

Endocrine Disruption and Indiana's Children¹

1. Background

The Environmental Protection Agency (EPA) has defined an endocrine disruptor as “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and /or behavior” (Crisp 1998). The normal endocrine system is the collection of organs (including the pituitary gland, thyroid gland, ovaries, testicles, and others) that release hormones. Hormones are chemical signals that carry instructions for basic functions to other parts of the body; these signals regulate the menstrual cycle, stimulate the development of male and female sex organs in the fetus, and regulate metabolism. An endocrine disruptor can interfere with the system in several ways. It can increase or decrease the effect of a hormonal signal, affect the tissues receiving the hormone signal by either blocking or mimicking the incoming hormone, alter the body's synthesis of the hormone, or alter its transportation throughout the body.

Endocrine disruption² has emerged as an important issue because interferences produced by these chemicals can produce potentially devastating outcomes and because exposures are potentially widespread. Given the breadth of this topic, this paper cannot be all inclusive. Instead, it will give an introduction to this emerging environmental health issue and summarize key evidence for some of the compounds most clearly implicated in endocrine disruption in humans, identify environmental risk factors and options to reduce exposures, briefly discuss the issue in the context of Indiana, identify regulations relevant to this issue, and provide recommendations for the future.

The concept of endocrine disruption has a relatively recent history. The first reports of chemical manipulation of the endocrine system date to the development of the synthetic estrogen diethylstilbestrol (DES) in the 1930s. During the 1950s, data began to accumulate that linked exposure to certain chemicals to endocrine disorders in animals including reports of DDT interfering with reproduction in birds. Rachel Carson brought attention to these effects in her landmark book Silent Spring in 1962. During the 1970s and 1980s there were many more reports of endocrine effects in wildlife, including declines in populations of sea mammals, abnormal mating behavior in birds, and demasculinization of male fish (Smolen & Colborn 1997). In 1991, Theo Colborn, a research scientist examining the effects of environmental contamination in the Great Lakes, convened the Wingspread Conference to bring together scientists from many disciplines for discussions of the relationship between environmental exposures and observed endocrine effects in humans and wildlife (Vogel 2004). The conference produced the Wingspread Consensus Statement which first introduced a detailed formulation of the endocrine disruption hypothesis.

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² The term most consistently used to describe chemicals that cause endocrine disruption is endocrine-disrupting chemical (EDC). Other terms that are used are endocrine modulator (industry) and hormonally active agents (see the National Academy of Science *Hormonally Active Agents in the Environment* (1999), Available at <http://lab.nap.edu/nap-cgi/discover.cgi?term=hormonally+active+agents>).

Since the Wingspread Conference, the scientific literature on endocrine disruption has been growing, and there are considerable data from studies in wildlife, laboratory animals, and in vitro. The evidence for human health effects is more limited, but expanding. The situation in humans is significantly more complicated than in laboratory animals because humans are simultaneously exposed to a multitude of compounds. Even so, there is a growing number of studies showing correlations in humans between exposures to certain chemicals and endocrine effects.

There is a growing suspicion that endocrine disruption in humans could be causing the rising incidence of certain endocrine disorders. Epidemiologic studies have documented an increasing incidence of certain endocrine disorders in humans, and these endocrine-linked conditions are similar in nature to endocrine disruption that has been documented in wildlife and laboratory animals. These conditions include: 1) dropping sperm counts over the past 60 years, 2) altered sex ratio in births in certain populations, 3) early puberty, 4) increasing incidence of birth defects of the male genitalia (hypospadias, undescended testis), and 5) increasing incidence of endocrine related tumors like breast cancer, testicular cancer, and prostate cancer (Raiser 2006).

Of particular concern is the potential for chemicals to cause transgenerational changes – that is, alterations that are passed from generation to generation. For example, there is now evidence that diethylstilbestrol (DES), which was prescribed to block miscarriage prior to its ban in the early 1970s, may have effects in the grandchildren of the women who took it. The DES daughters, who were exposed in utero when their mothers took DES, were found to be at increased risk for abnormal development of the reproductive system and for cancer of the vagina. The DES granddaughters do not have the anatomic abnormalities found in the DES daughters, but they have an increased risk for irregular periods and lower fertility (National Cancer Institute, 2006).

There are recent data implicating other endocrine disrupting compounds in changes that can lead to transgenerational effects (Crews & McLachlan 2006). DNA mutations with altered DNA sequence are inheritable, but there is now evidence that epigenetic changes to DNA, like methylation, that do not change the DNA sequence can also be inherited when they happen during certain stages of development. Epigenetic changes can alter the expression of genes and the timing of expression and may explain transgenerational effects of endocrine disruptors. The fungicide vinclozolin has been found to cause epigenetic changes and transgenerational health effects. Experiments in which pregnant rats were exposed to vinclozolin during the period of embryonic gonadal development show altered sperm formation and reduced sperm production in the male offspring. The same changes in sperm production were seen in the next three generations descended from the exposed rats even though those generations were not exposed to the vinclozolin. There had been a transgenerational effect. Changes in DNA methylation that may explain the transgenerational effects were found in the exposed rats and their descendents (Anway et al. 2005). The rats exposed in utero to vinclozolin also developed adult diseases in the prostate, testes, kidneys, and immune system. Some of those same changes were seen in their unexposed descendents for three generations (Anway, et al. 2006).

Endocrine disrupting chemicals can also affect multiple generations if they persist in the environment or in the exposed individual. Once released, some chemicals resist degradation and persist many years or even decades in the environment. Certain endocrine disrupting chemicals like polychlorinated biphenyls (PCBs) and the pesticide DDT have persisted decades after they were banned. Persistence increases the likelihood of exposure to multiple

generations over time. Many of these persistent chemicals also bioaccumulate, meaning they resist metabolism or excretion and accumulate in the body. Bioaccumulation allows the chemical to persist within an individual animal so it can be passed to the next animal along a food chain or from a mother to its offspring during gestation or nursing.

