

Final Report
To
Florida Harmful Algal Bloom Taskforce

Blue Green Algal Exposure, Drinking Water and Primary Liver Cancer

Investigators:

Lora E Fleming MD PhD MPH (Principal Investigator),
Carlos Rivero BS (GIS Investigator), John Burns MS (Consultant SJRWMD)

Sites:

NIEHS Marine and Freshwater Biomedical Sciences Center and GEOCORE Facility at the University of Miami Rosenstiel School of Marine and Atmospheric Studies, Key Virginia, FL with consulting agreement to St Johns River Water Management District (SJRWMD).

October 2000

ABSTRACT

The blue green algae or cyanobacteria represent a diverse group of organisms that produce potent natural toxins. There have been case reports of severe morbidity and mortality in domestic animals through drinking contaminated water. Although there has been little epidemiologic research on toxin effects in humans, a study by Yu et al (1995) found an increased association between primary liver cancer in humans and the use of surface drinking water sources. Surface drinking water supplies are particularly vulnerable to the growth of these organisms; in general, current US drinking water treatment practices do not monitor or treat for the blue green algal toxins.

This pilot study was an ecological study using a Geographic Information System (GIS) evaluation of the risk of primary liver cancer and proximity to a surface water treatment plant at the time of cancer diagnosis. The study linked all primary liver cancers diagnosed in Florida from 1981-1998 with environmental databases on sampling, drinking water sources and treatment plants. A significantly increased risk for primary liver cancer with residence at diagnosis within the distribution area of a surface water treatment plant was found compared to persons living in areas contiguous to the surface water treatment plants. However this increased risk was not seen in comparison to persons living in randomly selected ground water treatment areas or compared to the Florida cumulative incidence rate for the study period, using various comparison and GIS methodologies. These findings must be interpreted in light of significant issues of latency, high population mobility, and the lack of individual exposure information. Nevertheless, the issue of human health effects associated with the consumption of surface waters possibly contaminated by blue green algal toxins merits further investigation.

PURPOSE

The proposed study was an ecological study using a Geographic Information System (GIS) evaluation of the possible association of 1) residence at the time of diagnosis of primary liver cancer and 2) proximity to a surface water treatment plant for all primary liver cancers in Florida since 1981. The study used the Dept of Health (DOH) database of all primary liver cancer cases reported to the Florida Cancer Data System (FCDS) from 1981-1998, in conjunction with Florida Dept of Environmental Protection (DEP) and St Johns River Water Management District (SJRWMD) databases on drinking water sources and treatment plants. In addition, based on the GIS analysis results, directed drinking water samples could be collected from surface and other water sources tested specifically for the blue green algal toxins by the SJRWMD pre and post treatment. This ecological pilot study explored the possible risk of developing primary liver cancer associated with exposure to drinking water from surface water sources in Florida.

BACKGROUND

Primary Hepatocellular Carcinoma

Primary hepatocellular carcinoma (HCC), a malignant epithelial tumor, is the most prevalent type of liver cancer in the world. It is one of the three leading causes of cancer mortality, accounting for 250,000 to 1,000,000 deaths annually (London 1996) world wide, with a ratio of mortality to incidence of 0.98:1 (Pisani 1999). HCC is particularly a problem in the developing countries of the world where 81% of the world's cases are found (Parkin 1999a). In general, the international distribution of HCC correlates with the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. It is estimated that 54% of the world's HCC can be attributed to chronic HBV infection (Parkin 1999b) and 26% is related to HCV infection (Parkin 1999a).

Worldwide, the age-standardized incidence rate of primary liver cancer is 14.7/100,000 for males and 4.9/100,000 for females. For individuals living in developed countries versus those in developing countries, the age-standardized incidence rates are 7.6/100,000 and 17.9/100,000 for males, and 2.6/100,000 and 6.2/100,000 for females, respectively. Worldwide, HCC consistently demonstrates a male predominance (Parkin 1999a).

HCC incidence is increasing in the United States (Miller 1996, ACS 1998, Martin 1998, El Serag 1999). A recent study using data from the Surveillance, Epidemiology, and End Results (SEER), the US Vital Statistics, and the Department of Veteran Affairs, found a significant increase in the incidence of HCC in the US over the past two decades among persons age 40- to 60-years (El Serag 1999). In US children, however, the incidence rate of HCC decreased during 1975-1995 from 0.6/1 million to 0.2/1 million (Bulterys 1999).

In Florida since 1981, there has been a significant but not large increase in the average annual HCC incidence rate in Florida. The incidence of HCC in Florida was comparable to the US incidence with respect to average annual incidence and gender distribution. The average annual HCC incidence rates in Florida among male and female Hispanics and Blacks were consistently and significantly twice the rate of White males and females as standardized rate ratios. Males were at least twice as likely to have HCC compared to females in all 3 race-ethnic subpopulations (Shea 2000).

Cyanobacteria or Blue Green Algae

The Cyanobacteria or blue green algae are an ancient and ubiquitous family of photosynthetic organisms (Chorus 1999, Carmichael 1994, Falconer 1989, NHMRC 1994). These organisms are able to fix nitrogen, and are therefore an important part of the food chain. The cyanobacteria frequently are found growing on marine, brackish and fresh waters, including freshwater surface drinking sources, such as lakes and drinking water reservoirs. Similar to the marine algal blooms, cyanobacteria periodically will grow exuberantly, known as "blooms." The reasons for these blooms are not completely understood, but in some cases they

may be related to nutrients added naturally and through man-made sources (such as fertilizer runoff) (Philipp 1991, Carmichael 1993, Rapala 1997). These blooms can cause significant environmental impact due to the decrease in oxygen in the water, resulting in the die-off of fish and other organisms. Furthermore, similar to marine algal blooms or red tides, these blue green algal blooms can produce significant quantities of natural toxins, for reasons as yet unknown. When they produce these highly active natural biotoxins, these blue green algal blooms are known as a "harmful algal bloom (HAB)." To date at least 12 different species of Cyanobacteria have been shown to produce toxins, often several different toxins per species (Carmichael 1994). The main toxic cyanobacterial genera include *Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria*, and *Microcystis* (Carmichael 1993, NHMRC 1994, Chorus 1999).

Toxins

These toxins, along with those produced by the marine organisms such as dinoflagellates and diatoms, are highly toxic to many species. There is a wide spectrum of blue green algal toxins, predominantly affecting the nervous, hepatic and dermatologic systems (i.e. neurotoxic hepatotoxic and dermatotoxic).

The dermatotoxins include aplysiatoxins and lyngbyatoxin, and are often reported from marine cyanobacteria blooms. These are potent tumor promoters and protein kinase C activators. These toxins can cause severe dermatitis with only skin contact, as well as gastrointestinal inflammation with oral exposure (Chorus 1999).

The neurotoxins include: anatoxin a and anatoxin a (S) (both unique to the cyanobacteria), as well as saxitoxin and neosaxitoxin (also elaborated by marine dinoflagellates and associated with the human disease paralytic shellfish poisoning or PSP). Anatoxin a acts like the neurotransmitter acetylcholine except that it cannot be degraded by acetylcholinesterase; anatoxin a (S) is a natural organophosphate, binding to the acetylcholinesterase enzymes; the saxitoxins are sodium channel blockers. Singly or in mixtures, these cyanobacterial neurotoxins can cause death within minutes secondary to respiratory paralysis (Codd 1997, Carmichael 1994, Carmichael 1993).

The hepatotoxins are cyclic peptides, predominantly microcystins, nodularins, and cylindrospermopsin. Of note, these toxins are particularly toxic to the liver in part due to selective transport mechanisms that concentrate these toxins from the gut and blood into the liver cells; they damage the liver by deranging the cytoskeletal architecture of the hepatocytes. Cylindrospermopsin is a protein synthesis inhibitor, resulting in wide spread necrosis of the tissues of many organs. The microcystins and the nodularins are protein phosphatase inhibitors, as well as being potent tumor promoters in animals (similar to the carcinogen, okadaic acid, elaborated by marine dinoflagellates and associated with the human disease diarrhetic shellfish poisoning or DSP). The microcystins cause liver necrosis leading to death within hours to days (Elder 1993, Carmichael 1994, Humpage 1999, Yu 1995, Ohtani 1992, MacKintosh 1990, Repavich 1990, NHMRC 1994, Chorus 1999). At lower doses, enteritis and hepatitis are seen shortly after ingestion of these toxins in domestic and experimental animals.

The same cyanobacteria species can produce both neurotoxins and hepatotoxins, even during the same bloom; often the presence of the hepatotoxin is masked by the premature death of the animal due to the neurotoxin. In addition, there exist other toxins (including lipopolysaccharides, endotoxins and additional neurotoxins), as well as yet undescribed cyanobacterial toxins including additional tumor promoters (Falconer 1989, Codd 1997, Falconer 1994a, Falconer 1994b, Falconer 1996).

Animals

There have been frequent reports of thirsty domestic animals and wildlife consuming freshwater contaminated with toxic blue green algal blooms, and dying within minutes to days from acute neurotoxicity and/or hepatotoxicity (Jochimsen 1998, Elder 1993, Carmichael 1994, Codd 1997, Mahmood 1988, Carbis 1995, Negri 1995, Repavich 1990). Toxic blooms of cyanobacteria with associated animal poisonings have been reported in all continents except Antarctica (NHMRC 1994). Mammals and birds appear to be more susceptible to the blue green algal toxins than aquatic invertebrates and fish, with some species variability.

Prolonged morbidity and mortality have also been reported in animals exposed to blue green algae in the wild. For example, Carbis et al (1995) followed sheep exposed to *Microcystis aeruginosa* in a lake in Australia for 6 months; there was a 34% mortality over this period among the exposed sheep without clear etiology even after resolution of the initial liver toxicity observed during the first 3 weeks after microcystin exposure.

Experimentally, acute high dose administration of microcystin can lead to death from hepatoencephalopathy within hours, and chronic administration to mice of sublethal amounts of *Microcystis* extracts in drinking water results in increased mortality with chronic active liver disease even at fairly low doses and in relatively short time periods (Heinze 1999). Falconer et al (1992) gave intra peritoneal (ip) injections to mice of the gut and gut contents of boiled edible mussels from a water bloom of *Nodularia* in Western Australia. The cell density of the bloom in the water had been up to 100,000 cells/mL. The ip injections were lethal secondary to acute (within 24 hours) hepatotoxicity to 1 kg mice at 89 mg dry weight/kg, the *Nodularia* bloom LD₅₀ was 24.4 mg dry weight/kg. Based on these studies, Falconer et al (1992) conclude that edible mussels should not be collected for human consumption during a toxic blue green algal bloom.

Teratogenic activity has been demonstrated in mice with oral administration of *Microcystis* extracts; approximately 10% of otherwise normal neonatal mice had small brains with extensive hippocampal neuronal damage (Carmichael 1993, Astrachan 1980). Studies in cultured cells have also shown tumor promotion, and microcystins are preferentially taken up by hepatic cells, so that hepatic tumor promotion is likely (Falconer 1996, Carmichael 1994, Carmichael 1993, Sugimura 1986, Humpage 1999, Ito 1997). As noted above, the microcystins can cause tumor promotion in animals exposed to chronic low level non-lethal doses. Nishikawa et al (1992) showed that microcystins are powerful tumor promoters of hepatic liver tumors in rats mediated through the inhibition of protein phosphatase type 1 and type 2A activity (Hong-Bing 1996). Lyngbyatoxin A has been shown to be a potent tumor promoter in a two stage mouse skin carcinogenesis study by Fujiki et al (1984).

Humans

There are relatively few case reports and even fewer epidemiologic studies of the human health effects of the blue green algal toxins (Carmichael 1993, Jalaludin 1992, Falconer 1999, Chorus 1999). Humans can be exposed to the cyanobacteria and their toxins through direct skin contact or by drinking contaminated waters; other possible routes of exposure include inhalation of aerosol, consumption of contaminated food, and even through dialysis (Codd 1997, Chorus 1999). Occupational exposures for fishermen, watermen, and scientists, as well as recreational exposures, are both possible (Codd 1997, Baxter 1991, Philipp 1991).

Recently, there have been a host of articles concerning cyanobacteria as a source of chronic relapsing diarrhea, especially in travelers (both immunocompromised and not) to developing nations; the illness seems to be associated with the organisms rather than the toxins, and furthermore may actually be a separate group of organisms that are cyanobacterium-like (Soave 1986, Anon 1991, Hale 1994). Some researchers have even postulated a role for the blue green algae as a carrier or reservoir of the bacteria *Vibrio cholera*, the latter responsible for the human bacterial disease cholera (Islan 1994, Chorus 1999).

There are individual case reports of persons exposed through swimming to blue green algal blooms with skin irritation and allergic reactions (both dermatologic and respiratory) with continued positive reaction on skin testing (Falconer 1989, Carmichael 1993, Falconer 1999, Hashimoto 1974, NHMRC 1994, Chorus 1999). In particular, urticaria, blistering and even deep desquamation of skin in sensitive areas like the lips and under swimsuits has been reported, especially with *Lyngbya majuscula* in tropical areas. Consumption of or swimming in cyanobacterial toxin-contaminated waters has also yielded increased case reports of gastrointestinal symptoms, especially diarrhea (Billings 1981, Probert 1995). Turner et al (1990) reported 2 cases of pneumonia in healthy army recruits following probable inhalation from a canoe on waters with a blue green algal bloom of *Microcystis aeruginosa*; 16 other exposed recruits reported of a variety of gastrointestinal (hepatoenteritis), dermatologic and respiratory complaints (Turner 1990). In addition to

gastrointestinal and dermatologic symptoms, eye irritation, asthma, and “hay fever symptoms” have been reported repeatedly with contaminated recreational water exposure in the US, Canada, UK, and Australia (NHMRC 1994).

