EXPOSURE CONCENTRATIONS OF PHARMACEUTICAL ESTROGENS AND XENOESTROGENS IN MUNICIPAL WASTEWATER TREATMENT PLANT SOURCES, THE AQUATIC ENVIRONMENT AND AN AQUATIC HAZARD ASSESSMENT OF BISPHENOL-A: IMPLICATIONS FOR WILDLIFE AND PUBLIC HEALTH

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Humans are exposed daily to both pharmaceutical estrogens and xenoestrogens (PEXE) due to their presence in many household products, food products, soil, air, water and estrogen based medications. These PEXEs have been implicated in various human health outcomes, such as breast cancer in women and testicular dysgenesis syndrome including testicular cancer. They also can have adverse reproductive effects on aquatic wildlife through sex reversals, production of intersex individuals, alterations in mating, and prevention of gonadal maturation. There are many sources and types of PEXEs in air, water, soil, household products and food products, but the focus for this research is on the transport and fate of PEXEs from all media into surface water, especially through municipal waste water treatment plant (WWTP) sources. This dissertation consists of three related research papers. The first examines the sources and types of PEXEs in municipal WWTPs. The second documents and compares aquatic exposure concentrations of PEXEs to their Predicted No effect concentration (PNECs) to determine aquatic species protectiveness or risk. The third paper conducts an aquatic hazard assessment of the xenoestrogen, Bisphenol A (BPA).

The findings of the research suggest that PEXEs; contain compounds that can mimic estrogens, are mostly introduced into the environment through municipal WWTP effluent sources, and are discharged directly into rivers and lakes at environmentally relevant concentrations. Specifically, BPA, a compound widely used in plastics may be present in surface waters at hazardous concentrations that may present a risk for aquatic receptors. The public health significance of this research is that approximately sixty percent of Americans obtain their drinking water from surface water sources. Thus, understanding PEXEs and their concentrations present of WWTP effluents is imperative for environmental public health tracking of associated disease states, and in the regulation of fish or wildlife consumption from rivers and lakes. Further, to examine adverse health effects in the biotic aquatic system is to indirectly explore possible exposure and health effects on humans since species in the wild are sentinels for human exposure ("the canary in the mine"). Sentinel animals may provide early warning of potential risks before disease develops in human populations.

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PREFACE

It is with great pleasure that I offer my acknowledgement of the members of my dissertation committee for the guidance provided throughout the dissertation process. I would particularly like to thank Dr. Pitt, the Chairman of the Environmental and Occupational Health (EOH) Department in the Graduate School of Public Health for his unwavering support throughout my studies at the University of Pittsburgh. Special thanks to my advisor, Dr. Volz who meandered with me through "hills and valleys" throughout this process. I offer special thanks to all professors and staff at the University of Pittsburgh who along the path nudged and inspired me to go on.

I am thankful to my friends at the Graduate School of Public Health for their unwavering support and interest in my career. My immeasurable gratitude to my friends and colleagues who encouraged me during this "Mount Everest climb". I thank them for their understanding of my busy personal and professional schedule. I want to thank my parents who have long departed, but who have inspired me all my life. My brothers and sisters for always standing by me through innumerable storms and many happy moments during my study period. I would like to mention my unconditional love for my sons Richard and Erik for their patience, love and understanding throughout this process. Finally, my faith and thanks to my Creator for keeping me on this journey and many others to come. Let the games begin.

1.0 GENERAL INTRODUCTION

Low level exposure to pharmaceutical estrogens and xenoestrogens (PEXEs) is a concern that has emerged in recent research reports due to the PEXE's ability to mimic the naturally occurring estrogens and cause endocrine disruption by various mechanisms for both humans and wildlife. PEXEs (also called environmental estrogens) enter the aquatic environment mainly through waste water treatment plant (WWTP) effluents, due to inefficient removal rates during the wastewater treatment process. Accordingly, most of the reported adverse effects of PEXEs are found in the aquatic environment, particularly in rivers with a high charge of domestic and industrial wastewaters. Humans, particularly in the United States are also exposed daily to both pharmaceutical estrogens and xenoestrogens (PEXE) due to their presence in many household products, food products, soil, air, water and estrogen based medications. Xenoestrogens are synthetic substances that mimic or enhance the effect of estrogens. The estrogenic stimulation is an unintended side-effect of these agents or their metabolites. Xenoestrogens are part of a heterogeneous group of chemicals that are hormone or endocrine disruptors. PEXEs have been implicated in various human health outcomes, such as breast cancer in women and testicular dysgenesis syndrome including testicular cancer. Also, they can have adverse reproductive effects on aquatic wildlife through sex reversals, production of intersex individuals, alterations in mating, and prevention of gonadal maturation. There are many sources and types of PEXEs in air, water, soil, household products and food products, but the focus for this research is on the transport and fate of PEXEs from all media into surface water, especially through municipal waste water treatment plant (WWTP) sources; their

exposure concentration and impact on aquatic life and humans. This is important since approximately sixty percent of Americans obtain their drinking water from surface water sources. Further, to examine adverse health effects in the biotic aquatic system is to indirectly explore possible effects on humans and public health implications.

From a mechanistic perspective, endocrine disruption includes ligand-estrogen receptor interactions, plasma binding and uptake by tissue, chilarity, antiestrogenic activity of aryl hydrocarbon (AH) ligands and the antiandrogenic activity of certain PEXEs such as dichloro-diphenyl-trichloroethane **(**DDT). However, many substances including PEXEs can interact with the estrogen receptor (ER), albeit weakly, because of characteristics they share with two potent estrogenic substances, estradiol and diethylstilbestrol(Witorsch 2000). Primarily, these features are a ring structure (preferably aromatic and an unencumbered hydroxyl group) along with a hydrophobic center capable of interacting with the core of the binding domain of the estrogen receptor(Witorsch 2000). However, this affinity for the receptor does not explain the nature of the biological response (Witorsch 2000). An example of which is seen by a diversity of systemic biological effects exhibited by estradiol, tamoxifen, or raloxine, and by the antiestrogenic effect of DDT in the tiger salamander (Clark 1998). Different ER ligands give different profiles of biological response that appear to be influenced by tissue and species (Witorsch 2000). These distinct profiles are dictated by such factors as the ligand itself, the ER isoform, the genomic site of ER binding, and assembly of co-regulatory proteins associated with ER (Witorsch 2000). Among the other factors that influence the nature of endocrine disruption are the role of plasma binding on the delivery of environmental agents to the tissue, chirality of these agents, cross-talk between signaling systems (e.g., between AhR and ER), and alternate mechanisms

(e.g., antiandrogen effects). No doubt this is not a complete list of factors, since other aspects of ER were not addressed, such as the involvement of specie activation domains (AF-1 and AF-2) of the receptor in xenoestrogen action (Chang 1999; Gustafsson 2000).

While strides have been made in examining some wildlife and human effects, very few studies have examined and documented the effect of low level environmental concentrations of pharmaceutical estrogens and xenoestrogens (PEXEs) in aqueous media, particularly in waste water effluents, surface waters and, or drinking waters. Even fewer studies have examined; the most dominant PEXEs in WWTP effluents and relevant public health concerns, aquatic concentrations of PEXEs and compare them to a reference concentration to determine the relationship between environmental exposure concentration and possible risk or adverse effect for aquatic receptors, and the hazard assessment of the specific xenoestrogen Bisphenol A (BPA). With this background, this research; examines the concentration of the dominant steroidal estrogens in WWTP effluents and the possible public health effects on drinking water supply, determines and evaluates aquatic concentrations of PEXEs and calculates risks for both humans and wildlife, completes a hazard assessment of BPA. The significance of this research is that to know the class and concentration of the PEXEs in surface waters can lead to better regulation and management of PEXEs in WWTP effluents and hence drinking water supply thus protecting the public. Also, effective management and regulation of the most widely used chemical BPA to minimize its impact on the aquatic system.

1.1. GENERAL OVERVIEW OF RESEARCH PAPERS

This dissertation consists of three related research papers. The first research paper examines the sources and types of PEXEs in municipal WWTPs. We found that most PEXEs are introduced into the environment through municipal WWTP effluent sources due to inefficient removal rates during the wastewater treatment process. These effluents contain synthetic compounds; surfactants, flame retardants and halogenated hydrocarbons that can mimic estrogens; and are discharged directly into rivers and lakes. Advances in civilization coupled with rising population levels have resulted in an increasing need to treat and recycle available water resources. There are over 16,000 municipal WWTPs nationwide and over 75% of the nation's population is being served by centralized wastewater collection and treatment systems (USEPA 2004). The remaining population uses septic or other onsite systems (USEPA 2004), which have not been adequately studied for xenoestrogens release but, due to their high failure rate and lack of maintenance, could be considered potential non-point releasers of estrogenic compounds (Wright-Walters 2007).

In the United States surface water provides for 62% of the public water drinking supply (University of Michigan 2005). Thus, as rivers and lakes are used for water and food supply and recreation, and wastewater effluent usage increases, the presence and concentration of pharmaceutical estrogens and xenoestrogens in surface water becomes a valid public health concern. Additionally, many USA cities have significant combined sewer overflows (CSOs)

releasing untreated sewage directly into surface waters, thus increasing the amounts of xenoestrogens finding their way into drinking water supplies and commercial and subsistence fishing habitat. There is considerable evidence that fishes inhabiting waters that receive untreated municipal wastewaters or effluents from municipal WWTPs are exposed to chemicals that affect reproductive endocrine function(Kidd, Blanchfield et al. 2007). Male fishes downstream of some wastewater outfalls produce vitellogenin (VTG) (a protein normally synthesized by females during oocyte maturation) and early-stage eggs in their testes. This feminization has been attributed to the presence of estrogenic substances such as PEXEs. Laboratory studies have confirmed that environmental contaminants with endocrine disrupting properties (EDCs) such as PEXEs can disturb the development and expression of sexual characteristics in fish (Thorpe KL 2001; Sumpter 2003; Toft 2003), amphibians (Hayes, Collins et al. 2002; Hayes TB 2002), reptiles (Crain DA 1999; Willingham 1999; Willingham 2001), birds (Feyk LA 1998), and mammals (Gray LE 1994; Sharpe, Fisher et al. 1995). However, the extent to which the sexual characteristics and reproductive capabilities of natural populations are impacted by these EDCs is still not well understood.

Combinations of estrogenic compounds are present in municipal WWTP effluents but, the natural estrogens, 17β-estradiol (E2) and estrone (E1), and the synthetic E2 derivate 17α-ethinylestradiol (EE2) are most responsible for most estrogenic activity in WWTP effluents. Each xenoestrogen exhibits its own wildlife or human health risk, but synergistic effects could occur with xenoestrogen mixtures. Wildlife species are rarely exposed to single chemicals but instead are exposed to complex, fluctuating mixtures of contaminants that may act in various ways (Thorpe KL 2001; Silva E 2002; Sumpter 2003; Thorpe 2003) and that may induce combination effects (Rajapakse, Silva et al. 2002) via the same or different mechanisms. Less than 1 ng/L

EE2 can cause feminization of male fishes while 4 ng/L caused abnormal reproductive development (male fathead minnows) (Purdom 1994; Kidd, Blanchfield et al. 2007). E2 has been detected at concentrations from 1 ng/L to 80 ng/L in WWTP effluents(Desbrow and Waldock 1998). Total estrogenicity (E2 equivalents) of 147 ng/L has been measured in WWTP effluent(Furuichi 2004).

The second paper examines the various PEXEs and their environmentally relevant concentrations present in rivers, lakes, drinking water and effluents due to their reported adverse estrogenic effects in the aquatic environment. Most of the reported wildlife adverse effects are found in the aquatic environment, especially in rivers with a high charge of domestic and industrial wastewaters. Many species in the wild have experienced genetic and physical malfunctions, particularly fishes that live in waters that receive effluents from Industrial discharge; sewage treatment plants (WWTPs), CSOs and sanitary sewer overflows (SSOs). The phenomena ranges from subtle changes in the physiology and sexual behavior of fishes to permanently altered sexual differentiation and impairment of fertility. It is believed that this is due endocrine disrupting chemical(s) commonly referred to as xenoestrogens. Treated effluents from WWTPs are directly discharged into surface waters. In many cities there are many CSOs and SSOs that directly flow into river water and surface water. In the United States most of our drinking water is secured from surface waters. This second paper reports E1 concentration in surface water between 0.004-104 ng/L (Furuichi 2004; Viganò 2006), E2 concentration between 0.001-48 ng/L (Desbrow and Waldock 1998; Viganò 2006) and EE2 concentration between <0.1ng/L-17 µg/L. Nonylphenol and brominated biphenyls, a surfactant and flame retardant have been detected between 0.1-3.7 µg/L and 0.3-4.6 mg/kg (on suspended particles) respectively. Dibutyl phthalate has been reported at a concentration of 5-

10 ng/L in surface water and drinking water (Hyötyläinen 1997). Bisphenol A concentrations have been reported in drinking water from 300 pg/L to 2 ng/L and in river water from 500 pg/L up to 16 ng/L (Kuch 2001).

Knowing the environmentally relevant concentrations of the PEXE can provide information for determination of possible risks to these substances for both humans and wildlife. Also, this information can lead to better methodology for the protection of wildlife species and management of wastewater treatment plant effluent released PEXEs.

The third research paper focuses on one xenoestrogen Bisphenol A (BPA) and sets out to conduct an aquatic hazard assessment of BPA. Bisphenol A [BPA; 2,2-bis(4-hydroxyphenyl)propane] ,a xenoestrogen identified as an agonist of the estrogen receptor, is an industrially important chemical that is used as a primary raw material for the production of engineering plastics (*e.g.*, polycarbonate/epoxy resins), linings of food cans (*i.e.*, lacquer coatings), and dental composites/sealants. Despite its biodegradability and short half life, BPA has been implicated in various human and wildlife health endpoints such as infertility, impaired reproduction, precocious puberty, endometriosis or production of breast, vaginal, prostate, and uterine cancer. BPA has been identified in surface waters and, hence has been the subject of considerable research into its acute and chronic effects on aquatic organisms. An aquatic hazard assessment establishes a derived predicted no effect concentration (PNEC) below which it is assumed that the aquatic environment will not suffer adverse effects. This paper; 1) reviews literature on aquatic toxicity of BPA; 2) conducts and updates an aquatic hazard assessment for BPA using the weight of evidence approach, using ecologically relevant endpoints such as survival, growth and development, and reproductive success and 3)

compares the hazard assessment value(s) to environmental concentrations for BPA found in the aquatic environment to determine if there is sufficient protectiveness for the aquatic system, and discusses the relative contribution of aquatic sources to overall BPA exposures.

1.2. PUBLIC HEALTH SIGNIFICANCE

Species in the wild are sentinels for human exposure ("the canary in the mine"). Sentinel animals may provide early warning of potential risks before disease develops in human populations. Potential applications for sentinel species includes monitoring environmental media, identifying new exposures of potential concern as a result of observing changes in wild animal populations, and supporting risk assessment at several points in the process. Although it is unlikely that sentinel species data will be used as the sole determinative factor in assessing human health risks, the data can be useful for a weight-of-evidence approach in risk assessment decisions, for providing early warning of situations requiring further study, or for suggesting potential causes and effects. A key consideration for any application is to understand the mechanistic similarities and differences between toxicological effects in the sentinel species and humans.

Some species are a part of the human food chain and thus another route of exposure for humans to PEXEs. Understanding the contents of WWTP effluents, species and concentrations of PEXEs in surface water is imperative for environmental public health tracking of associated disease states, and in the regulation of fish or wildlife consumption from rivers and lakes.

Having an updated BPA aquatic hazard assessment will help to determine risks for both humans and wildlife populations from environmentally relevant concentrations of BPA. Further, it will foster the development of new policies and regulations regarding the production and proper management of BPA in the aquatic environment.

2.0 MUNICIPAL WASTEWATER CONCENTRATIONS OF PHARMACEUTICAL AND XENO- ESTROGENS: WILDLIFE AND HUMAN HEALTH IMPLICATIONS

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2.1. ABSTRACT

Most pharmaceutical estrogens and xenoestrogens are introduced into the environment through municipal waste water treatment plant (WWTP) effluent sources. These effluents contain synthetic compounds; surfactants, flame retardants and halogenated hydrocarbons that can mimic estrogens; and are discharged directly into rivers and lakes. As rivers and lakes are used for water and food supply, and recreation, and wastewater effluent usage increases, the presence and concentration of xenoestrogens in surface water becomes a valid public health concern. Additionally, many USA cities have significant combined sewer overflows releasing untreated sewage directly into surface waters, thus increasing the amounts of xenoestrogens finding their way into drinking water supplies and commercial and subsistence fishing habitat.

In the United States, humans are exposed daily to both pharmaceutical and xenoestrogens which have been implicated in various human health outcomes, such as breast cancer in women and testicular dysgenesis syndrome including testicular cancer. Also, they can have adverse reproductive effects in aquatic wildlife through sex reversals, production of intersex individuals, alterations in mating, and prevention of gonadal maturation. Combinations of estrogenic compounds are present in municipal WWTP effluents but, the natural estrogens, 17β-estradiol (E2) and estrone (E1), and the synthetic E2 derivate 17α-ethinylestradiol (EE2) are most responsible for in vitro estrogenic activity. Each xenoestrogen exhibits its own wildlife or human health risk, but synergistic effects could occur with xenoestrogen mixtures. Less than 1 ng/L EE2 can cause feminization of male fishes, 4 ng/L caused abnormal reproductive development (male fathead minnows). E2 has been detected at concentrations from 1 ng/L to

80 ng/L. Total estrogenicity (E2 equivalents) of 147 ng/L has been measured in WWTP effluent. Nonylphenol, a surfactant and brominated biphenyls, a flame retardant have been detected between 0.1-3.7 µg/L and 0.3-4.6 mg/kg (on suspended particles) respectively.

Understanding the species and xenoestrogen concentrations in surface water is imperative for environmental public health tracking of associated disease states. Such research will determine the necessity for utilizing limited and competing public financial resources to invest in technology to remove xenoestrogens from surface waters and, in regulation of fish or wildlife consumption from our rivers and lakes.

2.2. INTRODUCTION

Most pharmaceutical estrogens and xenoestrogens (PEXES) are introduced directly into surface waters through municipal wastewater treatment plant (WWTP) effluent sources, also called sewage treatment works (STW) (Daughton 1999; Norris 2007). The low concentrations of individual pharmaceutical estrogens (possibly exceeding the catabolic enzyme affinities of sewage microbiota), coupled with their metabolic "novelty," (increase polarity) leads to incomplete removal from STWs (Daughton 1999). The focus on PEXEs has been on their interaction with the hormone receptors and the subsequent regulation of target genes. Following binding to the hormone receptor, PEXEs may either stimulate or inhibit gene transcription in a manner similar to the natural hormone or they may inactivate gene transcription by forming receptor-ligand complexes with conformations that are unfavorable for activation (EUR 1996). Some substances have however, been found to exert both agonistic and antagonistic effects on endocrine receptors (EUR 1996). Compounds having different mechanisms of action may cause similar biological changes. For instance, antagonists to the

androgen receptor may give effects similar to those caused by estrogen receptor agonists. Besides interaction with hormone receptors, PEXEs may interfere with transport proteins, alter the synthesis and biotransformation of hormones, have direct toxic effects on the gonads or have adverse effects on the hypothalamus, the pituitary or endocrine glands.

Municipal wastewaters are a complex mixture containing estrogens and estrogen mimics called xenoestrogens, (Kidd, Blanchfield et al. 2007) natural and synthetic xenobiotics, household and agricultural chemicals, pharmaceuticals, hormones, and other compounds, many of which remain unidentified (Stevens JL 2003). The majority of natural and pharmaceutical estrogens excreted by humans as well as xenoestrogens from numerous domestic and municipal sources (e.g., detergents, plastics, cosmetics,) enter WWTPs (Norris 2007). STWs receiving domestic and pharmaceutical waste release a complex (and ill-defined) mixture of natural and synthetic chemicals into the aquatic environment, due to their partial or complete resistance to biodegradation during the treatment process (Desbrow and Waldock 1998). Most of these compounds are retained in biosolids and a smaller portion typically appears in the wastewater effluent depending on the chemical and the type of treatment and retention times. Currently, more than half of the biosolids produced by municipal wastewater treatment systems is applied to land as a soil conditioner or fertilizer and the remaining solids are incinerated or landfilled (USEPA 2004; King, Ballereau et al. 2006). These disposal practices provide numerous routes for xenoestrogen reentry into environmental media and ultimately surface water. The use of biosolids as a soil conditioner and fertilizer allows for pharmaceutical estrogens and xenoestrogens exposure through the food supply chain and also reentry into surface water systems through run off, and contaminated groundwater outflow. Through incineration, compounds such as dioxins and furans are released into the air and may be

deposited in watersheds through wet and dry deposition. Thus, there are many routes of reentry of xenoestrogens in and attached to the surface of biosolids from WWTPs possibly, increasing their environmental concentration and exposure routes for both humans and animals. There are over 16,000 municipal WWTPs nationwide and over 75% of the nation's population is being served by centralized wastewater collection and treatment systems. The remaining population uses septic or other onsite systems(USEPA 2004), which have not been adequately studied for xenoestrogens release. However, due to their high failure rate and lack of maintenance, could be considered potential non-point releasers of estrogenic compounds. Therefore, there can be an extremely varied mixture of pharmaceutical estrogens and xenoestrogens reentering surface waters possibly contaminating municipal drinking water supplies. But, what are the environmental concentrations of these compounds and are these concentrations significant enough to cause harm to human and wildlife health. Is human pharmaceutical estrogen and xenoestrogen exposure a valid public health concern?

