l	2.3	4.0	4.5	25.4	31.6	30.8	33.2	33.2	Not applicable
Chromium as Cr	mg/l	0.030	0.025	0.040	0.070	0.035	0.035	0.020	0.020	0.001
Cobalt as Co	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Copper as Cu	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.002
Iron as Fe	mg/l	4.5	2.6	21.3	1.1	0.6	0.7	ND	ND	0.3
Lead as Pb	mg/l	0.090	0.080	0.085	ND	ND	ND	ND	ND	0.001
Lithium as Li	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Magnesium as Mg	mg/l	3.1	3.8	3.2	28.6	27.4	27.0	28.4	28.4	Not applicable
Manganese as Mn	mg/l	ND	ND	0.1	ND	ND	0.2	ND	ND	Not available
Mercury as Hg	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Molybdenum as Mo	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.073
Nickel as Ni	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.025
Nitrate as NO3	mg/l	0.7	0.5	0.9	0.6	0.7	0.7	0.4	0.4	Not available
Nitrite as NO2	mg/l	0.5	0.5	2.2	1.0	0.7	0.6	0.6	0.6	0.06
Phosphorus as P	mg/l	0.0	0.2	0.1	0.1	0.7	0.9	0.4	0.4	Not applicable
Potassium as K	mg/l	1.2	2.8	7.4	12.2	19.0	19.2	11.4	11.4	Not applicable
Selenium as Se	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.001
Silica as Si	mg/l	8.7	4.7	49.2	5.5	5.8	4.8	4.3	4.3	Not applicable
Silver as Ag	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.0001
Sodium as Na	mg/l	2.4	2.8	1.3	63.6	106.4	115.8	55.2	55.2	Not applicable
Strontium as Sr	mg/l	ND	0.0	0.0	0.1	0.2	0.2	0.2	0.2	Not available
Sulphate as SO4	mg/l	3.0	3.2	6.2	23.6	29.5	25.7	26.7	26.7	Not applicable

Elements and ions	Units:	R1	R3	R6	E1	E3	E4	E6	E8	Guideline**
Sulphur as S	mg/l	1.6	2.0	2.1	9.1	10.7	10.4	9.6	9.6	Not available
Thallium as Tl	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.0008
Tin as Sb	mg/l	0.2	ND	0.2	ND	0.2	0.6	0.6	0.6	Not available
Titanium as Ti	mg/l	0.1	0.1	0.6	0.1	0.0	ND	ND	ND	Not available
Vanadium as V	mg/l	0.0	ND	0.1	ND	ND	0.0	ND	ND	Not available
Zinc as Zn	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	0.03
Zirconium as Zr	mg/l	ND	ND	ND	ND	ND	ND	ND	ND	Not available
Conductivity @25°C	mS/m	5.0	6.7	5.9	59.8	88.8	88.5	47.5	47.5	Not applicable
pH @25°C		7.7	7.1	7.1	8.5	8.0	8.0	8.5	8.5	6.5-9.0
Total Hardness *	mg/l	18.2	25.0	24.6	182.0	191.4	188.2	200.4	200.4	Not applicable
SAR	meq/l	0.244	0.248	0.114	2.16	3.64	3.6	1.68	1.68	Not applicable
CLASS		C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1-C.2.S.1	C.1.S.1-C.3.S.1	C.1.S.1-C.3.S.1	C.1.S.1-C.2.S.1	C.1.S.1-C.2.S.1	Not applicable

**CWQG. 1999. Canadian Water Quality Guidelines (and updates). Ottawa, ON: Task Force on Water Quality.

Table 3.7: Concentrations of elements in sediments at reference and experimental sites.

Element	Units:	R1	R3	R6	E1	E3	E4	E6	E8
Arsenic as As	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	7.01	ND	100.50	7.51	20.00	22.00	19.00	57.50
Copper as Cu	g/100g	ND	0.01	0.01	0.06	0.01	0.17	0.03	0.02
Iron as Fe	g/100g	2.89	3.16	1.05	7.06	5.06	2.61	5.05	9.38
Lead as Pb	mg/kg	0.31	0.18	ND	ND	0.02	ND	ND	0.10
Mercury as Hg	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	0.03	0.04	0.02	ND	ND	ND	0.01	0.01
Nickel as Ni	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ND	ND	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ND	ND	ND	ND	ND	ND	0.01	ND
Zinc as Zn	g/100g	ND	ND	ND	0.25	0.09	0.03	ND	ND

3.4 Discussion

The mean temperature recorded during this study corresponds very well with the ten-year mean for both experimental and reference sites. Rainfall figures, however, were exceptionally high with more than double the long-term average recorded for the months of November and December 2001. This resulted in a delayed corn-planting season. Also, the experimental sites were flushed for several days during these two months. All experimental sites overflowed at least until the end of January 2002. It is expected that concentrations of pesticides in the experimental sites would have been higher if less rainfall had occurred.

In the summer rainfall part of South Africa, corn is usually planted in late October – early November. Triazines herbicides are usually applied pre-emergence as a water-dispersed spray or in liquid fertilizers and again shortly after planting. Corn is usually treated with atrazine and terbuthylazine in a 1:1 mixture with a total application equal to 1 to 1.5 times the recommended dose (Solomon *et al.* 2002). According to farmers, atrazine and other triazines are being used in smaller quantities now compared to the early 1980's. During these years (1982 – 1984), between than 843 tons and 2 934 tons of atrazine was sold annually in South Africa (Hassett *et al.* 1987).

Due to the exceptionally high rainfall, the 2001 – 2002 planting season was delayed by about a month, with some farmers planting until mid January 2002. The delayed planting season resulted in much more corn being planted than was anticipated as the planting season for sunflower, groundnuts and other crops was over by the time the farmers could get into the fields.

The greater concentrations of atrazine and other triazines in sites E1 and especially E8 were expected as the farmer adjacent to site E8 applied higher rates of these herbicides. Sites E3 and E4 were overflowing for a longer period and atrazine concentrations were relatively low. Site E6 is a very large dam with

reasonably high levels of atrazine. The presence of terbuthylazine at reference site R6 was surprising, as no crops were being grown in the catchment area of this site. A possible explanation could be that the herbicide was used on the premises of the Naschem factory to eradicate weeds on the facilities boundaries and in firebreaks. The very low atrazine concentrations measured in the reference sites are probably the result of wet or dry deposition from nearby use areas. None of the sediment samples contained any atrazine or other triazines. Metabolites deisopropylatrazine (DIA) and deethylatrazine (DEA) followed the general trend observed for atrazine but metabolite diaminochlorotriazine (DACT) was detected in varying concentrations.

Atrazine is used in combination with various other herbicides including terbuthylazine, S-metolachlor and simazine. However, in the study area, atrazine was the predominant active ingredient.

Reinhardt *et al.* (1997) conducted a study on soil types and, of the 23 soil samples taken throughout the corn production area in South Africa, the Viljoenskroon area had the fourth highest percentage of sand, namely 81%. Due to the surface run-off and lateral movement, especially in sandy soils, water-bodies in corn production areas usually contain residues of atrazine and other triazines. A study in 1991 showed the presence of atrazine in groundwater and water-bodies ranging from small farm dams to large dams. Concentrations in groundwater approached 14 µg/L (Jansen van Rensburg 1991). In another study conducted during 1992, atrazine was detected in concentrations ranging from 0.73 to 14.97 µg/L in surface water and 0.49 to 3.89 µg/L in groundwater (Pick *et al.* 1992). The high rainfall and prolonged overflowing of the dams probably diluted atrazine concentrations in the water-bodies during my study. Frogs living in these dams were most likely exposed to greater concentrations atrazine and other triazine studying the years prior to this study.

None of the elements detected in the elemental scans occurred in concentrations exceeding published water quality guidelines and are unlikely to have adverse effects on *X. laevis*.

CHAPTER 4

CHAPTER 4

GONADAL RESPONSE OF *Xenopus laevis* LARVAE EXPOSED TO ATRAZINE MICROCOSMS

4.1 Introduction

According to Diana *et al.* (2000) a mounting body of evidence suggests that many amphibian species, across a wide spectrum of habitat types around the world, have experienced substantial declines in number and distribution over the last few years. Although several factors, including agrochemicals and habitat destruction, have been suggested to contribute to declines in several species (Carey *et al.* 1995; Hecnar 1995; Daszak *et al.* 1999, Kiesecker *et al.* 2001), the ultimate cause of these declines are likely to be multiple and varied (Diana *et al.* 2000).

Changes in morphology and physiology, accompanied by habitat shifts, result from an irreversible set of processes referred to as metamorphosis (McNabb *et al.* 1999). Among vertebrates, true metamorphosis occurs only in amphibians (most frogs) and fishes. The process of metamorphosis in amphibians has the potential for detecting chemical perturbation of development for the following reasons:

- Metamorphosis is under hormonal control.
- There is a robust ecological literature on the topic.
- It is easy to observe and quantify the change in gross morphology.
- The larval forms are generally aquatic and potentially exposed to chemical agents.
- Processes at this stage are relatively independent of maternally derived chemical influences.

- It is possible to study the effects of chemical perturbation on morphology using laboratory and semi-field conditions where exposure can be experimentally controlled.

Because the tissue-differentiation associated with reproduction is plastic during a critical period, many factors can influence gonadal development, including genotypic sex, hormones, or environmental factors (Chang *et al.* 1996; Donaldson 1996). Gonadal differentiation can also be indirectly influenced by changes in the enzymes that control steroid synthesis and metabolism (Piferrer *et al.* 1994). Likewise, environmental chemicals that act as hormone mimics or antagonists present in during a critical period of development can theoretically cause alterations in the reproductive system.

Exposure to steroids at stages outside the critical windows of sex determination and differentiation may also affect sexual development. However, longer exposure time and/or higher concentrations are generally required to cause an effect (Di Giulio *et al.* 1999). For example, stimulation of reproductive tract growth and inhibition of testes development appear to be characteristic of exposure of adult fish to exogenous estrogens and their mimics (Di Giulio *et al.* 1999).

Hayes *et al.* (2002b) investigated the effects of *X. laevis* larvae exposed from hatching to metamorphosis at atrazine concentrations from 0.1 to 200 µg/L. No effect on larval growth, developmental rate, mortality, time to metamorphosis, or size at metamorphosis in females or males were observed (Hayes *et al.* 2002b). However, it was reported that atrazine reduced the size of the male laryngeal dilator muscle at concentrations $\geq$ 1 µg/L. Atrazine exposures as small as 0.1 µg/L also were reported to induce gonadal anomalies in males (Hayes *et al.* 2002a). Another study using a different strain of *X. laevis* failed to demonstrate any effects of atrazine on laryngeal dilator muscle; however, animals exposed to 25 µg/L atrazine were observed to have significantly increased frequencies of discontinuous gonads (Carr *et al.* 2003). Exposure of *X. laevis,* larvae from 48-

72 h up to completion of metamorphosis, to atrazine at concentrations of 1, 10, and 25 µg/L showed no effects on post-hatching of treatment groups compared to reference groups. In both the reference and experimental groups, the hatching success was greater than 90% (Carr *et al.* 2003). Based on the gonadal morphology of the animals in the 25 µg/L exposure group, there were animals that showed intersex. Although the *X. laevis* in this group had gonads that were different in shape, size, and pigmentation from the reference group, the histological evaluation revealed that most of the intersex *X. laevis* had gonads that could be identified as either male or female (Carr *et al.* 2003). Coady *et al.* (2003) did a similar study where post-metamorphic *X. laevis* were exposed to atrazine at concentrations of 0.1, 1, 10, and 25 µg/L. Based on the gross morphology of the gonads, atrazine did not cause any concentration dependent effects on the gonad development or the frequency of gonadal anomalies (Coady *et al.* 2003).

According to a laboratory study conducted by Allran (2001), there were no effects observed in the development rate, percent metamorphosis, time to metamorphosis, mass of metamorphosis, and hematocrit values in *R. pipiens* larvae that have been exposed to atrazine at concentrations as high as 20 and 200 µg/L throughout metamorphosis. The conclusion was made that the concentrations of atrazine and nitrate compounds that are readily found in the natural environment, pose no significant threat to *R. pipiens* larvae.

The observations made on frogs in the laboratory raised the question of whether such effects can be observed in frog populations under field conditions at environmentally realistic exposures. It has been suggested that the occurrence of intersex in frogs of various habitats are commonly found at low frequencies and, occasionally at high frequencies (Solomon *et al.* 2003). Although many community-based toxicology studies have been performed to identify direct and indirect effects of atrazine, few have involved amphibians (Diana *et al.* 2000).

Rather than designing studies around standardized conditions, such as laboratory regulated temperature and photoperiods, it may be more productive to evaluate contaminant effects on the adaptations within the environment of the species. In this way, differences between adaptations and environment are acknowledged as part of the study design, for instance with the use of semi-natural microcosm.

With these observations in mind, the goal of this study was to determine the effect, if any, of atrazine at different concentrations on the number of days to metamorphosis and gonadal development of *X. laevis* up to Stage 66.

4.2 Materials and Methods

4.2.1 Study Design

During March 2002, twelve microcosms were constructed outdoors at the experimental facility of the Potchefstroom University, South Africa. The microcosms were adapted from pre-existing earthworm culture systems. Each microcosm was 2.25 m long, 1.2 m wide and 1.0 m deep and was lined with a polyethylene membrane. A 20 cm high wall was constructed around each microcosm to prevent surface water runoff entering the microcosms. The bottoms of the microcosms were covered with a three-centimeter layer of sandy soil previously used in earthworm cultures and filled with tap water to a depth of 0.4 m. Each microcosm held 1,100 L. A marker was attached to the side of each microcosm to indicate the initial water level. The water level was maintained throughout the study by adding additional tap water. Very little rain fell during the study period and water levels did not increase above the initial mark. Microcosms were covered with hail netting (nylon mesh 1 cm x 1 cm) fixed on metal frames. This was done to keep predators out such as dragonflies and birds. Macrophytes (*Ceratophylum*) from field sites were introduced and microcosms were allowed to stabilize for five months. In August 2002, 12 microcosms were randomly allocated in three sets of four. Due to the layout of the facility, all microcosms did not receive the same amount of sunlight. Microcosms 1, 2, 7 and 8 were cooler, because of the shade of a tree. For this reason, microcosms were numbered randomly to have warmer and cooler microcosms for each atrazine concentration (Fig. 4.1). One set of three microcosms received no atrazine and served as references. A stock solution was prepared by dissolving 100 mg atrazine in 1 L analytical grade methanol. Three microcosms per concentration were treated with atrazine to achieve initial concentrations of 1 µg/L, 10 µg/L, and 25 µg/L respectively (Fig. 4.1). The exposure study started at the end of August 2002.

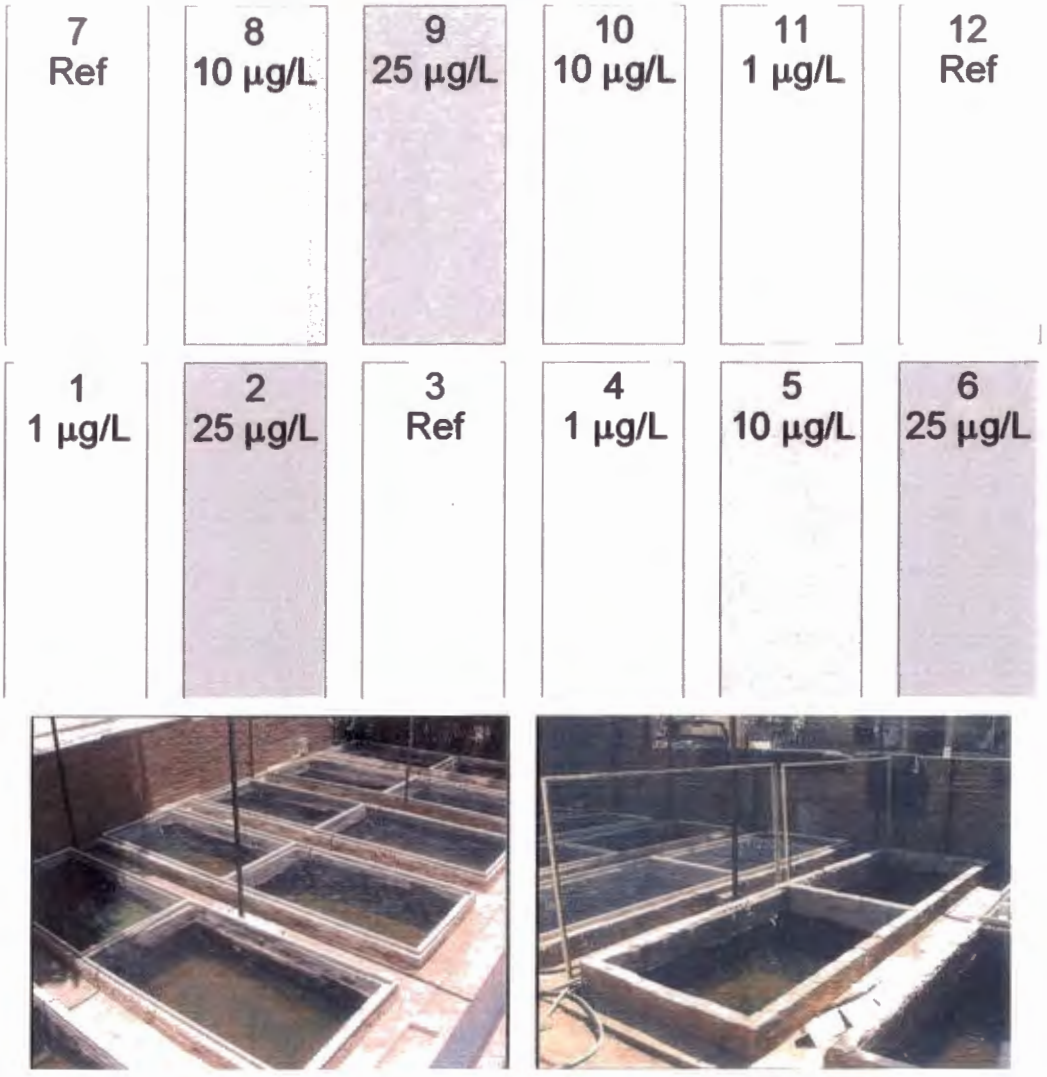

Figure 4.1: Experimental layout of microcosms. Four sets of three replicates per exposure were randomly placed.

Seven pairs of reproductive adult *X. laevis* were captured at an earth-walled dam 16 km to the north of the city of Potchefstroom, South Africa (Site R6 in Smith *et al.* 2003). This site was selected because of the absence of atrazine use in the watershed. On 28 August 2002, spawning was induced using commercially available chorionic gonadotrophin (Pregnyl). The male frogs were injected subcutaneous in the dorsal lymph sack for three consecutive days, while the females were injected on days two and three only (Table 4.1) (Van Wyk *et al.* 1984).

Table 4.1: Amount of Pregnyl injected in male and female *X. laevis* adults to induce spawning (Van Wyk *et al.* 1984).

DAY	DOSE FOR	
	Male	**Female**
1	250 I.U.	-
2	250 I.U.	50 I.U.
3	250 I.U.	500 I.U.

Males and females were placed together as pairs into breeding tanks (300 X 240 X 240 mm) after receiving the last Pregnyl treatment. The tank was fitted with a raised mesh floor to protect the eggs, and placed in a dark breeding room at ca. 30°C. The day after spawning, frogs were removed from the tank and the water with the embryos aerated. After hatching, the young tadpoles adhered to the side of the tank until day three when they started swimming freely and feeding.

Due to logistical time constraints, tadpoles from the same batch were added to the microcosm in two sets. Tadpoles were first released into the 12 microcosms on 5 September 2002. One hundred actively swimming four-day old tadpoles were siphoned from the breeding tanks to glass jars using a glass tube connected to a silicone tube. This process was repeated until each of the 12 jars contained 600 tadpoles. An additional 200 tadpoles were also released into each of the microcosms on 12 September 2002, to achieve the target population of 800 tadpoles. The 800 tadpoles per microcosm were well below the maximum density of 1 tadpole/L for *X. laevis* tadpole to reach metamorphosis successfully (Weldon 1999).

4.2.2 Data Collection

Water quality parameters were recorded on a weekly basis using a YSI 556 multi-probe system data-logger. The measurements included dissolved oxygen, conductivity, pH, air, and water temperatures. These measurements were taken every Thursday mid-morning in the middle of each microcosm at a depth of 10 cm. Bi-weekly, 1-L water-samples were collected from each microcosm to measure atrazine concentrations. This was done from 5 September 2002 to 12 December 2002. Mr. Peet Jansen Van Rensburg performed these analyses at the microbiology laboratories of the Potchefstroom University. For the phase extractions (Eisenreich *et al.* 1994), a 500-mL unfilterd water sample (measured in a volumetric flask) was extracted on pre-conditioned 500 mg C18 –SPE cartridges. The cartridges were cleaned prior to use with 3 mL of 85% DCM, 15% MeOH solution (v:v). Just prior to isolation, cartridges were conditioned ~ 2 mL of MeOH, followed by ~2 mL of Milli Q water. Solvents and water were drawn through the SPE cartridge under vacuum. Samples were interfaced to the SPE cartridge using Teflon tubing. The lower end of the extraction cartridge was connected to the vacuum, and sample water drawn through the cartridge at ~ 20 mL/min. Flow rate was controlled by adjusting the vacuum. Teflon tubing was cleaned before each extraction with Methanol and Milli-Q water.

After extraction, the cartridges were vacuumed to dryness. Analytes were extracted from the SPE cartridge by passing 7 mL of 85% dichloromethane, 15% methanol (v/v) solution, followed by 2 mL of methanol through the cartridge by gravity. The small amount of solvent remaining in the cartridge after extraction was forced through with air using a syringe. Samples were dried using a gentle stream of dry nitrogen gas. Methanol and then acetone were used successively to aid in the removal of water. The sample was reduced to dryness and reconstituted in 1.0 mL of acetone for GC/MSD analysis. A vortex mixer was used for 10-15 seconds to enhance dissolution of the residue.

All analyses were performed using a Agilent model 6890 series II^{plus} gas chromatograph interfaced to a 5973 mass selective detector (GC/MSD) operated in the selected ion monitoring (SIM) mode. The MSD transfer lines were maintained at 280 °C, and tuning was performed on a daily basis with PFTBA to ensure accurate mass calibration. The GCs were equipped for splitless injection and Agilent HP-5 or J&W DB-5 capillary columns (0.25 mm i.d. 30 m, 0.25-µm film thickness) were used for the separation. Electronic pressure programming was utilized in conjunction with a temperature program.

An elemental scan was conducted on one water-sample and one sediment-sample. To reduce costs, sub-samples from each microcosm were mixed and a representative pooled sample was analysed by SGS Laboratory (P.O. Box 5472, Halfway House 1685, South Africa).

4.2.3 Feeding

Algae and zooplankton flourished in the microcosms, but supplementary food (Complete Rabbit Pellets), supplied by Epol (Table 4.2), was given once a week and later twice a week. The food supplement per microcosm consisted of 20 g of pulverised rabbit pellets, homogenized in 500 ml of tap water.

Table 4.2: Composition of the complete rabbit pellets.

Protein	160 g/kg
Moisture	120 g/kg
Fat	25 g/kg
Fiber	170 g/kg
Calcium	18 g/kg
Phosphorus	7 g/kg

4.2.4 Development of Metamorphs

Extensive mortality occurred in microcosm 1 and 8 (1 and 10 µg/L atrazine, respectively) early in the study. The cause of the mortality could not be identified, however, microcosm 1 was shaded and it is possible that the tadpoles received a temperature shock when introduced into the microcosm. Tadpoles in microcosm 8 (10 µg/L) developed very slowly and were not yet at stage 66 during the period that the metamorphs were sampled. These two microcosms were excluded from the study. Metamorphs (Nieuwkoop & Faber stage 66 larvae that had completed metamorphosis) were removed from the microcosms every second day from November 2002 until mid January 2003. Metamorphs were initially captured with an aquarium net during the day. Later *X. laevis* bucket traps baited with marrowbones and ox-liver were used to catch metamorphs more efficiently. To avoid contamination, traps and nets were thoroughly rinsed with tap water before being used in the next microcosm. The metamorphs were then placed in clearly marked bottles and transported to the laboratory for further studies. A total of 600 frogs were collected; 150 frogs per treatment. As it was not always possible to obtain 50 specimens from each microcosm, this total was for the more than 75 metamorphs that were collected from any particular microcosm.

Collected specimens were anaesthetized through immersion in a 1:1000 dilution of 3-amino-benzoic-acid-ethyl-ester (MS-222). The date of completion of metamorphosis was recorded and body-mass was determined using an electronic Sartorius BP210S scale (0.0001 g accuracy). Snout-vent length was measured to the nearest 0.1 mm by means of a Teflon Vernier Caliper. After all the data were collected, a small cut was made on the abdomen to allow penetration of the fixative and a tag with the date and identification number was attached to the right hind leg of the frog. Specimens were fixed in Bouin's for 48 h, rinsed in water and transferred to 70 % EtOH. All the frogs were then dissected to expose the gonads for gross morphology. Frogs were sexed and the

gonads digitally photographed using a Nikon Coolpix 900 digital camera fitted on a Nikon SMX 1500 dissecting microscope.

4.2.5 Histology of Metamorphs

After completion of the gross morphology of all the 600 sampled metamorphs, 54 specimens were selected based on the anomalies found during the gross morphological examinations. These, as well as a further 150 more randomly selected specimens, were used for histological examination. Random numbers were generated using Microsoft Excel.

Gonads were surgically removed by dissection and prepared for histology. The preserved tissues were dehydrated in graded alcohols and embedded in paraffin. Seriall sections of 6 µm thick were made and routinely stained with Meyer hematoxylin and eosin (Fig. 4.2).

After staining and fixation, all the slides (1078 slides) were examined using a Nikon Alphaphot compound microscope. Every section was examined for any anomalies and noted in a catalog with specific reference to the specimen number, slide number, ribbon number and section number.

Figure 4.2: Serially sections were made using a Reichert-Jung 2050 microtome.

4.2.6 Statistical Analysis

Statistical analyses were performed with the help of R.B. Sielken and L.R. Holden*. The statistical program, Sigma Plot was used to calculate standard deviations, 95% confidence values and also to prepare all the graphs.

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Suite 230, 3833 Texas Ave, Bryan TX

4.3 Results

4.3.1 Monitoring of Atrazine Concentrations in the Microcosms

Atrazine concentrations in the various experimental microcosms remained consistent throughout the study and it was not necessary to add more atrazine at any stage (Fig. 4.3). On one occasion, atrazine was detected in one of the reference microcosms (no.3) at a concentration equal to the method detection limit (0.1 µg/L). The reason for this is not certain, however, it was most likely due to contamination in the laboratory, as atrazine was not detected before or after this event. Raw data are enclosed as Appendix 6. The temperature, pH, conductivity and dissolved oxygen (DO) are shown in Figures 4.4 – 4.7.

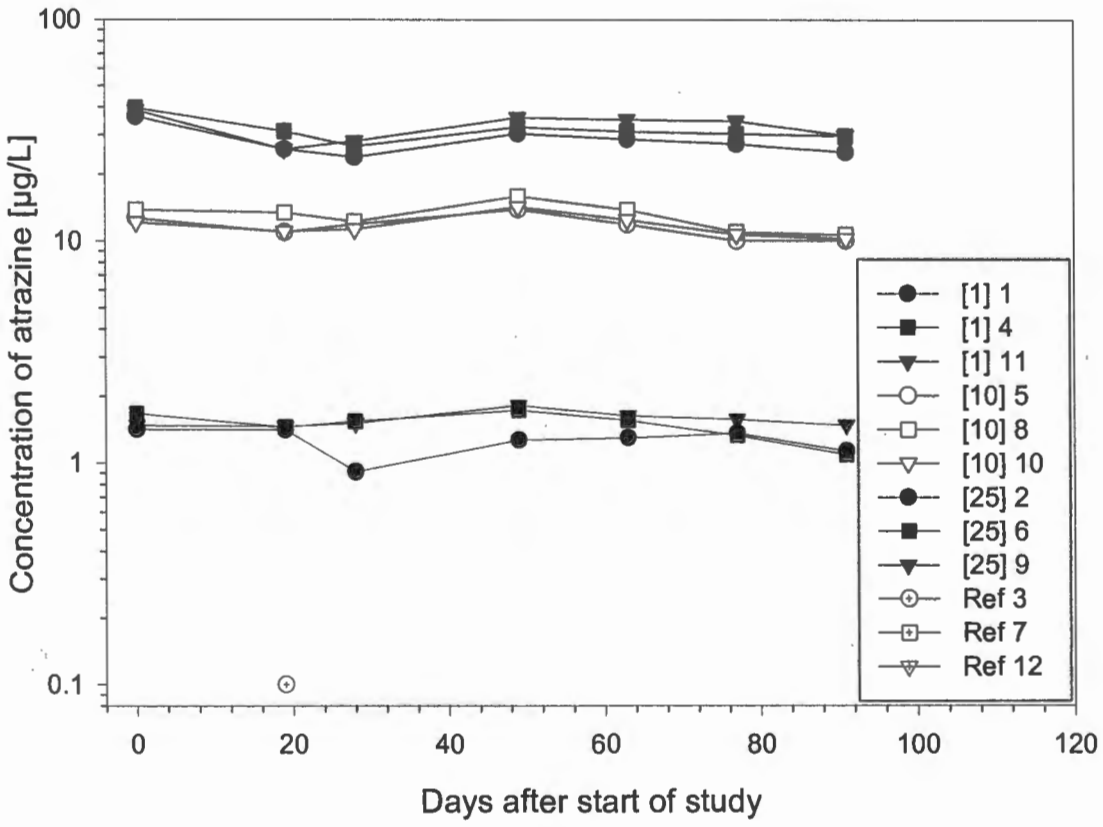

Figure 4.3: Atrazine concentrations as measured in microcosms.

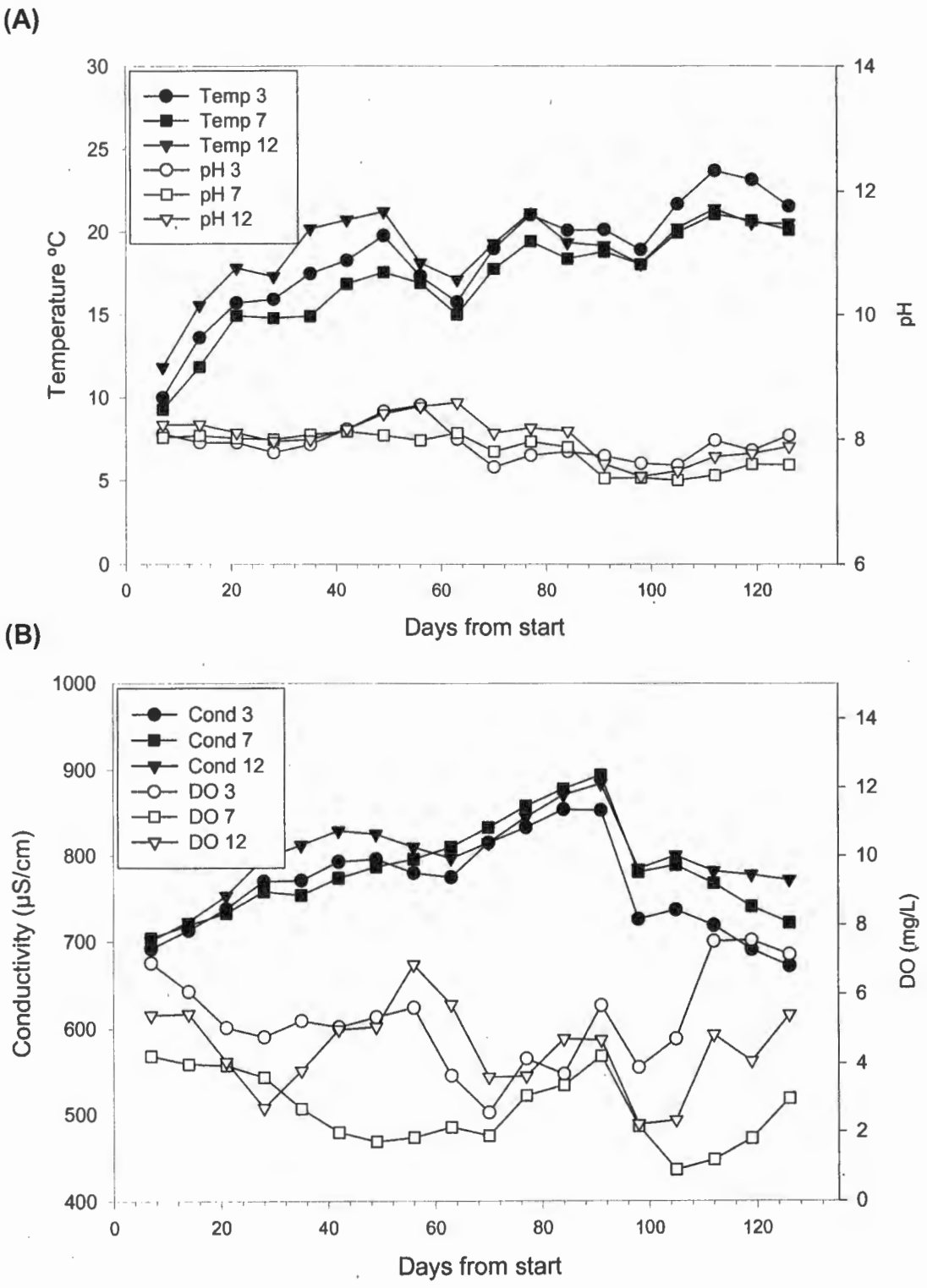

Figure 4.4: Graph **(A)** showing temperature and pH, Graph **(B)** conductivity, and DO of reference microcosms.

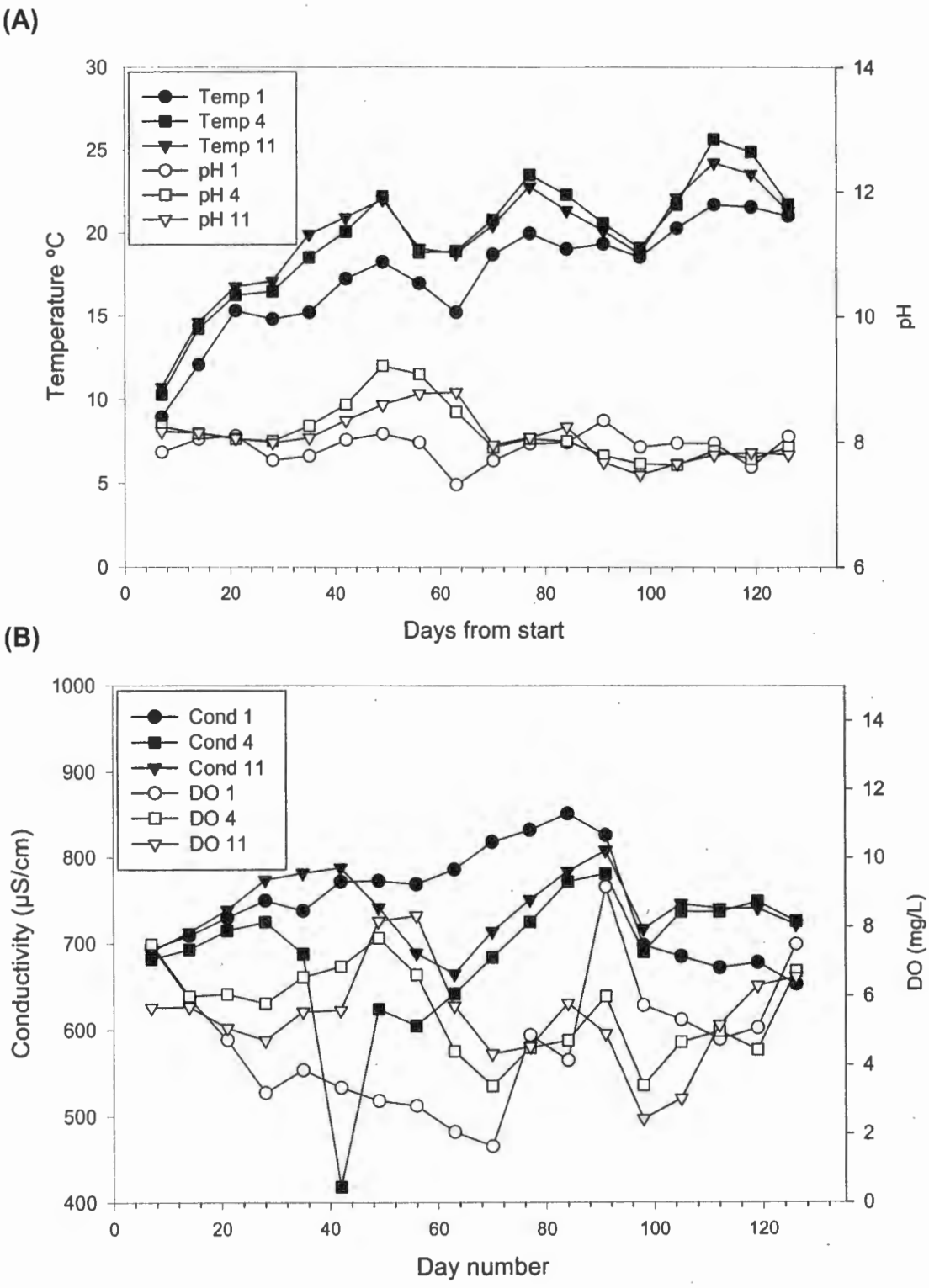

Figure 4.5: Graph **(A)** showing temperature and pH, Graph **(B)** conductivity, and DO of [1 µg/l] microcosms.

