U.S. Fish & Wildlife Service

ECOLOGICAL HAZARD ASSESSMENT OF CONTAMINANTS OF EMERGING CONCERN IN THE U.S. GREAT LAKES BASIN

Part A (Volume I of II):

Screening Assessment of Relative Hazard to Fish from Surface Water Exposures to Fourteen Contaminants of Emerging Concern in the U.S. Great Lakes Basin

December 2019

Biological Technical Publication

BTP-R3019-2021

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ISBN-978-1-938956-12-6 Electronic ISSN:2160-9497
Biological Technical Publications online:
http://digitalmedia.fws.gov/cdm/search/collection/document/searchterm/Biological%20Technical%20Publications/field/collec/mode/exact/conn/and/order/nosort

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Executive Summary

The purpose of this ecological hazard assessment (EHA) is to characterize the nature and degree of hazard to fish in U.S. Great Lakes waterways from exposure to contaminants of emerging concern (CECs). This EHA translates CEC concentrations in surface water into hazard to fish, where 'hazard' is defined as the potential for biological impacts. CECs comprise a large, diverse, and growing group of anthropogenic chemicals, mostly found in commercial products. They include chemical classes such as pharmaceuticals, veterinary drugs, personal care products, flame retardants, hormones, new pesticides, and other chemical groups. They are ubiquitous in freshwater systems and some are toxic to fish in laboratory assays at environmentally relevant concentrations.

The problem of assessing potential for impacts to fish from exposure to CECs on a regional scale is complex. Every year, thousands of CECs are produced and used in the U.S. - many in enormous quantities. Emerging contaminants enter the environment by numerous routes, such as non-point source runoff, aerial deposition, and point sources like wastewater treatment plants (WWTPs) and combined sewer overflows (CSOs). There are thousands of CEC sources and emerging contaminants are diverse in their chemistry and uses, resulting in a variety of fate and transport pathways and various uptake and accumulation scenarios in fish.

We addressed this complexity using a systematic approach. This assessment is broadly structured as a conventional EHA with familiar components: problem formulation, exposure assessment, toxicity assessment, hazard characterization, and uncertainty analysis. Using this framework, we assessed hazard to fish at each of 24 project locations. Due to the regional scale of the project, we provide a summary and synthesis of hazard across project locations to identify large-scale patterns. We also evaluated the spatial relation between point sources (WWTPs and CSOs) and the nature and degree of CEC hazards to fish.

A predominant finding in the environmental monitoring literature is that, as a group, CECs are ubiquitous. In the EHA exposure assessment, we analyzed CEC concentrations in over 500 water grab samples collected at 195 sampling sites distributed among 24 project locations in a variety of waterbodies across the U.S. Great Lakes basin. All samples were collected during 2010-2014. Out of more than 150 analytes, this assessment focuses on 14 CECs that were detected relatively frequently in preliminary sampling, that represent a variety of chemical use categories, and for which screening values could be developed from the ecotoxicology literature. There were over 7,000 water concentration data points for the 14 CECs. Only aqueous concentrations were evaluated – either measured aqueous or estimated from total concentration.

Evidence provided in published ecotoxicology assays is unequivocal – exposure to environmentally relevant aqueous concentrations of many CECs produces adverse effects in freshwater fish. In the EHA toxicity assessment, we developed screening values (SVs) for 14 CECs and up to 12 effect categories. A SV is an aqueous CEC concentration in water that has toxicological meaning. Published toxicological effects were grouped into the following categories: behavioral, developmental, growth, mortality, reproductive, circulatory/ blood constituents, endocrine, genotoxicity, gross pathology, histopathology, neurological, and physiological/metabolic. For each CEC, pairs of SVs were developed for each effect category that had sufficient published ecotoxicity data. Pairs of 'Mean' SVs[1], combined across effect categories, were also calculated for each CEC. A total of 82 pairs of SVs was derived. A SV pair is comprised of:

- SV_{LOW} - a CEC concentration in water below which no impacts to fish are anticipated, and
- SV_{HIGH} - a CEC concentration above which adverse impacts to fish are expected.

Relative strength of ecotoxicity information was also assessed for each SV to augment hazard interpretation.

[1] 'Mean' SVs were computed as the geometric mean of component effect-specific SV values divided by an uncertainty factor.

In the hazard characterization, we combined CEC exposure concentration information with SV information to describe potential for impacts to fish within each of the 24 project locations. Hazard scores were assigned to each CEC concentration in water based on SV_{LOW} and SV_{HIGH} exceedances. Numeric scores correspond to relative hazard levels: 1 = negligible hazard; 2 = low hazard; 3 = high hazard. We applied the array of 82 CEC SV pairs to each of >500 water samples, generating a total of >40,000 hazard scores. Drawing on this hazard score database, each project location's hazard assessment includes:

- A hazard brief that highlights CEC hazards to fish,
- Hazard rankings of effect categories, CECs, and sampling sites,
- Summary of strength of evidence,
- An evaluation of point source influence on hazard, and
- Maps of relative hazard to fish for high-hazard effect categories.

Hazard to fish due to the 14 CECs varied widely among the 24 project locations. There was clear and convincing evidence of CEC-related hazards at St. Louis River/Bay (MN), North Shore Channel (IL), Little Calumet River (IL), Maumee River (OH), Cuyahoga River (OH) and Tinkers Creek (OH). There was little to no evidence of hazard to fish from exposure to aqueous concentrations of the 14 CECs at Waupaca Chain O' Lakes (WI), St. Clair River (MI), River Raisin (MI), and Ashtabula River (OH). We found statistically significant evidence of elevated hazard downstream of point sources in St. Louis River/Bay (MN), Fox River/Green Bay (WI), Saginaw River (MI), Maumee River (OH), and Tinkers Creek (OH). There were no significant point source effects in 11 locations, and no statistical evaluation was possible for eight locations. Finally, inspection of the results dataset indicated no consistent correspondence between degree of CEC-related hazard and spatial overlap with a Great Lakes Area of Concern.

In addition to location-specific assessments, project-wide patterns of CEC-related hazard to fish were identified in terms of CECs and effect categories. Project-wide hazard to fish varied widely among the 14 CECs. There was clear and convincing evidence of widespread hazards to fish from exposure to DEET, estrone, ibuprofen and venlafaxine in water, while there was little or no evidence of hazards to fish from androstenedione and lidocaine exposures. There was significant evidence of elevated hazard downstream of point sources for carbamazepine, diphenhydramine, estrone, HHCB, ibuprofen and venlafaxine, and no evidence for lidocaine and β-sitosterol.

Project-wide hazard to fish also varied widely between effect categories. We found clear and convincing evidence of widespread and biologically important hazards to fish for developmental, reproductive and physiological/ metabolic effects, and little evidence of hazards to fish for growth, gross pathology and neurological effects. There was statistically significant evidence of increased hazard downstream of point sources at ≥3 project locations for behavioral, mortality, reproductive, endocrine and physiological/ metabolic effects, and no evidence for growth and gross pathology effects.

We have demonstrated that CEC-related hazard to fish is widespread in waterbodies of the U.S. Great Lakes Basin, although overall hazard is likely underestimated in this EHA. Methods for estimating aqueous CEC concentrations and scoring hazard were deliberately biased towards low hazard. We negatively skewed bias in our overall approach to avoid false positives, which strengthens the defensibility of positive findings. The 14 CECs were selected based on detection frequency in preliminary sampling, not toxic potency, so they are not necessarily among the most toxic of the >150 CECs we analyzed in surface water samples. The available ecotoxicity information for the 14 CECs is largely incomplete, and for a few of the 14 CECs it is quite limited. In some cases, elevated hazard was observed even at sampling sites with no known CEC point sources.

There are at least two important caveats to the hazard findings in this EHA:

- The problem of evaluating the potential for CEC impacts to Great Lakes basin fish is complex. This is due to a very large number and variety of potential CECs exposures and adverse effects, as well as a wide variety of waterbodies, fish species and CEC sources, a wide range of CEC chemical properties, and huge spatial scale. As a pragmatic necessity, the scope of our project encompasses a relatively narrow subset of information about CECs and the regional system, requiring a number of simplifying assumptions that introduce uncertainty into the assessment.
- The absence of an observed CEC-related hazard at a site does not equate with a conclusion that CEC-related hazards are actually absent. As more CEC SV pairs are developed and applied in EHAs, confidence in negative hazard findings will likewise increase.

Despite uncertainties, we found that the nature and intensity of hazard to fish from exposure to the 14 CECs varies between project locations and between sampling sites within locations. That is, spatio-temporal variability in CEC concentrations, spatial variability in the characteristics of waterbodies including the distribution of CEC sources, and

variable ratios between CEC concentrations result in variable potential among effect categories for hazard to fish.

At this time, there is no simple litmus test for natural resource managers to apply at specific locations that would sufficiently characterize hazard to fish from the set of 14 CECs considered in this EHA, let alone from all CECs. Nevertheless, the overall finding that elevated CEC exposure likely impacts fish populations is strongly supported by the evidence provided in this EHA, and we provide the following broad natural resource management implications of this finding:

- Management of aquatic natural resources can no longer ignore emerging contaminants in planning and decision-making;
- Assessment of relative hazard from CECs may improve prioritization among potential project sites for management actions such as reintroductions, habitat restoration, etc.; and
- The hazard assessment process illustrated in this EHA provides natural resource researchers and managers a means to compare potential for various types of CEC-related impacts in fish among alternative management scenarios.

Acknowledgements

We are grateful to the many people and organizations who contributed to tasks and activities related to this assessment. The USFWS Great Lakes CEC ecological hazard assessment and CEC screening value development were funded by the Great Lakes Restoration Initiative through the USFWS CEC Team. We thank the USFWS CEC Project management team led by Dr. Annette Trowbridge and Dr. Stephanie Hummel (USFWS – Region 3 Regional Office), without whose efforts this project would not have been possible. The authors are grateful to numerous USFWS colleagues and volunteers from the Great Lakes states who helped complete field work associated with this project during 2010-2014 (alphabetically listed):

New York:	Sandie Doran, Justin Ecret, Andy Lowell, Robyn Niver, Noelle Rayman, Megan Patch, Amy Roe, Scott Schlueter, David Stilwell, Tim Sullivan, Emily Van Wyk, John Wiley;
Pennsylvania:	Christina Cappelli, Jenny Johnson, Michael Penn;
Ohio:	Jeromy Applegate, Sarah Bowman, Melanie Cota, David DeVault, Jenny Finfera, David Henry, Mary Knapp, Keith Lott, Deborah Millsap, Evan Millsap, Sarah Millsap, Kevin Tloczynski;
Michigan:	James Boase, Andrew Briggs, Justin Chiotti;
Wisconsin:	Stewart Cogswell, Betsy Galbraith, Anthony Rieth, Marian Shaffer, Darin Simpkins, Tim Strakosh, Ted Treska, Sara Warner;
Minnesota:	Rich Davis, Glenn Miller, Nick Utrup

We are grateful to USGS staff, including Mike Menheer, Molly McCool, and Jeff Ziegeweid for collecting paired water and sediment samples, and Kathy Lee, Sue Langer and Sarah Elliott for data processing and compilation. We appreciate technical assistance from Mark Brigham at USGS-Upper Midwest Water Science Center in calculating aqueous concentrations from total concentration. We thank Michelle (Lutz) Nott (USGS - Wisconsin Water Science Center) for GIS shapefiles pertinent to our analysis of point sources, and Andy Lowell (USFWS-New York Field Office) for patient and expert advice on data sources and map-making using GIS. We thank Dr. Vicki Blazer (USGS-Leetown Science Center) and her crew during 2010-2012, and Dr. Heiko Schoenfuss and Linnea Thomas (St. Cloud State University, MN) during 2013-2014 for coordinating fish health assessments with water/sediment sampling.

We appreciate analytical laboratory services provided Dr. Edward Furlong and Bill Foreman and their staff at the National Water Quality Lab (USGS-Denver) who provided measured chemical concentrations in surface water samples and data QA/QC, and we thank SGS AXYS for providing detection limits of emerging contaminants. We gratefully acknowledge statistical advice provided by Dr. Timothy Robinson (University of Wyoming-Laramie) and Dr. John Sweka (USFWS-Northeast Fishery Center - Lamar, PA).

We appreciate the help of the following individuals, non-governmental organizations, and other federal and state agencies for their assistance during field work (in no particular order): Dr. Glenn Johnson at the State University of New York at Potsdam, Thomas and Helen Pahler (Norwood, NY), Bayside Boat and Tackle (Rochester, NY), Marlands Shore Association (Rochester, NY), Village of Tupper Lake WWTP, Village of Potsdam WWTP, NYS Department of Environmental Conservation, Toledo Yacht Club, Ashtabula Yacht Club, Midwest Biological Institute, Northeast Ohio Regional Sewer District, Ohio EPA, Southerly WWTP, Akron WWTP, Bedford WWTP, City of Solon Water Reclamation Department, Akron Water Supply, Cuyahoga Valley National Park, LaDue Reservoir, USEPA ORD, Green Bay Yacht Club, Metropolitan Water Reclamation District of Greater Chicago, Minnesota Land Trust, Minnesota Department of Natural Resources, and the Fond du Lac Band of Lake Superior Chippewa.

We are very grateful to the following inter-agency colleagues who provided expert technical review: Dr. Donald Tillitt (USGS-Columbia Environmental Research Center), Dr. Mark Jankowski (USEPA-Seattle), Sarah Elliott (USGS-Upper Midwest Water Science Center) and Mark Brigham (USGS-Upper Midwest Water Science Center). Their suggestions and critique greatly improved content and communication. Finally, we thank Mark Newcastle of the USFWS Branch of Printing and Publishing and Jon H. Soeder for their patient and professional guidance and implementation through multiple iterations of formatting and editing on the road to publication.

Acronym List

(This list includes acronyms that are used in the Attachments)

AHTN	Acetyl hexamethyl tetralin
AOC	Area of Concern
AOP	Adverse Outcome Pathway
BPA	Bisphenol A
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
CAFO	Confined Animal Feeding Operation
CEC	Contaminant of Emerging Concern
CECWG	Chemicals of Emerging Concern Working Group
CSO	Combined Sewer Overflow
DEET	Diethyl-meta-toluamide, N,N-
DL	Detection Limit
DO	Dissolved oxygen
DOC	Dissolved Organic Carbon
EC	Effect Concentration
ECCC	Environment and Climate Change Canada
ECB	European Chemicals Bureau
ECOSAR	Ecological Structure Activity Relationships
EHA	Ecological Hazard Assessment
ERA	Ecological Risk Assessment
ESRI	Environmental Systems Research Institute
FDA	Food and Drug Administration
GIS	Geographic Information System
GLRI	Great Lakes Restoration Initiative
GLWQA	Great Lakes Water Quality Agreement
HHCB	Hexahydrohexamethyl-cyclopentabenzopyran
HI	Hazard Index
HQ	Hazard Quotient
IJC	International Joint Commission
K_d	Sediment-Water Partitioning Coefficient
K_{oc}	Organic Carbon Partitioning Coefficient
K_{ow}	Octanol-Water Partitioning Coefficient
LC	Lethal Concentration
LLBDM	Little Lake Butte des Morts
LOAEC	Lowest Observed Adverse Effect Concentration
LOEC	Lowest Observed Effect Concentration
MDL	Method Detection Limit
NHD	National Hydrography Dataset
NOAEC	No Observed Adverse Effect Concentration
NOEC	No Observed Effect Concentration
NPDES	National Pollution Discharge Elimination System
NSAID	Non-Steroidal Anti-Inflammatory Drug
OECD	Organization for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbon
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
POC	Particulate Organic Carbon
QSAR	Quantitative Structure Activity Relationship
QSPR	Quantitative Structure Property Relationship
REAOC	Rochester Embayment Area of Concern
SAS	Statistical Analysis Software
SLERA	Screening Level Ecological Risk Assessment
SPSS	Statistical Package for the Social Sciences
SSD	Species Sensitivity Distribution
SSRI	Selective Serotonin Reuptake Inhibitor
STORET	STOrage and RETreival (USEPA water quality database)
SV	Screening Value
TBEP	Tris(2-butoxyethyl)phosphate
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UF	Uncertainty Factor
UNEP	United Nations Environmental Programme
USCB	U.S. Census Bureau
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
WWTP	Wastewater Treatment Plant

Chapter 1 - Introduction

Great Lakes fish have enormous intrinsic, ecological, cultural, and economic value for residents of the United States, Canada, and Original Nations. Emerging stresses to Great Lakes basin aquatic ecosystems potentially impact the livelihood, health, traditional practices, or recreation for millions of people, and provide fresh challenges to natural resource managers who work to maintain, conserve, and restore aquatic ecosystems including thriving native fish populations. Emerging stressors include climate change (Cohen 1986, Meisner et al. 1987, Hill and Magnuson 1990, Magnuson et al. 1997, Casselman 2002, Trumpickas et al. 2015, Collingsworth et al. 2017), invasive species (USEPA 2018a), proliferation of harmful or nuisance algae blooms (Michalak et al. 2013, EC 2014), and an ever increasing variety and quantity of contaminant of emerging concern (CEC) loadings (CECWG 2011).

The Great Lakes International Joint Commission (IJC) Chemicals of Emerging Concern Work Group[2] provides the following description of CECs (CECWG 2011):

> *Chemicals of emerging concern include new compounds that have gained entry into the environment or those that have been recently characterized due to increases in concentrations in the environment or improvements in analytical techniques. In the United States and Canada few of these compounds have regulations governing their release. Of concern is the uncertainty of potential adverse effects on wildlife and humans due to chronic exposure to low concentrations of these compounds.*

Contaminants of emerging concern include a wide variety of chemicals in commerce (Chapman 2006, Howard and Muir 2010, Howard and Muir 2011, UNEP 2010), including pharmaceuticals, veterinary drugs, personal care products, flame retardants, surfactants, plasticizers, hormones, fragrances, new biocides, and other categories. Very few CECs are regulated with respect to their release into the environment (CECWG 2009), yet a very large number of these compounds (thousands) were specifically designed to be biologically active.

Emerging contaminants are recognized as a global issue (UNEP 2010, UNEP 2013), and assessing and mitigating potential ecological effects from CEC loadings in the Great Lakes Basin is of significant interest to the IJC (CECWG 2011). There are tens of thousands of CECs (CECWG 2009; Diamond et al. 2011), which are discharged into aquatic systems via human, industrial, and agricultural waste streams (Wilde et al. 2000, Kjaer et al. 2007, Arnon et al. 2008, Petrovic et al. 2008, Snow et al. 2009, Phillips et al. 2010, Schultz et al. 2010, Song et al. 2010, Zhao et al. 2010a, Clarke and Smith 2011, Khan and Lee 2012, Phillips et al. 2012, Venkatesan et al. 2012, Cavallin et al. 2014, Fairbairn et al. 2014, Fairbairn et al. 2015, Bradley et al. 2016, Bradley et al. 2017). Large-scale monitoring programs have been conducted to survey CECs in surface waters, sediments and fish tissue:

- across the U.S. (Smith and Muir 1998, Kolpin et al. 2002, Ramirez et al. 2009, Glassmeyer et al. 2017);
- in Canada (Kleywegt et al. 2007, Newbold et al. 2007, Kleywegt et al. 2011); and
- in the Great Lakes Basin (Metcalfe et al. 2003, Klecka et al. 2010, Crimmins et al. 2012, Lee et al. 2012, Baldwin et al. 2016b, Choy et al. 2017, Elliott et al. 2017).

Laboratory analytical methods have been established for only a small fraction of CECs and their derivatives. Nevertheless, commonly detected CECs in environmental media include pharmaceuticals, natural and synthetic hormones, personal care and household products, veterinary medicines, flame retardants, new pesticides and other chemicals in domestic commercial products. Relative to their number and diversity, there is little information concerning their environmental fate, ecotoxic effects, or the potential for CECs to impact fish and wildlife inhabiting aquatic ecosystems or to disrupt human uses (such as fish consumption).

The vast majority of CECs are unregulated as environmental pollutants in the U.S. Efforts to prioritize across thousands of CECs to focus research and regulation have relied primarily on quantitative

[2] Throughout this document, the terms "contaminant of emerging concern", "chemical of emerging concern", and "emerging contaminant" are used interchangeably.

structure property relationship (QSPR) and quantitative structure activity relationship (QSAR) models (Hong et al. 2002, Sanderson et al. 2003, Walker et al. 2004, Diamond et al. 2010, Howard and Muir 2010, Howard and Muir 2011). However, during the past 15 years there has been a proliferation of peer-reviewed, empirical ecotoxicological studies showing effects in aquatic organisms from water-borne CECs at environmentally relevant exposure concentrations. This, coupled with method development for analyzing CECs in environmental media (e.g., USGS 2014; USEPA 2016a, AXYS 2018), has provided an opportunity to conduct ecological hazard assessments (EHAs)[3] for a limited set of CECs based on familiar exposure-level to effect-level comparisons. Ecological hazard assessments of CECs have been conducted previously in research (e.g., Ferrari et al. 2003, Bound and Voulvoulis 2004), resource management (e.g., Zhao et al. 2010b), regulatory applications (e.g., von der Ohe et al. 2011) and other contexts (Table 1-1).

Since 2014, the U.S. Fish and Wildlife Service (USFWS) CEC Project Team (USFWS staff in offices in Great Lakes states) has developed screening values (SVs) for CECs detected frequently in surface water in order to assess hazard consistently across all project locations (Gefell et al. 2019). A fish ecotoxicological database was compiled to derive CEC SVs (Gefell et al. 2019) that includes a very large number of effect endpoints that were not evaluated in the USFWS resident fish health assessments[4] (Blazer et al. 2014a, Blazer et al. 2014b, Thomas et al. 2017, Blazer et al. 2018, Jorgenson et al. 2018) or in previous CEC EHAs that assess hazards to fish (Table 1-1). The set of SVs evaluated in this EHA spans 12 effect categories (reproductive, mortality, endocrine, etc.), providing for a degree of power and flexibility in biological hazard interpretation not previously reported.

We define 'hazard' as the potential for adverse biological impacts. This document describes an assessment of hazard in fish from exposures to 14 CECs in surface water sampled during 2010-2014 at 195 sites, among 24 project locations, distributed across the U.S. Great Lakes Basin. The CEC EHA was conducted under the statutory authority of the Great Lakes Fish and Wildlife Restoration Act, and was funded through the Great Lakes Restoration Initiative (GLRI). The GLRI has developed Action Plans to focus restoration efforts on priority topics. Objectives and commitments specified in the Toxic Substances and Areas of Concern focus area of the GLRI Action Plan II (USEPA 2014a) include:

Objective

- Increase knowledge about contaminants in Great Lakes fish and wildlife

Commitments

- Reduce human exposure to contaminants from Great Lakes fish consumption
- Identify emerging contaminants and assess impacts on Great Lakes fish and wildlife

The USFWS CEC Team developed a CEC Project Five-Year Planning Document to further address and focus the vision of the GLRI Action Plan II regarding emerging contaminants. Three of the five goals in the USFWS five-year plan are partially fulfilled by this EHA:

1. Determine CECs of greatest concern & predict the tributaries with the greatest CEC occurrence and concentration within the Great Lakes Basin,
2. Determine if exposure to commonly occurring CECs and their mixtures in target biota cause changes in population relevant parameters in organisms, and potentially at the population level, and
3. Make a preliminary population relevant risk determination for CECs and their mixtures based on exposure and effects data.

This document is the first segment (Part A) of a two-part Great Lakes CEC Ecological Hazard Assessment. Part A provides a detailed assessment of the potential for biological impacts in fish from aqueous exposures to 14 commonly detected CECs in surface water. Part B (Gefell et al. 2019) is a companion document that provides a detailed description of resources for conducting CEC EHAs, including surface water SVs for the 14 CECs used in this hazard assessment and details their derivation. Together, these two documents pertain to the second and third USFWS project elements listed above.

Other projects conducted by the USFWS CEC project team and partners have addressed these goals via various approaches. Since 2010, the team - along with agency and academic partners - has engaged in field and laboratory studies concerning CEC effects in aquatic biota. Project conducted during 2010-2014 included health assessments of resident fish and quantification of CECs in various media at sites distributed across the Great Lakes Basin. Several studies pertain to the

[3]Chemical ecological risk assessments (ERAs) are simplified analyses of the potential for chemical impacts in complex ecological systems. In risk assessment, the word "risk" denotes a quantitative estimate of the probability of adverse effects, given the exposures, while the word "hazard" denotes a more qualitative potential to cause harm (Suter 1993). In this sense, an assessment that is often called a screening level ERA (SLERA) is also known as an ecological hazard assessment (EHA), which is the term we have used throughout this document.

[4]Comparisons between findings from the fish health assessment component of the USFWS Great Lakes CEC project and effect-specific hazards predicted in this EHA will be discussed in a subsequent document; preliminary analyses indicate a reasonably strong spatial correspondence.

first goal listed above, providing CEC concentrations in surface water that comprise this EHA's exposure assessment (Lee et al. 2012, Lee et al. 2015, Choy et al. 2017, Elliott et al. 2017). The CEC project team has addressed the second goal in field and laboratory effects assessments. Concurrent with media sampling, we conducted resident fish health surveys during 2010-2014 and caged fish tests during 2013-2014 to evaluate whether fish health is associated spatio-temporally with CEC concentrations in water or sediment. Fish health survey scope, methods and implementation were adjusted between the 2010-2012 (Blazer et al. 2014a, Blazer et al. 2014b, Blazer et al. 2018) and 2013-2014 periods (Thomas et al. 2017, Jorgenson et al. 2018). The third goal listed above was addressed with multigenerational laboratory studies and flow-through streamside exposure studies conducted to evaluate survival, growth, reproductive, gross pathology, histopathology and physiological effects in fish exposed to CEC mixtures of known concentrations (Wang 2017, Cipoletti 2018, Cipoletti et al. 2019, Schoenfuss et al. 2019). All of these studies have observed some level of impacts and changes in biological effects endpoints which could indicate detrimental effects on population recovery and sustainability over the long term. They indicate that CECs pose some level of hazard to fish in the field. An EHA is a valuable, versatile, and cost-effective tool that natural resource managers may apply to better understand the potential for chemical-related hazards in the areas they manage.

This CEC EHA addresses all three of the USFWS Five-Year Plan goals listed above, augmenting the USFWS fish health surveys, streamside exposures and laboratory studies by:

- Providing a methodologically consistent analysis of CEC hazard in fish using ambient water samples collected from all 24 project locations throughout 2010-2014;

- Determining hazard in 12 distinct effect categories – many of which were not included in the USFWS fish health studies, laboratory studies, or streamside exposure studies, or in previous EHAs; and
- Evaluating the potential for CEC-related impacts in the fish community as a whole, including sensitive species, rather than a few targeted species.

Assessment approaches are diverse among previously published CEC hazard assessments in aquatic systems (Table 1-1). Some of the studies were limited by considering only a few CECs, and others by considering only acute effects information in the toxicity assessment. Methods for developing a toxicity reference value were highly variable across the set of hazard assessments (see Table 1-1), and exposure assessments often included modeled CEC concentrations rather than measured values. Effect categories considered in previous CEC EHAs are largely limited to survival, growth, development, and reproduction.

This assessment refines and expands on previous approaches to CEC EHAs. First, in the exposure assessment we based our exposure estimates on measured concentrations, significantly reducing the uncertainty inherent in previous EHAs that used modeled Predicted Exposure Concentrations (PECs) to represent exposure. Our empirical exposure database is extensive, including thousands of chemical analyses from over 500 surface water samples collected at 195 water sampling sites distributed among 24 project locations across the US Great Lakes Basin. Second, in the toxicity assessment we developed 82 unique pairs of SVs in 12 individual effect categories for 14 CECs from a wide variety of chemical and human use groups, as opposed to a single SV per chemical representing only a few effect categories as in previous EHAs. Finally, we developed a hazard characterization methodology in which each water sample was evaluated for a suite of potential biological impacts using all available SV pairs. We developed hazard scoring, ranking, and spatially explicit hazard maps to enhance interpretation, and utilized statistical tests and project-wide tallies of exceedances to evaluate spatial correspondence between CEC-related hazard to fish and CEC point sources. Finally, we identified project-wide patterns in CEC-related hazard to Great Lakes Basin fish.

Text boxes throughout this document call out some key points, but are not intended to summarize exhaustively all important information and findings. Subsequent chapters are arranged according to the components of a conventional ecological hazard assessment:

- Problem Formulation (Chapter 2),
- Exposure Assessment (Chapter 3),
- Toxicity Assessment (Chapter 4),
- Hazard Characterization (Chapter 5) with a Hazard Summary/Synthesis (Chapter 6), and
- Uncertainty Assessment (Chapter 7).

Table 1-1. Summary of existing aquatic ecological hazard assessment (EHA) hazard quotients (HQs) for categories of contaminants of emerging concern (CECs) (alphabetical by primary author). Column headers correspond with the conventional elements of an EHA.

CEC Category	CEC Subcategory	CECs	Exposure Assessment	Toxicity Assessment	Hazard Characterization	Hazard Findings	Reference
Personal Care	Fragrance – polycyclic musks	Acetyl hexamethyl tetralin (AHTN), Hexahydrohexamethyl -cyclopentabenzopyran (HHCB)	Measured concentrations in water	Predicted No Effect Concentration (PNEC)[5] for growth in algae, reproductive effects in daphnia and growth or early life stage effects in fish	Hazard Quotient (HQ)[6]: Measured Conc/PNEC	HQ < 1 for both CECs	Balk and Ford, 1999
Pharmaceuticals	Selective serotonin reuptake inhibitor (SSRI)	Fluoxetine	Measured in water	50% Effect Concentration (EC_{50}) or Lowest Observed Effect Concentration (LOEC) values for algae, invertebrates, and fish. The selected screening value was a LOEC for growth inhibition in algae.	HQ: Measured Conc. / Effect Conc.	HQ < 1	Brooks et al. 2003
Pharmaceuticals	22 therapeutic use groups	27 CECs	Predicted Exposure Concentration (PEC)[7] estimated in water	PNEC derived from EC_{50} or chronic No Observed Effect Conc. (NOEC) values adjusted using safety factors for the most sensitive aquatic species among at least three trophic levels	HQ: PEC/PNEC	Some evidence of chronic aquatic hazard for 9 CECs	Carlsson et al. 2006
Pharmaceuticals	SSRI	Paroxetine (PA) and its principal metabolite	PEC estimated in water	PNEC derived from Microtox and daphnia EC_{50} values	HQ: PEC/PNEC	HQ < 1	Cunningham et al. 2004
Personal Care	Fragrance – polycyclic musks	HHCB	Estimated concentrations	PNEC based on lowest chronic NOEC value among algae, daphnia and fish with safety factor applied	Monte Carlo estimates of HQ: Estimated Concentration / NOEC	HQ < 1	Federle et al. (no date)
Pharmaceuticals	Antiepileptic, lipid regulator, non-steroidal anti-inflammatory (NSAID)	Carbamazepine, Clofibric acid, Diclofenac	PEC and some measured concentrations	PNEC based on acute EC_{50}, NOEC or LOEC values for motility, growth, reproduction or mortality from bioassays in bacteria, algae, invertebrates and fish	HQ: PEC or measured Concentration / PNEC	Carbamazepine HQ > 1; other HQs < 1	Ferrari et al. 2003

[5] PNEC values are chemical-specific Predicted No Effect Concentrations of water exposures in various taxonomic groups sometimes based on a percentile of species sensitivity distributions; the specific groups of organisms included in deriving PNECs varies between EHAs

[6] Hazard Quotients (HQs) are unitless ratios of a chemical exposure concentration divided by a toxicity threshold concentration, or, other defined toxicity reference concentration. An HQ is an index of relative hazard in a defined set of organisms from a specified exposure to a particular chemical contaminant. In the field of ecological risk assessment, the word "risk" refers to a quantitative estimate of the probability of specific adverse effects, given the exposures, whereas the word "hazard" implies a more qualitative potential to cause harm (Suter 1993). Values of HQ greater than 1 indicate an elevated potential for biological impacts, while values less than 1 suggest a low potential for harm.

[7] PEC – Predicted Exposure Concentration in water based on chemical characteristics of the CEC

Table 1-1 (continued).

CEC Category	CEC Subcategory	CECs	Exposure Assessment	Toxicity Assessment	Hazard Characterization	Hazard Findings	Reference
Pharmaceuticals	Antiepileptic, lipid regulator, NSAID, antibiotics, antihypertensive	Carbamazepine, Clofibric acid, Diclofenac, Ofloxacin, Propranolol, Sulfamethazole	PEC	Acute PNECs based on growth EC_{50} data in algae, and chronic PNECs based on growth, reproduction, and mortality NOECs in algae, invertebrates and fish	HQ: PEC/PNEC	All HQs < 1	Ferrari et al. 2004
Pharmaceuticals	Analgesics, NSAIDs, lipid regulators, psychiatric drugs, antihistamines, antiulcer agents, antibiotics and β-blockers	29 CECs	Measured in water	PNEC values derived from EC_{50} values in algae, daphnia and fish divided by an assessment factor	HQ: Measured Concentration / PNEC	Some CEC HQ values > 1	Ginebreda et al. 2009
Pharmaceuticals	Antibiotics	Ciprofloxacin, Mecillinam, Trimethoprim	PEC	PNEC values based on EC_{50} or acute NOEC values in bacteria, algae, cyanobacteria, invertebrates or fish	HQ: PEC/PNEC	Ciprofloxacin HQ > 1	Halling-Sorensen et al. 2000
Pharmaceuticals	Analgesic, anti-inflamatories, β-blockers, Antiepileptics	Ibuprofen, Diclofenac, Naproxen, Ketoprofen, Propanolol and Carbamazepine	Measured in water	Acute PNECs based on EC_{50} values in bacteria, algae, invertebrates and fish	HQ: Measured Concentration / PNEC	Concentration > PNEC for Ibuprofen, Diclofenac, Naproxen, Ketoprofen and Carbamazepine	Hernando et al. 2006
Pharmaceuticals	14 therapeutic use groups	23 CECs	PEC	PNECs for 11 CECs were derived from published assay data (not described in detail). PNECs for another 12 CECs were estimated using the ECOSAR model[8].	HQ: PEC/PNEC	HQ > 1 for: paracetamol, amoxicillin, oxytetracycline, mefenamic acid	Jones et al. 2002
Personal Care	Antimicrobial	Triclosan	Estimated using both fugacity model and some measured values	Species sensitivity distribution (SSD) inclusive of EC_x or NOEC values for algae, cyanobacteria, macrophytes, invertebrates, amphibians and fish. Effects included survival and reproduction in fish, and growth, reproduction and survival in the other taxa.	Comparison of distributions: 95^{th} %ile of exposure concentrations compared against 5^{th} %ile of SSD	Exposure 95^{th} %ile consistently below 5^{th} %ile of SSD; adverse effects expected to be unlikely	Lyndall et al. 2010

[8] The Ecological Structure Activity Relationships (ECOSAR) predictive model for estimating aquatic toxicity based on structural characteristics of chemicals.

Table 1-1 (continued).

CEC Category	CEC Subcategory	CECs	Exposure Assessment	Toxicity Assessment	Hazard Characterization	Hazard Findings	Reference
Veterinary Medicines	Veterinary Antibiotics	11 CECs	Measured in water	PNECs were developed from the lowest acute EC_{50} or NOEC values in bacteria, algae, invertebrates, and fish obtained from assay results or from the literature, divided by an assessment factor to adjust for exposure duration.	HQ: Measured Concentration /PNEC	HQ > 1 for 5 antibiotics	Park and Choi 2008
Personal Care	Antimicrobial	Triclosan	Estimated using a dilution model	Lowest acute toxicity values (acute NOECs and EC_x values) for triclosan in algae, macrophytes, invertebrates, and fish.	HQ: Modeled Exposure / Toxicity Value	No HQs ≥ 1	Reiss et al. 2002
Pharmaceuticals	Unspecified	29 CECs	Distribution of measured concentrations	Acute effect concentration (EC_{50}) for fish, daphnids, and algae, and chronic EC_{50} values for all model species were estimated with ECOSAR, a Quantitative Structure-Activity Relationship (QSAR) model	HQ: Highest Measured Concentration / Lowest Estimated Effect Concentration	No acute exposure hazards identified	Sanderson et al. 2003
Pharmaceuticals	SSRIs	Citalopram, Sertraline, Fluoxetine	Estimated concentrations of all three SSRIs, and their sum	PNECs developed from NOEC ranges with uncertainty factors applied	HQ: ∑Concentrations / PNECs	All HQ < 1	Styrishave et al. 2011
Plastics	Plasticizer	Bisphenol A	Measured samples in water	A PNEC was derived as the 5th percentile of chronic effect concentrations (EC_{50}, LC_{50}, NOEC, LOEC) for survival, growth, development, and reproduction effects in algae, invertebrates, amphibians, fish	HQ: Measured Concentration / PNEC	Some exceedances of PNECs	Wright-Walters et al. 2011
Pharmaceuticals	NSAIDs, lipid regulators, antiepileptics	14 CECs	Measured concentrations	PNECs for eight detected CECs were calculated from the lowest chronic NOECs for species in three trophic levels, adjusted with assessment factors.	HQ: Maximum measured concentration / PNEC	Diclofenac HQ > 1	Zhao et al. 2010b

Chapter 2 - Problem Formulation

2.1 Problem Statement

Emerging contaminants are ubiquitous in sampled waterways of the U.S. Great Lakes basin, and throughout the U.S. (Smith and Muir 1998, Kolpin et al. 2002, Rameriz et al. 2009, Klecka et al. 2010, Lee et al. 2011, Lee et al. 2012, Lee et al. 2015, Kostich et al. 2013, Deo 2014, Lee et al. 2015, Elliott et al. 2017), possibly representing an enormous unevaluated stress on, and hazard to, fish populations. There is also a substantial and growing published body of knowledge indicating that many CECs are toxic to fish in laboratory studies at environmentally relevant concentrations in water.

However, the relevance of CEC concentrations to natural resource management decision-making in waterways of the Great Lakes basin, such as in fisheries management, has not been clearly illustrated. Basic understandings such as the nature and extent of biological hazards to fish from environmental CEC exposures are underdeveloped.

2.2 EHA Purpose

The purpose of this EHA is to provide an ecotoxicological interpretation of surface water concentrations of a limited set of CECs measured at 195 sampling sites across the U.S. Great Lakes Basin between 2010 and 2014. We evaluated:

- whether ecological hazard to fish due to exposure to 14 CECs could be discerned in the Great Lakes surface water CEC dataset,
- nature and extent of that hazard, and
- spatial relationships between ecological hazard and mapped CEC point sources.

Additional purposes of this EHA include:

- provide an illustration of how to apply CEC SVs as hazard interpretive tools,
- rank CECs and sampled sites in terms of potential for direct impacts to fish, and
- identify project-wide patterns in hazard from CEC exposure.

2.3 Scale of the Issue

The potential importance of CECs to fish populations and communities in the Great Lakes Basin is reflected partly in the enormous scale of the issue, which is illustrated in the following subsections.

Some Key Points…

Problem Formulation

- **Purpose:** This Problem Formulation section describes the issues and bounds the scope of the hazard assessment.
- **Problem Statement:** CECs are ubiquitous in freshwater systems and are toxic to aquatic life in lab tests at environmentally relevant concentrations. However, knowledge about the distribution and nature of CEC hazards in waterways is underdeveloped.
- **Scale of the Issue**:
  - *Geography*: U.S. Great Lakes basin includes approximately 120,000 river miles of waterways.
  - *Chemicals*: Tens of thousands of CECs, many produced in high quantities annually. Over 2,300 WWTPs and CSOs discharge to waterways in the U.S. Great Lakes basin.
- **Scope of the Assessment:**
  - *Geography:* 24 project locations distributed among waterbodies in the U.S. Great Lakes basin.
  - *Waterbodies:* A wide variety of lotic and lentic systems including the nearshore in Great Lakes near large municipalities.
  - *Ecological Receptors:* Freshwater fish
  - *Temporal Scope:* 2010-2014. Years and seasons in which sampling occurred varied considerably between project locations, but there was substantially less variability between sampling sites within locations.
  - *Contaminants:* 14 CECs from a variety of chemical use classes that were detected relatively frequently in preliminary sampling and for which SVs could be developed.

2.3.1 Geographic Scale

The land area of the U.S. Great Lakes watershed is approximately 305,100 km^2 (117,800 mi^2), as computed using ArcGIS (ESRI 2018). The surface area and volume of the Great Lakes themselves are 244,106 km^2 (94,250 mi^2) and 22,812 km^3 (5473 mi^3), respectively (USCB 2012). Potential fish habitat within the U.S. Great Lakes watershed includes approximately 120,000 miles of rivers, streams and canals, including embedded wetlands, natural lakes and human-made impoundments (Figure 2-1). In the Great Lakes themselves, nearshore fish may be exposed to CECs in coastal wetlands and littoral zones that extend along approximately 8,724 km (5,421 mi) of shoreline in the United States alone (USCB 2012), not including islands or the shoreline of the St. Lawrence River.

2.3.2 Chemical Scale

This project characterizes hazard to fish from CEC exposures and explores spatial relationships between ecological hazard in fish and WWTP and CSO point sources of CECs. There are tens of thousands of CEC parent compounds in commercial products (CECWG 2009; Diamond et al. 2011) - with the number increasing annually - as well as countless metabolites and environmental degradants that are discharged from point sources. For many CECs, annual productionand usage rates are reported in tens, hundreds, or thousands of *tons per year* in the U.S. and Europe during the past few decades, such as the examples provided in Table 2-1. CEC ecotoxicity, by comparison, is reported in terms of micrograms (ug) or nanograms (ng) per L of water, where one ton ≈ 907,200,000,000 ug = 907,200,000,000,000 ng.

Emerging contaminants enter waterways via a variety of point and non-point pathways. Non-point sources include: direct runoff from urban impervious surfaces and agricultural fields; barnyards, confined animal feeding operations (CAFOs) and field-applied sewage sludge biosolids; leaching to groundwater from CAFO lagoons, sanitary lagoons and landfills; and aerial deposition (Wilde et al. 2000, Buxton and Kolpin 2002, La Guardia et al. 2004, Schwartz et al. 2004, Arnon et al. 2008, Petrovic et al. 2008, Song et al. 2010, Zhao et al. 2010a, Clarke and Smith 2011, Luo et al. 2011, Kahn and Lee 2012, Lu et al. 2012, Deo 2014, Ferrey et al. 2018).

Point sources include: wastewater treatment plant (WWTP) discharges; combined sewer overflows (CSOs); stormwater sewer pipe discharges; pharmaceutical and other chemical manufacturing plants and other permitted National Pollutant Discharge Elimination System (NPDES) discharges; and unreported, unpermitted discharges (Heberer 2002, Metcalfe et al. 2003, Gross et al. 2004, Kim et al. 2009, Phillips et al. 2010, Radke et al. 2010, Venkatesan et al. 2012, Barber et al. 2013, Arlos et al. 2014, Du et al 2014, Li et al. 2016, Zha et al. 2017).

Very few WWTPs are equipped for the intentional removal of CECs, and there is no treatment of any kind for waters discharged at CSOs. Common treatment technologies employed at WWTPs are designed principally to remove pathogens and/or nutrients from wastewater streams, not trace organics such as CECs. Although current wastewater treatment technologies may incidentally remove some parent CECs and metabolites, in the process it is also possible that in some cases they produce CEC degradants or reaction products that are much more potent than the original compound. For instance, the probable transformation of triclosan to chlorinated phenols and dioxins (ECCC 2016).

Mapped WWTP sources in the U.S. Great Lakes Basin (Figure 2-2) discharge CECs continuously and CSO inputs of CECs (Figure 2-3) are episodic, occurring during high flow events. Both types are long-term CEC discharge points.

Table 2-1. A few examples of CEC quantities produced or consumed annually in developed countries during the past several decades.

CEC Category	CEC	Country	Year	Quantity[9] Produced (P) or Consumed (C) per year	Source
Pharmaceutical	Ibuprofen	Germany	1995	105 tons (C)	PubChem database
	Ibuprofen	United Kingdom	2002	179 tons (C)	Jones et al. 2002
	Carbamazepine	Germany	1995	80 tons (C)	PubChem database
	Carbamazepine	United Kingdom	2002	40 tons (C)	Jones et al. 2002
Veterinary Drugs	Tetracyclines, Penicillins, Sulfonamides, Aminoglycosides, Lincosamides, Cephalosporins	USA	2009	14,500 tons (C)	FDA 2010
Insecticide	Imidicloprid	USA	2014	1,000 tons (C)	USGS Pesticide National Synthesis Project
	Imidicloprid	USA	2015	400 tons (C)	
Plasticizer	Bisphenol A	USA	2004	768,000 metric tonnes (P)	PubChem database
Personal Care	Triclosan	USA	1998	>500 tons (P)	PubChem database

[b] 1 metric tonne = 1000 kg; 1 ton = 2000 pounds; 1 metric tonne ≈ 1.1 tons.

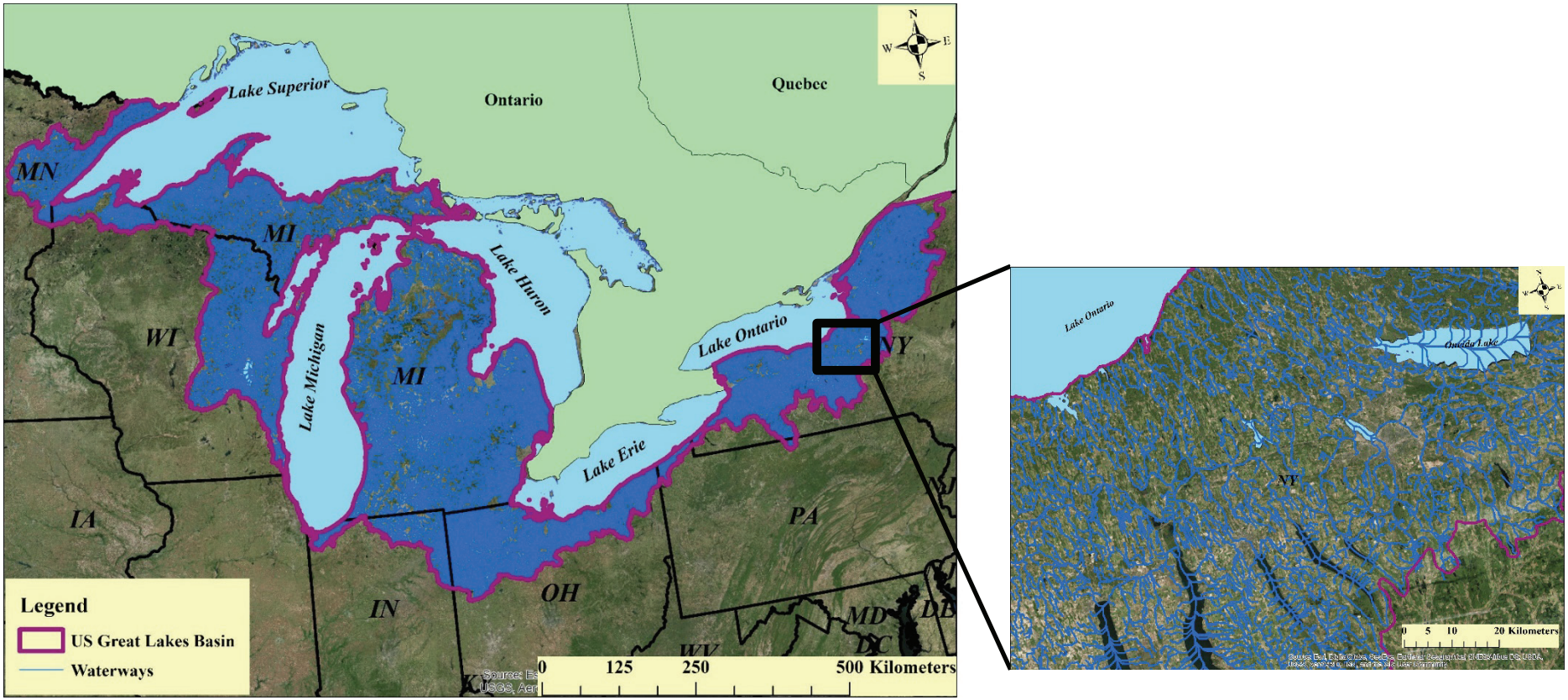


Figure 2-1. The dense network of waterways (depicted in blue) in the U.S. Great Lakes watershed totals approximately 120,000 miles (≈ 193,000 km) of rivers, streams and canals, including embedded inland lakes, reservoirs, and wetlands (source: National Hydrography Dataset (NHD Plus 2)). *Inset:* Central New York is magnified to show detailed complexity and density of Great Lakes basin waterways.

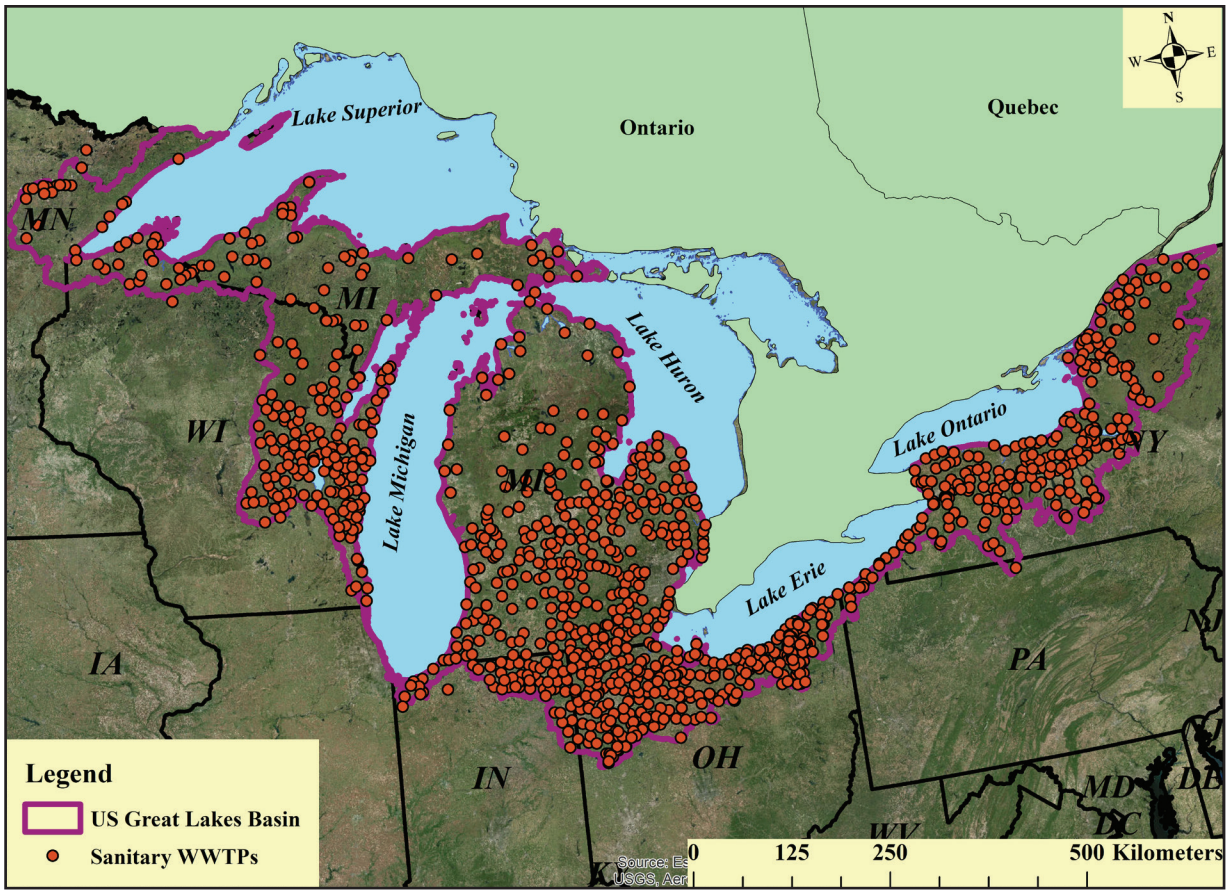


Figure 2-2. Distribution of more than 1,100 wastewater treatment plants (WWTPs) in the U.S. Great Lakes Basin (geographic data sources provided in section 5.3.4.2).

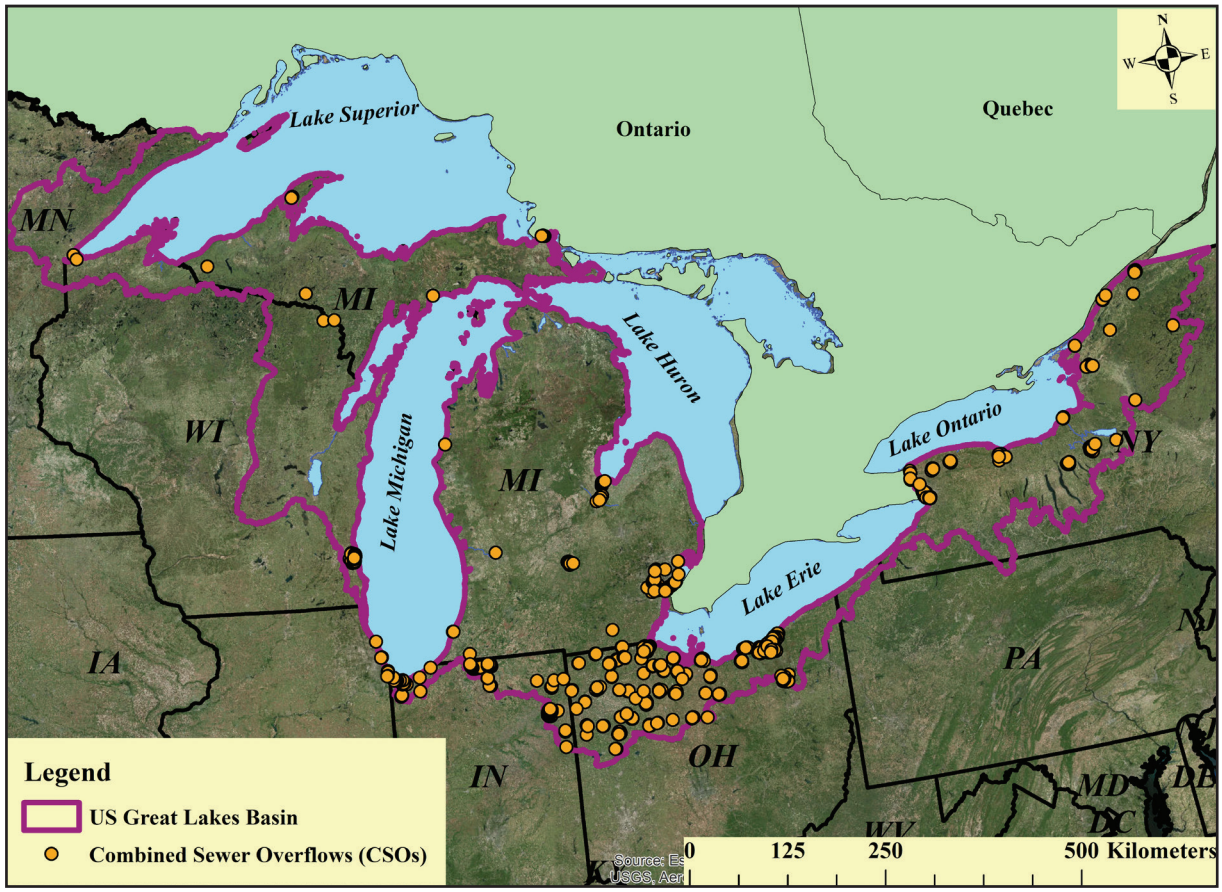


Figure 2-3. Distribution of more than 1,250 CSOs in the U.S. Great Lakes Basin; CSOs tend to occur in close proximity to each other relative to the scale of this map, so many dots overlap (geographic data sources provided in section 5.3.4.2).

2.4 EHA Project Scope

2.4.1 Geography

This EHA evaluates potential for biological impacts in fish from exposure to 14 aqueous CECs in 24 project locations distributed across the U.S. Great Lakes Basin (Figure 2-4). Nearly all of the water samples were collected within major tributaries. The relatively few samples that were collected in the Great Lakes proper were in the vicinity of tributary mouths, mostly near urban centers. Samples were collected in rivers, creeks, ponds, natural lakes, reservoirs, impoundments, and riparian wetlands within tributary river systems, canals and channelized rivers, and at Great Lakes near shore sites and coastal embayments. Within project locations, the size of the entire sampled reach ranged from a few kilometers to over 100 kilometers and the number of sampling sites within the project location ranged from two to 32. Further details on project locations and sampling sites within project locations are provided in Attachment A2.

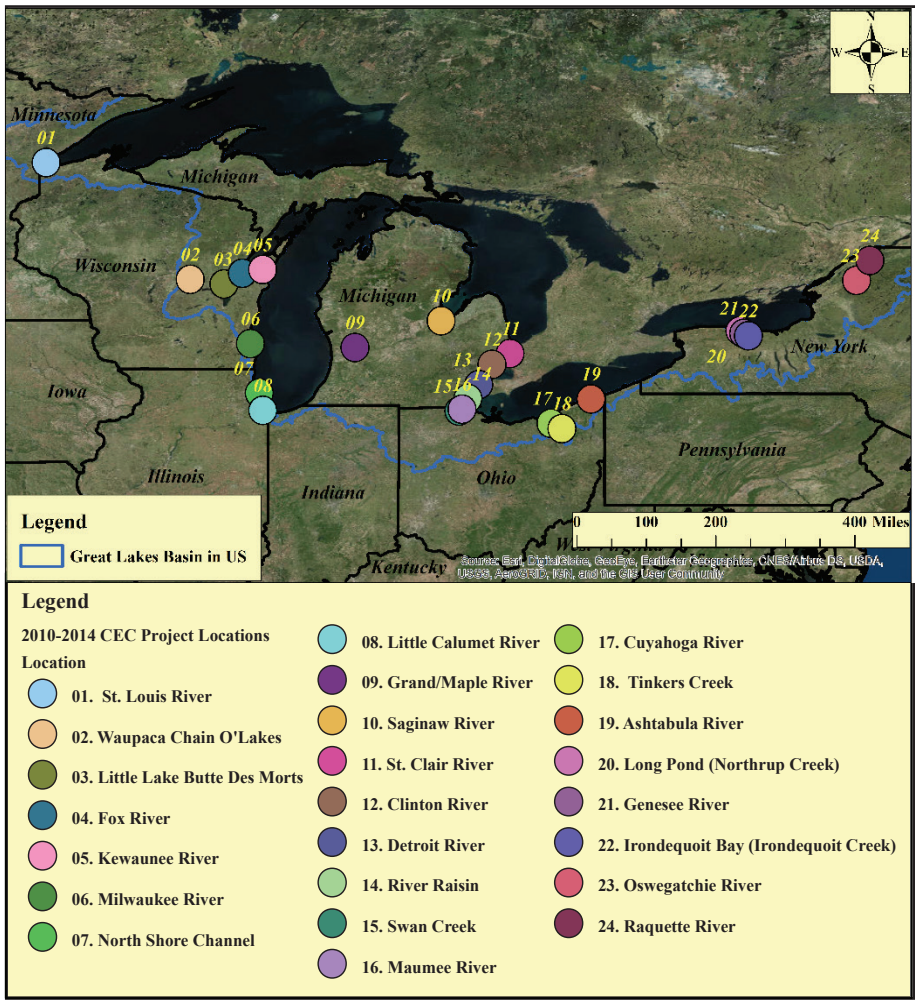


Figure 2-4. Spatial distribution of 24 project locations across the U.S. Great Lakes Basin. The list is ordered approximately west to east, from the St. Louis River in MN, to the Raquette River in NY.

Surface water sampling during 2010-2012 was originally designed to determine the nature and extent of CEC contamination in waterways of the basin (Choy et al. 2017, Lee et al. 2012, Lee et al. 2015). Many of the samples collected during 2010-2012 accompanied field assessments of fish health (Blazer et al. 2014a, Blazer et al. 2014b, Blazer et al. 2018) with the intention of exploring relationships between CEC contamination and biological effects in resident fish[10]. Surface water samples were collected at each site where fish health assessments were conducted. During this period, nine out of 12 project locations were placed in Great Lakes coastal urban centers where the likelihood of detecting CECs and the potential for observing associated biological effects were maximized. These nine urban project locations overlapped a Great Lakes Area of Concern (AOC)[11] within the area sampled. An additional two embayment locations were sampled that were adjacent to, but did not overlap, an AOC (Long Pond and Irondequoit Bay) and a reference location with no mapped CEC point sources was also sampled (Waupaca Chain O'Lakes).

During 2013-2014, a set of 12 different project locations was selected to expand CEC occurrence and fish health assessments to areas where CEC contamination

[10] This project was not initially designed to evaluate relationships between findings of the field fish health assessment and the results of this EHA, but the co-occurrence of fish health assessment findings and water CEC analytical results make this type of analysis feasible. An analysis testing the validity of EHA hazard results using fish health data will be reported elsewhere.

[11] The Great Lakes Water Quality Agreement (GLWQA) between the United States and Canada addresses the degradation of the physical, chemical, and biological integrity of the Great Lakes. In the agreement, first signed in 1972, each country committed to work toward restoration of the Great Lakes Basin. The GLWQA of 1987 (Annex 2) identified locations that have serious contamination and degradation issues to a greater degree than in the rest of the Great Lakes, and designated these locations as Areas of Concern.

was likely present but the confounding presence of significant legacy contamination that invariably occurs in major cities was reduced (Elliott et al. 2017, Thomas et al. 2017, Jorgenson et al. 2018).

Surface water samples were collected at each sampling site where resident fish were collected for the health assessments during 2010-2014, at each sampling site where fish were caged for in situ exposures for a known time period during 2013-2014, and often at additional sampling sites within the same project locations. Water samples were also obtained at project locations that were candidates for fish health assessments but where no fish were ultimately collected (e.g., Oswegatchie River in New York).

2.4.2 Temporal Scope

Whenever fish health assessments were conducted during 2010-2014, water sampling was also conducted. During the spring and early summer of each year, a traveling team of biologists conducted fish health assessments sequentially at a number of project locations distributed widely across the U.S. Great Lakes Basin. The fish health biologist teams differed, and waterbodies sampled differed, between the periods 2010-2012 and 2013-2014. In addition, not all surface water samples used in this EHA were collocated, or were contemporaneous, with fish assessments. Surface water samples for CEC analysis were collected in one or more seasons per year – spring, summer or fall. As a result, the dates of water sampling varied between project locations, and sometimes among sampling sites within locations. See sampling calendar by project location in Section 3.4 (Table 3-1), and sampling schedule by sampling site in Attachment A1 (Table A-2).

2.4.3 Ecological Receptors

This EHA evaluates hazard in fish at the low tolerance end of the chemical sensitivity spectrum in order to identify potential for impacts to populations of resident species (including vulnerable species) and the potential for impacts to fish communities through loss or suppression of populations. Our focus on fish was pragmatic. There is a substantial body of ecotoxicity studies on CEC effects in fish available in the peer-reviewed literature. While it is likely that additional aquatic taxa are impacted by CECs in water or sediment, we located very little ecotoxicity information on CECs in other aquatic taxa – so screening values could not be developed for taxa other than fish at this time. Ideally, we would like to assess threats of CEC contamination to all native and naturalized aquatic species within the U.S. Great Lakes Basin. However, even focusing on fish, there are least 160 fish species resident in the basin (Hubbs and Lagler 2004) representing a wide range of life histories and presumably a range of chemical sensitivities. Moreover, controlled ecotoxicological assays have been conducted with only a few dozen obligate or facultative freshwater fish species. Still fewer fish species have been tested for effects from CEC exposure. Therefore, we derived SVs designed to identify hazard in fish at the low tolerance (high sensitivity) end of the chemical sensitivity spectrum by applying interspecies sensitivity uncertainty factors during SV development (see Section 3.3 *in* Gefell et al. 2019).

2.4.4 Contaminants

Biological CEC-related effects in resident fish are due to the diverse, simultaneous and cumulative influence of many CECs in ambient waters. Ultimately, we would like to be able to evaluate the contribution of as many CECs as possible to the total CEC-related hazard to fish. However, there are tens of thousands of chemicals in commerce that have been referred to as CECs (e.g., CECWG 2009, Howard and Muir 2010, Diamond et al. 2011, Howard and Muir 2011), and standard analytical methods for detection in water have been developed for only a few hundred (e.g., USGS 2014, USEPA 2016a, AXYS 2018). Some CECs are consistently detected in ambient water samples, while others are rarely detected. Among frequently detected CECs, relatively few are represented in the fish ecotoxicity literature.

