NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION

SCIENCE ADVISORY BOARD

FINAL REPORT

Report of the Public Health Standing Committee on Estrogenic Compounds in the New Jersey Environment

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ABBREVIATION

Entry	Definition
4NP	4-nonyl phenol, a branched alkylated phenol used in detergents, with known endocrine disrupting properties
BPA	Bis-phenol A: a well-studied, synthetic chemical widely used in plastics, which has endocrine disrupting properties. It is approved for food and drink containers, which is the main route of human exposure.
CEC	Contaminants of emerging concern
DES	Diethyl-Stilbestrol a synthetic, non-steroidal with strong estrogenic activity
DRBC	Delaware River Basin Commission
DWTP	Drinking water treatment plant
E1	Estrone, a naturally -occurring estrogen
E2	17β-estradiol, the primary female hormone, which also serves as a reference for comparing the estrogenic potency of other chemicals
E3	Estriol, a naturally -occurring estrogen
EDC	Endocrine disrupting chemical: a blanket term for any substance, particularly exogenous and synthetic substance that interacts with any part of the endocrine system and alters some function
EE2	17α-ethinyl estradiol: a synthetic estrogen incorporated into many fertility regulating products (birth control pills).
ERα and ERβ	Estrogen Receptor alpha and beta
EU	European Union
GPER	G-protein coupled estrogen receptor`
HRPT	human-relevant potency threshold
IARC	International Agency for Research on Cancer
MDL	Method Detection Limit or Limit of Detection is a signal significantly above any signal from a blank . The lowest concentration of a chemical that can be reliably detected as different from zero.
MOE	Margin of Exposure as risk assessment term often used in reference to food intake, analogous to use of reference dose and hazard quotient. Note that higher values of MOE indicate greater safety whereas higher values of hazard index indicates greater hazard.
MTD	Minimum Therapeutic Dose (in the context of the WHO 2012 report), or alternatively used as abbreviation for Maximum Tolerated Dose
Pascal	Unit of Pressure: 1 Pa=the force of 1 Newton per sq meter; 1 Pa=0.007 mmHg
POTW	Publicly Owned Treatment Works, in reference to wastewater treatment plants
PQL	Pꞇactical quantitation level
S-EDC	Synthetic endocrine disrupting chemical as opposed to a natural (N-EDC) endocrine disruptor
SDWA	Safe Drinking Water Act
UCMR3	Unregulated Contaminant Monitoring Rule 3, the USEPA's effort to collect data from public drinking water systems on Chemicals of emerging concern (CECs) including estrogenic chemicals
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WWTP	Wastewater treatment plant

EXECUTIVE SUMMARY

Since the 1990s, the broad field of Endocrine Disrupting Chemicals (EDCs) has become a major theme in environmental health and toxicology. Pioneering evidence of the significance and mechanisms of EDCs included observations on feminization of male fish by estrogen-active substances in waters and food chains, and the reproductive failure of predatory birds (e.g. eagles, peregrines). Estrogenic and anti-estrogenic effects have been extensively studied. Natural human estrogens and metabolites as well as pharmaceutical estrogens (particularly in birth control pills) are introduced daily into wastewater streams, pass through wastewater treatment plants (WWTP) usually unaffected, and enter surface water systems, posing the potential contamination of drinking water and interfering with the maximal beneficial uses of New Jersey's waters. These substances are supplemented by industrial chemicals and natural products with varying degrees of hormone activity.

The Public Health Standing Committee was asked to address several questions regarding the occurrence, pathways, and significance of EDCs in New Jersey Waters. The charge did not include toxicologic evaluations of various EDCs but focused on their occurrence in the environment and the potential for human exposure. The Committee focused its attention on estrogenic activity, emphasizing natural hormones (estradiol, estrone, estriol), a pharmaceutical (ethynyl estradiol), and two industrial EDCs (nonylphenol, bisphenol A). Most estrogenic and antiestrogenic activity is mediated by binding to estrogen receptors (ER-alpha or ERα, and ER-beta or Erβ). The estrogenic potency of an EDCs is largely related to its affinity for these receptors, and chemicals can be ranked by their affinity for estrogen receptors, relative to the receptor affinity of 17β estradiol (E2), the main naturally occurring hormone in humans and most animals.

CHARGE QUESTIONS TO THE PHSC

1. ***What does the current science indicate in terms of adverse human health effects?***
2. ***What are the routes of human exposure?***
3. ***What has been done since the joint monitoring efforts conducted with USGS circa 2008 and should such efforts be resumed?***
4. ***Is this issue a concern for New Jersey?***
5. ***How does this concern compare to that of other emerging contaminants?***

CHAPTER 1: *Summary Response to Charge Question 1*

The Committee addressed Charge Question 1 in several parts. Estrogens have hormonal effects acting via the estrogen receptors but are also implicated as carcinogens based on many human epidemiologic studies. Effects of estrogens have been documented in multiple wildlife species and ecosystems, while effects on humans are suspected, but less well documented. The story of diethylstilbestrol (DES), once widely used in obstetrics to prevent miscarriages, is the clearest example of estrogenic effects in humans. Women who had been exposed to DES as fetuses have a high rate of clear cell cancer of the vagina. Male DES-babies also had increased risk of urogenital abnormalities. In comparison assays, DES estrogenic activity was greater than that of estradiol (E2).

The DES analogy has limited application because 1) it is no longer administered to people although veterinary uses continue in some countries where it may be detected in water, 2) it was administered in relatively huge doses (gram doses) over a long period of time. By contrast natural hormonal levels are measured in pg/ml (picograms/ml) concentrations.

BISPHENOL A, an industrial chemical, is widespread in the environment and has documented estrogenic effects, albeit much lower activity than E2. BPA is approved for container material on direct contact with food and water. Exposure is mainly due to leaching of BPA from plastic containers into food and drink, rather than from drinking water contaminated by BPA. Effects on humans are hotly debated. There are numerous online and published information resources on reducing exposure to BPA. Concentrations of BPA in drinking water sources are low, and although ingestion of leached BPA in water is a significant pathway, drinking tap water, in the usual sense, does not appear to be a significant pathway in New Jersey. The Committee concluded that bisphenol A is an endocrine disruptor, however, there are very few data points, and more monitoring is warranted.

The toxicodynamics of BPA and other EDCs is complex and controversial. This is in part because hormones are involved in signaling and therefore do not adhere to simple stable conditions where reactions are predicated by concentration. The literature offers many examples where EDC effects do not appear to follow dose-response relationships, and where presumed effects occur long after measured exposure. These important considerations were beyond the Committee's scope.

NONYLPHENOLs are industrial chemicals used as antioxidants, industrial oil additives, and in detergents, plastics, and personal care products. They are detected widely in food and environmental media. Nonylphenols are lipophilic and tend to accumulate in biota. 4-nonylphenol has received the most attention. Their reproductive toxicity in birds and fish and other organisms have been widely reported. They can cross the placental barrier (Jiang et al. 2022), are estrogen mimics, and bind to ERα and ERβ. There are few epidemiologic reports on adequate study populations. Numerous discussions of synthetic endocrine disruptors focus on nonylphenols generally and 4-nonylphenol in particular, as examples of adverse reproductive effects and other endocrine effects on biota. The Committee concluded that there is strong evidence that 4NP is an endocrine disruptor.

CANCER is addressed as a significant part of the environmental estrogen story because of multiple studies linking natural estrogens with breast cancer and other cancers. Life cycle factors such as early menarche, late menopause, and no pregnancies, are risk factors for certain

cancers, and that lifetime endogenous estrogen exposure is a risk factor for to breast and other gynecologic cancers. Multiple mechanisms have been put forth, including the stimulation of clonal expansion of pre-cancerous cells. Males are at risk as well. The risk of testicular cancer is about doubled in males with *in utero* exposure to estrogens (DES, oral contraceptives, estrogen therapy).

As the EDC story and environmental estrogens evolved there were reasons to be skeptical. The big question: could the adult human female body respond to low levels of exogenous estrogens when it is bathed in endogenous estrogens for much of the lifespan? Can physiological processes detect the signal of environmental estrogens over the clamor of natural estrogen activity? The answer is clearly YES in fish and "probably yes" in humans, particularly at points in the life cycle, when natural hormone levels are low. Studies on puberty and on the onset of breast cancer provide a clue. Pre-pubertal girls have much lower levels of total estrogens (< 30 pg/ml) than they will have as adults of reproductive age (up to 400 pg/ml) and are vulnerable to developmental effects. In the 40s and after menopause, when natural estrogen levels are declining, exogenous estrogens pose a cancer risk.

Several reviewers of the document stressed the importance of food packaging as a source of EDC exposure. The Committee recognized this but confined its deliberations to water-related exposures as charged.

The World Health Organization (WHO) 2012 Report

The Committee was asked to address a report issued by the World Health Organization (WHO, 2012) summarizing water data mainly on pharmaceuticals from various European countries. WHO (2012) concluded, "The substantial margins[1] of safety for individual compounds suggest that appreciable adverse impacts on human health are very unlikely at current levels of exposure in drinking-water."

Despite the absence of estrogens in the WHO report, the Committee approached the report conclusion as a null hypothesis, looking for evidence that it might not apply in North America or in the 2020s.

Caldwell et al (2010) arrived at a similar conclusion to WHO using a different approach. They calculated that any estrogenic exposure from drinking water would be much lower for children than typical exposure to estrogenic compounds in food, particularly milk. However, the water concentrations they used were modelled, not measured.

The Committee reviewed data on New Jersey waters and did not find any "positive" studies on surface water concentrations to warrant rejecting the WHO conclusion vis-à-vis estrogenic activity. The WHO conclusion was based on limited, non-systematic data and existing New Jersey data is likewise scattered. The Committee assembled existing data from New Jersey waters and advocated more systematic sampling.

[1] The Margins of Exposure (MOE) were mainly greater than 1,000, when comparing drinking water concentrations with the Minimal Therapeutic Dose. (WHO 2012: p ix). Note that using an MOE approach, a higher value is safer. This is directly opposite to the Hazard Index approach, used, for example, by EPA, where a higher value is less safe. Both processes compare average daily intake to some criteria value, such as the Reference Dose.

CHAPTER 2: *Summary Response to Charge Question 2*

Chapter 2 reviews the exposure pathways for EDCs. Drinking water is the most prevalent exposure pathway. Oral exposure through diet is also an important route to consider. The major pathway for bisphenol A, for example, is leaching from plastic food packaging; however, that exposure is beyond the scope of this report. Some fish diets, particularly frequent ingestion, can add significantly to estrogen exposure. The natural estrogens E1, E2, E3 could be detected in fish muscle, sometimes increased by two or three orders of magnitude over the concentrations in the source water for the fish. Fish content is thus a reflection of water concentrations as well as a source itself. Unlike mercury, for example, there is currently no guidance as to which fish may be high or low in estrogen. There are limited data on estrogen levels in New Jersey fish. However, except for subsistence fishing and some recreational fishing, most fish eaten in NJ comes from out of state commercial sources. People who eat large fish meals frequently could be at risk of consuming various chemicals including EDCs. There is no database (as far as we can tell) to guide consumers vis-à-vis estrogen content; other than female fish usually having higher levels than male fish. Based on a single study, carnivorous fish do not show the order of magnitude bioconcentration factor for estrogen as shown by mercury in the food chain.

This report does not address the many natural estrogens in foods (phytoestrogens). For example, zearalanonen is a fungal product, a mycoestrogen, which contaminates grain and therefore food products. This substance, through the ingestion of contaminated grain, has been implicated as a significant environmental estrogen, impacting pubertal development.

As noted in the charge question above, the SAB was tasked with determining if dermal exposure, as well as inhalation exposure to EDCs, would pose human health concerns. Inhalation is an important pathway for volatile chemicals, and a secondary pathway for chemicals in water that can be inhaled during showering. Due to the physical properties of most natural and synthetic estrogens, inhalation, even while showering, is likely to be negligible. Estrogens themselves have low volatility and are not likely to be encountered airborne. Moreover, even a hot shower should not volatilize estrogens significantly. For other compounds, particularly smaller, organic molecules, volatility would have to be examined on a case-by-case basis.

Dermal absorption, however, while taking a bath or swimming can be a significant secondary pathway for some compounds under certain circumstances. Percutaneous absorption potential of water-borne EDCs was assessed based on permeability coefficient, octanol/water partitioning coefficient (K_{ow}), and molecular weight. It was determined that E2 and EE could have significant dermal uptake from a bath or shower, or from swimming in contaminated surface water.

Evaluation of inhalation and dermal exposure to water-borne estrogenic compounds has determined that inhalation exposure is negligible while percutaneous exposure may be significant relative to the drinking water exposure pathway. Consequently, in scenarios where drinking water consumption represents a significant exposure, it may also be necessary to evaluate the contribution from dermal exposure.

In conclusion, the drinking water ingestion pathway is the main concern for EDCs in surface water. Inhalation is likely to be negligible for EDCs, even in hot showers. Dermal uptake of E2 and EE could be significant for bathing, showering, and swimming.

Chapter 3: *Summary Response to Charge Question 3*

Chapter 3 discusses two efforts to monitor estrogen and other hormonally active substances in New Jersey waters since 2008, one by the Delaware River Basin Commission (DRBC) and another by the USGS and NJDEP. The chapter tabulates and summarizes data from these studies, from the Stackelberg et al. (2007) study, and drinking water testing data for New Jersey from 2013-2016 compiled by the USEPA. These data are compared to health risk assessment information from de Aquino et al. (2021). Finally, the chapter makes recommendations for future monitoring of New Jersey waters.

Highlights of the comparison of data from these various monitoring efforts are:

- Many EDCs were detected in wastewater treatment plant effluent, and when present were typically in greater concentrations than in receiving water bodies and water before or after drinking water treatment.
- Some but not all substances appeared in lower concentrations at lower detection frequencies in wastewater plant outfalls than in inflows, indicating the potential for some removal by standard wastewater treatment process.
- Finished drinking water samples typically showed lower concentrations or detection frequencies of these substances than untreated waters, indicating that conventional drinking water treatment further reduce concentrations of some (but not all) of these substances.
- Twelve substances were detected in at least one finished drinking water sample; of these, only three had risk-based comparison values[2] from de Aquino (2021). In all three, drinking water concentrations in New Jersey were far below comparison values.

The chapter concludes that water sampling to date, while informative, has not been adequate to characterize the degree to which these EDCs occur throughout the wastewaters, drinking water source waters, and finished drinking waters in New Jersey.

The chapter includes a recommendation for an integrated survey of inflows to and outflows from wastewater treatment plants, ambient water upstream and downstream from wastewater treatment plant discharge points, and at intakes and finished water of drinking water treatment plants.

Chapter 4: *Summary Response to Charge Question 4*

While the question, "is this issue a concern in New Jersey," the database does not lend itself to a clear and direct answer. As a result, the Committee chose to address the question by highlighting the advancements analytic chemistry continues to make towards greater sensitivity

[2] *The guideline values (GV) from de Aquino, 2021 were estimated from the calculated acceptable daily intake (ADI) data, which itself is based on either a lowest daily therapeutic dose (LDTD), lowest observed adverse effect level (LOAEL), or no observed adverse effect level (NOAEL) divided by an appropriate uncertainty factor (UF).*

Following GV calculations de Aquino estimated a margin of exposure (ME) which takes into account the occurrence of a given EDC in the drinking water data.

for a wide variety of analytes while simultaneously making gains in accuracy, precision, and efficiency. Progress in that field includes pre-concentration of the analyte and matrix interference mitigation as well as analytic instrument enhancements.

While measurement science makes progress, regulators are much more constrained, often making decisions based on risk assessments, so that drinking water regulations are largely relicts of the 1990s, and the instrumentation and methods reference described in many of the regulations, has often not kept pace with current capabilities. Often this lag between capability and regulatory monitoring results from a failure to revisit or update compliance methods. Progress in the past decade has largely focused on mass spectroscopy (MS/MS) and especially liquid chromatography/mass spectrometry (LC/MS). These instruments are often still too complicated or too expensive for many commercial laboratories, responsible for compliance monitoring. However, these constraints may change in the near-term future.