2.3. THE PROBLEM

In the United States humans are exposed daily to xenoestrogens in food (e.g., phytoestrogens, various pesticides) and from contact with detergents [e.g., nonylphenols (NP)] and ingestion of plastic additives from plastic bottles, metal beverage can linings and food packaging (e.g., phthalates, bisphenol A (BPA). In addition, many personal care products (e.g., shampoos, cosmetics, aftershave lotions) contain xenoestrogens such as phthalates, NP and BPA. Most of these pharmaceutical and xenoestrogens are introduced into the environment via municipal WWTPs. Treated WWTP effluents are directly discharged in rivers and lakes. A recent

publication by the U.S. Geological Survey reported that reproductive hormones and estrogenic alkylphenols were present in 40% and 70%, respectively, of the surveyed U.S. surface waters (USEPA 2001). Thus, as rivers and lakes are used for municipal water sources, to help produce our food supply and for recreation, and as wastewater effluent water reuse increases, the presence and concentration of xenoestrogens in surface water becomes a valid public health concern. Advances in civilization coupled with rising population levels have resulted in an increasing need to treat and recycle available water resources. In the United States surface water provides for 62% of the public water drinking supply (University of Michigan 2005). Irrigation remains the largest use of freshwater in the United States and totaled 137 Bgal/d for 2000. Since 1950, irrigation has accounted for about 65% of total water withdrawals, excluding those for thermoelectric power. Historically, more surface water than ground water has been used for irrigation (Hutson Susan S. 2004). Following use, water is returned to the aquatic environment, usually via STWs of varying processes and performance, which improves its quality, but it has a high probability of being withdrawn downstream for municipal or industrial reuse. In US cities with a high population density, the volume of effluent discharged from STWs can be considerable, sometimes contributing up to 50% of the flow of a river, a figure that can rise as high as 90% in periods of low rainfall (Routledge, Sheahan et al. 1998).

STWs continuously receive a complex mixture of industrial, domestic, and agricultural wastewater containing a load of synthetic and natural chemical compounds. It has been demonstrated that, because of incomplete removal or conversion to an active form during the process of sewage treatment, pharmaceutical estrogens and xenoestrogens are released into surface water like rivers, lakes, and seas or adsorbed to sewage sludge or sediment (Liney,

Hagger et al. 2006). These chemicals are found in low parts per trillion in the aquatic environment (Ternes, Kreckel et al. 1999).

2.4. MECHANISM OF ACTIONS FOR ESTROGENIC AND ANTIESTROGENIC COMPOUNDS

The ER, like other members of the nuclear receptor superfamily (Evans 1988), is a ligand-inducible transcription factor. Two subtypes of the ER are known to date, the ERα (Green S 1986) and ERβ (Kuiper 1996; Mueller 2004), and both receptors have a distinct tissue distribution and play a distinct role in physiology (Mueller 2004). The estrogenic or antiestrogenic activity of any chemical is due to the its capability of interacting with the estrogen receptor (ER) (Mueller 2004), and the ER plays a pivotal role in development and neoplasia as a ligand-inducible transcription factor that regulates genes that are involved in cell proliferation and differentiation (Tsai M-J 1994). Since the ER is an important transcription factor in cell proliferation and differentiation, any disruption of the ER signaling pathways may contribute to infertility, developmental abnormalities, or endocrine cancer seen in wildlife and humans (Mueller 2004). Accordingly, observed adverse health effects might be linked to the exposure of chemicals with estrogenic or antiestrogenic activities (Colborn T 1992). Regulatory agencies and the scientific community have therefore put a lot of effort into identifying the estrogenic potential of synthetic and natural compounds (Gray LE 1994; Gray, Bartol et al. 2001). Endocrine-active compounds may also interfere with other signaling systems, most notably the androgen and thyroid hormone system, steroidogenesis, and in part the arylhydrocarbon (Ah) receptor (Gray LE 1994; Safe 1998). Impairment of endocrine function by exogenous compounds affects predominantly the estrogen, androgen, or thyroid hormone

system. The effects on these endocrine systems are mediated by their specific nuclear receptor, that is, the ER, androgen (AR), or thyroid hormone receptor (TR), and the molecular mechanism as described for the ER is conserved for these steroid hormone receptors (Mangelsdorf 1995). Thus any testing strategy for endocrine-active compounds should comprises therefore ER, AR, and TR action (Gray 1997; Gray 2002). Another important endpoint is steroidogenesis, since endocrine-active compounds could also impair enzymes that regulate steroid synthesis like aromatase (Gray 2002; O'Connor, Marty et al. 2002). The thyroid system is often impaired by imbalance of hormone synthesis and disrupted regulation of thyroid hormones; in addition, the TR often functions as a transcriptional repressor (Hu 2000).

2.5. IMPACT ON THE POPULATION

Both pharmaceutical estrogens and xenoestrogens have been implicated in various human health outcomes, such as breast cancer in women and testicular dysgenesis syndrome including testicular cancer (Giwercman 1993; Carlsen 1995; Toppari 1996). Also, they can have adverse reproductive effects in aquatic wildlife through sex reversals, production of intersex individuals, alterations in mating, and prevention of gonadal maturation.

2.5.1. Human Effects

Xenoestrogens have been implicated in a variety of medical problems. Foremost is the concern that xenoestrogens as false messengers disrupt the process of reproduction. Reproductive issues, which are of concern in humans are fetal exposure (perhaps leading to hypospadias),

decreased reproductive ability in men (i.e. decrease in sperm numbers and abnormal sperm shapes) and testicular carcinoma in situ. Another issue is the potential effect of xenoestrogens on oncogenes , specifically, it is implicated in breast cancer in women (Giwercman 1993; Carlsen 1995; Toppari 1996; Körner and Hagenmaier 2001), endometriosis (Adlercreutz 1995), heart disease (Meyer 2001), osteoporosis (Meyer 2001) and Alzheimer's disease (Meyer 2001). It is important to note that a recent comprehensive literature survey of 48 endocrine disrupting chemicals (EDCs) revealed that 79% of these EDCs were also carcinogenic or mutagenic, 52% were also immunotoxic, and 50% were also neurotoxic (Choi SM 2004). Both 4-*tert*-nonylphenol (4-NP) and BPA, for example, are contaminants found at appreciable concentrations in the aquatic environment that can cause endocrine disruption by interacting with both the estrogen receptor as agonists (Gaido KW 1997) and the androgen receptor (Sohoni 1998). In addition, 4-NP can disrupt steroidogenesis in the liver and can interfere with the dynamic control of follicle-stimulating hormone release from the pituitary (Harris CA 2001).

2.5.2. Wildlife Effects

Assessing whether any pharmaceutical estrogens or xenoestrogens pose a threat to the natural environment requires balancing information on its potency against observed environmental concentrations (Colborn T 1993). In many instances it is difficult to assign causality because of the complexity of environmental contaminants and the lack of analytical data that document contaminant levels during critical windows of exposure (Safe 2000). Nevertheless, there have been several incidents in wildlife populations that strongly correlate with exposure to specific industrial chemicals. This includes altered sex determination in alligators in Lake Apopka, Florida, exposed to a spill of organochlorine pesticides from a chemical waste site (Guillette LJ

Jr 1994; Guillette LJ Jr 1995; Guillette LJ Jr 1996). Several studies on wildlife populations have documented adverse effects that correlate with exposure to one or more putative endocrine-disrupting chemicals (Giesy JP 1994; Sumpter and Jobling 1995; Jobling 2003). Reproductive abnormalities have been observed in several wildlife populations living in polluted areas. (Guillette LJ Jr 1994; Guillette LJ Jr 1995; Guillette LJ Jr 1996; Jobling S 1998).

There is considerable evidence that fishes inhabiting waters that receive untreated municipal wastewaters or effluents from municipal WWTPs are exposed to chemicals that affect reproductive endocrine function (Kidd, Blanchfield et al. 2007). Estrogenic effects of treated wastewater, released into the aquatic environment, were first verified by Purdom et al., in 1994 (Purdom 1994). Purdom and colleagues reported that STW effluent was estrogenic to fish, causing feminization. The STW effluents tested were mainly domestic (rather than industrial) in source, indicating that the estrogenic component(s) were likely to be domestic in origin and were probably common to most STWs (Desbrow and Waldock 1998). Male fishes downstream of some wastewater outfalls produce vitellogenin (VTG) (a protein normally synthesized by females during oocyte maturation) and early-stage eggs in their testes, and this feminization has been attributed to the presence of estrogenic substances such as natural estrogens [17β- estradiol (E2), the synthetic estrogen used in birth-control pills 17α-ethinylestradiol (EE2), or weaker estrogen mimics such as NP in the water (Kidd, Blanchfield et al. 2007). Recent studies have also shown that concentrations of 4-NP and BPA that inhibit gonadal development and reproductive function in fish can also cause damage to the kidneys (as a consequence of VTG induction), and decreased body weight and induce stressed behavior (Magliulo L 2002). DNA damage in barnacles has also been reported (Atienzar FA 2002). Similarly, steroid estrogens that are known to be present in WWTP effluents (Desbrow and

Waldock 1998) and to cause feminizing effects in fish have been reported to be genotoxic in mammals both in cell lines and *in vivo* (Nutter LM 1991; Banerjee SK 1994; Han XL 1994; Nutter LM 1994). In laboratory studies, it has been confirmed that environmental contaminants with endocrine disrupting properties (EDCs) can disturb the development and expression of sexual characteristics in fish (Gimeno et al. 1996; Gray and Metcalfe 1997), amphibians (Hayes TB 2002), reptiles (Crain DA 1999; Willingham 1999), birds (Feyk LA 1998), and mammals (Gray LE 1994; Sharpe, Fisher et al. 1995). However, the extent to which the sexual characteristics and reproductive capabilities of natural populations are impacted by these EDCs is still not well understood.

2.6. SOURCE OF THE PROBLEM

2.6.1. Municipal Wastewater Treatment Plants and the Clean Water Act

Potable water utilities select a treatment train that is most appropriate for the contaminants found in their source water. The most commonly used processes include flocculation, sedimentation, filtration, and disinfection for surface water. Some treatment trains also include ion exchange and adsorption techniques (USEPA 2001). These conventional processes, according to the EPA are inefficient for substantially reducing certain pesticide concentrations and other EDCs including pharmaceutical and xenoestrogens from source water (USEPA 2001). Additionally, the Clean Water Act, amended in 1972 which addresses WWTP releases and the Safe Drinking Water Act (SDWA), first enacted in 1974 and amended in 1986 and 1996, which regulates contaminants in public water supplies does not have any provisions for removal or testing of xenoestrogens or pharmaceutical estrogens. Furthermore, many USA cities such as Pittsburgh and Los Angeles have significant combined sewer overflows releasing untreated

sewage directly into surface waters, thus increasing the amounts and concentrations of xenoestrogens finding their way into drinking water supplies, and commercial and subsistence fishing habitat. Many of the earliest sewer systems were combined sewers, designed to collect both sanitary wastewater and storm water runoff in a single system. These combined sewer systems were designed to provide storm drainage from streets and roofs to prevent flooding in cities. Later, lines were added to carry domestic wastewater away from homes and businesses. Early sanitarians thought that these combined systems provided adequate health protection. We now know that the overflows designed to release excess flow during rains also release pathogens and other pollutants (USEPA 2004).

2.7. CONCENTRATIONS IN THE ENVIRONMENT

Steroid estrogens have the potential to exert estrogenic effects in the low ng/L level, whereas alkylphenolic compounds are estrogenic at µg/L concentrations (Routledge, Sheahan et al. 1998). Natural and synthetic hormones are frequently detected in STW effluents and receiving surface waters with concentrations ranging from pg/L to ng/ L (Belfroid 1999; Baronti 2000; Kuch 2001), whereas alkylphenolic compounds are found in concentrations up to µg/L (Bolz, Hagenmaier et al. 2001; Stachel, Ehrhorn et al. 2003; Jin, Huang et al. 2004).

Ternes et al., (Ternes, Bonerz et al. 2007) were able to show in aerobic batch experiments that steroid conjugates such as glucuronides of E2 are rapidly cleaved in contact with activated sludge, and thus, the active form of the estrogen is released. To date, estrogenic effects on aquatic wildlife have not been conclusively linked to only one particular compound, but some chemicals are mainly responsible for higher estrogenicity indexes. Among them, the

natural estrogens estrone (E1) and E2, and the exogenous, EE2, the active ingredient in oral contraceptive pills, possess the highest estrogenicity indexes. Apart from these steroids, alkylphenols such as 4-tert-octylphenol and the technical isomer mixture of 4-NP, both breakdown products of nonionic surfactants (Desbrow and Waldock 1998), and BPA, a widely used monomer for epoxy resins, show estrogenic potentials of approximately 4 orders of magnitude lower than E2 (Ternes, Kreckel et al. 1999). Also representatives of the groups of PCBs, dioxins, phytoestrogens, pesticides, preservatives, antioxidants, or phthalic esters contribute to the daily exogenous burden of humans and wildlife with hormonally active agents (Belfroid 1999; Larsson 1999 ; Thorpe 2003; Zhou 2007).

Due to their incomplete breakdown in current municipal WWTP processes (Ternes, Kreckel et al. 1999), natural and synthetic estrogens can be found in the aquatic environment at low parts per trillion concentrations , typically at less than 5 ng/L (Belfroid 1999; Larsson 1999 ; Zhou 2007). WWTP effluents contain mixtures of individual estrogens and their mimics that differ in their ability to elicit estrogenic responses (Thorpe 2003). Combinations of estrogenic compounds are present in municipal WWTP effluents but the natural estrogens, E1 and E2 , and the synthetic E2 derivate EE2 are most responsible for most estrogenic activity in WWTP effluents (Thorpe 2003). Each xenoestrogen exhibits its own wildlife or human health risk, but synergistic effects could occur with xenoestrogen mixtures. Wildlife species are rarely exposed to single chemicals but instead are exposed to complex, fluctuating mixtures of contaminants that may act in various ways (Thorpe KL 2001; Silva E 2002; Sumpter 2003; Thorpe 2003) and that may induce combination effects (Rajapakse, Silva et al. 2002) via the same or different mechanisms. Less than 1 ng/L EE2 can cause feminization of male fishes, 4 ng/L caused abnormal reproductive development in male fathead minnows. E2 has been detected at

concentrations from 1 ng/L to 80 ng/L in surface water. Total estrogenicity (E2 equivalents) of 147 ng/L and 17 ng/L has been measured in final WWTP effluent and surface water respectively. NP and brominated biphenyls, a flame retardant have been detected between 0.1-3.7 µg/L and 0.3-4.6 mg/kg (on suspended particles) respectively and at 6-135 ng/L in river water and 2-15 ng/L in drinking water (Kuch 2001). Diethyl hexyl phthalate has been detected in sewage sludge between 15-50 ng/g (Petrovic and Barcelo 2000) and at 5-10 ng/L in surface and drinking water (Hyötyläinen 1997). Di butyl phthalate has been reported at a concentration of 5-10 ng/L in surface water and drinking water (Hyötyläinen 1997). Bisphenol A concentrations have been reported in drinking water from 300 pg/L to 2 ng/L and in river water from 500 pg/L up to 16 ng/L (Kuch 2001).

2.8. CONCLUSION

Understanding the species and concentrations of pharmaceutical and xenoestrogen in WWTP effluent, septic leachate, groundwater and surface water is imperative for environmental public health tracking of associated disease states. The current environmental concentrations of both pharmaceutical and xenoestrogens seem to be adequate to cause harmful health effects for human and wildlife and thus, human exposure to these compounds is a very valid public health concern.

Further research is needed to determine specific associations between disease states and pharmaceutical estrogen and or xenoestrogen exposure. Through the Toxic Substance Control Act (TSCA) the USEPA provides a very limited and inadequate framework to evaluate the estrogenic potential of new and existing chemical substances, intermediates and products. The TSCA program does this, as do all modern chemical evaluation programs, by

evaluating each chemical singly. It does not allow for testing the natural combinations of environmentally relevant concentrations of pharmaceutical and xenoestrogens and resulting synergies that may take place. Through modification of the CWA discharges such as WWTP effluents must be regulated due to their estrogenic potential. Additionally, through the SDWA the concentrations and number of potential estrogenic compounds present in our drinking water can be regulated. Most studies that have quantitatively measured pharmaceutical estrogens or xenoestrogens in WWTP effluents or surface water use a detection limit (DL) of 0.02 ng/L thus concentrations below this level are not measured. Yet, it is evident from the physical and reproductive malformations of fishes in these waters that there are possible health effects from concentrations below these DLs. Thus, quantitative methodologies must be established that can measure both xenoestrogen and pharmaceutical estrogenic concentration well below these levels. Estrogenic assays such as proliferation of MCF-7 human breast cancer cells (an estrogen receptor positive cell line), can be used to evaluate combined chemical estrogenicity. Naturalistic wildlife and holistic ecosystem effects studies also provide valuable risk information concerning cumulative estrogenicity at the level of a system or population, but these studies are few. Additionally, an important aspect of the total risk to humans and the environment from ingestion of xenoestrogens is the profusion of substances with estrogenic activity that are being introduced into water through municipal (household) sources or industrial processes. The EPA must incorporate appropriate methodologies to evaluate total estrogenic and other risk from environmental mixtures of the many commonly found pharmaceutical estrogens and xenoestrogens. We propose that categories of estrogens and their mimics be created for testing purposes that take into account their bioavailability, their probable uses and likely disposal paths. Ecological and other methods must be developed to

assess their likely combined impacts on living receptors in the aquatic environment through the use of sentinel species. Such research will determine the necessity for utilizing limited and competing public financial resources to invest in technology to remove pharmaceutical estrogens and xenoestrogens from surface waters and, in regulation of fish or wildlife consumption from our rivers and lakes.

3.0 ENVIRONMENTAL CONCENTRATIONS OF PHARMACEUTICAL ESTROGENS AND XENOESTROGEN IN SURFACE WATER: IMPLICATIONS FOR WILDLIFE AND HUMAN EXPOSURE

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3.1. PROBLEM STATEMENT

Low level exposure to pharmaceutical estrogens and xenoestrogens (PEXEs) is a concern that has emerged in recent research reports due to the PEXE's ability to mimic the naturally occurring estrogens and cause endocrine disruption for both humans and wildlife. PEXEs (also called environmental estrogens) enter the aquatic environment mainly through waste water treatment plant (WWTP) effluents, due to inefficient removal rates during the wastewater treatment process (Ternes, Kreckel et al. 1999). Accordingly, most of the reported adverse effects of PEXEs are found in the aquatic environment, particularly in rivers with a high charge of domestic and industrial wastewaters.

Many species in the wild have experienced genetic and physical malfunctions, particularly fishes that live in waters that receive effluents from Industrial discharge, waste water treatment plants (WWTPs), combine sewer overflows (CSOs) and sanitary sewer overflows (SSOs) (Jobling and Tyler 2003; Thorpe 2003; Sumpter and Johnson 2005). The phenomena ranges from subtle changes in the physiology and sexual behavior of fishes to permanently altered sexual differentiation and impairment of fertility (Jobling 2003; Jobling and Tyler 2003). It is believed that this is due to endocrine disrupting chemical(s) commonly referred to as xenoestrogens. It is believed that these xenoestrogens may disrupt the ER signaling pathway and contribute to infertility, developmental abnormalities, or endocrine cancer seen in wildlife and humans. Xenoestrogens have also been implicated in adverse health outcomes in humans such as testicular dysgenesis including testicular cancer and breast cancer in women.

In a large number of cities in the United States there are many CSOs and SSOs that flow directly into river and surface waters. Additionally, treated effluents from WWTPs are directly discharged into surface waters. Research has shown that PEXEs are present in WWTP effluents and river water. More specifically, published studies have reported that the naturally occurring pharmaceutical estrogens estrone (E1), 17β-estradiol (E2), estriol (E3) and the synthetic hormone 17α-ethinylestradiol (EE2); classified as a subclass, steroidal estrogens are the dominant estrogens found in WWTPs, while the xenoestrogens, Bisphenol A, alkyl phenoxylates and phthalate esters are the dominant xenoestrogens present in WWTP (Kuch 2001; Espejo, Valter et al. 2002; Sole et.al. 2005). Thus, with most of the drinking water in the United States being secured from surface waters, there exist a legitimate public health concern for the presence of these PEXEs in aqueous media and hence exposure to humans and wildlife. With the background, the aim of this paper is; 1) to establish environmental concentrations of these PEXEs in aqueous media through an extensive literature search; 2) Compare the found environmental concentrations of PEXEs in aqueous media to the predicted no effect concentration (PNEC), (a reference value) for each compound, to determine protectiveness or presumed risk for aquatic species; 3) to determine the implications for wildlife and humans.