For this EHA, our approach was to develop surface water CEC screening values for a chemically diverse set of relatively frequently detected CECs. Surface water samples collected during 2010-2012 were analyzed for a total of 114 CECs, including alkylphenols, flavors, fragrances, hormones, pharmaceuticals, plasticizers, flame retardants, sterols and other CECs; legacy PAHs and pesticides were also analyzed (Lee et al. 2012, Lee et al. 2015, Choy et al. 2017). Water samples collected during 2013-2014 were analyzed for a total of 199 chemicals – mostly CECs, but also some PAHs and pesticides (Elliott et al. 2017). Nearly all of the water analytes were parent compounds. Few CEC metabolites or environmental degradants were analyzed, although it seems likely that they represent the bulk of CEC-related contamination in freshwater systems since each parent compound probably yields at least two daughter compounds when metabolized or degraded. We identified 25 of the most frequently detected CECs in our surface water samples collected during 2010-2012. Among these, at least minimally sufficient ecotoxicity information was published to derive surface water SVs for 14 CECs representing a variety of CEC categories (Table 2-2) (see Gefell et al. 2019).

This EHA evaluates the potential for biological impacts in fish from aqueous exposures to these 14 CECs. Although this subset of CECs is distributed among several chemical and human use categories (Table 2-2), the degree to which this set of 14 CECs is representative of CECs as a huge and diverse group is uncertain. Hence, our findings and conclusions pertain only to these CECs, and we caution against extrapolation to other categories of CECs or other individual CECs based solely on the findings reported in this EHA.

Table 2-2. Emerging contaminants evaluated for potential impacts to fish in the Great Lakes CEC EHA (CAS Number and Use Sources: ChemIDPlus, PubMed, PubChem).

Common Chemical Na me	CAS Number	CEC Human Use Category
Androstene-3,17-dione, 4-	63-05-8	Hormone
Bisphenol A	80-05-7	Plasticizer
Carbamazepine	298-46-4	Pharmaceutical - Human - anticonvulsant
Citalopram	59729-33-8	Pharmaceutical - Human - antidepressant
Diethyl-meta-toluamide, N,N- (DEET)	134-62-3	Insect Repellant - Active Ingredient
Diphenhydramine	58-73-1	Pharmaceutical - Human - antihistamine
Estrone	53-16-7	Hormone
Hexahydrohexamethylcyclopentabenzopyran (HHCB)	1222-05-5	Fragrance - Musk
Ibuprofen	15687-27-1	Pharmaceutical - Human - analgesic
Lidocaine	137-58-6	Pharmaceutical - Human – anesthetic and antiarrhythmic
Sitosterol, beta-	83-46-5	Phyto-Hormone
Triclosan	3380-34-5	Personal Care - antibacterial
Tris(2-butoxyethyl)phosphate (TBEP)	78-51-3	Flame Retardant
Venlafaxine	93413-69-5	Pharmaceutical - Human - antidepressant

Chapter 3 - Exposure Assessment

This exposure assessment describes the rationale and methodologies employed to provide CEC concentration data used to characterize hazard to fish at sampled sites.

3.1 Exposure Pathway

One of the first steps in conventional chemical exposure assessment is to identify pathways by which target chemicals move through the environment from the chemical source to eventual uptake by potential receptors. Route of chemical uptake (e.g., dietary ingestion or direct absorption) is a key feature of an aquatic exposure pathway. In this EHA, we considered fish exposures to aqueous chemicals in surface water via passage through gills or skin, and incidental ingestion of water. Surface water CEC concentration was represented as aqueous CEC (i.e., filtered, or dissolved) in surface water. Concentration of aqueous chemical is the principal metric of exposure reported in published laboratory studies of CEC effects in fish (summarized in Gefell et al. 2019). We did not locate any studies evaluating CEC effects in fish after exposure by trophic uptake or after exposure of fish to CECs in sediment. However, trophic uptake of CECs occurs to varying degrees in aquatic systems, depending on CEC chemical properties and water quality characteristics. Bioaccumulation occurs when the rate of total uptake (direct uptake plus trophic uptake) of a chemical outpaces its elimination and metabolism after absorption. This EHA did not account for the trophic uptake exposure route. Nevertheless, the relative potential for bioaccumulation among the 14 CECs may be characterized by their log(K_{ow}) values[12] (Table 3-3), where higher log(K_{ow}) indicates greater potential for partitioning to fish from water increasing the likelihood of bioaccumulation.

3.2 Datasets

Concentrations of CECs in surface water were measured in water samples collected at 195 sampling sites distributed among 24 project locations across the U.S. Great Lakes Basin (Figure 3-1). The surface water analytical dataset used in this CEC exposure assessment was developed during a series of field projects coordinated and implemented by USFWS and the U.S. Geological Survey (USGS) during 2010-2014 (Lee et al. 2012, Lee et al. 2015, Choy et al. 2017, Elliott et al. 2017). All surface water samples were analyzed for CECs at the USGS National Water Quality Laboratory in Denver, Colorado. For samples collected during 2010-2012, sample site rationale (see Attachment A2) and CEC sampling and analytical methods are described in Choy et al. (2017) and Lee et al. (2012, 2015), while 2013-2014 sampling and chemical analyses are described in Elliott et al. (2017).

Concentrations of pharmaceuticals in samples collected during 2013-2014 were determined in filtered water. All other CEC concentrations were measured in unfiltered water (Table 3-2), from which dissolved CEC estimates were computed as described below.

Sample site mapping was conducted using ArcGIS 10.5 for desktop (ESRI 2018). Coordinates for water sampling sites were used as presented in Choy et al. (2017) and Elliott et al. (2017), with minor supplementation or modification as needed using desktop digitizing tools in ArcGIS.

Some Key Points…

Exposure Assessment

- **Purpose:** This Exposure Assessment provides the surface water CEC concentration dataset to be analyzed for hazard to fish
- **Field Sampling Scope**:
  - *Chemicals*: Measured concentrations of hundreds of CECs were determined in water samples
  - *Spatial*: 195 sampling sites among 24 project locations spanning the US Great Lakes basin
  - *Samples*: >500 surface water grab samples
- **Final CEC Exposure Dataset:**
  - *Dataset:* >7,000 water concentration data points for 14 commonly detected CECs
  - *Medium:* Only dissolved concentrations were evaluated - either measured dissolved concentration, or estimated from measured total concentration

[12]The log(K_{ow}) is the common logarithm of the octanol-water partitioning coefficient. It is a chemical characteristic commonly used to gauge potential for bioaccumulation, where higher values (e.g., >3) indicate a greater potential for a significant fraction of CEC in water to partition to forage organisms or suspended particles.

3.3 Spatial Information Relevant to Exposure Assessment

Surface water sampling was originally conducted for purposes other than an EHA. Water CEC concentration data were generated to explore associations with health impairments in resident or caged fish and to provide information on the extent of CEC contamination in the Great Lakes Basin. Within each project location, we sought to identify surface water sampling sites that balanced information value, accessibility, safety, and sampling and analytical costs.

Spatial data relevant to describing fish exposures to aqueous CECs at each of the 24 project locations, including sample site maps, are compiled in Attachment A2. Each of the 24 summaries contains the following elements:

- A map showing project location and general information about each project location within the U.S. Great Lakes Basin
- A list of surface water sampling sites for CEC analysis, which includes:
  - o Site names,
  - o Total number of surface water sampling events at each site during 2010-2014 (further detail on sampling schedule for sampling sites is provided in Attachment A1, Table A-2), and
  - o A flag (the character ">" and bold text) indicating sites that we considered to be influenced by point sources (mapped WWTPs and CSOs) - the remaining sites were considered relatively uninfluenced (Attachment A1, Table A-2).
- A sampling map, showing:
  - o Labeled sampling sites,
  - o Point sources (WWTPs and CSOs), and
  - o Locations of sites where Total Suspended Solids (TSS) had been determined historically, and reported in the U.S. EPA STORET database. The TSS data were used to estimate aqueous CEC from measured total CEC concentration (see Section 3.5 and Attachment A1).
  - o For very large project locations, supplemental maps may be included that zoom in on specific reaches.

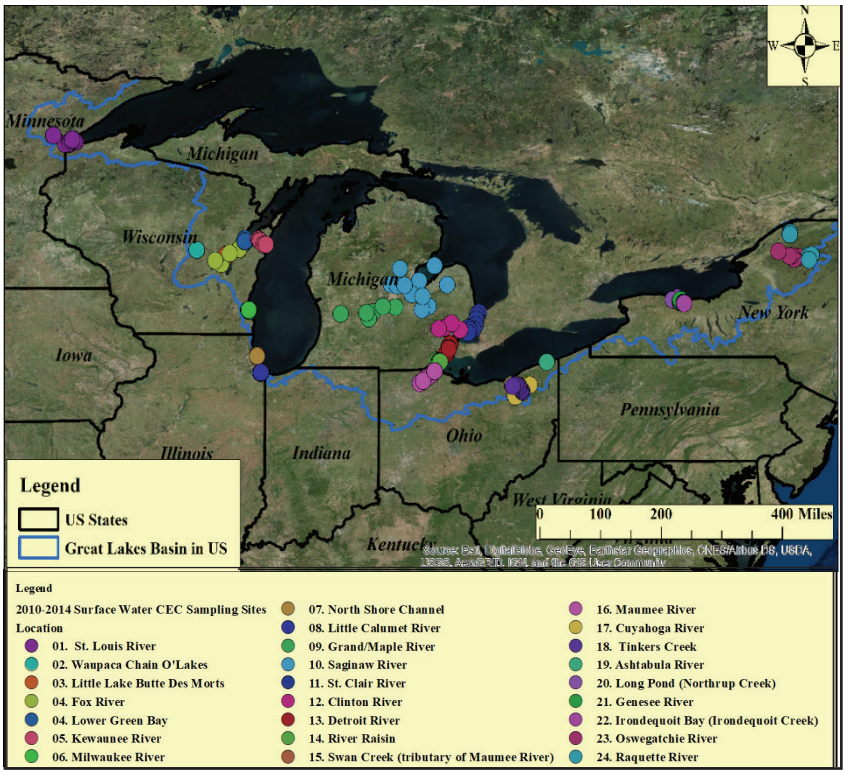


Figure 3-1. Spatial distribution of 195 surface water sampling sites at 24 project locations across the U.S. Great Lakes Basin. Multiple dots of the same color represent individual sampling sites within the same project location. The list is ordered west to east, from the St. Louis River in MN, to the Raquette River in NY.

3.4 Sampling Calendar

The calendar timing of surface water sampling was generally coincidental to field mobilizations for pre- and/or post-spawn fish health surveys in multiple fish species (Blazer et al. 2014a, Blazer et al. 2014b, Thomas et al. 2017, Blazer et al. 2018, Jorgenson et al. 2018). Where multiple water samples were planned for a given sampling site, an attempt was made to distribute sampling times over the daylight hours.

One set of 12 project locations was sampled during 2010-2012 (Choy et al. 2017) and a nearly separate set of 12 locations was sampled during 2013-2014 (Elliott et al. 2017) with little spatio-temporal overlap (Table 3-1). In general, project locations that overlap a Great Lakes Area of Concern were sampled during 2010-2012, and other project locations were sampled during 2013-2014. Since most water sampling was associated with field biological effects studies in pre-spawn fish, specific months of sampling were reasonably consistent across project locations within individual years during 2010-2012, and also during 2013-2014, but months sampled often varied between sampling years within project locations (Table 3-1). Water samples were collected between April and October, providing some information relevant to seasonality of CEC concentrations.

3.5 Estimating Aqueous CEC from Total CEC in Surface Water

Clear interpretation of hazard to fish from exposures to water-borne CECs requires that surface water CEC concentrations are expressed as aqueous CEC (μg/L),

Table 3-1. Great Lakes Basin oroject locations and sampling schedule for surface water CEC data included in the EHA exposure assessment. Cell contacts are months sampled (01 = December), by year. Individual sampling sites within a project location may nit have been sampled in each month indicated. Blank cells indicate either that no surface were collected, or collected samples were excluded from the EHA.

Project Locations (ordered West to East)	Area of Concern	Surface Water Sampling Schedule (Year/Month)				
		2010	2011	2012	2013	2014
1. St. Louis River/Bay, MN	Y	09	08,09	05,09		
2. Waupaca Chain O'Lakes, WI	N			04		
3. Little Lake Butte Des Mortes, WI	N				08	05,08
4. Fox River/Green Bay, WI	Y	10	06	04	08	05,08
5. Kewaunee River, WI	N				05	04,08
6. Milwaukee River, WI	Y		06			
7. North Shore Channel, IL	N					04,05,09
8. Little Calumet River, IL	N					04,05,09
9. Grand River/Maple River, MI	N				05	04,08
10. Saginaw River Watershed, MI	Y					04,05,08
11. St. Clair River, MI	Y				08	
12. Clinton River, MI	Y				06	
13. Detroit River, MI	Y	10	04,05	05		
14. River Raisin, MI	Y			05		
15. Swan Creek, OH	Y	10	04	09		
16. Maumee River, OH	Y		04,05	04,09		
17. Cuyahoga River, OH	Y				06	04,05,08
18. Tinkers Creek, OH	Y				08	04,08
19. Ashtabula River, OH	Y		04			
20. Long Pond, NY [13]	N			04		
21. Genesee River, NY	Y	09	05			
22. Irondequoit Bay, NY [13]	N			04		
23. Oswegatchie River, NY	N				08,10	05,08
24. Raquette River, NY	N				06,07	05,08

for all samples. In the hazard characterization (Chapter 5), exposure concentrations of 14 CECs were compared against SVs to estimate relative hazard of surface water CECs in fish. All of the SVs were developed from laboratory studies in which exposure was expressed as aqueous CEC concentration (μg/L) (Chapter 4), yet CEC analyses of our surface water samples yielded either total (unfiltered) or aqueous (filtered) concentrations (Table 3-2). Exposure concentrations and SVs should be matched with respect to CEC concentration units, medium (water or sediment), and chemical phase (aqueous, particulate, or total) for accurate interpretation of hazard. We converted water CEC from total concentration to aqueous concentration where necessary.

In the exposure dataset, concentration data were flagged as non-detect if the concentration fell below the associated USGS method detection limit (MDL)[14] or reporting limit, as appropriate – all others were considered detected. Detected aqueous concentrations in filtered surface water samples were obtained for most pharmaceuticals during 2013-2014 (Table 3-2) and were used unadjusted in the hazard assessment. However, most records of CEC concentration in the dataset were reported in terms of total (unfiltered) concentration (Table 3-2). In any sample for a given CEC, total CEC concentration is the sum of dissolved and particulate phase concentrations. The relationship between dissolved and particulate chemical concentrations in surface waters is complex and dynamic (USEPA 1999), but may be represented simply as the balance between rate of dissolution and rate of adsorption to (or absorption into) particles. These rates are related to many factors, including:

- chemical properties (e.g., octanol-water partitioning coefficient ($\log K_{ow}$), organic carbon-water partition coefficient ($\log K_{oc}$), and water solubility),
- suspended particles (e.g., total suspended solids (TSS) and fraction organic carbon in particles), and
- surface water properties (e.g., DO and temperature).

Sediment-water partitioning coefficients (K_d; sediment sorption coefficient) have been developed for many environmental contaminants to facilitate interconversion between aqueous (dissolved) and particulate chemical phases:

$K_{d\text{-Chemical A}}$ (L/kg) = [particulate-sorbed Chemical A] (μg/kg) / [dissolved Chemical A] (μg/L)

Application of measured or estimated Kd values to convert from one phase to the other assumes rates of adsorption and dissolution are in equilibrium (USEPA 1999). Published reports of CEC K_d values that were measured under ambient conditions are rare; most of

[13]While not within the current boundary of the Rochester Embayment Area of Concern (REAOC), Long Pond and Irondequoit Bay are both immediately adjacent to, and upstream of, the AOC and were originally within the AOC boundary as depicted in the REAOC Remedial Action Plan. Also, loadings from municipal CEC point sources to these project locations had ceased by the time of sampling due to WWTP facility closures, so it is likely that CEC detections and associated hazards were from historical inputs.

[14]For the purposes of subsequent discussions in this document, the term 'DL' will be considered inclusive of method detection limit and reporting limit, whichever applies.

the K_d constants reported in the literature or databases were measured in the laboratory under particular static conditions or were estimated from chemical properties using models.

The purpose of deriving K_d values was to apply them in estimating aqueous (dissolved; filtered) CEC concentration from measured total (unfiltered) concentration, which includes both dissolved and particulate phases of CEC. Total suspended solids (TSS) and other factors, such as aerobic state, can influence K_d values (USEPA 1999). We adjusted K_d only for TSS and percent organic carbon (fOC) in suspended particles. We are confident in assuming that water samples were collected under aerobic conditions because of the following: all samples were collected in the water column; sampling sites were primarily located in flowing areas of rivers or streams, or in relatively high energy nearshore areas in lentic systems; and most samples were collected during relatively high turbulence periods during or shortly after spring high flow conditions. The sample-specific value for K_d may also be influenced by water temperature; implications for not explicitly accounting for temperature are discussed in Chapter 7 – Uncertainty Analysis.

Among the 14 CECs, measured K_d values[15] for aerobic conditions after a 14-day equilibration period were located in the literature for carbamazepine, DEET, and ibuprofen (Conkle et al. 2012). For these and the other 11 CECs with surface water SVs, K_d values were derived from published K_{ow} or K_{oc} values. CEC-specific input data for estimating K_d values are provided in Table 3-3. Attachment A1 provides the rationale, calculation methods, and references for K_d estimation, and Table A-4 provides month-specific K_d values for the 14 CECs used to estimate sample-specific aqueous CEC. Where both measured and estimated K_d values are available we selected the greater value between them, which conservatively would result in a lower estimated aqueous CEC concentration used in hazard characterization.

Detected total concentrations in unfiltered samples were recorded for all analyzed CECs in 2010-2012 surface water samples and in non-pharmaceutical analytes during 2013-2014 (Table 3-2). We estimated aqueous (dissolved; filtered) CEC concentrations from the unfiltered CEC concentrations as follows:

$$CEC_{aq} = CEC_u \ / \ (TSS*K_d) + 1$$

Where,

CEC_{aq}	= aqueous phase CEC $_{concentration}$ (filtered water) (ug $CEC_{aqueous}$/L)
CEC_u	= unfiltered water (total) CEC concentration (ug CEC_{total}/L)
TSS	= total suspended solids (kg particles/L)
K_d	= sorption coefficient (L/kg particles)

Details on the derivation of this equation from published information are provided in Attachment A1.

[15]The USFWS is collaborating with USGS and others to develop empirical, ambient sediment-water partitioning coefficients (i.e, K_d; sorption coefficient) for inter-conversion between CEC concentration in dissolved phase and total concentration in surface water, but the work is in process at this time.

Table 3-2. Filtration status of surface water samples analyzed for emerging contaminants (from Choy et al. 2017 and Elliott et al. 2017). In some cases, neither filtered nor unfiltered concentrations were available (NA).

Chemical Name	2010	2011	2012	2013[16]	2014
Androstene-3,17-dione, 4-	Unfiltered	Unfiltered		Unfiltered	Unfiltered
Bisphenol A	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Carbamazepine	Unfiltered	Unfiltered	Unfiltered	***filtered***	***filtered***
Citalopram	Unfiltered	Unfiltered	Unfiltered	***filtered***	***filtered***
Diethyl-meta-tolumide,N,N- (DEET	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Diphenhydramine	Unfiltered	Unfiltered	Unfiltered	***filtered***	***filtered***
Estrone		Unfiltered	Unfiltered	Unfiltered	Unfiltered
Hexahydrohexamethyl Cyclopentabenzopyran (HHCB)	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Ibuprofen	Unfiltered[17]	Unfiltered	Unfiltered	Unfiltered[18]	NA
Lidocaine	Unfiltered	Unfiltered	Unfiltered	***filtered***	***filtered***
Sitosterol, beta-	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Triclosan	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Tris (2-butoxyethyl) phosphate (TBEP)	Unfiltered	Unfiltered	Unfiltered	Unfiltered	Unfiltered
Venlafaxine	Unfiltered	Unfiltered	Unfiltered	***filtered***	***filtered***

Table 3-3. Measured and estimated octanol-water partition coefficients ($Log_{10}K_{ow}$) and organic carbon partitioning coefficients (K_{oc}) used for estimating values of sorption coefficients (K_d) for 14 CECs; Attachment A provides details on how these input data were used to derive K_d estimates. NA = not applicable due to published $\mathbf{Log_{10}K_{ow}}$.[19]

Chemical Name	K_d Derivation Method	Input Data for estimating K_d			Source of Input Data
		$Log_{10}(K_{ow})$	$Log_{10}(K_{oc})$[19]	K_{oc}	
Androstene-3,17-dione, 4-	estimated from logKow	2.75	2.26	180.4	ChemIDPlus 2018
Bisphenol A	estimated from logKoc	NA	3.69	4,898	Roberts et al 2014
Carbamazepine	measured	2.45	2.02	104.4	Conkle et al 2012
Citalopram	estimated from logKow	3.5	2.85	708.0	PubChem 2018
DEET	measured	2.18	1.80	63.79	Conkle et al 2012
Diphenhydramine	estimated from logKow	3.27	2.67	465.5	ChemIDPlus 2018
Estrone	estimated from logKoc	NA	3.5	3162	Roberts et al 2014
HHCB	estimated from logKow	5.9	4.75	56,313	PubChem 2018
Ibuprofen	measured	3.97	3.22	1,668	Conkle et al 2012
Lidocaine	estimated from logKow	2.44	2.01	102.5	ChemIDPlus 2018
Sitosterol, beta-	estimated from logKow	9.65	7.72	52,509,151	ChemIDPlus 2018
TBEP	estimated from logKow	3.75	3.05	1,117	ChemIDPlus 2018
Triclosan	estimated from logKoc	NA	3.56	3,631	Roberts et al 2014
Venlafaxine	estimated from logKow	3.2	2.61	409.7	PubChem 2018

[16]For samples collected on August 15-16, 2013 at Tinker's Creek, both filtered and unfiltered analyses were conducted for carbamazepine, citalopram, diphenhydramine, lidocaine and venlafaxine. There was no apparent consistent pattern in relative magnitudes of estimated concentrations using the two methods. We used only the filtered results in this hazard assessment.

[17]In 2010, unfiltered ibuprofen results were reported for a limited number of samples collected from St. Louis River/Bay, Fox River/ Green Bay, and Detroit River.

[18]In 2013, unfiltered ibuprofen results were reported only for samples collected at the Tinkers Creek project location.

[19]Where a reported empirical $\log_{10}K_{oc}$ was provided in the literature it would be used preferentially to compute K_d; $\log_{10}K_{ow}$ would be considered not applicable (NA) in these cases. Where necessary, $\log_{10}K_{oc}$ was computed from $\log_{10}K_{ow}$ as $(0.7919 * \log_{10}K_{ow}) + 0.0784$; see Attachment A for details.

Chapter 4 - Toxicity Assessment

4.1 Background

The principal goal of the CEC EHA toxicity assessment was to develop defensible empirical surface water SVs[20] that are useful for distinguishing relative hazard to fish among sampling sites, among CECs, and relative to CEC sources. A secondary goal was to provide a strength of evidence evaluation for each SV in terms of the quantity of fish ecotoxicity information used in the derivation.

Historically, toxicity assessments for chemical hazard assessment typically have produced a single screening value for each chemical (see Table 1-1 *in* Gefell et al. 2019). These single screening values represented threshold concentrations for adverse effects across multiple effect categories (e.g., a combination of reproductive, growth, and survival effects) and also often across multiple taxa (e.g., all aquatic organisms). Less often, chemical-specific species sensitivity distributions are used to represent toxicity but usually are limited to specific effect categories such as survival and reproduction or limited with respect to the specific toxicity metric (e.g., LC_{50}, EC_{10}, LOAEC, etc).

Conventional hazard characterization generates a single hazard quotient (HQ) for each chemical of potential concern, computed by taking the ratio of a single representative exposure point concentration to a single SV for each chemical. The comparison of a single screening value to a single exposure point concentration results in one of only two possible findings. Either the SV is exceeded by the exposure concentration or not. The hazard interpretation of this dichotomous finding hinges on the intended use of the screening value as either a "lower bound" or an "upper bound" relative hazard threshold.

A "lower bound" SV is used to identify assessment elements that may be excluded from further scrutiny in the ecological risk assessment process, such as specific chemicals of concern, sampling locations, receptor taxa, exposure routes, or chemical uses (such as in future pesticide uses). If measured or predicted surface water concentrations fall below this "lower bound" SV, then ecological hazard is not expected and the scope of the assessment may be reduced accordingly. Examples of lower bound SVs include those used for screening level risk assessments at U.S. Superfund sites (e.g., USEPA 2018b) and the European Union's probable no effect concentrations (PNECs) (ECB 2003). The lower bound SV in this EHA is called the 'SV_{LOW}'.

The other type of SV includes values such as water quality criteria (e.g., USEPA 1993, USEPA 1995, USEPA 2018c), and is an "upper bound" SV. Exceedances of an upper bound SV are associated with an expectation of hazard in the sampled waterbody that, under certain statutory or regulatory authorities, trigger management actions if a sufficient number and/or magnitude of excursions is observed. The upper bound SV in this EHA is called the 'SV_{HIGH}'.

In the conventional paradigm, a HQ is computed for each chemical. If the EHA involves multiple chemicals with screening values, overall hazard is represented as a hazard index (HI). The HI is conventionally calculated as the sum of HQ values

Some Key Points…

Toxicity Assessment

- **Purpose:** Develop defensible empirical surface water screening values (SVs) that are useful for distinguishing relative hazard to fish among sampling sites, among CECs, and relative to CEC sources.
- **Overall Approach:** Expand on the conventional Toxicity Assessment paradigm to provide increased power and flexibility in the interpretation of CEC hazards to freshwater fish.
- **Screening Values**: SVs were developed for 14 CECs and up to 12 effect categories. A total of 82 pairs of SVs was derived. For each CEC, pairs of SVs were developed for each effect category with sufficient ecotoxicity data. Pairs of mean SVs were also calculated for each CEC. Each CEC-specific SV pair is comprised of:
  - SV_{LOW} - a CEC concentration in water below which significant impacts to fish are not anticipated.
  - SV_{HIGH} - a CEC concentration above which adverse impacts in fish are expected.
- **Strength of Information**: A breadth of information score was assigned to each SV based on quantity of information in the supporting ecotoxicity dataset. Strength of ecotoxicity information was used to interpret confidence in hazard findings.

[20]The CEC SVs are not statutory or regulatory standards, criteria or benchmarks, and also should not be construed as absolute toxicity thresholds.

across the chemicals under consideration, and is used in comparisons of overall hazard from multiple chemical exposures between spatial areas or between time periods at a particular site.

4.2 CEC Screening Values

This section highlights the principal characteristics of the CEC SVs that were used in this EHA to interpret the ecotoxicological significance of emerging contaminant exposures in freshwater fish. The SVs were derived from single-chemical exposures, only. Emerging contaminant SV derivation is thoroughly described in Gefell et al. (2019). An array of SVs (Table 4-1) provides interpretive flexibility for characterizing the potential for biological impacts in resident fish from aqueous CEC exposures. Hazard scores generated from application of the SV array (see Section 5.2) can be used to rank hazard and prioritize among alternative research and resource management actions concerning the potential for CEC impacts in freshwater systems, and to focus ecological risk assessments on locations and CECs associated with elevated hazard.

We developed SVs for 12 individual effect categories (behavioral, developmental, growth, mortality, reproductive, circulatory/blood constituents, endocrine, genotoxicity, gross pathology, histopathology, neurological, physiological/metabolic), as availability of appropriate published data allowed. Two sets of SVs were developed for each CEC: "comprehensive" and "population-relevant". Comprehensive SV values incorporate information on all adverse effects reported in the literature for a given effect category. Population-relevant SVs incorporate a subset of adverse effects that are most directly relevant to population-level effects and are used to focus EHAs on the potential for impacts to fish populations. We define population-relevant SVs as those that were derived from exposure-response data concerning effect endpoints that are related to survival (or, mortality), propagation, or growth. Further, we considered population-relevant effect endpoints to be those that are relatively easily incorporated into a quantitative, mechanistic population dynamics model. The concept of "population-relevance" is discussed further in Gefell et al. (2019). Population-relevant effect endpoints are associated with behavioral, developmental, growth, mortality, and reproductive effect categories.

In contrast, derivation of comprehensive SVs utilized all No Observed Adverse Effect Concentrations (NOAECs) and Lowest Observed Adverse Effect Concentrations (LOAECs) for any adverse effect endpoint, not only the population-relevant endpoints. Hence, for example, reproductive exposure-effect data used to derive the population-relevant reproductive SVs are a subset of the reproductive exposure-effect data that were utilized to derive the comprehensive reproductive SVs.

A pair of effect-specific SVs was developed for each CEC, a SV_{HIGH} paired with a SV_{LOW}, for each effect category with sufficient data reported in the literature (Figures 4-1 and 4-2):

- The SV_{LOW} is a CEC concentration in water below which significant adverse impacts to contaminant-sensitive fish species are not anticipated.
- The SV_{HIGH} is a water concentration above which adverse impacts in fish are expected in sensitive fish species.

Comprehensive and population-relevant mean SVs were also developed. Historically, surface water SVs developed under statutory or regulatory guidelines often consisted of a single chronic aquatic SV per individual chemical that is appropriate for screening for potential effects in fish from persistent surface water exposures. Following this precedent, we generated overall mean SVs for each CEC (Figure 4-1; Tables 4-2 and 4-3). For each of the 14 CECs considered in this EHA, comprehensive mean SV_{LOW} values were computed as the geometric mean of effect-specific comprehensive SV_{LOW} values divided by a database adequacy uncertainty factor (UF_{Data}).

For example, for venlafaxine we developed effect-specific comprehensive SV_{LOW} values for seven effect categories, ranging in magnitude from 6.81×10^{-5} to 1.28×10^{-2} μg/L (see Gefell et al. 2019)[21]. We computed the geometric mean of those seven values to be 0.000638 μg/L, and for computing a comprehensive SV_{LOW}, the UF_{Data} for venlafaxine had been assigned a value of 1. Venlafaxine comprehensive mean SV_{LOW} = (0.000638 μg/L / 1) = 0.000638 μg/L.

Comprehensive mean SV_{HIGH} values were generated as the geometric means of effect-specific SV_{HIGH} values divided by a UF_{Data}, and population-relevant mean SV_{HIGH} and mean SV_{LOW} values were produced similarly (Gefell et al. 2019).

The basis for assigning UF_{Data} values for a CEC was the number of effect categories for which a LOAEC or NOAEC was available (see Section 3.7 in Gefell

[21]Several SVs derived in Gefell et al. (2019) were not included in Table 4-1, including comprehensive effect-specific SVs developed for behavioral, developmental, growth, mortality, and reproductive effect categories. These comprehensive SVs incorporated some published ecotoxicity information for effect endpoints that were not considered population-relevant. Comprehensive behavioral, developmental, growth, mortality, and reproductive effect categories were excluded from this EHA to reduce confusion with population-relevant SVs derived for the same effect categories. The "population-relevance" of effect endpoints and the distinction between comprehensive and population-relevant SVs are described in Gefell et al. (2019).

et al. 2019). A high value for UF_{Data} reflects a low number of component effect-specific SVs and results in up to a five-fold reduction in the mean SV value. We developed a rubric for assigning UF_{Data} values (see Section 3.7 in Gefell et al. 2019). For the venlafaxine example above, we had developed a SV_{LOW} for seven effect categories, a relatively high number, so the assigned UF_{Data} value was '1'.

The CEC SVs were consistently derived using empirical data from published, controlled laboratory studies (Gefell et al. 2019). Data incorporated into SV derivations were required to meet explicit inclusion criteria. Published NOAECs were the empirical basis for developing SV_{LOW} values. We applied uncertainty factors (UFs) to individual NOAEC values reported in the literature to obtain distributions of SV_{LOW} point estimates, from which the effect-specific SV_{LOW} values were derived (see Chapter 4 in Gefell et al. 2019). Similarly, SV_{HIGH} values were developed from LOAECs by applying UFs. The UFs were derived and applied to account for several sources of uncertainty in these extrapolations: chemical exposure complexity, LOAEC-to-NOAEC extrapolation, exposure duration, inter-species sensitivity, intra-species sensitivity, and database adequacy. The rationale and methods used to derive the uncertainty factors are detailed in Gefell et al. (2019).

The CEC SVs are intended to be applied as pairs (SV_{HIGH} and SV_{LOW}) to score relative hazard. Up to a total of 82 SV pairs were applied to each water sample evaluated in this EHA (Table 4-1; Figure 4-2). We characterized hazard from CEC exposure in fish at 195 sampling sites across the Great Lakes Basin by applying the entire array of SV pairs. Although SV_{HIGH} and SV_{LOW} values vary over several orders of magnitude between CECs and effect categories (Figure 4-2), exposure concentrations are also variable (both spatially and temporally) and concentrations of different CECs can vary independently of each other - so it is not always the case that CECs with the lowest SV values are associated with greatest hazard.

4.3 SV Strength of Information

The purpose of this section is to provide a guide to consistent interpretation of relative strength of evidence used in the toxicity assessment for this EHA. There is uncertainty in hazard characterization related to the quality and quantity of information brought to bear in both the exposure and toxicity assessments. The principal features of the toxicity assessment are SVs, and our uncertainty in the SVs is inversely related to the strength of the underlying ecotoxicity information from the literature.

4.3.1 Breadth of Ecotoxicity Information for SVs

We developed breadth of information scores for each SV in order to facilitate strength of evidence communication and comparisons across CECs and effect categories during hazard characterization (Chapter 5). To simplify this analysis, information *quality* does not factor into our strength of evidence evaluation for SVs. We assumed that the quality of the ecotoxicity information used to derive SVs is adequate across all CECs and effect categories. We based this assumption on the fact that we applied quality assurance guidelines, study inclusion criteria, and data entry guidance to literature data during development of effect-specific SVs. In short, ecotoxicity information used to derive SVs was limited to data reported in the peer-reviewed primary literature that satisfied explicit study design and reporting attributes (see Chapters 2 and 3 in Gefell et al. 2019).

The *quantity* of reliable data therefore formed the basis of scoring individual SVs for breadth of information. Information scores for individual SVs ranged from 1 to 5 with the following interpretations regarding the ecotoxicity dataset: 5 = robust, 4 = broad, 3 = moderate, 2 = limited, 1 = sparse, 0 = No Data. These ordinal scores reflect only the breadth of information that was reported in the literature in ecotoxicity assays.

Effect-specific SVs and mean SVs were scored for breadth of information using different methods because they were derived differently (refer to Section 4.3 *in* Gefell et al. 2019). For effect-specific SVs, we scored breadth of information using the rubric provided in Table 4-4. The rubric was applied to data summarized in Attachment C concerning numbers of fish species and effect endpoints by SV effect category and CEC.

Mean SVs are geometric means of component effect-specific SVs, adjusted with a database adequacy UF to reflect the robustness of the ecotoxicity data used for its derivation (Figure 4-1). Confidence in each mean SV has already been evaluated in Gefell et al. (2019) using a rubric that assigns "confidence level" (High, Moderate, Low, Very Low) based on three measures of representation in the literature database:

- total number of effect categories represented,
- number of represented effect categories with three or more SV point estimates, and
- number of fish species represented.

For each mean SV, a breadth of information score (5, 4, 2, or 1) was assigned based on these "confidence levels", as follows:

"Confidence Level" for Mean SVs (as reported in Gefell et al. 2019)	Breadth of Information Score (used in this EHA)
High	5
Moderate	4
Low	2
Very Low	1

The breadth of information scores for population-relevant mean SVs are provided in Table 4-5; scores for comprehensive mean SVs are provided in Table 4-6.

4.3.2 Variability in Breadth of Information Scores

Our confidence in SVs varied between CECs and between effect categories due to differences in the robustness of the underlying ecotoxicity database. This begs the questions - in which CEC- and effect-specific SVs are we most confident? Least confident? We used variability in the breadth of information scores to rank our confidence among SVs across CECs, and across effect categories.

Ranking Confidence Among Effect Categories

Our confidence in SVs varied among effect categories. For each CEC, information in the ecotoxicity literature was sufficient to develop SVs only for certain effect categories (Table 4-1). For effect categories with a SV pair, we applied a rubric to score breadth of information for the individual SVs; the rubric is based on total numbers of fish species tested and effect endpoints evaluated (Table 4-4). Score assignments using the rubric range from 1 to 5. While the range of possible score values was arbitrary, it adequately provides a gross ordinal index of information quantity for each SV.

In general, the ecotoxicological dataset is more robust for SVs of population-relevant effect categories than for SVs of comprehensive effect categories, as indicated by effect-specific average scores (Tables 4-5 and 4-6). The breadth of information scores for population-relevant effect categories (Table 4-5) suggests greater confidence in population-relevant SV_{LOW} values than SV_{HIGH} values, for most effect categories. Average breadth of information scores were consistently higher for the SV_{LOW} than for the SV_{HIGH} (left margin of Table 4-5). This suggests that the quantity and/or diversity of available NOAEC information (used to derive SV_{LOW}) was consistently greater than the available LOAEC information (used to derive SV_{HIGH}) with respect to fish species tested and effect endpoints considered.

In summary, we sorted population-relevant effect categories by degree of confidence using averages of breadth of information scores, computed across the 14 CECs by effect category (Table 4-5).

Breadth of Ecotoxicity Information	Order of Confidence in Population-relevant SVs: Highest to Lowest
Robust Dataset ↓ Sparse Dataset	Mean Population-relevant SV Developmental Behavioral Reproductive Mortality Growth

In contrast to population-relevant effect categories, there is little discrepancy in mean breadth of information scores between the SV_{LOW} and SV_{HIGH} for comprehensive effect categories (left margin of Table 4-6). Comprehensive effect categories were sorted by degree of confidence based on effect-specific average scores provided in Table 4-6:

Breadth of Ecotoxicity Information	Order of Confidence in Comprehensive SVs: Highest to Lowest
Robust Dataset ↓ Sparse Dataset	Mean Comprehensive SV Circulatory/Blood Constituents Endocrine Physiological/Metabolic Histopathology Genotoxicity = Gross Pathology = Neurological

Ranking Confidence Among Emerging Contaminants

We ranked CECs for breadth of information using CEC-specific averages of scores, computed across effect categories (Tables 4-5 and 4-6). With few exceptions, the averages of breadth of information scores for CEC-specific, population-relevant SV_{LOW} values were greater than those for corresponding SV_{HIGH} values (see bottom margin in Table 4-5).

Population-relevant SVs in at least one effect category were developed for each CEC, so CEC-specific mean breadth of information scores were computed across effect categories for each of the 14 CECs. In summary, we ranked CECs in decreasing order of mean breadth of ecotoxicity information (see bottom margin in Table 4-5), as follows:

Breadth of Ecotoxicity Information	CEC
Robust Dataset ↓ Sparse Dataset	Bisphenol A Carbamazepine Triclosan Ibuprofen Estrone HHCB = β-Sitosterol Venlafaxine TBEP Citalopram Diphenhydramine DEET Androstenedione = Lidocaine

In this EHA, effect-specific comprehensive SVs are used for eight CECs, and mean comprehensive SVs for all 14 CECs were used (Table 4-1). We computed CEC-specific mean breadth of information scores across effect categories (see bottom margin in Table 4-6), and then ranked CECs in decreasing order of breadth of ecotoxicity information used to derive SVs, as follows:

Breadth of Ecotoxicity Information	CEC
Robust Dataset ↓ Sparse Dataset	DEET Ibuprofen Carbamazepine Venlafaxine Bisphenol A = Estrone = HHCB = β-Sitosterol

Table 4-1. A total of 82 SV pairs were applied to each surface water sample included in this EHA. Cells marked with an 'X' indicate that a SV pair is available. Section 4.2 describes the distinction between 'comprehensive' and 'population-relevant' SVs, and presents how mean SVs were computed from effect-specific SVs.

Effect Category		4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramie	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
POPULATION-RELEVANT SVs															
Population-relevant Mean SVs		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Effect-Specific SVs	Behavioral		X	X	X		X	X	X		X	X	X	X	X
	Developmental		X	X			X	X	X	X		X	X	X	
	Growth	X	X	X					X						
	Mortality		X	X		X			X	X			X	X	X
	Reproductive		X	X				X		X				X	X
COMPREHENSIVE SVs															
Comprehensive Mean SVs [23]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Effect-Specific SVs	Circulatory/ Blood Constituents			X		X				X		X			
	Endocrine					X				X					X
	Genotoxicity									X					
	Gross Pathology		X												
	Histopathology			X											X
	Neurological			X											
	Physiological / Metabolism			X				X	X	X					

[23]For six CECs (androstenedione, citalopram, diphenhydramine, lidocaine, TBEP, and triclosan) comprehensive mean SVs were applied in this EHA even though no *comprehensive* effect-specific SVs were used for those CECs. In Gefell et al. (2019), SVs were derived for *comprehensive* behavioral, developmental, growth, mortality, and reproductive effect categories, as well as *population-relevant* behavioral, developmental, growth, mortality, and reproductive effect categories. The difference is that comprehensive SV versions of these effect categories incorporated LOAECs and NOAECs for effect endpoints that were not considered to be population-relevant (for example, altered plasma vitellogenin). *Comprehensive* behavioral, developmental, growth, mortality, and reproductive SVs were included in computations of comprehensive mean SVs (Gefell et al. 2019), but were excluded in this EHA as effect-specific SVs.

Table 4-2a. Comprehensive SV_{HIGH} values: Aqueous concentrations of CECs in surface water (μg/L) above which it is reasonable to expect adverse effects in freshwater fish, based on currently available published literature on CEC ecotoxicity in fish. The mean comprehensive SV_{HIGH} = (geometric mean of effect-specific SVs) / UF_{DATA}. A blank cell indicates that data were not sufficient to generate an effect-specific SV estimate. Comprehensive SV_{HIGH} values in this table with no complimentary SV_{LOW} value were not included in this EHA.

Chemical Name	Comprehensive Mean[24] SV_{HIGH} (geometric mean of Effect-Specific SVs / UF_{DATA})	Database Adequacy UF (UF_{DATA})	Effect-Specific Comprehensive SV_{HIGH} Estimates (µg/L)						
			Circulatory/ Blood Constituents	Endocrine	Genotoxicity	Gross Pathology	Histopathology	Neurological	Physiology/ Metabolism
Androstene-3,17-dione, 4-	8.52E-01	5							
Bisphenol A	1.18E+02	1				4.92E+01			
Carbamazepine	1.39E+02	1	4.36E+02				1.89E-01	4.36E+02	3.06E+02
Citalopram	2.22E-01	5							
DEET	2.20E+01	3	1.89E+02	7.58E-02					
Diphenhydramine	1.26E+00	5							
Estrone	6.65E-03	3							2.05E-03
HHCB	2.13E+01	1							2.84E+00
Ibuprofen	1.05E+01	1	5.38E+03	1.89E+02	1.26E-02				1.89E+02
Lidocaine	8.90E+02	5							
Sitosterol, β-	1.84E+01	3	1.42E+01						
Triclosan	1.28E+01	3							
TBEP	2.67E+02	3							
Venlafaxine	1.55E-01	1		2.53E-02			1.89E-01		

[24]Mean SVs were derived as the geometric mean of component effect-specific SVs divided by a Database Adequacy uncertainty factor, as described in Gefell et al. (2019). In addition to the effect-specific SVs provided in this table, comprehensive SVs have also been developed for behavioral, developmental, growth, mortality, and reproductive effect categories. They were excluded from this EHA to reduce confusion with population-relevant SVs of the same effect categories. Population-relevance of effect endpoints is discussed in Gefell et al. (2019). Comprehensive mean SVs were derived from all effect categories, including those not shown in this table.

Table 4-2b. Comprehensive SV_{LOW} values: Aqueous concentrations of CECs in surface water (μg/L) below which it is reasonable to expect no significant adverse effects freshwater fish, based on currently available published literature on CEC ecotoxicity in fish. The mean comprehensive SV_{LOW} = (geometric mean of effect-specific SVs) / UF_{DATA}. A blank cell indicates that literature information was not sufficient to generate an effect-specific SV estimate. Comprehensive SV_{LOW} values in this table with no complimentary SV_{HIGH} value were not included in the EHA.

Chemical Name	Comprehensive Mean[25] SV_{LOW} (geometric mean of Effect-Specific SVs / UF_{DATA}) (µg/L)	Database Adequacy UF (UF_{DATA})	Effect-Specific Comprehensive SV_{LOW} Estimates (µg/L)						
			Circulatory/ Blood Constituents	Endocrine	Genotoxicity	Gross Pathology	Histopathology	Neurological	Physiology/ Metabolism
Androstene-3,17-dione, 4-	2.04E-04	5							
Bisphenol A	3.18E-02	1				2.70E-01			
Carbamazepine	8.65E-03	1	2.55E-03				5.11E-04	2.55E-03	2.55E-03
Citalopram	1.02E-04	5							
DEET	2.36E-02	1	1.28E-01	3.40E-04			1.28E+00		1.28E+00
Diphenhydramine	8.46E-03	5							
Estrone	1.44E-05	1							1.38E-07
HHCB	6.49E-02	1							1.92E-03
Ibuprofen	1.53E-02	1	1.45E+01	5.11E-01	5.65E-05		2.55E-01		5.11E-01
Lidocaine	2.40E+00	5							
Sitosterol, β-	6.04E-02	1	3.19E-02						
Triclosan	2.54E-03	1			2.98E-01		1.15E-03		
TBEP	4.48E-01	3							
Venlafaxine	6.38E-04	1		6.81E-05			5.11E-04		

[25]Mean SVs were derived as the geometric mean of component effect-specific SVs divided by a Database Adequacy uncertainty factor, as described in Gefell et al. (2019). In addition to the effect-specific SVs provided in this table, comprehensive SVs have also been developed for behavioral, developmental, growth, mortality, and reproductive effect categories. They were excluded from this EHA to reduce confusion with population-relevant SVs of the same effect categories. Population-relevance of effect endpoints is discussed in Gefell et al. (2019).

Table 4-3a. Population-relevant SV_{HIGH} values: Aqueous concentrations of CECs in surface water (μg/L) above which it is reasonable to expect adverse effects in freshwater fish populations, based on currently available published literature on CEC toxicity in fish. The mean population-relevant SV_{HIGH} = (geometric mean of effect-specific SVs) / UF_{DATA}. A blank cell indicates that literature information was insufficient to generate an effect-specific SV estimate. Population-relevant SV_{HIGH} values in this table with no complimentary SV_{LOW} value were not included in the EHA.

Chemical Name	Population-relevant Mean SV_{HIGH} (geometric mean of Effect-Specific SVs / UF_{DATA}) (μg/L)	Database Adequacy UF (UF_{DATA})	Effect-Specific Population-relevant SV_{HIGH} Estimates (μg/L)				
			Behavioral	Developmental	Growth	Mortality	Reproductive
Androstene-3,17-dione, 4-	3.23E+00	5			1.62E+01		
Bisphenol A	1.77E+02	1	6.06E+02	8.83E+01	2.59E+02	6.80E+02	1.83E+01
Carbamazepine	2.99E+02	1	4.13E+02	7.07E+02	7.19E+02	1.33E+03	8.52E+00
Citalopram	2.37E-01	5	1.19E+00				
DEET	7.10E+03	3				2.13E+04	
Diphenhydramine	3.35E+00	2	2.26E+00	1.98E+01			
Estrone	1.86E-02	2	2.02E-02	7.54E-02			3.39E-02
HHCB	6.02E+01	1	3.68E+01	5.66E+01	7.94E+01	7.94E+01	
Ibuprofen	8.22E-01	2		2.70E-01		5.78E+01	2.85E-01
Lidocaine	9.49E+02	5	4.75E+03				
Sitosterol, β-	1.01E+02	2	2.02E+01	2.02E+03			
Triclosan	4.08E+01	1	2.28E+01	7.38E+01		4.04E+01	4.08E+01
TBEP	5.11E+02	2	1.75E+03	1.62E+03		3.77E+02	
Venlafaxine	2.85E-01	2	7.44E-01			6.16E-02	4.04E+00

Table 4-3b. Population-relevant SV_{LOW} values: Aqueous concentrations of CECs in surface water (µg/L) below which it is reasonable to expect no significant adverse effects in freshwater fish populations, based on currently available published literature on CEC toxicity in fish. The mean population-relevant SV_{LOW} = (geometric mean of effect-specific SVs) / UF_{DATA}. A blank cell indicates that literature information was not sufficient to generate an effect-specific SV estimate. Population-relevant SV_{LOW} values in this table with no complimentary SV_{HIGH} value were not included in the EHA.

Chemical Name	Population-relevant Mean SV_{LOW} (geometric mean of Effect-Specific SVs / UF_{DATA}) (µg/L)	Database Adequacy UF (UF_{DATA})	Effect-Specific Population-relevant SV_{LOW} Estimates (µg/L)				
			Behavioral	Developmental	Growth	Mortality	Reproductive
Androstene-3,17-dione, 4-	1.27E-03	2			2.54E-02		2.54E-04
Bisphenol A	6.22E-02	1	3.82E+00	6.36E-04	1.27E-01	1.65E+00	1.82E-03
Carbamazepine	7.88E-02	1	2.54E-02	7.25E-03	2.29E+00	5.66E+00	1.27E-03
Citalopram	2.54E-04	5	1.27E-03				
DEET	1.27E-03	2			2.54E-03	2.54E-03	2.54E-03
Diphenhydramine	5.27E-02	2	3.56E-02	3.12E-01			
Estrone	1.15E-04	1	3.18E-05	3.18E-05		1.68E-03	1.02E-04
HHCB	9.10E-01	1	5.91E-01	8.65E-01	1.16E+00	1.16E+00	
Ibuprofen	4.17E-03	1	8.65E+00	6.36E-05	2.83E-02	6.36E-04	1.27E-04
Lidocaine	5.98E+00	5	2.99E+01				
Sitosterol, β-	1.71E-01	1	3.18E-03	9.48E+00	4.77E-01	3.18E+00	3.18E-03
Triclosan	2.86E-03	1	2.86E-03	2.86E-03	2.86E-03	2.86E-03	2.86E-03
TBEP	1.67E+00	2	1.65E+01	9.54E-01		2.37E+00	
Venlafaxine	2.60E-03	1	1.27E-03	3.18E-02	7.02E-03	6.47E-04	6.47E-04

Table 4-4. Rubric for assigning scores to the breadth of ecotoxicity information used to derive an effect-specific screening value (SV). Total number of effect endpoints is a count of the endpoints evaluated among all published assays that were used in the derivation of an effect-specific SV. Total number of fish species is a count of species tested that were included among the assays used to derive a SV. Breadth of fish ecotoxicity information score key: 5 = robust, 4 = broad, 3 = moderate, 2 = limited, 1 = sparse.

Total Number of Unique Effect Endpoints Tested[26] [27]	Total Number of Fish Species Tested					
	1	2	3	4	5	>5
1	1	1	1	1	1	1
2	1	1	1	1	1	2
3	1	1	2	2	2	3
4	2	2	2	3	3	3
5	2	2	3	3	3	4
6	2	3	3	3	4	4
7	3	3	3	4	4	5
8	3	4	4	4	5	5
>8	3	4	4	5	5	5

[26]In some cases, effect endpoints were summarized as groups of related effect endpoints in Gefell et al. (2019) (e.g., reproductive hormones in plasma) but were counted here as a single endpoint.

[27]For the mortality effect category, there is only one effect endpoint but it is extreme, so breadth of ecotoxicity information score = number of fish species, where ≥5 species = score of 5 assigned.

Table 4-5. Breadth of the ecotoxicity information used to derive population-relevant screening values. For effect-specific SVs, information scores were assigned using the rubric in Table 4-4. The rubric was applied to data presented in Attachment C. Gray shading indicates that no SV pair is available. Breadth of fish ecotoxicity information scoring key: 5 = robust, 4 = broad, 3 = moderate, 2 = limited, and 1 = sparse.

Population-relevant Effect Category	SV Type (SV_{HIGH} or SV_{LOW})	Mean by Effect Category Across 14 CECS (range of possible values: 0.07 to 5)	Breadth of Information Scores by CEC and SV													
			4-Androstenedione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta	TBEP	Triclosan	Venlafaxine
Population-relevant Mean SVs	SV_{HIGH}	**1.82**	1	5	5	1	1	1	2	2	4	1	2	1	4	1
	SV_{LOW}	**2.86**	1	5	4	1	1	1	4	2	4	1	5	2	5	4
Behavioral	SV_{HIGH}	**1.6**		1	4	3		1	1	2		1	2	1	5	1
	SV_{LOW}	**1.6**		1	4	4		1	1	2		1	2	1	5	1
Developmental	SV_{HIGH}	**2.1**		5	4			1	4	2	4		4	2	3	
	SV_{LOW}	**2.4**		5	4			1	4	4	4		5	2	5	
Growth	SV_{HIGH}	**0.3**	1	1	1					1						
	SV_{LOW}	**0.4**	1	1	2					1						
Mortality	SV_{HIGH}	**0.8**		2	1		1			1	3			1	1	1
	SV_{LOW}	**1.2**		5	2		2			1	3			1	2	1
Reproductive	SV_{HIGH}	**1.3**		5	3				3		4				1	2
	SV_{LOW}	**1.6**		5	3				3		4				3	4
Mean by CEC Across 5 Effect-Specific Categories (range of possible values: 0.2 to 5)	**SV_{HIGH}**		**0.2**	**2.8**	**2.6**	**0.6**	**0.2**	**0.4**	**1.6**	**1.2**	**2.2**	**0.2**	**1.2**	**0.8**	**2**	**0.8**
	SV_{LOW}		**0.2**	**3.4**	**3**	**0.8**	**0.4**	**0.4**	**1.6**	**1.6**	**2.2**	**0.2**	**1.4**	**0.8**	**3**	**1.2**

Table 4-6. Breadth of the ecotoxicity information used to derive comprehensive effect-specific screening values. Cell contents are breadth of information scores for SVs. For effect-specific SVs, scores were assigned using the rubric in Table 4-4 using data in Attachment C. Gray shading indicates that no SV pair is available to score. Breadth of fish ecotoxicity information scoring key: 5 = robust, 4 = broad, 3 = moderate, 2 = limited, and 1 = sparse.

Comprehensive Effect Category	SV Type (SV_{HIGH} or SV_{LOW})	Mean by Effect Category Across 14 CECS (range of possible values: 0.07 to 5)	Breadth of Information Scores by CEC and SV													
			4-Androstenedione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta	TBEP	Triclosan	Venlafaxine
Comprehensive Mean SVs	SV_{HIGH}	**1.93**	1	4	4	1	1	1	2	2	4	1	1	1	2	2
	SV_{LOW}	**2.57**	1	4	4	1	4	1	2	2	5	1	4	1	4	2
Circulatory/ Blood Constituents	SV_{HIGH}	**0.6**			1		3				3		1			
	SV_{LOW}	**0.6**			1		3				3		1			
Endocrine	SV_{HIGH}	**0.36**					3				1					1
	SV_{LOW}	**0.36**					3				1					1
Genotoxicity	SV_{HIGH}	**0.07**									1					
	SV_{LOW}	**0.07**									1					
Gross Pathology	SV_{HIGH}	**0.07**		1												
	SV_{LOW}	**0.07**		1												
Histopathology	SV_{HIGH}	**0.14**			1											1
	SV_{LOW}	**0.14**			1											1
Neurological	SV_{HIGH}	**0.07**			1											
	SV_{LOW}	**0.07**			1											
Physiological/Metabolic	SV_{HIGH}	**0.24**			1				1	1	1					
	SV_{LOW}	**0.24**			2				1	1	1					
Mean by CEC Across 7 Specific Effect Categories (range of possible values: 0.2 to 5)	**SV_{HIGH}**			**0.1**	**0.6**		**0.9**		**0.1**	**0.1**	**0.7**		**0.1**			**0.3**
	SV_{LOW}			**0.1**	**0.7**		**0.9**		**0.1**	**0.1**	**0.7**		**0.1**			**0.3**

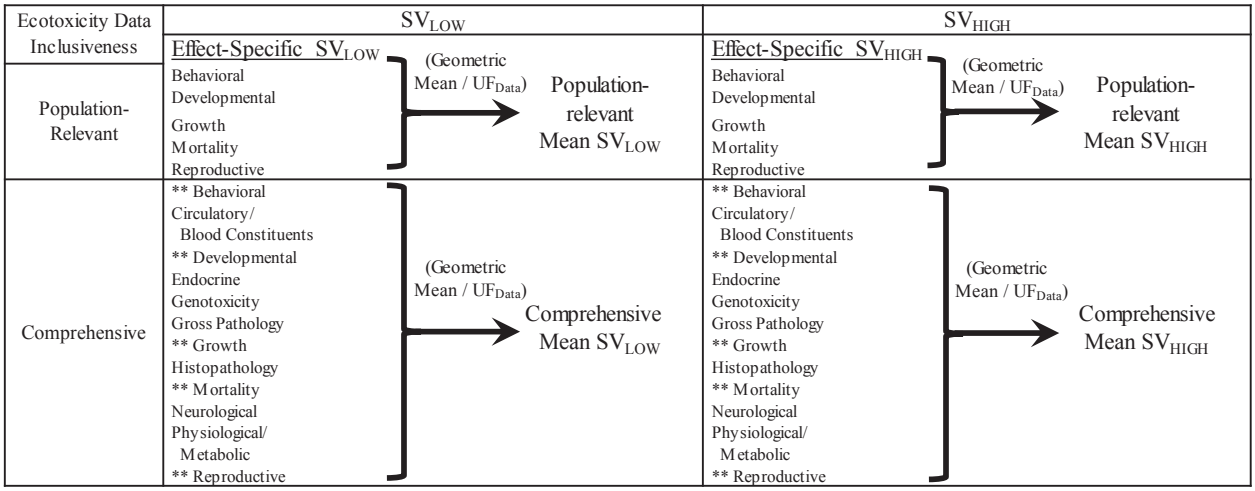


Figure 4-1. Various types of surface water screening values (SVs) for evaluating hazard from aqueous CEC exposures in fish, and their inter-relationships. The database adequacy uncertainty factor (UF_{Data}) accounts for differences in breadth of ecotoxicity information incorporated into mean SVs. Screening values and uncertainty factors are summarized in Chapter 4 and described in detail in Gefell et al. (2019).

** Gefell et al. (2019) incorporated all 12 comprehensive effect categories into computing *comprehensive mean SV* values, which we utilized unadjusted in this EHA. However, in this EHA we **did not use** *comprehensive effect-specific SVs* for behavioral, developmental, growth, mortality, and reproductive effect categories, which incorporated data for effect endpoints that we did not consider to be population-relevant. We did, however, use *population-relevant effect specific SVs* in this EHA for behavioral, developmental, growth, mortality, and reproductive effects.

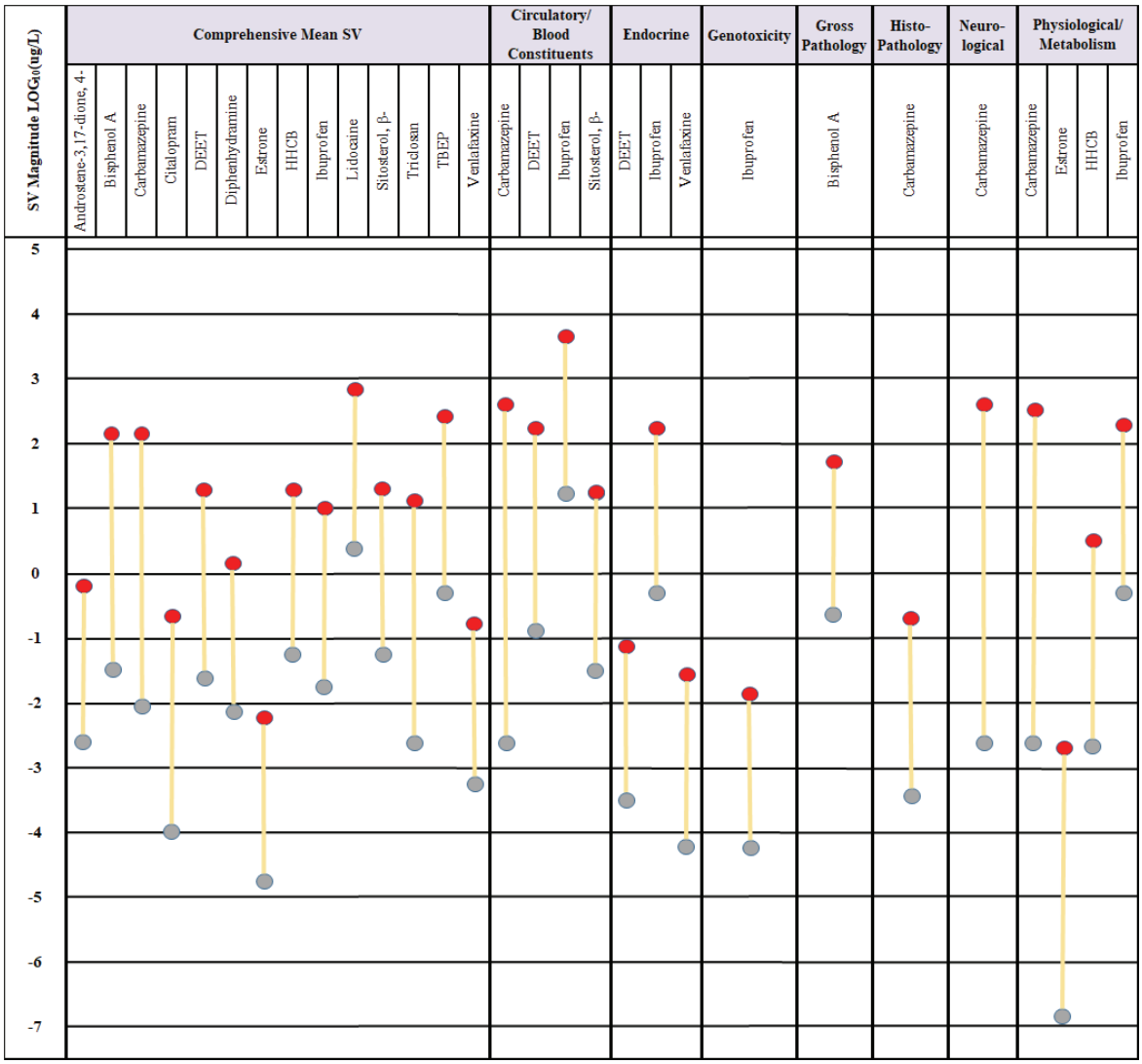


Figure 4-2a. Comprehensive SVs – illustration of relative magnitudes. Red dots indicate SV_{HIGH} values, grey dots are SV_{LOW} values, and yellow lines indicate the range of concentrations between the two SV values. Hazard scoring assigns a hazard score of 3 to surface water sample concentrations exceeding the SV_{HIGH}, a score of 1 to concentrations below the SV_{LOW}, and a score of 2 to concentrations falling between the SVs (further details in Section 5.2). CECs excluded under an effect category had insufficient literature information to derive a pair of SVs.

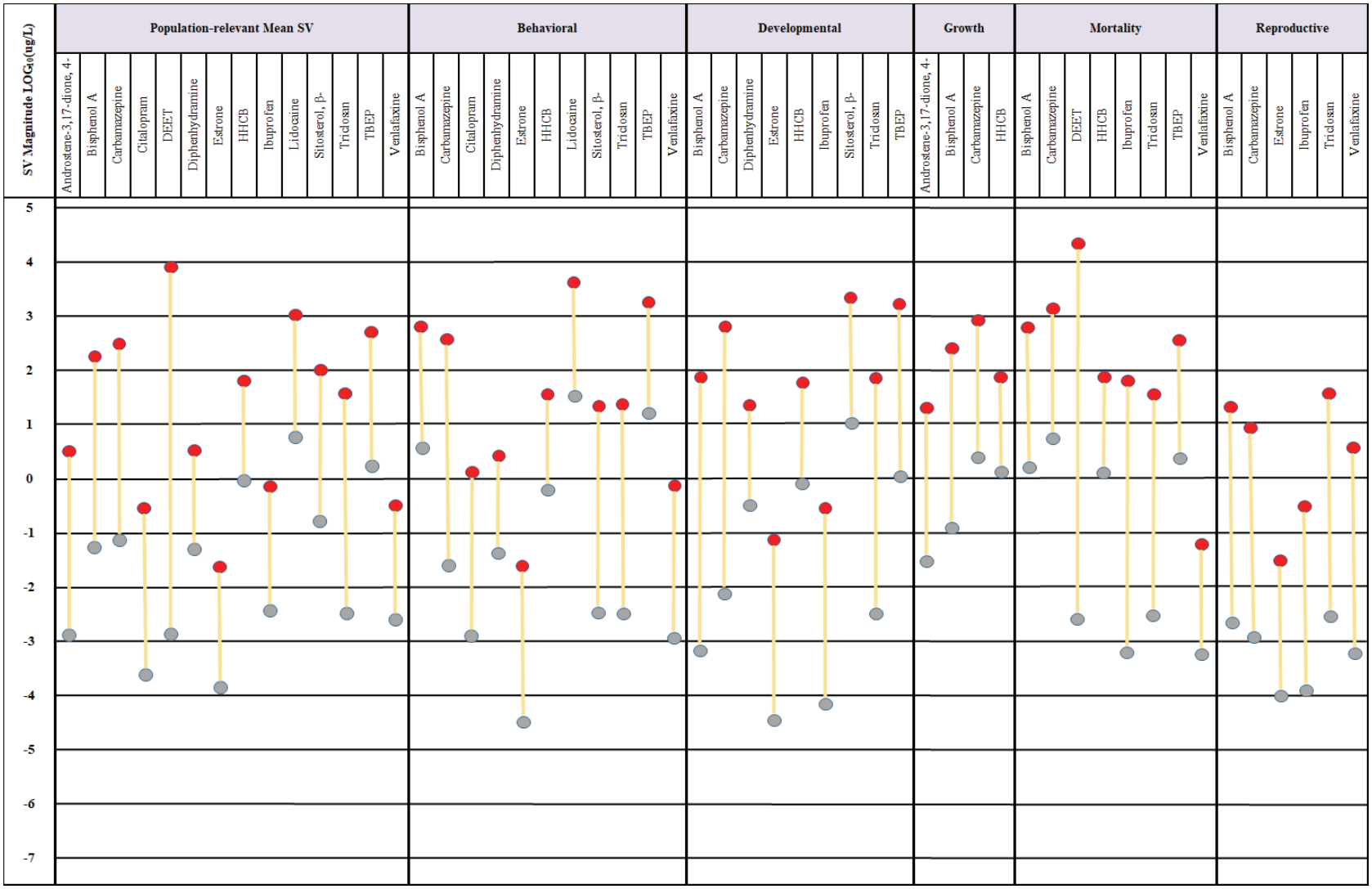


Figure 4-2b. Population-relevant SVs – illustration of relative magnitudes. Red dots indicate SV_{HIGH} values, grey dots are SV_{LOW} values, and yellow lines indicate the range of concentrations between the two SV values. Hazard scoring assigns a hazard score of 3 to surface water sample concentrations exceeding the SV_{HIGH}, a score of 1 to concentrations below the SV_{LOW}, and a score of 2 to concentrations falling between the SVs (further details in Section 5.2). CECs that do not appear under an effect category in this diagram had insufficient literature information to derive a pair of SVs.

