2.1. Environmental estrogens

In recent days, the impact of Endocrine Disrupting Chemicals (EDC) on human health and wildlife has been attracting an ever increasing amount of scientific and public interest. Well documented field studies have indicated significant changes occurring in the reproductive physiology of wild life populations and there are numerous evidences where humans or wildlife have been exposed to potential EDC suggesting changing patterns and trends in human reproductive health. Authors now believe that exposures to these endocrine active compounds can be plausibly linked to effects such as birth defects of reproductive organs, changes in behavior, reductions in sperm counts, and altered cell differentiation leading to the occurrence of cancer in reproductive organs.

The mindset among scientific workers started to change abruptly following a series of publications suggesting that some natural and man-made chemicals were threatening the reproductive capability and intelligence of future generations of humans and wildlife (Colborn et al., 1996, 1993). Of particular impact was the book entitled Our Stolen Future, Are We Threatening Our Fertility, Intelligence and Survival?—A Scientific Story written by (Colborn et al., 1996). This and other authors proposed that many Endocrine active compounds (EACs) elicited effects at doses far lower than toxicities caused by other modes of action (MOAs) and thus required special regulation. Altered programming can result in numerous adverse consequences in estrogen-target tissues, some of which may not be apparent until later in life. In mammals, chemicals having estrogenic activity can produce many health-related problems, such as early puberty in females, reduced sperm counts, altered functions of reproductive organs, altered sex-specific behaviors, and increased rates of some breast, ovarian, testicular, and prostate
2.2. Historical perspective

The presence and effects of EDCs is not a new phenomenon. The scientific community acknowledged that certain synthetic chemicals are capable of interfering with body’s development and function (Colborn et al., 1992). It was known as early as 1949, that crop dusters handling DDT (dichlorodiphenyltrichloroethane) frequently had reduced sperm counts. DDT was shown to produce characteristically estrogenic responses in the reproductive tracts of rats and birds (Bitman et al., 1970). The tragic use of the synthetic estrogen Diethylstilbestrol, or DES, by pregnant women from the late 1940s until 1971 to prevent miscarriages resulted in infertility and increased rates of vaginal clear cell adenocarcinomas in daughters (Colborn et al., 1993). In herring gulls, studies beginning

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in the mid-1960s in Lake Michigan suggested that environmental contaminants were adversely and, in some cases, severely affecting hatching success. The identification of EDCs has come through many avenues of investigation, including chance discoveries while working with various various chemicals and cell lines to detailed structure/activity investigations that test specific compounds for their ability to produce a response in cell lines or whole animals. Responses used to detect endocrine active compounds typically include cell proliferation (i.e., in breast cancer cell cultures), binding to the estrogen receptor, and production of specific proteins in cell cultures or whole animals. Examples of apparent reproductive endocrine disruption in aquatic organisms are, in some areas, disturbingly numerous and include intersex fish (male and female gonadal characteristics in gonochoristic or normally separate sex fish) (Jobling et al., 1998), elevated levels of a female egg protein in male fish (Lye et al., 1998; Janssen et al., 1997; Folmar et al., 1996), and degeneration of gonadal tissue (Lye et al., 1998; Janseen et al., 1997). In Lake Apopka in Florida, a dramatic decline in the alligator population has been attributed to a spill of DDT, dicofol, and possibly 1, 2-dibromo-3chloropropane (Risebrough, 1999). Feminization and masculinization as well as embryonic deformities in gulls have also been linked to PCBs and halogenated aromatic hydrocarbons. Interestingly, it appears that some of the effects seen in fish-eating birds in the Great Lakes are on the decrease (Fox, 1993).

2.3. EDC—Debates and discussions

There has been a vigorous debate within the scientific and regulatory communities regarding the extent of endocrine disruption in the environment, the importance of synthetic chemicals relative to naturally occurring EDCs, and the relevance of endocrine disrupters compared to an organism’s internal suite of hormones. Some argue that wildlife populations have already been significantly impacted by EDCs, resulting in decreased fertility in fish and shellfish, demasculinization and feminization of male fish, birds, and mammals, and masculinization of female fish and birds (Colborn et al., 1996). Others, however, are doubtful that EDCs in most situations are likely to have a
2.4. Mechanism of action of endocrine disruptors

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major impact. They point out that EDCs typically bind to receptors (e.g., the estrogen receptor) hundreds or thousands of times more weakly than endogenous hormones. An argument has also been made, in the case of environmental estrogens that the occurrence of natural and synthetic antiestrogens in the environment would act to negate the effect of environmental estrogens. While there is considerable and ongoing debate on the occurrence and significance of endocrine disruption, there is little disagreement over the need to better understand the impacts EDCs may be having in the environment.

2.4. Mechanism of action of endocrine disruptors

An endocrine disrupting chemical can affect the endocrine system of an organism in a wide variety of ways.

- **Altering Hormone Metabolism**

  Some chemicals do not directly interfere with hormones or their receptors, but upset the balance of hormones by interfering with their metabolism, i.e., their synthesis or natural breakdown and elimination from the body. For instance, the organochlorine pesticides DDE, atrazine and kepone have been found to alter the metabolism of estrogen (Bradlow et al., 1995).

- **Affects on Hormonal Control**

  The main control centre for the hormone system is the pituitary gland in the brain. The pituitary receives signals from, the hypothalamus, which constantly monitors the levels of hormones in the blood. Some hormone-disrupting chemicals appear to affect the brain’s control of the hormone system, either by their direct impact on steroid hormone levels, or indirectly by affecting the activities of the brain in other ways (Colborn et al., 1996).

- **Ah Receptor**

  The Ah receptor is not a hormone receptor but another sort of receptor in cells. Some chemicals, notably the dioxins and certain PCBs, can bind to these receptors and in so doing trigger many different biological effects, among which can be the disruption of hormones. It is thought that, this is how these chemicals can cause anti-estrogenic effects.
and alter levels of thyroid hormones (Safe and Krishnan, 1995). Besides the dioxins and PCBs, there are other chlorinated and non-chlorinated chemicals which are suspected of being able to bind to the Ah receptor (Giesy et al., 1994).

- **Binding and activating the oestrogen receptor (therefore acting as an estrogen)**
  By imitating the female hormone 17\(\beta\)-oestradiol. It has been found that if several chemicals that can bind and activate the estrogen receptor (Sumpter and Jobling, 2005).

- **Binding but not activating the estrogen receptor (therefore acting as an anti-estrogen), blocking Hormone Receptors**
  Some chemicals bind to hormone receptors and block them. This prevents hormones from binding to the receptors and exerting their normal biological effects (McLachlan, 1993). A few chemicals are known which can block the male sex hormone, the "androgen" receptor. These include the pesticides vinclozolin and DDE, which is the breakdown product of DDT (Kelce et al., 1994; 1995, Gray et al., 1994). These chemicals are known as "anti-androgenic chemicals".

- **Binding other receptors**
  There are many other receptors involved in the hormonal system, for example the androgen receptors. Substances like DDT metabolite p,p'-DDE or vinclozoline metabolites, block the androgen receptors, ie, the receptors for the male hormone testosterone. This binding can either activate the receptor, or inactivate it (Greim, 2004).

- **Modifying the metabolism of natural hormones**
  Some chemicals, such as lindane and atrazine, can affect the metabolic pathway of estradiol, producing more estrogenic metabolites. Other chemicals activate enzymes which speed up the metabolism of hormones, so disrupting their natural state. There are specific enzymes to metabolize estrogens. These enzymes break down estrogen rapidly to a form where they can no longer bind their receptor. However, if this enzyme is affected by a xenoestrogen, this metabolism will be reduced (Toppari et al., 1996).

- **Inhibiting hormone synthesis**
  EDCs can affect natural hormone production by interfering with the hormonal systems by inhibiting hormone synthesis. (Hotchkiss et al., 2008).
2.5. Types and sources of EDCs

EDCs include a diverse group of synthetic industrial and agricultural chemicals and even some naturally occurring compounds (Santodonato, 1997). They include industrial intermediates such as BPA (Celius et al., 1999; Sumpter and Jobling, 1995; Yamamoto et al., 2001) and 4-nonylphenol (White et al., 1994; Fujimoto et al., 2002; Okai et al., 2004), PAHs (Santodonato, 1997; Thomas, 1988), pesticides such as the insecticides endosulfan (Chakravorty et al., 1992; Briz et al., 2011), carbofuran (Sukumar and Karpagaganapathy, 1992), lindane (α-HCH) (Celius et al., 1999; El-mubarak and Huisingh, 2001), DDT and the herbicide atrazine (Hayes et al., 2011), PCBs (McGovern, 2006), and a number of metals including lead (Thomas, 1988), cadmium (Kime et al., 1996; Ruby et al., 2000), and mercury (Rurangwa et al., 1998).

Many of the chemicals currently known to interact with the estrogen receptor can be found in the effluent from sewage treatment plants (STPs) (Jobling et al., 1998). Although the efficacy or potency of all these compounds is less than that of the endogenous hormones, there is concern that some have the ability to bioaccumulate to active or harmful levels over time. As noted by Santodonato (1997), a number of estrogenic EDCs or their primary oxidative metabolites, share a common structural relationship with the phenolic A ring in 17β-estradiol. Alkylphenols and alkylphenol polyethoxylates, or APEs, have received much of the recent attention because of their estrogenic effects in laboratory studies, and their presence in the aquatic environment. APEs are used as surfactants in many applications from soaps and detergents to pesticide formulations. Once in the environment, microbial degradation results in loss of the ethoxylates, eventually leaving the more persistent alkylphenol (e.g., 4-nonylphenol). Jobling et al. (1998) have shown that exposure to alkylphenolic compounds result in the synthesis of vitellogenin in male fish. APEs enter the aquatic environment via discharges from STPs, textile, and pulp and paper mills (White et al., 1994; McAdam et al., 2011). 4-Nonylphenol, one of the degradation products of the nonylphenol polyethoxylates, has been the subject of numerous studies because of its estrogenic effects and presence in the environment. Much of the polycarbonate eventually winds up in landfills. Bisphenol A
appears to be an estrogen mimic, with a demonstrated affinity for rat ER (Krishnan et al., 1993).

