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The Impact of Endocrine-disrupting Chemicals on Wildlife Conservation

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May 6, 2016

A thesis submitted to the faculty of the Environmental Studies Program in partial fulfillment of the graduation requirements for the Degree of Bachelor of Arts with Honors in Environmental Studies

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) are chemicals that interfere with any part of hormone function, and are present in the environment due to human production, use, and disposal. EDC exposure in wildlife and effects on wildlife health have emerged as growing topics of research in recent decades, but how EDCs affect wildlife conservation is less explored. This study assesses the current state of the science of EDC effects in wildlife, and gauges how conservation scientists and practitioners view the impact of EDCs on wildlife conservation.

First, I completed a literature review of the effects of EDCs in wildlife, specifically looking at effects in all vertebrate taxa. Then, to address how conservationists view EDCs and wildlife conservation, I designed and distributed a survey to determine conservationist knowledge of EDCs, attitude towards EDCs, and practice of researching EDCs in wildlife. The majority of the 116 respondents were familiar with EDCs, but most were unaware of specific EDCs and physiological effects of EDCs in many taxa. Overall, respondents felt EDCs were a significant concern for wildlife conservation, but not in comparison to other threats. A majority of respondents believe EDCs should be a priority in their field of study, but only 24 respondents are studying or planning to study the effects of EDCs in wildlife.

The diversity and magnitude of wildlife species potentially at risk for the effects of EDCs mandates action, including precautionary measures to prevent further exposure in wildlife. Although a subset of survey respondents were actively aware of EDC effects in wildlife, most conservationists that responded are not informed enough and are too ambivalent to address the growing severity of EDC impacts on wildlife conservation.

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INTRODUCTION

Overview of Study

Wildlife play an integral role in maintaining functioning ecosystems, and certain species, such as top predators, have a disproportionally large impact on an ecosystem compared to their abundance (Chapin et al. 2000). Oftentimes, these critical species are threatened by human practices (Chapin et al. 2000). Global extinction of wildlife species is happening at an unnaturally fast rate due to immense human change to the global environment (Chapin et al. 2000, Barnosky et al. 2011). Decreases in wildlife have widespread effects, including ecosystem failure and economic consequences (Chapin et al. 2000). One method for addressing wildlife vulnerability and extinction is wildlife conservation, the practice of protecting wildlife and their habitats (Chapin et al. 2000).

An area of limited research is the impact of endocrine-disrupting chemicals (EDCs) on wildlife conservation. EDCs are chemicals that disrupt any part of hormone action, and may negatively affect physiological systems including reproductive and neurological systems (Gore et al. 2015). Many studies have shown evidence of exposure and effects of EDCs on wildlife (Gore et al. 2015), but few have focused on how EDCs could influence wildlife conservation decisions. This study seeks to determine what the current state of the science is of EDCs in wildlife conservation, and to determine if conservationists are aware of or are mitigating the effects of EDCs in wildlife.

I addressed these questions in two parts. First, I completed a literature analysis of effects of EDCs in wildlife, with a focus on vertebrates and species of conservation concern, to determine what data gaps exist. Second, I conducted a survey to assess how conservation scientists and practitioners view the importance of EDCs for wildlife conservation. The purpose of surveying is to gain a sense of current knowledge and awareness of EDCs in those people who are involved in conservation on a continual basis. Additionally, the survey gauges how many conservationists are researching EDCs or mitigating the effects of EDCs in wildlife. Conservation scientists and practitioners research species of conservation concern or implement conservation management

practices, so it is important to know how they perceive EDCs. These two study components were combined to address the impact of EDCs on wildlife conservation.

State of the Science of EDC Exposure and Effects

The Endocrine System

The endocrine system influences many aspects of life, including reproduction, metabolism, osmoregulation, embryonic development, growth, metamorphosis, and digestion. The system is present in all vertebrates and includes all endocrine glands, hormones, and target tissues. Hormones are carried through the blood, and their effects are localized to specific target tissues that have specialized hormone receptors. The endocrine system plays a crucial role in how an animal interacts with its environment because of the interrelationships among hormones, environmental cues, and physiological systems (Kardong 2015). In one example in vertebrates, environmental cues stimulate the nervous system, which alters the endocrine system to stimulate or inhibit certain hormone signals (Kardong 2015). The release or inhibition of hormones causes physiological and behavioral changes in the animal, changing how they interact with their environment, which can “feed forward” this cycle (Figure 1). The endocrine system can also be affected by social cues, such as changes in another animal’s behavior (Dietert 2014, Kardong 2015) and by environmental pollutants (Bergman et al. 2012, Dietert 2014, Gore et al. 2015).

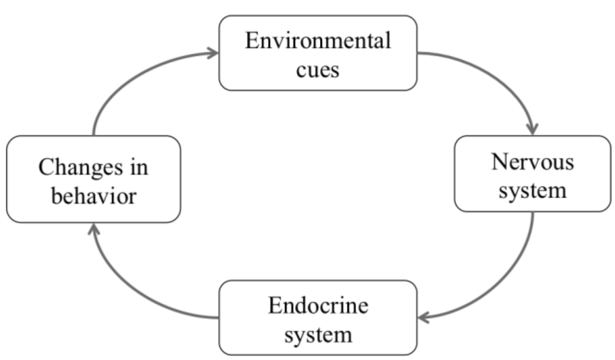

Figure 1. Environmental and hormonal feedback loop. (Kardong 2015)

The endocrine system is fairly well conserved among vertebrates. Major glands include the thyroid, ultimobranchial and parathyroid, adrenal gland, pancreatic islets, pituitary gland, gonads, and pineal gland (Kardong 2015), all of which play critical roles in vertebrate function as described in Table 1. Failure or malfunction of the major endocrine glands can lead to serious health impacts and eventually death (Table 1) (Evans 2011, Kardong 2015). For example, all birds, mammals, fishes, reptiles, and amphibians need a properly functioning thyroid gland for normal growth (Kardong 2015).

Endocrine-disrupting Chemicals (EDCs)

Certain chemicals that are environmental pollutants or are used in industrial practices have the property of being endocrine-disrupting chemicals. According to The Endocrine Society, a 100 year-old society of leading endocrinology professionals who maintain six peer-reviewed endocrinology journals, EDCs are "exogenous chemical(s), or mixtures of chemicals, that interfere with any aspect of hormone action" (Gore et al. 2015). EDCs are chemicals generated by human production, use, and disposal that enter the environment either intentionally or unintentionally (EPA 2015). For example, EDC sources include heavy metals, phytochemicals, byproducts of industry, chemical, and manufacturing practices, and agricultural chemicals (Dietert 2014).

The Endocrine Disruption Exchange (TEDX), an international nonprofit committed to studying EDCs, currently lists 1,038 chemicals that are potential endocrine disruptors (TEDX 2015), including bisphenol A (BPA), phthalates, and polychlorinated biphenyls (PCBs). Chemicals are included on the TEDX list when at least one peer-reviewed study shows endocrine system effects due to the chemical (TEDX 2015). In contrast, for the US Environmental Protection Agency (EPA) to list a chemical as a potential endocrine disruptor the EPA must test it in 11 assays, with limited consideration of preexisting studies (EPA 2015). The EPA has listed only 18 chemicals as potential EDCs since the start of the Endocrine Disruptor Screening Program (EDSP) in 1998 (EPA 2015). Although, the study of EDCs has greatly advanced since concerns over effects first arose in the 1950s (Hotchkiss et al. 2008), only a fraction of all EDCs in the environment are known (Bergman et al. 2012). Some EDCs are persistent in the

Table 1. Overview of major hormone producers and pathways in vertebrates. Based on information in Kardong 2015.

Gland	Hormone	Function in vertebrates	Presence in vertebrates	Disruption causes
Thyroid	Thyroxine/tetraiodothyronine (T4) hormone and triiodothyronine (T3) hormone	In endotherms, T3 and T4 control metabolism and can elevate oxygen consumption and heat production by tissues, as well as increase plasma membranes in mitochondria. In ectotherms, target tissues will only respond to thyroid hormones at certain warmer temperatures. Birds, mammals, reptiles, and fishes all need normal levels of thyroid hormones for growth. Thyroid hormones also stimulate molting, and control gonad maturation. In amphibians, T3 and T4 stop the growth of larvae and start metamorphosis.	Found in all vertebrates	Problems with thermoregulation, metabolism, development, and reproduction.
Ultimobranchial/parathyroid	Calcitonin hormone and parathyroid hormone	Regulates calcium. Calcium in the blood controls bone/egg/antler density and skeletal muscle spasms. The mechanism for calcium control in tetrapods is debated, but involves the intestines, kidneys, and bones.	The ultimobranchial gland is separate from the thyroid in fishes, amphibians, reptiles, and birds, but in mammals it is part of the thyroid. Mammals, amphibians, reptiles, and birds have parathyroid glands, but fish do not.	Problems with egg shell and bone density, and muscle function.
Adrenal gland	Corticosteroid hormones and mineralocorticoid hormones	In vertebrates: Controls osmoregulation and metabolism, and stress responses including emergency life history states. In mammals specifically: Controls osmoregulation through water reabsorption and sodium transport by the kidney. Glucocorticoids control metabolism of carbohydrates, and establish emergency life history state. Estrogens, androgens, and progestogens control reproduction. Epinephrine and norepinephrine help control stress response.	Found in all vertebrates	Problems with osmoregulation. Inability to react to stress. In mammals, disruption of placental estrogens ends gestation and causes premature birth.

Pancreatic islets	Insulin, glucagon, and somatostatin hormones	Insulin controls the overall metabolism of carbohydrates, fats, and proteins. Glucagon is critical for metabolic regulation in herbivores and fasting carnivores, and is critical for regulating digestion in birds and lizards. Somatostatin controls insulin and glucagon levels.	Found in all vertebrates	Problems with metabolism, including hypoglycemia and hyperglycemia.
Pituitary gland	Source of a variety of hormones, including vasopressin, oxytocin, growth hormone, prolactin, thyrotropin, gonadotropins, corticotropin/adrenocorticotropic hormone, and melanophore-stimulating hormone.	Controls the stimulation or inhibition of hormones in multiple other glands, including thyroid, gonad, and adrenal glands. Also controls blood pressure, osmoregulatory hormones, growth, and melanin distribution. In mammals specifically: Controls contractions during parturition and mammary gland activation. In birds specifically: Controls premigratory fattening, brood patches, and crop milk. In fish specifically: Controls osmoregulation, especially in anadromous fish.	Found in all vertebrates	Problems with most body systems, including but not limited to issues with osmoregulation, blood pressure, development, and reproduction.
Gonads	Androgens including testosterone, estrogens, progestogens	Hormones support gamete production and secondary sex characteristics.	Found in all vertebrates	Problems with reproduction, including infertility.
Pineal gland	Melatonin	Detects changes in photoperiod and regulates the reproductive cycle in many vertebrates. Affects skin color in lower vertebrates, and might influence the circadian rhythm of reptiles and birds.	Found in all vertebrates	Much is unknown, but may include problems with temporal cycling, courtship behavior, thyroid activity, and the immune system.

environment while others are not; however, persistence may not be important because of continual exposure to EDCs and cumulative effects of all EDCs taken together (Daughton and Ternes 1999). EDCs are ubiquitous throughout the world, because they travel both in the atmosphere and through the ocean (Annamalai and Namasivayam 2015). Nowhere on earth is uncontaminated (Lyons 2006).

Major exposure routes for EDCs are through three major pathways: oral ingestion, respiratory inhalation, or dermal absorption (Evans 2011). Mammals have two more pathways for EDC transfer from mother to offspring: transplacental diffusion and lactation (Bernanke and Kohler 2008, Evans 2011, Bergman et al. 2012a, Vethaak and Legler 2013). The method of exposure can determine which physiological systems are affected (Evans 2011). For example, if the EDC is orally ingested, it is more likely to impact the liver than if dermally absorbed (Evans 2011).

EDCs can interfere with endocrine pathways at many points, including hormone synthesis, transport, secretion, binding, and elimination of natural hormones (Dietert 2014) EDCs, particularly organochlorines, can mimic hormones and bind to receptors, inhibiting natural hormones (Jenssen 2005). Normally, the maximum effect of a hormone occurs when not all receptors are being used, also known as functional receptor saturation (Zoeller et al. 2012). Because of functional receptor saturation, large amounts of a hormone can actually decrease the desired effect, while small amounts can leave receptors open and have a bigger impact (Zoeller et al. 2012). This difference leads to nonlinear response curves, including nonmonotonic dose response curves (NMDRCs) (Zoeller et al. 2012). In NMDRCs, there may not be a lower threshold and any amount of hormone, or EDC, can have an effect (Zoeller et al. 2012).

The actions of hormones change throughout life, between males and females, and across different tissues and organs, so the effects of EDCs vary (Zoeller et al. 2012). Hormone action during development is often permanent, but the effects may not be observed until later life stages (Zoeller et al. 2012). This trend is also true of EDCs (Zoeller et al. 2012). Fetal exposure and exposure of young animals is particularly important due to their sensitivity at early life stages (Zoeller et al. 2012). Some of the most pronounced relationships of EDCs with the endocrine system include with the

thyroid hormone system, and with sex steroid hormones and cortisol (Jenssen 2005). These relationships can be impacted during early life stages (Jenssen 2005).

Exposure to EDCs and Effects on Humans

Much is currently known about EDC effects on humans. EDCs have been studied and are known to influence obesity and diabetes (effects on adipogenesis), female reproduction (abnormal puberty, irregular cyclicity, reduced fertility/infertility, polycystic ovarian syndrome, endometriosis, fibroids, preterm birth/adverse birth outcomes), male reproduction (cryptorchidism, hypospadias, poor semen quality, ultrastructural testicular abnormalities), cancers (prostrate, breast, endometrial, ovarian), altered thyroid hormone levels, and neurodevelopmental and neuroendocrine behavior (Gore et al. 2015). Effects of EDCs on female and male reproduction can lead to infertility, particularly in men (Gore et al. 2015). Much of what is known about EDC effects on humans comes from lab studies using rodents. Hunt et al. (2009) analyzed studies of BPA in both lab animals and humans, and suggested that studies in rodents are fairly valid predictors of effects in humans.

One of the best-studied examples of EDC effects in humans is diethylstilbestrol (DES), a synthetic estrogen. From 1938 until 1971, DES was commonly prescribed to pregnant women to prevent miscarriages, premature births, and other complications (CDC 2012). However, the U.S. Food and Drug Administration (FDA) recommended physicians stop prescribing DES in 1971 after it was learned that it caused complications for female offspring (CDC). If mothers were given DES during their first trimester of pregnancy, their daughters had a higher incidence of breast cancer, clear cell adenocarcinoma of the vagina and cervix, and reproductive anomalies (Veurink et al. 2005, Zoeller et al. 2012). Later it was learned that women prescribed DES had an increased risk for breast cancer (CDC), and sons exposed in utero to DES had an increased risk of testicular abnormalities (Palmer et al. 2009). Preliminary studies have shown effects of DES in grandchildren of women who took DES while pregnant, including decreased fertility and increased cancer risk (Titus-Ernstoff et al. 2006, Titus-Ernstoff et al. 2008, Titus-Ernstoff et al. 2010). This area of transgenerational effects of EDCs as well as neuroendocrine disruption are areas of growing research (León-Olea et

al. 2014). There is growing evidence for neuro-EDC connections to decreased fecundity, neurodegeneration, and cardiac disease in humans (León-Olea et al. 2014)

Exposure to EDCs and Effects on Wildlife

EDC exposures in wildlife and effects on wildlife health have emerged as growing topics of research in the past decade (Dietert 2014). Ample evidence shows EDC exposure in wildlife (Wang and Zhou 2013). The summary of the 2012 *State of the Science of Endocrine Disrupting Chemicals* report by the World Health Organization (WHO) and United Nations Environment Program (UNEP) states there is enough evidence to conclude that EDCs have negatively affected certain wildlife species (Bergman et al. 2012). Because EDCs can have diverse effects on multiple parts of the endocrine system and therefore affect a wide variety of physiological systems, there are many means of studying EDCs in wildlife (Jobling and Tyler 2006). For example, wildlife toxicology is the study of effects of environmental contaminants on wildlife health, behavior, and reproduction, while ecotoxicology is the study of chemical effects on the environment, both aquatic and terrestrial, as it relates to wildlife (Kendall et al. 2010).

Studies can also focus on varied aspects of EDCs in wildlife from exposure routes to effects, at an individual or a population level. Another method is biomonitoring, or measuring the burden of a certain EDC or group of EDCs in an organism (Bernanke and Kohler 2008). Biomonitoring can help determine if EDCs are bioaccumulating or biomagnifying, where the burden of EDCs increases as tropic levels increase due to consumption of contaminated prey (Bernanke and Kohler 2008). Effects of EDCs can also be analyzed through changes in genes and gene products (Walker and Gore 2011).

There are two main methods of studying EDCs in vertebrates, “bottom-up” and “top-down” studies (Bernanke and Kohler 2008). In “bottom-up” studies, animals are deliberately exposed to an EDC and then are studied throughout development for effects (Bernanke and Kohler 2008). “Bottom-up” studies can effectively link a particular EDC to observed effects, but are not effective in determining population or greater ecological effects (Bernanke and Kohler 2008). In “top-down” studies, populations are studied for changes in reproductive output that could potentially be due to effects of EDCs

(Bernanke and Kohler 2008). In this case, it is difficult to link EDCs to effects but correlations may be useful (Bernanke and Kohler 2008).

Given that nowhere on earth is uncontaminated by EDCS (Lyons 2006), EDC exposure in wildlife is a global problem, as highlighted by studies of animals in many parts of the world (Vos et al. 2000). For example, marine animals have been studied and found to have EDC exposure in many oceans (Vos et al. 2000). Reviews as early as 1998 of chemical studies in wildlife cite EDCs as a potential problem that could impair reproductive function and other physiological systems in all taxa of wildlife (Tyler et al. 1998, Fossi et al. 1999). EDCs pose a significant threat to wildlife because of their ability to affect reproduction, brain function, and the immune system, all of which are crucial for wildlife survival (Lyons 2006). However, most wildlife studies only associate EDCs with effects, and causality is difficult to determine and verify (Vos et al. 2000, Jobling and Tyler 2006). Some studies with causally linked effects include masculinization in marine snails due to tributylin (TBT) exposure, egg thinning in raptors due to DDT/DDE exposure, multiple effects in fish species, impaired reproductive and immune function in Baltic and gray seals due to PCB, and pesticide-induced reproductive harm in alligators (Table 2) (Vos et al. 2000, Vethaak and Legler 2013).

Aquatic environments are often considered the ultimate sink for EDCs due to continual human release of EDCs in water bodies and the ability for EDCs to migrate throughout the water (Wang and Zhou 2013). The greatest number of conclusive individual and population level causal effects of EDCs are in fish species due to exposures in aquatic environments (Wang and Zhou 2013). However, another exposure route, particularly for mammals and birds, is through food ingestion (Vethaak and Legler 2013). It is well known that global food chains are contaminated with many chemical contaminants in the environment.

Table 2. Species in which causally linked evidence to EDCs exists (Damstra et al. 2002, Hotchkiss et al. 2008, Vethaak and Legler 2013).