Chapter 5 - Hazard Characterization

5.1 Background

5.1.1 Conventional Hazard Characterization

Hazard characterization combines exposure information with toxicity information to describe hazard to biota (Figure 5-1). In the conventional paradigm, information is usually aggregated[28] in both the exposure and toxicity assessments prior to hazard characterization. For instance, exposure typically is depicted by one representative exposure point concentration for each contaminant of interest - computed as the mean, median, maximum or other descriptive statistical metric of measured samples. In prospective hazard assessments exposure has been represented by modeled chemical concentration point estimates, such as in evaluating hazard for potential future uses of a new pesticide where measured exposure concentrations are not available. Less often, exposure is a distribution of measured concentrations for use in a probabilistic assessment, developed from multiple measurements at sampling sites. In conventional hazard characterizations, a single chemical-specific toxicity reference value is compared against a single exposure point concentration. Depending on the purpose of an EHA, screening values are either a 'lower bound' screening value used to screen out assessment elements (e.g., CECs, sampling areas) associated with negligible expectation of hazard, or an 'upper bound' screening value used to identify assessment elements with high expectation of hazard (see Table 1-1 *in* Gefell et al. 2019).

5.1.2 CEC EHA Approach

5.1.2.1 Exposure

In the exposure assessment (Chapter 3), we did not aggregate the chemical concentration data into a single value, such as a statistical metric, or represent exposure as distributions of concentrations. Instead, each measured concentration from each sample was treated as an individual instance of exposure to be carried through the assessment, resulting in an exposure array of concentrations of a given CEC from all sampling events at each site (N events per site = 1 to 19). The complete exposure array included concentrations of 14 CECs for each sampling event at each of 195 sampling sites distributed across 24 project locations (Figure 3-1), totaling over 7,000 CEC exposure data points.

Some Key Points…

Hazard Characterization

- **Purpose:** The Hazard Characterization combines exposure information (Chapter 3) with toxicity information (Chapter 4) to describe potential for CEC-related impacts to fish within each Project Location.
- **Overall Approach:** Expand on conventional Hazard Characterization methods to provide increased power and flexibility in the interpretation of CEC hazards to Great Lakes fish.
- **Scope:** Hazard was scored in each of >500 water samples collected from 195 sites across the U.S. Great Lakes basin (Chapter 3), using an array of 82 pairs of SVs for 14 CECs and 12 effect categories (Chapter 4). A total of >40,000 hazard scores were generated.
- **Hazard Scoring**: Hazard scores were assigned to CEC concentrations in water based on SV exceedances. Numeric scores correspond to relative hazard levels: 1 = negligible hazard; 2 = low hazard; 3 = high hazard.
- **Hazard Mapping:** Distribution of mean and maximum hazard at sampling sites is illustrated within project locations using a color gradient: grey = negligible hazard; yellow = low hazard; red = high hazard.
- **Point Source Evaluation:** The potential for CEC point sources to increase hazard was evaluated within project locations by comparing hazard scores at sites upstream of WWTPs or CSOs versus hazard scores at downstream sites.

[28] We use the term "aggregate" in the sense of simplify, boil down, or condense from multiple measurements or a large amount of information into a single number derived from, and representing, the whole dataset.

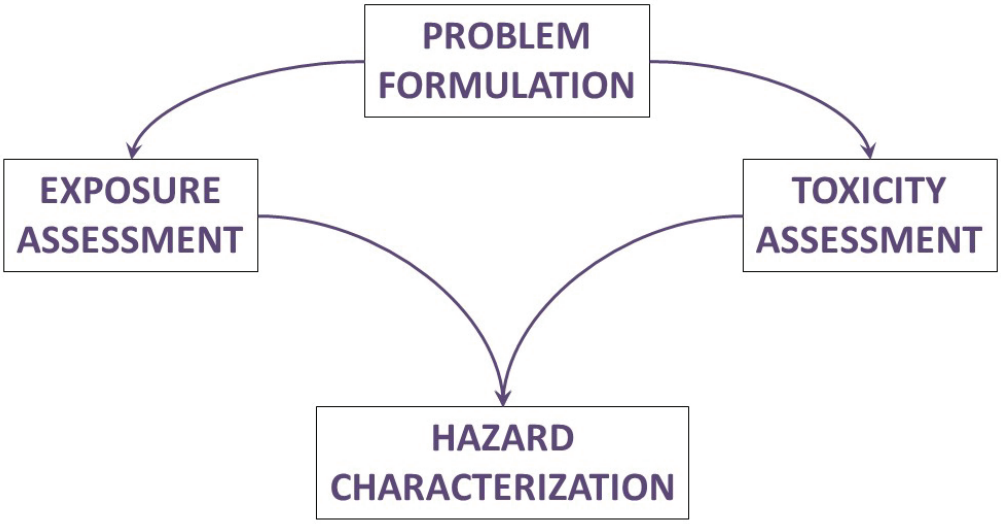


Figure 5-1. Conventional elements of a chemical ecological hazard assessment.

5.1.2.2 Toxicity

The toxicity assessment (Chapter 4) was also an expansion of the conventional paradigm. Toxicity was represented not by a single SV, but by *pairs* of SVs (SV_{HIGH} and SV_{LOW}) (see Section 4.2), both of which are applied to characterize hazard. A total of 82 pairs of SVs were derived for the 14 CECs, with unique pairs of SV values for a number of individual effect categories per CEC as well as mean SVs (Tables 4-2 and 4-3) (Gefell et al. 2019). The breadth of information used to develop each SV was provided in Section 4.3.

5.1.2.3 Hazard Computations

For each CEC, we assigned relative hazard scores ranging from 1 to 3 by effect category using effect-specific SV pairs, in contrast to conventional dichotomous hazard characterization using a single screening value per CEC. For comprehensive effect categories, we scored hazard in each water sample using a total of 16 effect-specific SV pairs, as well as comprehensive mean SVs for all 14 CECs (Table 4-1). For population-relevant effect categories, we scored hazard related to the 14 CECs using an array of 38 pairs of effect-specific SVs as well as CEC-specific population-relevant mean SVs (Table 4-1).

We applied this array of 82 SV pairs to each water sample, with each SV pair providing one hazard score for that sample. In order to interpret hazard, we grouped hazard score results by project location, sampling site, CEC, effect category, or orientation to point sources. This manner of aggregating hazard information obtained from individually scored sampling events ensured that important hazard information was retained that otherwise might be lost if exposure concentrations first had been aggregated (e.g., arithmetic mean, geometric mean, median, or upper percentile concentration). The discussion below and Figure 5-2 elaborate on these points.

5.2 Hazard Scoring

Hazard scores were generated for each exposure data point. An exposure data point is the aqueous concentration of one CEC obtained in one water sample during one sampling event. We defined a sampling event as the act of collecting a single physical water sample, which is uniquely identified by a specific date/time at a specific sampling site. For each water sample, a set of up to 82 hazard scores was developed (Table 4-1).

Hazard scores are ordinal data[29], indicating relative magnitude of hazard. Each pair of CEC-specific screening values defined three ranges of exposure concentrations that have toxicological interpretations. The concentration range associated with the lowest relative hazard was defined as all non-detects[30], plus detected exposure concentrations less than the SV_{LOW}. These were assigned a hazard score of '1' [31], which carries an expectation of 'negligible hazard' to fish. 'High hazard' was associated with exposure concentrations that exceed the SV_{HIGH}. These were

[29]Ordinal data have values that have an indisputable order with respect to some characteristic but have no fixed unit of measure (Statistical Package for the Social Sciences (SPSS) - https://www.spss-tutorials.com/measurement-levels/#ordinal-variable).

[30]Assigning all non-detects a hazard score of 1 simplifies hazard scoring, but also introduces uncertainty in the form of likely hazard underestimation (see Section 7.3.2 and Figure 7-2).

[31]The exposure concentration range associated with lowest hazard was assigned a hazard score of 1, rather than 0, in order to avoid the misimpression that we are certain that no hazard exists within that exposure range – uncertainties related to potential for false negative hazard findings are discussed in Chapter 7.

assigned a score of '3', which means a hazard to fish is expected. Concentrations falling between the SVs were assigned a hazard score of '2', representing observations of 'low hazard'. If an absolute toxicity threshold exists it likely falls between the SV_{LOW} and SV_{HIGH}, but its specific value is unknown and probably varies for a given CEC due to variables such as fish species, fish life stage, presence of other stressors, and exposure conditions.

This EHA evaluated hazard at 195 sampling sites, and the number of water samples collected per site ranged from 1 to 19. Each sampling event produced one water sample that was analyzed for hundreds of CECs, including the 14 subject CECs. Each sample was analyzed in hazard characterization using the complete array of 82 SV pairs (Table 4-1). Thus, tens of thousands of individual hazard scores were generated using these methods, each uniquely identified by sampling location, sampling site, sampling event (date/time), CEC, and effect category.

5.3 Interpreting Hazard Score Results

The purpose of this section is to provide guidance for interpreting hazard scores so that results of this EHA are communicated clearly and consistently.

5.3.1 Terminology

Publication inclusion/exclusion criteria that were common across the three empirical UF databases were:

Hazard Levels
Throughout this document, we use the following terms[32] to indicate different levels of our expectation of adverse biological impacts in resident fish species from exposure to one or more of the 14 CECs addressed in this EHA:

- "Negligible hazard" indicates a low expectation of impacts to fish (hazard score = 1), where the SV_{LOW} was not exceeded.
- "Low hazard" is used to indicate an intermediate expectation of impacts to resident fish (hazard score = 2), where the SV_{LOW} was exceeded but the SV_{HIGH} was not exceeded.
- "High hazard" is used to identify instances where there is a high expectation of biological impacts in resident fish species (hazard score = 3). This term indicates that a SV_{HIGH} value was exceeded by a measured or estimated[33] aqueous CEC concentration in surface water.

Among these designations, we have greatest confidence in the high hazard interpretation, since there is little potential for false positive exceedances of the SV_{HIGH} by detected CECs, and the SV_{HIGH} values themselves were derived from LOAECs reported from controlled experiments. The greatest uncertainty is associated with the "negligible hazard" designation, since there are a number of opportunities for false negative hazard findings using the methods in this EHA (see Chapter 7 – Uncertainty Analysis). This has important implications for interpreting and using hazard scores generated in this EHA. Generally, where hazard is indicated we are reasonably confident in the finding, but where hazard is contraindicated we are less confident in the finding.

CEC Source Influence at Sampling Sites
One purpose of this EHA was to evaluate spatial associations between hazard level and CEC point sources. We applied the following terms to sampling sites based on orientation to point sources, to indicate whether CEC loadings from point sources could adversely influence resident fish at the sampling site:

- "Influenced" – in lotic systems the sampling site is proximally *downstream* of a mapped point source, or in lentic systems the sites is *proximal* to a point source; and
- "Uninfluenced" – in lotic systems, the sampling site is *upstream* or *distally downstream* of a mapped point source, or in lentic systems the site is *distant* from a point source.

Further discussion of terms relating sampling sites to CEC point sources is provided in Section 5.3.4.1.

5.3.2 Relative Importance of Hazard Findings

The importance of a particular SV exceedance, or non-exceedance, should be interpreted in context. Were there exceedances of other SVs (different CECs and/or effect categories) in the same sample? Were there exceedances of the same SV, or other SVs, in other samples collected at the site? Was this the only sample collected at the site, or one of many?

In this EHA, positive and negative findings are interpreted differently. An exceedance of a SV_{HIGH} is considered a demonstrative positive finding – that is, it is a hazard determination of a specific type of hazard to fish (e.g., endocrine, reproductive, etc.) due to exposure to one of the CECs. The exceedance of a SV_{LOW} is considered a tentative (or, possible) positive finding. For exceedances of either the SV_{HIGH} or SV_{LOW}, we provide information to interpret the biological importance of the SV exceedance[34]. For instance, it may be appropriate to consider an

[32]Throughout this EHA, the generic term "elevated hazard" indicates that hazard was not negligible – that is, either high hazard or low hazard was observed.

[33]See Section 3.5 and Attachment A1 for discussion of how we estimated aqueous CEC concentration from total CEC concentration in water.

[34]In Section 6.4, we provide lists of CEC-specific effect endpoints evaluated in published assays that had been incorporated into SV derivations (summarized from Gefell et al. (2019).

exceedance of a reproductive SV_{HIGH} that was derived from data concerning multiple effect endpoints related to reproductive function in multiple fish species reported in many studies as more biologically important than an exceedance of a reproductive SV_{HIGH} based solely on gene expression of one reproductive hormone in one species determined in one published assay. Both sets are considered adverse reproductive endpoints, and both sets are appropriate to derive reproductive SVs. However, exceedance of the first SV *demonstrates* potential for impacts to reproductive functionality, while exceedance of the second SV - also a positive finding of reproductive hazard - *suggests* potential for reproductive function impacts.

The frequency of SV exceedances of the same SV pair provides additional insight. For example, it seems reasonable to interpret numerous exceedances of a SV_{HIGH} of the same type in many sampling events spanning multiple seasons and times of day as evidence of sustained high hazard at a sampling site. A few SV_{HIGH} exceedances among many sampling events may be interpreted as evidence for intermittent high hazard (see Figure 5-3), while a single SV exceedance from the only sampling event at a site provides tentative, or minimal, evidence of hazard.

For a non-exceedance of a SV - a hazard non-determination - the negative finding is considered provisional. For negative hazard findings, the absence of evidence of hazard does not necessarily equate with evidence of an absence of hazard. Our confidence in a negative finding is related to the number of effect endpoints that were evaluated in the literature and formed the basis for the SVs, and with the number of additional non-exceedances observed at the site for the same SV pair. A negative finding in a single sample of a SV that was based on a single effect endpoint from a single assay is more suspect (i.e., it is more likely a false negative finding) than repeated non-exceedances in many samples of a particular SV that incorporated assay results on many effect endpoints in multiple fish species. Yet, conceptually, even strong evidence of a negative finding is not definitive, since there are always untested effect endpoints with unknown relative sensitivity, that possibly are more sensitive.

5.3.3 Hazard Mapping within Project Locations: Color Codes and Symbols

The purpose of this section is to introduce the techniques we used to depict hazard spatially, applicable to all of the project location hazard maps in Section 5.4[35]. Maps illustrating mean and maximum hazard were generated by project location for effect categories with at least one high hazard score observation (see Table 5-1). We computed means of hazard scores across samples and CECs solely for the purpose of illustrating relative hazard within project locations. Mean hazard at a sampling site was computed from indiviudal hazard scores that resulted from a comparison of a single CEC concentration at one site collected at a point in time (date and time) to the corresponding CEC-specific SV pair for a given effect category. In each water sample, one of three possible scores was assigned to each CEC concentration for each effect category with a SV pair:

- 3 = CEC aqueous concentration > SV_{HIGH} (high hazard),
- 2 = CEC aqueous concentration falls between the SV_{LOW} and SV_{HIGH} (low hazard), and
- 1 = CEC aqueous concentration < SV_{LOW} (negligible hazard).

Statistical tests for differences in hazard between groups of sampling sites were non-parametric, which did not compare means of hazard scores. For the following reasons, we used mean values to map hazard across CECs rather than a summation such as the conventional hazard index (HI):

- the number of CECs scored for hazard can vary between sites, so sums of hazard scores would be positively biased at sites with higher numbers of CECs scored, and
- hazard was scored for each sample collected at each site and the number of samples per site varied, which would also result in hazard score summations that were biased high at sites with larger numbers of samples

Mean hazard is depicted by large dots on the maps. Maximum hazard scores were also depicted at each sampling site; they are depicted as small dots, usually embedded within the dots depicting mean scores.

Since we did not aggregate exposure information, we were able to retain information related to intermittent elevated hazard at sites by mapping means and maxima. For a given CEC and effect category, each of the three possible hazard scores (1, 2, or 3) is related to a *range* of exposure concentrations (Figures 4-2 and 5-2). If we were to apply hazard scoring to a single representative exposure point concentration (e.g., arithmetic mean, geometric mean, median, or high percentile concentration), each site would receive a single whole number hazard score for each CEC. However, even the lowest hazard score of 1 that was computed from a single exposure concentration could mask elevated hazard observable in individual sampling events (Figure 5-2).

It is important to retain intermittent high-hazard event information in the EHA. Intermittent exposure in fish has been associated with equal or greater magnitude of adverse effects than continuous exposure to comparable concentrations over an equivalent calendar time period (e.g., Curtis et al. 1985, Diamond et al. 2006, Handy 1994, Jarvinen et al. 1988, Little et al. 1993, Panter et al. 2000, Seim et al. 1984, Siddens et al. 1986).

[35]Methods for depicting mean hazard described in this section are not relevant to project-wide hazard maps provided in Chapter 6, in which only maximum hazard score (by CEC or effect category) is depicted for each of the 195 sampling sites included in this EHA.

In order to unmask intermittent elevated hazard events, our approach was first to obtain CEC- and effect-specific hazard scores for individual sampling events, and then compute effect-specific mean hazard across CECs, by site. Steps to obtain hazard score means for a given effect category (e.g., developmental effects) - at a specific sampling site - were the following (illustrated in Figure 5-3):

1. Apply effect-specific CEC SVs (e.g., developmental SVs) to water concentration data to score hazard for each CEC in each water sample:
    a. Obtain concentration data for each CEC in each surface water sample (i.e., each sampling event) (Elliott et al. 2017, Lee et al. 2012, Lee et al. 2015),
    b. Obtain surface water CEC SVs for screening hazards to freshwater fish (Chapter 4; also see Gefell et al. 2019),
    c. Obtain CEC-specific hazard scores by applying SVs to water concentration data - see Hazard Scoring in Section 5.2.

2. Obtain CEC-specific mean hazard scores within each effect category, averaged across water samples (i.e., sampling events), by site:

    a. For example, there were five surface water sampling events at the 'HogIsland' sampling site in the St. Louis River project location (see Attachment A, Section A.2.1). At the HogIsland site, the mean Developmental hazard score for Bisphenol A (BPA) was computed as:

$$Mean\ BPA\ Hazard\ Score_{HogIsland} = \frac{\sum_{n=1}^{5} Event\_n\_Score\_BPA}{5}$$

    b. There are a total of nine CECs with developmental SVs (Table 4-1). Continuing the HogIsland site example, CEC-specific mean developmental hazard scores were also obtained for each of the other eight CECs with developmental SVs– carbamazepine, diphenhydramine, estrone, HHCB, ibuprofen, β-sitosterol, TBEP and triclosan.

3. Obtain effect-specific mean hazard scores at each sampling site, averaged across CECs:

    a. Continuing the example, the overall mean hazard score for developmental effects at the HogIsland sampling site was computed across the nine CECs as:

$$Mean\ Developmental\ Hazard\ Score_{HogIsland} = \frac{\sum_{n=1}^{9} CEC\_n\_Mean\ Score}{9}$$

In a hypothetical case where we obtained an overall mean score of '1' for developmental effects at a sampling site, that would indicate that all developmental hazard scores – for all CECs and all sampling events - had a value of 1. That is, all exposure concentrations measured at the site were below the developmental SV_{LOW} for all CECs. For a site with many sampling events, an overall mean score of '1' for developmental effects would be relatively strong evidence for negligible developmental hazard at that site from exposure to the 9 CECs with developmental SVs.

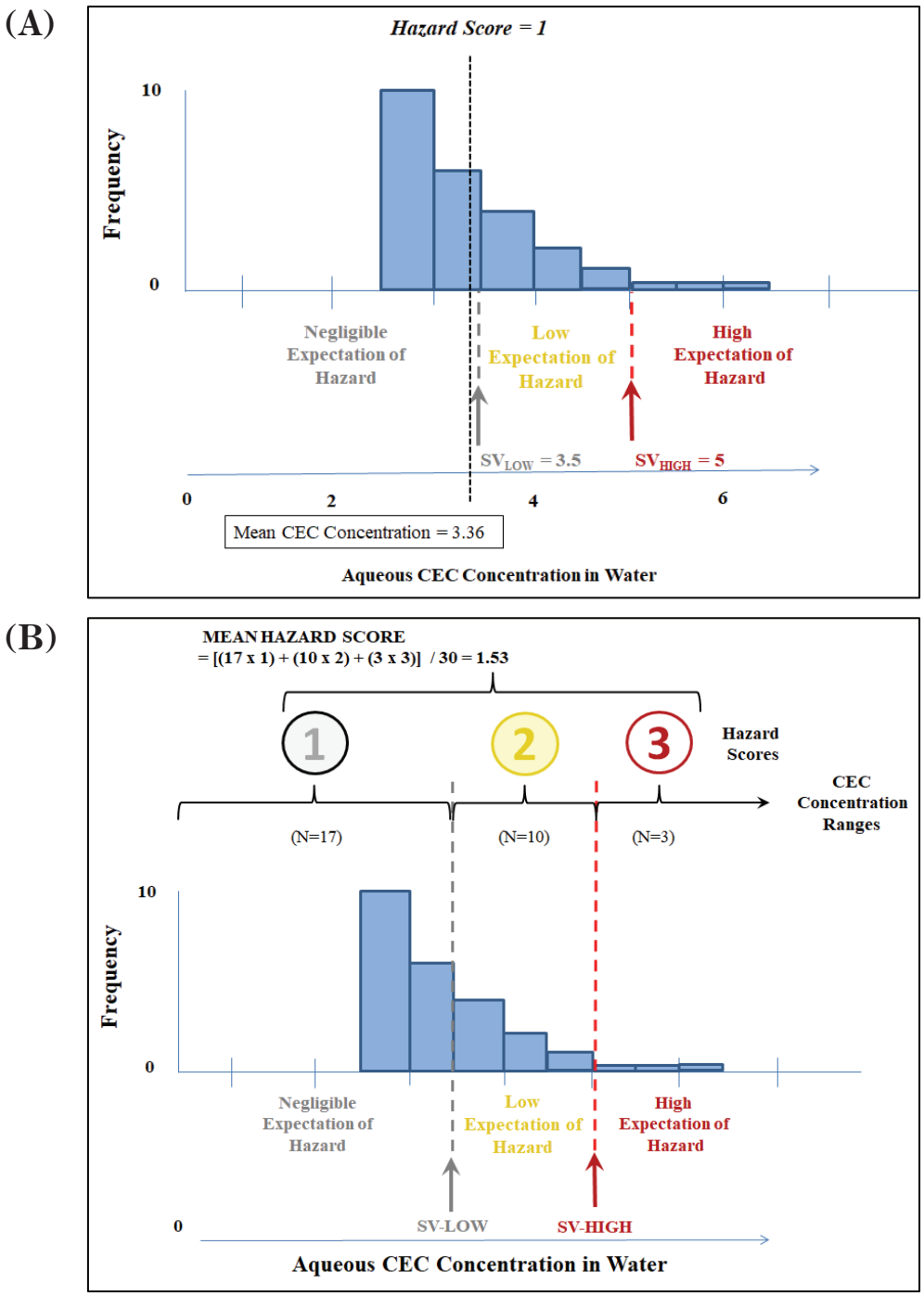


Figure 5-2. Illustration of unmasking intermittent high hazard for hazard mapping. High hazard can be masked (a) when using the conventional method of first taking the mean of an exposure concentration distribution and then characterizing degree of hazard associated with the mean concentration – in this case, the hazard score of the hypothetical mean exposure concentration equals 1. Whereas, (b) the mean of hazard scores assigned to each of 30 individual sampling event concentrations is 1.53, which indicates the presence of several observations of elevated hazard. Means of hazard scores are computed in this EHA solely for the purpose of mapping spatial distribution of relative hazard.

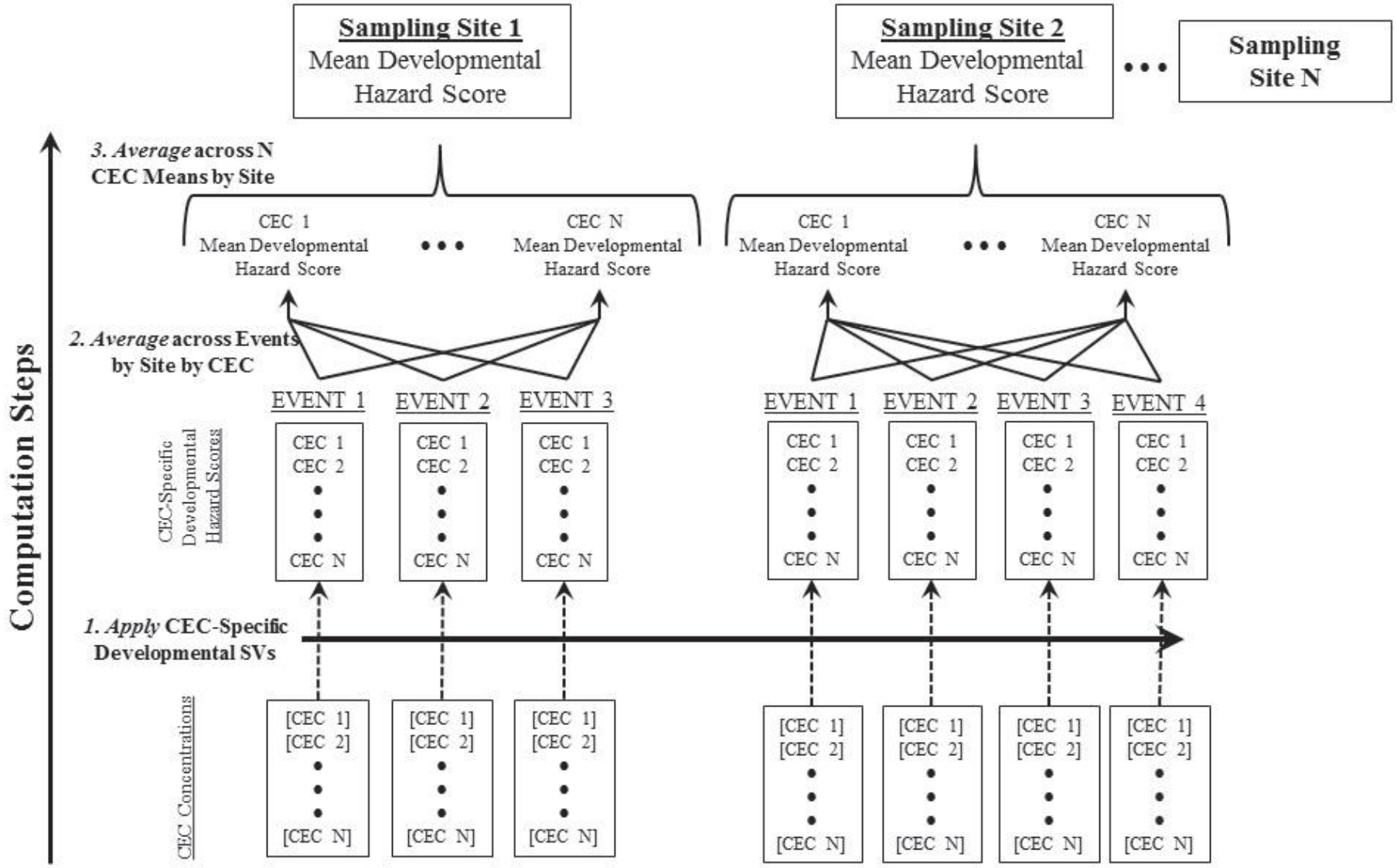


Figure 5-3. Computation steps (arranged bottom to top) to develop site-specific hazard scores for mapping mean hazard by effect category in Section 5.4. This schematic example illustrates how mapped mean developmental hazard scores were computed from CEC concentration data from several sampling events per sampling site.

Mapped mean effect-specific hazard is represented at sampling sites on a color gradient (Figure 5-4) illustrating degree of expectation of biological impacts in fish. Sites with an overall mean score of 1 were depicted with a large dark gray circle. Whereas, a mean hazard score of 3 indicates that all exposure concentrations for all CECs exceeded the SV_{HIGH} at that sampling site so there is a high expectation of hazard to fish, which is depicted with a large red circle. The color gradient between the extreme mean hazard score values of 1 and 3 is intended simply to provide quick, qualitative visualization of relative hazard differences between sites. For instance, a deep orange color illustrates that a larger *fraction of sampling events and/or CECs* showed high hazard (SV_{HIGH} exceeded) than if the color was a lighter orange. The numeric intervals for mean hazard on the color gradient are simply convenient bins that provide a reasonably gradual hazard gradient; they are not intended to indicate biological meaning other than a rough indication of *relative* hazard.

Depicting effect-specific mean hazard in this way, we can use the maps to distinguish at a glance between a mean hazard of 1 (meaning the concentration at each sampling event fell below the SV_{LOW}) and a hazard score of, say 1.3 or 1.7, which means that the SV_{LOW} was exceeded at the sampling site for some fraction of sampling events. Average hazard scores greater than 2 (e.g., 2.25, 2.63) are depicted by shades of orange, and are interpreted to mean that at least one exceedance of a SV_{HIGH} occurred at the site.

Mapped maximum effect-specific hazard at each site is depicted by a smaller dot, superimposed on the larger mean hazard dot, using a simplified color gradient of only 3 possible values:

- Dark Gray = 1 (negligible hazard expectation);
- Yellow = 2 (low hazard expectation), and
- Red = 3 (high hazard expectation).

Maximum effect-specific hazard is the highest hazard score observed at the site for any sampling event, for the CECs that have SVs for the given effect category. At sampling sites where there is only one sampling event, only that hazard value is depicted, as the "maximum".

Figure 5-4. Hazard-related color gradient for interpreting hazard maps.

Table 5-1. Highlights of hazard scoring results presented in Section 5.4 and Attachment B. Hazard maps were generated for project locations and effect categories where high hazard (hazard score = 3) was observed in at least one sample. Cell contents reflect exceedances of maximum observed hazard scores for each effect category in each project location: Red X = at least one high hazard observation (an exceedance of a SV_{HIGH}) for the indicated effect category and project location; black dot = at least one exceedance of a SV_{LOW}; blank cell = no SV_{LOW} was exceeded.

Project Location	Comprehensive								Population-relevant					
		Effect-Specific SVs								Effect-Specific SVs				
	Comprehensive Mean SVs	Circulatory / Blood Constituents	Endocrine	Genotoxicity	Gross Pathology	Histopathology	Neurological	Physiological/ Metabolic	Population-relevant Mean SVs	Behavioral	Developmental	Growth	Mortality	Reproductive
01. St. Louis River/Bay	•	•	X	X	•	•	•	X	X	•	X	•	X	X
02. Waupaca Chain O' Lakes	•	•	X					•	•	•	•		•	•
03. Little Lake Butte des Morts	•	•	X			•	•	X	•	•	•		•	•
04. Fox River/Green Bay	•	•	X	X		•	•	•	•	•	•		•	•
05. Kewaunee River	•	•	X			•	•	•	•	•	•		•	•
06. Milwaukee River	•	•	X	X		•	•	•	•	•	X		•	X
07. North Shore Channel	X	•	X		•	X	•	X	•	•	•	•	X	•
08. Little Calumet River	X	•	X		•	•	•	X	•	•	•	•	X	•
09. Grand River/Maple River	•	•	X			•	•	•	•	•	•		•	•
10. Saginaw River	•	•	X			•	•	•	•	•	•		•	•
11. St. Clair River	•		•					•	•	•	•		•	•
12. Clinton River	•	•	X			•	•	•	•	•	•		•	•
13. Detroit River	•	•	•	X		•	•	X	•	•	X		•	X
14. River Raisin	•		•					•	•	•	•		•	•
15. Swan Creek	X	•	•	X				•	X	•	X		•	X
16. Maumee River	X	•	X	X		•	•	X	•	•	X	•	X	X
17. Cuyahoga River	X	•	X		•	X	•	X	•	•	•	•	X	•
18. Tinkers Creek	X	•	X	X		X	•	X	X	•	X	•	X	X
19. Ashtabula River		•	•						•	•			•	
20. Long Pond	•	•	•					•	•	•	•		•	•
21. Genesee River	•		•					•	•	•	•		•	•
22. Irondequoit Bay	•	•	•					•	•	•	•		•	•
23. Oswegatchie River	•	•	•					•	•	•	•		•	•
24. Raquette River	•	•	X			•	•	X	•	•	•		•	•

5.3.4 Hazard Associated with CEC Point Sources

Within each project location, we analyzed whether hazard at sampling sites that are considered influenced by CEC point sources is greater than hazard at uninfluenced sites. Our premise is that the spatial orientation of a sampling site to nearby point sources is related to the potential for fish to experience CEC-related biological impacts at the site. Concentrations of many CECs are known to be greater downstream of point sources such as WWTPs and CSOs, compared to upstream (Choy et al. 2017, Deo 2014, Kolpin et al. 2002, Lee et al. 2011, Metcalfe et al. 2003, Petrovic et al. 2008, Phillips et al. 2010, Phillips et al. 2012, Saussereau et al. 2013, Venkatesan et al. 2012). In this EHA, we utilized pairs of SVs and a hazard scoring process to translate CEC concentration data into relative hazard data. We utilized both quantitative and qualitative methods to evaluate whether hazard to fish expressed as hazard scores is related to orientation of sampling sites relative to CEC point sources.

5.3.4.1 Sampling Site Groups: Point Source CEC-Influenced vs. Uninfluenced

For the purposes of this assessment, we assigned a designation to each of the 195 sampling sites indicating whether the site was potentially biologically *influenced* or relatively *uninfluenced* by aqueous CECs. Designations were made by inspecting the spatial orientation of each individual sampling site relative to mapped point sources (Attachment A2) using ArcMAP (ESRI 2018). The CEC influence status of each sampling site to the nearest point source is provided in Attachment A1, Table A-2. The following procedure to assign CEC influence status to sites is liable to underrepresent CEC influence, and in that sense, is conservative.

For lotic systems, a buffer circle with a 4 km radius was drawn around each point source to assign CEC influence status to sampling sites. "Influenced" sampling sites were within the 4 km buffer and downstream (DS) of a CEC point source (mapped WWTP or CSO), while "uninfluenced" sites were either upstream (US) or further than 4 linear kilometers downstream (DS distal) of the nearest point source. The choice of a single 4km buffer distance in lotic systems for all CECs was somewhat arbitrary, but it was also conservative; the rationale is as follows. Environmental degradation rate in the water column differs between CECs, so concentration attenuation rates and transport distances downstream of point sources in lotic systems also differs between CECs. Hence, theoretically, the buffer distance that we have used to designate sites as "influenced" versus "uninfluenced" should vary with CEC. We know from a number of field studies that low attenuation of CEC concentration in surface water, or an absence of attenuation, occurs for some CECs during downstream transport over river distances greater than 4km from sources (e.g., Gross et al. 2004, Fono et al. 2006, Lin et al. 2006, Radke et al. 2010, Kunkel and Radke 2011, Luo et al. 2011, Writer et al. 2012, Barber et al. 2013, Writer et al. 2013, Zha et al. 2017). Based on this literature, we anticipate that sometimes the surface water concentrations of certain CECs at sites labelled as "uninfluenced" (due to greater than 4km downstream distance from a point source) may be similar to concentrations at sites in the CEC "influenced" group. In these cases, we risk committing a Type II statistical error, where the statistical test of difference in hazard score between the two groups may show no difference, when in fact there may be an actual difference in hazard that would be identified statistically if the "uninfluenced" group was defined less conservatively.

In lentic systems, sampling sites that were within a 1km buffer of a point source (proximal) were considered potentially "influenced" by CECs, while sampling sites greater than1 km from a point source (distal) were considered relatively "uninfluenced". As with the lotic systems, we intended this distance to be conservatively short in order to avoid overstating potential impacts. The CEC influence status of each of the 195 sampling sites to the nearest point source is provided in Attachment A1, Table A-2.

5.3.4.2 Point Source Spatial Data

Assigning "influenced" and "uninfluenced" designations to each sampling site was conducted using ArcGIS 10.5 for desktop (ESRI 2018). We developed CEC point source shapefiles with WWTP and CSO coordinates obtained from several sources. The WWTP base layer was provided by the USGS Upper Midwest Water Science Center - metadata concerning the layer is provided in a supplemental information file (Baldwin et al. 2016a), associated with Baldwin et al. (2016b). We supplemented this WWTP base layer with a small number of points obtained from an online USEPA database[39] of WWTP information in the following project locations: Clinton River, North Shore Channel, Long Pond, Genesee River, and Raquette River. The CSO base layer was developed by the USFWS CEC Team for watershed modeling by combining individual layers of up-to-date data (as of 2017) provided by state natural resource departments and major municipalities in Wisconsin, Illinois, Indiana, Michigan, Ohio, and New York[40].

5.3.4.3 Quantitative Analysis: Statistical Test

One way that we explored whether CEC-related hazard to fish is altered by CEC point sources was through statistical testing. Within project locations, we compared hazard scores between CEC-influenced

[39]USEPA Facility Registry Dataset web page for wastewater treatment plants: https://catalog.data.gov/dataset/epa-facility-registry-service-frs-wastewater-treatment-plants.

[40]We located no CSOs in the state of Minnesota (Choy et al. 2017).

and uninfluenced site groups using the two-sided nonparametric Wilcoxon test for ordinal data in SAS for Windows 9.4 (PROC NPAR1WAY, WILCOXON analysis option, WILCOXON output option, p-value = P2WIL). Statistical comparisons were conducted for each possible CEC and effect category combination (Table 4-1) at two levels of resolution – site and sample. The principal hazard comparisons were made between sites in CEC-influenced areas versus sites in uninfluenced areas within individual project locations, where hazard at each individual site was represented by a single value. Two statistical comparisons were conducted – one using the maximum hazard value at each site, and one using the median value of samples collected at each site. Results of site hazard comparisons within project locations are provided in Section 5.4. The statistical power to discern hazard differences between site groups was related positively to the total numbers of sites in each group. Statistically significant differences between site groups were identified where $p < 0.1$. Sixteen of 24 project locations had sufficient numbers of sites in each group to perform the statistical analysis (Table 5-2).

While hazard differences between point source CEC-influenced and uninfluenced *sites* was of primary interest, we performed a secondary statistical hazard score comparison between *samples* collected in influenced and uninfluenced areas of the project locations. An advantage to comparing groups comprised of sample-specific data is that available hazard score results were fully utilized. Many of the project locations had very few sites in either point source CEC-influenced or uninfluenced areas (or both), and so the power to detect hazard differences related to point sources was severely limited. The advantage to using all samples collected at each site is that the total sample size was increased – in some cases substantially – so statistical power to detect hazard differences between point source CEC-influenced and uninfluenced regions within project locations was greater for comparisons using sample data than for site comparisons. A disadvantage is that interpretation of the sample-based results is likely subject to bias from pseudo-replication, or, within-site autocorrelation of hazard results. Because of this limitation, results for hazard comparisons using all sample data are considered supplementary and were not utilized for interpretation of hazard in this EHA, but for completeness they are reported in Attachment D.

We visually inspected hazard maps to augment statistical analyses. In eight project locations where statistical tests were precluded due to insufficient sample size (Table 5-2), the only means of interpreting spatial distribution of hazard potentially related to point sources was a qualitative inspection of hazard maps.

5.3.4.4 Qualitative Analysis: Tallies of SV_{HIGH} and SV_{LOW} Exceedances

We also applied a qualitative approach to evaluating point source influences. We inspected the occurrence rate of elevated hazard among samples and sites in point source CEC-influenced versus uninfluenced regions of each project location. In this EHA, contaminant concentration data from a total of 195 sampling stations in 24 project locations were utilized. Among these, 110 sampling sites were designated as point source CEC-influenced and 85 sites were considered uninfluenced by CECs from mapped WWTP and CSO point sources. Data from a total of 533 surface water sampling events were evaluated. Among these, there were 335 sampling events at sites designated as point source CEC-influenced, and 198 events at sites identified as uninfluenced.

For each project location, we provide charts showing fractions of samples and of sites that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category, and also fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups. As with the quantitative analysis, a qualitative comparison of SV exceedance tallies between CEC-influenced and uninfluenced sites was not possible for certain project locations (Table 5-2). We had derived SVs for comprehensive behavioral, developmental, growth, mortality and reproductive effect categories (see Gefell et al. 2019), which included exposure-response information for effect endpoints that *were not* considered population-relevant. However, we did not include them in this EHA, in order to avoid confusion with population-relevant effect categories of the same names. Hence, project location-specific comparisons between CEC-influenced sites and uninfluenced sites presented throughout Section 5.4 *do not include* these five comprehensive effect categories in the tallies of SV_{HIGH} or SV_{LOW} exceedances.

Table 5-2. Project locations where a statistical comparison of hazard scores between point source CEC-influenced and uninfluenced sites was possible (italics, shading) or was not possible.

Project Location	Sample Size (N)		Statistical Comparison Possible
	"Point Source CEC-Influenced" Sample Sites	"Uninfluenced" Sample Sites	
01. St. Louis River/Bay	*16*	*16*	*Y*
02. Waupaca Chain O'Lakes	0	2	N
03. Little Lake Butte des Morts	*3*	*2*	*Y*
04. Fox River/Green Bay	*8*	*7*	*Y*
05. Kewaunee River	*3*	*2*	*Y*
06. Milwaukee River	4	0	N
07. North Shore Channel	3	0	N
08. Little Calumet River	3	0	N
09. Grand River/Maple River	*2*	*4*	*Y*
10. Saginaw River	*6*	*8*	*Y*
11. St. Clair River	*5*	*3*	*Y*
12. Clinton River	*2*	*3*	*Y*
13. Detroit River	*5*	*5*	*Y*
14. River Raisin	*2*	*2*	*Y*
15. Swan Creek	13	0	N
16. Maumee River	*16*	*4*	*Y*
17. Cuyahoga River	*3*	*2*	*Y*
18. Tinkers Creek	*6*	*3*	*Y*
19. Ashtabula River	0	3	N
20. Long Pond	0	6	N
21. Genesee River	*4*	*2*	*Y*
22. Irondequoit Bay	0	6	N
23. Oswegatchie River	*2*	*3*	*Y*
24. Raquette River	*3*	*3*	*Y*

5.4 Ecological Hazard Analysis by Project Location

This section presents detailed hazard characterization results for each of the 24 project locations. Each location assessment includes the following five subsections:

1. A hazard brief that highlights CEC hazards to fish (see below and refer to Sections 5.2 and 5.3),
2. Hazard rankings of effect categories, CECs, and sampling sites (see below),
3. Summary of strength of evidence indicating high hazard (see below and refer to Section 4.3),
4. Comparisons of hazard scores between sites influenced by CEC point sources and uninfluenced sites (refer to Section 5.3.4):
   - Quantitative analysis using non-parametric statistical techniques and
   - Qualitative analysis of SV exceedance tallies,
5. Project location maps of relative hazard to fish for high-hazard effect categories (refer to Section 5.3.3).

All of the above analyses were based on hazard scores (see Section 5.2). Hazard scores were generated by effect category and CEC at each sampling site and for each sampling event, and were computed using two groups of SVs: comprehensive and population-relevant (Tables 4-2 and 4-3, respectively). The concept of "population-relevance" is described in depth in Gefell et al. (2019). In short, we define "population-relevant" SVs as those derived from exposure-response information concerning effect endpoints that are relatively easily related to survival (or, mortality), propagation, or growth, where quantitative laboratory data could be incorporated into a quantitative, mechanistic population dynamics model.

Some Key Points…

Location-Specific Hazards

- **Overall:** Hazard to fish due to14 CECs varied widely between 24 project locations
- **Greatest Evidence of Hazards**: Clear and convincing evidence of hazards was observed at St. Louis River/Bay, North Shore Channel, Little Calumet River, Maumee River, Cuyahoga River, Tinkers Creek
- **Least Evidence of Hazards**: Little evidence of hazard was observed at Waupaca Chain O' Lakes, St. Clair River, River Raisin, Ashtabula River
- **Elevated hazard downstream of point sources**: Significant evidence was observed for St. Louis River/Bay, Fox River/Green Bay, Saginaw River, Maumee River, Tinkers Creek; no evidence for 11 locations ; no statistical evaluation possible for 8 locations
- **Areas of Concern:** No clear correspondence between degree of CEC-related hazard and spatial overlap with an Area of Concern

Hazard Ranking. The purpose of the hazard ranking analysis was to identify factors that are the greatest contributors to overall (mean) hazard within each project location. For each project location, we ranked mean hazard among the elements of three factors: effect categories, CECs, and sampling sites. Within each project location, hazard scores for all sampling events were averaged by effect category, by CEC, or by sampling site. We computed the average of hazard scores in each element of a factor to illustrate relative hazard between elements within the factor. For instance, the mean of hazard scores was computed *within* each effect category in order to visualize relative hazard differences *among* effect categories. The means of hazard scores were not used in the non-parametric statistical comparison tests described in Section 5.3.4 or in Attachment D, and mean hazard scores should not be misconstrued as indicators of absolute degree of hazard.

The advantage of ranking relative hazard based on the mean hazard score rather than the median is that the mean score incorporates information from the entire distribution of individual hazard scores, while the median is simply a single point (50th percentile) within the distribution. There is a common concern with using the mean rather median to represent central tendency in statistical tests involving skewed distributions, which is that the occurrence of a few very high values in the distribution could bias the mean score toward the high end of the distribution – giving inflated weight to very high values. However, in this EHA hazard scores are integers falling within the relatively narrow range from 1 to 3, which greatly reduces the degree of this potential bias. Mean hazard scores were also selected in favor of sums of scores because the number of group elements (e.g., number of effect categories with SVs, and/or number of CECs or samples analyzed) and hence the number of hazard scores available for summing, may differ between CECs, between effect categories, or between sampling sites.

Hazard scores are ordinal data. They are integer values representing categories of hazard associated with ranges of exposure concentrations. Because they are not quantitative measures of biological impact, small differences in mean hazard values do not necessarily indicate real differences in underlying biological impact between groups. In order to reduce false precision in the hazard characterization, we assigned mean hazard scores to one of 10 hazard "bins" (Table 5-3). Hazard bins were inversely ranked from 10 to 1, where the greatest possible hazard rank (10) corresponds with the highest possible mean hazard score bin (2.8 - 3.0). Relatively high numeric rank broadly indicates greater expectation of biological impacts in resident fish. For each project location, hazard ranks were used simply to provide a visual comparison of relative overall hazard. For example, hazard ranks for each CEC were used in comparisons among CECs. Mean hazard scores were ordered high to low in each of the ranking charts in this section.

Table 5-3. Hazard ranks were assigned according to defined ranges, or bins, of mean hazard scores. Inverse ranking was utilized, where the highest possible values of mean hazard scores were assigned the highest numeric rank.

Mean Hazard Score Bins	Ranks of Mean Hazard Score Bins
2.8-3.0	10
2.6-2.8	9
2.4-2.6	8
2.2-2.4	7
2.0-2.2	6
1.8-2.0	5
1.6-1.8	4
1.4-1.6	3
1.2-1.4	2
1.0-1.2	1

Strength of Evidence. In each project location, strength of evidence in exposure and toxicity assessments were evaluated in terms of breadth of available information. Strength of evidence was evaluated only for effect categories where high hazard is observed in at least one sample. Breadth of exposure information was evaluated in terms of total number of sampling sites, sampling site spatial distribution and coverage, and sample sizes per sampling site. Breadth of ecotoxicity information was evaluated based on numbers of effect endpoints and fish species evaluated in published assays. The sufficiency of the ecotoxicological literature for deriving SVs varies between effect categories. For instance, only one CEC-specific pair of SVs was derived for each of the genotoxicity and gross pathology effect categories due to large gaps in the database of published ecotoxicity assays in fish, whereas 11 of the 14 CECs had sufficient published information to derive SVs for the behavioral effect category (Table 4-1).

Effect-Specific Hazard Maps. Site-specific mean and maximum hazard scores were mapped by effect category to spatially represent the distribution of relative hazard and types of hazard as related to CEC point sources (see details in Section 5.3.3). Effect-specific hazard maps were generated only for effect categories where a hazard score of 3 was observed for one or more CECs in at least one sampling event (Table 5-1; Attachment B). Wherever sites were sampled more than once, mean hazard score is depicted by large color-coded dots. Effect-specific mean hazard scores at each sampling site were computed by averaging CEC-specific hazard scores within effect categories, across sampling events. Mean hazard scores were used only for illustration purposes; they were not used for statistical analyses. The advantage of using mean rather than median scores for hazard illustration is discussed above under *Hazard Ranking.* For each effect category, the maximum hazard score at the site is the highest score for any CEC in any sample collected at the site. Maximum hazard is depicted at all sites, including those sampled only once.

The following subsections provide results of ecological hazard assessments conducted within each of the 24 project locations, listed here in geographic order from west to east (see Figure 2-4).

5.4.1 St. Louis River/Bay (MN, WI)

The St. Louis River main stem is approximately 309 km (192 mi) long, draining a watershed of ~9,410 km^2 (3,630 mi^2) and discharging into Lake Superior at the westernmost tip of the lake, between Duluth, MN and Superior, WI. The project location is in the lower ~90 km of the river main stem and the St. Louis and Superior Bays. Several large CEC point sources are located in the lower watershed. Sixteen of the 32 sampling sites were designated as CEC-'influenced', and 16 'uninfluenced', based on their down-gradient proximity to mapped CEC point sources. Sites were sampled one to five times during the following six sampling periods: September 2010, August or September 2011, or April, May or September 2012.

There is clear and convincing evidence of CEC-related hazards to fish at this project location. Observations of high mortality, developmental, and reproductive hazard (where SV_{HIGH} values were exceeded) indicate high potential of population-level impacts to sensitive resident fish species locally within the St. Louis and Superior Bays. High potential for adverse physiological/metabolic, endocrine, and genotoxicity effects in fish at sites in the St. Louis and Superior Bays were also observed. High hazard is attributed principally to ibuprofen, DEET, and venlafaxine, with estrone also contributing. Low hazard (exceedances of SV_{LOW} values) is spatially more widespread including at upriver sites, especially for DEET. SV_{LOW} exceedances were associated with 13 of 14 CECs and each of the 14 effect categories.

Considering all hazard data from the entire project location, endocrine and genotoxicity ranked highest among effect categories. DEET followed by ibuprofen, venlafaxine, and estrone ranked highest among CECs, and sites downstream of WWTPs ranked highest in overall hazard relative to other sampling sites. Strength of ecotoxicity information contributing to high hazard observations was greatest for developmental and reproductive effects associated with ibuprofen exposure and moderately strong for DEET endocrine effects; exposure information contributing to high hazard observations was also strong.

Statistical analysis of hazard associated with point sources indicated significantly higher ($p < 0.1$) overall hazard in point source CEC-influenced sites than in uninfluenced sites for four comprehensive effect categories and four population-relevant effect categories; in no case was overall hazard higher upstream of mapped point sources than below. Qualitatively, the total numbers of SV_{HIGH} and SV_{LOW} exceedances at point source CEC-influenced sites were dramatically higher than at uninfluenced sites, with more than half of sampling events and sampling sites resulting in at least one exceedance of a SV_{HIGH} at CEC-influenced sites. Maps confirm these results, clearly showing higher mean hazard below a WWTP than above for several effect categories. Refer to Section 5.3.3 for an explanation of color scales in hazard maps and descriptive terms.

Some Key Points…

St. Louis River/Bay (MN, WI)

- **Overall:** Clear and convincing evidence of CEC-related hazards to fish
- **High Hazard**:
  - 86 occurrences involving 41% of sampling events and 34% of sites
  - *CECs:* Ibuprofen, DEET, Venlafaxine, Estrone
  - *Effect Categories*: Mortality, Reproductive, Developmental, Endocrine, Genotoxicity, Physiological/Metabolic
- **Low Hazard**:
  - 1,138 occurrences involving 93% of sampling events and 97% of sites
  - *CECs:* 13 of 14
  - *Effect Categories*: 12 of 12
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources
- **Exposure Dataset**: Robust
  - 32 sampling sites with wide spatial distribution
  - 1 to 5 samples per site
  - 99 total samples

5.4.1.1 Hazard Brief for St. Louis River/Bay

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants
All effect categories and 13 of 14 CECs showed an exceedance of at least one SV_{LOW} and/or SV_{HIGH}. St. Louis River/Bay effect-specific hazard maps (Section 5.4.1.5) are provided for the following effect categories, for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Population-relevant mean SV, Developmental, Mortality, Reproductive
- Comprehensive: Endocrine, Genotoxicity, Physiological/Metabolic.

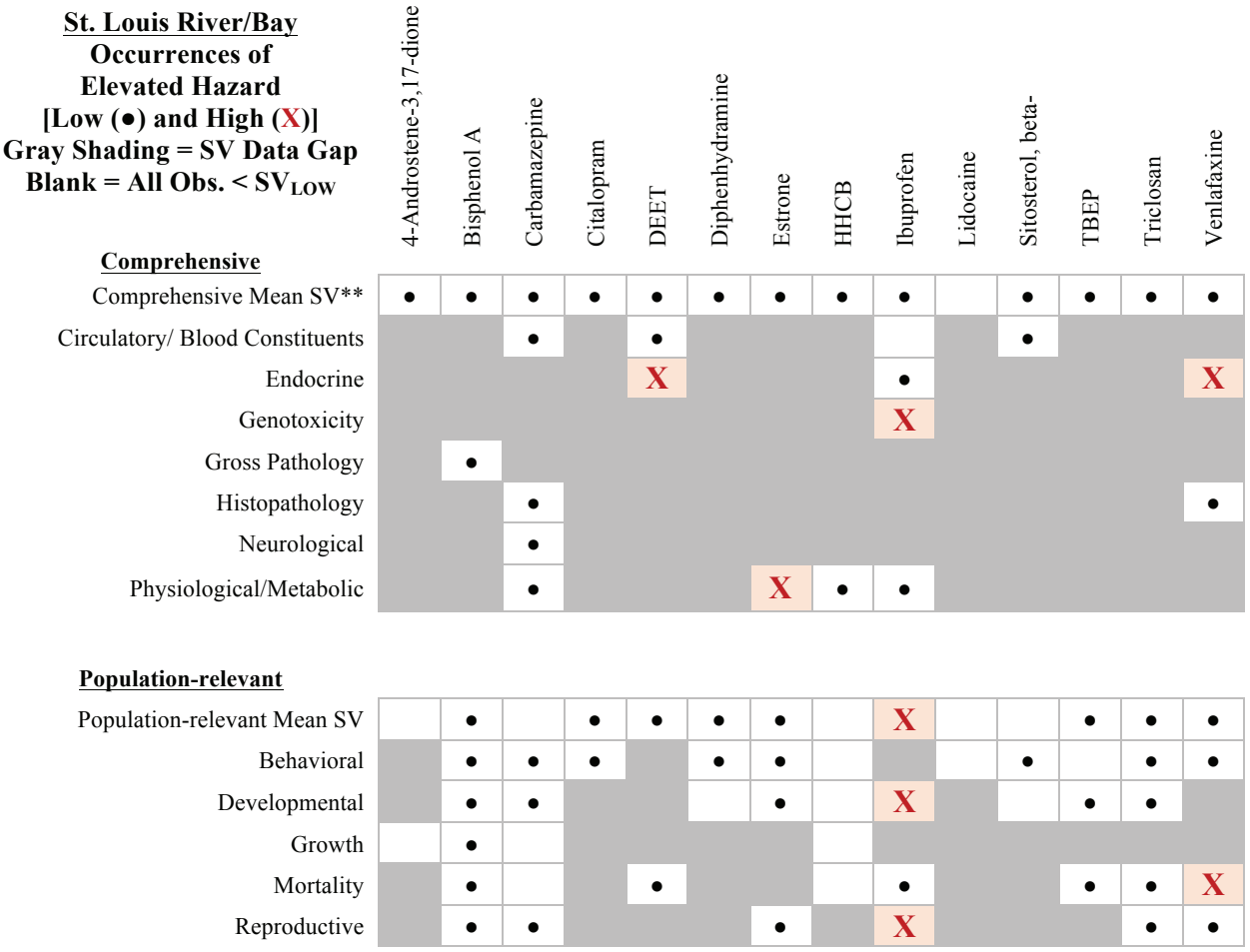
The following additional Effect Categories showed exceedances of SV_{LOW} values:

- Population-relevant: Behavioral, Growth
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Gross Pathology, Histopathology, Neurological

Among CECs evaluated, ibuprofen accounted for the greatest number of effect categories that have at least one observation of high hazard at the St. Louis River project location, followed by venlafaxine, DEET, and estrone. SV_{LOW} exceedances were observed for nine additional CECs. Only lidocaine concentrations did not exceed any screening value.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-1.

St. Louis River/Bay
Occurrences of Elevated Hazard
[Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA in order to eliminate confusion with population-relevant SVs for the same effect categories. The comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

High hazard related to at least one effect category was observed at sampling sites in the St. Louis Bay and Superior Bay, which receive inputs from several major WWTPs as well as CSOs and which are protected with a barrier that limits mixing of embayment waters with Lake Superior. Among the 32 sampling sites at St. Louis River/Bay, high hazard was observed in at least one sample at 11 different sites. Ten of these sites were identified as influenced by mapped point sources for the purpose of statistical analysis. The five STB-WLSSD sites associated with a WWTP had the most SV_{HIGH} exceedances (multiple CECs and effect categories) despite only one sample collected at three of the five sites.

St. Louis River/Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

01. > BlatnikBr (2)	**09. > HogIsland (5)**	17. **> STB-MP-4 (1)**	25. STR-FDL-2 (1)
02. CloquetDW (2)	10. MudLk (2)	18. **> STB-MP-5 (1)**	26. STR-FDL-3 (1)
03. CloquetUP (2)	11. NekukId (2)	19. **> STB-MP-6 (1)**	27. STR-FDL-4 (1)
04. CloughId (2)	**12. > RicesPt (8)**	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**	28. STR-FDL-5 (1)
05. > EriePr (8)	**13. > SMTP (5–10)**	**21. > STB-WLSSD-2 (1)**	29. STR-FDL-6 (1)
06. FDL (4)	14. **> STB-MP-1 (1)**	**22. > STB-WLSSD-3 (1)**	30. TallasId (2)
07. FondDu (2)	15. **> STB-MP-2 (1)**	**23. > STB-WLSSD-5 (1)**	**31. > WLSSD-distal (19)**
08. GrassyPt (6)	16. **> STB-MP-3 (1)**	24. STR-FDL-1 (1)	32. WireMi (2)

5.4.1.2 Hazard Rankings for St. Louis River/Bay

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites. A description of the ranking process, including the rationale for ranking mean rather than median hazard are provided in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Observations falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Overall, hazard at this site appears to be dominated by endocrine and genotoxicity hazard, although at least one occurrence of high hazard was observed for mortality, reproductive, developmental, and physiologic/metabolic effect categories.

Effect category ranks based on bins (see Table 5-3) for effect-specific mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	St. Louis River/ Bay Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.608	1.6-1.8	4
Genotoxicity	1.490	1.4-1.6	3
Mortality	1.196	1.0-1.2	1
Physiological/Metabolic	1.167	1.0-1.2	1
Population-relevant Mean SV	1.144	1.0-1.2	1
Reproductive	1.138	1.0-1.2	1
Comprehensive Mean SV	1.128	1.0-1.2	1
Histopathology	1.120	1.0-1.2	1
Behavioral	1.086	1.0-1.2	1
Circulatory/Blood Constituents	1.085	1.0-1.2	1
Developmental	1.076	1.0-1.2	1
Gross Pathology	1.018	1.0-1.2	1
Neurological	1.012	1.0-1.2	1
Growth	1.011	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	St. Louis River/ Bay CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.727	1.6-1.8	4
Venlafaxine	1.266	1.2-1.4	2
Ibuprofen	1.224	1.2-1.4	2
Estrone	1.213	1.2-1.4	2
Diphenhydramine	1.112	1.0-1.2	1
Citalopram	1.084	1.0-1.2	1
Sitosterol, beta-	1.079	1.0-1.2	1
TBEP	1.075	1.0-1.2	1
Triclosan	1.065	1.0-1.2	1
HHCB	1.050	1.0-1.2	1
Bisphenol A	1.042	1.0-1.2	1
4-Androstene-3,17-dione	1.014	1.0-1.2	1
Carbamazepine	1.009	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

Sampling Sites[41]

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (Bold ">" = site designated as point source CEC-influenced)	St. Louis River/ Bay Site-Specific Mean Hazard Score (range of mean: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> STB-WLSSD-5	1.537	1.4-1.6	3
> STB-WLSSD-3	1.488	1.4-1.6	3
> STB-WLSSD-2	1.384	1.2-1.4	2
> STB-WLSSD-4/ WLSSD-DISTAL	1.348	1.2-1.4	2
> STB-WLSSD-1	1.330	1.2-1.4	2
> SMTP	1.313	1.2-1.4	2
> BlatnikBr	1.201	1.2-1.4	2
> HogIsland	1.127	1.0-1.2	1
WireMi	1.085	1.0-1.2	1
> RicesPt	1.074	1.0-1.2	1
> STB-MP-4	1.055	1.0-1.2	1
> EriePr	1.044	1.0-1.2	1
NekukId	1.043	1.0-1.2	1
> STB-MP-1	1.041	1.0-1.2	1
> STB-MP-2	1.041	1.0-1.2	1
> STB-MP-3	1.041	1.0-1.2	1
> STB-MP-5	1.041	1.0-1.2	1
> STB-MP-6	1.041	1.0-1.2	1
CloquetDW	1.037	1.0-1.2	1
CloquetUP	1.037	1.0-1.2	1
CloughId	1.037	1.0-1.2	1
FondDu	1.037	1.0-1.2	1
GrassyPt	1.037	1.0-1.2	1
MudLk	1.037	1.0-1.2	1
TallasId	1.037	1.0-1.2	1
FDL	1.027	1.0-1.2	1

5.4.1.3 Breadth of Information Indicating High Hazard at St. Louis River/Bay

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization, focused on observations of high hazard.

- *Exposure:* Overall, spatial coverage of the project location and total number of sampling sites are excellent. Expectation of high hazard is indicated only at embayment sites that are near and downgradient of known point sources. Exposure information is fairly strong for these sites; most were sampled five or more times representing various seasons and times of day (Attachment A1, Table A-2). Most exceedences of SV_{LOW} values also occurred in embayment sites. Collectively, evidence for negligible hazard upstream of St. Louis Bay, where no mapped point sources of CECs were identified, is moderately strong due to a large number of sampling sites. A majority of sampling sites (about 20) are located in the fluvial part of this project location, and all of these sites indicated no high hazard. However, almost all of these sites were sampled only one or two times, and most of them only in the autumn. It is possible that evidence of high hazard would be identified at the upriver sites with additional sampling in various seasons and times of day.

[41]STR-FDL-1 through STR-FDL-6 are six individual sites distinct from the site designated as 'FDL' that are in very close proximity to FDL (see Figure A2-1b in Attachment A), and were lumped with FDL in the calculation of mean hazard score for this sampling site hazard ranking table.