Whereas heavy metals and many organics (i.e., volatile and semi-volatile organic compounds) can be measured reliably at low levels, steroid hormones such as E2 pose challenges for quantitation in water. They are often not retained on many of the general use solid phase sample preparation columns, and they do not always ionize efficiently, making sensitive detection difficult. Evaluating their potency as endocrine disruptors presents additional challenges as they are organic substances bound to receptors on tissues of great complexity and tenacity.

Chapter 5: *Summary Response to Charge Question 5*

Charge 5 requests a comparison among contaminants of emerging concern (CECs), many of which are only recently identified, lack comprehensive data on occurrence and/or toxicity, represent threats to human health or ecology of uncertain magnitude and are currently unregulated. The Committee believes that given the current paucity of data, it would be premature to go directly to risk assessments for each compound. It may be necessary to go to full risk assessments and management applications for multiple compounds on the CEC list provided to the Committee by the NJDEP. Hence, we suggest conceptual site models as an initial step. That is consistent with risk assessment.

A consistent preliminary assessment process would help DEP use existing information in order to set initial priorities. A good deal of this effort will be devoted to using existing data and prioritizing the collection of new data that will address epistemic (lack of knowledge) and aleatory (random events) forms of uncertainty. Whether the focus is on CECs in general or estrogenic compounds in particular, the approach should be directed to three connected parts of risk assessment: (1) characteristics of the potential hazards; (2) transport pathways that could lead to exposures; and (3) characteristics of persons likely to be exposed.

The literature offers field-tested applications of Conceptual Site Models (CSM) focusing on individual substances and/or on individual sites while identifying gaps in current knowledge. A Conceptual Site Model (CSM) is a representation of the chemical, physical and biological processes that condition how and when contaminants move from sources through the air, soil and water to people and other receptors (U.S.EPA, 2011; NJDEP, 2019). A CSM for a CEC would describe the sources (where and how the chemical could or does enter the environment), the

pathways or potential pathways through the environment, and the receptors (humans or ecologic) and health endpoints such as organ toxicity, reproductive toxicity, or cancer). At each step, expert judgment of toxicologists, environmental scientists, and others can be used to determine whether a CEC should move up the list because new data and judgements indicate that it is likely to pose a hazard to a group somewhere, or likely to be dismissed because of lack of evidence of toxic hazard for similar compounds. Expert judgement is not always entirely dependable since experts bring their own experiences, interpretations, and risk tolerance or aversiveness to risk scenarios. But consensus among experts with long and broad experience is likely to be a valuable part of any decision process.

Conclusions and/or Recommendations:

The endocrine system, with its network of glands and hormones, plays critical roles in the life cycle (growth, development, and all aspects of reproduction) and on a daily basis (regulating energy production, blood pressure, blood sugar, and calcium) among other functions. Some functions are tightly regulated, others, more loosely. In any case, dysregulation due to the absence, deficiency or excess of particular signals, may require treatment. Many such conditions arise spontaneously or "naturally" in individuals. There are many naturally occurring conditions of endocrine dysregulation that require treatment, without invoking environmental EDCs. A wide variety of chemicals—natural and synthetic—are employed to restore or optimize endocrine function.

Exogenous substances and endocrine disruptive chemicals (EDCs) are encountered mainly in food and water and are blamed for causing many ill effects. The Committee focused on the evidence that significant exposure to significant levels of EDCs is a public health concern in New Jersey at this time due to excretion into wastewater and ultimate contamination of drinking water. A registry of EDC levels in edible fish, comparable to that available for methylmercury, would be useful.

Exogenous estrogens may enter wastewater, pass through water treatment plants (WTPs) with little modification, travel to surface water and end up in drinking water. These compounds are not routinely monitored by NJDEP as part of its routine surface and ground water monitoring programs, due to the absence of federal criteria recommendations and state regulatory standards. The question is should they be monitored and if so to what extent.

The Committee did not find evidence that EDCs, natural or synthetic, are causing widespread or significant public health effects. Ecologic impacts were not part of the charge.

However, the Committee recognized the possibility of local exceedances that might arise from particular industrial or agricultural sources, historic waste sites, or other sources that could significantly contaminate wastewater and find their way into drinking water sources.

With possible local exceptions, the Committee did not find evidence that estrogens in the New Jersey environment occur with sufficient concentration or frequency to represent a hazard to human health. What little evidence exists is mainly on pharmaceuticals, not on natural or prescription estrogens. However, lack of evidence of harm does not equal evidence of no harm.

The volume of sampling data is still very small. The WHO (2012) conclusion may have contributed to a lack of interest in measuring these compounds. This SAB Committee's report should not be construed as discounting the value of sampling. Rather, the Committee

recommends systematic periodic sampling of effluent immediately downstream from New Jersey's wastewater treatment plants. The recurrent theme is that data on EDCs in New Jersey waters (and indeed anywhere) is too sparse.

Recommended considerations for future monitoring are detailed in Chapter 3.

Briefly, we recommend that the NJDEP consider conducting an integrated sampling of water media that were examined in prior surveys. Systematic sampling of three systems, should be taken at inflows to and outflows from wastewater treatment plants (WWTPs), the receiving body of water downstream of these discharge points, and at intakes and finished water of drinking water treatment plants utilizing that downstream source water (see Figure 3.1 for a conceptual diagram).

Sampling should be conducted at different times of year and/or at different flow levels of source waters. Alternatively, the survey might focus on a time of year and flow level that could be considered a "worst case" scenario for Phase 1 contaminants.

Phase I sampling should include (but not necessarily be limited to) the EDCs addressed in this study (E1, E2, E3, EE, BPA, NP).

A survey should target those watersheds and locations where sex hormone-related chemicals would be expected to occur within the State of New Jersey, for example where the configuration of WWTP discharges, source water, and downstream DWTP intakes shown in Figure 3.1 occurs. NJDEP could focus initial sampling and analysis on finished drinking water and select watersheds for follow-up sampling based on the results of that initial effort.

The timing of sampling among the sample points within watersheds might be lagged based on expected flow times, so that one can look at effectiveness of removal of contaminants through the WWTP and DWTP processes and dilution factors from outfall through transit in source waters to drinking water intakes. A lack of consistency in contaminant presence or concentration from WWTP inflow through DWTP finished water could indicate that there are sources other than WWTPs of Phase 1 contaminants in source waters, such as non-point source runoff into rivers from agricultural land, for example.

REPORT OF THE PUBLIC HEALTH STANDING COMMITTEE ON ESTROGENIC COMPOUNDS IN NEW JERSEY WATERS

BACKGROUND

Among many new environmental health concerns raised in the last quarter century is the presence of endocrine disrupting chemicals (EDC) and various pharmaceuticals and pharmacologically active metabolites in drinking water and food. EDCs include natural and synthetic substances of slight to great hormonal potency. The initial charge to the Committee included pharmaceuticals, but the Committee decided to defer consideration of pharmaceuticals and focus on EDCs, which are substances that mimic, block or otherwise disrupt one or more of the many endocrine activities of the body throughout the life cycle. More specifically the Committee focused on natural and synthetic estrogens and substances with estrogenic properties. Estrogenic effects occur throughout the human life cycle, in females more than in males, and play critical roles in development, maturation, metabolism, reproduction, bone and muscle physiology and aging.

These chemicals with estrogenic or anti-estrogenic properties can enter the environment in several ways (residential or commercial wastewater, historic landfill leachate, industrial effluents, livestock wastes). Most wastewater is subject to treatment, which may reduce the estrogenic properties as treatment plant effluent is discharged to surface water (e.g., rivers Conley et al. (2016). Treatment plant effluent may retain estrogenic activity and ultimately can find a way into drinking water supplies. Drinking water treatment systems vary in whether they alter, affect, or reduce estrogenic activity in their output. Public drinking water is subject to various treatments, such as chlorination (Schenck et al. 2012), while private wells typically access untreated groundwater. As will be described below, the amount of estrogenic activity in drinking water is highly variable and is generally uncertain.

Long before the term “endocrine disruptor” was coined by a 1971 Wingspread Conference (Colburn et al. 1993), the effects were recognized in the dramatic disappearance of iconic birds such as the Bald Eagle, Peregrine Falcon, and Brown Pelican (Hickey 1968). In many places these species experienced total reproductive failure (Rudd, 1970), leading to population decline and extinctions. Long-lived adult birds continued to migrate south, year after year, but immature birds were nowhere to be found. Reproductive failure attributable to abnormally thin-shelled eggs that collapsed during incubation was eventually connected to the anti-estrogenic effects on eggshell production (Porter & Wiemeyer 1969, Peakall 1970a) of the novel pesticide, DDT (dichloro-diphenyl-trichloroethane). Ecotoxicologists described bioamplification of DDT in the food chain with consequences particularly for top level predators such as the eagle, falcon, and pelican (Hickey 1969). The decline of these predatory species began simultaneously in North America and Europe, reported already by the mid-1950s, followed quickly on the introduction and widespread use of DDT. Experiments with DDT in birds showed lowered levels of estradiol (E2) and inhibition of calcium transfer from blood to eggshell (Peakall 1970b).

Although most uses of DDT in the United States were banned in 1972, it took more than a decade to recognize EDCs as widespread in the environment. Synthetic chemicals were found to affect various aspects of the endocrine system in aquatic organisms, terrestrial wildlife, and quite likely in humans (Colburn et al. 1993). *Our Stolen Future* (Colburn et al.1997) documented the status of knowledge in the mid-1990s and is credited as creating the field of endocrine

disruption. Recognition of EDCs and EDC research swelled in the 1990s and has remained a priority of environmental toxicology.

NJDEP's Responsibility

Given its mandate (e.g., under the Clean Water Act, NJDEP 2021), in the early 2000s, DEP began to focus on "contaminants of emerging concern," including pharmaceuticals and hormone disrupting chemicals in drinking water (NJDEP 2003). Estrogenic substances, both environmental estrogens such as bisphenol-A (BPA) and pharmaceutical estrogens such ethinylestradiol (EE2) often used in oral contraceptives, have been studied, particularly in Europe and China. The NJDEP charged the Public Health Standing Committee (PHSC) of its Science Advisory Board (SAB) to consider current knowledge and research needs, focusing on possible monitoring and/or regulatory action to consider the human risk of estrogenic compounds and pharmaceuticals in water. For this report, the Committee narrowed its focus to estrogenic active substances. An extensively researched background document (NJDEP 2022) served as a resource for the Committee.

Contaminants of emerging concern (CEC's) are, by definition, not currently regulated. Ledoux (2001) provided a detailed background paper for the NJDEP signaling that agency's entry into the EDC arena. The EDCs of regulatory concern are primarily made by humans. A 2002 international conference reviewed many studies of EDCs in aquatic organisms, terrestrial wildlife, and humans. The consensus recognized strong evidence for toxicity and adverse developmental and reproductive effects in fish and other aquatic organisms, while cautiously recommending extensive study to evaluate and document human reproductive effects (Miyamoto and Burger 2003). A consensus on human health risk was elusive at the time and remains so today. Most of Miyamoto & Burger's (2003) research priorities remain relevant two decades after their publication.

Endocrine Disruptor Overview

Endocrine disruptor is a blanket term applied to a substance that alters some function of the endocrine system through some hormonally related mechanism which can include:

1. Mimicking or opposing the action of hormones, usually by activating or blocking specific hormone receptors
2. Increasing or decreasing the production, release, or breakdown of natural hormones
3. Increasing, decreasing, or altering the function of hormone receptors
4. Altering the interactions between and consequences of binding of hormones to receptors and the subsequent recruitment of functional complexes.

Endocrine disruptors can enhance hormonal reaction to reach toxic levels or interfere with hormonal reactions to disrupt development, behavior, metabolism, and reproduction. Most endocrine disruption attention has focused on sex hormones, particularly estrogens, although thyroid systems, for example, are subject to dysregulation or disruption (see *PHSC Report on Perchlorate (PHSC 2020)*).

The Committee focused on substances with estrogenic activity. The hormonal mechanisms are mediated primarily by binding to nuclear hormone estrogen receptors ERα and ERβ, which are ligand-activated transcription factors, and a G protein-coupled estrogen receptor,

GPER, which activates various signaling pathways implicated in both carcinogenesis and cancer prevention studies (Arterburn & Prossnitz 2023).

For the purpose of the Committee's research and reflection, EDC effects can be divided into those related to endocrine activity and those related to cancer. Many EDCs and/or metabolites have been tested for carcinogenicity. Some, even including the natural estradiol (E2), test positive in some assays. Fortunately, E2 is not a strong carcinogen. For some compounds results are inconsistent, positive in some assays and negative in others. Some carcinogenic effects are hormonal, for example, stimulating clonal expansion of cancer cells in the breast. Some carcinogenic effects may be due to promoting oxidative damage. Endocrine disturbance in organisms including humans can occur at any stage from gametogenesis to fertilization from embryonic development to adulthood, and may affect many aspects of development, reproductive biology and behavior including libido and secondary sex characteristics, fertility, and ability to sustain a pregnancy.

Based on available studies, for the most part the individual estrogenic compounds are present in waters at very low (ppb or ppt) concentrations, which are assumed by some to be subthreshold concentrations, but interactions such as additivity or synergism need to be considered as a possibility. It may seem obvious that such low concentrations of exogenous "estrogens" are too low to impact a system that already has its own endogenous estrogens. However, endogenous estrogens function at levels measured in pg/mg (ppb) concentrations. EPA (2023) has published tables on "risk-based screening levels" for EDCs and many other compounds in drinking water.

The exogenous estrogens may enter wastewater, pass through water treatment plants (WTPs) with little modification, travel to surface water and end up in drinking water. These compounds are not routinely monitored by DEP as part of NJDEP's routine surface and ground water monitoring programs because of the absence of federal criteria recommendations and state regulatory standards. The question is should they be monitored and if so to what extent.

Many substances exert some estrogenic/anti-estrogenic activity. Industrial chemicals such as bis-phenol A have received extensive study compared to naturally occurring hormones that are excreted daily into the wastewater system. Ecological effects have been widely studied (Miyamoto and Burger 2003) and captured the attention of NJDEP (Nadeau 2001??) at a time when effects on humans were sparsely researched and widely doubted.

To facilitate discussion, the Committee focused on the naturally occurring hormones: estradiol (E2), estriol (E3), and Estrone (E1) as well as the synthetic ethinylestradiol, (EE2) commonly incorporated in oral contraceptives. Two synthetic, industrial compounds: bisphenol A (BPA) and 4-nonylphenol were included on the priority list as well.

The World Health Organization Report (2012)

In 2012, regarding CECs, the WHO (2012), concluded that "[t]he substantial margins of safety for individual compounds suggest that appreciable adverse impacts on human health are very unlikely at current levels of exposure in drinking-water." However, the WHO acknowledged the lack of impact studies on aquatic life and ecosystems and recommended "where specific circumstances, such as a catchment survey, indicate a potential for elevated concentrations of pharmaceuticals in the water cycle (surface water, groundwater, wastewater

effluent and drinking-water), relevant stakeholders could undertake targeted, well-designed and quality-controlled investigative studies to obtain more information to assess potential health risks arising from exposure through drinking-water. If necessary, screening values could be developed". Caldwell et al. (2010) reached a similar conclusion from a different perspective, arguing that any effect of estrogenicity in water would be dwarfed by the exposure to these compounds in milk. However, their conclusion was based on modelled concentrations.

The NJDEP asked the Public Health Standing Committee (PHSC) of its Science Advisory Board to determine whether the WHO (2012) conclusion was correct and applicable to New Jersey in the 2020's and was adequately protective of public health and the environment. The charge had five parts each of which was assigned to a subgroup of the PHSC, and each is addressed in chapters 1 to 5.

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CHAPTER 1: RESPONSE TO CHARGE QUESTION 1

Question 1 - What does the current science indicate in terms of adverse human health effects?