Newer evidence examining endocrine effects suggests that chemicals can have adverse health effects at concentrations orders of magnitude lower than previous toxicity studies have shown. This is an important finding from the perspective of screening chemicals for potential health effects. Normally hormones exert effects at exceedingly low concentrations, and similarly, endocrine disruptors have been documented to have effects at very low doses. In fact, some recent data challenge the results of previous toxicological studies that tested relatively high doses of chemicals and found “no observed effect levels” (a level of exposure below which the observed effect is no longer detected). These earlier toxicological studies, however, often were not looking for endocrine effects or the effect may not have been present at the higher doses being studied. Some endocrine effects are now known to decrease at higher doses, a phenomenon that is believed to occur because the chemical overwhelms the hormonal signaling mechanism, causing it to shut down. Some recent studies are reporting endocrine effects at concentrations 1000-fold less or lower than previous no observed effect levels (Welshons, et al., 2006, Colburn, et al., 1997).

In children, and especially in the fetus, normal development is dependent on normal hormonal signaling and the timing of those signals. As one example of this sensitivity, endogenous estrogen levels are very low in prepubertal children. However, estrogen receptors are present in sensitive tissues throughout childhood, so those tissues are capable of receiving estrogen signals. Theoretically then, even small amounts of exogenous estrogens could have significant influence, and many endocrine disruptors have estrogenic effects. The exogenous compound would not have to exceed the endogenous estrogen level by much to trigger effects in the estrogen sensitive tissues. The FDA estimated daily physiologic production rates for sex steroids in children including estrogen in 1999 and those rates are used in risk assessments concerning estrogenic compounds. The assumption is that exposures below the normal production rate would not have an adverse effect. Some researchers have found that children's normal estrogen levels are lower than previously believed and are concerned that the FDA's rate estimate might be too high (Aksglaede, et al., 2006).

Some endocrine disrupting chemicals are widely used and some are present in everyday objects such as food, drinking water, plastic food containers, toys, cosmetics and pesticides. Given their extensive use, there is considerable potential for exposure. Government agencies in the U.S. and Europe are prioritizing work on endocrine disruptors with the greatest likelihood of exposure.

The British Institute for Environment and Health reviewed published literature and governmental websites in 2005 and compiled a list of 539 manmade chemicals that had been suggested to be endocrine disruptors (Institute for Environmental Health, 2005). The European Commission sponsored a series of studies to prioritize work on endocrine disruptors and found similar numbers, generating a list of 564 compounds that had been reported to be possible endocrine disruptors. Their list included 18 groups of pesticides, 18 groups of industrial chemicals, metals, and other substances. Working from this list, BKH Consulting Engineers, the contractor for the European Commission, evaluated each chemical looking for those with long environmental persistence or high volume

production. Of the 146 compounds that qualified, 66 compounds (Appendix A) had significant data supporting their categorization as an endocrine disruptor. Appendix B lists the endocrine effects in humans, wildlife or laboratory animals that have been associated with these compounds (European Commission, 2007).

Appendix C provides a more detailed summary of documented endocrine effects of a few of the more prevalent chemicals: bisphenol A, PCBs, organochlorine pesticides, phthalates, phytoestrogens, dioxins, brominated flame retardants, atrazine, and triclosan.

In 2002, the International Program on Chemical Safety (IPCS) concluded that our knowledge about endocrine disrupting chemicals (EDC) and our understanding of the threat these chemicals pose to humans and wildlife is incomplete (World Health Organization, 2002), but “overall, the biological plausibility of possible damage to certain human functions (particularly reproductive and developing systems) from exposure to EDCs seems strong when viewed against the background of known influences of endogenous and exogenous hormones on many of these processes. Furthermore, the evidence of adverse outcomes in wildlife and laboratory animals exposed to EDCs substantiates human concerns.” (World Health Organization, 2002)

2. Environmental Risk Factors

Endocrine-disrupting compounds have diverse routes of exposure. They can be inhaled and absorbed through the lungs, absorbed through the skin, or ingested with food or water or exposure can occur through a combination of these routes. Endocrine disruptors in the air can be produced by industrial emissions or from volatilization. The use of personal care products on the skin can result in absorption of phthalates and similar compounds. Food can carry pesticides or pick up compounds from packaging, such as bisphenol A. Water supplies can be contaminated by discharges from industries, effluent from water treatment plants, or storm water runoff. Water treatment plant effluent includes pharmaceuticals such as estrogens from birth control pills and replacement therapy and thyroid hormone from replacement therapy. Ethynylestradiol or EE2, the most commonly used estrogen in birth control pills is eliminated from the body with urine thus raising concerns about environmental contamination through waste water (Raiser et al., 2006). Children are at higher risk compared to adults - they eat more food and drink more water per pound of body weight, breathe more air per pound of body weight, and have higher skin surface area to weight ratios. The developing physiologic systems of the fetus or child are also much more vulnerable than mature systems in adults.

3. Extent of the Problem in Indiana

There are very few Indiana-specific studies of endocrine disruption, but it is safe to assume that Indiana would have exposures similar to the rest of the country for many endocrine disruptors. General data on exposure to bisphenol A, dioxins, and brominated flame retardants will be summarized first followed by a summary of exposure studies that are specific to Indiana.

3.1. Exposure to Endocrine Disruptors in the U.S. and Other Countries

Bisphenol A. There is evidence of extensive, low level exposure to bisphenol A, a compound commonly found in plastics. It has been reported in serum at concentrations of 1.5 ng/mL in adult men and 0.64 ng/mL in adult women. Levels of bisphenol A in amniotic fluid have been reported to be five times higher than maternal serum. Bisphenol A has also been reported in human milk. In one study of 394 adults, 95% of urine samples had detectable bisphenol A. (Maffini et al., 2006). Polycarbonate baby bottles have been shown to leach bisphenol A (Gibson, 2007).