There are many pharmaceutical estrogens but the focus of this paper is the steroidal estrogens subclass E1, E2, E3, EE2 and the xenoestrogens Bisphenol A, alkyl phenoxylates and phthalate esters. Determining the environmental concentrations of PEXEs present in surface waters are imperative for environmental public health tracking of associated environmental exposures and possible disease states. Such research will determine the necessity for utilizing limited and competing public financial resources to invest in technology to remove PEXEs from

surface waters and, in regulation of fish and, or wildlife consumption from our rivers and lakes. Also, it could be the initial step to link specific concentrations of PEXEs with disease states.

3.2. INTRODUCTION

Exposure to low levels of steroidal estrogens and xenoestrogens is a concern that has emerged in recent research reports due to their ability to mimic the naturally occurring estrogens. While strides have been made in examining some wildlife and human effects, very few studies have examined and documented the effect of low level environmental concentrations of pharmaceutical estrogens and xenoestrogens (PEXEs) in aqueous media, particularly in surface waters and, or drinking waters. Even fewer studies have examined the aquatic concentrations of PEXEs and compare them to a reference concentration to determine the relationship between environmental exposure concentration and possible risk or adverse effect for aquatic receptors. The steroidal estrogens in focus in this paper estrone (E1), estradiol (E2) and estriol (E3) are naturally occurring hormones while the synthetic hormone 17α-ethinylestradiol (EE2) and the xenoestrogens are chemicals or foreign compounds that mimic the behavior of natural hormones and bind to estrogen receptor sites of both humans and animals. PEXEs exposure can occur through various routes and their doses can be administered through absorption, ingestion, or injection. In recent years estrogen has been found to be; important for a healthy life for both animals and humans, and controls many other functions besides the reproductive system. Thus, research studying the effects of excessive PEXEs levels on both animals and humans are being conducted.

Since the initial observation by Allen and Doisy (1923) of estrogen-induced vaginal epithelial cell cornification in the ovariectomized or immature female rodent, a wide variety of non-steroidal chemicals have been shown to mimic this action, as well as other known estrogenic actions such as enlargement and/or growth of rodent uteri or avian oviducts. Such compounds have been referred to as estrogen mimics, xenoestrogens, or environmental estrogens. In 1993, Colborn et al., hypothesized that prenatal or early postnatal exposure to the large amounts of industrial-derived endocrine-disrupting chemicals that have been released into the environment since World War II could result in permanent and irreversible damage to wildlife and humans. The discussion about effects on human health is still controversial.

PEXEs enter the aquatic environment mainly through sewage treatment plant (WWTP) effluents due to inefficient removal rates during the wastewater treatment process. Accordingly, most of the reported adverse effects are found in the aquatic environment, especially in rivers with a high charge of domestic and industrial wastewaters.

Many species in the wild have experienced genetic and physical malfunctions, particularly fishes that live in waters that receive effluents from Industrial discharge, WWTPs, combine sewer overflows (CSOs) and sanitary sewer overflows (SSOs). The phenomena ranges from subtle changes in the physiology and sexual behavior of fishes to permanently altered sexual differentiation and impairment of fertility. It is believed that this is due endocrine disrupting chemical(s) commonly referred to as xenoestrogens. PEXEs have also been implicated in adverse health outcomes in humans such as breast cancer in women and testicular dysgenesis including testicular cancer. Treated effluents from WWTPs are directly

discharged into surface waters and in many cities in the United States, there are many CSOs and SSOs that directly flow into river water and surface water. Additionally, in the United States most of our drinking water is secured from surface waters. Published studies have reported that the dominant PEXEs in aqueous media are E1, E2, E3, EE2, Bisphenol A (BPA), Phthalate esters and alkylphenoxylates (Spengler, Korner et al. 2001; Huggett 2003; Williams 2003; Tan, Hawker et al. 2007). Thus, the aim of this paper is to document and determine the various environmentally relevant concentrations of these PEXEs that are present in aqueous media by reviewing published studies from various databases. Knowing the concentrations of PEXEs that are present in the environment and correlating these concentrations with their documented Predicted no effect concentrations (PNEC) will eventually**;** determine vulnerability of aqueous receptors to adverse health effects, establish possible association between exposure concentration and disease states**,** lead to policies and regulations to govern both xenoestrogens and pharmaceutical estrogens in the environment and aquatic media. The following questions can then be answered; what then are the implications of xenoestrogens in our surface waters and our drinking water supply? Is there a public health problem? Is there a need for concern? Do they contribute to disease states for the public?

3.3. ROUTES OF ENTRY OF PHARMACOLOGICAL ESTROGENS AND XENOESTROGENS INTO THE ENVIRONMENT-CONCEPTUAL MODELS OF SOURCES, TRANSPORT AND EXPOSURE

Most PEXEs enter the aquatic environment through WWTP effluents (Daughton 1999; Norris 2007). The low concentrations of individual PEXEs (possibly exceeding the catabolic enzyme affinities of sewage microbiota), coupled with their metabolic "novelty," (increase polarity) leads to their incomplete removal from WWTPs (Daughton 1999). PEXEs are also introduced into the aquatic environment through other point sources such as, combined sewer overflows (CSOs) and sanitary sewer overflows (SSOs). Also, other nonpoint sources (e.g., land fill leachate, runoff from amended soils, and street dust) have been identified as contributing additional amounts of these chemicals and metabolites (Yamamoto and Miyachi 2000; Yamamoto and Liljestrand 2003). It is therefore expected that such a widespread load of hormonally active chemicals may threaten aquatic organisms, particularly fishes, whose reproductive system and gonad development are sensitive to these pollutants (Gimeno et al. 1996; Gray et al. 1997). Below is a diagram (Figure 3-1) which is a conceptual model showing the fate and transport of PEXEs into the aquatic environment. Once these PEXEs enter the aquatic environment through various exposure routes, such as dermal or ingestion; aquatic ecological receptors, terrestrial ecological receptors and humans are exposed to them (Figure 3-1).

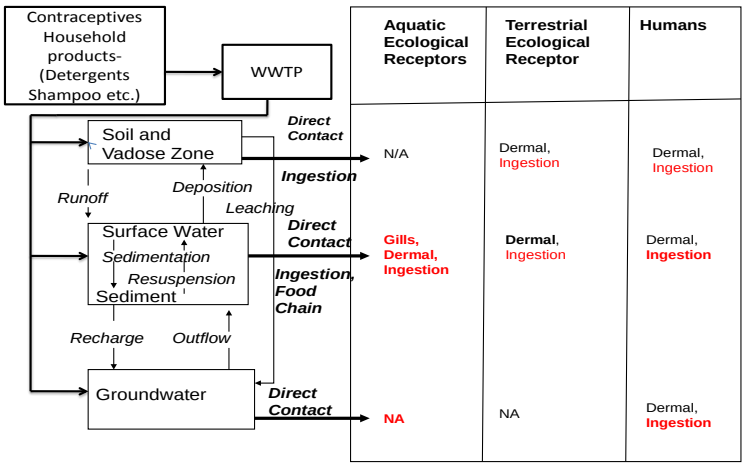

Figure 3-1 Conceptual Model Showing exposure to ecological and human receptors from household chemicals and WWTP effluents

3.4. DEFINITIONS

3.4.1. Endocrine Disruption (ED) and Pharmaceutical Estrogens and Xenoestrogens (PEXEs)

At the European workshop on the impact of endocrine disrupters on human health and wildlife, held in December 1996 in Weybridge, UK, (EUR 1996) it was agreed that "An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function". It was also agreed that "a potential endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism". As these definitions suggest it is important to develop *in vivo* systems for determination of candidate chemicals that are new or presently on the market and can be assumed to be in surface waters, mixed waste streams such as municipal

wastewater and biosolids, while *in vitro* systems would be helpful in screening new and current chemicals substances as well as assessing the effects of mixtures of potential endocrine disrupting compounds.

Endocrine Disrupting Chemicals (EDCs) are a heterogeneous class of chemicals, both man-made and natural, which are present in the environment and have the potential to alter the endocrine system of organisms (Colborn T 1993; Anway 2005). PEXEs are a part of this class of compounds. The presence of PEXEs in the environment as evidenced by the appearance of intersex fishes in many rivers and lakes in Europe, Canada and USA is a major emerging concern for regulators and policymakers, researchers, public health professionals and the general public as these chemicals have been implicated in various health endpoints, notably cancer (Anway 2005). The focus on EDCs has been on their interaction with the hormone receptors and the subsequent regulation of target genes. Following binding to the hormone receptor, EDCs may either stimulate or inhibit gene transcription in a manner similar to the natural hormone or they may inactivate gene transcription by forming receptor-ligand complexes with conformations that are unfavorable for activation (EUR 1996). Some substances have, however, been found to exert both agonistic and antagonistic effects on endocrine receptors (EUR 1996). Compounds having different mechanisms of action may cause similar biological changes. For instance, antagonists to the androgen receptor may give effects similar to those caused by estrogen receptor agonists. Besides interaction with hormone receptors, EDCs may interfere with transport proteins, alter the synthesis and biotransformation of hormones, have direct toxic effects on the gonads or have adverse effects on the hypothalamus, the pituitary or endocrine glands.

Many EDCs are classified as 'xenoestrogens' because their action mimics that of estrogen hormones. Xenoestrogens are chemicals that are man-made or produced outside of the body, and are generally synthesized chemicals, although there are plant synthesized xenoestrogens-also called phytoestrogens. Xenoestrogens are also often referred to as estrogen mimics, or environmental estrogens. Thus, xenoestrogens are a special subset of EDCs. The first reported evidence of for the effects of excess estrogen exposure came in 1923 from observations by Allen and Doisy (Allen and Doisy 1983) of estrogen-induced vaginal epithelial cell cornification in the ovariectomized and immature female rodent (Allen and Doisy, 1923). Since then, a wide variety of non-steroidal chemicals have been shown to mimic this action, as well as other known estrogenic actions such as enlargement and/or growth of rodent uteri or avian oviducts (Cook 1933; Cook 1933.; Dodds 1936; Reid 1944; Bitman 1968; Eroschenko 1981).

In 1991 a hypothesis was formulated to suggest that numerous xenobiotic chemicals used in everyday commerce or natural chemicals released into the environment by human activity had the potential to disrupt the endocrine system of wildlife and humans at ecologically relevant concentrations. This hypothesis has become known as the endocrine-disrupting contaminants (EDCs) hypothesis (Colborn T 1992). Colborn (Colborn T 1993) pointed out that large amounts of industrial-derived endocrine-disrupting chemicals have been released into the environment since world War II, and further hypothesized that prenatal or early postnatal exposure to these compounds could result in permanent and irreversible damage to wildlife and humans.

In many well documented and published cases the reproduction of wildlife has been adversely affected by EDCs (Purdom 1994; Routledge, Sheahan et al. 1998; Tyler, Jobling et al. 1998). But, the discussion about environmental effects of EDCs on human health is still controversial (Safe 2004); although it is hypothesized that EDCs are associated with decreased male reproductive capacity (Carlsen 1995; Toppari 1996; Safe 2000; Safe 2004).

Pharmaceutical estrogens such as the ones discussed in this paper, E1, E2, E3, EE2 have the potential to exert estrogenic effects in the low ng/L level. EE2 which is the synthetic birth control pill exerts estrogenic activity in WWTP effluents and surface water (Desbrow and Waldock 1998). PEXEs enter surface water mainly through WWTP effluents due to the inefficient removal rates during the wastewater treatment process (Ternes, Kreckel et al. 1999). Accordingly, most of the reported wildlife effects of PEXEs are found in the aquatic organisms, especially in rivers with a high charge of domestic including sewer overflows and industrial wastewaters (Ternes, Kreckel et al. 1999) .

Endocrine disruption (ED) is widespread in freshwater aquatic receptor populations and has been reported in numerous parts of the world (Jobling and Tyler 2003). The ED phenomena ranges from subtle changes in the physiology and sexual behavior of fish to permanently altered sexual differentiation and impairment of fertility (Jobling 2003). EDC disruption such as demasculinization has also been reported in other aquatic receptors such as amphibians (Hayes TB 2002) and in terrestrial reptiles (Crain DA 1999; Willingham 1999).

Assessing whether any EDC poses a threat to a receptor in the natural environment requires balancing information on its potency against observed environmental concentrations (Jobling 2003). In many instances it is difficult to assign causality because of the complexity of

environmental contaminant mixtures and the lack of analytical data that document contaminant levels during critical windows of exposure (Safe 2000). Nevertheless, there have been several incidents in wildlife populations that strongly correlate with exposure to specific industrial chemicals; this includes demasculinization of alligators in Lake Apopka, Florida, exposed to a spill of organochlorine pesticides from a chemical waste site (Guillette LJ Jr 1994; Guillette LJ Jr 1995; Guillette LJ Jr 1996). Several studies on wildlife populations have documented adverse effects that correlate with exposure to one or more putative endocrine-disrupting chemicals (Giesy JP 1994; Guillette LJ Jr 1994; Birnbaum 1995; Fry 1995; Guillette LJ Jr 1995; Sumpter JP 1995; Guillette LJ Jr 1996; de Solla SR 1998). Also, reproductive abnormalities have been observed in several wildlife populations living in polluted areas (Guillette LJ Jr 1994; Guillette LJ Jr 1995; Guillette LJ Jr 1996; Jobling S 1998).

In laboratory studies, it has been confirmed that EDCs can disturb the development and expression of sexual characteristics in fish (Gray 1997; Gimeno 1998; Gray and Ostby 1998), amphibians (Hayes, Collins et al. 2002), reptiles (Crain and Guillette 1998; Crain, Spiteri et al. 1999; Willingham 1999; Willingham 2001), birds (Feyk LA 1998), and mammals (Gray LE 1994; Sharpe, Fisher et al. 1995). However, the extent to which the sexual characteristics and reproductive capabilities of natural populations are impacted by these EDCs is still not well understood.

Reasonable progress has been made to identify and quantify the main estrogenic chemicals present in environmental waters. In Japanese rivers Nakada et al., (Nakada, Nyunoya et al. 2004) found that the natural estrogens E1 and E2 represented more than 98% of the total estrogen equivalent concentration (EEQ) in the WWTP effluent, and the

contribution of phenolic compounds to total EEQ was less than 2%. In contrast Desbrow et al., (Desbrow and Waldock 1998) identified the natural estrogens E1 and E2, and the synthetic steroid estrogen EE2, as the major contributors to the estrogenic activity of some WWTP effluents. Many other studies (Spengler, Korner et al. 2001; Huggett 2003; Williams 2003; Tan, Hawker et al. 2007) have subsequently supported these conclusions that the natural and synthetic steroidal estrogens contribute most to estrogenic activity in WWTP effluents. This is of importance since WWTP effluents flow directly into lakes and rivers. However, there are some locations, often apparently associated with particular industries such as the textile industry, where alkylphenolic chemicals (such as nonylphenol and its ethoxylates) appear to contribute significantly to the total estrogenicity of a river or effluent (Kuch 2001; Espejo, Valter et al. 2002; Sole et.al. 2005).

3.4.2. **Steroid Hormones**

The steroid hormone family consists of different groups that are recognized by their physiological functions. There are three systems. They include the female secondary characteristic inducing hormones, estrogens (mainly E2, but also E1 and E3); the male counterpart, androgens (mainly testosterone and its derivative 5α-dihydrotestosterone) and the progestins essential for pregnancy (progesterone, 17-hydroxyprogesterone and 17,20-dihydroxyprogesterone). In addition to regulating reproductive systems, the sex hormones also have effects on many other functions, such as growth, hemoglobin production and calcium metabolism in the skeleton. Besides being synthesized in gonads, a number of steroid hormones are synthesized by the adrenal cortex. Small amounts of adrenal sex hormones and glucocorticoids are produced in zona reticularis. The major part of the adrenal sex steroids are

androgens but small amounts of estrogens and progesterone are also produced. Adrenal androgens are of little physiological importance in the male, but in adult women they are thought to play a role for the sex drive. When secreted in abnormal amounts, as in patients with congenital enzyme deficiencies in the adrenal gland or in patients with adrenal tumors, they have effects that depend on the sex and age of the individual. In prepubertal males and in females the effects can be dramatic. Females may develop a beard, a masculine pattern of body hair distribution and the clitoris may grow to resemble a small penis.

3.4.3. **Xenoestrogens**

Xenoestrogens are synthetic substances that differ from those produced by living organisms and mimic or enhance the effect of estrogens. The estrogenic stimulation is an unintended side-effect of these agents or their metabolites. Xenoestrogens are part of a heterogeneous group of chemicals that are hormone or endocrine disruptors. They differ from phytoestrogens (estrogenic substances from plants), mycoestrogens (estrogenic substances from fungi), and pharmacological estrogens (estrogenic action is intended). There are several classes of xenoestrogens such as the alkylphenoxylates, brominated compounds and phthalate esters. External estrogens from a variety of sources may have a cumulative effect upon living organisms, and xenoestrogens may be part of a larger picture of a process of estrogenization of the environment. Xenoestrogens have only been recently (less than 70 years) introduced into the environment since their initial production by industrial, agricultural, and chemical companies.

The ubiquitous presence of such estrogenic substances is a significant health concern, both individually and for a population. Life relies on the transmission of biochemical

information to the next generation, and the presence of xenoestrogens may interfere with this transgenerational information process through "chemical confusion" (Vidaeff AC 2005).

Xenoestrogens have been implicated in a variety of medical problems. Foremost is the concern that xenoestrogens as false messengers disrupt the process of reproduction. Studies have implicated observations of disturbances in wildlife with estrogenic exposure. Reproductive issues which are of concerns in humans are fetal exposure (perhaps leading to hypospadias) and decreased reproductive ability in men (i.e. decrease in sperm numbers). Another issue of concern is the potential effect of xenoestrogens on oncogenes, specifically in relation to breast cancer (http://www.ourstolenfuture.org/NewScience/oncompounds/bisphenola/bpauses.htm 2007).

There are many synthetic chemicals used daily that are shown to have unintended estrogenic effects. Some are; 4-Methylbenzylidine camphor (4-MBC) (sunscreen lotions) , hydroxy-anisole butyrate (food preservation), atrazine (weedkiller), bisphenol-A (food preservation, plasticizers), di (2-ethylhexyl)phthalate (DEHP), dieldrin (insecticide), DDT (insecticide), hexachlorocyclohexane (insecticide), endosulfan (insecticide), erythrosine, Red Dye No.3, heptachlor, methoxychlor (insecticide), Polychlorinated biphenyls,(PCB)s (lubricants, adhesives, paints), p-nonylphenol (in PVC products and byproduct from detergents and spermicides), parabens (lotions), phenosulfothiazine, phthalates (plastic softener).

3.5. PUBLISHED STUDIES REPORTING ENDOCRINE DISRUPTION

3.5.1. Wildlife Studies

The study of the problem of PEXEs and their effect on wildlife is difficult and complex. Transgenerational effects are difficult to prove, effects may be multifactorial, and the large

variety of substances in question with the absence of unexposed controls do not lend themselves to easy interpretation. Believers that environmental estrogen disruption is a major health hazard are opposed by detractors who argue that observed effects are spurious and inconsistent, or that the quantities of the agents are too low to have any effect. A 2005 study by Belcher and coworkers demonstrated that even very low levels of a xenoestrogen, in this case BPA could affect fetal neural development more than higher levels (Zsarnovszky, Le et al. 2005), indicating that classical models where dose equals response may not be applicable in susceptible tissue**.** Wildlife species are rarely exposed to single chemicals but instead are exposed to complex, fluctuating mixtures of contaminants that may act in various ways (Thorpe KL 2001; Silva E 2002; Sumpter 2003; Thorpe 2003) and that may induce combination effects (Rajapakse, Silva et al. 2002) by same or different mechanisms. Most of the field observations noted have been confirmed in the laboratory where large doses of environmental estrogens have produced reproductive abnormalities in exposed animals (Colborn T 1993; Safe 2000).

The effects of environmental endocrine disruptors on wildlife populations are being extensively investigated; adverse developmental and reproductive effects have been primarily linked to organochlorine compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), as well as alkylphenols derived from alkylphenol ethoxylate (AE) surfactants. Persistent organochlorine pollutants (POPs), including both pesticides such as DDT/DDE and PCBs, were among the first industrial compounds identified in the environment (Colborn T 1993). The use and production of DDT and PCBs were restricted and banned in most countries in the 1970s; however, these compounds are still the most abundant POPs in most wildlife and human samples, even though their

concentrations have significantly decreased over the past 30 years (Safe 2000). This finding is attributed to the improvement in analytical techniques and instrumentation, an ever-increasing number of structurally diverse POPs have been detected in environmental samples at low concentrations (Kutz FW 1991; Giesy JP 1994; Vallack HW 1998).