Polycyclic aromatic hydrocarbons, or PAHs, are found in fossil fuels such as oil and coal and are released into the environment through combustion, surface runoff, oil spills, recreational boating and shipping, municipal waste effluents and atmospheric deposition (Kime, 1998). In urban areas it is thought that the majority of PAHs are the result of atmospheric deposition from the combustion of fossil fuels (McElroy et al., 1989). Santodonato (1997) noted that while PAHs may function as weak ER agonists, they are expected to bind preferentially to the Ah receptor, triggering the induction of Ah-responsive genes which can lead to an anti estrogenic effect. Dioxins are EDCs not intentionally manufactured, but are typically formed and released through industrial activities such as chlorine bleaching at pulp and paper mills, chlorination at waste and drinking water treatment plants, and from municipal solid waste and industrial incinerator emissions. Anderson et al., (1996) have shown in vitro that both the dioxin TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) and the Furan (2,3,4,7,8-pentachlorodibenzofuran) are estrogen antagonists. Polychlorinated biphenyls (PCBs) were manufactured and used widely as coolants and lubricants in transformers, capacitors, and other electrical equipment. There are no known natural sources of PCBs. They have the ability to bioaccumulate and result in related health concerns. PCBs generally appear to produce anti-estrogenic and possibly anti-androgenic responses. Phthalate esters are used in the manufacture of polyvinyl chloride as a softening agent and have been shown to be somewhat estrogenic. Compounds identified to date in descending order of potency include butylbenzyl phthalate (BBP), dibutyl phthalate, di-isobutyl phthalate, and diethyl phthalate (Harris et al., 1997; Parveen et al., 2009).

Pesticides are used to control a wide variety of insect and plant pests. They are usually applied as a formulation containing the active ingredient, along with other materials, such as solvents, wetting agents or carriers (Pait et al., 1992). Some active ingredients have the potential to impact the endocrine system, as do some of the surfactants used in the formulations. The carbamate insecticide carbofuran, has been shown to inhibit oocyte development in at least one species of fish (Sukumaran and
Karpagaganapathy, 1992). Tennant et al. (1994), working with rats, concluded that atrazine is also an endocrine disruptor. Crain et al. (1997) showed that atrazine has the ability to stimulate production of the enzyme aromatase which converts androgens to estrogens, and presumably could interfere with sexual differentiation and development. Atrazine is the most commonly detected pesticide contaminant of ground water, surface water, and precipitation and among other effects alters male reproductive tissues when animals are exposed during development. Atrazine demasculinizes male gonads producing testicular lesions associated with reduced germ cell numbers in teleost fish, amphibians, reptiles, and mammals, and induces partial and/or complete feminization in fish, amphibians, and reptiles. (Hayes et al., 2011)

DDT, the pesticide banned in early 1970s, has been shown to induce production of the egg protein vitellogenin in primary fish hepatocytes (Celius et al., 1999; Patisaul and Adewale, 2009). The metabolites of DDT also appear capable of impacting the endocrine system. Donohoe and Curtis, (1996) have shown that o’p’-DDE is estrogenic, however, p,p’-DDE, the dominant persistent metabolite of DDT, is not estrogenic using a variety of assays (Safe, 1995; Donohoe and Curtis, 1996). Kelce et al. (1995) while confirming that it had little ability to bind to the estrogen receptor in rats, found that p,p’-DDE was a potent androgen antagonist in male rats. Kime (1999) has pointed out that trace elements which induce the production of metallothioneins in the liver or gonads might disrupt gamete production by disturbing normal zinc homeostasis, essential for the development of both eggs and sperm. Cadmium, at aqueous concentrations of 50 ppm, has been shown to significantly decrease sperm motility (Kime et al., 1996). Mercury has been shown to have a major impact on sperm motility at a concentration of only 1 ppb (Rurangwa et al., 1998), and to have a direct effect on the egg micropyle, preventing entry of sperm (Khan and Weis, 1993). In rainbow trout, exposure to lead resulted in smaller oocytes (eggs) (Ruby et al., 2000).

A number of phytoestrogens, including genistein, daidzein and enterodiol are known to affect the endocrine system. An infertility syndrome in sheep, known as clover disease, can be found in animals grazing on subterranean clover (Cheek et al., 1998).
Fungi are also known to produce several toxins which can affect the endocrine system (Celius et al., 1999). One of these is the mycotoxin zearalenone produced by Fusarium, a common contaminant in cereals and other plant products. Effluents from pulp and paper mills can affect reproductive function in fish (Tremblay and Van Der Kraak, 1998; Jobling and Tyler, 2003). One of the compounds that could be responsible is β sitosterol, a major by-product of wood pulp delignification, which appears to be activated or produced in the presence of Mycobacterium smegmatis (Bortone and Cody, 1999; MacLatchy et al., 1997). In addition to the compound types discussed above, there is growing acknowledgment that natural and synthetic estrogens, when present, are likely responsible for at least some of the endocrine-related effects seen in fish, particularly near Sewage treatment plants (STPs) (Harries et al., 1999; Larsson et al., 1999; Barber et al., 2007). The synthetic estrogen 17α-ethinylestradiol used in oral contraceptives, has been found in STP effluents along with 17β-estradiol and the E₂ metabolites estrone and estriol. In 2002, the U.S. Geological Survey (USGS) published results from its first national reconnaissance of pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, finding that a wide range of chemicals are present in most streams, and that substantial levels of hormones, detergent metabolites (APEOs), plasticizers such as phthalates, and nonprescription drugs are common (Kolpin et al., 2002). The sources of these chemicals are varied, but a large contribution is made by discharge of wastewater effluent from sewage treatment facilities, particularly for chemicals such as pharmaceuticals intended for humans (Daughton and Ternes, 1999).

Another source of endocrine disruptors to aquatic environments includes the runoff from the solid precipitate of sewage treatment, originally called sewage sludge, now euphemistically known as biosolids. A number of studies have shown that biosolids contain substantial concentrations of heavy metals, phthalates, various hormones, and alkylphenol ethoxylates; when applied to agricultural fields or in forests, runoff removes a fraction of the adsorbed contaminants, thus providing a mechanism for transport to
waterways (EPA, 1990; Baronti et al., 2000; de Jonge, 2002). There is evidence that many of these chemicals do not break-down into harmless compounds. Evidence exists showing that methylation of inorganic mercury is performed by microorganisms (Drexel, et al., 2002, King et al., 2001). Bacteria convert non-hormonal compounds into hormonally-active substances (Panter, et al., 1999; McLachlan, 2001), and estrogens from septic fields and high-estrogen groundwater sources migrate to marine environments (Atkinson et al., 2003).

2.6. Reported evidences of endocrine disruption in animals

2.6.1. Evidences in invertebrates

There are several evidences that environmental pollutants are interfering with the endocrine system of invertebrates. A widespread development of male sexual organs in female marine gastropods, such as dog whelk has been attributed to the exposure to tributyltin (TBT) and other organotin compounds such as triphenyltin (TPT), constituents of marine- antifouling paints (Fent, 1996). The imposition of male sexual organs on females often results in sterilization of females, leading to population declines in many harbours and coastal waters. In addition to organotin compounds other groups of compounds have been related to physiological disturbances in invertebrate species that might be endocrine related. Female marine snails in the NE Pacific have male genitals. N. lamellosa snails in the NE Pacific have been found to have egg retention problems. Dog-whelk snails in the UK have larger penises and some are sterile (WHO-IPCS, 2002). Heavy metals have been shown to have significant impacts on crustaceans (molting, growth and reproduction) and echinoderms (oogenesis and gonadal development) (Oberdorster and Cheek, 2009). Various pesticides disturb endocrine processes in invertebrates at concentrations considerably below those that are overtly metabolically toxic. Triazine herbicides disturbed the fecundity and growth in Daphnia pulex, impaired reproduction and development in the malacostran Gammarus fasciatus, and reduced frequency of molting in D. pulex. The organochlorine 1,1,1-trichloro-2,2-bis[4-chlorophenyl]ethane (DDT), an insecticide, caused a substantial reduction in fecundity in the snail Lymnaea stagnalis (Woin and Bronmark, 1992).
Deformities of the mouth parts and other head capsule features of chironomid larvae have been suggested to arise as a consequence of exposure of the organisms to pesticides and heavy metals. Nonylphenol decreased the fecundity in *Daphnia magna* (Baldwin *et al.*, 1997), induced cyprid major protein, which resembles vitellin, in the juvenile barnacle *Balanus Amphitrite* (Billinghurst *et al.*, 2000), delayed the development of the oyster *Crassotrea virginica* (Nice *et al.*, 2000). The exposure to the xeno-estrogen bisphenol A (BPA) induced asexual reproduction while suppressing sexual reproduction in *Hydra oligactis* (Fukuhori *et al.*, 2005)

### 2.6.2. Effect on Fishes

Endocrine disruption has been reported in freshwater fish populations around the world. This phenomenon ranges from subtle changes in the physiology and sexual behavior of fish to permanently altered sexual differentiation and impairment of fertility. Endocrine disruption in fishes are disturbingly numerous and includes intersex fish (male and female gonadal characteristics in gonochoristic or normally separate sex fish), elevated levels of a female egg protein vitellogenin in male fish (Jobling and Sumpter, 1995), normally found at extremely low levels in males, and degeneration of gonadal tissue (Leino *et al.*, 2003). Not surprisingly intersex males have very low sperm counts and were less fertile than their normal counterparts.