Dioxin. The levels of dioxin in the U.S. environment have been declining for the last 30 years because of restrictions on manmade sources; it is estimated that quantifiable industrial emissions of dioxins are more than 90% less than 1987 levels. Because dioxins accumulate in fatty tissues, exposure is usually through food containing animal fat. A diet including low fat meats and low fat dairy products helps reduce exposure. (U.S. Food and Drug Administration, 2006)

Flame Retardants. The demand for brominated flame retardants, primarily polybrominated diphenyl ethers or PBDEs, in the U.S. has increased dramatically over the past two decades partly because of fire safety laws. U.S. demand in 2001 accounted for 80% of use globally at 36,500 tons, nearly double what it was in 1990 (Fischer, 2006).

Levels of brominated flame retardants in blood, food, and breast milk have also been rising. In one study the total PBDE level in human serum pooled from 100 individuals in Texas in 2003 was 61.7 parts per billion of lipid (nanograms per gram of lipid); whereas, in archived pooled serum from Texas in 1973 PBDEs were undetectable. Blood levels from 39 individuals in Mississippi and New York ranged from 4.6 to 365.5 parts per billion lipid with a mean of 52.6. The authors stated that these levels were the highest reported worldwide as of that date (Schechter et al., 2005).

Schechter and colleagues (2003) reported that levels of total PBDE in the breast milk of 47 lactating women in Texas ranged from 6.2 to 419 parts per billion of lipid with a mean of 73.9. These U.S. levels were 10 to 100 times higher than in European breast milk (Schechter et al., 2003).

The chief routes of exposure to PBDEs are not certain, but PBDE levels have been identified in food, in indoor air, and in household dust (Rudel et al., 2003; Betts 2004). A "market basket" survey of foods primarily of animal origin showed levels that were higher in the U.S. than those reported in foods in Spain or Japan (Schechter, et al., 2004).

3.2 Exposure to Endocrine Disruptors in Indiana

There has been one study of levels of flame retardant in Indiana. Blood levels of brominated flame retardants (PBDEs) were measured in a study of twelve mothers and their newborn babies (Mazdai et al., 2003). The levels were the same in each mother and her newborn suggesting that the flame retardants readily crossed the placenta to the fetus. The blood levels ranged from 14 to 580 ng/g (parts per billion) of lipid, which is 20 to 106-fold higher than the levels reported in Swedish mothers and their infants in a separate study from 2003 and 20-fold higher than in Norwegian blood samples from the late 1990s. No association was identified between the PBDE levels and birth weight or thyroid hormone level.

Polychlorinated biphenyls or PCBs contaminate many of Indiana's surface waters and accumulate in fish. The Fish Consumption Advisory (Indiana State Department of Health, 2006) warns that the PCB levels in fish from multiple locations around the state are high enough to limit the consumption of the contaminated species. At a dozen locations around Indiana, the Advisory states that the fish should never be consumed because of contamination levels. PCB levels in fish from the Little Miami River were reported by the U.S. Geologic Survey to be among the highest detected in the U.S. (Rowe et al., 2004).

Exposure to PCBs in Monroe County, Indiana, was investigated in the 1980s, and elevated levels were found in people who had been occupationally exposed. A few people with non-occupational exposures to waste sites containing PCBs also had elevated levels, but as a group their levels were statistically not significantly different from people who were unexposed (Stehr-Green et al., 1986).

Given its extensive agriculture, Indiana has the potential for contamination of water by agricultural chemicals. According to the National Pesticide Use Database (National Center for Food & Agricultural Policy, 2004), Indiana used a total of over 28 million pounds of pesticides in 1997, the most recent year for which the database had information. Corn and soybeans, both planted extensively in Indiana, are the two most heavily treated U.S. crops accounting for 41% of total pesticide use nationwide. Alachlor, atrazine, cyanazine, metolachlor, and simazine are five relatively water soluble herbicides that are used extensively in Indiana, and these are the most commonly detected herbicides in Indiana surface waters (Frankenberger & Homes, 1998).

Atrazine is the herbicide most commonly used on cropland and it is the most heavily used pesticide in Indiana with an average of over 6.5 million pounds applied per year. More than 80% of Indiana's corn acreage is treated with atrazine (National Agricultural Statistics Service). Once dissolved in water, atrazine is slow to break down. Atrazine has been found in drinking water in Indiana, and it peaks in the spring with levels that have occasionally exceeded the U.S. drinking water standard of three parts per billion (3 µg/L or micrograms per liter). Forty-seven percent of finished samples from Indiana public water systems had detectable levels of atrazine (Purdue Extension, 2004).

Levels of agricultural chemicals have been assessed in Indiana in the White River watershed and the Great and Little Miami River Basins by the US Geologic Survey. The White River watershed was studied from 1991 to 1995; atrazine was present in all samples and levels were as high as 11 µg/L. Metolachlor was the second most frequently detected pesticide. It was present in the majority of samples and the maximum level was 4.9 µg/L (U.S. Geologic Survey, 1995; Rowe et al., 2004)

Testing of the Great and Little Miami River Basins by the U.S. Geologic Survey from 1999 to 2001 found one or more pesticides in every water sample with most samples containing mixtures of 10 to 20 pesticides. Samples collected during runoff events contained as many as 31 pesticides. The median for the sum of herbicide concentrations in the Great Miami River at Hamilton was 0.33 µg/L, similar to levels in mixed use streams in other parts of the Corn Belt. The median in streams draining agricultural land along the Miami rivers was 0.98 µg/L. (Rowe et al. 2004)

4. Applicable Statutes and Regulations

Since endocrine disruptors are a diverse collection of compounds, they are not regulated as a group, but there has been regulation of some specific compounds and federal regulation requiring study of endocrine disruption.