Species	Locations	Contaminant	Effect	Strength of Evidence
Mammals				
Seals	Baltic sea; Wadden sea	PCBs (polychlorinated biphenyls) and metabolites	Reproductive failure and consequent population decline	Moderate
Seals	Wadden Sea	TCDD (dioxin)-like PAHs	Lowered immune competence likely contributing to mass mortalities	Moderate
Otter/mink	Great Lakes, USA; Nordic countries, Europe	PCBs (polychlorinated biphenyls)	Reproductive failure	Moderate
Birds				
Predatory birds, numerous species (e.g. bald eagle, osprey, peregrines, guillemot)	Europe, North America	DDT/DDE	Eggshell thinning, breeding failure, population declines	Strong
Reptiles				
Alligator	Florida, USA	DDT/DDE and other organochlorine pesticides	Demasculinization and decline in number of juveniles	Moderate
Amphibians				
Numerous frog species	USA	Atrazine and certain pesticides	Likely contributing to global population declines	Moderate
Fish				
Numerous freshwater and estuarine species	Worldwide	Estrogenic contaminants from sewage effluent	Feminization and likely population impacts in some species	Strong
Poeciliid species (e.g. mosquitofish, white sucker, mummichoq)	Canada, USA, and Europe	Paper mill, pulp mill effluent	Masculinization with possible population impacts	Strong
Invertebrates				
Numerous marine snail species	Worldwide	TBT (tributylin)	Reproductive failure with population declines in many species worldwide	Strong

Inhalation of atmospheric EDCs is another exposure route, which is an emerging area of research (Annamalai and Namasivayam 2015). Major EDCs in the atmosphere include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants (BFRs), dioxins, alkylphenols (APs), perfluorinated chemicals (PFCs), pesticides, and phthalates (Annamalai and Namasivayam 2015). Effects of all of these EDCs when inhaled have been studied either in the lab or in situ (Annamalai and Namasivayam 2015).

There are many inherent difficulties of studying EDC effects on reproduction in wildlife (Hunt et al. 2009). Environmental exposures to EDCs may affect individual species differently, as inter-species genetic differences can cause differences in response (Hunt et al. 2009). This difference in response is also true for individual animals of the same species due to intra-species genetic differences (Hunt et al. 2009). Abnormal reproductive development in males is linked to EDCs in many taxa (Edwards et al. 2005). Some vertebrate species have a greater innate reproductive plasticity, which makes them more vulnerable to effects like testicular dysgenesis syndrome than other species (Edwards et al. 2005). This vulnerability begins as early as the sexually undifferentiated embryo stage, when bipotential reproductive tissues are extremely sensitive to environmental pressures, and when study *in situ* of wildlife is almost impossible (Edwards et al. 2005).

A study by Giesy and Kannan (2001) looked at the global wildlife burden of perfluorooctane sulfonate (PFOS). PFOS is a breakdown product of perfluorooctanesulfonyl fluoride (POSF), a chemical used as a surface protector in carpets, leather, paper, packaging, fabric, and upholstery (Giesy and Kannan 2001). They found that PFOS was widespread in the global environment, and found residues in all fish, bird, and marine mammal species studied (Giesy and Kannan 2001). Both mink and bald eagle PFOS levels were higher than their diet would imply, suggesting the potential for PFOS to bioaccumulate (Giesy and Kannan 2001). Giesy and Kannan (2001) also found that the PFOS levels were higher in animals near urban areas than rural areas. For example, PFOS levels were higher in aquatic and marine mammals in the Great Lakes (USA) and the Mediterranean Sea (Europe) than in remote parts of the Arctic Ocean (Giesy and Kannan 2001).

EDC Impacts on Specific Vertebrate Taxa

Evidence exists of EDC exposure and effects in all major vertebrate taxa, including fish, amphibians, reptiles, birds, and mammals. Through literature analysis, Bernanke and Kohler (2008) and Vethaak and Legler (2013) evaluate the potential for EDCs to impact different wildlife taxa (Table 3).

Fish

Fish are frequently exposed to EDCs due to the contaminated aquatic environments in which they reside (Wang and Zhou 2013). Fish are particularly sensitive to anthropogenic chemicals because of their tendency to uptake EDCs like PCBs and PAHs through their gills and skin (Bernanke and Kohler 2008). There is global evidence that EDCs are compromising the physiology and sexual behavior of fish, including impacts on sexual differentiation and impairment of fertility (Bernanke and Kohler 2008). Some studies show evidence of EDC effects at a population level, but more evidence is needed for effects on population declines (Bernanke and Kohler 2008).

Amphibians

Amphibian populations are declining worldwide (Hayes et al. 2006, Bernanke and Kohler 2008). There is not causative evidence that EDCs are responsible for this decline (Bernanke and Kohler 2008), but EDCs greatly impact individual amphibians, especially their reproductive capabilities (Hayes et al. 2006). However, similarly to fish, more studies are needed at the population level (Bernanke and Kohler 2008, Vethaak and Legler 2013). Hayes et al. (2006) looked at pesticide impacts on frogs, and studied what hadn’t been studied before: low doses and effects of combined pesticides. They found that mixtures of pesticides had greater effects (Hayes et al. 2006), and found that both low doses and combined pesticides were a problem that needs to be studied more (Hayes et al. 2006).

Table 3. Field evidence of EDC effects in different wildlife taxa (Vethaak and Legler 2013).

Mammals
Altered steroid, thyroid, retinol, and cortisol levels
Features of masculinization
Reproductive impairment, sterility
Impairment of immune system, increased disease susceptibility
Nonreproductive disorders including tumors and skeletal deformities

Birds
Eggshell thinning
Changes in thyroid, retinol, and glutathione homeostasis
Lowered reproductive success
Lowered plasma corticosterone concentrations
Lowered immune function
Altered behavior

Amphibians and reptiles
Hermaphroditism/feminizing effects
Features of demasculinization
Altered steroid levels and thyroid activity
Impaired gonadal development
Reduced hatching success, poor egg viability and suvival
Increased risks for parasitic infestations

Fish
Feminization, masculinization
Changes in steroid levels, retinol, and thyroid hormone status
Induction of VTG and gonadal abnormalities (e.g. intersex)
Decreased fertility, compromised reproductive performance
Inhibition of gonadal development
Thyroid hyperplasia
Immunosuppression

Invertebrates
Masculinization (inposex/intersex)
Increased levels of VTG-related proteins
Feminization effects, demasculinization
Reproductive impairment, decreased fecundity and spawning, sterility
Interference with molting
Developmental/reproductive abnormalities

Reptiles

Reptiles have few field studies of EDCs in comparison to other taxa (Vethaak and Legler 2013), and most EDC work regarding reptiles is done in labs (Bernanke and Kohler 2008). One exception is the study of American alligators. Following a major chemical spill into Lake Apopka, Florida, the alligator population had a 90% decline in juveniles, and adults of both sexes experienced reproductive impairment (Crain et al. 1997, Vethaak and Legler 2013). Reptiles are also not as well studied as other taxa for certain EDC effects on reproduction and physiology (Neuman-Lee et al. 2015). Endocrine systems are highly divergent across the reptile class, making them a difficult group in which to extrapolate the effects of EDCs (Bernanke and Kohler 2008). For example, Neuman-Lee et al. (2015) cite few studies of PBDE effects on reptiles prior to their 2015 lab study of garter snakes, in which they found physiological effects of PBDEs on both mother garter snakes and the offspring, including increased size and higher thyroid follicular height (Neuman-Lee et al. 2015). No biomonitoring for PBDEs is occurring in wild garter snakes (Neuman-Lee et al. 2015). More reptile species than those currently being studied are likely impacted by EDCs, due to the widespread distribution of EDCs (Bernanke and Kohler 2008).

Birds

Wild bird populations are declining, and EDCs are a possible stressor (Bernanke and Kohler 2008). Ample evidence exists to show EDCs are a major concern for birds (Bernanke and Kohler 2008), particularly on reproductive systems, with the organochlorine pesticide DDT the best-studied example (Bernanke and Kohler 2008). The first major concern for EDCs in birds occurred with widespread use of DDT in the US and elsewhere in the 1950s and 1960s, causing thinning of eggshells and chick mortality (Luzardo et al. 2014). A 1972 ban on DDT has reduced this burden, but recent studies reveal birds are still being exposed to DDT as a residual environmental contaminant and other EDCs (Luzardo et al. 2014). Moore et al. (2014) ran a controlled lab experiment and found that zebra finches exposed to methylmercury throughout their life could not mount a corticosterone hormone response compared to non-exposed zebra

finches. Stress responses driven by corticosterone are important for predator avoidance and other behaviors, so inability to mount a stress response is concerning for zebra finch survival (Moore et al. 2014). Luzardo et al. (2014) looked at 57 EDCs (23 organochlorine pesticides, 18 polychlorinated biphenyls, and 16 polycyclic aromatic hydrocarbons) in 102 birds of six different species. All birds sampled had high levels of organochlorine pesticides, including DDT, which is concerning given that DDT is banned worldwide except for limited uses in disease vector control (Luzardo et al. 2014). The levels of polychlorinated biphenyls and polycyclic aromatic hydrocarbons were low and comparable with human levels (Luzardo et al. 2014). Based on predictive models of toxicity, the PCB and PAH levels were not considered a health risk for any of the birds (Luzardo et al. 2014).

Mammals

Effects of EDC exposure in mammals are varied. It is likely that mammal populations have been impacted by EDCs, of particular concern because of the unique aspects of reproduction in mammals versus other taxa (Bernanke and Kohler 2008, Evans 2011, Bergman et al. 2012, Vethaak and Legler 2013). All mammals have mammary glands and typically placental nourishment of embryos, which means they have multiple means to transfer EDC body burdens to offspring (Bernanke and Kohler 2008, Evans 2011, Bergman et al. 2012, Vethaak and Legler 2013).

Suckling young gain part of the mother's burden of chemical contaminants through colostrum and milk via the mammary glands at a crucial time for development (Bartol and Bagnell 2012, Bergman et al. 2012). Both colostrum and milk naturally contain bioactive factors from the mother, which help neonates adapt and respond to the extrauterine environment they are born into (Bartol and Bagnell 2012). These bioactive factors, termed lactocrine signaling, are important for proper offspring development of somatic tissues (Bartol and Bagnell 2012). However, EDCs can disrupt the transfer of these bioactive factors, and pose both a short and long term health risk for the offspring (Bartol and Bagnell 2012).

Mammals are particularly susceptible to effects of EDCs if they are exposed during gestation and postnatal growth (Evans 2011). EDC disruption of uterine

development in the fetus and neonate can cause infertility and cancer as an adult, and potentially affect their offspring too (Spencer et al. 2012). Mammals undergo uterine adenogenesis, or morphological differentiation of the uterine glands after birth, a postnatal event that is vulnerable to effects of EDCs (Bazer et al. 2012). Ewe lambs exposed to progestin for 56 days following birth did not have proper development of uterine glands (Bazer et al. 2012). When they became adults, they were unable to exhibit typical estrous cycles or maintain pregnancy (Bazer et al. 2012).

A large number of studies have shown evidence for EDC effects on marine mammals, with fewer studies on freshwater and terrestrial mammals (Jenssen 2005, Fillman et al. 2007, Bernanke and Kohler 2008, Vethaak and Legler 2013). Studies of PCB effects on mink and otters in the Great Lakes comprise most studies of EDCs and freshwater mammals (Vethaak and Legler 2013). For terrestrial mammals, some studies of reproductive dysfunctions or disorders in endangered top predators suggest an association with EDCs, including studies of cryptorchidism and sperm abnormalities in Florida panthers (Buergelt et al. 2002, Vethaak and Legler 2013). However, these "top-down" studies were not causally conclusive (Vethaak and Legler 2013). Laboratory studies are often of mammals, and have revealed extensive evidence that environmental halogenated chemicals have thyroid hormone-disrupting action in rats and mice (Shimizu 2014). Many halogenated chemicals are lipophilic and so can bioaccumulate in mammals (Shimizu 2014). In a review of EDC effects on laboratory mammals, Manfo et al. (2014) found that pesticides, heavy metals, phthalates, and dioxins can lead to disorders of testicular function and development of male sexual characteristics in rats and mice.

Based on studies showing effects of EDCs on other wild taxa, it seems surprising that wild mammals would be exempt from EDC effects although few studies exist (Kumar and Holt 2014). Kumar and Holt (2014) cite subtle changes to mammalian reproduction that could have cascading effects. For example, if due to EDCs oocyte and Sertoli cell production decreased in mammalian fetuses they could have lowered gamete production, and ultimately lowered fertility (Kumar and Holt 2014). This would be undesirable for already threatened populations (Kumar and Holt 2014). However, these changes are likely undetectable in wild and threatened species, because these species are less frequently studied invasively (Kumar and Holt 2014).

Biomagnification of many persistent organic pollutants (POPs), such as organochlorines, is particularly high for marine endothermic mammals, and they are among most vulnerable to climate change (Jenssen 2005). Many seal and polar bear studies have found increased organochlorine exposure, including PCBs and DDT, and decreased thyroid hormone levels (Jenssen 2005). In addition, POPs effect on cortisol levels might inhibit physiological processes and leave marine mammals more vulnerable to environmental stressors like climate change or habitat loss (Jenssen 2005). Fillman et al. (2007) studied reproductive failures in South American fur seals and concluded there is a suspected association between reproductive failure and bioaccumulation of PCBs, DDT, and other organochlorines in maternal body tissues.

Reproduction

Of all physiological systems, EDC effects on the reproductive system are likely the largest concern for vulnerable species, because their populations are already reproductively unstable. In mammals, the best evidence of reproductive disruption comes from marine mammals and a few freshwater or terrestrial mammals (Table 4).

A great variety of bird species have been studied for reproductive effects of EDCs including but not limited to raptors, gulls, herons, starlings, and swallows (Vos et al. 2000, Vethaak and Legler 2013). Reproductive effects found include eggshell thinning, reduced hatchability, lower breeding success, developmental impairment, feminization, impaired ovarian function, and reproductive failure, due to organochlorines (DDT/DDE and PCBs), PBDEs, PAHs, and heavy metals (Vos et al. 2000, Vethaak and Legler 2013). Similarly, a number of fish species have been studied for the reproductive effects of EDCs, both in the wild and in laboratories (Vos et al. 2000, Vethaak and Legler 2013). Gonadal abnormalities and general reproductive impairment are the most common reproductive effects found in fish, but effects span from feminization and masculinization to infertility (Vethaak and Legler 2013). Studies have documented the effects EDCs have on the reproductive success of many amphibians and some reptiles (Table 5).

Table 4. Studied reproductive effects of EDCs in wild mammals (Vos et al. 2000, Vethaak and Legler 2013, and references therein)

Species	EDC	Effect	Source
Baltic gray seals	Organochlorines	Sterility, uterine stenosis, uterine smooth muscle tumors	Vethaak and Legler 2013
Beluga whales	PCBs, dieldrin (organochlorine), dioxins	Decreased fecundity, hermaphroditism	Vos et al. 2000, Vethaak and Legler 2013
Black and brown bear	Unknown	Masculinization	Vos et al. 2000
Dall's porpoises	PCBs, DDT/DDE	Reduced testosterone levels	Vos et al. 2000
European otter	PCBs	Reproductive impairment	Vos et al. 2000, Vethaak and Legler 2013
Harbor seals	PCBs and metabolites	Decreased fecundity, implantation failure	Vos et al. 2000, Vethaak and Legler 2013
Mink and Otter	Dioxins	Reproductive effects	Vos et al. 2000, Vethaak and Legler 2013
Panther	Mercury, DDT/DDE, PCBs	Cryptorchidism	Vos et al. 2000, Vethaak and Legler 2013
Polar bear	PCBs, DDT/DDE	Masculinization	Vos et al. 2000, Vethaak and Legler 2013
Ringed seals	PCBs	Sterility	Vos et al. 2000, Vethaak and Legler 2013
Saimaa ringed seal	Mercury	Decreased population size	Vos et al. 2000
Sea lions	DDT-like compounds	Premature pupping	Vos et al. 2000

Table 5. Studied reproductive effects of EDCs in reptiles and amphibians (Vos et al. 2000, Vethaak and Legler 2013, and references therein)

Species	EDC	Effect	Source
American alligator	DDT/DDE	Low hatching rates, abnormal gonadal morphology and sex steroid concentrations	Vos et al. 2000, Vethaak and Legler 2013
Multiple frog species including: American leopard frogs, cricket frogs, and green frogs	Agricultural pesticides (organochlorines)	Feminization, impaired fertility, altered gonadal function, and others	Vethaak and Legler 2013
Red-eared turtles	DDT/DDE	Generation of female hormones in males	Vethaak and Legler 2013
Snapping turtles	Organochlorines	Decreasing hatching success, feminization	Vos et al. 2000, Vethaak and Legler 2013

Cancers

One health impact of EDCs is cancer promotion. Multiple EDCs, such as PAHs, are reported as carcinogens (Annamalai and Namasivayam 2015). Cancers are a concern for wildlife morbidity and mortality, and McAloose and Newton (2009) reported an increase in benign and malignant tumors in marine animals from 1989 to 2009. However, it is difficult to study cancer prevalence in wild populations, due to the invasive nature of diagnosis (McAloose and Newton 2009). Existing studies focus on isolated populations faced with cancer burdens to give relevant insight (McAloose and Newton 2009). For example, facial cancer threatens Tasmanian devils with extinction (McAloose and Newton 2009, Pye et al. 2016).

Cancer incidence is higher in wildlife in environments contaminated with anthropogenic chemicals (McAloose and Newton 2009). Martineau et al. (2002) analyzed dead Beluga whales in the St. Lawrence estuary, Quebec, Canada from 1983-1999, and they found that cancers accounted for 18% of deaths and cancers were found in 27% of all whales analyzed. This is a rate much higher than that of any other cetaceans (Martineau et al. 2002). The St. Lawrence estuary is highly polluted by polycyclic

aromatic hydrocarbons (PAHs) from aluminum smelters (Martineau et al. 2002). The researchers hypothesized the increase in cancer in belugas could be due to the PAH contamination, as human cancers in the area were also epidemiologically linked to the PAH contamination (Martineau et al. 2002). Ylitalo et al. (2005) found that sea lions with genital carcinomas had levels of polychlorinated biphenyls (PCBs) that were 85% higher than in sea lions that did not have genital carcinomas. Early recognition of wildlife cancers and causes could help drive better mitigation and policy (McAloose and Newton 2009).

Immune System Impacts

Avcedo-Whitehouse and Duffus (2009) focused on how the immune system could be challenged in a rapidly changing environment. They determined that because hormones control many immune system processes, there is great potential for EDCs to affect normal immune system processes (Avcedo-Whitehouse and Duffus 2009). EDCs affect stress responses in wildlife, influencing the hormone corticosteroid, which plays into immune system health (Pottinger 2003).

Threatened and Vulnerable Species

EDCs are of particular concern for threatened species, because of their vulnerable population status (Kumar and Holt 2014). These contaminants are another stress for wildlife in addition to better-known concerns like human population growth, habitat degradation, and climate change (Kumar and Holt 2014). It is challenging to demonstrate a clear link between EDC effects and population declines, but The Endocrine Society's *State of the Science of Endocrine Disrupting Chemicals* suggests it is likely but not proven that EDCs are involved in current wildlife declines (Bergman et al. 2012). The report also claims that declines in populations of raptors, seals, and snails are "extremely likely" to have occurred due to EDCs, while certain species have rebounded due to improved regulation of chemicals (Bergman et al. 2012).

Top predators are often threatened species worldwide, and due to their high trophic status also tend to accumulate any EDCs present in their food webs (Fossi et al. 1999). Accumulation of EDCs places species at potentially high risk for adverse effects

(Fossi et al. 1999); thus, more methods are needed to assess EDC exposure, protect, and conserve threatened top predators and other endangered species (Fossi et al. 1999). However, methods must be noninvasive and avoid further harm to already vulnerable species (Fossi et al. 1999).