Biologists around the world have accumulated substantial evidence of reproductive endocrine disruption in wild fish, particularly in waters receiving sewage treatment plant or pulp mill effluents (Jobling and Tyler, 2003; Mills and Chichester, 2005). The exposure is related to reduced gonad weight, dysfunction of steroid synthesis and metabolism, decreased plasma steroid levels, masculinisation (including secondary sex characteristics and behavior), altered sex ratio, reduced egg production and hatchability, delayed sexual maturity, reduced capability to spawn, and increased vitellogenin expression (Cheek, 2006). Recently, it has been established that roach (*Rutilus rutilus*) exposed to treated sewage effluent in UK rivers, have a reduced reproductive capacity (Beresford *et al.*, 2004). Male rainbow trout in Britain have vitellogenin, a phospholipoprotein synthesized in female livers in response to estrogen
and retarded testicular growth. In Florida, female mosquito fish are masculinized with male sex organs and try to mate with other females. Pregnant female mosquitofish with gonopodia displayed male-like courtship behavior toward other females and successfully delivered live young (Howell et al., 1980). Interestingly, male mosquitofish living in the same effluent-receiving stream showed apparent precocious sexual development, growing a gonopodium at a smaller body size than males in an unexposed population (Howell et al., 1980). Chemicals present in the effluent from a bleached Kraft pulp mill at Terrace Bay, Ontario, contribute to endocrine dysfunction and delayed reproduction in white sucker fish in the surrounding environment (Munkittrick et al., 1998, Karels et al., 2001; Sepulveda et al., 2001).

2.6.3. Effect on Amphibians

The impact of endocrine-disrupting chemicals in the environment is of special concern in amphibians, which are declining globally (Stuart et al., 2004; Wang et al., 2004). A report showed a strong association between DDT spraying and effects on a local population of western spotted frogs (Rana pretiosa) (Patla and Keinath, 2005). It was observed that a population of spring peepers (Pseudacris crucifer) at Point Pelee National Park, Canada, had appreciable levels of DDT, DDE, DDD, and dieldrin (Russell et al., 1995). Exposure to chemicals that influence endocrine function contributes to the increased prevalence of limb malformations in North American frog populations (WHO-IPCS, 2002). Environmental contamination probably accounted for the historical and geographical trends in the gonadal alterations (intersexuality) observed in frogs and likely contributed to the decline of the species. Nearly 3% of cricket frogs (Acris crepitans) collected in Illinois during 1993-95 had intersex gonads—either testes with large oocytes or even a complete testis and a complete ovary (instead of two complete testes or two complete ovaries) (Reeder et al., 2005). Juvenile cricket frogs captured from PCB (polychlorinated biphenyl) and PCDF (polychlorinated dibenzofuran)-contaminated sites had a heavily male-skewed sex ratio (58-75% male) relative to the expected female-skewed sex ratio (20-40% males) of juveniles caught at reference sites (Reeder et al., 1998). Wild frogs in the US suffer from sexual disruption (Reeder et al., 2005). Ten to
ninety percent of male northern leopard frogs collected in 2001 in the upper Midwest also had eggs in the testis (Hayes et al., 2003).

2.6.4. Effect on Reptiles

A presumed pesticide spill in Lake Apopka (Florida, USA) provides a well-publicized example of potential EDC effects on the decrease in alligator population (Guillette et al., 1994). Alligators at Lake Apopka showed a suite of gonadal deformities that correlated with abnormal plasma steroid levels. They had a variety of sex organ and other developmental abnormalities attributed to exposure to high levels of various organochlorine contaminants that can affect the endocrine balance. Even though several explanations have been proposed, the precise cause of the changes in the alligators remains unknown. Male alligators in Lake Apopka were demasculinized. Their phalluses were smaller (1/3 to 1/2) than normal, they had poorly organized seminiferous tubules, and many were lined with a cuboidal epithelium. In addition, alterations to sperm cells were identifiable by the presence of elongated, bar-shaped nuclei. They had low levels of both testosterone and estrogen, but there was more estrogen than testosterone. Female alligators were super feminized with a much larger estrogen to testosterone ratio than normal and abnormal ovaries (Guillette et al., 1994). Some follicles were found to be polyovular (consisting of three or four oocytes), and selected oocytes were polynuclear (possessing two or three nuclei each). The red-eared turtle population in Lake Apopka had demasculinized males and hatchlings with abnormal hormone patterns. The hatchlings were either females with normal ovaries or intersexed (there were no normal males) and they had an increased risk of mortality. The population also experienced a reduced hatching rate. The developmental abnormalities observed in common snapping turtles (Chelydra serpentina serpentinia) in the Great Lakes–St. Lawrence River basin (Bishop et al., 1998) offer another possible example of the effects of EDCs in reptiles. Adult male snapping turtles captured in the Great Lakes region bear high body burdens of PCBs, DDE, and other organochlorines and have shorter, female-like precloacal lengths—the distance between the posterior tip of the plastron (the ventral shell) and the cloaca—compared to males with low body burdens of organochlorines. Normally, males have
longer precloacal lengths than females. Hatchling males reared from wild-caught, contaminant-containing eggs displayed the same ambiguous precloacal length, indicating that developmental exposure is probably responsible (de Solla et al., 2002). Likewise, several environmental chemicals (PCBs, trans-nonachlor, cis-nonachlor, chlordane, and p,p'-DDE) have been shown to alter turtle sex determination. In addition, PCBs and chlordane also alter steroid hormone profiles of hatchling turtles.

Male sex reversal of wild-caught eggs exposed to chemical contaminants in the laboratory has been demonstrated in alligators (o,p'- and p,p'-DDE, Matter et al., 1998) and Caiman (bisphenol A, Stoker et al., 2003), but did not occur in wild-caught green sea turtle (Chelonia mydas) hatchlings with p,p'-DDE burdens in egg yolk (Podreka et al., 1998). Laboratory experiments with farm-raised red-eared slider turtles (Trachemys scripta) showed that steroidal estrogens, hydroxylated PCBs, chlordane, p,p'-DDE, trans-nonachlor and Aroclor 1242 switched some, but not all males to females (Willingham et al., 2000). Steroidal estrogens, hydroxylated PCBs, trans-nonachlor, and p,p'-DDE can bind estrogen receptors in the alligator uterus (Vonier et al., 1996), suggesting a mechanism for contaminant-induced feminization. Adult yellow-blotched map turtles (Graptemys flavimaculata) from Southern Mississippi, USA have PCBs and DDTs in their liver, fat, and muscle (Kannan et al., 2000) and males captured from a contaminated site had lower circulating testosterone than males from a reference site. A small percentage of the exposed males had female-like levels of estrogen, but no changes in external sex characteristics were present (Shelby and Mendonca, 2001). In the laboratory, hatchling male red-eared slider turtles that had been dosed with chlordane and Aroclor 1242 (a commercial PCB mixture) had lower blood levels of testosterone and females had lower progesterone and testosterone than vehicle-dosed controls. Estrogen was non-detectable in both the normal and chemical-exposed young turtles (Willingham et al., 2000). Juvenile alligators exposed to different intensities of environmental contamination had different rates of testosterone metabolism, suggesting that contaminants may enhance testosterone hydroxylation (disposal), indirectly lowering circulating testosterone levels (Gunderson et al., 2001).
2.6.5. Effect on Birds

Since the 1950s, fish-eating and predatory bird populations have suffered a variety of health problems due to organochlorine pollutants, including poor reproductive success, growth retardation, and goiter. Predatory and fish-eating birds are burdened with a stew of organochlorines, with p,p'-DDE and PCBs being the most common. Eggshell thinning and altered sex organ development have been observed in birds of prey exposed to the pesticide DDT, resulting in severe population declines. Reproductive failure in bald eagles (*Haliaeetus leucocephalus*), brown pelicans (*Pelecanus occidentalis*), gulls (*Larus occidentalis, Larus argentatus*), and other birds of prey during the 1970s through the early 1990s was mainly due to eggshell thinning caused by high body burdens of p,p'-DDE (Lundholm, 1997). One well-accepted explanation is that p,p'-DDE blocks the prostaglandin signaling that stimulates the eggshell gland to deposit calcium in the shell (Lundholm, 1997; Bowerman *et al.*, 2000; Dawson, 2000).

Three types of endocrine disruption have been investigated in wild birds: reproductive disruption, thyroid disruption, and glucocorticoid disruption. In the laboratory, p,p'-DDE and other DDT metabolites stimulated primordial germ cells to migrate into a more female-like position in the testes of male Western gull (*L. occidentalis*) embryos. At hatching, some male embryos even had oviducts (Fry and Toone, 1981). To test whether early signs of feminization might indicate continued abnormal sexual development, Hart *et al.* (2003) studied a common tern (*Sterna hirundo*) breeding colony in Buzzard’s Bay, MA. Many of these birds feed near New Bedford Harbor, a site heavily contaminated with PCBs, and their eggs have very high PCB concentrations, similar to those found in bald eagles incapable of reproducing. In the 1970s, herring gulls (*L. argentatus*) in the great lakes region had goiters. This abnormality was associated with heavy organochlorine contamination in the fish upon which the gulls were feeding and led to the hypothesis that contaminant exposure upset thyroid hormone homeostasis (Mcnabb and Fox, 2003). Herring gulls in the great lakes had compromised glucocorticoid status. Higher burdens of PCBs, PCDDs (polychlorinated dibenzodioxins), and PCDFs in the yolk sacs of 26 day old (two days
pre-hatching) embryos were significantly linked to lower plasma corticosterone concentrations and suppressed gluconeogenic and lipogenic enzymes (Lorenzen et al., 1999).

A group of embryonic abnormalities directly related to contaminant exposure in some fish-eating birds has been defined as the specific syndrome GLEMEDS. GLEMEDS involves a consistent pattern of subcutaneous edema, beak malformations, cardiac edema, and skeletal malformations and particularly abnormalities that are of ectodermal origin. An abnormality that has been characterized well in cormorants (crossed-bill syndrome) has been correlated with concentrations of different polychlorinated halogens in bird eggs. The expression of this syndrome is the result of the deposition of coplanar PCB congeners in the eggs by the maternal bioaccumulation of the compounds that were present in their fish based diet. (Gilbertson et al., 1991)

Exposure to mercury pollution could be hitting some wild birds' reproductive prospects hard by causing males to pair with other males. American white ibises (Eudocimus albus) from south Florida that consumed methyl mercury (MeHg), the most toxic and easily absorbed form of mercury found in the environment, were more likely to engage in same-sex pairings, a phenomenon unknown in wild populations of this species with no exposure to the pollutant (Frederick and Jayasena, 2010). Feminization and masculinization as well as embryonic deformities in gulls have also been linked to PCBs and halogenated aromatic hydrocarbons.