The Food Quality Protection Act passed by Congress in 1996 requires the EPA to establish a program to screen pesticides for potential endocrine effects, specifically interference with the function of male, female and thyroid hormones. The Act also requires testing of any substance that might have a cumulative effect to that of a pesticide, if there is sufficient evidence of exposure.

In response to the Food Quality Protection Act, the EPA convened the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC) composed of scientists, chemical manufacturers, health and environmental advocates. The Committee published its recommendations in October of 1998, and the EPA has relied heavily on those recommendations in establishing the Endocrine Disruptor Screening Program. The program has since been working to prioritize the chemicals of concern and to develop screening methods (Schierow & Buck, 2001; U.S.EPA, 2006a).

The 1996 Safe Drinking Water Act Amendments authorize the screening of compounds found in drinking water for endocrine effects. The screening is to be conducted by the manufacturers of the compounds. Both the Food Quality Protection Act and Safe Drinking Water Act authorize the EPA to act to protect the public health from substances found to have damaging effects. The National Science and Technology Council's Committee on the Environment and Natural Resources formed an Endocrine Disruptor Working Group to coordinate federal response to concerns about endocrine disruptors. (Schierow & Buck, 2001)

Some individual endocrine disruptors are regulated or coming under regulation. The production of PCBs was banned in the United States after 1979 (U.S. EPA 2007). After findings of rising PBDE levels in breast milk, a voluntary ban on the flame retardant penta-PBDE began in Europe in the mid-1990s followed by the European Union's formal ban of all uses of penta-PBDE in 2004 (Birnbaum & Staskal 2004). California was the first state to ban the penta- and octa- forms of PBDEs (California Department of Toxic Substances Control, 2006) followed shortly by the U.S. as a whole. Only deca-PBDE is currently being manufactured and sold in the U.S.; the other PBDE compounds were banned in 2004 (Schechter et al., 2005; U.S. EPA, March 2006). The city of San Francisco banned bisphenol A in toys as of December of 2006, and there have been attempts to make the ban statewide (Kay, 2007).

Atrazine has come under close scrutiny and limits have been imposed in the U.S. and abroad on its purchase and use. In October of 2003 the European Union announced a ban on the use of atrazine (Weiss, 2004). In the U.S. atrazine is a restricted use pesticide, which means its purchase and use are restricted to trained applicators. The EPA has also restricted where and how it can be used (U.S. EPA, November 2006).

Under the Safe Drinking Water Act, the EPA has set the Maximum Contaminant Level (an enforceable standard) for atrazine in drinking water at 3 µg/L (3 parts per billion). Communities in which atrazine and its metabolites exceed an annual average of 2.6 parts per billion must comply with the Atrazine Monitoring Program. Eleven public water systems in Indiana are currently in the Atrazine Monitoring Program; these include Indianapolis (Eagle Creek), Santee Utilities, Batesville, Bedford, Fort Wayne, Jasper, Logansport, Stucker Fork, Versailles,

Westport, and Winslow. Starting in 2004, water systems in the program that exceed a 90-day rolling average must develop and implement a watershed mitigation plan. If the 90-day average is exceeded a second time within five years, atrazine use in the watershed will be canceled (Purdue Extension, 2004; U.S. EPA, November 2006).

Other federal laws that could potentially pertain to endocrine disruptors include: 1) The Federal Food, Drug, and Cosmetics Act (FFDCA) section 408(p) which provides the EPA authority to require testing of all pesticide chemicals or any substance that might act cumulatively with a pesticide (21 U.S.C. 346(a)(p)) 2) the Toxic Substances Control Act (TSCA) which provides authority for the EPA to require testing of certain chemicals, provided certain hazard and/or exposure-based findings are made (15 U.S.C. § 2603) 3) the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides the EPA with authority to require testing of pesticides if EPA determines that additional data are required to maintain an existing registration [7 U.S.C. § 136a(c)(2)(B)]. (U.S. EPA, 2006c)

Other state actions that pertain to endocrine disruptors include California's Proposition 65, which was enacted by ballot initiative. It requires the governor to publish annually a list of chemicals known to cause reproductive harm, birth defects or cancer. (California Office of Environmental Health Hazard Assessment, 2003)

5. Recommendations

For many endocrine disruptors, data collection and regulation initiatives at the national level will pertain to Indiana. For some chemicals of particular concern because of patterns of use or exposure, Indiana could explore independent means to reduce risk. Generally the means available for approaching risk reduction for endocrine disruptors include education, research, and regulatory options such as:

- Educate the public about ways to reduce exposure - for example, promoting awareness of fish advisories
- Encourage voluntary reductions in use of endocrine-disrupting chemicals
- Encourage voluntary withdrawals of products known to contain endocrine-disrupting chemicals from the market
- Conduct research studies to identify exposures and prioritize chemicals of concern
- Promote alternatives to the chemical or chemicals in question
- Provide subsidies or incentives for use of alternatives - for example, tax incentives for organic farming
- Inform the public through right-to-know requirements in product labeling
- Require strict liability of those selling or using chemicals with damaging effects
- Restrict use of selected chemicals
- Ban use of chemicals/products
- Support efforts to require thorough health testing prior to allowing a chemical on the market (a national rather than state-level option)

There are significant gaps in our knowledge about the compounds that could have endocrine effects in humans and of the extent of exposures, but credible bodies such as the World Health Organization (WHO 2002) have concluded that there is sufficient information to warrant concern and to make this a research priority. For

many compounds, there is evidence of the potential for harm to children from the in vitro or animal studies and for some compounds there is evidence of widespread human exposure. In both cases, steps should be taken to limit children's exposure before damage occurs, to act in a precautionary manner and prevent harm. The Prague Declaration, issued in May 2005 by over 100 scientists convened to discuss research on endocrine disruption, called for a precautionary approach in regulation: "In view of the magnitude of the potential risks associated with endocrine disruptors, we strongly believe that scientific uncertainty should not delay precautionary action on reducing the exposures to and the risks from endocrine disruptors." (Prague Workshop, 2005)

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Appendix A

The European Commission published a report in 2000 that ranked chemicals as potential endocrine disruptors based on a review of the scientific literature. From an initial list of 564 compounds with published concerns about endocrine disruption, 146 were either highly persistent once released to the environment or produced in high volume. From the 146 persistent /high volume compounds, 66 compounds were selected because each had at least one study showing endocrine effects in humans or animals (Annex 15).