Conversions of total CEC to aqueous CEC (Section 3.5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is moderate, based on a good spatial distribution of USEPA STORET water quality sampling sites throughout the St. Louis River/Bay project location (Figure A2-1a in Attachment A2). The project location was subdivided into 3 segments – upper river, lower river, and embayments – and CEC aqueous concentration was estimated separately based on TSS data from within each of those segments. However, splitting the location into three segments reduced the numbers of USEPA STORET sites in each area so confidence in representativeness of empirical TSS values is limited.

- *Ecotoxicity:* Among observations of high hazard for ibuprofen, DEET, estrone, and venlafaxine, the breadth of applicable ecotoxicity information was as follows (Tables 4-5 and 4-6):
    - o Ibuprofen
        - Broad - Population-relevant mean SVs
        - Broad - Developmental
        - Broad - Reproductive
        - Sparse - Genotoxicity
    - o DEET
        - Moderate - Endocrine
    - o Estrone
        - Sparse - Physiological/Metabolic
    - o Venlafaxine
        - Sparse - Endocrine
        - Sparse - Mortality

5.4.1.4 St. Louis River/Bay Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores between sites that are suspected to be CEC-'influenced' by discharges from point sources and sites that are expected to be relatively 'uninfluenced' by CEC point sources. For each CEC and effect category evaluated in this EHA, we quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis (methods provided in Section 5.3.4).

Site-specific maximum and/or median hazard scores were significantly higher in the point source CEC-influenced site group as compared to uninfluenced sites in eight effect categories, for either DEET or ibuprofen. Supporting qualitative data indicated a higher incidence of both SV_{HIGH} and SV_{LOW} exceedances at sites expected to be influenced by CECs as compared to uninfluenced sites (Table 6.3). The following chart identifies those analyses with statistically significant differences between site groups:

When *all* hazard scores for each site in the St. Louis River/Bay location, not just maximum or median scores, are included in the statistical analysis[42], additional significant differences ($p < 0.1$) are observed for the following CEC-effect category combinations (Attachment D):

- Bisphenol A – Population-relevant mean SV, Behavioral, Developmental, Growth, Mortality, and Reproductive
- DEET - Comprehensive mean SV
- HHCB – Physiological/Metabolic
- β-Sitosterol – Behavioral
- TBEP – Comprehensive mean SV

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
DEET	Circulatory/Blood Constituents	Y	N
	Endocrine	Y	N
Ibuprofen	Comprehensive mean SV	Y	Y
	Population-relevant mean SV	Y	N
	Developmental	Y	N
	Genotoxicity	Y	N
	Mortality	Y	N
	Reproductive	Y	N

[42]Cautionary caveats are provided in Attachment D regarding the use of all hazard scores (rather than just maximum or median score) from each site for statistical comparisons of hazard to fish between CEC-'influenced' and 'uninfluenced' sites.

A summary of the qualitative comparison between site groups is provided in the following chart. Shown are fractions of samples and of sites at the St. Louis River/Bay location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. Data are provided in the following chart for point source CEC-influenced versus uninfluenced site groups.

There was a total of 85 exceedances of effect-specific SV_{HIGH} values, including at least one each for DEET, estrone, ibuprofen and venlafaxine at 10 of the 16 CEC-influenced sites. In stark contrast, there was only one SV_{HIGH} exceedance, for venlafaxine, among the 16 uninfluenced sites.

Similarly, among the 16 CEC-influenced sites, there was a total of 1044 exceedances of various effect-specific SV_{LOW} values, including at least one each for 12 of the 14 CECs. Only ibuprofen and lidocaine had no SV_{LOW} exceedances. Whereas, among the 16 uninfluenced sites there was a total of 94 exceedances, attributable only to 4-androstene-3,17-dione, β-sitosterol or DEET.

St. Louis River/Bay Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	85	0.6 (40/67)	0.63 (10/16)	1044	0.96 (64/67)	1.0 (16/16)
Uninfluenced Sites	1	0.03 (1/32)	0.06 (1/16)	94	0.88 (28/32)	0.94 (15/16)

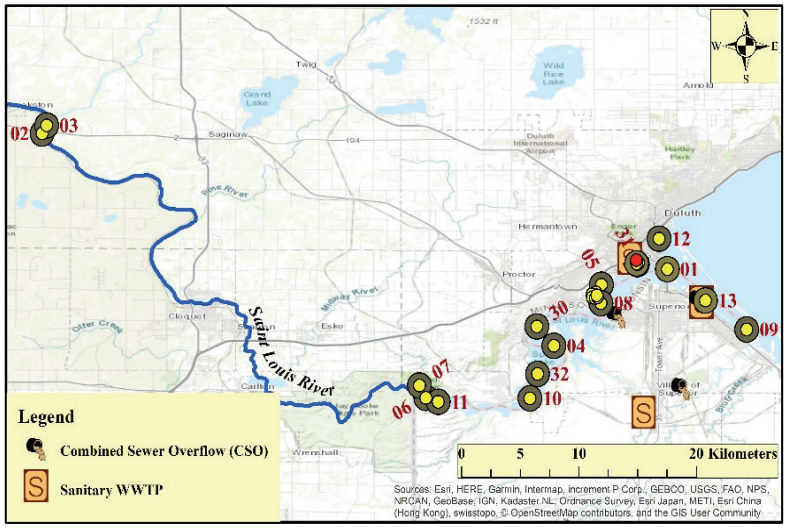
* Includes exceedances in all 14 effect categories considered in this EHA, but excludes exceedances of SVs in the c*omprehensive* behavioral, developmental, growth, mortality and reproductive effect categories that were described in Gefell et al. 2019.

5.4.1.5 St. Louis River/Bay Hazard Maps

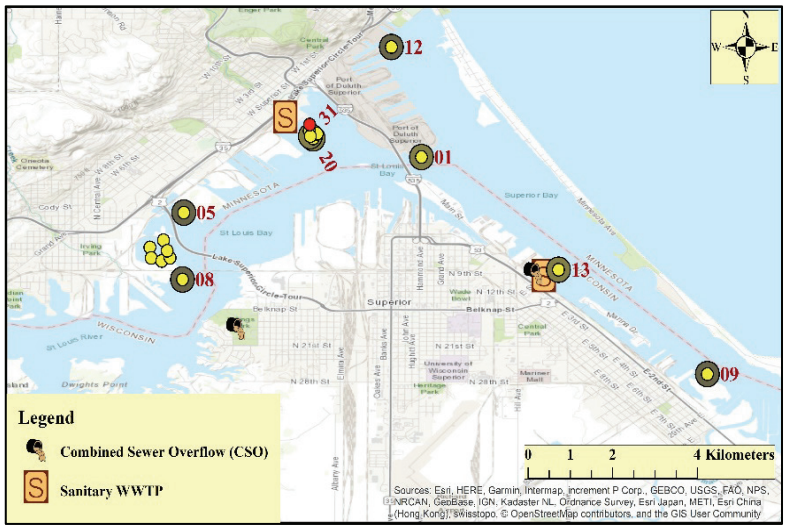
In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Population-relevant Mean SV

(A)



(B)



KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites (N) = Total Number of sampling events per site Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source	
01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

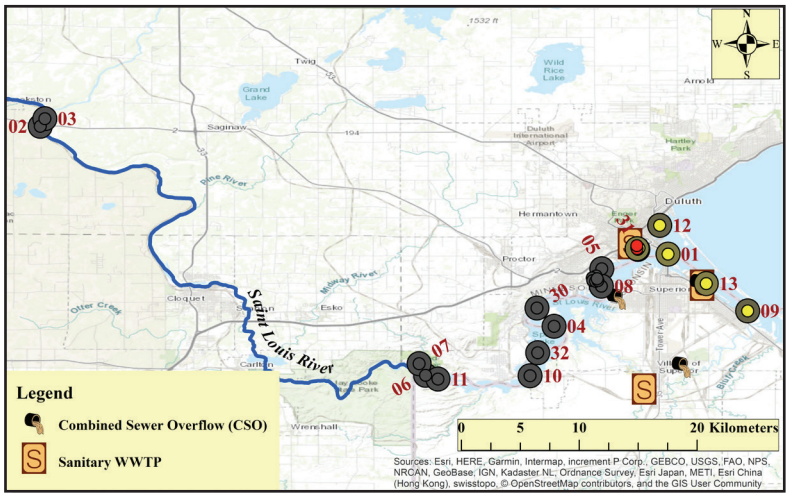
Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

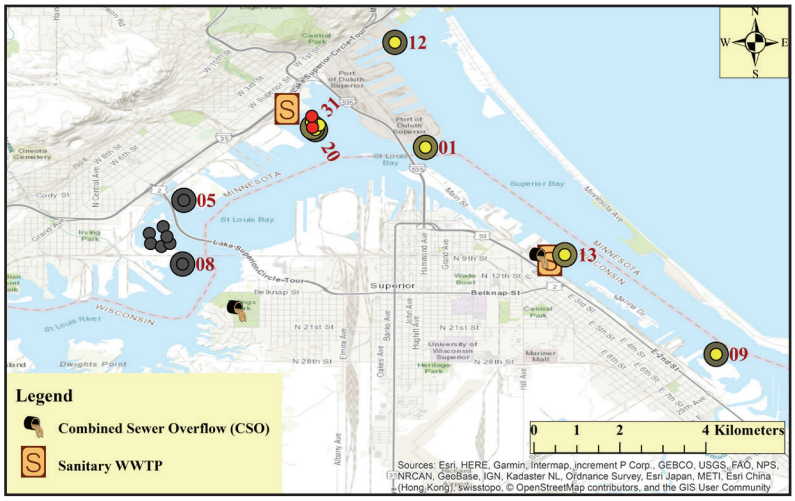
Figure 5-5. Population-relevant Mean SV Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Developmental Hazard

(A)



(B)



KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

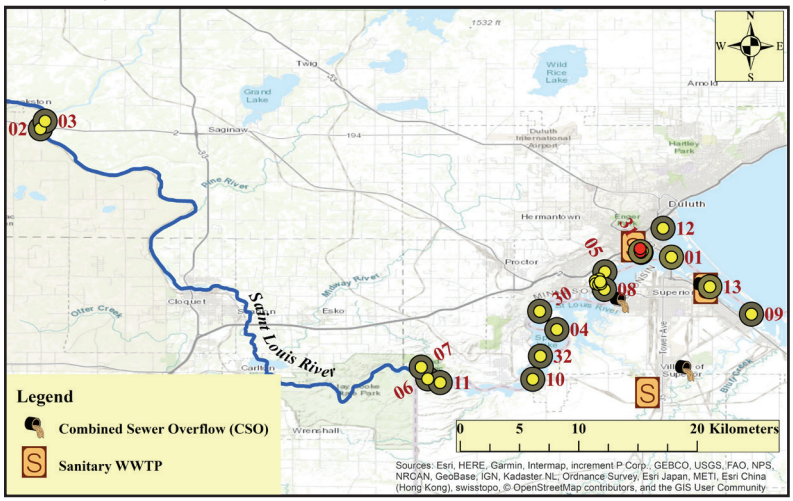
Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

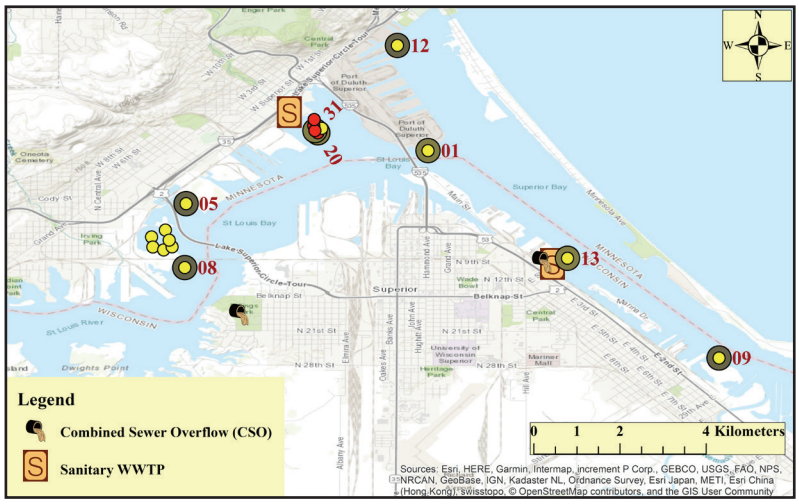
Figure 5-6. Developmental Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Mortality Hazard

(A)



(B)



KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

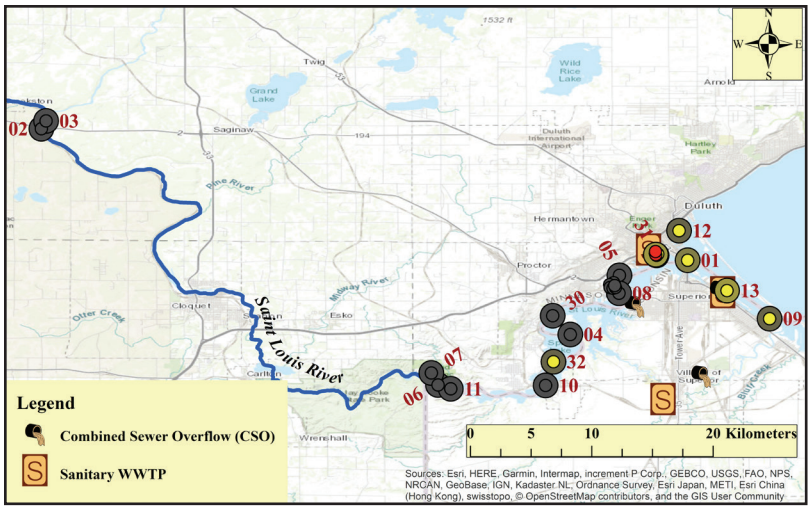
Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

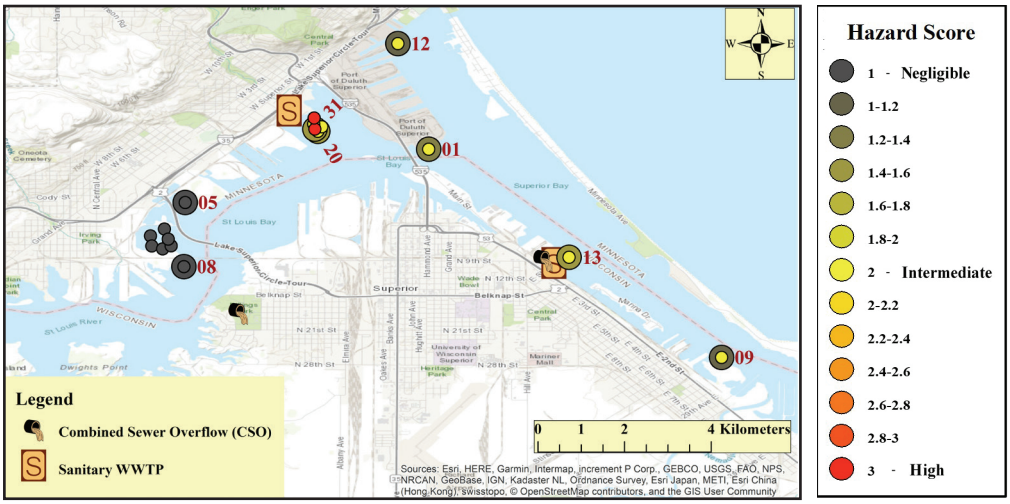
Figure 5-7. Mortality Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Reproductive Hazard

(A)



(B)



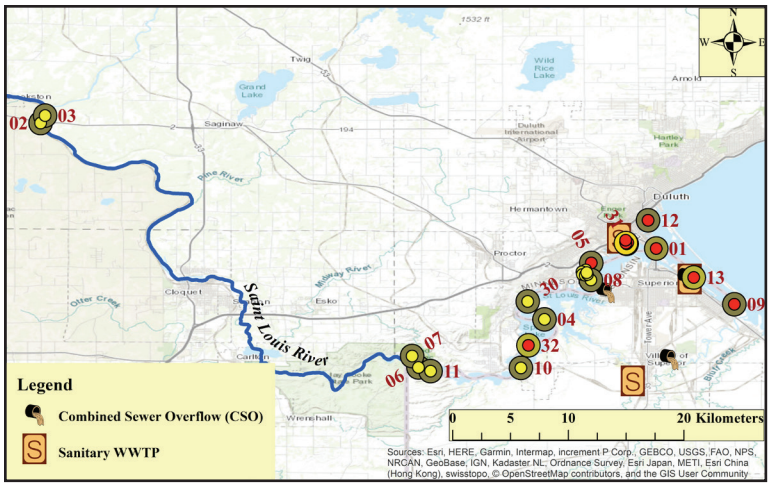
KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites (N) = Total Number of sampling events per site Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source	
01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

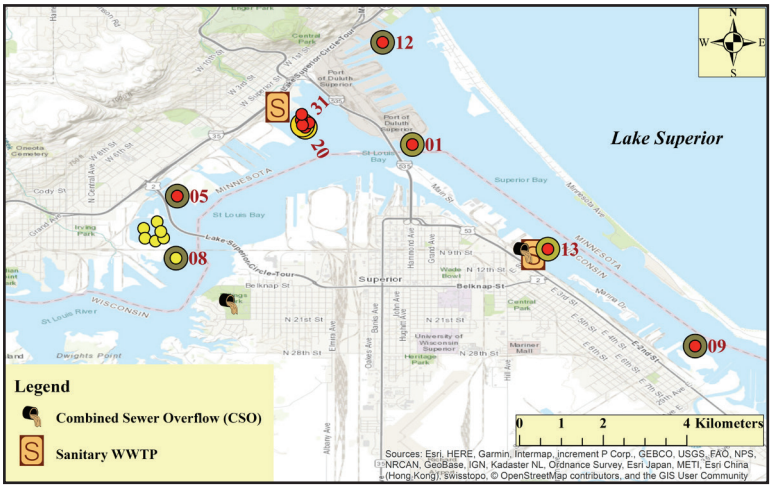
Figure 5-8. Reproductive Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Endocrine Hazard

(A)



(B)



KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

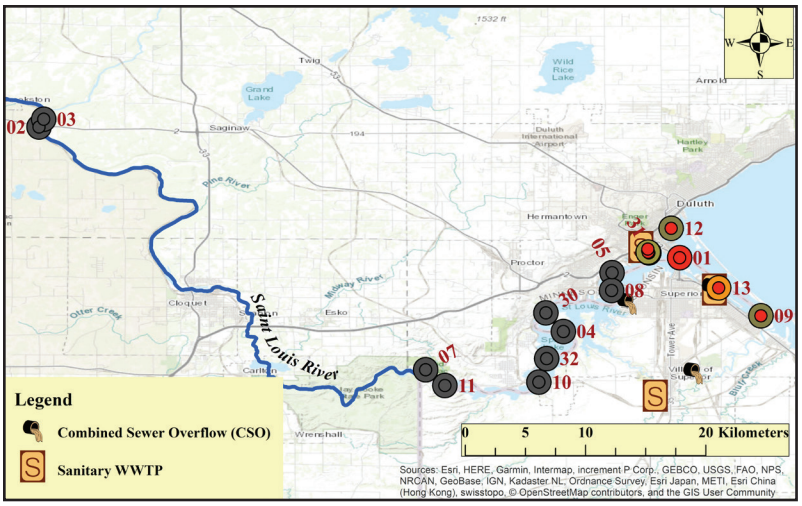
Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

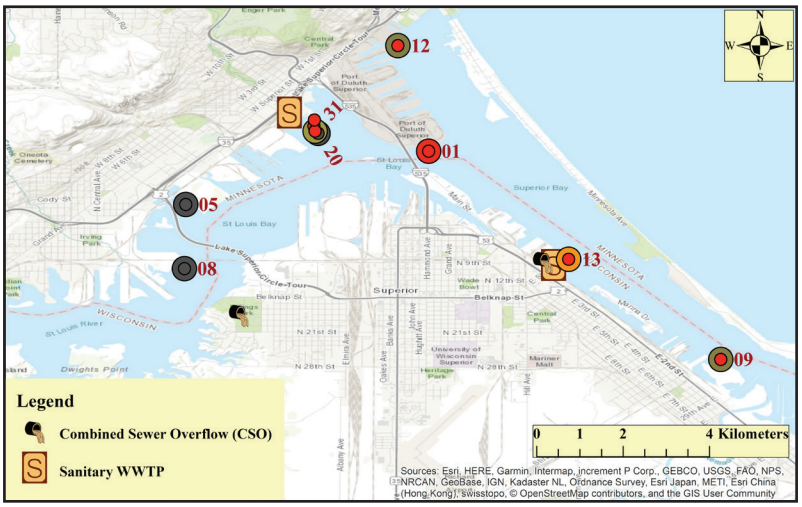
Figure 5-9 Endocrine Hazard Maps for the St. Louis River and St. Louis and Superior Bays.
(A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Genotoxicity Hazard

(A)



(B)



KEY

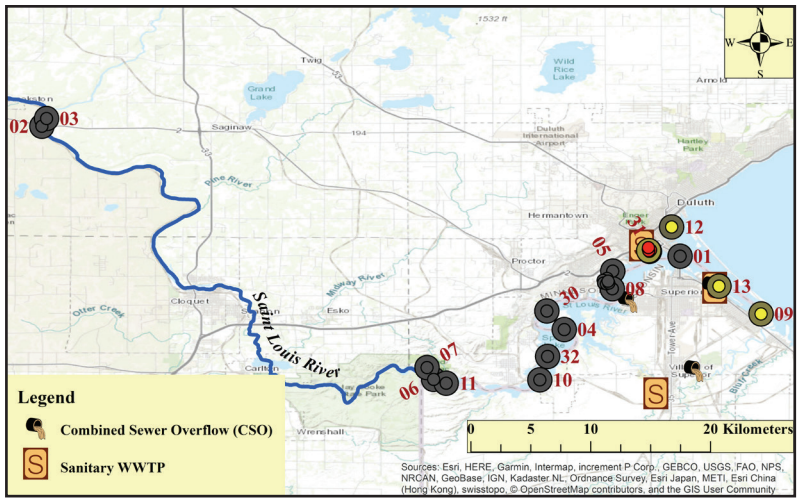
St. Louis River/Bay Project Location – Surface Water Sampling Sites (N) = Total Number of sampling events per site Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source	
01. > BlatnikBr (2)	**17. > STB-MP-4 (1)**
02. CloquetDW (2)	**18. > STB-MP-5 (1)**
03. CloquetUP (2)	**19. > STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

Hazard Score
1 - Negligible
1-1.2
1.2-1.4
1.4-1.6
1.6-1.8
1.8-2
2 - Intermediate
2-2.2
2.2-2.4
2.4-2.6
2.6-2.8
2.8-3
3 - High

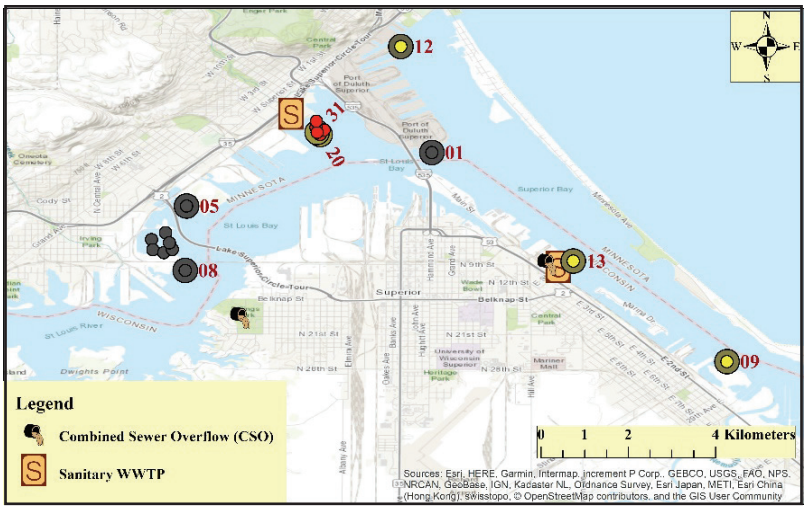
Figure 5-10. Genotoxicity Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

Physiological/Metabolic Hazard

(A)



(B)



KEY

St. Louis River/Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

01. > BlatnikBr (2)	17. **> STB-MP-4 (1)**
02. CloquetDW (2)	18. **> STB-MP-5 (1)**
03. CloquetUP (2)	19. **> STB-MP-6 (1)**
04. CloughId (2)	**20. > STB-WLSSD-1/ WLSSD-Far Dist (6)**
05. > EriePr (8)	**21. > STB-WLSSD-2 (1)**
06. FDL (4)	**22. > STB-WLSSD-3 (1)**
07. FondDu (2)	**23. > STB-WLSSD-5 (1)**
08. GrassyPt (6)	24. STR-FDL-1 (1)
09. > HogIsland (5)	25. STR-FDL-2 (1)
10. MudLk (2)	26. STR-FDL-3 (1)
11. NekukId (2)	27. STR-FDL-4 (1)
12. > RicesPt (8)	28. STR-FDL-5 (1)
13. > SMTP (5–10)	29. STR-FDL-6 (1)
14. > STB-MP-1 (1)	30. TallasId (2)
15. > STB-MP-2 (1)	**31. > WLSSD-distal (19)**
16. > STB-MP-3 (1)	32. WireMi (2)

Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

Figure 5-11. Physiological/Metabolic Hazard Maps for the St. Louis River and St. Louis and Superior Bays. (A) Entire Project Location; (B) Embayment Sites. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1.

5.4.2 Waupaca Chain O'Lakes (WI)

The Waupaca Chain O'Lakes in Wisconsin are headwater lakes of the Crystal River, a tributary of the Waupaca River that empties into the Fox River upstream of Lake Winnebago. The two sampling sites were located in 207 acre Rainbow Lake, were designated as 'uninfluenced' by mapped CEC point sources, and were sampled once each in April 2012. There is little evidence of CEC-related hazards to fish possible due in part to the very limited exposure information. Nevertheless, elevated CEC-related hazard - including high potential for adverse endocrine effects from DEET - was observed at the Waupaca Chain O'Lakes project location. Given these results, it is reasonable to expect that a more robust exposure dataset may result in additional evidence of CEC-related hazards.

5.4.2.1 Hazard Brief for Waupaca Chain O'Lakes

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

High hazard was indicated only for the endocrine hazard category (Table 5-1) at this project location. Among CECs evaluated, DEET alone accounted for high hazard observations at both sampling sites. Estrone had the greatest variety of SV_{LOW} exceedances including behavioral, developmental, and reproductive effects, suggesting possible population level impacts in intolerant fish species.

Some Key Points…

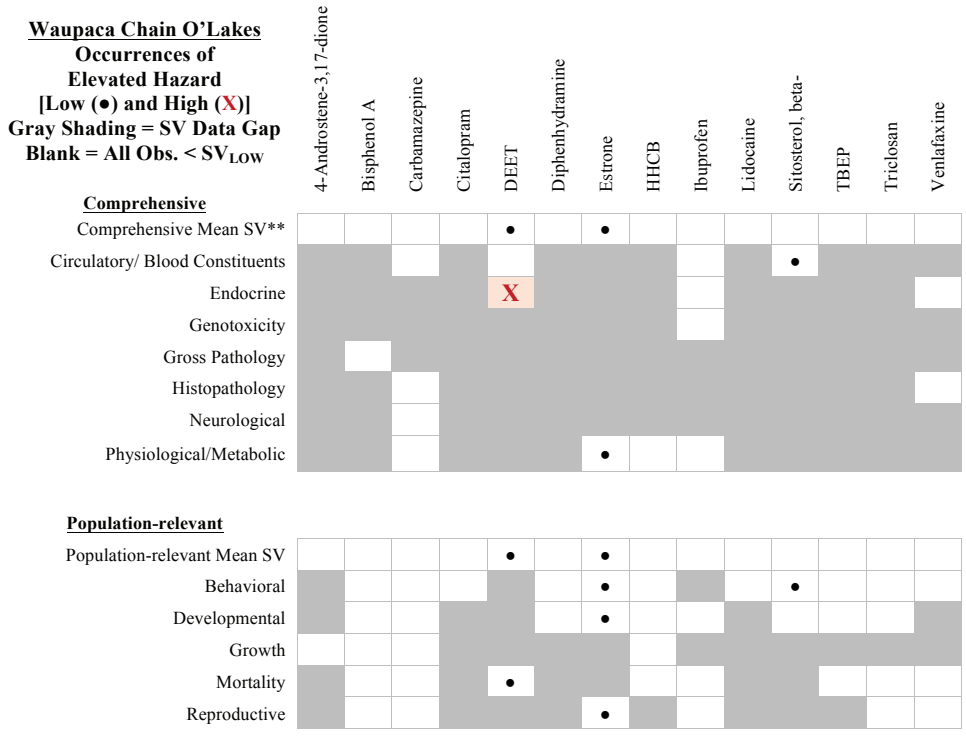
Waupaca Chain O'Lakes (WI)

- **Overall:** Little evidence of CEC-related hazards in fish
- **High Hazard**:
  - 2 occurrences involving 100% of sampling events and sites
  - *CECs:* DEET
  - *Effect Categories*: Endocrine
- **Low Hazard**:
  - 15 occurrences involving 100% of sampling events and sites
  - *CECs:* DEET, Estrone, β-Sitosterol
  - *Effect Categories*: 7 of 12
- **Point Source Analysis:** No evaluation; no mapped CEC point sources within project location
- **Exposure Dataset**: Minimal
  - 2 sites
  - 1 sample per site
  - 2 total samples

SV_{LOW} exceedances occurred in the following Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Physiological/ Metabolic

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-2.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

5.4.2.2 Hazard Rankings for Waupaca Chain O'Lakes

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Observations falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Waupaca Chain O'Lakes Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.667	1.6-1.8	4
Behavioral	1.136	1.0-1.2	1
Circulatory/Blood Constituents	1.125	1.0-1.2	1
Mortality	1.125	1.0-1.2	1
Physiological/Metabolic	1.125	1.0-1.2	1
Comprehensive Mean SV	1.107	1.0-1.2	1
Population-relevant Mean SV	1.107	1.0-1.2	1
Reproductive	1.083	1.0-1.2	1
Developmental	1.056	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Waupaca Chain O'Lakes CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	2	1.8-2.0	5
Estrone	1.5	1.4-1.6	3
Sitosterol, beta-	1.3	1.2-1.4	2
4-Androstene-3,17-dione	1	1.0-1.2	1
Bisphenol A	1	1.0-1.2	1
Carbamazepine	1	1.0-1.2	1
Citalopram	1	1.0-1.2	1
Diphenhydramine	1	1.0-1.2	1
HHCB	1	1.0-1.2	1
Ibuprofen	1	1.0-1.2	1
Lidocaine	1	1.0-1.2	1
TBEP	1	1.0-1.2	1
Triclosan	1	1.0-1.2	1
Venlafaxine	1	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across Effect Categories and CECs) are as follows (neither sampling site was designated as a 'CEC-influenced' site.):

Sampling Site	Waupaca Chain O'Lakes Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
COL-2	1.159	1.0-1.2	1
COL-1	1.073	1.0-1.2	1

5.4.2.3. Breadth of Information Indicating High Hazard at Waupaca Chain O'Lakes

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was associated with observations of high hazard.

- *Exposure:* Two sampling sites and only one sample per site provided minimal spatio-temporal coverage of the project location. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is moderate, based on four USEPA STORET water quality sampling sites located in and near the project location at the headwaters of the Crystal River in the Fox River watershed (Figure A2-2 in Attachment A2).
- *Ecotoxicity:* Breadth of ecotoxicity information for deriving DEET SVs in the Endocrine effect category is moderate (Table 4-6).

5.4.2.4 Waupaca Chain O'Lakes Point Source Analysis

Neither of the two sampling sites was influenced by a mapped CEC point source and sample size was 1 at each site, so no statistical comparisons between point source CEC-influenced and uninfluenced sites were possible. Qualitative evidence (chart below) indicates some potential for impacts.

Waupaca Chain O'Lakes Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	NA	NA	NA	NA	NA	NA
Uninfluenced Sites	2	1.0 (2/2)	1.0 (2/2)	15	1.0 (2/2)	1.0 (2/2)

* Includes exceedances in all 14 effect categories considered in this EHA, but excludes exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories that were described in Gefell et al. 2019.

5.4.2.5 Waupaca Chain O'Lakes Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3

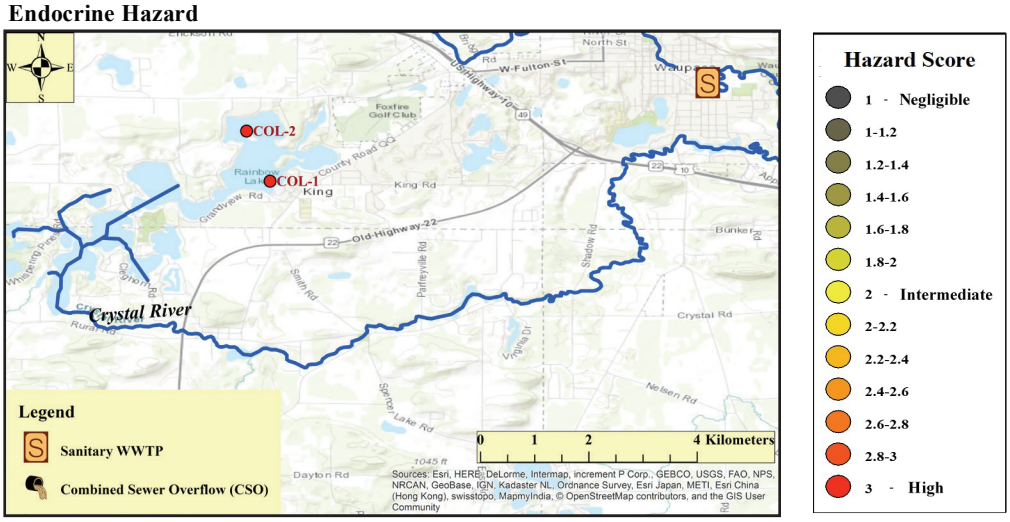


Figure 5-12. Endocrine Hazard Map for the Waupaca Chain O'Lakes. Waupaca Chain O'Lakes project location is in the Fox River watershed. Sample size was one (1) at both sampling sites; no mean hazard scores were available.

5.4.3 Little Lake Butte des Morts (LLBDM) (WI)

Little Lake Butte des Morts is a shallow 1200 acre lake embedded within the Fox River system in Wisconsin, just downstream of Lake Winnebago. Four of the five sampling sites were designated as CEC-'influenced', and one 'uninfluenced', based on their down-gradient proximity to mapped CEC point sources. Either four or eight samples were analyzed from individual sampling sites, and the timing of surface water sampling was the same at each of the five sites: August 2013, and May and August 2014.

Assessment results provide substantial evidence of hazard to fish from exposure to the 14 CECs in the Little Lake Butte des Morts project location. A reasonably strong exposure dataset provided evidence for elevated hazard observed at each sampling site (including a wide range of effect categories and CECs), where nearly all hazard observations were exceedances of a SV_{LOW}, indicating low hazard. There is also limited evidence for high hazard (SV_{HIGH} exceedance), observed at two of the five sampling sites: LLB-2 (1/8 events showed high physiological/metabolic hazard from estrone) and LLB-3 (2/8 events showed high endocrine hazard from DEET). Concentrations of eight additional CECs exceeded at least one SV_{LOW}, but concentrations of diphenhydramine, lidocaine and TBEP did not exceed any screening value. While there was a total of only three SV_{HIGH} exceedances, SV_{LOW} exceedances totaling 316 were pervasive.

Five of the six highest ranked effect categories were comprehensive type effects, led by neurological, histopathology and endocrine hazards. DEET-related hazard ranked highest among CECs, followed by estrone, carbamazepine, venlafaxine, and beta-sitosterol. Hazard at the four sites identified as CEC-influenced ranked higher than hazard at the one uninfluenced site, and LLB-3 – the site closest to an upstream WWTP – ranked at the top for hazard to fish. While the strength of exposure data was relatively strong considering the small size of the project location, the breadth of ecotoxicity data associated with observations of high hazard was only sparse to moderate.

There were no statistically significant differences in hazard scores between point source CEC-influenced and uninfluenced site groups, but these results

Some Key Points…

Little Lake Butte des Morts (WI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard**:
  - 3 occurrences involving 11% of sampling events and 40% of sites
  - *CECs:* DEET, Estrone
  - *Effect Categories*: Endocrine, Physiological/ Metabolic
- **Low Hazard**:
  - 316 occurrences involving 100% of sampling events and sites
  - *CECs:* 10 of 13 (Ibuprofen data not available)
  - *Effect Categories*: 9 of 12
- **Point Source Analysis:** No evidence of elevated hazard downstream of point sources
- **Exposure Dataset**: Substantial
  - 5 sites
  - 4 to 8 samples per site
  - 28 total samples

should be considered tentative considering the small numbers of sites in each group. Qualitatively, all three observations of SV_{HIGH} exceedance occurred at CEC-influenced sites, while at least one SV_{LOW} exceedance occurred in 27 of all 28 sampling events and at all five sites. Hazard maps illustrate high hazard in proximity to WWTPs.

5.4.3.1 Hazard Brief for LLBDM

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

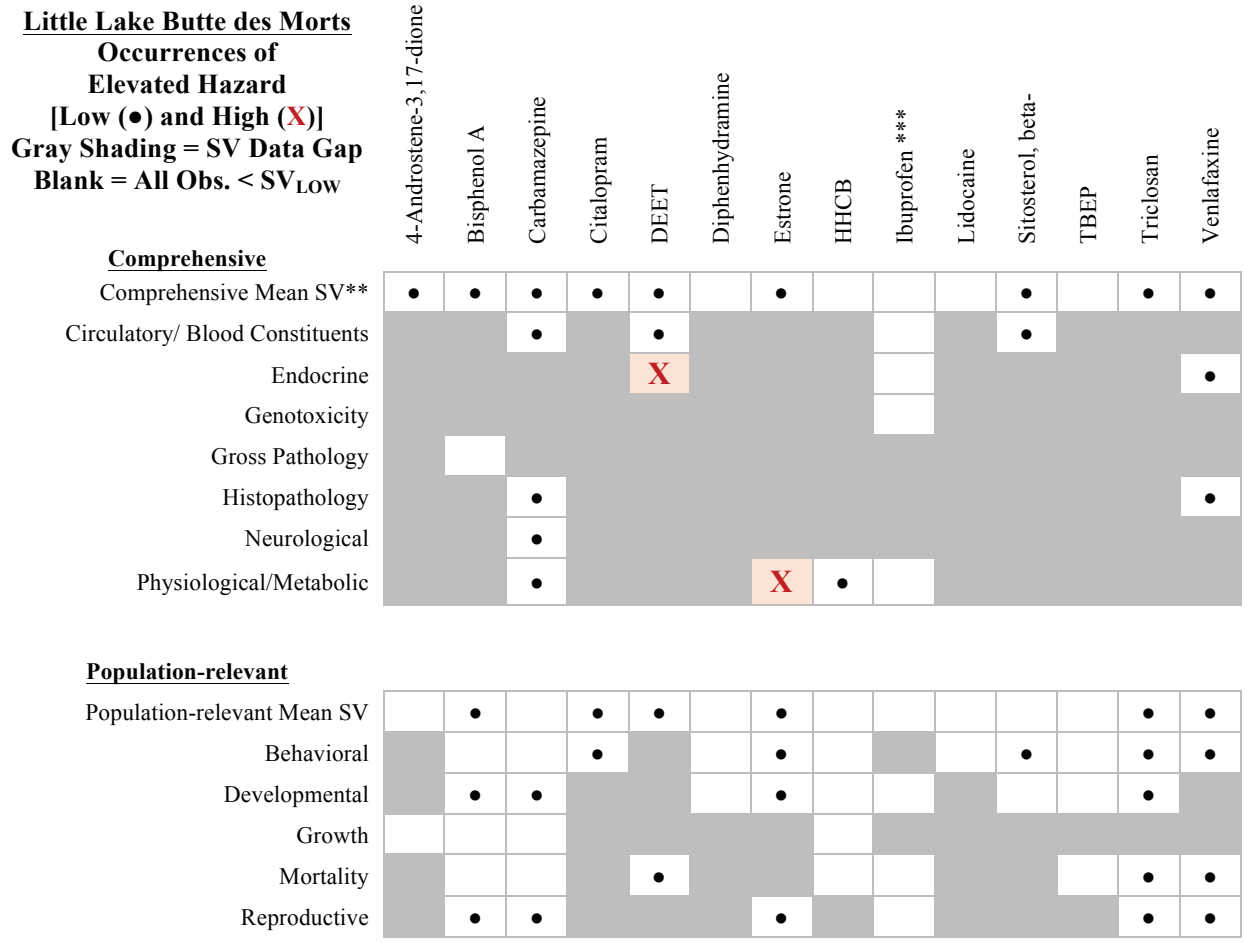
Effect Categories and Emerging Contaminants
LLBDM effect-specific hazard maps (Section 5.4.3.5) are provided for the following effect categories for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: None
- Comprehensive: Endocrine, Physiological/ Metabolic

SV_{LOW} exceedances also occurred in the following Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-3.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites
Mapped LLBDM sampling sites that were evaluated for hazard to fish were:

Little Lake Butte des Morts Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. LLB-1 (4)
2. **> LLB-2 (8)**
3. **> LLB-3 (8)**
1. **> LLB-4 (4)**
2. **> LLB-5 (4)**

5.4.3.2 Hazard Rankings for LLBDM

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Observations falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[43]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	LLBDM Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Neurological	1.575	1.4-1.6	3
Histopathology	1.475	1.4-1.6	3
Endocrine	1.438	1.4-1.6	3
Physiological/Metabolic	1.400	1.2-1.4	2
Circulatory/Blood Constituents	1.342	1.2-1.4	2
Reproductive	1.288	1.2-1.4	2
Mortality	1.132	1.0-1.2	1
Behavioral	1.125	1.0-1.2	1
Comprehensive Mean SV	1.101	1.0-1.2	1
Population-relevant Mean SV	1.084	1.0-1.2	1
Developmental	1.073	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

[43]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

Emerging Contaminants[44]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	LLBDM CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.410	1.4-1.6	3
Estrone	1.304	1.2-1.4	2
Carbamazepine	1.291	1.2-1.4	2
Venlafaxine	1.246	1.2-1.4	2
Sitosterol, beta-	1.235	1.2-1.4	2
Triclosan	1.100	1.0-1.2	1
HHCB	1.043	1.0-1.2	1
Bisphenol A	1.041	1.0-1.2	1
4-Androstene-3,17-dione	1.025	1.0-1.2	1
Citalopram	1.025	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1

Sampling Sites
Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	LLBDM Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> LLB-3 (8)	1.256	1.2-1.4	2
> LLB-5 (4)	1.163	1.0-1.2	1
> LLB-4 (4)	1.149	1.0-1.2	1
> LLB-2 (8)	1.107	1.0-1.2	1
LLB-1 (4)	1.103	1.0-1.2	1

5.4.3.3. Breadth of Information Indicating High Hazard at LLBDM

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* Spatial coverage of the project location and number of sampling sites was moderately strong, given the relatively small size of the project area. High hazard is indicated at two sites (LLB-2 and LLB-3), which are near and downgradient of known point sources. Exposure information is relatively strong for these sites, which were sampled eight times representing various seasons and times of day over a two-year period (Attachment A1, Table A-2).

  Exposure information is less strong at sites where no SV_{HIGH} was exceeded, including at two sites downstream of a mapped WWTP. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is relatively high, based on an adequate number and distribution of USEPA STORET water quality sampling sites at this project location (Figure A2-3 in Attachment A2).

- *Ecotoxicity:* Breadth of ecotoxicity information supporting observations of high hazard was as follows (Tables 4-5, 4-6):
  - o DEET
    Moderate – Endocrine
  - o Estrone
    Sparse – Physiological/Metabolic

[44]Analytical data for ibuprofen was not included in the exposure database for this project location.

5.4.3.4 LLBDM Point Source Analysis

In this section, we provide results of quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). At LLBDM, there were no statistically significant differences in site-specific maximum or median hazard scores between upstream, uninfluenced sites and the point source CEC-influenced downstream sites. Also, no differences were observed when all hazard scores for each site in the LLBDM location, not just maximum or median scores, are included in the statistical analysis[45] (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the LLBDM location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Although a difference between site groups in tallies of SV_{HIGH} exceedances was observed at LLBDM, the single site in the uninfluenced group limits our confidence in this result. There was a total of three SV_{HIGH} exceedances among CEC-influenced sites, observed in three different sampling events at two sites, and attributed to DEET and estrone. There were no SV_{HIGH} exceedances at the uninfluenced site. Also, among the four CEC-influenced sites, there was a total of 284 exceedances of various effect-specific SV_{LOW} values, attributed to 10 of the 14 CECs.

Observations of SV_{HIGH} exceedances occurred at the two influenced sites closest to WWTPs. However, at LLB-1 (the upstream-most site at this project location), five CECs exceeded SV_{LOW} values in 11 effect categories, suggesting either an unmapped point source near this site or the waters of Lake Winnebago contribute substantial CEC loadings to the LLBDM project location.

LLBDM Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	3	0.13 (3/24)	0.5 (2/4)	284	0.96 (23/24)	1.0 (4/4)
Uninfluenced Sites	0	0.0 (0/4)	0.0 (0/1)	32	1.0 (4/4)	1.0 (1/1)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[45]Cautionary caveats regarding this approach are described in Attachment D.

5.4.3.5 LLBDM Hazard Maps

In this section, we provide hazard maps by effect category, interpreted as described in Section 5.3.3.

Endocrine Hazard

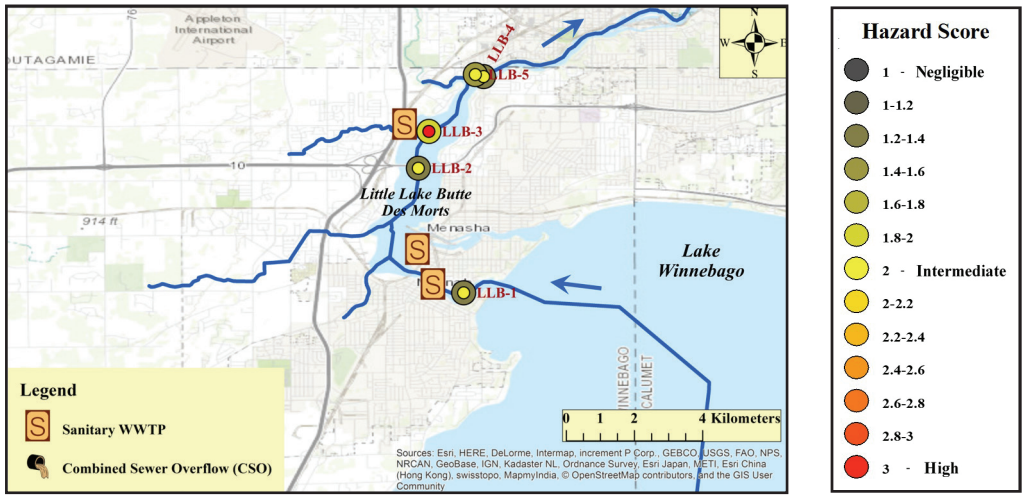


Figure 5-13. Endocrine Hazard Map for Little Lake Butte Des Morts. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Physiological/Metabolic Hazard

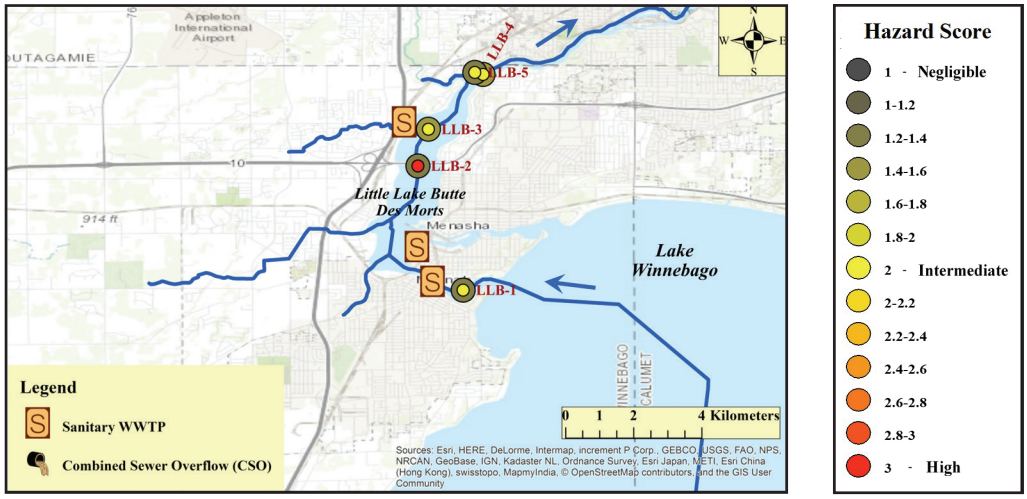


Figure 5-14. Physiological/Metabolic Hazard Map for Little Lake Butte Des Morts. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

5.4.4 Fox River/Green Bay (WI)

This project location is comprised of several distinct waterbodies: approximately the lower 100 km (62 mi) of the 320 km (200 mi) Fox River main stem, downstream of Little Lake Butte des Morts in Wisconsin; the southernmost part of Green Bay of Lake Michigan; and a couple of reference sites upstream of Lake Winnebago. Eight of the 15 sampling sites were designated as CEC-'influenced', and seven 'uninfluenced', based on their orientation and proximity to mapped CEC point sources. Sites were sampled one to eight times during one or more (up to three) of the following sampling events: October 2010, June 2011, April 2012, August 2013, or May and August in 2014.

There is substantial evidence of CEC-related hazards. High hazard to fish from exposures to DEET (endocrine effects), ibuprofen (genotoxicity), or venlafaxine (endocrine effects) was observed only seven times at five out of 15 sampling sites, three of which were designated as point source CEC-influenced. The 337 SV_{LOW} exceedances occurred in a variety of effect categories due to carbamazepine, estrone, and venlafaxine exposure, and were spatially widespread.

The hazard rank for endocrine effects was greatest among effect categories and the top five ranked mean hazard scores were in comprehensive effect categories. Mean hazard scores for DEET and β-sitosterol ranked highest among CECs, and seven of the top eight ranked sampling sites had been designated as CEC-influenced due to proximity to WWTPs. Confidence in the quantity and distribution of exposure assessment data is high, but the breadth of ecotoxicity information associated with high hazard observations is sparse to moderate.

Statistically significantly higher maximum hazard score ($p < 0.1$) was observed in the CEC-influenced site group compared to the uninfluenced site group for 10 different effect categories and three CECs. Most of these statistical differences are apparently due principally to a 3-fold higher incidence of SV_{LOW} exceedances in the CEC-influenced group. Although the tally of SV_{HIGH} exceedance is also greater in the CEC-influenced group, the total number of SV_{HIGH} exceedances is low and not likely to be driving the statistical differences. Endocrine and genotoxicity hazard maps illustrate a close spatial correspondence between WWTPs and the occurrence of high hazard.

Some Key Points…

Fox River / Green Bay (WI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard**:
  - 7 occurrences involving 16% of sampling events and 33% of sites
  - *CECs:* DEET, Ibuprofen, Venlafaxine
  - *Effect Categories*: Endocrine, Genotoxicity
- **Low Hazard**:
  - 337 occurrences involving 100% of sampling events and sites
  - *CECs:* 12 of 14
  - *Effect Categories*: 10 of 12
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources
- **Exposure Dataset**: Substantial
  - 15 sites
  - 1 to 8 samples per site
  - 37 total samples

5.4.4.1 Hazard Brief for Fox River/Green Bay

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

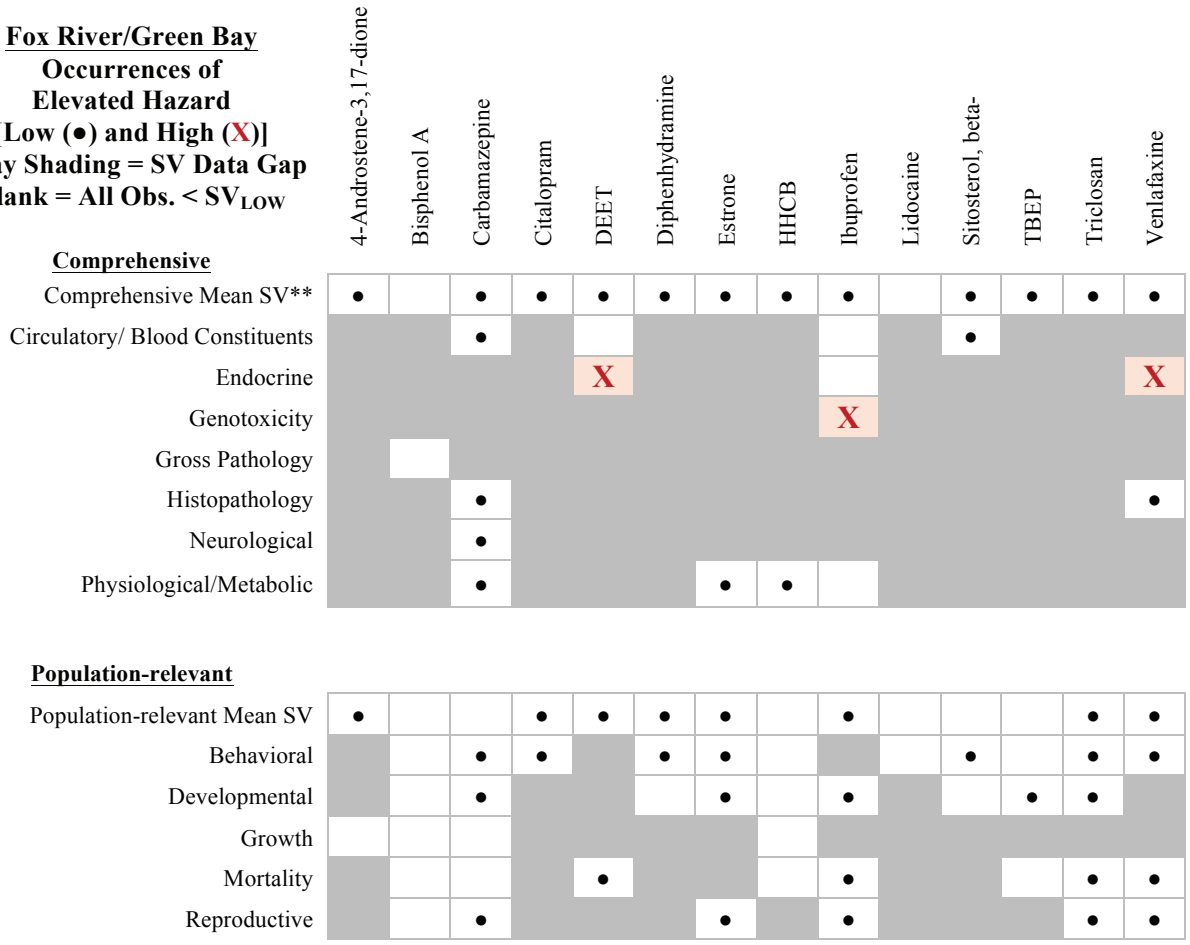
Fox River/Green Bay effect-specific hazard maps (Section 5.4.4.5) are provided for endocrine and genotoxicity effects, the only effect categories for which relatively high hazard was observed (Table 5-1). There were SV_{LOW} exceedances in additional effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/Metabolic.

High hazard potential for biological impacts was attributable to DEET, ibuprofen, and venlafaxine exposures, while a SV_{LOW} was exceeded for 12 of the 14 CECs evaluated. Only bisphenol-A and lidocaine had no screening value exceedances.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-4.

Fox River/Green Bay
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

High hazard (highlighted in red, below) was observed at three sites identified as point source CEC-influenced by mapped point sources, and at two uninfluenced sites. At least one SV_{LOW} was exceeded at all 15 sites.

Fox River/Green Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> DPERE-9 (3)**
2. **EASTR-10 (3)**
3. **> FXR-1 (1)**
4. **> FXR-13 (4)**
5. **> FXR-14 (1)**
6. **> FXR-2 (1)**
7. **> FXR-3 (1)**
8. FXR-4 (1)
9. FXR-5 (2)
10. FXR-6 (1)
11. **> GRBAY-12 (3)**
12. LBM-1 (3)
13. LKP-1 (3)
14. **> LLB-6 (8)**
15. **PRGAM-11 (2)**

5.4.4.2 Hazard Rankings for Fox River/Green Bay

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Observations falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Fox River/Green Bay Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.407	1.4-1.6	3
Circulatory/Blood Constituents	1.184	1.0-1.2	1
Histopathology	1.160	1.0-1.2	1
Physiological/Metabolic	1.145	1.0-1.2	1
Neurological	1.136	1.0-1.2	1
Mortality	1.135	1.0-1.2	1
Genotoxicity	1.111	1.0-1.2	1
Behavioral	1.108	1.0-1.2	1
Comprehensive Mean SV	1.103	1.0-1.2	1
Population-relevant Mean SV	1.091	1.0-1.2	1
Reproductive	1.086	1.0-1.2	1
Developmental	1.031	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Fox River/Green Bay CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.661	1.6-1.8	4
Sitosterol, beta-	1.310	1.2-1.4	2
Venlafaxine	1.148	1.0-1.2	1
Estrone	1.133	1.0-1.2	1
Carbamazepine	1.080	1.0-1.2	1
Citalopram	1.044	1.0-1.2	1
HHCB	1.044	1.0-1.2	1
Ibuprofen	1.043	1.0-1.2	1
Triclosan	1.025	1.0-1.2	1
Diphenhydramine	1.017	1.0-1.2	1
4-Androstene-3,17-dione	1.015	1.0-1.2	1
TBEP	1.009	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Fox River/Green Bay Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> DPERE-9	1.252	1.2-1.4	2
> GRBAY-12	1.163	1.0-1.2	1
> FXR-1	1.159	1.0-1.2	1
> FXR-13	1.155	1.0-1.2	1
EASTR-10	1.142	1.0-1.2	1
> LLB-6	1.132	1.0-1.2	1
> FXR-2	1.085	1.0-1.2	1
> FXR-3	1.085	1.0-1.2	1
PRGAM-11	1.079	1.0-1.2	1
> FXR-14	1.073	1.0-1.2	1
FXR-4	1.073	1.0-1.2	1
LBM-1	1.068	1.0-1.2	1
FXR-5	1.067	1.0-1.2	1
FXR-6	1.049	1.0-1.2	1
LKP-1	1.018	1.0-1.2	1

5.4.4.3. Breadth of Information Indicating High Hazard at Fox River/Green Bay

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* Spatial coverage across the project location and total number of sampling sites are good. High endocrine hazard was observed at four sites and high genotoxicity hazard at two sites (Attachment B, Table B-4a). However, three of the four sites with high endocrine hazard from DEET and/or venlafaxine exposure (PRGAM-11, DPERE-9 and FXR-1) and the two sites with high genotoxicity hazard from ibuprofen exposure were sampled only 1 to 3 times, so repeatability of observed elevated hazard is unknown. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is relatively high, based on a robust USEPA STORET water quality dataset with a large number of sampling sites that are well-distributed across the project location (Figure A2-4a in Attachment A2).

- *Ecotoxicity:* Among observations of high hazard for ibuprofen, DEET and venlafaxine, breadth of ecotoxicity information was as follows (see Tables 4-5 and 4-6):
    - o Ibuprofen
        Sparse – Genotoxicity
    - o DEET
        Moderate – Endocrine
    - o Venlafaxine
        Sparse - Endocrine

5.4.4.4 Fox River/Green Bay Point Source Analysis

Statistically significantly higher maximum hazard score ($p < 0.1$) was observed in the point source CEC-influenced site group compared to the uninfluenced site group in six comprehensive effect categories and four population-relevant effect categories, for carbamazepine, HHCB, or venlafaxine, with seven of 12 significant differences attributed to venlafaxine. The qualitative analysis shows that SV_{LOW} exceedances likely drove the observations of significant differences in maximum hazard.

In this section, we provide results of quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (methods in Section 5.3.4); the following chart provides the statistical results summary.

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
Carbamazepine	Circulatory/Blood Constituents	Y	N
	Neurological	Y	N
	Physiological/Metabolic	Y	N
HHCB	Physiological/Metabolic	Y	Y
Venlafaxine	Comprehensive mean SV	Y	N
	Population-relevant mean SV	Y	N
	Behavioral	Y	N
	Endocrine	Y	N
	Histopathology	Y	N
	Mortality	Y	N
	Reproductive	Y	N

When *all* hazard scores for each site in the Fox River/ Green Bay location, not just maximum or median scores, are included in the statistical analysis[46], an additional significant difference ($p < 0.1$) is observed for histopathology effects associated with carbamazepine exposure (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Fox River/Green Bay location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Fox River/ Green Bay Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	5	0.18 (4/22)	0.38 (3/8)	253	1.0 (22/22)	1.0 (8/8)
Uninfluenced Sites	2	0.13 (2/15)	0.29 (2/7)	84	1.0 (15/15)	1.0 (7/7)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

While the chart indicates that the numbers of affected samples and sites appeared little different between the two site groups, the greater gross numbers of exceedances at CEC-influenced sites indicated greater hazard than uninfluenced sites. There were five SV_{HIGH} exceedances in the CEC-influenced site group from DEET, ibuprofen and venlafaxine, and two in the uninfluenced site group. There was a total of 253 exceedances of various effect-specific SV_{LOW} values among the eight CEC-influenced sites, implicating 11 of the 14 CECs. In contrast, the total number of SV_{LOW} exceedances among the seven uninfluenced sites was 84.

The occurrence of SV_{HIGH} exceedances at EASTR-10 and PRGAM-11, two sites we had identified as "uninfluenced" based on distance from mapped CEC point sources, may be due to unmapped point sources just upstream of these sites. This is plausible since both sites are in the urban lower Fox River. The SV_{HIGH} for DEET endocrine hazard was exceeded in both point source influenced and uninfluenced sites and SV_{LOW} exceedances were widespread, suggesting aerial deposition, which could explain why DEET-related endocrine hazard was not statistically different between groups.

[46]Cautionary caveats regarding this approach are described in Attachment D.