2.6.6. Effect on Mammals

Marine mammals throughout the world’s oceans, including seals, sea lions, porpoises, dolphins, and some whales have high concentrations of organochlorine pollutants such as PCBs and pesticides stored in their blubber (Le Boeuf et al., 2002, Fossi et al., 2003). There is ample evidence that populations of Baltic ringed seals (Phoca hispida botnica) and gray seals (Halichoerus grypus) have declined markedly over the past 100 years. Wild grey seals (Halichoerus grypus) off the Scottish coast have polybrominated diphenyl ethers (PBDEs, a type of flame-retardant) in their blubber and unlike the PCB case, seals with more PBDEs have higher thyroid hormone levels (Hall et
The association between PCB body burden and reproductive and thyroid disruption is consistent across species. In a heavily contaminated population of polar bears in Svalbard, Norway hormonal changes are linked with the total amount of PCBs and DDEs in the blood - female bears with higher contaminant burdens have higher blood levels of progesterone, but similar estrogen compared to females with low contaminant burdens (Haave et al., 2003). More heavily contaminated male bears have lower blood testosterone levels (Oskam et al., 2003). Regardless of age and sex, bears with higher contaminant burdens have lower blood levels of cortisol (the predominant stress hormone) and thyroid hormones (Oskam et al., 2004; Skaare et al., 2001).

Female hamsters exposed to Kepone show masculinization and no feminization and they try to mate with other females. American mink have undergone reproductive failures. Beluga whales in Quebec have abnormal reproductive rates, an increased number of hermaphrodites, and an increased frequency of thyroid lesions (De Guise et al., 1995; Mikaelian et al., 2003). In Alberta, Canada, there are incidences of masculinization (i.e., pseudo-hermaphroditism) of black and brown bears. Interestingly adult male white-footed mice (Peromyscus leucopus) inhabiting a PCB- and cadmium-contaminated area had significantly lower relative testis weights (Batty et al., 1990).

2.7. Worrying trends in humans

The possibility that environmental exposure to chemicals might affect human reproduction is not new. However, the hypothesis that environmental chemicals acting as EDCs could be causative agents of changes in population-based, reproductive health trends is relatively recent. Changes occurring in various human reproductive health statistics, particularly changes in temporal and geographical trends, play a key role in the debate about possible effects of exposure to EDCs.

2.7.1. Effects on reproductive systems

Exposure to endocrine-disrupting chemicals may be linked to increases in reproductive disorders in humans. It is known that exposure in the womb to a man-made synthetic estrogen drug causes a range of reproductive problems in humans and in
laboratory animals. Studies on animals also show that exposure to estrogenic chemicals and other endocrine disrupting chemicals cause very similar effects. From such evidence it has been suggested that exposure to endocrine-disrupting chemicals could be partly or wholly responsible for increases in the incidence of male reproductive disorders which have been recorded in many countries over the past 20-50 years. There is also considerable evidence from laboratory animal studies of the adverse effects of exposure of males to estrogenic and anti androgenic chemicals during critical periods of development. Effects induced by exposure to 17β-estradiol and other estrogenic chemicals during the period of male reproductive tract development include reduced testis and epididymis weight, reduced sperm numbers and motility, increased prostate weight, and delayed puberty. Principal manifestations of developmental exposure to antiandrogens include reduced anogenital distance, hypospadias, retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production. It has been suggested that early exposure to EDCs could cause abnormalities of the genital tract as well as reduce sperm production and induce testis cancer (WHO-IPCS, 2002).

2.7.2. Reproductive tract abnormalities:

In utero exposure to hormonally active chemicals is contributing to the observed increase in the incidence of male reproductive tract abnormalities. Increases in the incidence of cryptorchidism (failure of the testis to descend into the scrotum) and hypospadias (urethral opening along the shaft of the penis) have been reported (Chilvers et al., 1984; Aho et al., 2000). Normal development of the male reproductive tract is dependent on the expression and action of Müllerian inhibiting substance and androgens (testosterone and dihydrotestosterone) during fetal development. Since development of the male reproductive tract is under sex hormone control, changes in the incidence of hypospadias and cryptorchidism could therefore be considered as likely markers of endocrine disturbance.
2.7.3. Declining semen quality and sperm counts

Premature sexual development is linked to exposure to estrogenic contaminants. Global reductions in human semen quality over time are related to increasing exposure to estrogenic, antiandrogenic or other as yet unidentified chemicals, during critical phases of testicular development. A number of studies from different parts of the world have shown significant declines in sperm count and semen volume in men over time. In 1992, a report suggested that sperm count had fallen by 50% in 50 years, following an analysis of results from 61 previous studies which were undertaken between 1938 and 1991 in several different countries (Carlsen et al., 1992). Subsequent studies carried out in Paris, Belgium and Scotland (UK) has also indicated that, sperm counts have declined (Auger et al., 1995; Irvine et al., 1996). These studies reported that sperm count has decreased over the past 20 years at a rate of 2% per year. Furthermore, the studies showed that it was not only sperm count, but other measures of sperm quality which have declined. The percentage of motile sperm in semen has decreased and the numbers of physically abnormal sperm has increased. Preliminary findings of a study in Bangalore, India, also suggest sperm count has decreased in the last 5 years (Mehta and Kumar, 1997).

2.7.4. Endometriosis

Endometriosis is an estrogen dependent disease in which cells that line the uterus (endometrial cells) begin to grow in the pelvis and abdomen, most commonly on the ovaries, the outer surface of the uterus, the intestines or the ligaments that support the uterus. The growth of this tissue is estrogen stimulated and occurs in approximately 14% of women (WHO-IPCS, 2002). A study conducted in Belgium had reported high frequency of endometriosis implicating pollutions and dioxins. Endocrine disruption mediated by exposure to TCDD was reported from Italy (Bois and Eskenazi, 1994). There is evidence that in utero exposure in humans to DES results in an increased relative risk (Missmer et al., 2004). Most striking are the observations of rhesus monkeys administered different doses of TCDD and their subsequent development of endometriosis (Rier et al., 93; 2001). Although this study had low sample size and confi-
2.7. Worrying trends in humans

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Underlying variables that brought into question the relationship between endometriosis and TCDD (Woodruff et al., 2008). Another study revealed that adult exposure of cynomolgus monkey to TCDD promotes growth and survival of endometriosis implants (Yang et al., 2000). Similar data were obtained in rodent models of endometriosis in which human endometrium is transplanted into mouse and rat peritoneum, and the established lesions grew larger when animals were exposed to TCDD in utero and as adults (Nayyar et al., 2007), underscoring the estrogen (and EDC) dependence of this disorder. There are also correlative findings of phthalate levels in plasma and endometriosis. High plasma concentrations of di-(2-ethylhexyl)- phthalate in women with endometriosis, and an association of phthalate esters with endometriosis was found among Indian women (Reddy et al., 2006).

2.7.5. Precocious puberty

Precocious puberty has been reported in children exposed to environmental contaminants. Premature breast bud development was reported in young girls in Puerto Rico with exposure to phthalate esters (Colon et al., 2000). In Michigan premature puberty was recently reported in young girls with exposure to brominated diphenyl ethers. The age of puberty onset has been suggested to be decreasing over at least two decades. However, the extent and persistence of the decline remains controversial.

2.7.6. Effects on sex ratio

The number of human males, relative to females, has been declining for several decades. The cause of this change is unknown, however environmental chemical exposure is suspected to play a role. A study of offspring born from 1978 to 1990 in the Netherlands revealed an increase in daughters when men had workplace exposure to pesticides. Other occupational exposures reported to be associated with a change in the sex ratio have included working in the aluminum industry as "carbon setters", "anode setters" or "carbon changers" and exposure to waste anesthetic gases. Changes in the sex ratio have been reported following the accidental release of dioxin into the environment.
in Seveso, Italy. It has been suggested that the sex ratio is a potential sentinel marker for population health analysis (Davis et al., 1998). Declining sex ratios (fewer males) have been recorded for a number of regions including Canada, the United States, The Netherlands, and Denmark. Additional information shows apparent declines in the sex ratio in Sweden, Germany, Norway, and Finland (WHO-IPCS, 2002).

2.7.7. Fecundity and fertility

An increasing body of evidence suggests that environmental exposures are adversely influencing female fecundity and fertility. Exposure to environmental EDCs has been linked to earlier menarche and precocious puberty. Consumption of either fish or cooking oil contaminated with polychlorinated biphenyls (PCBs) has been associated with alterations in menstruation. DDT recently was associated with a significant 2-fold increase risk in early pregnancy loss among women enrolled in a preconception prospective pregnancy study. With regard to fertility end points, two of three authors have reported a reduction in the secondary sex ratio associated with consumption of PCB-contaminated marine fish or serum PCBs. (Buck Louis et al., 2006).

2.7.8. Effects on Nervous systems

Numerous studies in experimental laboratory animals, principally rats, show that low levels of EDC exposure during gestation and early postnatal life, the critical period for brain sexual differentiation, cause permanent morphological changes in sexually dimorphic, steroid-sensitive hypothalamic–limbic brain regions. As reviewed by Dickerson and Gore (2007), much research has focused on developmental effects of EDCs on subregions of the hypothalamus–preoptic systems that control reproductive physiology and behavior in adulthood. With regard to pesticides that act on the brain, both organophosphate and the insecticidal carbamate pesticides can reduce acetylcholinesterase (enzyme) activity, and hence block nerve impulses. This effect may be linked to the suppression of the brain’s release of hormones that stimulate the gonads
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[(the gonadotrophic hormones, which are follicle stimulating hormone (FSH) and leutinizing hormone (LH)].