Annex 15. List of 66 substances with classification high, medium or low exposure concern

NR	CASNR	Name	HPV/pers.	ECO	HUM	Total	Concern
11	12789-03-6	Chlordane	Highly Pers	2	1	1	High
12	57-74-9	Chlordane (cis- and trans-)	Highly Pers	2	1	1	High
20	143-50-0	Kepone = Chlordecone	Highly Pers	2	1	1	High
21	2385-85-5	Mirex	Highly Pers	2	1	1	High
24	8001-35-2	Toxaphene = Camphechlor	Highly Pers	2	1	1	High
42	50-29-3	DDT (technical) = clofenotane	HPV	1	1	1	High
56	50-29-3	p,p'-DDT = clofenotane	HPV	1	1	1	High
57	3563-45-9	Tetrachloro DDT = 1,1,1,2-Tetrachloro-2,2-bis(4-chlorophenyl)ethane	Highly Pers	1	2	1	High
63	50471-44-8	Vinclozolin	HPV	3	1	1	High
69	12427-38-2	Maneb	HPV	3	1	1	High
70	137-42-8	Metam Sodium	HPV	3	1	1	High
73	137-26-8	Thiram	HPV	3	1	1	High
74	12122-67-7	Zineb	HPV	3	1	1	High
78	58-89-9	Gamma-HCH = Lindane	HPV	2	1	1	High
87	330-55-2	Limuron (Lorox)	HPV	3	1	1	High
142	1912-24-9	Atrazine	HPV	2	1	1	High
163	34256-82-1	Acetochlor	HPV	3	1	1	High
164	15972-60-8	Alachlor	HPV	2	1	1	High
191	100-42-5	Styrene	HPV	3	1	1	High
198	118-74-1	Hexachlorobenzene = HCB	HPV	3	1	1	High
270	85-68-7	Butylbenzylphthalate (BBP)	HPV	3	1	1	High
279	117-81-7	Di-(2-ethylhexyl)phthalate (DEHP) = Dioctylphthalate (DOP)	HPV	3	1	1	High
286	84-74-2	Di-n-butylphthalate (DBP)	HPV	3	1	1	High
326	80-05-7	2,2-Bis(4-hydroxyphenyl)propan = 4,4'-isopropylidenediphenol = Bisphenol A	HPV	1	1	1	High
396	1336-36-3	PCB	Pers.		1	1	High
408	35065-27-1	PCB153	Pers.		1	1	High
410	32774-16-6	PCB169	Pers.		1	1	High
417	2437-79-8	PCB47	Pers.		1	1	High
422	32598-13-3	PCB77	Pers.		1	1	High
427	53469-21-9	Aroclor 1242	Highly Pers		1	1	High
428	12672-29-6	Aroclor 1248	Pers.		1	1	High
429	11097-69-1	Aroclor 1254	Highly Pers		1	1	High
430	11096-82-5	Aroclor 1260	Pers.		1	1	High
438	59536-65-1	PBBs = Brominated Biphenyls (mixed group of 209 Congeners)	Pers.		1	1	High
467	40321-76-4	1,2,3,7,8-Pentachlorodibenzodioxin	Pers.		1	1	High
472	No CAS 140	2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	Pers.		1	1	High
487	57117-31-4	2,3,4,7,8-Pentachlorodibenzofuran	Pers.		1	1	High
525	688-73-3	Tributyltin	Metal	1	2	1	High
526	No CAS 050	Tributyltin compounds	Metal	1	2	1	High
527	56-35-9	Tributyltin oxide = bis(tributyltin) oxide	HPV/ Metal	1	2	1	High
504	26354-18-7	2-propenoic acid, 2-methyl-, methyl ester = Stannane, tributylmeacrylate	Metal	1	2	1	High
512	No CAS100	Methoxyethylacrylate tnbutyltin, copolymer	Metal	1	2	1	High
514	4342-30-7	Phenol, 2-[[[tributylstannyloxy]carbonyl	Metal	1	2	1	High
515	4342-36-3	Stannane, (benzoyloxy)tributyl-	Metal	1	2	1	High
516	4782-29-0	Stannane, [1,2-phenylenebis(carbonyloxy)	Metal	1	2	1	High
517	36631-23-9	Stannane, tributyl = Tributyltin naphthalate	Metal	1	2	1	High

Key to Annex 15:

HPV/pers - high production volume/ environmentally persistent

ECO, HUM, and total - categories for wildlife (ECO), humans (HUM), and overall (total) based on the strength of scientific evidence for endocrine disruption; 'Total' was set at category 1 for any compound that was category 1 for either human or wildlife

category 1 - At least one study with evidence of endocrine disruption in an intact organism

category 2 - Potential for endocrine disruption based on in vitro data, effects in vivo that may or may not be endocrine, chemical relationship to a known category 1 or 2 compound

category 3 - Evidence of not being an endocrine disruptor or insufficient data available

Concern - high, medium, or low concern for human exposure based on chemical properties, bioaccumulating potential, degradation in the environment, use, production volume, emissions, and known environmental levels

Source: BKH Consulting Engineers (2000)

Appendix B

Table 3.4 from the BKH (2000) report provides documented endocrine effects for the compounds in Appendix B.