5.4.4.5 Fox River/Green Bay Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Endocrine Hazard

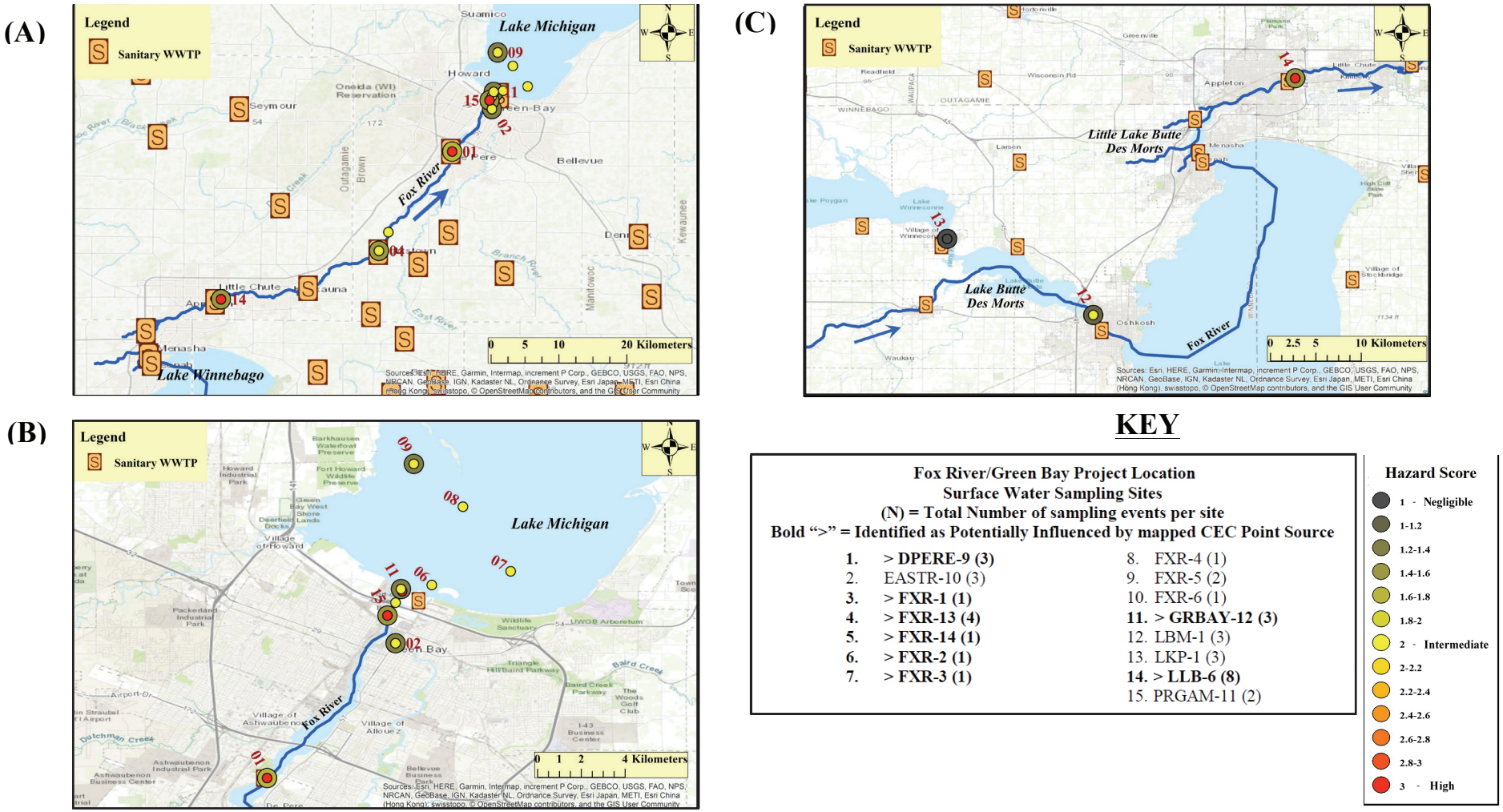
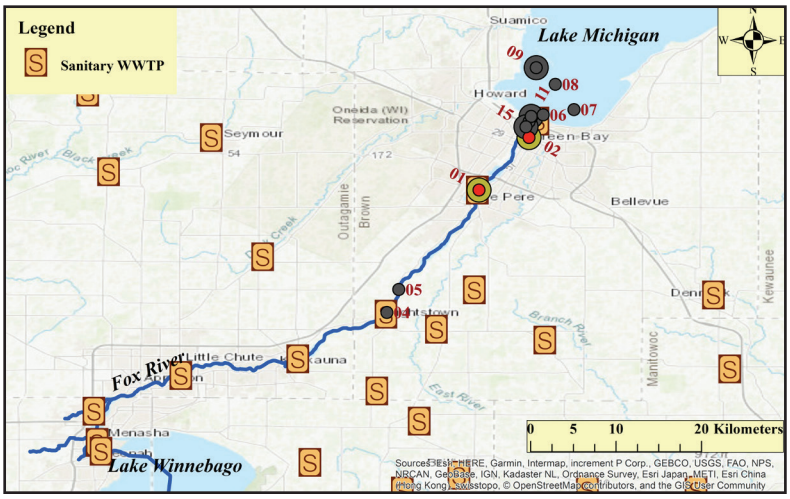


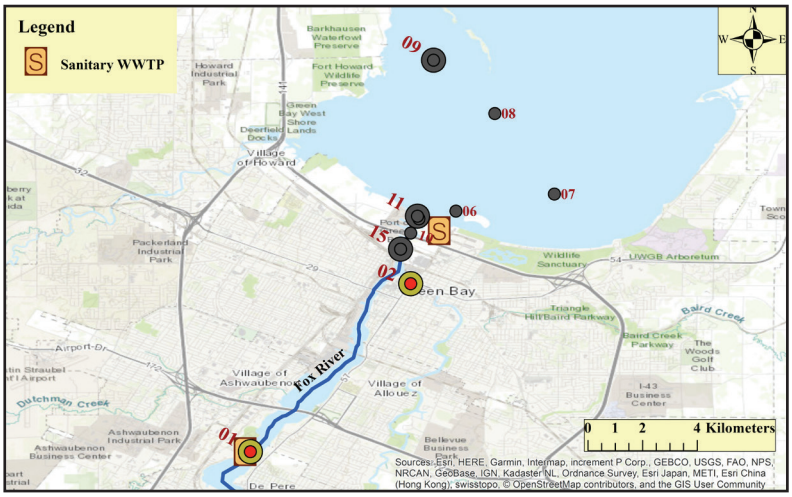
Figure 5-15. Endocrine Hazard Maps for the Fox River and Green Bay. (A) Entire project location; (B) Lower River; (C) Upper River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

Genotoxicity Hazard

(A)



(B)



KEY

Fox River/Green Bay Project Location
Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. > DPERE-9 (3)	8. FXR-4 (1)
2. EASTR-10 (3)	9. FXR-5 (2)
3. > FXR-1 (1)	10. FXR-6 (1)
4. > FXR-13 (4)	**11. > GRBAY-12 (3)**
5. > FXR-14 (1)	12. LBM-1 (3)
6. > FXR-2 (1)	13. LKP-1 (3)
7. > FXR-3 (1)	**14. > LLB-6 (8)**
	15. PRGAM-11 (2)

Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

Figure 5-16. Genotoxicity Hazard Maps for the Fox River and Green Bay. (A) Entire project location; (B) Lower River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. No genotoxicity hazard scores were available for the upper river. Arrows indicate river flow direction.

5.4.5 Kewaunee River (WI)

The Kewaunee River flows approximately 45 km (28 mi), west to east, along the southern end of the Kewaunee Peninsula in Lake Michigan. Three of the five sampling sites were designated as CEC-'influenced', and two 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Sites were sampled a total of between three and five times each, during the following sampling events: May 2013, and April and August 2014.

Observations of elevated hazard in the Kewaunee River are distributed throughout the project location, providing some evidence of hazards to fish. While high hazard is limited to two observations of endocrine hazard from DEET exposure, low hazard is pervasive and varied, largely attributed to carbamazepine, estrone, β-sitosterol and venlafaxine in 9 of 12 effect categories. There was a total of 128 SV_{LOW} exceedances at this location.

Among effect categories, endocrine, histopathology and circulatory/blood constituent hazards ranked highest, while hazards to fish from exposure to β-sitosterol and DEET ranked highest among the CECs considered in this EHA. The downstream-most CEC sampling site ranked highest among sampling sites for overall hazard to fish (Section 5.4.5.2). The strength of ecotoxicity information associated with observations of high hazard is moderate, but limitations in exposure data may significantly constrain our overall confidence in hazard results at this project location.

There were no statistically significant differences in maximum or median hazard scores between CEC sampling sites designated as CEC-influenced and sites considered to be uninfluenced. The statistical result is consistent with a low number of high hazard observations throughout the project location. However, there was a five-fold increase in SV_{LOW} exceedances at CEC-influenced sites compared to uninfluenced sites.

5.4.5.1 Hazard Brief for Kewaunee River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

Kewaunee River (WI)

- **Overall:** Some evidence of hazards to fish
- **High Hazard**:
  - 2 occurrences involving 11% of sampling events and 40% of sites
  - CECs: DEET
  - *Effect Categories*: Endocrine
- **Low Hazard**:
  - 128 occurrences involving 89% of sampling events and 100% of sites
  - CECs: 7 of 13 (no Ibuprofen data)
  - *Effect Categories*: 9 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure Dataset**: Substantial
  - 5 sites
  - 3 to 5 samples per site
  - 19 total samples

Effect Categories and Emerging Contaminants

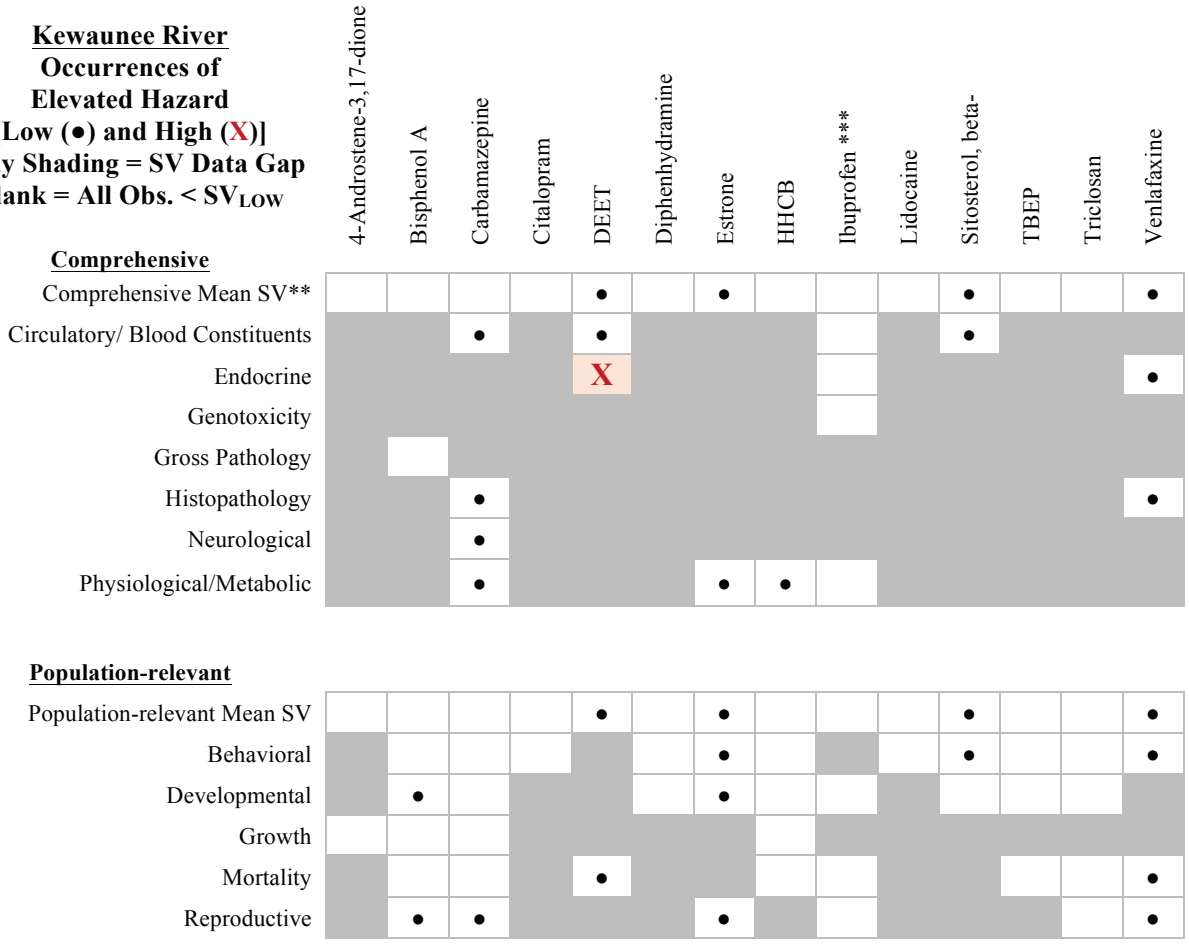
An effect-specific hazard map is provided only for the endocrine hazard category at the Kewaunee River location (Section 5.4.5.5), where relatively high hazard was observed in at least one sample. At least one SV_{LOW} value was exceeded in the following additional effect categories (Table 5-1):

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/ Metabolic.

DEET accounted for each of the two observations of high endocrine hazard, one at a site designated as CEC-influenced and the other at an uninfluenced site. Exceedances of SV_{LOW} values were attributed to DEET as well as to bisphenol A, carbamazepine, estrone, HHCB, β-sitosterol and venlafaxine.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-5.

Kewaunee River
Occurrences of
Elevated Hazard
[Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

The two observations of DEET endocrine high hazard occurred at two middle reach sites, one designated as influenced by CEC point sources and the other uninfluenced.

Kewaunee River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. KWE-1 (3)
2. **> KWE-2 (3)**
3. **> KWE-3 (5)**
4. KWE-4 (3)
5. **> KWE-5 (5)**

5.4.5.2 Hazard Rankings for Kewaunee River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Observations falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[47]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Kewaunee River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.273	1.2-1.4	2
Histopathology	1.220	1.2-1.4	2
Circulatory/Blood Constituents	1.209	1.2-1.4	2
Physiological/Metabolic	1.164	1.0-1.2	1
Reproductive	1.145	1.0-1.2	1
Neurological	1.080	1.0-1.2	1
Behavioral	1.075	1.0-1.2	1
Mortality	1.063	1.0-1.2	1
Comprehensive Mean SV	1.054	1.0-1.2	1
Developmental	1.046	1.0-1.2	1
Population-relevant Mean SV	1.042	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants[48]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Kewaunee River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Sitosterol, beta-	1.251	1.2-1.4	2
DEET	1.232	1.2-1.4	2
Estrone	1.187	1.0-1.2	1
Venlafaxine	1.137	1.0-1.2	1
Carbamazepine	1.065	1.0-1.2	1
Bisphenol A	1.045	1.0-1.2	1
HHCB	1.032	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

[47]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[48] Ibuprofen was not included in the exposure database for this project location

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Kewaunee River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> KWE-5	1.189	1.0-1.2	1
> KWE-3	1.071	1.0-1.2	1
KWE-4	1.059	1.0-1.2	1
> KWE-2	1.046	1.0-1.2	1
KWE-1	1.032	1.0-1.2	1

5.4.5.3. Breadth of Information Indicating High Hazard at Kewaunee River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Two observations of high endocrine hazard occurred at two different sites. Coverage of the river by sampling sites is fair; greater than 10 km separates the two sampling sites where the greatest potential for impact to fish was observed. Strength of evidence is moderate for one site downstream of a WWTP (KWE-3), which was sampled five times in spring and summer at various times of day over two years. The other site with high endocrine hazard (KWE-4) is far downstream of the nearest point source and was sampled only three times, so repeatability of observed elevated hazard is unknown. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is moderate to low. A relatively small USEPA STORET water quality dataset with a small number of sampling sites is clustered in the downstream half of the river (Figure A2-5 in Attachment A2). If TSS in the upper river differs appreciably from the lower river, then the conversion of CEC concentration data collected at downstream CEC sampling sites may be more accurate than at upstream CEC sampling sites.

- *Ecotoxicity*: The breadth of ecotoxicity information for the DEET endocrine SVs is moderate (Table 4-6).

5.4.5.4 Kewaunee River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (methods provided in Section 5.3.4). There were no statistically significant differences ($\alpha = 0.10$) between site groups, whether site maxima or site medians were compared – possibly due to small numbers of sampling sites in each group. However, when all hazard scores for each site in the Kewaunee River location - not just maximum or median scores - are included in the statistical analysis[49], significant differences ($p < 0.1$) are observed for reproductive and histopathology effects associated with carbamazepine exposure (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Kewaunee River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups. These qualitative results indicate essentially no difference between point source CEC-influenced and uninfluenced site groups with respect to occurrence of SV_{HIGH} or SV_{LOW} exceedances.

[49]Cautionary caveats regarding this approach are described in Attachment D.

Kewaunee River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	1	0.08 (1/13)	0.33 (1/3)	108	0.85 (11/13)	1.0 (3/3)
Uninfluenced Sites	1	0.17 (1/6)	0.5 (1/2)	20	1.0 (6/6)	1.0 (2/2)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There was one exceedance of a DEET endocrine SV_{HIGH} in each of the site groups. The occurrence of a DEET SV_{HIGH} exceedance at "uninfluenced" KWE-4 may be due to an unmapped point source just upstream of that site or because DEET may have been aerially deposited relatively heavily across the Kewaunee River project location.

Among the three CEC-influenced sites, there was a total of 108 exceedances of various effect-specific SV_{LOW} values for bisphenol A, carbamazepine, DEET, estrone, HHCB, β-sitosterol and venlafaxine, whereas, at the two uninfluenced sites there were only 20 SV_{LOW} exceedances attributed to bisphenol A, DEET, HHCB and β-sitosterol. The most upstream site (KWE-1) and the other site designated as uninfluenced had one SV_{LOW} exceedance each for β-sitosterol (a plant sterol), bisphenol-A and DEET.

5.4.5.5 Kewaunee River Hazard Map

In this section, we provide a hazard map for the one effect category with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Endocrine Hazard

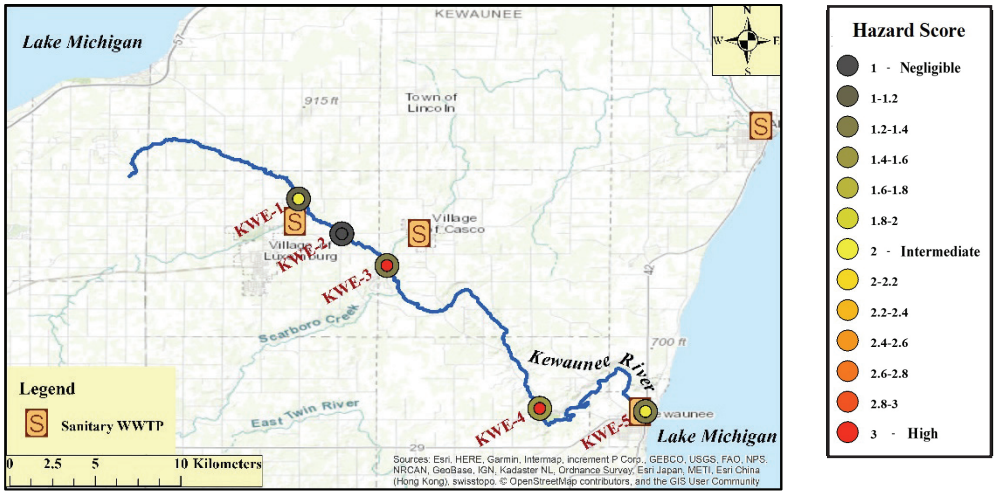


Figure 5-17. Endocrine Hazard Map for the Kewaunee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

5.4.6 Milwaukee River (WI)

This urban project location includes the lowermost reaches and confluence of the Menomonee River (116 mi; 187 km), Kinnikinnic River (22 mi; 35 km), and Milwaukee River (104 mi; 167 km) in the city of Milwaukee, WI, and a Lake Michigan sampling site immediately adjacent to the mouth of the watershed. All four sampling sites were designated as CEC-'influenced' based on their proximity to mapped CEC point sources, and all four sites were sampled twice during June 2011 to measure surface water CEC concentrations.

Elevated hazard was observed at all four CEC sampling sites in this watershed river network; no uninfluenced upstream sites were available to contrast against the substantial evidence of hazard to fish at this location. Ibuprofen accounted for all observations of developmental, reproductive, and genotoxicity high hazard at this project location, with DEET and venlafaxine contributing to high endocrine hazard. Seven additional CECs contributed to SV_{LOW} exceedances in eight additional effect categories. There were 12 high hazard observations (SV_{HIGH} exceedances) and 106 SV_{LOW} exceedances, affecting all four sampling sites and all eight sampling events.

Comprehensive effect categories accounted for seven of the eight highest ranked effect-specific hazards. Although accounting for few high hazard observations, DEET ranked well above the next highest ranked CECs, which were estrone, β-sitosterol, venlafaxine and citalopram. A sampling site in the near shore of Lake Michigan near the river mouth (JINSLA-15), which is proximate to a major WWTP discharge, ranked higher for mean hazard to fish than the other three sites. Substantial evidence of CEC-related hazard was observed despite a limited exposure dataset, and confidence in the ecotoxicity information associated with observations of high hazard from ibuprofen exposure is high, from DEET exposure is moderate, and from venlafaxine exposure is low.

No statistical analysis of hazard associated with point sources was conducted, since all sampling sites had been designated as point source CEC-influenced.

Some Key Points…

Milwaukee River (WI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard**:
  - 12 occurrences involving 100% of sampling events and sites
  - *CECs:* DEET, Ibuprofen, Venlafaxine
  - *Effect Categories*: Reproductive, Developmental, Endocrine, Genotoxicity
- **Low Hazard**:
  - 106 occurrences involving 100% of sampling events and sites
  - *CECs:* 10 of 14
  - *Effect Categories*: 10 of 12
- **Point Source Analysis:** No evaluation; all sites were considered influenced by CECs
- **Exposure Dataset**: Limited
  - 4 sites
  - 2 samples per site
  - 8 total samples

5.4.6.1 Hazard Brief for Milwaukee River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants
Milwaukee River effect-specific hazard maps (Section 5.4.6.5) are provided for the following effect categories for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

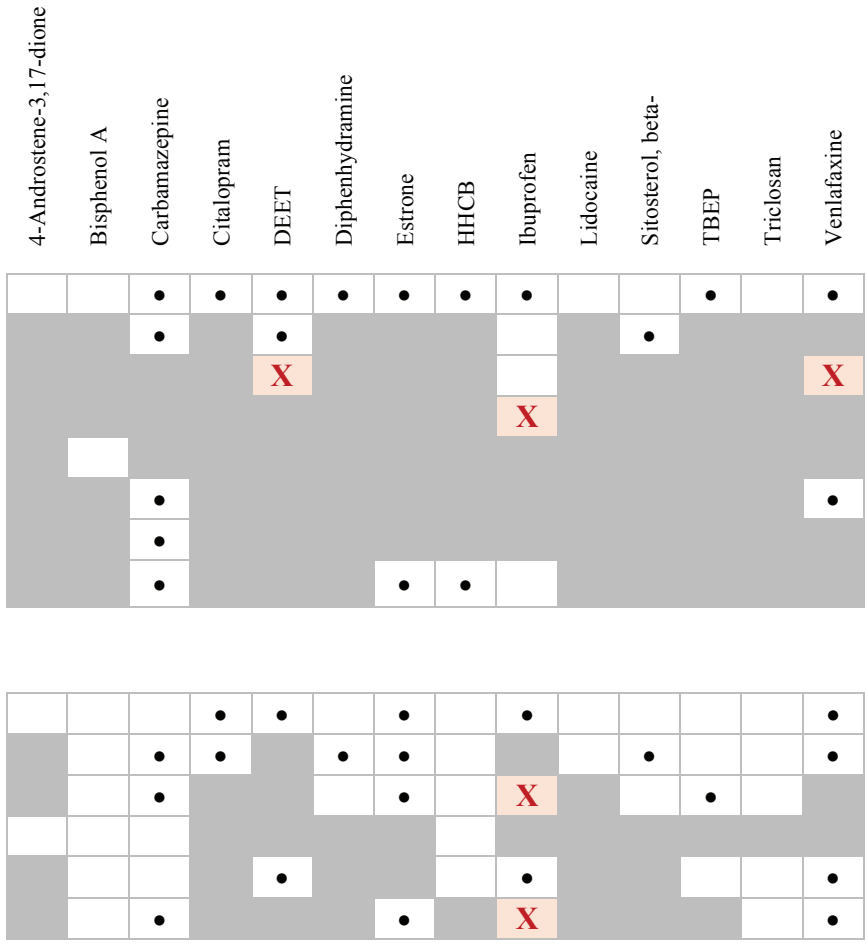
- Population-relevant: Developmental, Reproductive
- Comprehensive: Endocrine, Genotoxicity

Exceedances of at least one SV_{LOW} were observed in additional effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Mortality
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/Metabolic.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-6.

Milwaukee River
Occurrences of
Elevated Hazard
[Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive Mean SV values used in this EHA.

Sampling Sites

High hazard was observed in all four sampling sites at the Milwaukee River location, despite only two samples analyzed at each site. All four sites are influenced by mapped CEC point sources. More than half of the 57 observations (identified by unique combinations of sampling site-effect category-CEC) of SV_{LOW} exceedances occurred at JISLA-15, near the WWTP discharge into Lake Michigan, adjacent to the mouth of the river system.

Milwaukee River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> JISLA-15 (2)**
2. **> KINI-17 (2)**
3. **> MENMR-13 (2)**
4. **> MILWR-14 (2)**

5.4.6.2 Hazard Rankings for Milwaukee River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Milwaukee River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.792	1.6-1.8	4
Circulatory/Blood Constituents	1.313	1.2-1.4	2
Genotoxicity	1.250	1.2-1.4	2
Histopathology	1.250	1.2-1.4	2
Neurological	1.250	1.2-1.4	2
Physiological/Metabolic	1.219	1.2-1.4	2
Behavioral	1.216	1.2-1.4	2
Comprehensive Mean SV	1.205	1.2-1.4	2
Reproductive	1.188	1.0-1.2	1
Mortality	1.172	1.0-1.2	1
Population-relevant Mean SV	1.143	1.0-1.2	1
Developmental	1.111	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Milwaukee River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	2.075	2.0-2.2	6
Estrone	1.375	1.2-1.4	2
Sitosterol, beta-	1.300	1.2-1.4	2
Venlafaxine	1.286	1.2-1.4	2
Citalopram	1.250	1.2-1.4	2
Carbamazepine	1.182	1.0-1.2	1
Diphenhydramine	1.125	1.0-1.2	1
Ibuprofen	1.125	1.0-1.2	1
TBEP	1.075	1.0-1.2	1
HHCB	1.054	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Milwaukee River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> JISLA-15	1.427	1.4-1.6	3
> KINNI-17	1.146	1.0-1.2	1
> MENMR-13	1.085	1.0-1.2	1
> MILWR-14	1.134	1.0-1.2	1

5.4.6.3. Breadth of Information Indicating High Hazard at Milwaukee River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Spatial coverage of the urban Milwaukee area was adequate, with one sampling site in each of the three converging rivers and one at the mouth of the river at Lake Michigan. However, sample size was only two at each site so repeatability of high hazard observations is unknown. No uninfluenced sites were included from any of the rivers to serve as reference. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the TSS data supporting the concentration conversion is high. A relatively robust USEPA STORET water quality dataset is available from numerous water quality sampling sites clustered in the lower rivers (Figure A2-6 in Attachment A2).

- *Ecotoxicity*: Among observations of high hazard for ibuprofen, DEET and venlafaxine, breadth of applicable ecotoxicity information was as follows (Tables 4-5 and 4-6):

  - Ibuprofen
    - Broad - Developmental
    - Broad – Reproductive
    - Sparse - Genotoxicity
  - DEET
    - Moderate - Endocrine
  - Venlafaxine
    - Sparse - Endocrine

5.4.6.4 Milwaukee River Point Source Analysis

All four sampling sites were identified as point source CEC-influenced, so no comparison of hazard between CEC-influenced sites and uninfluenced sites was possible.

A summary of hazard occurrence at the four point source CEC-influenced sites is provided in the chart below. Shown are fractions of samples and of sites at the Milwaukee River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having at least one SV_{LOW} exceedance.

All samples and all sites had at least one SV_{HIGH} exceedance and at least one SV_{LOW} exceedance. There was a total of 12 exceedances of various effect-specific SV_{HIGH} values attributed to DEET, ibuprofen and venlafaxine, and a total of 106 SV_{LOW} exceedances attributed to eight of the 14 CECs considered in this EHA.

Milwaukee River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	12	1.0 (8/8)	1.0 (4/4)	106	1.0 (8/8)	1.0 (4/4)
Uninfluenced Sites	NA	NA	NA	NA	NA	NA

* Excluding exceedances of SVs in the c*omprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.6.5 Milwaukee River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Developmental Hazard

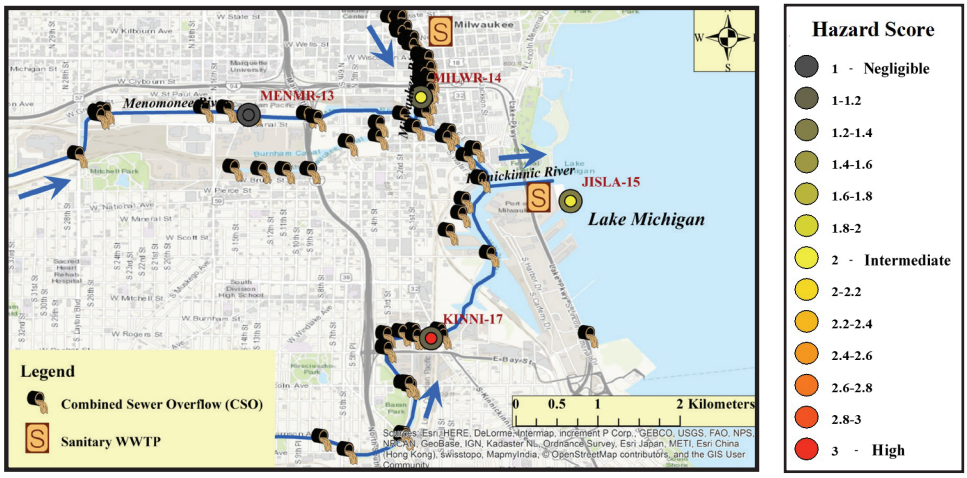


Figure 5-18. Developmental Hazard Map for the Milwaukee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Reproductive Hazard

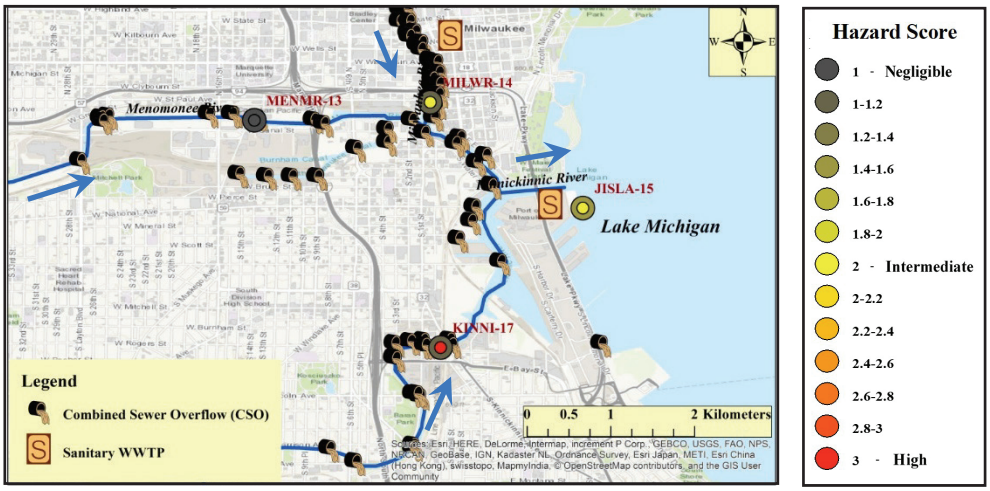


Figure 5-19. Reproductive Hazard Map for the Milwaukee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Endocrine Hazard

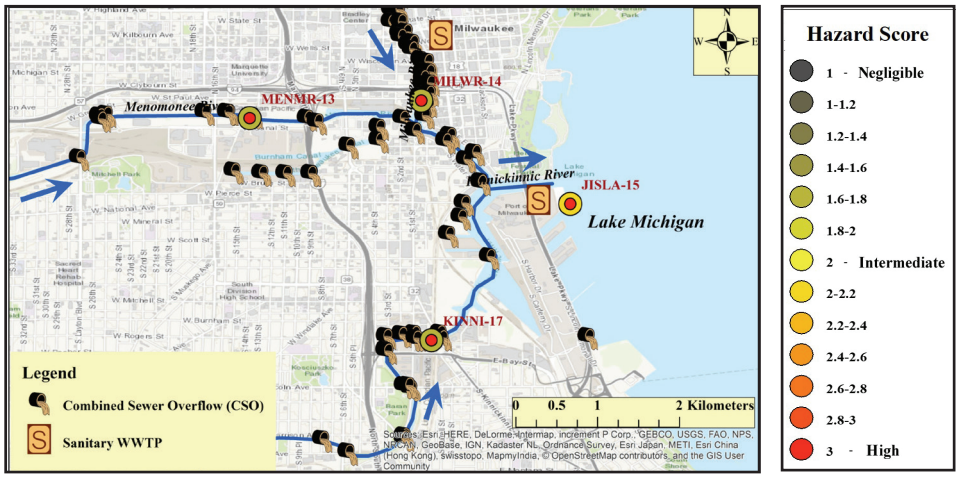


Figure 5-20. Endocrine Hazard Map for the Milwaukee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Genotoxicity Hazard

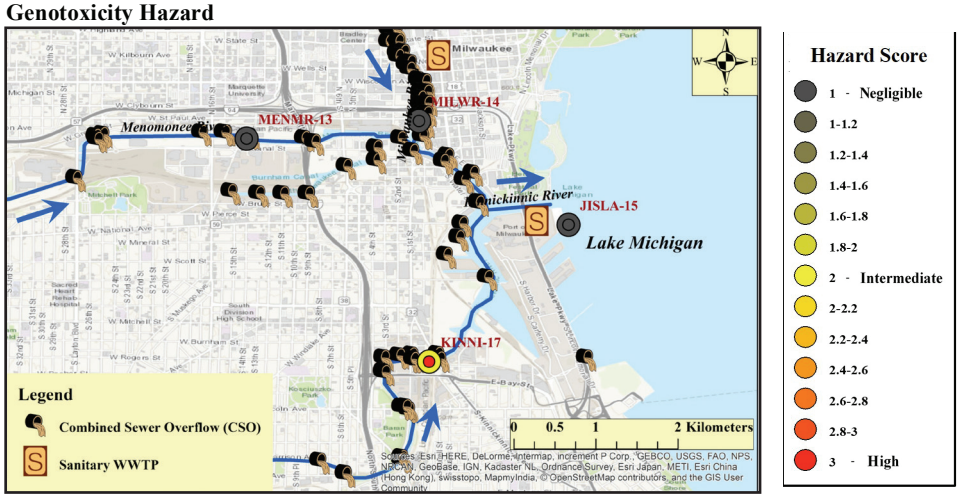


Figure 5-21. Genotoxicity Hazard Map for the Milwaukee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

5.4.7 North Shore Channel (IL)

The North Shore Channel is a sanitary and shipping channel in the city of Chicago, IL, that flows approximately 12 km (7.5 mi) from Lake Michigan to the Chicago River. All three sampling sites were designated as CEC-'influenced', based on their proximity to several mapped CEC point sources. Sites were sampled for surface water CEC concentration measurements a total of three or six times during the months of April, May and September 2014.

This EHA provides clear and compelling evidence indicating that fish in the North Shore Channel project location are impacted by CECs. High hazards (SV_{HIGH} exceedances) were associated with five effect categories and four CECs, including high mortality hazard from venlafaxine exposure. There was a total of 43 SV_{HIGH} exceedances and 475 SV_{LOW} exceedances, affecting all three sampling sites and all sampling events. SV_{LOW} exceedances were observed for every effect category except genotoxicity, and for each of the 13 CECs with exposure information[50] except lidocaine.

Among effect categories, six of the top seven hazard score ranks (based on mean hazard scores) were attributed to comprehensive effect categories, with ranks ranging from '4' (on a scale of 1 to 10) to a rank of '8' for endocrine effects - the highest hazard rank achieved for any effect category in this EHA. Venlafaxine and estrone ranked highest among CECs, followed by DEET, carbamazepine, triclosan and HHCB.

All three sampling sites are point source CEC-influenced, so no statistical comparison with uninfluenced sites is possible. Although no uninfluenced upstream sites were available to contrast against the solid evidence of hazard to fish at the North Shore Channel, the upstream most sampling site (CHI-112), which is upstream of the WWTP, was ranked lower for overall hazard than the other two sampling sites which received effluent from the WWTP and several additional CSOs.

Due to data gaps in the literature, the ecotoxicity information incorporated into SVs associated with observations of high hazard was generally 'sparse' to 'limited', except for moderate breadth of evidence incorporated into DEET endocrine SVs, and confidence in exposure information is moderate. However, the collective evidence of elevated hazard in multiple effect categories associated with several CECs at all sampled sites – despite ecotoxicity data gaps – provides strong confidence in the conclusion that fish are impacted at this location.

5.4.7.1 Hazard Brief for North Shore Channel

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

North Shore Channel (IL)

- **Overall:** Clear and convincing evidence of CEC-related hazards to fish
- **High Hazard**:
  - 43 occurrences, involving 92% of sampling events, and 100% of sites
  - *CECs:* Venlafaxine, Estrone, DEET, Carbamazepine
  - *Effect Categories*: Mortality, Endocrine, Histopathology, Physiological/Metabolic
- **Low Hazard Observations**:
  - 475 occurrences involving 100% of sampling events and sites
  - *CECs:* 12 of 13 (no Ibuprofen data)
  - *Effect Categories*: 11 of 12
- **Point Source Analysis:** No evaluation; all sites are CEC-influenced
- **Exposure Dataset**: Substantial, considering the size of the location
  - 3 sites
  - 3 to 6 samples per site
  - 12 total samples

Effect Categories and Emerging Contaminants

North Shore Channel effect-specific hazard maps (Section 5.4.7.5) are provided for the following effect categories, for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Mortality
- Comprehensive: Comprehensive mean SV, Endocrine, Histopathology, Physiological/ Metabolic.

Exceedances of SV_{LOW} values occurred in additional Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Growth, Reproductive
- Comprehensive: Circulatory/Blood Constituents, Gross Pathology, Neurological.

Among CECs evaluated, venlafaxine accounted for most of the observations of high hazard at the North Shore Channel project location, with DEET, carbamazepine and estrone also contributing. Concentrations of eight additional CECs exceeded at least one SV_{LOW} value. No screening value was exceeded by concentrations of ibuprofen or lidocaine.

The following chart indicates CECs and Effect Categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-7.

[50]No water concentration data for ibuprofen was available at this location.

North Shore Channel
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●	●	●	●	●	●	X	●			●	●	●	X
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									X
Genotoxicity														
Gross Pathology		●												
Histopathology			X											X
Neurological			●											
Physiological/Metabolic			●				X	●						
Population-relevant														
Population-relevant Mean SV	●	●	●	●	●	●	●	●			●	●	●	●
Behavioral			●	●		●	●	●			●		●	●
Developmental		●	●			●	●	●				●	●	
Growth		●						●						
Mortality					●			●				●	●	X
Reproductive		●	●				●						●	●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

High hazard was observed at all three sampling sites at North Shore Channel location, with CHI-36 accounting for eight of the 17 observations. Although all three sites are downstream of CEC point sources, CHI-36 is the furthest downstream of the three sites and is influenced by the most point sources including the WWTP. The site furthest upstream, CHI-112, is influenced by numerous CSOs but not the WWTP and accounts for only three of 17 observations of high hazard.

The following are mapped North Shore Channel sampling sites evaluated for hazard to fish:

North Shore Channel Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> CHI-112 (3)**
2. **> CHI-36 (3)**
3. **> CHI-RP4 (6)**

5.4.7.2 Hazard Rankings for North Shore Channel

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[51]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	North Shore Channel Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	2.583	2.4-2.6	8
Physiological/Metabolic	2.148	2.0-2.2	6
Histopathology	2.083	2.0-2.2	6
Neurological	1.889	1.8-2.0	5
Circulatory/Blood Constituents	1.704	1.6-1.8	4
Reproductive	1.700	1.6-1.8	4
Comprehensive Mean SV	1.654	1.6-1.8	4
Mortality	1.571	1.4-1.6	3
Developmental	1.528	1.4-1.6	3
Population-relevant Mean SV	1.504	1.4-1.6	3
Behavioral	1.485	1.4-1.6	3
Growth	1.125	1.0-1.2	1
Gross Pathology	1.028	1.0-1.2	1

Emerging Contaminants[52]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	North Shore Channel CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Venlafaxine	2.103	2.0-2.2	6
Estrone	2.019	2.0-2.2	6
DEET	1.989	1.8-2.0	5
Carbamazepine	1.722	1.6-1.8	4
Triclosan	1.722	1.6-1.8	4
HHCB	1.667	1.6-1.8	4
Diphenhydramine	1.458	1.4-1.6	3
TBEP	1.433	1.4-1.6	3
4-Androstene-3,17-dione	1.426	1.4-1.6	3
Sitosterol, beta-	1.411	1.4-1.6	3
Citalopram	1.222	1.2-1.4	2
Bisphenol A	1.087	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

[51] Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[52] Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	North Shore Channel Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> CHI-36	1.708	1.6-1.8	4
> CHI-RP4	1.695	1.6-1.8	4
> CHI-112	1.422	1.4-1.6	3

5.4.7.3. Breadth of Information Indicating High Hazard at North Shore Channel

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* Spatial coverage of sampling sites in this urban site is adequate. Although there are only three sites, the North Shore Channel is not long and is lined fairly evenly with CEC point sources throughout its length (Figure A2-7 in Attachment A2). Sample sizes per site were low, but CEC-related hazard to fish clearly was evident nevertheless. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was moderate to high. Although there are only three USEPA STORET water quality sampling sites (Figure A2-7 in Attachment A2), this project location is not large and the TSS dataset includes more than 15 data points for 11 of 12 calendar months (Figure A-1b in Attachment A1).

- *Ecotoxicity:* Despite generally sparse ecotoxicity information used to derive the SVs, there were a number of observations of high hazard - for four CECs over five effect categories. Breadth of ecotoxicity information for the SVs was as follows (see Tables 4-5 and 4-6):
  - o Carbamazepine
    Sparse - Histopathology
  - o DEET
    Moderate – Endocrine
  - o Estrone
    Limited – Comprehensive mean SVs
    Sparse – Physiological/Metabolic
  - o Venlafaxine
    Limited – Comprehensive mean SVs
    Sparse – Endocrine
    Sparse – Histopathology
    Sparse – Mortality

5.4.7.4 North Shore Channel Point Source Analysis

All three sampling sites were identified as influenced by mapped point sources, so no CEC point source influenced-uninfluenced comparison was possible.

A summary of hazard occurrence at the three point source CEC-influenced sites is provided in the chart below. Shown are fractions of samples and of sites at the North Shore Channel location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having at least one SV_{LOW} exceedance.

All three sites and all but one sample had at least one SV_{HIGH} exceedance, and all samples and all three sites had at least one SV_{LOW} exceedance. There was a total of 43 exceedances of various effect-specific SV_{HIGH} values at the North Shore Channel location, attributed to carbamazepine, DEET, estrone and venlafaxine. There was a total of 475 exceedances of effect-specific SV_{LOW} values attributable to 12 of the 14 CECs.

North Shore Channel Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	43	0.92 (11/12)	1.0 (3/3)	475	1.0 (12/12)	1.0 (3/3)
Uninfluenced Sites	NA	NA	NA	NA	NA	NA

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.7.5 North Shore Channel Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Mortality Hazard

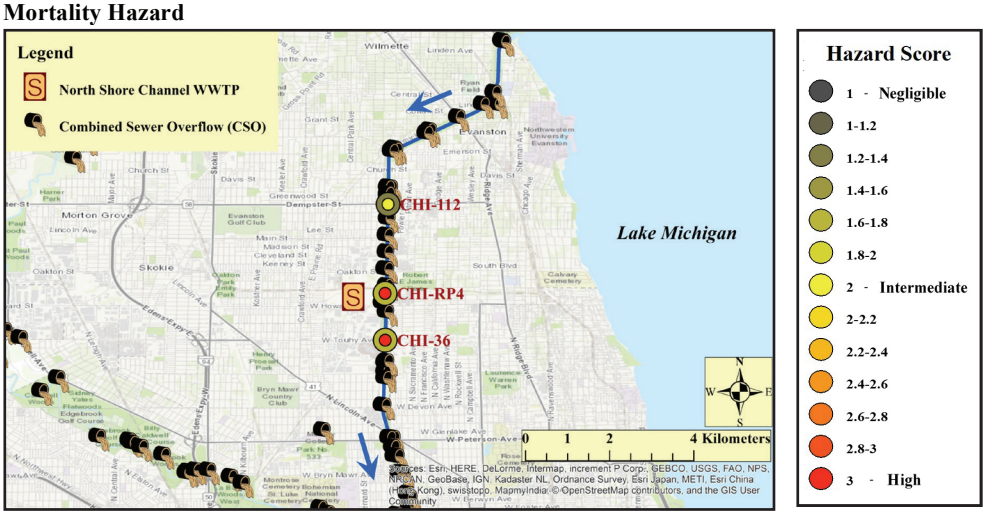


Figure 5-21. Mortality Hazard Map for the North Shore Channel. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Comprehensive Mean SV Hazard

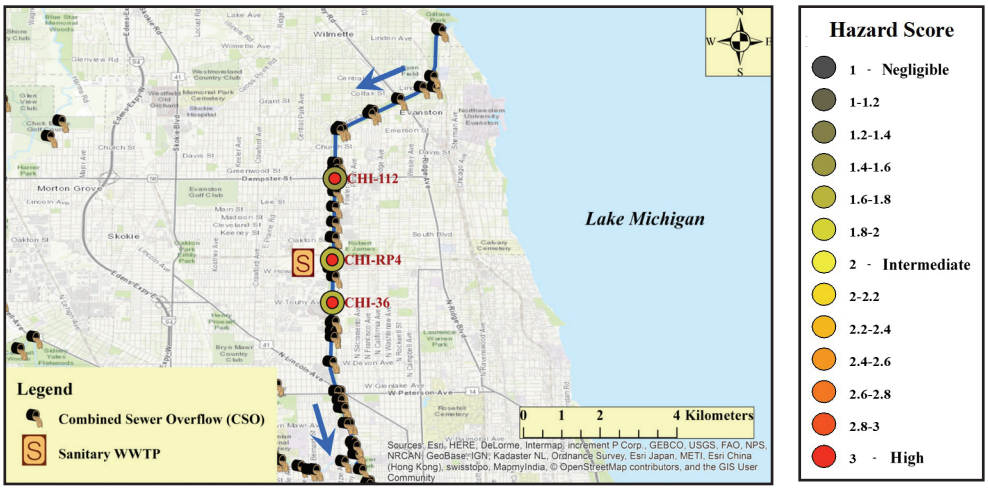


Figure 5-23. Comprehensive Mean SV Hazard Map for the North Shore Channel. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Endocrine Hazard

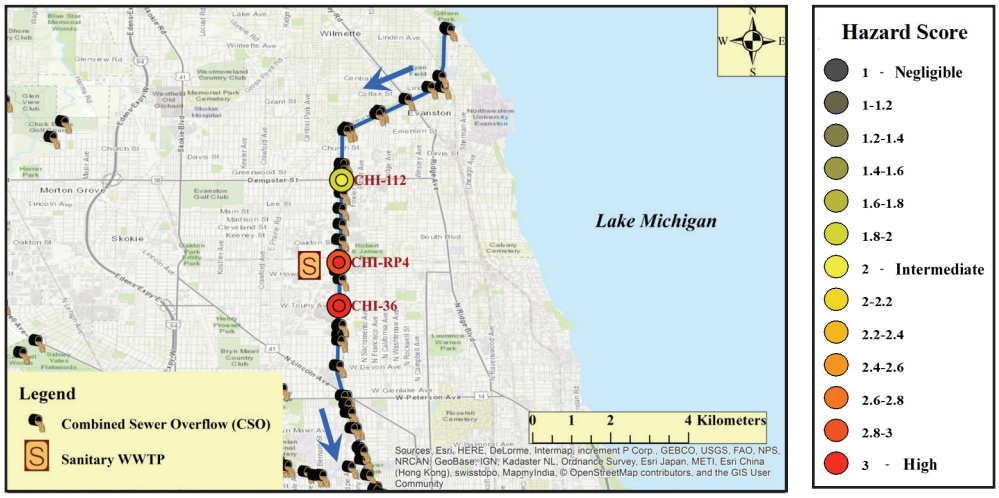


Figure 5-24. Endocrine Hazard Map for the North Shore Channel. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

Histopathology Hazard

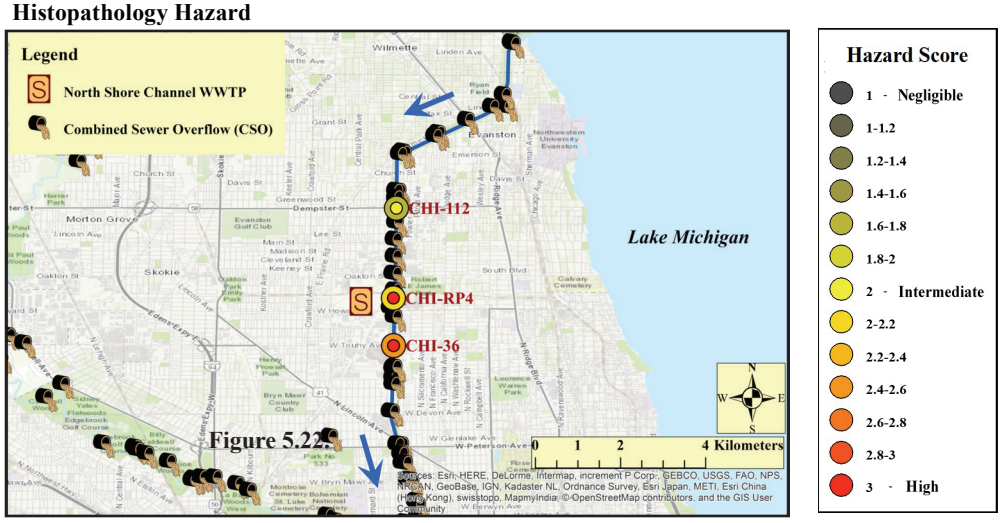


Figure 5.25. Histopathology Hazard Map for the North Shore Channel. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

Physiological/Metabolic Hazard

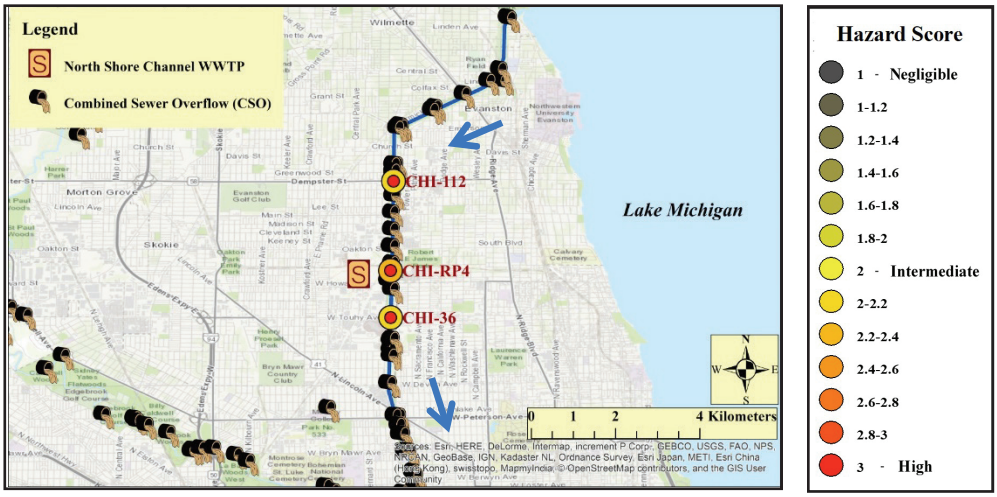


Figure 5-26. Physiological/Metabolic Hazard Map for the North Shore Channel. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

5.4.8 Little Calumet River (IL)

The Little Calumet River connects Lake Michigan with the Calumet-Saganashkee Channel in the city of Chicago, IL. All three sampling sites were designated as CEC-'influenced', based on their proximity to several mapped CEC point sources. Sites were sampled for surface water CEC concentration measurements a total of three or six times during the months of April, May and September 2014.

There is clear and compelling evidence indicating that fish in the Little Calumet River project location are impacted by CECs. High hazard (SV_{HIGH} exceedance) was observed in four effect categories associated with exposure to four CECs, while SV_{LOW} exceedances were observed in all effect categories except genotoxicity, and associated with all 13 CECs with exposure information - except lidocaine. There was a total of 25 SV_{HIGH} exceedances and 432 SV_{LOW} exceedances, affecting all sampling sites and all sampling events.

Among effect categories, five of the top six hazard score ranks (based on mean hazard scores) were attributed to comprehensive effect categories, with ranks ranging from '4' (on a scale of 1 to 10) to a rank of '8' for endocrine effects - the highest hazard rank achieved for any effect category in this EHA. Venlafaxine and DEET ranked highest among CECs, both of which were associated with high endocrine hazard, followed by estrone and carbamazepine.

The breadth of ecotoxicity information incorporated into SVs associated with observations of high hazard was generally 'sparse' to 'limited', except for moderate breadth of evidence incorporated into DEET endocrine SVs; confidence in exposure information is low. However, the collective evidence of elevated hazard in multiple effect categories associated with several CECs at all sampling sites – despite ecotoxicity data gaps - supports the conclusion that fish are impacted at this location. All three sampling sites are point source CEC-influenced, so no statistical comparison with uninfluenced sites is possible.

5.4.8.1 Hazard Brief for Little Calumet River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

Little Calumet River (IL)

- **Overall:** Clear and convincing evidence of CEC-related hazards to fish
- **High Hazard**:
  - 25 occurrences, involving 75% of sampling events and 67% of sites
  - *CECs:* DEET, Estrone, Venlafaxine
  - *Effect Categories*: Mortality, Endocrine, Physiological/Metabolic
- **Low Hazard**:
  - 432 occurrences, involving 100% of sampling events and sites
  - *CECs:* 12 of 13 (no Ibuprofen data available)
  - *Effect Categories*: 11 of 12
- **Point Source Analysis:** No evaluation; all sites are CEC-influenced
- **Exposure Dataset**: Substantial, considering the size of the location
  - 3 sites
  - 3 to 6 samples per site
  - 12 total samples

Effect Categories and Emerging Contaminants

Little Calumet River effect-specific hazard maps (Section 5.4.8.5) are provided for the following effect categories for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Mortality
- Comprehensive: Comprehensive mean SV, Endocrine, Physiological/Metabolic.

Venlafaxine, estrone, and DEET accounted for all eight observations of high potential for biological impacts in fish.

Exceedances of SV_{LOW} values occurred in additional Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Growth, Reproductive
- Comprehensive: Circulatory/Blood Constituents, Gross Pathology, Histopathology, Neurological.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-8.

Little Calumet River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●	●	●	●	●	●	X	●			●	●	●	●
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									X
Genotoxicity														
Gross Pathology		●												
Histopathology			●											●
Neurological			●											
Physiological/Metabolic			●				X	●						
Population-relevant														
Population-relevant Mean SV	●	●	●	●	●	●	●	●					●	●
Behavioral			●	●		●	●	●			●		●	●
Developmental		●	●				●	●				●	●	
Growth		●												
Mortality					●								●	X
Reproductive		●	●				●						●	●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

There were eight observations of high hazard at the Little Calumet River location, evenly distributed between CHI-76 and CHI-RP7. The following mapped Little Calumet River sampling sites were evaluated for hazard to fish:

Little Calumet River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> CHI-56 (3)**
2. **> CHI-76 (3)**
3. **> CHI-RP7 (6)**

5.4.8.2 Hazard Rankings for Little Calumet River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[53]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Little Calumet River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	2.556	2.4-2.6	8
Physiological/Metabolic	1.963	1.8-2.0	5
Histopathology	1.944	1.8-2.0	5
Neurological	1.889	1.8-2.0	5
Reproductive	1.711	1.6-1.8	4
Circulatory/Blood Constituents	1.648	1.6-1.8	4
Comprehensive Mean SV	1.560	1.4-1.6	3
Behavioral	1.414	1.4-1.6	3
Population-relevant Mean SV	1.376	1.2-1.4	2
Mortality	1.349	1.2-1.4	2
Developmental	1.347	1.2-1.4	2
Gross Pathology	1.306	1.2-1.4	2
Growth	1.125	1.0-1.2	1

Emerging Contaminants[54]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Little Calumet River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Venlafaxine	1.992	1.8-2.0	5
DEET	1.956	1.8-2.0	5
Estrone	1.667	1.6-1.8	4
Carbamazepine	1.646	1.6-1.8	4
Triclosan	1.500	1.4-1.6	3
Bisphenol A	1.406	1.4-1.6	3
Diphenhydramine	1.389	1.2-1.4	2
HHCB	1.373	1.2-1.4	2
Sitosterol, beta-	1.367	1.2-1.4	2
4-Androstene-3,17-dione	1.259	1.2-1.4	2
Citalopram	1.222	1.2-1.4	2
TBEP	1.100	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

[53]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[54]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Little Calumet River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> CHI-76	1.648	1.6-1.8	4
> CHI-RP7	1.622	1.6-1.8	4
> CHI-56	1.251	1.2-1.4	2

5.4.8.3. Breadth of Information Indicating High Hazard at Little Calumet River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* Spatial coverage of the Little Calumet River was limited, with all three sampling sites clustered within a 5 km stretch of the river (Figure A2-8 in Attachment A2). Temporal coverage was fair – one or two water samples were collected in three seasons during one year (Attachment A1, Table A-2). Despite the presence of numerous point sources, sample sizes were inadequate to detect high hazard at one of the three sites in this system, where all sampling sites are potentially influenced by CEC loadings were from intermittent CSO discharges. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was limited. Although there is only one USEPA STORET water quality sampling site with TSS data near the project location (Figure A2-8 in Attachment A2), that site had been sampled between five and seven times in each of 10 calendar months (Figure A-1b in Attachment A1).
- *Ecotoxicity:* Breadth of ecotoxicity information for DEET, estrone, and venlafaxine was as follows (see Tables 4-5 and 4-6):
  - o DEET
    Moderate - Endocrine
  - o Estrone
    Limited –Comprehensive mean SV
    Sparse - Physiological/Metabolic
  - o Venlafaxine
    Sparse - Endocrine
    Sparse - Mortality

5.4.8.4 Little Calumet River Point Source Analysis

All three sampling sites were considered CEC-'influenced', so no CEC point source influenced-uninfluenced comparison was possible.

A summary of hazard occurrence at the three point source CEC-influenced sites is provided in the chart below. Shown are fractions of samples and of sites at the Little Calumet River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having at least one SV_{LOW} exceedance.

Two of the three sites and nine of 12 samples had at least one SV_{HIGH} exceedance, while all samples and all three sites had at least one SV_{LOW} exceedance. There were 25 exceedances of Effect-specific SV_{HIGH} values for DEET, estrone and venlafaxine, and there were 432 exceedances of various Effect-specific SV_{LOW} values for 12 of the 14 CECs.

North Shore Channel Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	25	0.75 (9/12)	0.67 (2/3)	432	1.0 (12/12)	1.0 (3/3)
Uninfluenced Sites	NA	NA	NA	NA	NA	NA

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.8.5 Little Calumet River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Mortality Hazard

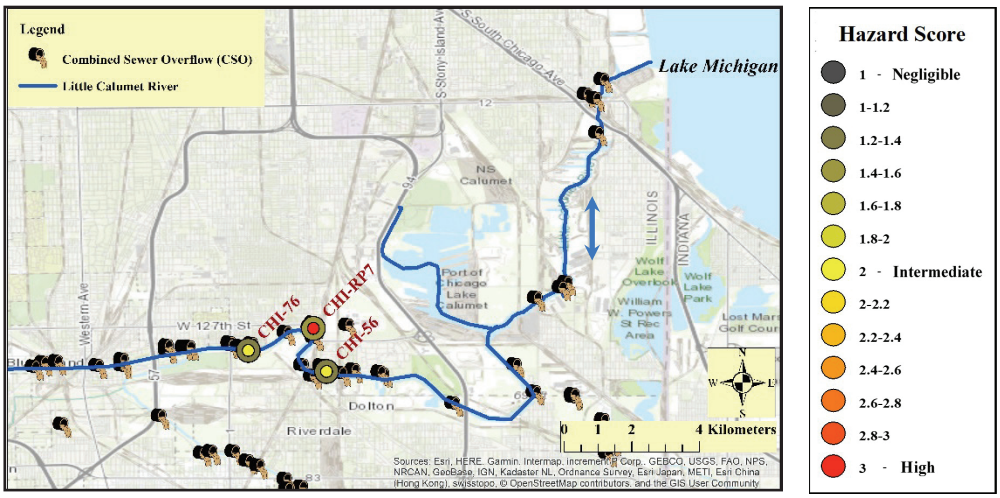


Figure 5-27. Mortality Hazard Map for the Little Calumet River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrow indicates bidirectional flow in this waterbody.

Comprehensive Mean SV Hazard

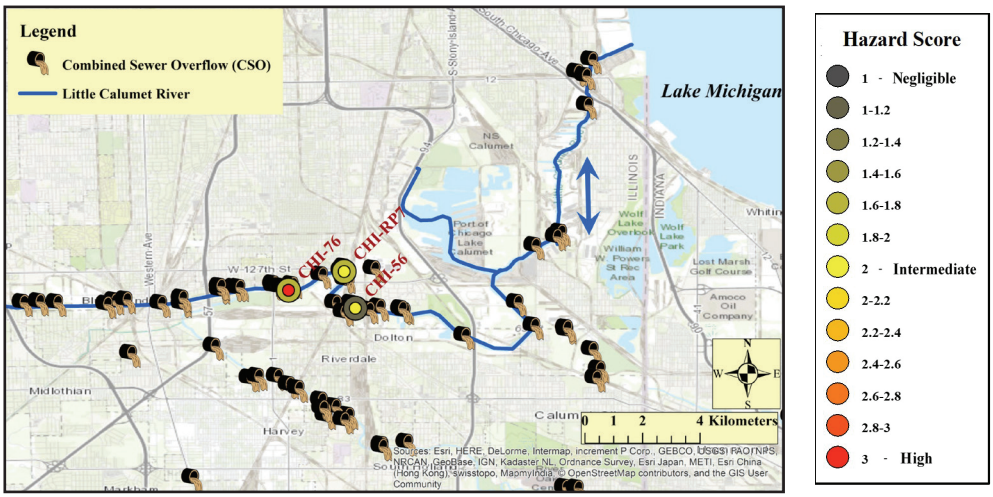


Figure 5-28. Comprehensive Mean SV Hazard Map for the Little Calumet River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrow indicates bidirectional flow in this waterbody.

Endocrine Hazard

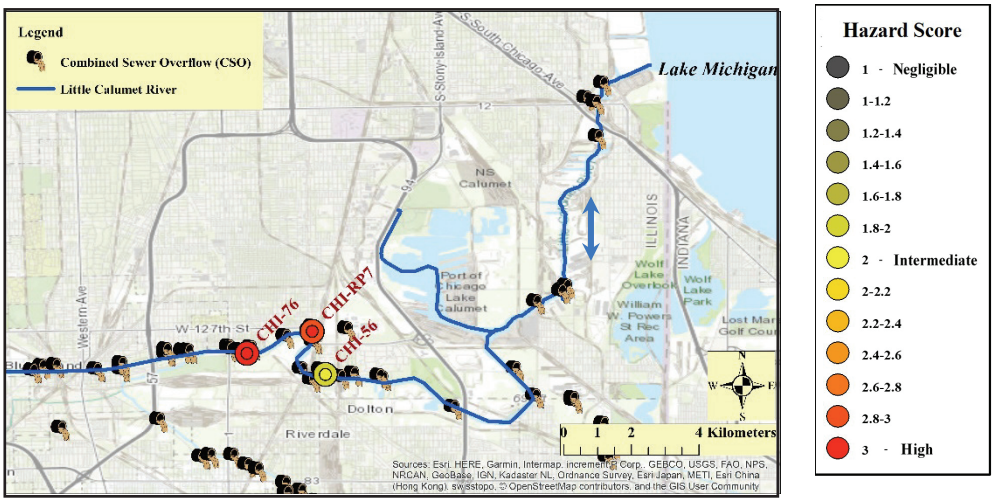


Figure 5-29. Endocrine Hazard Map for the Little Calumet River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrow indicates bidirectional flow in this waterbody.

Physiological/Metabolic Hazard

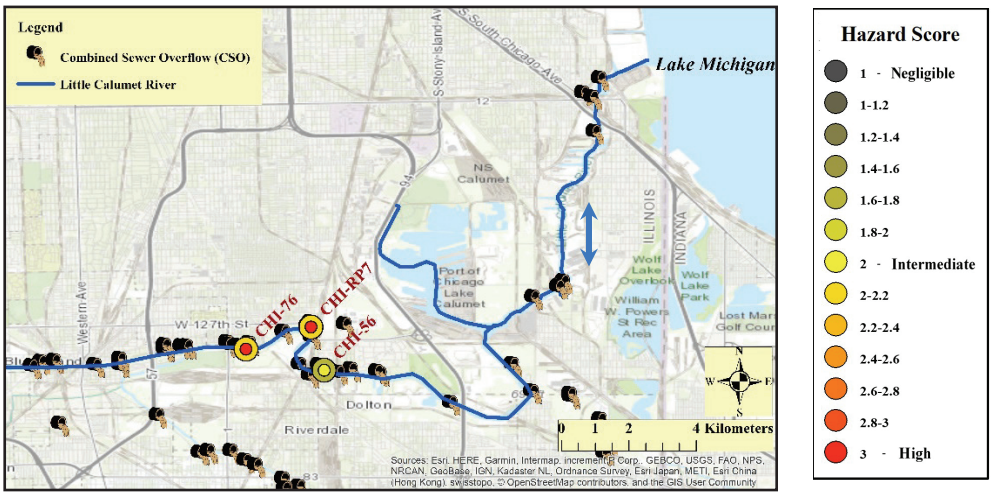


Figure 5-30. Physiological/Metabolic Hazard Map for the Little Calumet River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrow indicates bidirectional flow in this waterbody.

5.4.9 Grand River/Maple River (MI)

The Grand River flows westward, traversing several hundred kilometers across the lower peninsula of Michigan, draining a ~14,420 km^2 (5,570 mi^2) watershed to the east shore of Lake Michigan. The Maple River (74 mi; 119 km) is a major tributary of the Grand River. The project area extends over a 200 km (125 mi) middle river reach, where two of the six sampling sites were designated as CEC-'influenced' and four 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Sites were sampled for surface water CEC concentration measurements during two or three of the following sampling events: May 2013, April 2014 or August 2014.

There is substantial evidence that biological impacts to fish from CEC exposures occur at this project location. Elevated hazard (predominantly SV_{LOW} exceedances) was observed for 10 of the 13 CECs with exposure information, and in all effect categories except genotoxicity, gross pathology and growth. There was a total of 273 SV_{LOW} exceedances at this location, with at least one occurrence for each sampling event and at each sampling site. Occurrence of high hazard (SV_{HIGH} exceedance) was limited to endocrine effects associated with DEET exposure. However, the strength of ecotoxicity information associated with observations of high hazard is moderate, and considering the large area covered in this project location, it is notable that high hazard was observed at four of the six sampling sites – two point source CEC-influenced sites and two uninfluenced sites.