The nervous system plays an integrative role along with the endocrine and immune systems in orchestrating important physiologic functions of the body. These integrative functions are critical for normal development, cognitive functions, and behavior. A number of environmental estrogens have been shown to cause neurotoxic effects (Lee et al., 2007). A variety of adverse health effects have been observed ranging from motor impairment and memory loss to subtle behavioral changes. Of particular concern are the potential effects of exposures on the developing nervous system, because both the nature and adversity of the outcome may depend on the time window during which chemical exposure occurs and may result in irreversible neurobehavioral changes later in life. The complexity of the nervous system as well as its integrative nature offers multiple potential target sites that may be disrupted through a variety of mechanisms, including endocrine-disrupting mechanisms. Chemical induced effects may be direct, that is, due to an agent or its metabolites acting directly on sites in the nervous system; or indirect, that is, due to agents or metabolites that produce their effects primarily by interacting with sites outside the nervous system. Effects on the nervous system include slightly reduced IQ, attention deficits, poorer memory and slight adverse effects on psychomotor and neurological function.

2.7.9. Effects on Immune system

Interaction between the immune and endocrine systems is well documented and therefore it has been suggested that the immune system may be susceptible to endocrine disruption. The immune system can be affected by the direct actions of chemicals on specific target components of this system characterized by immune suppression, which can lead to decreased resistance to microbial agents or immune enhancement, leading to allergy. Toxic responses may occur when the immune system acts as a passive target of chemical insults, leading to altered immune function. Toxicity may also arise when the immune system responds to the antigenic specificity of the chemical as part of a specific
immune response, that is, hypersensitivity or allergy. Evidence from studies on DES raises the possibility that exposure to estrogentic or other endocrine-disrupting chemicals during development, could alter development of immunity and lead to permanent changes in immune system function in later life. Animal studies do show that exposure to some estrogentic chemicals during development, including DDT and chlordane, cause suppression of the immune system (Holladay and Luster, 1996).

2.7.10. Effects on lactation

Research indicates that exposure to some endocrine disrupters throughout life could be associated to the duration of lactation in women. Lactation in women can be affected by pharmacological doses of oral contraceptives. The impact of DDE on women’s ability to lactate has been investigated in North Carolina, US, and also in northern Mexico where DDT has been heavily used in agriculture. Both studies found that women with higher levels of DDE in their breast milk lactated for shorter time periods than women with lower levels. The main reason why women lactated for shorter times was because they produced insufficient milk to continue breast feeding. Researchers think that DDE could be inhibiting lactation because of its estrogen-like effects. The study concluded that exposure to DDE, and possibly to other estrogentic pollutant chemicals, may therefore be contributing to lactation failure throughout the world (Gladen and Rogan, 1995).

2.7.11. Spontaneous Abortion

Environmental chemicals in our environment may be causing spontaneous abortions in exposed women. There is evidence that organochlorine and carbamate pesticides cross the placenta and possibly cause fetal death. In a study of couples living and working on Ontario farms, increased miscarriage rates were observed when certain pesticides (atrazine, glyphosate, 2,4-D, 2,4-DB, MCPA, carbaryl, thiocarbanates, and insecticides), were applied in the 3-month window of time before conception (Arbuckle et al., 2001). Pesticides associated with increased risk of miscarriage when exposure
occurred during the first trimester of pregnancy were atrazine, dicamba, and 2,4-D. Several studies have also reported increased risk of miscarriage in occupations associated with agriculture (e.g., gardeners, greenhouse workers, veterinarians). Women exposed to hexachlorobenzene (HCB) as children, who developed severe porphyria cutanea tarda, have been followed for approximately 40 years. During a review of the reproductive outcomes of these women, an unexpected finding was the association of high serum concentrations of HCB with high rates of spontaneous abortion. Other heavy metals such as mercury may also be fetotoxic. There is suggestive evidence that women exposed to substances produced by molds, some of which are highly estrogenic, in grain have a 2-fold increase in risk of spontaneous abortion in addition to estrogen-related cancer. A number of reports have shown that chemical contaminants are detectable in human serum and ovarian follicular fluid of both pregnant and non-pregnant women, respectively. Contaminants such as herbicides, lead, mercury, dioxins, drugs, and tobacco smoke by-products have also been measured in seminal fluid. These data suggest that potential toxic agents are present in the body and in some cases may achieve concentrations in target tissues sufficient to induce an adverse effect.

2.8. EDC link to incidence of cancers

Increases in the incidence of certain cancers in many parts of the industrialized world are often cited as evidence that widespread exposure of the general population to EDCs has had adverse impacts on human health. Of particular concern are the observed increased incidences of cancers at hormonally sensitive sites, such as breast (Vom Saal et al., 1998), and testis (Adami et al., 1994). Uterus and prostate are also another two hormonally active sites for cancer. These increases cannot be adequately explained by improved diagnostic techniques, and it has been argued that these trends coincide roughly with the increasing use and release of industrial chemicals into the environment. Furthermore, these concerns are also based on plausible mechanisms of action because both human and experimental model studies that have demonstrated that these cancers are either dependent on or modulated by the hormonal milieu. Chemicals are thought to act as tumor initiators, as tumor promoters, or as both. In this context, EDCs with estrogenic
activity are generally regarded as tumor promoters. The U.S. National Toxicology Program has recommended that estrogen be added to the list of "potential human carcinogens." The uterus is highly responsive to hormonal alterations. Cancer of the uterus is more common in developed countries, with a similar pattern of hormonal risk factors as breast cancer. Exposures to BPA in adulthood also enhance the rate of growth and proliferation of existing hormone-sensitive mammary tumors, suggesting multiple mechanisms by which BPA may affect breast cancer development (Lozada, 2011). There is clear evidence that unopposed estrogen is the major risk factor for endometrial cancer.

2.9. Human exposure

Exposure to man-made endocrine-disrupting chemicals is presently unavoidable because of their widespread presence in the environment. For the general population, by far the largest exposure comes from foods that are contaminated with these chemicals. As a result of the widespread occurrence of many endocrine-disrupting chemicals in the environment, most foodstuffs are contaminated by some of them. Since many of the chemicals are soluble in fat and/or bioaccumulate, the highest levels are found in meat, fish and dairy products. It has been estimated for the persistent organochlorine chemicals for example, that about 80% of our intake of these chemicals come from these foodstuffs (Hall, 1992). Some individuals may also be exposed to endocrine disrupters as a result of handling chemicals at work.

2.9.1. Food and Drinking Water

Aside from chemicals that have accumulated in foods, other dietary exposure may come from pesticide residues which remain on sprayed crops and contaminate drinking water. A review of pesticides in the US reported that fruit and vegetable crops receive most of the pesticides applied to agricultural crops, and the contamination rate with pesticides is higher for fruits than for any other commodities (Culliney et al. 1992). In The Netherlands, residues of several endocrine-disrupting pesticides were found to be present in foods that were tested, including carbaryl, dicofoil, endosulfan, lindane and vinclozolin (Health Inspector for Public Health, The Netherlands, 1995). Another study revealed that 33.2 % of fruit and vegetables produced in the Netherlands, and 59.4 % of
imported foods, contained detectable levels of pesticide residues (van Klaveren, 1997). Pesticides can leach from sprayed land into watercourses which are used for drinking water supplies. Monitoring has shown that the most commonly detected pesticides in surface waters include several endocrine disrupters, namely organochlorine insecticides lindane, endosulfan and dieldrin, and the fungicide vinclozolin.

2.9.2. Food Packaging and Processing

Some EDCs are used in the production of food packaging materials. Several studies have shown that these chemicals can leach out of packaging into food they are in contact with. BPA, an estrogenic chemical, is present in lacquer coatings which are used to line the inside of some food cans. Tests on tins of peas, artichokes, green beans, mixed vegetables, corn and mushrooms found the liquid surrounding vegetables in food cans had estrogenic properties due to BPA (Brotons et al., 1995). BPA is also present in polycarbonate plastic which is widely used for packaging of food and drinks. BPA leaches out in trace amount from resins and polycarbonates plastics of food packages (Knaak and Sullivan, 1966; Krishnan et al., 1993). Research indicates that some phthalates, including DBP and BBP which are estrogenic, are present in foods from general environmental contamination (MAFF, 1995; 1996). DBP has also been found in printing inks used in plastic food packaging and on paper and board packaging (Nerin, 1993; MAFF, 1995). Studies show that DBP can migrate from the packaging into a wide variety of foods it is in contact with (Nerin, 1993; MAFF, 1995).

2.9.3. PVC articles and toys

A number of endocrine disruptors have been reported in food contact articles and baby toys mainly during the second half of the 1990s. Phthalate esters used in the production of various plastics including PVCs are among the most common industrial chemicals. Their ubiquity in the environment and tendency to bioconcentrate in animal fat are well known. Some children’s toys are made from soft PVC plastic which contains phthalate plasticizers (Meek and Chan, 1994, Vinkelsoe et al., 1997). A recent study
conducted by the Danish Environment Protection Agency, found that a number of phthalates could leach from three brands of teethers, which could potentially expose infants to these chemicals. The phthalates included BBP and DBP which are estrogenic. In addition, another estrogenic chemical, nonylphenol, was found to leach from the teethers (Vinkelsoe et al., 1997). Research by Harris et al. (1997) has demonstrated that the isomeric phthalate DINP (disononyl phthalate), commonly used at high concentrations in consumer products such as PVC toys, can also show weak estrogenic activity in vitro with human breast cancer cell lines. DEP (diethyl phthalate) also showed some activity. Bisphenol A, nonylphenol, phthalates, styrene dimers and trimers, and their transition are used in the manufacture of PVC articles and toys (Kawamura, 2010).

2.10. Fish species are sensitive indicators of endocrine disruption

In the last decade, studies have increasingly identified sex-reversal of many fish species living in waters found to be contaminated with various EDCs (Jobling et al., 1998). The finding that 5% of roach fish (Rutilus rutilus, a common cyprinid) living downstream from a U.K. sewage treatment plant were hermaphroditic was a surprise to researchers, who noted that only two hermaphroditic roach fish had ever been reported in the literature, one in 1965 and one in 1979 (Sumpter and Jobling, 1995). Because it is known that the synthesis of vitellogenin, a liver protein used for creating egg yolks in female fish, is mainly under the control of the sex hormone 17β-estradiol, researchers suspected the presence of an estrogen of some sort in the effluent of the sewage treatment facility (Purdom et al., 1994).