Charge 1 had several components to consider, the main focus of which is a very wide-ranging question. We divide adverse effects of endocrine disrupting chemicals (EDC) into two categories. A) Effects related to the hormonal activity (including antagonistic effects, and B) Cancer. There are other common toxic modes of action leading to adverse endpoints that are not addressed by this report. Indeed, there is extensive literature warning about all the bad things that EDCs can do to the body. EDCs are known to affect a wide variety of hormone systems which provides biological plausibility for linking EDCs with altered reproductive endpoints (sperm counts, fertility), developmental abnormalities, and conditions such as early puberty and endometriosis. Impacts on immune function and nervous system function have occurred and cancer is often mentioned. However, documenting effects in humans is challenging as EPA "explains" in ending is March 2023 Fact Sheet: "considerable scientific uncertainty remains regarding the actual causes of such effects. Nevertheless, there is little doubt that small disturbances in endocrine function, particularly during certain highly sensitive stages of the lifecycle (e.g., development, pregnancy, lactation) can lead to profound and lasting effects."

Effects related to the hormonal activity can occur at any stage in the life cycle: gametogenesis, fertilization, embryogenesis, fetal development, infancy, adolescence, adulthood, including reproductive and post-reproductive years. The mechanisms of action area vary. Primary actions of estrogenic compounds are mediated through binding to estrogen receptors (ERα and ERβ) and a G protein-coupled estrogen receptor (GPER). In addition to receptor binding, an EDC can increase or inhibit production, release or degradation of hormones, and can modify the various functions of the hormones. EDCs can increase or decrease the number of receptors or can compete for receptors. Thus, hormonal action is not simply a matter of toxicokinetics and circulating hormone-activity levels.

Binding to the ERα and ERβ receptors can have different effects in different contexts and in different tissues. The estrogen receptor complex activates transcriptional processes and/or signaling events that influence gene expression and thus many different responses (Fuentes & Silveyra 2019). The binding affinity of a substance for the receptors, particularly to ERα has been measured with several methods and offers clues to the whether a compound will exert or inhibit an estrogen response. The Committee searched for studies that compared the estrogenic potency of different substances (Blair et al. 2000). A variety of sources were identified. Compounds were compared to the potency or receptor affinity of estradiol (E2). The results changed depending on the assay system used, but the relationships among chemicals changed little. Thus diethylstilbestrol (DES) had higher affinity than E2 in several studies, though the relative binding was 30% stronger (Borgert et al. 2018) or 400% stronger (Blair et al. 2000).

Borgert et al. (2018) from Europe summarizes published data from many sources to arrive at a human-relevant potency threshold (HRPT). They extracted data on the median ERα affinity relative to E2 and proposed a ranking based on the mode of action, for example affinity for ERα. They conclude that compounds with a relative affinity of 10E-04 relative to E2 (i.e. 10,000-fold lower) were unlikely to influence any adverse effects in humans, at least via that mode of action.

Table 1.1 Relative affinity for ER*α* compared to beta-estradiol (E2) (comparing published median values) (after Borgert et al. 2018). This is only part of the Borgert list.

SUBSTANCE	RELATIVE ER*α* AFFINITY	CATEGORY
17β-estradiol (E2)	1.0	Natural
Estrone (E1)	0.029	Natural
Estriol (E3)	0.083	Natural
17α-estradiol	0.026	Pharmaceutical
17α-ethinylestradiol (EE2)	1.3	Pharmaceutical
Diethylstilbestrol (DES)	1.4	Pharmaceutical
Tamoxifen	8.3E-06	Pharmaceutical
Zearalenone	0.34	Botanical[a] (Mycoestrogen)
Genistein	4.5E-04	Botanical, (Phytoestrogen)
Testosterone	7.1E-06	Androgen[b]

a = 27 botanicals are listed in the Borgert table, mostly in the range of 10E-5 or 10E-6 relative potency. Zearalenone is an exception.
b = 4 androgens are listed, all in the E-06 range.

DIETHYLSTILBESTROL

There are numerous reports on how humans *could be* harmed by EDCs, and numerous studies document a wide variety of effects in rodents, lending credence to the proposed risks to humans. The Committee searched for documented EDC effects in humans.

First and foremost is the story of diethylstilbestrol (DES), a synthetic drug that was used from the 1940s to 1971 to treat a variety of gynecological and obstetrical disorders, particularly to sustain pregnancies threatened by impending miscarriage (Veurink et al. 2005). Apart from questionable efficacy, the prenatal exposure to DES was identified as a cause of a unique clear cell vaginal cancer in the girls identified as DES daughters who had been exposed to DES in utero. Moreover, DES sons also manifested urogenital developmental defects (Victoria Better Health 2023). This was entirely a clinical human EDC event, not an environmental exposure. However, it raised the suspicion that similar EDC relationships exist.

However, before trying to generalize from the DES experience to environmental estrogens it is important to consider the issue of the potency and the dosage scale. DES is a more potent estrogen than estradiol (Bogert et al. 2018) based on ERα receptor binding affinity. DES was also administered in relatively huge doses for prolonged periods as illustrated in the box below.

Excerpts from a treatment protocol for DES to prevent miscarriage (Karnacky, 1950):

"For mild spotting "*100 mg stilbestrol are given every 15 minutes until symptoms are relieved and then 25 mg. three times daily for one week. Twenty-five mg. are given every morning until the eighth month."* Impending miscarriage: *"Give 250 to 1,000 mg. by mouth every 15 minutes until pain, cramps, and bleeding stop, followed by 250 to 500 mg. intramuscularly about every third day for 2 to 4 weeks."*

Such dosages are not likely to be matched for a contaminant in drinking water.

The DES story provides a well-studied account of the severe human impact of a synthetic EDC. Other examples of human EDC effects are limited, are mostly observational, and most do not have a clear mode of action.

BISPHENOL A

Bisphenol A (BPA) is a high-volume industrial compound, nearly ubiquitous in the environment. It is identified as an EDC (Bao et al. 2020). In observational studies human exposure to BPA has been associated with organ toxicity, obesity, diabetes, cardiovascular disease, and a recent prospective study links elevated BPA levels with increased all-cause mortality (Bao et al. 2020). Although recognized as an EDC, it is not clear how many of these adverse effects are endocrine-mediated. Alternate mechanisms for increased mortality "remain to be elucidated"- (Bao et al. 2020). BPA is an archetype of a non-hormonal endocrine active substance.

BPA is widely used in a variety of products, including food packaging, canned goods, baby bottle soothers, reusable cups, medical devices, household objects, and various other consumer products around the world (Qi et al. 2024). Although it does not have the steroid structure of an estrogen hormone it can form a configuration that binds to estrogen receptors, thereby influencing endocrine activities. It is also implicated in affecting thyroid function. Fetal life and infancy are the most vulnerable periods. Although FDA continues to authorize use of BPA for food contact, the FDA joins with many organizations and websites explaining why and how to reduce exposure to plasticizers including BPA.

Exposure to Bisphenol A appears to be mainly due to its leaching from plastics into food and drink. In April 2023, the European Food Safety Agency lowered its Tolerable Daily Intake (TDI) to 0.2 ng/kg-body weight/day (a 20,000-fold reduction from its 2015 TDI). This new TDI is often exceeded (Marchiandi et al. 2024).

BPA may be present in drinking water sources, typically at levels below 1 ppb (EPA) and Minnesota has set a Guidance Value of 20 ppb. (Minnesota, 2014). In New Jersey BPA was detected in about half of wastewater samples (maximum = 0.44 ppb) and Stackelberg et al (2007) reported detecting BPA in 2 of 12 finished water samples (maximum=0.22ppb). Based on the very limited data it appears that drinking water is not likely to be a widespread source of BPA exposure in New Jersey, or perhaps elsewhere. However, BPA should be part of any water monitoring program.

The Committee determined that there is strong support for multiple EDC actions of bisphenol A including via estrogen receptor binding, but that clear evidence in of toxic effects in humans is obscured by the universality of exposure to bisphenol A and by the presence of other compounds with similar mode of action.

4-NONYLPHENOL

The xenobiotics, nonylphenols, including 4-nonylphenol (4NP), are industrial chemicals used as antioxidants, industrial oil additives and in detergents, plastics, and personal care products (Soares et al. 2008).

They are found widely in food and environmental media. Nonylphenols are lipophilic and tend to accumulate in biota. Their reproductive toxicity in birds and fish and other organisms have been widely reported. They can cross the placental barrier. (Jiang et al. 2022), are estrogen mimics, and bind to ERα and ERβ.

There are few epidemiologic reports on adequate study populations. However, Jiang et al. (2022) reported on preterm births in a nested case-control analysis of 515 mother-neonate pairs within a prospective study cohort between 2015 and 2018 in a Guangxi, China population. Preterm birth was elevated for higher exposure to nonylphenols as a group and for 4NP specifically. Exposure was assessed through UPLC-MS from venous blood during the first trimester.

Numerous discussions of endocrine disruptors focus on nonylphenols generally and 4-nonylphenol in particular as examples of adverse reproductive effects and other endocrine effects on biota. The Committee concludes that there is strong support for endocrine disruption by 4NP. Like BPA, 4-nonylphenol has been detected in New Jersey waters.

All wastewater samples tested by USGS/NJDEP (2013), before and after treatment, detected 4NP up to 10 and 3.7 ppb respectively. Stackelberg et al. (2007) found 4NP in 25% and 8% of raw and finished wastewater, respectively, with concentrations up to about 1 ppb. The comparison value proposed by de Aquino et al. is 90 ug/L (ppb). The US guidance value for drinking water is 28 ppb, and Minnesota has recommended a limit of 20 ppb.

OTHER ENVIRONMENTAL ESTROGENS

The mycoestrogen zearalenone occurs in the diet from moldy grain consumed by livestock. Dietary exposure to zearalenone was reported associated with slower growth and delayed pubertal development in adolescent girls in New Jersey (Rivera-Nuñez et al. (2019). This compound has a high receptor binding affinity.

A meta-analysis of studies of males with *in utero* exposure to estrogens found a doubling of risk of testicular cancer, whether the exposure was DES, oral contraceptives, or other estrogen treatment. Cryptorchidism and hypospadias, a well-publicized syndrome, was significantly elevated in DES-sons, but risk was only about 30% increased ($P>0.05$) for other pre-natal estrogen exposures (Martin et al. 2007).

ESTROGENS AND CANCER

Some estrogenic compounds have tested positive in one or more cancer assays and are classified as human carcinogens. More are considered possible carcinogens. Some estrogenic compounds also have anti-cancer activity. The hormone-cancer relationship is complex, and the very large Nurses Health Studies have provided some evidence regarding both exogenous hormones (oral contraceptives) and endogenous hormones. Epidemiologic studies identified high estrogen as a risk factor for breast cancer (Rice et al. 2016). Early menarche and late menopause indicated a high lifetime estrogen level, particularly if uninterrupted by pregnancies (Lambe et al. 1996). This evidence was originally crudely observational but has now been confirmed in many studies (Davis & Bradlow 1995). Whether the hormone initiates the cancer or feeds and stimulates clonal expansion is unclear, but estrogens as carcinogenic is now widely accepted. Most breast cancers are identified as "receptor positive", such that estrogen stimulates cell proliferation, and anti-estrogens are first line treatment. Studies of hormone replacement therapy provided evidence that estrogens could increase uterine cancer as well (Rice et al.2016)

IARC, the International Agency for Research on Cancer, publishes detailed monographs on carcinogens and carcinogenesis. An IARC (2007) monograph addressed estrogen-progesterone combination contraceptives and hormone replacement therapy, reviewing many studies in the process. IARC concluded that there was sufficient evidence that this combination was carcinogenic both as a contraceptive and hormone replacement.

DIETARY ESTROGENS

The use or avoidance of phytoestrogens or dietary estrogens for their beneficial (hormone-replacement) or adverse (endocrine-disruptive) effects has engendered numerous opinion pieces and some studies. This is outside the Committee Charge which focused on water. In general, the phytoestrogen level in most diets is below an expected physiologic effect level. The potent mycoestrogen, zearalenone is an exception (Rivera-Núñez 2019).

EXOGENOUS ESTROGENS AGAINST THE NATURAL ESTROGEN BACKGROUND

One of the central questions in understanding environmental estrogens is whether and when exogenous compounds: xenoestrogens, phytoestrogens, pesticides, or any EDC can exert a detectable signal against the background of natural estrogens. At pharmacologic doses, as in oral contraceptives, the exogenous signal is sufficient to increase the cancer risk. At the much lower "environmental doses" the evidence is unclear. If there were strong effects, we would probably know them by now. Epidemiologic approaches are limited by the need for large samples sizes and long (very long) follow up periods. Much attention has focused on estrogens and breast cancer which commonly occurs around age 40, the life phase at which natural estrogen level in women is declining Normal serum E2 levels are: 30 to 400 pg/mL for premenopausal women (influenced by monthly cycle and pregnancy); 0 to 30 pg/mL for postmenopausal women. and 10 to 50 pg/mL for men. Pre-pubertal children's levels typically are below 30 pg/mL (Mayo Clinic 2023).

Exogenous estrogen would be more likely to have detectable effects in children, in post-menopausal females, and in males, where endogenous estrogen levels are relatively low (< 50 pg/ml). Therefore, it is uncertain whether breast cancers in women of reproductive age are caused or stimulated by exogenous estrogen.

The main carcinogenic mechanism of estrogenic compounds is estrogen-receptor mediated. Total estrogenic activity was studied in the Nurses' Health Study using a bioassay which reported that breast cancer risk was significantly greater (86%) in the highest vs lowest quartile of total estrogenic activity. However, this association was entirely explained by the estrone, estradiol, and estrone sulfate concentration in the blood, meaning that the contribution, if any, of xenoestrogens was not detectable against the background of physiologic hormones (Holder et al. 2022).

Most literature about endocrine disruptions focuses on the hormone-related effects which are well-documented in fish, but much less so in humans. From a risk and regulation perspective, it is likely that carcinogenicity, if it occurs, will occur at a much lower dose than significant hormonal effects, and would be the regulatory driver, for example, of setting an MCL.

DEFINITION OF MARGIN OF EXPOSURE (MOE) vs HAZARD INDEX

The principle is akin to the use of the HAZARD INDEX (HI), which is a value that can be used to determine the health concerns associated with exposure to chemical mixtures. The HI places the exposure level in the numerator and the benchmark or reference dose in the denominator. Therefore, the greater the value of the HI, the greater the potential hazard.

$$\mathbf{HI} = \frac{\mathbf{Exposure}}{\mathbf{Reference\ Dose}}$$

The principle of MARGIN OF EXPOSURE (MOE) is used more prevalently in food safety and cosmetic safety. The MOE uses a NOAEL/NOEC, the benchmark dose obtained from toxicology studies, in the numerator, and the dose, or the daily absorbed dose per kg body weight, in the denominator. Therefore, the greater the value of MOE, the "safer" the exposure scenario.

$$\mathbf{MOE} = \frac{\mathbf{NOAEL}}{\mathbf{Dose}}$$

An acceptable MOE for a NOAEL/NOEC-based assessment is 100 and for a LOAEL/LOEC-based assessment add an additional factor of 10 to give an acceptable MOE of 1,000 for a LOAEL/LOEMC-based assessment. In other words, a MOE >100 is considered protective, however if a LOAEL is used, then a MOE>1,000 is needed to be considered protective.

THE WHO 2012 REPORT

Charge question 1 goes on to ask the SAB to comment on a report published in 2012 by the World Health Organization (2012), specifically asking whether the current science supports the WHO's conclusion. In 2012, the WHO summarized water sampling data from various European countries and concluded "The substantial margins of safety for individual compounds suggest that appreciable adverse impacts on human health are very unlikely at current levels of exposure in drinking-water." The Margins of Exposure were mainly greater than 1000 comparing drinking water concentrations with the Minimal Therapeutic Dose. (WHO 2012:p ix). (MTD=in this case is minimal therapeutic dose rather than Maximal Tolerated Dose).