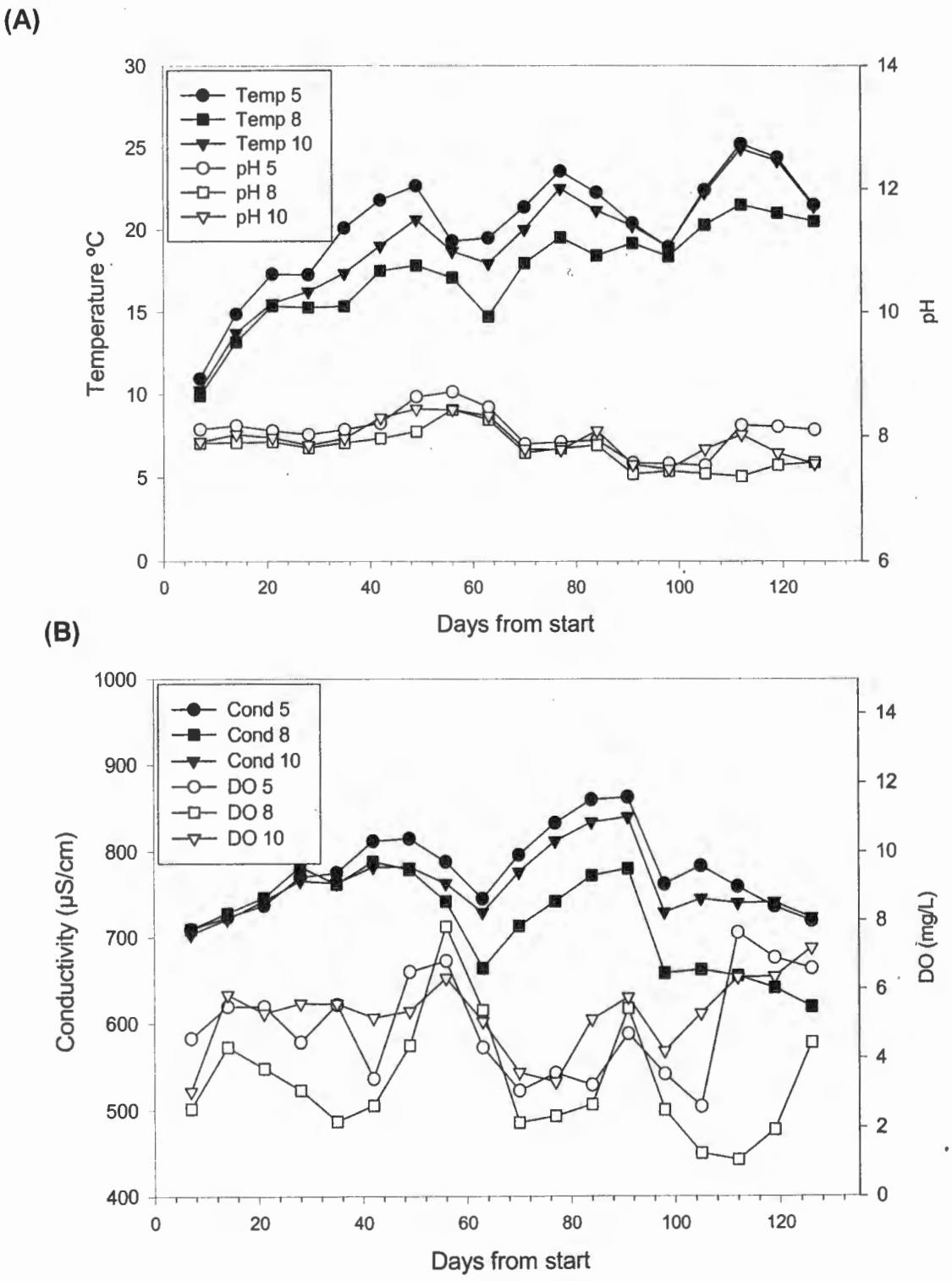

Figure 4.6: Graph **(A)** showing temperature and pH, Graph **(B)** conductivity, and DO of [10 µg/l] microcosms.

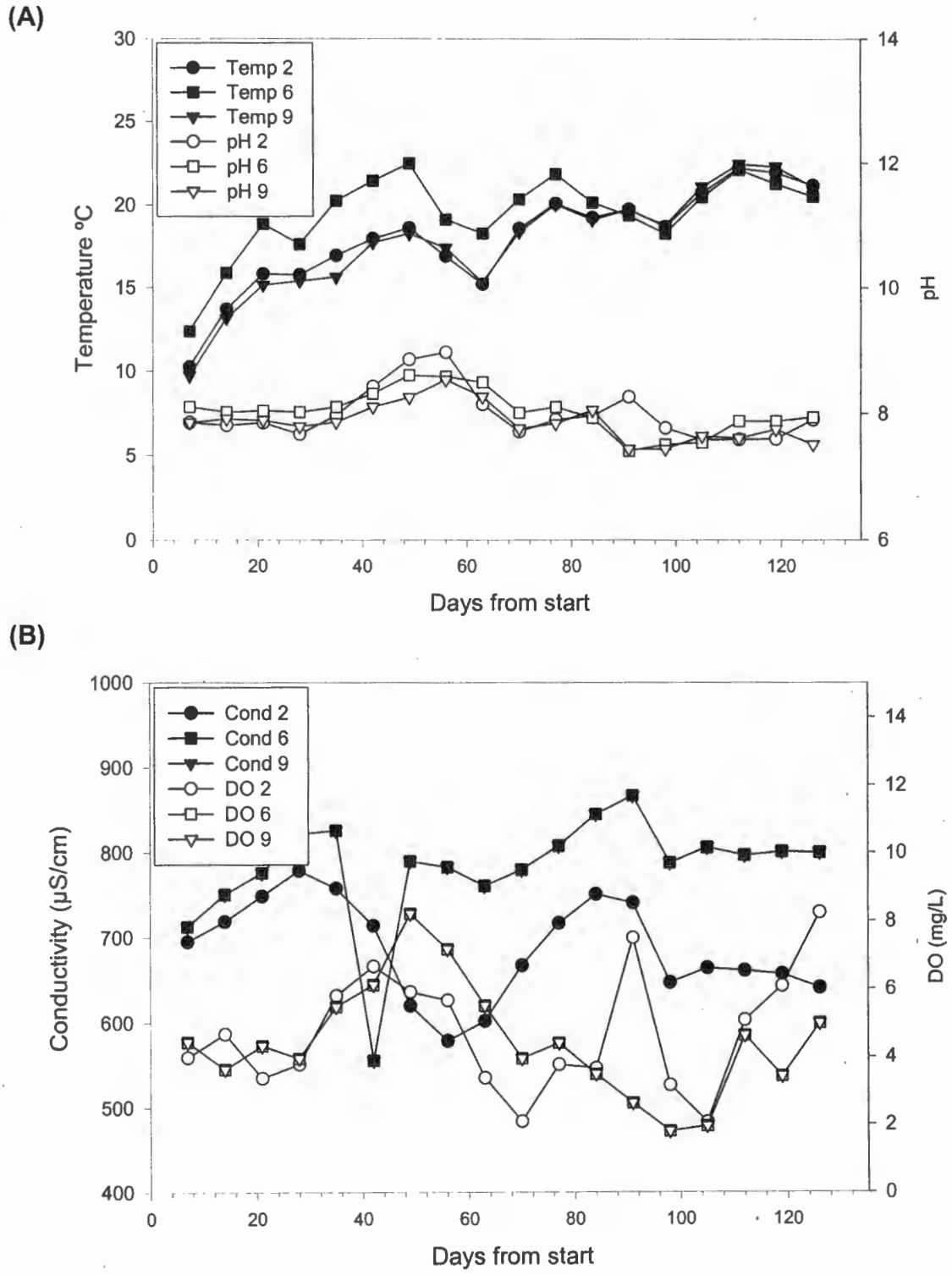

Figure 4.7: Graph **(A)** showing temperature and pH, Graph **(B)** conductivity, and DO of [25 µg/l] microcosms.

4.3.2 Elements scan of Water and Sediment of Microcosms

The element scan revealed high concentrations of calcium, magnesium, sodium and sulphur (Table 4.3).

Table 4.3: Elements, as detected in water and sediment scans (mg/L).

Elements	Water	Sediment	Guideline**
Aluminum (Al)	0.18	2.87	0.005 - 0.1
Arsenic (As)	<0.12	<0.12	0.005
Boron (B)	0.03	0.04	Not available
Barium (Ba)	0.03	0.32	Not available
Beryllium (Be)	<0.01	<0.01	Not available
Bismuth (Bi)	<0.12	<0.12	Not available
Calcium (Ca)	47.99	71.41	Not available
Cadmium (Cd)	<0.02	<0.02	0.000017
Cobalt (Co)	<0.05	<0.05	Not available
Chrome (Cr)	<0.04	<0.04	0.001
Copper (Cu)	<0.02	<0.02	0.002
Iron (Fe)	0.03	3.85	0.3
Potassium (K)	5.38	7.79	Not available
Lithium (Li)	<0.01	<0.01	Not available
Magnesium (Mg)	48.15	74.27	Not available
Manganese (Mn)	0.69	7.07	Not available
Molybdenum (Mo)	<0.04	<0.04	0.073
Sodium (Na)	35.25	46.37	Not available
Nickel (Ni)	<0.06	<0.06	0.025
Phosphorus (P)	<0.18	<0.18	Not available
Lead (Pb)	<0.14	<0.14	0.001
Sulfur (S)	29.62	0.39	Not available
Antimony (Sb)	<0.18	<0.18	Not available
Silicon (S)	1.19	9.55	Not available
Tin (Sn)	<0.15	<0.15	Not available
Strontium (Si)	0.06	0.14	Not available
Titanium (Ti)	<0.01	0.13	Not available
Vanadium (V)	<0.02	<0.02	Not available
Zinc (Zn)	<0.01	0.04	0.03
Zirconium (Zr)	<0.02	<0.02	Not available
Nitrate	0.31	-	Not available
Nitrite	<0.5	-	0.06

**CWQG. 1999. Canadian Water Quality Guidelines (and updates). Ottawa, ON: Task Force on Water Quality.

4.3.3 Development of *X. laevis* tadpoles and Gross Morphology

Tadpoles developed well in spite of the fact that the microcosms were colder than natural water-bodies. Tadpoles schooled normally and appeared to be under no stress (Fig. 4.8). No obvious tank effects were observed. Tadpoles developed except for the early mortality in microcosm 1 and slow development in microcosm 8. The first metamorphs reached developmental stage 66 in the 10th week of development (Fig. 4.9). From then on the number of frogs reaching stage 66 increased gradually. Collection of metamorphs was terminated in week 19 when the target numbers were met. Week 16 and especially 17 do not reflect a decrease in the numbers of frogs reaching stage 66, but rather reflect collecting effort (Christmas time).

Figure 4.8: Photograph showing schooling of tadpoles in one of the microcosms.

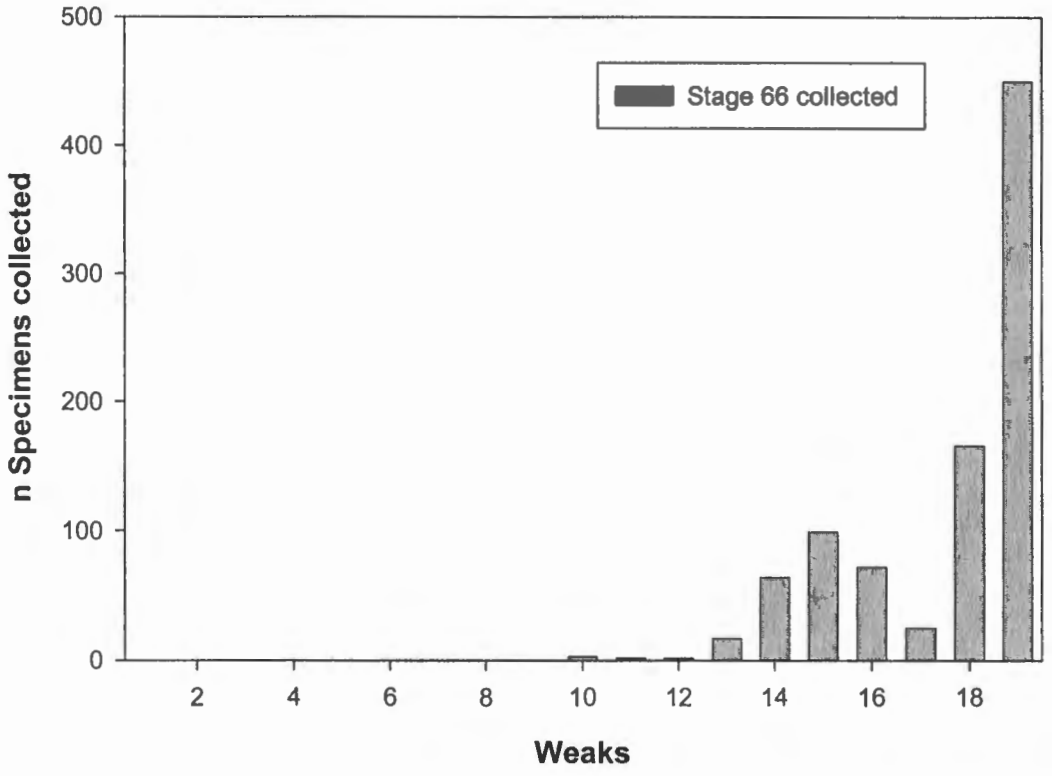

Figure 4.9: Cumulative number of NF stage-66 *X. laevis* collected over the exposure period.

All 600 specimens were examined visually and under a dissecting microscope during gross morphological assessment for gonadal anomalies. Pending histological examination, anomalies were calculated based on total number of metamorphs at each treatment concentration. Of the total number of frogs exposed to 1 µg/L atrazine, 1.33% showed gonadal anomalies compared to 0.67% from the 10 µg/L exposure, 3.33% from the 25 µg/L and 4.0% from the reference microcosms (Table 4.4 & Fig. 4.10) No inter-sex gonads were observed. All of the gonadal anomalies were observed only in males.

Table 4.4: Number of frogs examined with percentage of gonadal anomalies.

Nominal concentration (measured) in µg/L	Number of -				Percentage gonadal anomalies	
	Frogs collected	Males	Females	Gonadal anomalies	Total frogs	Males
Ref (0 - 0.1)	150	68	82	6	4.0	8.8
1 (0.91 - 1.82)	150	72	78	2	1.3	2.8
10 (10 - 15.9)	150	59	91	1	0.7	1.7
25 (23.8 - 39.7)	150	70	80	5	3.3	7.1

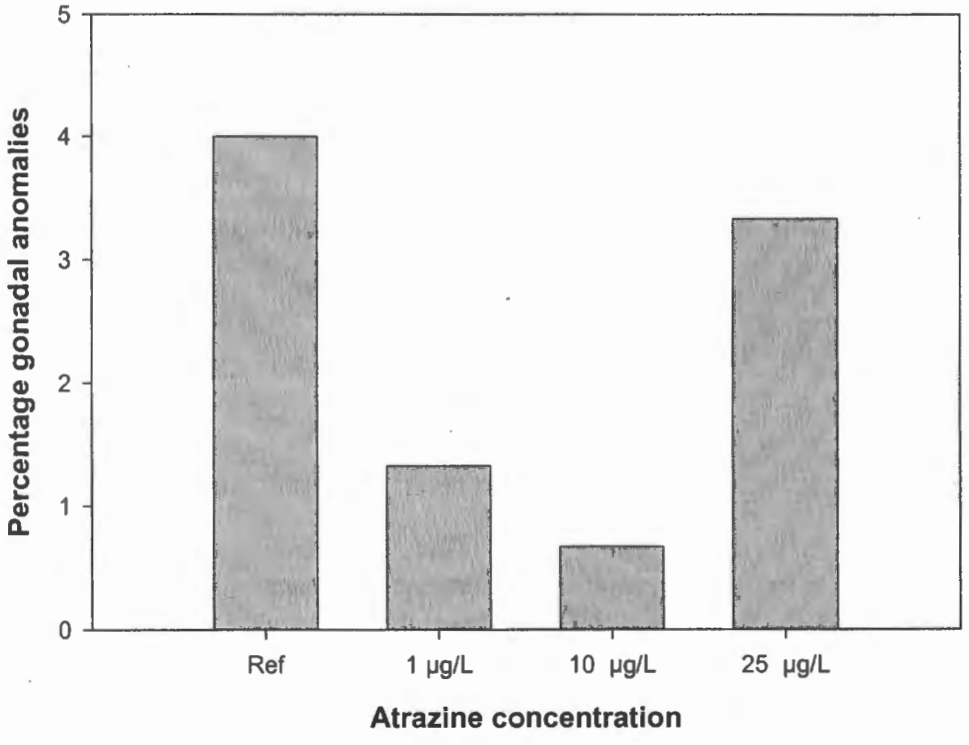

Figure 4.10: Graph showing percentage gonadal anomalies observed in male and female frogs based on gross morphology.

4.3.4 Histological Examination

A total of 214 randomly selected specimens from the control and the three atrazine concentrations were histologically examined. Off these, 45 were from the reference ponds (51.1% male and 48.9% female), 54 from 1 µg/L (55.6% male and 44.4% female), 51 from 10 µg/L (33.3% male and 66.7% female), and 58 from 25 µg/L (53.5% male and 46.5% female) (Fig. 4.11). During the preparation for histology, six specimens were lost due to an error in the preparation procedures, and thus were not included in the statistical analyses.

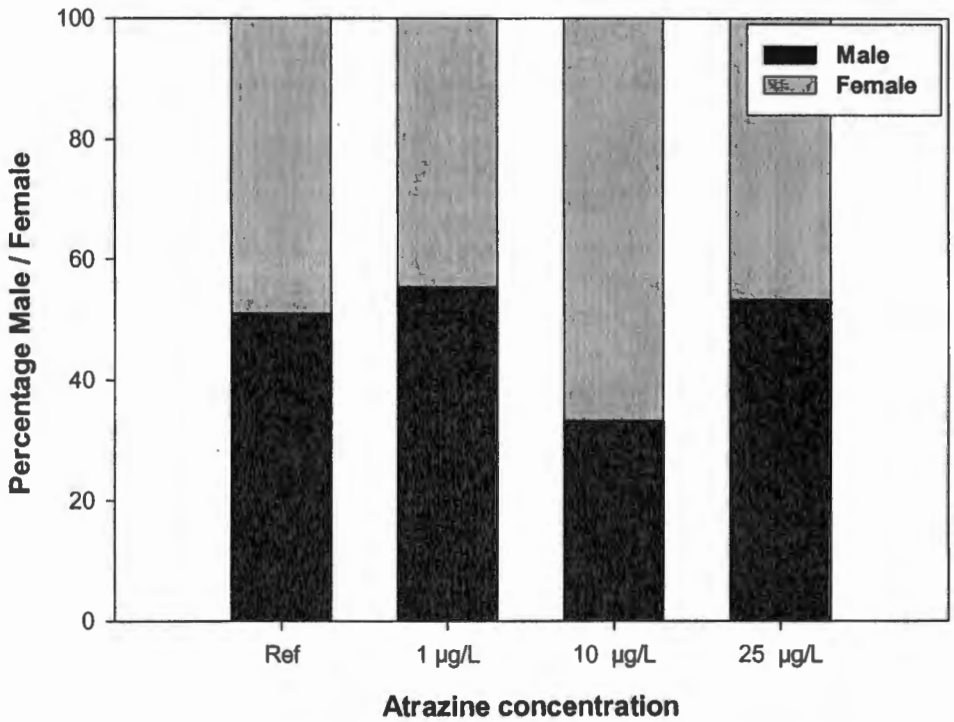

Figure 4.11: Percentage of male and female metamorphs histological examined.

The only gonadal anomaly observed was testicular oocytes. These oocytes were identified as stage 1 oocytes, because within the nucleus the chromosomes have assumed the lampbrush configuration (Fig. 4.12). The extra nucleoli are also

sequestered at the outer border of the nucleus. The oocytes were small and varied in diameter from 28.6 µm to 39.7 µm.

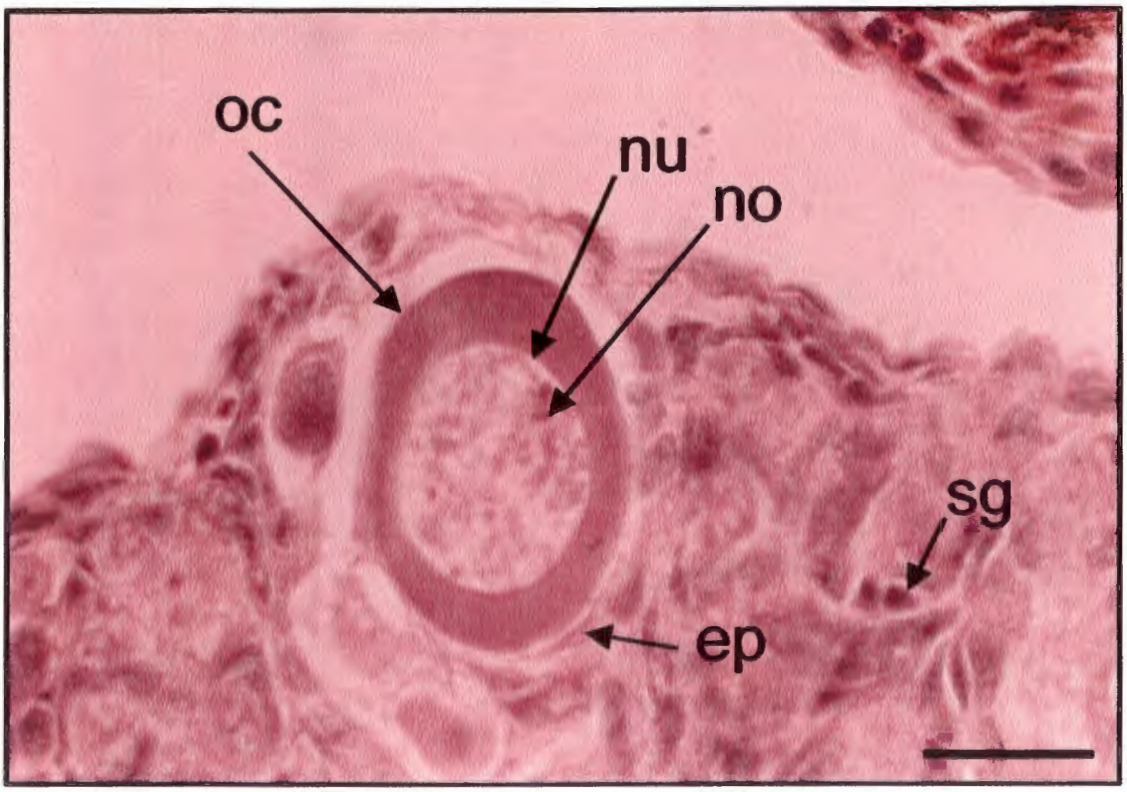

Figure 4.12: Photomicrograph showing a testicular oocyte.

Annotations: ep, epithelial cell; oc, oocyte; no, nucleolus; nu, nucleus; sg, spermatogonium.

(Scale bar = 20 µm)

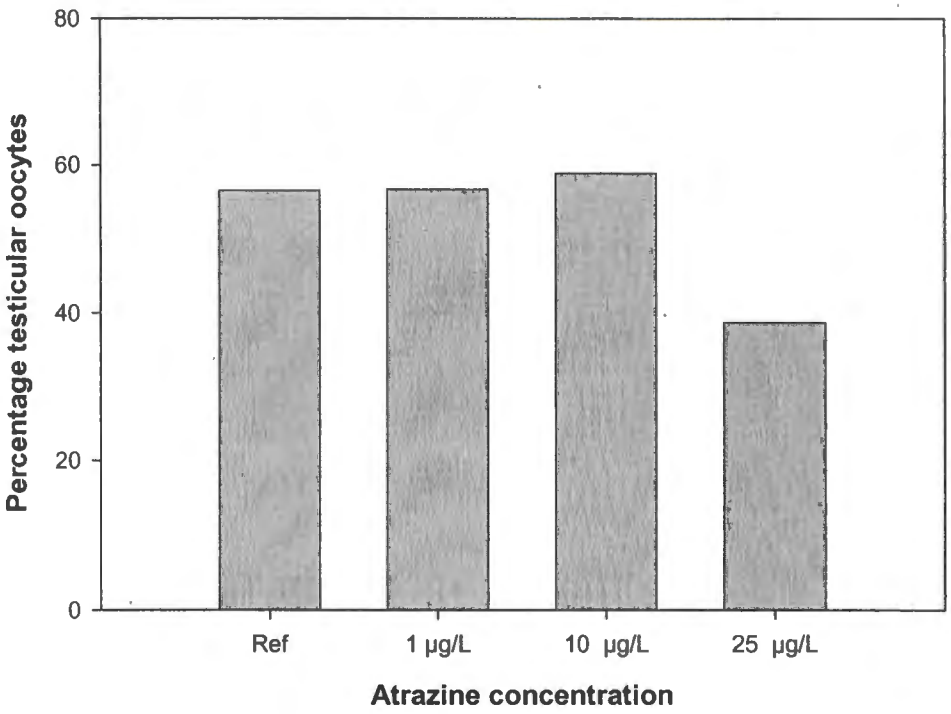

Figure 4.13: Percentage of frogs with testicular oocytes found at the different atrazine concentrations based on the histological examination.

Histological examination revealed the percentage of individuals with testicular oocytes to be 56.5% in the reference group, compared to 56.7% in the 1 µg/L group, 58.8% in the 10 µg/L group, and 38.7% in the 25 µg/L group (Fig. 4.13).

No gonadal anomalies were observed in any of the females. Testicular oocytes found in the testes were clear. Figure 4.14 shows a complete range of sections from beginning to end to give an idea of how small the detection range of these oocytes is.

A chi-square test of homogeneity (i.e. equality) was performed. The null-hypothesis that the percentage of frogs with testicular oocytes does not differ between different microcosms was supported. The chi-square value was 2.9482 with 3 degrees of freedom. This gave a non-significant p-value of p=0.3997

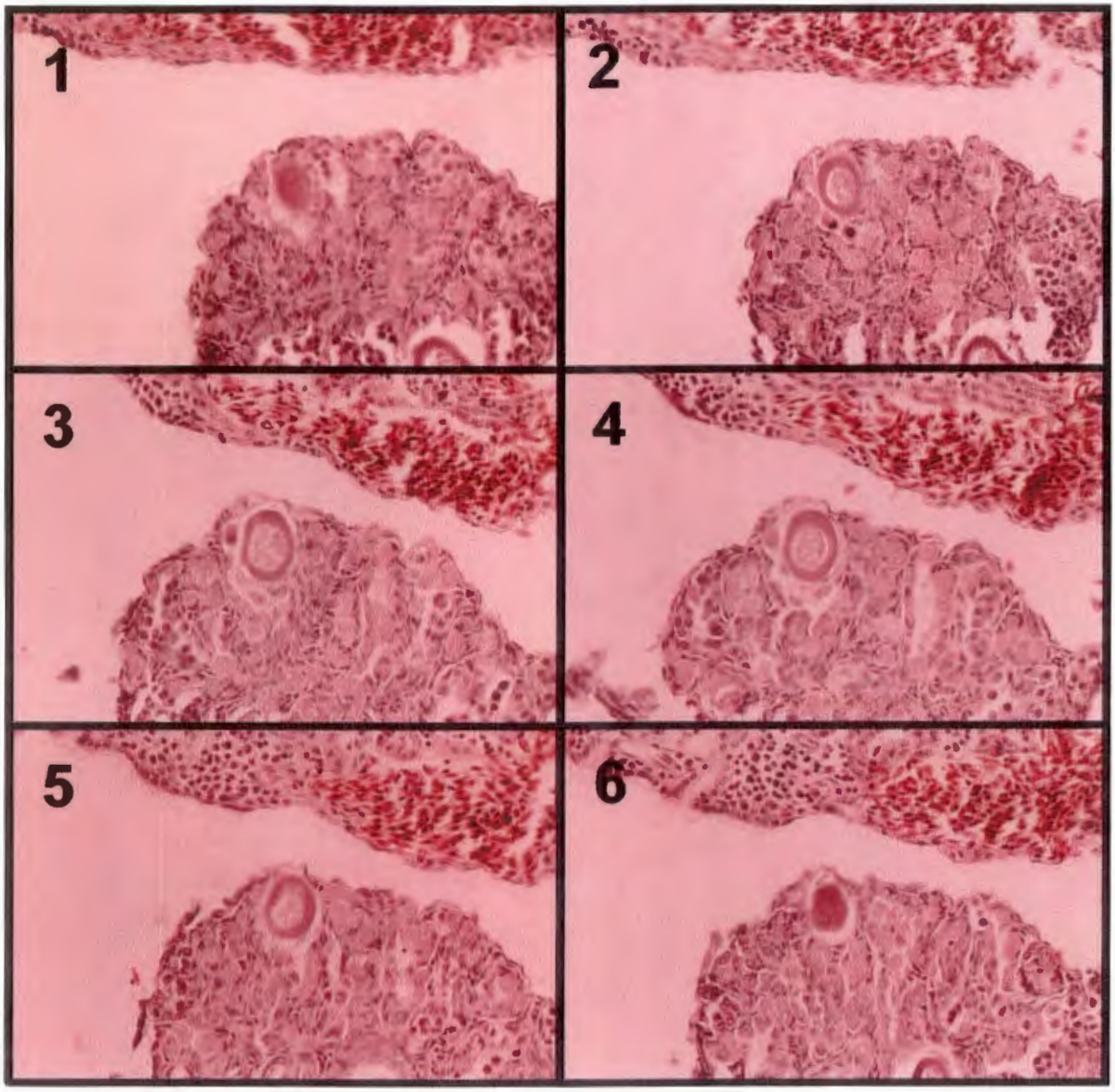

Figure 4.14: Series of histological sections (six sections) showing the oocyte, from beginning to end, in the testicular tissue at 6 µm.

Oocytes were found in one of the testes, and in a number of specimens, in both of the testes. This trend was observed in the control and at all the atrazine concentrations. In the reference group, the majority had oocytes in both of the testes (53.8%), while in the 1 µg/L group, 35.3% had oocytes in both of the testes. In the 10 µg/L group 60% had oocytes in both testes, and in the 25 µg/L group half of the specimens had oocytes in both of the testes Fig.4. 15). A chi-square test of homogeneity across the atrazine (and reference) concentrations gave a chi-square value of 1.8734 with 3 degrees of freedom and non-significant p-value of 0.5991.

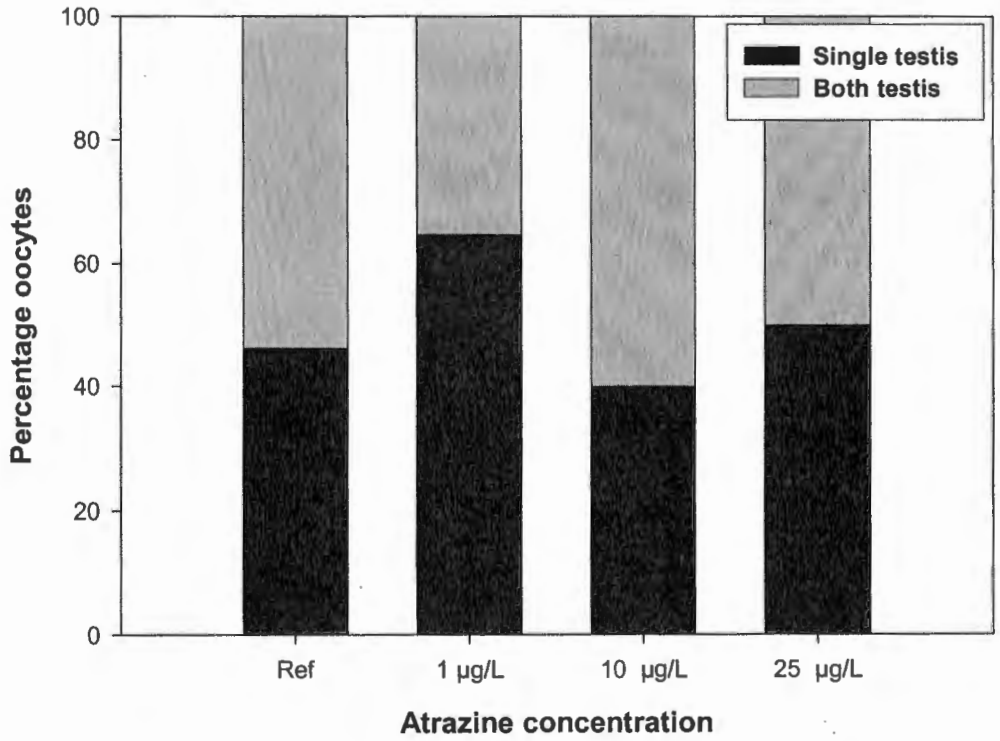

Figure 4.15: Percentage of frogs with oocytes in one or both testes at the different atrazine concentrations based on the histological examination.

The distribution of numbers of oocytes in the different concentration groups (Fig. 4.16). The mean number of oocytes per specimen varied from 8.5% for the 10

µg/L treatments to 11.08% for the 25 µg/L treatments. The reference group and 1 µg/L concentrations, were 9.5 and 9.8 respectively. The null-hypothesis that the number of testicular oocytes was the same for all sites was supported using a Kruskal-Wallis Test ($p < 0.2742$).

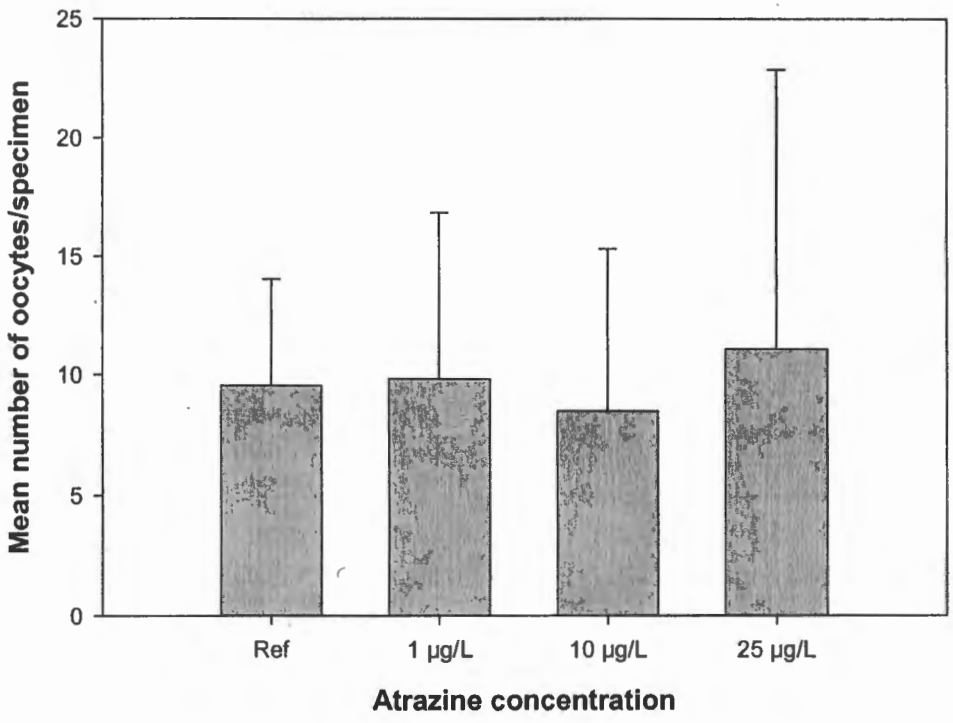

Figure 4.16: Mean number of oocytes found per specimen in the different atrazine concentrations (Bars represent 95% confidence values).

In most of the cases where oocytes were found in testicular tissue, there were more than one oocytes present (Fig. 4.17). The majority of affected specimens had between 2 – 10 oocytes in the testis (Ref: 46.2%, 1 µg/L: 64.7%, 10 µg/L: 60%, and 25 µg/L: 41.7%).