The major estrogenic components found in British Rivers receiving domestic treatment effluents were the natural hormones E2 and E1, with minor amounts of the birth control pill ingredient 17α-ethinylestradiol. Jobling et al.,(Jobling, Williams et al. 2006) found that both the incidence and the severity of intersex in wild roach were significantly correlated with the predicted concentrations E1 and E2 and the synthetic contraceptive pill EE2. Additionally, the predicted steroid estrogen exposure was less well correlated with the plasma vitellogenin concentration measured in the same fish (Jobling, Williams et al. 2006).

Liney et al., (Liney, Hagger et al. 2006) found that early life-stage roach, *Rutilus rutilus,* exposed to treated wastewater effluent reported induced feminization of male roach, measured as vitellogenin induction and histological alteration to gonads, also reported statistically significant alterations in kidney development (tubule diameter), modulated immune function (differential cell count, total number of thrombocytes), and genotoxic damage (micronucleus induction and single-strand breaks in gill and blood cells). More importantly, they also found that genotoxic and immunotoxic effects occurred at concentrations of wastewater effluent lower than those required to induce physical defects. WWTP effluents contain a mixture of natural and synthetic xenobiotics, household and agricultural chemicals, pharmaceuticals, hormones, and other compounds, many of which remain unidentified (Stevens JL 2003). Liney et al., reported that several studies have correlated exposure to WWTP

effluent with alterations in sex steroid hormone levels in adult and juvenile fish (Folmar LC 1996; Folmar LC 2001a; Hecker M 2002), impaired gonadal development in adults and juveniles (Hemming, Waller et al. 2001; Jobling, Coey et al. 2002; Sheahan, Brighty et al. 2002), altered sexual differentiation in early life stages (Rodgers-Gray, Jobling et al. 2001), and induction of the egg-yolk precursor protein vitellogenin (VTG) in adult male and juvenile fish of both sexes (Purdom 1994; Rodgers-Gray, Jobling et al. 2001; Rodgers-Gray, Smith et al. 2004). These effects have been associated with the presence of chemical contaminants in the effluents that act as estrogen receptor agonists, including natural and synthetic steroids (Desbrow and Waldock 1998; Routledge, Sheahan et al. 1998), alkylphenol polyethoxylates (Jobling and Horn 1996; Seki M 2003), and phthalates and pesticides (Jobling, Reynolds et al. 1995; Ankley 1998; Christiansen, Kinnberg et al. 2000). These disorders, however, may not necessarily be a consequence of estrogenic effects alone.

3.5.2. **Laboratory Studies**

Strong support for the endocrine-disruptor hypothesis has come from laboratory animal studies where increasing numbers of synthetic chemicals have been shown to exhibit estrogenic/antiestrogenic, androgenic/ antiandrogenic, and other endocrine like activities . Studies on in utero exposure to the estrogenic drug diethylstilbestrol (DES) have served as an important model for delineating problems associated with exposure to estrogenic compounds in both animal models and in humans; DES-induced effects on the male and female reproductive tracts strongly support the endocrine-disruptor hypothesis (Colborn T 1992; Colborn T 1993; Anway 2005). vom Saal et al., (vom Saal 2005a) reported that fetal exposure to

low doses of BPA (2 or 20 pg/kg/day) resulted in increased prostate weight in the male offspring.

In laboratory studies, it has been confirmed that environmental contaminants with endocrine disrupting properties (EDCs) can disturb the development and expression of sexual characteristics in fish (Gimeno et al. 1996; Gray and Metcalfe 1997), amphibians (Hayes et al.2002), reptiles (Crain et al. 1999; Willingham and Crews 1999; Willingham et al. 2000), birds (Feyk and Giesy 1998), and mammals (Gray et al. 1994, Sharpe et al. 1995).

Low doses of xenoestrogens can cause major reproductive deficits in the experimental animals as reported by Fusani et al in 2007 in a study where male and female rats were exposed to low doses of the pure estrogen, ethinylestradiol during development, by oral administration to their mothers during pregnancy and lactation, and to them until puberty, evaluated the effects of the exposure on development and reproductive physiology of individuals, and on fertility and fecundity of pairs in which both members had been exposed to the same treatment(Fusani, Della Seta et al. 2007). They concluded that environmentally relevant doses of xenoestrogens which have no evident physiological effects can alter the reproductive success of exposed pairs in natural populations.(Fusani, Della Seta et al. 2007).

3.6. METHOD

An extensive literature search of all available data bases such as EBSCO, Pubmed and scientific journals was conducted. The criteria for the search were; find any reported concentration of PEXEs in focus in the study in aqueous matrix from the environment, including all surface water types as well as effluents entering surface waters; studies were not restricted to any one

country, they could be used from any part of the world; studies must be peer reviewed and published. Sediment concentrations were recorded (Table 3-1) if they were found in the studies, but they were not included in the analysis (Table 3-2) as they were not the focus of this study. Studies that were selected for use based on the criteria established above were recorded in Table 3-2 by study country, which is the country of origin for the study; chemical of concern, which is the chemical(s) found in the study; environmental concentration found or reported, which is the concentration of the chemical found; matrix, which is type of aqueous media, that is, river water, effluents etc., and results, which is the reported result(s) of the study. Maximum environmental concentration (MxEC) and minimum environmental concentrations (MnEC) for the PEXEs of concern in this study were determined from Table 3-1 for WWTP effluents, industrial effluents, drinking water and river water and tabulated along with their PNECs (Table 3-2). Measured environmental concentrations (MECs) of PEXEs in WWTP effluents, river water, drinking water and industrial effluents were used together with predicted no observed effect concentrations (PNECs) obtained from peer-reviewed literature, to calculate risk quotients expressed as MEC/PNEC ratios (Lindberg and Bjorklund 2007), Table 3-2. The risk quotient was used as an indicator to determine protectiveness or possible risk for the aquatic species at environmentally relevant concentrations.

3.7. RESULTS

The literature reports different and varied concentrations of PEXEs in the aquatic environment. Twenty five (25) studies were found matching the criteria established in the methodology Section and tabulated in Table 3-1. The following are the findings; Viganò et. al., ((Vigano,

Mandich et al. 2006; Viganò 2006) embarked upon a study to determine the concentrations of PEXEs along the river Po, a major waterway in Italy. They reported environmental concentrations of the steroidal estrogens (E1, E2, E3, EE2), OP, NP and BPA at the upper, middle and lower portions of the confluence River Lambro (a polluted tributary in the middle of the river Po). They reported mean concentrations of estrogens and xenoestrogens in water samples (n = 9) collected from the middle River Po upstream and downstream (22 km) of the confluence of the River Lambro as well as from the tributary. Mean steroidal concentration ranged from 0.001µg/L to 0.047µg/L with EE2 being detected in one sample at 0.002µg/L. Mean NP concentration ranged from 0.003µg/L to 0.269µg/L, while mean OP concentration ranged from 0.010ng/L to 0.014ng/L. Mean BPA concentration ranged from 0.270µg/L to 0.302µg/L, mean tOP concentration ranged from 0.015µg/L to 0.073µg/L; while sediment concentration of NP ranged from 3.89ng/g to 120ng/g and tOP concentration from 3.73ng/g to 6.09ng/g.

Shappell (Shappell 2006) reported that in a study to determine estrogenic activity in regional water samples taken from the states of North Dakota and Minnesota, samples were taken from wetland and ponds surrounding agricultural land (in use and inactive), river water and municipal wastewater lagoons. Estrogenicity, expressed as estradiol equivalents (EEq) was 5.2 x10^{-13} *M* EEq for wetland and ponds, 5.8 x 10^{-13} *M* EEq for river water, 7.6 x 10^{-12} *M* EEq for municipal waste water, and 7.7 x 10^{-12} *M* EEq found in lagoons. They concluded that estrogenic activity in surrounding wetlands and ponds from different agricultural land uses was not different with the highest activity found downstream from municipal wastewater treatment effluent discharge sites, in winter when river flow was lowest. Further, Dorabawila et al.,

(Dorabawila and Gupta 2005) tested surface water samples from ponds, rivers (Wicomico, Manokin and Pocomoke), sewage treatment plants (WWTPs), and coastal bays (Assawoman, Monie, Chincoteague, and Tangier Sound – Chesapeake Bay) on the Eastern Shore of Maryland for E2. In river waters-E2 concentration ranged from 1.9ng/L to 6.0 ng/L; E2 concentration in all the coastal bays tested was 2.3ng/L to 3.2ng/L.

Sole et al., (Sole et.al. 2005) examined and analyzed water composites of WWTP influents, effluents, sludge, river water and sediment for synthetic and natural estrogen over a seven-month period in two tributaries of the Llobregat River in northeastern Spain. They reported that natural and synthetic estrogens occurred in the water and sediments analyzed but, in the ng/L and µg/kg range, respectively. Sole and colleagues reported concentrations of up to 31 µg/L for nonylphenol ethoxylates (NPEOs), 15 µg/L for nonylphenol (NP), and 35 µg/L for nonylphenoxy carboxylate (NPE1C) in river water downstream of WWTPs. Concentrations of xenoestrogens found in river sediment ranged from 10µ/kg to 820 µg/kg of NPEOs and from 22µ/kg to 645 µg/kg for NP.

In 2005 Beck et al., (Beck 2005) investigated estrogenic compounds in German coastal surface water off the Baltic sea. They determined concentrations for E1, E2, E3, EE2 (all steroidal estrogens) and three xenoestrogens (NP, 4-*tert*-OP, BPA) in coastal marine waters were 0.1µg/L to 17µg/L for the steroidal estrogens, 0.22µg/L to 5.4µg/L for BPA, 4.2µg/L to 6.1ug/L for NP and 0.11µg to 0.6µg/L for 4-tert-OP.

Pawlowski and colleagues (Pawlowski 2004) determined estrogenicity of solid phase-extracted water samples from two municipal WWTP effluents and from water from the river Rhine. They reported that estrogenic activity was lower in river water than in WWTP effluents.

Estrogenic activity was detected in the effluents of both WWTPs with values of 0.242 +/- 0.038 nM (65.96 +/- 10.4 ng/L) and 0.125 +/- 0.026 nM EEqs (34.1 +/- 7.18 ng/L). In river water, the total estrogenic activity of steroidal estrogens was equal to 0.014 nM EEq (3.8 ng/L).

Nakada et al., (Nakada, Nyunoya et al. 2004) in their study to identify estrogenic compounds in WWTP effluents discharged to the Tamagawa River in Tokyo, Japan, reported that; E1 and E2 were the dominant environmental estrogens in WWTP effluents. They concluded that a significant contribution to estrogenic activities stems from unidentified components in the effluents. Also, the averaged concentrations of NP, BPA, E1, and E2 were 564 ± 127ng/L, 27 ± 19ng/L, 33 ± 11ng/L, and 4.6 ± 3.0 ng/L, respectively. Based on the concentration and relative potency of these compounds, the natural estrogens E1 and E2 represented more than 98% of the total estrogen equivalent concentration (EEq) in the WWTP effluent, while the contribution of phenolic compounds to total EEq was less than 2%. Also results by Furuichi et al., (Furuichi 2004; Furuichi, Kannan et al. 2006) from water samples taken from various locations in the Tamagawa River in Tokyo, Japan characterized E1 and E2 as the major contributors to estrogenic activity in river water. Interestingly, Nakada, (Nakada, Nyunoya et al. 2004) in the previous study reported this same conclusion for WWTP effluents flowing into this river. Furuichi and colleagues reported concentration ranges for; E1 between 17.1ng/L to 107.6ng/L, E2 between 2.6ng/L and 14.7ng/L, EE2 less than 0.02ng/L (<0.02) which is the detection limit (DL), BPA between 16.5ng/L and 150ng/L, NP and OP at concentrations in the range of 51.6ng/L–147ng/L and 6.9ng/L–81.9 ng/L, respectively.

Kawaguchi et al., 2004 (Kawaguchi, Inoue et al. 2004) investigated the upstream, midstream and downstream portions of same Tamagawa river for phenolic xenoestrogens

and found the following concentration ranges; 2,4- dichlorophenol (DCP) 29.8pg/ml to 81.4pg/ml, 4-tert butylphenol (BP) 7.2pg/ml to 26.8pg/ml, OP, <2pg/ml to 19.2pg/ml, NP, 37.6pg/ml to 57.9pg/ml, pentachlorophenol (PCP)<10pg/ml which is the detection limit, BPA, 41.5pg/ml to 72.2pg/ml. The researchers determined that the concentrations of the detected compounds were higher in the downstream samples than in the upstream samples and considered that the contamination came from the drainages for homes and industries.

In 2003 (Pawlowski, Ternes et al. 2003) investigated the estrogenic activities of two municipal sewage treatment plant effluents and of the Rhine river water. Chemical analysis of representative water samples identified steroidal estrogens up to 5.6 ng/L for E2, 19 ng/L for E1 as well as 1.5 ng/L for EE2; the Rhine river contained 3.9 ng/L E2. Thus, the authors concluded that, WWTP effluents and Rhine water contained biologically relevant concentrations of estrogenic compounds.

Laganà 2004 (Laganà, Bacaloni et al. 2004) verified the occurrence of endocrine disrupters in environmental samples of sewage influents and effluents of an Italian WWTP and the river Tiber. Alkylphenols were detected in effluents in the range 13–36 ng/L for bisphenol A and up to 1 µg/L for nonylphenol. Estrogens were determined in effluents at levels below 30 ng/L. Analysis of river (Tiber) receiving effluent waters revealed high quantities of bisphenol A in a range of (15–29 ng/L) and nonylphenol (up to 1.2 µg/L), whereas the presence of all the other compounds were at levels of a few ng/L.

Céspedes et. al.,(Céspedes, Petrovic et al. 2004) determined the estrogenic activity in surface water. Water samples were collected from several different points in seven Portugal rivers. They utilized an integrated chemical ecological approach plotting a dose response curve

and extrapolating the concentration at fifty percent effective concentration (EC50). The authors reported EC50 values for the natural and synthetic (E1, E2, EE2, E3) as 40ng/L to 2120ng/L, alkylphenol (OP, NP, NP1EO) as 80µg/L to 2995µg/L and BPA as 2.3mg/L to 7mg/L.

In another study in the United Kingdom, Williams et al.,(Williams 2003) measured the concentration of E1, E2 and EE2 in WWTP effluents, river water from the Nene and Lea upstream and downstream from WWTP and in the riverbed sediment to determine steroid estrogen profile along river stretches arising from WWTP discharges. The following were the concentrations found; in WWTP effluents-E1 (<0.4 - 12.2 ng/L), E2 (<0.4-4.3 ng/L), EE2 (<0.4-3.4 ng/L);, in river water-E1 concentration on sediments ranged from <40ng/kg-388ng/kg and E1 concentration in river water was <0.4-2.5ng/L.

Hugget et. al., (Huggett 2003) reported PEXEs concentrations in WWTP effluents from New York. They reported E1 concentrations ranged from below detection (≤1 ng/L) to 42 ng/L, and E2 concentrations varied from no detection to 20 ng/L while NP was identified in each of the four New York samples at concentrations ranging from 12 to 79 µg/L.

Espejo et. al., (Espejo, Valter et al. 2002) determined 18 isomeric 4-nonylphenols and 4-*tert*.-octylphenol in wastewater from WWTP in Switzerland and reported that the average concentration of free alkylphenols in the wastewater from the sewage plant in Aïre, Geneva (Switzerland) ranged from 1.0 to 6.8 µg/l (average 2.5 µg/L).

Fromme et al (Fromme, Kuchler et al. 2002) measured the xenoestrogens, bisphenol A (BPA), bisphenol F (BPF), butylbenzyl phthalate (BBP), dibutyl phthalate (DBP), and di(2-ethylhexyl)phthalate (DEHP), in various media (surface water, sediments, sewage treatment

plant effluents, sewage sludge, dump water, liquid manure) in order to understand exposure to these compounds in different environments. They reported that BPA measurements showed low concentrations from 0.0005 to 0.41µg/L in surface water, in sewage effluents from 0.018 to 0.702µg/L, in sediments from 0.01 to 0.19mg/kg and in sewage sludge from 0.004 to 1.363mg/kg. DEHP dominated the phthalate concentrations, which ranged from 0.33 to 97.8µg/L (surface water), 1.74 to 182µg/L (sewage effluents), 27.9 to 154mg/kg (sewage sludge) and 0.21 to 8.44mg/kg (sediment).

Körner et. al., 2001(Körner 2001) investigated the presence of estrogenic substances in the water of the small streams Körsch (Kö) and Krähenbach (Kr), southwest Germany, by chemical and biological analysis. The authors postulated that because a large proportion of the Kö water near its mouth consisted of WWTP effluents, the impact of WWTPs on levels of estrogens in surface water is an environmental issue of concern. Further, they reported that in July 1996, water samples were taken from Kr and Kö (four sites) and tested in the E-Screen assay with human MCF-7 breast cancer cells and all Kö samples induced estrogen dependent cell proliferation, resulting in EEq concentration between 3.3ng/L and 9.7 ng/L while the Kr water showed no effect. Also, in 1998/99 eight samples taken from Kö (near its mouth) and nine samples taken from Kr were collected and tested in the E-Screen after solid phase extraction. Estrogenicity was detectable in three Kr samples but Kö samples had a median EEq of 3.1 ng/L (range: 1.2–42 ng/L). GC/MS analysis revealed differences in the levels of E2 and E1 between the two streams. E2 was detectable in five Kö samples only (0.7–1.8 ng/L). E1 was found in the Kö from 2.5 to 38 ng/L (median: 7.6 ng/L) and in the Kr between 0.8 and 22 ng/L (median: 1.7 ng/L).

Peñalver et al., (Peñalver 2002) measured concentration of phthalate esters in river water and industrial effluents in the Ebro river, Spain. They reported di-n-butylphathalate at 0.4µg/L and di-ethylhexylphthalate at 3.2µg/L.

Kuch et al., (Kuch 2001) in a study to determine endocrine disrupting phenolic and estrogenic compounds in surface and drinking water found the following concentrations in drinking water; BPA-300 pg/L to 2 ng/L NP-2 to 15 ng/L, OP-150 pg/L to 5 ng/L, Steroid hormones-100 pg/L to 2 ng/L and in river water the following concentration; BPA-500 pg/L up to 16 ng/L, NP-2 to 15 ng/L, OP-150 pg/L to 5 ng/L. Xenoestrogens were also found in drinking water by Japanese researchers (Inoue, Yoshie et al. 2002). They found trace concentrations of BPA-0.08ng/ml, NP-0.05ng/ml, and OP-0.04ng/ml in three drinking water samples (bought in Tokyo groceries). In addition, xenoestrogens were detected in Japanese river water in a range of 0.01–0.17 ng/ml.

In 2000, Sole et al., (Sole, Lopez de Alda et al. 2000) determined the concentration of DES-43ng/L in WWTP influent and 34 ng/L in WWTP effluent while NP levels ranged from 6 µg/L to 343 µg/L in the WWTPs and from non-detected to 644 µg/L in the receiving waters; for the Llobregat river.

Corcia et. al.,(Corcia 2000) in a study on the occurrence and abundance of dicarboxylated metabolites of NP polyethoxylate surfactant in WWTP effluent in Italy, reported that relative abundances of nonyl phenol ethyoxylates (A_9PE), nonyl phenol ethyoxlates carboxylates (A_9PECs), and dicarboxy ethoxylates (CAPECs) found were 10 ± 2, 24 ± 5, and 66 ± 7 respectively. This study was carried out for 4 months and they reported that on the average, CAPEC amounts having one ethoxy unit ($CAPE_2Cs$) were almost double those of species having

only a phenoxy acid moiety ($CAPE_1Cs$). CAPEC species having more than four ethoxy units were never detected.

Desbrow et al., (Desbrow and Waldock 1998) reported the following steroid estrogen at the following concentration; E1(1- 76 ng/L), E2(1- 48 ng/L), EE2 (<1 to 7 ng/L) in WWTP effluents that are being discharged into British rivers.

In 1997 Castillo (M. Castillo 1997) and colleagues investigated industrial effluents for phthalate esters and found a concentration of 0.16 to 54.4µg/L. Field(Field and Reed 1996) investigated the concentration of NPECs in paper mill effluents (industrial) as well as WWTP effluents received by the Fox river, Wisconsin and also NPEC concentration from this river. NPEC concentration range for paper mill effluents was 18 to 1270µg/L; 140 to 273µg/L for WWTP effluents; 2.0 to 13.8µg/L for river water. The authors reported that water from other rivers in the Eastern United States was analyzed and the NPEC concentration ranged from 1.4 to 13.5µg/L for these rivers.