In general, the few epidemiologic studies available have been performed after a significant community exposure event. With a long history of episodes of possible adverse health effects in animals and humans in Australia, Pilotto et al (1997) studied the effects in South Australia of exposure to blue green algae as a result of recreational water activities. They used a serial symptom questionnaire on a large sample (777 “exposed” and 75 “unexposed”), as well as water sampling for cyanobacteria and toxin. Although there was no difference in the type and quantity of symptoms reported acutely, the Investigators found a significant trend to increasing symptom occurrence with duration of exposure, and a symptom dose response that correlated with exposure to 5000 cells per ml for more than one hour; however, symptoms did not correlate with the presence of hepatotoxins in the water. The Investigators suggested that the current safety threshold for exposure of 20,000 cells per mL may be too high. El Saadi et al (1995) performed a case control study in 11 South Australian towns along the Murray River, a cyanobacterial historic epicenter, using gastrointestinal and dermatologic cases and controls with similar town distributions. Persons who drank the river water, even after chlorination, were significantly more likely to have gastrointestinal symptoms, while those using river water for domestic purposes were significantly more likely to have both gastrointestinal and dermatologic symptoms, compared with persons using rainwater. Furthermore, there was a correlation with report of symptoms and mean log cyanobacterial cell counts.

Seasonal gastroenteritis has been reported worldwide and may be related to the consumption of contaminated drinking water (Carmichael 1993, Volterra 1993, Codd 1984, Falconer 1999). Some of the first reports of adverse health effects from exposure to the blue green algae were by Veldee (1931) when an estimated 9000 persons out of a population of 60,000 in Charleston (West Virginia) reported acute gastroenteritis after a period of low rain fall and reportedly contaminated drinking water; other outbreaks were seen along the Ohio River in the same year (Tisdale 1931). Lipp and Erb (1976) reported that 62% of the population of 8000 of Sewickley (Pennsylvania) suffered from acute gastroenteritis; the reservoir was found to be contaminated by *Schizothrix calcicola*. In 1988, severe gastroenteritis was reported in Brazil after the flooding of a newly constructed dam and reservoir with 2000 cases and 88 deaths (particularly children) over a 42 day period; cases were restricted to the areas supplied by drinking water from the reservoir and had only consumed boiled water with negative bacterial and viral cultures, and *Anabaena* and *Microcystis* blooms were present (Chorus 1999, Teixeira 1993).

Liver enzymes, especially GGT, have also been found to be increased after consumption of drinking water contaminated with Microcystis toxins in Australia. Other Australian episodes have included a severe outbreak of hepatoenteritis after drinking water with a novel cyanobacterial toxin contamination on Palm Island in Queensland (Australia) (Falconer 1983, Carmichael 1993, Bourke 1983, Probert 1995, El Saadi 1995, Chorus 1999). In this particular episode, the drinking water reservoir had been dosed with copper sulphate to remove a persistent cyanobacteria bloom of *Cylindrospermopsis raciborskii*, leading to lysis of the algal cells and substantial release of toxins into the drinking water. Reportedly some of the children were critically ill with severe hepatoenteritis and kidney failure, and 150 persons (140 children) were ultimately hospitalized. Subsequent research identified the cytotoxic cylindrospermopsin as well as other toxins as the probable cause of the outbreak. In another study by Falconer (1994) in different area of Australia with a similar situation of cyanobacterial toxin contamination of a drinking water supply after the use of copper sulfate, clinical liver function data were examined. There was a statistically significant increase in the liver enzyme GGT in persons drinking from the contaminated reservoir only during the period of bloom and cell lysis compared to all others in the same area with different water supplies. GGT has also been used as an effective marker for liver injury in experimental animal studies with microcystin exposure (Falconer 1994a, Falconer 1994b, Chorus 1999).

A recent and infamous outbreak occurred in Brazil when over 100 patients on kidney dialysis developed visual disturbances, nausea and vomiting, followed by 50 deaths from acute liver failure. Apparently the dialysis water was contaminated with blue green algal toxins; microcystins produced by cyanobacteria were subsequently identified in the water and in the human tissues, as well as inadequate water treatment procedures leading to the contamination (Jochimsen 1998).

Pilotto et al (1999) attempted to look at perinatal outcome and the possible relationship with cyanobacterial contamination of drinking water in an ecological study. The investigators examined the perinatal outcome (prematurity, low birth weight and very low birth weight, and congenital defects detected at birth) for 32,700 singleton live newborns of non-Aboriginal mothers from 1992-94 in South Eastern Australia; exposure data were based on weekly cell counts from 29 drinking water storage sites for the 156 towns in the same area (percentage of time occurrence and average cell counts), and the mother's address at the time of the newborn's birth. This work was based on the concern raised by laboratory animal studies showing impaired fetal development (especially neurologic) and low birth weight after exposure to untreated reservoir water sampled during a bloom, as well as fetal mortality, small fetuses, and congenital malformation with injection of microcystins into pregnant rats. Although there were statistically significant associations with particular exposure levels and particular birth outcomes (especially the very low birth weight category and exposure during the first trimester with percentage of time occurrence, and congenital malformations with average cell counts), there was an overall lack of dose response; similar results were seen for the whole gestation and the last 12 weeks of gestation. The authors concluded that their ecological study did not provide clear evidence for an association. However, as they pointed out, there were no individual drinking water exposure data and in areas with frequent known cyanobacterial contamination, systematic avoidance of drinking water can be common.

Cancer

Yu et al and others (1989a, 1989b, 1995, Junshi 1990, Chorus 1999) have studied the possible relationship between the consumption of surface drinking water (pond, ditch, river vs well water or deep well) and an increased risk for primary hepatic cancer (as well as chronic gastrointestinal diseases) in China. China has an extremely high rate of primary liver cancer, previously associated with hepatitis B and aflatoxin exposures (Yu 1995). However, reportedly large epidemiologic studies in 1973 and in 1983 were performed in Haimen, Quidong and Nanhui Counties (Guangxi province, China) to evaluate drinking water source, exposure and risk of primary hepatic cancer. These studies found not only a significantly increased risk of primary liver cancer in areas of high surface drinking water consumption (SIR=2.6) compared with areas of non-surface drinking water consumption (SIR=0.34), but also a strong dose response relationship. Reportedly, changing from pond/ditch to deep well (at least 200 m) water in Quidong lead to a stabilization with subsequent decrease of the mortality rate from primary hepatic cancer, while in Haimen where there was no change, the liver cancer mortality rates continued to increase during the same time period; in an area where there was a mixture of well and river water, there was no significant change in the mortality rate during this time period. Monitoring studies using a sensitive ELISA test from microcystins revealed high levels of microcystins, as well as the presence of blue green algae, in the surface as opposed to other drinking water sources (Ueno 1996). On average the surface water sources contained 130 pg/ml of microcystins compared to the well samples (the vast majority less than 49 pg/ml) (Falconer 1996).

Ito et al (1997) was able to induce neoplastic nodular formation in mouse liver by repeated ip injections of sublethal dose (20 ug/kg) microcystin LR without the use of an initiator; however, repeated oral administration of a sublethal dose (80 ug/kg) did not result in nodular formation. Ueno et al (1996) postulated that the combined effects of a potent hepatocarcinogen such as aflatoxin from the diet with intermittent microcystin intake through drinking water could explain the high rates of primary liver cancer associated with surface drinking water source in this area. Yu (1995) reported on the results of experiments with male F-344 rat exposed to different mixtures of aflatoxin, deep well water, and pond/ditch water after partial hepatectomy. The results showed significant increase in the gamma-glutamyl transferase (GGT) liver enzyme in rats exposed to aflatoxins and pond/ditch water compared to the other groups including control.

Yu (1995) postulated that microcystins are promoters with a synergistic effect between microcystins and aflatoxins for primary hepatocellular carcinoma. As a result of this work, the Chinese government reportedly urged their people to use deep water wells or minimally granular activated carbon filtration for their drinking water, as well as other interventions (i.e. hepatitis B vaccine and shifting to rice instead of corn to avoid aflatoxins), to prevent primary liver cancer in China.

Treatment

In general, the only treatment available for exposure to the blue green algal toxins is supportive medical treatment after complete removal from exposure (Chorus 1999). If the exposure was oral, administration of activated carbon to decrease gut absorption may be efficacious if given within hours of exposure. Artificial respiration with exposure to the neurotoxins (such as saxitoxin) should also be considered (NHMRC 1994). Based on past outbreaks, monitoring of volume, electrolytes, liver and kidney function should all be considered in the case of acute gastroenteritis associated with some of the blue green algal toxins.

Although no specific treatments exist for the cyanobacterial toxins, Nagata et al (1995) have created at least 6 monoclonal antibodies (Mabs) to microcystin LR isolated from *Microcystis aeruginosa*. Given acutely, these MABs showed a protective effect on the hepatotoxicity and inhibition of protein phosphatase of microcystin LR *in vitro* and *in vivo* in a dose dependent manner.

Of note, activated carbon given to experimental animals pre-treatment was not an effective antidote for preventing effects from subsequent microcystin administration (Mereish 1989, Beasley 1989). Hermansky et al (1991) used a variety of chemoprotectants in pre-treatment prior to exposure of experimental mice to a lethal dose of microcystin LR (100 ug/kg). Phenobarbital (but not the calcium channel blockers or water soluble anti-oxidants) provided partial protection, while the hydrophobic anti-oxidants (such as Vitamin E and silymarin), glutathione active compounds (such as glutathione), and immunosuppressive agents (such as rifampin and cyclosporin A) provided significant protection if given 48 hours prior to exposure to microcystin in laboratory animals.

Prevention

Due to their significant potential toxicity and the lack of specific treatment modalities available, the best treatment for the health effects of the blue green algae is the prevention of exposure to the blue green algal toxins. Therefore, monitoring for these toxins in surface drinking and recreational waters, as well as other exposure venues, is crucial in the prevention of human health effects from the blue green algal toxins (NHMRC 1994, Chorus 1999). For example, recent monitoring studies in Florida (SJRWMD 2000) of recreational and surface drinking water supplies with active algal blooms, found 87/167 samples (75 individual water bodies) with significant levels of toxin producing blue green algae. All of these samples had positive identification of blue green algal toxins, with 80% lethal in mice. Monitoring should include visual monitoring for blooms, cell counts and identification, and toxin identification and toxicity testing; other monitoring indices have also been used, including phosphorus levels in the water, as well as surveillance of health effects in human and animal populations (Chorus 1999).

Falconer (1994a) recommended 20,000 cells/ml sampled in the top meter of open water as the maximum safe level of cyanobacteria in recreational waters. Nevertheless, Falconer warned that if the bloom is toxic, swallowing or bathing in these waters should be considered hazardous. Chorus et al (1999) used data from Pilotto et al (1997) to derive a guideline for acute non cumulative health effects resulting in discomfort, not serious, health outcomes. Significantly increased odds ratios for eye irritation, rash and gastrointestinal symptoms were associated with water contact for more than 1 hour above 5000 cyanobacterial cells/mL and for persons bathing in water with 5000-20,000 cells/mL. Pilotto et al (1997) suggested that the current safety threshold for exposure of 20,000 cells per mL might be too high based on their results.

With monitoring programs, response programs must be established based on the results of regular monitoring. Australia and the UK have attempted to develop such monitoring and response programs for

surface drinking water sources (NHMRC 1994, Jones 1993, Burch 1993) with alert levels and corresponding responses based on the number of cyanobacterial cells per ml in routine sampling. For example, Burch et al (1993) and Chorus et al (1999) proposed: Alert Levels 1 (cells 500-2000 cells/mL or offensive odor or taste); Level 2 (potentially toxic cells 2000-15,000 cells/mL for 2-3 consecutive samples or confirmed toxic bloom, persistent odor/taste, and obvious bloom); and Level 3 (persistent high numbers widespread, toxic, cells >15,000 cell/mL for toxic species, persistent bloom, and only partial success of control measures). Level 1 is associated with increased monitoring; Level 2 results in media information release and consultation with health authorities, as well as bloom control measures (such as booms, activated carbon); and Level 3 results in the same actions as Level 2 as well as possible declaration of water as unsafe for consumption and provision of safe drinking water alternatives after consultation with health authorities. Subsequent health surveillance and evaluation may be necessary, especially if exposure is suspected. These authors recommend that separate guidelines should be developed for recreational and occupational use of potentially contaminated surface waters based on the probability and severity of potential health effect development from exposure to cyanobacterial toxins (Bartram and Rees 1999, Chorus 1999). In areas of endemic toxic blue algal blooms, public education and awareness plans should be considered (Chorus 1999), including issues such as avoidance of occupational and recreational exposure, description of possible health effects, and warnings that boiling water will not destroy the cyanobacterial toxins.

In general, the information available is considered inadequate for the calculation of a tolerable daily intake (TDI) for the majority of the cyanobacterial toxins (Chorus 1999). In particular, data are not available on metabolic disposition, acute and subacute toxicity, repeated administration, developmental effects, and carcinogenicity and genotoxicity. In such cases, a TDI can be derived using the lowest observed adverse effect level (LOAEL) or the no observed adverse effect level (NOAEL) from experimental animal data divided by appropriate safety and uncertainty factors, as described in the Addendum to the World Health Organization Guidelines for Drinking Water Quality (WHO 1988). A study by Fawell et al (1994, Chorus 1999) derived a NOAEL of 40 ug/kg body weight per day in a mice gavage study with a 1000 fold uncertainty factor (egg. intra-species, inter-species, limitations of database) resulting in a provisional TDI of 0.04 ug/kg body weight per day of microcystin LR. Falconer (1994a, 1994b) used the following 10 fold safety factors: use of subchronic data applied to lifetime risk, use of pig data applied to humans, use of intra-human variation, and tumor promotion risk; therefore he applied a 10,000 overall safety factor. He used subchronic exposure data in pigs that showed a lowest observed effect level of 280 ug/kg/day, and an assumption of 2 liters water intake per day by a 60 kg adult. This led him to a provisional TDI of 0.067ug/kg body weight per day. The WHO (1998) adopted a provisional guideline (TDI x body weight x proportion of total daily intake of the contaminant ingested from drinking water divided by the daily water intake in liters) for microcystin LR of 1.0 ug/L.

Special exposure circumstances (such as dialysis water) may necessitate even stricter control levels (Chorus 1999). Use of potentially contaminated water for irrigation is controversial since not only can the irrigation aerosol cause potential harm through skin and respiratory contact, but there is limited evidence that terrestrial plants, including food crops, can take up microcystins (MacKintosh 1990, Chorus 1999).