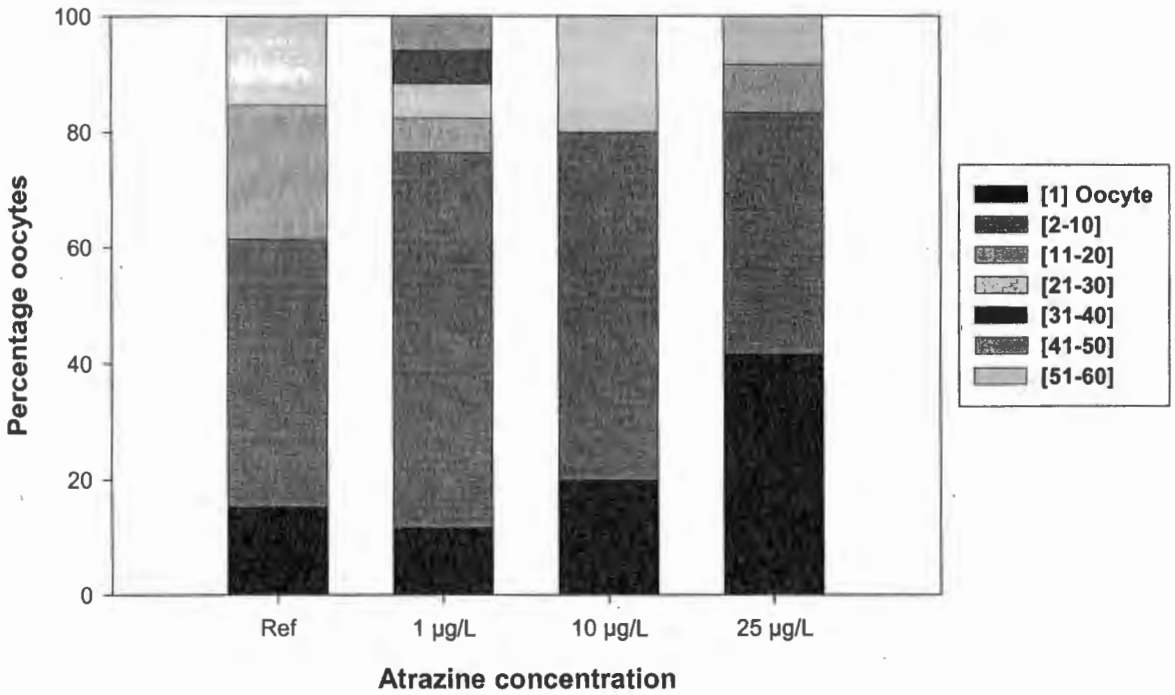

Figure 4.17: Percentage oocytes found in one of the categories specified.

4.4 Discussion

In this study, the first metamorphs reached developmental stage 66 in week 10. From week 13 onwards, greater numbers of frogs completed metamorphosis. Water temperatures in the microcosms were generally low and varied from 10°C in the first week to 20°C in week 10. Under controlled temperature conditions of 20° - 25°C, *X. laevis* tadpoles take only about 58 days to complete metamorphosis (Nieuwkoop *et al.* 1967). In its natural habitat, with fluctuating temperatures that may drop significantly at night in shallow waters, time to metamorphosis for *X. laevis* ranges from eight to nine weeks (Balinsky 1969). The slower development was most likely as a result of low water temperature. Ponds were covered with hail netting and partially shaded by trees and a building. Furthermore ponds were uniformly deep and thus did not have shallower parts where water temperature would be higher as is the case in natural ponds. Balinsky (1969) cited that the lethal temperature ranges for *X. laevis* embryos are higher than 35°C and lower than 10°C.

No inter-sex gonads were observed during gross morphological examination. The frequency of gonadal anomalies in the form of discontinuous testis observed was low and varied between 1.7% and 7.1% in frogs from the exposed microcosms and 8.8% in the reference microcosms. Most of the gonadal anomalies in the reference microcosms were observed in microcosm 12 and were not related to the single (and likely erroneous) detection of atrazine in reference microcosm 3. A total of 67 specimens were collected in microcosm 3. The gonadal anomalies observed in the exposed microcosms showed no concentration-responses to atrazine. Also, no gonadal anomalies were observed in frogs identified as females. Based on gross morphology, atrazine had no adverse effect on gonadal development at mean concentrations ranging from 1.4 to 30.8 μg/L under semi-field conditions.

According to the histological examination that was done on the gonads of the selected specimens, the total number of anomalies found in all four of the atrazine concentrations did not show clear statistical differences between the different concentrations and no concentration response. The highest percentage anomalies were found in the 10 µg/L concentration group, and the lowest percentage in the 25 µg/L group. Thus the highest percentage anomalies were not observed, as suspected at the 25 µg/L group, but rather an even distribution over the control and exposed groups occurred.

If one takes into account that the 10 µg/L concentration group had the least number of males, and all the gonadal anomalies were only observed in males, one can argue that if more males were histologically examined, this group might have had an even higher percentage of gonadal anomalies.

Although Hayes *et al.* (2001; 2002) have suggested that atrazine causes an increase in gonadal anomalies in *X. laevis* and other frogs, our results suggest that both gonadal anomalies and testicular oocytes occur in both exposed and control frogs. In addition, there was no concentration response between the frequency of oocytes and the total number of oocytes in individual frogs. The reasons for this difference is not clear, however, our observations are based on larger numbers of frogs the exposures of which were well characterized during the study. When considering the entire body of evidence presented in this thesis, and that in the literature, there is no compelling evidence to suggest that atrazine causes gonadal anomalies in *X. laevis*.

CHAPTER 5

CHAPTER 5

GENERAL DISCUSSION

5.1 Overview and Results

Several ecotoxicological and ecological risk assessment studies on the effects of atrazine on amphibians (Solomon *et al.* 1996) have been conducted and the results published in the scientific peer-reviewed literature (Sparling *et al.* 2000; Blaustein *et al.* 2003). Other than traditional toxicity studies with mortality and growth as endpoints, few studies have been conducted on potential developmental and reproductive effects in amphibians (Hayes *et al.* 2002a, b; 2003). The present study was designed to establish the effect of atrazine, if any, on natural occurring *X. laevis* populations and to control most of the confounders found in the natural environment, with a microcosm study.

In designing the present study it was hypothesized that:

1. Atrazine does not have adverse effects on *X. laevis* populations in corn-growing areas where atrazine is being used, when compared with *X. laevis* populations in areas with no atrazine use.
2. Based on previous findings, it would be expected to observe gonadal anomalies at atrazine concentrations of 25 µg/L or higher. To test this, semi-natural microcosms were designed and *X. laevis* exposed to various atrazine concentrations (0; 1, 10 and 25 µg/L).

This study was designed using a phased approach:
The first phase compared the age-size-class structure of *X. laevis* populations in corn-growing areas versus non-corn-growing areas. Possible sites were identified and evaluated for their suitability as reference or experimental sites. A mark and recapture study revealed that robust populations of *X. laevis* occurred

in all three reference and five experimental sites. The mass, snout-vent length, sex ratio, and age structure of all the frogs that were collected were compared. The mass and the snout-vent length data both showed that the female frogs were overall much larger than the male frogs, a normal occurrence in *X. laevis* (Kelley 1996). In addition, the mean snout-vent length at both the reference and experimental sites were also not different for male and female individuals.

Focusing on the age profile of the frogs, the mean age at reference sites was not different from mean age at experimental sites. Therefore there were no significant differences between the *X. laevis* populations in the reference and experimental sites.

In the second phase (Chapter 2), the water quality, and in particular the triazine levels were measured over a period of one field season. The rainfall figures were exceptionally high, with more than double the long-term (10 year) mean recorded for the months of November and December 2001. The highest concentrations of atrazine were measured in experimental sites E1 and E8, while E3 and E4 overflowed for a longer period and atrazine concentrations were relatively low. In reference site R6, a low atrazine concentration was measured. This may have been due atmospheric contamination. In spite of the fact that difficulties were experienced, this study provided a unique set of data that will serve in future as a reference for further ecotoxicological studies.

No atrazine was detected in any of the sediment samples and none of the elements detected in the elemental scans occurred in concentrations exceeding published water quality guidelines. These were unlikely to have had adverse effects on *X. laevis*.

In the third phase, *X. laevis* were collected at both the reference and experimental sites. From these frogs the necessary tissues were removed and send to the United States for analyses. Frozen blood plasma and gonads

collected for hormone and enzymatic activity were shipped to Michigan State University (East Lansing, MI, USA) while gonads and larynxes were shipped to Texas Tech University (Lubbock, LX, USA) for histological analyses. These results are not presented in this thesis but a draft of a paper for publication on the hormone analyses is attached as Appendix 3.

The fourth phase (Chapter 4), a microcosm study was undertaken to establish the effect of pure atrazine on developing *X. laevis*. Most of the frogs reached developmental stage 66 between weeks 13 and 19. This is slightly slower that described by Balinsky (1969), but within the normal range according to Kelley (1996). The slower development was most likely a result of low water temperature. Ponds were covered with hail netting and partially shaded by trees and a building. Furthermore, ponds were uniformly deep and thus did not have shallower parts where water temperature would have been higher, as is the case with natural ponds.

Based on the gross morphology of the 600 specimens examined that were exposed at different levels in the microcosms, the reference group had the highest percentage of gonadal (testis and ovaries) anomalies (4.0%), followed by the 25 µg/L group with 3.33% (Table 4.4). Of these 600 specimens, 214 were randomly selected for gonadal histological studies. Serial sections and examination of all sections revealed the percentage of individuals with testicular oocytes present to be 56.5% in the reference group, compared with 56.7% in the 1 µg/L group, 58.8% in the 10 µg/L group, and 38.7% in the 25 µg/L atrazine exposed group. No significant statistical differences were found ($p = 0.3997$, chi-square value) when comparing the reference and exposed groups. These oocytes where found in both testes. The majority of specimens with testicular oocytes had a single oocyte but some had as many as 58 oocytes. The largest number of oocytes was found in the 25 µg/L group but there was no statistical significant difference between treatment groups.

During the study described in this thesis, none of the female adults or female metamorphs collected during the 2001-2002 field studies showed any gross gonadal anomalies. At all sites, a low percentage of males showed gross gonadal anomalies. This varied between discontinuous testes, single testes and uneven testes size. These anomalies are not uncommon in nature, and have been observed at a frequency of < 5% in *X. laevis* and various other anuran species from areas where atrazine has not been used in the past (Du Preez personal communication).

5.2 Conclusions

The results obtained from this study support Hypothesis 1, which stated that the levels of atrazine observed in natural corn growing areas where *X. laevis* breeds, do not have negative effects, as measured by this study, on *X. laevis* populations.

Hypothesis 2, which stated that gonadal anomalies might be expected on *X. laevis* frogs at atrazine concentrations of at least 25 µg/L, in semi-natural microcosms, was not supported by the data obtained from the present microcosm study.

As in the case with similar studies investigating natural and semi-natural exposures, some inherent causl-effect related uncertainties are to be expected, but cannot be avoided.

- Exposure of natural occurring *X. laevis* populations are likely to be to a cocktail of different xenobiotic chemicals, and / or to chemicals with raised environmental concentrations due to anthropogenic activities, such as heavy metals. To test for this confounder, water samples were collected on a weekly basis and sediment samples collected on a bi-weekly basis,

from both the reference and experimental sites, to scan for selected agrochemicals and other water quality criteria. Therefore, the chemicals that were present in all the sites during this study are known and described (Chapter 3).

- Movements of *X. laevis* between various sites might have played a part in the population structure, as well as causing the frogs to be exposed to variable atrazine levels. In the present study, a number of individuals from each site were marked and no movements between sites were observed, during the population characterization part of the study (Chapter 2).

- *X. laevis* frogs, that appear to be normal on a gross morphological basis and histological examination, might not necessarily function normally on a physiological or genetic level. This is very difficult to investigate, because to examine a specimen on histological level, it must be sacrificed. However, the *X. laevis* populations of the exposed and reference sites showed no differences on population densities and other population characteristics (Chapter 2, *X. laevis* Population Profile).

- The occurrence of the exceptionally high rainfall during this study period (2001-2002) might have had a diluting effect on the atrazine levels in the experimental sites, and had a possible diluting effect. Additionally, rain borne atrazine and leaching might have introduced atrazine into the reference sites. However, because the atrazine levels were regularly measured at all the sites, the actual exposure levels are known. While the concentrations in the field sites were not as great as may be expected in years of normal rainfall, they were greater than those reported to cause gonadal anomalies in laboratory studies in *X. laevis* (Hayes et al, 2002a) and in the *R. pipiens* in the laboratory and field (Hayes 2002b; 2003). These conclusions that atrazine had no adverse effects on gonadal development in *X. laevis* are further supported by the results of the semi-

field microcosm studies. Here developing frogs were exposed to atrazine-only at measured concentrations of up to 30 µg/L and showed no increase in gonadal anomalies and no concentration-response in terms of the frequency of gonadal anomalies.

- The natural occurring *X. laevis* populations might have built up a level of resistance to certain chemicals, including natural and anthropogenic endocrine disrupting chemicals. Because atrazine is the most widely used herbicide in the catchment area of the experimental sites (Chapter 3, Characteristics of experimental sites), and has possibly been used in this area since 1956, this might be the case. It might be argued that the levels in the reference sites indicate an almost complete absence during the study period, but historic contamination of these sites, and therefore the exposure history of the resident populations are not known. In the case of the reference sites, significant exposure is however unlikely, since the reference area is almost exclusively a cattle farming area, due to topography and poor soil conditions. In addition, the parental frogs used in the microcosm study were all collected from a reference site that had no measurable levels of atrazine. The combination of the known atrazine history of the reference sites (Chapter 2), together with the lack of measurable effects observed in the microcosm study (Chapter 4), and the degree of correspondence between the observed effects of the naturally (Appendix 2) and controlled exposed frogs (Appendix 2), provides enough confidence to state that historical selection was not a significant confounder for this study.

The importance of atrazine as an herbicide in South Africa, and for that matter the rest of the world, cannot be overemphasized. Atrazine is a very cost-effective herbicide and is widely used in various parts of the world, mainly for the production of corn, an important crop in terms of human and animal nutrition. If atrazine use were to be restricted, it would have significant financial implications

for the farming community. Alternative products are more expensive and not necessarily without environmental risks.

5.3 Recommendations and Further studies

A study of atrazine and its effect on frogs has many facets. Only some of these could be investigated during the present study, leaving much scope for further studies. Future investigations should concentrate on the following:

- At this stage little is known of the biology of most amphibian species including *X. laevis*; so these evaluations will demand additional ecological and life history information.

- The specific biochemical mechanism by which pesticides could alter sexual differentiation in frogs is poorly understood at present. This will need to be addressed.

- The effect of hermaphroditic individuals on a given anuran population remains to be investigated. Whether phenotypic males with intersex gonads can (1) vocalize effectively (2) are attractive to normal females as well as to phenotypic females with intersex gonads; and (3) collectively perform the behavioral requirements necessary for proper mating, including the release of functional sperm and ova, are unknown (Murphy *et al.* 2000).

- How genetic sex relates to phenotypic sex in hermaphroditic frogs also remains to be studied. Such investigations would be greatly facilitated by identification of genetic markers for sexing male and female amphibians. The influence of contaminants and other environmental stressors on

reproductive determination, differentiation, physiology, and behavior remain to be studied adequately.

- Amphibians, especially *X. laevis* is a good model organism for field and microcosm studies on the effects of contaminants in surface waters because they are 100% aquatic and would thus be exposed in all stages of their life-cycle. They could thus facilitate the identification of substances such as developmental or reproductive toxicants that may only affect anurans at sensitive stages of development. Over time, amphibians as a group have developed a wide range of exquisite strategies for resisting environmental insults. Toxicology studies need to consider these mechanisms and do further studies on them.

CHAPTER 6

CHAPTER 6

Summary

It has been reported that exposure of *Xenopus laevis* to atrazine in laboratory studies increase the incidence of intersex gonads and induced changes in plasma testosterone concentrations. However, the response to atrazine observed in laboratory tests with *X. laevis* has yet to be observed in exposed field populations. The aim of this was to ascertain whether exposure to atrazine and related triazines has caused endocrine responses in *X. laevis* under field and semi-natural microcosm conditions in South Africa. Corn is grown intensively in the north western part of South Africa. Atrazine concentrations in the experimental sites ranged from 1.5 – 11.6 µg/L and in reference sites from 0.05 – 0.69 µg/L during the field use season. Starting in November 2001, weekly and later bi-weekly samples of water were taken to characterize exposure of *X. laevis* populations to atrazine and related triazines. Tissue samples were taken from frogs for hormonal, gonadal, and aromatase activity. No gonadal abnormalities were observed in females. In male frogs a few frogs with only one testis were observed but no correlation was found between abnormalities and atrazine exposure. No hermaphrodite specimens were observed.

A microcosm study with three replicates of atrazine concentrations 0, 1, 10 and 25 µg/L was designed. *X. laevis* tadpoles were exposed until completion of metamorphosis. 150 specimens per concentration were dissected. Based on gross morphology, no hermaphrodite specimens were observed. Deformed testes were observed but showed no dose response to atrazine. In reference ponds 4.00% of males had deformed testes. 1.335, 0.67% and 3.33% of males showed deformities at 1, 10 and 25 µg/L atrazine respectively. No deformed ovaries were observed in females. 214 specimens were randomly selected for histological examination and serially sectioned. Results obtained supported the findings of the gross morphology that gonadal anomalies showed no dose

response to atrazine. Histological examination revealed the percentage of individuals with gonadal anomalies to be 56.5% in the reference group, compared to the 56.7% in the 1 µg/L group, 58.8% in the 10 µg/L group, and 38.7% in the 25 µg/L group.

OPSOMMING

Verslag is gelewer dat die blootstelling van *Xenopus laevis* aan atrasien in laboratoriumstudies die voorkoms van interseks gonades verhoog en veranderinge in plasma testosteroon konsentrasies voortbring. Nietemin, die reaksie tot atrasien waargeneem in laboratorium toetsings met *X. laevis* moet nog waargeneem word onder veld bevolkings. Die doel van hierdie studie was om te bepaal of die blootstelling aan atrasien en verwante triasiene, endokriene reaksies uitlok by *X. laevis* onder veld- en semi-natuurlike mikrokosmos toestande in Suid-Afrika. Mielies word intensief geplant in die noord-westelike dele van Suid-Afrika. Atrasien konsentrasies in die eksperimentele damme het gewissel van 1.5 - 11.6 µg/L en in kontrole damme vanaf 0.05 - 0.69 µg/L tydens die veldseisoen. Beginnende November 2001, is weeklikse en later twee-weeklikse monsters van die water geneem om die blootstelling van *X. laevis* bevolkings se reaksie tot atrasien en ander triasiene te karakteriseer. Weefselmonsters is geneem van die paddas vir hormonale, gonadale, en aromatase aktiwiteite. Geen gonadale afwykings is waargeneem in wyfie paddas nie. By manlike paddas is 'n paar paddas met slegs een testis waargeneem, maar geen korrelasie is gevind tussen abnormaliteite en atrasien blootstelling nie. Geen hermafroditiese voorbeelde is waargeneem nie.

'n Mikrokosmos studie met drie replikate van atrasien konsentrasies 0, 1, 10 en 25 µg/L is ontwerp. *X. laevis* paddavissies is blootgestel tot en met die voltooiing van metamorfose. 150 Voorbeelde per konsentrasie is gedissekteer. Gebasseer op gros morfologie, is geen hermafroditiese voorbeelde waargeneem nie. Misvormde testikels is waargeneem, maar het geen dosis reaksie tot atrasien aangetoon nie. In verwysingsdamme het 4.00% van die manlike spesies afwykende testikels getoon. 1.33%, 0.67% en 3.33% van die manlike spesies het afwykings getoon by 1, 10 en 25 µg/L onderskeidelik. Geen misvormde ovaria is waargeneem in die wyfie paddas nie. 214 Voorbeelde is lukraak geselekteer vir histologiese ondersoeke en is reeksgewys gepartikuleer. Resultate verkry het die bevindings van die gros morfologie ondersteun dat gonadale anomaliteite geen

dosis reaksie getoon het tot atrasien nei. Histologiese ondersoeke het getoon dat die persentasie individue met gonadale anomaliteite 56.5% is in die verwysingsgroep, vergelykend met 56.7% in die 1 µg/L groep. 58.8% in die 10 µg/L groep en 38.7% in die 25 µg/L.

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APPENDICES

APPENDICES

APPENDIX 1

Poster, SETAC North America, 2002

Exposure characterisation and response to field exposures of *Xenopus laevis* to atrazine and related triazines in South African corn growing regions

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ABSTRACT

It has been reported that the exposure of Xenopus to atrazine in laboratory studies increased the incidence of inter-sex gonads and induced changes in plasma testosterone concentrations. However, the responses to atrazine observed in laboratory tests with *X. laevis* are yet to be observed in exposed field populations. The aim of this study was to ascertain whether exposure to atrazine and related triazines has caused endocrine responses in *X. laevis* under field conditions in South Africa (SA). Metamorphs and adult *X. laevis* were collected and examined. No gonadal abnormalities were observed in females. A few male frogs with only one testis were observed but no correlation was found between abnormalities and atrazine exposure. No indication of inter-sex was observed in any of the examined specimens.

INTRODUCTION

A recent laboratory study reported that atrazine exposures (0.1 to 200 µg/L) not only decreased laryngeal size in males but also increased the incidence of inter-sex gonads in *X. laevis*. The aim of this study was to ascertain whether exposure to atrazine and related triazines has caused endocrine responses in *X. laevis* under field conditions in South Africa

METHODOLOGY

- Mark and recapture studies were done to determine population sizes.
- Age determination was done through scelotochronology.
- Weekly and later two-weekly water samples were analized for atrazine, metabolites,other triazines and other pesticides.
- Sexually-mature adults were trapped over a period of 48 hours.
- Frogs were observed for ectromelia and ectrodactyly.
- Sub-samples of males and females were randomly taken from each site and held in individual containers for 48 hours to allow recovery from trapping stress.
- Blood samples were collected.
- Following euthanization with MS-222, frogs were weighed, measured and dissected.
- After a low-power inspection and photography of gonads, one gonad of each specimen was flash-frozen with liquid nitrogen and stored at -20°C.
- The other gonad and the larynx were fixed in Bouin's fixative and preserved in 70% EtOH for histological examination. Histological examinations are still ongoing.

RESULTS

Table1: Estimated frog numbers

	Site no	Estimated population size
Reference sites	R1	316
	R3	360
	R6	950
Experimental sites	E1	370
	E3	1218
	E4	354
	E6	909
	E8	216

Figure 1: Sex ratio of frogs

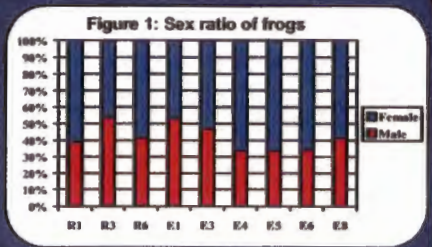


Figure 2: Rainfall at experimental sites

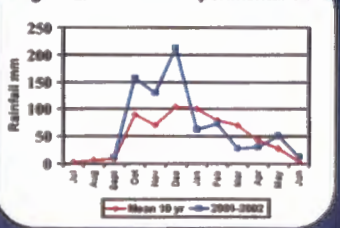
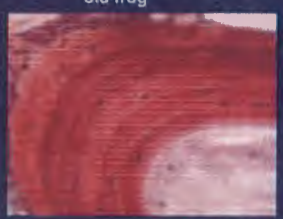


Figure 3: Growth rings of a 6-year old frog



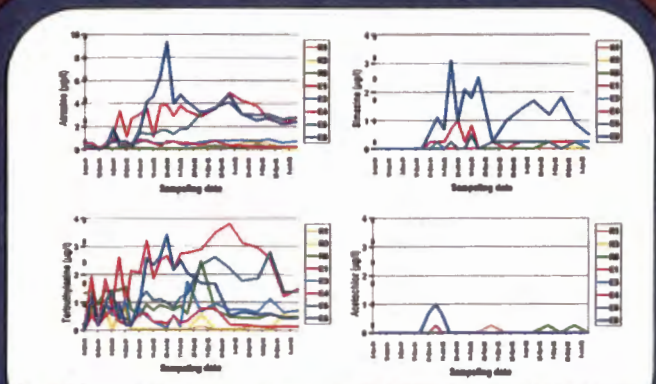


Figure 4: Sampling concentrations of atrazine, simazine, terbuthylazine and acetochlor.

Table 2: Number of frogs examined with percentage of gonadal abnormalities

		Experimental sites	Reference sites
Xenopus metamorphs	Number examined	168	61
	% gonadal abnormalities	0%	0%
Adult male Xenopus	Number examined	43	57
	% Testis abnormalities	4.7%	3.5%
Adult female Xenopus	Number examined	48	69
	% Ovary abnormalities	0%	0%

E3 E3 R3

Figure5: Testis abnormalities observed in both experimental (E) and reference (R) sites.

CONCLUSIONS

- Populations of *X. laevis* were present at all sites and had similar age-size classes. Obvious population-level effects were not observed.
- Exposures to pesticides used in corn production have occurred in the 2001-2002 use season and because of the historic use of herbicides in the region, exposure most likely occurred in previous years.
- Because of above-average rainfall at the sites, atrazine exposure concentrations in 2001-2002 were likely lower than in previous years.
- The adult *Xenopus* examined were most likely exposed to greater concentrations in years prior to 2001-2002.
- No abnormalities of the ovaries were observed.
- Males showed the same percentage (approximately 4%) deformities of the testes in both exposed and reference ponds.

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APPENDIX 2

Poster, Pesticide Conference Cape Town, 2003

Microcosm study to evaluate the effect of atrazine exposures on African Clawed Frog (*Xenopus laevis*) tadpoles

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SUMMARY

A microcosm study with three replicates of atrazine concentrations 0, 1, 10 and 25 µg/L was designed. Xenopus tadpoles were exposed until completion of metamorphosis. 150 specimens per concentration were dissected and the gonads photographed. Based on gross morphology, no inter-sex specimens were observed. Deformed testes were observed but showed no dose response to atrazine. In reference ponds 4.00% of males had deformed testes. 1.33%, 0.67% and 3.33% of males showed deformaties at 1, 10 and 25 µg/L atrazine respectively. No deformed ovaries were observed in females.

INTRODUCTION

It has been reported that the exposure of *Xenopus* to atrazine in laboratory studies increased the incidents of inter-sex gonads and induced changes in plasma testosterone concentrations. The aim of this study was to ascertain whether exposure to atrazine had any effect on *Xenopus* tadpoles in a semi-natural environment in microcosm ponds.

METHODOLOGY

- Twelve ponds (2.25m X 1.2m X 0.4m) were used during this study.
- Ponds were lined with polyethelene membrane and provided with a sediment bottom.
- Water plants from dams where Clawed Frogs occur naturally were introduced into each pond.
- Three ponds per concentration were treated with atrazine concentrations of 1, 10 and 25 µg/L respectively, and three ponds had no atrazine.
- Tadpoles were obtained via induced spawning of six pairs of mature Clawed Frogs.
- Eight hundred randomly selected, 48 h tadpoles, were released into each of the ponds.
- Development of tadpoles and the water level in the ponds were monitored weekly.
- On a two-weekly basis the atrazine concentration in each pond was verified (GC-MS).
- Metamorphs were removed from ponds as individuals completed metamorphosis, anaesthetized with 3-amino benzoic acid ethyl ester (MS-222), snout-vent length measured, body-mass determined, individually numbered and fixed in Boun's fixative.
- Gonads were examined for gross morphological abnormalities and digitally photographed.

EXPERIMENTAL LAYOUT

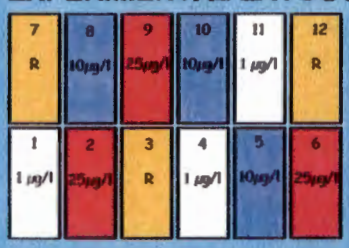

Figure 1: Images showing the experimental layout and specimens

RESULTS

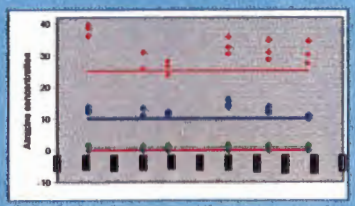

Figure 2: Atrazine concentrations as measured

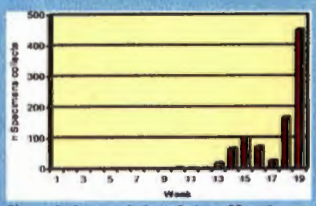

Figure 3: Accumulation of stage 66 metamorphs

Table 2: Number of frogs examined with percentage of gonadal abnormalities

		Males	Females
Reference ponds	Number examined	68	82
	% gonadal abnormalities	4.00	0
1 µg/L Atrazine	Number examined	72	78
	% gonadal abnormalities	1.33	0
10 µg/L Atrazine	Number examined	59	91
	% gonadal abnormalities	0.67	0
25 µg/L Atrazine	Number examined	70	80
	% gonadal abnormalities	3.33	0

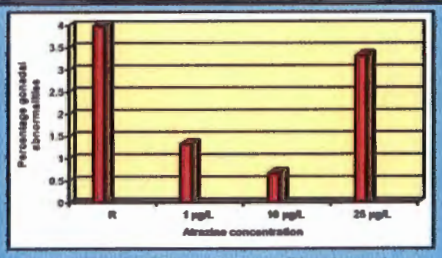

Figure 4: Percentage gonadal abnormalities observed

Figure 5: Normal testes and ovaries

Figure 6: Deformed testes observed

CONCLUSIONS

- No gonadal abnormalities were observed in females
- Males showed a low percentage (below 4%) deformities of the testes in both exposed and reference ponds.
- No inter-sex gonads were observed.
- Atrazine does not appear to affect the gonadal development of *Xenopus* at the concentrations used in this study.

ACKNOWLEDGMENTS

We would like to acknowledge Ms. C. Combrink and Mr. J. Legoete for technical assistance, Mr Peet Jansen van Rensburg for chemical analyses and the Potchefstroom University for research facilities.

APPENDIX 3

Draft of Hormonal Article

Plasma Testosterone and Estradiol Concentrations and Gonadal Aromatase Activities in African Clawed frogs (*Xenopus laevis*) From the Corn-Growing Region of South Africa.

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Synopsis: Environmental effects of exposure to triazines on estrogen synthesis

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ABSTRACT

African clawed frogs (*Xenopus laevis* [L.]) were collected from a range of environments with different exposure profiles for atrazine and related pesticides as well as edaphic parameters in the vicinity of Potchefstroom, South Africa. Frogs were surveyed for possible effects of exposure to traizine herbicides, including atrazine as well as other pesticides and metals on plasma testosterone (T) and 17β-estradiol (E2) concentrations, gonadal aromatase activity, and gonad growth (GSI). Plasma concentrations of both T and E2 varied among locations, but were not correlated to measured accessory factors such as temperature, pH, dissolved oxygen, or conductivity. The greatest median plasma T concentrations (males: 1.9 ng/ml; females: 1.6 ng/ml) occurred in frogs inhabitating areas where corn was not grown as compared to the median values from areas in the vicinity of corn fields (males: 0.4 ng/ml; females: 0.1 ng/ml) (t-test probabilities; males: $p = 0.548$, females: $p = 0.002$). Median E2 concentrations were also greater in frogs collected from the non-agricultural region (males: 0.3 ng/ml; females: 2.8 g/ml) than those in frogs from the corn growing area (males: 0.2 ng/ml; females: 0.5 ng/ml) (t-test probabilities; males: $p = 0.732$, females: $p = 0.018$). Because there was some exposure to agricultural chemicals at both regions, and because there were simultaneous exposures to multiple chemicals, a regression analysis was employed. Statistically significant negative correlations were observed between plasma T concentrations of females and concentrations of atrazine, deisopropylatrazine, deethylatrazine and tertbutylazine and diaminochlorotriazine in males. Plasma E2 in females exhibited a statistically significant negative correlation with atrazine and deethylatrazine. No significant correlations were observed between gonadal aromatase activity or GSI and any of the agricultural chemicals measured. Aromatase activity was not detectable in the testes at most of the sites. Median aromatase activities in ovaries

varied among sampling sites ranging from 7 to >3000 times greater than those in males. Although exposure to agricultural inputs did not affect aromatase activities effects of atrazine or co-applied pesticides on sex steroid homeostatsis can not be excluded at this point.

Keywords: amphibians, aromatase, agriculture, frog, triazines

INTRODUCTION

Considerable interest and controversy have recently surrounded the issue of potential endocrine disrupting effects of agricultural chemicals on development and reproduction in amphibians particularly in habitats adjacent to application (Clark et al, 1999), and have been suggested as one of multiple potential causes for declines in populations of frogs that have occurred in several areas since the 1960s (Houlahan et al, 2000). In particular, concern has been raised about the widely used broadleaf herbicide, atrazine (6-chloro-4-ethylamino-6-isopropyl-amino-*s*-triazine) (Hayes et al, 2002a; Hayes et al, 2002b; Hayes et al, 2003; Kiesecker, 2002; Reeder et al, 1998). Since the early 1960s, atrazine has been used extensively in corn-growing regions of many areas of the world, including North America and South Africa (USEPA, 2001). Although less atrazine is now being used and it is being used in combination with other chemicals, it is still the most widely used herbicide in the United States and and probably the world for the last four decades (Hopenhayen-Rich et al., 2002). Atrazine is not predicted to have measurable effects on the survival or growth of most fish amphibians and invertebrates at the concentrations of atrazine found in the environment (Torres and O'Flaherty, 1976; Kirby and Sheahan, 1994; Solomon et al., 1996; Detenbeck et al., 1996; Howe et al., 1998; Diana et al., 2000). Atrazine does not have a high potential to bio-accumulate or biomagnify (Solomon et al. 1996) but is, persistent in the environment with an estimated half-life in water ranging from 40-240 d (Giddings et al., 2000) and field dissipation half-lives ranging from 8-99 d (Novartis, 2000). As a result of both its widespread use and persistence, atrazine has been widely detected in surface waters and rainfall with peak concentrations ranging up to approximately 20 μg/L (Giddings et al., 2000, Solomon et al., 1996).

Recently, several studies have suggested that atrazine may disrupt the endocrine systems of frogs (Hayes et al, 2002a; Hayes et al, 2002b; Tavera-Mendoza et al, 2002a; Tavera-Mendoza et al, 2002b). It has been reported that environmentally relevant concentrations of atrazine, as small as 0.1 μg/L, caused both gonadal abnormalities and demasculinization of the larynx, as indicated by reduced size of the laryngeal dilator muscle, in male *X. laevis* exposed from hatching through completion of metamorphosis (Hayes et al., 2002a). However, other studies with *X. laevis* have not found effects of atrazine on the size of the larengeal dilator muscle, gonadal development or plasma hormone concentrations (Carr et al., 2003; Coady et al., 2003a & b). It has been hypothesized by Hayes et al., (2002a) that atrazine can affect development of frogs, including *X. laevis* by induction of aromatase (*CYP*-19 gene) activity. Aromatase is the enzyme that catalyzes the aromatization of T to E2. Atrazine has been reported to up-regulate the activity of the CYP-19 gene in a human adrenocarcinoma (H295R) cell line (Sanderson et al., 2001). This hypothesis was proposed to explain the observation of smaller laryngeal dilator muscle in male *X. laevis* exposed to atrazine than those of unexposed individuals (Hayes et al., 2002a). Since laryngeal development is an androgen-dependent process and a decrease in plasma concentration of plasma T (Hayes et al. (2002a) those authors proposed that up-regulation of aromatase resulted in a decrease in plasma T, which then resulted in a smaller laryngeal dilator muscle. To date no direct evidence of the induction of aromatase activity in anuran species due to atrazine exposure or related pesticides has been presented (Hayes et al, 2002a; Villeneuve et al, 2002; Coady et al, 2002).

The present study was conducted to test the hypothesis that atrazine or related triazines might affect aromatization of T to E2 under field conditions. Specifically, aromatase activities in the gonads and plasma concentrations of T and E2 were measured in blood

plasma of adult male and female *X. laevis* from corn growing areas in the Potchefstroom region of South Africa where it is known that atrazine concentrations are greater than in areas in an adjacent watershed, where corn is not grown.