Arctic Species

The Arctic lands and oceans are a focal spot for EDC accumulation due to ocean and atmospheric currents (Jenssen 2005). Arctic residents are adapted to the extreme environmental conditions, so if EDCs alter the ability of resident species to breed, molt, or migrate, there could be large implications beyond the scope of the effects in other climates (Jenssen 2005). EDC effects in Arctic species are well studied (Fox 2001, Annamalai and Namasivayam 2015), including the effects of perfluorocarbons (PFCs) on plankton, shrimp, arctic cod, birds, narwhal, beluga, polar bears, mink, and arctic fox (Annamalai and Namasivayam 2015). Fox (2001) summarized the effects of EDCs on wildlife in Canada, and found studies of evidence of endocrine disruption in 16 bird species, three terrestrial mammals, one whale, one reptile, and several amphibians. Effects included decreased reproductive success, gross congenital abnormalities and interference with developmental or regenerational processes; thyroid dysfunction; metabolic abnormalities; feminization or demasculinization; alterations in sex ratios; altered brain development and behavior; and altered immune function (Fox 2001).

Transgenerational Epigenetics

An area of limited research is the transgenerational effect of EDCs (Fossi et al. 1999, Walker and Gore 2011, Dietert 2014, Schwindt 2015). Transgenerational epigenetic inheritance transfers non-genomic changes to DNA structure from the parent's environmental exposures on to future generations (Walker and Gore 2011, Dietert 2014). EDCs are actually the most studied group of chemicals for transgenerational epigenetics (Dietert 2014). In humans, transgenerational epigenetics of EDC exposure in one generation may influence disease risk in subsequent generations that were not exposed (Dietert 2014). Transgenerational inheritance of EDC effects is important because if offspring and successive generations take up the altered phenotypes there could be

evolutionary impacts (Schwindt 2015). This area of transgenerational epigenetics is still understudied, and transgenerational effects are still unclear in both aquatic and terrestrial species (Schwindt 2015).

Tools for Future and Current Research

Most endocrinology knowledge is gathered from captive populations, but a need exists to determine the endocrinology of wild populations as well for EDC analysis (Kersey and Dehnhard 2014). Comparative endocrinology, the study of hormone signaling across a variety of taxa, can be an effective tool in wildlife conservation, and could be useful in determining effects of EDCs between taxa (Tubbs et al. 2014). The endocrine system is thought to be fairly well conserved across vertebrates, and considered well conserved across mammals, including humans (Gore et al. 2015). Comparative physiology is often needed to extrapolate results to other species that can't be studied (Kendall et al. 2010), and comparative phylogenetic approaches can be used to develop understanding of effects of EDCs and commonalities in mechanisms between taxa (Jobling and Tyler 2006).

A broader understanding of the diversity of reproductive mechanisms across species is necessary for comparative endocrinology of effects of EDCs (Tan et al. 2009, Kersey and Denhard 2014, Kumar and Holt 2014). African wild dogs (*Lycaon pictus*) are listed as endangered on the IUCN Red List due to overall population decline (IUCN 2012), and therefore any other health stressors, such as EDCs, could be of concern. Due to the lack of information about the reproductive biology and endocrinology of African wild dogs, accurate modeling using data from other species could prove difficult (Van den Berghe et al. 2012, Van der Weyde et al. 2015, Van der Weyde et al. 2016).

It is well known that mammals can pass EDCs through the placenta and milk to their offspring (Bernanke and Kohler 2008, Evans 2011, Bergman et al. 2012, Vethaak and Legler 2013), but less is known about mother to offspring transfer in other non-mammalian species that use semi-placental strategies (Lyons and Adams 2015). In a study of scalloped hammerhead sharks (*Sphyrna lewini*), Lyons and Adams (2015) investigated EDC transfer between mothers who use a yolk-sac placental strategy and offspring. The researchers found that females offloaded 0.03-2.3% of hepatic EDC-

contaminant load to offspring while pregnant via pseudo-placental contaminant transfer (Lyons and Adams 2015).

Hormones activate other processes through hormone-specific receptors at the cell level. In a study of estrogen receptor binding and activation in Southern White and Greater One-horned Rhinos, Tubbs et al. (2014) determined that exogenous dietary phytoestrogens may play a role in the reproductive failure of captive Southern White Rhinos. They found that in vitro analysis of endocrinology was as effective as in vivo analysis, which if often difficult or impossible to conduct (Tubbs et al. 2014). Using in vitro analysis could increase the number of species for which comparative endocrinology is a possibility, which would enable better management of wild and captive endangered species (Tubbs et al. 2014).

Another means of assessing the effects of EDCs without invasive procedures is *in silico*, or computer generated, modeling, either on an individual level or an interspecies level (Gurney 2006, Jobling and Tyler 2006). Gurney (2006) developed strategic models of populations and individuals exposed to EDCs. Although the models were not as accurate as plasma or fecal analysis of EDC effects at the individual level, they provided insight into population viability statistics. Gurney (2006) determined that population viability is more dependent on female reproductive output, especially the number of female offspring produced, than male reproductive ability when all individuals are exposed and affected by EDCs. *In silico* modeling can also be used for comparative endocrinology. McRobb et al. (2014) compared ligand binding sites across humans, amphibians, and fish to determine how conserved the sites were. The intention was to decide if the amphibian and fish species commonly used for EDC testing were apt models for humans, and analysis revealed that the hormone binding sites were well conserved. Given that mammalian endocrine systems are well conserved between species (Gore et al. 2015), McRobb et al.'s finding is important for other mammals. This method of modeling will be useful for determining how to test potential endocrine disruptors, as well as comparing endocrine physiology between species (McRobb et al. 2014). However, better tools should continually be developed for extrapolation across and between individuals, species, and populations (Tan et al. 2009).

Jorgenson et al. (2015) investigated whether laboratory species are a good model for endangered species in the wild, particularly for testing the effects of estrogens. They compared the endangered Rio Grande silvery minnow (*Hybognathus amarus*) against common experimental species fathead minnow (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) in response to 17ß-estradiol exposure. They measured EDC effects through larval predator-escape performance, survival, juvenile sex ratios, and whole-body vitellogenin concentration (Jorgenson et al. 2015). Jorgenson et al. (2015) found that the endangered Rio Grande silvery minnow was more sensitive to estradiol exposure than either of the common toxicological study species. Their findings point to a need for more phylogenetic variety within laboratory species, however they state that surrogate species should still be used for testing and creating regulations so as to not affect endangered species more than necessary (Jorgenson et al. 2015). Ylitalo et al. (2008) looked at the blood and blubber organochlorine concentrations in four Hawaiian monk seal subpopulations. Out of 158 seals tested, very few had organochlorine levels above thresholds considered safe for marine mammals in toxicology models (Ylitalo et al. 2008). However, the researchers stated Hawaiian monk seals may be more sensitive to organochlorines and other EDCs than the marine mammals used to create the toxicity models and thresholds (Ylitalo et al. 2008). Lyons (2006) also cites certain mollusk species that are more sensitive to EDCs than the typical laboratory species used.

Behavior can be used as a proxy for an animal's health, and therefore animal behavior could serve as a noninvasive assay for EDC exposure (Clotfelter et al. 2004). In situ determination of EDC exposure is difficult for most species and sometimes impossible for rare species, so using noninvasive behavioral indicators of EDC contamination would advance the study of EDCs in the wild (Clotfelter et al. 2004). Animal behavior could also help researchers understand how EDC exposure could have population level effects or community level consequences (Clotfelter et al. 2004). More studies that bridge the research gap between behavior impairment and changes in reproductive success that cause population declines are needed (Clotfelter et al. 2004). For example, clear evidence suggests foraging and antipredator behavior can have large impacts on population and community dynamics, but it is very hard to see EDC effects

when looking on a large scale at a couple of species' predator and prey relationships (Clotfelter et al. 2004).

Shenoy and Crowley (2011) looked at effects of EDCs on male mating signals. For most vertebrates, male mating signals are important cues for female mate choice (Shenoy and Crowley 2011). Androgen hormones in males control mating signals, and oestrogens control female response (Shenoy and Crowley 2011). Altering these pathways can have implications for mating system ecology (Shenoy and Crowley 2011). If male signaling is affected, then over time reduced reliability on the signal can lead to reduced preference for that signal by females (Shenoy and Crowley 2011). Reduced preference by females could influence the evolution of new traits to signal mate quality (Shenoy and Crowley 2011). Multi-generational signal disruption could lead to population or community level effects, and eventually evolutionary implications (Shenoy and Crowley 2011). At the extreme, there could be genetic differences between EDC affected populations and unexposed populations, leading to speciation (Shenoy and Crowley 2011). Shenoy and Crowley (2011) recommend more field studies, long-term mesocosm studies, and modeling for more conclusive evidence of the theories they propose.

Policy and Regulation of EDCs

Two main regulatory bodies are responsible for regulation and protection of wildlife in the United States: the U.S. Fish and Wildlife Service (FWS) and the National Oceanic and Atmospheric Association's National Marine Fisheries Service (NOAA NMFS). Both FWS and NMFS work to protect all wildlife species, including endangered species. Marine mammals and fish fall under the jurisdiction of NMFS while all other species are under the jurisdiction of FWS (FWS 2016). The Endangered Species Act (ESA) and Marine Mammal Protection Act (MMPA) protect many of these species (FWS 2016, NOAA 2016). Nowhere in the FWS website are endocrine-disrupting chemicals mentioned, although pesticides, some of which are EDCs, are mentioned.

On the NOAA webpage, the NMFS Marine Mammal Health and Stranding Response Program (MMHSRP) describes "toxic substances" as a threat to marine mammals and includes a biomonitoring program and tissue bank for storing tissue samples. The tissue bank acts as an avenue to retroactively analyze contaminant levels in

the tissues of certain indicator species: Atlantic bottlenose dolphins, Atlantic white-sided dolphins, pilot whales, and harbor porpoises (NOAA 2016).

The Environmental Protection Agency (EPA) regulates EDCs in the U.S. (EPA 2016). In 1998, the EPA's Endocrine Disruption Screening Program (EDSP) was created, and now decides which chemicals are EDCs and should be regulated as EDCs under current laws (EPA 2016). This is significant because the agencies protecting and regulating wildlife, NOAA and FWS, are not the agencies in charge of EDC policy and regulation.

Bergman et al. (2012) state no widely supported system for evaluating the strength of relationship between EDCs and adverse health outcomes exists worldwide. Better tests are needed to start identifying EDCs even before looking at effect levels (Lyons 2006). Policy makers need to use the principles of endocrinology in making policy, especially context dependent hormones that can act at very low doses and exhibit nonlinear and non-monotonic dose-response curves (NMDRCs) (Zoeller et al. 2012, Vandenberg et al. 2013). Vandenberg et al. (2013) state that the current testing protocol and policy does not account for life stage dependence of EDCs and is too limited of an approach. Hotchkiss et al. (2008) support this finding and advise incorporating solutions to problems of variability, reproducibility, and biological significance. There is a need to focus on effects from cumulative exposure or exposure to multiple EDCs at low doses at once (Hayes et al. 2006, Lyons 2006, Hotchkiss et al. 2008, Tan et al. 2009), and a need for more wildlife field studies that span multiple years (Jobling and Tyler 2006).

Many studies have called for urgent national and international policy to limit EDC production (Annamalai and Namasivayam 2015), and for interdisciplinary collaboration (Lyons 2006, Wang and Zhou 2013). Academic and peer-reviewed evidence of EDC effects needs to be utilized by public and wildlife health agencies (Zoeller et al. 2012). Scientists need to be involved in connecting findings to policy (Lyons 2006). There exists a need to focus on sensitive species that can be used as sentinel species in the wild (Lyons 2006). The potential transgenerational effects of EDCs could have large implications on populations and even evolution, so studies have called for proactive policy in preventing future exposure to EDCs (Walker and Gore 2011).

Proof of harm or safety of EDCs is incredibly difficult to measure, and a direct link between EDC exposure and population decline is challenging to prove (Bernanke and Kohler 2008). Jobling and Tyler (2006) state better communication of risks of EDCs would enable policy decisions about wildlife and EDCs to be protective. Prevention and precaution could help ensure wildlife safety (Jobling and Tyler 2006, Gore et al. 2015), and long term studies on wildlife population level would help fill data gaps (Bernanke and Kohler 2008). Jobling and Tyler (2006) state, a "major challenge faced by environmental biologists is the need to place endocrine disruption into context with other environmental pressure faced by our wildlife populations, for example, global warming," and making national and international policy reflect this challenge.

International and Global Issues

The Stockholm Convention aims to limit and control the use of POPs, many of which are EDCs, worldwide in order to protect human health and the environment, and 180 countries are part of the agreement (UNEP 2008). The convention entered into force in 2004, and has helped reduce global use of POPs (UNEP 2008). However, certain POPs are still in use and many POPs are long term legacy pollutants still in the environment. DDT is still used in certain countries for mosquito-borne disease control (EPA 2016), which is an approved use under the Stockholm Convention (UNEP 2008). Concerns still exist about DDT remaining in the environment, and Luzardo et al. (2014) can still find DDT levels of concern in bird species in Spain. Lyons (2006) suggests worldwide control of EDCs employ a precautionary approach, instead of the standard reactionary method (Lyons 2006).

METHODS

Survey Design

The survey (Appendix A) was designed using *Qualtrics*, an online survey platform. The survey consisted of 20 core questions plus free response options for 10 questions (Table 6). Many questions were multi-part questions. Formats included multiple-choice questions, a rank order question, and free response questions. Seven of the multiple-choice questions utilized a five-point Likert scale for responses, and six of the multiple-choice questions also provided a free response option for explanation.

The survey was designed to address three areas for conservationists: knowledge of EDCs, attitude towards EDCs, and practice. Practice addressed what respondents know is actively being researched or practiced regarding EDCs in wildlife. Question order was designed to minimize bias from previous questions while maintaining a logical flow, so questions from knowledge, attitude, and practice were interspersed. The knowledge section includes questions 1, 4, 5, 6, and 11. These questions asked respondents to describe their knowledge of EDCs in general (Q1), EDC effects in seven different taxa (Q4), nine different EDC effects (Q5), nine different EDCs (Q6), and critical data gaps (Q11). The attitude section includes questions 2, 3, 7, and 9. These questions gaged respondents' attitudes about EDCs in context with other conservation concerns (Q2), EDCs alone as a conservation concern (Q3), the importance of EDCs in their work (Q7), and their perspective on EDCs as a research priority (Q9). The practice section includes questions 13, 15, 17, 18, and 19. These questions assessed if respondents were currently conducting research on wildlife and EDCs (Q13), planning to conduct research (Q15), knew of other persons conducting research (Q17), could name species being studied for EDC exposure or effects (Q18), and knew of any interventions (Q19).

The remaining six questions gathered background information about respondents. Questions 21, 27, 28, and 29 asked about respondent occupation, means of accessing the survey, age, and educational attainment, respectively. Questions 30 and 32 asked if respondents wanted a copy of the final report and if they would be willing to be contacted for further questions.

Table 6. Survey questions.

Question number	Question
Q1	How familiar are you with the environmental pollutants known as “endocrine-disrupting chemicals” (EDCs)?
Q2	Please rank the following concerns for wildlife conservation from most (1) to least (7) important. Endocrine-disrupting chemicals (EDCs), Human population growth, Habitat loss, Climate change, Disease, Invasive species, Wildlife trafficking, hunting, and poaching
Q3	For endocrine-disrupting chemicals (EDCs) specifically, how significant do you believe their effect is on wildlife reproduction, behavior, or conservation?
Q4	How familiar are you with the effects of endocrine-disrupting chemicals (EDCs) in the following taxa? Humans, Amphibians, Fish, Birds, Reptiles, Marine mammals, Terrestrial mammals (excluding humans)
Q5	How familiar are you with the following potential effects of endocrine-disrupting chemicals (EDCs) in wildlife listed in the table below? Female reproductive impacts, Male reproductive impacts, Tumors, Thyroid impacts, Neurological function impacts, Developmental impacts, Metabolic function impacts, Immune system impacts, Behavioral impacts, Other
Q6	How familiar are you with each of the endocrine-disrupting chemicals (EDCs) listed below in terms of possible effects on wildlife? DDT, Atrazine, Other pesticides, Dioxins, Polychlorinated biphenyls (PCBs), Polybrominated diethyl ethers (PBDEs), Bisphenol A (BPA), Phthalates, Residual pharmaceuticals, Other
Q7	Are endocrine-disrupting chemical (EDC) effects on wildlife important in your specific work?
Q9	Should endocrine-disrupting chemical (EDC) effects on wildlife conservation be more of a research priority in your field?
Q11	Can you identify 2 critical data gaps that, in your opinion, need to be filled to improve knowledge of how endocrine-disrupting chemicals (EDCs) may affect wildlife conservation?
Q13	Are you currently conducting any research regarding endocrine-disrupting chemicals (EDCs) and their effects on wildlife?
Q15	Are you planning to conduct any research regarding endocrine-disrupting chemicals (EDCs) in the next 5 years?
Q17	Do you know of other researchers or practitioners studying endocrine-disrupting chemical (EDC) effects on wildlife?
Q18	Which species, if any, are being studied for endocrine-disrupting chemical (EDC) exposure or effects?
Q19	Do you know of any interventions being implemented to prevent endocrine-disrupting chemical (EDC) exposure in wildlife?
Q21	What is your primary professional affiliation?
Q27	How did you receive or access this survey?
Q28	What is your age?
Q29	Please select all degrees completed.
Q30	Would you like a copy of my final report?
Q32	Would you be willing to be contacted for further questions?

Survey Distribution

The survey was electronically distributed using *Qualtrics* to reach as many conservation scientists and practitioners as possible. The survey was posted to the Society for Conservation Biology bulletin board, and sent three times to the ECOLOG-L listserv maintained by the University of Maryland for the Ecological Society of America. The ECOLOG-L listserv reached many academic respondents, and in additional, specific colleagues of faculty at Colby College who work in conservation received the survey via email.

Zoos that received an Association of Zoos and Aquariums (AZA) Conservation Grants Funds Award in either 2014 or 2015 (determined from the AZA website) were sent the survey and asked to distribute it to their conservation practitioners. These zoos included the Smithsonian Conservation Biology Institute at the National Zoo, San Diego Zoo Wildlife Conservancy, Lincoln Park Zoo Conservation and Science Centers, Cleveland Metroparks Zoo, Oregon Zoo, Omaha's Henry Doorly Zoo and Aquarium, Cincinnati Zoo Center for Research of Endangered Wildlife, Minnesota Zoo, Atlanta Zoo, San Francisco Zoo, Los Angeles Zoo, Zoo Miami, Denver Zoo, Knoxville Zoo, Saint Louis Zoo Institute for Conservation Medicine, and Point Defiance Zoo and Aquarium. Fort Worth Zoo and Oakland Zoo were also awarded funds, but did not have contact information available to the public and therefore were not sent the survey. Additionally, the survey was posted to the Conservation Breeding Specialist Group (CBSG) on LinkedIn, and was sent to the World Association of Zoos and Aquariums (WAZA) Conservation Director.

For NGOs, the survey was distributed to five top wildlife conservation NGOs with publicly available contact information: World Wildlife Fund, National Wildlife Federation, Wildlife Conservation Network, Global Wildlife Conservation, and African Wildlife Foundation. For conservationists working for government agencies, the survey was sent to US Fish and Wildlife Service regions with available contact information. These regions included the Pacific Region (1), the Southwest Region (2), the Midwest Region (3), the Northeast Region (5), the Mountain-Prairie Region (6), and the Alaska Region (7). The Southeast Region (4) and Pacific Southwest Region (8) did not provide contact information. Furthermore, the California Department of Fish and Wildlife

(Wildlife Branch) and Maine Department of Inland Fisheries and Wildlife were also sent the survey.

Data Analysis

The survey data was analyzed using *Qualtrics*, *Excel*, *Stata*, and *R Studio.* Multiple-choice questions with a five-point Likert scale were reassigned values 1 through 5, where 1 corresponds to the most negative response and 5 corresponds to the most positive response. Standard deviations or relative frequency statistics were calculated for all multiple-choice questions. Contingency tables were used to cross-tabulate responses in one question against another. Overall familiarity with EDCs (Q1), occupation (Q21), and age (Q27) were used as variables in contingency tables. Free responses were categorized in a manner appropriate for each question.