Barriers that reduce exposure to cyanobacterial blooms at “critical control points” are the first step in prevention, especially for surface drinking water sources (Chorus 1999). Of note, algacides, especially copper sulfate, can be added to water supplies to control toxic blooms, but acutely this leads to cell lysis and substantial release of the toxins into the water, as well as the possibility of copper toxicity, thus exacerbating the potential for health effects (Chorus 1999, Carmichael 1993, Falconer 1999, NHMRC 1994). Therefore, removal of intact cells is recommended (Chorus 1999). Activated carbon, chlorination and ozonation in conjunction with other water treatment practices have all been used in the treatment of drinking water supplies with potential blue green algal contamination (NHMRC 1994, Jones 1993, Chorus 1999). The use of activated carbon treatment during active blooms will decrease, but not necessarily eliminate, levels of cyanobacterial toxins in drinking water (Chorus 1999; J Burns SJRWMD, FL, verbal communication). This is of particular concern when the toxin is a potential carcinogen, since low level chronic exposure may

predispose to the development of cancer (Chorus 1999, Carmichael 1993, NHMRC 1994). Changing drinking water sources and technology to groundwater should be explored (Chorus 1999).

Finally, there is the possibility of exposure to these toxins through the consumption of contaminated food (Prepas 1997, Falconer 1992). In addition to the issue of terrestrial plant toxin uptake mentioned above, based on examination and experimentation of the gut contents of mussels during a bloom of *Microcystis* in Australia, Falconer et al (1992) concluded that edible mussels should not be collected for human consumption during a toxic blue green algal bloom.

Cyanobacteria (particularly *Spirulina*) have been used as food and alleged therapeutic agent in the US, Canada, Mexico, and India (NHMRC 1994, Chorus 1999). Although most cyanobacteria species are non toxic, the etiology and conditions for cyanobacteria toxin production are not well understood, nor have the health risks and benefits of long term consumption of cyanobacteria been studied. Therefore, monitoring for toxicity in cyanobacteria used purposely for human consumption is recommended.

Summary

In summary, blue green algal are ubiquitous in surface waters throughout the year in subtropical climates such as Florida, and they are associated with frequent toxic blooms. Both occupationally and recreationally humans can be exposed via dermal and aerosol routes, as well as through consumption of drinking water and possibly contaminated foods. The human health effects associated with the blue green algal toxins are predominantly by inference from their known health effects in a wide variety of organisms, especially neurotoxicity, hepatotoxicity and tumor promotion.

Short term and long term health effects have not been thoroughly evaluated in persons with occupational, recreational and consumption exposure to blue green algae and their toxins (NHMRC 1994). Current drinking water treatment practices in the US do not regularly monitor, or necessarily remove these toxins from the drinking water since this would involve extremely expensive measures (J Burns, SFWMD, verbal communication, Falconer 1989, Volterra 1993, Falconer 1999, Heinze 1999). Even with treatment, low level chronic exposure to the carcinogenic hepatotoxins are possible in persons consuming drinking water derived from surface water drinking plants in Florida and other parts of the US.

METHODS

The pilot study was an ecological study linking incident cases of primary liver cancer diagnosed Florida from 1981-1998 by their place of residence at the time of cancer diagnosis with environmental and geographic data on drinking water plants and sources in a GIS model.

Human Health Data

The Human Subjects Protocol was constructed and approved by the University of Miami School of Medicine Human Subjects Committee (Protocol #99/054) and by the Florida Dept of Health.

The study population was defined as all cases of primary hepatocellular carcinoma diagnosed in the state of Florida between 1981 and 1998 in Florida's incident cancer registry, the Florida Cancer Data System (FCDS) database. Because FCDS has been independently estimated to capture more than 95% of all incident cancer cases in Florida, this project was considered to be population-based. Hepatocellular carcinomas were defined as those cancer cases with the appropriate SEER ICD-0 diagnostic codes, as described below.

Cancer incidence data are submitted to FCDS from all hospitals, laboratories, ambulatory surgical centers, and radiation therapy centers in Florida. These data are subjected to verification and validation procedures before entry into the FCDS database. The International Classification of Diseases for Oncology, 2nd edition

(ICD-O-2), is used for coding the topology and morphology of tumors. The data set analyzed in this project was extracted using SQL queries of the FCDS Oracle database.

The operational definition for the dependent variable, “case of primary liver cancer”, was all records with the following ICD-0-2 codes:

- Hepatocellular carcinoma, NOS: ICD-O-2 code 8170;
- Hepatocellular carcinoma, fibrolamellar: ICD-O-2 code 8171;
- Combined hepatocellular carcinoma and cholangiocarcinoma: ICD-O-2 code 8180.

The request for the use of the Florida Cancer Data System (FCDS) database of hepatocellular cancer cases (anonymous with geocode) from 1981-1998 was approved by the Florida Dept of Health, and the file was constructed and received from the FCDS. All of these data were incorporated into the GIS database in tabular and ArcView shapefile formats.

Exposure Data

From Florida DEP and other websites, geo-referenced information (GPS) was acquired for the deep ground water wells, as well as the administrative addresses for their respective treatment plants. However, apparently due to data sensitivity issues, it was not possible to acquire the actual location of the deep ground water treatment plants in geographic coordinates, despite considerable efforts on the part of various colleagues (including HAB Taskforce members).

Drs John Burns and Chris Williams of SJRWMD provided geocoded addresses of the actual surface water treatment plants, as well as accurate hardcopy maps of the water distribution service areas from the 18 major surface water treatment plants in Florida. These maps were digitized using the UTM coordinate system (NAD 83) within ArcView GIS.

These data formed the base data layers within the GIS database.

GIS Methods

The geocoded data (residence at time of diagnosis) of all cases of hepatocellular carcinoma from the FCDS database included the following variables: age, date of birth, gender, and race-ethnic information. In addition, racial, ethnic, and socioeconomic data from 1990 were acquired from the U.S. Census bureau. This information was mapped above the base layers described in the previous section.

To compare surface water treatment areas to ground water treatment areas, the following GIS analyses were performed. The geographic center of the actual surface water distribution service area was used as the hypothetical location of the surface water treatment plant in lieu of the actual location of the plant due to the significant distance between the treatment plant and its corresponding service area. The immediate area surrounding the actual location of the surface water treatment plants was not an accurate representation of the population served by such plants. In other words, the treatment plants were far removed from their actual service areas (perhaps to avoid potential anthropogenic influence upon the surface water source).

The average size plus two standard deviations of the surface water service areas was determined and used to identify the standard area of influence for the initial analyses. A circular buffer with an area equal to the standard area of influence was created and applied to each hypothetical surface water treatment facility (Figure 1).

A hypothetical center for each ground water treatment plant was determined based on the average location of its corresponding wells. This was due to the absence of positional information for the groundwater treatment plants. This calculation was based on the assumption that the ground water wells can be located within the service area due to a minimal risk of anthropogenic influence. In addition, the service area boundaries for

the treatment facilities were also unknown. Therefore, the same circular buffer used for the surface water treatment facilities was applied to each ground water facility (Figure 2).

The geographic intersection between the 1990 Census block group data and the standard area of influence for each ground and surface water facility were determined. The demographic profile and population parameters for each area were calculated from this intersection and used as approximate denominator data (Figure 3). In an attempt to control for confounding bias, four (4) sets of eighteen (18) ground water study areas (controls) were randomly selected from groups matching the following characteristics:

1. Random;
2. Similar Median Income/Rent to Surface Water areas;
3. Similar Race-Ethnic Distribution to Surface Water Areas;
4. Similar Median Income/Rent and Race-Ethnic Distribution to Surface Water areas.

To compare populations contiguous to the surface water treatment service areas, the following 2 contiguous buffer analyses were performed. For both these analyses, it was assumed that individuals living within the surface water treatment areas would be exposed to contaminated water significantly more than those individuals living just outside of the service area. For both the actual and circular buffer analyses, it was assumed that the areas immediately surrounding the core areas would be similar in demographic characteristics and lifestyle patterns. These assumptions were the basis for creating contiguous buffers around the core domains whose areas were (approximately) equal to that of their individual representative surface treatment area cores (Figure 4).

In the first analysis, the actual service area of each surface treatment facilities was used to delineate the exposure domain. An unique contiguous actual buffer control area was determined for each surface treatment facility and applied within the geospatial information system to create contiguous buffer control areas approximating the actual surface water treatment service area in shape and area.

An additional evaluation was developed to determine whether or not the shape of the service area was a significant factor in the distribution of the disease, as well as in an attempt to create a more accurate buffer zone of the actual service area. A circular domain equal in area to the service boundary of its representative treatment facility was created. As with the first analysis using the actual service area of the surface water treatment facility, the representative circular areas were identified as core areas where exposure to contaminated water was expected to be highest while their contiguous circular buffer control areas were expected to contain persons similar with regards to confounding variables but without exposure to surface water. The circular buffers were readily created using the following equation:

$$\Pi r_{\text{large}}^2 - \Pi r_{\text{core}}^2 = \text{Area}_{\text{core}} = \Pi r_{\text{core}}^2$$

$$\Pi r_{\text{large}}^2 = 2 \Pi r_{\text{core}}^2$$

$$r_{\text{large}}^2 = 2 r_{\text{core}}^2$$

$$r_{\text{large}} = \sqrt{(2 r_{\text{core}}^2)}$$

The result of this computation was a circular area with a radius equal to r_{large} and an area twice that of the original core area. Thus, the buffer radius for each circular core area could be calculated by subtracting the radius of the circular core area from the radius of the larger area. A unique buffer radius was determined for each surface treatment facility and applied within the geospatial information system to create circular buffers whose were equal to its core (Figure 5).

As would be expected, there were no geographic overlaps between the actual surface water treatment plants. Where potential overlaps of either circular area or the contiguous circular buffer or the contiguous actual

buffers occurred, the GIS selected the first area mapped such that there were no double counting of cancer cases or underlying population in the analysis. Of note, the GIS created geographic centers of the 18 surface treatment plants remain the same throughout the analyses. However as noted below, the circumscribed denominator populations (and cancer cases) change dramatically from analysis to analysis, therefore comparison between the absolute incidence rates of the different analyses for the 18 surface water treatment sites would be inappropriate.

Statistical Methods

Throughout the analyses, cumulative incidence rates were calculated to allow for comparisons between the different geographic spaces modeled in the GIS.

The hepatocellular carcinoma cases from 1981 through 1998 were spatially joined to their corresponding service area; initial cumulative incidence rates (and confidence intervals) were calculated for each of the Surface Water Areas and for the randomly selected Ground Water Areas for the entire relevant population. In addition, the initial pooled hepatocellular cumulative cancer incidence rates for all study groups (surface, and ground water areas) were also calculated. A similar process was repeated for the 2 contiguous buffered area analyses. Results were expressed as age-standardized incidence rates using the 1970 US standard million population and Florida population projections (1990). Age-adjusted rates were calculated based on the 1990 Census age groups. The standard errors for the age-adjusted rates were calculated after the method of Breslow and Day (1982)(Howe 1996).

The age specific rate per hundred thousand, S (i,t), for age group i over t years was calculated by the following:

$$S(i,t) = \frac{d(i,t)}{p(i,t)} \times 10^6$$

where d (i,t) is the incidence in age group i over t years, p(i,t) is the population in group i over t years, t is the number of years observed, and i is a particular age group in 1990 Census age distribution intervals.

The age-adjusted rate per hundred thousand A(t), over t years was calculated by the following:

$$A(t) = \sum_{i=1}^n \frac{S(i,t) \times P(i)}{\sum_{i=1}^n P(i)}$$

where P(i) = the number in age group i in the standard population and all others as above. The standard error (S.E.) of the age-adjusted rates was calculated as a standard Poisson variation, as follows:

$$S.E. = \sqrt{v}$$

where the variant “v” was calculated according to the method of Breslow and Day (1982)(Howe 1996):

$$v = \sum_{i=1}^n [S(i,t) / p(i,t)] \times w(i)$$

with the weight “w” calculated:

$$w = \frac{1}{n}$$

$$w(i) = [P(i) / \sum_{i=1} P(i)]^2$$

For the individual cumulative incidence rates, the Mann Whitney Rank Sum Test was used to compare the Surface Water Treatment Areas to the different comparison groups of the Ground Water Areas and to the contiguous buffered areas (Glantz 1987). The incidence rates for the pooled Surface Treatment Areas were compared to the rates for the pooled Ground Water Areas and the pooled contiguous buffered areas, and expressed as Standardized Rate Ratios (SRR) with 95% confidence intervals (Breslow 1982, Howe 1996).

The data were analyzed using proprietary programs of the FCDS and Microsoft Excel 97 software, and ESRI ArcView GIS Version 3.2 software.

RESULTS

There were 4741 incident cases of hepatocellular carcinoma from 1981 through 1998 in Florida, with an age adjusted cumulative incidence rate for HCC of 1.41/100,000 (SE 0.0003) (Figure 6). There were 18 Surface Water Treatment Areas (see Figure 7).

Surface vs Ground Water Analyses

There were 6614 Ground Water Well Areas (see Figure 7). For the first set of analyses comparing the Study Surface Water Treatment Areas to the Ground Water Control Areas, there were a total of 461 cases of hepatocellular carcinoma residing at the time of cancer diagnosis in the 18 Surface Water Treatment Areas combined. The individual Surface Water Treatment Area cases ranged from 0 cases of hepatocellular carcinoma in 3 areas to 113 cases, and the individual cumulative age-adjusted cancer rates for hepatocellular carcinoma ranged from 0 (due to the lack of hepatocellular carcinoma cases in 3 areas) to 3.08/100,000 (SE 0.0188) based on 5 cases of cancer. The pooled Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 1.13 (SE 0.00075) for 1981-1998. As a comparison, the cumulative age adjusted cancer rate of hepatocellular carcinoma for all of Florida for the same time period was greater at 1.41/100,000 (Table 1).

There were 1249 total study created ground water treatment areas after the GIS manipulations described above. The results of the random selection from pooling of the Ground Water Areas matched with the Surface Water Areas based on median income and median monthly rent, and race/ethnic distribution for the 4 control Ground Water Areas are displayed in Table 2 and Figure 8. **Control 1** was simply a random sample of 18 areas from a pool of 1249 ground water treatment plants. **Control 2** was a random sample of 18 areas from a pool of 1176 ground water treatment plants selected based on median income (\$14,750.36-\$132,934.8) and median monthly rent (\$241.58-\$619.68). **Control 3** was a random sample of 18 areas from a pool of 1246 ground water treatment plants selected based on percent Black (0-66.39%) and percent Hispanic (0-43.68%). **Control 4** was a random sample of 18 areas from a pool of 1174 ground water treatment plants selected based on the requirements for both Control 2 and Control 3. There was potential overlap between the different control groups.