Note that in the 2012 report, the WHO acknowledged the general lack of monitoring data on chemicals in water. The WHO report focused mainly on pharmaceuticals. It included E1, E2, EE and tamoxifen, but not BAP or other industrial EDCs or pesticides.

The "current science" described in the charge question presumably refers to new information since about 2010. This could include:

a) new and more systematic information on the occurrence and concentrations of estrogenic compounds in New Jersey waters (*see Chapter 3*),
b) new information regarding toxicity and dose-response curves or thresholds for any of the hormones or pharmaceuticals under consideration, and/or
c) new information on how other states, agencies or countries are responding to estrogenic substances in drinking water (exposure could include dermal from bathing and inhalation from showering) including any screening levels, maximum contaminant levels (*see Chapter 2 for routes of exposure*).

Our reading of the WHO document is that it had limited sampling data for several drugs from several countries based on "ad hoc sampling". WHO compared modeled concentrations to the Minimal Therapeutic Dose. The WHO (2012) concluded that there was no justification for expensive routine water monitoring programs for drugs in general. However, WHO did allow for special circumstances or special programs to obtain data. The WHO report was a literature review aimed at pharmaceuticals with only peripheral attention to estrogens. We interpret the WHO suggestions to be mixed messages.

Caldwell et al (2010) arrived at a similar conclusion using a different approach. They calculated that any estrogenic exposure from drinking water would be much lower for children, particularly, than typical exposure to estrogenic compounds in food, particularly milk. However, the water concentrations they used were modelled not measured.

The findings of Caldwell et al (2010) are supported in an unusual editorial by 19 editors, published simultaneously in eight toxicology journals (Autrup et al. 2020). This is an informative review focused mainly on Europe. They noted that synthetic EDCs (S-EDC) (have so much lower potency than natural N-EDCs (e.g. phytoestrogens) and natural or pharmacological hormones, that one would need to ingest a thousand-fold higher amount of the S-EDC to compete with the N-EDCs. They provide several sources of data on relative potency suggesting

that an exogenous S-EDC molecule hardly has a chance to find a vacant receptor. Their skepticism is e, well-documented, and intended to put to bed regulatory concerns about S-EDCs ingestion in Europe. However, it is an opinion piece, republished multiple times, and should be interpreted cautiously.

EPA (2024) publishes a database which includes screening levels of about 800 chemicals. Bisphenol A is on the list with a RfD=0.05 mg/kg-d. For residential tap water, the screening levels for a child (corresponding to a Hazard Quotient of 1.0) is 770 ug/L. For DES the ingestion screening level is based on a cancer risk of 10E-06, yielding a screening level of 2.2E-04. E1,E2,E3,EE and nonylphenol are not on the screening list.

When the natural estrogens E1, E2, E3 were detected in fish muscle, they exceeded the drinking water guidance levels published by de Aquino (2021), sometimes by two or more orders of magnitude. For BPA and 4NP, exposure from ingestion of fish tissue concentrations in some instances exceeded and in others were lower than the de Aquino et al. guidelines for drinking water.

After describing controversial aspects of all harmful effects attributed to EDC's, EPA concluded that "Nevertheless, there is little doubt that small disturbances in endocrine function, particularly during certain highly sensitive stages of the lifecycle (e.g., development, pregnancy, lactation) can lead to profound and lasting effects."

Charge question 1 asks for the SAB's input on additional justification for the Department to develop screening levels for estrogenic compounds and pharmaceuticals, or certain classes of such contaminants, as recommended by the 2020 SAB report. The Committee concluded that at present the information on environmental occurrence of estrogen active chemicals in New Jersey waters is too sparse for confidence. The Committee reviewed the values arrived at by de Aquino et al (2021) and considered applying a Threshold of Toxicological Concern approach (Yamada et al 2021).

However, the Committee ultimately concluded that current information on environmental occurrence of estrogen active chemicals in New Jersey waters is too sparse to draw definitive conclusions. No evidence that environmental estrogen activities in New Jersey waters reaches concentrations that pose a human health risk. Nor, however, is there sufficient information to be confident in that observation. Therefore, we propose a targeted monitoring effort to better risk-inform decision-makers.

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CHAPTER 2: RESPONSE TO CHARGE QUESTION 2

Question 2 -What are the routes of human exposure?

To address this charge, the committee took two approaches to answer the question:

- First, the primary exposure pathway is ingestion, particularly of drinking water and fish consumption. Could fish from New Jersey waters constitute a significant ingestion route for New Jersey residents to endocrine disruptors?

- Second, could the inhalation and dermal routes of human exposure constitute significant exposure pathways to EDCs for NJ residents?

FIRST APPROACH STRATEGY FOR EVALUATING HUMAN EXPOSURES:

Ingestion (Oral) Exposure Pathway

Drinking water would be the primary exposure pathway to EDCs, however data on EDCs in New Jersey drinking water sources is sparse (see chapter 3). Likewise, there is very limited data on fish consumption as a source of EDC exposure.

To approach the first question, we surveyed the literature on relationships of concentrations of estrogenic substances in fish muscle with the concentrations of these substances in the ambient surface waters from which the fish were taken. Only fish muscle tissues were included in the analyses because those are the tissues most likely to be ingested.

XENOBIOTICS IN FISH

Eating fish and seafood is a major pathway for exposure to methylmercury (MeHg) and polychlorinated biphenyls. It is also a pathway for ingestion of industrial chemicals. These include per- and poly-fluorinated alkyl (PFAS) compounds and short-chain chlorinated paraffins (SCCP)s

Risk communication and risk management include dissemination of information, posting or closing of certain waters to fishing. Whereas EPA/FDA have a database on MeHg in many types of seafood, there is as yet no comparable database for the CECs (PFAS, SCCA, EDCs) in fish.

The following estrogenic indicator substances were surveyed:

- Natural estrogens: estrone (E1), 17-beta estradiol (E2), and estriol (E3)
- Synthetic estrogen: (for birth control and livestock uses) 17-alpha ethinylestradiol (EE2)
- Common industrial xenobiotics with estrogenic properties: bisphenol A (BPA) and 4-nonylphenol. (4NP)

Several authors had noted the lack of data on hormone residues in fish tissue: Xu et al (2006), Wang et al. (2012), Jakimska et al. (2013), and Guedes-Alonso et al. (2017). The analysis of water is relatively simple, compared to the complexity of the food matrix and the low concentrations to be quantified (in the order of ng/g) (Guedes-Alonso et al.,2014). The lack of data on concentrations of estrogen-active substances in fish tissue partly reflects analytic challenges only recently overcome with applications of LC-MS/MS. Separating the hormone active steroids from other steroids and the complex organic matrix of muscle tissue remains challenging.

Observations

We reviewed publications in peer-reviewed journals which reported concentrations of any of these six substances in fish muscle or in whole fish. We found reports from China, the Netherlands, and Middle East/ North Africa, published 2002-2022. Although numerous studies reported concentrations of one or more of the indicator substances in fish muscle, only three publications (Belfroid, 2002; Chen, 2012; Lv, 2019) compared fish muscle concentrations to their source water concentrations.

As indicated below (Table 1) the ratios of concentrations in fish muscle to ambient water indicate the occurrence of ***bioconcentration*** of these substances in fish muscle from source surface water. Bioconcentration factors of two to three orders of magnitude were consistently observed in those reports for the estrogenic substances assayed.

FISH CONSUMPTION

According to USEPA (2011) the mean fish consumption for consumers in the Middle Atlantic States is 16.2 g/day (0.6 oz). and the 95th percentile is 47.8 g/day (1.7 oz). For subsistence fishing EPA uses 142 g/day (5.0 oz) as the 95th percentile.

From the data compiled, we conclude that it is possible for residents of New Jersey to ingest a hazardous level of E1, E2, E3, BPA and 4NP from fish if the bioconcentration of the substance, in combination with the quantities of fish consumed, yields ingestion levels exceeding the health guidelines. Fish may pose a risk, if their source water have concentrations of estrogenic substances exceeding health guidelines for ingestion of those substances

- by ≥ 123x for most of the population,
- by ≥ 42x for the highest 5th percentile of consumers, or
- by ≥ 14x for subsistence fishers.

Although such exceedances have not been documented in New Jersey waters to date, the bioconcentration observations of estrogenic substances in fish muscle noted above suggest the possibility of such exposure levels.

Conclusions Regarding Drinking Water and Fish Consumption

Based on the publications available to date, ingestion of drinking water appears to be the route of greatest potential for environmental population exposure; ingestion of fish, however, may be a secondary route for some fish species and from some water bodies.

The EPA (2023) and FDA (2024) recommended fish consumption of 227-340 g/week (8-12 oz/week or 1.1-17 oz/day). To be clear, this is recommended for healthy eating, not for avoidance. Currently, there are no data available suggesting that fish derived from New Jersey waters have high concentrations of natural estrogens or estrogenic xenobiotics. However, as noted in the response to the charge regarding documentation of concentrations in New Jersey waters, monitoring for these substances has been limited, and further testing of surface water is recommended and planned.

With the exception of subsistence fishers and recreational salt-water fishers, most fish consumed in New Jersey comes from out-of-state sources. Freshwater fishing is usually associated with catch-and-release.

Table 2.1: Bioconcentration factors in fish from ambient surface water (Ratios of fish muscle to water concentrations) for indicator estrogenic substances in two publications from China *(rounded to 3 statistical figures)*

Reference and Fish Type	Substance	Mean fish muscle concentration ppb (mg/L)	Ambient water concentration ppb (mg/L)	Bioconcentration factor = Ratio of fish muscle to water
Chen, 2012 Tilapia	E1 (estrone)	0.80	0.0092	87.0
	E2 (17 beta estradiol)	1.40	<0.00477	>293
	E3 (estriol)	0.45	<0.00127	>354
	EE2 (17 alpha ethinylestradiol)	not detected	not detected	N/A
Lv, 2019 Various Species	BPA (bisphenol A)	30.8	0.8	38.5
	4NP* (4-nonylphenol)	238	1.03	232
	E1	0.42	0.003	140
	BPA	141	0.08	1,075
	4NP	48.8	0.5	97.6

*Calculations for 4-nonylphenol yielded an approximate hazard index of 0.16 for daily ingestion of 8 oz portions of fish with the 4-NP concentrations of 238 ng/g.

Additional Pertinent Observations on Fish from the Literature

In the one publication (Zhou, 2019) that compared fish muscle concentrations in herbivorous, omnivorous, and carnivorous fish, there was little indication of biomagnification for estrogens. BPA was highest in carnivorous fish but only fourfold-above herbivorous fish. Vine (2009) concurs that a predator fish (Pike) in England does not appear to biomagnify estrogens from organisms lower in the food chain. Belfroid (2002) evaluating bisphenol A only, found up to 330 ppb BPA in various surface water samples but only 1-11 ppb in fish muscle, concluding that this chemical does not bioamplify and therefore does not pose risk of estrogenic effects via fish ingestion.

Recently, Mheidli (2022) conducted a review of pharmaceuticals in water and fish tissue using 69 published studies from 21 countries comprising the region "Middle East and North Africa, MENA". The review included the four estrogens listed above (E1,E2,E3,EE2) while BPA and 4NP, which are not pharmaceuticals, were not included. Water, but not fish, concentrations were assessed. Risk quotients and bioconcentration factors were utilized to conclude that drinking water and fish consumption, separately and combined, comprise potential human risks for the three naturally- occurring estrogens, E1, E2, and E3, but not for the synthetic estrogen, EE2. A major limitation of this review is the combination of very heterogenous monitoring studies with different analytic tools and statistical approaches.

SECOND APPROACH STRATEGY FOR EVALUATING HUMAN EXPOSURES:

Inhalation and Dermal Exposure Pathways

The primary exposure pathway associated with the presence of estrogenic compounds in New Jersey public water systems is from direct consumption of potable water. However, potable water is also used for bathing which could introduce secondary exposure pathways from inhalation of volatized compounds (e.g., shower) and/or dermal exposure (e.g., bath). In addition, New Jersey waters (rivers and lakes) are used for recreational purposes that could pose inhalation/dermal exposure potential from activities such as swimming and other water sports.

The physio-chemical properties of a compound will influence its potential for inhalation or dermal exposure. For the inhalation pathway, a compound's volatility will be the primary determinant of exposure potential. Volatility is mainly a function of lipophilicity and low molecular weight. Generally, the same factors (octanol/water coefficient and low molecular weight) control a compound's ability to penetrate the skin and be absorbed percutaneously.

Overview of Screening Paradigm

As previously noted, consumption of drinking water is presumed to be the primary route of exposure to water-borne estrogenic compounds. Accordingly, the process for screening the potential significance of inhalation and dermal exposure pathways is through a relative source contribution with drinking water, rather than an absolute estimation of exposure. The first step is to set the exposure parameters for the drinking water pathway. A conventional approach would be to evaluate an adult (e.g., 70 kg) using an upper-bound estimate of daily water consumption (2 liters) (EPA-RAGS, 1989). Ingestion dose (mg/kg-day) can then be calculated for a potable water source with a known concentration of an estrogenic compound. EPA recommends that if the daily dose of a chemical from either the inhalation or dermal exposure pathway is minimal (e.g., < 10%) relative to water ingestion, it would be eliminated from further consideration. For example, EPA's Dermal Exposure Assessment Guidance (RAGS Part E, 2004) recommends screening out the dermal exposure pathway if dermal dose is less than 10% of dose from water ingestion.

Inhalation

Following the above paradigm, airborne concentrations of a specific estrogenic compound could be modeled under a common exposure scenario (e.g., showering). An event-based dose could be estimated and compared to the dose from a standardized drinking water

exposure scenario. While this approach would constitute an effective screening tool, it could prove arduous when used to screen multiple chemicals or when exposure scenarios vary. A more simplified approach would be to use a combination of boiling point (BP), vapor pressure (VP) and carbon number to assess volatility of water-borne chemicals.

Generally speaking, VOCs are defined by specific chemical characteristics [3,4]:

- Chemicals with BP > 216 ^{o}C have limited volatility; VOC are defined as a chemical with a BP < 250 ^{o}C
- Chemicals with a VP < 0.1 mm Hg are considered non-volatile
- Chemicals with more than a 12-carbon chain have limited volatility

The criteria above relating carbon chain length to volatility applies to aliphatic chemicals, for which estrogenic compounds (having mostly aromatic structures) generally do not belong. Therefore, carbon chain length should be eliminated as a screening criterion; hence, any compound with either a BP > 250 ^{o}C or a VP < 0.1 mm Hg will be considered of limited volatility and screened out of an inhalation exposure pathway. Referring to Table 2, the vapor pressures of the estrogenic compounds listed are well below the screening value of 0.1 mm Hg (1 Pa = 133 mm Hg). Typical shower temperatures are about 40C, with 50C being a dangerously hot shower. We conclude that inhalation during showering is a negligible pathway for estrogenic compounds.

Dermal Absorption

It is noted that pharmaceutical formulations delivering estrogenic compounds to the systemic circulation through gels and skin patches are designed accordingly and possess biokinetic profiles distinct from the assessment of transdermal absorption of waterborne estrogenic compounds in an environmental milieu. Transdermal formulations are engineered to deliver a constant and predictable amount of drug to the circulation. Estrogen replacement therapy for post-menopausal women is particularly well suited for the use of gels and skin patches. Some clinical uses of estradiol involve dermal application from a concentrated formulation, that is much higher (17,000 mg/L) than relevant environmental concentrations. From a dermal pathway perspective, environmental exposure to estrogenic agents in water at much lower concentrations are less predictable; hence, a paradigm for assessment is outlined below.