Table 3.4 Endocrine Disrupting effects observed in category 1 substances (↑: Increase; ↓: Decrease)

Name	Effects
Chlordanes (2)*	Testicular toxicity
Kepone (Chlordecone)	Sperm development ↓
Mirex	Testis descent ↓
Toxaphene	Thyroid tumours ↑
DDTs (3)*	Oestrus cycle ↑; Ovulation ↓ Eggshell thickness ↓; Uterus weight ↑
Vinclozolin	Testis weight ↓; Testosterone levels ↓; Sexual potency ↓; Sex organs malformation ↑
Maneb	Thyroid hormone synthesis ↓
Metam Sodium	Neuroendocrine Pituitary effects
Thiram	Thyroid hormone synthesis ↓
Zineb	Thyroid hormone synthesis ↓
Gamma-HCH Lindane	Testis weight ↓; Vaginal opening ↓; Uterus weight ↓
Limuron	Sex organs weight ↓
Amitrol	Thyroid hormone synthesis ↓
Atrazine	Pseudopregnancies ↑; Estrous cycle irregular; Androgen receptors ↓
Acetochlor	Thyroid hormone levels ↓
Alachlor	Thyroid hormone levels ↓
Nitrofen	Thyroid effects
Hexachlorobenzene	Testicular effects; Ovarian effects; Testosterone levels
Tributyltin compounds (18)*	Imposex
Triphenyltin (2)*	Imposex
Tri-n-propyltin (TPrT)	Imposex
Tetrabutyltin (TTBT)	Imposex**
4-tert-Octylphenol	Vaginal opening ↑; Uterus weight ↑
Phenol, nonyl-	Uterus weight ↑; Testis weight ↓; Vitellogenin level ↑
Butylbenzylphthalate (BBP)	Testes weight ↓; Sperm production ↓; Testosterone levels ↓
Di-(2-ethylhexyl) phthalate (DEHP)	Testes weight ↓; Sex organs weight ↓; Sperm production ↓; Testosterone levels ↓; Ovarian weight ↓
Di-n-butylphthalate (DBP)	Testicular atrophy; Prostate atrophy
Bisphenol A	Skewed sex ratio; Prostate size ↑; Prolactin secretion ↑; Persistent vaginal cornification; Vaginal opening ↑
PCBs (9)*	Thyroid effects; Uterus weight ↑; Endometriosis; Progesterone receptors ↑; Uterus weight ↓; Uterus weight ↑; T4 plasma levels ↓; Estrous cycle length ↑
PBBs = Brominated Biphenyls	Thyroid hormone levels ↓; Sex hormone levels ↓
Dioxins/Furans (3)*	Hepatic AHH induction; Uterus weight ↓; Sperm number ↓; Thyroid effects; Neoplasms ***
3,4-Dichloroaniline	Androgen synthesis
4-Nitrotoluene	Uterus weight
Styrene	Prolactin secretion ↑; Pituitary effects
Resorcinol	T4/T3 metabolism ↓; Thyroid effects

* In between brackets the number of individual substances of the group, is given.

** Tetrabutyltin is debutylated to TBT in both vertebrates and invertebrates. Therefore same effects as TBT

*** Due to structural analogy all 2,3,7,8-substituted congeners have been categorised in category 1

Source: BKH Consulting Engineers (2000)

Appendix C

This appendix is intended to provide a sampling of some of the data that have been collected on some of the most prevalent endocrine disrupting compounds. It is not an exhaustive literature review on each compound, nor does it include all potential endocrine disruptors.

Bisphenol A

What is Bisphenol A? Bisphenol A is used in the manufacturing of polycarbonate plastics and epoxy resins including many food and beverage containers and dental materials.

Animal and in vitro data. In vitro, bisphenol A binds estrogen receptors. In rats it has estrogenic effects including advancing puberty, disrupting estrous cycling, increasing uterine weight and having an association with polycystic ovaries or altered mammary gland architecture depending on the timing of exposure, prenatal versus postnatal (Raiser et al., 2006). Female mice and rats have shown increased body weights if they were born to dams exposed to low doses of bisphenol A. Prenatal exposure to bisphenol A induced changes in gross and microscopic anatomy of mouse ovaries, and increasing levels of exposure correlated with increasing incidence of chromosomal disturbances in the oocytes. Findings in the mouse female have also included decreased weight of the vagina, alterations in the endometrium, and increased uterine weight (Maffini et al., 2006). The effect of bisphenol A on the uterus was found to be one million-fold higher in prenatally exposed mice as compared to those exposed in the prepubertal period postnatally. Prenatal exposure was also associated with significantly earlier first estrus and altered patterns of estrous cycling. Parts of the rat brain that normally differ between the genders have shown alteration following prenatal exposure. Perinatal exposure of female mice has been shown to alter mammary gland morphology such that the glands in virgin mice resembled those of pregnant mice. Male mice exposed in utero showed increased anogenital distance, increased prostatic size, decreased epididymal weight, and decreased sperm production in adulthood (Maffini et al., 2006). Most of the effects of bisphenol A seen in rodents occur at doses below the level designated safe for humans by the U.S. Food and Drug Administration (FDA) of 50 micrograms per kilogram body weight per day. The FDA safe levels were based on studies in the 1980s which examined effects at doses more than 1000-fold higher. (Welshons, et al., 2006)

Data in humans There are relatively few studies of bisphenol A effects in humans, but estrogenic effects similar to those in rodents have been seen. One study found that women who suffered miscarriages had higher blood levels of bisphenol A than women who carried their pregnancies to term, and some of the miscarried pregnancies showed genetic abnormalities (Maffini et al. 2006). There are other studies that have found a relationship between bisphenol A levels and obesity, polycystic ovary syndrome, and endometrial hyperplasia (an overgrowth of the lining of the uterus, considered pre-neoplastic) (Welshons, et al., 2006)

PCBs

What are PCBs? Polychlorinated biphenyls or PCBs have been widely used in many applications including dyes, carbonless copy paper, as an insulator in electrical equipment, and as a plasticizer in paints, plastics and rubber products. More than 1.5 billion pounds were made in the U.S. before 1977 when manufacturing PCBs was banned (U.S. EPA, 2007). They are very slow to degrade, persisting decades in the environment, and they tend to accumulate in the fatty tissues of the animals exposed to them. Human exposure is most often from food, and PCBs are present in higher concentration in foods containing animal fats like dairy products and fish (Centers for Disease Control and Prevention, 2005).