The Mann Whitney Rank Sum Test as applied to the individual incidence rates for the 18 surface water areas in comparison with the incidence rates of the 4 different groups of randomly selected matched ground water treatment control areas (Table 3). There were no significant differences (at $p < 0.05$) between the incidence rates by the Rank Test. Therefore, in this analysis, the risk of having hepatocellular carcinoma was not statistically different between persons living in the study surface water areas and persons living in the control ground water areas.

As noted above, the pooled Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 1.13 (SE 0.00075) for 1981-1998; all of the control ground water area pooled

cumulative age-adjusted cancer rate for hepatocellular carcinoma were greater, ranging from 1.15-1.41/100,000 for 1981-1988. The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled Surface Water Treatment Areas vs pooled Ground Water Treatment Control Areas ranged from 0.80 to 0.98 with statistically significant 95% confidence intervals (Table 4). The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled Surface Water Treatment Areas vs general Florida population was 0.80 with statistically significant 95% confidence intervals (Table 4). Therefore, in this analysis, the risk of having hepatocellular carcinoma was statistically significantly less for all persons living in the study surface water areas compared with all persons living in the 4 groups of study created control ground water treatment areas and compared to the general Florida population.

Contiguous Buffer Analyses

As stated above, for both the actual and circular buffer analyses, it was assumed that the areas immediately surrounding the core areas would be similar in demographic characteristics and lifestyle patterns. For the contiguous circular buffer control analysis, this assumption was the basis for creating buffers around the core domains whose areas were equal to that of their representative cores. However, this same technique was applied to the actual service areas with less than fair results. Although the circular buffers were not exactly the same size as their core counterparts, the differences in area can be explained by the approximation of π (see table 5). The inability to extend the precision of this constant to more decimal places (20+) resulted in differences in the calculated areas, particularly in the case of the actual contiguous buffer control areas. Furthermore, this circular buffer technique did not apply very well to the polygonal service areas due to the irregularities encountered in their shapes resulting in possible misclassification of surface water exposed cases being included in the non surface water contiguous circular buffer control areas (see Figure 9). Nevertheless, both these contiguous buffer techniques created comparison populations with similar race-ethnic and socio-economic backgrounds (Table 6).

For the analyses comparing the actual surface Water Treatment Areas to their contiguous buffered control areas, there were a total of 335 cases of hepatocellular carcinoma residing at the time of cancer diagnosis in the 18 actual Surface Water Treatment Areas combined. The individual Surface Water Treatment Area cases ranged from 0 cases of hepatocellular carcinoma in 4 areas to 103 cases, and the individual cumulative age-adjusted cancer rates for hepatocellular carcinoma ranged from 0 (due to the lack of hepatocellular carcinoma cases in 4 areas) to 4.88/100,000 based on 3 cases of cancer. The pooled actual Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 1.15 (SE 0.0009) for 1981-1998; the pooled contiguous actual buffer control zone cumulative age-adjusted cancer rate for hepatocellular carcinoma was 0.83 (SE 0.0011) for 1981-1998. As a comparison, the cumulative age adjusted cancer rate of hepatocellular carcinoma for all of Florida for the same time period was greater at 1.41/100,000 (Table 7).

For the actual service areas with their buffers, the Mann Whitney Rank Sum Test as applied to the individual incidence rates for the 18 actual surface water areas in comparison with the incidence rates of the contiguous buffer control group (Table 8). There was a significant difference (i.e. $p < 0.02$ between the incidence rates by the Rank Test. Therefore, in this analysis, the risk of having hepatocellular carcinoma was significantly higher for persons living in the actual surface water areas compared to persons living in actual contiguous presumably unexposed buffer control areas.

As noted above, the pooled actual Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 1.15 (SE 0.0009) for 1981-1998. The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled actual Surface Water Treatment Areas vs the pooled contiguous buffer control area was 1.39 with statistically significant 95% confidence intervals (Table 9). The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled actual Surface Water Treatment Areas vs general Florida population was 0.82 with statistically significant 95% confidence intervals (Table 9). Therefore, in this analysis, the risk of having hepatocellular carcinoma was statistically significantly greater for all persons living in the actual surface water area compared with all persons living in the actual contiguous buffered area, but statistically significantly less compared to the general Florida population.

For the analyses comparing the circular surface Water Treatment Areas to their contiguous buffered control areas, there were a total of 299 cases of hepatocellular carcinoma in the 18 circular Surface Water Treatment Areas combined. The individual Surface Water Treatment Area cases ranged from 0 cases of hepatocellular carcinoma in 4 areas to 99 cases, and the individual cumulative age-adjusted cancer rates for hepatocellular carcinoma ranged from 0 (due to the lack of hepatocellular carcinoma cases in 4 areas) to 2.73/100,000 based on 4 cases of cancer. The pooled circular Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 0.99 (SE 0.0017) for 1981-1998; the pooled contiguous circular buffer zone cumulative age-adjusted cancer rate for hepatocellular carcinoma was 1.15 (SE 0.0014) for 1981-1998. As a comparison, the cumulative age adjusted cancer rate of hepatocellular carcinoma for all of Florida for the same time period was greater at 1.41/100,000 (Table 7).

For the actual service areas with their buffers, the Mann Whitney Rank Sum Test as applied to the individual incidence rates for the 18 actual surface water areas in comparison with the incidence rates of the contiguous buffer control group (Table 8). There was no significant difference ($p < 0.05$) between the incidence rates by the Rank Test. Therefore, in this analysis, the risk of having hepatocellular carcinoma was not statistically different between persons living in the circular surface water areas and persons living in contiguous presumably unexposed buffer control areas.

As noted above, the pooled actual Surface Water Treatment Area cumulative age-adjusted cancer rate for hepatocellular carcinoma was 0.99 (SE 0.0017) for 1981-1998. The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled actual Surface Water Treatment Areas vs the pooled contiguous buffer control area was 0.85 with statistically significant 95% confidence intervals (Table 9). The Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled circular Surface Water Treatment Areas vs general Florida population was 0.70 with statistically significant 95% confidence intervals (Table 9). Therefore, in this analysis, the risk of having hepatocellular carcinoma was statistically significantly less for all persons living in the circular surface water area compared with all persons living in the circular contiguous buffered area as well as compared to the general Florida population.

CONCLUSIONS

In this pilot ecological study using a GIS analysis, the risk of hepatocellular carcinoma was significantly associated with residence at the time of diagnosis in a Surface Water Treatment Area of Distribution when comparing actual service areas to GIS created actual contiguous buffer control areas. However, additional analyses comparisons with the general Florida population, with circular buffer zones and with randomly selected study-generated ground water did not find an elevated association between risk of HCC and presumed exposure to surface drinking water. The majority of the individual cumulative incidence rates and the cumulative pooled incidence rate for hepatocellular carcinoma for Surface Water Treatment Plants were less than the cumulative incidence rate for hepatocellular carcinoma of all of the selected Ground Water Area comparison groups and for the Florida Population as a whole during the same time period.

The NIEHS Center/University of Miami Investigators have provided a ranking of the initial cumulative incidence rates for hepatocellular carcinoma from 1981-1998 around Surface Water Distribution areas to the SJRWMD Investigators to direct their water sampling and testing efforts. This testing will involve pre/post treatment testing during active algal blooms of a subsample of Surface Water Treatment plants; these data will be incorporated into the GIS analysis. In addition, Dr John Burns of SJRWMD traveled to Australia to meet with scientific colleagues concerning the testing, treatment and epidemiologic study of the blue green algae and their toxins; he will communicate his findings to the Harmful Algal Bloom Taskforce (**Appendix**).

Study Limitations

This study was an ecological study of the possible association between primary liver cancer and location of surface drinking water plants. As an ecologic study, this study can only be considered to be hypothesis-

generating; it can not prove or disprove an etiological association. The Investigators note that the limitations of this study described below must be considered in its interpretation regardless of the results.

With regards to exposure, this study assumed that the place of residence at the time of cancer diagnosis was the same place of residence at the time of cancer initiation; this was a major assumption given the significant mobility of the Florida population and the potential 15-25 year latency from exposure to disease diagnosis of primary liver cancer. Furthermore, the use of the exposure measure of proximity of residence at the time of cancer diagnosis to drinking water treatment plants was only a surrogate for exposure. It is possible that persons could have lived close to a drinking water treatment plant and not necessarily utilized the treatment plant as their major source of drinking water (egg. Individual well water and bottled water). Furthermore, it is not known if treated surface drinking water contaminated with blue green algal toxins actually reached the tap consumer, nor if it was a constant or more likely intermittent exposure. In addition, due to the lack of geocoded data for the Ground Water treatment plants, assumptions were made in the GIS modeling with regards to the hypothesized location of the ground water treatment facilities. In future analyses, it would be important to obtain and incorporate the correct geographic position of the ground water treatment plants.

With regards to disease and population, the statistical analyses were limited by the relatively small number of cases for each individual treatment area despite the long time period. The use of the 1990 Census data for the population denominator assumed relative stability of the population over the 18 year period from 1981-1998. Furthermore, the use of 1990 Census data even on the Census Block level did not eliminate possible confounding from race/ethnic or socio economic class and other confounding variables.

In addition to those limitations already mentioned, with regards to the comparison of study created surface and ground water treatment areas, there were a number of assumptions and possible biases. The use of a large radius could have lead to misclassification of cancer cases to include unexposed as well as exposed cases for the surface treatment areas, biasing the risk towards the null (Figure 3). This was not as important an issue for the study created ground water treatment areas since they were selected not to overlap with the study-created surface water treatment areas.

Finally with regards to the contiguous buffer analyses, in addition to small numbers of cases, there were several limitations (as well as assumptions). It was assumed that populations living contiguous to, but outside of, surface water treatment areas would not be exposed to surface water, but would be similar in terms of confounding variables. In particular for the actual contiguous control buffer, it was difficult using GIS to create an area equal to the actual surface treatment area (Table 5). However, the use of rates rather than cancer cases for comparison purposes should eliminate this bias (except in situations where there were small numbers of cancer cases in a small geographic area). For the circular contiguous buffer control analysis, there was the possibility of misclassification of exposed cancer cases into non exposed cases (Figure 9), leading to a bias towards the null of risk. Therefore, in the context of the above described limitations, the use of the actual surface water treatment distribution areas should be expected to give the most accurate evaluation of HCC risk for persons possibly exposed to surface drinking water.

RECOMMENDATIONS

This pilot study did show an association between possible exposure to surface drinking water and the risk of hepatocellular carcinoma in Florida, although not in comparison to ground water or the general Florida population. Although only a hypothesis generating study, the exposures and possible health effects (both acute and chronic) of the blue green algae need to be evaluated more extensively in Florida, particularly given the results of the St John River Water Management Cyanobacterial Survey (SJRWMD 2000).

Future studies should focus on establishing the actual exposure at the tap of consumers by sampling of tap water supplied by a surface water treatment plant during a toxic bloom. In addition, more acute symptoms

and health effects (such as liver enzyme elevations) should be evaluated in those persons with established exposure, and this cohort of exposed and possibly effected persons should be followed over time to evaluate exposure and chronic health effects. Other confounding causes of both liver damage and HCC should be evaluated, such as ethanol, solvent exposure, aflatoxins, and viral hepatitis B and C. With regards to the specific issue of hepatocellular carcinoma, although a cancer associated with a high mortality, a case control study of persons with hepatocellular carcinoma compared to control persons with out this cancer to evaluate their lifetime history of drinking, occupational and recreational surface water exposure (as well as confounding information such as viral hepatitis and alcohol consumption) would be of interest, particularly among persons who have lived in potentially high risk areas such as Florida. Evaluation of tumor tissue and other body fluids from persons with hepatocellular carcinoma for microcystin exposure, as well as confounders such as aflatoxins and viral hepatitis, could also be performed.

Finally, in addition to exposure monitoring and health surveillance for the blue green algae and their toxins, prevention of both the exposure and potential health effects needs to be a primary focus of Florida's scientific and public health community involved in the study and prevention of the harmful algal blooms.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding of the Florida Marine Research Institute (FMRI) and the Florida Harmful Algal Bloom Taskforce for this study. The authors received considerable scientific support for this study from John Stinn MMA, Kathleen Shea MPH, Alan Rowan MS, Chris Williams MS, and Steven Wiersma MD MPH, as well as members of the Harmful Algal Bloom Taskforce and the St Johns River Water Management District. Ms. Gayl van de Bogart and Ms. Jill Tincher provided significant administrative support.

These data were presented preliminarily at the May 9, 2000 Meeting of the HAB Taskforce at the Florida Marine Research Institute in St Petersburg, Florida. In addition, these data were presented at the Annual Meeting of the North American Association of Central Cancer Registries (New Orleans, April 2000), the Annual Florida Epidemiology Meeting (Gainesville, FL in August 2000), the Annual Florida Cancer Data System Meeting (Melbourne, FL in August 2000), the Annual National Institute of Environmental Health Sciences Director's Meeting (Detroit, MI in October 2000), and NALMS Conference (Miami, FL in November 2000).

REFERENCES

American Cancer Society (ACS). The liver cancer resource center: liver cancer overview [monograph online] 1998. <http://www3.cancer.org/cancerinfo/documents/overviews/liveover.aspect=25>. (11 Jun 98).

Anon. Outbreaks of diarrheal illness associated with cyanobacteria (blue green algae) like bodies – Chicago and Nepal, 1989 and 1990. *MMWR* 1991;40:325-327.

Astrachan NB, Archer BG, Hilbelink DR. Evaluation of the subacute toxicity and teratogenicity of anatoxin a. *Toxicon* 1980;18:684-688.

Bartram J, Rees G. *Recreational Water Monitoring*. London: E & FN Spon, 1999.

Baxter PJ. Toxic marine and freshwater algae: an occupational hazard? *Br J Ind Med* 1991;49:505-506.

Beasley VR, Dahlem AM, Cook WO et al. Diagnostic and clinically important aspects of cyanobacterial (blue green algae) toxicosis. *J Vet Diagn Invest* 1989;1:359-365.

Billings WH. Water associated human illness in Northeast Pennsylvania and its suspected association with blue green algae blooms. In: Carmichael WW Ed. *The Water Environment: Algal Toxins and Health*. New York: Plenum Press, 1981, pgs 243-255.