The approach for evaluating the dermal exposure pathway for water-borne chemicals reflects EPA's Risk Assessment Guidance for Superfund (Part E):Dermal Exposure Assessment, 2004. Absorption of water-borne chemicals through the skin is influenced by contaminant concentration, contact time with the skin and surface area contact. However, the single most important parameter is the permeability coefficient (K_p - cm/hr) of the chemical which is a

[3] As defined by California Air Resources Board (CARB) Low Vapor Pressure – Volatile Organic Compound Research, https://ww2.arb.ca.gov/our-work/programs/consumer-products-program/complying-regulations/lvp-voc-research (Last Accessed 3/3/2025)

[4] As defined by the US EPA, Techinical Overview of Volatile Organic Compounds. https://www.epa.gov/indoor-air-quality-iaq/technical-overview-volatile-organic-compounds#:~:text=A%20VOC%20is%20any%20organic,5%2C%206%2C%207 (Last Accessed 3/3/2025)

function of the chemicals octanol/water partitioning coefficient (K_{ow}) and MW. Equation 1 below relates the permeability coefficient (K_p) to octanol/water coefficient (K_{ow}) and MW.

A simplified approach would be to screen dermal exposure potential using the K_p value of approximately 0.01 cm/hr - see Section 3 and Appendix A of RAGS Part E (2004). That is for a chemical with a Kp less than 0.01 cm/hr the dermal pathway would be screened.

Equation 1.1: *K_p Equation*

$$\log K_p = -2.80 + 0.66 \log K_{ow} - 0.0056\, MW$$

where:
K_p = Dermal permeability coefficient of compound in water (cm/hr)
K_{ow} = Octanol/water partition coefficient (dimensionless)
MW = Molecular weight (g/mole)

Table 2.2: Permeability coefficient (K_p) Calculation for example estrogens

Name	**Estrone**	**Estradiol**	**Estriol**	**Ethinylestradiol**
Molecular Weight (g)	270.37	272.3	288.39	296.4
Vapor Pressure (Pa)	3E-08	3E-08	9E-13	6E-09
Solubility (mg l^{-1})	13	13	13	4.8
Henry's Constant (Pa m^3 mol^{-1})	6.2E-7	6.3E-7	2.0E-11	3.8E-7
Log K_{ow}	3.43	3.94	2.81	4.15
Calculated Kp (cm/hr)	0.009	0.02	0.002	0.02

Based on the Kp calculations above, estradiol and ethinylestradiol having Kp values > 0.01 cm/hr could contribute a significant percent (> 10%) of the dermal exposure pathway. Accordingly, evaluation of the dermal r exposure pathway, for example for bathing would be indicated in determining the significance of total multi-pathway exposure for these substances.

Summary

Since there is a dearth of information documenting estrogenic substances from dietary exposures, including from fish, estimations were extrapolated from published reports on concentrations in edible fish and their source waters. Evaluation of data pertinent to potential exposure via fish consumption suggests that this route could constitute hazardous exposures to water-borne estrogenic compounds to people eating fish caught from New Jersey waters. Biomagnification of estrogenic substances in carnivorous fish has not been supported in the literature thus far, but this process could be revisited if future data from drinking and surface water monitoring in New Jersey suggest a greater likelihood of this exposure route than is currently indicated. The applicable scenarios would entail concentrations of these chemicals down to two orders of magnitude below health guidelines and/or extremely high levels of fish consumption. Those scenarios remain theoretical until or unless there is evidence of surface water concentrations in New Jersey involving such exposures.

Evaluation of inhalation and dermal exposure to water-borne estrogenic compounds has determined that inhalation exposure is negligible while percutaneous exposure may be significant *relative* to the drinking water exposure pathway. Consequently, in scenarios where drinking water consumption represents a significant exposure, it may also be necessary to evaluate the contribution from dermal exposure.

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CHAPTER 3: RESPONSE TO CHARGE QUESTION 3

Question 3 - What has been done since the joint monitoring efforts conducted with USGS circa 2008 and should such efforts be resumed?

Introduction

This section comprises: 1) a detailed discussion of two monitoring efforts conducted on New Jersey waters since 2008, one by the Delaware River Basin Commission (DRBC, 2013), and one jointly by the U.S. Geological Survey (USGS) and the New Jersey Department of Environmental Protection (NJDEP) (USGS/NJDEP, 2018); 2) a summary of a paper by de Aquino et al. (2021) that describes a monitoring study in Brazil and a human health risk assessment; 3) a compilation and interpretation of data from the first monitoring effort in New Jersey by USGS and NJDEP (Stackelberg et al., 2004), the two newer studies, and the U.S. Environmental Protection Agency's Unregulated Contaminants Monitoring Rule 3 (UCMR3) testing from 2013-2016 (USEPA, n.d.); and 4) recommendations for future monitoring.

Summary of two studies conducted in New Jersey since 2008, of sex hormone-related chemicals and other compounds in water

Since the first joint monitoring effort by USGS and NJDEP (Stackelberg, 2007), two monitoring efforts in New Jersey waters that included sex hormone-related compounds have been performed. These monitoring efforts include a) sampling for contaminants of emerging concern (CEC) in the tidal Delaware River basin by the DRBC (2013); and b) occurrence of CECs in wastewater and sludge from three publicly owned treatment works in New Jersey (USGS/NJDEP, 2018). Another monitoring study looked specifically at antibiotics (Gibs et al., 2013), and is briefly summarized in the Appendix Tables. Other large-scale evaluations for hormones, pharmaceuticals, and/or pesticide metabolites in groundwater across the U.S. have been conducted; these papers are summarized in the Appendix Tables (Bexfield, 2019; Mahler, 2021).

a. Contaminants of Emerging Concern in the Tidal Delaware River, Pilot Monitoring Survey 2007-2009

The Delaware River Basin Commission (DRBC) published a document entitled *Contaminants of Emerging Concern in the Tidal Delaware River* (2013). The compounds included in that evaluation included pharmaceuticals, hormones, and sterols, perfluoroalkyl and polyfluoroalkyl substances, and polybrominated diphenyl ethers collected from the tidally influenced portions of Delaware River from Trenton to the head of Delaware Bay over a three-year period (2007- 2009).

Analytical samples were analyzed using different methods appropriate for the specific compounds. Pharmaceuticals were analyzed using liquid chromatography/electrospray ionization/tandem mass spectroscopy (LC/ESI-MS/MS) in positive and negative ion modes. Sterols and Hormones were analyzed using gas chromatography (GC) and low-resolution MS. Perfluoroalkyls were analyzed using LC MS/MS. Polybrominated diphenyl ethers were analyzed using GC and high-resolution

MS. Nonylphenols were analyzed using GC MS. Bisphenol A was analyzed using LC MS/MS.

Fifty-seven (57) pharmaceutical compounds were detected during the evaluation in the nanogram per liter (ng/L) range. Ten compounds detected in all three years include azithromycin, caffeine, carbamazepine, clarithromycin, codeine, dehydronifedipine, diltiazem, diphenhydramine, erythromycin-hydrate, and fluoxetine. The DRBC concluded that the following 15 pharmaceutical compounds should be considered priority pollutants for future monitoring and assessment in surface waters of the tidal Delaware River: acetaminophen, carbamazepine, clarithromycin, codeine, dehydronifedipine, erythromycin-hydrate, fluoxetine, gemfibrozil, 2-hydroxyibuprofen, ibuprofen, lincomycin, metformin, sulfamethoxazole, thiabendazole, and triclocarban.

Sterols and hormones were both included in the DRBC evaluation in 2007 and 2008. However, fecal sterols (coprostanol, epicoprostanol, cholestanol) and desmosterol as well as the plant sterols (campesterol, stigmasterol and beta-sitosterol) were detected. DRBC concluded that the presence of fecal sterols indicates the presence of human sewage but are not major contributors to ecotoxicity in the river. Therefore, in 2009, only hormones were analyzed. Hormones detected in 2007, 2008 and 2009 at low concentrations included estrone, norethindrone, 17-α- ethynylestradiol, desogestrel and testosterone. DRBC calculated hazard quotients of 0.2 (long-term exposure) and 0.07 (short-term exposure) for estrone in the Delaware River. The EPA has listed the following 12 hormones which may require regulation under the Safe Drinking Water Act (SDWA) on the Contaminant Candidate List 3 (CCL3) and/or Unregulated Contaminants Monitoring Rule 3 (UCMR3): 17- alpha estradiol, equilenin, equilin, 17-beta estradiol, estriol, estone, 17-alpha ethynylestradiol, mestranol, norethindron, testosterone and 4-androstene-3,17-dione.

Perfluoroalkyl and polyfluoroalkyl substances were detected in the DRBC evaluation in the ng/L range. The DRBC evaluation tested for 13 substances; all but two substances (perfluorododecanoic acid and perfluorooctanesulfonamides) were detected in the evaluation.

Polybrominated diphenyl ethers were evaluated for the DRBC study. Forty-six (46) individual congeners (grouped compounds with similar structures) were evaluated. The homologs with the maximum concentrations were decabromodiphenyl ethers and nonabromodiphenyl ethers. The most dominant (in frequency and concentration) homolog was decabromodiphenyl ethers detected in the range of 2,090 to 7,630 pg/L. Nonabromodiphenyl ethers, pentabromodiphenyl ethers, and tetrabromodiphenyl ethers were predominant at different testing locations in the range of 29 to 161 pg/L. Overall, total polybrominated diphenyl ethers (tPBDE) were detected at concentrations in the range of 87 to 9,376 pg/L.

Nonylphenol ethoxylates are surfactants used in detergents and other industrial applications. Nonylphenols are found in the environment as decay products of nonylphenol ethoxylates. Nonylphenols are considered more toxic than nonylphenol

ethoxylates. Although nonylphenols are regulated, their toxicity is still emerging in the area of estrogenic effects. The concentrations of nonylphenols measured by the DRBC had a maximum of 0.0876 µg/l, which IS considered below a protective level. Because of its widespread occurrence and the evolving knowledge of its ecotoxicity, DRBC characterized nonylphenols as a contaminant of emerging concern.

b. A Reconnaissance of Contaminants of Emerging Concern in Wastewater and Sludge from Three Publicly Owned Treatment Works in New Jersey

The Committee reviewed the 2018 report between NJDEP Division of Science, Research and Environmental Health and the USGS New Jersey Water Science Center entitled *A Reconnaissance of Contaminants of Emerging Concern in Wastewater and Sludge from Three Publicly Owned Treatment Works in New Jersey* (2018). The Report evaluated CECs from different classes of synthetic contaminants including pharmaceuticals, pesticides, sterols and hormones, flavors and fragrances, alkylphenol ethoxylates, polyaromatic hydrocarbons, and per- and polyfluorinated alkyl substances. . Wastewater, landfill leachate, and sludge taken from three New Jersey regions were evaluated for multiple contaminants to determine the likelihood of occurrence in various types of developed areas including residential, commercial, industrial (including two areas with pharmaceutical processing), and hospital/retirement areas. Analytical methods that were used, like the DRBC evaluation, were compound specific.

Fifty-five (55) individual pharmaceutical compounds, classified into seven categories, were evaluated for this joint study. The seven classifications are stimulants, pain relievers, mood stabilizers, antimicrobials, opioids/barbiturates/muscle relaxers/anti-coagulants, anti-coagulants/blood pressure medications/antihistamines and an anti-retroviral therapy drug.

For pharmaceuticals, nine wastewater compounds were detected at each sampling location. Wastewater treatment plants reduced the number and concentration of pharmaceuticals detected, but all sludge samples contained at least five pharmaceuticals' compounds at detectable levels.

Twelve pesticide/herbicide compounds were included in this class of samples. Five of the 12 compounds were detected in wastewater samples. The large variation in instrument reporting levels for the sludge samples confused the interpretation of that data. The compound thiabendazole, an antihelminthic, was the only substance detected in the sludge samples.

The wastewater and sludge samples retrieved for the study were analyzed for five sterols and seventeen hormones. The hormones detected included those typically related to androgen and estrogen hormone replacement therapy. At least four of the seventeen hormones included in the sampling were detected at each location, with many locations having at least nine hormones detected. Two androgen hormones were detected at the highest concentrations and in the most locations.

One estrogenic compound was detected in a single wastewater sample. The authors reported that analysis for sterols appeared to be compromised by matrix effects, resulting in three of the five compounds being near the reporting level (RL). Therefore, even for results that were well above the RL, the results for all sterols were qualified as "estimated". For both the sterols and hormones, occasionally the analysis was not able to produce a reportable result for treated effluents. Therefore, a proper evaluation of the treated effluent concentrations was not obtained.

Wastewater, treated water, and landfill leachate were analyzed for thirteen compounds that were classified as flavors and fragrances. Sludge samples were analyzed for ten of these compounds including 3-methyl-1h-indole (skatole), camphor, and menthol. Many of these compounds can occur naturally or through the synthetic manufacture and use of these compounds. The twenty wastewater sampling locations contained detectable levels of between eight and twelve of the compounds. The concentration of the flavors and fragrances in these wastewater samples was generally low, save for three compounds; 5-methyl-1h-benzotriazole (BHA), menthol, and methyl salicylate which were detected at higher levels. Menthol was detected at every wastewater sampling location, except one. The number of flavors and fragrances, as well as the magnitude of the detection was reduced in the treated effluent.

Fourteen phenols and alkylphenol ethoxylate compounds were measured in wastewater during this study. These compounds include alkylphenols, alkylphenol ethoxylates, phosphates, and bisphenol A which are used in the manufacture of resins, polymers, fire-retardants and surfactants. Many of these compounds are known for their estrogenic activity. Across the study area, each sample of untreated wastewater contained detectable levels of between five and ten of the fourteen phenols and alkylphenol ethoxylate compounds. The most commonly detected analytes included benzophenone, and tris(2-butoxyethyl) phosphate. Only three phenols and alkylphenol ethoxylate compounds were detected in the effluent, and at lower levels than were measured in the influent. The three samples of landfill leachate contained between four and nine detectable phenols and alkylphenol ethoxylate compounds. Concentrations of these compounds were typically comparable to those found in other wastewater samples, except bisphenol A which was at low concentrations in the wastewater samples but were found at greater concentrations in the landfill leachate.

Seventeen polycyclic aromatic hydrocarbon (PAH) compounds were measured in the wastewater, and fifteen PAHs were quantified in sludge for this evaluation. The PAH compounds include anthracene, benzo(a)pyrene, phenol, and naphthalene and can form during the incomplete combustion of hydrocarbons as well as from manufacturing processes containing these chemicals. Several of these compounds have New Jersey groundwater quality criteria or surface water criteria. Detectable levels of at least three of the PAH compounds were found in all wastewater samples. The most commonly detected compounds were phenol and p-cresol, which were found in every wastewater sample collected. Overall, the p-cresol concentrations were higher before treatment and showed markedly lower results in the effluent. Between 8 and 11 of the PAHs were

detected in the sludge samples collected from the three POTWs. PAHs with the highest concentrations in the dried materials were typically p-cresol, and 9,10-anthraquinone.

For wastewater, the two locations with detectable levels of a perfluorinated compound included an industrial area with pharmaceutical processing, and a retirement community location. The influent to the wastewater treatment plant also contained detectable levels of perfluorinated compounds. Overall, levels of compounds in the influent were not always different than the levels in the effluent, suggesting that traditional wastewater treatment has not removed perfluorinated compounds. For sludge, between seven and ten of the thirteen perfluorinated compounds were detected in the analysis of dried and wet filter cakes, respectively.

A common theme between the various reports and articles researched for this charge is the need for an increased understanding of the concentrations and loads that the human population is contributing to the environment.

Summary of de Aquino Paper and its Relevance

The aim of de Aquino et. al. (2021), was to set a priority list of pharmaceuticals and endocrine disrupting compounds (P&EDCs) in Brazilian water by using a quantitative chemical risk assessment (QCRA).

A list of P&EDCs to be used in the QCRA was prepared using the following criteria:

- Top 20 selling pharmaceutical active ingredients and associations of active ingredients in Brazil from the "2017 Pharmaceutical Market Statistical Yearbook";
- Antibiotics that have controlled sales;
- Occurrence in surface, ground, and/or drinking water through literature review. (Foreign data was used to compare values published in Brazilian studies).