MATERIALS AND METHODS

Sampling locations

A total of eight sites were studied in two adjacent regions in the vicinity of Potchefstroom, South Africa (SA) (DuPreez et al., 2003). One region was in the Viljoenskroon area which is a corn-growing area (CGR), where predominantly corn (*Zea mays*) is cultivated. The other area, in an adjacent watershed near Potchefstroom where corn is not cultivated, was designated as a non corn-growing area (NCGR). The sites were selected to give a range of concentrations of atrazine and other pesticides used in corn production that were likely to runoff into farm ponds adjacent to the fields (DuPreez et al., 2003). Compounds of major interest were atrazine, its degradation products desethylated atrazine (DEA), desisopropyl atrazine (DIA), diaminochlorotriazine (DACT), and tertbutylazine, as well as simazine and acetochlor. During the sampling period, various environmental parameters were measured at each field site to describe the habitat, including a brief description of vegetation and water depth (Smith et al., 2003).

Non corn growing area (NCGR): Site NCGR3 is a small heavily vegetated site with clear water of medium depth. Secci depth was 32cm. Sites NCGR1 and NCGR6 are large, earth-walled farm ponds with muddy water and Secci depths of 11.5cm and 6.5 cm respectively. Vegetation was limited to the periphery of the ponds. Conductivity varied between 47µS/cm for site NCGR1 and NCGR3 and 38µS/cm for site NCGR6. The pH varied between 5.1 for site NCGR3 and 8.8 for site NCGR6. (DuPreez et al, 2003). The pH was slightly alkaline, ranging from 7.3 for site NCGR3 to 8.3 for site

NCGR1. Site NCGR3 dries up frequently, while sites NCGR1 and NCGR6 are semi-permanent ponds. All three sites have fairly large catchment areas and sites NCGR1 and NCGR3 both have springs flowing into these sites.

Corn growing area (CGR): Areas of the ponds in the Viljoenskroon corn growing region (CGR) ranged from small, with a surface area of 2400m^2 (sites CGR4 and CGR8), to medium size of 4,700 m^2 (site CGR1), to large 46,000 m^2 (site CGR3) and 68,000m^2 (site CGR6). All sites had vegetation around the periphery and had floating aquatic plants. Conductivity ranged from 210 (site CGR3) to 1567 µS/cm (site CGR4). The pH ranged from 7.2 (site CGR3) to 10.0 (site CGR1). The pH was alkaline for all sites ranging from 8.2 (site CGR4) to 10.8 (site CGR8). All sites had clear water with light penetrating to the bottom in sites CGR1, CGR3, CGR4 and CGR8. Site CGR6 had a Secci depth of 207cm. Water and sediment grab samples were analyzed for the presence of atrazine, related triazines, other pesticides, and metals.

Frog collection

Wild *X. laevis* of both sexes were collected after the rainy season in April and May 2002. The only exception was location CGR8 where frogs were collected at 5 different times during the year (April, May, June, August and September) as a result of difficulty experienced in trapping enough frogs at this site. At all other sites sampling took place once or twice within a few days. Corn in this region of SA is usually planted in October/November and is treated with atrazine and related triazines. Frogs were collected using 10 bucket traps per site baited with liver and marrow bones. Traps were collected two days later and the frogs transferred to a container labeled with a number corresponding to the site. Frogs were then transported to the laboratory at Potchefstroom University, South Africa.

Blood and tissue collection

After capture, frogs were kept individually in laboratory water in 2L plastic containers for 48 h to recover from capture stress before blood and gonad tissue were collected. Blood was collected by cardiac puncture with syringes coated with 7.5% EDTA to prevent clotting. Blood samples were centrifuged at 2,000 x g for three minutes at room temperature to separate out the plasma fraction. Plasma was stored at –80°C until further analyses. Gonads were removed, measured and weighed. One gonad was fixed in 10% neutral buffered formalin or Bouins's for 48 hr then transferred to ethanol before preparation for sectioning and staining for histological examination. The second gonad of each specimen was snap frozen in liquid nitrogen for subsequent biochemical analyses. The gonadosomatic index (GSI) was calculated (Equation 1).

GSI (%) = gonad weight / body weight x 100 (1)

Biochemical Analyses (laboratory)

Steroid hormones: Frozen plasma samples were thawed on ice, and T and E2 extracted twice with diethyl ether (5 mL) in glass tubes. The solvent extract was separated from the water phase by centrifugation at 2000 x g for 10 min and transferred into small glass vials. The solvent was evaporated under a nitrogen stream, and the residue was dissolved in phosgel buffer, and either immediately measured or frozen at –80°C for later hormone determination. Concentrations of E2 and T in blood plasma were measured by competitive ELISA as described by Cuisset et al. (1994) with modifications (Hecker et al, 2002). In this competitive ELISA plasma steroid competes with acetylcholinesterase labeled steroid for the binding site on the polyclonal rabbit anti-steroid antibody. Antiserum to T was obtained from Dr. D. E. Kime (Sheffield, UK). Cross reactivities of the T antiserum are described in Nash et al. (2000). The antiserum to E2 (Cayman Chemical, Ann Arbor, MI) cross-reacted with estradiol-3-glucoronide (17%), estrone (4%), estriol (0.57%), T (0.1%) and 5α-dihydrotestosterone

(0.1%). For all other steroids cross reactivities were less than 0.1%. The steroid ELISAs were performed using COSTAR high binding plates (COSTAR). The working ranges of these assays were determined as follows:

testosterone: 0.78 - 800 pg/well

17ß-estradiol: 0.78 - 800 pg/well

CYP19 aromatase activity: Aromatase activity was measured following the protocol of Lephart and Simpson (1991) with minor modifications (Sanderson et al., 2000). Less than 0.5 g of gonadal tissue was homogenized in 600 µL of ice cold gonad buffer (50 mM KPO_4, 1 mM EDTA, 10 mM glucose-6-phosphate, pH 7.4). The homogenate was incubated with 21.33 nM ^{3}H-androst-4-ene-3,17-dione (25.9 Ci/nmol; Lot No. 3467-067; New England Nuclear; NET-926), 0.5 IU/ml glucose-6-phosphate (Sigma Cat. # G6378), and 1 mM NADP (Sigma Cat. # N-0505) at 37°C and 5% CO_2 for 120 min. Tritiated water released from each sample was extracted and counted for 2 min using a liquid scintillation counter. Aromatase activity was expressed in pmoles of androstenedione converted per h per mg protein. The specificity of the reaction for the substrate was determined in a competitive test with non-labeled androstenedione. Addition of large amounts of androstenedione reduced tritiated water formation to the levels found in the tissue blanks, which demonstrated that the activity being measured was specific for aromatase (data not shown).

Statistical analyses

Because of the nature of the parameters measured, several statistical models were used for data analyses. Data for males and females analyzed separately. The study was designed to be analyzed by both fixed-effects models and by regression types of statistics. This type of analysis was necessitated because the specific locations could not be classified as exposed or unexposed. There was a range of concentrations of the

primary residues of concern, with greater concentrations in the CGR. However, residues were also detected in the NCGR. Because most of the investigated parameters were not normally distributed (one sample Kolmogorov-Smirnov test) statistical comparisons were made between the median values of plasma T and E2 concentrations as well as the E2/T ratio, the GSI and gonadal aromatase activity. Differences between the means of the exposure groups (means of the different medians at the single sites of one group) were tested using a two-sided, two-sample t-test assuming equal variances. Because a primary objective of this study was to investigate the relationships between exposure to atrazine and or its' degradation products and other triazine herbicides, correlations between atrazine or concentrations or degradation products and each parameter were also investigated. When necessary, data were log-transformed to approximate a normal distribution before calculating linear regressions and Pearson correlation coefficients. Physiological parameters such as plasma T and E2 or aromatase activity react quickly to environmental changes, within as little as a few hours, days or weeks. Therefore, the 4-wk weighted means (4WM) of the period before collecting frogs at the individual sites for pesticide concentrations and environmental co-factors were used for the calculations of significances and correlations/regressions in this study. Values were considered to be statistically significant when $p < 0.05$.

RESULTS

Pesticide Concentrations

Concentrations of pesticide residues varied among locations and among seasons. With the exception of site CGR8, the greatest concentrations of atrazine in pond water were observed at the end of the rainy season (March through May) (Du Preez et al., 2003). The greatest mean atrazine concentrations (4WM) were measured at sites CGR1 (4.1 µg/L), CGR6 (3.9 µg/L) and CGR8 (3.5 µg/L) (Table 1). Concentrations of

atrazine at locations NCGR1, NCGR3, NCGR6 and CGR4 were low but in most cases detectable. At NCGR4, 4WM concentrations of pesticides were as low as in water from the reference sites. Statistically significant linear relationships were observed between log atrazine concentration (4WM) and the log concentrations of its degradation product DIA ($r = 0.829$; $p = 0.011$) and DEA ($r = 0.905$; $p = 0.002$). Tertbutylazine concentrations between 2.4 and 3.7 µg/L were measured at CGR1 and CGR6.

The log of concentrations of the triazines DIA ($r = 0.770$; $p = 0.025$), DEA ($r = 0.746$; $p = 0.034$) and atrazine ($r = 0.802$; $p = 0.017$) were positively correlated with the pH of the water at the sampling sites (Table 2). The log DIA concentration was positively correlated with log temperature ($r = 0.801$; $p = 0.017$), and the log visual depth ($r = 0.814$; $p = 0.014$) (Table 2). No other relationships were observed between pesticide concentrations and environmental co-factors.

Physiological responses

GSI: The means of the median GSI values for both female (t-test: $p = 0.0640$) and male (t-test: $p = 0.0725$) *X. laevis* from the CGR sites were greater than the female and male means from the NCGR (Figure 1, Table 3). Among the sites in the corn growing area CGR6 had the least median GSI in males and females that was similar to those in the NCGR. The GSI of female *X. laevis* from CGR1 and CGR8 were more variable than that at other locations. In general, less variations among individuals of both sexes was observed in the NCGR where the median GSI values were less than that for almost all other locations (Figure 1). The GSI of males ($r = 0.732$; $p = 0.039$) as well as the log GSI of females ($r = -0.847$; $p = 0.008$) showed a significant positive relationship with the log conductivity (logCond) (Table 4). Furthermore, a negative correlation was observed for the GSI in males ($r = 0.817$; $p = 0.013$) and the log GSI in females ($r = -0.736$; $p = 0.037$) with the log visual depth (logDepth) (Table 4).

Testosterone: The median plasma T concentrations of females were significantly greater at the NCGR sites than at the CGR sites (t-test: $p = 0.0180$). The median plasma T concentrations of males were not significantly different between the CGR and NCGR. The greatest median T concentrations in plasma of males were 3.9 ng/ml (CGR4), 2.3 x 10^3 pg/ml (NCGR3), and 1.9 x 10^3 pg/ml (NCGR6) (Figure 2). Similar plasma T concentrations in females were observed only at NCGR1 (2.0 x 10^3 pg/ml) and NCGR6 (1.6 x 10^3 pg/ml). At all other sites Median plasma T concentrations of females were less than 0.6 ng/ml (Figure 2). Plasma T concentrations of females were negatively correlated with the logarithm of the concentrations of atrazine ($r = -0.919$; $p = 0.001$), tertbutylazine ($r = -0.763$; $p = 0.028$), DIA ($r = -0.755$; $p = 0.030$), and DEA ($r = -0.863$; $p = 0.006$), but not DACT concentrations (Table 4). There Plasma T concentration was not correlated with the the 4WM concentration of atrazine ($r = -0.554$; $p = 0.154$) (Table 4). There was, however, a significant negative correlation between the log of plasma T concentrations in males and the log of the DACT concentration ($r = -0.831$; $p = 0.011$) (Table 4). In male *X. laevis* there was a significant negative correlation between the pH and the log T ($r = -0.823$; $p = 0.012$) (Table 5). No correlations with other co-factors could be observed for males or for females.

Estradiol: In general, median concentrations of CGR2 in the plasma of females from the CGR were significantly less than those of females from the NCGR (t-test: $p = 0.0018$), but there was no significant difference for males (Table 3; Figure 2). The greatest median E2 concentrations occurred in males from sampling sites NCGR6 (4.8 ng/ml) and CGR4 (3.4 ng/ml). At all other sites plasma E2 concentrations of males were less than 0.3 ng/ml. In females the greatest plasma E2 concentrations occurred in *X. laevis* from the NCGR (2.7-4.4 ng/ml) (Figure 2). In both males and females plasma E2 concentrations at the sites CGR4 were similar to those in the NCGR, while at all

other CGR sites E2 concentrations were less than in the NCGR (Figure 2) Concentrations of E2 in the plasma of both males and females were more variable in sites of the NCGR than those from the CGR. A similar degree of variability as for females at the NCGR sites was observed in male *X. laevis* collected at locations CGR1 and CGR4. In females plasma E2 was negatively correlated with 4WM atrazine concentration ($r = -0.806$; $p = 0.016$), and DEA ($r = -0.768$; $p = 0.026$) (Table 4).

E2/T ratio: E2/T ratios of both males and females were not significantly different between the CGR and NCGR and regardless of whether the comparisons were conducted on the basis of medians or means (Table 3). Except for site NCGR6 for both males and females and CGR8 for females there were no significant differences in the median E2/T ratios (Figure 2). In general, E2/T ratios were slightly greater in females than in males. Within-population variation was greater at NCGR6 for both sexes, at NCGR3 for males, and CGR8 for females than at most other locations. The distribution of E2/T ratios of males from CGR8 was skewed and with greater values within the upper quartile. The only significant correlation between the E2/T ratio for males was with DIA ($r = -0.830$; $p = 0.011$) (Table 4). There were no significant relationships observed for the E2/T ratio for females and any of the triazine residues.

There were significant negative correlations between log E2/T ratio of male *X. laevis* and both log temperature ($r = 0.810$; $p = 0.015$) and dissolved oxygen (DO) ($r = 0.717$; $p = 0.045$) (Table 5). No significant correlations were observed for the E2/T in females (Table 5).

CYP19 aromatase activity: There was no significant difference in the median aromatase activity in ovaries between the CGR and the NCGR (Table 3). In most testes from most sites except NCGR3 and CGR4 no gonadal aromatase activity was detectable (Figure 1). When aromatase activity was measurable in males, values ranged from 7 to

53 fmol/h/mg protein. In males from these locations median aromatase activities in testes were 7- (CGR4) to 14-fold (NCGR3) less than that in ovaries of females collected at the same sites. The greatest aromatase activities in ovaries were observed at NCGR3 ($1.9x10^2$ fmol/h/mg protein), CGR1 ($1.2x10^2$ fmol/h/mg protein) and CGR8 ($3.2x10^2$ fmol/h/mg protein) (Figure 1). In both testes and ovaries, greater median activities were characterized by proportionately greater variabilities (Figure 1). The greatest variability was observed in ovaries of *X. laevis* from CGR8 where sampling occurred over an extended time period (April through September). The only accessory factor with which log ovarian aromatase activity was correlated was the log of 4WM water temperature ($r = 0.786$; $p = 0.021$) (Table 2). Aromatase activity was not correlated with atrazine concentration, but was positively correlated with the log of the DIA concentration ($r= 0.857$; $p= 0.007$) (Table 4). No statistically significant correlations between the log CYP19 aromatase activity in males and concentrations of pesticides or environmental co-factors were observed because no aromatase activity was detectable at most of the sampling sites.

Relationships between physiological parameters: There were statistically significant positive correlations between the log T and log E2 concentrations in both males ($r = 0.757$; $p < 0.001$) and females ($r = 0.868$; $p < 0.001$) (Table 6). Both sexes also exhibited positive correlations between log E2 and log E2/T ratio (males: $r = 0.804$; $p < 0.001$; females: $r = 0.582$; $p < 0.001$) (Table 6). However, neither aromatase activity nor GSI were correlated with plasma concentrations of T or E2 in either males or females.

DISCUSSION

Atrazine concentrations measured in water of most of the ponds that are located in the CGR of the Viljoenskroon region were similar to those reported for a variety of waters from the Midwest, USA, exposed to runoff from agricultural lands (Brady et al, 1995;

Richards and Baker, 1998). Although less than peak concentrations that can be observed during extreme exposure scenarios such as storm runoffs, these concentrations reflect a typical exposure situation for many water-bodies. In the current study, trace amounts of atrazine and other triazines were also detected in water from the non-corn growing areas. This is likely due to atmospheric deposition from wet and dry fall into water-bodies in non-agricultural areas (Rawn et al., 1998). Atrazine concentrations in the Potchefstroom region, SA, display typical exposure scenarios likely to occur in many different areas of the world where modern agriculture is practiced. Furthermore, the fact that most concentrations of degradation products were strongly correlated with atrazine suggests that calculation of the 4WM atrazine concentration was an appropriate and accurate way to gauge atrazine levels over time at these sites.

While several studies have been conducted in which *X. laevis* were exposed to atrazine under laboratory conditions (Hayes et al, 2002a; Carr et al., 2003; Coady et al, 2003a&b) no studies had been undertaken to investigate the effects of atrazine on *X. laevis* in their natural habitat. Studies on other species such as the leopard frog (*Rana pipiens*) and the cricket frog (*Acris crepitans*) have been conducted to investigate possible impacts of atrazine in the wild (Hayes et al., 2002b; Reeder, 1998). Although these studies found an increase in the occurrence of intersex in male frogs from areas of more intense agriculture, there was no significant correlation between concentrations of atrazine and degree of response. Therefore, the present results may be of general importance for the evaluation of atrazine exposure in our surrounding environments.

In recent studies, in which female tadpoles of the African clawed frog (*X. laevis*) were exposed to 21 μg/L atrazine for 48 h, a reduced number of primary germ cells (20 %) compared to controls (2 %) (Tavera-Mendoza et al. 2002a) was obseved. The same exposure resulted in a 57% reduction in testicular volume, a 70% reduction in primary

spermatogonial cell nests, a 74% decline in nursing cells, testicular resorption among 70% of exposed animals, and failed testicular development in 10% of animals were observed in male tadpoles (Tavera-Mendoza et al. 2002b). Assuming exposure to atrazine in the ponds in South Africa investigated in our study was similar to that in the studies of Taverna-Mendoza, similar effects on the gonads would be expected: a decrease in the GSI. But instead, an increase in the GSI was observed for both male and female frogs collected at the CGR where they had been exposed to greater concentrations of atrazine. However, maximum atrazine concentrations at the agricultural sites were more than 5-fold less than those tested in the Tavera-Mendoza et al studies (2002a; 2002b). A possible explanation for the differences in GSI could be differences in food resources that would allow the frogs from the CGR to expend more energy on gonad growth. This could be a consequence of general eutrophication in agricultural areas that may lead to increased primary production, and subsequent to an increase in the biomass of aquatic invertebrates. However, differences in food availability and production were not determined in this study. It has to be elucidated whether the increase in gonad growth is a response to edaphic conditions and whether it has any effect on the populations of *X. laevis* at these sites (Smith et al., 2003b). In conclusion, there is no evidence from the reported data that exposure to atrazine or degradation products had a negative impact on gonad growth under field conditions.

Plasma concentrations of T in both males and females from the NCGR were similar to those reported for *X. laevis* in a laboratory study during winter but were less than those in males during summer (Kang et al., 1995). It was hypothesized by the authors of the laboratory study that low levels of T in males were due to sexual inactivity during winter while females had consistently lesser plasma T concentrations during the whole year. Since the frogs investigated in our study were caught after the breeding season the

low plasma T titers observed under field conditions were likely due to result from sexual inactivity that is typical for the recovery phase after spawning. Estradiol concentrations in plasma of female *X. laevis* are similar to those observed for females from a different study (Tobias et al., 1998). The variation in concentrations of T of males and females observed in our study and that of E2 in females is comparable to that observed in other studies (Kang et al., 1995; Tobias et al., 1998). Males in this study exhibited greater E2 concentrations than those reported for males from other studies (Tobias et al., 1998). The reason for this is unknown. However, plasma E2 concentrations reported by Tobias et al (1998) were measured at a different time, and none of the frogs in that study had completed a breeding cycle. Since different stages in the reproductive cycle are characterized by dramatic changes in sex steroids, and frogs in our study were investigated at a different time than those in the Tobias at al (1998) study it is difficult to directly compare these studies. A different study on the European green frog (*Rana esculenta*) found plasma E2 concentrations in males that were similar to those observed in our study (Paolucci et al., 1990). Furthermore, it is known that during puberty male mammals exhibit greater plasma concentrations of estrogen that are similar to those in females (reviewed in Sharpe, 1998). Greater concentrations of estrogens in males seem to occur frequently in some species, and therefore, it can not be excluded that the E2 levels in male frogs reported in this study reflect a typical phenomenon in male *X. laevis*.

It is known that estrogens derived from the testis play an important role in the negative feedback regulation of follicle stimulating hormone (FSH) (Finkelstein et al, 1991; Bagatell et al, 1994). In our study, frogs were collected in the post-spawning period when FSH would be expected to be down-regulated, and therefore, it would be possible that the elevated E2 concentrations measured in males resulted from the negative

feedback control of gonadotropin secretion. However, there is no direct evidence for this type of regulatory mechanism from the presented data, and it would require further research to test for this hypothesis.

The variability in concentrations of plasma T and E2 observed in our study is comparable to that observed in two laboratory studies (Kang et al, 1995; Tobias et al, 1998). Furthermore, frogs were collected after the spawning period when physiological processes are known to change dramatically. Therefore, the variability observed in our study is likely to reflect the normal situation.

The reason for the differences in plasma concentrations of T and E2 in female *X. laevis* is unknown at this time. There were statistically significant differences between the CGR and NCGR regions and a statistically significant negative correlation between both T and E2 concentrations in females and atrazine, most of its degradation products or overall chlorotriazine concentrations. There was no significant correlation between plasma sex steroids in males and atrazine. However, plasma concentrations of T in males were significantly negatively correlated with DACT ($p = 0.011$) and overall chlorotriazine concentrations ($p=0.1$).

Few studies have reported effects of atrazine on plasma E2 concentrations. A study in which juvenile *X. laevis* that had been exposed to a range of atrazine concentrations, up to 25 ug/L from 72 h post-hatch until 2 to 3 mo post-metamorphosis found no statistically significant differences in plasma T concentrations among atrazine treatments and controls (Coady et al, 2003a). There were also no statistically significant effects of waterborne atrazine exposure on plasma concentrations of E2 in females. However, in males exposed to 1.0 ug/L plasma concentrations of E2 were less than those of controls, but not at greater or lesser doses, such that there was no consistent dose-response relationship (Coady et al., 2003b). It is not possible from the correlation

alone to establish causality between exposure to atrazine and plasma concentrations of T or E2.

It has been hypothesized that atrazine affects sexual differentiation in both sexes by inducing aromatase. Such a change in aromatase activity could result in lesser concentrations of T, which could result in demasculinization of males or, through inappropriate synthesis and secretion of E2, resulting in feminization of males (Hayes et al, 2002b). Such a mechanism was deemed plausible, based on *in vitro* studies on human and fish cell lines (Sanderson et al., 2000; Sanderson et al., 2001) but until the field studies on which we report here, the hypothesis had not been tested *in vivo* in amphibians under field conditions. There were neither differences in aromatase activities among locations or significant correlations between exposure to atrazine and aromatase activity nor increases in plasma estradiol levels. This result does not support the hypothesis suggested by Hayes et al (2002a) that atrazine causes an increase in the production of E2 by inducing aromatase activity.

The only chemical that seemed to be correlated with aromatase activity was DIA and then only for females. In males, there was no significant correlation between atrazine concentrations and plasma concentrations of T or the E2/T ratio, which would be expected if there was a causal relationship between atrazine exposure and an aromatase-mediated response. There was, however a significant negative correlation between the E2/T ratio of males and DIA. In the H295R, human adenocarcinoma cell line DIA significantly increased *CYP-19* mRNA expression as well as aromatase activity (Sanderson et. al., 2001). The observed relationship between aromatase activity and DIA was not consistent with published literature or with the hypothesis that atrazine or its degradation products were responsible for changes in plasma T concentrations through an aromatase-mediated process. The observed trend in plasma E2 concentration

was in the opposite direction that would be expected if aromatase activity was up-regulated. Furthermore, DIA concentrations were significantly correlated with temperature so temperature could have had a direct or indirect effect on aromatase activity. Thus, it is unlikely that there was an effect on aromatase activity that was due to atrazine or its degrqadation products and it also seems unlikely that there was a strong case for decreased plasma T in males that was associated with the presence of atrazine. In fact, the significant positive correlation between E2 and T in both males and females indicates that both E2 and T are equally affected, and therefore, the observed effects may be due to a more general mechanism such as increased metabolism. A study of Atlantic salmon (*Salmo salar*) hypothesized that atrazine can affect T concentrations by increasing metabolic breakdown of the hormone (Moore and Waring, 1998). However, no E2 measurements were made in that study. Increased metabolic activity is a common response to exposure of environmental pollutants as well as being a natural phenomenon in the recrudescence cycle. Thus, it cannot be excluded as a possible cause for the observed decrease in plasma sex steroids.

Another hypothesis is that atrazine or its degradation products may affect the endocrine system at the level of the central nervous system (CNS). Atrazine has been found to affect serum luteinizing hormone (LH) and prolactin concentrations in Sprague-Dawley and Long-Evans female rats by altering the hypothalamic control of these hormones (Cooper et al., 2000). Similar conclusions were drawn in a recent paper by Stoker et al. (2002) that suggests effects of atrazine degradation products on puberty and thyroid function in male Wistar rats are via actions on the CNS control of the pituitary-gonadal axis. However, the doses at which atrazine caused the observed effects were much greater than those observed in this study (100 – 200 mg/Kg oral administration), and, because of atrazine's low bioconcentration factor, are unlikely to reflect realistic

exposure scenarios to atrazine in the environment. Thus, again, it is unlikely that atrazine would cause the observed decreases in plasma hormone concentrations.

Although significant negative correlations were observed between exposure to atrazine and its degradation product DACT and plasma concentrations of sex steroids in wild *X. laevis*, it is impossible from this study to conclude the exact cause or accuracy of these differences. The CGR were characterized by intensive agriculture and a variety of different chemicals, including herbicides, fungicides, insecticides, and fertilizers were applied. Thus, to determine a possible causative agent, more detailed studies would be necessary to determine the effects of both chemical and edaphic factors.

In conclusion, the current study provides evidence that the plasma concentrations of both T and E2 in females are less in the CGR than in the NCGR where concentrations of pesticides, including atrazine were greater. However, the observed effects on steroid homeostasis in wild frogs does not seem to negatively affect gonad growth as stated by two different studies (Tavera-Mendoza et al., 2002a&b). There is no evidence that these differences were due to induction of aromatase as has been previously suggested (Hayes et al. 2002a&b). The data presented here demonstrate that, if there is an effect of atrazine on the endocrine system, it is unlikely to be via direct alteration of aromatase enzyme activity. Because frogs in the two areas were exposed to other factors, both chemical and physical, it is impossible to determine the extent and via what mode of action atrazine my affect the endocrine systems of *X. laevis*. To adequately test this hypothesis, the critical mechanism of action needs to be established, so that an appropriate biomarker can be developed for use in definitive laboratory studies and in field monitoring for atrazine-specific responses.

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TABLES AND FIGURES

Table 1: Concentrations of triazine herbicides and degradation products (μg/L) at non corn growing regions (NCGR) corn growing areas (CGR) in SA. Values represent values of two individual samplings that were conducted within a time period in 2002 four weeks before collecting *X. laevis*.

	Date (all 2001)	Simazine (mg/L)	DACT (mg/L)	DIA (mg/L)	DEA (mg/L)	Atrazine (mg/L)	Tertbutylaz. (mg/L)
NCGR1	2-Apr	<0.5	3.91	0.23	0.18	0.28	<0.1
	15-Apr	<0.5	<0.1	0.27	<0.1	0.26	<0.1
	mean[1]	0.25	1.98	0.25	0.12	0.27	0.05
NCGR3	18-Mar	<0.5	<0.1	0.30	0.15	0.36	<0.1
	2-Apr	<0.5	<0.1	0.61	<0.1	0.57	<0.1
	mean[1]	0.25	0.05	0.46	0.1	0.47	0.05
NCGR6	1-May	<0.5	<0.1	0.13	0.12	<0.1	0.51
	13-May	<0.5	<0.1	0.15	0.13	0.2	0.59
	mean[1]	0.25	0.05	0.14	0.13	0.13	0.55
CGR1	15-Apr	<0.5	<0.1	0.7	1.29	4.14	3.66
	1-May	<0.5	<0.1	0.93	1.16	3.5	2.95
	mean[1]	0.25	0.05	0.82	1.23	3.82	3.31
CGR3	2-Apr	<0.5	0.38	0.47	0.43	1.05	0.97
	15-Apr	<0.5	<0.1	0.28	0.38	1.01	1.07
	mean[1]	0.25	0.22	0.38	0.41	1.03	1.02
CGR4	2-Apr	<0.5	<0.1	0.18	0.11	0.32	0.19
	15-Apr	<0.5	<0.1	0.64	<0.1	0.29	0.24
	mean[1]	0.25	0.05	0.41	0.08	0.31	0.22
CGR6	15-Apr	<0.5	5.45	0.49	1.09	3.78	2.46
	1-May	<0.5	<0.1	0.32	0.90	3.90	2.40
	mean[1]	0.25	2.75	0.41	0.10	3.84	2.43
CGR8	15-Apr	1.70	1.24	0.70	1.04	3.53	0.86
	1-May	1.20	<0.1	0.88	0.91	3.12	0.72
	mean[1]	1.45	0.65	0.79	0.975	3.33	0.79

mean[1]: When concentration below detection limit ½ of the detection limit was used for the calculations

Table 2: Pearson correlation coefficients (r) and Bonferroni probabilties (p) between water triazine herbicides and their degradation products (4WM) concentrations and environmental co-factors measured at the different sampling sites in the Potchefstroom region, SA. Bold numbers = regression significant at a level of $p < 0.05$. Positive (+) and negative correlation coefficients are indicated.

		logSimazine	logDACT	logDIA	logDEA	logAtrazine	logTerbutyl
logTemp (°C)	r	+ 0.244	+ 0.202	+ **0.801**	+ 0.246	+ 0.565	- 0.132
	p	0.560	0.632	**0.017**	0.557	0.144	0.756
PH	r	+ 0.361	- 0.160	+ **0.770**	+ **0.746**	+ **0.802**	+ 0.627
	p	0.379	0.706	**0.025**	**0.034**	**0.017**	0.096
logCond (µS/cm)	r	+ 0.165	+ 0.002	+ 0.556	+ 0.420	+ 0.539	+ 0.541
	p	0.696	0.997	0.152	0.300	0.169	0.166
DO (mg/L)	r	+ 0.317	- 0.106	+ 0.587	+ 0.128	+ 0.246	- 0.115
	p	0.445	0.803	0.126	0.762	0.558	0.786
logDepth (cm)	r	- 0.584	- 0.204	- **0.814**	- 0.426	- 0.650	- 0.246
	p	0.129	0.629	**0.014**	0.293	0.081	0.558

Table 3: Differences between the median of the non-corn-growing area (NCGR) and the corn-growing area (CGR) (calculated from site medians) (t-test). Values given represent the probability of making a Type I error (p).

Sex Steroid	females	males
Testosterone	0.0180	0.7317
Estradiol	0.0018	0.5479
E2/T ratio	0.8388	0.1754
GSI	0.0640	0.0725
Aromatase	0.6712	0.6538

Table 4: Pearson correlation coefficients (r) and Bonferroni probabilties (p) between biomarker responses of male and female *X. laevis.* Average values (4WM) of triazine herbicides and their degradation products in water measured in the month prior to collection of frogs at the different sampling sites in the Potchefstroom region, SA. Positive and negative correlations are indicated by + and -, respectively. Bold numbers = significant regressions ($p < 0.05$). N = 8.

Males		GSI	LogT	logE2	logE2/T	Log Aromatase
LogDACT	r	- 0.325	- **0.831**	- 0.699	- 0.154	
	p	0.432	**0.011**	0.054	0.717	
LogDIA	r	+ 0.648	- 0.151	- 0.459	- **0.830**	
	p	0.061	0.722	0.253	**0.011**	
LogDEA	r	+ 0.171	- 0.618	- 0.501	- 0.460	
	p	0.686	0.102	0.206	0.251	
Log Atrazine	r	+ 0.336	- 0.554	- 0.608	- 0.628	
	p	0.431	0.154	0.110	0.095	
Log Tertbutylazine	r	+ 0.182	- 0.12	- 0.014	- 0.010	
	p	0.666	0.453	0.973	0.981	
Females		LogGSI	LogT	LogE2	logE2/T	Log Aromatase
LogDACT	r	- 0.191	- 0.082	- 0.461	- 0.157	+ 0.171
	p	0.650	0.847	0.250	0.710	0.686
LogDIA	r	+ 0.543	- **0.755**	- 0.486	+ 0.019	+ **0.857**
	p	0.173	**0.030**	0.222	0.964	**0.007**
LogDEA	r	+ 0.166	- **0.863**	- **0.768**	+ 0.231	+ 0.280
	p	0.695	**0.006**	**0.026**	0.582	0.501
Log Atrazine	r	+ 0.318	- **0.919**	- **0.806**	+ 0.019	+ 0.610
	p	0.443	**0.001**	**0.016**	0.964	0.109
Log Tertbutylazine	r	+ 0.258	- **0.763**	- 0.684	+ 0.428	- 0.173
	p	0.537	**0.028**	0.061	0.291	0.682

Table 5: Pearson correlation coefficients (r) and Bonferroni probabilties (p) between the median biomarker responses in male and female *X. laevis* and the average values (4WM) of environmental co-factors measured in the month prior to collection of frogs at the different sampling sites in the Potchefstroom region, SA. Positive and negative correlations are indicated by + and -, respectively. Bold numbers = significant regressions ($p < 0.05$). N = 8.

Males		GSI	logT	logE2	logE2/T	Log Aromatase
logTemp (°C)	r	+ 0.593	- 0.232	- 0.601	- **0.810**	
	p	0.121	0.581	0.115	**0.015**	
PH	r	+ 0.394	- 0.060	+ 0.153	- 0.442	
	p	0.334	0.887	0.717	0,273	
logCond (µS/cm)	r	+ **0.732**	- 0.007	- 0.089	- 0.182	
	p	**0.039**	0.986	0.833	0.666	
DO (mg/L)	r	+ 0.400	- 0.095	- 0.494	- **0.717**	
	p	0.326	0.822	0.285	**0.045**	
logDepth (cm)	r	- **0.817**	+ 0.084	+ 0.294	+ 0.589	
	p	**0.013**	0.843	0.551	0.124	
Females		logGSI	logT	logE2	logE2/T	Log Aromatase
logTemp (°C)	r	+ 0.579	- 0.483	- 0.412	- 0.426	+ **0.786**
	p	0.133	0.225	0.310	0.293	**0.021**
PH	r	+ 0.258	- 0.823	- 0.472	+ 0.308	+ 0.522
	p	0.537	0.012	0.237	0.458	0.185
logCond (µS/cm)	r	+ **0.847**	- 0.442	- 0.685	+ 0.124	+ 0.237
	p	**0.008**	0.072	0.061	0.769	0.573
DO (mg/L)	r	- 0.032	- 0.156	- 0.182	+ 0.200	- 0.583
	p	0.941	0.711	0.666	0.635	0.129
logDepth (cm)	r	- **0.736**	+ 0.623	+ 0.611	- 0.187	- 0.704
	p	**0.037**	0.099	0.108	0.658	0.051

Table 6: Pearson correlation coefficients (r) and Bonferroni probabilties (p) between the different biomarkers measured in male and female *X. laevis* from the different sampling sites in the Potchefstroom region, SA. Top values represent the r values, with associated p values given below. Positive and negative correlations are indicated by + and -, respectively. Bold numbers = significant regressions ($p < 0.05$).

Males		GSI	logT	logE2	logE2/T	Log Aromatase
GSI	R^2		+ 0.065	- 0.084	- 0.166	
	p		0.533	0.427	0.114	
	n		93	92	92	
logT [pg/ml]	R^2			+ **0.757**	+ 0.201	
	p			**<0.001**	0.059	
	n			**92**	89	
logE2 [pg/ml]	R^2				+ **0.804**	
	p				**<0.001**	
	n				**89**	
females		LogGSI	logT	logE2	logE2/T	Log Aromatase
logGSI	R^2		- 0.114	- 0.114	- 0.076	+ 0.074
	p		0.257	0.252	0.460	0.456
	n		101	102	96	105
logT [pg/ml]	R^2			+ **0.868**	+ 0.088	- 0.003
	p			**<0.001**	0.394	0.975
	n			**99**	96	99
logE2 [pg/ml]	R^2				+ **0.582**	- 0.047
	p				**<0.001**	0.643
	n				**96**	99
logE2/T	R^2					- 0.093
	p					0.375
	n					94

Figure 1: Gonadosomatic index (GSI) (A & B) and aromatase activity (C & D) of male (A & C) and female (B & D) *X. laevis* from the Potchefstroom region of SA. Solid dots = water atrazine concentrations. Bold horizontal lines = group means of medians for NCGR sites and CGR sites. Box Plots: The center vertical line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges (hinges) at the first and third quartiles. The absolute value of the difference between the values of the two hinges is called Hspread. Lower inner fence = lower hinge - (1.5 • (Hspread)); Upper inner fence = upper hinge + (1.5 • (Hspread)); Lower outer fence =lower hinge - (3 • (Hspread)); Upper outer fence = upper hinge + (3 • (Hspread)). Values between the inner and outer fences are plotted with asterisks. Values beyond the outer fences, called far outside values, are plotted with empty circles.