Animal and in vitro data In vitro, certain PCBs have been shown to increase the production of GnRH (gonadotropin releasing hormone), a hormone involved in regulating reproduction (Raiser et al., 2006). Animal studies have found estrogenic effects and disruption of reproduction. In rats prenatal exposure led to increased uterine weight (Raiser et al., 2006).

Data in humans. Studies have found that PCBs produce effects on reproduction similar to some of the effects in animals, including estrogenic effects in males and disruption of reproduction. Women working with PCBs were more likely to deliver babies prematurely and with a decreased birth weight (U.S. EPA, 2006b). Men exposed to

PCBs prior to age 20 have been shown to have a lower chance of fathering male offspring (del Rio Gomez et al. 2002). PCBs are classified as probable human carcinogens and have been associated with an increased risk of breast cancer in some studies (Centers for Disease Control and Prevention, 2005).

Results regarding the potential impact of PCBs on puberty are mixed. A study in Belgium noted a significant delay in puberty in boys with high serum levels of PCBs; whereas, a North Carolina study found no association with pubertal timing for boys or girls and two other studies in Michigan found no association for girls (Raiser et al. 2006).

Since estrogen is known to reduce lactation, it was hypothesized that the estrogenic effects of PCBs and DDE (a breakdown product of DDT) could interfere with milk production. Studies in North Carolina and Mexico found that women with the highest levels of DDE and PCBs in their breastmilk breastfed less than 40% as long as women with lowest levels. In one study girls with higher prenatal exposure to PCBs were heavier for their height. Several studies have found altered gender ratios in births in areas suffering high PCB and dioxin contamination with lower birth rates for males (Rogan & Ragan 2003). PCB exposure has also been associated with decreases in thyroid hormone in animals and humans (U.S. EPA 2006b).

Organochlorine pesticides

What are organochlorine pesticides? They are chlorine-containing pesticides, mostly insecticides. They tend to be very persistent in the environment with some lasting decades after they are released. They also tend to bioaccumulate so that an animal or person can have levels thousands of times higher than their surroundings.

Many organochlorine pesticides were banned in the U.S. in the 1970s and 1980s including DDT, aldrin, dieldrin, toxaphene, and heptachlor. Some remain in use including lindane, endosulfan, dicofol, methoxychlor and pentachlorophenol (National Agricultural Statistics Service).

Animal and in vitro data. There are innumerable studies of organochlorine pesticides documenting toxicity in the nervous system, immune system and carcinogenicity in certain animal species, but we will give a sampling of some of the effects seen in the reproductive and endocrine systems. In vitro DDT has been shown to interact with the receptor for the thyroid stimulating hormone (Rossi, M., et al., 2007). Studies of lindane exposure in rodents demonstrated endocrine effects including delayed puberty, reduced pituitary and uterine size and disrupted estrous cycling (Raiser et al., 2006). DDT exposure in rodents has been associated with offspring survival, reduced fertility, reduced fetal weight, increased nipples occurring on males, and alterations in the reproductive organs including reduced weight of the prostate and testes and reduced anogenital distance (Agency for Toxic Substances and Disease Registry, 2002).

Data in humans. The risk of breast cancer was shown to increase with increasing concentrations of serum DDT in women who were exposed during childhood (Cohn 2003). Prenatal exposure to DDE (a breakdown product of DDT) has been associated with increased height and weight at age 14 in boys (Rogan & Ragan 2003).

DDT and its metabolites and degradation products have been associated with effects on human reproduction. Blood levels of DDE have been associated with altered timing of puberty in some studies but not in others (Raiser et al. 2006; Rogan & Ragan 2003). Girls whose mothers had higher DDT blood levels at the time of delivery were found 28 - 31 years later to have a 32% lower probability of pregnancy per 10 ug/L increase in DDT (Cohn, et al., 2003). Perry and associates (2006) closely studied a group of Chinese women 20 to 34 years old and found that higher levels of total DDT and metabolites were associated with higher risk of pregnancy loss and shortened menstrual cycles. They also found that higher DDT levels were associated with lower levels of estrogen and progesterone during key portions of the menstrual cycle (Perry, et al., 2006).

Phthalates

What are phthalates? These chemicals are plasticizers used in many products including personal care products, cosmetics, toys, medical devices, and food packaging.

Animal and in vitro data. Some phthalates have been shown to have estrogenic effects in rodents, but there is also evidence of anti-androgenic effects. In vitro, phthalates are known to interact with a type of nuclear receptor called peroxisome proliferator-activated receptor (PPAR) which can affect gene expression. Some studies have documented altered gene expression in pathways for the synthesis of steroid hormones and gene regulation in the presence of certain phthalates. Animal models have shown that males exposed at critical periods to phthalates while in utero can have reproductive abnormalities including reduced ano-genital distance (distance between the anus and genitalia), hypospadias (abnormally developed penis), cryptorchidism (undescended testicles), lower sperm counts in adulthood, and malformations of the epididymis, vas deferens, seminal vesicles, and prostate (Latini et al., 2006).

Data in humans. In humans, phthalate exposure has been associated with reduced semen quality and concentration (Duty et al., 2003). Male infants born to women with elevated phthalate exposure during pregnancy were found to have shortened ano-genital distance and higher risk for cryptorchidism (Swan et al., 2005). Another study looked at phthalates in breast milk and found that the levels correlated with reduced androgen activity in male infants. The anti-androgen action of phthalates is believed to be due to altered gene expression for proteins involved in testosterone synthesis thus reducing its synthesis (Lottrup et al., 2006; Main et al., 2006).