Mann-Whitney U tests (with a 95% confidence interval) were used to assess if overall familiarity with EDCs (Q1), significance of EDCs for wildlife (Q3), importance in respondents' work (Q7), occupation (Q21), and age (Q27) affected responses. Respondents aged 20-30 were compared to respondents over the age of 30. This separation was chosen because the youngest category of respondents likely gained more information about EDCs during schooling than older respondents, due to the increase in EDC research in the past 30 years. For Q21, respondents affiliated with an academic institution were compared against all other affiliations. For Q1, Q3, and Q7, the three lower likert responses (1-3) were compared against the two top responses (4 and 5).

RESULTS

A 20-question survey (Appendix A) was administered to conservation practitioners and 116 people responded. Eighty-five of the 116 respondents completed the survey. Results are described below in four areas: characteristics of survey respondents, knowledge, attitude, and practice.

Characteristics of Survey Respondents

Sixty-four respondents described how they became aware of the survey. Sixty-six percent of respondents accessed the survey through a listserv, most often the ECOLOG-L listserv moderated by the University of Maryland for the Ecological Society of America. Thirty-three percent of respondents accessed the survey through emails directly, and one respondent accessed the survey through the Wildlife Disease Association Facebook group. Respondents accessed the survey from many locations within North America (Figure 2), and a few respondents accessed the survey in Europe and Australia.

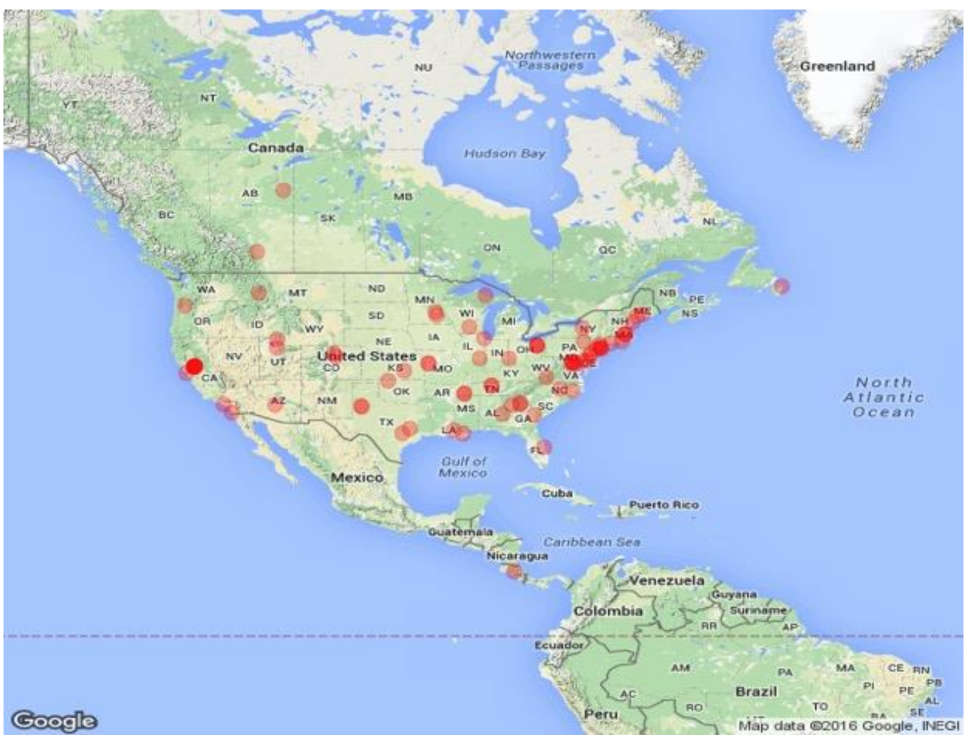

Figure 2. Geographic distribution of responses in North America. Not shown are responses in Europe and Australia.

Eighty-one respondents stated their primary professional affiliation or occupation (Table 7). The majority of respondents were associated with a university or college, but persons from zoos, NGOs, and government agencies also responded.

Table 7. Primary professional affiliation of respondents. (n=81).

Affiliation	Count	Percent (%)
University/college	50	62
Government	13	16
Non-governmental organization	8	10
Zoo	7	9
Other	3	4
Total	81	100

Of the 50 respondents associated with a college or a university 49 provided a description of their role. Forty-one percent of respondents were students pursuing undergraduate through professional degrees, while 31% of respondents were faculty at a university or college (Figure 3).

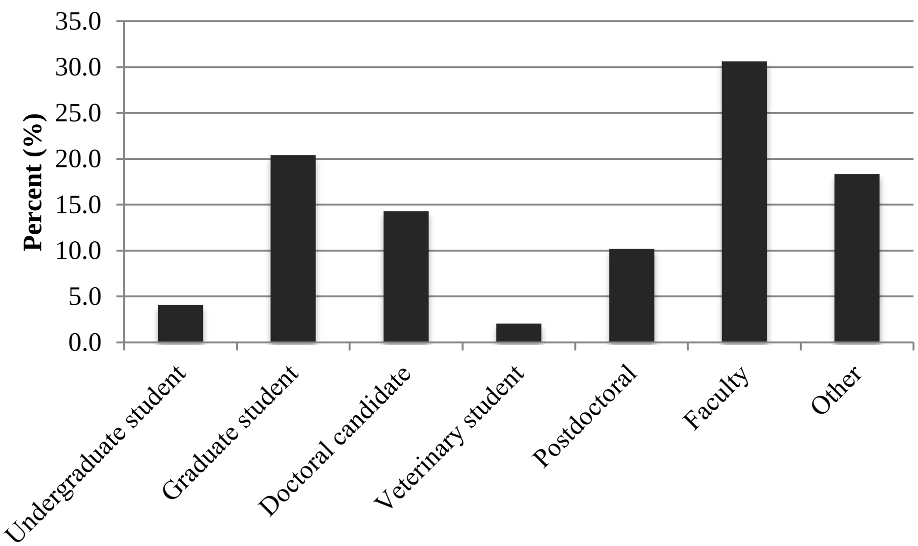

Figure 3. Categorization of respondent's explanations of their affiliations with a university or college. (n=49)

Respondents affiliated with zoos, NGOs, and government agencies also provided a description of their role. Of the seven respondents affiliated with a zoo, six respondents were research scientists and one respondent was the Director of Conservation (Figure 4).

Of the eight respondents affiliated with a NGO, four respondents coordinate policy, two conduct research, and two work on other aspects (Figure 4). Of the 13 government respondents, six respondents work on science and seven work on policy (Figure 4).

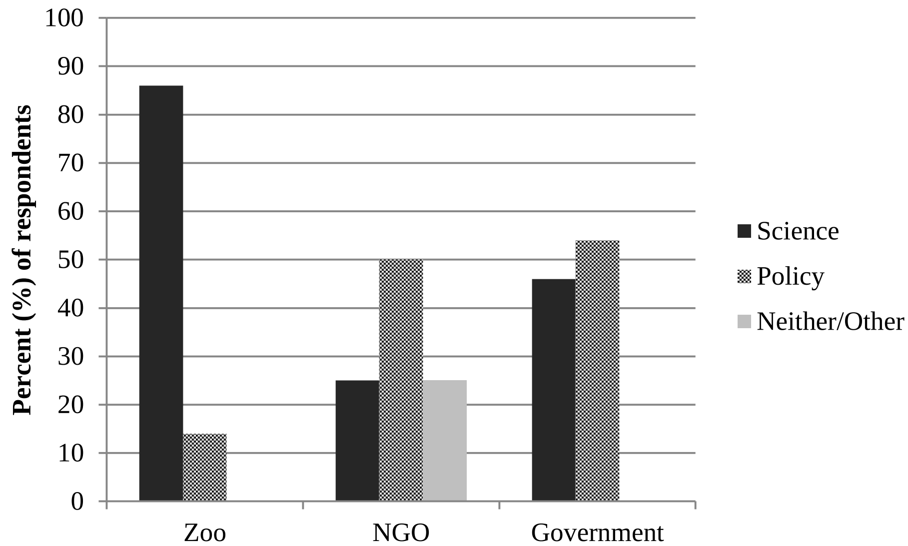

Figure 4. Categorization of respondent's explanations of their role with affiliated zoos, NGOs, and government. (n=31)

Sixty four percent of respondents were between the ages of 20 and 40 years old, while respondents older than 40 years old accounted for 36% of the total (Figure 5). Age categories over 50 were combined for contingency table analysis with other questions.

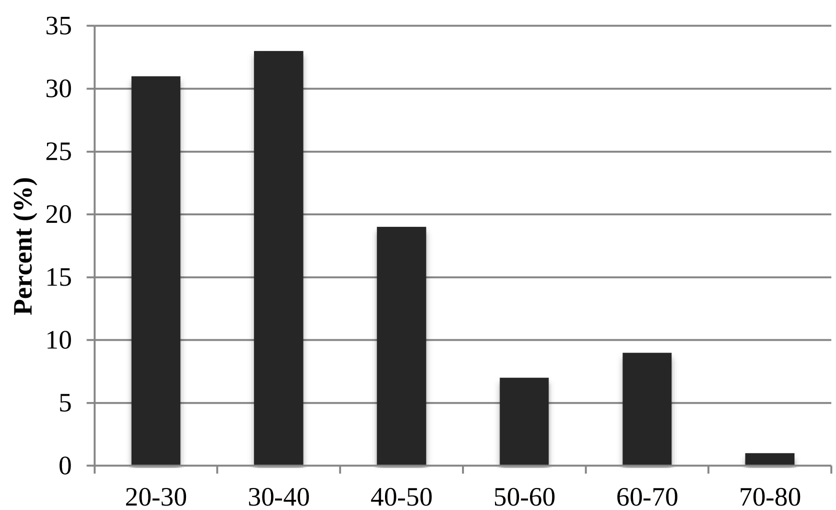

Figure 5. Age of survey respondents. (n=81)

Question 29 asked respondents about their highest level of education (Figure 6). The most common response was completion of a PhD, which was 46% of respondents, followed by 30% with a Master's degree, and 14% with a Bachelor's degree. Few respondents selected completion of a DVM or completion of another type of degree. No respondents reported completion of a MD or JD.

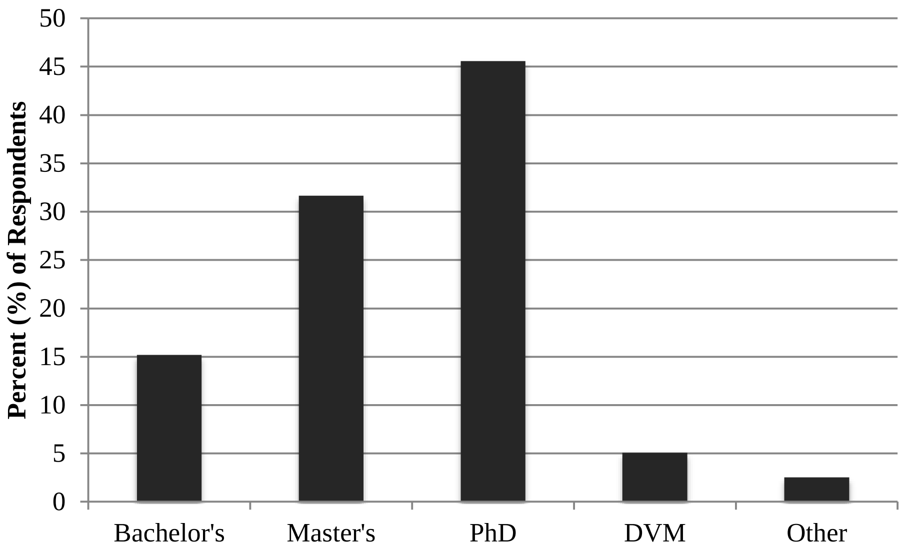

Figure 6. Educational attainment of respondents. (n=79)

Knowledge

Respondents' knowledge of EDC effects on wildlife was assessed through questions 1, 4, 5, 6, and 11, as described in section 2.1 Survey Design. In Q1, respondents stated their familiarity with EDCs in general. The majority of respondents selected they were either 'moderately familiar' or 'extremely familiar' with EDCs, and very few respondents cited a complete unfamiliarity with EDCs (Table 8). Responses in Q1 were used as a variable in contingency tables with all other questions.

Table 8. Q1 - Survey respondent familiarity with endocrine-disrupting chemicals (EDCs). (n=92)

Answer	Count	Percent (%)
Moderately familiar	25	27
Extremely familiar	24	26
Somewhat familiar	21	23
Slightly familiar	17	18
Not at all familiar	5	5

Familiarity with EDCs was varied within occupation categories (Q21). A majority of respondents who selected NGO (Q21) as their primary professional affiliation claimed they were ‘moderately familiar’ with EDCs in Q1. Sixty-three percent of university/college respondents and 62% of government respondents selected either ‘moderately familiar’ or ‘extremely familiar’ regarding EDCs. Zoo respondents were split between ‘slightly familiar’ (29%), ‘somewhat familiar’ (43%) and ‘extremely familiar’ (29%) with EDCs. There was no difference in how respondents rated their familiarity with EDCs among the different age groups (Q28).

Question 4 asked respondents to determine how familiar they were with EDC effects in various taxa. Respondents ranked familiarity with EDCs in humans, amphibians, fish, birds, reptiles, marine mammals, and terrestrial mammals (excluding humans) on a scale from ‘not at all familiar’ (1) to ‘extremely familiar’ (5). Amphibians and fish both have a mean value greater than 3 or ‘somewhat familiar,’ showing respondents were most familiar with effects of EDCs in those two taxa (Table 9). Respondents were less familiar with effects in humans, reptiles, birds, marine mammals, and terrestrial mammals in that order (mean ≤ 3) (Table 9 and Figure 7). Standard deviations were fairly consistent for each taxon. The standard deviation for terrestrial mammals was smaller than other taxa, showing the consistency in which respondents ranked their lack of familiarity with that taxon.

Table 9. Q4 How familiar are you with the effects of endocrine-disrupting chemicals (EDCs) in the following taxa? (n=90)

Taxa	Mean	Standard deviation
Amphibians	3.29	1.26
Fish	3.12	1.35
Humans	2.91	1.24
Reptiles	2.59	1.41
Birds	2.53	1.32
Marine mammals	2.44	1.30
Terrestrial mammals (excluding humans)	2.33	1.18

Polygon size in Figure 7 corresponds to the percent of respondents that selected that level of familiarity. Fewer respondents selected ‘extremely familiar’ overall than

other responses. Only 12% of respondents selected ‘extremely familiar’ for any of the taxa, as shown by the smaller light blue polygon representing ‘extremely familiar’ responses in Figure 7. On average the other familiarity responses were selected 22% of the time, as show by the larger similarly sized polygons in Figure 7. Vertices of each polygon show the percent of respondents that chose that level of familiarity for that taxon. For example, the purple polygon shows 24% of respondents selected ‘moderately familiar’ in regards to amphibians.

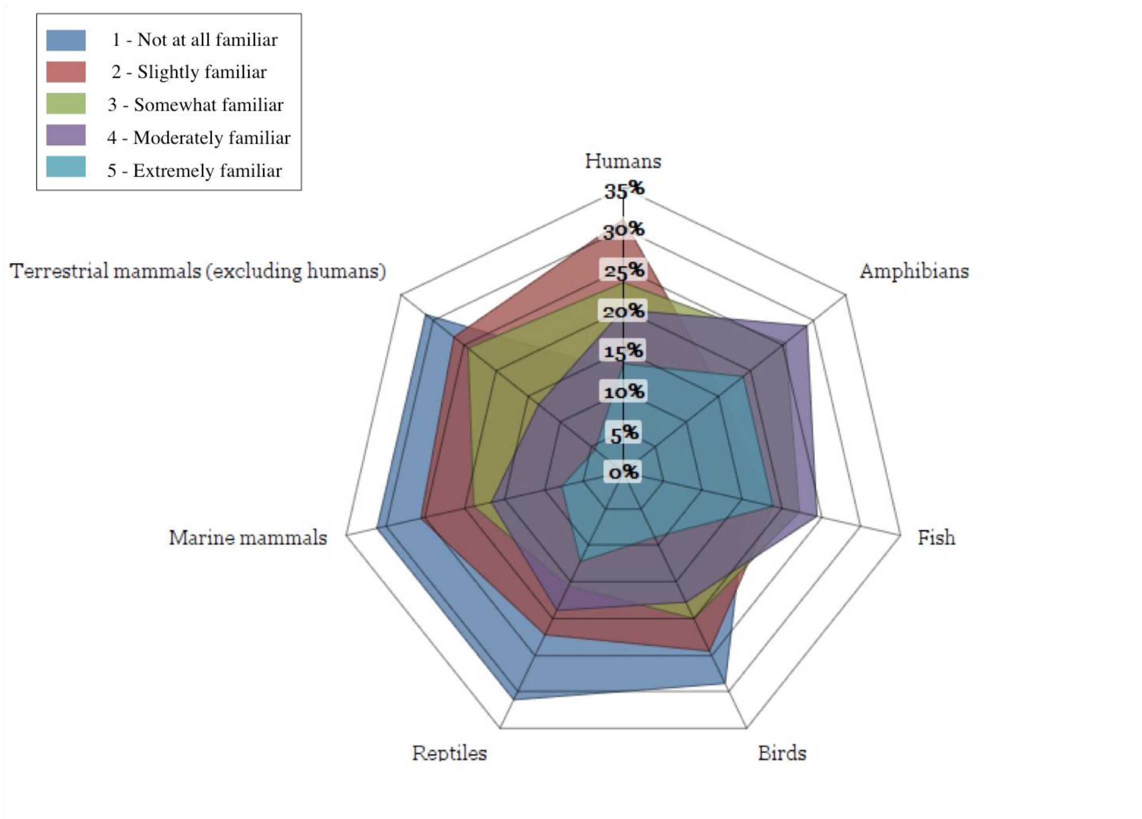

Figure 7. Q4 – How familiar are you with the effects of endocrine-disrupting chemicals (EDCs) in the following taxa? (n=90)

Comparing overall familiarity with EDCs stated in Q1 with familiarity in Q4 of EDC effects in specific taxa yields interesting results. For those who were ‘extremely familiar’ with effects of EDCs (Q1), the majority responded ‘extremely familiar’ with EDC effects in humans, amphibians, and fish, but responses were varied for birds, reptiles, marine mammals, and terrestrial mammals (excluding humans). For all taxa, the majority of those who responded ‘not at all familiar’ with EDCs in Q1 also said they

were either ‘not at all familiar’ or ‘slightly familiar’ with taxa-specific effects, a result to be expected. None of these respondents ranked their familiarity above ‘slightly significant’ in any taxa. Similarly, respondents who selected ‘slightly familiar’ in Q1 tended to respond with less familiarity in each of the taxa.

Familiarity with effects of EDCs in different taxa (Q4) varied little across occupations (Q21). Familiarity with each of the different taxa followed the same trends in each occupation as in the overall responses. Age (Q28) made no difference in respondents’ familiarity, except with amphibians. The majority of respondents age 50-80 stated they were ‘moderately familiar’ with the effects of EDCs in amphibians. Besides that one exception, familiarity with each of the different taxa was varied in each age group.

Question 5 asked respondents to rate their familiarity with specific physiological effects of EDCs on wildlife on a scale from ‘not at all familiar’ (1) to ‘extremely familiar’ (5). Respondents were most familiar with male reproductive impacts, female reproductive impacts, and developmental impacts (mean ≥ 3) (Table 10). Respondents were less familiar with behavioral impacts, immune system impacts, thyroid impacts, metabolic function impacts, tumors, and neurological function impacts (mean ≤ 3) (Figure 8 and Table 10). Standard deviations were fairly consistent for each effect, showing a spread of about 1.3. Standard deviations were smaller for female and male reproductive impacts reaffirming the consistency in which respondents were most familiar with those effects.