Figure 2: Plasma T concentrations (A & B), plasma E2 concentrations (C & D), and E2/T ratios (E & F) in male (A, C & E) and female (B, D & F) *X. laevis* from the Potchefstroom region in SA. Solid dots = water atrazine concentrations at the sampling sites. Bold horizontal lines = group means of medians for NCGR sites and CGR sites. See legend of figure 1 for an explanation of the box plots. * = Group median at CGR sites significantly different from that at C sites (NCGR) (T females [B]: $p = 0.018$; E2 females [D]: $p=0.0018$).

Figure 1

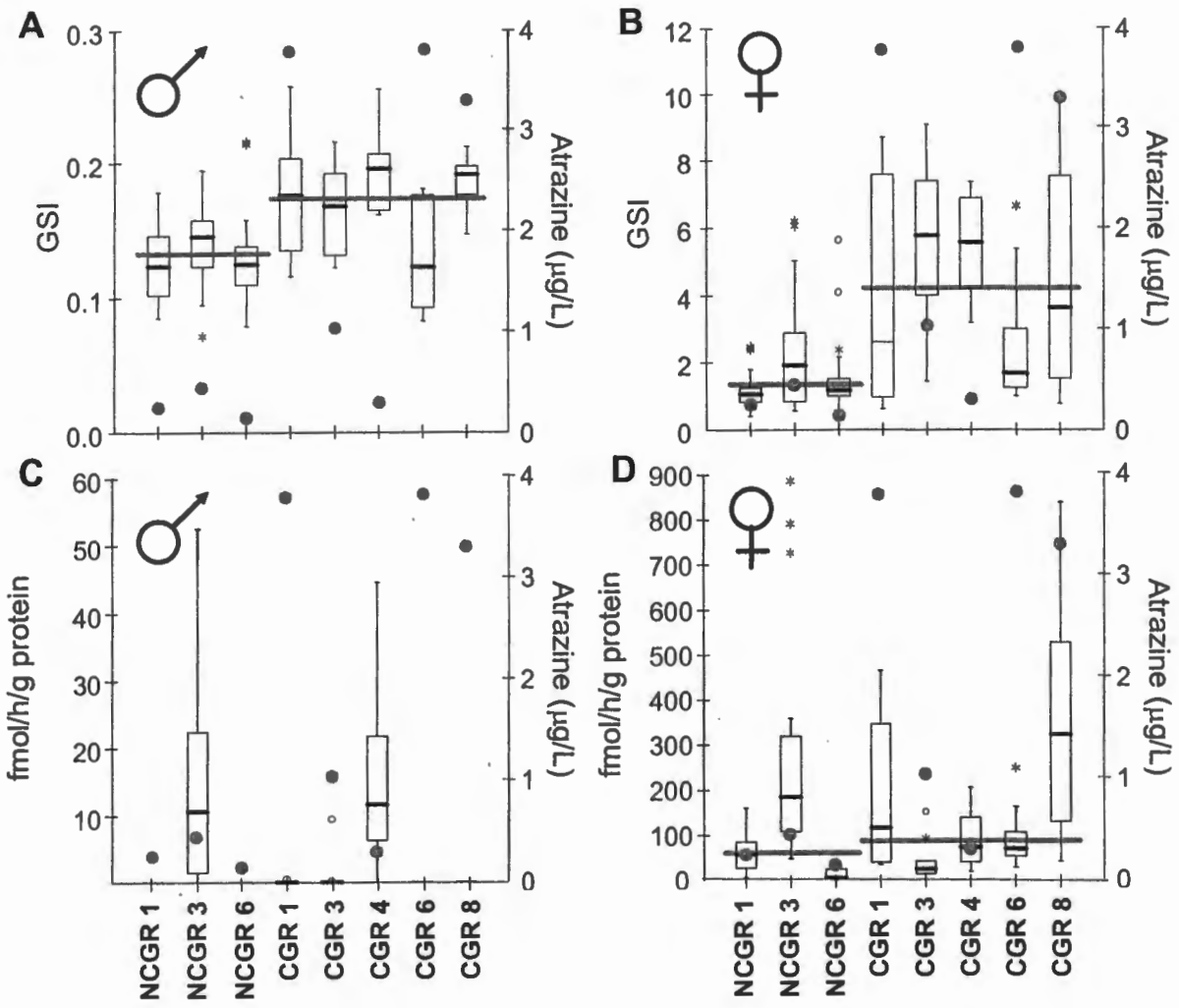
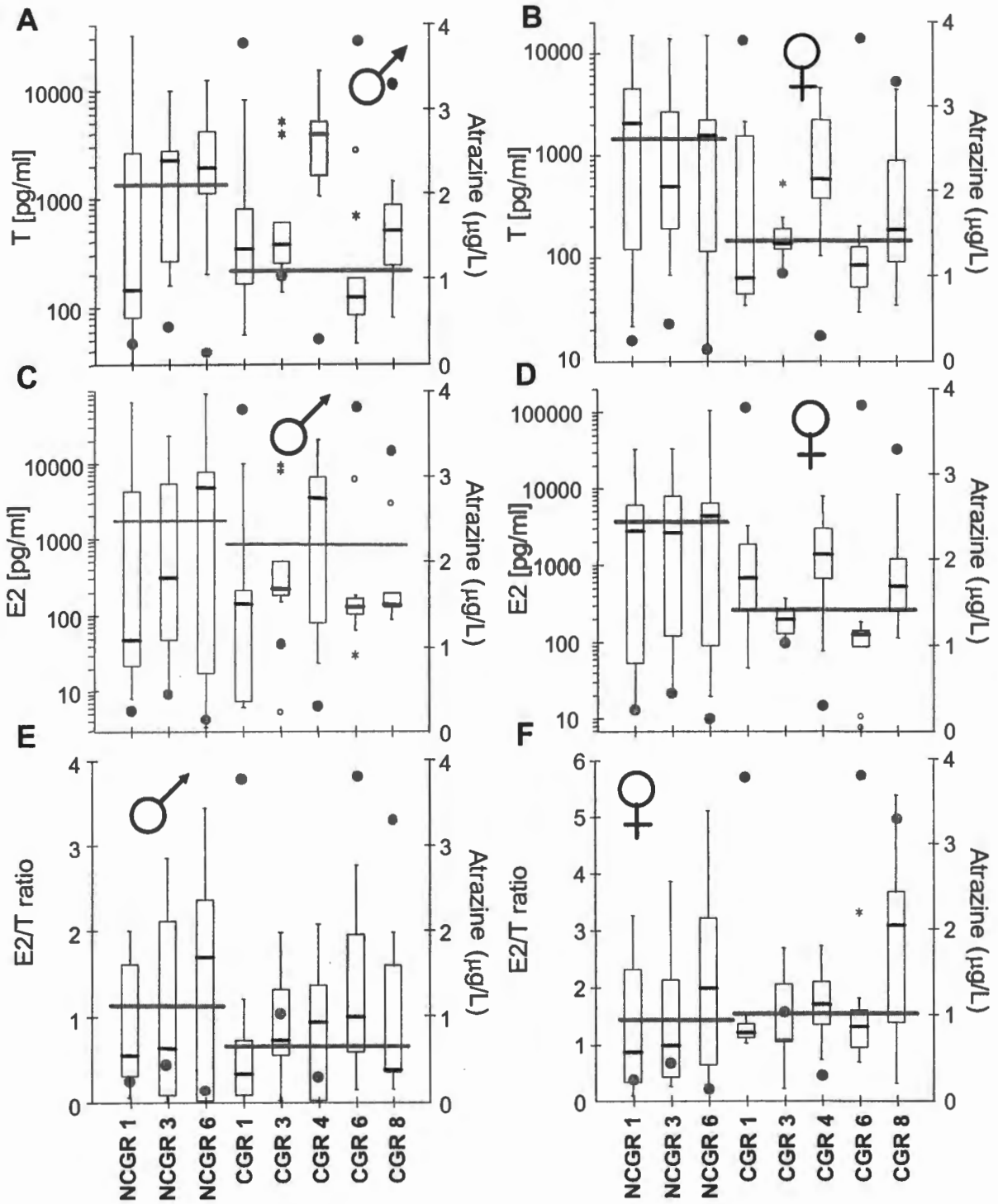

A
B
C
D
GSI
fmol/h/g protein
Atrazine (µg/L)
NCGR 1
NCGR 3
NCGR 6
CGR 1
CGR 3
CGR 4
CGR 6
CGR 8

Figure 2


A
B
C
D
E
F
T [pg/ml]
E2 [pg/ml]
E2/T ratio
Atrazine (µg/L)
NCGR 1
NCGR 3
NCGR 6
CGR 1
CGR 3
CGR 4
CGR 6
CGR 8

APPENDIX 4

Total Data Summary for Chapter 2

Appendix 4: Total Data Summary for Chapter 2

Site R1

	No	Sex	Mass	S-V length	Age
	1	F	141.4	110.8	
	2	F	204.5	125.8	
	3	F	22.7	57.4	
	4	F	21.5	60.3	2
	5	F	42.3	75.3	
	6	F	25.4	60.7	4
	7	F	93.8	94.1	
	9	F	68.8	88	2
	11	F	36.4	73.1	4
	12	F	151.5	115	2
	14	F	48.7	53.3	2
	16	F	17.9	57.4	3
	17	F	20.1	61	
	18	F	18.9	58.5	
	19	F	39.1	71.9	
	20	F	28.9	54.9	4
	21	F	33.9	71.5	3
	22	F	22.1	60	
	23	F	65.4	83.3	
	24	F	38.6	70.1	
	25	F	26.3	64.8	4
	26	F	27.8	65.4	3
	27	F	122.6	119.2	2
	28	F	22.5	58.2	1
	29	F	36.8	70.3	2
	30	F	13.4	50.1	
	31	F	108.1	105.2	6
	33	F	19.2	56.5	
	34	F	79.1	92.7	
	35	F	81.9	97.3	2
	38	F	13.8	51.4	2
	39	F	103.7	101	
	40	F	33	69.5	
	41	F	20.7	60.2	
	43	F	22.5	62	4
	44	F	8.5	44.2	1
	46	F	7.6	41.5	4
	48	F	25.7	60.4	
	50	F	12.9	52.6	1
	51	F	106.9	99.4	
	52	F	20.8	61	2
	55	F	58.2	88.3	
	56	F	38.6	77.4	2

Appendix 4: Total Data Summary for Chapter 2

	59	F	70.4	88.5	2
	60	F	22.2	62	2
	62	F	17.3	56	3
	65	F	28.7	66.2	
	74	F	16.5	56.8	1
	76	F	29.9	67.9	
	81	F	8.8	39.6	1
	85	F	21.9	59.2	
	87	F	55.9	81.7	3
	88	F	40.4	76.4	3
	90	F	32.7	71.2	
	91	F	14.4	51.9	3
	93	F	57.5	87	2
	94	F	17.1	59.5	2
	95	F	20.7	61.7	
	96	F	22.6	61.3	3
	98	F	84.3	92.7	
	99	F	29.6	76.9	2
n	**61**				
Mean			**44.97**	**71.60**	**2.54**
SD			**39.57**	**19.54**	**1.12**
Min			**7.60**	**33.90**	**-2.00**
Max			**204.5**	**125.8**	**6.00**
	8	M	41.7	73.1	5
	10	M	15.6	53.1	3
	13	M	22.9	66.8	4
	15	M	12.4	49.8	
	32	M	35.9	72.2	3
	36	M	20.5	59	1
	37	M	26.7	61.5	
	42	M	30.8	67.5	
	45	M	14.7	50.6	
	47	M	22.8	62.3	
	49	M	15.7	51.2	2
	53	M	20.5	59.6	
	54	M	16.5	56	4
	57	M	31.9	65.1	
	58	M	13.5	46.3	
	61	M	14.1	52.2	
	63	M	20.4	56.4	4
	64	M	11.5	49	
	66	M	18.3	58.4	2
	67	M	17	57.4	3
	68	M	16.5	57.7	4
	69	M	9.4	43	
	70	M	15.7	52.2	
	71	M	17.6	52.6	2

	72	M	8.3	42.3	2
	73	M	20.5	57.6	2
	75	M	7.4	41.5	
	77	M	16	53.5	4
	78	M	11.3	46.2	1
	79	M	15.8	55.5	
	80	M	17.1	55.4	2
	82	M	23.5	59.6	
	83	M	12.9	50.2	1
	84	M	10.5	48.2	1
	86	M	16.1	54	2
	89	M	24.1	65.2	3
	92	M	14	50.1	5
	97	M	22.3	58.6	
	100	M	21.9	62.4	3
n	**39**				
Mean			**18.57**	**55.73**	**2.74**
SD			**7.35**	**7.64**	**1.25**
Min			**7.40**	**41.50**	**1.00**
Max			**41.7**	**73.1**	**5.00**

Site R3

	No	Sex	Mass	S-V length	Age
	1	F	35.2	70.8	
	2	F	46.2	73.8	
	3	F	46.1	78.3	
	7	F	52.6	80.3	2
	9	F	35.8	71.8	
	13	F	38.2	71.3	1
	15	F	35.9	69	
	16	F	9.2	43.1	
	20	F	15.4	44.2	
	24	F	82.6	49.7	
	25	F	39.3	73.9	4
	27	F	60.1	78.8	1
	29	F	46.2	78	2
	32	F	49.1	82.5	3
	33	F	40.6	76.8	2
	35	F	117.8	105.2	1
	36	F	62.8	85	
	39	F	39.4	75.9	1
	40	F	39.8	74.9	3
	41	F	32.8	70.5	1
	43	F	41.2	71.6	2
	44	F	42.7	75	
	45	F	13.7	50.6	1
	46	F	71.8	90.6	2
	48	F	32.1	69.4	2
	49	F	32.1	68.9	2
	50	F	39.9	75.2	2
	52	F	49.3	78.6	1
	53	F	8.3	43.3	
	63	F	12.2	46.8	1
	67	F	12.8	49.4	1
	71	F	10.3	44.4	1
	81	F	60	85.4	2
	82	F	11.8	47.7	1
	85	F	6.1	38.8	
	88	F	29.2	66.1	2
	89	F	11.2	45.3	1
	90	F	23.5	64.1	3
	92	F	40.3	78.4	
	93	F	7.9	42.4	
	94	F	37.8	75.4	
	96	F	8.5	44.8	1
	97	F	23.2	63.4	

	98	F	7.9	44.4	
	99	F	10.3	44.9	
n	**45**				
Mean			**34.87**	**65.75**	**1.70**
SD			**22.73**	**16.18**	**0.82**
Min			**6.10**	**38.80**	**1.00**
Max			**117.8**	**105.2**	**4.00**
	22	J	3.3	30.2	1
	75	J	3.4	31.6	1
	100	J	4.7	35	1
n	**3**				
Mean			**3.80**	**32.27**	**1.00**
SD			**0.78**	**2.47**	**0.00**
Min			**3.30**	**30.20**	**1.00**
Max			**4.7**	**35**	**1.00**
	4	M	13.9	48.1	
	5	M	19.9	53.4	
	6	M	14.9	50.5	
	8	M	11.8	45.5	1
	10	M	12.6	47.4	1
	11	M	11.3	45.7	1
	12	M	21	58.4	
	14	M	16.2	52	
	17	M	14.4	50.1	1
	18	M	10.9	47.5	1
	19	M	13.3	51.4	
	21	M	22.4	61.8	
	23	M	13.3	47.8	1
	26	M	12.5	49	1
	28	M	12	47.8	1
	30	M	7.6	43	1
	31	M	11.5	48.4	1
	34	M	11.5	45.8	1
	37	M	14.2	48.3	1
	38	M	13	49.2	1
	42	M	24.3	64.2	2
	47	M	28.8	68.8	2
	51	M	9.8	43.3	1
	54	M	29.8	65.5	1
	55	M	29	66	1
	56	M	13	48.6	1
	57	M	8.2	43.5	
	58	M	6.9	39.5	1
	59	M	40.1	71.2	1
	60	M	31.7	66.7	2

Appendix 4: Total Data Summary for Chapter 2

	61	M	27.2	66.2	2
	62	M	13.3	48.9	1
	64	M	13.5	48.6	
	65	M	27.7	64.4	
	66	M	10.2	44.5	1
	68	M	12.5	47.7	1
	69	M	12.1	48.4	
	70	M	13	48.3	1
	72	M	11	46.5	1
	73	M	8.4	41.2	
	74	M	11.8	51.2	
	76	M	13.6	49.5	1
	77	M	24.5	56.9	2
	78	M	65.2	85.6	4
	79	M	13.7	51.9	
	80	M	24	58.7	
	83	M	12	47.8	1
	84	M	13	48.8	
	86	M	18.1	55.8	
	87	M	9.8	45.6	1
	91	M	25.9	62.3	
	95	M	20.3	58.3	2
n	**52**				
Mean			**17.32**	**52.80**	**1.26**
SD			**9.95**	**9.09**	**0.62**
Min			**6.90**	**39.50**	**1.00**
Max			**65.2**	**85.6**	**4.00**

Site R6

	No	Sex	Mass	S-V length	Age
	4	F	16.8	50.2	
	5	F	7.1	38.3	2
	6	F	27.9	67.3	1
	9	F	6.3	39.2	3
	10	F	32.9	68.2	1
	11	F	30.2	68	
	12	F	32.3	65.7	
	13	F	30.1	68.7	1
	14	F	32	69.4	1
	16	F	18.3	58.7	1
	17	F	13.7	49.8	1
	18	F	32.6	66.3	1
	19	F	20.4	58.2	2
	20	F	21.2	52.5	
	21	F	26.4	64.8	1
	22	F	31.9	69	1
	24	F	51.4	77	1
	25	F	46.4	79	1
	26	F	20	56.9	
	31	F	32	69	1
	32	F	98	100	
	34	F	11.6	45.2	1
	35	F	11.2	45.8	3
	36	F	36.1	69.2	1
	38	F	20.7	61.2	
	39	F	43.6	73.8	2
	41	F	38	73.6	1
	42	F	25.7	63.9	1
	43	F	34.7	71.2	1
	47	F	25.2	67.4	1
	50	F	7.1	41.7	3
	51	F	48	76.5	
	53	F	13.6	53.3	1
	54	F	142.1	113.1	
	56	F	96.3	100.1	1
	58	F	26.8	65.7	
	59	F	72.9	95.8	1
	60	F	28.2	64.2	1
	61	F	10.9	43.4	
	62	F	142.5	108.9	2
	65	F	29.7	64.1	1
	67	F	32.2	66.8	2
	69	F	42.6	70.6	1

	70	F	42.8	76.5	1
	73	F	8.7	41	2
	74	F	111.3	107	1
	75	F	32.6	71.2	1
	77	F	33.6	72.6	1
	80	F	19.6	59	2
	84	F	49.7	74.2	
	85	F	48	80.1	2
	87	F	11	45.3	1
	88	F	10.2	44.6	
	92	F	75.9	94.1	2
	94	F	28.4	67.9	
	98	F	51	80	2
	99	F	50.7	77.6	2
	100	F	41	72.5	1
n	**58**				
Mean			**37.62**	**67.85**	**1.39**
SD			**29.66**	**17.30**	**0.62**
Min			**6.30**	**38.30**	**1.00**
Max			**142.5**	**113.1**	**3.00**
	15	J	6.4	37.5	
	1	M	10.4	46.1	1
	2	M	19.4	60.8	1
	3	M	40.6	67.9	2
	7	M	34	68.4	
	8	M	7.1	38.9	
	23	M	33.3	66.7	1
	27	M	22.5	61.6	
	28	M	70	82.7	3
	29	M	57.7	83	4
	30	M	30.9	69	2
	33	M	21.4	57.1	1
	37	M	66.2	84.5	1
	40	M	46.5	75.8	
	44	M	22.7	59.7	1
	45	M	14.7	49.6	2
	46	M	27.9	64.9	1
	48	M	19	57.2	1
	49	M	8.1	44.2	3
	52	M	62.5	81.6	2
	55	M	20.9	59.4	1
	57	M	47.1	76.6	3
	63	M	50	76.1	1
	64	M	43	76.3	2
	66	M	31.7	66.2	1
	68	M	18	57.3	1

Appendix 4: Total Data Summary for Chapter 2

	71	M	39.9	74.3	1
	72	M	22.9	54.9	1
	76	M	57.3	84.7	
	78	M	24.4	63.7	1
	79	M	28.3	68	
	81	M	28.9	68	5
	82	M	18	53.2	
	83	M	9.5	45.6	1
	86	M	20.8	58.8	
	89	M	15.2	51.7	2
	90	M	12.8	45.6	2
	91	M	9.8	43.4	2
	93	M	29.6	63.3	1
	95	M	26.3	63.4	1
	96	M	10.2	46.3	
	97	M	20.3	60	1
n	**41**				
Mean			**29.26**	**62.84**	**1.66**
SD			**16.78**	**12.42**	**1.00**
Min			**7.10**	**38.90**	**1.00**
Max			**70**	**84.7**	**5.00**

Appendix 4: Total Data Summary for Chapter 2

Site E1

	No	Sex	Mass	S-V length	Age
	3	F	21.9	58.7	2
	4	F	30.0	69.2	2
	6	F	10.5	44.9	1
	7	F	15.7	53.8	3
	8	F	12.4	50.2	1
	9	F	15.1	53.4	3
	11	F	17.9	57.4	1
	13	F	15.6	50.4	1
	15	F	17.6	55.2	8
	17	F	16.3	54.6	2
	20	F	18.4	57.5	2
	21	F	24.5	61.0	1
	23	F	18.3	60.6	1
	24	F	14.4	51.6	1
	27	F	31.9	69.4	1
	28	F	17.5	52.3	
	29	F	14.4	53.2	
	32	F	26.9	61.8	
	33	F	16.5	56.2	1
	37	F	8.0	41.2	5
	39	F	8.1	39.2	
	40	F	23.1	66.0	1
	47	F	23.6	63.2	3
	49	F	14.1	53.3	1
	54	F	7.3	39.2	
	55	F	18.1	55.2	1
	56	F	37.3	76.9	
	58	F	14.6	56.7	1
	60	F	14.2	52.4	
	61	F	24.1	64.2	
	62	F	16.7	57.5	1
	66	F	13.7	50.0	4
	68	F	51.8	83.4	4
	79	F	16.7	55.2	
	81	F	17.6	57.1	1
	83	F	9.9	45.0	2
	86	F	19.5	58.5	1
	87	F	124.8	105.2	
	88	F	18.5	56.6	
	91	F	19.4	56.5	1
	92	F	12.9	52.2	
	94	F	26.3	65.6	
	95	F	11.3	49.2	

Appendix 4: Total Data Summary for Chapter 2

	97	F	9.5	43.1	2
	98	F	18.6	55.7	2
n	**45**				
Mean			**20.79**	**57.10**	**1.97**
SD			**17.80**	**11.43**	**1.56**
Min			**7.30**	**39.20**	**1.00**
Max			**124.8**	**105.2**	**8.00**
	34	J	6.3	40.0	1
	38	J	3.0	30.6	1
	50	J	5.3	36.5	1
	85	J	2.1	27.9	1
n	**4**				
Mean			**4.18**	**33.75**	**1.00**
SD			**1.96**	**5.50**	**0.00**
Min			**2.10**	**27.90**	**1.00**
Max			**6.3**	**40**	**1.00**
	1	M	31.1	63.2	2
	2	M	49.0	79.6	1
	5	M	9.7	45.2	4
	10	M	11.0	47.5	3
	12	M	13.6	49.2	
	14	M	8.2	39.8	6
	16	M	10.0	43.5	
	18	M	13.1	47.9	2
	19	M	5.3	35.6	
	22	M	8.3	43.3	2
	25	M	13.1	48.6	3
	26	M	15.5	51.7	
	30	M	14.6	49.3	1
	31	M	29.1	64.8	
	35	M	12.5	48.6	
	36	M	19.7	54.2	1
	41	M	12.1	59.6	3
	42	M	12.4	49.8	
	43	M	10.9	49.6	1
	44	M	15.8	50.7	2
	45	M	12.7	47.2	1
	46	M	9.8	46.5	
	48	M	15.9	53.5	1
	51	M	12.7	47.4	
	52	M	5.7	37.0	1
	53	M	11.5	47.2	2
	57	M	12.3	49.6	

	59	M	8.9	47.1	1
	63	M	40.7	74.8	1
	64	M	19.8	56.0	2
	65	M	8.1	44.0	
	67	M	14.1	52.6	1
	69	M	17.8	52.6	1
	70	M	11.6	47.6	4
	71	M	16.9	55.0	4
	72	M	10.0	44.7	5
	73	M	17.9	55.4	
	74	M	8.7	42.5	1
	75	M	11.1	47.3	3
	76	M	10.1	45.6	1
	77	M	12.5	46.6	5
	78	M	13.6	51.0	
	80	M	12.4	48.8	4
	82	M	14.0	52.6	
	84	M	14.5	52.5	1
	89	M	10.8	45.6	1
	90	M	7.7	43.3	2
	93	M	13.8	50.5	1
	96	M	18.6	53.2	2
	99	M	17.7	54.2	
	100	M	13.8	49.0	
n	**51**				
Mean			**14.52**	**50.25**	**2.17**
SD			**7.86**	**7.79**	**1.42**
Min			**5.30**	**35.60**	**1.00**
Max			**49.0**	**79.6**	**6.00**

Site E3

	No	Sex	Age	S-V length	Age
	1	F	6.9	38.2	1
	2	F	100.2	100.4	2
	3	F	102.3	104.8	
	5	F	45.1	73.2	2
	7	F	13.8	50.3	
	8	F	24	50.4	2
	9	F	31.7	67.1	
	10	F	13	51	2
	13	F	12.1	48.5	
	15	F	20.4	59.1	
	16	F	30.9	69.5	
	19	F	14.7	51.3	
	20	F	12.1	47.9	
	22	F	134	105.1	2
	24	F	14.1	49.2	1
	25	F	23.6	60.3	
	26	F	11.9	50.3	2
	27	F	12.9	47.9	
	28	F	16.4	56	
	29	F	17	53.7	
	30	F	9.6	45.2	
	33	F	16.4	50.4	
	38	F	18.7	56	1
	40	F	20	58.3	2
	41	F	16	54.2	
	43	F	10.2	47	
	46	F	14.7	52.2	
	47	F	96	97.1	1
	49	F	24	61.5	
	50	F	11	44.3	
	52	F	36	69.3	1
	53	F	14.7	50.8	
	54	F	30.6	66.7	
	57	F	26.4	60.3	
	63	F	88	93.8	
	64	F	10.7	47.1	1
	67	F	70.4	85.8	
	68	F	18	55.3	
	69	F	12.5	50.5	
	70	F	15.8	44.9	
	73	F	24	61.3	
	76	F	15.8	52.8	2

	78	F	99.7	99.8	
	79	F	12	49.3	1
	84	F	13.5	50.3	
	85	F	55.4	84.6	
	88	F	9.8	47	3
	90	F	13.7	50.3	1
	92	F	13.4	51.4	1
	93	F	11.7	50.4	1
	98	F	5.7	36.4	1
	99	F	28.2	69.2	1
	100	F	51.4	82.2	
n	**53**				
Mean			**29.64**	**60.56**	**1.48**
SD			**29.72**	**17.68**	**0.60**
Min			**5.70**	**36.40**	**1.00**
Max			**134**	**105.1**	**3.00**
	4	M	49.1	75.8	3
	6	M	31.4	64.9	1
	11	M	16	55.3	1
	12	M	21.6	58.6	
	14	M	7.8	43.2	2
	17	M	20.7	57.8	3
	18	M	11.6	44.8	
	21	M	15.2	43.2	1
	23	M	10.6	45.2	
	31	M	10.8	45.8	
	32	M	7.3	48.7	
	34	M	13.2	51	1
	35	M	13.4	48.3	1
	36	M	8.9	39.5	
	37	M	21.7	59.2	3
	39	M	13	47.7	
	42	M	13.9	50.5	
	44	M	16.1	53	
	45	M	12.2	47.5	
	48	M	10.5	43.2	
	51	M	11.4	45.2	
	55	M	13.5	50.5	
	56	M	15.7	52.9	
	58	M	15.3	51.5	1
	59	M	13.8	48	2
	60	M	12.2	45.3	1
	61	M	10.1	43.6	
	62	M	14.2	48.3	2
	65	M	30.7	66.9	
	66	M	10.3	45.6	
	71	M	10.5	45.2	

Appendix 4: Total Data Summary for Chapter 2

	72	M	9.7	46.4	
	74	M	11	46.5	1
	75	M	13.4	49	
	77	M	17.8	59.8	
	80	M	23.9	58.8	1
	81	M	21.6	57.8	
	82	M	19	56.1	
	83	M	9.3	43.6	
	86	M	13.9	53.3	1
	87	M	24.5	61.2	1
	89	M	9.3	42.2	1
	91	M	12.7	49.5	
	94	M	15.6	54.5	4
	95	M	13.2	45.9	
	96	M	12.1	47.8	2
	97	M	12.2	47	4
n	**47**				
Mean			**15.36**	**50.76**	**1.76**
SD			**7.37**	**7.33**	**1.04**
Min			**7.30**	**39.50**	**1.00**
Max			**49.1**	**75.8**	**4.00**

Site E4

	No	Sex	Mass	S-V length	Age
	1	F	120	107	
	2	F	21.3	58.4	4
	3	F	23.1	63	
	4	F	24.3	62	3
	5	F	30.4	76.6	3
	7	F	23.6	61.8	
	8	F	23.3	60.1	2
	9	F	35.1	68.1	3
	10	F	21.4	55.7	1
	11	F	55.7	88.6	6
	13	F	18.9	56.2	3
	15	F	38	70.6	
	16	F	49.2	82.3	4
	17	F	84.8	95.4	4
	18	F	23.3	63.1	
	20	F	22	62.6	3
	22	F	39.9	76.2	1
	23	F	31.3	70.7	3
	26	F	30.2	67.4	2
	28	F	14.3	54.3	1
	30	F	34.6	74.2	3
	31	F	22	60.4	1
	32	F	58.4	84.7	3
	35	F	21.9	64.5	1
	36	F	109	108.1	4
	37	F	30.6	68.2	1
	39	F	24.3	61.3	2
	40	F	24.6	64.5	
	42	F	19.2	59.3	3
	44	F	38.7	70.8	
	45	F	36.3	72.1	
	46	F	33.7	68.1	
	47	F	20.5	60.4	1
	48	F	20.1	64.9	3
	50	F	25.3	64.4	2
	51	F	26.4	67.4	3
	52	F	18.9	58	2
	53	F	20	59.2	2
	55	F	35.2	68.1	2
	56	F	34.5	70	3
	58	F	22.7	67.9	2
	59	F	29.6	65.4	3
	61	F	15.4	51.2	1

Appendix 4: Total Data Summary for Chapter 2

n	**43**				
Mean			**33.77**	**68.68**	**2.50**
SD			**22.24**	**12.47**	**1.16**
Min			**14.30**	**51.20**	**1.00**
Max			**120**	**108.1**	**6.00**
	62	J	4.3	33.6	1
	63	J	3.6	32.2	1
	64	J	4.2	32.2	1
n	**3**				
Mean			**4.03**	**32.67**	**1.00**
SD			**0.38**	**0.81**	**0.00**
Min			**3.60**	**32.20**	**1.00**
Max			**4.3**	**33.6**	**1.00**
	6	M	17	56.1	4
	12	M	22.7	65.8	
	14	M	16.8	55.3	1
	19	M	16.5	55	1
	21	M	18.2	54.5	3
	24	M	43.8	75.3	1
	25	M	17	51.1	5
	27	M	23.7	61.3	3
	29	M	22.4	60.5	1
	33	M	14.4	51	
	34	M	15.8	54	3
	38	M	22	59	
	41	M	14.2	50.4	4
	43	M	21.5	59.2	4
	49	M	17.6	55.3	3
	54	M	17	50.9	2
	57	M	23.9	61.9	
	60	M	18.8	54.2	
	65	M	24.5	61.4	3
	66	M	31.3	67.1	
	67	M	30.1	63.7	2
	68	M	14.7	50.4	4
n	**22**				
Mean			**21.09**	**57.88**	**2.75**
SD			**6.93**	**6.38**	**1.29**
Min			**14.20**	**50.40**	**1.00**
Max			**43.8**	**75.3**	**5.00**

Site E5

	No	Sex	Mass	S-V length
	1	F	18.7	58.4
	4	F	20	57.5
	5	F	6.3	36.1
	7	F	19.8	59.4
	8	F	17.8	57.3
	10	F	10.7	46.2
	11	F	75.2	94.2
	14	F	14.1	50
	15	F	70.7	90.5
	17	F	18.4	57.5
	19	F	19.4	59
	21	F	194.5	128.6
	22	F	20.1	60.1
	25	F	18.7	55.1
	26	F	11.3	48.5
	27	F	52.9	84.6
	28	F	28.8	67.3
	29	F	23.5	62
	30	F	30.5	69.4
	32	F	32.2	64.7
	33	F	21.2	60
	37	F	12.8	52
	38	F	14.6	51.1
	39	F	16.8	57.2
	41	F	12	50.3
	42	F	11.9	47.2
	44	F	12.4	52.1
	46	F	18.2	56.5
	47	F	11.6	44.3
	49	F	18.3	55.5
	50	F	15.1	51.8
	51	F	8.6	42
	52	F	20.3	61.6
	53	F	17.2	55.4
	56	F	24.9	61.2
	57	F	25.5	63.9
	58	F	10.5	44.3
	60	F	34.4	72.6
	61	F	36.9	76.9
	62	F	27.3	68.2
	63	F	23.1	60.6
	64	F	34.2	67.4
	65	F	19.6	58.5

	68	F	65.3	87.3
	69	F	15.9	52.8
	71	F	46.3	77
	72	F	66.7	89
	73	F	43.6	73.6
	74	F	25.3	64.8
	76	F	16.3	52.1
	77	F	14.4	49.7
n	**51**			
Mean			**28.33**	**62.03**
SD			**28.76**	**15.95**
Min			**6.30**	**36.10**
Max			**194.5**	**128.6**
	2	M	14.6	52.3
	3	M	17.3	54.3
	6	M	10.4	45.9
	9	M	11.9	48.4
	12	M	22	53.4
	13	M	16.3	55
	16	M	22.2	59.3
	18	M	18.6	54.5
	20	M	16.6	56
	23	M	10.5	45.1
	24	M	12.2	48.1
	31	M	8	43.8
	34	M	19.4	56
	35	M	13.5	49
	36	M	24	58.4
	40	M	11.6	47.3
	43	M	13.3	49
	45	M	24	60
	48	M	8.2	43.4
	54	M	29.5	68.2
	55	M	11.6	45.4
	59	M	12.3	47.7
	66	M	10.7	43.3
	67	M	15.1	51.8
	70	M	14.2	50.2
	75	M	8.8	43.2
n	**26**			
Mean			**15.26**	**51.12**
SD			**5.52**	**6.22**
Min			**8.00**	**43.20**
Max			**29.5**	**68.2**

Site E6

	No	Sex	Mass	S-V length	Age
	4	F	10.1	47.3	
	5	F	10.1	42.5	2
	6	F	9	44.1	2
	7	F	51.1	79.1	3
	8	F	22.6	60.6	1
	9	F	14.9	51.7	
	10	F	72.5	91.9	5
	12	F	29.1	63.8	3
	13	F	23.6	63.2	1
	14	F	27.9	65.1	
	16	F	16.4	53.5	2
	17	F	47.2	79.5	
	18	F	53.3	83.6	1
	19	F	47	77.9	3
	21	F	12	48.2	4
	22	F	23.2	60.5	
	23	F	15	49.5	1
	24	F	36.8	70.4	
	27	F	21.8	56.2	
	28	F	21.6	60	6
	30	F	16.5	53.9	3
	32	F	14.3	50.9	
	35	F	25.9	59.8	2
	36	F	24.8	61.7	2
	37	F	13.3	51	1
	38	F	26.9	65.5	2
	41	F	95.6	99.3	
	43	F	36.5	70.5	
	44	F	13.5	47.9	2
	45	F	21.8	58.9	
	47	F	18.7	59.2	2
	48	F	17.7	55.5	4
	50	F	23.3	59.6	
	52	F	26.5	65.5	1
	54	F	20.7	59.3	2
	56	F	87.2	95.2	5
	57	F	44.5	73.8	
	58	F	18.1	56	2
	63	F	15.2	51.1	1
	64	F	25.7	62.1	2
	65	F	12.2	45.4	1
	66	F	33.1	69.9	3
	67	F	18.3	56.7	4