Bourke ATC, Hawes RB, Nielson A, Stallman ND. An outbreak of hepatoenteritis (the Palm Island mystery disease) possibly caused by algal intoxication [abstract]. *Toxicon Suppl* 1983;45-48.

Breslow NE, Day NE. *Statistical methods in cancer research*. Vol.2. Lyon, France: IARC Statistical Publications, 1982: 59.

Burch MD. The development of an alert levels and response framework for the management of blue green algal blooms. In: *Blue Green Algal Blooms: New Developments in Research and Management*. A symposium convened by the Australian Center for Water Quality Research and the University of Adelaide, 17th February 1993, Adelaide, SA (quoted pg 74 in NHMRC 1994 Report).

Bulterys M, Goodman MT, Smith MA, Buckley JD. Hepatic tumors. In: Ries LAG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL et al (editors). *Cancer Incidence and Survival Among Children and Adolescents: United States SEER Program 1975-1995*, 1999. Bethesda, MD: National Cancer Institute, SEER Program. NIH Pub. No. 99-4649.

Carbis CR, Waldron DL, Mitchell GF, Anderson JW, McCauley I. Recovery of hepatic function and latent mortalities in sheep exposed to the blue green alga *Microcystis aeruginosa*. *Veterinary Record* 1995;137:12-15.

Carmichael WW, Falconer IR. Diseases related to freshwater blue green algal toxins, and control measures. In: IR Falconer, ed. *Algal Toxins in Seafood and Drinking Water*. Academic Press, 1993, pgs 187-209.

Carmichael WW. The toxins of cyanobacteria. *Scientific Am Jan* 1994:78-86.

Chorus I, Bartram J, Eds. *Toxic Cyanobacteria in Water: a Guide to their Public Health Consequences, Monitoring and Management*. London: E & FN Spon, 1999.

Codd GA, Ward CJ, Bell SG. Cyanobacterial toxins: Occurrence, modes of action, health effects and exposure routes. *Arch Tox Supp* 1997;19:399-410.

Codd GA. Toxins of freshwater cyanobacteria. *Microbiol Sci* 1984;1:48-52.

El Saadi O, Esterman AJ, Cameron S, Roder DM. Murray River water, raised cyanobacterial cell counts, and gastrointestinal and dermatological symptoms. *Med J Australia* 1995;162:122-125.

El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; 340: 745-750.

Elder GH, Hunter PR, Codd GA. Hazardous freshwater cyanobacteria (blue green algae). *Lancet* 1993;341:1519-1520.

Falconer IR, Beresford AM, Runnegar MTC. Evidence of liver damage by toxin from a bloom of blue green alga, *Microcystis aeruginosa*. *Med J Australia* 1983;1:511-514.

Falconer IR, Burch MD, Steffensen DA, Choice M, Coverdale OR. Toxicity of the blue green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. *Env Tox Water Quality* 1994b;9:131-139.

Falconer IR, Choice A, Hosja W. Toxicity of edible mussels (*Mytilus edulis*) growing naturally in an estuary during a water bloom of the blue green alga *Nodularia spumigena*. *Env Tox Water Quality* 1992;7:119-123.

Falconer IR, Humpage AR. Tumor promotion by cyanobacterial toxins. *Phycologia* 1996;35(6 supplement):74-79.

Falconer IR. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Env Toxicol* 1999;14:5-12.

Falconer IR. Effects on human health of some toxic cyanobacteria (blue green algae) in reservoirs, lakes and rivers. *Tox Assessment* 1989;4:175-184.

Falconer IR. Health problems from exposure to cyanobacteria and proposed safety guidelines for drinking and recreational water. *Royal Society of Chemistry* 1994a; 149:3-10. (from the 1st International symposium on detection methods for cyanobacterial (blue-green algal) toxins – 1993 Sep: Bath)

Fawell JK, James HA. Report FR 043/DoE 3728. Allen House, The Listons, Liston Road, Marlow, Bucks, SL7 1FD, UK, 1994.

Fijiki H, Sukanuma M, Hakii H et al. A two stage mouse skin carcinogenesis study of lyngbyatoxin A. *J Cancer Res* 1984;108:174-176.

Glantz SA. *Primer of Biostatistics*. New York.: McGraw Hill Inc, 1987.

Hale D, Aldeen W, Carroll K. Diarrhea associated with cyanobacterial like bodies in an immunocompetent host. *JAMA* 1994;271:144-145.

Hashimoto Y, Kamiya H, Yamazato K, Nozawa K. Occurrence of a toxic blue green alga inducing skin dermatitis in Okinawa. *Proceedings of the 4th International Symposium on Animal, Plant and Microbial Toxins*, Tokyo: 1974:333-338.

Heinze R. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. *Env Toxicol* 1999;14:57-60.

Hermansky SJ, Stohs SJ, Eldeen ZM et al. Evaluation of potential chemoprotectants against microcystin LR hepatotoxicity in mice. 1991;11(1):65-74.

Hong-Bing W, Hui-Gang Z. Promoting activity of microcystins extracted from water blooms in SHE cell transformation assay. *Biomed Env Sci* 1996;9:46-51.

Howe, H.L., Lehnherr, M., Derrick, L, editors. *Cancer incidence in North America, 1988-1992*. Sacramento, CA: North American Association of Central Cancer Registries; 1996.

Humpage AR, Falconer IR. Microcystin-LR and liver tumor promotion: effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. *Env Toxicol* 1999;14:61-75.

Islan MS, Drasar BS, Sak RB. Probable role of blue green algae in maintaining endemicity and seasonality of cholera in Bangladesh: a hypothesis. *J Diarrh Dis Res* 1994;12:245-256.

Ito E, Kondo F, Terao K, Harada K-I. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin LR. *Toxicon* 1997;35(9):1453-1457.

Jalaludin B, Smith W. Blue green algae (cyanobacteria). *Med J Australia* 1992;156:744.

Jochimsen EM, Carmichael WW, Jisi A et al. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *NEJM* 1998;338:873-888.

Jones GJ, Burch M, Falconer IR, Craig K. Cyanobacterial toxicity. In: *Technical Advisory Group Report, Algal Management Strategy*. Murray-Darling Basin Commission, Canberra, Australia, 1993, pgs 17-32.

Junshi C, Campbell TC, Junyao L, Peto R. *Diet, Life Style and Mortality in China*. Oxford, England: Oxford University Press, 1990.

Lipp EC, Erb J. Gastrointestinal illness at Sewikley PA. *J AM Water Works Assoc* 1976;68:606-610.

London WT, McGlynn KA. Liver cancer. In: Schottenfeld D, Fraumeni JF. *Cancer epidemiology and prevention*. 5th ed. New York: Oxford University Press, 1996:772-793.

MacKintosh C, Beattie KA, Klumpp S, Cohen P, Codd GA. Cyanobacterial microcystin LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *Fed Euro Biochem Soc Let* 1990;264(2):187-192.

Mahmood NA, Carmichael WW, Pfahler D. Anticholinesterase poisonings in dogs from a cyanobacterial (blue green algae) bloom dominated by *Anabaena flos-aquae*. *Am J Vet Res* 1988;49(4):500-503.

Martin P. Hepatocellular carcinoma: risk factors and natural history. *Liver Transpl Surg*. 1998 Sep;4(5 Suppl 1):S87-91.

Mereish KA, Solow R. Interaction of microcystin LR with SuperChar: water decontamination and therapy. 1989;27(4&5): 271-280.

Miller BA, Kolonel LN, Bernstein L, Young, Jr. JL, Swanson GM, West D, et al. (eds.). *Racial/Ethnic Patterns of Cancer in the United States 1988-1992*. Bethesda, MD: National Cancer Institute;1996. NIH Pub. No. 96-4104.

Nagata S, Soutome H, Tsutsumi T et al. Novel monoclonal antibodies against microcystin and their protective activity for hepatotoxicity. *Natural Tox* 1995;3:78-86.

National Health and Medical Research Council (NHMRC). *Health Effects of Toxic Cyanobacteria (Blue Green Algae)*. Canberra, Australia: Australian Government Publishing Service, 1994.

Negri AP, Jones GJ, Hindmarsh M. Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium *Anabaena circinalis*. *Toxicon* 1995;33(10):1321-1329.

Nishikawa-Matushima R, Ohta T, Nishiwaki S et al. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin LR. *J Cancer Res Clin Oncol* 1992;118:420-424.

Ohtani L, Moore RE, Runnegar MTC. Cylindrospermopsin: a potent hepatotoxin from the blue green alga *Cylindrospermopsis raciborskii*. *J Am Chem Soc* 1992;114(20):7941-7942.

Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999a; 80: 827-841.

Parkin DM, Pisani P, Munoz N, Ferlay J. The global health burden of infection associated cancers. *Cancer Surv* 1999b; 33:5-33.

Philipp R, Rowland MGM, Baxter PJ, McKenzie C, Bell RH. Health risks from exposure to algae. *CDR (London: England Rev)* 1991;1:R67-68.

Pilotto LS, Douglas RM, Burch MD et al. Health effects of exposure to cyanobacteria (blue green algae) during recreational water activities. *Australian N Zealand J Pub Health* 1997;21:562-566.

Pilotto LS, Kliewer EV, Davies RD, Burch MD, Attewell RG. Cyanobacterial (blue green algae) contamination in drinking water and perinatal outcomes. *Australian New Zealand J Pub Health* 1999;23:154-158.

Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 major cancers in 1990. *Int J Cancer* 1999; 83:18-29.

Prepas EE, Kotak BG, Campbell LM et al. Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam *Anodonta grandis simpsoniana*. *Can J Fish Aquat Sci* 1997;54:41-46.

Probert CSJ, Robinson RJ, Jayanthi V, Mayberry JF. Microcystin hepatitis. *Arch Gastroenterol* 1995;32:199.

Rapala J, Sivonen K, Lyra C, Niemela SI. Variation of microcystin, cyanobacterial hepatotoxins, in *Anabaena* spp as a function of growth stimulation. *App Env Microbiol* 1997;63:2206-2212.

Repavich W, Sonzogni WC, Standridge JH, Wedepohl RE, Meisner LE. Cyanobacteria (blue green algae) in Wisconsin waters: acute and chronic toxicity. *Water Res* 1990;24(2):225-231.

Shea K. *Hepatocellular Carcinoma in Florida*. Masters Project in Public Health, University of Miami School of Medicine, 2000.

Soave R, Dube JP, Ramos LJ, Tummings M. A new intestinal pathogen [abstract]? Clin Res 1986;34:533A.

St Johns River Water Management District (SJRWMD). Cyanobacteria Survey Project. SJRWMD. Jacksonville, FL: 2000.

Sugimara T. Studies on environmental chemical carcinogenesis in Japan. Science 1986;233:312-318.

Teixera MGLC, Costa MCN, Carvalho VLP, Pereira MS, Hage E. Gastroenteritis epidemic in the area of the Itaparica Dam, Bahia, Brazil. Bulletin PAHO 1993;27:244-253.

Tisdale J. Epidemic of intestinal disorders in Charleston (West Virginia) occurring simultaneously with unprecedented water supply conditions. Am J Pub Health 1931;21:198-200.

Turner PC, Gammie AJ, Hollinrake K, Codd GA. Pneumonia associated with contact with cyanobacteria. Br Med J 1990;300:1440-1441.

Ueno Y, Nagat S, Tsutsumi T et al. Detection of microcystins, a blue green algal hepatotoxin, in drinking water sampled in Hiamen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogen 1996;17:1317-1321.

Veldee MV. An epidemiological study of suspected water-borne gastroenteritis. Am J Publ Health 1931;21(9):1227-1235.

Volterra L. Sanitary implications associated with the use of eutrophic freshwater. Ann Inst Superiore di Sanita 1993;29:327-333.

World Health Organization (WHO). Guidelines for Drinking Water Quality. Geneva: WHO, 1988.

Yu S-J. Primary prevention of hepatocellular carcinoma. J Gastroenterol Hepatol 1995;10:674-682.

Yu S-Z, Chen Z-Q, Liu Y-K, Huang Z-Y, Zhao Y-F. The aflatoxins and contaminated water in the etiological study of primary liver cancer. In: Natori S, Hashimoto K, Ueno Y eds. Mycotoxins & Phycotoxins #88. Amsterdam: Elsevier, 1989a, pgs 37-44.

Yu S-Z. Drinking water and primary liver cancer. In: Tang Z-Y, Wu M-C, Xia S-S (eds). Primary Liver Cancer. Berlin: Spring Verlag, 1989b, pgs 30-37.