In order to test this hypothesis, fishes were placed in cages directly in the effluent of 28 sewage treatment plants around the country to determine if similar results were obtained; five other control groups were placed in cages and raised in trout farms, where the water is believed to be unpolluted. For the treatment fish raised in the sewage treatment plant effluent, at 13 of the 28 sites all the fish died due to poor effluent quality, but at the other 15 sites, in all cases there was a substantial increase in vitellogenin synthesis in both males and females (Sumpter and Jobling, 1995). Because the fish
vitellogenin increased dramatically, from 500 to 50,000 fold, they concluded that something in the effluent was acting as an estrogen, and thus altering development. Since female roach usually exhibit around a 1 million-fold increase in vitellogenin synthesis during the reproductive cycle, this is the basis for suggesting that fish are sensitive indicators of endocrine disrupting chemicals (Sumpter and Jobling, 1995).

As a result of these early findings, a significant amount of further study in the U.K. has been performed, indicating that wastewater effluent from sewage treatment plants is commonly estrogenic (Purdom et al., 1994) initially suggested that the likely sources were birth control pills (ethynylestradiol) or alkylphenol ethoxylates, deriving from surfactants and detergents that partially break-down during the sewage treatment process. A number of studies coupled observations of increased vitellogenin synthesis with laboratory studies aimed at identifying the source of the estrogen. Routledge et al. (1998) found that trout (Oncorhynchus mykiss) and roach fish exposed in vivo to 17β-estradiol or the alkylphenol ethoxylate, 4-tert-octylphenol, at environmentally relevant concentrations similar to those measured in U.K. streams, were able to induce similar increases in vitellogenin synthesis, evidence that these chemicals are contributing factors to the widespread sexual disruption seen in these fishes (Jobling et al., 1998). More recent studies have confirmed these findings, providing conclusive evidence of widespread sexual disruption of fishes in the U.K. (Williams et al., 2003). Few studies have examined U.S. fish for evidence of endocrine disruption; one study that did examine the question found that 84% of genetically male fish were sex-reversed in the Hanford Reach area of the Columbia River in Washington State (Nagler et al., 2001).

2.10.1. Endocrine system in fish: General idea

The endocrine system in fish consists of various glands located throughout the body which synthesize and secrete hormones to regulate an array of biological processes (Fig. 2.1 and 2.2). For example, the thyroid gland secretes the hormones thyroxine (T4) and triiodothyronine (T3), which are believed to aid fish in adapting to changes in temper
2.10. Fish species are sensitive indicators of endocrine disruption

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-ature and to osmotic stress. The pituitary gland in fish secretes a number of hormones which affect growth, osmoregulation, lipid metabolism and reproductive development and behavior, as well as controlling other endocrine glands (Bone et al., 1995). The corpuscles of Stannius secrete hypocalcin which is thought to be involved in calcium homeostasis and may also be involved in controlling the ratio of calcium to sodium and potassium in the plasma (Bone et al., 1995). As in humans, the fish pancreas secretes insulin which aids in glucose permeability. Secretion of glucagon, also by the pancreas, enables increased glycogen and lipid metabolism. Although most of what is currently known about the effects of EDCs involves reproduction and reproductive behavior, other areas of the endocrine system, such as the thyroid, may also be targets for EDCs.

Pituitary-Hypothalamus-Gonadal axis is one of the most studied pathways that can be affected by EDCs. The release of gonadotropin releasing hormone (GnRH) from the hypothalamus in response to a series of environmental cues in fish results in the production and release of gonadotropin hormones (GTH) from the pituitary gland. The gonadotropins released into the systemic circulation elicit increased androgen and estrogen production by the gonads (Bone et al., 1995). The major estrogen in female fish, 17β-estradiol, is produced primarily in the ovary by the follicular cells. In addition to their importance in eliciting reproductive behavior and the development and maintenance of secondary sex characteristics, the estrogens and androgens are involved in the production of gametes (Bone et al., 1995).

In oviparous or egg-laying fish, as in other egg-laying animals, the release of 17β-estradiol from the ovary leads to the synthesis of large amounts of vitellogenin by the hepatocytes (liver cells). This high density lipoprotein, the precursor of egg yolk, is then transported from the liver via the circulatory system and incorporated into developing oocytes (Anderson et al., 1996). Although estrogens are typically associated with females and androgens with males, that demarcation may not be a rigid one (Sharpe, 1997). Research indicates that both male and female vertebrates produce and use estrogens and androgens. It has become fairly well accepted that a minor role for estrogens exists in males, for example, in the regulation of GTH secretion by the pituitary gland. It is also possible that proper levels of estrogens in males may have more widespread effects, and may even be essential for fertility (Sharpe, 1997). Exposure of an organism, however, to levels of natural hormones or EDCs which overwhelm or interfere with the proper functioning of the endocrine system has the potential to seriously affect the health of an organism and its progeny.
Fig. 2.1. Schematic of endocrine system in teleost fish (Bond, 1979).

Fig. 2.2. Pituitary-hypothalamus-gonadal axis and the action of estradiol
(Adapted from Drean, 1994).
2.10. *Fish species are sensitive indicators of endocrine disruption*  
*Roy, 2011*

The most frequently and best understood types of EDCs are those that mimic estrogens (Gillesby and Zacharewski, 1998; Kime, 1999). A current model for the binding and action of 17β-estradiol is shown (Fig. 2.3). 17β-estradiol produced by the ovaries and transported via the circulatory system is passively taken up by the cell (e.g., hepatocyte) and then crosses the nuclear membrane. The unliganded estrogen receptor (ER) is maintained in an inactive conformation through interactions with a number of proteins, primarily heat shock proteins Hsp 59, 70, and 90 (Gillesby and Zacharewski, 1998). Following the binding of estrogen to the receptor, the heat shock proteins dissociate allowing the ER to change its conformation to the active form. Once activated, the receptor forms a homodimer complex which seeks out specific DNA segments, in this case the estrogen response elements (EREs). Binding of the complex to the ERE results in a rearrangement of the chromatin and transcription of the gene, followed by production of the target protein (Fig. 2.3). A compound able to bind to the estrogen receptor in the cell might very well result in transcription and pleiotropic responses potentially affecting numerous functions within the organism. In fish hepatocytes, the protein vitellogenin is produced via the pathway illustrated in Fig. 2.3.

While vitellogenin is normally associated with female fish, male fish also possess the hepatocyte ER and can synthesize vitellogenin when exposed to 17β-estradiol or to estrogen mimics. The production of this egg protein in oviparous fish, particularly in males, has become an important biomarker in the investigation of EDCs, both in the laboratory and in the field. Normally, vitellogenin in males is either absent or at very low concentrations (Sumpter and Jobling, 1995; Panter *et al.*, 1998). In females vitellogenin is taken up by the ovaries, in male fish vitellogenin produced as a result of exposure to estrogens or to estrogen mimics is only slowly metabolized, making it a valuable biomarker. Currently, however, the ecological significance of elevated levels of vitellogenin in fish, particularly in males, is unclear (Jobling *et al.*, 1998). Although the mode of action of estrogenic compounds on the production of vitellogenin in male fish is fairly well understood, there still remains much uncertainty as to how EDCs impact the overall development of aquatic organisms.
In most cases, timing of exposure during development seems to be critical. It appears there is a labile period when fish are most susceptible to endocrine perturbation occurring just after hatching or at a juvenile stage, the time prior to morphological sex differentiation (Jobling et al., 1998). Sex reversal in fish by treating eggs or larvae with 17β-estradiol or testosterone has found widespread use in aquaculture (Yamazaki, 1983). There is also some evidence that adult gonadal organization can be affected, but these changes appear to be reversible once the EDC is removed.

Fig. 2.3. Mechanism of action of the estrogen receptor (Adapted from Gillesby and Zacharewski, 1998).
Table 2.1. Selected endocrine glands and hormone action in fish (Adapted from Bone et al., 1995).

<table>
<thead>
<tr>
<th>Gland/Hormone</th>
<th>Target Organ</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pituitary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolactin</td>
<td>Various</td>
<td>Osmoregulation, reproduction, growth, lipid metabolism, metabolism</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Various</td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid</td>
<td>Stimulation of growth</td>
</tr>
<tr>
<td>Gonadotropic hormone</td>
<td>Gonads</td>
<td>Stimulation of thyroxin, Stimulation of gonads</td>
</tr>
<tr>
<td>Isotocin, Mesotocin</td>
<td>Blood vessels</td>
<td>Constricts gill blood vessels, systemic vasodilation</td>
</tr>
<tr>
<td><strong>Thyroid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroxin</td>
<td>Many</td>
<td>Adaptation to environmental changes such as temperature or osmotic changes</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Gills and kidney</td>
<td>Regulation of calcium metabolism.</td>
</tr>
<tr>
<td><strong>Corpuscles of Stannius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcin</td>
<td>Gills</td>
<td>Calcium homeostasis</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>All cells</td>
<td>Increases glucose permeability</td>
</tr>
<tr>
<td>Glucagon</td>
<td>All cells</td>
<td>Glycogen and lipid metabolism</td>
</tr>
<tr>
<td><strong>Chromaffin tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenaline</td>
<td>Circulation</td>
<td>Gill vasodilation, system vasoconstriction</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Circulation</td>
<td>Increase heart and glucose metabolism</td>
</tr>
<tr>
<td><strong>Intrarrenal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Gills, kidney</td>
<td>Stress response, osmoregulation</td>
</tr>
<tr>
<td><strong>Gonads</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androgens and estrogens</td>
<td>Many including brain</td>
<td>Reproductive status and behavior; also of other fish (as pheromones)</td>
</tr>
</tbody>
</table>
2.11. BISPHENOL A – an endocrine disruptor of repute

Chemical nature: (Aldrich, 2001)

Synonyms: 4,4'-(1-methylethyldene)bisphenol
4,4'-isopropylidenediphenol
2,2'-bis (4-hydroxyphenyl)propane

Molecular formula: C\textsubscript{15}H\textsubscript{16}O\textsubscript{2}

Purity: 99%

Molecular weight: 228.29

Chemical constituents: Carbon 78.92%
Hydrogen 7.07%
Oxygen 14.02%

Melting Point: 150-155° (solidification range)

Boiling Point: 220°/4 mm

Physical property: Irritant

 Decay: above 8 mm pressure when heated above 220°

Solubility: Insoluble in water. Soluble in aqueous alkaline Solutions, alcohol and acetone. Slightly soluble in CCl\textsubscript{4}
2.11. BISPHENOL A – an endocrine disruptor of repute

Fig. 2.4. Structural similarities of Bisphenol A and its derivatives with 17β-estradiol

BPA- Bisphenol A
Bis-DMA-Bisphenol A dimethacrylate
Bis-GMA-bisphenol A diglycidylether methacrylate
BADGE- Bisphenol A diglycidylethe

2.11.1. General Overview

Bisphenol A (BPA) is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles. The first evidence that BPA and alkylphenols could be estrogenic was published in the 1930s on the basis of feeding experiments with BPA and 4-propylphenol to ovariectomised rats (Dodds and Lawson, 1936).
Recent studies have shown that it can leach out of certain products, including the plastic lining of cans used for food, polycarbonate babies' bottles and tableware, and white dental fillings and sealants. Because BPA is an unstable polymer and is also lipophilic (fat-seeking), it can leach into canned foods (Noonan, 2011), infant formula and other food products (Schecter, 2010), especially when heated (Brotons, 1995). Once in food, BPA can move quickly into people, a particular concern for women of childbearing age and young children.