Four comprehensive effect categories achieved the highest hazard ranks among all effect categories at this location, with endocrine hazard ranked highest. DEET was ranked highest among CECs for overall hazard to fish, followed by venlafaxine and citalopram – two antidepressants. Sampling site overall hazard ranking did not correspond well with designations based on point source proximity (CEC-influenced vs. uninfluenced), which suggests the presence of CEC sources not explicitly accounted for in this EHA, such as aerial deposition, urban or agricultural runoff, or unmapped WWTPs, CSOs or other point sources.

Statistical analysis of point source influence on CEC hazard showed no significant differences ($p < 0.1$) between point source CEC-influenced sites ($n = 2$) and uninfluenced sites ($n = 4$). Qualitative analysis of tallies of exceedances also showed no difference between the site groups in fraction of sampling events and fraction of sites with SV_{LOW} exceedances. However, fractions of sites and events with SV_{HIGH} exceedances was greater in the CEC-influenced group compared with the uninfluenced group, although total number of SV_{HIGH} exceedances in each site group was two.

Some Key Points…

Grand River / Maple River (MI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard**:
  - 4 occurrences, involving 24% of sampling events and 67% of sites
  - *CECs:* DEET
  - *Effect Categories*: Endocrine
- **Low Hazard**:
  - 273 occurrences, involving 100% of sampling events and sites
  - *CECs:* 10 of 13 (no Ibuprofen data available)
  - *Effect Categories*: 9 of 12
- **Point Source Analysis:** Limited evidence; high hazard more prevalent downstream of point sources
- **Exposure Dataset**: Limited, considering the large extent of the project location
  - 6 sites
  - 2 or 3 samples per site
  - 17 total samples

5.4.9.1 Hazard Brief for Grand River/Maple River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

An effect-specific hazard map (Section 5.4.9.5) is provided only for the endocrine hazard category at the Grand River/Maple River location, where relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1). All hazard scores were ≤ 2 for all of the other effect categories. The DEET concentration exceeded the endocrine SV_{HIGH} in one out of three surface water samples collected at each of four sites among the six Grand River/Maple River sampling sites (Attachment B, Table B-9). No other SV_{HIGH} exceedances were observed.

Exceedances of SV_{LOW} values occurred in additional Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/ Metabolic.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-9.

Grand River/Maple River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●		●	●	●		●				●	●		●
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									●
Genotoxicity														
Gross Pathology														
Histopathology			●											●
Neurological			●											
Physiological/Metabolic			●				●	●						
Population-relevant														
Population-relevant Mean SV				●	●		●				●			●
Behavioral			●	●			●				●			●
Developmental		●	●				●							
Growth														
Mortality					●									●
Reproductive		●	●				●							●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

The DEET SV_{HIGH} exceedances occurred at two sites identified as influenced by mapped CEC point sources and two uninfluenced sites.

Grand River / Maple River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. GRAND-3 (3)
2. GRAND-4 (2)
3. GRAND-5 (3)
4. **> GRAND-6 (3)**
5. MAPLE-1 (3)
6. **> MAPLE-2 (3)**

5.4.9.2 Hazard Rankings for Grand River/Maple River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[55]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Grand River/Maple River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.847	1.8-2.0	5
Neurological	1.667	1.6-1.8	4
Histopathology	1.625	1.6-1.8	4
Circulatory/Blood Constituents	1.444	1.4-1.6	3
Physiological/Metabolic	1.389	1.2-1.4	2
Reproductive	1.300	1.2-1.4	2
Mortality	1.210	1.2-1.4	2
Comprehensive Mean SV	1.203	1.2-1.4	2
Population-relevant Mean SV	1.167	1.0-1.2	1
Behavioral	1.146	1.0-1.2	1
Developmental	1.083	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminant[56]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Grand River/Maple River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.778	1.6-1.8	4
Venlafaxine	1.528	1.4-1.6	3
Citalopram	1.417	1.4-1.6	3
Carbamazepine	1.374	1.2-1.4	2
Sitosterol, beta-	1.267	1.2-1.4	2
Estrone	1.167	1.0-1.2	1
HHCB	1.048	1.0-1.2	1
TBEP	1.044	1.0-1.2	1
Bisphenol A	1.035	1.0-1.2	1
4-Androstene-3,17-dione	1.019	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

[55]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[56]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categoriesand CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Grand River/Maple River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
GRAND-3	1.338	1.2-1.4	2
> GRAND-6	1.315	1.2-1.4	2
GRAND-5	1.224	1.2-1.4	2
GRAND-4	1.205	1.2-1.4	2
MAPLE-1	1.123	1.0-1.2	1
> MAPLE-2	1.123	1.0-1.2	1

5.4.9.3. Breadth of Information Indicating High Hazard at Grand River/Maple River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Spatial coverage of this huge river system was sparse, given the enormous size of the project location. Only six sampling sites distributed over hundreds of kilometers of river (Figure A2-9 in Attachment A2) and numerous opportunities for upstream-downstream sampling of WWTPs were unexploited. Although sample sizes were low, the temporal distribution of samples was moderate with the sampling schedule covering two seasons over two years. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was limited due to very few USEPA STORET water quality sampling sites with TSS data distributed over hundreds of kilometers of river within the project location (Figure A2-9 in Attachment A2). Due to potential differences in geomorphology and water quality over the watershed, this project location was subdivided into three segments – upper Grand River, and lower Grand River, and Maple River - and CEC aqueous concentration was estimated separately based on TSS data representative of each segment.

- *Ecotoxicity*: All observations of high hazard were exceedances of the DEET endocrine SV_{HIGH}, which is based on a moderately strong ecotoxicity dataset (Table 4-6).

5.4.9.4 Grand River/Maple River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). No statistically significant differences in hazard scores ($\alpha = 0.1$) were observed between point source CEC-influenced and uninfluenced sites. Likewise, no significant differences are observed when all hazard scores for each site in the Grand River/ Maple River location - not just maximum or median scores - are included in the statistical analysis[57] (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Grand River/Maple River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

High hazard was evenly split between point source CEC-influenced and uninfluenced sites, with a total of four SV_{HIGH} exceedances – two in each site group - all attributed to DEET. Although we mapped endocrine effects, endocrine effect hazard was not statistically different between the two groups. The lack of a statistically significant difference between sites groups is likely affected by few observations of high hazard, few sites in each group, and relatively wide-spread distribution of high DEET hazard, which in turn may be due to unmapped CEC point sources or aerial deposition.

[57]Cautionary caveats regarding this approach are described in Attachment D.

Examination of SV_{LOW} exceedances also does not reveal an association between proximity to an upstream mapped CEC point source and occurrence of elevated hazard. There was a total of 94 exceedances of various effect-specific SV_{LOW} values in the two CEC-influenced sites, and 179 exceedances of the SV_{LOW} for nine CECs observed at the four "uninfluenced" sites. These results suggest the presence of unmapped CEC point sources, and/or non-point CEC inputs to the system.

Grand River/ Maple River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	2	0.33 (2/6)	1.0 (2/2)	94	1.0 (6/6)	1.0 (2/2)
Uninfluenced Sites	2	0.18 (2/11)	0.5 (2/4)	179	1.0 (11/11)	1.0 (4/4)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.9.5 Grand River/Maple River Hazard Map

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Endocrine Hazard

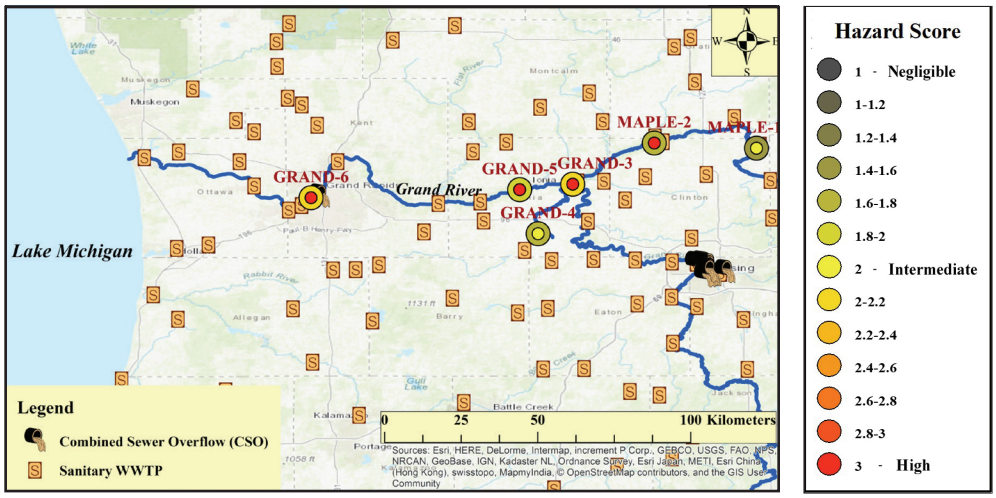


Figure 5-31. Endocrine Hazard Map for the Grand River / Maple River Watershed. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

5.4.10 Saginaw River (MI)

The Saginaw River project location is comprised of a network of tributary rivers that converge to form the Saginaw River in central Michigan, which discharges into Lake Huron at the city of Saginaw, MI. The Saginaw River watershed area is 8,595 mi^2 (22,260 km^2). Six of the 14 sampling sites were designated as CEC-'influenced', and eight 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Each of the sites was sampled for surface water CEC concentration measurements once in April 2013, and once in August 2014.

There is substantial evidence that hazards to fish from CEC exposures are present at this location. Elevated hazard (predominantly SV_{LOW} exceedances) was observed for 10 of the 13 CECs with exposure information (except diphenhydramine, lidocaine and triclosan), and in all effect categories except genotoxicity, gross pathology and growth. Elevated hazard was broadly distributed at this location. There was a total of 370 SV_{LOW} exceedances at this location, with at least one occurrence for 27/28 sampling events and 13/14 sites. However, occurrence of high hazard (SV_{HIGH} exceedance) was limited to endocrine effects associated with DEET exposure at only five out of 14 sampling sites (three CEC-influenced and two uninfluenced); the strength of ecotoxicity information associated with these observations of high hazard is moderate.

Among effect categories, five of the top six hazard score ranks (based on mean hazard scores) were attributed to comprehensive effect categories, with endocrine hazard ranked highest. Venlafaxine and DEET ranked highest for overall hazard among the 13 CECs, followed by carbamazepine, estrone and β-sitosterol.

Some Key Points…

Saginaw River (MI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard:**
  - 5 occurrences, involving 18% of sampling events, and 36% of sites
  - *CECs:* DEET
  - *Effects Categories*: Endocrine
- **Low Hazard:**
  - 370 occurrences, involving 96% of sampling events, and 93% of sites
  - *CECs*: 10 of 13 (no Ibuprofen data)
  - *Effect Categories:* 9 of 12
- **Point Sources Analysis:** Significant evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Substantial
  - 14 sampling sites
  - 2 samples per site
  - 28 total samples

Site-specific maximum and/or median hazard scores associated with four CECs and five effect categories were statistically significantly higher in the CEC-influenced site group compared to uninfluenced sites. Qualitative analysis of exceedance tallies support these findings. Although there was little difference between site groups in numbers of SV_{HIGH} exceedances, there was nearly double the incidence of SV_{LOW} exceedances at CEC-influenced sites (35.7 exceedances per site) compared with uninfluenced sites (19.5 exceedances per site).

5.4.10.1 Hazard Brief for Saginaw River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

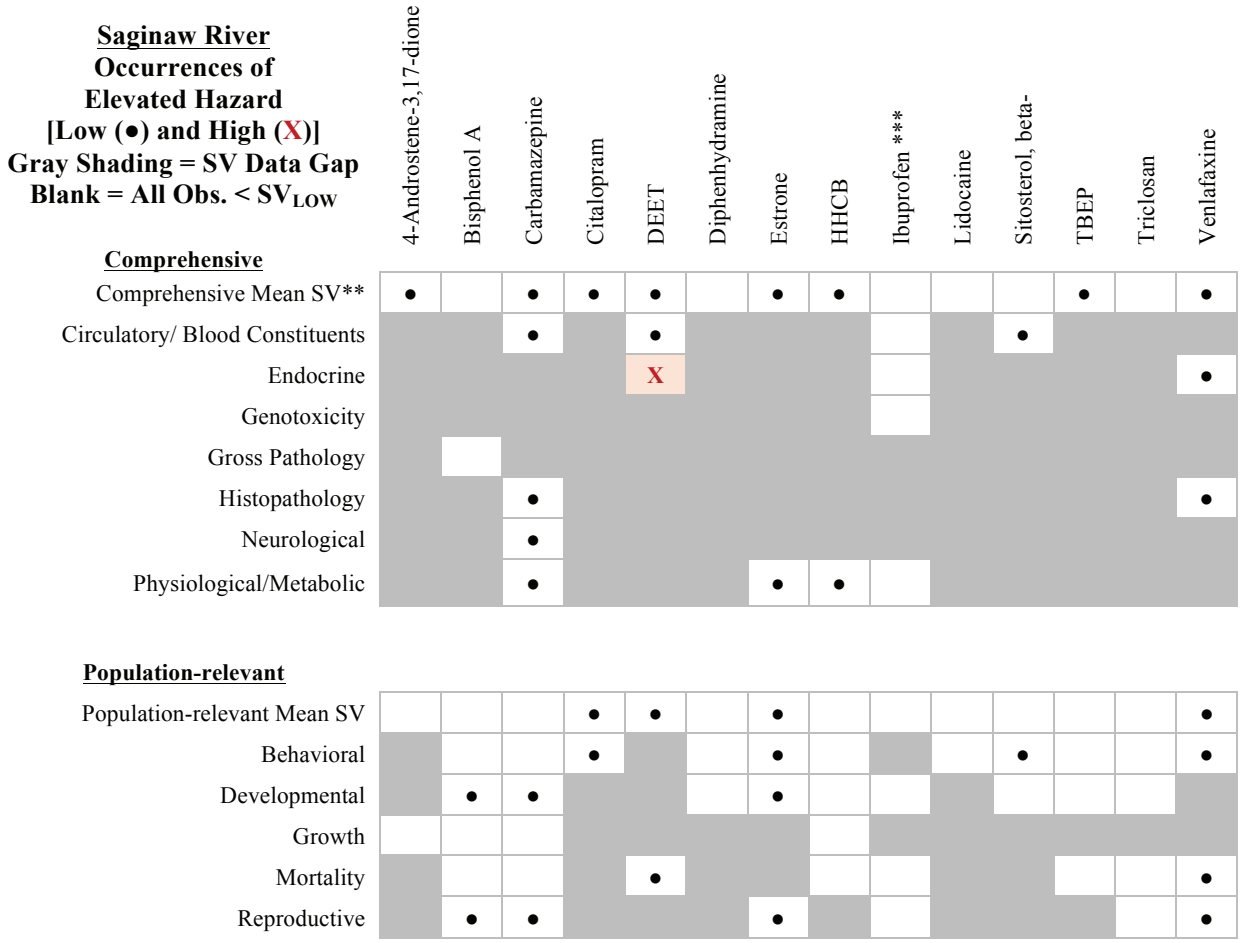
Effect Categories and Emerging Contaminants
An effect-specific hazard map (Section 5.4.10.5) is provided only for the endocrine hazard category at the Saginaw River location, where relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1). All hazard scores were ≤ 2 for all of the other effect categories.

Concentration of DEET exceeded the endocrine SV_{HIGH} in one out of the two surface water samples, at five of 14 sampling sites (see Attachment B, Table B-10). No other SV_{HIGH} exceedances were observed.

Exceedances of SV_{LOW} values occurred in additional Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Growth, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histological, Neurological, Physiological/ Metabolic.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-10.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

The DEET SV_{HIGH} exceedances occurred at three sites identified as point source CEC-influenced and two uninfluenced sites, distributed widely over the project location.

Saginaw River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. SGNR-1 (2)	**4. > SGNR-12 (2)**	**7. > SGNR-2 (2)**	**10. > SGNR-5 (2)**	13. SGNR-8 (2)
2. > SGNR-10 (2)	5. SGNR-13 (2)	8. SGNR-3 (2)	11. SGNR-6 (2)	**14. > SGNR-9 (2)**
3. > SGNR-11 (2)	6. SGNR-14 (2)	9. SGNR-4 (2)	12. SGNR-7 (2)	

5.4.10.2 Hazard Rankings for Saginaw River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[58]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Saginaw River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.732	1.6-1.8	4
Histopathology	1.589	1.4-1.6	3
Neurological	1.536	1.4-1.6	3
Physiological/Metabolic	1.369	1.2-1.4	2
Circulatory/Blood Constituents	1.321	1.2-1.4	2
Reproductive	1.300	1.2-1.4	2
Mortality	1.184	1.0-1.2	1
Behavioral	1.143	1.0-1.2	1
Comprehensive Mean SV	1.143	1.0-1.2	1
Population-relevant Mean SV	1.118	1.0-1.2	1
Developmental	1.063	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants[59]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Saginaw River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.543	1.4-1.6	3
Venlafaxine	1.541	1.4-1.6	3
Carbamazepine	1.289	1.2-1.4	2
Estrone	1.286	1.2-1.4	2
Sitosterol, beta-	1.207	1.2-1.4	2
Citalopram	1.131	1.0-1.2	1
HHCB	1.051	1.0-1.2	1
TBEP	1.036	1.0-1.2	1
4-Androstene-3,17-dione	1.024	1.0-1.2	1
Bisphenol A	1.009	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

[58]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[59]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Saginaw River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> SGNR-12	1.356	1.2-1.4	2
> SGNR-11	1.308	1.2-1.4	2
> SGNR-10	1.301	1.2-1.4	2
SGNR-3	1.281	1.2-1.4	2
SGNR-13	1.274	1.2-1.4	2
> SGNR-2	1.267	1.2-1.4	2
SGNR-7	1.151	1.0-1.2	1
> SGNR-9	1.151	1.0-1.2	1
SGNR-8	1.137	1.0-1.2	1
> SGNR-5	1.123	1.0-1.2	1
SGNR-4	1.116	1.0-1.2	1
SGNR-1	1.075	1.0-1.2	1
SGNR-6	1.034	1.0-1.2	1
SGNR-14	1.014	1.0-1.2	1

5.4.10.3. Breadth of Information Indicating High Hazard at Saginaw River

- *Exposure:* There was good distribution of sites in this river system - a total of 14 sampling sites were spaced evenly between several rivers and included good representation of both influenced and uninfluenced sites (Figure A2-10 in Attachment A2). However, false negative results are likely at a number of sites due to very small sample numbers, and coverage of the entire project location was only fair due to large distances between sampling sites (>50 km in some cases). Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only a single USEPA STORET water quality sampling site with TSS data located in the lower Saginaw River to represent the entire watershed (Figure A2-10 in Attachment A2).
- *Ecotoxicity:* All observations of high hazard were exceedances of DEET endocrine SVs, which are based on a moderately strong ecotoxicity dataset (Table 4-6).

The evidence suggesting an absence of high hazard for other CECs and effect categories is weak. Only two surface water samples per site were analyzed for CECs – only one sample each in April and August, and only during 2014 (Attachment A1, Table A-2).

5.4.10.4 Saginaw River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4); the following chart provides the results summary.

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
Carbamazepine	Histopathology	N	Y
	Reproductive	N	Y
Citalopram	Comprehensive mean SV	Y	Y
	Population-relevant mean SV	Y	Y
HHCB	Physiological/Metabolic	Y	Y
TBEP	Comprehensive mean SV	Y	Y

When *all* hazard scores for each site in the Saginaw River location, not just maximum or median scores, are included in the statistical analysis[60], additional significant differences ($p < 0.1$) are observed for behavioral effects associated with citalopram and venlafaxine exposures (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Saginaw River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Saginaw River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	3	0.25 (3/12)	0.5 (3/6)	214	1.0 (12/12)	0.83 (5/6)
Uninfluenced Sites	2	0.13 (2/16)	0.25 (2/8)	156	0.94 (15/16)	1.0 (8/8)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There was a total of three SV_{HIGH} exceedances at three of the six sites designated as CEC-influenced, and two SV_{HIGH} exceedances at two of the uninfluenced sites – all five SV_{HIGH} exceedances were attributed to DEET. However, when SV_{LOW} exceedances are considered, there is evidence of elevated hazard at sampling sites downstream of mapped CEC point sources. At the six CEC-influenced sites, there was a total of 214 Effect-specific SV_{LOW} exceedances, but a total of 156 SV_{LOW} exceedances at the eight uninfluenced sites. SV_{LOW} exceedances in both groups were attributed to 9 of the 14 CECs considered in this EHA.

[60]Cautionary caveats regarding this approach are described in Attachment D.

5.4.10.5 Saginaw River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

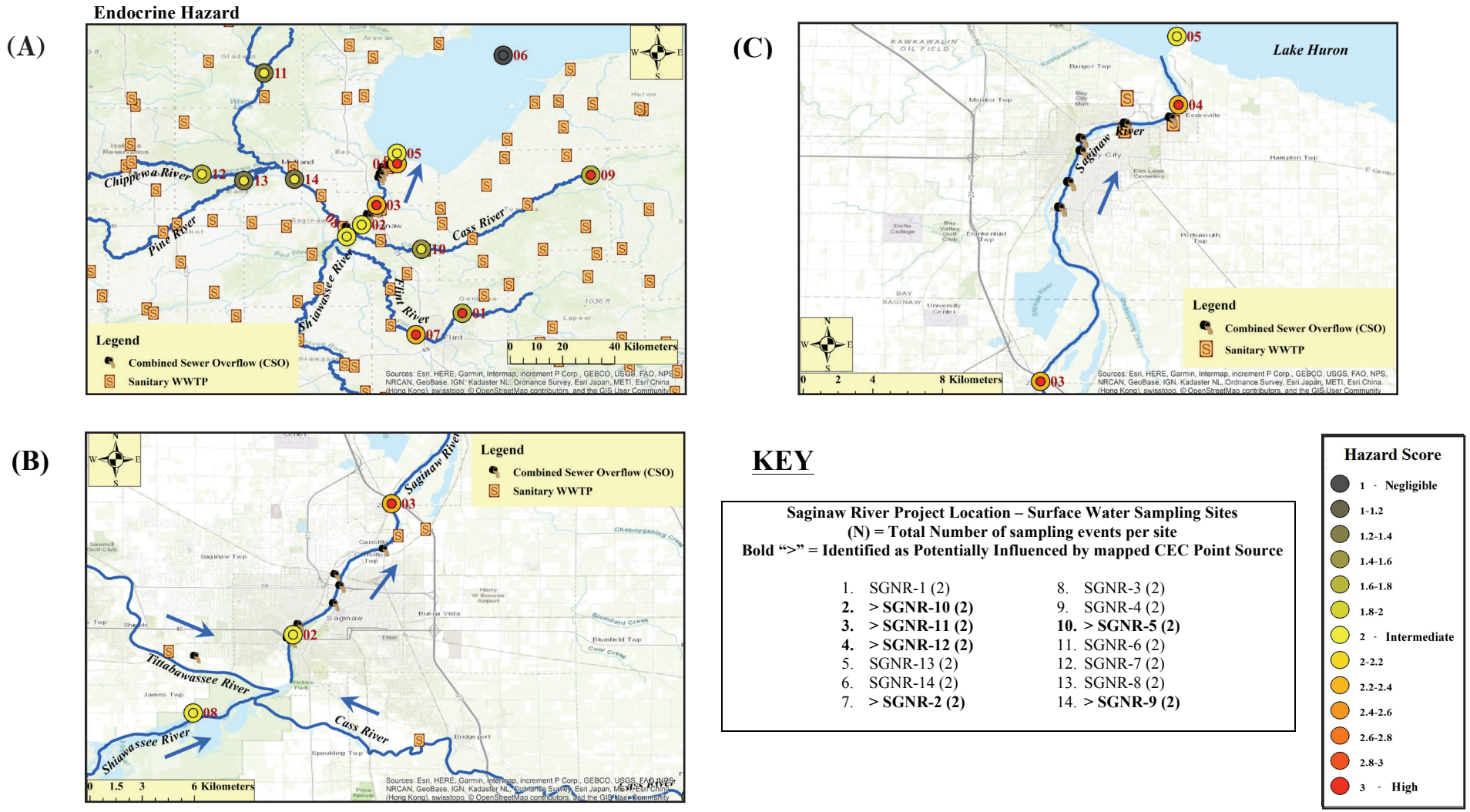


Figure 5-32. Endocrine Hazard Map for the Saginaw River Watershed. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) River confluence at center of watershed; (C) Lower Saginaw River. Arrows indicate river flow direction.

5.4.11 St. Clair River (MI)

The St. Clair River connects Lake Huron to Lake St. Clair, forming part of the U.S.-Canada border. Mapped WWTPs are distributed fairly evenly along the 65 km (40.5 mi) length of the river, and sampling sites were selected in proximity to several of these. Five of the eight sampling sites were designated as CEC-'influenced', and three 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Each of the sites was sampled one or two times for surface water CEC concentration measurements during August, 2013.

There is little evidence of CEC-related impacts to fish at the St. Clair River compared to most of the other project locations considered in this EHA. Hazard occurrence is limited to a total of 20 observations of low hazard (SV_{LOW} exceedance) associated with four CECs; there were no SV_{HIGH} exceedances. Hazard ranks (based on mean hazard scores) for all effect categories, CECs, and sampling sites were '1' – the lowest possible rank. There were no statistically significant differences in site-specific maximum or median hazard scores between CEC-influenced and uninfluenced site groups, for any CEC-effect category combination.

All samples were collected during August of 2013 in only one or two sampling events per site, sample sizes were very small at each sampling site, and there were approximately 10km distance gaps between sites in the main river stem. Despite these exposure dataset limitations, SV_{LOW} exceedances were observed at five of the eight sampling sites and in six effect categories, including in all six effect categories for estrone. These observations suggest that a more robust exposure dataset could reveal greater evidence of CEC-related hazards to fish at this location.

Some Key Points…

St. Clair River (MI)

- **Overall:** Little evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 20 occurrences, involving 64% sampling events, and 75% of sites
  - *CECs*: Androstenedione, DEET, Estrone, β-Sitosterol
  - *Effect Categories*: 6 of 12
- **Point Source Analysis:** No evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Limited
  - 8 sampling sites
  - 1-2 samples per site
  - 11 total samples

5.4.11.1 Hazard Brief for St. Clair River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

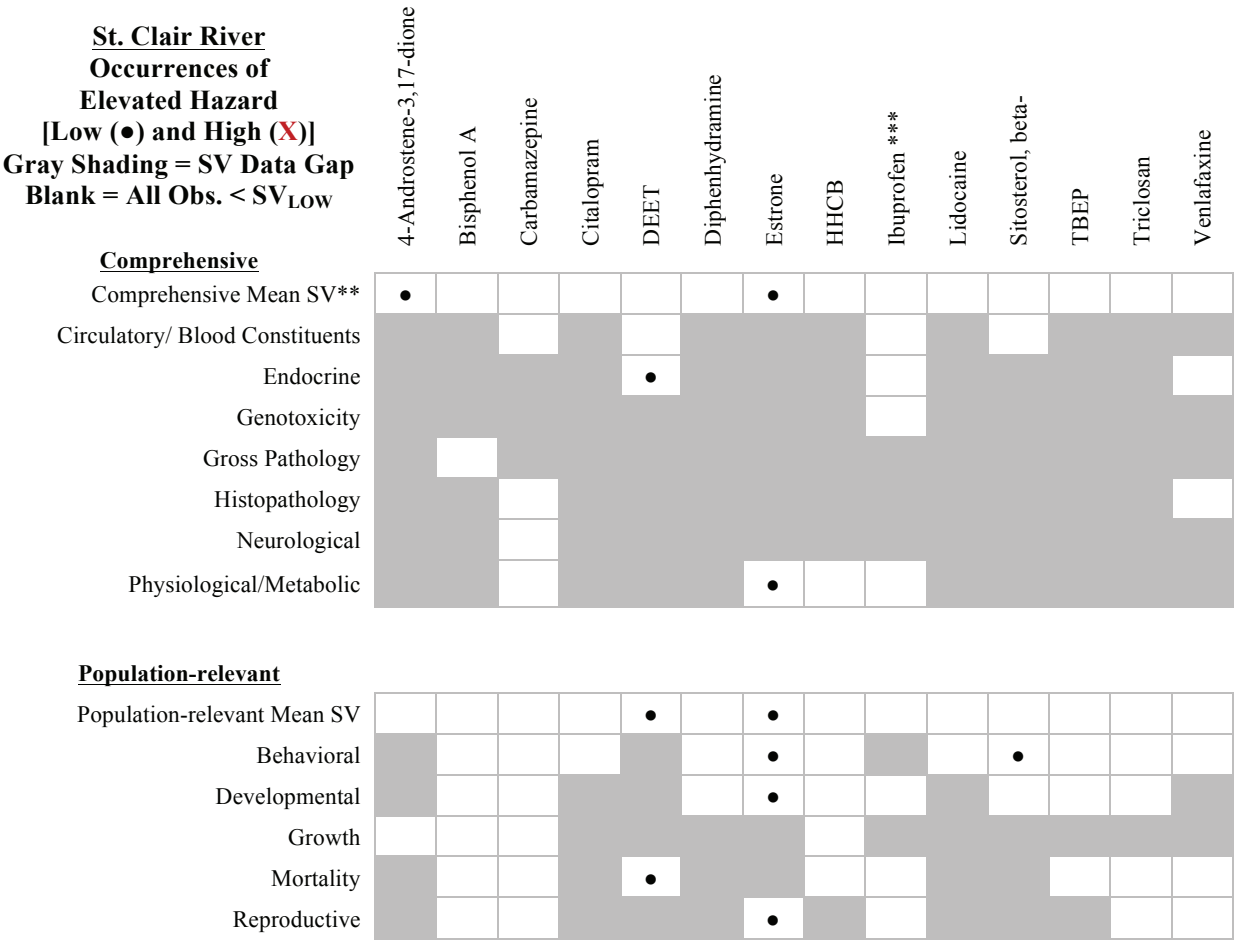
No exceedance of a SV_{HIGH} was observed at this project location (Table 5-1).

The SV_{LOW} was exceeded in these hazard categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Endocrine, Physiological/Metabolic.

Surface water concentrations of androstenedione, DEET, estrone and β-sitosterol exceeded a SV_{LOW} a total of 20 times.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Appendix B, Table B-11.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

There were no observations of high hazard at St. Clair River sampling sites evaluated for hazard to fish, but SV_{LOW} values were exceeded at six of the eight sampling sites.

St. Clair River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. > SCR-1 (2)	**3. > SCR-3 (2)**	**5. > SCR-5 (2)**	7. SCR-7 (1)
2. > SCR-2 (1)	**4. > SCR-4 (1)**	6. SCR-6 (1)	8. SCR-8 (1)

5.4.11.2 Hazard Rankings for St. Clair River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[61]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	St. Clair River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.125	1.0-1.2	1
Physiological/Metabolic	1.042	1.0-1.2	1
Comprehensive Mean SV	1.038	1.0-1.2	1
Mortality	1.036	1.0-1.2	1
Population-relevant Mean SV	1.029	1.0-1.2	1
Reproductive	1.025	1.0-1.2	1
Behavioral	1.017	1.0-1.2	1
Developmental	1.016	1.0-1.2	1
Circulatory/Blood Constituents	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1

Emerging Contaminants[62]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	St. Clair River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.150	1.0-1.2	1
4-Androstene-3,17-dione	1.125	1.0-1.2	1
Estrone	1.125	1.0-1.2	1
Sitosterol, beta-	1.013	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
HHCB	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

[61] Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[62] Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	St. Clair River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
SCR-8	1.123	1.0-1.2	1
> SCR-1	1.027	1.0-1.2	1
>SCR-3	1.027	1.0-1.2	1
> SCR-4	1.014	1.0-1.2	1
SCR-7	1.014	1.0-1.2	1
> SCR-5	1.007	1.0-1.2	1
> SCR-2	1.000	1.0-1.2	1
SCR-6	1.000	1.0-1.2	1

5.4.11.3. Breadth of Information Indicating High Hazard at St. Clair River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no exceedances of SV_{HIGH} values. However, the evidence suggesting an absence of high hazard is weak. Only one or two surface water samples per site were analyzed for CECs, and all samples were collected during only one month in 2013. Additional potential for false negative findings are due to gaps in the fish ecotoxicity database as discussed above in Section 5.3.2. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was limited due to only two USEPA STORET water quality sampling sites with TSS data, one in the upper St. Clair River and the other in the lower river separated by approximately 40km (Figure A2-11 in Attachment A2).

5.4.11.4 St. Clair River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4).

There were no statistically significant differences in site-specific maximum or median hazard scores between point source influenced and uninfluenced site groups at the St. Clair River location. Likewise, no significant differences are observed when all hazard scores for each site - not just maximum or median scores - are included in the statistical analysis[63] (Attachment D). These negative statistical findings may be due to small total sample size in the uninfluenced site group (n = 3), an absence of SV_{HIGH} exceedances, and a proportional distribution of SV_{LOW} exceedances between influenced and uninfluenced sites.

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the St. Clair River location that have at least one observation of a SV_{LOW} exceedance, for any CEC and effect category; these numbers are provided for point source CEC-influenced versus uninfluenced site groups.

No SV_{HIGH} exceedances were observed at any sampling site. There was a total of 10 exceedances of various SV_{LOW} values for 4-androstene-3,17-dione, DEET, and β-sitosterol at CEC-influenced sites, and 10 SV_{LOW} exceedances at uninfluenced sites, attributed to 4-androstene-3,17-dione, DEET, and estrone.

[63]Cautionary caveats regarding this approach are described in Attachment D.

St. Clair River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	0	0.0 (0/8)	0.0 (0/5)	10	0.63 (5/8)	0.8 (4/5)
Uninfluenced Sites	0	0.0 (0/3)	0.0 (0/3)	10	0.67 (2/3)	0.67 (2/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.11.5 St. Clair River Hazard Maps

There were no exceedances of SV_{HIGH} values; no hazard maps were generated.

5.4.12 Clinton River (MI)

The Clinton River flows 134 km (83 mi) eastward across the lower peninsula of Michigan along the northern edge of greater Detroit, discharging into Lake St. Clair. Two of the five sampling sites were designated as CEC-'influenced', and three 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Each of the sites was sampled twice for surface water CEC concentration measurements during June, 2013.

There is some evidence of CEC-related hazard to fish at this project location, despite only two water samples per site. Elevated hazard (predominantly SV_{LOW} exceedances) was observed for six of the 13 CECs with exposure information, and in all effect categories except genotoxicity, gross pathology and growth. There was a total of 139 SV_{LOW} exceedances at this location, with at least one occurrence for each sampling event and at each sampling site. Occurrence of high hazard was limited to two exceedances of the DEET endocrine SV_{HIGH}, and the strength of ecotoxicity information associated with DEET endocrine SVs is moderate.

Five comprehensive effect categories ranked high for hazard to fish, with endocrine hazard ranking highest, followed by neurological and physiological/ metabolic. Among CECs, DEET ranked far higher than the next most hazardous CECs, which were estrone, carbamazepine and venlafaxine. The two downstream-most sampling sites ranked highest for hazard to fish among the five sites. Although there were no statistically significant differences in site-specific maximum or median hazard scores between CEC-influenced and uninfluenced site groups, the incidence of SV_{LOW} exceedances averaged 37 per site in the CEC-influenced group and 21.7 per site in the uninfluenced group.

5.4.12.1 Hazard Brief for Clinton River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

Clinton River (MI)

- **Overall:** Some evidence of hazards to fish
- **High Hazard:**
  - 2 occurrences, involving 20% of the sampling events, and 40% of sites
  - *CECs*: DEET
  - *Effect Categories*: Endocrine
- **Low Hazard:**
  - 139 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: 6 of 13 (no Ibuprofen data)
  - *Effect Categories*: 9 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Limited
  - 5 sampling sites
  - 2 samples per site
  - 10 total samples

Effect Categories and Emerging Contaminants

The endocrine effect category had SV_{HIGH} exceedances attributable to DEET in one of two samples collected at each of two sites (Section 5.4.12.5); there were no other observations of high hazard at the Clinton River location (Table 5-1).

However, SV_{LOW} values were exceeded in additional effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/ Metabolic.

There were 139 instances where the surface water concentrations of carbamazepine, DEET, estrone, HHCB, β-sitosterol, and venlafaxine exceeded SV_{LOW} values. Although only two samples were analyzed at each site, there were 47 instances where both samples exceeded a SV_{LOW}.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-12.

Clinton River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**			●		●		●	●						●
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									●
Genotoxicity														
Gross Pathology														
Histopathology			●											●
Neurological			●											
Physiological/Metabolic			●				●	●						
Population-relevant														
Population-relevant Mean SV					●		●							●
Behavioral			●				●				●			●
Developmental			●				●							
Growth														
Mortality					●									●
Reproductive			●				●							●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

There were only two observations of high hazard at Clinton River sampling sites evaluated for hazard to fish (red-highlighted in chart below), but SV_{LOW} values were exceeded at all five sampling sites.

Clinton River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> CLI-1-DOWN (2)**
2. CLI-2-CAPT (2)
3. **> CLI-3-WWTP (2)**
4. CLI-4-STONY (2)
5. CLI-5-CASS (2)

5.4.12.2 Hazard Rankings for Clinton River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[64]

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Clinton River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.750	1.6-1.8	4
Neurological	1.500	1.4-1.6	3
Physiological/Metabolic	1.467	1.4-1.6	3
Histopathology	1.400	1.2-1.4	2
Circulatory/Blood Constituents	1.300	1.2-1.4	2
Reproductive	1.240	1.2-1.4	2
Comprehensive Mean SV	1.192	1.0-1.2	1
Mortality	1.186	1.0-1.2	1
Behavioral	1.145	1.0-1.2	1
Population-relevant Mean SV	1.131	1.0-1.2	1
Developmental	1.113	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants[65]

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Clinton River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.860	1.8-2.0	5
Estrone	1.400	1.2-1.4	2
Carbamazepine	1.345	1.2-1.4	2
Venlafaxine	1.300	1.2-1.4	2
Sitosterol, beta-	1.160	1.0-1.2	1
HHCB	1.129	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

[64]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[65]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Clinton River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> CLI-1-DOWN	1.342	1.2-1.4	2
CLI-2-CAPT	1.253	1.2-1.4	2
> CLI-3-WWTP	1.178	1.0-1.2	1
CLI-4-STONY	1.144	1.0-1.2	1
CLI-5-CASS	1.062	1.0-1.2	1

5.4.12.3 Breadth of Information Indicating High Hazard at Clinton River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* Spatio-temporal coverage of this system was limited, with only five sampling sites distributed over several dozen kilometers of river each of which was sampled only twice. Despite very small sample sizes, high hazard in a couple of samples and a large number of SV_{LOW} exceedances were observed. These findings suggest that better temporal distribution and a greater number of samples would likely have resulted in more high-hazard observations. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was moderate due to five USEPA STORET water quality sampling sites with TSS data distributed between the upper and lower river within the project location (Figure A2-12 in Attachment A2); collective TSS sample size totaled >20 in each of nine calendar months (Figure A-1b in Attachment A1).
- *Ecotoxicity:* Both observations of high hazard were exceedances of DEET endocrine SVs, which are based on a moderately strong ecotoxicity dataset (Table 4-6).

The evidence suggesting an absence of high hazard is weak. Only two surface water samples per site were analyzed for CECs, and all samples were collected during one month in 2013. Additional potential for false negative findings is due to gaps in the fish ecotoxicity database as discussed above in Section 5.3.2.

5.4.12.4 Clinton River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences for any CEC or effect category, which may be due to very small numbers of sites in both site groups and an even distribution of SV_{HIGH} and SV_{LOW} exceedances between the groups. Likewise, no significant differences are observed when all hazard scores for each site - not just maximum or median scores - are included in the statistical analysis[66] (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Clinton River location that have at least one observation of a SV_{HIGH} exceedance,

Clinton River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	1	0.25 (1/4)	0.5 (1/2)	74	1.0 (4/4)	1.0 (2/2)
Uninfluenced Sites	1	0.17 (1/6)	0.33 (1/3)	65	1.0 (6/6)	1.0 (3/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[66]Cautionary caveats regarding this approach are described in Attachment D.

for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

There was one endocrine SV_{HIGH} exceedance in each site group associated with DEET. Among CEC-influenced and uninfluenced sites,there were totals of 74 and 65 SV_{LOW} exceedances, respectively. In both groups, SV_{LOW} exceedances were attributable to carbamazepine, DEET, estrone, HHCB, β-sitosterol and venlafaxine.

The upstream most sampling site (CLI-5-CASS) was identified as uninfluenced by mapped point sources. Nevertheless, it showed five exceedances of DEET SV_{LOW} values and three exceedances of β-sitosterol SV_{LOW} values. The widespread elevated hazard to fish from DEET exposure may be due to aerial deposition. Exceedances of the SV_{LOW} for six and five CECs (including pharmaceuticals) were observed at CLI-1-DOWN and CLI-3-WWTP, respectively – both of which were identified as influenced by point sources. CLI-2-CAPT, an uninfluenced site far downstream of mapped point sources, also showed one SV_{HIGH} exceedance and SV_{LOW} exceedances in five CECs suggesting either an unmapped upstream WWTP or CSO in relatively close proximity, other upstream point or non-point sources, or little attenuation of CEC concentrations during transport from far upstream mapped point sources.

5.4.12.5 Clinton River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Endocrine Hazard

Figure 5-33. Endocrine Hazard Map for the Clinton River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.

5.4.13 Detroit River (MI)

The Detroit River serves as part of the border between U.S. and Canada, draining southward from Lake St. Clair into northwest Lake Erie and receiving numerous CEC point source inputs distributed along its 45 km (28 mi) length from urban areas on both sides of the border. Five of the 10 sampling sites were designated as CEC-'influenced', and five 'uninfluenced', based on their downstream proximity to mapped CEC point sources. Sites were sampled one or two times for surface water CEC concentration measurements during one or two of the following sampling events: October 2010, April or May 2011, or May 2012.

There is substantial evidence of a high potential for impacts to fish in this project location, despite very low sample numbers at each of the 10 sampling sites. There were 14 observations of high hazard (SV_{HIGH} exceedances), including physiological/metabolic hazard from exposure to estrone, and genotoxicity,

Some Key Points…

Detroit River (MI)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard:**
  - 14 occurrences, involving 24% of sampling events and 20% of sites
  - *CECs*: Estrone, Ibuprofen
  - *Effect Categories*: Genotoxicity, Physiological/Metabolic, Developmental, Reproductive
- **Low Hazard:**
  - 176 occurrences, involving 88% of sampling events, and 100% of sampling sites
  - *CECs*: 10 of 14
  - *Effect Categories:* 10 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Substantial
  - 10 sampling sites
  - 1-2 samples per site
  - 17 total samples

reproductive, and developmental hazards attributed to ibuprofen. SV_{LOW} exceedances totaled 176, and occurred at each of the 10 sampling sites and in 16 of 17 sampling events, involving 10 of the 14 CECs and all effect categories except gross pathology and growth. Overall strength of evidence was low in the exposure dataset, but the breadth of ecotoxicity information was 'broad' for ibuprofen reproductive and developmental SVs.

In this project location, genotoxicity and physiological/metabolic ranked highest among effect categories for overall hazard to fish, and estrone ranked highest among CECs, followed by DEET, triclosan and ibuprofen. There was no evidence of hazard differences between CEC-influenced and uninfluenced sampling sites, although hazard maps illustrate that elevated hazard is more prevalent at downstream sites.

5.4.13.1 Hazard Brief for Detroit River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

High hazard was observed in four effect categories (Table 5-1), which were mapped in Section 5.4.13.5:

- Population-relevant: Developmental, Reproductive
- Comprehensive: Genotoxicity, Physiological/Metabolic.

Additional effect categories showed exceedances of the SV_{LOW}:

- Population-relevant: Population-relevant mean SV, Behavioral, Mortality
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine, Histopathology, Neurological

Ibuprofen accounted for 12 of the 14 observations of high hazard, while SV_{LOW} values for 10 of the 14 CECs were exceeded.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-13.

Detroit River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●		●		●	●	●	●	●		●	●	●	
Circulatory/ Blood Constituents			●								●			
Endocrine					●				●					
Genotoxicity									X					
Gross Pathology														
Histopathology			●											
Neurological			●											
Physiological/Metabolic			●				X	●	●					
Population-relevant														
Population-relevant Mean SV	●				●		●		●			●	●	
Behavioral							●				●		●	
Developmental			●				●		X			●	●	
Growth														
Mortality					●				●				●	
Reproductive			●				●		X				●	

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

High hazard (red-highlighted below) was observed at only two sampling sites, but exceedances of SV_{LOW} values were observed at all 10 sites.

Detroit River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. > DTR-1 (2)	**4. > DTR-4 (1)**	**7. > GROSIL-3 (2)**	10. WYAND-2 (2)
2. > DTR-2 (2)	5. DTR-5 (1)	8. PTHENN-1 (2)	
3. DTR-3 (2)	6. DTR-6 (1)	**9. > TRENTN-4 (2)**	

5.4.13.2 Hazard Rankings for Detroit River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Detroit River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Genotoxicity	1.400	1.2-1.4	2
Physiological/Metabolic	1.338	1.2-1.4	2
Reproductive	1.200	1.0-1.2	1
Endocrine	1.183	1.0-1.2	1
Comprehensive Mean SV	1.157	1.0-1.2	1
Developmental	1.144	1.0-1.2	1
Mortality	1.125	1.0-1.2	1
Population-relevant Mean SV	1.107	1.0-1.2	1
Neurological	1.100	1.0-1.2	1
Behavioral	1.073	1.0-1.2	1
Circulatory/Blood Constituents	1.050	1.0-1.2	1
Histopathology	1.050	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Detroit River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Estrone	1.417	1.4-1.6	3
DEET	1.370	1.2-1.4	2
Triclosan	1.300	1.2-1.4	2
Ibuprofen	1.211	1.2-1.4	2
HHCB	1.129	1.0-1.2	1
4-Androstene-3,17-dione	1.083	1.0-1.2	1
TBEP	1.070	1.0-1.2	1
Carbamazepine	1.064	1.0-1.2	1
Diphenhydramine	1.050	1.0-1.2	1
Sitosterol, beta-	1.050	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Detroit River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
WYAND-2	1.451	1.4-1.6	3
> TRENTN-4	1.427	1.4-1.6	3
PTHENN-1	1.116	1.0-1.2	1
> DTR-2	1.104	1.0-1.2	1
> DTR-4	1.098	1.0-1.2	1
> GROSIL-3	1.055	1.0-1.2	1
> DTR-1	1.024	1.0-1.2	1
DTR-5	1.012	1.0-1.2	1
DTR-6	1.012	1.0-1.2	1
DTR-3	1.006	1.0-1.2	1

5.4.13.3 Breadth of Information Indicating High Hazard at Detroit River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* There was good coverage of sampling sites along the bottom two thirds of the Detroit River, which is affected by numerous WWTPs (Figure A2-13, Attachment A2). However, very small sample numbers per site (Table A-2, Attachment A1) and an absence of sampling sites in the heavily CSO-influenced upper third of the river increase the likelihood of false negative high hazard results. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only one USEPA STORET water quality sampling site with TSS data located in the lower Detroit River to represent the entire 45 km river (Figure A2-13 in Attachment A2); however, TSS sample size was >10 in each of eight calendar months (Figure A-1b in Attachment A1).

- *Ecotoxicity*: Among observations of high hazard for ibuprofen and estrone, the breadth of applicable ecotoxicity information was as follows (Tables 4-5 and 4-6):
    - o Ibuprofen
        - Broad - Developmental
        - Broad – Reproductive
        - Sparse - Genotoxicity
    - o Estrone
        - Sparse - Physiological/Metabolic

5.4.13.4 Detroit River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences between site groups, for any CEC or effect category. Likewise, no significant differences are observed when all hazard scores for each site - not just maximum or median scores - are included in the statistical analysis[67] (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Detroit River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Occurrence of SV_{HIGH} and SV_{LOW} exceedances were evenly distributed between the site groups. Among the five CEC-influenced sampling sites, there were six exceedances of various effect-specific SV_{HIGH} values for ibuprofen, in two sampling events. Similarly, there were eight exceedances of SV_{HIGH} values for estrone and ibuprofen in two events among the five uninfluenced sites. Total exceedances of SV_{LOW} values among CEC-influenced and uninfluenced sites numbered 96 and 80, respectively; all attributed to nine of the 14 CECs.

Although three of the sites designated as "uninfluenced" for this analysis (DTR-3, DTR-5, DTR-6) were far downstream of mapped WWTP and CSO point sources, there was an HHCB SV_{LOW} exceedance at all three sites. In addition, the "uninfluenced" WYAND-2 site accounted for nine SV_{HIGH} exceedances for estrone and ibuprofen, and 42 SV_{LOW} exceedances for various effect categories and eight CECs. These observations suggest either very high loadings of these CECs upstream of that site, high environmental CEC persistence in the water column with little attenuation from upstream WWTP or CSOs, and/or there are unmapped sources less than 4km upstream.

Detroit River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	6	0.22 (2/9)	0.2 (1/5)	96	0.89 (8/9)	1.0 (5/5)
Uninfluenced Sites	8	0.25 (2/8)	0.2 (1/5)	80	0.88 (7/8)	1.0 (5/5)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[67]Cautionary caveats regarding this approach are described in Attachment D.

5.4.13.5 Detroit River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Developmental Hazard

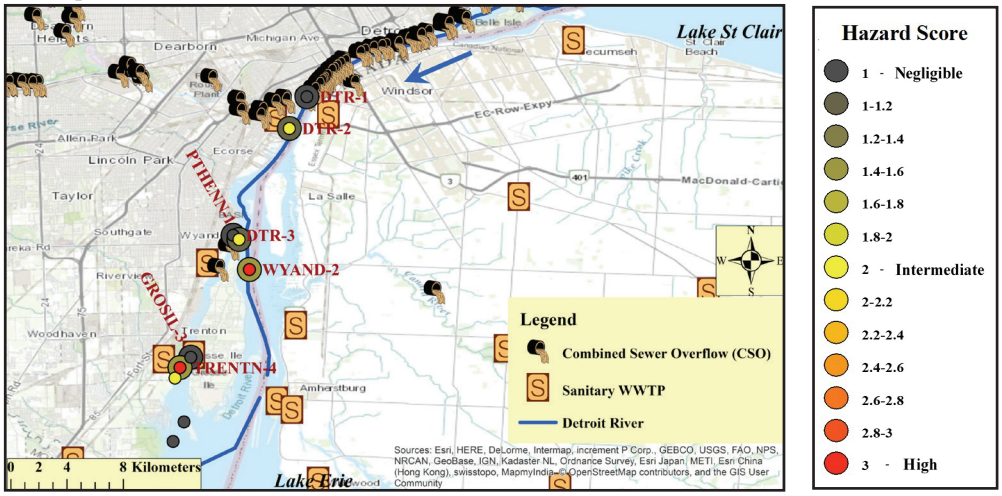


Figure 5-34. Developmental Hazard Map for the Detroit River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. The arrow indicates direction of flow.

Reproductive Hazard

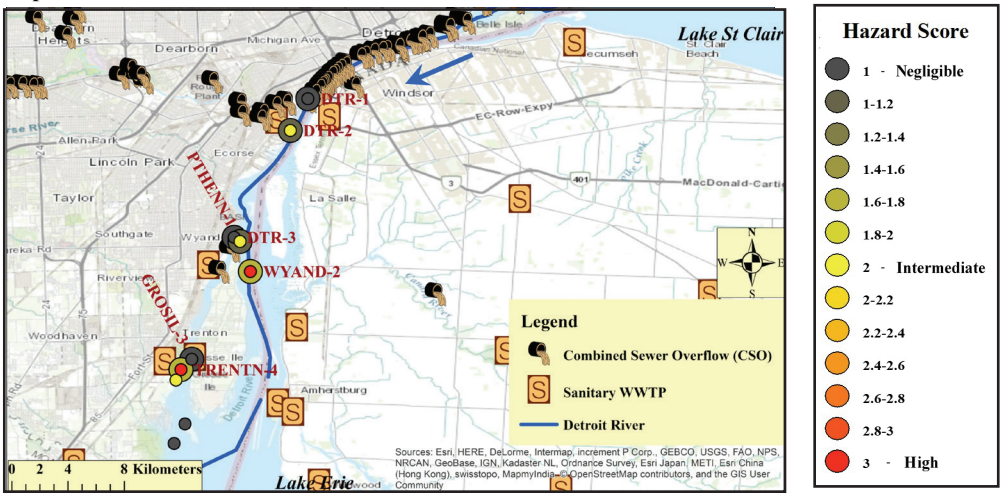


Figure 5-35 Reproductive Hazard Map for the Detroit River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate direction of flow.

Genotoxicity Hazard

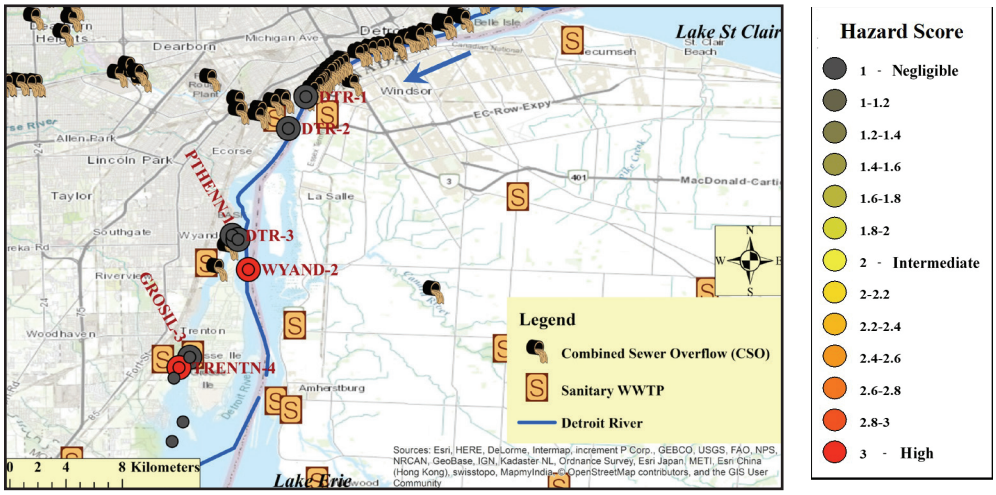


Figure 5-36. Genotoxicity Hazard Map for the Detroit River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. The arrow indicates direction of flow.

Physiological/Metabolic Hazard

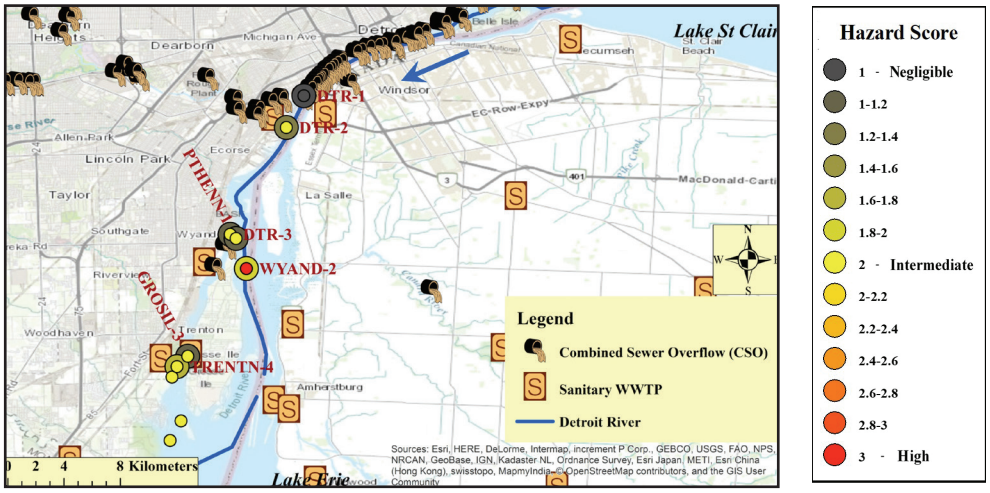


Figure 5-37. Physiological/Metabolic Hazard Map for the Detroit River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate direction of flow.

5.4.14 River Raisin (MI)

The River Raisin flows 220 km (136 mi) eastward through agricultural and industrial areas in the south east corner of Michigan's lower peninsula into the west shore of Lake Erie at the city of Monroe. Two of the four sampling sites were designated as CEC-'influenced', and two 'uninfluenced', based on their proximity to mapped CEC point sources. Each site was sampled once for surface water CEC concentration measurements during May 2012.

There is relatively little evidence to indicate CEC-related elevated hazard at this location, possibly due in part to a very sparse exposure dataset, comprised of a single sample collected in May of 2012 at each of only four sampling sites clustered near the mouth of the river. In spite of these limitations, there were 22 SV_{LOW} exceedances observed in six individual effect categories (as well as the Population-relevant and Comprehensive Mean SVs), attributed to DEET, estrone and HHCB exposure and involving all four sampling sites. Given these results, it is reasonable to expect that a more robust CEC exposure dataset may result in additional evidence of hazard to fish.

At this project location, the highest ranked effect categories for overall hazard to fish were endocrine and physiological/metabolic hazards, and the highest ranked CECs were DEET and estrone. There was no evidence of a hazard difference between point source CEC-influenced sites and uninfluenced sites.

5.4.14.1 Hazard Brief for River Raisin

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

River Raisin (MI)

- **Overall:** Little evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard Observations:**
  - 22 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: DEET, Estrone, HHCB
  - *Effect Categories:* 6 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Minimal
  - 4 sampling sites
  - 1 sample per site
  - 4 total samples

Effect Categories and Emerging Contaminants

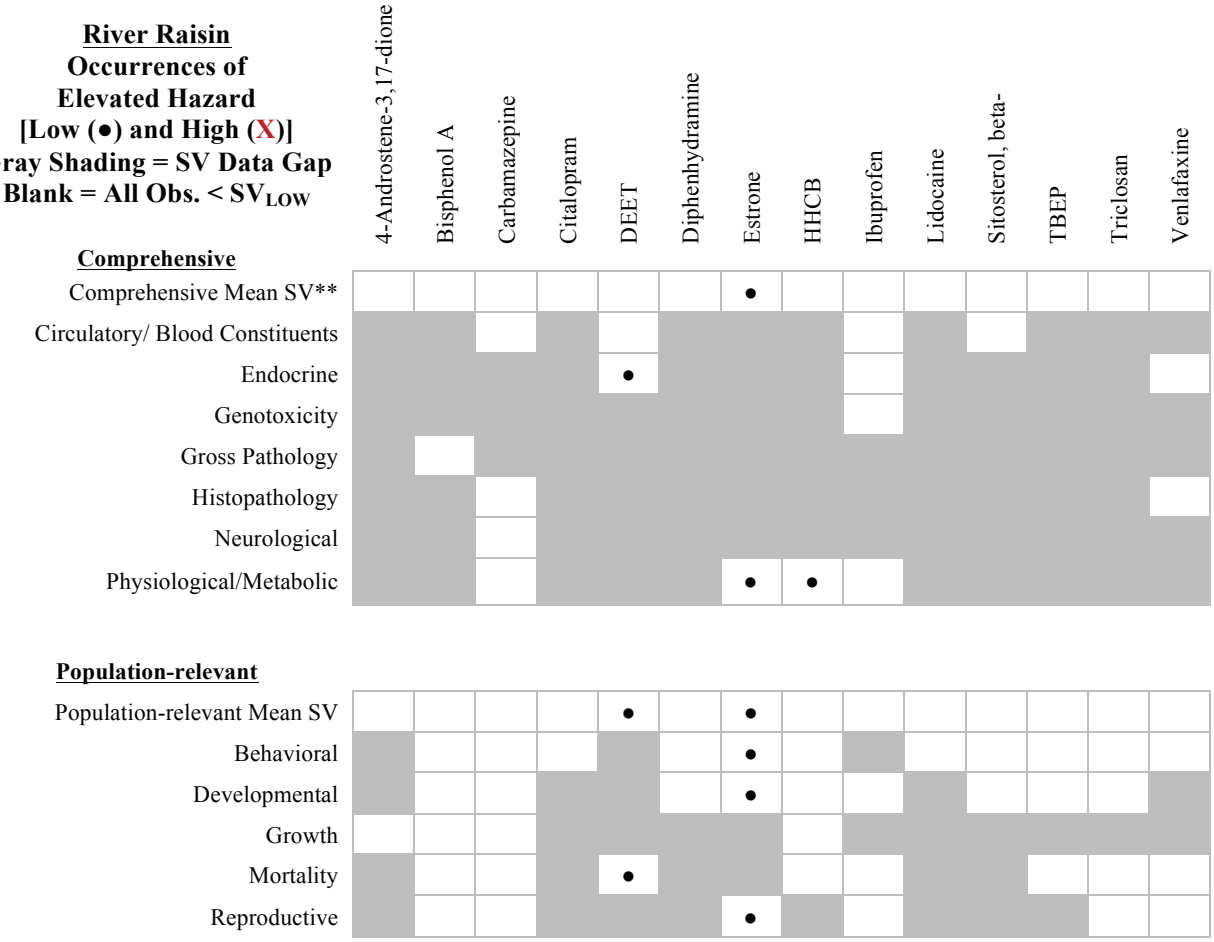
There were no exceedances of SV_{HIGH} at the River Raisin project location, possibly because only one water sample was collected at each site. However, there were SV_{LOW} exceedances in the following effect categories in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Endocrine, Physiological/Metabolic.

DEET accounted for 12 of the 22 SV_{LOW} exceedances, with the rest distributed evenly between estrone and HHCB.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-14

River Raisin
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

All four sites showed several SV_{LOW} exceedances, but there were no observations of high hazard.

River Raisin Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. RRR-1 (1)
2. RRR-2 (1)
3. **> RRR-3 (1)**
4. **> RRR-4 (1)**

5.4.14.2 Hazard Rankings for River Raisin

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	River Raisin Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.333	1.2-1.4	2
Physiological/Metabolic	1.313	1.2-1.4	2
Mortality	1.125	1.0-1.2	1
Population-relevant Mean SV	1.089	1.0-1.2	1
Reproductive	1.042	1.0-1.2	1
Developmental	1.028	1.0-1.2	1
Behavioral	1.023	1.0-1.2	1
Comprehensive Mean SV	1.018	1.0-1.2	1
Circulatory/Blood Constituents	1.000	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	River Raisin CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.600	1.4-1.6	3
Estrone	1.250	1.2-1.4	2
HHCB	1.143	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Ibuprofen	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Sitosterol, beta-	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	River Raisin Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> RRR-4	1.122	1.0-1.2	1
RRR-1	1.049	1.0-1.2	1
RRR-2	1.049	1.0-1.2	1
> RRR-3	1.049	1.0-1.2	1

5.4.14.3 Breadth of Information Indicating High Hazard at River Raisin

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no exceedances of any SV_{HIGH} at this project location (Table 5-1). However, the evidence suggesting an absence of high hazard is weak. Only one surface water sample per site was analyzed for CECs, and all samples were collected in the spring of 2012 during high flow (Attachment A, Table A-2). Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only a single USEPA STORET water quality sampling site with TSS data located in the lower River Raisin to represent the entire project location (Figure A2-14 in Attachment A2).

Additional potential for false negative hazard findings due to gaps in the fish ecotoxicity database is discussed above in Section 5.3.2.

5.4.14.4 River Raisin Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences in hazard scores between point source influenced and uninfluenced site groups at the River Raisin location. Likewise, no significant differences are observed when all hazard scores for each site - not just maximum or median scores - are included in the statistical analysis[68] (Attachment D). These negative statistical findings may be due to very small total sample size in both site groups (n = 2), an absence of SV_{HIGH} exceedances, and a proportional distribution of total SV_{LOW} exceedances between influenced and uninfluenced sites (see chart below).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the River Raisin location that have at least one observation of a SV_{LOW} exceedance, for any CEC and effect category; these numbers are provided for point source CEC-influenced versus uninfluenced site groups.

The River Raisin water sampling dataset is sparse, and so of limited use for addressing whether CEC point sources affect ecological hazard in fish at this location. Nevertheless, among CEC-influenced and uninfluenced sites, there were totals of 14 and 8 exceedances of SV_{LOW} values, respectively. The downstream most sampling site (RRR-4) had more than twice as many total SV_{LOW} exceedances than any of the other three sites, and is the only site with estrone SV_{LOW} exceedances. HHCB and DEET exceedances were evenly distributed between the point source influenced and uninfluenced site groups. There were no exceedances of SV_{HIGH} values.

River Raisin Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	0	0.0 (0/2)	0.0 (0/2)	14	1.0 (2/2)	1.0 (2/2)
Uninfluenced Sites	0	0.0 (0/2)	0.0 (0/2)	8	1.0 (2/2)	1.0 (2/2)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[68]Cautionary caveats regarding this approach are described in Attachment D.