TABLES

Table 1. Age Adjusted Incidence Rates for Liver Cancer (Hepatocellular Carcinoma) around study created Florida Surface Water and Ground Water Treatment Plants

Table 2. Population Characteristics (1990 Census) for study created Surface Water Treatment Areas vs Ground Water Treatment Control Areas

Table 3. Mann Whitney Rank Sum Test of Incidence Rates for Study Surface Water Areas vs for Ground Water Control Areas

Table 4. Pooled Age Adjusted Incidence Rates and Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled study Surface Water Treatment Areas vs pooled Ground Water Treatment Control Areas

Table 5. Differences in area (square meters) for each of the spatial domains in the contiguous buffer analysis

Table 6. Population Characteristics (1990 Census) for Actual Surface Water Treatment Areas and Contiguous Control Buffer Areas

Table 7. Age Adjusted Incidence Rates for Liver Cancer (Hepatocellular Carcinoma) around actual and circular Florida Surface Water Treatment Plants compared to Contiguous Buffer Controls areas

Table 8. Mann Whitney Rank Sum Test of Incidence Rates for Actual Surface Water Areas vs for Contiguous Buffered Control Areas

Table 9. Pooled Age Adjusted Incidence Rates and Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled Surface Water Treatment Areas vs pooled Contiguous Buffered Control Areas

FIGURES

Figure 1. Example of Surface Water Treatment Plants locations and Actual Service Area Boundaries with Study Surface Water Treatment Plants and Study Areas of Influence

Figure 2. Example of Actual Ground Water Wells with Study Ground Water Treatment Plant and Study Area of Influence

Figure 3. Example of Cancer Cases, Surface Water Treatment Plant locations and Actual Service Area Boundaries with Study Surface Water Treatment Plants and Study Areas of Influence

Figure 4. Example of Actual Surface Treatment Areas with actual Contiguous Buffer Control Area Approximations

Figure 5. Example of Circular Surface Treatment Areas with Circular Contiguous Buffer Approximations

Figure 6. Hepatocellular Carcinoma Cases in Florida (1981-1998) with 1990 Census Block Population Density Background

Figure 7. Location of Study Surface Water Treatment Areas and Study Ground Water Well Areas

Figure 8. Sites of 18 Study Surface Water Areas and 4 sets of 18 Randomly Selected Matched Ground Water Control Areas

Figure 9. Example of both Actual and Circular Surface Water Treatment Areas and their Contiguous Actual and Circular Buffer Control Approximations, with HCC patients

Table 1. Age Adjusted Incidence Rates for Liver Cancer (Hepatocellular Carcinoma) around study created Florida Surface Water and Ground Water Treatment Plants

Area Number First Analysis	Surface Water Cancer Cases	Surface Water Rates+	Control 1 Cases	Control 1 rates+	Control 2 Cases	Control 2 rates+	Control 3 cases	Control 3 rates+	Control 4 cases	Control 4 rates+
1	9	2.13	4	0.87	23	0.86	124	0.99	6	1.21
2	26	1.23	54	1.23	8	0.88	16	0.73	52	0.86
3	95	1.01	7	1.22	15	1.11	40	1.67	10	1.33
4	46	0.97	6	0.77	6	1.67	35	1.38	21	1.14
5	66	1.02	9	0.60	50	1.20	307	1.70	18	1.41
6	17	1.93	14	0.76	258	1.66	3	1.48	39	1.18
7	20	0.69	8	1.22	72	1.71	18	1.27	22	2.44
8	26	1.17	45	1.61	29	0.87	140	1.33	1	0.41
9	7	0.75	7	0.92	102	1.43	17	1.32	23	1.61
10	10	1.43	7	1.54	29	1.37	21	1.91	27	1.36
11	113	1.44	19	1.28	2	0.71	0	0	10	1.28
12	18	1.54	2	4.85	0	0	0	0	0	0
13	0	0	79	1.45	51	1.81	24	1.29	4	0.60
14	1	0.48	4	0.85	1	0.94	7	1.42	4	0.86
15	5	3.08	14	0.81	2	0.78	98	2.08	4	1.43
16	0	0	0	0	10	0.80	41	2.11	5	1.20
17	3	0.75	0	0	2	1.95	10	0.80	2	0.46
18	0	0	0	0	0	0	0	0	14	1.17
Overall	461	1.13	280	1.20	660	1.41	901	1.40	262	1.15

+Age Adjusted Rates = number of HCC cases/100,000 (1981-1998)

Bold=>State of Florida Age Adjusted Cumulative Cancer Rate (1981-1998) = 1.41/100,000

Table 2. Population Characteristics (1990 Census) for study created Surface Water Treatment Areas vs Ground Water Treatment Control Areas

	Treatment	Control 1	Control 2	Control 3	Control 4
Cases of Liver Cancer	461.00	280.00	660.00	901.00	262.00
Mean Population	82162.89	51027.89	109328.83	143702.78	58813.72
Min Population	93.00	439.00	2673.00	4246.00	4492.00
Max Population	462053.00	284927.00	668731.00	713002.00	323578.00
SD Population	122798.18	69071.44	168352.03	201147.88	74620.08
Mean Median Value	73842.58	72118.54	64324.29	71404.84	66898.20
Min Median Value	27730.00	32800.00	36700.00	37715.38	33737.50
Max Median Value	149874.38	156537.29	103592.59	108592.55	114706.50
SD Median Value	29546.11	23724.24	19998.60	20881.36	19372.35
Mean Median Rent	430.62	392.60	389.26	417.23	391.17
Min Median Rent	274.05	283.25	245.50	235.08	244.75
Max Median Rent	618.50	574.37	574.47	545.44	583.69
SD Median Rent	94.52	77.31	97.04	96.65	94.90
Mean Percent Black	21.03	8.98	14.36	13.85	11.39
Min Percent Black	1.64	0.00	0.52	0.90	1.31
Max Percent Black	67.20	24.74	33.60	51.95	34.93
SD Percent Black	22.68	7.09	9.07	11.80	7.65
Mean Percent Hispanic	12.52	4.94	3.77	3.63	4.47
Min Percent Hispanic	0.89	0.18	0.64	0.67	0.70
Max Percent Hispanic	67.38	32.96	14.13	9.29	15.82
SD Percent Hispanic	15.58	7.37	3.45	2.30	4.28

Control 1 = random sample from a pool of 1249 ground water treatment plants.

Control 2 = random sample from a pool of 1176 ground water treatment plants selected based on median income (\$14,750.36-\$132,934.8) and median monthly rent (\$241.58-\$619.68).

Control 3 = random sample from a pool of 1246 ground water treatment plants selected based on percent Black (0-66.39%) and percent Hispanic (0-43.68%).

Control 4 = random sample from a pool of 1174 ground water treatment plants selected based on the requirements for both Control 2 and Control 3.

Table 3. Mann Whitney Rank Sum Test of Incidence Rates for Study Surface Water Areas vs for Ground Water Control Areas

	Surface Water Rank	Control Rank	Significance
Control 1	338.0	328.0	NS
Control 2	323.5	342.5	NS
Control 3	311.5	354.5	NS
Control 4	341.0	325.0	NS

NS= not significant at $p < 0.05$

Table 4. Pooled Age Adjusted Incidence Rates and Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled study Surface Water Treatment Areas vs pooled Ground Water Treatment Control Areas

	Age Adj Rate*	SE	SRR=Surface /Control (95% Confidence Intervals)	SRR=Surface or Control/Florida (95% Confidence Intervals)
Surface Water	1.13	0.00075		0.80 (0.79-0.81)
Control 1	1.19	0.00099	0.95 (0.94-0.96)	0.84 (0.84-0.86)
Control 2	1.41	0.00076	0.80 (0.80-0.01)	1.00 (0.99-1.00)
Control 3	1.39	0.00066	0.81(0.80-0.82)	0.99 (0.98-0.99)
Control 4	1.15	0.00098	0.98 (0.98-0.00)	0.82 (0.81-0.83)

*State of Florida Age Adjusted Cumulative Cancer Rate (1981-1998) = 1.41/100,000 (SE 0.0003)

Table 5. Differences in area (square meters) for each of the spatial domains in the contiguous buffer analysis

Site	Service Area	Service Buffer	Circular Core	Circular Buffer
1	256503286.5	527902385.5	255210785.7	254686250.8
2	66264242.29	81284225.07	65930225.99	65794712.12
3	217139118.9	330508172.8	216044875.8	215600891.6
4	89623823.1	146262313.9	89172008.09	88988749.38
5	18124788.19	40984757.37	18033411.5	17301837.05
6	18390191.46	31944696.66	18297442.79	18259830.21
7	5488222.871	11855188.53	5460530.054	5449306.552
8	188025325.1	299729440	187077557.2	186693080.5
9	8799255.441	10751675.92	8754901.402	8550687.138
10	10429384.96	11953140.75	10376796.89	10355472.77
11	38905142.57	52216630.5	38709125.13	38629566.69
12	155208586.7	245391006.6	154426266.4	154108870.5
13	150789657.3	187476583.9	150029720.1	122823212.9
14	139401814.6	88608647.54	138699137.5	84364723.08
15	576458739.4	782879147.7	573553394.8	572374617.5
16	59739915.71	19254783.71	59438944.61	25818324.11
17	267532905.2	367884385.3	266184841.7	255492399
18	385429332.2	511338186.3	383486598.3	334938779.7

Table 6. Population Characteristics (1990 Census) for Actual Surface Water Treatment Areas and Contiguous Control Buffer Areas

	Actual Surface Treatment Area	Contiguous Control Buffer Area	Circular Surface Treatment	Circular Contiguous Control Buffer Area
Cases of Liver Cancer	335	104	299	138
Mean Population	60362.5	27451.3	61940.5	26810.3
Min Population	161	316	198	187
Max Population	447904	205909	485953	180557
SD Population	105256.7	55433.7	114060.2	47637.2
Mean Median Value	76545.9	82079.9	76493.6	79211.4
Min Median Value	37962	39600	38477	39600
Max Median Value	204246	192750	203600	188830
SD Median Value	40800.4	36446.6	47183.5	36842.6
Mean Median Rent	424.1	44.9	430.2	428.8
Min Median Rent	256	275	284	270
Max Median Rent	644	716	735	644
SD Median Rent	105.8	105.3	113.9	109.3
Mean Percent Black	25.4	22.2	24.5	22.4
Min Percent Black	0	0.3	0	0.3
Max Percent Black	70	69	71	67
SD Percent Black	26.4	26.1	27.1	25.8
Mean Percent Hispanic	8.9	9.6	8.7	9.2
Min Percent Hispanic	0	0.3	0	0.7
Max Percent Hispanic	31	30	31	32
SD Percent Hispanic	8.6	9.6	9.3	9.4

Table 7. Age Adjusted Incidence Rates for Liver Cancer (Hepatocellular Carcinoma) around actual and circular Florida Surface Water Treatment Plants compared to Contiguous Buffer Controls areas

Area number Second Analysis	Cancer Cases with actual area	Rates+ with actual area	Cancer cases of actual buffer control	Rates+ with actual buffer control	Cancer Cases with circular area estimate	Rates+ with circular area estimate	Cancer cases with circular buffer control	Rates+ with circular buffer Control
1	5	1.37	1	3.21	4	1.75	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	10	1.36	3	2.01	9	1.28	2	1.79
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	51	0.51	39	0.99	1	0.99	0	0
8	3	4.88	2	5.23	1	2.67	2	6.83
9	3	1.24	0	0	1	0.32	2	6.99
10	28	1.40	36	1.02	99	1.01	46	1.57
11	28	1.41	1	0.26	22	1.37	3	0.59
12	41	1.08	5	0.61	34	1.08	9	0.79
13	39	0.96	10	1.04	33	0.63	17	1.03
14	9	2.13	1	0.65	4	2.72	5	1.69
15	39	1.72	0	0	39	1.39	48	1.64
16	8	0.43	6	0.34	16	0.53	4	0.49
17	25	1.31	0	0.5	15	0.94	0	0
18	21	1.05	0	0	21	1.08	0	0
Overall	335	1.16	104	0.83	299	0.99	138	1.16

+Age Adjusted Rates = number of HCC cases/100,000 (1981-1998)

*>State of Florida Age Adjusted Cumulative Cancer Rate (1981-1998) = 1.41/100,000

Table 8. Mann Whitney Rank Sum Test of Incidence Rates for Actual Surface Water Areas vs for Contiguous Buffered Control Areas

	Surface Water Area Rank	Contiguous Buffer Control Rank	Significance
Actual	366	234	0.02
Circular	332	268	NS

NS= not significant at $p < 0.05$

Table 9. Pooled Age Adjusted Incidence Rates and Standardized Rate Ratios (SRR) of Hepatocellular Carcinoma for pooled Surface Water Treatment Areas vs pooled Contiguous Buffered Control Areas

	Age Adj Rate*	SE	SRR=Surface /Control (95% Confidence Intervals)	SRR=Surface or Buffer/Florida (95% Confidence Intervals)
Actual Surface Water Area	1.15	0.0009		0.82 (0.81-0.83)
Contiguous Buffer Control	0.83	0.0011	1.39 (1.38-1.40)	0.59 (0.58-0.60)
Circular Surface Water Area	0.99	0.0017		0.70 (0.69-0.71)
Contiguous Circular Buffer Control	1.16	0.0014	0.85 (0.84-0.85)	0.82 (0.80-0.83)

*State of Florida Age Adjusted Cumulative Cancer Rate (1981-1998) = 1.41/100,000 (SE 0.0003)

Figure 1. Example of Surface Water Treatment Plants locations and Actual Service Area Boundaries with Study Surface Water Treatment Plants and Study Areas of Influence

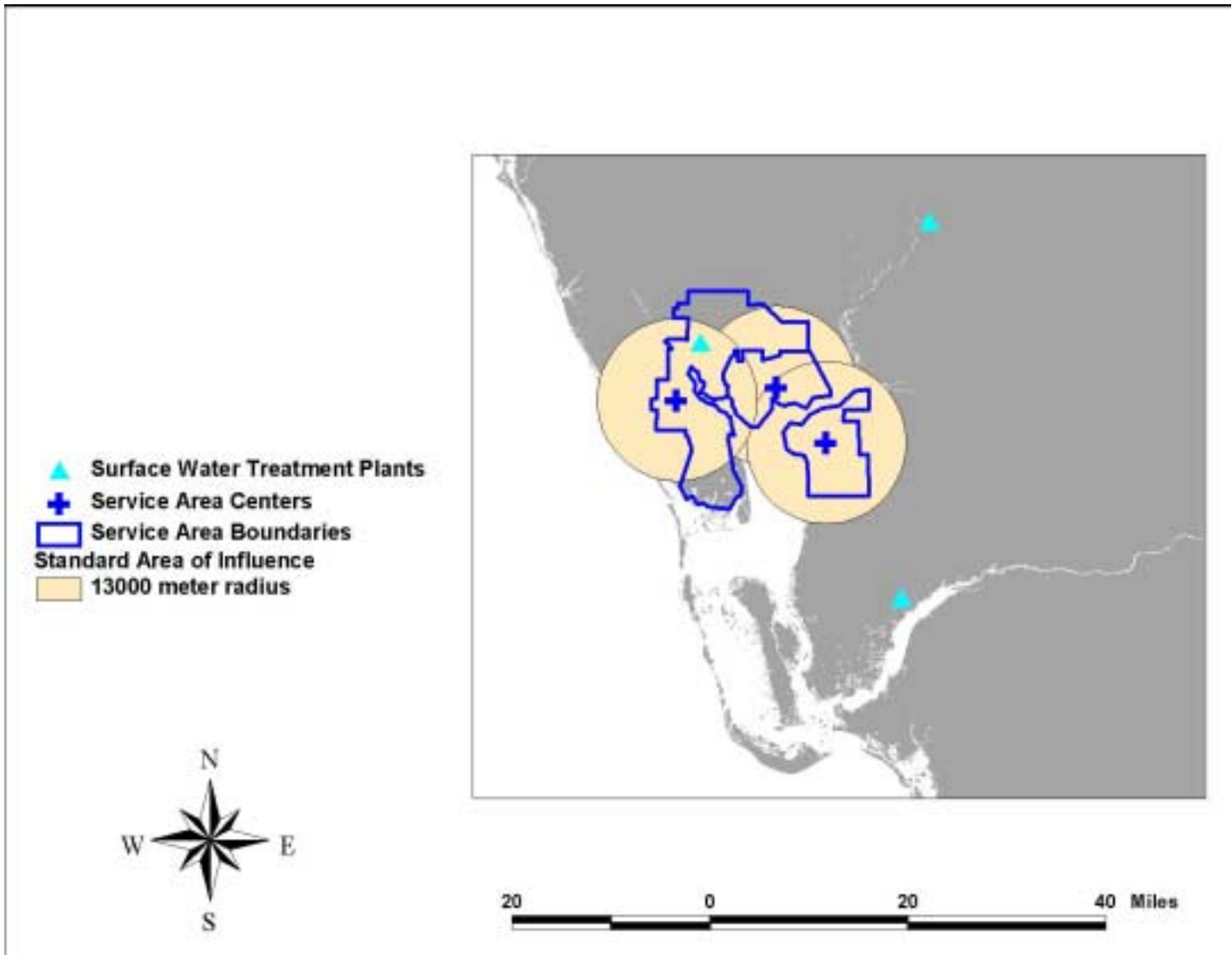


Figure 2. Example of Actual Ground Water Wells with Study Ground Water Treatment Plant and Study Area of Influence