Two recent studies have explored the effects of increased ingestion of food and drink packaged in EDC-containing sources. Both found rapid (within a few days to a week) increases in BPA levels in urine and blood samples taken from subjects who intentionally increased their intake of common foods and drinks packaged in BPA containing containers (Carwile, 2009). BPA exhibits an estrogen-mimetic action at concentrations as low as 2-5 mg/l. Low levels of BPA have also been found to cause biological effects, and its mode of action appears to mimic that of the female hormone, estrogen. BPA therefore belongs to a group of chemicals termed “hormone disruptors” or “endocrine disruptors” that are able to disrupt the chemical messenger system in the body.

2.11.2. Production

World production capacity of this compound was 1 million tons in the 1980s, and more than 2.2 million tons in 2009. In 2003, U.S. consumption was 856,000 tons, 72% of which was used to make polycarbonate plastic and 21% going into epoxy resins. Bisphenol A was first synthesized by the Russian chemist A.P. Dianin in 1891. This compound is synthesized by the condensation of acetone with two equivalents of phenol. The reaction is catalyzed by a strong acid, such as hydrochloric acid (HCl) or a sulfonated polystyrene resin.
2.11.3. Uses of BPA

About 65% of the BPA produced is used to make polycarbonate, and approximately 25% is used in epoxy resin production. The remaining 10% is used in other products such as speciality resins and in the manufacture of flame retardants, such as tetrabromobisphenol A (European Chemical News 1999). BPA is therefore used in the manufacture of a great variety of products including: compact disks, food can linings, thermal (fax) paper, safety helmets, bullet resistant laminate, plastic windows, car parts, adhesives, protective coatings, powder paints, polycarbonate bottles and containers (including returnable milk and water bottles) and the sheathing of electrical and electronic parts (Staples, 1998). BPA is also used in PVC production and processing, where it may be used as a reaction inhibitor, and as an anti-oxidant.

2.11.4. Environmental Occurrence

BPA has been found in lakes, rivers, and the ocean, as well as in sediments and soils. BPA in water bodies is most frequently the result of its presence in municipal wastewater discharges and in leachate from landfills. While most reported levels in fresh water are low, <1 µg/l, some of the higher levels reported in the environment have caused adverse effects in laboratory experiments. Studies conducted around population centers have found BPA at concentrations nearing the Lowest Observed Effect Levels (LOEL).

2.11.5. Environmental Contamination and Exposure

The primary route of BPA contamination in the aquatic environment is effluent from wastewater treatment plants and landfill sites (Kang et al., 2007a). Wastewaters from kraft pulp, printing paper, and packing-board paper plants contain high concentration of BPA (Rigol et al., 2004). BPA was also found in wastewater from waste paper recycling plants, which use thermal and printing paper as raw material (Rigol et al., 2002). Migration from BPA - based products is closely related to BPA contamination of domestic sewage (Kang et al., 2007b; Yamamoto and Yasuhara, 1999). Effluents
containing BPA after leachate treatment are a source of BPA contamination in the aquatic environment (Yamamoto et al., 2001). BPA levels in 4 landfill leachates ranged from 15 to 5400 µg/l; after treatment, levels in the effluent ranged from 0.5 - 5.1 µg/l (Yamamoto et al., 2001). BPA can migrate from BPA-based products into the aquatic environment and can leach into water from plastic wastes. Yamamoto and Yasuhara. (1999) reported that BPA leached from waste plastics, such as polyvinyl chloride (PVC) products and synthetic leather, reaching aqueous concentrations of 1.98–139 µg/l. BPA migration from PVC hoses used for drainage, watering and sprinkling ranged from 4 to 1730 µg/l (Yamamoto et al., 2001). BPA leached into the water from an epoxy-resin tank, reaching a concentration of 7.8 µg/l (Yeo and Kang, 2006).

BPA has been investigated in marine waters in one study. At 28 locations around the Singapore coastline BPA was detected in most samples with a maximum concentration of 2.47 µg/l found at one site while > 70% of the samples from other locations contained less than 0.4 µg/l (Basheer et al., 2004). The mean maximum concentration is higher than most of those reported from the freshwater locations; however, it is known that BPA persists longer in seawater than in freshwater. BPA in surface river water can be adsorbed to sediments, based on the Koc values (314–1524) for BPA (Howard, 1989). BPA levels in river water in the United States, Germany, Japan, Spain, China, and the Netherlands, were 21 µg/l or less, while levels in sediment were generally higher than in water, ranging from <0.5 to 1630 µg/kg. BPA in anaerobic or semi-aerobic sediment environments can persist for a prolonged period of time. Since BPA persists longer in seawater than freshwater, BPA contamination is potentially higher in marine than in freshwater organisms. (Kang et al., 2007 a).

2.11.6. Human Exposure sources

With regard to possible effects in humans, alarm bells really started to ring in 1997, when one research team, fronted by Fred vom Saal and Wade Welshons, found that even very low levels of BPA could cause harmful effects. Human exposure can arise from a number of sources, particularly from the direct contact of food with BPA.
containing plastics. BPA leaching from the plastic material used to line food and drink cans has received particular attention. Other exposure routes that are a focus of attention include BPA leaching from babies’ feeding bottles, and BPA and related compounds leaching from dental fillings and sealants. A recent Health Canada study found that the majority of canned soft drinks it tested had low, but measurable levels of BPA (Lang et al., 2008).

With regard to water pollution, a UK study has highlighted the potential for bisphenol-related substances to be present in drinking water via the materials used in the supply system. It underlines the need to control and reduce initial exposures from newly installed products, but the study was unable to accurately determine worst case exposures. Polycarbonates (made with BPA) and polysulphones (made with BPA and bisphenol S) are used to produce bottles for the storage of mineral waters, and so bottled water is also a potential source of BPA (Fielding et al., 1999). Wine stored in plastic bottles may also be contaminated to some extent (Larroque et al., 1989). BPA is widely used as a color developer in thermal paper. Thermal paper is ubiquitous in daily life due to its use in cash register receipts, so opportunities for human contact abound (Mendum et al., 2010). With regard to raw water contamination, the potential for BPA to contaminate groundwater, before it is put into the supply system, has not been fully explored. Contamination may potentially result from disposal of contaminated sludge on land, or from leachate from landfill sites, but this needs investigation.

2.11.7. Environmental Fate, Transport, and Metabolism

About 25 percent of an environmental release of BPA would be found in soil, 25 percent in sediment and 50 percent in water with less than 1 percent in biota (Staples et al., 1998). Plants can rapidly absorb BPA through their roots from water and metabolize it to several glycosidic compounds. Glycosylation, the main route of BPA metabolism in plants, leads to loss of estrogenicity of the parent compound. BPA mono- and di-b-D-glucopyranosides show reduced or no estrogenic activity in vitro tests (Morohoshi et al., 2003). Two oxidative enzymes, peroxidase and polyphenol oxidase, are associated with BPA metabolism (Kang et al., 2007 a).
Photolysis and photo-oxidation are the main non-biological pathways of BPA. It breakdown in the aquatic environment. Photodegradation of BPA is slow in pure water, but can be accelerated in the presence of: a) dissolved organic matter, including humic and fulvic acid (Zhan, 2006), b) reactive oxygen species, including hydroxyl radicals, peroxyl radicals and singlet oxygen (Sajiki, 2003; Zhan, 2006), and/or c) ions, including ferric and nitrate ions (Zhan, 2006; Zhou, 2004). BPA has been found in a number of market seafood species. In Singapore, Basheer et al. (2004) found 13.3 – 213.1 μg/kg ww of BPA in prawn, crab, blood cockle, white clam, squid, and fish purchased from local supermarkets, indicating the potential for human exposure by eating contaminated seafood.

2.11.8. Laboratory studies

Workers have shown that exposure to even lower levels of BPA can be harmful. For example, Oehlmann and colleagues have found that BPA, at levels down to 1μg/l, can cause dramatic effects in female freshwater ramshorn snails (Marisa cornuarietis). BPA apparently stimulates egg production and causes swelling of the female sexual glands which results in blocked ducts. This blockage prevents the eggs from being transported, so that the egg containing gland can be put under so much pressure that it bursts, and effectively these snails are sterilized. For example, two to three days after they had hatched, Kloas et al. (1999) exposed the tadpoles of frogs (Xenopus laevis) to BPA for 12 weeks. They found that at a BPA exposure level of 23μg/l there was a significant increase in the number of females, and that a concentration of 2.3μg/l BPA caused a smaller, but not significant, increase in the number of females. 22 such an alteration in sex-ratios may have an important effect on population levels.