Table 10. Q5 - How familiar are you with the following potential effects of endocrine-disrupting chemicals (EDCs) in wildlife? (n=82-84)

Effect	Mean	Standard deviation
Male reproductive impacts	3.45	1.19
Female reproductive impacts	3.23	1.17
Developmental impacts	3.18	1.28
Behavioral impacts	2.52	1.38
Immune system impacts	2.48	1.33
Thyroid impacts	2.44	1.36
Metabolic function impacts	2.41	1.25
Tumors	2.40	1.25
Neurological function impacts	2.37	1.30

Fewer respondents selected ‘extremely familiar’ overall than other responses. Only 12% of respondents selected ‘extremely familiar’ for any of the physiological effects, as shown by the smaller light blue polygon representing ‘extremely familiar’ responses in Figure 8. On average ‘moderately familiar’ and ‘somewhat familiar’ were selected 19% of the time, while ‘slightly familiar’ and ‘not at all familiar’ were selected 25% of the time, as show by the larger similarly sized polygons in Figure 8.

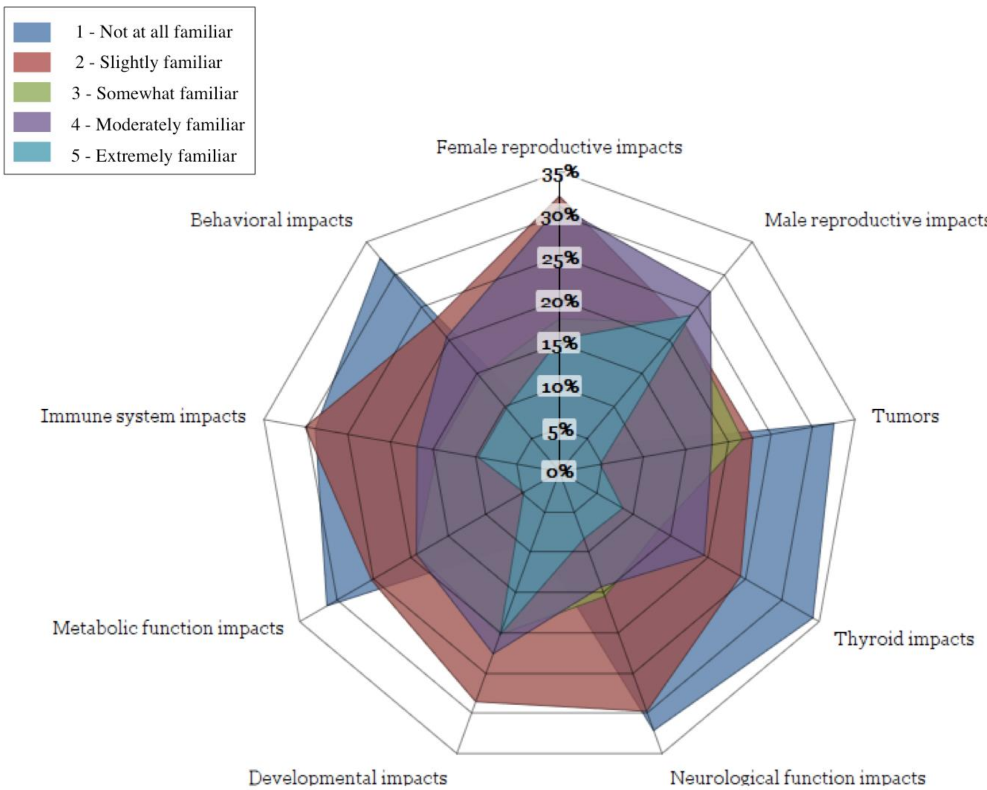

Figure 8. Q5 - How familiar are you with the following potential effects of endocrine-disrupting chemicals (EDCs) in wildlife? (n=82-84)

Comparing overall familiarity with EDCs stated in Q1 with familiarity in Q5 of different effects of EDCs also shows potential trends. ‘Moderately familiar’ and ‘extremely familiar’ respondents in Q1 were more familiar with female and male reproductive impacts than those who cited less familiarity with EDCs in general. For respondents who selected ‘moderately familiar’ in Q1, the majority also selected ‘moderately familiar’ with both female and male reproductive impacts in Q5. Similarly, for respondents who selected ‘extremely familiar’ in Q1, the majority also selected

‘extremely familiar’ with both female and male reproductive impacts. The same trend is true with developmental impacts. For those respondents who answered ‘moderately familiar’ and ‘extremely familiar’ in Q1, familiarity was varied with tumors, thyroid impacts, neurological function impacts, developmental impacts, metabolic function impacts, immune system impacts, and behavioral impacts. Respondents who selected ‘somewhat familiar’ in Q1 had varied responses for all effects in Q5. Respondents in the ‘not at all familiar’ category in Q1 didn’t respond above ‘slightly familiar’ for any potential effect in Q5. The majority for ‘not at all familiar’ respondents in Q1 was always either ‘not at all familiar’ or ‘slightly familiar’ for every potential effect in Q5. Again, respondents who selected ‘slightly familiar’ in Q1 tended to respond with less familiarity to each potential effect in Q5.

Familiarity with potential effects of EDCs (Q5) varied little depending on occupation (Q21). Familiarity with each of the different effects followed the same trends in each occupation as in the overall responses. Age (Q28) made no difference in respondents’ familiarity with physiological effects, except in two instances. The majority of respondents age 40-50 were ‘not at all familiar’ with thyroid impacts, and the majority of respondents age 50-80 were ‘slightly familiar’ with metabolic function impacts. Besides those exceptions, familiarity with each of the different physiological effects was varied in each age group.

In Question 6 respondents rated their familiarity with the effects of a list of EDCs that included DDT, atrazine and other pesticides, dioxins, PCBs, PBDEs, BPA, phthalates, and residual pharmaceuticals. Respondents were most familiar with DDT, atrazine, PCBs, other pesticides, and residual pharmaceuticals (mean ≥ 3) (Figure 9 and Table 11). Respondents were less familiar with BPA, dioxins, PBDEs, and phthalates (mean ≤ 3) (Figure 9 and Table 11). Standard deviations were fairly consistent for each EDC, showing a spread of about 1.3. One exemption is the standard deviation was much smaller for DDT reaffirming the consistency in which respondents were most familiar with DDT.

Table 11. Q6 - How familiar are you with each of the endocrine-disrupting chemicals listed below in terms of possible effects on wildlife? (n=81-83)

EDC	Mean	Standard deviation
DDT	3.78	0.98
Atrazine	3.23	1.49
Polychlorinated biphenyls (PCBs)	3.07	1.34
Other pesticides	3.04	1.38
Residual pharmaceuticals	3.00	1.21
Bisphenol A (BPA)	2.88	1.38
Dioxins	2.73	1.34
Polybrominated diethyl ethers (PBDEs)	2.63	1.46
Phthalates	2.58	1.34

Fewer respondents selected ‘extremely familiar’ overall than other responses, and ‘moderately familiar’ was selected the most. Only 17% of respondents selected ‘extremely familiar’ for any of the EDCs, as shown by the smaller light blue polygon representing ‘extremely familiar’ responses in Figure 9. ‘Moderately familiar’ was selected 25% of the time, while ‘somewhat familiar’ was selected 19% of the time. Both ‘slightly familiar’ and ‘not at all familiar’ were selected 20% of the time.

Respondents who stated they were less familiar with EDCs in general (Q1) were also less familiar with specific EDCs (Q6). For DDT, all of the respondents that were ‘extremely familiar’ with EDCs were either ‘moderately familiar’ or ‘extremely familiar’ with DDT. The other Q1 categories were varied in familiarity with DDT. Similarly, the majority of respondents that were ‘extremely familiar’ with EDCs in Q1 were ‘extremely familiar’ with atrazine as an EDC with possible effects on wildlife, and were also ‘extremely familiar’ with other pesticides. For dioxins, the majority of respondents who were ‘slightly familiar’ with EDCs in general (Q1) were ‘not at all familiar’ with dioxins. The opposite was true for those respondents who were ‘extremely familiar’ with EDCs in Q1, as they were ‘moderately familiar’ with dioxins. Levels of familiarity with dioxins were varied in respondents of other overall EDC familiarity. Responses regarding familiarity with PCBs, PBDEs, BPA, phthalates, and residual pharmaceuticals were varied across all Q1 responses.

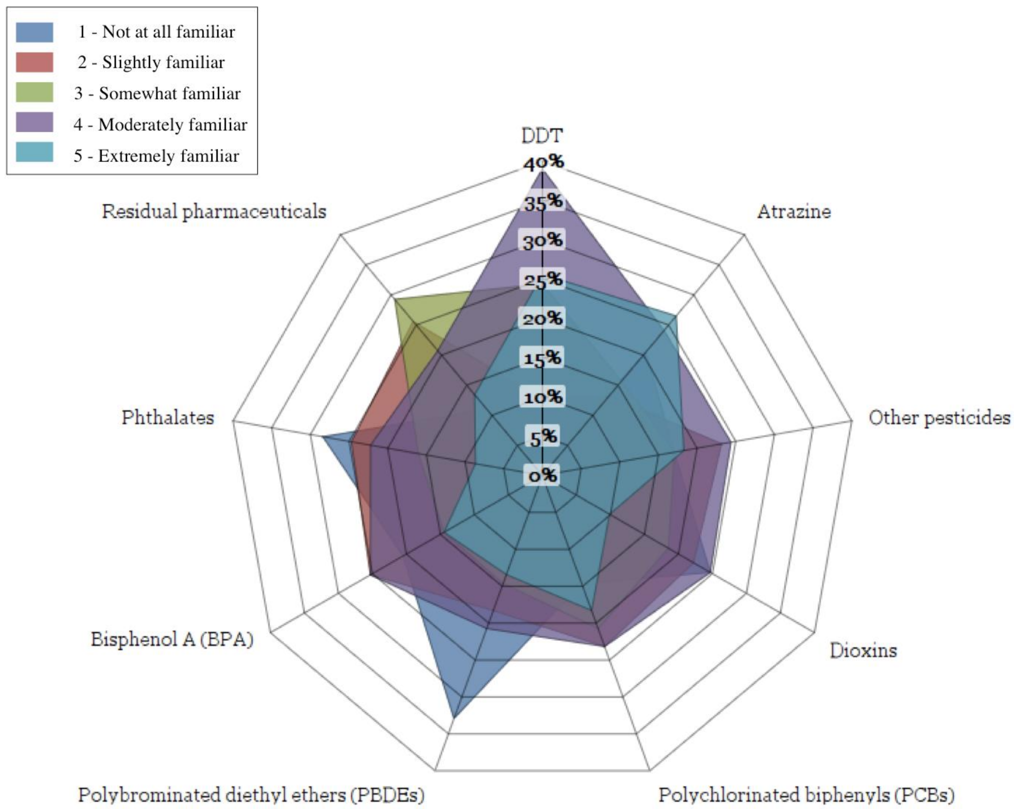

Figure 9. Q6 - How familiar are you with each of the endocrine-disrupting chemicals (EDCs) listed below in terms of possible effects on wildlife? (n=81-83)

Familiarity with certain EDCs (Q6) varied for some occupations (Q21). The majority of government respondents were 'moderately familiar' with DDT, and 'not at all familiar' with phthalates. The majority of NGO respondents were 'slightly familiar' with other pesticides, and 'somewhat familiar' with phthalates and residual pharmaceuticals. Occupation made no difference in zoo and university or college respondents' familiarity with certain EDCs. Age (Q28) made no difference in respondents' familiarity with certain EDCs, except in two instances. The majority of respondents age 50-80 were 'moderately familiar' with DDT, and were 'slightly familiar' with BPA. Besides those exceptions, familiarity with each of the different EDCs was varied in each age group.

Question 11 asked respondents if they could identify two critical data gaps that need to be filled to improve knowledge of how EDCs may affect wildlife conservation. Sixty percent of respondents affirmed their ability to identify data gaps, while 40% said

no. Of the respondents who said yes, 98% provided an explanation of two critical data gaps. These free responses were grouped into six categories (Figure 10).

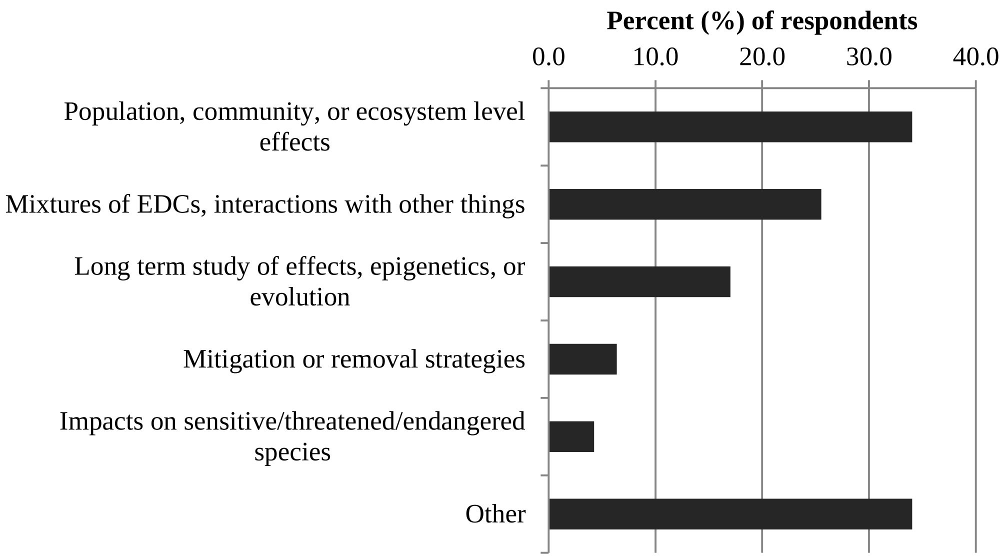

Figure 10. Identification of critical data gaps in understanding how EDCs affect wildlife conservation. (n=80)

Thirty-four percent of respondents cited population, community, or whole ecosystem level effects as a data gap. Twenty-six percent of respondents said mixtures of multiple EDCs or interactions of EDCs with other environmental factors was a data gap, and 17% of respondents said the need for long-term study of effects including multigenerational effects was a data gap. Three respondents stated a gap in knowledge of mitigation or removal strategies for EDCs in the environment and in wildlife, and two respondents stated a need for more research regarding EDCs and sensitive or threatened species (Figure 10). Twenty percent of respondents provided data gaps that did not fit into these six categories, and were categorized as ‘Other’ (Table 12).

Table 12. ‘Other’ critical data gaps from Figure 10 in understanding how EDCs affect wildlife conservation. (n=16)

‘Other’ data gaps
Ranking of chemicals in terms of presence in aquatic habitats. Better estimates of the environmentally relevant amounts.
understanding [sic] routes of contaminant exposure/accumulation and knowing where the chemicals move once inside the body to better understand which organ systems may be most affected
1 - actual physiological effect of these chemicals at low levels. 2 - the actual levels of these pollutants in the environment, with particular emphasis on local ecosystems
(These might be gaps in my knowledge, not gaps in the research field): Sources of endocrine disruptors for terrestrial animals; rural vs [sic] urban gradient of impacts.
impacts [sic] on offspring sex ratios, impacts on reproductive success
I believe pollution and agriculture had much more "downstream" effects than what we currently know.
levels [sic] of exposure and types of exposure
The biochemical effects on metabolic processes and what concentrations are harmful
education/awareness [sic]; baseline levels
Waste treatment and non-point source pollution.
background [sic] levels of endocrine disrupting contaminants in raptors, and levels at which behavioral abnormalities are induced.
The effect of metabolites, the effect not only on reproduction but development (especially in mammals).
More definitive research, acceptance of their effects as solid science so that impacts may be evaluated
Do wildlife that frequently use natural reserve areas close to agricultural plantations have greater effects from EDCs? What would be an appropriate buffer size between agricultural areas and natural areas where the application of EDC is prohibited or restricted in certification programs (related to the findings of first question)?
What are the relative effects of phytoestrogens in the development of ovo-testes in male fish. [sic] What are the effects of poorly studied hormones (progesterone, andosterone) and retinoic acid on reproduction and immunity. What are the molecular effects of EDCs on the responses of macrophages and dendritic cells to pathogen exposure
Lack of in vivo studies to directly assess physiological outcomes of exposure, lack of environmental contaminant loads, particularly for emerging EDCs

Knowledge of EDC data gaps (Q11) correlates with overall familiarity of EDCs (Q1) (Figure 11). Respondents who answered ‘not at all familiar,’ ‘slightly familiar,’ or ‘somewhat familiar’ in Q1 knew of data gaps significantly less frequently than respondents who answered ‘moderately familiar’ or ‘extremely familiar’ in Q1 ($p < 0.001$).

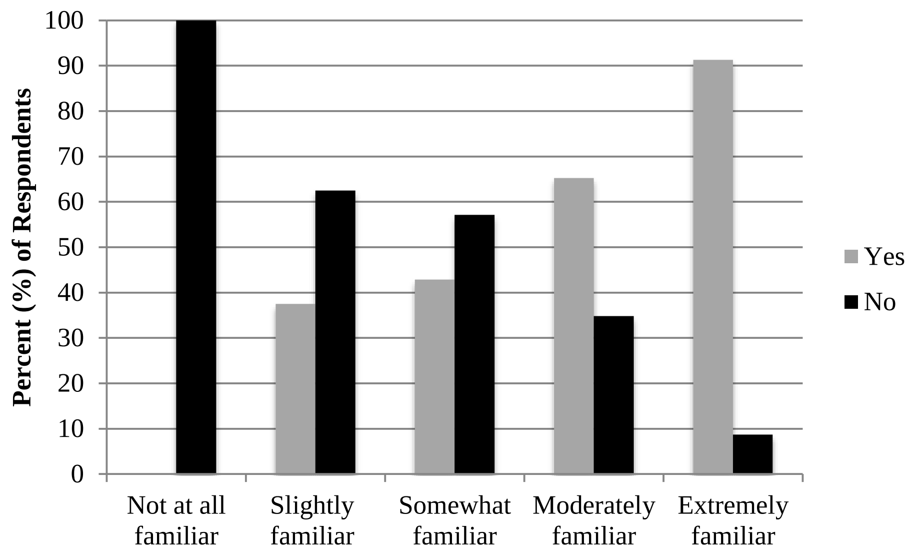

Figure 11. Ability to identify two critical data gaps (Q11) based on overall familiarity with EDCs (Q1). (n=79)

In terms of occupation, the majority of 'University/college,' 'NGO,' and 'Government' respondents stated they could identify two data gaps, while 'Zoo' respondents were the opposite (Figure 12). Only 33% of 'Zoo' respondents stated they could identify two data gaps, and the majority stated they could not identify data gaps.