Table 3-1 Found Concentration of Pharmaceutical Estrogens and Xenoestrogens

Study Country	**Chemical of concern**	**Environmental Conc. Found (reported)**	**Matrix**	**Study Objective**	**Results**
Viganò et. al., 2006 Italy	E1,E2,E3, EE2, OP, tOP, NP, BPA	Mean concentrations <u>River water</u> <u>Upper stream</u> E1, 0.004 ± 0.003µg/L E2, 0.001 ± 0.002µg/L E3, 0.004 ± 0.004µg/L EE2, ND NP, 0.003 ± 0.008µg/L OP, 0.014 ± 0.015µg/L BPA, 0.270 ± 0.330µg/L tOP, 0.015 ± 0.007µg/L <u>Downstream</u> E1, 0.006 ± 0.006µg/L E2, 0.001± 0.001µg/L E3, 0.006± 0.008µg/L EE2, ND OP, 0.010 ± 0.0020µg/L NP, 0.011 ± 0.024µg/L BPA, 0.302 ± 0.245µg/L tOP-0.019 ± 0.007µg/L <u>River Lambro</u> E1, 0.047 ± 0.024µg/L E2, 0.004 ± 0.002µg/L E3, 0.050 ± 0.062µg/L EE2, 0.002 ± 0.004µg/L NP, 0.269 ± 0.0091µg/L OP, 0.012 ± 0.008µg/L BPA, 0.494 ±0.297µg/L tOP, 0.073 ± 0.020µg/L <u>River Sediment</u> NP, 3.89 ± 120 ng/g tOP, 3.73 ± 6.09ng/g	River water Sediment	Investigate concentrations of PEXEs in the Upper and Middle section of the Po River	Natural and xenoestrogens were detected in water and sediment samples

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Shappell, 2006 US	Estrogenicity Determination using estradiol equivalents (EEq)	5.2 x10^{-13} *M* EEq.-wetland and ponds; 5.8 x 10^{-13} *M* EEq.-river water 7.6 x 10^{-12} *M* EEq-municipal waste water 7.7 x 10^{-12} *M* EEq-lagoons	Wetlands, agricultural ponds, river water, wastewater lagoons	Determine Estrogenic activity of regional water samples from various locations.	No difference in estrogenic activity in surrounding wetlands and ponds from different land uses. The highest activity was found downstream from municipal WWTP effluent discharge sites, in winter when river flow was lowest
Dorabawila et. al., 2005 USA	E2	In river waters- E2, (1.9- 6.0) ng/ L E2 concentrations in all the coastal bays tested were 2.3-3.2 ng/L	Surface water WWTP Coastal bay	Surface water samples from ponds, rivers, WWTPs, and coastal bays on the Eastern Shore of Maryland were analyzed for E2.	Highest E2 concentrations in river waters were observed Immediately downstream of WWTPs.
Sole et.al., 2005 Spain	NP, NP ethoxylates	<u>In river water downstream of WWTPs</u> NPEOs-31µg/L, NP-15µg/L NPE1C- 35µg/ L <u>In river sediment</u> NPEOs -10 to 820µ/kg NP -22 to 645µg/kg	River water River sediment	Chemical analysis of natural and synthetic estrogenic concentration in two tributaries of the Llobregat River (NE Spain).	Nonylphenol and nonylphenol ethoxylates the water and sediments in the µg/L and µg/kg range, respectively.
Beck et. al., 2005 Germany	E1, E2, E3, , EE2, NP, 4*t*OP, BPA	E1, (0.1-0.5)µg/L E2-,ND(<0.3)ng/L E3, ND(1.0)ng/L EE2,(1.7-17)µg/L BPA,(0.22-5.4)µg/L NP, (4.2-6.1)µg/L 4tOP, (0.11-0.6)µg/L	Marine waters	Determine concentrations of naturally occurring estrogens (E1, E2, E3,), one synthetic hormone (EE2) and three xenoestrogens (NP, 4-*tert*-OP, BPA in coastal marine waters.	E1, EE2, BPA, NP and 4-*t*-OP were found. E1 and EE2 in the range of effect concentrations (reported in the literature) for aquatic organisms
Ternes et. al., 2004 Germany	Estrogenicity as E2 equivalents	<u>WWTP effluents</u> 0.242 +/- 0.038 nM (65.96 +/- 10.4 ng/l) and 0.125 +/- 0.026 nM EEqs (34.1 +/- 7.18 ng/l) <u>River water</u> 0.014 nM EEq (3.8 ng/L).	WWTP effluents, River water	Determine estrogenicity of solid phase-extracted water samples from two municipal sewage treatment plant effluents and river Rhine water.	In river Rhine water, estrogenic activity was lower, however, displaying significant differences between the left and right bank of the river.

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Nakada et. al., 2004 Japan	NP, BPA, E1, E2	Average concentrations of NP, BPA, E1, and E2 were 564 ± 127, 27 ± 19, 33 ± 11, and 4.6 ± 3.0 ng/L, respectively.	WWTP effluent	Identify the dominant contributors to estrogenic activity in environmental waters	E1 and E2 were the dominant environmental estrogens in the WWTP effluent,
Furuichi et. al., 2004 Japan	E1 E2 EE2 BPA NP, OP	E1,(17.1-107.6)ng/L E2, (2.6-14.7)ng/L EE2 (<0.2)ng/L BPA(16.5-150)ng/L NP (78-147)ng/L OP(6.9-81.9)ng/L	River water	To quantitatively characterize the substances contributing to estrogenic activity in river water,	E1 & E2 were the major contributors to the estrogenic activity in the Tama River.
Kawaguchi et. al., 2004. Japan	2,4-DCP, 4-*tert*-BP, 4-*tert*- (OP), NP, PCP, BPA	2,4-DCP, (29.8-81.4)pg/ml BP, (7.2-26.8)pg/ml OP, (-<2-19.2)pg/ml NP, (37.6-57.9)pg/ml PCP, (<10)pg/ml BPA, (41.5-72.2)pg/ml	River water	River water was sampled from three sites (upstream, midstream and downstream) of Tama River, Tokyo, Japan	2,4-DCP, BP, OP, NP and BPA were detected in the river water samples. Concentrations were higher in the downstream samples than in the upstream samples.
Pawlowski et. al., 2004 Germany	E1, E2, EE2.	WWTP effluents E2-, 5.6 ng/L E1-19 ng/L EE2- 1.5 ng/L River water E2-3.9ng/L	WWTP effluents River water	To investigate the estrogenic activities of two municipal sewage treatment plant	WWTP effluents contained E1, E2 and EE2 while river water contained E2 only.

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Laganà et. al., 2004 Italy	BPA, NP, E2, E1, E3, EE2	Influents E1,(15-60)ng/L, E2, (10-31)ng/L E3, (23-48)ng/L EE2-ND BPA, (332-339)ng/L NP, (4194-8768)ng/L Effluents E1, (5-30)ng/L E2, (3-8)ng/L E3, (ND-1)ng/L EE2, ND BPA, (13-36ng/L NP, (1120-2235)ng/L River water E1, (5-12)ng/L E2, (2-6)ng/L E3, (2-5)ng/L EE2, (ND-1)ng/L BPA, (15-29)ng/L NP, (1289-1466)ng/L	WWTP Influents, WWTP Effluents, River water	Determine trace amounts of estrogenic compounds in sewage and surface waters.	E1, E2, E3, BPA and NP were present in both influent and effluents and river water. EE2 was only detected in river water.
Cespedes et. al., 2004 Portugal	Natural and synthetic (E1, E2, EE2, E3), alkylphenol (OP, NP, NP1EO) BPA	Natural and synthetic 40-2120ng/L Alkylphenol 80-2995 µg/L BPA 2.3-7mg/L These are EC50 values	Surface water	Determination of estrogenicity in natural waters.	Estrogenic activity observed was mainly attributed to the presence of alkylphenolic compounds.
Williams et. al., 2003 UK	E1, E2, EE2	WWTP effluents E1 (<0.4 - 12.2) ng/L. E2 (<0.4-4.3) ng/L, EE2, (<0.4-3.4) ng/L Sediments- E1, (<40ng/kg-388)ng/kg River water E1, (<0.4-2.5)ng/L	STW Effluents River water River sediment	To measure conc. of E1, E2, EE2 in WWTP effluents, in the water column and in the bed sediment of the River Nene and the River Lea, U.K., upstream and downstream of WWTP.	E1 was detected at the highest concentration and in almost all samples from the three WWTP effluents.
Huggett et. al., 2003 USA	E1, E2, NP	E1, (≤1 ng/l- 42 ng/L), E2, (≤1 ng/l -200 ng/L. NP, (12 to 790 µg/L	Municipal wastewater	Determine concentrations of PEXEs in WWTP	E1, E2 and NP were the PEXEs found.

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Espejo et. al., 2002 Switzerland	NP OP	Ranges from 1.0 to 6.8 µg/L(average 2.5 µg/L) for free alkyl phenols, "bonded" 4-alkylphenols can reach about 0.66 mg/L	Wastewater	Determination of 18 isomeric 4-nonylphenols and 4-*tert*.-octylphenol in wastewater	The average concentration of free alkylphenols in the wastewater of the sewage plant in Aïre, Geneva (Switzerland) ranges from 1.0 to 6.8 µg/L (average 2.5 µg/L).
Fromme et. al., 2002 Germany	BPA, BPF, BBP, DBP, DEHP,	BPA, (0.0005- 0.41µg/L) in surface water, in sewage BPA, (0.018 to 0.702µg/L) in effluents BPA, (0.01 to 0.19mg/kg) from, in sediments and BPA, (0.004 to 1.363mg/kg). in sewage sludge DEHP,(0.33 to 97.8µg/L) in surface water), DEHP, (1.74- 182µg/L) in sewage effluents, DEHP, (27.9 to 154 mg/kg in sewage sludge) DEHP, (0.21 8.44mg/kg in sediment	Surface water, sediments, sewage treatment plant effluents, sewage sludge, dump water, liquid manure	BPA, BPF, BBP, DBP, and DEHP, were measured in various compartments (surface water, sediments, sewage treatment plant effluents, sewage sludge, dump water, liquid manure) in order to understand exposure to these compounds in different environments	Measured concentrations of BPF were clearly lower than BPA in all environmental media. DEHP dominated the phthalate concentrations, DBP was found only in minor concentrations and BBP, only in a few samples in low amounts.
Körner et. al., 2001 Germany	E1, E2	E2, (0.7–1.8) ng/L E1, Kö (2.5- 38) ng/L (median: 7.6)ng/L E1, Kr (0.8 - 22)ng/L) (median: 1.7 ng/L)	River water	Determine the presence of estrogenic substances in the water of the small streams Körsch (Kö) and Krähenbach (Kr), southwest Germany, by chemical and biological analysis.	Low levels of xenoestrogens found.
Peñalver et. al., 2001 Spain	DnBP, DEH).	DnBP-0.4 µg/ L DEHP-3.2 µg/L	River water Industrial effluent	Analysis of water from the Ebro river and the industrial port of Tarragona.	Phthalate esters concentrations were in the microgram range.

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Kuch et. al., 2001 Germany	BPA , 4-tert-OP, NP, E1, E2 EE2	Drinking water BPA, (300 pg/L - 2 ng/L) NP, (2 to 15) ng/L OP,(150 pg/L- 5 ng/L) Steroid hormones, (100 pg/L to 2 ng/L) River water BPA,(500 pg/L- 16 ng/L) NP, (2 to 15) ng/L OP,(150 pg/L -5 ng/L)	Drinking water River water	Determine BPA , 4-tert-OP, NP, E1, E2 EE2 concentrations in water.	Environmental EDCs are not completely removed in the process of sewage treatment but are carried over into the general aquatic environment. After ground passage, they can eventually be found in drinking water.
Inoue et. al., 2001 Japan	DCP, BP, OP, NP, BPA	River water DCP, 0.01ng/ml BP, (0.01-0.05)ng/ml OP-0.17ng/L NP, (0.04-0.013)ng/ml BPA, (0.01-0.13)ng/ml Drinking water BPA-0.08ng/ml NP-0.05ng/ml OP-0.04ng/ml	River water Drinking water	Determination on phenolic xenoestrogens in river water and drinking water	Trace concentrations of BPA, NP, and OP were detected in three drinking water samples (bought in Tokyo groceries). Xenoestrogens were detected in all Japanese river water at (range: 0.01–0.17 ng ml^{-1}).
Sole et. al., 2000 Spain	NP, DES	DES-43ng/L in WWTP influents and 34 ng/L in effluents NP levels ranged from 6 to 343 g/L in the WWTP effluents. NP ranged from non-detected to 644 g/L in the receiving waters	WWTP effluents WWTP influents River water	To determine both presence and effects of such compounds in two tributaries of the Llobregat river (NE Spain).	In the natural environment WWTPs discharge into rivers a very heterogeneous mixture of chemicals, some of them estrogenic and others antiestrogenic
Corcia et. al., 2000 Italy	Alkylphenol polyethoxylates (APEs) Dicarboxylated metabolites (CAPECs) of A_9PE surfactants	relative abundances of A_9PE_{1-2}, A_9PECs, and CAPECs were found to be respectively 10 ± 2, 24 ± 5, and 66 ± 7.	WWTP effluents	To monitor monthly CAPECs and the other A_9PE metabolites in effluents of five activated sludge sewage treatment plants for 4 months.	On the average, CAPEC amounts having one ethoxy unit ($CAPE_2Cs$) were almost double those of species having only a phenoxy acid moiety ($CAPE_1Cs$). CAPEC species having more than four ethoxy units were never detected.
Desbrow et. al., 1998 UK.	E1, E2, EE2	E1(1- 76 ng/L) E2(1- 48 ng/L) EE2 (<1 - 7 ng/L)	WWTP effluent	To isolate and identify the major estrogenic chemicals present in seven WWTP effluents, receiving primarily domestic effluent, discharging into British rivers.	Three sterols (E1, E2, EE2) were isolated from estrogenic fractions of sewage extracts.

Table 3-1 continued

Study Country	Chemical of concern	Environmental Conc. Found (reported)	Matrix	Study Objective	Results
Castillo et. al., 1997 Spain	Phthalate esters	0.16 - 54.4 µg/L.	Industrial effluents	Characterization of complex mixtures of organic contaminants present in various industrial effluents	The developed protocol permitted unequivocal identification phthalate esters
Field et. al., 1996 USA	NPEC	Paper mill 18 -1270 µg/L WWTP effluents 140 -273 µg/L R iver water 2.0-13.8µg/L	Paper mill effluents WWTP effluents River water	To assess NPECs in surface waters impacted by municipal and industrial effluents.	NPECs detected in paper mill and municipal sewage effluents.

APE- Alkylphenol polyethoxylates, EE2-17α-ethinyl estradiol, E1- estrone, E2-estradiol, E3-estriol, EEq-estrogen equivalent, DCP- dichlorophenol, BP-butyl phenol, OP-Octyl phenol, NP-Nonyl phenol, NPEO- Nonylphenol ethoxylates, HCS- hexachlorocyclohexane, HCB- hexachlorobenzene

MnEC for E1 in river water was 4ng/L while MxEC was 108ng/L with a risk ratio of 35, MnEC for E1 in WWTP effluents was <0.1ng/L while MxEC was 76ng/L with a risk ratio of 25. PNEC for E1 was 3ng/L, Table 3-2. MnEC for E2 in river water was 0.7ng/L while MxEC was 14.7ng/L with a risk ratio of 15, MnEC for E2 in WWTP effluents was <0.4ng/L while MxEC was 200ng/L with a risk ratio of 200 and a PNEC of 1ng/L, Table 3-2. MnEC for EE2 in river water was <0.2ng/L while MxEC was 4ng/L with a risk ratio of 40, MnEC for EE2 in WWTP effluents was <0.4ng/L while MxEC was 7ng/L with a risk ratio of 70 and a PNEC of 0.1ng/L, Table 3-2. MnEC for E3 in river water was 44ng/L while MxEC was 50ng/L with a risk ratio of 50, MnEC or E3 in WWTP effluents was <1ng/L while MxEC was 1ng/L with a risk ratio of 1 and a PNEC of 1ng/L, Table 2. MnEC for combined steroidal estrogens in drinking water was 100pg/L while MxEC was 2ng/L with a risk ratio of 3-2, and a PNEC of 1ng/L, Table 3-2. MnEC for NP in river water was 0.003µg/L while MxEC was 1466ng/L with a risk ratio of 1, MnEC or NP in WWTP effluents was 1.1µg/L, while MxEC was 790µg/L, with a risk ratio of 1, MnEC or NP in drinking water was 2ng/L while MxEC was 50ng/L, with a risk ratio of 0.05 and a PNEC of 1.0ng/L (Environment Canada), Table 3-2. MnEC for NPEC in river water was <0.2µg/L while MxEC was 270µg/L with a risk ratio of 2, MnEC for NPEC in WWTP effluents was 140µg/L, while MxEC was 273µg/L, with a risk ratio of 2, MnEC or NPEC in drinking water was 2ng/L while MxEC was 35µg/L, with a risk ratio of 0.31 and a PNEC of 110µg/L Table 3-2. MnEC for OP in river water was 0.011µg/L while MxEC was 0.0819µg/L with a risk ratio of 0.0007. MnEC for OP in WWTP effluents was 1µg/L, while MxEC was 6.8µg/L, with a risk ratio of 0.06. MnEC or OP in drinking water was 150pg/L while MxEC was 0.04µg/L, with a risk ratio of 0.0003 and a PNEC of 110µg/L, Table 3-2. MnEC for BPA in river water was 0.0005µg/L while MxEC was 0.494µg/L

with a risk ratio of 0.003. MnEC for BPA in WWTP effluents was 13ng/L while MxEC was 0.036µg/L, with a risk ratio of 0.0002, and a PNEC of 160µg/L Table 3-2. MnEC for phthalate esters in industrial effluents was 0.16µg/L while MxEC was 54.4µg/L. Specifically, there was a risk ratio of <1 with a PNEC of 3109µg/L; for DMP; a risk ratio of <1 with a PNEC of 865µg/L for DEP; a risk ratio of 1.3 with a PNEC of 43µg/L; a risk ratio of 1.4 for BBP with a PNEC of 38µg/L; and a risk ratio of 21µg/L with a PNEC 2.49µg/L. According to the literature, if the exposure concentration exceeds the effect concentration (MxEC>PNEC), then an ecological risk is suspected (Lindberg, Bjorklund et al. 2007). However, it should be mentioned that the actual ecological risk should be lower than the one estimated due to effects such as dilution of effluent wastewater in the recipient water bodies. Based on the literature, if the risk ratio is >1, then an ecological risk is suspected(Lindberg, Bjorklund et al. 2007). Using risk ratios calculated, E1, E2, E3 and EE2 present an ecological risk for aquatic organisms in river water and WWTP effluents. Combined steroidal estrogens posed a risk for drinking water used in this study. NP presents a risk for WWTP effluents, whereas NP presented no risk for river water and drinking water. NPEC presents a risk for industrial effluents and WWTP effluents, but presented no risk for drinking water. OP presented no risk for river water, WWTP or drinking water. BPA presented no risk for river water and WWTP effluents when the PNEC provided by Staples (2002) is used. However, using the value of 0.01µg/L found in the third paper of this research, BPA presents a risk to organisms present in WWTP effluents and river water. The following phthalate esters; DEHP, DIDP and DINP presented risks in industrial effluents.