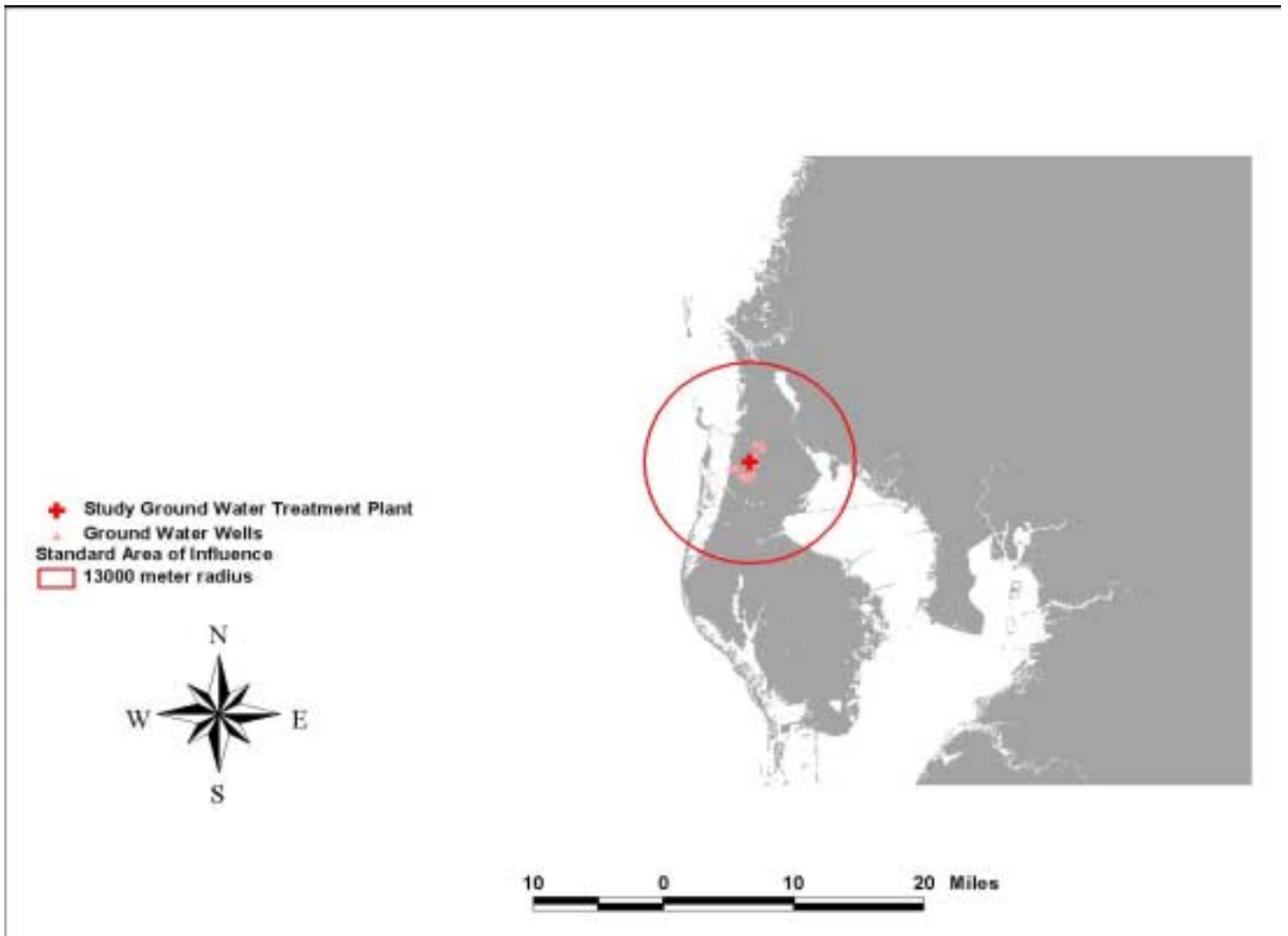


Figure 3. Example of Cancer Cases, Surface Water Treatment Plant locations and Actual Service Area Boundaries with Study Surface Water Treatment Plants and Study Areas of Influence

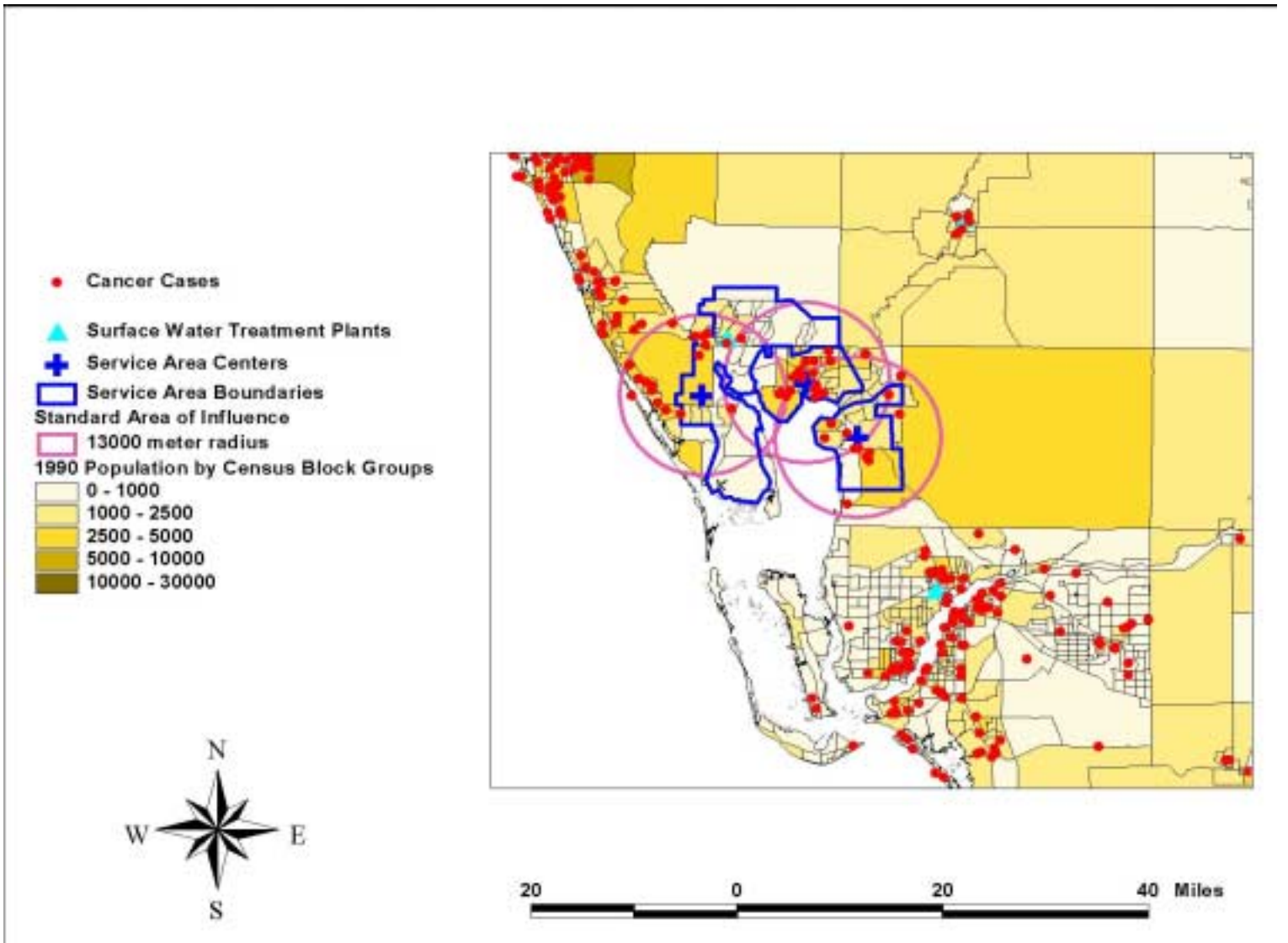


Figure 4. Example of Actual Surface Treatment Areas with actual Contiguous Buffer Control Area Approximations.

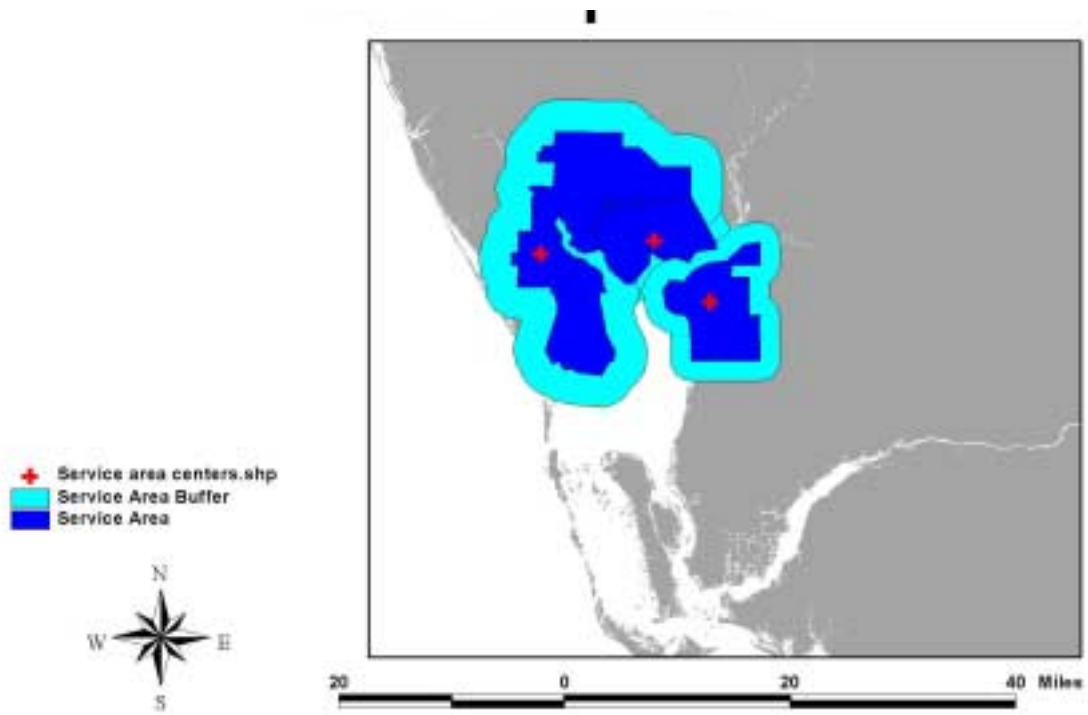


Figure 5. Example of Circular Surface Treatment Areas with Circular Contiguous Buffer Approximations

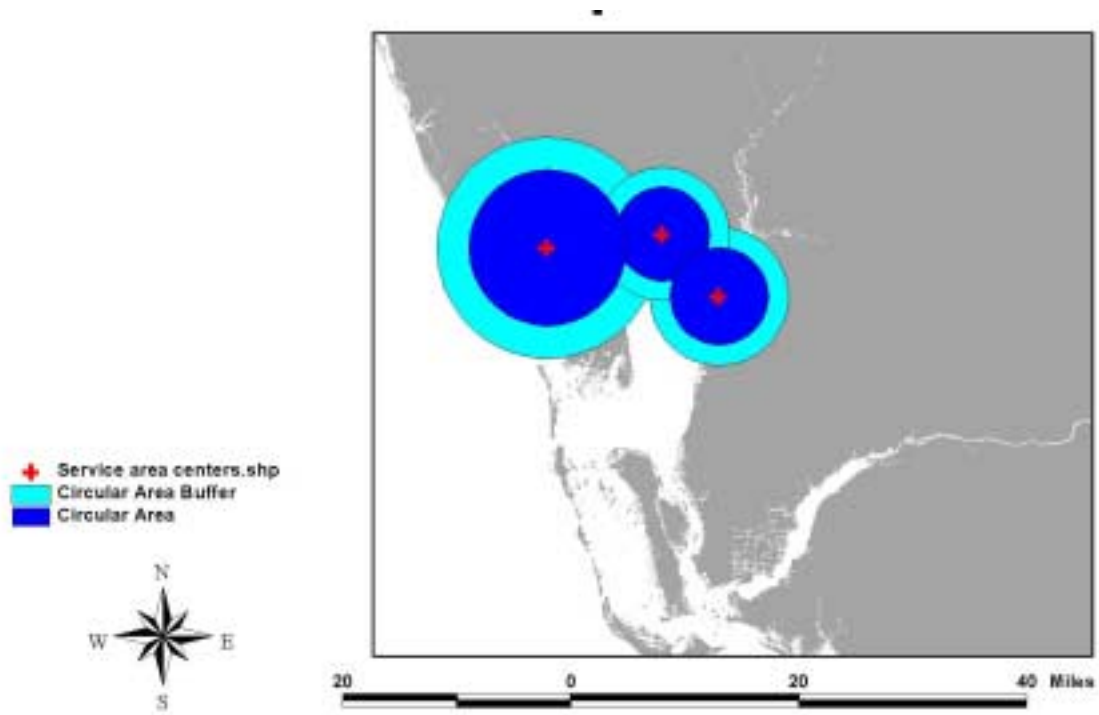


Figure 6. Hepatocellular Carcinoma Cases in Florida (1981-1998) with 1990 Census Block Population Density Background