5.4.14.5 River Raisin Hazard Maps

Since there were no exceedances of SV_{HIGH} values, no hazard maps were generated.

5.4.15 Swan Creek (OH)

Swan Creek is a major tributary of the Maumee River, draining over 530 km² (204 mi²) of predominantly agricultural and urban/residential areas in northeast Ohio as it flows eastward to its confluence with the river near downtown Toledo, OH. The project location on Swan Creek is limited to an urban reach near the confluence with the Maumee River, where CEC point sources influence each sampling site. All thirteen of the sampling sites were designated as CEC-'influenced' based on their downstream proximity to mapped CEC point sources, and each site was sampled one or two times for surface water CEC concentration measurements. Timing of sampling varied among sites at the Swan Creek project location; sampling occurred October 2010, April/May 2011, or September 2012.

Substantial evidence of CEC-related hazard to fish is present at this project location. Elevated hazard was observed in eight effect categories, including high hazard attributed to ibuprofen for genotoxicity, developmental, and reproductive effects, as well as exceedances of comprehensive mean SV_{HIGH} and population-relevant mean SV_{HIGH}. The breadth of ecotoxicity information brought to bear in observations of high hazard was 'broad' to 'robust' for ibuprofen SVs in each effect category except genotoxicity. There was a total of seven occurrences of high hazard (SV_{HIGH} exceedance), but there were 145 SV_{LOW} exceedances - attributable to six CECs in eight effect categories – with at least one observation of low hazard in each sampling event and at each site.

Although ibuprofen was the only CEC with observations of high hazard at this project location, DEET ranked highest among the effect categories for overall mean hazard to fish, followed by ibuprofen and estrone. Effect categories that ranked highest for hazard to fish were genotoxicity and endocrine effects; all other categories were assigned the lowest possible hazard rank of '1' (on a scale of 1 to 10).

No statistical analysis of hazard associated with point sources was conducted, since all sampling sites had been designated as point source CEC-influenced.

Some Key Points…

Swan Creek (OH)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard:**
  - 7 occurrences, involving 16% of sampling events, and 23% of sites
  - *CECs:* Ibuprofen
  - *Effect Categories*: Genotoxicity, Developmental, Reproductive
- **Low Hazard:**
  - 145 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs:* 6 of 14
  - *Effect Categories*: 8 of 12
- **Point Source Analysis:** No evaluation, all sites CEC-influenced
- **Exposure Dataset:** Substantial
  - 13 sampling sites
  - 1-3 samples per site
  - 19 total samples

5.4.15.1 Hazard Brief for Swan Creek

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

Swan Creek effect-specific hazard maps (Section 5.4.15.5) are provided for the following effect categories for which relatively high hazard (SV_{HIGH} exceedance) was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Population-relevant mean SV, Developmental, Reproductive
- Comprehensive: Comprehensive mean SV, Genotoxicity

The following additional effect categories showed exceedances of SV_{LOW} values:

- Population-relevant: Behavioral, Mortality
- Comprehensive: Circulatory/Blood Constituents, Endocrine, Physiological/ Metabolic

Among CECs evaluated, ibuprofen accounted for all of the high hazard observations at the Swan Creek project location.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-15.

Swan Creek
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**					●		●		X			●		
Circulatory/ Blood Constituents									●		●			
Endocrine					●				●					
Genotoxicity									X					
Gross Pathology														
Histopathology														
Neurological														
Physiological/Metabolic							●	●	●					
Population-relevant														
Population-relevant Mean SV					●		●		X					
Behavioral							●				●			
Developmental							●		X					
Growth														
Mortality					●				●					
Reproductive							●		X					

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

Five of the seven ibuprofen high hazard observations occurred at the SWC-08 site, in five different effect categories. Red-highlighted sites had at least one observation of high hazard:

Swan Creek Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> SWC-01 (1)**
2. **> SWC-02 (1)**
3. **> SWC-03 (1)**
4. **> SWC-04 (1)**
5. **> SWC-05 (1)**
6. **> SWC-06 (1)**
7. **> SWC-07 (2)**
8. **> SWC-08 (2)**
9. **> SWC-09 (3)**
10. **> SWC-10 (2)**
11. **> SWC-11 (2)**
12. **> SWC-12 (1)**
13. **> SWC-CP-8 (1)**

5.4.15.2 Hazard Rankings for Swan Creek

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Swan Creek Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Genotoxicity	2.000	1.8-2.0	5
Endocrine	1.438	1.4-1.6	3
Physiological/Metabolic	1.181	1.0-1.2	1
Mortality	1.165	1.0-1.2	1
Reproductive	1.131	1.0-1.2	1
Population-relevant Mean SV	1.128	1.0-1.2	1
Comprehensive Mean SV	1.119	1.0-1.2	1
Developmental	1.085	1.0-1.2	1
Behavioral	1.058	1.0-1.2	1
Circulatory/Blood Constituents	1.044	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Swan Creek - CEC Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.762	1.6-1.8	4
Ibuprofen	1.519	1.4-1.6	3
Estrone	1.410	1.4-1.6	3
Sitosterol, beta-	1.062	1.0-1.2	1
HHCB	1.020	1.0-1.2	1
TBEP	1.015	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (All sites designated as point source influenced)	Swan Creek Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
SWC-8	1.220	1.2-1.4	2
SWC-11	1.213	1.2-1.4	2
SWC-7	1.146	1.0-1.2	1
SWC-1	1.137	1.0-1.2	1
SWC-4	1.137	1.0-1.2	1
SWC-10	1.128	1.0-1.2	1
SWC-6	1.123	1.0-1.2	1
SWC-9	1.077	1.0-1.2	1
SWC-12	1.068	1.0-1.2	1
SWC-5	1.068	1.0-1.2	1
SWC-CP-8	1.061	1.0-1.2	1
SWC-2	1.055	1.0-1.2	1
SWC-3	1.055	1.0-1.2	1

5.4.15.3 Breadth of Information Indicating High Hazard at Swan Creek

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure:* A sufficient number of sampling sites are well-distributed along a large section of creek providing excellent coverage of this project location. However, the combination of very small sample sizes and the fact that CEC inputs in this system are due solely to intermittent CSO discharges contribute to the potential for false negatives for high hazard. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was low due to only two USEPA STORET water quality sampling sites with TSS data located in lower Swan Creek to represent the entire project location (Figure A2-15 in Attachment A2).
- *Ecotoxicity:* All observations of high hazard were related to ibuprofen exposure, with the following breadth of ecotoxicity information (see Tables 4-5 and 4-6):
  - o Ibuprofen
    - Broad - Population-relevant mean SV
    - Broad - Developmental
    - Broad - Reproductive
    - Broad - Comprehensive mean SV
    - Sparse - Genotoxicity

The evidence suggesting an absence of high hazard for other CECs and effect categories is weak. Most sampling sites had only one surface water sample, and no more than three samples per site were analyzed for CECs. Additional potential for false negative findings due to gaps in the fish ecotoxicity database are discussed in Section 5.3.2.

5.4.15.4 Swan Creek Point Source Analysis

All 13 sampling sites were identified as CEC-influenced by mapped WWTP or CSO point sources, so no CEC point source influenced-uninfluenced comparison was possible.

A summary of hazard occurrence at the 13 sites is provided in the chart below. Shown are fractions of samples and of sites at the Swan Creek location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having at least one SV_{LOW} exceedance.

Three samples with a total of seven SV_{HIGH} exceedances occurred at three different sites in the downstream half of the project location (SWC-7, SWC-8, and SWC-11), and all were attributed to ibuprofen. There was a total of 145 exceedances of various Effect-specific SV_{LOW} values, and all samples at all sites had at least one CEC that exceeded a SV_{LOW}. There were SV_{LOW} exceedances in one or more samples for DEET, estrone, HHCB, ibuprofen, β-sitosterol, and TBEP.

Swan Creek Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	7	0.16 (3/19)	0.23 (3/13)	145	1.0 (19/19)	1.0 (13/13)
Uninfluenced Sites	NA	NA	NA	NA	NA	NA

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.15.5 Swan Creek Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Population-relevant Mean SV Hazard

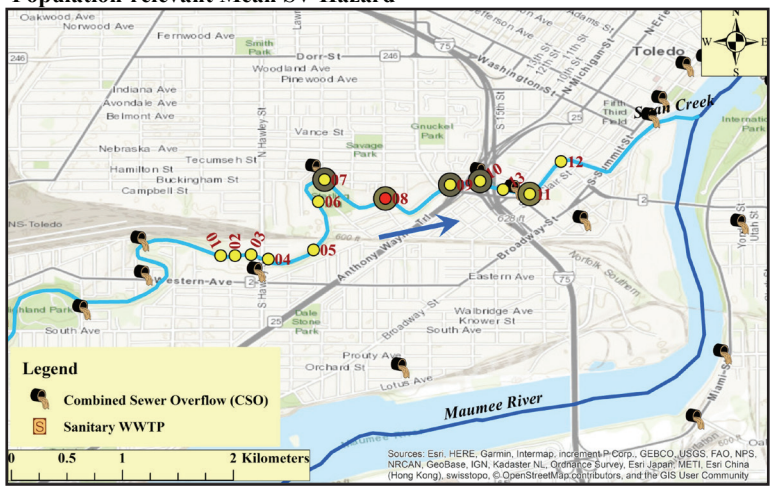


Figure 5-38. Population-relevant Mean SV Hazard Map for Swan Creek.

Reproductive Hazard

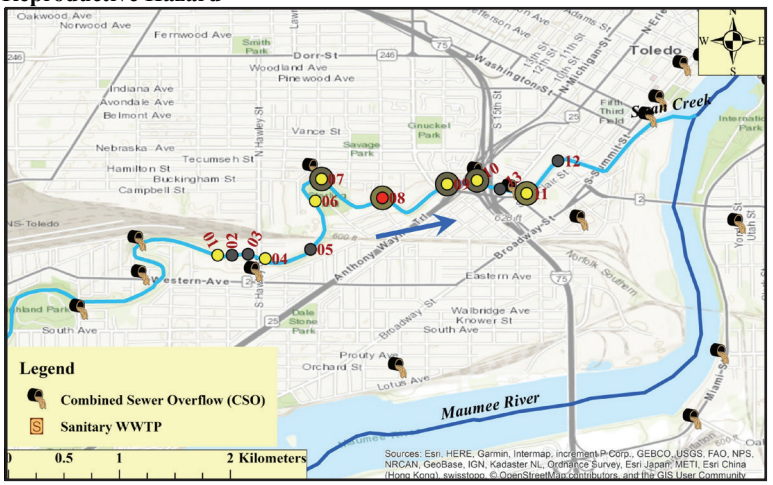


Figure 5-40. Reproductive Hazard Map for Swan Creek.

Developmental Hazard

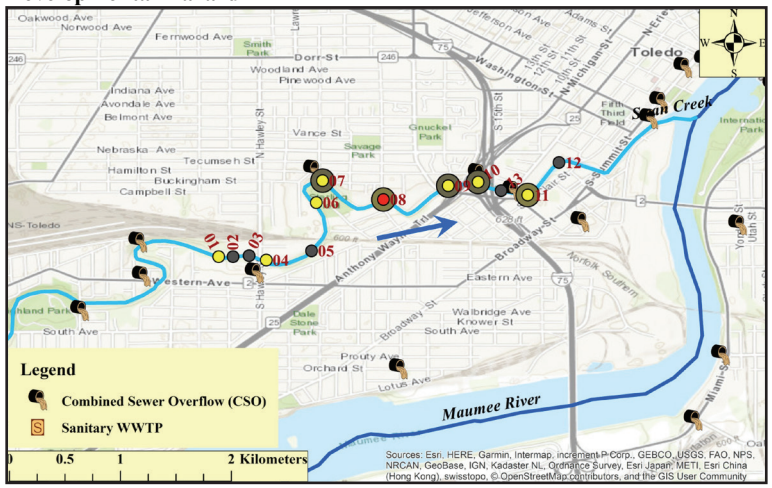


Figure 5-39. Developmental Hazard Map for Swan Creek.

KEY

Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate flow direction.

Swan Creek Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. > SWC-01 (1)
2. > SWC-02 (1)
3. > SWC-03 (1)
4. > SWC-04 (1)
5. > SWC-05 (1)
6. > SWC-06 (1)
7. >SWC-07 (2)
8. > SWC-08 (2)
9. > SWC-09 (3)
10. > SWC-10 (2)
11. > SWC-11 (2)
12. > SWC-12 (1)
13. > SWC-CP-8 (1)

Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

Comprehensive Mean SV Hazard

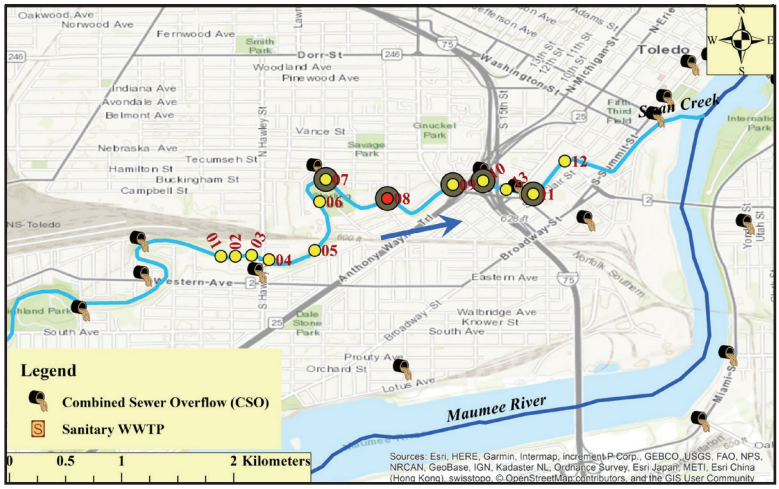


Figure 5-41. Comprehensive Mean SV Hazard Map for Swan Creek.

Genotoxicity Hazard

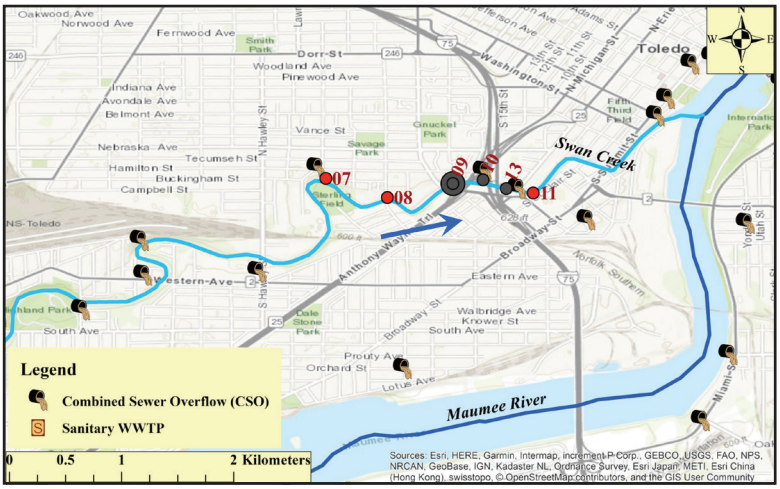


Figure 5-42. Genotoxicity Hazard Map for Swan Creek.

KEY

Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate flow direction.

Swan Creek Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. **> SWC-01 (1)**
2. **> SWC-02 (1)**
3. **> SWC-03 (1)**
4. **> SWC-04 (1)**
5. **> SWC-05 (1)**
6. **> SWC-06 (1)**
7. **>SWC-07 (2)**
8. **> SWC-08 (2)**
9. **> SWC-09 (3)**
10. **> SWC-10 (2)**
11. **> SWC-11 (2)**
12. **> SWC-12 (1)**
13. **> SWC-CP-8 (1)**

Hazard Score

- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

5.4.16 Maumee River (OH)

The Maumee River flows over 220 km (137 mi) northeastwardly from Indiana through northern Ohio to discharge into the southwestern corner of Lake Erie at Toledo, OH. The project location is in the lowermost ~50 km of the river, with land use shifting from upriver agricultural areas to the heavily urban/industrial area near its mouth. Sixteen of the 20 sampling sites were designated as CEC-'influenced', and four 'uninfluenced', based on their downstream proximity to mapped CEC point sources. There was considerable variation in the timing of sampling between sampling sites. Sites were sampled between one and five times for surface water CEC concentration measurements, during one or more (up to three) of the following sampling events: April or May 2011, or April or September 2012.

The preponderance of evidence indicates that CEC-related impacts to fish occur in the lower Maumee River. The incidence of SV_{HIGH} exceedance is 40 in the lower ~10 km of the river, below the confluence with Swan Creek. High hazard occurred at 45% of the project location sampling sites and 45% of sampling events, involving high potential for mortality as well as reproductive, developmental, endocrine, genotoxicity, and physiological/metabolic effects in fish. High hazards are associated with DEET, estrone, ibuprofen and venlafaxine exposures. The strength of ecotoxicity information contributing to observations of high hazard was greatest for developmental and reproductive effects associated with ibuprofen exposures and moderately strong for DEET-related endocrine effects.

Low hazard (SV_{LOW} exceedance) - indicating a possibility of impacts - was ubiquitous, with an incidence of 721 and occurring at all 20 sampling sites and in 40/41 sampling events, averaging 36 SV_{LOW} exceedances per site. At this location, low hazard occurred for every effect category except Gross Pathology and was associated with each CEC except lidocaine. The breadth of implicated effects and the very high incidence and broad spatial distribution of SV_{LOW} exceedances suggest that ecologically significant CEC-related impacts to fish are pervasive in the Maumee River project location.

The highest potential for impacts (as indicated by ranking overall mean hazard) was observed with neurological effects, followed by histopathology and endocrine effects; the highest ranked population-relevant hazards were associated with reproductive and behavioral effects. DEET ranked highest among CECs for overall mean hazard to fish at this location, followed by carbamazepine, venlafaxine and citalopram. Eight of the top nine hazard-ranked sampling sites had been designated as point source CEC-influenced sites.

Statistical analysis of site-specific maximum a and median hazard as related to point sources indicated significantly higher ($p < 0.1$) overall hazard in point source CEC-influenced sites than in uninfluenced

Some Key Points…

Maumee River (OH)

- **Overall:** Clear and convincing evidence of hazards to fish
- **High Hazard:**
  - 40 occurrences, involving 44% of sampling events, and 45% of sites
  - *CECs*: DEET, Estrone, Ibuprofen, Venlafaxine
  - *Effect Categories:* Endocrine, Genotoxicity, Physiological/Metabolic, Developmental, Mortality, Reproductive
- **Low Hazard**:
  - 722 occurrences, involving 98% of sampling events, and 100% of sites
  - *CECs*: 13 of 14
  - *Effect Categories*: 11 of 12
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Robust
  - 20 sampling sites
  - 1-5 sample per site
  - 41 total samples

sites for two comprehensive effect categories and four population-relevant effect categories; in no case was overall hazard higher above mapped point sources than below. Qualitatively, there were more than five times as many SV_{HIGH} exceedances per site at point source CEC-influenced sites than at uninfluenced sites.

5.4.16.1 Hazard Brief for Maumee River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

Maumee River effect-specific hazard maps (Section 5.4.16.5) are provided for the following effect categories for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Endocrine, Genotoxicity, Physiological/Metabolic.

The following additional effect categories showed exceedances of SV_{LOW} values:

- Population-relevant: Population-relevant mean SV, Behavioral, Growth
- Comprehensive: Circulatory/Blood Constituents, Histopathology, Neurological

Among CECs evaluated, the pharmaceuticals ibuprofen and venlafaxine accounted for most of the observations of high hazard at the Maumee River project location, with DEET and estrone also contributing. Gross pathology was the only effect category with no exceedances of SV_{HIGH} or SV_{LOW}.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-16.

Maumee River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●	●	●	●	●	●	X	●	●		●	●	●	X
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X				●					X
Genotoxicity									X					
Gross Pathology														
Histopathology			●											●
Neurological			●											
Physiological/Metabolic			●				X	●	●					
Population-relevant														
Population-relevant Mean SV	●	●	●	●	●	●	●		●			●	●	●
Behavioral			●	●		●	●				●		●	●
Developmental		●	●				●		X			●	●	
Growth		●												
Mortality					●				●				●	X
Reproductive		●	●				●		X				●	●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

Among the 20 sampling sites at Maumee River, high hazard was observed in at least one sample at nine sites (red-highlighted in list below). Seven of the high hazard sites were identified as influenced by mapped point sources. An additional 11 sites showed exceedances of at least one SV_{LOW} value (Attachment B, Table B-16).

Maumee River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. CLARKO-6 (2)	**6. > MAU-DS-PB-WWTP (2)**	**11. > MAU-N-EW-PB-WWTP (1)**	**16. > MAU-WAT-1 (1)**
2. > MAU-BVP (1)	**7. > MAU-DS-WWTP (1)**	12. MAU-PB-WWTP (1)	**17. > MAU-WAT-2 (1)**
3. > MAU-CSO-68 (1)	**8. > MAU-Distal-DS-WWTP (5)**	**13. > MAU-TOL-WWTP (1)**	**18. > MX-WWTP (5)**
4. > MAU-CSO-9 (2)	**9. > MAU-GR-1 (1)**	14. MAU-US-CSO-9 (1)	**19. > SWANC-5 (5)**
5. > MAU-DS-CSO-9 (1)	**10. > MAU-LASALLE (1)**	15. MAU-US-WWTP (5)	**20. > TOLEDO-7 (3)**

5.4.16.2 Hazard Rankings for Maumee River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Maumee River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Neurological	1.760	1.6-1.8	4
Histopathology	1.573	1.4-1.6	3
Endocrine	1.559	1.4-1.6	3
Physiological/Metabolic	1.309	1.2-1.4	2
Circulatory/Blood Constituents	1.284	1.2-1.4	2
Reproductive	1.256	1.2-1.4	2
Comprehensive Mean SV	1.245	1.2-1.4	2
Behavioral	1.215	1.2-1.4	2
Mortality	1.198	1.0-1.2	1
Population-relevant Mean SV	1.154	1.0-1.2	1
Devvelopmental	1.132	1.0-1.2	1
Genotoxicity	1.120	1.0-1.2	1
Growth	1.003	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Maumee River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.722	1.6-1.8	4
Carbamazepine	1.552	1.4-1.6	3
Venlafaxine	1.428	1.4-1.6	3
Citalopram	1.390	1.2-1.4	2
Triclosan	1.193	1.0-1.2	1
Diphenhydramine	1.157	1.0-1.2	1
Sitosterol, beta-	1.149	1.0-1.2	1
HHCB	1.090	1.0-1.2	1
Estrone	1.065	1.0-1.2	1
Ibuprofen	1.061	1.0-1.2	1
TBEP	1.050	1.0-1.2	1
4-Androstene-3,17-dione	1.015	1.0-1.2	1
Bisphenol A	1.011	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Maumee River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> TOLEDO-7	1.482	1.4-1.6	3
> MAU-LASALLE	1.476	1.4-1.6	3
> MAU-TOL-WWTP	1.402	1.4-1.6	3
> MX-WWTP	1.371	1.2-1.4	2
> MAU-DS-WWTP	1.305	1.2-1.4	2
> MAU-Distal-DS-WWTP	1.305	1.2-1.4	2
> MAU-DS-PB-WWTP	1.293	1.2-1.4	2
MAU-PB-WWTP	1.280	1.2-1.4	2
> MAU-N-EW-PB-WWTP	1.256	1.2-1.4	2
> MAU-BVP	1.195	1.0-1.2	1
MAU-US-WWTP	1.185	1.0-1.2	1
> MAU-CSO-9	1.152	1.0-1.2	1
> MAU-CSO-68	1.146	1.0-1.2	1
> MAU-DS-CSO-9	1.146	1.0-1.2	1
MAU-US-CSO-9	1.146	1.0-1.2	1
> SWANC-5	1.093	1.0-1.2	1
CLARKO-6	1.079	1.0-1.2	1
> MAU-WAT-1	1.061	1.0-1.2	1
> MAU-GR-1	1.049	1.0-1.2	1
> MAU-WAT-2	1.049	1.0-1.2	1

5.4.16.3 Breadth of Information Indicating High Hazard at Maumee River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Spatial coverage of this project location by 20 sampling sites is excellent, with adequate representation of point source influenced and uninfluenced sites. However, all but two of the 20 sites were sampled only in one month of one year and all but five of the sites had ≤2 surface water samples analyzed (12 were sampled only once). Greater numbers of samples at each site representing various seasons and times of day may have resulted in significantly more observations of high hazard. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was high due to numerous USEPA STORET water quality sampling sites with TSS data distributed throughout the Maumee River project location (Figure A2-16 in Attachment A2).
- *Ecotoxicity:* Among observations of high hazard for ibuprofen, DEET, estrone and venlafaxine, breadth of applicable ecotoxicity information was as follows (Tables 4-5 and 4-6):
  - Ibuprofen
    - Broad - Developmental
    - Broad – Reproductive
    - Sparse - Genotoxicity
  - DEET
    - Moderate - Endocrine
  - Estrone
    - Limited - Comprehensive mean SV
    - Sparse - Physiological/Metabolic
  - Venlafaxine
    - Limited - Comprehensive mean SV
    - Sparse - Endocrine
    - Sparse - Mortality

5.4.16.4 Maumee River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4); the following chart provides the results summary.

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
4-Androstene-3,17-dione	Comprehensive mean SV	N	Y
Citalopram	Comprehensive mean SV	Y	Y
	Population-relevant mean SV	Y	Y
	Behavioral	Y	Y
Diphenhydramine	Comprehensive mean SV	Y	Y
Estrone	Comprehensive mean SV	N	Y
	Population-relevant mean SV	N	Y
	Behavioral	N	Y
	Developmental	N	Y
	Physiologic/Metabolic	N	Y
	Reproductive	N	Y
HHCB	Physiological/Metabolic	Y	Y

When *all* hazard scores for each site in the Maumee River location, not just maximum or median scores, are included in the statistical analysis[69], additional significant differences ($p < 0.1$) are observed for behavioral effects associated with citalopram exposure and for the comprehensive mean SV associated with HHCB exposure (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Maumee River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Maumee River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	38	0.5 (16/32)	0.44 (7/16)	602	0.97 (31/32)	1.0 (16/16)
Uninfluenced Sites	2	0.22 (2/9)	0.5 (2/4)	120	1.0 (9/9)	1.0 (4/4)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There was a total of 38 exceedances of various effect-specific SV_{HIGH} values for DEET, estrone, ibuprofen and venlafaxine, in 16 of the 32 samples collected at point source CEC-influenced sampling sites; all of these SV_{HIGH} exceedances occurred at seven of the 16 sites – six of which occur in the middle or lower reaches of the project location. In contrast, among the four uninfluenced sites, there was a total of only two exceedances of a SV_{HIGH} – both for DEET – in two of the nine samples.

There was a total of 602 exceedances of various effect-specific SV_{LOW} values, at least one of which occurred in each sample at each of the 16 CEC-influenced sites, with 12 of the 14 CECs considered in this EHA (all but ibuprofen and lidocaine) represented among the SV_{LOW} exceedances. At uninfluenced sites, there was a total of 120 exceedances of various effect-specific SV_{LOW} values for 4-androstene-3,17-dione, carbamazepine, DEET, estrone, β-sitosterol and venlafaxine, with at least one SV_{LOW} exceedance at all four sites uninfluenced by WWTP or CSO point sources.

[69]Cautionary caveats regarding this approach are described in Attachment D.

5.4.16.5 Maumee River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

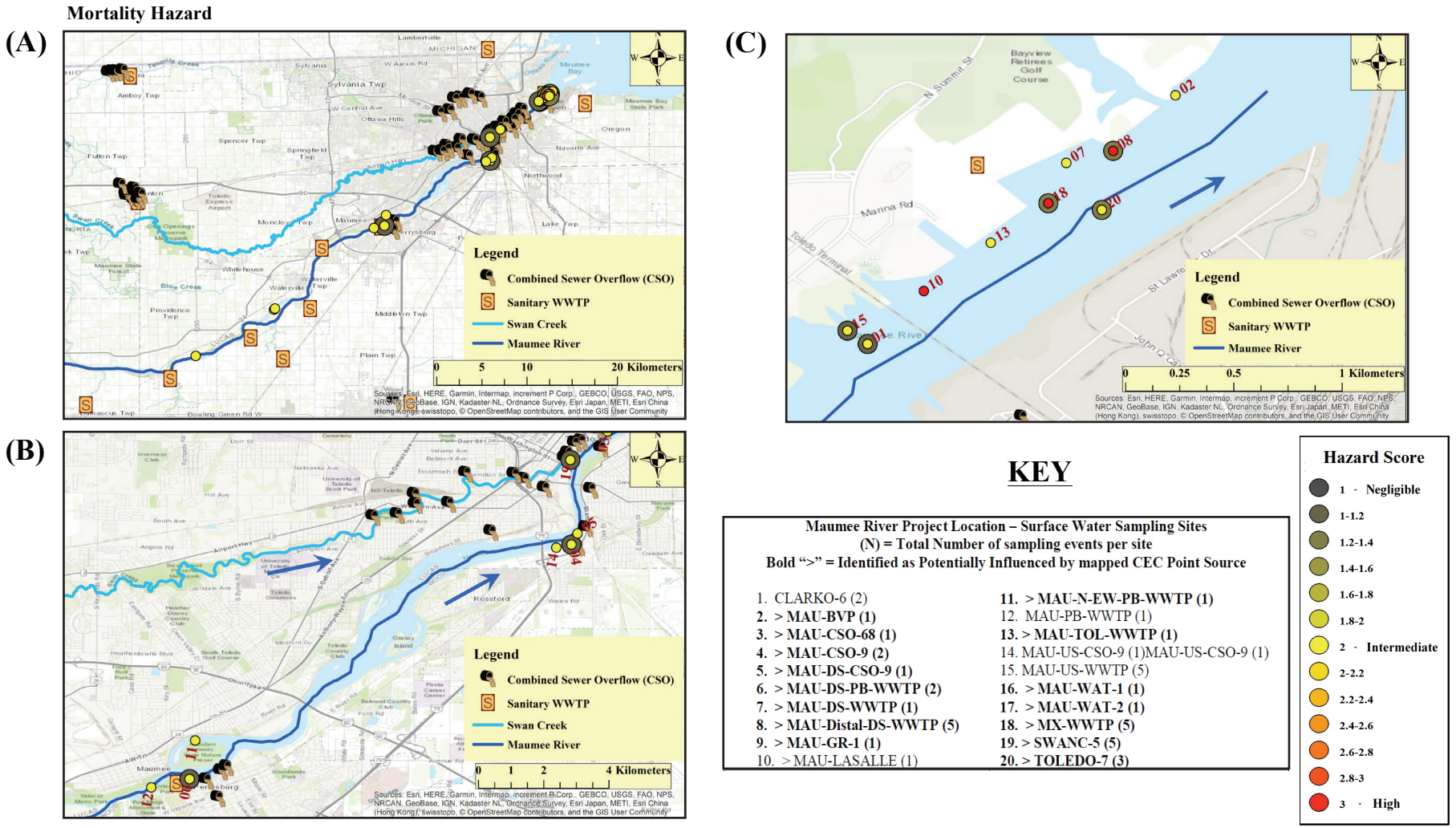


Figure 5-43. Mortality Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

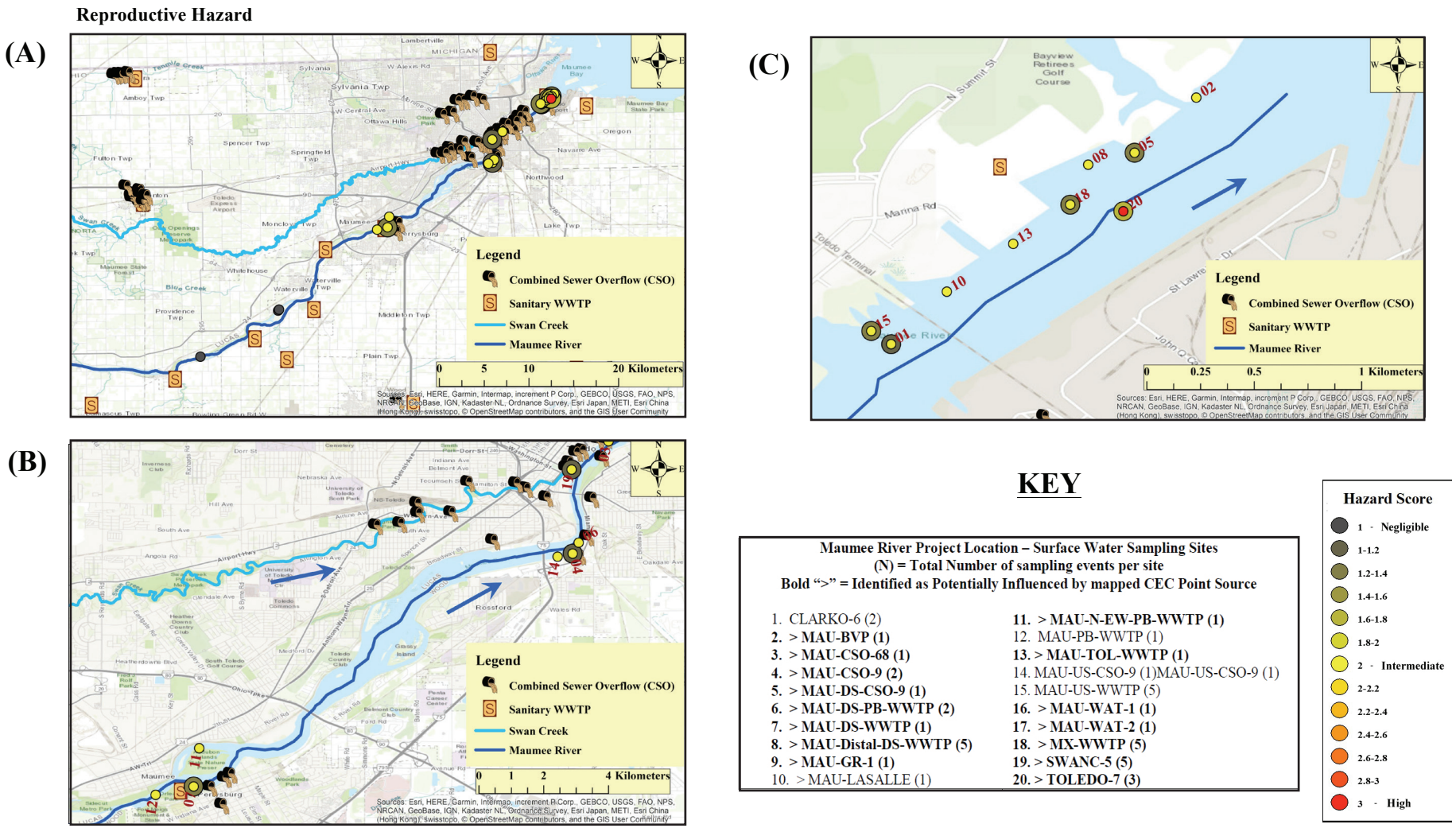


Figure 5-44. Reproductive Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

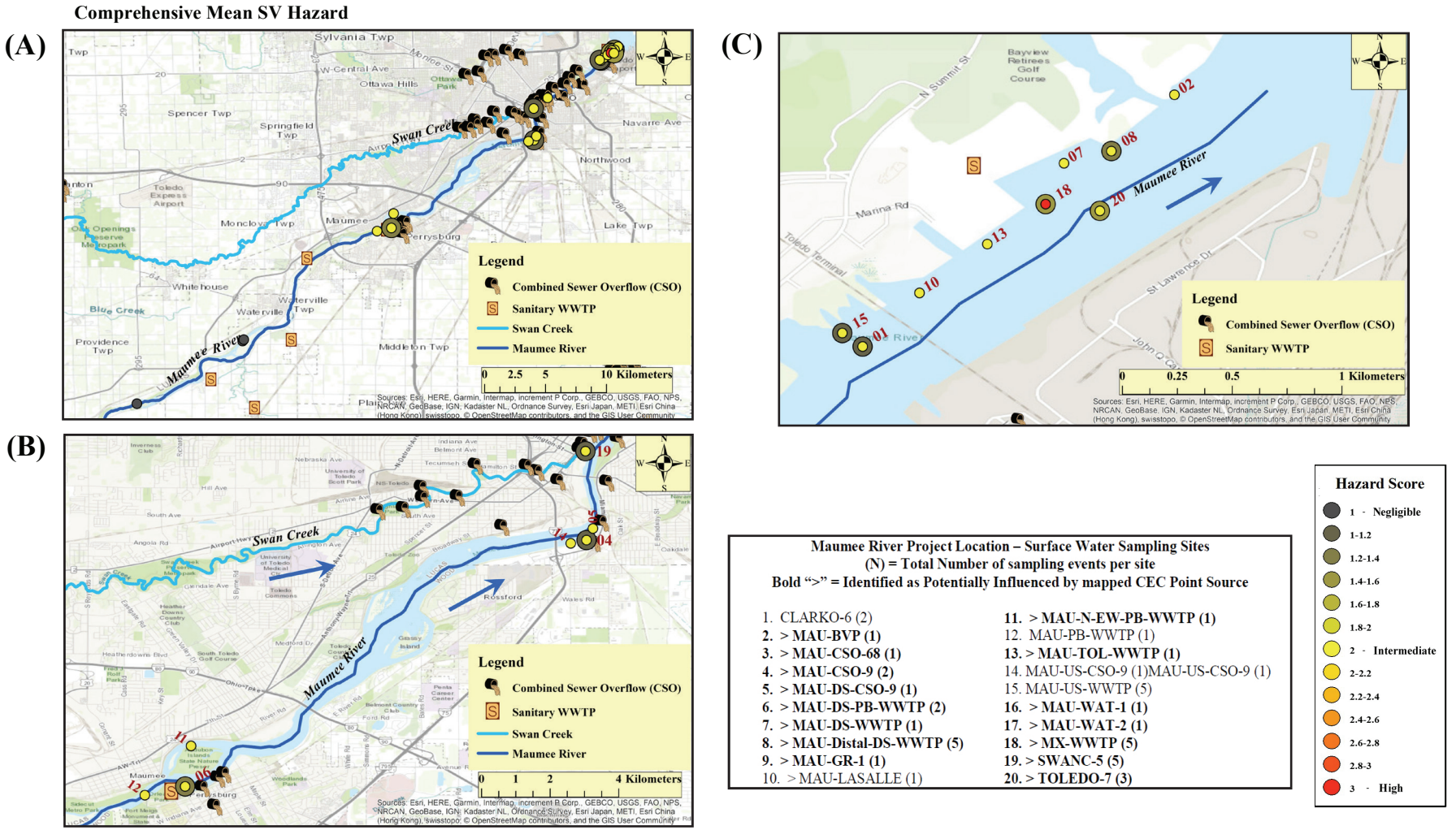
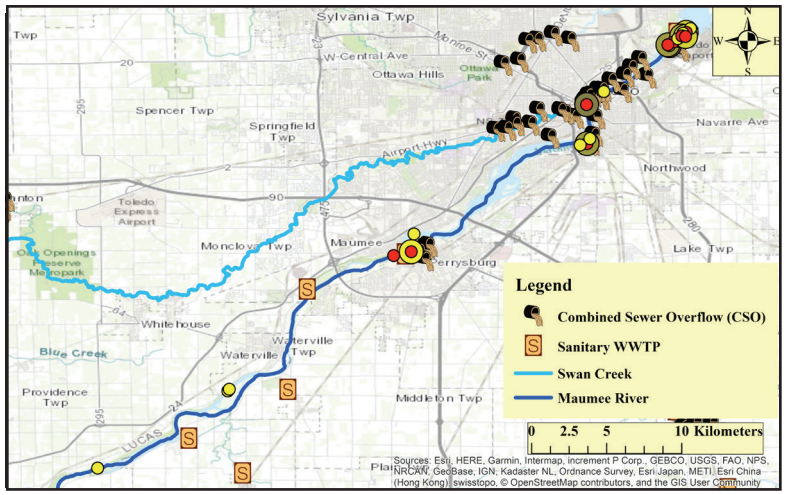


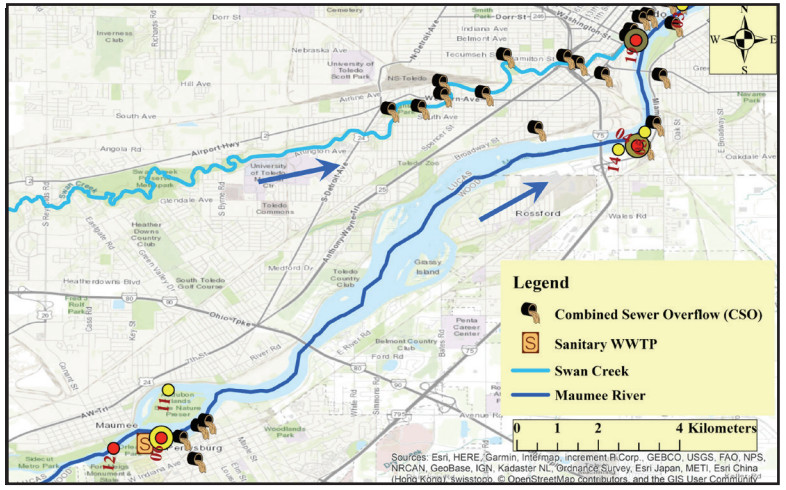
Figure 5-45. Comprehensive Mean SV Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

Endocrine Hazard

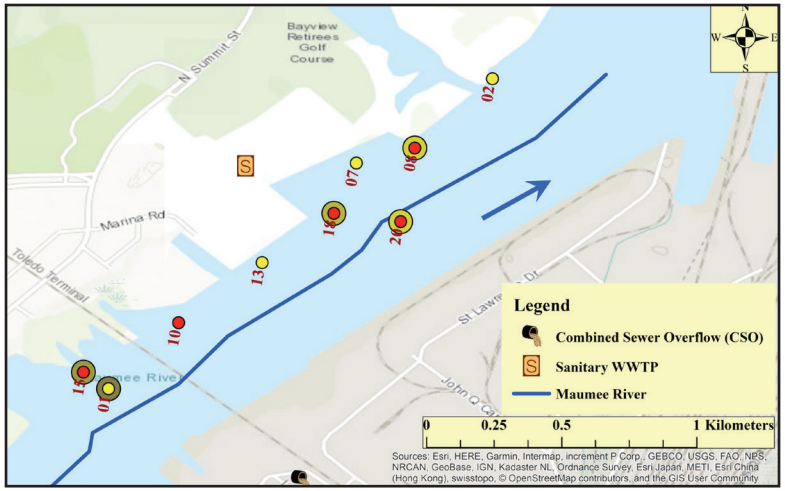
(A)



(B)



(C)



KEY

Maumee River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. CLARKO-6 (2)
2. **> MAU-BVP (1)**
3. **> MAU-CSO-68 (1)**
4. **> MAU-CSO-9 (2)**
5. **> MAU-DS-CSO-9 (1)**
6. **> MAU-DS-PB-WWTP (2)**
7. **> MAU-DS-WWTP (1)**
8. **> MAU-Distal-DS-WWTP (5)**
9. **> MAU-GR-1 (1)**
10. > MAU-LASALLE (1)
11. **> MAU-N-EW-PB-WWTP (1)**
12. MAU-PB-WWTP (1)
13. **> MAU-TOL-WWTP (1)**
14. MAU-US-CSO-9 (1)MAU-US-CSO-9 (1)
15. MAU-US-WWTP (5)
16. **> MAU-WAT-1 (1)**
17. **> MAU-WAT-2 (1)**
18. **> MX-WWTP (5)**
19. **> SWANC-5 (5)**
20. **> TOLEDO-7 (3)**

Hazard Score
- 1 - Negligible
- 1-1.2
- 1.2-1.4
- 1.4-1.6
- 1.6-1.8
- 1.8-2
- 2 - Intermediate
- 2-2.2
- 2.2-2.4
- 2.4-2.6
- 2.6-2.8
- 2.8-3
- 3 - High

Figure 5-46. Endocrine Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

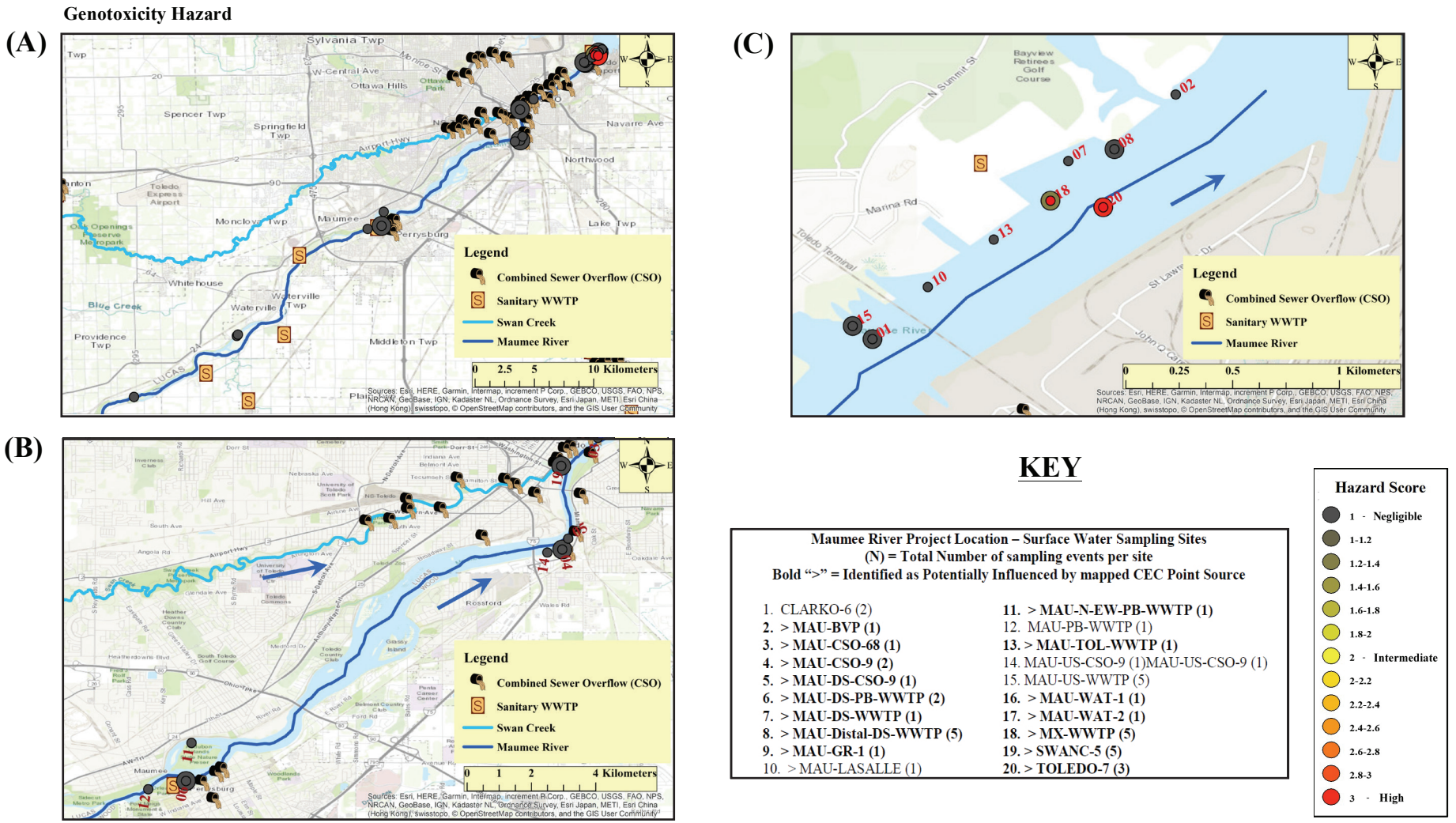


Figure 5-47. Genotoxicity Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

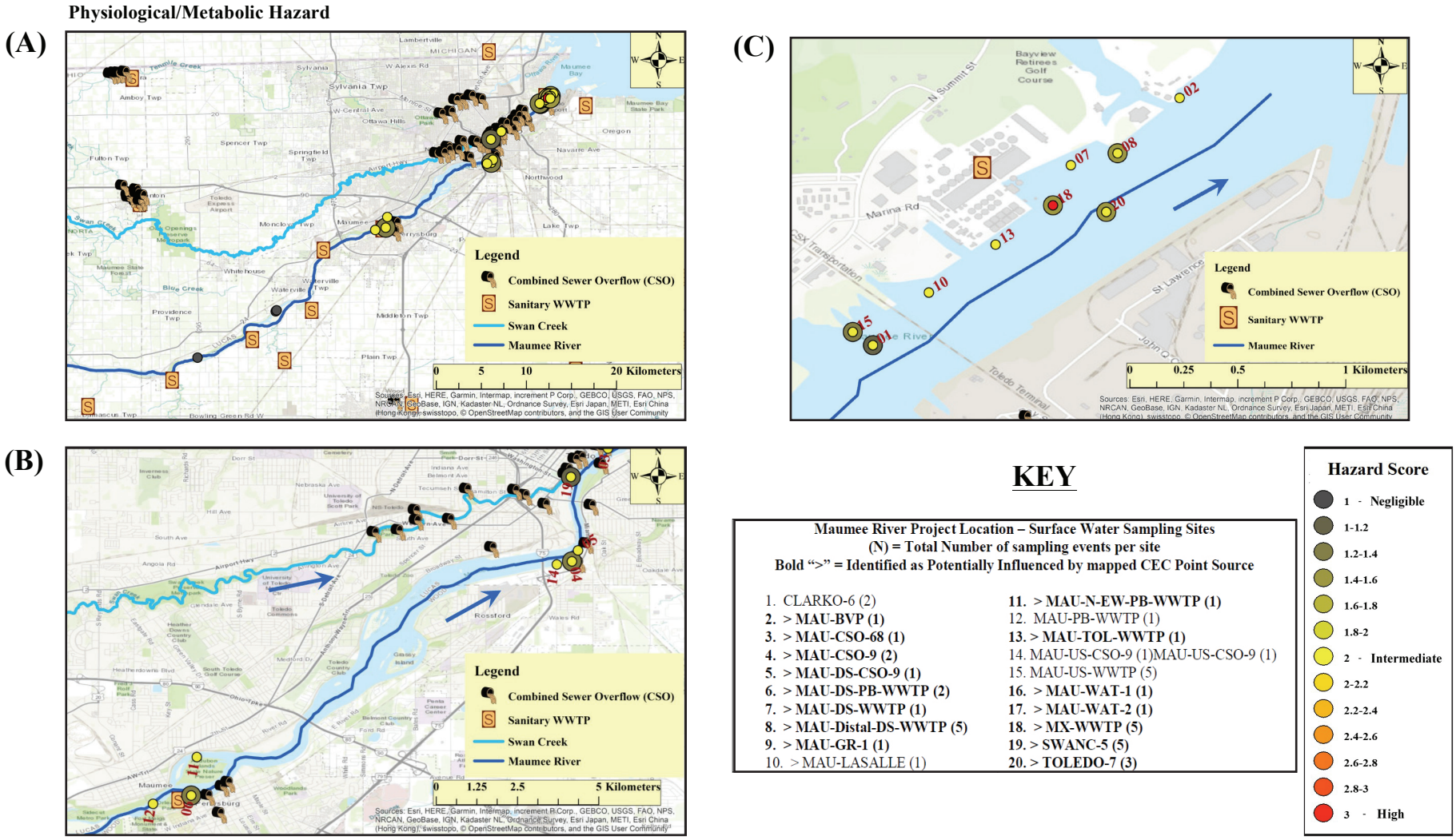


Figure 5-48. Physiological/Metabolic Hazard Map for the Maumee River. (A) Entire project location; (B) Middle Maumee River; (C) Lower Maumee River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. A small dot not paired with a large circle indicates sample size = 1. Arrows indicate river flow direction.

5.4.17 Cuyahoga River (OH)

The Cuyahoga River flows 137 km (85 miles) from its headwaters to the south shore of Lake Erie at Cleveland, OH, draining a 2100 km^2 (810 mi^2) watershed with mixed agricultural and urban/industrial inputs. The project location focuses on the lower ~60 km of the river main stem, with an additional upstream sampling site in a tributary impoundment. Three of the five sampling sites were designated as CEC-'influenced' and two 'uninfluenced' based on their downstream proximity to mapped WWTP or CSO point sources. Between five and eight samples were collected at the sites; each site was sampled at least once during each of the following sampling events: June 2013, and April, May and August 2014.

There is clear and convincing evidence that CEC exposures significantly impact fish in the lower Cuyahoga River. High hazard (SV_{HIGH} exceedance) was observed 33 times for an average occurrence rate of 11 exceedances per site at the three downstream-most sites - including high potential for mortality in fish from venlafaxine exposure, as well as high potential for endocrine, histopathology, and physiological/metabolic effects from venlafaxine, DEET, and estrone exposures. Over half of the 28 sampling events had at least one SV_{HIGH} exceedance. Out of a total of 2044 hazard scores generated for this project location, 700 (more than 1/3) indicated low hazard (SV_{LOW} exceedance), which involved all five sampling sites, 27/28 sampling events, all effect categories except Genotoxicity, and all CECs scored for hazard at this location except lidocaine.

At the Cuyahoga River location, five of the six effect categories that ranked highest for overall mean hazard were comprehensive effects, with endocrine hazard at the top. Among population-relevant effect categories, reproductive effects ranked highest for hazard to fish. Venlafaxine ranked highest among CECs for overall hazard to fish, followed by DEET and carbamazepine.

There were no statistically significant differences ($p < 0.1$) in site-specific maximum or median hazard scores between point source CEC-influenced and uninfluenced site groups, likely due to high incidence of elevated hazard (SV_{HIGH} and/or SV_{LOW} exceedances) throughout this location. The occurrence of hazard at sites distant or upstream from mapped WWTPs or CSOs suggests the presence of non-point CEC sources and/or additional point sources in this system.

Some Key Points…

Cuyahoga River (OH)

- **Overall:** Clear and convincing evidence of hazards to fish
- **High Hazard:**
  - 33 occurrences, involving 54% of sampling events, and 60% of sites
  - *CECs*: DEET, Estrone, Venlafaxine
  - *Effect Categories*: Endocrine, Histopathology, Physiological/Metabolic, Mortality
- **Low Hazard:**
  - 700 occurrences, involving 96% of sampling events, and 100% of sites
  - *CECs*: 12 of 13 (no Ibuprofen data)
  - *Effect Categories*: 11 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure Dataset:** Substantial
  - 5 sampling sites
  - 5-8 samples per site
  - 28 total samples

5.4.17.1 Hazard Brief for Cuyahoga River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

Cuyahoga River effect-specific hazard maps (Section 5.4.17.5) are provided for the following effect categories for which relatively high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Mortality
- Comprehensive: Comprehensive mean SV, Endocrine, Histopathology, Physiological/Metabolic.

The following additional effect categories showed exceedances of SV_{LOW} values:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Growth, Reproductive
- Comprehensive: Circulatory/Blood Constituents, Gross Pathology, Neurological

Among CECs evaluated, venlafaxine accounted for the greatest diversity of high hazard observations at the Cuyahoga River project location, with DEET and estrone also contributing. All CECs except lidocaine had exceedances of a SV_{LOW} at this project location.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-17.

Cuyahoga River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dior	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●	●	●	●	●	●	●	●			●	●	●	X
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									X
Genotoxicity														
Gross Pathology		●												
Histopathology			●											X
Neurological			●											
Physiological/Metabolic			●				X	●						
Population-relevant														
Population-relevant Mean SV	●	●	●	●	●	●	●					●	●	●
Behavioral			●	●		●	●	●			●		●	●
Developmental		●	●			●	●					●	●	
Growth		●												
Mortality					●								●	X
Reproductive		●	●				●						●	●

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

High hazard was observed at three of the five sampling sites (red-highlighted in the list below), including sites identified as both influenced and uninfluenced by mapped point sources. Concentrations of CECs at CUY-3-WWTP resulted in high hazard observations in five effect categories. Hazard attenuation with downstream distance from a point source is evident between CUY-3-WWTP and CUY-2-BOLANZ in the hazard maps below, with the exception of endocrine hazard.

Cuyahoga River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. CUY-1-ROCK (5)
2. CUY-2-BOLANZ (5)
3. **> CUY-3-WWTP (8)**
4. **> CUY-4-UP (5)**
5. **> CUY-5-LaDUE (5)**

5.4.17.2 Hazard Rankings for Cuyahoga River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[70]
Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Cuyahoga River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	2.150	2.0-2.2	6
Neurological	1.800	1.6-1.8	4
Histopathology	1.793	1.6-1.8	4
Physiological/Metabolic	1.677	1.6-1.8	4
Reproductive	1.447	1.4-1.6	3
Circulatory/Blood Constituents	1.447	1.4-1.6	3
Comprehensive Mean SV	1.388	1.2-1.4	2
Mortality	1.289	1.2-1.4	2
Behavioral	1.276	1.2-1.4	2
Population-relevant Mean SV	1.234	1.2-1.4	2
Developmental	1.199	1.0-1.2	1
Gross Pathology	1.025	1.0-1.2	1
Growth	1.019	1.0-1.2	1

Emerging Contaminants[71]
CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Cuyahoga River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Venlafaxine	1.853	1.8-2.0	5
DEET	1.790	1.6-1.8	4
Carbamazepine	1.551	1.4-1.6	3
Estrone	1.356	1.2-1.4	2
Citalopram	1.335	1.2-1.4	2
Sitosterol, beta-	1.221	1.2-1.4	2
Triclosan	1.220	1.2-1.4	2
HHCB	1.209	1.2-1.4	2
Diphenhydramine	1.181	1.0-1.2	1
TBEP	1.141	1.0-1.2	1
Bisphenol A	1.068	1.0-1.2	1
4-Androstene-3,17-dione	1.050	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

[70]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.
[71]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Cuyahoga River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> CUY-3-WWTP	1.576	1.4-1.6	3
CUY-2-BOLANZ	1.407	1.4-1.6	3
CUY-1-ROCK	1.373	1.2-1.4	2
> CUY-4-UP	1.286	1.2-1.4	2
> CUY-5-LaDUE	1.079	1.0-1.2	1

5.4.17.3 Breadth of Information Indicating High Hazard at Cuyahoga River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Spatial coverage of the project location by sampling sites was poor, with only five sites distributed over approximately 100 km of river and omitting upstream-downstream sampling at numerous WWTPs (Figure A2-17, Attachment A2). However, sample sizes and temporal coverage at each of the five sites was good – a total of five to eight samples were collected at various times of day in four different months over a two-year period (Attachment A1, Table A-2). Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was high due to numerous USEPA STORET water quality sampling sites with TSS data distributed throughout the Cuyahoga River project location (Figure A2-17 in Attachment A2).
- *Ecotoxicity*: Among observations of high hazard for DEET, estrone and venlafaxine, breadth of applicable ecotoxicity information was as follows (see Tables 4-5 and 4-6):
  - o DEET
    - Moderate – Endocrine
    - Sparse - Histopathology
    - Sparse - Mortality
  - o Estrone
    - Sparse - Physiological/Metabolic
  - o Venlafaxine
    - Sparse - Endocrine

5.4.17.4 Cuyahoga River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences observed, which may be due to small numbers of sites in both groups, and the presence of SV_{HIGH} exceedances in both groups. However, when *all* hazard scores for each site in the Cuyahoga River location, not just maximum or median scores, are included in the statistical analysis[72], additional significant differences ($p < 0.1$) are observed for the following CEC-effect category combinations (Attachment D):

- Carbamazepine – Population-relevant mean SV
- Diphenhydramine – Population-relevant mean SV, Behavioral
- β-Sitosterol – Behavioral, Circulatory/Blood Constituents
- Venlafaxine - Mortality

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Cuyahoga River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Cuyahoga River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	25	0.44 (8/18)	0.33 (1/3)	428	0.94 (17/18)	1.0 (3/3)
Uninfluenced Sites	8	0.70 (7/10)	1.0 (2/2)	272	1.0 (10/10)	1.0 (2/2)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[72]Cautionary caveats regarding this approach are described in Attachment D.

All 25 observations of various effect-specific SV_{HIGH} exceedances in the CEC-influenced site group occurred at one site (CUY-3-WWTP) over eight sampling events; at least one exceedance of a SV_{HIGH} value for DEET, estrone, and venlafaxine was observed. Among these, most were observations of exceedances of endocrine SV_{HIGH} values for DEET and venlafaxine, while a single estrone exceedance of the physiological/metabolic SV_{HIGH} value was observed. High endocrine hazard at the two "uninfluenced" sites (CUY-1-ROCK and CUY-2-BOLANZ) was attributed to DEET and venlafaxine, and contributed to the absence of a significant difference in endocrine hazard scores. Both of these sites were distally downstream (>4km) of the nearest mapped CEC point source, and so were classified as uninfluenced by CECs from WWTPs or CSOs for the purpose of grouping for statistical testing. The high endocrine hazard at these sites may have been due to the combined inputs from known point sources far upstream in the river, an unmapped point source, non-point sources such as aerial deposition (DEET), and, for CUY-1-ROCK, inputs from a heavily CEC-contaminated tributary (Tinkers Creek).

There were 428 exceedances of various effect-specific SV_{LOW} values in 17 of 18 sampling events conducted across the three CEC-influenced sites, including at least one exceedance for 12 of the 14 CECs (except ibuprofen and lidocaine) and for each effect category but growth and genotoxicity. There were also a large number (n = 272) and high diversity (11 of 14 CECs and all but two effect categories) of SV_{LOW} exceedances at the two sites "uninfluenced" by WWTPs and CSOs.

5.4.17.5 Cuyahoga River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

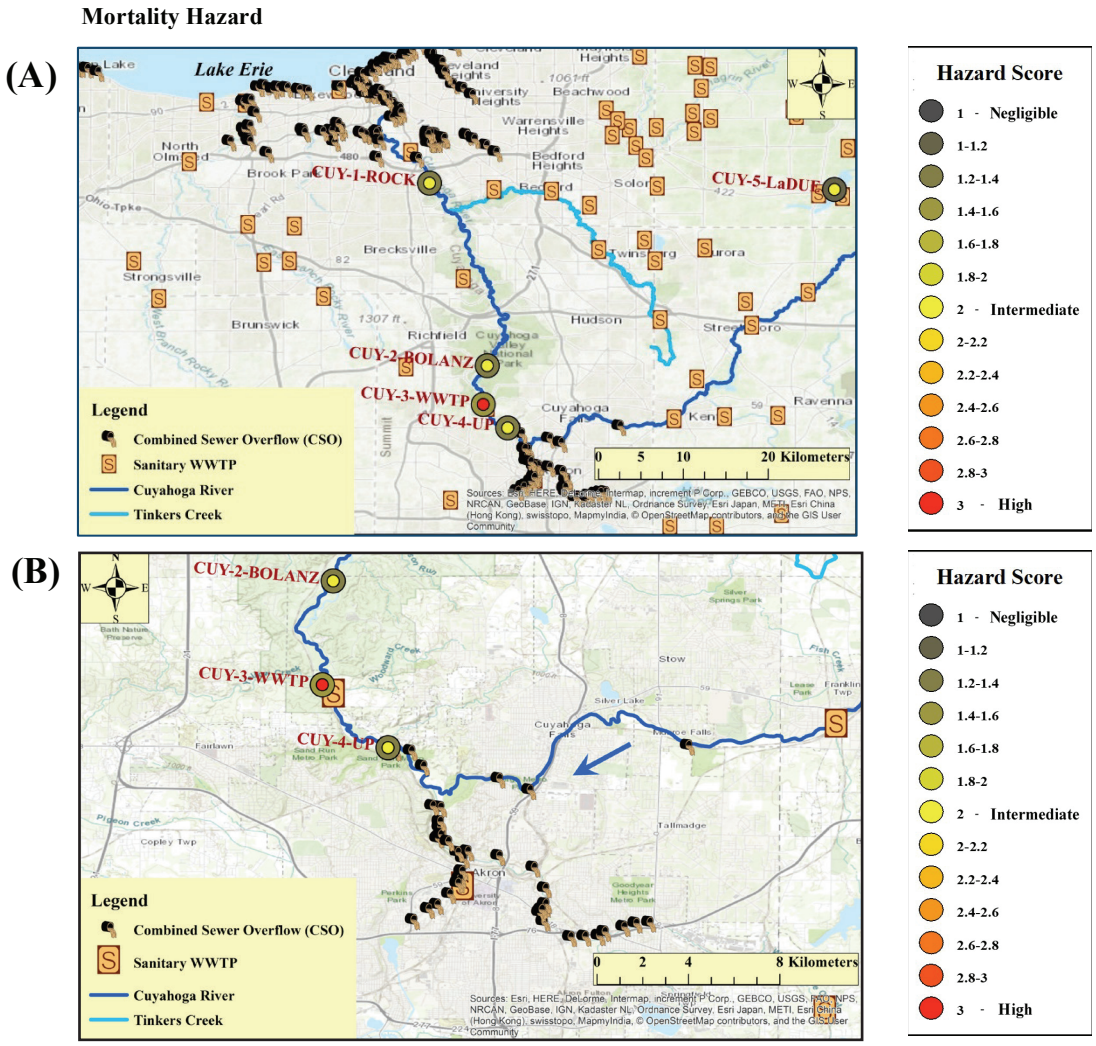


Figure 5-49. Mortality Hazard Map for the Cuyahoga River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Middle Cuyahoga River. Arrows indicate river flow direction.