Table 3-2 Minimum and Maximum Environmentally Relevant Concentrations of Pharmaceutical Estrogens and Xenoestrogens and their Predicted No Effect Concentrations and Risk Ratio

Compound	PNEC	Minimum Environmental concentration (MnEC) found	Maximum Environmental Concentration (MxEC)found	Risk Ratio (MxEC/PNEC)	Matrix
E1	3ng/L[+]	4ng/L Viganò et. al., 2006	108ng/L Furuichi et. al., 2004	35	River water
		<0.ng/L Williams et. al., 2003	76ng/L Desbrow et. al., 1998	25	WWTP effluents
E2	1ng/L[+]	0.7ng/L Körner et. al., 2001	14.7ng/L Furuichi et. al., 2004	15	River water
		<0.4ng/L Williams et. al., 2003	200ng/L Huggett et. al., 2003	200	WWTP effluents
EE2	0.1ng/L[+]	<0.2ng/L Furuichi et. al., 2004	4ng/L Viganò et. al., 2006	40	River water
		<0.4ng/L Williams et. al., 2003	7ng/L Desbrow et. al., 1998	70	WWTP effluents
E3	1ng/L[++]	44ng/L Viganò et. al., 2006	50ng/L Viganò et. al., 2006	50	River wáter
		<1ng/L Beck 2005	1ng/L Lagana et. al., 2006	1	WWTP effluents
Combined Steroidal estrogens	1ng/L EEQ[+]	100pg Kuch et. al., 2001	2ng/L Kuch et. al., 2001	2	Drinking water

Table 3-2 continued

Compound	PNEC	Minimum Environmental concentration (MnEC) found	Maximum Environmental Concentration (MxEC)found	Risk Ratio (MxEC/PNEC)	Matrix
NP	1.0ug/L** 0.33ug/L*	0.003ug/L Viganò et. al., 2006	1466ng/L Laganà et. al., 2004	1	River water
		1.1ug/L Laganà et. al.,2006	790ug/L Hugget et. al., 2003	790	WTTP effluents
		2ng/L Kutch et. al., 2001	50ng/L (0.05ug/L) Inoue et.al., 2001	0.05	Drinking water
NPEC	110ug/L** (AE)	<0.2ug/L Field et. al., 1996	270ug/L Field et. al.,1996	2	Industrial effluents
		140µg/L Field et al., 1996	273µg/L Field et. al., 1996	2	WWTP effluents
		2µg/L Field et. al., 1996	35µg/L Sole et. al., 2005	0.31	River water
OP	110ug/L**	0.011ug/L Viganò et. al., 2006	81.9ng/L (0.0819ug/L) Furuichi et. al., 2004.	0.0007	River water
		1ug/L Espejo et. al., 2002	6.8ug/L Espejo et. al.,2002	0.06	WWTP effluents
		150pg/L Kuch et. al., 2001	40ng/L Ioue et. al., 2001	0.0003	Drinking water
BPA	100µg/L (Staples 2002) 0.01µg/L***	0.0005µg/L Fromme et. al., 2002	(0.494ug/L) Viganò et. al., 2006	0.00494 49.4***	River water
		13ng/L Laganà et al.,	36ng/L (0.036ug/L)	0.00036 3.6***	WWTP effluents

Table 3-2 continued

Compound	PNEC	Minimum Environmental concentration (MnEC) found	Maximum Environmental Concentration (MxEC)found	Risk Ratio (MxEC/PNEC)	Matrix
		2004	Laganà et al., 2004		
Phthalate esters	DMP-3109ug/L DEP-865ug/L DBP-43ug/L BBP-38ug/L DEHP-2.49ug/L (Staples 2003)	0.16µg/L Field et. al., 1996	54.4µg/L Field et. al., 1996	<1 <1 1.3 1.4 21	Industrial effluents

DMP-dimethylphthalate,DEP-diethylphthalate,DBP-di-n-butylphthalate,BBP-butylbenzylphthalate, DIEHP-diethylhexylphthalate. PNEC-[+]Environment Agency (England & Wales), *EU, **Environment Canada **http://www.ec.gc.ca/TOXICS/docs/npe/notice/en/NPE_alts.cfm, [++]-combined value used, ***taken from Paper 3

3.8. DISCUSSION AND CONCLUSION

The results of the study suggest that the aquatic receptors are not sufficiently protected from the steroidal estrogens E1, E2, E3 and EE2. Since these compounds are found in mixtures and not necessarily as single components, a combined steroidal PNEC value was also reported. All the steroidal estrogens of concern in this study reported aqueous environmental concentrations above their individual as well as combined PNECs (risk ratio >1), which again suggests that the aquatic receptors in these aqueous media are not sufficiently protected. Aquatic receptors exposed to NP in river water and drinking water environments were not at risk while aquatic receptors exposed to NP in WWTP effluents were at risk. Some phthalate esters and NPECs reported concentrations above their PNECs which suggest that the aquatic receptors in these environments are not sufficiently protected from compounds. Organisms in both river water and WWTP effluent environments may be at risk to BPA exposure. The data suggest that aquatic receptors exposed to OP concentrations in these environments are protected from adverse environmental effects such as endocrine disruption. This is evidenced by PNEC values for these compounds that are below the environmental concentration range found and risk ratio <1.

There is sufficient evidence that aquatic organisms exposed to PEXEs are at risk to elicit a public health concern and hence a debate. A recent publication by the U.S. Geological Survey reported that reproductive hormones and estrogenic alkylphenols were present in 40% and 70%, respectively, of the surveyed U.S. surface waters (USEPA 2001). Thus, as rivers and lakes

are used for municipal water sources, to help produce our food supply and for recreation, and as wastewater effluent water reuse increases, the presence and concentration of xenoestrogens in surface water becomes a valid public health concern. Advances in civilization coupled with rising population levels have resulted in an increasing need to treat and recycle available water resources. In the United States surface water provides for 62% of the public water drinking supply (University of Michigan 2005). Following use, water is returned to the aquatic environment, usually via WWTPs of varying processes and performance, which improves its quality, but it has a high probability of being withdrawn downstream for municipal or industrial reuse. In US cities with a high population density, the volume of effluent discharged from WWTPs can be considerable, sometimes contributing up to 50% of the flow of a river, a figure that can rise as high as 90% in periods of low rainfall (Routledge, Sheahan et al. 1998). There are over 16,000 municipal WWTPs nationwide and over 75% of the nation's population is being served by centralized wastewater collection and treatment systems. The remaining population uses septic or other onsite systems. (USEPA 2004), which have not been adequately studied for xenoestrogens release but, due to their high failure rate and lack of maintenance, could be considered potential non-point releasers of estrogenic compounds (Wright-Walters 2007). This means that humans are likely to consume PEXEs from drinking water since its source is from surface water. Problematic for humans is the fact that some aquatic receptors are a part of the food chain and so there is the likelihood that humans can be exposed to PEXEs via the ingestion route.

There is strong evidence obtained from laboratory studies showing the potential PEXEs to cause endocrine disruption at environmental concentration exposure levels. In wildlife

populations, associations have been reported between reproductive and developmental effects and endocrine-disrupting chemicals. In the aquatic environment, effects have been observed in mammals, birds, reptiles, fish, and mollusks from Europe, North America, and other areas. There is strong evidence that xenoestrogens are present in our environment at concentrations that are harmful to wildlife and humans. There is even stronger evidence that xenoestrogens are present in surface waters at these harmful concentrations and hence a valid public health concern since surface water is, eventually used for drinking water. There is also evidence of human effects in vitro and very strong evidence of low dose effects in rats and mice. CDC has reported BPA and octyl phenol concentrations in 95% of human samples. Also, it is shown from research that most xenoestrogens enter surface water through municipal waste water treatment plants and the most dominant xenoestrogens present in WWTPs are the steroidal estrogens with the birth control pill EE2 being the most harmful. Detractors may argue that the there may be association but not causation.

The risk ratios reported in this study cannot be ignored as there is enough evidence that indicates that aquatic receptors are at risk to PEXEs and some of these aquatic receptors are part of the human food chain. There is enough evidence to elicit at least a national public health debate as recent CDC research has shown xenoestrogens are present in 95% of the US population. Further research is needed to investigate or link health effects of humans and aquatic organism to the PEXE environmental concentrations. Based on the current and available information there should be policies and regulations put in place as part of the Clean Water Act, Toxic Substance Control Act, and Food and Drug Administration to regulate

manufacture, sale and use of PEXEs in our environment and also the use of alternative chemicals that are not endocrine disruptors.

3.9. ENVIRONMENTAL PUBLIC HEALTH TRACKING NETWORK IMPLICATIONS

The CDC describes the EPHT as the ongoing collection, integration, analysis, and interpretation of data about the following factors; environmental hazards, exposure to environmental hazards, health effects potentially related to exposure to environmental hazards (CDC.gov). The goal of environmental public health tracking is to protect communities by providing information to federal, state, and local agencies. These agencies, in turn, will use this information to plan, apply, and evaluate public health actions to prevent and control environmentally related diseases.

The compilation of PEXE exposure data can serve as a link between association, causation and disease states. It will help to bridge the gap between data trends and disease states. Data generated from this process can be analyzed and make statistical inferences at the population level. The problem of PEXE exposure is an emerging one as far as it being recognized by researchers or scientist, but clearly humans as well as wildlife have been exposed at least for the last 70years; human data are still sparse but there is a wealth of wildlife exposure data. However, based on available registries information, a spatial association between PEXE exposures and disease states can be made for different classes of PEXEs, and in essence a screening process leading to a hypothesis for further studies by public health professionals. A

causal link between PEXEs and specific disease states is yet to be developed or established however, having all exposure data in one central location and tracking such data allows the Public health regulators, practioners and educators to make sound decisions with regards to communities, populations or sub-populations.

4.0 AN UPDATED WEIGHT OF EVIDENCE APPROACH TO THE AQUATIC HAZARD ASSESSMENT OF BISPHENOL A

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4.1. ABSTRACT

An aquatic hazard assessment establishes a derived predicted no effect concentration (PNEC) below which it is assumed that aquatic organisms will not suffer adverse effects from exposure to a chemical. An aquatic hazard assessment of the endocrine disruptor Bisphenol A [BPA; 2, 2-bis (4-hydroxyphenyl) propane] was conducted using a weight of evidence approach, using the ecotoxicological endpoints of survival, growth and development and reproduction. New evidence has emerged that suggests that the aquatic system may not be sufficiently protected from adverse effects of BPA exposure at the current PNEC value of 100 µg/L. It is with this background that; 1) An aquatic hazard assessment for BPA using a weight of evidence approach, was conducted, 2) A PNEC value was derived using a non parametric hazardous concentration for 5% of the specie (HC_5) approach and, 3) The derived BPA hazard assessment values were compared to aquatic environmental concentrations for BPA to determine, sufficient protectiveness from BPA exposure for aquatic species. A total of 61 studies yielded 94 no observed effect concentration (NOEC) and a toxicity dataset, which suggests that the aquatic effects of mortality, growth and development and reproduction are most likely to occur between the concentrations of 0.0483 µg/L and 2,280 µg/L. This finding is within the range for aquatic adverse estrogenic effects reported in the literature. A PNEC of 0.06 µg/L was calculated. The 95% confidence interval was found to be (0.02, 3.40) µg/L. Thus, using the weight of evidence approach based on repeated measurements of these endpoints, the results indicate that currently observed BPA concentrations in surface waters exceed this newly

derived PNEC value of 0.06 µg/L. This indicates that some aquatic receptors may be at risk for adverse effects on survival, growth and development and reproduction from BPA exposure at environmentally relevant concentrations.

4.2. INTRODUCTION

A hazard assessment is one of the components of risk assessment (USEPA 1998), (Figure 4.1). Although the definitions of risk differ among users of risk assessment methodologies, the basics of risk assessment related to the aquatic environment are universal in that they comprise a comparison of the exposure of (a part of) the ecosystem to a chemical with the sensitivity of the ecosystem for this chemical (Suter 2003; Suter 2006). The exposure is often represented by a Predicted Environmental Concentration (PEC) or a Measured Environmental Concentration (MEC) (Suter 2003; Suter 2006) and can be obtained by actual field measurements (monitoring data) or by estimations using environmental fate and transport models. The ecosystem sensitivity is often expressed as a Predicted No Effect Concentration (PNEC) which is usually derived from toxicity tests. A comparison of the PEC or MEC and the PNEC, or the PEC/PNEC or MEC/PNEC ratios, are widely accepted and applied endpoints in aquatic risk assessment models intended for screening and hazard characterization (USEPA 1998; Suter 2006). When the process of Environmental Risk Assessment (ERA) is applied to a chemical exposure, such as , Bisphenol A [BPA; 2, 2-bis (4-hydroxyphenyl) propane], the assessment is based on a

comparison of the exposure of (a part of) the ecosystem to BPA with the sensitivity of (the same part of) the ecosystem for BPA (Suter 2003; Suter 2006).

Assumptions are made concerning the aquatic environment which allow, however uncertain, an extrapolation to be made from single-species short-term toxicity data to ecosystem effects (Smrchek 1993; Smrchek 1998). It is assumed that: 1) Ecosystem sensitivity depends on the most sensitive species, and 2) The protection level for ecosystem structure is sufficient for the protection of community function (Smrchek 1993; Smrchek 1998; Burton 2002; EC 2003; Suter 2003; Suter 2006). These two assumptions have important consequences. By establishing which specie is the most sensitive to the toxic effects of a chemical in the laboratory, extrapolation can subsequently be based on the data from that specie (Smrchek 1993; Smrchek 1998; Suter 2003; Suter 2006). Furthermore, the functioning of any ecosystem in which that specie exists is protected, provided the structure is not sufficiently distorted as to cause an imbalance. It is generally assumed and accepted that protection of the most sensitive species should protect structure, and hence function of the ecosystem (Smrchek 1993; Smrchek 1998; EU 2003; Suter 2003; Suter 2006; Walker 2006)

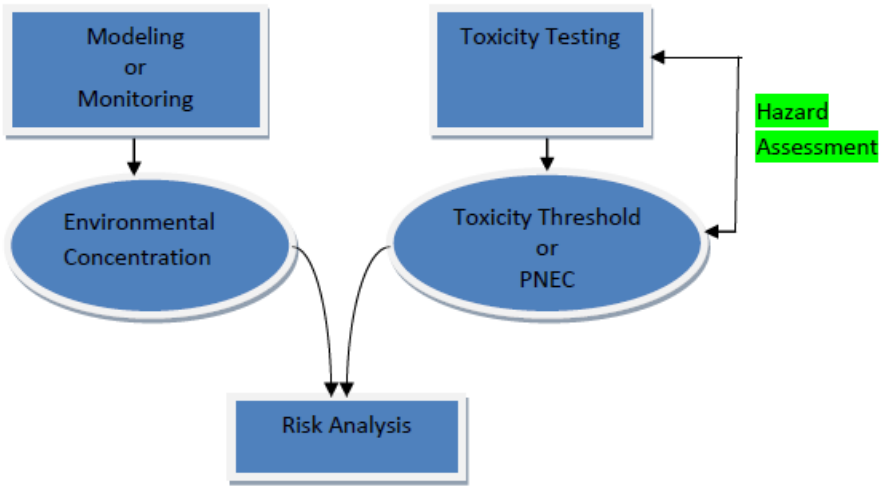
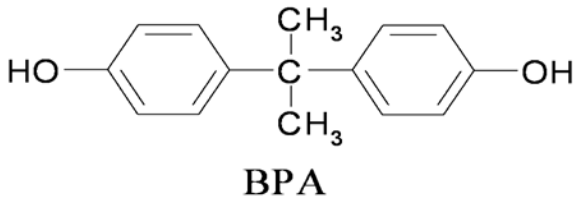


Figure 4-1 General framework for environmental risk assessment, showing the hazard assessment process based on the comparison of an environmental concentration with the sensitivity of the environment

4.3. BPA

Bisphenol A [BPA; 2, 2-bis(4-hydroxyphenyl)propane], is one of the highest-production-volume (HPV) chemicals in the world with total production capacity greater than 3.7 million metric tons (m.t.)/year (SRI 2007). Global demand for BPA is expected to increase 6–7%/yr (SRI 2007).



BPA

Figure 4-2 Molecular Structure of BPA

Chemically, BPA is an organic compound composed of two phenol rings connected by a methyl bridge, with two methyl functional groups attached to the bridge (Figure 4-2). Bisphenol A is mainly used in the production of epoxy resins and polycarbonate plastics (Kang 2006; Kang 2007). Most companies use proprietary methods to synthesize BPA (Kang 2006), but BPA may be synthesized by the condensation of acetone with phenol (EU 2003). Two grades of BPA are produced: one for the manufacture of epoxy resins and a higher purity for polycarbonate production (EU 2003). BPA resins have been widely used in linings of metal cans used to preserve food (MacLusky 2005). The U.S. Food and Drug Administration listed BPA as an indirect food additive for use only as a component of adhesives under the U.S. Code of Federal Regulations, Title 21 part 17.105 (USDA 2006). Since 2001, the BPA-epoxy resin films used to line beverage cans have been largely replaced with polyethylene terephthalate films (Miyamoto 2006).

The physical-chemical properties and transport characteristics of BPA control its distribution and ultimate fate in the environment. BPA is a moderately water-soluble compound (300 mg/L) at ambient temperatures that dissociates under alkaline conditions (pKa = 9.6 to 11.3) (Staples 1998). BPA has been found to be readily biodegradable, achieving 81 to 93% mineralization to carbon dioxide in 28 days. Studies have shown that BPA is rapidly degraded in surface waters taken from diverse geographies in the United States and Europe (Kang 2007). BPA in natural river water was biodegraded with measured half-lives of 0.5 to 3 days (Staples 1998; Staples 2002; Klečka 2005). In summary, BPA's physical, chemical, and environmental fate characteristics influence the nature of its presence in aquatic systems, and need to be considered when conducting or evaluating ecotoxicological studies.

4.3.1. BPA Migration into the Aquatic Environment

BPA has been the subject of considerable aquatic toxicity testing in recent years. BPA discharge into the aquatic environment occurs from the migration of BPA-based products (processing and production) into rivers and marine waters but, the primary route of BPA contamination in the aquatic environment is through effluent from wastewater treatment plants and landfill sites (Kang 2007). This is due to its incomplete removal during the sewage treatment process (Fürhacker 2000; Lee 2000b). It is difficult for aquatic organisms (microorganisms, planktons, plants, invertebrates, and vertebrates) to escape toxic and endocrine-disruptive effects by BPA (Kang 2007) due to its transmission system to the ecosystem as seen in Figure 4-3 which is a conceptual model showing BPA exposure into the environment.

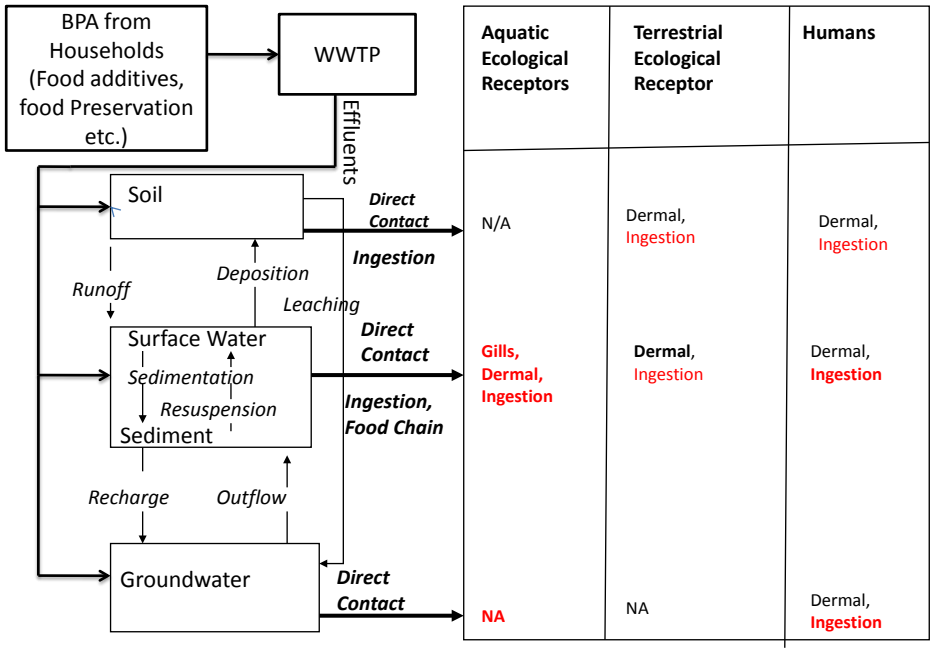


Figure 4-3 BPA Exposure to Human and Ecological Receptors

4.4. BPA CONCENTRATIONS IN THE AQUATIC ENVIRONMENT

BPA levels in the aquatic environment according to the literature range from 0.0005 μg/L to 8 μg/L. (Table 4-1). Although BPA levels in river water near wastewater treatment plants and or landfills can be high, downstream they can be decreased to levels below detection limits (Kim 2004; Kang 2006b). This could be due to dilution effects, biodegradation by microorganisms and/or photolysis and hydrolysis reactions. The acute toxicity of BPA to aquatic organisms varies from slight to moderate, that is, 1000-10000 μg/L for freshwater and marine species (Alexander 1998).

Table 4-1 BPA Concentrations in the Aquatic Environment

River water (μg/L)	Country	Reference
<1.0-8.0	United States	Staples et al., 2000(Staples and Harris 2000)
<0.05-0.272	Germany	Bolz et al., 2001(Bolz 2001)
0.0005-0.014*	Germany	Kuch and Ballschmiter, 2001
0.009-0.776	Germany	Heemken et al., 2001
0.0005-0.41	Germany	Fromme et al., 2002
0.042-0.092	Germany	Stachel et al., 2003
0.02-0.015	Japan	Takahashi et al., 2003
<0.005-0.08	Japan	Kawahata et al., 2004
0.01-1.4	Japan	JMC, 1999
<0.2-1.9	Japan	Matsumoto 1982
<0.09	Japan	Matsumoto 1977
0.5-0.9	Japan	Kang and Kondo 2006
0.03-0.083	China	Jin et al., 2004
<0.012-0.33	The Netherlands	Belfroid et al., 2002
0.0088-1	The Netherlands	Vethaak et al., 2005

*Median concentration

4.5. HAZARD ASSESSMENT COMPONENTS

The environmental hazard assessment of chemicals consists of the identification of the effects that a chemical may have on organisms in the environment (Smrchek 1993; Zeeman 1993). Typical regulatory toxicity studies used in hazard assessments follow conventional acute tests (ASTM 1980; USEPA 1982 ; ECETTC 1996) and longer-term testing using conventional early life stage (OECD 1982) or life cycle test methods (USEPA 1986; OECD 1996). Effects are expressed in terms of the acute and chronic toxicity of a chemical on the exposed organisms (USEPA 1998). These are generally given as either the lethal concentration (LC) or as the effective concentration (EC) that describe the type and seriousness of the effect for a known concentration of a chemical. For the acute values, the LC_{50} (lethality or mortality) (EC_{50}) (non-lethal/lethal effects) refers to the concentration that results in 50 percent of the test organisms affected at the end of the specified exposure period in a toxicity test (USEPA 1982 ; USEPA 1998; Calow 2003). The chronic values represent the concentration of the chemical that results in no statistically significant sub-lethal effects on the test organism following an extended or chronic exposure (USEPA 1998; Calow 2003; Suter 2003; Suter 2006). Two other measures of responses are the lowest observed effect concentration (LOEC), which describes the lowest tested concentration that shows a statistically significant difference from an unexposed control group, and the no observed effect concentration (NOEC), which represents the highest tested concentration not showing statistically significant differences from an unexposed control group (EC 1996; USEPA 1998; Suter 2006). When the effective concentrations for a range of species for a chemical are tabulated, the tabulation is called a hazard profile or toxicity profile (USEPA

1998). The hazard assessment includes an effects assessment for the endpoints; survival, growth and development, and reproduction.