Appendix 4: Total Data Summary for Chapter 2

	68	F	23.1	59.3	
	69	F	19.8	60	1
	70	F	62.6	86.4	
	72	F	30.3	65.2	
	73	F	22	58.6	
	74	F	10.1	44.1	
	75	F	26.1	62.1	
	77	F	53.5	79	1
	78	F	24.5	64.5	2
	81	F	22.7	60.5	
	82	F	43.8	79.1	2
	84	F	29.9	67.4	2
	85	F	12.2	49.4	
	86	F	46.9	76.3	
	89	F	17.1	54.2	2
	90	F	25.1	61	3
	92	F	28	68.7	
	93	F	48.6	78.7	
	94	F	31.4	70	
	96	F	19	55	
	98	F	16.5	53.5	
	100	F	30.8	65.2	
n	**65**				
Mean			**28.61**	**63.01**	**2.32**
SD			**17.65**	**12.54**	**1.27**
Min			**9.00**	**42.50**	**1.00**
Max			**95.6**	**99.3**	**6.00**
	46	J	6.8	37.7	
	1	M	25	55.1	1
	2	M	14.7	50.5	1
	3	M	20	54.4	2
	11	M	19.4	53.2	3
	15	M	13.8	51.1	
	20	M	18.9	53.3	2
	25	M	17.7	57.1	
	26	M	9.9	41.1	2
	29	M	17.2	54.4	1
	31	M	12	47.4	1
	33	M	27.5	61.5	2
	34	M	15.6	48.3	
	39	M	16.3	50.9	
	40	M	18.7	57.3	3
	42	M	19.4	55.2	
	49	M	10.6	45.7	1
	51	M	13.4	47.4	1
	53	M	15.9	50.8	2

	55	M	12.8	59.2	2
	59	M	35.8	69.9	1
	60	M	12.7	46.9	2
	61	M	30	65.8	1
	62	M	19.8	54.1	3
	71	M	23.8	59.1	4
	76	M	13.6	48.9	1
	79	M	19	56.2	
	80	M	16.2	53.6	
	83	M	20.3	58.6	
	87	M	27.3	66.5	1
	88	M	22.3	59.2	
	91	M	20	55.8	5
	95	M	17.2	52	1
	97	M	17	51.6	
	99	M	15.9	52.8	
n	**34**				
Mean			**18.52**	**54.26**	**1.87**
SD			**5.66**	**6.08**	**1.10**
Min			**9.90**	**41.10**	**1.00**
Max			**35.8**	**69.9**	**5.00**

Site E8

	No	Sex	Mass	S-V length	Age
	1	F	17.1	55.5	6
	2	F	24.5	59.5	
	3	F	35.8	74.4	2
	4	F	32.8	68.3	
	5	F	21.3	55.5	
	8	F	70.6	90	
	9	F	20.4	60.2	2
	10	F	12.9	47.6	2
	14	F	18.8	55.5	2
	16	F	21.6	57.6	2
	18	F	22.4	60	2
	19	F	13.3	51.5	1
	22	F	24.9	71.3	
	23	F	64.8	88.6	2
	24	F	20.7	57.2	3
	25	F	44.1	77	2
	31	F	20.2	59.8	2
	32	F	22.4	64.2	
	33	F	16.1	54.2	1
	34	F	16.3	54.9	2
	36	F	23	58.1	4
	37	F	15.7	50.5	2
	38	F	19	57.6	2
	39	F	25.5	62.3	
	40	F	40.3	73.1	1
	41	F	16.8	53.5	1
	42	F	32.6	69.3	
	44	F	26.1	61	3
	45	F	52	82.3	4
	49	F	19.2	58	
	50	F	45.6	75.2	1
	52	F	19.2	58.6	2
	53	F	16.3	53.5	
	54	F	27.4	64.2	3
	56	F	14.4	51.1	4
	59	F	18.2	57.4	2
	65	F	27.3	61.2	2
	67	F	8.4	41.4	1
	69	F	21.4	63.4	4
	70	F	21.7	59.7	1
	72	F	18.7	52.4	
	75	F	38	67.7	1
	76	F	32.4	71	1

	78	F	53	67.8	1
	79	F	17.5	54.9	1
n	**45**				
Mean			**26.46**	**61.96**	**2.12**
SD			**13.57**	**10.21**	**1.17**
Min			**8.40**	**41.40**	**1.00**
Max			**70.6**	**90**	**6.00**
	51	J	3.6	32.2	1
	58	J	6.5	39	
	60	J	7.1	40.4	
	81	J	6.5	37.2	
	82	J	7.3	37.1	
	83	J	5.2	32.1	1
n	**6**				
Mean			**6.03**	**36.33**	**1.00**
SD			**1.40**	**3.46**	**0.00**
Min			**3.60**	**32.10**	**1.00**
Max			**7.3**	**40.4**	**1.00**
	6	M	9.2	40.5	
	7	M	20.5	56.6	
	11	M	41.7	75.7	
	12	M	27.8	64.6	2
	13	M	23.6	60.8	3
	15	M	28.6	63.5	5
	17	M	16.2	53.5	
	20	M	18.3	56.1	2
	21	M	11.3	49.4	
	26	M	24.1	62.2	2
	27	M	17.5	51.8	2
	28	M	11.3	46.3	2
	29	M	20	56.2	4
	30	M	16	50.6	2
	35	M	12.3	48	
	43	M	11	45.9	3
	46	M	15.9	59.9	3
	47	M	16.6	53.3	2
	48	M	16.6	52.7	2
	55	M	19.8	55.6	
	57	M	17.2	55.3	
	61	M	27.1	65.7	
	62	M	15.5	54	
	63	M	18.8	53.5	
	64	M	13.1	50	2
	66	M	14.2	50	1
	68	M	23.7	57.3	2
	71	M	16	53.4	2

	73	M	16.8	51	2
	74	M	10.7	43.3	5
	77	M	17.4	53.8	
	80	M	11.8	45.9	1
n	**32**				
Mean			**18.14**	**54.26**	**2.45**
SD			**6.66**	**7.17**	**1.10**
Min			**9.20**	**40.50**	**1.00**
Max			**41.7**	**75.7**	**5.00**

Summary of data for sites

Site	R1	R3	R6	E1	E3	E4	E5	E6	E8
Total number of frogs	100.0	100.0	100.0	100.0	100.0	68.0	77.0	100.0	83.0
n Females	61.0	45.0	58.0	45.0	53.0	43.0	51.0	65.0	45.0
n Males	39.0	52.0	41.0	51.0	47.0	22.0	26.0	34.0	32.0
n Juveniles	0.0	3.0	1.0	4.0	0.0	3.0	0.0	1.0	6.0
MASS									
Mean mass F	45.0	34.9	37.6	20.8	29.6	33.8	28.3	28.6	26.5
Min mass F	7.6	6.1	6.3	7.3	5.7	14.3	6.3	9.0	8.4
Max mass F	204.5	117.8	142.5	124.8	13.4	120.0	194.5	95.6	70.6
Mean mass M	18.6	17.3	29.3	14.5	15.4	21.1	15.3	18.5	18.1
Min mass M	7.4	6.9	7.1	5.3	7.3	14.2	8.0	9.9	9.2
Max mass M	41.7	65.2	70.0	49.0	49.1	43.8	29.5	35.8	41.7
Mean mass J		3.8		4.2		4.0			6.0
Min mass J		3.3		2.1		3.6			3.6
Max mass J		4.7		6.3		4.3			7.3
SNOUT-VENT LENGTH									
Mean S-V length F	71.6	65.8	67.9	57.1	60.6	68.7	62.0	63.0	62.0
Min S-V length F	33.9	38.8	38.3	39.2	36.4	51.2	36.1	42.5	41.4
Max S-V length F	125.8	105.2	113.1	105.2	105.1	108.1	128.6	99.3	90.0
Mean S-V length M	55.7	52.8	62.8	50.3	50.8	57.9	51.1	54.3	54.3
Min S-V length M	41.5	39.5	38.9	35.6	39.5	50.4	43.2	41.1	40.5
Max S-V length M	73.1	85.6	84.7	79.6	75.8	75.3	68.2	69.9	75.7
Mean S-V length J		32.3		33.8		32.7			36.3
Min S-V length J		30.2		27.9		32.2			32.1
Max S-V length J		35.0		40.0		33.6			40.4
AGE									
1 year	9	43	49	34	24	14		21	14
2 years	22	16	14	15	12	11		21	27
3 years	13	3	6	9	4	18		9	6
4 years	10	2	1	6	2	8		4	5
5 years	1	0	1	3	0	1		4	2
6 years	1	0	0	1	0	1		1	1
7 years	0	0	0	0	0	0		0	0
8 years	0	0	0	1	0	0		0	0
Total number	56	64	71	69	42	53		60	55
Mean age	2.55	1.44	1.46	2.09	1.62	2.51		2.20	2.22

APPENDIX 5

Total Data Summary for Chapter 3

Total data summaries Reference site R1

Site R1 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	19.40	23.00	20.00	20.79	24.62	22.88	23.40	22.40	23.17	22.97	24.64
pH		7.19	7.62	7.08	7.17	6.88	7.12	6.88	7.40	8.04	7.80	7.08
Cond	µS/cm	41.00	39.00	40.00	37.00	42.00	44.00	45.00	44.00	45.00	43.00	47.00
DO Conc	mg/l	7.20	7.62	6.17	4.93	4.61	4.68	3.90	3.97	3.98	4.26	2.91
Rainfall	mm	0.00	46.00	17.00	33.00	37.00	0.00	12.00	8.00	55.00	0.00	5.00
Depth ref	cm	29.30	31.50	36.60	40.00	40.00	37.40	37.50	37.50	40.02	39.40	36.10
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.19	0.41	0.12	1.57	1.66	<0.1	0.57	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	<0.1	0.13	0.12	0.15	<0.1	<0.1	0.16	0.47	0.14	0.13	0.42
G30033 (DEA)	µg/l	<0.1	<0.1	0.24	<0.1	<0.1	0.12	0.18	0.10	0.36	0.12	0.16
Atrazine LL	µg/l	0.40	0.30	0.10	0.32	<0.1	0.38	0.41	0.28	0.27	0.14	0.23
Atrazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.38	<0.1	<0.1	<0.1	<0.1
Terbuthylazine LL	µg/l	<0.1	1.92	0.46	1.90	<0.1	2.11	<0.1	<0.1	<0.1	<0.1	<0.1
Terbuthylazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.39	<0.1	<0.1	<0.1	<0.1

Site R1 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.19	0.41	0.12	1.57	1.66	0.05	0.57	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.05	0.13	0.12	0.15	0.05	0.05	0.16	0.47	0.14	0.13	0.42
G30033 (DEA)	µg/l	0.05	0.05	0.24	0.05	0.05	0.12	0.18	0.10	0.36	0.12	0.16
Atrazine LL	µg/l	0.40	0.30	0.10	0.32	0.05	0.38	0.41	0.28	0.27	0.14	0.23
Atrazine SPE	µg/l	0.05	0.05	0.05	0.05	0.05	0.05	0.38	0.05	0.05	0.05	0.05
Atrazine mean	µg/l	0.23	0.18	0.08	0.18	0.05	0.22	0.39	0.16	0.16	0.10	0.14
Terbuthylazine LL	µg/l	0.05	1.92	0.46	1.90	0.05	2.11	0.05	0.05	0.05	0.05	0.05
Terbuthylazine SPE	µg/l	0.05	0.05	0.05	0.05	0.05	0.05	2.39	0.05	0.05	0.05	0.05
Terbuthylazine mean	µg/l	0.05	0.99	0.26	0.97	0.05	1.08	1.22	0.05	0.05	0.05	0.05

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		1.32	2.86	0.85	10.99	11.63	0.35	2.83	0.45	0.35	0.35
G28279 (DIA) days	µg/l		0.88	0.83	1.04	0.35	0.35	1.11	2.34	1.29	0.92	2.95
G30033 (DEA) days	µg/l		0.35	1.67	0.35	0.35	0.86	1.25	0.50	3.24	0.85	1.14
Atrazine mean days	µg/l		1.23	0.53	1.28	0.35	1.52	2.75	0.82	1.43	0.67	0.98
Terbuthylazine mean days	µg/l		6.91	1.79	6.82	0.35	7.55	8.54	0.25	0.45	0.35	0.35

Summary data for Site C1 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	6.88	0.05	1.32
G28279 (DIA)	0.47	0.05	0.20
G30033 (DEA)	0.60	0.05	0.13
Atrazine	0.39	0.05	0.15
Terbuthylazine	1.22	0.05	0.20

Reference site R1 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
22.86	21.60	23.93	22.63	21.19	24.13	21.38	20.40	18.39	18.07	14.17	12.87	9.30
8.21	7.30	5.61	8.12	8.31	8.56	8.40	8.12	6.66	7.52	8.21	8.03	8.40
44.00	43.00	44.00	42.00	40.00	45.00	43.00	42.00	42.00	44.00	42.00	40.00	45.00
2.80	5.92	6.54	6.40	6.57	5.20	5.78	5.25	5.40	4.10	3.11	5.36	2.84
39.00	45.00	0.00	35.00	47.00	20.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00
38.00	42.00	43.00	43.00	42.50	40.00	40.00	38.00	39.00	36.00	35.00	33.00	32.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	0.50	0.50	2.33	3.49	<0.1	3.91	<0.1	<0.1	<0.1	6.88	1.75
0.25	0.15	0.27	0.20	0.33	0.11	0.26	0.23	0.27	<0.1	0.16	0.40	0.24
0.60	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.18	<0.1	0.14	0.12	0.19
0.24	0.23	0.13	0.10	0.19	0.26	0.34	0.28	0.26	<0.1	0.26	0.32	0.35
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.21	0.26
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	0.50	0.50	2.33	3.49	0.05	3.91	0.05	0.05	0.05	6.88	1.75
0.25	0.15	0.27	0.20	0.33	0.11	0.26	0.23	0.27	0.05	0.16	0.40	0.24
0.60	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.18	0.05	0.14	0.12	0.19
0.24	0.23	0.13	0.10	0.19	0.26	0.34	0.28	0.26	0.05	0.26	0.32	0.35
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
0.15	0.14	0.09	0.08	0.12	0.16	0.20	0.17	0.16	0.05	0.16	0.19	0.20
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.21	0.26
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.13	0.16

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	0.35	3.50	3.50	16.28	48.89	0.70	58.59	0.65	0.80	0.60	96.31	24.50	1.32	concentration
1.75	1.04	1.92	1.40	2.31	1.54	3.70	3.47	3.52	0.80	1.92	5.56	3.37	0.20	
4.20	0.35	0.35	0.35	0.35	0.70	0.70	0.75	2.31	0.80	1.67	1.69	2.62	0.13	
1.02	0.99	0.61	0.53	0.84	2.17	2.75	2.48	2.03	0.80	1.87	2.60	2.77	0.15	
0.35	0.35	0.35	0.35	0.35	0.70	0.70	0.75	0.65	0.80	0.60	1.85	2.19	0.20	

Reference site R3

Site R3 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	16.50	23.40	21.80	19.35	26.64	23.66	24.50	22.62	23.29	23.24	22.00
pH		6.40	6.71	6.26	6.30	6.60	6.63	5.07	6.74	7.59	7.11	6.64
Cond	µS/cm	57.00	57.00	68.00	45.00	75.00	90.00	96.00	94.00	84.00	86.00	67.00
DO Conc	mg/l	2.75	8.32	4.25	2.80	4.40	1.70	3.32	2.20	3.46	3.07	1.12
Rainfall	mm	2.50	15.70	60.60	21.00	78.70	4.60	47.90	12.80	5.00	0.00	61.50
Depth ref	cm	17.40	30.30	39.00	42.50	96.60	86.50	95.50	84.00	94.00	79.70	63.00
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.10	2.89	0.15	1.25	2.69	<0.1	0.21	0.76	0.93	<0.1
G28279 (DIA)	µg/l	<0.1	0.10	<0.1	0.17	<0.1	0.30	0.34	0.13	0.19	<0.1	0.20
G30033 (DEA)	µg/l	<0.1	0.12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.13	0.53
Atrazine LL	µg/l	0.46	0.43	<0.1	0.35	<0.1	0.29	<0.1	<0.1	0.21	0.12	0.26
Atrazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.39	<0.1	<0.1	<0.1	<0.1
Terbuthylazine LL	µg/l	<0.1	2.79	0.48	2.12	<0.1	1.56	<0.1	<0.1	<0.1	<0.1	<0.1
Terbuthylazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.17	<0.1	<0.1	<0.1	<0.1

Site R3 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.10	2.89	0.15	1.25	2.69	0.05	0.21	0.76	0.93	0.05
G28279 (DIA)	µg/l	0.05	0.10	0.05	0.17	0.05	0.30	0.34	0.13	0.19	0.05	0.20
G30033 (DEA)	µg/l	0.05	0.12	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.13	0.53
Atrazine LL	µg/l	0.46	0.43	0.05	0.35	0.05	0.29	0.05	0.05	0.21	0.12	0.26
Atrazine SPE	µg/l	0.05	0.05	0.05	0.05	0.05	0.05	0.39	0.05	0.05	0.05	0.05
Atrazine mean	µg/l	0.25	0.24	0.05	0.20	0.05	0.17	0.22	0.05	0.13	0.08	0.16
Terbuthylazine LL	µg/l	0.05	2.79	0.48	2.12	0.05	1.56	0.05	0.05	0.05	0.05	0.05
Terbuthylazine SPE	µg/l	0.05	0.05	0.05	0.05	0.05	0.05	2.17	0.05	0.05	0.05	0.05
Terbuthylazine mean	µg/l	0.05	1.42	0.26	1.08	0.05	0.81	1.11	0.05	0.05	0.05	0.05

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		0.71	20.22	1.02	8.75	18.84	0.35	1.03	6.84	6.51	0.35
G28279 (DIA) days	µg/l		0.71	0.35	1.22	0.35	2.10	2.39	0.63	1.72	0.35	1.43
G30033 (DEA) days	µg/l		0.81	0.35	0.35	0.35	0.35	0.35	0.25	0.45	0.91	3.74
Atrazine mean days	µg/l		1.68	0.35	1.39	0.35	1.19	1.53	0.25	1.17	0.59	1.09
Terbuthylazine mean days	µg/l		9.95	1.84	7.59	0.35	5.64	7.76	0.25	0.45	0.35	0.35

Summary data for Site R3 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	7.38	0.05	1.28
G28279 (DIA)	1.34	0.05	0.29
G30033 (DEA)	2.21	0.05	0.37
Atrazine	0.84	0.05	0.25
Terbuthylazine	1.42	0.05	0.37

Reference site R3 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
21.84	19.99	20.73	20.37	18.37	23.56	18.82	20.55	16.65	15.62	10.32	10.37	11.63
7.30	6.89	5.23	8.06	8.04	8.26	8.27	8.50	6.45	8.19	8.73	8.59	8.56
55.00	46.00	44.00	43.00	54.00	48.00	48.00	51.00	59.00	48.00	49.00	37.00	38.00
2.18	1.69	3.97	4.07	2.49	5.00	1.21	5.89	3.27	3.09	2.49	5.25	2.36
6.50	46.20	6.00	54.00	73.00	12.50	2.50	0.00	14.00	0.00	2.80	7.50	74.10
48.50	42.10	38.00	39.50	52.00	56.00	42.00	38.00	39.00	33.00	31.00	30.00	28.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	3.49	7.38	4.43	2.79	3.52	<0.1	<0.1	<0.1	<0.1	<0.1	0.22	2.14
<0.1	0.23	0.14	0.19	0.12	0.13	0.30	0.61	1.34	0.44	0.31	<0.1	0.22
<0.1	0.12	0.11	0.10	<0.1	<0.1	<0.1	0.15	1.91	2.21	<0.1	0.12	0.17
0.16	0.15	0.17	0.11	0.19	<0.1	0.57	0.36	<0.1	<0.1	<0.1	<0.1	<0.1
<0.1	<0.1	<0.1	0.11	<0.1	<0.1	<0.1	<0.1	1.44	1.62	1.45	<0.1	<0.1
<0.1	<0.1	<0.1	<0.1	<0.1	1.63	<0.1	<0.1	<0.1	<0.1	<0.1	1.74	2.20
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	3.49	7.38	4.43	2.79	3.52	0.05	0.05	0.05	0.05	0.05	0.22	2.14
0.05	0.23	0.14	0.19	0.12	0.13	0.30	0.61	1.34	0.44	0.31	0.05	0.22
0.05	0.12	0.11	0.10	0.05	0.05	0.05	0.15	1.91	2.21	0.05	0.12	0.17
0.16	0.15	0.17	0.11	0.19	0.05	0.57	0.36	0.05	0.05	0.05	0.05	0.05
0.05	0.05	0.05	0.11	0.05	0.05	0.05	0.05	1.44	1.62	1.45	0.05	0.05
0.11	0.10	0.11	0.11	0.12	0.05	0.31	0.20	0.74	0.84	0.75	0.05	0.05
0.05	0.05	0.05	0.05	0.05	1.63	0.05	0.05	0.05	0.05	0.05	1.74	2.20
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
0.05	0.05	0.05	0.05	0.05	0.84	0.05	0.05	0.05	0.05	0.05	0.90	1.12

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	24.43	51.65	31.01	19.50	49.31	0.70	0.75	0.65	0.80	0.60	3.08	29.92	1.28	concentration
0.35	1.62	0.97	1.33	0.84	1.83	4.19	9.20	17.39	7.01	3.72	0.70	3.05	0.29	
0.35	0.87	0.77	0.70	0.35	0.70	0.70	2.28	24.79	35.38	0.60	1.72	2.42	0.37	
0.75	0.71	0.78	0.77	0.84	0.70	4.35	3.06	9.67	13.39	9.01	0.70	0.70	0.25	
0.35	0.35	0.35	0.35	0.35	11.77	0.70	0.75	0.65	0.80	0.60	12.56	15.74	0.37	

Reference site R6

Site R6 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	18.50	21.80	20.50	20.80	22.13	23.02	23.80	21.69	22.02	22.39	22.48
pH		6.81	7.92	7.20	7.01	6.29	6.58	6.75	6.77	7.27	8.41	6.48
Cond	µS/cm	62.00	57.00	56.00	56.00	42.00	49.00	52.00	49.00	38.00	40.00	47.00
DO Conc	mg/l	6.85	7.78	6.77	3.82	3.67	3.93	3.06	3.36	3.77	3.09	2.45
Rainfall	mm	2.50	15.70	60.60	21.00	78.70	4.60	47.90	12.80	5.00	0.00	61.50
Depth ref	cm	36.70	84.40	88.00	83.20	151.50	141.50	139.50	145.00	138.00	129.00	106.20
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.14	1.54	0.14	1.62	3.31	<0.1	0.64	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	<0.1	0.16	<0.1	<0.1	0.18	0.34	0.45	0.13	0.17	<0.1	<0.1
G30033 (DEA)	µg/l	<0.1	0.12	<0.1	0.11	<0.1	<0.1	0.12	0.12	<0.1	<0.1	<0.1
Atrazine LL	µg/l	0.69	0.56	0.12	0.51	0.10	0.38	0.35	<0.1	0.15	0.19	0.11
Atrazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	0.14	0.14	0.35	0.18	<0.1	0.11	<0.1
Terbuthylazine LL	µg/l	1.84	1.68	0.88	1.52	0.94	1.58	2.67	0.94	1.46	1.57	0.90
Terbuthylazine SPE	µg/l	1.16	2.25	1.68	1.67	1.55	1.25	1.45	1.11	<0.1	0.94	1.00

Site R6 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.14	1.54	0.14	1.62	3.31	0.05	0.64	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.05	0.16	0.05	0.05	0.18	0.34	0.45	0.13	0.17	0.05	0.05
G30033 (DEA)	µg/l	0.05	0.12	0.05	0.11	0.05	0.05	0.12	0.12	0.05	0.05	0.05
Atrazine LL	µg/l	0.69	0.56	0.12	0.51	0.10	0.38	0.35	0.05	0.15	0.19	0.11
Atrazine SPE	µg/l	0.05	0.05	0.05	0.05	0.14	0.14	0.35	0.18	0.05	0.11	0.05
Atrazine mean	µg/l	0.37	0.31	0.09	0.28	0.12	0.26	0.35	0.11	0.10	0.15	0.08
Terbuthylazine LL	µg/l	1.84	1.68	0.88	1.52	0.94	1.58	2.67	0.94	1.46	1.57	0.90
Terbuthylazine SPE	µg/l	1.16	2.25	1.68	1.67	1.55	1.25	1.45	1.11	0.05	0.94	1.00
Terbuthylazine mean	µg/l	1.50	1.96	1.28	1.60	1.25	1.42	2.06	1.03	0.76	1.26	0.95

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		0.97	10.79	0.98	11.34	23.16	0.35	3.19	0.45	0.35	0.35
G28279 (DIA) days	µg/l		1.09	0.35	0.35	1.26	2.38	3.14	0.65	1.53	0.35	0.35
G30033 (DEA) days	µg/l		0.86	0.35	0.78	0.35	0.35	0.86	0.62	0.45	0.35	0.35
Atrazine mean days	µg/l		2.14	0.60	1.96	0.83	1.84	2.46	0.57	0.90	1.05	0.56
Terbuthylazine mean days	µg/l		13.72	8.97	11.17	8.72	9.91	14.42	5.13	6.80	8.79	6.65

Summary data for Site R6 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	6.82	0.05	1.23
G28279 (DIA)	0.45	0.05	0.18
G30033 (DEA)	0.38	0.05	0.12
Atrazine	0.47	0.08	0.19
Terbuthylazine	2.06	0.50	0.91

Reference site R6 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
22.43	20.95	23.48	22.06	19.96	23.47	20.33	20.06	18.33	17.92	11.44	12.31	10.63
8.02	6.42	7.29	7.62	8.14	8.18	7.82	8.73	6.58	7.45	8.48	8.83	8.69
49.00	48.00	53.00	51.00	47.00	54.00	54.00	57.00	53.00	54.00	45.00	45.00	39.00
2.63	6.68	6.33	7.22	6.07	5.13	5.27	3.05	5.22	3.18	2.89	3.24	2.67
6.50	46.20	6.00	54.00	73.00	12.50	2.50	0.00	14.00	0.00	2.80	7.50	74.10
122.00	120.00	124.00	121.00	142.00	121.00	108.00	97.00	89.00	88.00	69.00	67.00	67.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	1.23	6.82	1.97	0.58	3.67	<0.1	1.09	<0.1	<0.1	<0.1	3.75	1.25
0.15	0.13	0.16	0.13	0.20	0.17	0.25	0.22	0.22	0.13	0.15	0.23	0.15
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.16	0.21	0.12	0.13	0.23	0.38
0.13	0.16	0.15	0.10	0.15	0.26	0.38	0.12	0.19	<0.1	0.20	0.28	0.40
<0.1	0.11	0.12	0.12	<0.1	<0.1	<0.1	0.12	0.14	0.89	<0.1	<0.1	<0.1
0.94	1.03	0.81	0.85	0.69	0.71	0.88	0.63	0.59	0.48	0.78	0.80	0.76
0.81	1.19	0.70	0.89	0.88	0.50	1.02	0.57	0.73	0.51	0.59	0.32	0.43

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	1.23	6.82	1.97	0.58	3.67	0.05	1.09	0.05	0.05	0.05	3.75	1.25
0.15	0.13	0.16	0.13	0.20	0.17	0.25	0.22	0.22	0.13	0.15	0.23	0.15
0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.16	0.21	0.12	0.13	0.23	0.38
0.13	0.16	0.15	0.10	0.15	0.26	0.38	0.12	0.19	0.05	0.20	0.28	0.40
0.05	0.11	0.12	0.12	0.05	0.05	0.05	0.12	0.14	0.89	0.05	0.05	0.05
0.09	0.14	0.13	0.11	0.10	0.16	0.22	0.12	0.16	0.47	0.13	0.17	0.22
0.94	1.03	0.81	0.85	0.69	0.71	0.88	0.63	0.59	0.48	0.78	0.80	0.76
0.81	1.19	0.70	0.89	0.88	0.50	1.02	0.57	0.73	0.51	0.59	0.32	0.43
0.88	1.11	0.75	0.87	0.78	0.60	0.95	0.60	0.66	0.50	0.69	0.56	0.59

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	8.61	47.76	13.79	4.07	51.37	0.70	16.34	0.65	0.80	0.60	52.47	17.57	1.23	concentration
1.05	0.88	1.13	0.91	1.40	2.44	3.46	3.36	2.85	2.08	1.80	3.15	2.07	0.18	
0.35	0.35	0.35	0.35	0.35	0.70	0.70	2.40	2.76	1.86	1.61	3.26	5.31	0.12	
0.62	0.95	0.94	0.77	0.68	2.19	3.02	1.81	2.10	7.52	1.52	2.34	3.12	0.19	
6.15	7.77	5.26	6.09	5.46	8.46	13.30	8.93	8.52	7.94	8.23	7.84	8.31	0.91	

Experimental site E1

Site E1 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	26.10	27.50	24.00	21.20	26.86	24.59	25.50	22.66	25.76	26.15	27.15
pH		8.51	8.01	8.69	8.14	8.34	7.64	8.49	9.23	8.33	8.49	8.66
Cond	µS/cm	767.00	801.00	882.00	470.00	712.00	369.00	536.00	507.00	341.00	339.00	405.00
DO Conc	mg/l	7.51	5.12	10.38	4.86	4.45	2.78	3.87	3.03	4.15	3.43	3.25
Rainfall	mm	38.00	30.00	7.00	74.00	11.00	42.00	35.00	34.00	40.00	0.00	2.00
Depth ref	cm	61.50	101.00	90.00	101.00	75.70	100.50	65.00	55.00	79.00	57.50	51.00
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	0.24	0.23	<0.1	0.11	0.80	1.04	<0.1	0.69	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	0.47	0.19	<0.1	0.36	0.11	0.25	0.15	0.43	0.16	0.13	0.27
G30033 (DEA)	µg/l	0.28	0.23	<0.1	0.31	0.28	0.37	0.43	0.34	0.30	0.41	0.51
Atrazine LL	µg/l	1.49	1.17	<0.1	1.25	2.76	4.65	<0.1	2.30	5.37	3.74	<0.1
Atrazine SPE	µg/l	0.25	0.26	0.19	0.19	0.79	4.56	0.38	3.38	2.76	5.24	<0.1
Terbuthylazine LL	µg/l	0.28	5.30	0.33	5.03	1.81	3.43	<0.1	1.83	3.49	4.52	4.84
Terbuthylazine SPE	µg/l	0.19	0.19	0.14	0.15	0.48	3.76	0.38	2.71	1.80	3.69	<0.1

Site E1 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.24	0.23	0.05	0.11	0.80	1.04	0.05	0.69	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.47	0.19	0.05	0.36	0.11	0.25	0.15	0.43	0.16	0.13	0.27
G30033 (DEA)	µg/l	0.28	0.23	0.05	0.31	0.28	0.37	0.43	0.34	0.30	0.41	0.51
Atrazine LL	µg/l	1.49	1.17	0.05	1.25	2.76	4.65	0.05	2.30	5.37	3.74	0.05
Atrazine SPE	µg/l	0.25	0.26	0.19	0.19	0.79	4.56	0.38	3.38	2.76	5.24	0.05
Atrazine mean	µg/l	0.87	0.71	0.12	0.72	1.78	4.60	0.21	2.84	4.06	4.49	0.05
Terbuthylazine LL	µg/l	0.28	5.30	0.33	5.03	1.81	3.43	0.05	1.83	3.49	4.52	4.84
Terbuthylazine SPE	µg/l	0.19	0.19	0.14	0.15	0.48	3.76	0.38	2.71	1.80	3.69	0.05
Terbuthylazine mean	µg/l	0.24	2.74	0.23	2.59	1.14	3.60	0.21	2.27	2.65	4.10	2.44

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		1.60	0.35	0.78	5.63	7.29	0.35	3.46	0.45	0.35	0.35
G28279 (DIA) days	µg/l		1.36	0.35	2.53	0.78	1.73	1.06	2.13	1.40	0.92	1.91
G30033 (DEA) days	µg/l		1.60	0.35	2.14	1.97	2.58	3.00	1.69	2.68	2.90	3.56
Atrazine mean days	µg/l		4.99	0.85	5.04	12.43	32.23	1.50	14.20	36.55	31.45	0.35
Terbuthylazine mean days	µg/l		19.21	1.63	18.10	8.01	25.17	1.50	11.36	23.82	28.72	17.11

Summary data for Site E1 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	1.00	0.25	0.31
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	5.20	0.05	1.14
G28279 (DIA)	0.93	0.05	0.44
G30033 (DEA)	1.66	0.05	0.85
Atrazine	5.47	0.05	3.18
Terbuthylazine	4.10	0.21	2.57

Experimental site E1 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
28.42	20.72	27.27	22.53	19.70	23.72	20.97	22.15	19.16	21.03	11.08	19.54	12.66
8.85	8.44	8.29	8.96	8.24	7.99	8.96	9.29	8.91	9.17	9.28	10.00	9.70
440.00	416.00	541.00	519.00	255.00	333.00	383.00	425.00	417.00	489.00	381.00	519.00	603.00
3.21	3.86	3.68	4.75	1.03	1.92	3.85	7.17	7.22	7.98	4.54	6.67	9.57
18.00	36.00	5.00	2.00	38.00	6.00	20.00	0.00	11.00	0.00	15.00	13.00	24.00
44.00	55.00	44.00	45.00	76.00	60.00	47.00	38.00	27.00	16.00	12.00	9.00	5.00
0.70	1.00	<0.5	0.80	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	4.95	<0.1	1.24	5.02	<0.1	1.14	<0.1	<0.1	<0.1	5.20	1.43
0.22	0.61	0.24	0.53	0.40	0.59	0.49	0.43	0.70	0.93	0.91	0.23	0.52
0.47	0.78	0.40	0.67	0.44	1.17	1.66	0.93	1.16	1.29	1.47	0.97	1.50
6.78	5.03	2.90	3.80	3.52	2.85	3.57	6.26	4.14	3.50	2.60	2.78	3.21
<0.1	4.79	3.12	3.94	3.45	2.53	3.85	4.69	5.39	4.01	3.80	2.95	3.50
4.18	3.05	2.03	2.50	2.54	2.51	3.28	4.36	2.94	2.87	3.70	1.57	1.93
<0.1	3.34	2.13	2.80	2.34	2.36	3.81	3.37	3.66	2.95	2.78	1.34	1.62

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.70	1.00	0.25	0.80	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	4.95	0.05	1.24	5.02	0.05	1.14	0.05	0.05	0.05	5.20	1.43
0.22	0.61	0.24	0.53	0.40	0.59	0.49	0.43	0.70	0.93	0.91	0.23	0.52
0.47	0.78	0.40	0.67	0.44	1.17	1.66	0.93	1.16	1.29	1.47	0.97	1.50
6.78	5.03	2.90	3.80	3.52	2.85	3.57	6.26	4.14	3.50	2.60	2.78	3.21
0.05	4.79	3.12	3.94	3.45	2.53	3.85	4.69	5.39	4.01	3.80	2.95	3.50
3.41	4.91	3.01	3.87	3.48	2.69	3.71	5.47	4.76	3.76	3.20	2.86	3.35
4.18	3.05	2.03	2.50	2.54	2.51	3.28	4.36	2.94	2.87	3.70	1.57	1.93
0.05	3.34	2.13	2.80	2.34	2.36	3.81	3.37	3.66	2.95	2.78	1.34	1.62
2.12	3.19	2.08	2.65	2.44	2.44	3.55	3.86	3.30	2.91	3.24	1.46	1.78

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
4.90	7.00	1.75	5.60	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.31	TWMC as a daily concentration
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	
0.35	0.35	34.64	0.35	8.69	70.31	0.70	17.04	0.65	0.80	0.60	72.76	20.00	1.14	
1.54	4.27	1.65	3.71	2.79	8.19	6.92	6.39	9.05	14.82	10.95	3.22	7.27	0.44	
3.29	5.45	2.79	4.69	3.09	16.43	23.24	13.95	15.07	20.70	17.68	13.59	20.99	0.85	
23.89	34.37	21.09	27.09	24.37	37.67	51.97	82.12	61.92	60.08	38.40	40.10	46.96	3.18	
14.81	22.35	14.57	18.55	17.07	34.13	49.67	57.92	42.91	46.56	38.89	20.38	24.89	2.57	