Subsequently independent scientists working in the USA and Germany have confirmed that relatively low levels of BPA can indeed cause effects on male animals exposed in the womb. A team of workers have reported effects on female offspring, indicating that animals exposed to low doses may come to puberty earlier (Howdeshel et al., 1999). Furthermore, other studies have reported changes in vaginal cells and the oestrous cycle in female mice exposed in the womb to relatively low levels of BPA (Markey et al., 1999). A Japanese study has suggested a direct effect on sperm.
production in rats given a low dose for just 6 days (Ohsako et al., 1999). Also, behavioral effects from early life exposures have now been identified (Farobollini et al., 1999). There are now several studies that strongly support the suggestion that BPA can cause effects at low doses, including effects on the female reproductive tract, on breast tissue (Colerangle and Roy, 1997) as well as effects on the testes, sperm production, and on the behaviour of both males and females. The research at Washington State University shows that BPA affects the earliest stages of egg production in the ovaries of developing mice fetuses suggesting that their offspring may suffer genetic defects in biological processes such as mitosis and DNA replication. This is an example of a “transgenerational” effect in that the grandchildren of the exposed animals are still at risk for adverse health effects (Lawson et al., 2011).

A new study by researchers at Uppsala University have shown that newborn mice that are exposed to BPA develop changes in their spontaneous behavior and evince poorer adaptation to new environments. They also showed hyperactivity as young adults. Their study also revealed that one of the brain’s most important signal systems, the cholinergic signal system, is affected by Bisphenol A and that the effect persisted into adulthood (Viberg et al., 2011).

Cell culture studies support animal evidence that BPA has dose-dependent effects. One recent study showed estrogen-like effects at extremely low concentrations; when somewhat higher concentrations were used, there were no effects on the cellular kinase pathway being studied. Even at a higher dose, BPA inhibited the effects of estradiol. Despite the wide ranges of doses used in the study, even the very highest was in the nanomolar region; for all conditions, they were low and in the environmentally relevant range of concentrations (Jeng, 2011). Recent data further suggests that BPA leads normal human breast cells to behave like cancer cells, and indicates that BPA may also make cells less responsive to the cancer-inhibiting effects of the anti-estrogen tamoxifen (Goodson, 2011).

### 2.11.9. Human studies

Only three studies associating BPA exposure with reproductive and developmental outcomes in humans were identified. In a study of 77 women, higher
serum BPA was found in women with a history of recurrent miscarriage than in controls (Sugiura-Ogasawara et al., 2005). In another study (Takeuchi et al., 2004) 19 women with polycystic ovary syndrome and 7 obese women were found to have higher serum BPA than 19 controls. Additionally, significant correlations were found between serum androgen measures and serum BPA. Another report from the same group (Takeuchi and Tsutsumi, 2002) found higher serum BPA in males than in either normal women or women with polycystic ovary syndrome and confirmed the correlation with testosterone across groups. A third study found lower concentrations of serum BPA in women with complex endometrial hyperplasia with malignant potential as compared to controls with normal endometrium or with simple endometrial hyperplasia of a benign nature (Hiroi et al., 2004).

2.11.10 Health effects

2.11.10.1. Effect on immune systems

The immune system is under tight, complex regulation to ensure that it continues to function at the optimal range. Existing data suggest that BPA could perturb this regulatory apparatus, leading to weakened defense capabilities or detrimental overstimulation of immune functions as an end result. It appears that BPA can either act directly or indirectly via the neuroendocrine system to affect the immune system. It has been known that both the thyroid and sex hormone neuroendocrine systems are immunoregulators. In an *in vitro* system, Yamashita et al. (2002) using immune cells from BALB/c mice demonstrated that BPA enhanced innate immune response by increasing cytokine production including tumor necrosis factor (TNF) and IL-1 in macrophages, and stimulated both T and B cells in adaptive responses. *In vivo*, BPA also enhanced Th1 or Th2 response, depending on the doses administered (Tian et al., 2003; Yoshino et al., 2003). In addition, prenatal exposure to BPA was shown to augment both Th1 and Th2 responses in adulthood (Yoshino et al., 2004).

2.11.10.2. Effect on Nervous system

BPA has both indirect and direct effects on the nervous system. Since gonadal hormones in conjunction with other neurotrophins regulate cell death, neuronal migration neurogenesis, and neurotransmitter plasticity, BPA, in disrupting sex hormone functions,
can affect brain development (Simerly, 2002). In disrupting thyroid functions, BPA can also affect the development of the nervous system because thyroid hormones play an important role in prenatal and neonatal development of the brain (Porterfield and Hendrich, 1993). Early hypothyroidism, for example, caused stunted dendritic growth in hippocampal CA3 neurons, resulting in cognitive effects including impaired memory, spatial perception, and attention problems (Schantz and Widholm, 2001). In addition, BPA may directly cause neurodegeneration. BPA was shown to produce oxidative stress and induce apoptosis in neuronal cells (Lin et al., 2006b). Experimental data from literature indicate that BPA has a significant impact on the dopaminergic system and hippocampal associated cognitive functions, as well as having a neurodegenerative effect.

2.11.10.3. Disruption of dopaminergic system

The dysfunction of dopaminergic systems has been associated with neuropsychiatric disorders such as Parkinson's disease, schizophrenia, attention deficit/hyperactivity (ADHD), and autism. Some evidence suggests that BPA can affect the dopaminergic systems via the endocrine mechanism. Prenatal and neonatal exposure to BPA was shown to alter D1 receptor expression and density in male mice, which in turn led to the enhancement of D1 receptor-dependent rewarding effect induced by methamphetamine (Suzuki et al., 2003). Laviola et al. (2005) observed that BPA affected the development of the dopamine pathways in a sex-linked manner. Prenatal exposure of mice to BPA appeared to have blunted the development of the dopaminergic reward pathway in the female offspring (but not the male offspring). The treated adult female offspring no longer displayed an amphetamine reinforced behavior.

2.11.10.4. Disrupted thyroid function.

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3) have diverse functions. They are essential to brain development, influence growth via stimulation of growth hormone, and regulate basal metabolic rates, as well as lipid and carbohydrate metabolism. Environmental chemicals can disrupt TH functions by preventing the biosynthesis via the inhibition of iodide uptake or thyroid peroxidase activity, interfering with the activity of transthyretin that transports of THs to target tissues, increasing the metabolism via deiodinases and uridine diphosphate glucuronyltransferase, or perturbing the
binding to thyroid hormone receptors (TRs) (Zoeller, 2007). Using rat liver cells, Moriyama et al. (2002) demonstrated that BPA interacted with both TRα and TRβ in inhibiting T3 stimulated response. The experimental findings led the authors to conclude that BPA could displace T3 from the TR and recruit a transcriptional repressor, resulting in gene suppression. BPA can antagonize T3 action at the transcriptional level. Some in vitro studies demonstrated that BPA is a TR antagonist (Kitamura et al., 2002; Moriyama et al., 2002), at least one study showed that BPA acts as an agonist (Ghisari and Bonefeld-Jorgensen, 2005). An in vivo study conducted by Iwamuro et al. (2003) found that BPA reduced the rate of metamorphosis in Xenopus. This finding seems to be consistent with the in vitro observation that BPA serves as a TR antagonist.

2.11.10.5. BPA and cancer

A 2008 review has concluded that perinatal exposure to low doses of BPA, alters breast development and increases breast cancer risk” (Brisken, 2008). Another review concluded that "animal experiments and epidemiological data strengthen the hypothesis that fetal exposure to xenoestrogens may be an underlying cause of the increased incidence of breast cancer observed over the last 50 years" (Soto et al., 2008). An in vitro study in 2009 has concluded that BPA is able to induce neoplastic transformation in human breast epithelial cells (Fernandez and Russo, 2009). One more study concluded that maternal oral exposure to low concentrations of BPA during lactation increases mammary carcinogenesis in a rodent model (Jenkins et al., 2009). A 2010 study with the mammary glands of the offspring of pregnant rats treated orally with 0, 25 or 250 μg BPA/kg body weight has found that key proteins involved in signaling pathways such as cellular proliferation were regulated at the protein level by BPA (Betancourt et al., 2010). Moreover it was found that BPA may reduce sensitivity to chemotherapy treatment of specific tumors (Lapensee et al., 2010).

2.11.10.6. DNA methylation and epigenetic alterations

Researchers at the Yale University School of Medicine reported that in utero exposure to BPA causes diminished methylation of the estrogen response element of the Hoxa10 gene. This finding suggests that permanent epigenetic alteration of estrogen response element sensitivity to estrogen may be a general mechanism by which endocrine
disrupting chemicals exert their actions. BPA binds to the estrogen receptor by tricking the cells' machinery. The \textit{Hoxa10} gene is a homeobox gene which controls uterine growth and development. A homeobox is a DNA sequence found in genes that are involved in the regulation of patterns of development. In this study, pregnant mice were treated with BPA. \textit{Hoxa10} and protein expression were increased by 25 percent in the reproductive tracts of mice exposed \textit{in utero}. DNA methylation of \textit{Hoxa10} was significantly reduced in both the promoter and intron regions of the gene after BPA exposure. The decrease in methylation led to an increase in the binding of the ER \( \alpha \) to the ERE of the gene (Bromer \textit{et al.}, 2010).

\textbf{2.12. CONCLUSION}

In assessing effects for both humans and wildlife, it is important to assess overall total exposure to "estrogenic" and other hormone disrupting substances. Although the evidence is limited, accumulating data are pointing to the potential role of endocrine disruptors either directly or indirectly. A concentrated and co-ordinated program of research is required to investigate the possible link between demonstrated trends in human reproductive health and human exposure to chemicals in the environment with known estrogenic or related hormonal activities.

Not only are several pesticides estrogenic, but also a number of other substances related to BPA have also been found to be estrogenic in test tube studies. Bisphenol A, bisphenol F, and bisphenol AF based plastics all represent a potential source of these active compounds. BADGE (Bisphenol A diglycidyl ether) is a starting substance of many epoxy resins used as internal can coatings, but it is also used as an additive, functioning as a stabiliser and plasticizer in blends of PVC and epoxy resins (vinyllic organosols), and as a performance enhancer of polyester-based internal can coatings. The present literature provides background information on the production and use of BPA, and summarizes the known exposure routes and research detailing the effects of BPA. Given the concerns about the effects of BPA and related substances, it is of paramount importance to identify and quantify all likely exposure routes.