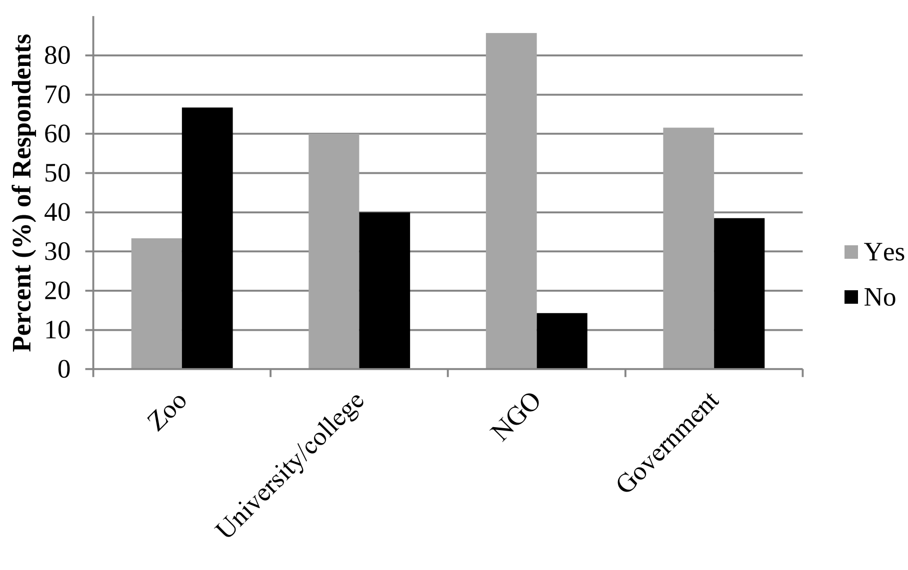

Figure 12. Ability to identify two critical data gaps (Q11) based on occupation (Q21). (n=79)

Sixty-three percent of respondents age 20-30 could not identify two critical data gaps, while the majority of other age groups could identify two critical data gaps (Figure 13). Respondents age 20-30 were significantly less likely to be able to identify data gaps than respondents over the age of 30 ($p = 0.006$).

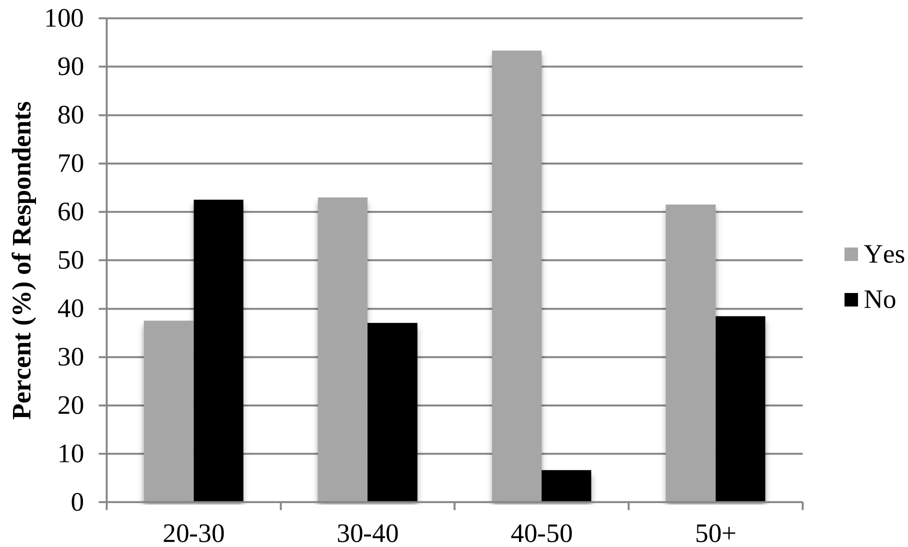

Figure 13. Ability to identify two critical data gaps (Q11) based on age (Q28). (n=79)

Attitudes

Question 2 asked respondents to rank seven concerns for wildlife conservation: EDCs, human population growth, habitat loss, climate change, disease, invasive species, and wildlife trafficking, hunting, and poaching (Table 13). Concerns were presented in a random order to respondents. Habitat loss was consistently ranked the top concern for wildlife conservation (mean = 1.66). Other top concerns included climate change and human population growth (mean ≤ 4). EDCs were ranked as a lesser concern for wildlife conservation, as were invasive species, disease, and wildlife trafficking, hunting, and poaching (mean ≥ 4).

Table 13. Q2 – Please rank the following concerns for wildlife conservation from most (1) to least (7) important. (n=92)

Conservation concern	Mean	Standard deviation
Habitat loss	1.66	1.10
Human population growth	3.00	1.81
Climate change	3.28	1.61
Invasive species	4.75	1.52
Endocrine-disrupting chemicals (EDCs)	4.84	1.54
Wildlife trafficking, hunting, and poaching	4.98	1.70
Disease	5.49	1.46

When comparing responses of Q2 to familiarity selected in Q1, the most prevalent trend across all categories of Q1 was the ranking of habitat loss. One hundred percent of respondents 'not at all familiar' with EDCs ranked habitat loss as the top threat (1) to wildlife conservation, and other Q1 categories were similar. Seventy-one percent of 'slightly familiar,' 43% of 'somewhat familiar,' 56% of 'moderately familiar,' and 52% of 'extremely familiar' respondents ranked habitat loss as the top threat to wildlife conservation. The lowest ranked threat to wildlife conservation was disease, which remained true across all Q1 familiarity categories. Disease was most commonly ranked as either the lowest threat (7) or second lowest (6) threat in 'not at all familiar' through 'extremely familiar' respondents of Q1. The remaining threats (EDCs, human population growth, climate change, and hunting) had varied ranks in all of the familiarity with EDC categories of Q1.

All occupations ranked concerns in a fairly similar manner. A majority of each occupation ranked habitat loss as the top concern (1) for wildlife conservation. For EDCs, rankings were varied in every occupation, and EDCs were often ranked lowly. Forty-three percent of zoo respondents and 46% of government respondents placed EDCs as their fifth concern for wildlife conservation.

Question 3 asked respondents to state how significant of an effect they believe EDCs have on wildlife reproduction, behavior, or conservation. Question 3 isolated the effects of EDCs without comparing them to other threats to conservation to determine attitude towards EDCs specifically. Only 18% of respondents thought EDCs were not very significant ('not at all significant,' 'slightly significant,' or 'neutral), while 51% of

respondents said ‘moderately significant’ and 31% said ‘extremely significant’ (Figure 14).

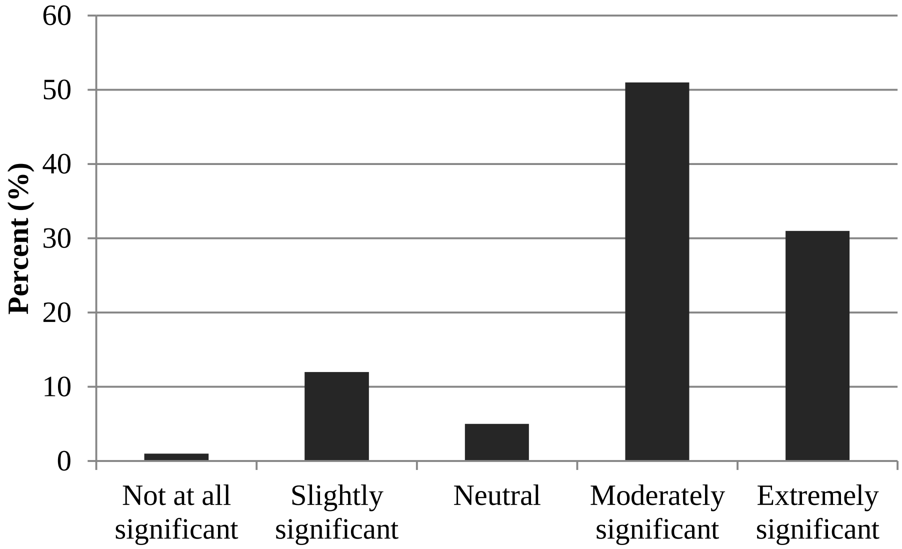

Figure 14. Q3 - For EDCs specifically, how significant do you believe their effect is on wildlife reproduction, behavior, or conservation?

Age was not a significant factor in how respondents answered Q3 ($p = 0.830$). Although not significant, respondents affiliated with an academic institution seem to rate EDC significance higher in Q3 than respondents with other primary professional affiliations ($p = 0.091$, Figure 15). How respondents answered in Q1 influenced their Q3 responses. Respondents who selected ‘not at all familiar,’ ‘slightly familiar,’ or ‘somewhat familiar’ in Q1 rated EDC significance in Q3 lower than respondents who selected ‘moderately familiar’ or ‘extremely familiar’ in Q1 ($p = 0.004$).

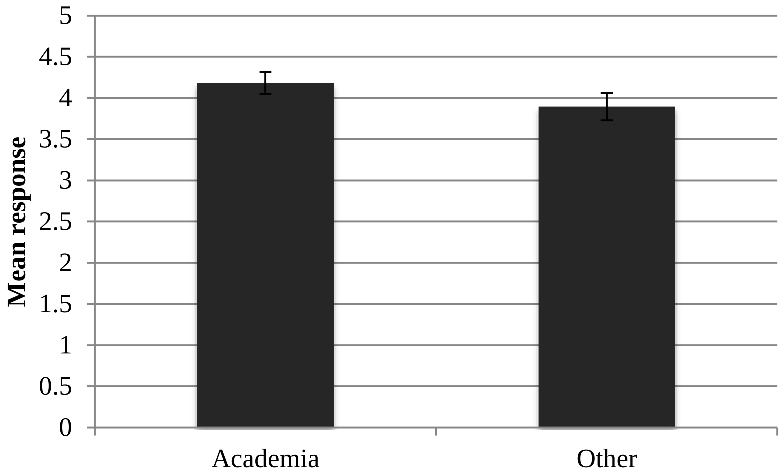

Figure 15. Mean response to Q3 by respondents primarily affiliated with academic institutions and other occupations. (n=79)

Question 7 asked respondents if EDC effects on wildlife were important in their own specific work. Responses ranged from ‘not at all important’ to ‘extremely important’ (Figure 16).

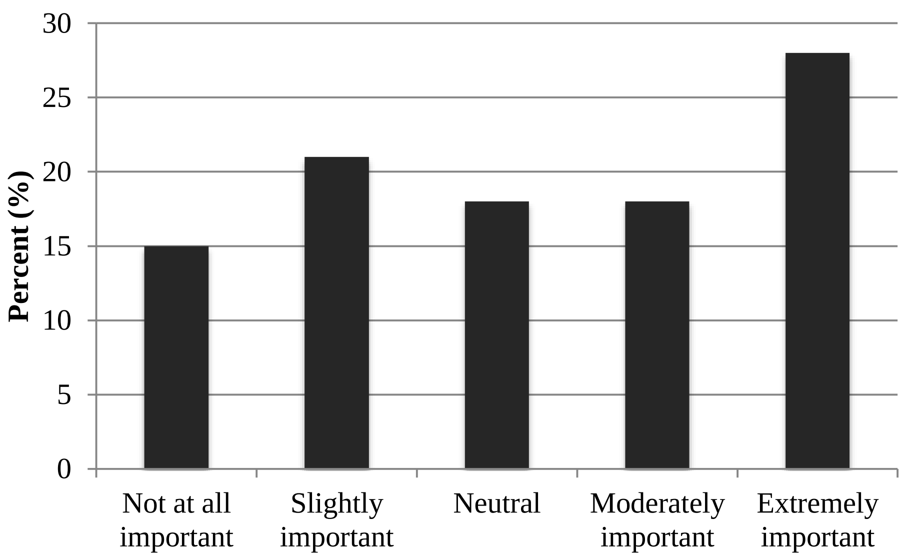

Figure 16. Q7 – Are endocrine-disrupting chemical (EDC) effects on wildlife important in your specific work? (n=82)

Moderate or extreme knowledge of EDCs (Q1) correlates with increased importance of EDCs in a respondent’s specific work ($p < 0.001$). Sixty-seven percent of respondents who selected ‘not at all familiar’ for general EDC familiarity (Q1) also selected ‘not at all important’ for importance of EDCs in their work. Seventy percent of

respondents that selected ‘extremely familiar’ for general EDC familiarity (Q1) also selected ‘extremely important’ for importance of EDCs in their work. Other levels of familiarity in Q1 were varied for the importance of EDCs in their specific work. Respondents aged 20-30 answered similarly to respondents over the age of 30 in how important EDCs were in their specific work ($p = 0.738$). Although not significant, respondents affiliated with an academic institution seem to rate EDC importance in their work higher in Q7 than respondents with other primary professional affiliations ($p = 0.065$, Figure 17). Respondents’ answers in Q3 did not influence responses in Q7 ($p = 0.1196$).

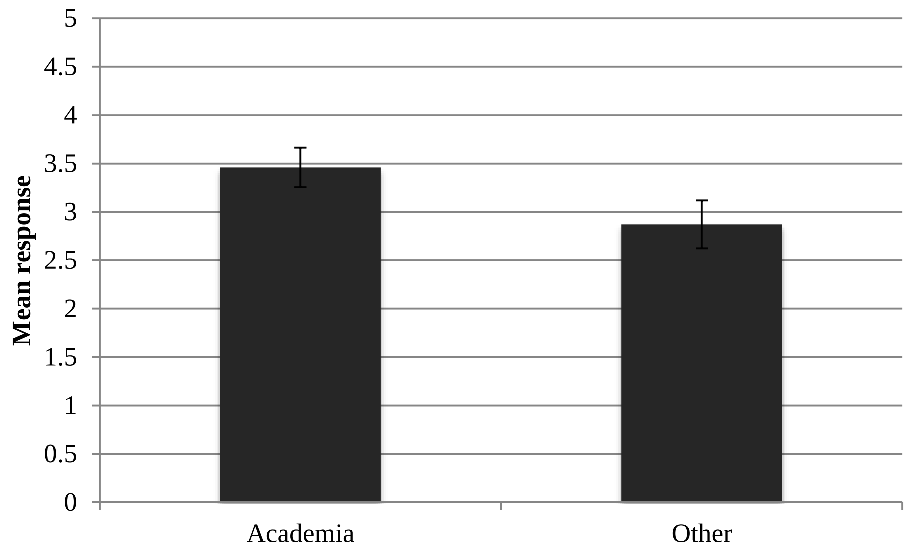

Figure 17. Mean response to Q7 by respondents primarily affiliated with academic institutions and other occupations. (n=79)

Respondents were given the option to provide an explanation for their answer to Q7. Twenty-nine respondents explained their attitude chosen in Q7 (Table 14). Themes presented in the explanations varied greatly from unimportance and lack of EDC knowledge to EDC effects on wildlife as the central focus of a respondent’s work. This variance was especially clear across different rankings.

Table 14. Text responses that accompanied the attitudes presented in Q7. (n=29)

Q7 Ranking	Explanation
1	I work on amphibians and reptiles in the Southwest. Aquatic systems are generally not linked to human or agricultural waste streams (or are upstream of them), and therefore EDCs have not been a priority concern.
	I work with plants
2	I work with amphibian diseases. I don't know any interaction between EDCs and disease but I know EDCs can have reproductive effects on amphibians.
	I don't deal with it at all, but it'd be interesting to see how it'd affect organismal responses to climate change.
	I work with insects and while EDC's are often related to pesticide use, this does not directly relate to my research in any way.
	I do environmental compliance and conservation planning, which most of the time do not evaluate in detail, the effects of EDCs
	I see them as important but feel I do not have enough information on which to take any action within my state.
	The threats from EDCs are mostly poorly understood and too hidden compared to other more obvious and immediate threats
	previous [sic] work on toxicology in bald eagles ... with more recent attention to PCBs & dioxins
	I am not studying EDCs specifically, but it is nearly impossible to eliminate them from any animals environment. Therefore there is always and potental [sic] effect of EDCs.
	targeted [sic] exploitation, road and bycatch mortality and general habitat loss are greater short-term concerns for freshwater turtles
3	In studies of amphibian disease, anything that compromises their immune system is important.
	I'm a stress physiologist (behavioral endocrinologist), but I work primarily on a highly habitat-specific species. Habitat changes will literally kill the population off before we see significant deleterious effects from pollution or pesticides.
	I am a student, so my focus is still relatively broad
	Hantavirus research
	I don't deal with this issue directly, but I expect I will run into this as my conservation and management work expands and as more is known about the effects of EDCs across various taxa (I work mostly with large carnivores, and I haven't heard of this issue in my study species).
4	I work for the USGS studying mussel and eels and water pollution (including EDCs) can definitely impacts both.
	Water quality
	I am more occupied with sources of acute exposure but understand that EDC effects are also important.
	Used to be more relevant when we worked with Bald Eagles; becoming more relevant as we move into more work with local amphibian species.
5	I am an amphibian conservation biologist and although I don't work with this group of chemicals I understand their importance.
	working [sic] with hormones and reproduction / behaviour
	An ecologist
	conduct [sic] ecological risk assessments
	PhD in Wildlife Disease
	conservation [sic] medicine research and global zoological conservation
	As a graduate student I hope to study the impacts of mercury in areas of illegal gold mining.
	I study the effects of endocrine disrupting chemicals on reproduction of endangered species for a living.
	I am an assistant professor in a Biology department and sub-lethal effects of EDCs on wildlife represents my lab's research interests.

In Question 9, respondents either agreed or disagreed with the concept that EDC effects on wildlife conservation should be more of a research priority in their field. Responses were strongly affirmative, as 81% of respondents selected either 'agree' or 'strongly agree' (Figure 18).

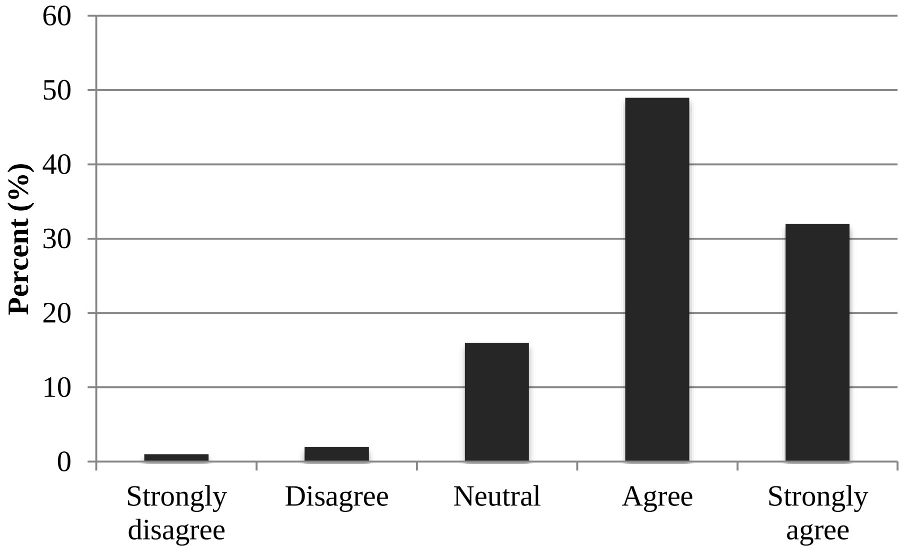

Figure 18. Q9 – Should endocrine-disrupting chemical (EDC) effects on wildlife conservation be more of a research priority in your field?

Both age (Q28) and occupation (Q21) made no difference in how respondents rated if EDCs should be more of a priority ($p_{age} = 0.1182$, $p_{occupation} = 0.5948$). However, respondents who selected 'not at all familiar,' 'slightly familiar,' or 'somewhat familiar' in Q1 rated EDCs as a research priority in Q9 significantly lower than respondents who selected 'moderately familiar' or 'extremely familiar' in Q1 (p = 0.010). Similarly, respondents who selected 'not at all significant,' 'slightly significant,' or 'somewhat significant' in Q3 rated EDCs as a research priority in Q9 significantly lower than respondents who selected 'moderately significant or 'extremely significant' in Q3 (p = 0.003). In addition, respondents who selected 'not at all important,' 'slightly significant,' or 'neutral' in Q7 rated EDCs as a research priority in Q9 significantly lower than respondents who selected 'moderately important' or 'extremely important' in Q7 (p = 0.008).

Nineteen respondents explained their attitude chosen in Q9. Common themes found in text responses included EDCs as an emerging concern and underestimation of EDCs as a concern (Table 15).