Dioxins

What are dioxins? Dioxins are a family of chlorine-containing organic compounds created as byproducts during the combustion of wastes, fuels, and during some chemical processes, including chlorine bleaching of paper and chemical manufacturing (U.S. Food and Drug Administration, 2006). They are persistent in the environment and are eliminated very slowly from the body, so they tend to bioaccumulate (Van den Berg et al., 2006).

Animal and in vitro data In vitro, dioxins have been found to produce an anti-estrogenic effect. One of the dioxins, TCDD, can cause a release of leutenizing hormone (LH) in vitro and release of LH and FSH (follicle stimulating hormone) in vivo in rats (Raiser et al. 2006). Both LH and FSH are hormones involved in regulation of reproduction. There has also been evidence that dioxins may interfere with thyroid function in fetal or young animals (Giacomini et al. 2006).

Prenatal dioxin exposure in rats has been associated with decreased sperm production, feminization of behavior, and reduced ovarian weight when they reach adulthood. Prenatal exposure of Rhesus monkeys has been associated with cognitive deficits and changes in social interaction (Charnley & Kimbrough, 2005).

Data in humans Usually exposure includes multiple forms of dioxins, and there is evidence that the effects can be additive, so the concept of measuring total dioxin-like activity has evolved (Van den Berg et al. 2006). In a Belgian study higher total dioxin-like activity in serum was correlated with urban dwelling versus rural and it correlated with delayed development of adult-stage breasts (Raiser et al., 2006). Though there have been studies suggesting an association between dioxins and thyroid function in humans, the evidence is not yet definitive (Giacomini et al., 2006).

After a high dose accidental exposure in Seveso, Italy, there was an altered sex ratio in births with fewer males than females born to parents who were exposed. A second incidence of accidental exposure occurred in Japan and Taiwan through contaminated rice oil. In the children who were exposed, the eruption of permanent teeth was delayed and there were delays in achievement of developmental milestones. Children born to exposed women were shorter and weighed less than those whose mothers were not exposed. One study found that dioxin levels in breast milk correlated with lower thyroid hormone levels in the mother and higher thyroid stimulating hormone levels in the infants, but whether lower or not, all of the levels were within the range of normal. (Charnley & Kimbrough, 2005)

Brominated Flame Retardants

What are brominated flame retardants? This class of compounds, which chiefly includes the polybrominated diphenyl ethers (PBDEs), is used in fabrics, upholstery, carpeting, furniture, electronics and a wide variety of other objects.

Animal and in vitro data In vitro studies have shown that some PBDEs and their metabolites reduce the activity of an enzyme involved in the synthesis of steroid hormones (Raloff, 2005) and can have estrogenic activity (Meerts, et al., 2001). PBDEs bioaccumulate so that animals higher in an affected food chain have higher concentrations (Birnbaum, 2004). In rats exposure was associated with delayed puberty in both males and females, behavioral changes, and altered thyroid function (Birnbaum, 2004).

Data in humans. While some data are available on levels of PBDEs in human blood and breast milk, there is very little published on health effects in humans. One study found an association between PBDEs in breast milk and undescended testes in newborn boys (Main, et al., 2007).

Atrazine

What is atrazine? Atrazine is a triazine herbicide that is used extensively in corn production to kill weeds. It is highly water soluble, so it is easily carried into surface and ground water by precipitation or irrigation.

Animal and in vitro data. There are data showing both anti-androgenic and pro-estrogenic effects of atrazine (Fan et al., 2007). Atrazine has been found to cause demasculinization of male frogs producing hermaphrodites at a level of 0.1 part per billion (Hayes et al. 2003). In rodents it is associated with increased risk for low sperm counts and breast and prostate cancers. Puberty was delayed in female rats exposed to atrazine at 20 parts per million or more (Laws et al., 2003). In pregnant animals, exposure to atrazine caused decreased fetal growth, birth defects, and high levels reduced fetal survival. (Agency for Toxic Substances & Disease Registry, 2007). Atrazine exposure increased the risk of mammary tumors in rats (Agency for Toxic Substances & Disease Registry, 2003)

Data in humans. Couples living on farms that use atrazine had an increased incidence in pre-term births, though it was difficult to exclude effects from other agricultural chemicals. Maternal exposure to atrazine in drinking water has also been associated with low birth weight and heart, urinary and limb defects (Agency for Toxic Substances & Disease Registry, 2003)

Triclosan

What is triclosan? Triclosan is the widely used anti-bacterial compound present in hand soaps, detergents and many other household products.

Animal and in vitro data. Triclosan has a chemical structure similar to thyroid hormone. At concentrations often found in the environment (below one part per billion), triclosan altered the response to thyroid hormone of bullfrogs (Veldoen et al., 2006). At higher levels, it had an estrogenic effect on male medaka (Ishibashi et al., 2004)

Data in humans Though no adverse effects have been documented in humans to date, triclosan exposure is nearly ubiquitous. One hundred percent of Swedish women in a recent study had detectable triclosan in their plasma and breast milk, with higher levels in those who used triclosan-containing personal care products (Allmyr, et al., 2006). Of human breast milk from California and Texas, 97% had detectable levels of triclosan (Dayan, 2007).

Phytoestrogens

Phytoestrogens are estrogenic compounds found naturally in food like genistein from soy and coumestrol from several vegetables. In rodents genistein has been found to alter the development of oocytes in the ovary and to increase or decrease uterine weight and advance or delay puberty depending on the timing of exposure. Coumestrol has been shown to advance puberty and inhibit the normal pulsatile release of leutenizing hormone (LH), a hormone involved in regulating estrus cycles. (Rasier et al., 2006)