Experimental site E3

Site E3 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	24.30	31.30	25.30	18.72	25.23	24.51	26.00	23.58	26.81	24.25	20.39
pH		8.21	9.05	8.95	7.68	7.51	7.19	7.34	7.31	8.04	8.54	8.36
Cond	µS/cm	1413.00	1498.00	948.00	210.00	798.00	440.00	643.00	617.00	548.00	668.00	652.00
DO Conc	mg/l	6.65	12.69	9.69	4.88	7.91	6.23	3.60	0.97	3.84	4.29	1.32
Rainfall	mm	0.00	27.00	34.00	58.00	39.00	83.00	35.00	17.00	50.00	0.00	0.00
Depth ref	cm	60.00	53.00	59.80	85.50	60.00	79.00	80.00	69.00	80.00	54.00	32.00
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.14	0.33	0.18	1.36	5.22	<0.1	5.96	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	0.11	0.18	<0.1	0.19	0.18	0.29	0.23	<0.1	0.41	0.15	0.25
G30033 (DEA)	µg/l	<0.1	0.13	<0.1	0.13	0.41	0.36	0.47	<0.1	0.12	0.20	0.47
Atrazine LL	µg/l	0.82	0.63	0.14	0.57	1.46	0.40	1.35	0.37	0.32	0.86	1.04
Atrazine SPE	µg/l	<0.1	0.16	0.16	0.16	0.69	0.92	0.38	0.84	1.46	0.92	0.65
Terbuthylazine LL	µg/l	<0.1	3.31	0.88	2.76	1.23	0.54	0.68	<0.1	0.88	0.72	0.53
Terbuthylazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	0.42	0.46	2.43	0.46	1.23	0.52	0.33

Site E3 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.14	0.33	0.18	1.36	5.22	0.05	5.96	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.11	0.18	0.05	0.19	0.18	0.29	0.23	0.05	0.41	0.15	0.25
G30033 (DEA)	µg/l	0.05	0.13	0.05	0.13	0.41	0.36	0.47	0.05	0.12	0.20	0.47
Atrazine LL	µg/l	.0.82	0.63	0.14	0.57	1.46	0.40	1.35	0.37	0.32	0.86	1.04
Atrazine SPE	µg/l	0.05	0.16	0.16	0.16	0.69	0.92	0.38	0.84	1.46	0.92	0.65
Atrazine mean	µg/l	0.43	0.40	0.15	0.37	1.08	0.66	0.87	0.60	0.89	0.89	0.84
Terbuthylazine LL	µg/l	0.05	3.31	0.88	2.76	1.23	0.54	0.68	0.05	0.88	0.72	0.53
Terbuthylazine SPE	µg/l	0.05	0.05	0.05	0.05	0.42	0.46	2.43	0.46	1.23	0.52	0.33
Terbuthylazine mean	µg/l	0.05	1.68	0.47	1.40	0.82	0.50	1.55	0.26	1.05	0.62	0.43

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		0.97	2.29	1.29	9.52	36.57	0.35	29.79	0.45	0.35	0.35
G28279 (DIA) days	µg/l		1.27	0.35	1.31	1.25	2.01	1.60	0.25	3.68	1.02	1.76
G30033 (DEA) days	µg/l		0.92	0.35	0.94	2.87	2.54	3.26	0.25	1.04	1.42	3.32
Atrazine mean days	µg/l		2.77	1.06	2.57	7.53	4.61	6.06	3.01	8.01	6.25	5.91
Terbuthylazine mean days	µg/l		11.75	3.26	9.83	5.76	3.50	10.87	1.28	9.49	4.33	3.03

Summary data for Site E3 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	5.96	0.05	1.13
G28279 (DIA)	0.81	0.05	0.30
G30033 (DEA)	0.64	0.05	0.35
Atrazine	1.19	0.15	0.82
Terbuthylazine	1.68	0.05	0.83

Experimental site E3 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
19.75	18.80	22.93	20.27	19.57	20.38	19.88	20.57	16.01	17.26	13.22	16.62	14.05
8.03	7.82	8.06	8.12	7.93	7.95	7.96	8.05	7.81	8.42	8.66	9.60	9.29
677.00	660.00	741.00	714.00	680.00	522.00	589.00	687.00	641.00	708.00	661.00	695.00	714.00
1.01	0.75	1.50	1.59	3.94	0.74	0.99	2.49	0.81	5.64	5.22	6.05	7.01
170.00	59.00	46.00	1.00	58.00	29.00	11.00	0.00	17.00	0.00	15.00	5.00	34.00
32.00	28.50	32.00	29.50	37.00	32.00	28.00	23.00	17.00	9.00	3.00	1.00	3.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	0.32	0.43	0.47	2.93	<0.1	0.38	<0.1	<0.1	<0.1	4.61	2.88
0.43	0.17	0.10	0.11	<0.1	0.20	0.26	0.28	0.47	0.58	0.49	<0.1	0.81
0.37	0.22	0.22	0.24	0.18	0.51	0.64	0.43	0.38	0.37	0.47	0.37	0.45
0.39	0.69	0.65	0.78	0.73	0.81	1.04	1.05	1.01	0.93	1.23	0.88	0.97
0.62	0.47	0.76	0.60	<0.1	0.89	1.06	0.98	1.19	1.02	1.15	0.64	0.89
<0.1	<0.1	0.34	0.10	0.37	0.81	1.07	0.96	0.95	0.83	1.73	1.12	1.03
0.27	0.21	0.39	0.28	<0.1	0.96	1.22	0.97	1.07	0.87	1.00	0.55	0.74

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	0.32	0.43	0.47	2.93	0.05	0.38	0.05	0.05	0.05	4.61	2.88
0.43	0.17	0.10	0.11	0.05	0.20	0.26	0.28	0.47	0.58	0.49	0.05	0.81
0.37	0.22	0.22	0.24	0.18	0.51	0.64	0.43	0.38	0.37	0.47	0.37	0.45
0.39	0.69	0.65	0.78	0.73	0.81	1.04	1.05	1.01	0.93	1.23	0.88	0.97
0.62	0.47	0.76	0.60	0.05	0.89	1.06	0.98	1.19	1.02	1.15	0.64	0.89
0.50	0.58	0.71	0.69	0.39	0.85	1.05	1.02	1.10	0.98	1.19	0.76	0.93
0.05	0.05	0.34	0.10	0.37	0.81	1.07	0.96	0.95	0.83	1.73	1.12	1.03
0.27	0.21	0.39	0.28	0.05	0.96	1.22	0.97	1.07	0.87	1.00	0.55	0.74
0.16	0.13	0.37	0.19	0.21	0.89	1.15	0.96	1.01	0.85	1.37	0.83	0.89

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	0.35	2.21	3.01	3.30	41.03	0.70	5.64	0.65	0.80	0.60	64.55	40.26	1.13	concentration
3.01	1.22	0.71	0.77	0.35	2.77	3.58	4.22	6.12	9.34	5.86	0.70	11.31	0.30	
2.60	1.53	1.52	1.68	1.26	7.07	8.99	6.38	4.98	5.92	5.58	5.19	6.34	0.35	
3.53	4.07	4.95	4.83	2.73	11.89	14.67	15.24	14.29	15.65	14.30	10.65	13.05	0.82	
1.10	0.90	2.57	1.33	1.47	12.40	16.03	14.45	13.09	13.62	16.40	11.68	12.44	0.83	

Experimental site E4

Site E4 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	26.60	28.30	24.90	21.33	29.96	24.99	28.06	25.13	26.01	24.54	22.21
pH		7.54	7.65	7.84	7.46	7.76	7.29	7.58	7.50	7.65	8.02	7.79
Cond	µS/cm	1331.00	1420.00	1485.00	811.00	853.00	364.00	651.00	631.00	550.00	671.00	704.00
DO Conc	mg/l	0.83	1.20	4.70	1.72	3.86	1.41	2.42	2.59	1.97	3.30	2.70
Rainfall	mm	0.00	27.00	34.00	58.00	39.00	83.00	35.00	17.00	50.00	0.00	0.00
Depth ref	cm	72.20	64.30	65.20	78.50	66.00	76.20	73.00	72.50	75.20	63.20	50.10
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.21	1.37	0.23	2.76	4.23	<0.1	4.59	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	0.10	0.11	<0.1	0.17	0.16	0.15	0.27	0.40	0.39	<0.1	0.16
G30033 (DEA)	µg/l	<0.1	0.14	<0.1	0.13	0.41	0.21	0.31	0.13	0.13	0.29	0.57
Atrazine LL	µg/l	0.18	0.33	0.48	0.30	1.96	0.33	0.95	0.17	1.37	0.97	0.88
Atrazine SPE	µg/l	0.13	0.11	0.11	0.12	0.69	0.74	0.50	0.87	0.96	0.91	0.83
Terbuthylazine LL	µg/l	<0.1	1.51	0.65	1.22	1.60	0.33	<0.1	<0.1	1.05	0.95	0.43
Terbuthylazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	0.47	0.33	0.36	0.43	0.50	0.55	0.44

Site E4 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.21	1.37	0.23	2.76	4.23	0.05	4.59	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.10	0.11	0.05	0.17	0.16	0.15	0.27	0.40	0.39	0.05	0.16
G30033 (DEA)	µg/l	0.05	0.14	0.05	0.13	0.41	0.21	0.31	0.13	0.13	0.29	0.57
Atrazine LL	µg/l	0.18	0.33	0.48	0.30	1.96	0.33	0.95	0.17	1.37	0.97	0.88
Atrazine SPE	µg/l	0.13	0.11	0.11	0.12	0.69	0.74	0.50	0.87	0.96	0.91	0.83
Atrazine mean	µg/l	0.16	0.22	0.30	0.21	1.33	0.54	0.72	0.52	1.16	0.94	0.85
Terbuthylazine LL	µg/l	0.05	1.51	0.65	1.22	1.60	0.33	0.05	0.05	1.05	0.95	0.43
Terbuthylazine SPE	µg/l	0.05	0.05	0.05	0.05	0.47	0.33	0.36	0.43	0.50	0.55	0.44
Terbuthylazine mean	µg/l	0.05	0.78	0.35	0.64	1.03	0.33	0.20	0.24	0.78	0.75	0.44

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		1.48	9.56	1.64	19.30	29.60	0.35	22.93	0.45	0.35	0.35
G28279 (DIA) days	µg/l		0.74	0.35	1.16	1.15	1.05	1.86	2.00	3.53	0.35	1.14
G30033 (DEA) days	µg/l		0.97	0.35	0.90	2.85	1.46	2.15	0.64	1.17	2.03	3.97
Atrazine mean days	µg/l		1.55	2.08	1.46	9.28	3.77	5.04	2.60	10.45	6.57	5.96
Terbuthylazine mean days	µg/l		5.46	2.44	4.46	7.23	2.33	1.43	1.21	6.99	5.26	3.05

Summary data for Site E4 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	4.59	0.05	1.00
G28279 (DIA)	0.69	0.05	0.26
G30033 (DEA)	0.57	0.05	0.19
Atrazine	1.33	0.14	0.48
Terbuthylazine	1.04	0.05	0.41

Experimental site E4 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
23.21	20.15	22.22	21.28	19.93	23.39	18.91	20.59	18.74	16.47	11.32	13.71	9.49
7.79	7.76	8.16	8.17	7.76	7.78	8.08	8.42	7.70	8.88	9.24	9.72	9.01
773.00	694.00	801.00	739.00	686.00	780.00	854.00	1182.00	1567.00	1000.00	948.00	1020.00	1320.00
1.93	4.20	4.60	3.21	3.10	3.12	1.44	4.87	4.94	4.16	4.85	6.68	6.42
170.00	59.00	46.00	1.00	58.00	29.00	11.00	0.00	17.00	0.00	15.00	5.00	34.00
44.50	47.00	45.00	43.50	47.00	42.00	32.00	17.00	12.00	8.00	7.00	4.00	3.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	2.78	0.98	0.46	3.79	<0.1	<0.1	<0.1	<0.1	<0.1	0.54	2.63
0.20	0.29	0.23	0.21	0.15	<0.1	<0.1	0.18	0.64	0.69	0.60	<0.1	0.25
0.18	0.15	0.23	0.20	0.21	0.21	0.21	0.11	<0.1	0.16	0.26	0.13	0.12
0.16	0.77	0.74	0.75	0.70	0.48	0.44	0.32	0.29	0.21	0.26	0.33	0.15
0.50	0.76	0.71	0.65	0.64	0.49	0.56	0.37	0.29	0.19	0.19	0.11	0.14
0.37	<0.1	0.44	0.35	0.39	1.82	1.74	0.14	<0.1	0.11	<0.1	<0.1	<0.1
0.26	0.43	0.39	0.41	0.33	0.27	0.31	0.19	0.24	<0.1	<0.1	<0.1	<0.1

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	2.78	0.98	0.46	3.79	0.05	0.05	0.05	0.05	0.05	0.54	2.63
0.20	0.29	0.23	0.21	0.15	0.05	0.05	0.18	0.64	0.69	0.60	0.05	0.25
0.18	0.15	0.23	0.20	0.21	0.21	0.21	0.11	0.05	0.16	0.26	0.13	0.12
0.16	0.77	0.74	0.75	0.70	0.48	0.44	0.32	0.29	0.21	0.26	0.33	0.15
0.50	0.76	0.71	0.65	0.64	0.49	0.56	0.37	0.29	0.19	0.19	0.11	0.14
0.33	0.76	0.73	0.70	0.67	0.48	0.50	0.35	0.29	0.20	0.22	0.22	0.14
0.37	0.05	0.44	0.35	0.39	1.82	1.74	0.14	0.05	0.11	0.05	0.05	0.05
0.26	0.43	0.39	0.41	0.33	0.27	0.31	0.19	0.24	0.05	0.05	0.05	0.05
0.31	0.24	0.41	0.38	0.36	1.04	1.02	0.17	0.15	0.08	0.05	0.05	0.05

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	0.35	19.43	6.86	3.24	53.02	0.70	0.75	0.65	0.80	0.60	7.52	36.86	1.00	concentration
1.40	2.04	1.64	1.47	1.08	0.70	0.70	2.67	8.31	11.01	7.16	0.70	3.54	0.26	
1.29	1.03	1.58	1.40	1.48	2.98	3.01	1.61	0.65	2.61	3.10	1.79	1.67	0.19	
2.32	5.34	5.08	4.90	4.70	6.78	7.02	5.20	3.76	3.17	2.66	3.03	2.02		
2.20	1.67	2.89	2.66	2.54	14.60	14.34	2.49	1.89	1.31	0.60	0.70	0.70		

Experimental site E6

Site E6 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	24.70	26.90	24.10	22.66	24.80	23.81	25.60	23.99	25.72	24.65	26.29
pH		8.27	8.35	8.42	7.97	7.77	7.32	7.78	8.39	7.56	8.47	8.77
Cond	µS/cm	792.00	814.00	806.00	827.00	568.00	353.00	471.00	528.00	366.00	465.00	526.00
DO Conc	mg/l	7.50	9.48	8.36	3.43	3.36	1.39	3.10	4.54	3.66	3.91	4.14
Rainfall	mm	16.00	38.00	41.50	33.00	73.00	34.00	36.00	6.00	101.00	1.00	5.00
Depth ref	cm	60.00	51.50	50.60	68.50	61.40	62.00	66.40	57.00	69.20	54.10	42.10
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
G28273 (DACT)	µg/l	<0.1	0.16	0.82	0.13	2.64	4.61	<0.1	0.35	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	<0.1	0.11	<0.1	0.14	0.40	0.86	0.77	<0.1	0.52	0.14	0.12
G30033 (DEA)	µg/l	<0.1	<0.1	<0.1	0.14	0.42	0.32	0.33	<0.1	0.18	0.24	0.44
Atrazine LL	µg/l	0.76	0.68	<0.1	0.61	4.46	0.78	0.84	0.42	2.86	1.87	2.08
Atrazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	1.07	0.55	0.81	0.51	1.26	1.96	1.81
Terbuthylazine LL	µg/l	<0.1	3.70	0.56	3.03	3.57	0.41	0.47	<0.1	2.06	2.39	1.55
Terbuthylazine SPE	µg/l	<0.1	<0.1	<0.1	<0.1	0.69	0.31	0.55	0.27	1.07	1.54	1.42

Site E6 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
G28273 (DACT)	µg/l	0.05	0.16	0.82	0.13	2.64	4.61	0.05	0.35	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.05	0.11	0.05	0.14	0.40	0.86	0.77	0.05	0.52	0.14	0.12
G30033 (DEA)	µg/l	0.05	0.05	0.05	0.14	0.42	0.32	0.33	0.05	0.18	0.24	0.44
Atrazine LL	µg/l	0.76	0.68	0.05	0.61	4.46	0.78	0.84	0.42	2.86	1.87	2.08
Atrazine SPE	µg/l	0.05	0.05	0.05	0.05	1.07	0.55	0.81	0.51	1.26	1.96	1.81
Atrazine mean	µg/l	0.40	0.37	0.05	0.33	2.77	0.67	0.82	0.46	2.06	1.91	1.94
Terbuthylazine LL	µg/l	0.05	3.70	0.56	3.03	3.57	0.41	0.47	0.05	2.06	2.39	1.55
Terbuthylazine SPE	µg/l	0.05	0.05	0.05	0.05	0.69	0.31	0.55	0.27	1.07	1.54	1.42
Terbuthylazine mean	µg/l	0.05	1.87	0.31	1.54	2.13	0.36	0.51	0.16	1.56	1.96	1.49

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	2.25	1.75	1.75
G28273 (DACT) days	µg/l		1.11	5.71	0.91	18.47	32.29	0.35	1.76	0.45	0.35	0.35
G28279 (DIA) days	µg/l		0.74	0.35	0.99	2.80	6.01	5.41	0.25	4.70	0.97	0.81
G30033 (DEA) days	µg/l		0.35	0.35	0.98	2.93	2.24	2.30	0.25	1.62	1.70	3.09
Atrazine mean days	µg/l		2.56	0.35	2.31	19.37	4.67	5.77	2.32	18.55	13.38	13.59
Terbuthylazine mean days	µg/l		13.11	2.14	10.77	14.90	2.53	3.55	0.79	14.08	13.75	10.40

Summary data for Site E6 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	0.25	0.25	0.25
Acetochlor	0.25	0.25	0.25
G28273 (DACT)	6.40	0.05	1.61
G28279 (DIA)	0.86	0.05	0.29
G30033 (DEA)	1.90	0.05	0.67
Atrazine	5.05	0.05	2.60
Terbuthylazine	3.58	0.05	1.78

Experimental site E6 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
27.15	21.38	27.11	25.15	23.53	24.76	22.65	21.93	18.68	17.97	11.95	13.52	9.91
9.21	8.11	9.36	9.23	8.16	8.68	8.94	9.13	8.04	9.26	9.32	9.56	9.22
530.00	516.00	545.00	540.00	566.00	494.00	458.00	457.00	436.00	417.00	366.00	382.00	362.00
3.56	3.84	5.80	6.52	5.88	5.33	4.29	5.67	3.99	2.59	3.71	7.33	5.79
18.00	68.00	3.00	45.00	43.00	69.00	6.00	1.00	20.00	1.00	20.00	27.00	40.00
35.80	38.50	37.50	45.00	67.00	40.00	33.00	28.00	29.00	22.00	18.00	17.00	22.00
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	3.17	1.21	6.40	2.32	<0.1	1.10	5.54	<0.1	<0.1	4.96	1.28
0.25	0.25	0.16	0.19	0.15	0.26	0.41	0.20	0.49	0.32	0.59	0.16	<0.1
0.17	0.38	0.35	0.32	0.28	1.18	1.90	0.69	1.09	0.90	1.11	0.71	1.02
1.41	2.13	1.44	1.80	1.58	2.51	4.11	4.39	3.78	3.90	3.83	3.03	3.36
1.82	2.17	1.36	1.98	1.47	2.89	3.93	5.71	4.42	3.87	3.79	3.29	3.35
1.00	1.27	0.83	1.25	0.89	1.78	2.88	2.25	2.18	2.43	4.43	1.81	1.86
1.15	1.42	0.78	1.10	0.83	2.09	2.87	2.78	2.46	2.40	2.73	1.41	1.48

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	3.17	1.21	6.40	2.32	0.05	1.10	5.54	0.05	0.05	4.96	1.28
0.25	0.25	0.16	0.19	0.15	0.26	0.41	0.20	0.49	0.32	0.59	0.16	0.05
0.17	0.38	0.35	0.32	0.28	1.18	1.90	0.69	1.09	0.90	1.11	0.71	1.02
1.41	2.13	1.44	1.80	1.58	2.51	4.11	4.39	3.78	3.90	3.83	3.03	3.36
1.82	2.17	1.36	1.98	1.47	2.89	3.93	5.71	4.42	3.87	3.79	3.29	3.35
1.61	2.15	1.40	1.89	1.53	2.70	4.02	5.05	4.10	3.89	3.81	3.16	3.35
1.00	1.27	0.83	1.25	0.89	1.78	2.88	2.25	2.18	2.43	4.43	1.81	1.86
1.15	1.42	0.78	1.10	0.83	2.09	2.87	2.78	2.46	2.40	2.73	1.41	1.48
1.07	1.35	0.80	1.18	0.86	1.94	2.87	2.51	2.32	2.41	3.58	1.61	1.67

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.25	daily
0.35	0.35	22.22	8.47	44.80	32.48	0.70	16.53	72.07	0.80	0.60	69.44	17.96	1.61	concentration
1.75	1.73	1.14	1.33	1.08	3.67	5.71	2.97	6.31	5.15	7.07	2.28	0.70	0.29	
1.17	2.67	2.44	2.24	1.98	16.48	26.63	10.41	14.11	14.35	13.37	9.91	14.27	0.67	
11.28	15.06	9.80	13.23	10.69	37.80	56.32	75.77	53.28	62.16	45.72	44.21	46.96	2.60	
7.52	9.42	5.63	8.23	6.01	27.13	40.24	37.70	30.17	38.62	42.97	22.55	23.34	1.78	

Experimental site E8

Site E8 raw data

Values presented as less than (<) are below the level of detection

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Temp	oC	26.10	26.10	25.00	19.42	29.60	23.86	23.70	21.67	27.87	25.30	25.13
pH		8.26	7.90	8.63	7.34	7.91	7.50	7.39	7.75	7.94	8.36	7.84
Cond	µS/cm	580.00	592.00	594.00	211.00	511.00	349.00	500.00	478.00	256.00	251.00	271.00
DO Conc	mg/l	5.80	5.03	10.08	3.96	4.13	3.63	1.32	1.16	4.21	4.50	0.70
Rainfall	mm	38.00	30.00	7.00	74.00	11.00	60.00	35.00	34.00	40.00	0.00	2.00
Depth ref	cm	84.00	97.30	87.00	104.80	89.00	87.50	77.50	63.00	80.05	67.30	47.00
Simazine	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.60	1.10	0.70
Acetochlor	µg/l	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.70	1.00	0.60
G28273 (DACT)	µg/l	0.26	0.27	1.80	0.11	1.20	8.16	<0.1	8.00	<0.1	<0.1	<0.1
G28279 (DIA)	µg/l	0.46	<0.1	0.11	0.25	0.17	0.70	0.61	0.55	0.36	0.12	0.47
G30033 (DEA)	µg/l	0.27	0.21	<0.1	0.18	0.39	0.21	0.26	<0.1	0.17	0.17	0.47
Atrazine LL	µg/l	0.98	0.70	<0.1	0.59	4.38	0.37	0.50	0.15	3.67	3.95	6.85
Atrazine SPE	µg/l	0.18	0.22	0.19	0.19	0.49	0.33	0.26	0.36	0.26	6.24	4.72
Terbuthylazine LL	µg/l	0.15	3.28	0.50	2.53	2.09	2.31	<0.1	<0.1	1.87	3.48	3.39
Terbuthylazine SPE	µg/l	<0.1	0.11	<0.1	0.11	0.30	0.24	0.26	0.24	0.26	2.95	2.75

Site E8 processed data

Values for pesticide concentrations less that the limit of detection (LOD) were assigned a value of half the LOD.

Date		5-Nov-01	12-Nov-01	19-Nov-01	26-Nov-01	3-Dec-01	10-Dec-01	17-Dec-01	22-Dec-01	31-Dec-01	7-Jan-02	14-Jan-02
Simazine	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.60	1.10	0.70
Acetochlor	µg/l	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.70	1.00	0.60
G28273 (DACT)	µg/l	0.26	0.27	1.80	0.11	1.20	8.16	0.05	8.00	0.05	0.05	0.05
G28279 (DIA)	µg/l	0.46	0.05	0.11	0.25	0.17	0.70	0.61	0.55	0.36	0.12	0.47
G30033 (DEA)	µg/l	0.27	0.21	0.05	0.18	0.39	0.21	0.26	0.05	0.17	0.17	0.47
Atrazine LL	µg/l	0.98	0.70	0.05	0.59	4.38	0.37	0.50	0.15	3.67	3.95	6.85
Atrazine SPE	µg/l	0.18	0.22	0.19	0.19	0.49	0.33	0.26	0.36	0.26	6.24	4.72
Atrazine mean	µg/l	0.58	0.46	0.12	0.39	2.43	0.35	0.38	0.25	1.96	5.09	5.78
Terbuthylazine LL	µg/l	0.15	3.28	0.50	2.53	2.09	2.31	0.05	0.05	1.87	3.48	3.39
Terbuthylazine SPE	µg/l	0.05	0.11	0.05	0.11	0.30	0.24	0.26	0.24	0.26	2.95	2.75
Terbuthylazine mean	µg/l	0.10	1.69	0.28	1.32	1.20	1.28	0.15	0.15	1.06	3.21	3.07

Values for each sampling period were assumed to represent the previous time period

Days between samples			7	7	7	7	7	7	5	9	7	7
Simazine days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	5.40	7.70	4.90
Acetochlor days	µg/l		1.75	1.75	1.75	1.75	1.75	1.75	1.25	6.30	7.00	4.20
G28273 (DACT) days	µg/l		1.90	12.57	0.78	8.41	57.13	0.35	39.98	0.45	0.35	0.35
G28279 (DIA) days	µg/l		0.35	0.74	1.72	1.16	4.92	4.28	2.75	3.22	0.85	3.26
G30033 (DEA) days	µg/l		1.50	0.35	1.27	2.76	1.48	1.81	0.25	1.54	1.22	3.30
Atrazine mean days	µg/l		3.19	0.85	2.72	17.04	2.46	2.63	1.27	17.64	35.65	40.48
Terbuthylazine mean days	µg/l		11.85	1.93	9.23	8.37	8.93	1.07	0.73	9.56	22.50	21.49

Summary data for Site E8 (all concentrations in µg/l)

	Max	Min	TWMC
Simazine	3.10	0.25	1.03
Acetochlor	1.00	0.25	0.30
G28273 (DACT)	8.16	0.05	1.42
G28279 (DIA)	0.88	0.05	0.44
G30033 (DEA)	1.25	0.05	0.57
Atrazine	9.34	0.12	3.40
Terbuthylazine	3.41	0.10	1.32

Experimental site E8 continued

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
25.71	21.31	27.76	25.70	24.05	28.52	24.34	22.23	19.00	21.50	11.24	11.23	11.78
8.23	7.68	8.12	8.44	8.08	8.33	8.55	8.95	8.41	9.10	8.95	8.96	8.55
307.00	340.00	396.00	415.00	439.00	478.00	435.00	403.00	382.00	407.00	372.00	449.00	445.00
2.93	4.42	6.53	10.37	9.70	7.47	6.76	7.84	4.67	7.26	4.01	2.60	7.00
18.00	36.00	5.00	2.00	38.00	6.00	20.00	0.00	11.00	0.00	15.00	13.00	24.00
44.50	56.00	42.50	45.00	63.00	50.00	34.00	14.00	12.00	10.00	9.00	7.00	6.00
3.10	1.00	2.10	1.80	2.50	<0.5	1.00	1.40	1.70	1.20	1.80	0.90	0.50
<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
<0.1	<0.1	0.24	0.40	0.30	3.52	<0.1	2.82	1.24	<0.1	<0.1	3.83	1.09
0.21	0.18	0.31	0.27	0.45	0.45	0.48	0.41	0.70	0.88	<0.1	0.59	0.82
0.13	0.44	0.39	0.42	0.37	0.96	1.25	0.69	1.04	0.91	<0.1	0.74	1.19
4.95	11.60	3.36	5.31	3.73	3.25	4.16	5.71	3.53	3.12	3.01	2.92	3.29
7.26	7.08	4.12	5.55	4.40	3.21	4.08	6.81	3.86	2.97	3.10	2.61	3.14
1.81	3.31	1.66	2.71	1.85	1.67	2.05	0.41	0.86	0.68	0.87	0.59	0.60
2.67	3.52	2.10	2.81	2.19	1.69	2.12	0.44	0.86	0.72	0.69	0.38	0.43

The values for the two methods of analysis Liquid-Liquid (LL) extraction and SPE extraction were averaged

21-Jan-02	28-Jan-02	4-Feb-02	11-Feb-02	18-Feb-02	4-Mar-02	18-Mar-02	2-Apr-02	15-Apr-02	1-May-02	13-May-02	27-May-02	10-Jun-02
3.10	1.00	2.10	1.80	2.50	0.25	1.00	1.40	1.70	1.20	1.80	0.90	0.50
0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.05	0.05	0.24	0.40	0.30	3.52	0.05	2.82	1.24	0.05	0.05	3.83	1.09
0.21	0.18	0.31	0.27	0.45	0.45	0.48	0.41	0.70	0.88	0.05	0.59	0.82
0.13	0.44	0.39	0.42	0.37	0.96	1.25	0.69	1.04	0.91	0.05	0.74	1.19
4.95	11.60	3.36	5.31	3.73	3.25	4.16	5.71	3.53	3.12	3.01	2.92	3.29
7.26	7.08	4.12	5.55	4.40	3.21	4.08	6.81	3.86	2.97	3.10	2.61	3.14
6.11	9.34	3.74	5.43	4.06	3.23	4.12	6.26	3.70	3.05	3.06	2.76	3.22
1.81	3.31	1.66	2.71	1.85	1.67	2.05	0.41	0.86	0.68	0.87	0.59	0.60
2.67	3.52	2.10	2.81	2.19	1.69	2.12	0.44	0.86	0.72	0.69	0.38	0.43
2.24	3.41	1.88	2.76	2.02	1.68	2.08	0.42	0.86	0.70	0.78	0.49	0.52

7	7	7	7	7	14	14	15	13	16	12	14	14	217	total days
21.70	7.00	14.70	12.60	17.50	3.50	14.00	21.00	22.10	19.20	21.60	12.60	7.00	1.03	TWMC as a
1.75	1.75	1.75	1.75	1.75	3.50	3.50	3.75	3.25	4.00	3.00	3.50	3.50	0.30	daily
0.35	0.35	1.67	2.80	2.12	49.24	0.70	42.32	16.09	0.80	0.60	53.56	15.30	1.42	concentration
1.47	1.26	2.15	1.89	3.16	6.26	6.75	6.12	9.11	14.10	0.60	8.20	11.52	0.44	
0.94	3.06	2.72	2.94	2.56	13.45	17.46	10.34	13.47	14.51	0.60	10.32	16.70	0.57	
42.74	65.39	26.16	38.01	28.45	45.21	57.67	93.92	48.04	48.72	36.66	38.70	45.01	3.40	
15.69	23.87	13.13	19.32	14.13	23.51	29.17	6.35	11.20	11.22	9.34	6.81	7.23	1.32	

Concentrations of elements and ions in water at reference site R1

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	17	4	2.8	3.7	2.4			6.0
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.04	ND	0.02	ND	ND			0.0
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	1.4	2.8	2.5	2.3	2.4			2.3
Chromium as Cr	mg/l	ICP	ND	0.04	0.02	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	8.8	ND	3.8	3.2	2			4.5
Lead as Pb	mg/l	GTA	ND	ND	ND	0.09	ND			0.1
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	2.4	3.3	3.3	3.1	3.3			3.1
Manganese as Mn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.98	ND	1.3	ND	0.3	ND	0.04	0.7
Nitrite as NO2	mg/l	IC	ND	1	0.3	ND	0.33	ND		0.5
Phosphorus as P	mg/l	ICP	ND	0.04	ND	ND	ND			0.0
Potassium as K	mg/l	ICP	1.8	1.5	0.9	<1.4	0.79			1.2
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	19	6.9	6.1	6.3	5.4			8.7
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	2.6	2.6	2.5	1.8	2.6			2.4
Strontium as Sr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sulphate as SO4	mg/l	IC	4.9	3.7	2	2.2	2			3.0
Sulphur as S	mg/l	ICP	3.3	1.4	1.4	0.2	ND			1.6
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.21			0.2
Titanium as Ti	mg/l	ICP	0.38	0.11	0.08	0.09	0.03			0.1
Vanadium as V	mg/l	ICP	ND	0.04	ND	ND	ND			0.0
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	4.55	5.29	4.92	4.85	5.14			5.0
pH @25°C		Electrometric	7.8	7.7	7.8	7.6	7.5			7.7
Total Hardness *	mg/l	Calculation	13	20	20	19	19			18.2
SAR	meq/l	Calculation	0.3	0.24	0.24	0.18	0.26			0.2
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1			C.1.S.1

*Means calculated with ND assumed to be zero. Mean for pH was calculated using the geometric mean.

**CWQG. 1999. Canadian Water Quality Guidelines (and updates). Ottawa, ON: Task Force on Water Quality

Guidelines of the Canadian Council of Resource and Environment Ministers. 1999.