Table 15. Text responses that accompanied the attitudes presented in Q9. (n=19)

Q9 Ranking	Text response
1	Absolutely. I work in conservation biology and I find that a great deal of the work that my colleagues and I do is undermined by the long term negative impacts of persistent EDCs. If we are successful in reducing pressures to species in respect to habitat loss with the creation of protected areas, or poaching from enforcement of take quotas/bans, we are unable to make these concrete strides and protections against EDC exposure.
3	It already is!
	My specific field is climate change, it'd be interesting to know how those interact but I haven't been exposed to areas of research that look at both simultaneously.
	These chemicals are already a research priority in my field
	Useful complementary info, but not the top priority in my field
4	Interactions often play big roles in disease outcomes
	May impact further study of the amphibian fungal disease we research.
	It already rather is. Contamination is sometimes a confounding variable in the same assays used to measure naturally occurring hormones (e.g., plastic lab equipment is largely a no-no because of synthetic estrogen contamination).
	I think the effects of developmental projects with relation to EDCs should be evaluated in environmental compliance documents.
	I suspect this is a much bigger issue that we realise. [sic] I am aware of some startling impacts of EDCs on fish, and I'm sure that is just the "canary in the coal mine".
	The threats from EDCs are mostly poorly understood and too hidden compared to other more obvious and immediate threats
	I feel that is should be priority, but I don't think it will be anytime soon. There is so much basic information, about biology, behaviors, etc, that is unknown in many endangered species that is needed before EDC research would be possible.
5	Although, as I mentioned above, EDCs are not a big issue for our work (...as far as we know), they are exceedingly important in a more general sense.
	impacts [sic] are under-estimated and therefore ignored in experimental design
	I am a scientist working on EDCs in Australia. endocrine [sic] disrupting contaminants are the biggest threat to wildlife and humans known to date.
	Better awareness and education needed
	If organisms are unable to procreate, this is just as important as any other wildlife research.
	I believe this issue will prove to be of even greater importance very soon.
	There is a lot of focus by fish and wildlife agencies on habitat loss, invasives [sic], and other larger factors. I think the significance of endocrine changes occurring due to human waste and pesticides is not as recognized as it should be and has the potential to alter our natural [sic] resources that we rely on much more than we anticipate.

Practice

Question 13 asked respondents if they were currently conducting any research regarding EDCs and their effects on wildlife. Eighty-one percent of respondents said they were not currently conducting research, but 19% did say they were currently conducting research. Fourteen of the 15 respondents who said they were conducting research provided a brief explanation of their research (Table 16 and Figure 19).

Table 16. Research regarding EDC effects on wildlife being conducted by respondents. (n=14)

Current research explanations
Modeling impacts of PCBs on marine mammal populations
I study the impacts of atrazine on somatic and gonadal development of amphibians, with emphasis latent effects of larval exposure (i.e., effects that become evident after metamorphosis, which have rarely been examined).
Estrogen in wastewater. Impacts on fish.
Indirectly - my research looks at the uptake and effects of pesticides in terrestrial phase amphibians. I don't directly measure endocrine impacts, but this certainly would be something that I could start investigating.
influences [sic] on behavior and evolutionary processes
amphibian [sic] health in an area with hydrocarbon extraction and upgrading
PCBs in plastic and its effect on fish health
Examining reptilian immune and stress response of several EDCs
i [sic] am the first to demonstratethat [sic] a single low dose of atrazine causes disruption to viviparous lizards exposed in utero. i [sic] am now investingating [sic]EDC effects on spermatogenesis and oxidative status in spem [sic] and ova.
effects [sic] of EDCs and pharmaceuticals on fish behavior (boldness, courtship, aggression, communication, behavioral consistency and variability)
I study the effects of pesticides on reproductive biology across taxa including amphibians, fish, and mice as a model for human health.
Mercury accumulation in birds and mammals
Looking at the effects of diet on captive white rhino reproduction and studying exposure of CA condors to legacy EDCs such as DDT
I am exploring the sub-lethal effects of estrogen and androgen mimics on fish morphology, development, behavior transgenerationally

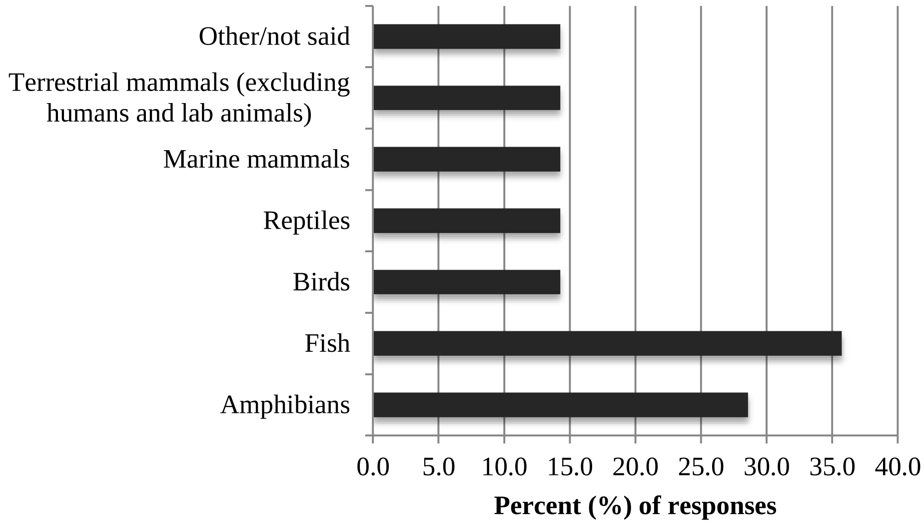

Figure 19. Wildlife taxa studied by respondents who said they conduct EDC research. (n=14)

The majority of respondents currently conducting research are not conducting research related to the most frequently stated data gaps in Q12. Six of the total 14 respondents are studying data gaps such as population level effects, multiple EDCs or interactions, multigenerational effects, or impacts on threatened species. Of all 14 respondents, the most common taxa of study are fish and amphibians (5 and 4 researchers, respectively). Birds, reptiles, marine mammals, and terrestrial mammals are less common. All were stated as research subjects by two respondents.

Question 15 asked respondents if they were planning to conduct any research regarding EDCs and their effects on wildlife in the next 5 years. Question 15 was provided only to those respondents who selected ‘no’ in Q13. Again, the majority of respondents stated they were not planning to conduct research, but 14% of respondents not currently conducting research have plans to do so in the next 5 years. Eight of the 9 respondents who have plans to conduct research in the next five years provided a brief explanation of their plans (Table 17).

Table 17. Respondents' future research plans regarding EDC effects on wildlife. (n=8)

Future research plans
Effect of chemicals up the food chain from insects to bats
Hermaphroditic fish
Plastic degradation and accumulation in parasitic animals
Looking at direct and indirect effects in terrestrial mammals
Effects of pharms/personal care products on fish reproduction and behavior
within [sic] confines of ecological risk assessment
plastics [sic] impacts in fish, impacts of wastewater on fish spawning
Not sure.

The majority of respondents planning to conduct research in the next five years regarding EDCs and wildlife conservation are not planning to research any of the most common data gaps gathered in Q12. Only two of the eight respondents stated research plans related to the data gaps of threatened species and population or ecosystem level effects. Three respondents were unclear about taxa of study, two proposed research with terrestrial mammals, and three proposed research with fish.

The majority of respondents who answered 'extremely familiar' in Q1 answered 'yes' that they were currently conducting research regarding EDCs and their effects on wildlife (Q13). The majority of all other familiarity categories in Q1 responded no to conducting research currently and answered no to plans to conduct research regarding EDCs and their effects on wildlife in the near future (Q15).

Only 'Zoo' and 'University/college' respondents responded that they were currently conducting research (Q13), and no respondent in 'NGO' or 'Government' answered affirmatively. The majority of respondents in each occupation category responded negatively to whether they were currently conducting research (Q13). Again, the majority of respondents in each occupation category responded negatively to whether they were planning to conduct research in the near future (Q15), but at least one respondent from each occupation category is planning to conduct research in the future regarding EDCs and effects on wildlife (Q15).

Question 17 asked respondents if they knew of other conservation researchers or practitioners studying EDC effects on wildlife. Seventy-one percent of respondents selected that they knew of other researchers or practitioners, and only 29% did not know of other researchers or practitioners studying EDCs and wildlife.

The majority of respondents who answered ‘slightly familiar,’ ‘moderately familiar,’ or ‘extremely familiar’ in Q1 knew of other researchers or practitioners studying EDC effects on wildlife. The majority of respondents in ‘not at all familiar’ or ‘somewhat familiar’ categories of Q1 did not know of other researchers or practitioners studying EDC effects on wildlife. The majority of respondents in all four occupation categories (Q21) and all four age categories (Q28) responded affirmatively to knowing other researchers or practitioners studying EDC effects on wildlife.

Question 18 asked respondents to list species they knew are currently being studied for either EDC exposure or effects. Categorization of text responses revealed that the most commonly known and stated taxa of study were amphibians and fish, mentioned in 44% and 39% of responses (Figure 20). Ten to 12 respondents each mentioned birds, reptiles, and marine mammals, while only four respondents mentioned terrestrial mammals (Figure 20).

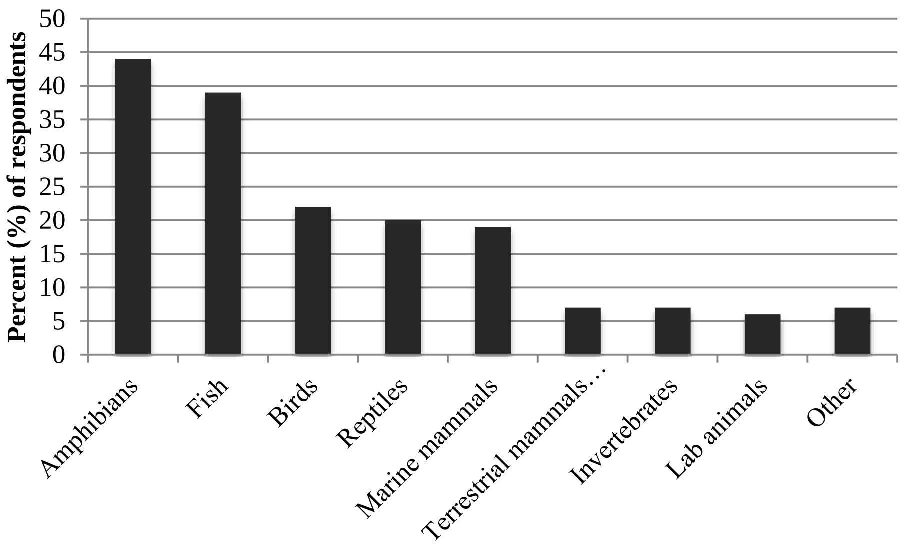

Figure 20. Q18 - Which species, if any, are being studied for endocrine-disrupting chemical (EDC) exposure or effects? ‘Terrestrial mammals’ excludes humans and laboratory animals. (n=54)

Question 19 asked respondents if they knew of any interventions being implemented to prevent EDC exposure in wildlife. Only 23% of respondents knew of interventions, while 78% were not aware of interventions. All 18 respondents who knew

of interventions provided a brief explanation of the interventions they were aware of (Table 18).

Table 18. Respondent explanations of interventions being implemented to prevent EDC exposure or effects in wildlife. (n=18)

Intervention explanations
I'm unsure whether it was specifically targeted to wildlife conservation, but Rwanda's (a country where ecotourism is very important economically) ban on plastic bags may reduce EDCs entering the environment
Bioremediation is an active field of research- can microbes degrade EDCs, and if so, can we use them effectively? 2. Vegetative buffer zones around aquatic habitats are used to reduce loading of aquatic EDCs.
atrazine [sic] has been limited
attempts [sic] made at water treatment facilities
policy [sic] and law.
Working to reduce effects of pesticides on raptors
chemical [sic] filters in waste water treatment plants in sacramento [sic], CA
Some EDCs are being phased out in many countries, especially when human impacts are found
Recent legislation banning/limiting plastic microbeads; historic bans on PCBs, PBDEs, and PFCs
Scientific literature suggesting possible links to multiple links to physiological impairments
superfund [sic] cleanups
bans [sic] on certain chemicals in some countries
BPA is in the limelight and companies ar [sic] working to remove it from shelves. I'm aware that even if/when BPA goes away, there are still dozens if not hundreds of others doing just the same thing and posing the same risks. BPA is posterchild [sic], a scapegoat.
letting poeple [sic] know the harm they can cause by flushing unused medicine and the effects of birth control hormones that make it into the water
policies [sic] related to disposal of pharmaceuticals; policies related to chemical fertilizers and lawn chemicals
Best management practices for waste water treatment that decrease nitrogen, phosphorus, nutrients, and sediment seem to decrease the presence of EDCs as well
Mitigation of contaminated sites, increased efficacy of wastewater treatment, etc.
Ban of DDT

The most common intervention cited by respondents was a legal ban on certain EDCs (Figure 21). EDCs mentioned as having bans or a control policy in place were atrazine and other pesticides, PCBs, PBDEs, PFCs, BPA, DDT, and pharmaceuticals. Other common interventions mentioned were wastewater treatment and bans on certain products containing EDCs. Only two respondents mentioned education or bioremediation as interventions, and five respondents stated other types of interventions. Both age (Q28) and occupation (Q21) made no difference in if respondents could identify interventions.

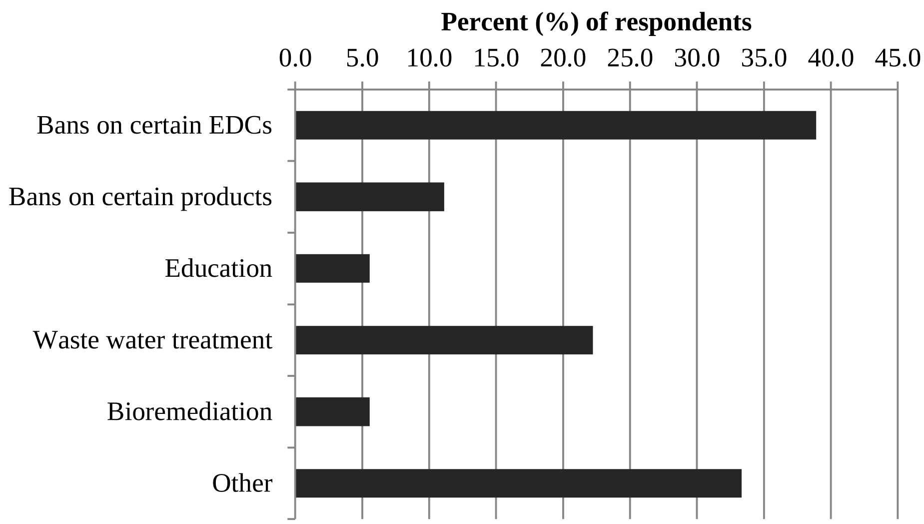

Figure 21. Categorization of respondent explanations of current interventions. (n=18)

DISCUSSION

Overall, 116 conservation scientists and practitioners responded to the survey, and provided valuable insight about how they view the impact of EDCs on wildlife conservation. Their responses revealed important information about knowledge of EDCs, attitudes towards EDCs, and the practice of researching EDCs in wildlife.

Biases and Limitations

Respondents were not selected at random, and took this survey by choice. Therefore, bias exists in those who chose to respond and therefore the responses they provided. Most respondents likely decided to take the survey because they had a greater interest in EDCs or were more informed about EDCs than the average conservation scientist or practitioner. However, the results of the survey show that even in this limited sample, awareness of and concern for EDCs varies greatly. Thus, the survey results are a useful representation of knowledge and attitudes among diverse conservation practitioners.

In addition, 41% of respondents were undergraduate, graduate, or doctoral students. There are many levels of experience within these categories, which this survey did not address. However, current students are likely more exposed to new techniques of study and topics during schooling than non-student respondents.

Knowledge

Most respondents stated they were familiar with EDCs in general, but survey questions revealed they are less familiar with specific EDC chemicals or specific EDC effects on wildlife. Those respondents who said they were most familiar with EDCs in general were more knowledgeable about other questions. When presented with a range of taxa, a list of physiological effects, and a list of EDCs, respondents were consistently familiar with only some choices on each list. Fewer respondents stated they were ‘extremely familiar’ with any of the taxa, effects, or EDCs.

Respondents were more familiar with the effects of EDCs in amphibians and fish than in reptiles, birds, marine mammals, or terrestrial mammals. This trend was true even for those respondents who were ‘extremely familiar’ with EDCs overall. Twelve of the 22 respondents in the ‘extremely familiar’ category are currently conducting research regarding EDC effects on wildlife, and so would likely be aware of the literature regarding EDCs and wildlife. Six out of these 12 are conducting research regarding amphibians or fish. This question revealed that even those conducting research are not familiar with effects of EDCs in reptiles, birds, and mammals. This unfamiliarity could be due to a shortage of conclusive studies in those taxa. Fifty-four respondents identified species or taxa they knew were being studied for effects of EDCs. The majority of respondents identified amphibians or fish as the taxa being studied, and few cited other taxa. This identification of amphibians and fish helps to explain why respondents were more familiar with effects of EDCs in amphibians and fish than other taxa.

In terms of effects of EDCs, respondents were familiar with female and male reproductive impacts and developmental impacts, and had limited familiarity any other effects provided. Only 12% of respondents selected ‘extremely familiar’ for any of the physiological effects. While studies have shown all of these effects in wildlife (Jobling and Tyler 2006, Bernanke and Kohler 2008, Vethaak and Legler 2013), a knowledge gap exists regarding impacts on tumors, thyroid, neurological, metabolic, immune, and behavioral systems. This gap could be due to a focus in the conservation community on studies that look directly at reproductive impacts or developmental impacts, as those are two areas of great concern for conservation of species.

In terms of certain EDCs, respondents were familiar with half of the chemicals in the list, including DDT, PCBs, atrazine and other pesticides, and residual pharmaceuticals. The EDCs presented to respondents represent a small fraction of all known endocrine disruptors, but this small group reveals the scope of respondents’ knowledge. Respondents are familiar with some more media prevalent and historically significant EDCS, like DDT and atrazine, but lack knowledge about other EDCs with equal potential to cause negative effects. This difference could influence how respondents’ understand the literature regarding a variety of EDCs.

Respondents cited many critical data gaps that need to be filled to improve knowledge of how EDCs affect wildlife conservation. Ability to identify data gaps was significantly correlated with respondent familiarity with EDCs in Q1. Multiple respondents cited important data gaps related to population or ecosystem level effects of EDCs, effects of exposure to EDC mixtures, and long term or multigenerational effects. These are commonly listed data gaps in the literature (Hayes et al. 2006, Jobling and Tyler 2006, Lyons 2006, Hotchkiss et al. 2008, Tan et al. 2009, Walker and Gore 2011, Dietert 2014, Schwindt 2015). A few respondents selected mitigation or removal strategies for EDCs in the environment and in wildlife as another data gap. One respondent said, "research needs to focus on ways to remove EDCs from the environment. [A]lternatives to EDCs need to be found so we can elimate [*sic*] wildlife exposure." This response shows mitigation of EDC effects and the removal of EDCs from the ecosystems is an area lacking in current research, but must be more of a priority.

A few respondents also mentioned a need for more studies of EDC effects on sensitive, threatened, or endangered species, however, no respondents stated the need for better knowledge of basic endocrinology in those vulnerable species. Little information exists about the endocrinology of rare or threatened species, particularly in the wild (Tubbs et al. 2014).

Respondents rarely showed differences in knowledge based on age, with one exception. Respondents age 20-30 were significantly less frequently able to identify two critical data gaps than all older respondents. However, it is unlikely this means younger respondents were less knowledgeable, because respondents aged 20-30 had the same familiarity as other ages in other questions. Occupation did not seem to influence respondents' knowledge.