The authors estimated Guideline Values (GV) for P&EDCs in drinking water using the following equation:

$$\text{GV (ug/L)} = \frac{\text{ADI (ug/kg/d)} * \text{BW (kg)}) * \text{AF}}{\text{V (L/d)}} \quad ,$$

where,

ADI= acceptable daily intake
BW = body weight (60 kg)
AF = Allocation Factor (proportion of ADI attributed to DW consumption)
V=daily water intake (2 liters / day)

The estimated GVs were then used to calculate the margin of exposure for drinking water consumption.

Acceptable Daily Intakes (ug/kg/d) were calculated as follows:

ADI = $\frac{Reference\ Dose\ (ug/kg/d)}{UF}$,
where,

References Dose were based on various QCRA approaches in the literature (LDTD, NOAEL, LOAEL, etc.)

UF = Uncertainty Factor

A UF of 1,000 was used for deriving the GV from LDTD based on the following:

- 10 to account for response changes in humans
- 10 to account for protection of sensitive sub-groups (children/infants)
- 10 to account for the fact that LDTD is not a no-effect level

Additional UFs were considered for deriving GVs from NOAEL or LOAEL based on the following:

- 10 to account for interspecies variation
- 10 to account for intraspecies variation
- 10 when using data from a subchronic study
- 10 when using a LOAEL instead of a NOAEL

In both approaches, additional safety factors were applied for each of the following:

- 10 to account for Cytotoxic pharmaceuticals considering the high toxicity level of these compounds
- 10 to account for Endocrine disruptors, considering the potential effects on hormonal function and fertility are unwanted by individuals not treated by these medications

Different Allocation Factors were adopted depending on the authorized use of the compound as follows:

- AF of 1.0 was adopted for pharmaceuticals prescribed only to humans based on the premise that such a CEC was widespread in the environment and unlikely to be found in food.
- AF of 0.1 was considered for the case of pharmaceuticals used for agriculture or veterinary purposes
- AF of 0.2 was used for natural estrogen hormones and compounds that mimic them
- AF of 0.6 was used for bisphenol A based on the "European Food Safety Authority" Study

Guidance Values (GVs) for known carcinogens were calculated based on the risk of the carcinogenicity formula below:

GV (ug/L) = $\frac{R*BW\ (kg)}{SF\ (kg*d)*V}$ * 1000(ug) / 1 (mg), where

R = lifetime cancer risk (10^{-4} or 10^{-6})
BW (60kg)
SF=Slope factor (California OEHHA)

V=average daily water intake (2 L/d)

Margin of Exposure (ME) was calculated using the following formula, ME = GV / OC, where

GV = the lowest GV estimated for a given CEC;
OC = occurrence of CEC in drinking water (maximum reported concentration in literature)

The ME represents how much the occurrence of the CEC is lower or higher than the GV and is interpreted as follows:

ME ≤ 1:	CEC found in DW at concentration greater than or equal to its GV. Therefore, represents a high risk to human health.
1< ME ≤ 10:	CEC found at concentration slightly lower than its GV. Therefore, considered an "alert" situation because the occurrence is at the same order of magnitude as the concentration that would represent a health risk to humans.
10 < ME ≤ 100:	CEC found in drinking water up to two orders of magnitude less than their GVs. Therefore, a moderate risk to human health.
100 < ME ≤ 1000:	CEC found in drinking water up to three orders of magnitude less than their GVs. Therefore, a low risk to human health.
ME > 1000:	CEC found in drinking water MORE than three orders of magnitude less than their GVs. Therefore, a negligible risk to human health.

Antimicrobial resistance induction was estimated by calculating a risk quotient (RQ) using predicted no effect concentrations (PNEC) using the following formula:

RQ = $\frac{MEC\ (\frac{ng}{L})}{PNEC\ (\frac{ng}{L})}$, interpreted as follows:

RQ ≥ 1 = high ecological risk
0.1 ≤ RQ <1 = Moderate ecological risk
RQ < 0.1 = Low risk

Based on observed concentrations of compounds in water in comparison to Guideline Values, de Aquino et al. made the following conclusions:

The following seven compounds were judged to have "alert" risk to human health status with (1<ME≤10):

- Trimethoprim
- Propanolol
- Estradiol
- Acetylsalicylic Acid
- Atorvastatin
- Diclofenac
- Ketoprofen

The following seven compounds showed a high risk to human health status with (ME<1):

- 17-alpha-ethinylestradiol
- 17-beta-estradiol
- Betamethasone
- Dexamethasone
- Prednisone
- Naproxen
- Estrone

The remaining CECs were judged to have a moderate to negligible risk to human health.

CONCLUSION

The de Aquino study covered many classes of pharmaceuticals. It is notable that E1, E2 and EE2 were in the highest health risk category, along with other commonly used steroid compounds. This finding suggests the importance of obtaining more data on these compounds in drinking water. De Aquino is a convenient reference going from concentrations to guidance values. The Committee considered them useful preliminary values but does not endorse these values nor the methodology.

Compilation and summary of analytical data for chemicals with sex hormone-related activity in New Jersey waters

Altogether, three surveys of chemicals with potential sex hormone-related activity (two of which are described above) are selected for summary tabulation in this section. These three studies, by Stackelberg et al. (2007), the DRBC (2012), and the USGS and NJDEP (2018), have provided important information regarding the presence and concentrations of these substances in water resources and associated media in New Jersey waters specifically. However, the surveys were conducted during different periods of time, examined different components of water resources, and cannot be considered either representative or comprehensive. Relevant aspects of each study will be briefly summarized here:

- Stackelberg et al. (2007) reported on a joint effort of the U.S. Geological Survey and the New Jersey Department of Environmental Protection to sample one conventional drinking water treatment plant at six points along the treatment process chain, from screened source water through the finished water as it entered the distribution system. Samples were taken in July and August of 2003. The drinking water treatment plant is located in an unspecified urban watershed, into which over 50 wastewater treatment plant discharge upstream.

- The Delaware River Basin Commission (DRBC 2012) survey monitored a variety of unregulated substances. DRBC sampled Delaware River water at six locations along its length from below Trenton to the Delaware Bay. Samples from 2007 and 2009 were taken in October, while samples from 2008 were taken in August.

- In 2010-2011, USGS and NJDEP conducted a survey of the sewer-sheds in three areas of New Jersey (NJDEP, 2018). One sewer-shed was characterized as “residential with small

commercial areas," one was "urbanized," and the third was "urban and industrial." Samples were taken in various locations in the sewer collection system; at the inflow to the wastewater treatment plant, at its outflow, and in solids removed from the treatment process (sludge). The time period of sample collection is reported as February 2010 to August 2011, but dates are not associated with sewer-sheds or sample points in the draft report.

In all three of these surveys, the target analytes included other types of unregulated chemical substances beyond those discussed in this report. There is also a fourth monitoring effort included in this summary:

- The U.S. Environmental Protection Agency published the Third Unregulated Contaminant Monitoring Rule (UCMR3) in 2012. Among other requirements, this federal rule required certain community water systems in the U.S. (all systems serving more than 100,000 people and a nationally--but not necessarily state-level--representative sample of systems serving fewer than 100,000 people) to test treated drinking water for seven hormones (referred to by USEPA as "List 2" substances). Sampling occurred in the period 2013-2016.

Table 3.1 summarizes the findings of the four data sources. Columns in the table present the findings of each monitoring effort. Columns are ordered with wastewater inflow and outfall sample data on the left, receiving bodies of water in the middle, and treated or finished drinking water to the right. Rows show data by specific sex hormone-active substances that were included in the NJDEP's "Phase 1 Contaminant List" which was meant to guide the Public Health Subcommittee's initial discussions (versions of September 20, 2022 and April 8, 2022). This list included hormones and other chemicals with estrogenic or androgenic activity. It is important to emphasize that the samples of wastewater, source water, and finished drinking water summarized in Table 3 were taken from different locations and at different times, and there were differences in the target analytes tested for in these media. (*See Text Box 3.1 for generalized conclusions.*)

We conclude that water sampling to date, while informative, has not been adequate to characterize the degree to which Phase 1 Contaminant List substances occur throughout the wastewaters, drinking water source waters, and finished drinking waters in New Jersey. While the UCMR 3 data are encouraging, particularly for large systems serving 100,000 people or more, it must be repeated that only a small number of water systems in New Jersey that serve fewer than that number had to test. It is not clear to what degree those smaller systems that were tested were representative of New Jersey systems.

Text Box 3.1: Generalizations made from the data presented in Table 3.1:

- ***Many Phase 1 Contaminant List substances were detected in inflows to wastewater treatment plants, based on data from USGS/NJDEP (2018).***

- ***Compared to other waters tested, concentrations of Phase 1 Contaminant List substances were typically highest in samples of these wastewater plant inflows.***

- ***Some substances appeared in lower concentrations at lower detection frequencies in wastewater plant outfalls than in inflows, indicating the potential for some removal by standard wastewater treatment process, while other substances did not show a concentration reduction.***

- ***With some exceptions, samples from sources waters (either the Delaware River samples from DRBC (2013) or the raw intake water samples from Stackelberg et al. (2007) showed lower concentrations and fewer detections of Phase 1 List substances.***

- ***Finished drinking water samples from Stackelberg et al. (2007) and the UCMR 3 survey showed lower concentrations or detections frequencies of many of these substances than untreated source waters, indicating that conventional drinking water treatment may reduce the concentrations of some (but not all) of these substances.***

- ***Twelve Phase 1 Contaminant List substances were detected in at least one finished drinking water sample from Stackelberg et al. (2007) and UCMR 3. Of these 12, only three had a Provisional Comparison Value (PVC) from the de Aquino (2021) study (listed as the GV in de Aquino). In these cases, treated drinking water concentrations were far below the PCV.***

Table 3.1: Analyte Detection Frequencies (DF) and maximum detections (all values in *micrograms per liter*) from principal surveys of Phase 1 contaminants in wastewater or drinking water samples in New Jersey. Phase 1 contaminant lists were provided by NJDEP to the Public Health Subcommittee and were meant to include hormones and other chemicals with estrogenic or androgenic activity. The right-most column indicates whether there is a Provisional Comparison Value for that substance in drinking water from de Aquino et al. (2021). Data sources and a key to abbreviations are listed at the end of the table.

Report	**USGS/NJDEP, 2018**		**DRBC, 2013**	**Stackelberg et al., 2007**		**USEPA-UCMR 3 Screening Survey (List 2)**	**Provisional Comparison Value (from de Aquino et al., 2021)**
Sampling Period	**2010-2011**		**2007-2009**	**2003**		**2013-2016**	
Medium Sampled / Analyte	**Wastewater treatment plant inflow to 2 or 3 plants**	**Wastewater treatment plant outfalls from 2 or 3 plants**	**Delaware River, annual samples at six locations from Trenton to Bay**	**Raw intake drinking water for 1 source**	**Finished drinking water from 1 source**	**Finished drinking water from 28 systems**	
Phase 1 Contaminants from NJDEP's September 20, 2022 List							
Sex Hormones							
17-α-Ethinylestradiol (EE2)	DF=67% Max=0.79	DF=33% Max=1.5	DF=22% Max=0.004	--	--	DF=0% RL=0.0009	0.003
Estrone (E1)	DF=67% Max=435	DF=33% Max=27.2	DF=6% Max=0.0013	--	--	DF=0% RL=0.002	0.078
17-α-Estradiol (17-α-E2)	DF=67% Max<0.80	DF=33% Max<0.80	DF=0% DL=0.001-0.004	--	--	--	--
17-β-Estradiol (E2)	DF=67% Max=7.4	DF=33% Max=8.3	DF=0% DL=0.0011-0.004	--	--	DF=0% RL=0.0004	0.008
Equilin	DF=67% Max<926	DF=33% Max<4.0	DF=0% DL=0.001-0.008	--	--	DF=0% RL=0.004	--
Equilenin	DF=67% Max=15	DF=33% Max<2.0	DF=0% DL=0.0008-0.0009	--	--	--	--
17-α-*dihydro*-Equilin	--	--	DF=0%	--	--	--	--

			DL=0.001-0.004				
Estriol (E3)	DF=67% Max=144	DF=33% Max<2.0	DF=0% DL=0.0034-0.016	--	--	DF=0% RL=0.0008	0.01
Diethylstilbestrol	DF=67% Max<0.8	DF=33% Max<0.8	--	--	--	--	--
Mestranol (meEE2)	DF=67% Max<0.8	DF=33% Max<0.8	DF=0% DL=0.0013-0.020	--	--	--	--
Norethindrone	DF=67% Max<0.8	DF=33% Max<0.8	DF=6% Max=0.0042	--	--	--	--
Progesterone	DF=67% Max=10.2	DF=33% Max<8.0	DF=0% DL=0.0008-0.024	--	--	--	--
4-Androstene-3,17-dione	DF=67% Max=222	DF=33% Max=52	DF=0% DL=0.002-0.011	--	--	DF=4% Max=0.0015	--
Cis-Androsterone	DF=67% Max=1,710	DF=33% Max=1,670	DF=0% DL=0.0055-0.081	--	--	--	--
Testosterone	DF=67% Max=47.2	DF=33% Max=37.1	DF=6% Max=0.0014	--	--	DF=4% Max=0.00097	--
11-keto-Testosterone	DF=67% Max=64	DF=33% Max=6.8	--	--	--	--	--
Dihydro-Testosterone	DF=67% Max=61	DF=33% Max=43	--	--	--	--	--
Epitestosterone	DF=67% Max<12	DF=33% Max=14	--	--	--	--	--
Organohalogens							
Methyl triclosan	--	--	--	--	--	--	--
Triclosan	DF=100%	DF=67%	--	DF=0%	DF=0%	--	150

	Max=3.8	Max=0.6		RL=1	RL=1		
Tris(2-chloroethyl) phosphate (TCEP)	--	--	--	DF=100% Max=0.12	DF=8% Max=0.05	--	--
Non-Halogenated Compounds							--
Bisphenol A	DF=50% Max=0.31	DF=50% Max=0.44	DF=0% DL=0.00005	DF=67% Max=0.36	DF=17% Max=0.22	--	72
4-Nonylphenol (4-NP), linear or branched	DF=100% Max=10	DF=100% Max=3.7	DF=100% Max=0.088	DF=25% Max=1.4	DF=8% Max=1.1	--	90 (Nonylphenol)
4-tert-Octylphenol (OP)	DF=100% Max=0.46	DF=100% Max=0.48	--	DF=0% RL=1	DF=0% RL=1	--	90 (Octylphenol)
Tributyl phosphate	DF=100% Max=0.37	DF=100% Max=0.33	--	DF=42% Max=0.14	DF=8% Max=0.18	--	--
Pesticide Transformation Products							
4-Hydroxychlorothalonil	--	--	--	--	--	--	--
Deethylatrazine (DEA)	--	--	--	--	--	--	--
Desulfinylfipronil	--	--	--	--	--	--	--
Fipronil amide	--	--	--	--	--	--	--
Fipronil sulfone	--	--	--	--	--	--	--
Additional Phase 1 Contaminants from NJDEP's April 8, 2022 List							
Acetominophen (Paracetamol)	DF=100% Max=91	DF=0% RL=0.64	DF=17% Max=0.11	DF=75% Max=0.12	DF=17% Max="0" (RL=0.036)	--	160
Acetyl hexamethyl tetrahydronaphthalene (AHTN, Tonalide)	DF=100% Max=0.52	DF=67% Max=0.24	--	DF=100% Max=0.2	DF=58% Max=0.068	--	--
Caffeine	DF=100% Max=78	DF=100% Max=0.33	DF=94% Max=0.24	DF=100% Max=0.19	DF=25% Max=0.06	--	--
Carbamazepine	DF=67% Max=0.45	DF=100% Max=0.33	DF=100% Max=0.067	DF=92% Max=0.6	DF=100% Max=0.14	--	--
Carbaryl	DF=0% RL=0.38-1.1	DF=0% RL=0.38	--	DF=50% Max=0.12	DF=0% RL=1	--	--