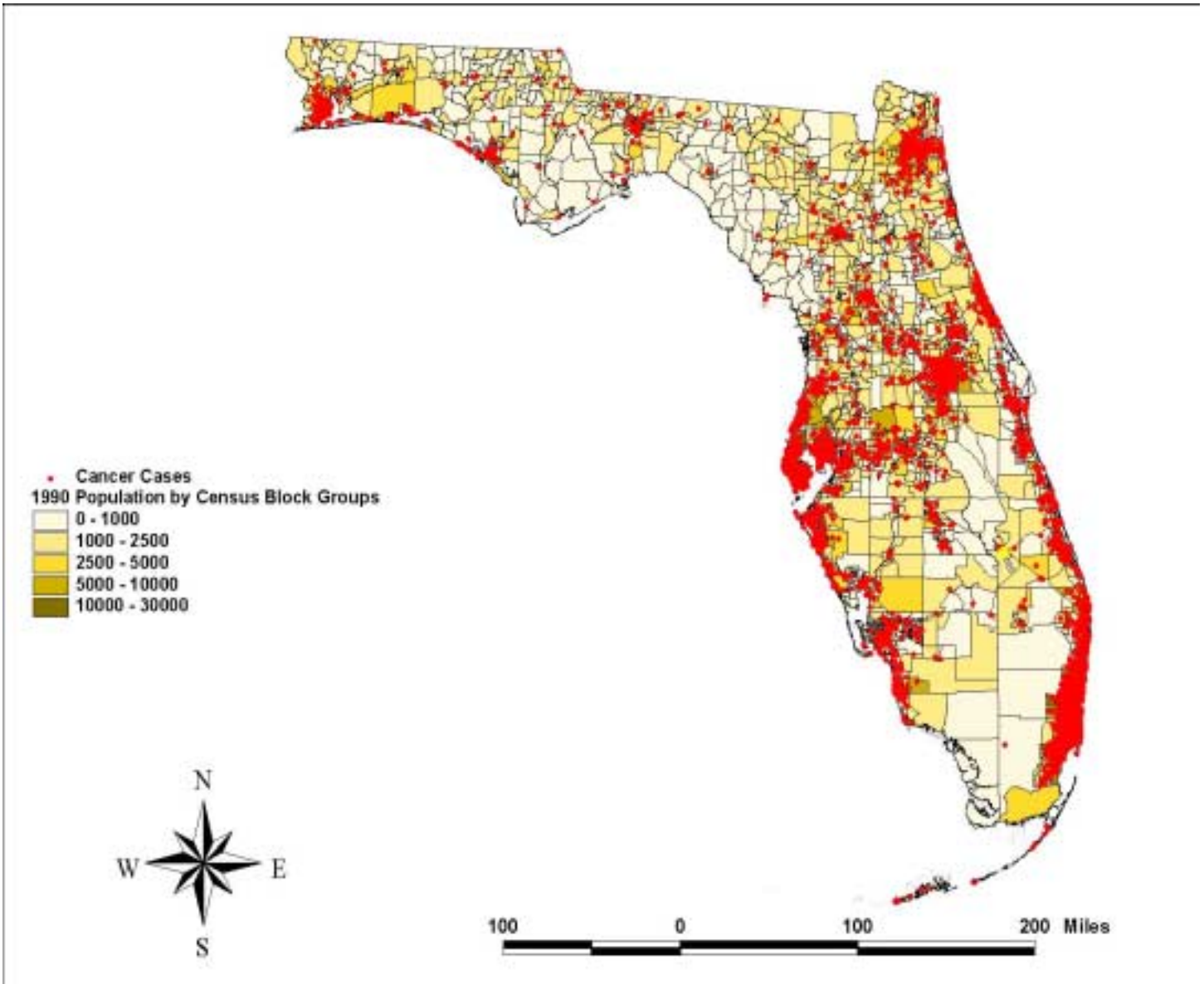


Figure 7. Location of Study Surface Water Treatment Areas and Study Ground Water Well Areas

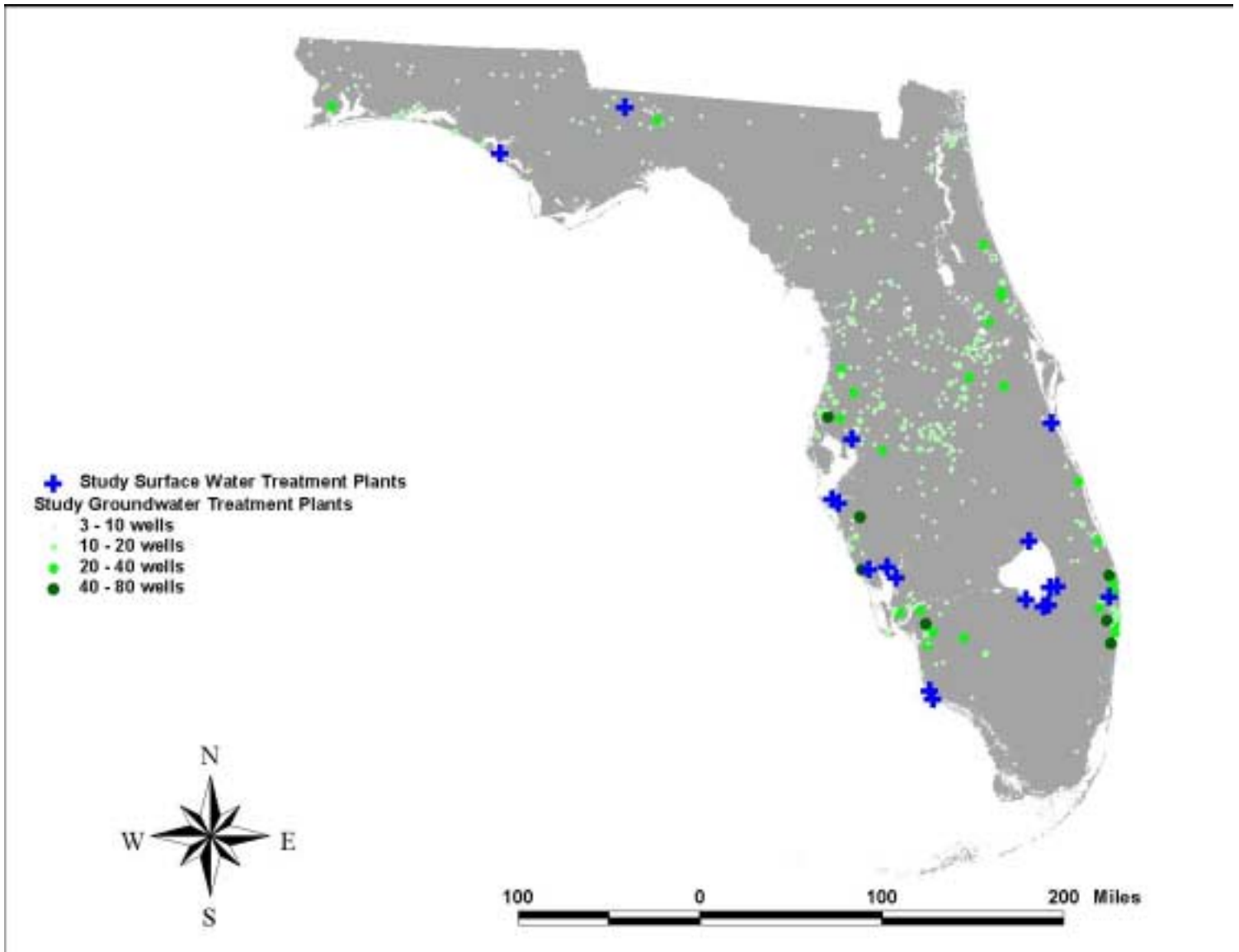


Figure 8. Sites of 18 Study Surface Water Areas and 4 sets of 18 Randomly Selected Matched Ground Water Control Areas

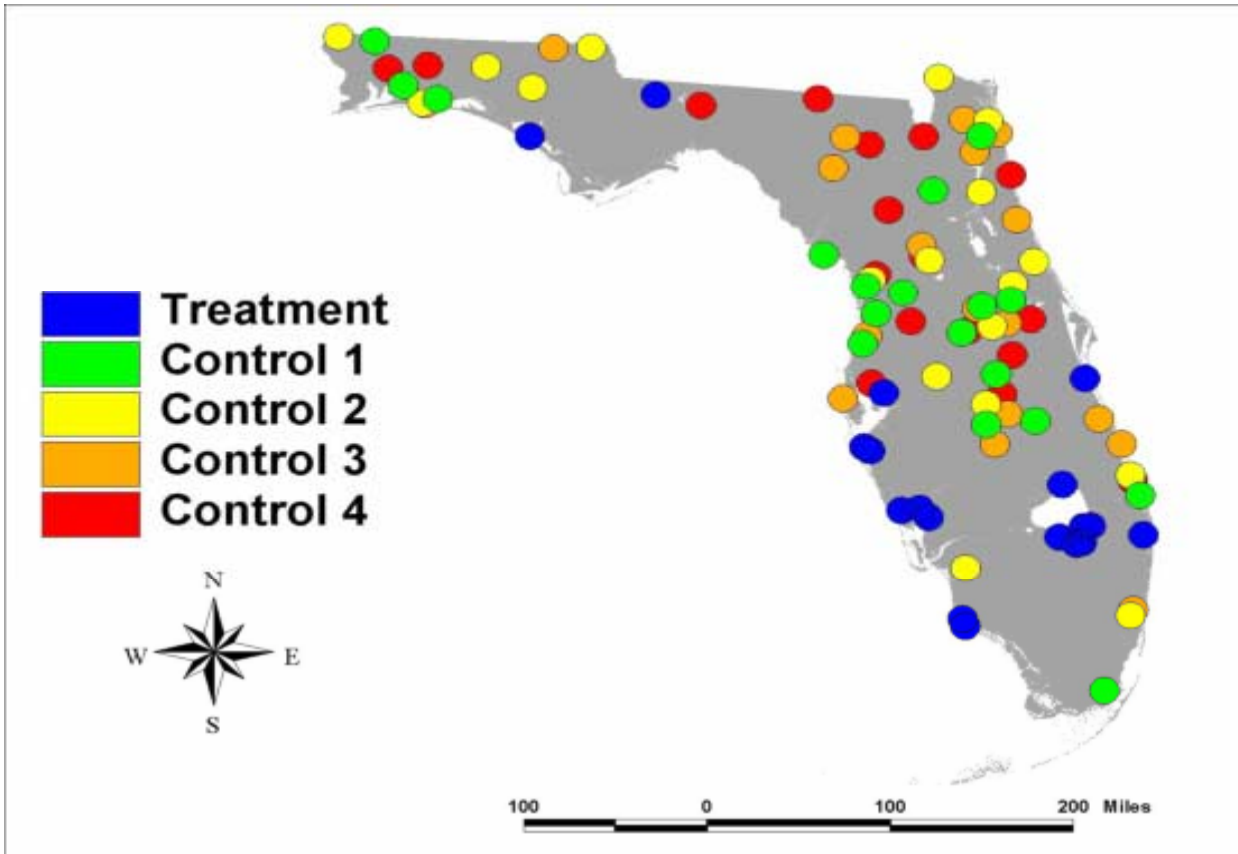
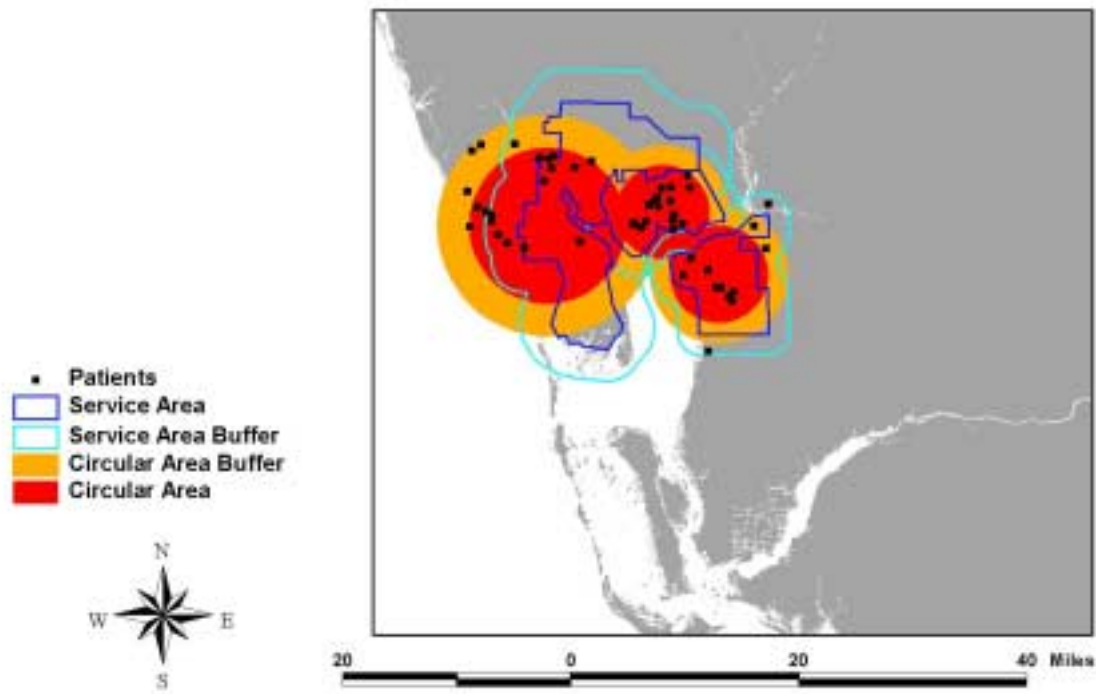


Figure 9. Example of both Actual and Circular Surface Water Treatment Areas and their Contiguous Actual and Circular Buffer Control Approximations, with HCC patients



Appendix: Report from John Burns, SJRWMD