Attitudes

EDCs in Wildlife Conservation

Respondents did not rank EDCs highly when given a list of threats to wildlife conservation. Habitat loss and human population growth were consistently ranked the top concerns for wildlife conservation, while EDCs were ranked as a lesser concern.

Although EDCS were a lesser concern, they were ranked higher than wildlife trafficking, hunting, and poaching, which is a well-known concern that often gets media attention. When asked about EDCs alone, the majority of respondents thought EDCs were a significant concern for wildlife conservation. Additionally, when asked how important EDCs are in their current work, responses were varied. But the majority of respondents either 'agreed' or 'strongly agreed' that EDCs should be more of a priority in their field.

Importance of EDCs in Respondents' Work

Respondents working in similar fields wrote conflicting explanations on whether EDC research is important for their work. One respondent selected 'not at all important' and stated, "I work with amphibian diseases. I don't know any interaction between EDCs and disease but I know EDCs can have reproductive effects on amphibians." Another respondent selected 'neutral' and stated, "In studies of amphibian disease, anything that compromises their immune system is important." Although both respondents study amphibian disease, their beliefs of the importance of EDCs in their work were different. An area of missing research is the interaction between EDCs, the immune system, and disease (Coppock 2011). This is important because of the overlap between immune and endocrine systems (Avcedo-Whitehouse and Duffus 2009). One respondent with a "PhD in Wildlife Disease" affirmed this potential by rating EDCs as 'extremely important' to their work.

Another respondent stated, "I work on amphibians and reptiles in the Southwest. Aquatic systems are generally not linked to human or agricultural waste streams (or are upstream of them), and therefore EDCs have not been a priority concern." Despite this assertion, EDCs are commonly found in all U.S. surface waters tested by the US Geological Survey (USGS 2002). Many studies have shown interactions between aquatic systems, EDCs, and amphibians (Hayes et al. 2006, Bernanke and Kohler 2008, Vethaak and Legler 2013). This response is concerning as it indicates EDCs may not be considered as a variable in studies when they should be. Another respondent rated EDCs as only 'slightly important' to their work but had conducted "previous work on toxicology in bald eagles … with more recent attention to PCBs & dioxins." In contrast, another respondent who said EDCs were only 'slightly important' for their work

acknowledged the overall importance of EDCs. They stated, “I am not studying EDCs specifically, but it is nearly impossible to eliminate them from any animals environment. Therefore there is always and potental [*sic*] effect of EDCs.”

Two respondents said “I see [EDCs] as important but feel I do not have enough information on which to take any action,” and “threats from EDCs are mostly poorly understood and too hidden compared to other more obvious and immediate threats.” Both these respondents rated EDCs as only ‘slightly important’ in their work, and while their responses show concern for EDCs, they are of a lesser importance than other conservation threats. Another respondent said “I work mostly with large carnivores, and I haven’t heard of this issue [EDCs] in my study species … but I expect I will run into this as my conservation and management work expands and as more is known about the effects of EDCs across various taxa.” EDCs are likely a greater concern for top predators due to bioaccumulation and their tendency to be threatened species (Fossi et al. 1999, Vethaak and Legler 2013).

Age did not influence the importance of EDCs in respondents’ work, but it is likely that occupation played a significant role. Additionally, general familiarity with EDCs played a very significant role in whether respondents thought EDCs were important to their work.

EDCs as a Research Priority in Respondents’ Fields

In response to whether EDCs should be a priority in their field one respondent said, “I work in conservation biology and I find that a great deal of the work that my colleagues and I do is undermined by the long term negative impacts of persistent EDCs. If we are successful in reducing pressures to species in respect to habitat loss with the creation of protected areas, or poaching from enforcement of take quotas/bans, we are unable to make these concrete strides and protections against EDC exposure.”

One respondent stated, “I suspect this is a much bigger issue that we realise. [*sic*] I am aware of some startling impacts of EDCs on fish, and I'm sure that is just the "canary in the coal mine".” Another respondent stated, “I feel that is [*sic*] should be priority, but I don't think it will be anytime soon. There is so much basic information, about biology, behaviors, etc, that is unknown in many endangered species that is needed

before EDC research would be possible," a claim supported by many studies calling for more basic research on endangered species' physiology, and in particular, endocrinology (Tyler et al. 1998, Jobling and Tyler 2006, Tan et al. 2009, Kendall et al. 2010, Coppock 2011, Spencer et al. 2012, Wang and Zhou 2013, Kumar and Holt 2014, Tubbs et al. 2014, Jorgenson et al. 2015).

Two respondents who were 'neutral' about whether EDCs should increase as a research priority in their field gave interesting explanations. One respondent said, "I haven't been exposed to areas of research that look at both [EDCs and climate change] simultaneously, [but] it'd be interesting to know how those interact," As climate change alters ecosystems and increases human pressure on many species, EDCs are likely to be another stressor that is not well-recognized in wildlife. Another respondent said that EDCs were "useful complementary info, but not the top priority in my field."

Two respondents who 'agreed' that EDCs should increase as a research priority in their field proposed two areas of study where EDCs should be taken into account. One respondent said, "contamination is sometimes a confounding variable" in studies and that plastic laboratory equipment is limited when studying hormones. Another respondent said, "I think the effects of developmental projects with relation to EDCs should be evaluated in environmental compliance documents," revealing a gap in policy where EDCs are left unchecked but need to be accounted for.

Multiple respondents 'strongly agreed' that EDCs should be an increasing priority in their field. One respondent said, "impacts are under-estimated and therefore ignored in experimental design," while another said, "I believe this issue will prove to be of even greater importance very soon." One respondent, who studies EDCs in wildlife, said, "endocrine disrupting contaminants are the biggest threat to wildlife and humans known to date." Another respondent said, "if organisms are unable to procreate [due to EDCs], this is just as important as any other wildlife research." Finally, one respondent stated, "I think the significance of endocrine changes occurring due to human waste and pesticides is not as recognized as it should be and has the potential to alter our natiral [*sic*] resources that we rely on much more than we anticipate."

Age and occupation did not influence respondents' attitudes in this section, but general EDC knowledge, belief about the significance of EDCs, and importance of EDCs in respondents' work all significantly influenced responses about the priority of EDCs.

Overall, some respondents are thinking about EDCs as a concern for wildlife conservation, but other concerns hold greater weight and EDCs are viewed as more of a problem to address in the future. A subset of respondents is more concerned about EDCs and feels action should be taken immediately for wildlife protection.

Practice

Twenty-four of the 116 total respondents are either currently conducting research or are planning to conduct research in the next five years related to the effects of EDCs on wildlife. The majority of both current and future research is not directed towards filling in critical data gaps outlined by other respondents or found in the literature. Most studies continue to focus on effects of a single EDC in more commonly studied taxa, including amphibians and fish. However, a quarter of respondents planning to conduct future research are planning to study effects of EDCs in terrestrial mammals, which helps fill a critical data gap. Eighty-seven percent of respondents conducting or planning to conduct research are affiliated with either a zoo or an academic institution, not a government agency or a NGO. Basic research may be less commonly conducted at NGOs or government agencies.

Most respondents know of another researcher studying the effects of EDCs on wildlife, although they are not conducting research themselves, and some still indicated that they know little about EDC effects. Interventions are important in preventing exposure to and effects of EDCs in wildlife, but only 18 respondents could state an intervention they knew of. Most interventions stated consist of legal bans on EDCs, which are not effective given the ample literature of persistent effects from these EDCs.

These findings are interesting because even though most respondents know of other researchers studying EDCs, there are many respondents who are unknowledgeable about the effects of EDCs in wildlife as evidenced in the previous sections. Knowledge of other researchers does not appear to translate into knowledge of the researcher's findings.

Conclusions and Future Directions

My survey has revealed that conservation scientists and practitioners view EDCs differently depending on the context in which EDCs are described. Many respondents see EDCs as a concern for wildlife conservation, but not in comparison to more prominent threats. Conservationists are familiar with the basics of endocrine disruption by chemicals; however, they are unaware of specific EDCs and physiological effects of EDCs in many taxa. Most conservation scientists and practitioners can cite critical data gaps in research regarding EDC effects on wildlife, but few are actively conducting research to close these gaps. Attitudes vary among respondents when deciding if EDCs were important in their specific work, even though most respondents view EDCs as a significant concern for wildlife reproduction, behavior, and conservation. Overall, most respondents believe EDCs should be a priority in their field of study, but more action is needed to make EDCs as a priority into a reality. Although a subset of survey respondents were actively aware of EDC effects in wildlife, most conservationists that responded are not informed enough and are too ambivalent to address the growing severity of EDC impacts on wildlife conservation. Respondents believe that EDCs are a threat to wildlife conservation, but they do not believe EDCs are an important factor in their work. The magnitude of concern in many free responses highlights EDCs as an emerging threat for wildlife conservation that needs to be addressed, but respondents' attitude dichotomy is problematic for prioritizing research about EDC impacts on wildlife conservation.

In the past 20 years, EDCs have become a major topic of research, and now there exists a breadth of information on the subject. For wildlife specifically, studies are scattered across different EDCs and taxa, and vary from tissue level to population level studies. Because of the diversity and timeframe of studies, multiple research gaps remain. Very few studies have looked at long-term effects of EDCs at a large scale, a key concern for wildlife population management. In the same regard, no comprehensive cause and effect at the population level has been studied. Most laboratory and field studies focus on one chemical at a time, but ample evidence indicates that wildlife are exposed to many EDCs, and studies should better reflect that. Because of the diversity and magnitude of species of wildlife, the one-chemical one-organism study approach is not going to work within the timeframe necessary to take precautionary or preventative action necessary for

wildlife protection. The diversity and magnitude of wildlife species potentially at risk and limitations of field and laboratory studies also necessitate improvements in computer modeling of multiple EDC effects at both the individual and population level. An impediment to modeling is the lack of basic information about the endocrinology of many species, especially those that are rare or threatened, which is necessary for extrapolating EDC effects. Gaining a sense of “baseline” endocrinology for many of these species may prove impossible, as they are likely already exposed to EDCs. Policy must reflect these knowledge gaps by employing a precautionary approach to limit EDC exposure in wildlife.

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APPENDIX A

Acronym glossary

Acronym	Definition
EDC	Endocrine-disrupting chemical
BPA	Bisphenol A
PCB	Polychlorinated biphenyl
TEDX	The Endocrine Disruption Exchange
EPA	US Environmental Protection Agency
NMDRC	Nonmonotonic dose response curve
DES	Diethylstilbestrol
FDA	US Food and Drug Administration
WHO	World Health Organization
UNEP	United Nations Environment Program
TBT	Tributylin
DDT/DDE	Dichlorodiphenyltrichloroethane/Dichlorodiphenyldichloroethylene
TCDD	Tetrachlorodibenzo-p-dioxin
OC	Organochlorine
PAH	Polycyclic aromatic hydrocarbon
BFR	Brominated flame retardant
AP	Alkylphenol
PFC	Perfluorocarbon
PFOS	Perfluorooctane sulfonate
POSF	Perfluorooctanesulfonyl fluoride
PBDE	Polybrominated diphenyl ether
POP	Persistant organic pollutant
FWS	US Fish and Wildlife Service
NOAA	National Oceanic and Atmospheric Association
NMFS	NOAA's National Marine Fisheries Service
ESA	Endangered Species Act
MMPA	Marine Mammal Protection Act
MMHSRP	Marine Mammal Health and Stranding Response Program
EDSP	Endocrine Disruptor Screening Program

APPENDIX B

Survey

Endocrine-disrupting chemicals (EDCs), such as those from plastics and pesticides, have been hypothesized to affect wildlife populations. This survey is part of an honors thesis project at Colby College (Waterville, Maine, USA) to assess how conservation scientists and practitioners view the relative importance of EDCs for wildlife conservation.

I would be grateful if you could answer the following questions regarding EDCs in conservation, as well as a few background questions. The survey should take you about 5-10 minutes to complete. Thank you for taking the time to share your expertise.

Your answers are confidential and will only be reported in the final synthesis in which all responses are anonymous. This survey was reviewed by the Institutional Review Board at Colby College and determined exempt under category 45 CFR 46.101(b)(2).

The following questions all reference endocrine-disrupting chemicals (EDCs). According to the U.S. National Institute of Environmental Health Sciences, EDCs "are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. "A growing number of studies suggest wildlife are being exposed to EDCs, but how EDCs affect wildlife health and wildlife conservation is less well-understood.

Q1 How familiar are you with the environmental pollutants known as "endocrine-disrupting chemicals" (EDCs)?

- ❍ Not at all familiar (1)
- ❍ Slightly familiar (2)
- ❍ Somewhat familiar (3)
- ❍ Moderately familiar (4)
- ❍ Extremely familiar (5)

Q2 Please rank the following concerns for wildlife conservation from most (1) to least (7) important.

______ Endocrine-disrupting chemicals (EDCs) (1)
______ Human population growth (2)
______ Habitat loss (3)
______ Climate change (4)
______ Disease (5)
______ Invasive species (6)
______ Wildlife trafficking, hunting, and poaching (7)

Q3 For endocrine-disrupting chemicals (EDCs) specifically, how significant do you believe their effect is on wildlife reproduction, behavior, or conservation?

- ❍ Not at all significant (1)
- ❍ Slightly significant (2)
- ❍ Neutral (3)
- ❍ Moderately significant (4)
- ❍ Extremely significant (5)

Q4 How familiar are you with the effects of endocrine-disrupting chemicals (EDCs) in the following taxa?

	Not at all familiar (1)	Slightly familiar (2)	Somewhat familiar (3)	Moderately familiar (4)	Extremely familiar (5)
Humans (1)	❍	❍	❍	❍	❍
Amphibians (2)	❍	❍	❍	❍	❍
Fish (3)	❍	❍	❍	❍	❍
Birds (4)	❍	❍	❍	❍	❍
Reptiles (5)	❍	❍	❍	❍	❍
Marine mammals (6)	❍	❍	❍	❍	❍
Terrestrial mammals (excluding humans) (7)	❍	❍	❍	❍	❍

Q5 How familiar are you with the following potential effects of endocrine-disrupting chemicals (EDCs) in wildlife listed in the table below?

	Not at all familiar (1)	Slightly familiar (2)	Somewhat familiar (3)	Moderately familiar (4)	Extremely familiar (5)
Female reproductive impacts (1)	❍	❍	❍	❍	❍
Male reproductive impacts (2)	❍	❍	❍	❍	❍
Tumors (3)	❍	❍	❍	❍	❍
Thyroid impacts (4)	❍	❍	❍	❍	❍
Neurological function impacts (5)	❍	❍	❍	❍	❍
Developmental impacts (6)	❍	❍	❍	❍	❍
Metabolic function impacts (7)	❍	❍	❍	❍	❍
Immune system impacts (8)	❍	❍	❍	❍	❍
Behavioral impacts (9)	❍	❍	❍	❍	❍
Other: (10)	❍	❍	❍	❍	❍

Q6 How familiar are you with each of the endocrine-disrupting chemicals (EDCs) listed below in terms of possible effects on wildlife?

	Not at all familiar (1)	Slightly familiar (2)	Somewhat familiar (3)	Moderately familiar (4)	Extremely familiar (5)
DDT (1)	❍	❍	❍	❍	❍
Atrazine (2)	❍	❍	❍	❍	❍
Other pesticides (3)	❍	❍	❍	❍	❍
Dioxins (4)	❍	❍	❍	❍	❍
Polychlorinated biphenyls (PCBs) (5)	❍	❍	❍	❍	❍
Polybrominated diethyl ethers (PBDEs) (6)	❍	❍	❍	❍	❍
Bisphenol A (BPA) (7)	❍	❍	❍	❍	❍
Phthalates (8)	❍	❍	❍	❍	❍
Residual pharmaceuticals (9)	❍	❍	❍	❍	❍
Other: (10)	❍	❍	❍	❍	❍

Q7 Are endocrine-disrupting chemical (EDC) effects on wildlife important in your specific work?

❍ Not at all important (1)
❍ Slightly important (2)
❍ Neutral (3)
❍ Moderately important (4)
❍ Extremely important (5)

Q8 Explain (optional):

Q9 Should endocrine-disrupting chemical (EDC) effects on wildlife conservation be more of a research priority in your field?
- Strongly disagree (1)
- Disagree (2)
- Neutral (3)
- Agree (4)
- Strongly agree (5)

Q10 Explain (optional):

Q11 Can you identify 2 critical data gaps that, in your opinion, need to be filled to improve knowledge of how endocrine-disrupting chemicals (EDCs) may affect wildlife conservation?
- Yes (1)
- No (2)

Answer If Can you identify 2 critical data gaps that, in your opinion, need to be filled to improve knowledge of how endocrine-disrupting chemicals (EDCs) may impact wildlife conservation?; Yes Is Selected

Q12 Please briefly state the 2 data gaps.

Q13 Are you currently conducting any research regarding endocrine-disrupting chemicals (EDCs) and their effects on wildlife?
- Yes (1)
- No (2)

Answer If Are you currently conducting any research regarding endocrine-disrupting chemical (EDC) impacts on wildlife? Yes Is Selected

Q14 Please briefly explain the research you are conducting.

Answer If Are you currently conducting any research regarding endocrine-disrupting chemical (EDC) impacts on wildlife? No Is Selected

Q15 Are you planning to conduct any research regarding endocrine-disrupting chemicals (EDCs) in the next 5 years?
- Yes (1)
- No (2)

Answer If Are you planning to conduct any research regarding endocrine-disrupting chemical (EDC) in the next 5 years? Yes Is Selected

Q16 Please briefly explain the research you plan to conduct.

Q17 Do you know of other researchers or practitioners studying endocrine-disrupting chemical (EDC) effects on wildlife?
- Yes (1)
- No (2)

Q18 Which species, if any, are being studied for endocrine-disrupting chemical (EDC) exposure or effects?

____

Q19 Do you know of any interventions being implemented to prevent endocrine-disrupting chemical (EDC) exposure in wildlife?
- Yes (1)
- No (2)

Answer If Do you know of any interventions being implemented to prevent endocrine-disrupting chemical (EDC) exposure in wildlife? Yes Is Selected

Q20 Please briefly explain.

Q21 What is your primary professional affiliation?
- Zoo (1)
- University/college (2)
- Non-governmental organization (3)
- Government (4)
- Other (5)

Answer If What is your primary professional affiliation? Zoo Is Selected

Q22 Please briefly describe your role.

Answer If What is your primary professional affiliation? University/college Is Selected

Q23 Please briefly describe your role.

Answer If What is your primary professional affiliation? Non-governmental organization Is Selected

Q24 Please briefly describe your role.

Answer If What is your primary professional affiliation? Government Is Selected

Q25 Please briefly describe your role.

Answer If What is your primary professional affiliation? Other Is Selected

Q26 Please briefly describe your primary professional affiliation and role.

Q27 How did you receive or access this survey?

____

Q28 What is your age?
- ❍ 20-30 (1)
- ❍ 30-40 (2)
- ❍ 40-50 (3)
- ❍ 50-60 (4)
- ❍ 60-70 (5)
- ❍ 70-80 (6)
- ❍ 80+ (7)

Q29 Please select all degrees completed.
- ❑ Bachelor's degree (1)
- ❑ Master's degree (2)
- ❑ PhD (3)
- ❑ DVM (4)
- ❑ MD (5)
- ❑ JD (6)
- ❑ Other: (7) ____________________

Q30 Would you like a copy of my final report?
- ❍ Yes (1)
- ❍ No (2)

Answer If Would you like a copy of my final report? Yes Is Selected

Q31 Email for distribution of final report:

Q32 Would you be willing to be contacted for further questions?
- ❍ Yes (1)
- ❍ No (2)

Answer If Would you be willing to be contacted for further questions? Yes Is Selected

Q33 Please provide the best way to contact you (include email address, phone number, etc.):