Acute and chronic toxicity data from laboratory studies with BPA can be used to support an ecological risk assessment for aquatic environment as there are numerous procedures to extrapolate effect data from the laboratory to the field (Chapman 1998). The extrapolation procedures attempt to address uncertainties between laboratory and field systems. The weight of evidence approach discussed below was used to address uncertainty in measurement of one or more endpoints (Chapman 1998). That is, the measurement of one or more endpoints numerous times, then assigning the greatest weight to results that are confirmed by combined evaluation of the procedures and approaches used. Applying this principle in the context of this hazard assessment of BPA, we looked at the concentration range at which NOEC and LOEC values were clustered. The concentration range for the LOEC and NOEC values is indicative of a range for acute and chronic toxicity of aquatic organisms to BPA.

Staples et. al.,(Staples 2002) reported a BPA chronic aquatic toxicity value of 160 µg/L through a hazard assessment. They found concentrations of BPA in surface water ranged from 0.001 to 0.10 µg/L. They concluded that comparing the weight of the evidence of the aquatic toxicity data that showed chronic effects, BPA is unlikely to cause adverse effects on aquatic populations or ecosystems. Since 2002 when Staples and colleagues completed their research, several relevant studies have been published, which indicate that the hazard assessment reported may not be sufficiently protective of the ecosystems and populations (Levy 2004; Fukuhori 2005; Lee 2007). It is with this background that this research was embarked upon.

4.6. WEIGHT OF EVIDENCE APPROACH

A weight-of-evidence evaluation takes into account the strengths and weaknesses of different measurement methods when determining whether the results show that a stressor has caused, or could cause, a harmful environmental effect (Burton 2002). A formal weight-of-evidence evaluation, whether qualitative or quantitative, can provide a framework for rigorous consideration of the strengths and weaknesses of various measurements, and of the nature of uncertainty associated with each of them (Bettinger 1995). As used in environmental studies, weight-of-evidence is the process of combining information from multiple lines of evidence to reach a conclusion about an environmental system or stressor (Burton 2002). Examples include the characterization of ecological risk posed by a hazardous waste site and the estimation of no-observed-effect-levels (NOECs) for chemicals from studies on different species or pathways. The process incorporates judgments about the quality, extent and congruence of the information in each level of effect (LOE) (Burton 2002). The weight-of-evidence evaluation procedure integrates the results of multiple measurements (endpoints) in environmental risk assessments. Multiple measurements are often used to evaluate each effect of concern. Applying a weight-of-evidence evaluation in a hazard assessment will promote systematic analysis of risk posed by the chemical of concern, and documentation of the evaluation will elucidate a risk assessor's thought process. It is important to recognize, however, that professional judgment may also be influenced by factors other than scientific knowledge and technical expertise.

Weight-of-evidence is reflected in three characteristics of measurement endpoints: a) the weight assigned to each measurement endpoint; b) the magnitude of response observed in

the measurement endpoint; and c) the concurrence among outcomes of multiple measurement endpoints(Burton 2002).

4.7. METHODOLOGY

The paper has three objectives. The first is to determine best available aquatic sensitivity data for BPA. To meet this objective a critical review of the available literature on BPA aquatic toxicity studies through 2008 was performed. The literature searches were performed using USEPA ECOTOX database, Pan Pesticide Database (PAN 2000), other relevant scientific databases such as EBSCO and peer reviewed journals. The current and updated European risk assessment report for BPA (EC 2008) was also thoroughly reviewed for studies that were not available elsewhere.

4.7.1. Studies Selection Process

Studies that reported the effects assessments endpoints of survival, growth and development, and reproduction were selected. These endpoints are predictive of population or eco-system level effects (Ankley 1998). All the studies collected from the research efforts were critically reviewed for suitability for use in risk assessment, following the criteria and procedures outlined in the E.U. Technical Guidance Document (EC 1996) and USEPA guidelines (USEPA 1998). Specific assessment criteria (Staples 2002) for deciding on the quality, usefulness and inclusion of the study included: 1) A thorough description of the experimental design, including

exposure regime and replication, 2)Analytical confirmation of test concentrations; 3) Description of ecologically relevant endpoints and all supplemental morphological information collected; 4)Use of test procedures that are based, at least generally, on internationally accepted procedures and practices. Newly developed test procedures must be able to be repeated, and meet all other required criteria. 5) Clear linkage of reported findings with the exact experimental design, and 6) Sufficient reporting of results, including system performance, toxicity results, and statistical methods employed to ascertain how the data support the conclusions that are drawn. Consideration was also given to whether the studies were conducted under E.U., U.S., and/or OECD principles of Good Laboratory Practices (GLP)(OECD 1982). Compliance with GLP provides some assurance that the data reflect the conduct and findings of the study; that the raw data will be retained for additional analyses, if necessary; that data are not mistakenly changed or omitted. This also assures that the study can be reconstructed from the raw data (OECD 1982). This does not mean that good research cannot be conducted in the absence of GLP, only that certain documentation procedures are in place to track the execution and reporting of an experiment (Staples 2002). Studies were included from any wildlife species that had been exposed to BPA and results are tabulated in Table 4-3 by species, endpoint, result, status and comments.

For the selection of a study for use in this analysis, the following guidance established by Staples (Staples 2002) was used; if all or most (four or more) of the guidance criteria appear to have been met, the study was designated as “valid” (Table 4-3), or generally consistent with current practices with E.U. risk assessments (UKEA 2001) or USEPA (USEPA 1998). Accordingly, when some (less than four), but not all, of the criteria were fulfilled, the studies were

designated as “use with care”. Experiments with insufficient information to allow proper evaluation or with other obvious flaws were designated as “not valid” (Table 4-3) and were unsuitable for the hazard assessment. An example of an obvious flaw is a long-term study of a readily biodegradable test substance conducted using a static test system where test chemical concentrations were not measured(Staples 1998). All studies designated as “valid” and “use with care” (Table 4-3) were deemed acceptable for use in this aquatic hazard assessment. Other studies deemed to be “not valid” were also presented. Studies that were determined to be valid were included in the analysis. The concentrations of BPA at NOEC and LOEC were included in the analysis to assess hazard. Studies with reported endpoints of a Fifty Percent Lethal Concentration (LC_{50}), Fifty Percent Effective Concentration (EC_{50}) or Twenty Five Percent Inhibition Concentration (IC_{25}) were included in our studies table (Table 4-3) but were not included in the analysis as those endpoints are not considered in completing a hazard assessment. However, in analyzing the data there were no LC_{50} or EC_{50} or IC_{25} values for any specie that were lower than any LOEC or NOEC for that specie.

4.7.2. **Update of the Hazard Assessment**

The second objective of this paper is to use the BPA sensitivity data collected from the initial research in objective one to conduct and update an aquatic hazard assessment for BPA using a weight-of-evidence approach using the ecologically relevant endpoints of survival, growth and development, and reproductive success. To accomplish this all NOEC and LOEC concentrations were ranked from high to low sensitivity (smallest to largest concentration) to determine the

lowest concentration (NOEC) at which there was no reported toxic effect or the lowest concentration (LOEC) at which there was a toxic effect. NOEC and LOECs for ecologically relevant endpoints were used to evaluate the survival, growth and development, and reproductive success of aquatic organisms (Suter 2006) following exposure to BPA.

The third objective is to compare the BPA concentration range of derived hazard assessment in objective two to the published BPA concentration range found in the aquatic environment, to determine if the aquatic system is sufficiently protected from possible adverse effects of BPA. To accomplish this task the data from Table 4-3 were used to graph a scatter plot showing BPA concentrations vs. Ecotoxicological Endpoints (Figure 4-3). Also, the NOEC and LOEC values were separated and log transformed to show where each clustered and the range of the sensitivity spread and are depicted in Figure 4-4 (BPA vs. NOEC) and Figure 4-5(BPA vs. LOEC values). Next, using NOEC values only, a PNEC (Equations 4-1 and 4-2) for BPA was calculated using the HC_5 approach, using a nonparametric HC_5 estimation previously described by van der Hoeven (van der Hoeven 2001), which is a modification of previous work by Leeuwen and Hermens (van Leeuwen 1995). The PNEC is an ecotoxicological measure for multiple species systems. It can be defined as the concentration below which a specified percentage of species in an ecosystem are expected to be protected (Pennington 2004). The HC_5 approach assumes that only 95% of the species can be protected so there is a 5% of the species that is affected by the chemical, in this case BPA. This HC_5 approach used in this analysis makes no assumption about the distribution of the dataset. A confidence limit [HC_5 (0.05)] for this HC_5 value was also calculated. See Equations 4-4 and 4-5. It is important to

stress that the HC_5 and $HC_5(0.05)$ estimates will often be much more affected by the non-randomness of the species set for which sensitivity data are available than by the choice of the statistical method (van der Hoeven 2001).

4.8. RESULTS

Sixty one (61) studies which represented twenty four (24) different species were reviewed and included in this analysis (Table 4-3). Thirteen (13) of these studies were deemed not valid and were not included in the analysis. A total of ninety four (94) LOEC and NOEC values were obtained from the studies (Table 4-3) deemed acceptable ("valid" or "use with care") for use, and included in the analysis, Table 4-3. BPA sensitivities ranged from 0.002 µg/L (growth NOEC) to 12500 µg/L (reproduction LOEC), Figure 4-3.

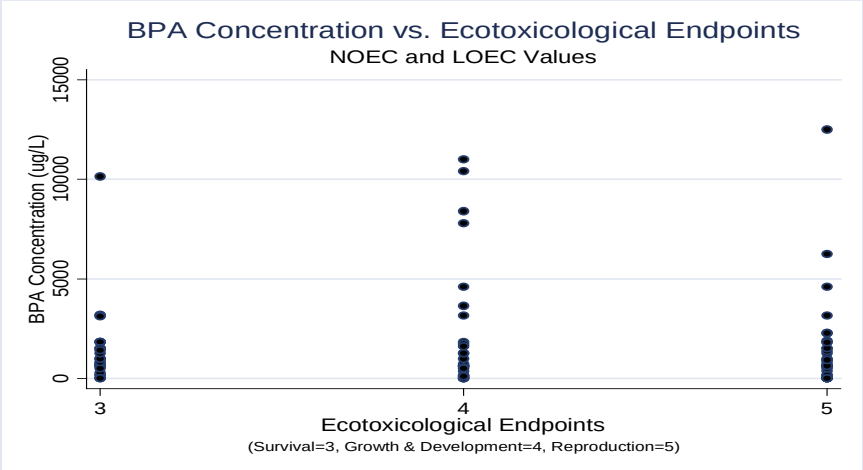


Figure 4-3 BPA Concentrations versus Ecotoxicological Endpoints

Individually, BPA sensitivities for the endpoint survival clustered between 2.4 µg/L and 1820 µg/L, while sensitivities for the endpoint growth and development clustered from 0.002 µg/L to 1820 µg/L and sensitivities for the endpoint reproduction clustered between 0.0079 µg/L and 2280 µg/L. The NOEC for BPA for all endpoints ranged from 0.002 µg/L to 10400 µg/L, see Table 4-3 and Figure 4-4. The LOEC for BPA for all endpoints ranged from 0.0483 µg/L to 12500 µg/L, see Table 4-3 and Figure 4-5. Thus, using the weight of evidence approach, the toxicity dataset for BPA suggests that adverse effects of mortality, growth and development and reproduction are most likely to occur between the concentration of 0.0483 µg/L and 2280 µg/L based on repeated measurements of these endpoints.

A PNEC was derived using a methodology proposed and utilized by van der Hoeven (van der Hoeven 2001). This approach, unlike the van Leewen's HC_5, makes no assumptions about the species sensitivity distribution. But, like van Leewen's HC_5 approach, it postulates that it can only protect a 95% of the species thus, there is a 5% of these species that cannot and will not be protected.

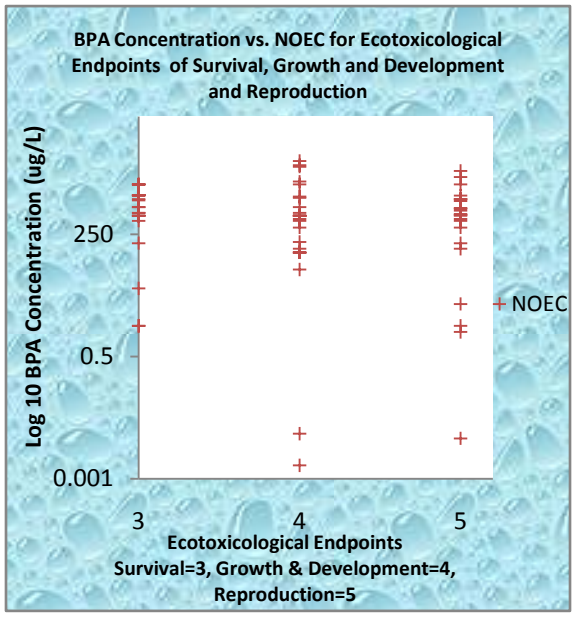


Figure 4-4 BPA Concentration vs. NOEC

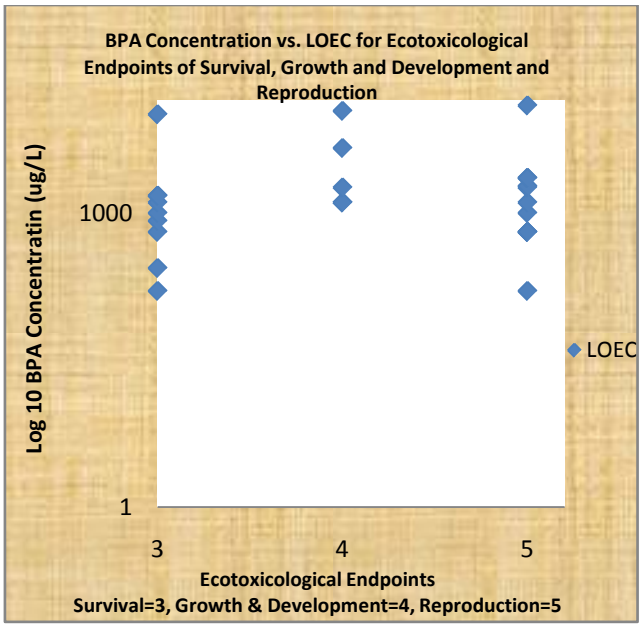


Figure 4-5 BPA Concentrations vs. LOEC

4.8.1. **Calculations**

The PNEC, which is the conservative estimate for HC_5, is the observed value with rank k and was determined as follows;

Determine the observed sensitivity

$$\boldsymbol{y = 0.05(n + 1) = 3.35,}$$ Equation 4-1 (van der Hoeven 2001)

Where ;

n is the total number of observations

Largest integer below $y = k, y = 3$

Then;

$$Z_k + (Z_{k+1} - Z_k)(y - k)$$
$$= 10^{-2+[0.244513-(-2)](3.35-3)}$$
$$= 10^{-1.21407}$$
$$= 0.061 \mu g / L = estimate\ of\ \ HC_5$$

Equation 4-2
(van der Hoeven 2001)

Next, determine the sensitivity at which the probability of underestimating is 0.05. This is also the lower bound estimate of a one-sided 95%CI.

$$Z_k + (Z_{k+1} - Z_k)(0.05 - q_{k,n}(\alpha)) / q_{k+1.n}(\alpha) - q_{k,n}(\alpha)$$
$$With\ n = 67,\ k = 1, \quad (Using\ lower\ bound\ est.\ for\ Z_1 = -2.69897,\ rank\ Z_2 = -2.1023)$$
$$q_k = 0.0437, \quad q_{k+1} = 0.0688\ \{inverse\ beta\ distribution\ with\ parameters$$
$$[k_1, (n + 1 - k_1)] \quad and \quad (k_1 + 1, n - k_1)\}$$
$$= 10^{-2.69897 + [-2.1023-(-2.69897)](0.05-0.0437)/(0.0688-0.0437)}$$
$$= 10^{-2.54923}$$
$$= 0.00282 \mu g/L$$

Equation 4-3 (van der Hoeven 2001)

To find the lower bound estimate for a two sided 95% CI

$$Z_k + (Z_{k+1} - Z_k)(0.05 - q_{k,n}(\alpha)) / q_{k+1.n}(\alpha) - q_{k,n}(\alpha)$$
$$10^{[-2.69897](0.05)/(0.0792)}$$
$$= 10^{-1.7039}$$
$$= 0.02 \mu g/L$$

Equation 4-4

(van der Hoeven 2001)

To find the upper bound estimate for the two sided 95% CI.

To find the upper bound of 95% *CI*

$$z_{k_2} - (z_{k_2} - z_{k_2-1})(q_{k_2,n}(\alpha) - 0.05)/(q_{k_2,n}(\alpha) - q_{k_2-1,n}(\alpha))$$

Where $k_2 = 8$ *and from ranks* $Z_{k_2} = 0.623249, \quad Z_{k_2-1} = 0.380211$

$q_{k_2} = 0.05381, \quad q_{k-1} = 0.04372$ {*inverse beta distributions with parameters* $[k_2, (n+1-k_2)]$ *and* $k_{2-1}, n+2-k_2$, (*indicates as* $q_{k_2,n}(\alpha)$ *and* $q_{k_2-1,n}(\alpha)$}

$$= 10^{0.623249-(0.623249-0.380211)(0.05381-0.05)/(0.05381-0.04372)}$$

$$= 10^{0.531478}$$

$$= 3.40 \mu g / L$$

Equation 4-5

(van der Hoeven 2001)

The HC_5 estimate (PNEC) is 0.06 µg/L. The sensitivity for which the probability of underestimating is 5% is 0.003 µg/L. The 95% confidence interval is (0.02, 3.4) µg/L. The reported concentration of BPA found in the aquatic environment is between 0.0005 µg/L to 8 µg/L (Kuch 2001; Belfroid 2002).

4.9. DISCUSSION

Comparing the results of the analysis with the environmental concentration of BPA suggest that some aquatic receptors are not sufficiently protected from the adverse effects of BPA exposure. The results indicate far more sensitivity for aquatic receptors to BPA exposure than had previously found. This is due to the availability of new published studies with sensitivities from the more sensitive taxonomic groups such as algae, cyanobacteria and insecta. The European risk assessment of BPA reports a PNEC five folds greater for whole freshwater. However, they noted that this PNEC may not have taken into consideration the full effects of BPA on snails. Snails are an extremely sensitive species to BPA and are included in our assessment. In

ecological assessment it is assumed that the sensitivity of the ecosystem (PNEC) is dependent on the most sensitive species and the ecosystem is protected if those species are present. Then, our research suggests that ecosystems that contain insecta, mollusc, crustacean and cnidarians are protected from adverse effects from exposure to BPA if this PNEC is utilized.

In conducting this hazard assessment there were a number of uncertainties and problems that were important and should be addressed. The assessment assumes that there will be different and varied species (greater than 8) in the experiments conducted. This assessment utilized twenty four (24) different species. All available sensitivities for all specie were included in our analysis as long as they met the initial assessment criteria. This means that several NOEC values for one species may have been utilized in the study. This adds uncertainty to the data set since, ideally a NOEC value for one specie should be utilized. Note however, in reality that several NOECs and LOECs are generated for the same or different endpoints and important also is the age of the specie. Younger species seemed to be more sensitive than older ones. The use of the NOEC and LOEC values incorporates uncertainty in the data set as the NOEC or LOEC result is strongly dependent on the experimental design. Depending on whether the number of concentrations tested is high or low, the NOEC or LOEC value may vary. In calculation of the PNEC using the HC_5 approach, without any assumptions about the distribution, it is assumed that the sampling is with replacement, although in reality it is clearly not the case. However, the difference is negligible when there are millions of species to draw from. Since the species dataset was not huge, this introduces some uncertainty into our analyses.

The statistical methodology assumes species *n* is from a random drawing from a complete set of sensitivity data for all existing species and the distribution is continuous and uniform. In reality this may not be true. The data reflect that vertebrates are overrepresented (Table 4-2) which may indicate a flaw in the statistical methodology which should be taken into account when using these results.

Table 4-2 The distribution over taxonomic groups of species with data available on the sensitivity to BPA

Taxonomic Group	Number of specie sensitivity data for BPA	
	No.	%
Plants algae	4	4.255319
Bacteria and Cyanobacteria	12	12.76596
Protozoa, rotifera, Cnidaria and mollusc		
Insecta	4	4.255319
Crustacea	6	6.382979
Vertebrates	63	67.02128
Amphibians	5	5.319149

Important also is that parametric methods give a reasonable good description of the data near the center of the distribution. However, in the tails they cannot be trusted to resemble the real distribution and the HC_5 by its nature is a tail value. This introduces another statistical uncertainty into the analysis. For this method to be valid the sample size must be greater than 19. Our sample size was 94 and valid.