Carbazole	DF=0% RL=0.03-0.32	DF=0% RL=0.03	--	DF=42% Max=0.072	DF=0% Max=0.5	--	--
Codeine	DF=67% Max=0.38	DF=67% Max=0.27	DF=83% Max=0.16	DF=8% Max=0.01	DF=8% Max=0.03	--	--
Diazinon	DF=0% RL=0.16-0.48	DF=0% RL=0.16	--	DF=50% Max=0.14	DF=0% RL=0.5	--	--
Fluoxetine	DF=0% RL=0.64-1.9	DF=0% RL=0.64	DF=17% Max=0.008	DF=0% RL=0.014	DF=0% RL=0.014	--	--
Hexahydrohexamethyl-cyclopentabenzopyran (HHCB, Galaxolide)	DF=100% Max=6.4	DF=100% Max=3.9	--	DF=92% Max=0.085	DF=0% RL=0.5	--	--
3-methyl-1H-indole (Skatole)	DF=100% Max=3.4	DF=33% Max=0.021	--	DF=0% RL=0.5	DF=0% RL=0.5	--	--
Metolachlor	DF=0% Max=0.08-0.54	DF=33% Max=0.019	--	DF=58% Max=0.11	DF=0% RL=0.5	--	--
Triphenyl phosphate	DF=100% Max=0.22	DF=100% Max=0.22	--	DF=75% Max=0.08	DF=0% RL=0.5	--	--
Tris(2-butoxyethyl) phosphate (TBEP)	DF=100% Max=54	DF=100% Max=8	--	DF=100% Max=0.57	DF=0% RL=0.5	--	--
Tris(dichloroisopropyl) phosphate (TDIP)	DF=100% Max=0.46	DF=100% Max=0.68	--	DF=100% Max=0.11	DF=17% Max=0.07	--	--

-- = Not reported as an analyte
DF = Detection frequency
RL = Reporting limit
DL = Detection limit

Data from USGS/NJDEP (2018) extracted from tables in Appendix A – Summary Statistics, and Appendix C – Area Results by Site Category
Data from DRBC (2013) extracted from Tables 4, 6-8, and 11, and Appendix B data tables.
Data from Stackelberg et al. (2007) extracted from Tables 1A and 1B
Data from USEPA-UCMR3 extracted from table downloaded from https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#3

Recommended considerations for future monitoring

We recommend that NJDEP consider conducting an integrated sampling of water media that were examined in the surveys summarized in Table 3.1. Samples should be taken at inflows to and outflows from wastewater treatment plants (WWTPs), the receiving body of water downstream of these discharge points, and at intakes and finished water of drinking water treatment plants utilizing that downstream source water (see Figure 3.1 for a conceptual diagram).

Sampling should be conducted at different times of year and/or different flow levels of source waters. Alternatively, the survey might focus on a time of year and flow level that could be considered a "worst case" scenario for Phase 1 contaminants.

The selection of Phase 1 contaminants for analysis needs to be considered. One approach is to utilize methods that capture a wide variety of potential contaminants; alternatively, another approach is to target specific compounds or classes of compounds that could serve as an initial screening tool, perhaps followed up with more comprehensive testing on a limited subset of locations.

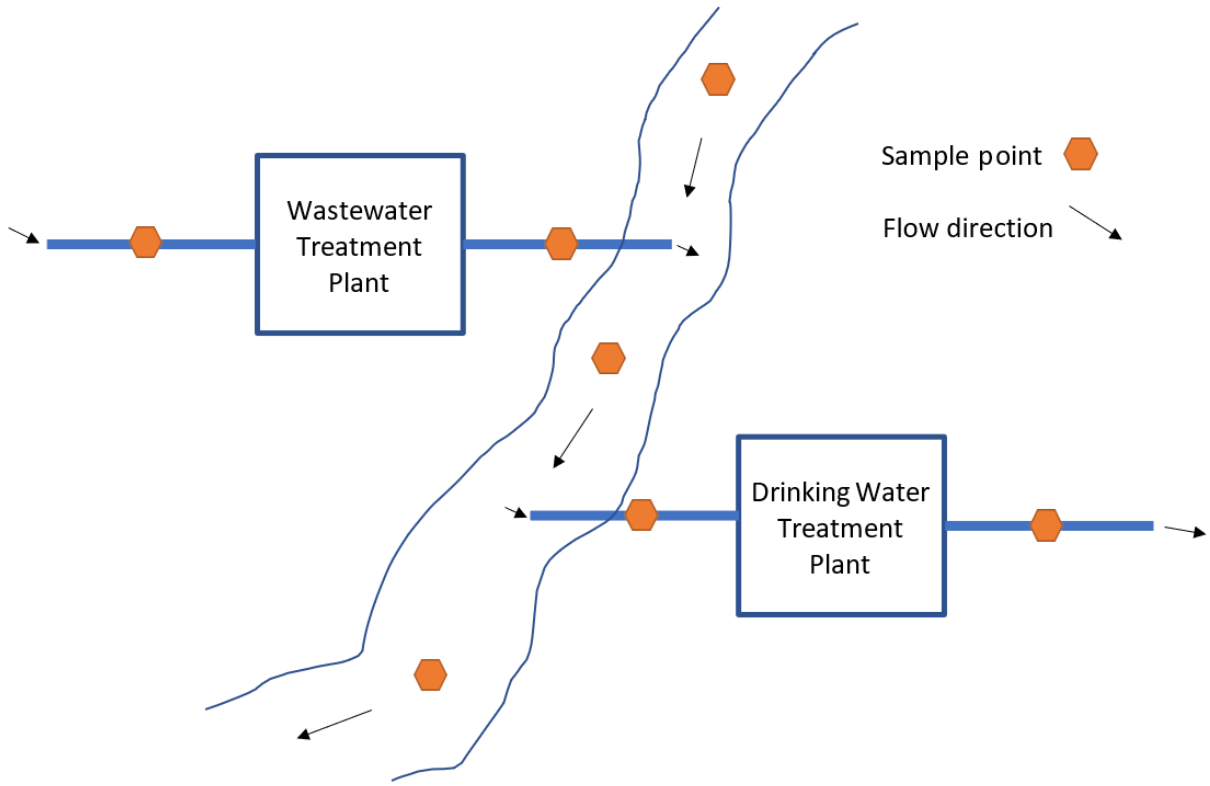
A survey should target those watersheds and locations where sex hormone-related chemicals would be expected to occur within the State of New Jersey, for example where the configuration of WWTP discharges, source water, and downstream DWTP intakes shown in Figure 3.1 occurs. NJDEP could focus initial sampling and analysis on finished drinking water and select watersheds for follow-up sampling based on the results of that initial effort.

The timing of sampling among the sample points within watersheds might be lagged based on expected flow times, so that one can look at effectiveness of removal of contaminants through the WWTP and DWTP processes and dilution factors from outfall through transit in source waters to drinking water intakes. A lack of consistency in contaminant presence or concentration from WWTP inflow through DWTP finished water could indicate that there are sources other than WWTPs of Phase 1 contaminants in source waters, such as non-point runoff into rivers from agricultural land, for example.

It should be noted that the NJDEP has already completed an integrated sampling approach like the one described here for a study of antibiotics in the Hohokus Brook/Saddle River watershed (Gibs et al. 2013).

See Chapter 5 for a discussion of how occurrence data could be used in the overall risk assessment framework.

Figure 3.1: Diagram of potential sample points in an integrated sampling effort to examine Phase 1 Contaminant List substances, from inflows at wastewater treatment plants through finished drinking water.



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Chapter 3 Appendix Tables. Additional details of monitoring efforts for chemicals of emerging concern (CECs).

***Contaminants of Emerging Concern*, DRBC, 2013**

Sample Type	Pharmaceutical Compounds	Sterols/ Hormones	Per- / poly-fluoroalkyl	Polybrominated Diphenyl Ethers	Nonylphenols
Surface Water (Delaware River)	15 pharmaceutical compounds should be considered priority pollutants: acetaminophen, carbamazepine, clarithromycin, codeine, dehydronifedipine, erythromycin-hydrate, fluoxetine, gemfibrozil, 2-hydroxyibuprofen, ibuprofen, lincomycin, metformin, sulfamethoxazole, thiabendazole, and triclocarban	DRBC calculated hazard quotients of 0.2 (long-term exposure) and 0.07 (short-term exposure) for estrone. The EPA has listed the following 12 hormones which may require regulation under the Safe Drinking Water Act (SDWA) on the Contaminant Candidate List 3 (CCL3) and/or Unregulated Contaminants Monitoring Rule 3 (UCMR3): 17- alpha estradiol, equilenin, equilin, 17-beta estradiol, estriol, estone, 17-alpha ethynylestradiol, mestranol, norethindron, testosterone and 4-androstene-3,17-dione.	11 of 13 compounds detected. Perfluorodecanoate (PFDA) Perfluorododecanoate (PFDoA) - ND Perfluoroheptanoate (PFHpA) Perfluorohexanoate (PFHxA) Perfluorononanoate (PFNA) Perfluorooctanoate (PFOA) Perfluoropentanoate (PFPeA) Perfluoroundecanoate (PFUnA) Perfluorobutanoate (PFBA) Perfluorobutanesulfonate (PFBS) Perfluorohexanesulfonate (PFHxS) Perfluorooctanesulfonate (PFOS) Perfluorooctane sulfonamide (PFOSA)-ND	The most dominant (in frequency and concentration) homolog was decabromodiphenyl ethers detected in the range of 2,090 to 7,630 pg/L. Nonabromodiphenyl ethers, pentabromodiphenyl ethers, and tetrabromodiphenyl ethers were predominant at different testing locations in the range of 29 to 161 pg/L. Overall, total polybrominated diphenyl ethers (tPBDE) were detected at concentrations in the range of 87 to 9,376 pg/L.	The concentrations of nonylphenols measured by the DRBC had a maximum of 0.0876 µg/l, which are considered below those that would be protective.

A Reconnaissance of Contaminants of Emerging Concern in Wastewater and Sludge from Three Publicly Owned Treatment Works in New Jersey, USGS/NJDEP, 2018, Unpublished

Sample Type	Pharmaceutical Compounds	Sterols/ Hormones	Per- / poly-fluoroalkyl	Polyaromatic Hydrocarbons	Phenols / Alkylphenol Oxylates	Pesticide / Herbicide	Flavors and Fragrances
Wastewater / Landfill Leachate / Sludge	9 compounds detected in wastewater at each location. All sludge samples contained at least 5 compounds	The wastewater and sludge samples were analyzed for 5 sterols and 17 hormones. The hormones detected were related to androgen and estrogen hormone replacement therapy. 4 of the 17 hormones were detected at each location, with many locations having 9 hormones detected. T	For wastewater, the two locations with detectable levels of a perfluorinated compound included an industrial area with pharmaceutical processing, and a retirement community location. The influent to the wastewater treatment plant also contained detectable levels of perfluorinated compounds. The levels in the influent were not always different than the levels in the effluent, suggesting that traditional wastewater treatment has not removed perfluorinated compounds. For sludge, between 7-10 of the 13 perfluorinated compounds were detected in the analysis of dried and wet filter cakes.	Detectable levels of at least three of the PAH compounds were found in all wastewater samples. The most commonly detected compounds were phenol and p-cresol, which were found in every wastewater sample collected. Overall, the p-cresol concentrations were higher before treatment and showed markedly lower results in the effluent. Between 8 and 11 of the PAHs were detected in the sludge samples.	Untreated wastewater contained between 5-10 of 14 compounds. The most common: benzophenone, and tris(2-butoxyethyl) phosphate. Three Phenols and Alkylphenol Ethoxylates compounds were detected in the effluent, and at lower levels than were measured in the influent. Landfill leachate samples contained between 4-9 detectable Phenols and Alkylphenol Ethoxylates compounds. Concentrations of these compounds were typically comparable to those found in other wastewater samples, except bisphenol A which was at low concentrations in the wastewater samples, but were found at greater concentrations in the landfill leachate.	5 of 12 detected in wastewater. Instrument reporting discrepancies for sludge samples. Thiabendazole only, was detected	Sludge samples were analyzed for 10 compounds including 3-methyl-1h-indole (Skatole), camphor, and menthol. The 20 wastewater sampling locations contained detectable levels of between eight and twelve of the compounds. The concentration of the flavors and fragrances in these wastewater samples was generally low, save for three compounds; 5-methyl-1h-benzotriazole (BHA), menthol, and methyl salicylate which were detected at higher levels. Menthol was detected at every wastewater sampling location, except one. The number of flavors and fragrances, as well as the magnitude of the detection was reduced in the treated effluent.

***Inclusion of Pesticide Transformation Products is the Key to Estimating Pesticide Exposure and Effects in Small US Streams*, Mahler et al., 2021**

Sample Type	Pesticide / Herbicide
Surface Water: 76-100 wadable Streams (Pacific NW, Coastal California, Midwest, NE, SE) from 2013-2017	Parent pesticides detected in 95% of the samples. Transformation products detected in 90% of the samples. Herbicide TPs detected more frequently than insecticide TPs. Potentially due to much higher mass application rates for herbicides vs. insecticides. Hazard quotients based on acute aquatic-life benchmarks for invertebrates, nonvascular plants, and vertebrate-centric molecular endpoints quantified the range of the potential risk. Concluded that potential toxicity to aquatic life exists.

***Hormones and Pharmaceuticals in Groundwater used as a Source of Drinking Water Across the United States*, Bexfield et al., 2019**

Sample Type	Pharmaceutical Compounds	Sterols/ Hormones
Drinking Water: 18 Principal Aquifers from 2013 - 2015. Spatially distributed, not at WWT source.	Generally occur in DW less frequently than VOCs. Most frequently detected: carbamazepine, sulfamethoxazole, 1,7-dimethylxanthine, and meprobamate. Concentrations ranged from 1.7 ng/L to 677 ng/L.	Generally occur in DW less frequently than VOCs. The 4 detected hormone compounds: bisphenol A, 4,4'-bisphenol F, cholesterol, and testosterone. Concentrations ranged from 3 ng/L to 570 ng/L.

Concluded that where detected, concentrations are not expected to have adverse human health effects.

***Occurrence and Partitioning of Antibiotic Compounds Found in the Water Column and Bottom Sediments from a Stream Receiving Two Wastewater Treatment Plant Effluents in Northern New Jersey, 2008* - Gibs et al., 2013**

Sample Type	Pharmaceutical Compounds
Surface Water and associated sediment from WWTP Effluent. Saddle River, Hohokus Brook, and Sprout Brook, Bergen County, NJ	Eight antibiotic compounds (azithromycin (maximum concentration 0.24 µg/L), ciprofloxacin (0.08 µg/L), enrofloxacin (0.015 µg/L), erythromycin (0.024 µg/L), ofloxacin (0.92 µg/L), sulfamethazine (0.018 µg/L), sulfamethoxazole (0.25 µg/L), trimethoprim (0.14 µg/L) and (erythromycin–H2O (0.84 µg/L)) were detected in the water samples from the sites downstream from the WWTP discharges. These concentrations decreased with increasing distance downstream from the WWTP discharges. Azithromycin, ciprofloxacin, ofloxacin, and trimethoprim were detected in stream-bottom sediments. The concentrations of three of the four compounds detected in sediments were highest at a sampling site located downstream from the WWTP discharges due to sorption and sediment transport.

CHAPTER 4: RESPONSE TO CHARGE QUESTION 4

Question 4 – Is this issue a concern for New Jersey? The Department asks the SAB to determine the hazardous nature (e.g., human health risks) of estrogenic compounds in NJ water.