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Concentrations of elements and ions in water at reference site R3

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	8.8	0.6	0.03	ND	0.38			2.5
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.12	0.13	ND	0.09	ND			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	1.7	5.4	4.7	3.9	4.5			4.0
Chromium as Cr	mg/l	ICP	ND	0.02	0.03	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	8.3	ND	1.4	0.32	0.47			2.6
Lead as Pb	mg/l	GTA	0.08	ND	ND	ND	ND			0.1
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	3	4.4	4.5	3.6	3.6			3.8
Manganese as Mn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.16	0.95	0.28	0.35	0.94	ND	ND	0.5
Nitrite as NO2	mg/l	IC	0.34	1.1	0.3	0.3	0.25	ND		0.5
Phosphorus as P	mg/l	ICP	ND	0.27	0.08	ND	ND			0.2
Potassium as K	mg/l	ICP	2.7	3.7	2	<1.4	ND			2.8
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	8.6	5.9	4.4	1.5	3.3			4.7
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	3	3	3.1	2.3	2.8			2.8
Strontium as Sr	mg/l	ICP	ND	ND	0.02	ND	ND			0.0
Sulphate as SO4	mg/l	IC	2	6.6	2.3	2.2	3			3.2
Sulphur as S	mg/l	ICP	3	2.4	1.4	1.6	1.6			2.0
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Titanium as Ti	mg/l	ICP	0.14	ND	ND	ND	ND			0.1
Vanadium as V	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	5.84	8.22	7.38	6.51	5.72			6.7
pH @25°C		Electrometric	6.9	7.6	7.5	6.6	6.9			7.1
Total Hardness *	mg/l	Calculation	16	32	26	25	26			25.0
SAR	meq/l	Calculation	0.33	0.23	0.24	0.2	0.24			0.2
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1			C.1.S.1

Concentrations of elements and ions in water at reference site R6

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	49	13	41	40	26			33.8
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.09	0.06	0.19	0.27	0.14			0.2
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	4.2	3.8	4.1	4.8	5.6			4.5
Chromium as Cr	mg/l	ICP	ND	0.05	0.03	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	28	ND	23	20	14			21.3
Lead as Pb	mg/l	GTA	0.07	ND	ND	0.1	ND			0.1
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	3.6	2.7	3	3.4	3.3			3.2
Manganese as Mn	mg/l	ICP	ND	ND	0.07	ND	ND			0.1
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	1.8	ND	1.2	0.63	0.81	ND	0.02	0.9
Nitrite as NO2	mg/l	IC	ND	2.2	ND	ND	ND	ND		2.2
Phosphorus as P	mg/l	ICP	ND	0.1	0.2	0.16	0.12			0.1
Potassium as K	mg/l	ICP	8.7	5.6	8.7	7.4	6.6			7.4
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	67	22	59	57	41			49.2
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	1.1	1.3	1.5	1	1.5			1.3
Strontium as Sr	mg/l	ICP	ND	ND	0.04	0.02	ND			0.0
Sulphate as SO4	mg/l	IC	13	7.2	3.2	3.4	4.2			6.2
Sulphur as S	mg/l	ICP	6.7	2.4	0.13	0.67	0.8			2.1
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.2			0.2
Titanium as Ti	mg/l	ICP	1.1	0.32	0.78	0.64	0.33			0.6
Vanadium as V	mg/l	ICP	ND	ND	0.06	ND	ND			0.1
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	6.55	5.27	4.94	6.73	5.97			5.9
pH @25°C		Electrometric	6.3	7.3	7.5	7	7.3			7.1
Total Hardness *	mg/l	Calculation	25	21	23	26	28			24.6
SAR	meq/l	Calculation	0.1	0.13	0.14	0.08	0.12			0.1
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1			C.1.S.1

Concentrations of elements and ions in water at experimental site E1

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	3	ND	2.2	ND	1.1			2.1
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.06	0.08	0.08	0.1	ND			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	0.02			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	32	30	24	20	21			25.4
Chromium as Cr	mg/l	ICP	ND	0.09	0.05	ND	ND			0.1
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	2.2	0.02	1.6	ND	0.38			1.1
Lead as Pb	mg/l	GTA	ND	ND	ND	ND	ND			ND
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	39	39	24	26	15			28.6
Manganese as Mn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.13	0.48	1.2	1.1	0.78	0.55	0.03	0.6
Nitrite as NO2	mg/l	IC	0.62	1.2	1.1	ND	ND	ND		1.0
Phosphorus as P	mg/l	ICP	0.1	0.13	0.12	0.09	ND			0.1
Potassium as K	mg/l	ICP	15	14	11	9	12			12.2
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	7.8	4.6	7.2	0.6	7.3			5.5
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	110	73	45	54	36			63.6
Strontium as Sr	mg/l	ICP	ND	0.08	0.17	0.12	0.12			0.1
Sulphate as SO4	mg/l	IC	60	27	11	8	12			23.6
Sulphur as S	mg/l	ICP	23	10	3.7	3.8	5			9.1
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Titanium as Ti	mg/l	ICP	0.21	ND	0.08	ND	ND			0.1
Vanadium as V	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	87.15	76.1	46.5	52.7	36.5			59.8
pH @25°C		Electrometric	8.7	8.5	8.4	8.9	7.9			8.5
Total Hardness *	mg/l	Calculation	241	235	159	157	118			182.0
SAR	meq/l	Calculation	3.1	2.9	1.5	1.9	1.4			2.2
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.2.S.1	C.2.S.1			C.1.S.1-C.2.S.1

Concentrations of elements and ions in water at experimental site E3

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	1.1	ND	ND	ND	0.1			0.6
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.08	ND	ND	0.04	ND			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	0.04	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	30	26	29	37	36			31.6
Chromium as Cr	mg/l	ICP	ND	0.02	0.05	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	1.1	0.07	ND	ND	ND			0.6
Lead as Pb	mg/l	GTA	0.12	ND	ND	ND	ND			ND
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	33	34	22	26	22			27.4
Manganese as Mn	mg/l	ICP	0.1	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	1.4	0.53	0.62	1.1	0.62	0.74	0.03	0.7
Nitrite as NO2	mg/l	IC	0.89	0.76	0.77	ND	0.36	ND		0.7
Phosphorus as P	mg/l	ICP	1.3	0.46	1.1	0.53	0.32			0.7
Potassium as K	mg/l	ICP	21	11	26	19	18			19.0
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	13	7.1	1.7	2.3	5			5.8
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	251	109	84	26	62			106.4
Strontium as Sr	mg/l	ICP	ND	0.23	0.21	0.19	0.26			0.2
Sulphate as SO4	mg/l	IC	85	34	8.6	7.1	13			29.5
Sulphur as S	mg/l	ICP	30	11	2.5	4	6			10.7
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.2			0.2
Titanium as Ti	mg/l	ICP	0.04	ND	ND	ND	ND			0.0
Vanadium as V	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	148	83.8	69	82.9	60.1			88.8
pH @25°C		Electrometric	7.9	8.4	8.4	7.5	7.9			8.0
Total Hardness *	mg/l	Calculation	211	204	163	199	180			191.4
SAR	meq/l	Calculation	7.5	3.3	2.8	2.6	2			3.6
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.3.S.1	C.2.S.1			C.1.S.1-C.3.S.1

Concentrations of elements and ions in water at experimental site E4

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	0.63	ND	ND	ND	ND			0.6
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.15	ND	0.12	0.05	0.15			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	34	27	29	34	30			30.8
Chromium as Cr	mg/l	ICP	ND	0.06	0.01	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	0.73	ND	ND	ND	ND			0.7
Lead as Pb	mg/l	GTA	0.09	ND	ND	ND	ND			ND
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	32	28	22	26	27			27.0
Manganese as Mn	mg/l	ICP	0.15	ND	ND	ND	ND			0.2
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.15	0.47	1.9	0.58	1	0.99	0.05	0.7
Nitrite as NO2	mg/l	IC	0.77	0.8	0.39	ND	0.5	ND		0.6
Phosphorus as P	mg/l	ICP	1.4	0.89	1	0.54	0.65			0.9
Potassium as K	mg/l	ICP	23	12	24	17	20			19.2
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	13	6.2	1.8	0.79	2.4			4.8
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	213	100	80	87	99			115.8
Strontium as Sr	mg/l	ICP	ND	0.19	0.18	0.24	0.3			0.2
Sulphate as SO4	mg/l	IC	64	22	6.3	23	13			25.7
Sulphur as S	mg/l	ICP	28	7.6	2.4	7.3	6.5			10.4
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.6			0.6
Titanium as Ti	mg/l	ICP	ND	ND	ND	ND	ND			ND
Vanadium as V	mg/l	ICP	ND	0.02	ND	ND	ND			0.0
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	134	77.8	67.9	83.5	79.5			88.5
pH @25°C		Electrometric	7.8	8.4	8.3	7.8	7.8			8.0
Total Hardness *	mg/l	Calculation	217	183	163	192	186			188.2
SAR	meq/l	Calculation	6.3	3.2	2.7	2.7	3.1			3.6
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.3.S.1	C.3.S.1			C.1.S.1-C.3.S.1

Concentrations of elements and ions in water at experimental site E6

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	0.11	ND	ND	ND	0.04			0.1
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.12	ND	0.09	0.05	ND			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	43	31	26	30	36			33.2
Chromium as Cr	mg/l	ICP	ND	0.02	ND	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	ND	ND	ND	ND	ND			ND
Lead as Pb	mg/l	GTA	ND	ND	ND	ND	ND			ND
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	48	27	19	22	26			28.4
Manganese as Mn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.17	ND	0.16	0.33	0.56	0.43	0.49	0.4
Nitrite as NO2	mg/l	IC	0.37	1.6	ND	0.31	0.31	ND		0.6
Phosphorus as P	mg/l	ICP	ND	0.64	0.59	0.26	0.07			0.4
Potassium as K	mg/l	ICP	10	11	17	12	7			11.4
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	7.3	7.3	1.7	1.2	3.8			4.3
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	78	52	56	54	36			55.2
Strontium as Sr	mg/l	ICP	ND	0.21	0.11	0.13	0.23			0.2
Sulphate as SO4	mg/l	IC	68	24	4.8	5.6	31			26.7
Sulphur as S	mg/l	ICP	27	7.3	1.3	1.6	11			9.6
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.6			0.6
Titanium as Ti	mg/l	ICP	ND	ND	ND	ND	ND			ND
Vanadium as V	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	81.7	55.8	51.2	0.57	48.1			47.5
pH @25°C		Electrometric	8.3	8.4	8.3	8.9	8.7			8.5
Total Hardness *	mg/l	Calculation	305	188	146	166	197			200.4
SAR	meq/l	Calculation	1.9	1.6	2	1.8	1.1			1.7
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1	C.2.S.1			C.1.S.1-C.2.S.1

Concentrations of elements and ions in water at experimental site E8

Elements and ions	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	May-02	Jun-02	Mean*
Aluminum as Al	mg/l	ICP	0.11	ND	ND	ND	0.04			0.1
Antimony as Sb	mg/l	ICP	ND	ND	ND	ND	ND			ND
Arsenic as As	mg/l	GTA	ND	ND	ND	ND	ND			ND
Barium as Ba	mg/l	ICP	0.12	ND	0.09	0.05	ND			0.1
Beryllium as Be	mg/l	ICP	ND	ND	ND	ND	ND			ND
Bismuth as Bi	mg/l	ICP	ND	ND	ND	ND	ND			ND
Boron as B	mg/l	ICP	ND	ND	ND	ND	ND			ND
Cadmium as Cd	mg/l	ICP	ND	ND	ND	ND	ND			ND
Calcium as Ca	mg/l	ICP	43	31	26	30	36			33.2
Chromium as Cr	mg/l	ICP	ND	0.02	ND	ND	ND			0.0
Cobalt as Co	mg/l	ICP	ND	ND	ND	ND	ND			ND
Copper as Cu	mg/l	ICP	ND	ND	ND	ND	ND			ND
Iron as Fe	mg/l	ICP	ND	ND	ND	ND	ND			ND
Lead as Pb	mg/l	GTA	ND	ND	ND	ND	ND			ND
Lithium as Li	mg/l	ICP	ND	ND	ND	ND	ND			ND
Magnesium as Mg	mg/l	ICP	48	27	19	22	26			28.4
Manganese as Mn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Mercury as Hg	mg/l	ICP	ND	ND	ND	ND	ND			ND
Molybdenum as Mo	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nickel as Ni	mg/l	ICP	ND	ND	ND	ND	ND			ND
Nitrate as NO3	mg/l	IC	0.17	ND	0.16	0.33	0.56	0.43	0.49	0.4
Nitrite as NO2	mg/l	IC	0.37	1.6	ND	0.31	0.31	ND		0.6
Phosphorus as P	mg/l	ICP	ND	0.64	0.59	0.26	0.07			0.4
Potassium as K	mg/l	ICP	10	11	17	12	7			11.4
Selenium as Se	mg/l	GTA	ND	ND	ND	ND	ND			ND
Silica as Si	mg/l	ICP	7.3	7.3	1.7	1.2	3.8			4.3
Silver as Ag	mg/l	ICP	ND	ND	ND	ND	ND			ND
Sodium as Na	mg/l	ICP	78	52	56	54	36			55.2
Strontium as Sr	mg/l	ICP	ND	0.21	0.11	0.13	0.23			0.2
Sulphate as SO4	mg/l	IC	68	24	4.8	5.6	31			26.7
Sulphur as S	mg/l	ICP	27	7.3	1.3	1.6	11			9.6
Thallium as Tl	mg/l	ICP	ND	ND	ND	ND	ND			ND
Tin as Sb	mg/l	ICP	ND	ND	ND	ND	0.6			0.6
Titanium as Ti	mg/l	ICP	ND	ND	ND	ND	ND			ND
Vanadium as V	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zinc as Zn	mg/l	ICP	ND	ND	ND	ND	ND			ND
Zirconium as Zr	mg/l	ICP	ND	ND	ND	ND	ND			ND
Conductivity @25°C	mS/m	Electrometric	81.7	55.8	51.2	0.57	48.1			47.5
pH @25°C		Electrometric	8.3	8.4	8.3	8.9	8.7			8.5
Total Hardness *	mg/l	Calculation	305	188	146	166	197			200.4
SAR	meq/l	Calculation	1.9	1.6	2	1.8	1.1			1.7
CLASS		Calculation	C.1.S.1	C.1.S.1	C.1.S.1	C.1.S.1	C.2.S.1			C.1.S.1-C.2.S.1

Concentrations of elements in sediment at reference site R1

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	0.02	ND	ND	ND	14	7.01
Copper as Cu	g/100g	ICP	ND	ND	ND	ND	ND	ND
Iron as Fe	g/100g	ICP	0.14	0.31	0.1	ND	11	2.89
Lead as Pb	mg/kg	GTA	ND	0.31	0.31	ND	ND	0.31
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	0.03	ND	ND	ND	0.03
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	ND	ND	ND	ND	ND

Concentrations of elements in sediment at reference site R3

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Copper as Cu	g/100g	ICP	ND	0.01	ND	ND	ND	0.01
Iron as Fe	g/100g	ICP	ND	0.17	0.32	ND	9	3.16
Lead as Pb	mg/kg	GTA	ND	0.18	0.18	ND	ND	0.18
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	0.04	ND	ND	ND	0.04
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	ND	ND	ND	ND	ND

Concentrations of elements in sediment at reference site R6

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	200	1	100.50
Copper as Cu	g/100g	ICP	0.01	0.01	ND	ND	ND	0.01
Iron as Fe	g/100g	ICP	0.03	ND	0.13	ND	3	1.05
Lead as Pb	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	0.02	ND	ND	ND	0.02
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	ND	ND	ND	ND	ND

Concentrations of elements in sediment at experimental site E1

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	0.02	ND	ND	15	7.51
Copper as Cu	g/100g	ICP	ND	0.06	ND	ND	ND	0.06
Iron as Fe	g/100g	ICP	0.04	0.38	0.11	0.79	34	7.06
Lead as Pb	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	0.25	ND	ND	ND	0.25

Concentrations of elements in sediment at experimental site E3

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	20	ND	20.00
Copper as Cu	g/100g	ICP	ND	0.009	ND	ND	ND	0.01
Iron as Fe	g/100g	ICP	0.14	0.96	0.14	ND	19	5.06
Lead as Pb	mg/kg	GTA	ND	0.02	ND	ND	ND	0.02
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	0.09	ND	ND	ND	0.09

Concentrations of elements in sediment at experimental site E4

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	ND	22	22.00
Copper as Cu	g/100g	ICP	ND	0.17	ND	ND	ND	0.17
Iron as Fe	g/100g	ICP	ND	0.75	0.09	ND	7	2.61
Lead as Pb	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	0.03	ND	ND	ND	0.03

Concentrations of elements in sediment at experimental site E6

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	ND	19	19.00
Copper as Cu	g/100g	ICP	ND	0.03	ND	ND	ND	0.03
Iron as Fe	g/100g	ICP	ND	0.04	0.1	ND	15	5.05
Lead as Pb	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	0.01	ND	ND	ND	0.01
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	0.01	ND	0.01
Zinc as Zn	g/100g	ICP	ND	ND	ND	ND	ND	ND

Concentrations of elements in sediment at experimental site E8

Element	Units:	Method Used	Nov-01	Dec-01	Jan-02	Feb-02	Mar-02	Mean
Arsenic as As	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Cadmium as Cd	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Chromium as Cr	mg/kg	ICP	ND	ND	ND	100	15	57.50
Copper as Cu	g/100g	ICP	ND	0.02	ND	ND	ND	0.02
Iron as Fe	g/100g	ICP	ND	1.06	0.07	ND	27	9.38
Lead as Pb	mg/kg	GTA	ND	0.1	0.1	ND	ND	0.10
Mercury as Hg	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Molybdenum as Mo	mg/kg	ICP	ND	0.01	ND	ND	ND	0.01
Nickel as Ni	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Selenium as Se	mg/kg	GTA	ND	ND	ND	ND	ND	ND
Silver as Ag	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Thallium as Tl	mg/kg	ICP	ND	ND	ND	ND	ND	ND
Zinc as Zn	g/100g	ICP	ND	ND	ND	ND	ND	ND

APPENDIX 6

Total Data Summary for Chapter 4

Snout Vent length of frogs (mm)

	Microcosm number and atrazine concentration									
	2	3	4	5	6	7	9	10	11	12
	25 µg/L	Ref	1 µg/L	10 µg/L	25 µg/L	Ref	25 µg/L	10 µg/L	1 µg/L	Ref
1.	16.20	21.2	18.30	15.40	19.20	34.70	18.30	18.90	11.60	17.20
2.	13.90	31.5	18.70	14.30	21.40	20.20	18.50	17.90	16.80	15.00
3.	21.20	20.3	17.60	17.20	16.40	20.40	17.20	18.00	15.60	20.10
4.	18.00	20.1	22.00	20.60	14.70	20.00	15.00	20.10	17.40	19.10
5.	18.50	21.4	20.30	17.40	17.40	17.20	15.00	17.70	18.60	17.10
6.	19.50	21.6	17.40	20.00	20.60	23.70	14.60	17.30	14.20	20.70
7.	19.30	21.4	20.50	17.60	17.10	20.10	15.20	18.90	15.70	15.60
8.	15.50	20.1	17.90	15.20	16.40	22.30	18.50	18.20	14.90	18.40
9.	18.30	19.3	18.00	20.60	14.70	24.50	16.00	17.60	16.70	24.80
10.	16.70	18.4	20.10	19.20	16.20	19.10	15.00	18.80	14.90	15.30
11.	20.40	20.3	17.50	17.40	13.70	16.70	16.80	17.50	19.70	16.20
12.	18.10	21.4	18.30	18.00	14.30	19.90	17.10	17.60	17.20	16.70
13.	17.40	15.5	22.60	17.80	13.70	30.60	16.10	16.80	13.60	20.10
14.	15.60	18.3	24.20	18.20	14.30	15.80	13.40	16.60	18.20	16.10
15.	19.90	19.9	21.20	17.10	16.00	20.50	17.70	17.40	15.90	17.20
16.	16.60	18.7	18.00	17.80	23.00	19.40	17.10	17.30	16.40	21.30
17.	20.80	18.7	19.10	17.00	14.50	26.50	16.10	17.40	16.20	18.30
18.	16.90	16.3	17.30	16.40	13.30	20.40	16.70	17.40	14.80	20.40
19.	16.10	18.9	16.10	15.70	15.80	26.60	18.10	17.30	18.90	19.30
20.	25.00	18.1	17.50	18.40	16.50	24.20	14.70	16.30	15.70	30.20
21.	16.30	16.2	18.70	26.30	16.10	25.10	14.70	17.00	16.70	35.10
22.	17.00	19.1	17.70	25.20	14.60	21.40	18.40	24.20	16.10	24.90
23.	16.40	17.5	16.70	20.40	14.00	-	24.40	17.60	16.30	27.10
24.	16.40	21.3	18.60	15.50	14.30	-	19.60	18.90	17.50	20.50
25.	15.90	19.4	18.50	17.70	22.40	-	20.50	18.30	15.20	25.60
26.	16.90	28.4	17.40	18.20	23.30	-	13.30	16.50	15.80	21.50
27.	18.90	22.1	17.30	15.30	23.30	-	13.40	17.90	16.50	35.40
28.	20.70	22.3	17.10	17.30	24.20	-	12.50	16.40	16.70	26.40
29.	18.70	22.1	22.20	27.50	20.80	-	-	16.20	17.20	21.10
30.	15.80	34.3	23.30	16.30	24.10	-	-	19.40	19.20	20.00
31.	16.80	21.8	17.70	18.30	21.00	-	-	16.80	17.20	21.30
32.	18.20	27.4	17.10	16.50	24.70	-	-	21.30	16.30	21.60
33.	18.00	18.5	17.10	22.30	17.10	-	-	22.70	16.70	21.40
34.	19.00	30.5	16.40	25.30	13.60	-	-	17.20	18.40	21.60
35.	16.50	21.4	16.00	18.40	22.30	-	-	16.40	19.20	23.30
36.	16.50	21.5	18.60	17.90	16.30	-	-	19.20	16.70	19.80
37.	16.80	22.7	18.80	18.40	16.40	-	-	17.30	17.60	22.30
38.	16.10	27.9	20.50	17.30	14.20	-	-	17.70	17.80	19.60
39.	17.50	23.3	17.20	19.30	14.80	-	-	17.00	16.70	23.10
40.	17.00	20.4	17.80	16.90	15.30	-	-	17.50	18.80	23.60

41.	16.90	28.1	19.30	18.20	24.20	-	-	18.10	16.80	20.70
42.	17.10	22.3	26.40	16.50	14.90	-	-	16.20	17.40	19.90
43.	17.00	23	21.90	27.30	16.50	-	-	18.20	19.40	27.30
44.	16.20	20.5	19.80	26.20	15.40	-	-	19.30	17.80	23.80
45.	16.20	19.1	17.20	16.30	18.40	-	-	16.30	19.90	20.20
46.	22.20	21	15.70	16.60	18.00	-	-	16.90	17.70	20.40
47.	20.90	19.8	18.10	17.40	15.80	-	-	17.60	16.20	24.40
48.	16.80	27.3	19.40	17.90	-	-	-	15.50	16.20	22.50
49.	17.60	25.4	15.50	16.80	-	-	-	16.20	18.80	16.20
50.	21.50	19.6	28.50	17.80	-	-	-	19.90	17.10	20.50
51.	25.90	19.6	21.80	18.50	-	-	-	18.30	17.10	22.70
52.	17.20	20.6	23.70	15.40	-	-	-	20.00	22.20	15.20
53.	28.90	22	21.50	18.30	-	-	-	14.50	18.60	21.70
54.	21.40	21.8	26.00	19.70	-	-	-	18.60	18.40	17.40
55.	17.90	20.9	20.20	17.20	-	-	-	17.20	18.30	21.50
56.	20.30	20.8	19.40	17.10	-	-	-	18.80	19.40	22.70
57.	23.10	23.3	19.40	19.20	-	-	-	18.20	17.30	19.70
58.	19.10	21.2	19.00	19.10	-	-	-	15.40	21.90	21.50
59.	23.20	19.6	20.50	17.80	-	-	-	19.50	19.30	22.00
60.	19.00	20.1	20.80	21.20	-	-	-	15.50	18.50	23.60
61.	22.30	22	21.20	19.90	-	-	-	19.20	18.70	22.40
62.	17.50	24.4	19.60	19.80	-	-	-	18.20	19.50	-
63.	19.50	22.3	19.90	17.80	-	-	-	19.30	19.10	-
64.	17.70	20.7	25.10	18.00	-	-	-	19.50	19.30	-
65.	18.40	21.2	18.50	21.20	-	-	-	19.00	21.00	-
66.	18.50	19.4	22.50	18.40	-	-	-	18.60	18.40	-
67.	17.30	21.4	21.50	25.40	-	-	-	22.00	19.30	-
68.	16.40	-	20.50	34.70	-	-	-	18.40	19.80	-
69.	18.20	-	27.10	29.30	-	-	-	19.90	17.10	-
70.	17.40	-	20.00	28.50	-	-	-	20.00	19.40	-
71.	17.10	-	20.60	26.80	-	-	-	18.20	19.80	-
72.	16.80	-	21.20	34.60	-	-	-	18.60	18.30	-
73.	16.40	-	19.70	18.10	-	-	-	19.10	18.40	-
74.	21.70	-	21.80	18.70	-	-	-	19.00	19.20	-
75.	17.40	-	19.50	18.20	-	-	-	19.50	18.10	-
Mean	18.40	21.60	19.73	19.54	17.56	22.24	16.57	18.12	17.59	21.32
Min	15.60	15.50	15.50	15.30	13.30	15.80	12.50	14.50	13.60	15.00
Max	28.90	34.30	28.50	34.70	24.70	34.70	24.40	24.20	22.20	35.40

Mass (g)

	Microcosm number and atrazine concentration									
	2	3	4	5	6	7	9	10	11	12
	25 µg/L	Ref	1 µg/L	10 µg/L	25 µg/L	Ref	25 µg/L	10 µg/L	1 µg/L	Ref
1.	0.38	1.00	0.60	0.44	0.90	4.46	0.58	0.78	0.13	0.70
2.	0.24	3.53	0.63	0.31	1.03	0.84	0.62	0.61	0.49	0.29
3.	0.98	0.82	0.53	0.49	0.47	0.83	0.48	0.62	0.45	0.86
4.	0.54	0.82	1.03	0.86	0.29	0.83	0.31	0.81	0.60	0.73
5.	0.66	1.00	0.92	0.56	0.48	0.52	0.31	0.61	0.71	0.52
6.	0.76	1.00	0.63	0.80	0.80	1.44	0.35	0.55	0.28	0.84
7.	0.71	1.04	0.93	0.54	0.48	0.96	0.32	0.78	0.37	0.69
8.	0.38	0.88	0.53	0.35	0.46	1.23	0.62	0.57	0.31	0.59
9.	0.74	0.83	0.53	0.93	0.30	1.68	0.40	0.56	0.46	1.48
10.	0.46	0.68	0.82	0.75	0.40	0.79	0.27	0.65	0.36	0.34
11.	0.92	0.92	0.48	0.50	0.25	0.43	0.45	0.56	0.76	0.41
12.	0.66	1.10	0.62	0.63	0.29	0.83	0.48	0.50	0.53	0.44
13.	0.61	0.34	1.16	0.56	0.25	3.35	0.37	0.49	0.25	0.83
14.	0.35	0.64	1.49	0.58	0.28	0.43	0.25	0.50	0.66	0.45
15.	0.80	0.74	1.01	0.74	0.40	0.98	0.53	0.49	0.43	0.51
16.	0.44	0.62	0.59	0.66	1.28	0.84	0.48	0.48	0.40	0.92
17.	1.02	0.58	0.77	0.58	0.33	1.96	0.42	0.51	0.35	0.65
18.	0.48	0.37	0.53	0.46	0.24	0.88	0.44	0.57	0.28	0.84
19.	0.41	0.69	0.42	0.47	0.38	2.09	0.57	0.54	0.71	0.70
20.	1.61	0.56	0.59	0.62	0.48	1.47	0.31	0.39	0.40	2.55
21.	0.45	0.36	0.71	1.98	0.49	1.69	0.32	0.52	0.44	4.68
22.	0.45	0.72	0.62	1.37	0.35	1.03	0.62	1.68	0.42	1.39
23.	0.43	0.51	0.50	0.92	0.27	-	1.69	0.53	0.42	2.01
24.	0.39	0.81	0.66	0.34	0.33	-	0.79	0.61	0.60	0.91
25.	0.39	0.63	0.65	0.51	1.13	-	0.89	0.69	0.33	1.67
26.	0.43	2.16	0.54	0.59	1.28	-	0.25	0.48	0.40	1.04
27.	0.62	1.11	0.59	0.36	1.17	-	0.24	0.53	0.48	4.40
28.	0.91	1.04	0.49	0.51	1.42	-	0.23	0.44	0.45	1.90
29.	0.61	1.04	1.10	2.22	0.86	-	-	0.41	0.51	0.97
30.	0.36	4.32	1.24	0.43	1.40	-	-	0.82	0.83	0.73
31.	0.42	1.03	0.62	0.61	0.94	-	-	0.46	0.54	0.96
32.	0.52	2.26	0.51	0.44	1.50	-	-	0.96	0.51	1.01
33.	0.52	0.60	0.52	1.06	0.46	-	-	1.19	0.51	1.00
34.	0.64	2.69	0.52	1.58	0.24	-	-	0.52	0.65	1.02
35.	0.44	1.03	0.43	0.63	1.16	-	-	0.38	0.86	1.34
36.	0.43	1.01	0.66	0.51	0.39	-	-	0.66	0.48	0.83
37.	0.41	1.21	0.60	0.61	0.40	-	-	0.48	0.62	1.14
38.	0.47	2.09	0.85	0.52	0.27	-	-	0.55	0.55	0.77
39.	0.46	1.41	0.50	0.78	0.33	-	-	0.49	0.51	1.30
40.	0.46	0.89	0.59	0.51	0.37	-	-	0.54	0.72	1.34

41.	0.46	2.14	0.71	0.62	1.42	-	-	0.59	0.58	0.95
42.	0.42	1.09	1.69	0.50	0.36	-	-	0.41	0.58	0.80
43.	0.44	1.27	0.99	2.01	0.50	-	-	0.56	0.72	1.89
44.	0.34	0.96	0.76	1.84	0.36	-	-	0.73	0.55	1.38
45.	0.37	0.72	0.50	0.45	0.56	-	-	0.38	0.77	0.84
46.	0.96	0.89	0.37	0.47	0.54	-	-	0.48	0.52	0.80
47.	0.86	0.71	0.58	0.56	0.35	-	-	0.56	0.38	1.28
48.	0.43	1.99	0.75	0.59	-	-	-	0.34	0.40	1.08
49.	0.48	1.50	0.67	0.52	-	-	-	0.41	0.64	0.42
50.	0.93	0.76	2.07	0.62	-	-	-	0.76	0.43	0.95
51.	1.69	0.77	0.84	0.85	-	-	-	0.56	0.47	1.18
52.	0.54	0.79	1.27	0.37	-	-	-	0.77	0.95	0.42
53.	2.78	1.05	1.15	0.52	-	-	-	0.29	0.61	1.05
54.	1.01	0.95	1.67	0.71	-	-	-	0.60	0.60	0.54
55.	0.57	0.83	0.92	0.49	-	-	-	0.49	0.57	1.00
56.	0.83	0.90	0.80	0.51	-	-	-	0.68	0.71	1.34
57.	1.35	1.18	0.86	0.60	-	-	-	0.64	0.53	0.83
58.	0.65	0.99	0.71	0.64	-	-	-	0.34	1.05	1.02
59.	1.24	0.81	0.97	0.54	-	-	-	0.67	0.82	1.17
60.	0.67	0.76	0.90	0.86	-	-	-	0.32	0.58	1.33
61.	1.35	1.14	1.02	0.82	-	-	-	0.67	0.69	1.05
62.	0.64	1.35	0.87	0.76	-	-	-	0.57	0.76	-
63.	0.71	1.30	0.81	0.52	-	-	-	0.72	0.72	-
64.	0.56	0.88	1.60	0.58	-	-	-	0.74	0.87	-
65.	0.63	1.26	0.75	0.74	-	-	-	0.78	1.01	-
66.	0.64	0.75	1.16	0.58	-	-	-	0.67	0.65	-
67.	0.58	0.98	1.06	1.59	-	-	-	1.06	0.76	-
68.	0.52	-	1.06	4.61	-	-	-	0.60	0.80	-
69.	0.62	-	1.77	2.32	-	-	-	0.81	0.54	-
70.	0.46	-	0.89	2.14	-	-	-	0.82	0.82	-
71.	0.52	-	0.84	1.93	-	-	-	0.67	0.85	-
72.	0.52	-	1.00	3.66	-	-	-	0.71	0.61	-
73.	0.49	-	0.92	0.58	-	-	-	0.74	0.67	-
74.	1.01	-	1.09	0.70	-	-	-	0.73	0.71	-
75.	0.56	-	0.82	0.56	-	-	-	0.77	0.64	-
Mean	0.66	1.10	0.83	0.86	0.61	1.34	0.48	0.62	0.58	1.10
Min	0.34	0.34	0.37	0.34	0.24	0.43	0.23	0.29	0.25	0.29
Max	2.78	4.32	2.07	4.61	1.50	4.46	1.69	1.68	1.05	4.68

Sex based on gross morphology

	Microcosm number and atrazine concentration									
	2	3	4	5	6	7	9	10	11	12
	25 µg/L	Ref	1 µg/L	10 µg/L	25 µg/L	Ref	25 µg/L	10 µg/L	1 µg/L	Ref
1.	F	F	F	F	M	F	F	F	F	M
2.	F	F	M	M	M	F	F	M	F	F
3.	F	M	M	M	F	M	F	M	F	F
4.	F	M	M	M	M	F	F	M	F	F
5.	F	F	M	F	M	F	F	F	F	F
6.	M	F	M	F	F	M	F	F	F	M
7.	M	F	M	F	F	M	F	F	M	F
8.	F	F	F	F	F	F	M	F	M	M
9.	F	F	F	M	F	F	M	F	F	M
10.	F	M	M	M	F	F	F	M	M	F
11.	M	F	M	F	M	F	M	M	F	F
12.	F	M	M	F	M	F	F	F	F	M
13.	F	F	F	M	M	M	M	F	F	F
14.	M	M	F	F	F	M	F	M	M	F
15.	M	F	F	F	F	F	M	F	F	F
16.	F	M	M	M	M	F	M	M	M	M
17.	F	F	F	F	F	M	F	F	M	F
18.	F	M	F	F	F	F	F	F	F	M
19.	M	F	M	F	F	M	M	F	M	F
20.	F	M	F	M	M	M	F	F	F	M
21.	M	F	F	F	F	M	F	F	F	F
22.	M	M	F	F	F	M	M	F	M	M
23.	F	M	F	M	F	-	M	F	M	F
24.	M	M	F	F	F	-	F	M	F	F
25.	F	F	M	F	M	-	F	F	M	M
26.	F	F	F	F	F	-	F	F	F	F
27.	F	F	M	F	F	-	F	F	F	M
28.	F	F	M	M	F	-	F	M	M	F
29.	F	F	M	F	M	-	-	F	F	F
30.	F	M	M	F	F	-	-	M	M	M
31.	M	M	F	M	M	-	-	F	F	F
32.	F	M	M	F	M	-	-	F	M	M
33.	M	F	F	F	M	-	-	M	M	F
34.	F	F	F	F	M	-	-	M	M	M
35.	M	M	M	F	M	-	-	M	F	M
36.	M	F	M	F	F	-	-	M	F	F
37.	M	F	F	M	M	-	-	M	M	F
38.	M	F	F	M	M	-	-	F	M	M
39.	F	F	M	F	M	-	-	F	F	F
40.	F	F	F	F	M	-	-	F	F	F

41.	M	M	F	F	M	-	-	F	F	M
42.	F	F	F	M	F	-	-	F	F	F
43.	F	F	M	M	M	-	-	F	M	M
44.	M	F	M	M	M	-	-	M	F	M
45.	M	F	M	F	M	-	-	M	M	M
46.	M	M	F	M	M	-	-	F	F	M
47.	M	F	F	F	M	-	-	F	M	M
48.	M	M	F	M	-	-	-	F	F	M
49.	M	F	F	F	-	-	-	F	F	M
50.	M	F	F	M	-	-	-	F	M	M
51.	F	F	F	M	-	-	-	M	M	M
52.	M	F	M	M	-	-	-	M	F	M
53.	F	M	F	F	-	-	-	F	M	F
54.	F	M	F	F	-	-	-	F	F	F
55.	F	F	M	M	-	-	-	F	M	M
56.	M	F	M	M	-	-	-	M	M	M
57.	M	F	F	F	-	-	-	F	M	M
58.	M	F	M	M	-	-	-	M	M	M
59.	M	M	F	F	-	-	-	M	F	F
60.	F	F	M	F	-	-	-	M	F	F
61.	M	M	F	F	-	-	-	F	F	M
62.	F	M	F	M	-	-	-	M	F	-
63.	F	M	M	M	-	-	-	F	M	-
64.	F	M	M	M	-	-	-	F	M	-
65.	M	F	M	F	-	-	-	F	F	-
66.	M	M	M	F	-	-	-	F	F	-
67.	F	F	M	F	-	-	-	F	M	-
68.	F	-	F	M	-	-	-	F	M	-
69.	M	-	M	F	-	-	-	M	M	-
70.	F	-	F	F	-	-	-	F	M	-
71.	F	-	F	M	-	-	-	M	F	-
72.	F	-	F	F	-	-	-	M	M	-
73.	M	-	M	M	-	-	-	F	F	-
74.	F	-	M	M	-	-	-	F	M	-
75.	M	-	F	M	-	-	-	M	M	-
n M	34	26	36	32	26	10	9	28	36	32
n F	41	41	39	43	21	12	19	47	39	29
M = male F = female										

Frogs showing gonadal anomalies based on gross morphology

	Microcosm number and atrazine concentration									
	2	3	4	5	6	7	9	10	11	12
	25 µg/L	Ref	1 µg/L	10 µg/L	25 µg/L	Ref	25 µg/L	10 µg/L	1 µg/L	Ref
1.										
2.										
3.										
4.										
5.					X					
6.										
7.	X									
8.										
9.										
10.										
11.										
12.										
13.										
14.										
15.										
16.		X								
17.										
18.										
19.									X	
20.										
21.										
22.										X
23.										
24.		X								
25.										
26.										
27.										
28.										
29.										
30.										
31.										
32.										
33.										
34.										
35.										
36.	X									
37.										
38.										
39.										
40.										

41.										
42.										
43.										
44.										
45.										X
46.										X
47.					X					
48.	X									
49.										
50.										X
51.										
52.										
53.								X		
54.										
55.										
56.										
57.										
58.										
59.										
60.										
61.										
62.										
63.										
64.										
65.										
66.										
67.										
68.										
69.									X	
70.										
71.										
72.										
73.										
74.										
75.										

Atrazine concentrations for microcosm experiment

Mic. no	Treatment	12-Sep	1-Oct	10-Oct	31-Oct	14-Nov	28-Nov	12-Dec	Avg	stdev
1	1	1.42	1.41	0.91	1.27	1.30	1.36	1.13	1.26	0.18
2	25	36.31	25.90	23.86	30.39	28.73	27.30	25.12	28.23	4.19
3	Ref	<0.1	0.10	<0.1	<0.1	<0.1	<0.1	<0.1	0.10	
4	1	1.67	1.45	1.54	1.73	1.56	1.33	1.09	1.48	0.22
5	10	12.67	10.93	11.87	13.88	11.89	10.02	10.03	11.61	1.41
6	25	39.73	31.12	26.67	32.47	31.00	30.25	29.56	31.54	4.04
7	Ref	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		
8	10	13.78	13.40	12.20	15.91	13.78	10.96	10.67	12.96	1.83
9	25	38.73	25.83	28.16	35.82	34.97	34.46	29.76	32.53	4.67
10	10	12.11	11.02	11.28	14.23	12.45	10.68	10.22	11.71	1.35
11	1	1.48	1.46	1.51	1.82	1.63	1.57	1.48	1.56	0.13
12	Ref	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		