The studies used in this research were performed in the laboratory and in extrapolating to the aquatic environment; uncertainties are introduced because laboratory results are not

true reflections of the field conditions since it is almost impossible to mimic field conditions exactly. Also, only one or two compounds were tested in laboratory analyses, but in the field aquatic species are exposed and continually bathe in a mixture or concoction of chemicals. Succinctly, these results are not always and probably never true reflections of the field thus, inserting more uncertainties into the analysis.

4.10. CONCLUSION

The results of this research suggest that the aquatic environment is not sufficiently protected from adverse effects of BPA exposure at the established environmental concentration of 0.0005 µg/L to 8 µg/L. This means that, it may be possible that aquatic receptors previously thought to be protected from the adverse effects of aquatic BPA exposure concentrations are actually at risk based on this new research data. It is also clear that some species are more sensitive that others and may require additional and further research.

More research is needed to understand the full effects of BPA exposure concentrations in the aquatic environment. Additional research should focus on both laboratory and field tests. In particular laboratory experiments that simulate the field environment should be a focus for better correlation of results

Different approaches other than the weight of evidence should be explored for completing a hazard assessment of BPA. There should be specific policies in place to; identify and or manage BPA and other xenoestrogens in the aquatic environment, protect the

ecosystem and the public. Review of current US environmental policies suggest that BPA would need to be regulated under several different Acts such as the Toxic Substance Control Act, Federal Insecticide, Fungicide and Rodenticide Act and Food and Drug Administration Act which complicates current regulation.

4.11. PUBLIC HEALTH SIGNIFICANCE

Species in the wild are sentinels for human exposure ("the canary in the mine"). Sentinel animals may provide early warning of potential risks before disease develops in human populations. Potential applications for sentinel species includes monitoring environmental media, identifying new exposures of potential concern as a result of observing changes in wild animal populations, and supporting risk assessment at several points in the process. Although it is unlikely that sentinel species data will be used as the sole determinative factor in assessing human health risks, the data can be useful for a weight-of-evidence approach in risk assessment decisions, for providing early warning of situations requiring further study, or for suggesting potential causes and effects.

Some species are a part of the human food chain and thus another route of exposure for humans to BPA. Understanding the species and concentrations of BPA in the aquatic environment is imperative for environmental public health tracking of associated disease states, and in the regulation of fish or wildlife consumption from rivers and lakes. Having an updated BPA aquatic hazard assessment will help to determine risks for both humans and

wildlife populations from environmentally relevant concentrations of BPA. Further, it will foster the development of new policies and regulations regarding the production and proper management of BPA in the aquatic environment.

4.12. AQUATIC ECOTOXICOLOGICAL STUDIES REVIEWED

Table 4-3 Table Showing Aquatic Ecotoxicological Studies with Endpoints of Survival, Growth and Development and Reproduction

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Jörg Oehlmann (Oehlmann 2006)	Ramshorn Snail (Gastropoda: Prosobranchia) (*Marisa cornuarietis)*	Egg production, reproduction Clutch production, reproduction	LOEC=0.0483, NOEC=0079,$EC_{10=}$0.0139, EC_{10}=0.0146	Valid	Mortality, numbers of eggs, clutches, and eggs per clutch in the tanks were recorded daily. GLP. Renewal test
Ishibashi (Ishibashia 2005)	Medaka (Oryzias latipes)	96h-survival: 14d- survival: 14d-hatching, reproduction:	LC_{50}=13900 LC_{50}=14800 LOEC=12500 NOEC=6250	Valid	Early Life stage Toxicity study
Fukuhori (Fukuhori 2005)	Primitive invertebrate (hydra olgactis)	Growth- Testes- male-35days, Female-50d-egg formation, reproduction:	NOEC=170 LOEC=1000	Valid	Standard protocol used-GLP
Koponen & Kukkonen (Koponen 2002)	Frog (*Rana Temporaria)*	20-d, abnormal development 72-h, mortality:	NOEC=10 LOEC=1000 LT_{50}=1000	Not Valid	Renewal- embryos used. No chemical analysis of exposure concentrations

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Lee & Choi (Lee 2007)	Diptera, Chironomidae (*Chironomus riparius)*)	Growth & development 48h-fresh wt. 24h-dry wt.	LC_{50}=6030	Valid	Short term study-Non-biting midges are the insects most found in freshwater ecosystems
Levy (Levy 2004)	Tadpoles (*xenopus laevis)*	120d-feminization, reproduction: 120d-sex reversal, reproduction:	LOEC=160 NOEC=7.3	Valid	GLP-Assessment of BPA on froglets and tadpoles
Bayer (Bayer 1999b)	Rainbow Trout (*Oncorhynchus mykiss)*	28-d growth	NOEC=3640 LOEC=11,000	Valid	Juvenile test OECD proposed guideline, measured conc. GLP
Alexander (Alexander 1998)	Atlantic silverside (marine) (*Mendia, menidia)*	96h- survival	96h-LC_{50}=9400	Valid	ASTM Method, flow through, measured concentration, non-GLP
Bayer AG 1999a (Bayer 1999a)	Zebra fish (*Branchydanio retio)*	14d-survival	NOEC=3200 LOEC=10150	Valid	OECD 204, measured concentrations, GLP
Fraünhofer (FIECE 2000)	Zebra fish (*Branchydanio retio)*	120d-fertillization rate; Mortality, egg production hatchability growth Time to onset of reproduction	NOEC=750 LOEC=1500 (EC_{10}=390) NOEC=1500	Use with care	Lifecycle test renewal, measured concentrations, F_o to F_1 to F_2, full details not available

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Yokota (Yokota 2000)	Medaka fish (*Oryzias latipes*)	60d- survival 60d-growth 60d-hatchability 60d-time to hatch 60d-sex ratio 60d-embryo survival	NOEC=1820 NOEC=355; LOEC=1820 NOEC=1820 NOEC=1820 NOEC=355, LOEC=1820 NOEC=1820	Valid	Modified OECD 210 ELS test measured concentrations, renewal, until hatch then flow-through non-GLP
Yokota (Yokota 2000)	Medaka fish (*Oryzias latipes*)	96h-survival	LC_{50}=13000	Valid	OECD 204, measured concentrations, non-GLP
Metcalfe (Metcalfe 2001)	Medaka fish (*Oryzias latipes*)	100d-post hatch; growth 100d-post hatch, sex ratio	NOEC= 120 NOEC=120	Valid	Renewal, measured concentrations, non-GLP
Shioda (Shioda 2000)	Medaka fish (*Oryzias latipes*)	14d-no. of eggs 14d-no. of hatched eggs	NOEC/LOEC=684/2280 NOEC/LOEC=684/2280	Valid	Renewal, males exposed for two weeks, bred with unexposed females in clean water, nominal study
Tabata (Tabata 2001)	Medaka fish (Oryzias latipes)	72h-survival 72h-survival	LC_{50}=7500 adults LC_{50}=5100 embryos	Use with care	General procedures describe renewal, nominal only
Tabata (Tabata 2000)	Medaka fish (Oryzias latipes)	100d-survival 100d-sex ratio	NOEC=100 NOEC= 100	Not valid	General procedures described-no statistics

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Alexander (Alexander 1998)	Fathead minnow (*Pimephales promelas)*	98d-survival	98hr-LC_{50}=4600-4700	Valid	ASTM Method, static & flow through, measured concentration, non-GLP
Caunter (Caunter 1999)	Fathead minnow (*Pimephales promelas)*	32-d post hatch survival 32-d post hatch, growth	NOEC=640 NOEC=640	Valid	Measured concentration. GLP
Caunter (Caunter 2000)	Fathead minnow (*Pimephales promelas)*	F_o 71-d, growth F_o egg production: F_1 egg hatchability F_1 60d, survival F_1 30-43d, growth F_1 egg production F_2 egg hatchability F_2 160-260-d, survival F_2 130-260d,growth	NOEC=640 LOEC=1280 NOEC=640 LOEC=1280 NOEC=160 LOEC=640 NOEC=640 NOEC=640 NOEC=160, LOEC=640 NOEC=16 LOEC=160 NOEC=640 NOEC=640	Valid	Multigenerational study
Browmer (Bowmer 1999)	Carp (*Cyprinus carpio)*	28d-survival: 28d-growth:	NOEC=740 NOEC=740	Use with care	Non-GLP -abstract only
Harbruge (Haubruge 2000)	Guppy (*Poecilia reticulata*)	21d -sperm count: 21d-gonad size: 21d-sperm length:	LOEC=274 NOEC=549 NOEC=549	Use with care	Non-standard procedure, non-GLP

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Kwak (Kwak 2001)	Swordtail Fish (*Xiphophorus helleri)*	96h-survival	96-h LC_{50}=17,930	USE WITH CARE	Non-standard - renewal
Kwak (Kwak 2001)	Swordtail Fish (*Xiphophorus helleri)*	60d-tail length, growth:	NOEC=0.2 LOEC=2.0	Not valid	Non-standard-static test, non-GLP
Pickford (Pickford 2000)	African Clawed Frog (*Xenopus Laevis)*	90-d mortality 90-d, growth& development 90d-sex ratio	NOEC=500 NOEC=500 NOEC=500	Valid	Measured conc. GLP
Kloas (Kloas 1999)	African Clawed Frog (*Xenopus Laevis)*	12-wk survival: 12-wk, growth& development: 12-wk-sex ratio:	NOEC=23 NOEC=23 NOEC=2.3 LOEC=23	Not valid	Larval development renewal non-GLP
Alexander (Alexander 1998)	Water flea (*Daphnia magna)*	48-immobilization:	EC_{50}=10,000	Valid	ASTM-non-GLP
Caspers (Caspers 1998)	Water flea (*Daphnia magna)*	21-d, survival: 21-d,molting success, growth: 21-d, reproduction:	NOEC=3160 NOEC=3160 NOEC=3160	Valid	Renewal, GLP
Alexander 1988 (Alexander 1998)	Mysid shrimp (marine) (*Mysidopsis bahia)*	96h-survival	LC_{50}=1100	Valid	Non-GLP

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Andersen (Anderson 2000)	Copepod (marine) (*Acartia tonsa)*	5-d, larval growth:	EC_{10}=100	Not valid	No report available
Kusk (Kusk 1999)	Copepod (marine) (*Acartia tonsa)*	48-h, survival:	LC_{50}=3400 to 5000	USE WITH CARE	General methods
Anderson (Andersen 1999)	Copepod (marine) (*Acartia tonsa)*	72-h, immobilization	EC_{50}=960	Not valid	No reported details
Anderson (Andersen 1999)	Copepod (marine) (*Acartia tonsa)*	12-d, stimulated production	Effect levels unstated	Not valid	Feed concentrations not reported
Watts (Watts 2001)	Chironomid (*Chironomus riparius)*	Life cycle test, emergence, eggs production, female and male gonadal malformations	Effect levels unstated	Not valid	BPA concentrations not stated or estimated
Oehlmann (Oehlmann 2000)	Prosobranch snails (*marisa cornuarietis)* *nucella lapillus*	Reports both feminization and virilization in females (*marisa*), feminization in males and females (*nucella*)	LOEC=1 No replicates, nominal concentration, egg production, sex organ malformation	Not valid	No replicates

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Alexander (Alexander 1998)	Green Algae (*Pseudokirchneriella Subcapitata)formerly Selenastrium capricornutum*	96h-, cell count, reproduction : chlorophyll a, growth:	96-h EC_{50}=2730 96-h EC_{10}=1360*NOEC=1360 96-h EC_{50}=3100 96-h EC_{10}=1360-1680	Valid	USEPA, non-GLP. Based on cell count and total cell volume.
Alexander (Alexander 1998)	Diatom (marine) (*Skeletonema costatum)*	96h-, cell count, reproduction : cell volume, growth:	96-h EC_{50}=1000 96-h EC_{10}=400-690 96-h EC_{50}=1800 96-h EC_{10}=400-690	Valid	USEPA, non-GLP
Segner (Segner 2003)	Zebra fish (*Danio rerio)*	Fertilization to adult-survival Fertilization success growth	LOEC=1500 NOEC=1500 NOEC=1500	Valid	European IDEA project-life cycle-life stage toxicity test
Watts et al., 2003	Zurich strain (male clone) *(H vulgaris)*	96h-mortality 72h-growth , reproduction	LC_{50}=6910 LOEC=1000, NOEC=4600	USE WITH CARE	Standard test-GLP
Watts (Watts 2001)	*G . pulex* juvenile adult pairs	240h-LC 24h-growth 24h-precop sep	LC_{50}-1490 LOEC=830 NOEC=0.01, NOEC=8400	USE WITH CARE	Standard test
Watts (Watts 2003)	C. riparius	240h Life cycle growth	LC_{50}-11510 NOEC=10400, LOEC=0.078 NOEC=100	Use with care	Standard test, F_1, F_2 generation

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Pascoe (Pascoe 2002)	Freshwater Cnidarian (*Hydra vulgaris)* male zurich strain	96h -mortality 72h-mortality 48h-mortality 24h-mortality 72h-growth 6wk-growth	LC_{50}=6900 LC_{50}=7400 LC_{50}=8900 LC_{50}=12400 NOEC=0.002 LOEC=4600 NOEC=42	Valid	Renewal test
Yang , (Yang 2005)	Black spotted frog (*Rana nigromaculata)*	15-60d-deformity development:	2-200	Not valid	Tadpoles 5dph-No description of endpoint
Andersen (Andersen 2001)	calanoid copepod (Acartia tonsa)	5d-development 2d- mortality	EC_{50}=550 LC_{50}-4200	Valid	Standard test
Tatarazako (Tatarazako 2002)	Water flea (*Ceriodaphnia dubia*)	6-7d-,progeny/count numbers reproduction	IC_{25}=3922, LOEC=1880; NOEC=940	Valid	Progeny/counting-GLP
Alexander (Alexander 1998)	Opossum shrimp (*Americamysis bahia*)	24h-mortality 48h-mortality 72h-mortality 96h-mortality	LC_{50}=3300 LC_{50}=1800 LC_{50}=1200 LC_{50}=1100	Valid	ASTM Method-non GLP
Kashiwada (Kashiwada, Ishikawa et al. 2002)	Medaka, high-eyes *(Oryzias latipes)*	72h-mortality embryo male female	IC_{50}=9000 LC_{50}=5100 LC_{50}=6800 LC_{50}=8300	Valid	GLP

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Warner (Warner and Jenkins 2007)	Fathead minnow (*Pimephales promelas)*	29-30d-development (abnormal) 29-30d-DVP(deformity) growth(length) survival(mortality)	NOEC=100 NOEC=1000 NOEC=1000 NOEC=1000	Valid	GLP
Lahnsteiner (Lahnsteiner 2005)	Brown Trout (*salmo trutto fario)*	3mths growth (volume) growth (weight) reproduction (circular cell) reproduction (germ cell)	NOEC=2.4 NOEC=2.4 NOEC=1.76 NOEC=2.4	Valid	Standard Procedures
Kinnberg (Kinnberg and Toft 2003)	Guppy (*Poecilia reticulata)*	30days (0% mortality)	NR-Zero=5000	Valid	Standard Procedures
Arukwe (Arukwe, Celius et al. 2000)	Atlantic salmon (*salmo salar*	2wk- mortality 7d-mortality	NR-Zero=5000 NR-Zero=125000	Valid	Standard procedures
Lysak (Lysak 1972)	Rainbow Trout donaldason trout (*Oncorhynchus mykiss)*	2d,mortality	LC_0=5000 LC_{100}=7000	Valid	GLP
Kang, (Kang, Yokota et al. 2002)	Medaka high eyes (*Oryzias latipes)*	3wk-,fecudity, reproduction fertility, reproduction reduction, survival growth:	NOEC=837 NOEC=1720 NOEC=3120	Valid	GLP

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Sohoni (Sohoni 2001)	Fathead Minnow (*Pimephales promelas)*	164d-length, growth: 72d-growth egg production, reproduction: F_1 hatchability:	LOEC=1280 LOEC=640 LOEC=1280 LOEC=640	Valid	Standard procedures
Hahn (Hahn 2002)	Midge (*Chironomus riparius)*	24h- survival, mortality:	100-3000	Not valid	No end point reported
Hill (Hill 2002; Hill 2002)	Sponge (poriferans) (*Heteromyenia)*	Abnormal development 9d-growth	NOEC=1600	Use with care	No end point reported
Stoker (Stoker, Sirosky et al. 2003)	Alligator *(Caiman latirostris)*	Sex reversal; reproduction	LOEC-1400	Not Valid	Topical application of BPA-Non-GLP
Oka (Oka, Adati et al. 2003)	Tadpole (*Xenopus laevis*)	Abnormal growth & development:	LOEC=4650	Not Valid	Embryos used. No analysis of test concentrations
Putt (Putt 2003)	Duckweed *Lemna gibba*	7d-growth	NOEC=7800	Valid	The static-renewal study was performed to GLP according to OECD Guideline 221

Table 4-3 continued

Study	Specie	Endpoint	Result (µg/L)	Status (valid/non-valid)	Comments
Springborn (Springborn Smithers 2006a)	Rotifers *Brachionus calyciflorus*	48h-reproduction	NOEC=1800	Valid	Study performed to GLP. NOEC based on measured concentration
Springborn (Springborn Smithers 2006b)	Scud *(Hyalella azteca)*	42d-reproduction	NOEC=490	Valid	This study was performed to GLP using US EPA guidelines and a full study report is available
Sayers (Sayers 2005)	Midge *Chironomus tentans*	98h-survival	NOEC=1400	Valid	The test was performed according to US EPA guidelines and was GLP compliant

d=day, h=hour, wk-week, *For algae studies it is generally accepted that a 72-hour (or longer) NOEC value can be considered as a chronic result-.if a long-term NOEC is not available then an EC_{10} obtained by extrapolation using appropriate statistics, such as probit analysis, can be considered as if it were a NOEC.

5.0 OVERALL SUMMARY AND CONCLUSION

The research has shown that there are various sources of PEXEs. It is also shown that most PEXEs enter the aqueous environment through point sources such as WWTPs due to their inefficient removal rates, and through nonpoint sources such as urban run offs. It is also shown that the steroidal estrogens E1, E2, E3 and the synthetic birth control pill EE2 are the dominant estrogens present in WWTP effluents, which flow directly into surface waters. Levels of BPA and alkyl phenoxylates tend to be present in high concentrations downstream from industrial point sources such as paper mills. PEXEs were also found in drinking water.

In the United States surface water provides 60% of the drinking water supply. Also, rivers and lakes are used for recreational purposes and food supply. This information is important as it suggests that both wildlife and humans are exposed to these PEXEs via different routes and sources. However, what is unsure, is the direct health impact(s) associated with such exposures for both humans and wildlife and or the synergistic or additive effects of these chemicals. This is partly so because in nature these PEXEs are in mixtures or concoctions but, most of the laboratory tests are done on a single chemical thus, the interpretation of the results for the field environment may be flawed.

This research suggests that aquatic organisms are at risk to PEXEs at environmentally relevant concentrations. But, there is some uncertainty as to the direct or indirect impact of these exposures to humans. Again, these chemicals are in mixtures in their native environment and most of the experiments are being done on single chemicals. The end user of this

information must therefore be aware that it may not be possible to infer or make an association that any or all adverse effects came from one or more chemicals or even which specific chemical(s).

More research is needed to address this issue of emerging contaminants in our aqueous environment. Exposure concentrations, exposure routes and mechanism of action(s) should be thoroughly investigated to determine exactly at what concentrations humans and wildlife are affected and specifically, the adverse effect caused at that concentration(s). In other words, Epidemiological studies should also be conducted to determine if there are associations of specific disease states with specific PEXEs. There must be more laboratory research into mixtures or concoctions of PEXEs that simulate the field environment so that better associations can be made between adverse effects and chemical(s). A compliment of PEXE field research must also be done to ensure the generation of direct results, which minimizes uncertainty and could be more valid. More toxicological studies should be conducted at environmentally relevant concentrations. There needs to be the use of standardized methods in the analysis of these compounds. Various and different methods were used in the analysis of PEXEs in the research studies reviewed. This presents somewhat of a quandary as some methods are considered more accurate than others. For example the use of mass spectrometry to measure a PEXE versus a biological method such as E-screen. Also, there may be the need to investigate the use of lower detection limits to measure PEXE concentrations in analytical methods. Until there are established or standardized methods, researchers may not be able to accurately quantitate true concentrations of these PEXEs present in our environment and draw equitable conclusions. However, the fact that these PEXEs are present

in our environment and are affecting wildlife and possibly humans cannot go unnoticed. This makes this issue a valid public health concern.

Until there are regulations or laws implemented to effectively deal with this issue of emerging contaminants and PEXEs, there are some precautions that can be taken. Multiple exposure can be minimized by use of alternative household products not containing PEXEs. The use of an alternative birth control pill should also be considered to eliminate EE2 from the aqueous environment. The use of safer alternative chemicals should be considered for the chemical industry. WWTP should be modified to increase their efficiency in removal rates for PEXEs and there should be mandatory testing of our water supply for PEXEs. Long term, effective regulations should be implemented to deal with the issue of emerging contaminants.

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