This committee decided to address this question directly, by trying to assess whether people in New Jersy will be at risk of adverse health effects as a consequence of exposure to endocrine disruptors. To estimate risk, the external dose and internal dose as well as the NOAEL and/or LOAEL for a chemical must be estimated or measured. NJDEP has created regulatory limits for chemicals classes such as PFAS that are health-based standards, and we expect this process to be repeated for other EDC emerging contaminants. The health-based standards were created using all relevant studies as well as uncertainty factors and assumptions of vulnerability for those potentially exposed, assumptions that provide a precautionary limit for possible exposure. The net result is a regulatory limit that is often more protective than those from the EPA and, unfortunately, generally too low for routine measurement with readily available analytical techniques (e.g. HPLC/MS). A dilemma is created when trying to evaluate whether residents are at risk, if the threshold concentration cannot be measured in the environment. In considering whether the regulatory threshold should be established based on the ability to measure the EDC in the environmental matrix (exposure pathway), or whether it should remain a health-based standard, the committee evaluated the history of measurement capabilities vs time and concluded that if the standards are health based, the method capabilities will eventually catch-up as evidence is provided below.

Previous methods for endocrine active chemical measurement

The evolution of analytical methodologies for measuring most organic based xenobiotics has closely followed regulatory guidelines or standards; and absent a lowering of standards required for regulatory compliance, the analytical methodologies have also remained somewhat stagnant. Many of the compounds originally identified as endocrine disruptors were linked to other negative health outcomes, such as suspected carcinogens (e.g., organochlorine pesticides). Quantifying these compounds was important as they were regulated for their non-EDC health impacts rather than their endocrine properties. Even after their carcinogenic properties were proposed to be related to their endocrine disruptive properties the cancer endpoints drove the improved sensitivity and lowering of detection levels. The organochlorine regulations were far more influential in developing and improving methods used for compliance monitoring.

Additionally, most of the methods accepted by or developed by EPA or states for compliance with EPA and/or other regulations are seriously outdated. Most last century methods have not seen significant upgrades with the corresponding increase in sensitivity in the last 30 years. Many of these methods were very specific as to the type of instrument and expected detection limits and other performance criteria and those methods have not kept pace with the current state-of-the-art capability. For example, there is no current method which recognizes the use of an Orbitrap mass spectrometer for the quantitation of many of the compounds. As regulations change, methods are adapted and improved to try to keep pace with the new regulatory limits and generally these methods are adaptations of literature reported capabilities, often created to remove the "non-detect" label from environmental samples collected in the field.

All environmental estrogenic chemicals, including polychlorinated hydroxybiphenyls, dichlorodiphenyltrichloroethane (DDT) and derivatives, alkylphenols, bisphenol A,

methoxychlor and chlordecone, compete with E2 for binding to both ER subtypes with a similar preference and degree. In most instances the relative binding affinities (RBA) are at least 1000-fold lower than that of E2. Some phytoestrogens such as coumestrol, genistein, apigenin, naringenin, and kaempferol have a higher affinity for ERβ than to ERα and compete with E2. Estrogenic chemicals stimulate the transcriptional activity of ERα and ERβ at concentrations of 100-1000 nm (Kuiper et al. 1998).

As detailed in Hall and McDonald (1999), the human estrogen receptors ERα and ERβ belong to the nuclear receptor superfamily of ligand-inducible transcription factors. The family of ligand-inducible transcription factors also include the receptors for steroids, thyroid hormone, retinoic acid, vitamin D, and orphan receptors for which no ligands have yet been identified (Hall and McDonald, 1999). The mechanism of action of ER is like that of other nuclear receptors creating difficulties in detection and quantitation of ER and further emphasizing the need of reliable methods to isolate and accurately quantitate this diverse set of compounds.

GC to GC/MS to LC to LC/MS transitions

In the early 1970's soon after the creation of the EPA, the initial methods for analysis of organic contaminants was performed by gas chromatography, with compounds containing halogens generally detected at the lowest levels, using an electron capture detector (ECD). Mass spectrometry as a GC universal detector was still in its earliest stages of development. The primary limitation in using a gas chromatographic separation method was the analyte's boiling point. Many of the compounds with endocrine disruptive properties were not volatile enough to be separated from one another and from the background sample matrix, using gas chromatographic separation, until the addition of some type of sensitive detection. Early iterations of a single quadrupole mass spectrometer became more sensitive for compounds that could not be detected with ECD as ECD was competitive in sensitivity with MS detection until the mid-1990s. To make analytes volatile enough for GC separation, a chemical modification (usually referred to as derivatization) was made to the analyte (Bowden et. Al, 2009). Also at that time, LC/MS was becoming a viable technique for detecting non-volatile or polar compounds.

Sensitivity improvements continued from the 1990s corresponding to improvements in MS capabilities and the evolution of new MS platforms (e.g. triple quad, ion trap/orbi trap and ToF platforms). Generally, EDCs are either non-volatile, or polar compounds and even the semi-volatile can be quantified by LC/MS techniques. While individual sensitivities are comparable for specific compounds classes many have recognized that LC/MS has significant sensitivity advantages to GC/MS (Omar et. al. 2016) but for many EDCs they may remain complementary techniques, (Krone et. al. 2010 and Grover et. al. 2009).

Gains in LC/MS sensitivities

While many of the sensitivities have been related to improvements in mass spectrometric platform technology, the continued improvements are reflected in only modest gains in sensitivity. While quantitative sensitivity may be approaching a technology limit, the isolation of analyte from matrix will continue to add modest improvements to those related to instrumental improvements. The bottom line is that method sensitivity continues to improve while not as dramatically as in the 1990 – 2015 period (Omar et. al. 2016).

Environmental samples vs biological samples

Environmental samples are generally easier to analyze with fewer interferences than biological samples. While environmental samples can be very "messy" the biological matrices are generally more challenging for separating specific EDC analytes than environmental matrices and therefore more difficult to separate matrix from analyte. The implication is that overall, the sensitivity for EDCs in a finished water sample or similar are better than those for biomonitoring samples. The net result is that the external exposures are a little easier to quantify than the internal exposures.

Availability of acceptable EPA methods

One resource that continues to lag the corresponding need, is the EPA methods required for compliance monitoring. Fortunately, as new compound classes are declared as EDCs and/or as other regulated compounds, new, more sensitive, EPA methods usually follow. What is really needed are updates to older methods for quantifying compounds whose regulatory levels were established decades ago. Newer threshold health effects (e.g. endocrine disruption) for compounds already with regulatory limits, are likely to be lower than those used to set the original regulatory limits. The net result will be as compound classes are reevaluated for an ED endpoint and possible regulation, they will need to rely on methods and technologies not capable of quantifying the new lower regulatory levels.

Availability of instrumentation

Another consideration for compliance monitoring is the reasonable availability of instruments capable of quantifying EDCs at the newer regulatory limits. If an instrument cost is significantly out of reach for labs responsible for compliance monitoring, it is unlikely to be purchased and used even if it is capable of achieving the sensitivity required. What generally happens is that the compliance lab eventually purchases the required instrument or waits for the sensitivity improvements in compound isolation or preconcentration techniques to catch up to the regulatory requirements.

Recognizing the need for preconcentration

Most new regulatory requirements for compounds with health effects like the recent limits on PFAS, have limits below what most methods can achieve without preconcentration of the analytes. Very good LC/MS methods can get to the sub ng/ml level but the PFAS limits in water are well below the ng/l into the pg/l range. To achieve these lower levels, most validated methods will rely on some type of preconcentration, usually a solid phase extraction followed by solvent blowdown. Using this technology, concentration factors of 1000x are easily achieved as long as a suitable solid phase column can be purchased; one that retains the analytes until they released quantitively by solvent washout. Alternatively, there are methods that employ direct preconcentration through blowdown of the original sample, but this is very time consuming and potentially labor intensive.

Alternative assays (Immunoassays)

There are "quantitative" assays that use antibody specific detection of certain analyte classes or even specific compounds. Enzyme Linked Immunosorbent Assay (ELISA) is often compared to LC/MS methods for sensitivity in various matrices. It uses enzymes to detect and

quantify immunosorbent reactions and is more often employed for the quantitation of analytes in clinical assays. It is seeing more recent application for environmental samples and has comparable sensitivities to those of LC/MS. While some argue that it is as rugged a technique as MS methods, the enzymatic chemistry, lack of a broad range of antibodies, lower end sensitivity differences and concomitant interferences make this a less used method for quantitation of environmental contaminants.

Other assays have been investigated to determine structurally diverse estrogens. Fang et al. (2000) examined the quantitative agreement between in vitro estrogen receptor competitive binding assays (ER binding assays), yeast-based reporter gene assays (yeast assays), and the MCF-7 cell proliferation assay (E-SCREEN assay). The assay performance was evaluated for relative sensitivity, detection of active/inactive chemicals, and estrogen/antiestrogen activities. The conclusions indicate that the ER binding assay was a "a good predictor for the two other assays when the antiestrogens were excluded" (Fang et al., 2000).

The question today for NJDEP, EPA and others: should regulatory limits, for example for drinking water, take into consideration the current analytical capabilities or be based exclusively on health effects? The new regulatory limits for PFAS are not readily measurable using today's instrumentation and direct analysis. The question about whether preconcentration methods are enough to ensure compliance with certainty is still open. As has been demonstrated in the past, however, technology generally catches up to regulatory limits, even though given today's technology, the greatest improvements in analytical sensitivity are already in the rearview mirror. While the instruments with the lowest level of detection (greatest sensitivity) may currently be too expensive for many commercial laboratories tasked with compliance measurement, history has demonstrated that the price of these instruments will come down and that until they do, the assays are likely to be very pricey. It may be more prudent to require less frequent testing, especially when there is a history of non-detect in a sample. ***It is the recommendation of this panel that health-based standards be used as the guidance for regulatory compliance even if they exceed the current capabilities of most if not all analytical laboratories, performing compliance testing.*** Technology will catch up.

"EPA's Endocrine Disruptor Screening Program (EDSP) uses a two-tiered approach to screen pesticides, chemicals, and environmental contaminants for their potential effect on estrogen, androgen and thyroid hormone systems," (EPA 2024). The efficiency and cost-effectiveness of first tier testing includes incorporation of high-throughput and computational toxicology to identify candidate chemicals, for example new pesticide components for tier 2 testing. Tier 2 can include various receptor binding assays.

EDCs will remain a major focus for analytical method improvement as they are currently "the" class of emerging contaminants. Technology will continue to improve, making lower levels in environmental samples easier to measure but there may always be catching up to try to measure lower health based regulatory levels.

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CHAPTER 5: RESPONSE TO CHARGE QUESTION 5

Question 5: How does this concern compare to that of other emerging contaminants?

Charge 5 requests a comparison among contaminants of emerging concern (CEC), many of which are only recently identified, lack comprehensive data on occurrence and/or toxicity, and represent threats to human health or ecology of uncertain magnitude. The Committee believes that given the current paucity of data, it would be premature to go directly to risk assessments for each compound. It may be necessary to go to full risk assessments and management applications for multiple compounds on the CEC list provided to the Committee by the NJDEP. Hence, we suggest a process that sets priorities based on a combination of quantitative and qualitative analyses and is consistent with risk assessment.

Risk Assessment and Conceptual Site Models

The risk assessment portion of risk analysis addresses three generic questions (Greenberg et al., 2020):

1. What can go wrong?
2. What are the chances that something with important consequences will go wrong?
3. What are the consequences if something does go wrong?

A consistent preliminary assessment process would help DEP use existing information to set initial priorities. A good deal of this effort will be devoted to using existing data and prioritizing the collection of new data that will address epistemic (degree of knowledge) and aleatory (random events) forms of uncertainty.

Whether the focus is on CEC's in general or estrogenic compounds in particular, the approach should be directed to three connected parts of risk assessment: (1) characteristics of the potential hazards; (2) transport pathways that could lead to exposures; and (3) characteristics of persons likely to be exposed.

The literature offers field-tested applications of Conceptual Site Models (CSM) focusing on individual substances and/or on individual sites while identifying gaps in current knowledge. For example, in 1994, the Committee on Remedial Action Priorities for Hazardous Waste Sites of the NRC examined a set of CSM models developed by the EPA, DOE, and DOD to prioritize which waste management sites should be ranked among the most in need of remediation (Committee on Remedial Action 1994). The models developed for each organization varied in data and methods were used. However, there was a similar inherent logic in each. More recently, the Consortium for Risks Evaluation with Stakeholder Participation (CRESP, 2018) created CSMs to help prioritize remediation options at Hanford (WA) and other nuclear defense sites. Each of these studies offers steps that can be adapted to the case of evaluating the priorities among CECs that have both spatial and temporal risk dimensions.

These studies are organized around principles for building credible models. At the heart of the challenge is understanding causal pathways (Buhlmann 2020; Gass 1983; NAS 2015). The following are four elements of a credible CSC model process:

1. Purpose: Specifying the purpose and intended users.
2. Establishing Credibility: Implementing peer and public review of the process, results, and communication of information.
3. Logic: Explaining the data, relationships among variables, including limitations of the data and methods, and validation of the process and model(s).
4. Uncertainty: Describing quality assurance of the data, uncertainty, and sensitivity of the model, and how these influence the results. For example, what will happen to the process as we become more able to measure lower levels of contamination in the environment, and build models that improve predictions of migration of contaminants?

A Conceptual Site Model for CECs

A Conceptual Site Model (CSM) is representation of the chemical, physical and biological processes that condition how and when contaminants move from sources through the air, soil and water to people and other receptors (U.S.EPA, 2011; NJDEP, 2019). A CSM for a CEC would describe the sources (where and how the chemical could or does enter the environment), the pathways or potential pathways through the environment, and the receptors (humans or ecologic) and particular health endpoints such as organ toxicity, reproductive toxicity, or cancer).

Building the Conceptual Site Model requires careful attention to the sources-pathways-receptors-outcome and the development of the CSM is essentially a status report on the CEC as a hazard at locations. For each pathway, the CSM may identify barriers that currently interdict the pathway (e.g., no "known connection" between a waste site and ground water), or barriers that may be instituted (for example, wastewater treatments) to minimize exposure.

It is likely that a suite of models is required (see the above case studies that use different approaches to avoid depending on a single model). Some processes may be more appropriate for leachate exposure, others for direct discharge into water bodies, and others for treated water.

The candidate CECs can be identified and then measured in environmental media, including wastewater, surface water, drinking water, fish tissue, and others that the DEP considers important to understand. The frequency and magnitude of CEC detection will be important to consider. A rarely detected CEC may be less important than one frequently detected. However, as noted above, with lowered detection levels, something "rarely detected" can become ubiquitous.

Qualitative or quantitative information on toxicity of a CEC will be important, but scarce or non-existent at first. A relative toxicity can be inferred from a combination of approaches applying basic principles of toxicology. These include structure-activity relationships, comparing the CEC with toxicity assessed for more familiar, well-studied compounds. Physical/chemical properties including structural groups, octanol-water partition coefficient, vapor pressure, complexation with macromolecules, genotoxicity are properties that may be inferred (at first) and then studied.

At each step expert judgment of toxicologists, environmental scientists, and others can be used to determine whether a CEC should move up the list because new data and judgements indicate that it is likely to pose a hazard to a group somewhere, or likely to be dismissed because of lack of evidence of toxic hazard for similar compounds. Expert judgement is not always entirely dependable since experts bring their own experiences and interpretations to risk scenarios. But consensus among experts with long and broad experience is likely to be a valuable part of any decision process.

Statistical analysis of detection frequencies and co-occurrences will likely lead to development of surrogate measures, whereby analysis of a subset of chemicals can be used to characterize the potential risks of exposure to different environmental media. For example, the OECD is considering managing a group of 20 PFAS chemicals *(Diderich & Moorghen, 2015).* This approach could be considered for groups of endocrine disruptors.

The CSM approach is typically linked to site specific plans to remediate Superfund and brownfield sites. But it need not be a single small site nor contaminants common to NPL sites. For example, the USGS has been concerned about the buildup of estrogenic compounds and the impact of these on ecological systems and drinking water. Their approach has been to establish monitoring stations and record data that allows them to look for trends and conduct studies on managing exposure (Furlong et al. 2014, Aris et al, 2014, Gordon et al. 2021, Smalling et al. 2021).

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