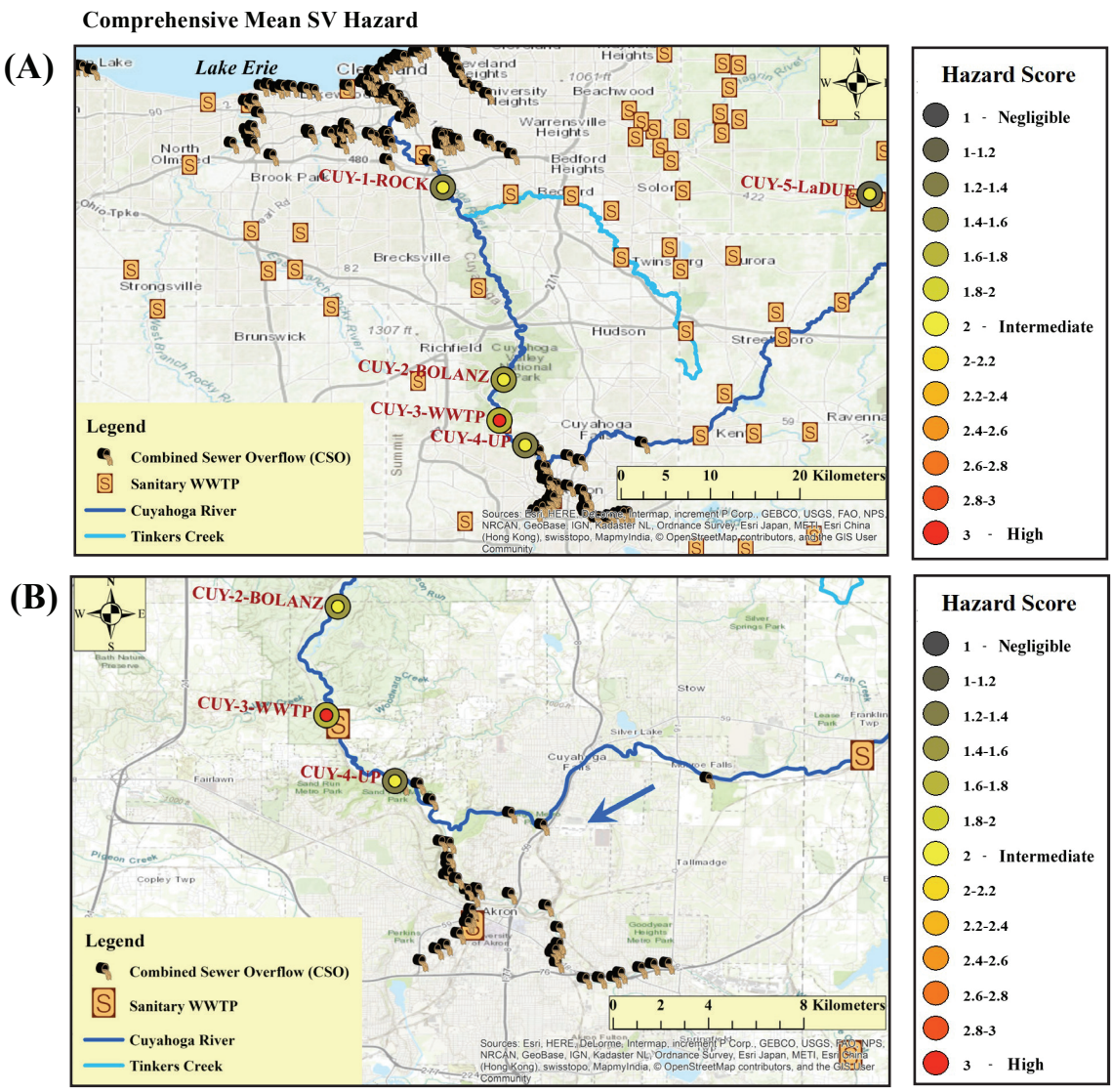


Figure 5-50. Comprehensive Mean SV Hazard Map for the Cuyahoga River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Middle Cuyahoga River. Arrows indicate river flow direction.

Endocrine Hazard

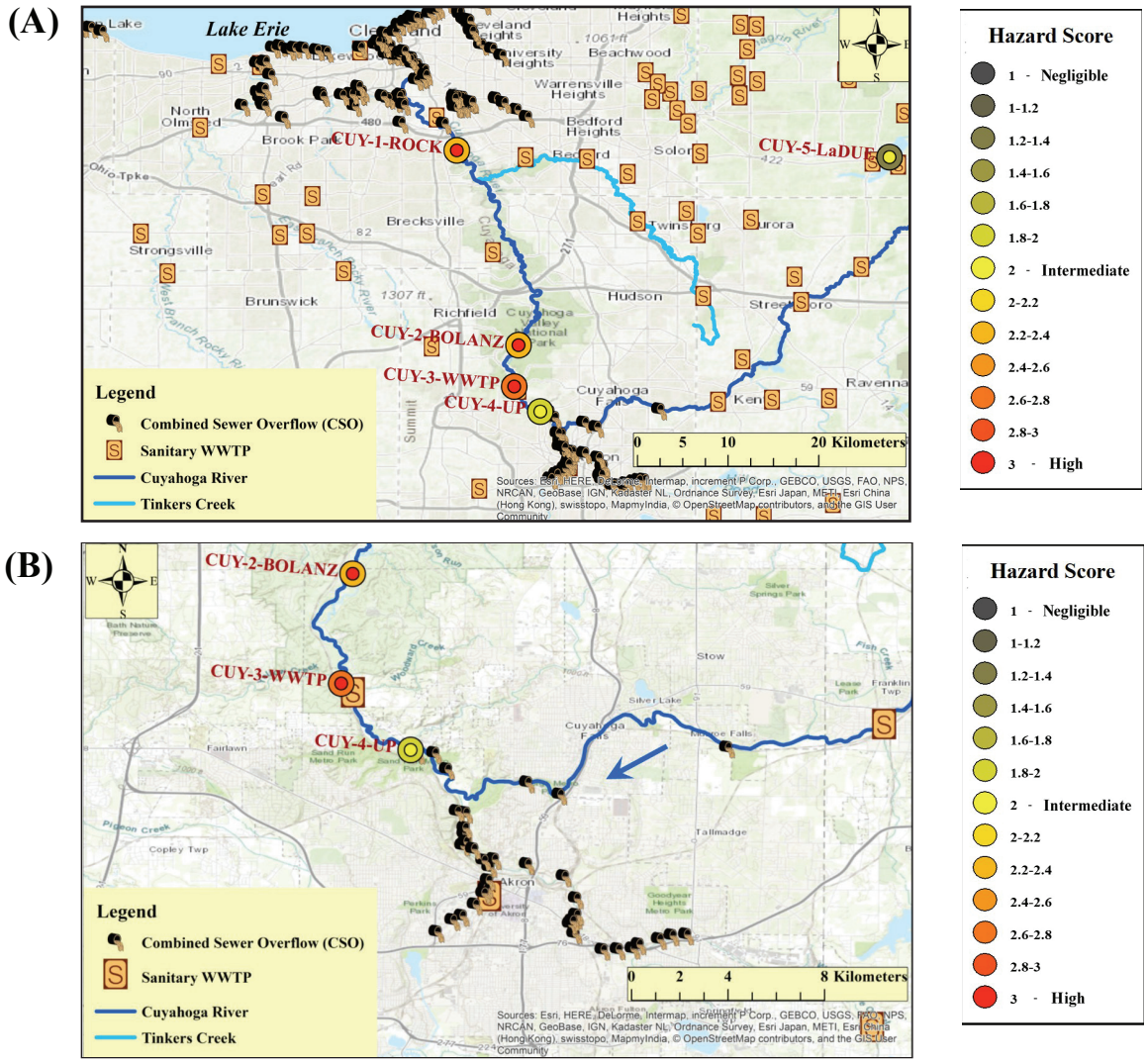


Figure 5-51. Endocrine Hazard Map for the Cuyahoga River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Middle Cuyahoga River. Arrows indicate river flow direction.

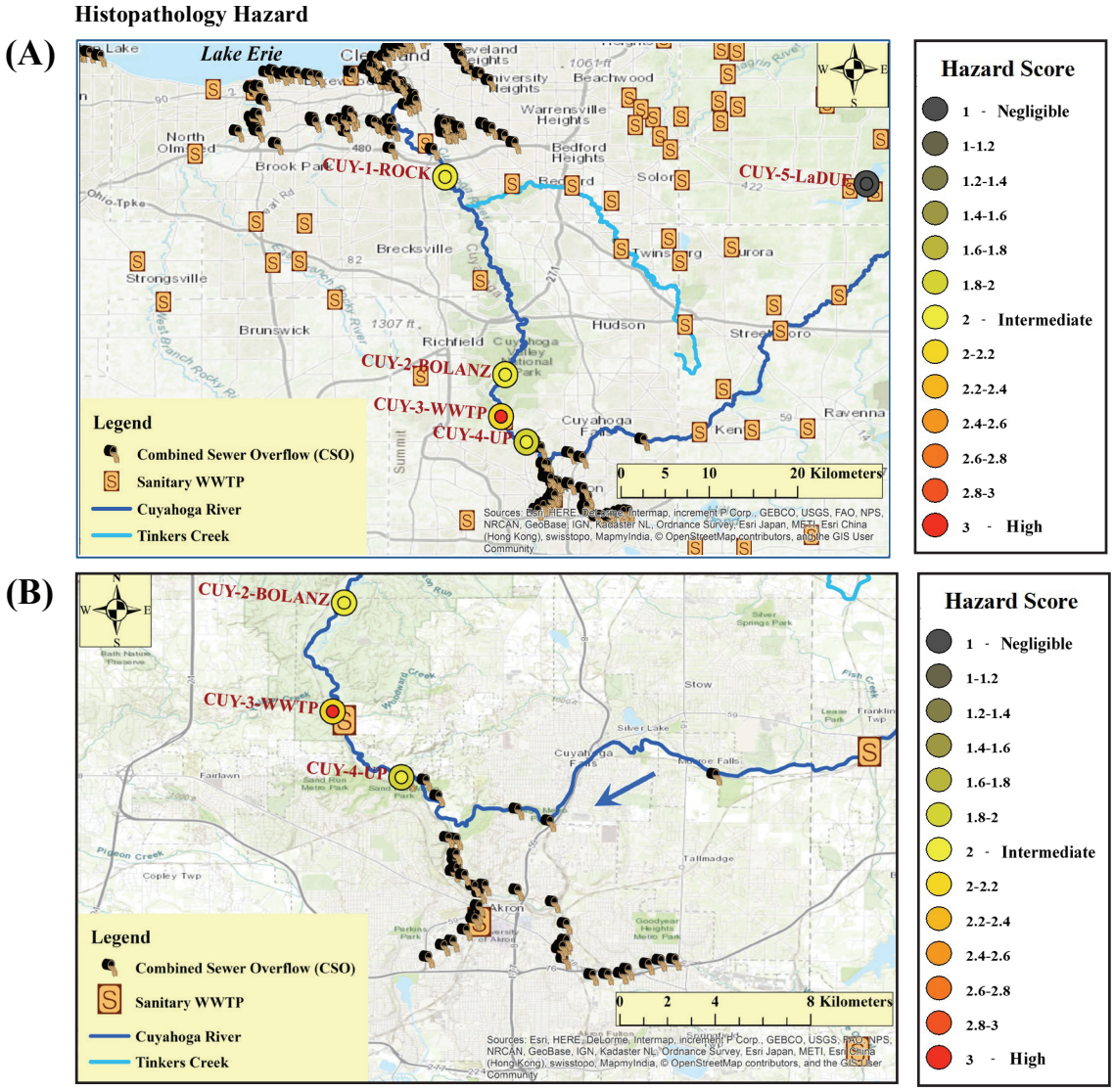


Figure 5-52. Histopathology Hazard Map for the Cuyahoga River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Middle Cuyahoga River. Arrows indicate river flow direction.

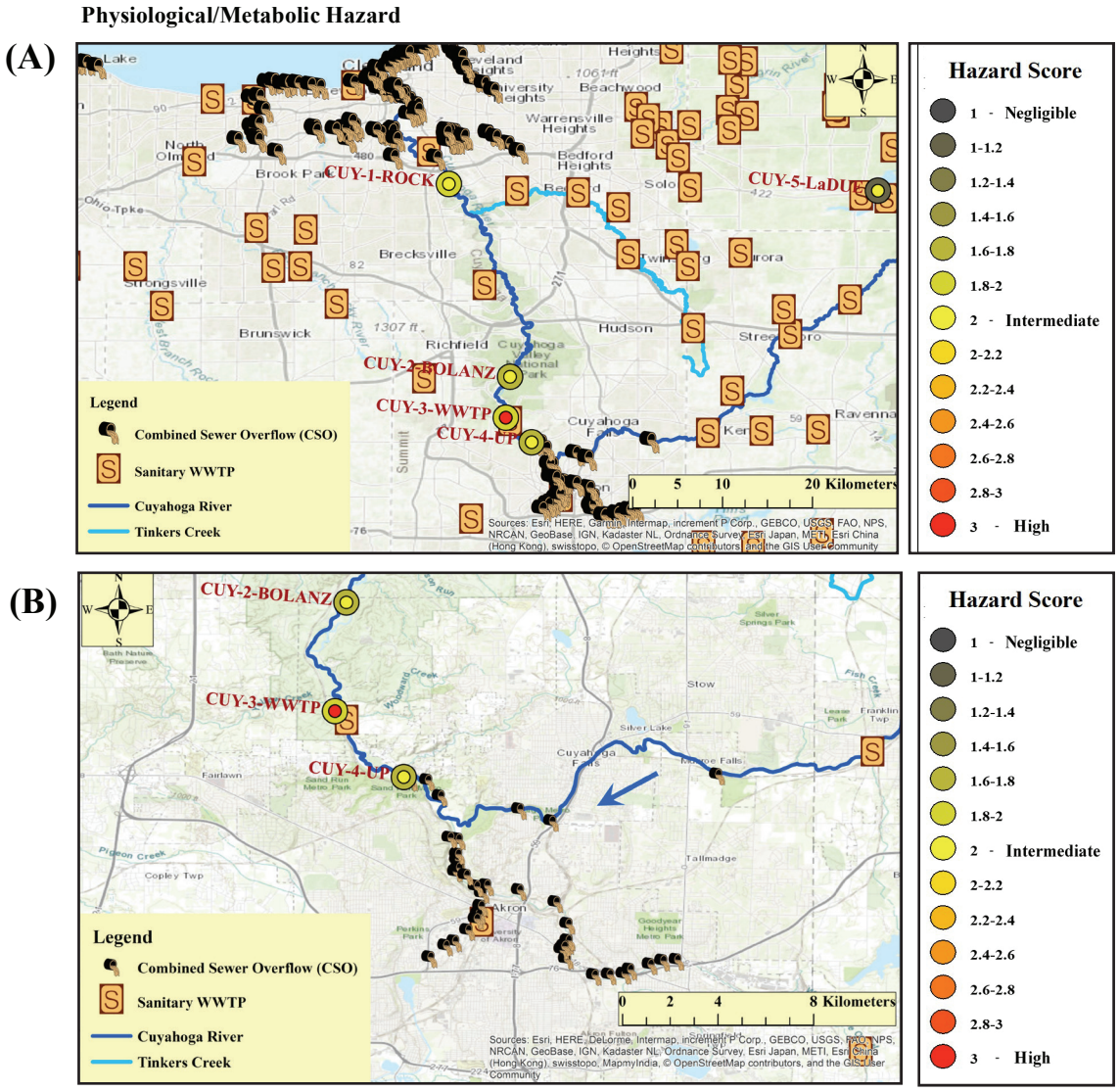


Figure 5-53. Physiological/Metabolic Hazard Map for the Cuyahoga River. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Middle Cuyahoga River. Arrows indicate river flow direction.

5.4.18 Tinkers Creek (OH)

Tinkers Creek is the largest tributary of the Cuyahoga River in north central Ohio, flowing 45.1 km (~28 mi) from its headwaters through primarily developed areas to the river confluence, and receiving inputs from numerous CEC point sources. The project location extends along the length of the creek. Six of the nine sampling sites were designated as CEC point source-'influenced', and three 'uninfluenced', based on downstream proximity to mapped WWTPs and CSOs. Either three or six surface water samples were collected at the sites, with at least one sample per site collected during each of three sampling periods: August 2013, and April and August 2014.

There is clear and convincing evidence that Tinkers Creek fish populations and communities are impacted by CECs. There was a total of 80 observations of high hazard involving eight of the nine sampling sites and 82% of the 33 sampling events, for an average of over 8 SV_{HIGH} exceedances per sampling site (Table 6-3). Population-relevant hazards include high potential for mortality associated with venlafaxine exposure and high reproductive and developmental hazard associated with ibuprofen. Additional high potential for impacts (SV_{HIGH} exceedances) were observed for endocrine hazards attributable to DEET and venlafaxine exposures, genotoxicity associated with exposure to ibuprofen, histopathology hazard attributable to carbamazepine and venlafaxine, and physiological/metabolic effects related to estrone exposure. Further, there were 101 SV_{LOW} exceedances *per site* and 27 SV_{LOW} exceedances per sampling event, totaling 911 observations of low hazard (Table 6-3). SV_{LOW} exceedances involved all nine sampling sites, 100% of sampling events, all effect categories except gross pathology, and all CECs except lidocaine and triclosan. The strength of ecotoxicological evidence brought to bear in the high hazard observations was high for ibuprofen developmental and reproductive SVs, and moderate for DEET endocrine SVs.

The top ranked effect categories based on overall mean hazard scores were histopathology, endocrine, and neurological. The highest ranked population-relevant effect category was reproductive effects. The top ranked CEC for overall mean hazard was venlafaxine, followed by DEET, citalopram and carbamazepine.

Site-specific maximum or median hazard scores were significantly ($p < 0.1$) greater in the point source CEC-influenced site group compared with uninfluenced sites for three CECs and four effect categories, including two population-relevant effects (Table 6-8). Overall, the qualitative evidence indicating CEC-related point source impacts is strong, with the tally of SV_{HIGH} exceedances (across all CECs and effect categories) averaging 3.1/sampling event and 12.5/site for CEC point source-influenced sites compared to 0.6/event and 1.7/site for uninfluenced sites. The prevalence of sampling events with a SV_{HIGH} exceedance and with a SV_{LOW} exceedance were approximately 6-fold and 2.5-fold higher, respectively, in the CEC point source-influenced site group compared to uninfluenced sites (Table 6-3).

Some Key Points…

Tinkers Creek (OH)

- **Overall:** Clear and convincing evidence of hazards to fish
- **High Hazard:**
  - 80 occurrences, involving 82% of sampling events, and 89% of sites
  - *CECs*: Carbamazepine, DEET, Estrone, Ibuprofen, Venlafaxine
  - *Effect Categories*: Endocrine, Genotoxicity, Histopathology, Physiologic/Metabolic, Developmental, Mortality, Reproductive
- **Low Hazard:**
  - 911 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: 12 of 14
  - *Effect Categories*: 11 of 12
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources
- **Exposure dataset:** Substantial
  - 9 sampling sites
  - 3 or 6 samples per site
  - 33 total samples

5.4.18.1 Hazard Brief for Tinkers Creek

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

Tinkers Creek had the greatest number of occurrences of relatively high hazard in the greatest diversity of effect categories among the 24 project locations. Effect-specific hazard maps (Section 5.4.18.5) are provided for the following effect categories for which high hazard was observed in at least one sample for at least one CEC (Table 5-1):

- Population-relevant: Population-relevant mean SV, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Endocrine, Genotoxicity, Histopathology, Physiological/Metabolic.

The following additional effect categories showed exceedances of SV_{LOW} values:

- Population-relevant: Behavioral, Growth
- Comprehensive: Circulatory/Blood Constituents, Neurological

Among CECs evaluated, the pharmaceuticals venlafaxine and ibuprofen accounted for most of the observations of high hazard at the Tinkers Creek project location, with carbamazepine, DEET and estrone also contributing. Each of the observations of high mortality hazard is attributable to venlafaxine exposure.

The following chart identifies 12 high hazard associations between CECs and effect categories, summarized from data presented in Attachment B, Table B-18.

Tinkers Creek
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}

	4-Androstene-3,17-dion	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	●	●	●	●	●	●	X	●	●			●		X
Circulatory/ Blood Constituents			●		●						●			
Endocrine					X									X
Genotoxicity									X					
Gross Pathology														
Histopathology			X											X
Neurological			●											
Physiological/Metabolic			●				X	●						
Population-relevant														
Population-relevant Mean SV	●	●	●	●	●	●	●	●	●			●		X
Behavioral			●	●		●	●	●			●	●		●
Developmental		●	●				●	●	X			●		
Growth								●						
Mortality					●			●	●			●		X
Reproductive		●	●				●		X					●

Sampling Sites

Among the nine sampling sites at Tinkers Creek, high hazard was observed in at least one sample at all sampling sites except the upstream-most site (TIC-1). Six of these sites are influenced by mapped point sources. High mortality hazard was surprisingly widespread, occurring at five of nine sampling sites and occurring consistently at each site, with all six samples analyzed at TIC-5 and at TIC-8 showing high mortality hazard from aqueous venlafaxine exposure (Attachment B, Table B-18). All sampling sites, including TIC-1, showed a variety of SV_{LOW} exceedances for several CECs.

Tinkers Creek Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. TIC-1 (3)
2. **> TIC-2 (3)**
3. TIC-3 (3)
4. **> TIC-4 (3)**
5. **> TIC-5 (6)**
6. **> TIC-6 (3)**
7. **> TIC-7 (3)**
8. **> TIC-8 (6)**
9. TIC-9 (3)

5.4.18.2 Hazard Rankings for Tinkers Creek

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Mean hazard scores falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins (Table 5-3) for mean hazard (averaged across CECs and sampling sites) are as follows:

Effect Category	Tinkers Creek Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Histopathology	1.944	1.8-2.0	5
Endocrine	1.938	1.8-2.0	5
Neurological	1.889	1.8-2.0	5
Physiological/Metabolic	1.551	1.4-1.6	3
Reproductive	1.432	1.4-1.6	3
Comprehensive Mean SV	1.413	1.4-1.6	3
Behavioral	1.337	1.2-1.4	2
Genotoxicity	1.333	1.2-1.4	2
Mortality	1.329	1.2-1.4	2
Circulatory/Blood Constituents	1.306	1.2-1.4	2
Population-relevant Mean SV	1.286	1.2-1.4	2
Developmental	1.220	1.2-1.4	2
Growth	1.009	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and sampling sites) are as follows:

CEC	Tinkers Creek CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Venlafaxine	2.071	2.0-2.2	6
DEET	1.807	1.8-2.0	5
Carbamazepine	1.672	1.6-1.8	4
Citalopram	1.617	1.6-1.8	4
Estrone	1.336	1.2-1.4	2
HHCB	1.294	1.2-1.4	2
TBEP	1.259	1.2-1.4	2
Diphenhydramine	1.222	1.2-1.4	2
Sitosterol, beta-	1.181	1.0-1.2	1
Ibuprofen	1.167	1.0-1.2	1
4-Androstene-3,17-dione	1.080	1.0-1.2	1
Bisphenol A	1.049	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins (Table 5-3) for mean hazard (averaged across effect categories and CECs) are as follows:

Sampling Site (bold ">" = site designated as point source influenced)	Tinkers Creek Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> TIC-8	1.654	1.6-1.8	4
> TIC-5	1.559	1.4-1.6	3
> TIC-6	1.376	1.2-1.4	2
> TIC-7	1.366	1.2-1.4	2
> TIC-4	1.354	1.2-1.4	2
TIC-3	1.325	1.2-1.4	2
TIC-9	1.321	1.2-1.4	2
> TIC-2	1.285	1.2-1.4	2
TIC-1	1.061	1.0-1.2	1

5.4.18.3 Breadth of Information Indicating High Hazard at Tinkers Creek

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: There was excellent coverage of the creek by the spatial distribution of nine sampling sites, including both point source CEC-influenced and uninfluenced sites. Generally, sample sizes were small but temporal coverage was good; samples were collected in three different months over a two-year period. Despite small sample sizes there were numerous and a wide variety of high hazard observations, suggesting this site is highly contaminated with CECs. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was high due to numerous USEPA STORET water quality sampling sites with TSS data distributed throughout the lower three quarters of the Tinkers Creek project location (Figure A2-18 in Attachment A2).
- *Ecotoxicity:* There were observations of high hazard for six CECs and nine effect categories despite generally low breadth of applicable ecotoxicity information, suggesting a highly contaminated project location.

Breadth of ecotoxicity information for high hazard observations was as follows (Tables 4-5 and 4-6):

- Carbamazepine
  - Sparse - Histopathology
- DEET
  - Moderate - Endocrine
- Estrone
  - Sparse - Physiological/Metabolic
  - Limited - Comprehensive mean SV
- Ibuprofen
  - Broad - Developmental
  - Broad - Reproductive
  - Sparse - Genotoxicity
- Venlafaxine
  - Limited - Comprehensive mean SV
  - Limited - Population-relevant mean SV
  - Sparse - Endocrine
  - Sparse - Histopathology
  - Sparse - Mortality

5.4.18.4 Tinkers Creek Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4); the following chart provides the results summary.

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
Citalopram	Behavioral	N	Y
Diphenhydramine	Comprehensive mean SV	Y	N
Venlafaxine	Endocrine	N	Y
	Mortality	Y	N

When *all* hazard scores for each site in the Tinkers Creek location, not just maximum or median scores, are included in the statistical analysis[73], additional significant differences ($p < 0.1$) are observed for the following CEC-effect category combinations (Attachment D):

- 4-Androstene-3,17-dione – Comprehensive mean SV
- Carbamazepine – Population-relevant mean SV, Behavioral, Developmental, Reproductive, Comprehensive mean SV, Circulatory/ Blood Constituents, Histopathology, Neurological, Physiological/Metabolic
- Diphenhydramine – Population-relevant mean SV, Behavioral
- Estrone - Population-relevant mean SV, Behavioral, Developmental, Reproductive, Comprehensive mean SV, Physiological/ Metabolic
- HHCB – Behavioral, Comprehensive mean SV, Physiological/Metabolic
- Venlafaxine - Population-relevant mean SV, Behavioral, Reproductive, Comprehensive mean SV, Histopathology

A summary of the qualitative comparison is provided in the following tallies. Shown are fractions of samples and of sites at the Tinkers Creek location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Tinkers Creek Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	75	0.96 (23/24)	1.0 (6/6)	723	1.0 (24/24)	1.0 (6/6)
Uninfluenced Sites	5	0.44 (4/9)	0.67 (2/3)	188	1.00 (9/9)	1.0 (3/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There was a total of 75 exceedances of various effect-specific SV_{HIGH} values for carbamazepine, DEET, estrone, ibuprofen and venlafaxine among the six CEC-influenced sites, and only five SV_{HIGH} exceedances for DEET and venlafaxine among the 3 uninfluenced sites. Various effect-specific SV_{LOW} values for 11 of the 14 CECs were exceeded 723 times in the CEC-influenced group, and 188 times for 10 CECs at sites "uninfluenced" by WWTPs and CSOs.

Two sites with high endocrine hazard from DEET and venlafaxine exposures (TIC-3 and TIC-9) had been a priori designated as "uninfluenced" due to distance from mapped point sources, suggesting the presence of unmapped point sources, limited attenuation of high CEC concentrations from point source upstream (>4 km), and/or non-point source inputs such as aerial deposition.

[73]Cautionary caveats regarding this approach are described in Attachment D.

5.4.18.5 Tinkers Creek Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

Population-relevant Mean SV Hazard

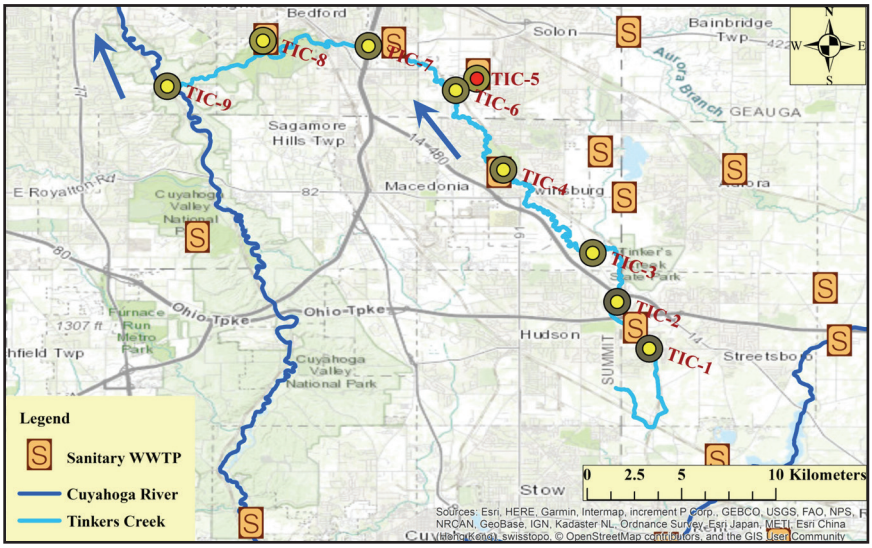


Figure 5-54. Population-relevant Mean SV Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Developmental Hazard

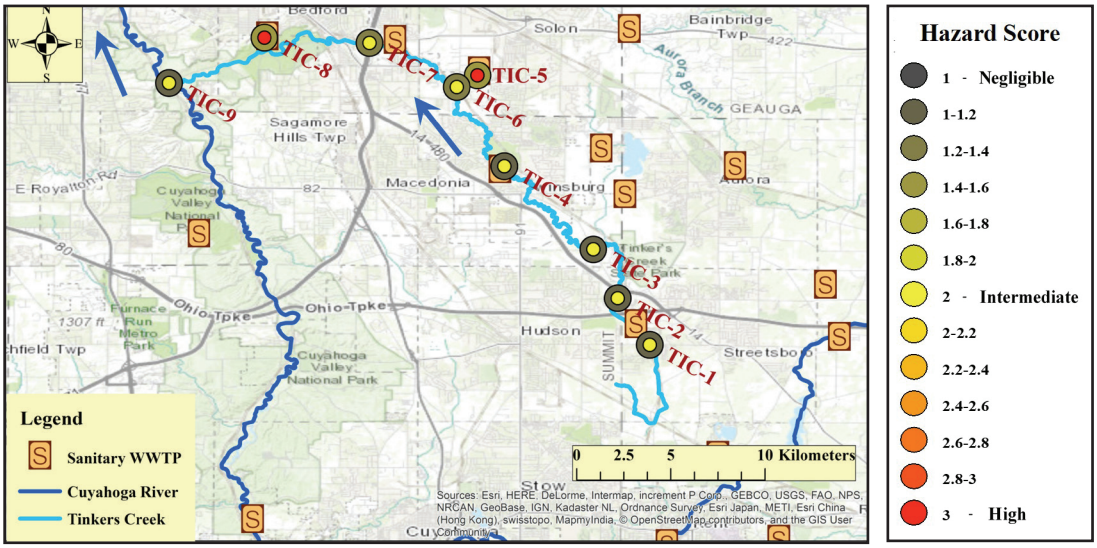


Figure 5-55. Developmental Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Mortality Hazard

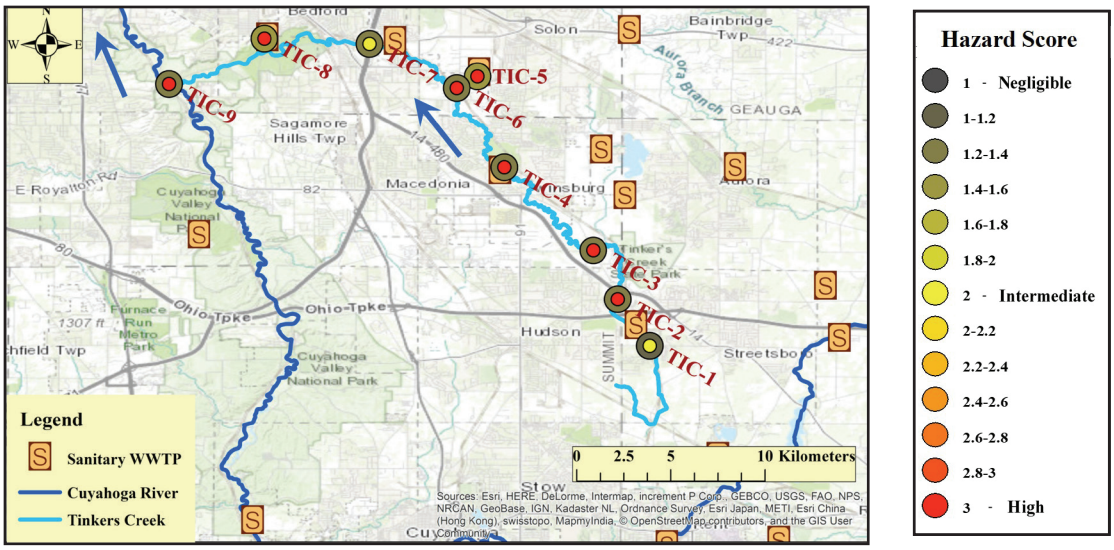


Figure 5-56. Mortality Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Reproductive Hazard

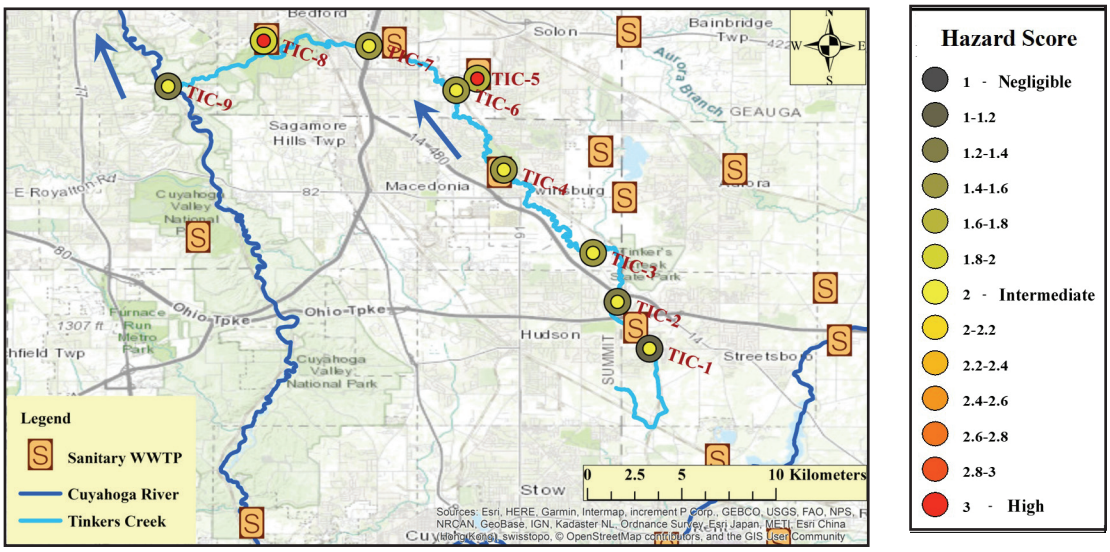


Figure 5-57. Reproductive Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Comprehensive Mean SV Hazard

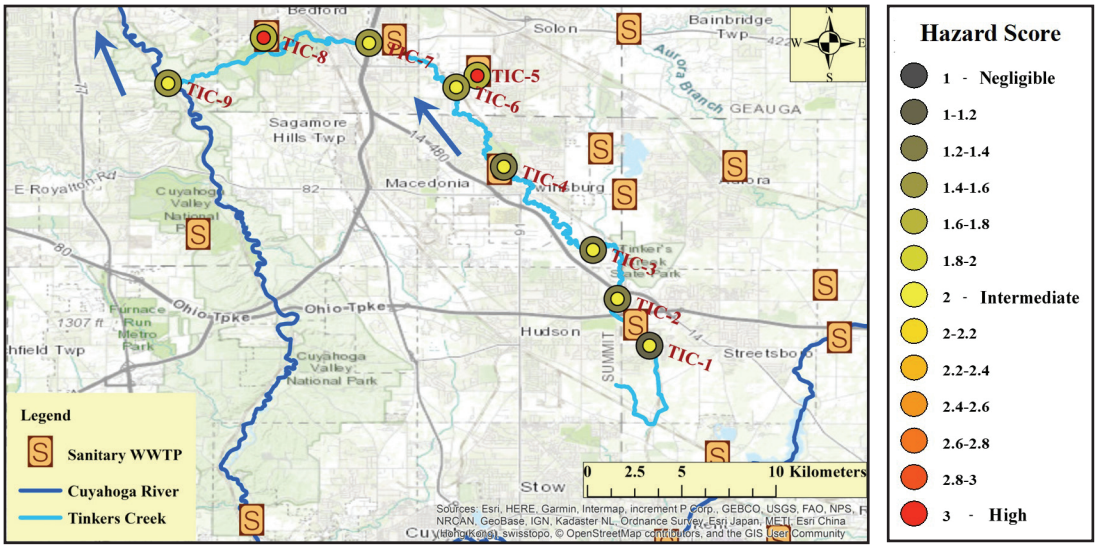


Figure 5-58. Comprehensive Mean SV Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Endocrine Hazard

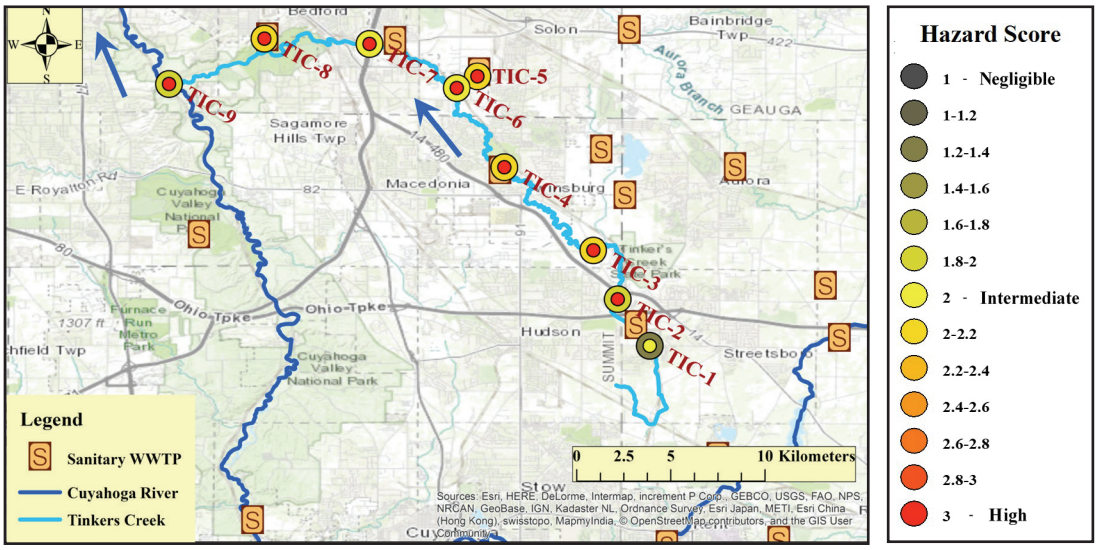


Figure 5-59. Endocrine Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Genotoxicity Hazard

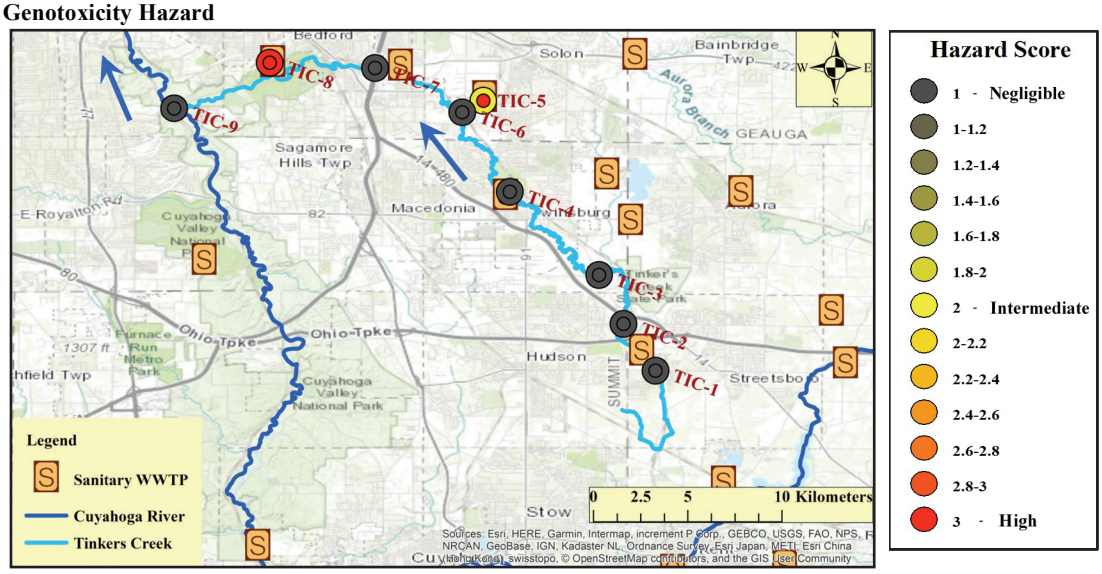


Figure 5-60. Genotoxicity Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Histopathology Hazard

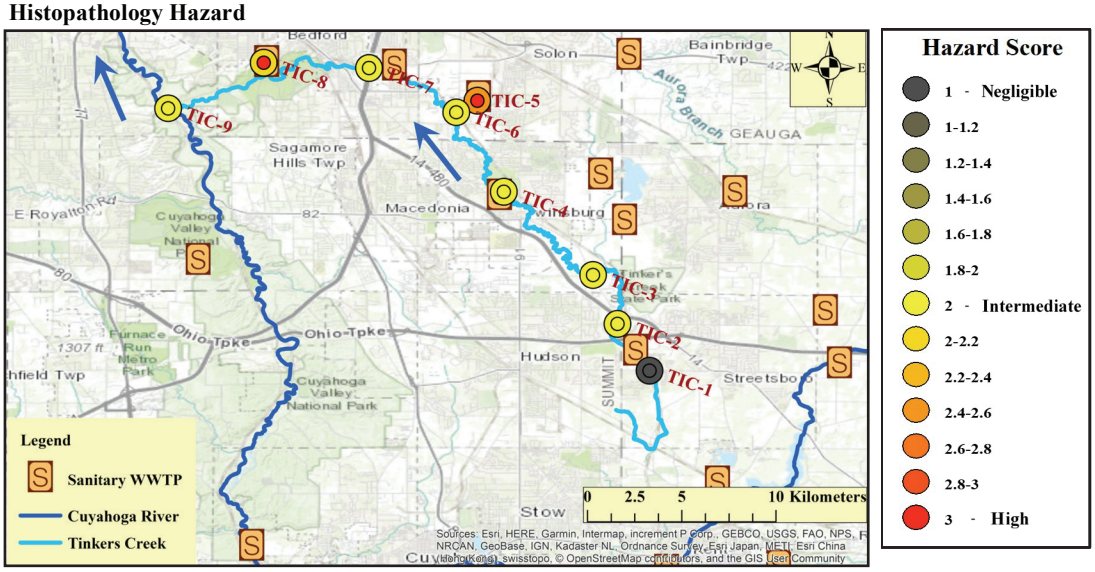


Figure 5-61. Histopathology Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

Physiological/Metabolic Hazard

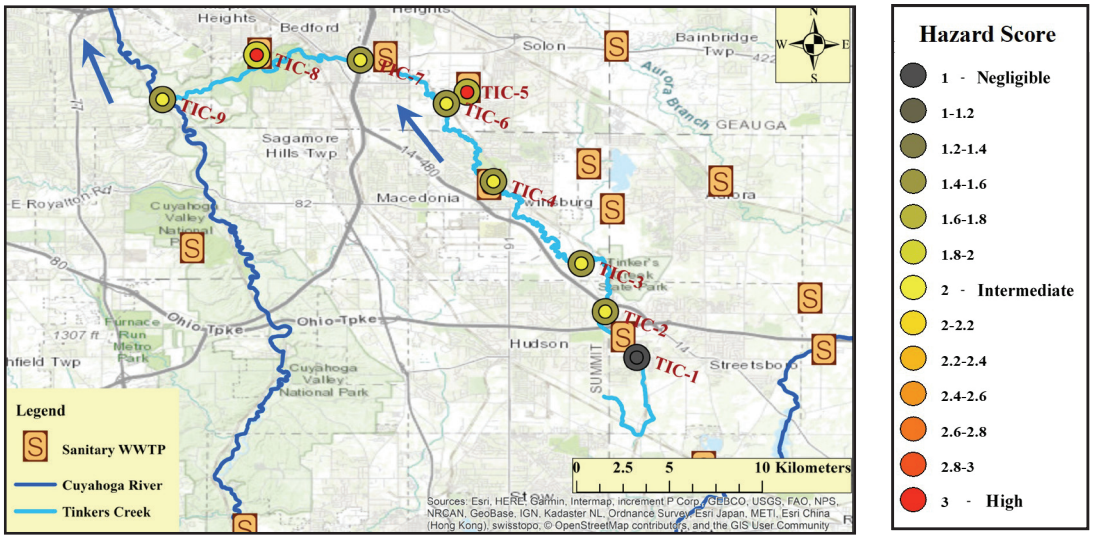


Figure 5-62. Physiological Metabolic Hazard Map for Tinkers Creek. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. Arrows indicate river flow direction.

5.4.19 Ashtabula River (OH)

The main stem of the Ashtabula River traverses approximately 64 km (40 mi) in northeastern Ohio, draining a relatively small watershed of 355 km² (137 mi²) of primarily agricultural lands and discharging into Lake Erie at Ashtabula, OH. The three sampling sites were all designated as 'uninfluenced' by mapped CEC point sources. Each site was sampled once in April 2011 – for a total of only three surface water grab samples collected at this project location.

The very sparse exposure dataset provided little evidence indicating hazard to fish at this project location. There were no SV_{HIGH} exceedances, and although an average of 4.3 SV_{LOW} exceedances occurred at the three sampling sites, they were limited to mortality and endocrine effects associated with DEET exposure, and behavioral and circulatory/blood constituent effects related to β-sitosterol exposure. Neither of these CECs is necessarily associated with WWTP or CSO point sources – DEET has been shown to be aerially transported with detectable concentrations in rain and snow, and β-sitosterol, a plant steroid hormone, enters waterbodies via multiple routes. It is unclear whether further sampling is likely to reveal additional hazards attributable to the 14 CECs considered in this EHA.

5.4.19.1 Hazard Brief for Ashtabula River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

Ashtabula River (OH)

- **Overall:** Little evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 13 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: DEET, β-Sitosterol
  - *Effect Categories*: 4 of 12
- **Point Source Analysis:** No evaluation; no CEC-influenced sites
- **Exposure dataset:** Minimal
  - 3 sampling sites
  - 1 sample per site
  - 3 total samples

Effect Categories and Emerging Contaminants

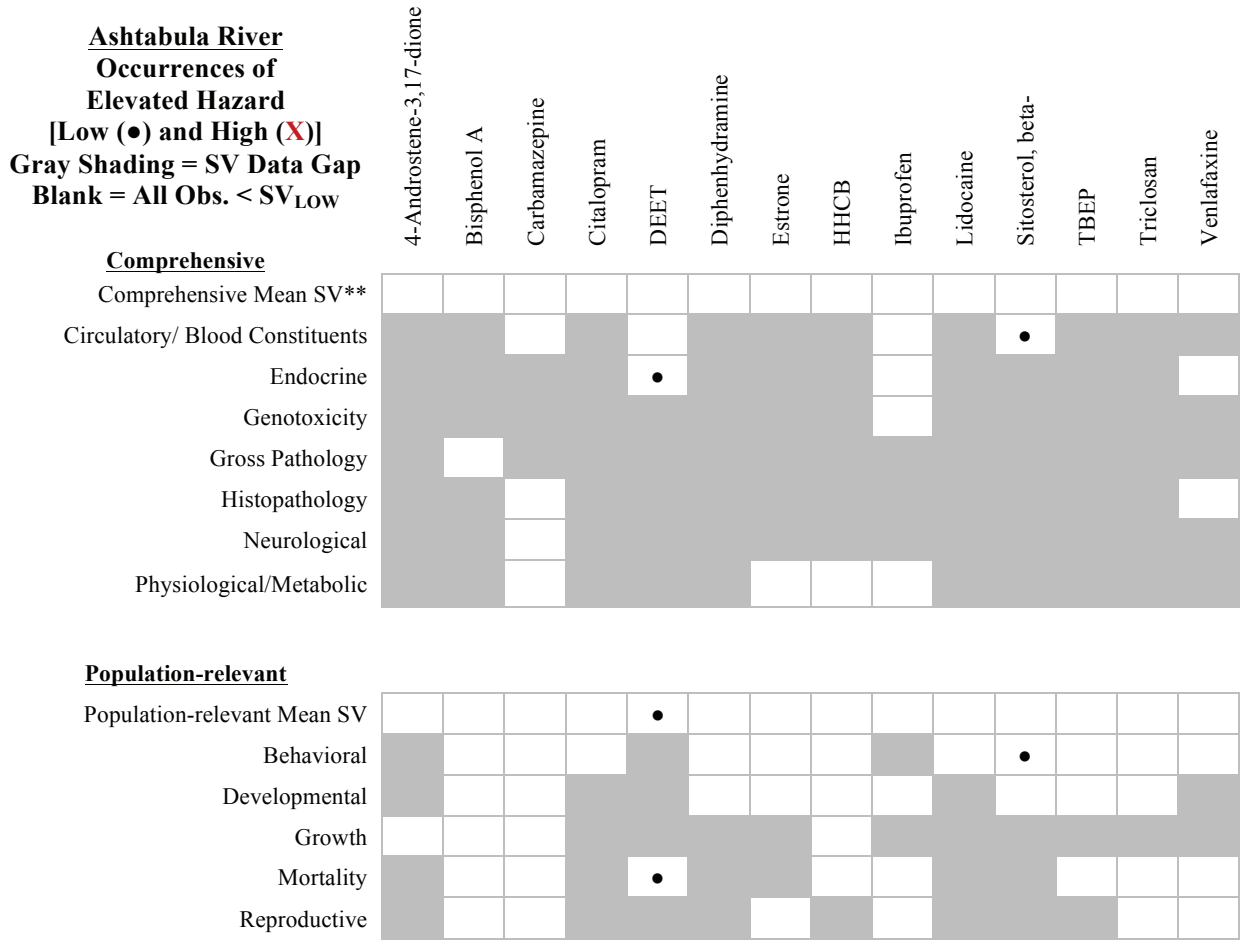
There were no exceedances of any SV_{HIGH} value for any effect category at the Ashtabula River project location (Attachment B; Table B-19), possibly because only one water sample was collected at each site; no hazard maps were produced.

SV_{LOW} values were exceeded in at least one sample for the following effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Mortality
- Comprehensive: Circulatory/Blood Constituents, Endocrine

All SV_{LOW} exceedances are attributable to DEET and β-sitosterol.

The following chart indicates CECs and Effect Categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-19.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

Exceedances of SV_{LOW} values were well-distributed among the three sampling sites. None of these sites was identified as potentially point source-influenced, and there were no observations of high hazard at this project location.

Ashtabula River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site

1. ASH-1 (1)
2. ASH-2 (1)
3. ASH -3 (1)

5.4.19.2 Hazard Rankings for Ashtabula River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Ashtabula River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.333	1.2-1.4	2
Circulatory/Blood Constituents	1.167	1.0-1.2	1
Mortality	1.125	1.0-1.2	1
Population-relevant Mean SV	1.071	1.0-1.2	1
Behavioral	1.061	1.0-1.2	1
Comprehensive Mean SV	1.000	1.0-1.2	1
Developmental	1.000	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1
Physiological/Metabolic	1.000	1.0-1.2	1
Reproductive	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Ashtabula River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.600	1.4-1.6	3
Sitosterol, beta-	1.267	1.2-1.4	2
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Estrone	1.000	1.0-1.2	1
HHCB	1.000	1.0-1.2	1
Ibuprofen	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (No site was designated as point source influenced)	Ashtabula River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
ASH-1	1.061	1.0-1.2	1
ASH-2	1.061	1.0-1.2	1
ASH-3	1.037	1.0-1.2	1

5.4.19.3. Breadth of Information Indicating High Hazard at Ashtabula River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no high hazard observations. However, the evidence suggesting an absence of high hazard is weak. There were only three sampling sites in the entire Ashtabula River watershed occurring relatively near each other in the lower river, and only one surface water sample per site was analyzed for CECs. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was high due numerous USEPA STORET water quality sampling sites with TSS data located in the lower Ashtabula to represent the entire project location (Figure A2-19 in Attachment A2). Potential for false negative findings due to gaps in the fish ecotoxicity database is discussed above in Section 5.3.2.

5.4.19.4 Ashtabula River Point Source Analysis

All three Ashtabula River sampling sites were designated as uninfluenced by point sources, so no point source CEC influenced-uninfluenced comparisons were possible.

A summary of hazard occurrence at the three " uninfluenced" sites is provided in the chart below. Shown are fractions of samples and of sites at the Ashtabula River location that have at least one observation of a SV_{LOW} exceedance, for any CEC and effect category.

Very low sample numbers severely limit the strength of evidence in the hazard assessment for this location, with only one sample analyzed at each of three uninfluenced sites. Nevertheless, there were 13 exceedances of SV_{LOW} values for DEET and β-sitosterol at all three sampling sites. There were no exceedances of SV_{HIGH} values.

Ashtabula River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	NA	NA	NA	NA	NA	NA
Uninfluenced Sites	0	0.0 (0/3)	0.0 (0/3)	13	1.0 (3/3)	1.0 (3/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.19.5 Ashtabula River Hazard Maps

There were no exceedances of any SV_{HIGH} value at the Ashtabula River project location, so no hazard maps were produced.

5.4.20 Long Pond (NY)

Long Pond is a 440 acre coastal impoundment on the south shore of Lake Ontario, just west of Rochester, NY, that received CEC inputs from a WWTP located on its principal tributary – Northrup Creek; the WWTP was decommissioned a few years prior to sampling. Northrup Creek drains a watershed of approximately 26.2 km^2 (10.1 mi^2). One purpose of sampling at this location was to determine whether residual CEC-related hazards could be observed following cessation of continuous point source CEC inputs. All six sampling sites were designated as 'uninfluenced' by actively discharging CEC point sources, and were sampled once each in April, 2012, for a total of only six surface water samples collected at this project location.

The minimal exposure dataset revealed some evidence of hazard to fish in Long Pond. Although there were no SV_{HIGH} exceedances, there was a total of 45 SV_{LOW} exceedances associated with five CECs and seven effect categories (plus the comprehensive mean SV and population-relevant mean SV), with at least one exceedance at each sampling site. The absence of CEC-'influenced' sampling sites precluded evaluation of point source-related hazard.

5.4.20.1 Hazard Brief for Long Pond

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Some Key Points…

Long Pond (NY)

- **Overall:** Some evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 45 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: 5 of 14
  - *Effect Categories*: 7 of 12
- **Point Source Analysis:** No evaluation; no CEC-influenced sites
- **Exposure dataset:** Minimal
  - 6 sampling sites
  - 1 sample per site
  - 6 total samples

Effect Categories and Emerging Contaminants

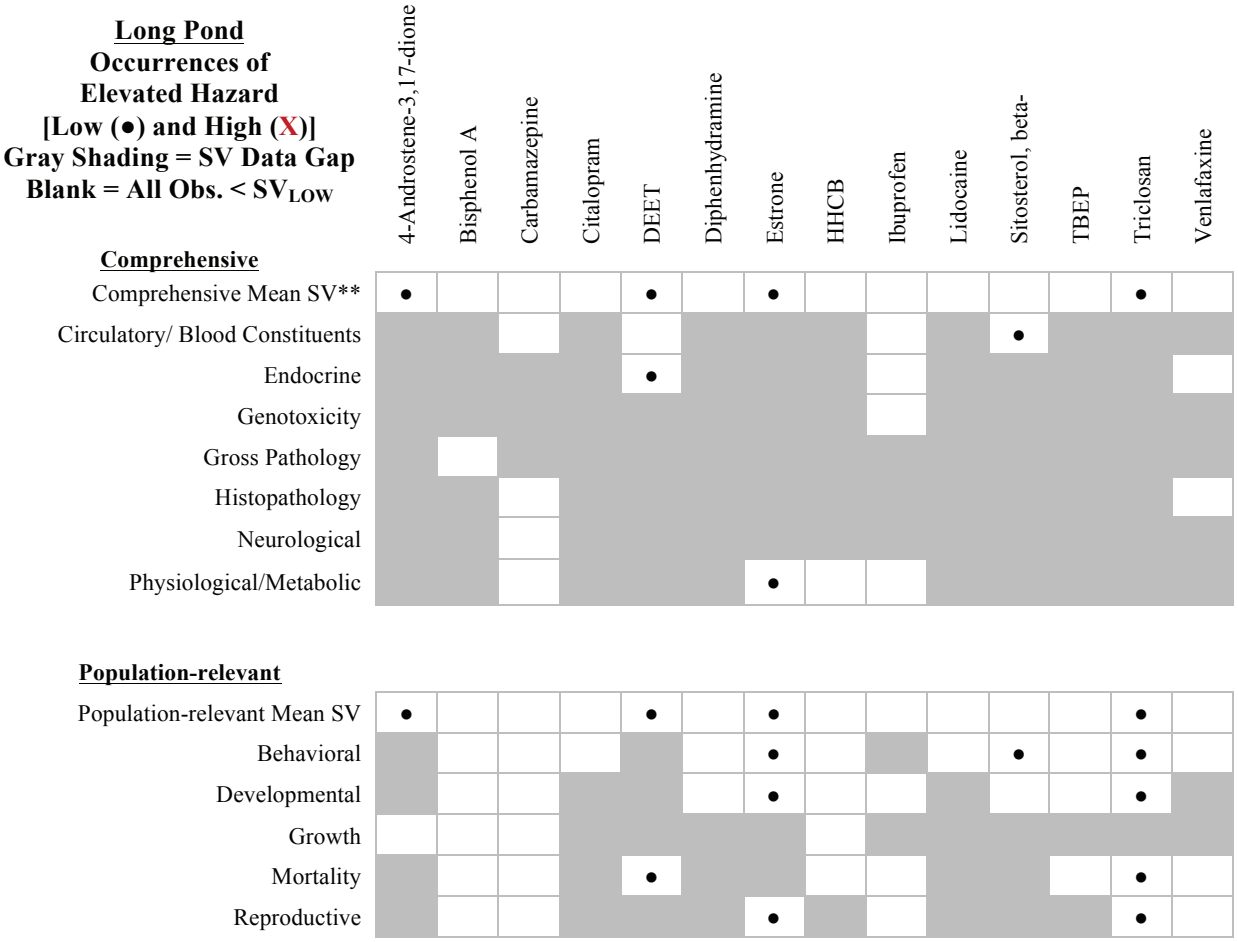
There were no exceedances of any SV_{HIGH} value for any effect category at the Long Pond project location (Attachment B; Table B-20); no hazard maps were produced.

SV_{LOW} values were exceeded in at least one sample for the following effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Development, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine, Physiological/Metabolic

DEET accounted for most of the SV_{LOW} exceedances at a number of sites, while low hazard from androstenedione, estrone, β-sitosterol and triclosan also occurred at Long Pond sampling sites.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-20.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

While all sampling sites showed SV_{LOW} exceedances, most exceedances occurred at LP-SOUTH (8 effect categories) and LP04 (9 effect categories) for multiple CECs. Triclosan and the two hormones exceeded screening values only at LP-SOUTH and LP04, respectively. At the other four sampling sites, exceedances were attributable only to DEET and β-sitosterol. None of these sites were identified as potentially CEC point source-influenced, and there were no observations of high hazard.

Long Pond Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site

1. LP01 (1)
2. LP02 (1)
3. LP04 (1)
4. LP06 (1)
5. LP06-REF (1)
6. LP-South (1)

5.4.20.2 Hazard Rankings for Long Pond

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Long Pond Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.333	1.2-1.4	2
Mortality	1.146	1.0-1.2	1
Behavioral	1.121	1.0-1.2	1
Population-relevant Mean SV	1.107	1.0-1.2	1
Comprehensive Mean SV	1.095	1.0-1.2	1
Circulatory/Blood Constituents	1.083	1.0-1.2	1
Reproductive	1.056	1.0-1.2	1
Physiological/Metabolic	1.042	1.0-1.2	1
Developmental	1.037	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Long Pond CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.767	1.6-1.8	4
Sitosterol, beta-	1.267	1.2-1.4	2
Estrone	1.167	1.0-1.2	1
Triclosan	1.167	1.0-1.2	1
4-Androstene-3,17-dione	1.111	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
HHCB	1.000	1.0-1.2	1
Ibuprofen	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (No site was designated as point source influenced)	Long Pond Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
LP04	1.171	1.0-1.2	1
LP_South	1.146	1.0-1.2	1
LP01	1.061	1.0-1.2	1
LP02	1.061	1.0-1.2	1
LP06	1.061	1.0-1.2	1
LP06-REF	1.049	1.0-1.2	1

5.4.20.3 Breadth of Information Indicating High Hazard at Long Pond

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no high hazard observations. However, the evidence suggesting an absence of high hazard is weak. Only one surface water sample per site was analyzed for CECs, and all samples were collected in the spring of 2012 during high flow. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the Long Pond TSS data available for this conversion was high due to monthly (May-September) TSS measurements during 2003-2009 (Makarewicz and Nowak 2010).

Additional potential for false negative findings is due to gaps in the fish ecotoxicity database as discussed above in Section 5.3.2.

5.4.20.4 Long Pond Point Source Analysis

All six Long Pond sampling sites were designated as uninfluenced by CEC point sources, so no uninfluenced comparisons were possible.

A summary of hazard occurrence at the six "uninfluenced" sites is provided in the chart below. Shown are fractions of samples and of sites at the Long Pond location that have at least one observation of a SV_{LOW} exceedance, for any CEC and effect category.

Very low sample numbers severely limits the strength of evidence in the hazard assessment for this location, with only one sample analyzed at each of six uninfluenced sites. Nevertheless, there was a total of 45 exceedances of SV_{LOW} values for 4-androstene-3,17-dione, DEET, estrone, β-sitosterol and triclosan at all six sampling sites. Only DEET and β-sitosterol SV_{LOW} values were exceeded at the reference site (LP06-REF). There were no exceedances of SV_{HIGH} values.

5.4.20.5 Long Pond Hazard Maps

There were no exceedances of any SV_{HIGH} value at the Long Pond project location; no hazard maps were produced.

Long Pond Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	NA	NA	NA	NA	NA	NA
Uninfluenced Sites	0	0.0 (0/6)	0.0 (0/6)	45	1.0 (6/6)	1.0 (6/6)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.21 Genesee River (NY)

The Genesee River flows north for 232 km (144 miles) from the Alleghany highlands in northern Pennsylvania to the south shore of Lake Ontario at Rochester, NY. The river drains an extensive watershed (2,372 mi^2; 6,146 km^2) with principally agricultural and small town CEC inputs, including the western Finger Lakes. However, the six sampling sites at this project location are limited to the lowermost six miles of the river main stem within the city of Rochester, which includes several CEC point sources. Four of the sampling sites were designated as CEC-'influenced', and two 'uninfluenced', based on downstream proximity to mapped CEC point sources. At three sites, only one surface water sample was collected during September 2010, and at the other three sites, one sample was collected during each of two sampling events - September 2010 and May 2011.

There is limited evidence of CEC-related hazard at this location, with no observations of SV_{HIGH} exceedance and a total of 36 SV_{LOW} exceedances, averaging six per site. SV_{LOW} exceedances in six effect categories (plus the comprehensive mean SV and population-relevant mean SV) were associated with exposures to DEET, estrone, HHCB and β-sitosterol. Estrone ranked highest among the 14 CECs for overall mean hazard to fish.

A statistical test comparing site-specific maximum and median hazard scores between the CEC-influenced site group and uninfluenced sites revealed no significant ($p < 0.1$) hazard differences between the groups. Qualitative inspection of SV_{HIGH} and SV_{LOW} tallies likewise revealed no appreciable difference in overall hazard between the site groups.

The sparse exposure dataset may explain, at least partly, the low incidence and breadth of observed hazard, as well as the lack of difference in hazard between CEC-influenced and uninfluenced sites.

Some Key Points…

Genesee River (NY)

- **Overall:** Some evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 36 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: DEET, Estrone, HHCB, β-Sitosterol
  - *Effect Categories*: 6 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
  Exposure dataset: Limited
  - 6 sampling sites
  - 1 or 2 samples per site
  - 9 total samples

5.4.21.1 Hazard Brief for Genesee River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

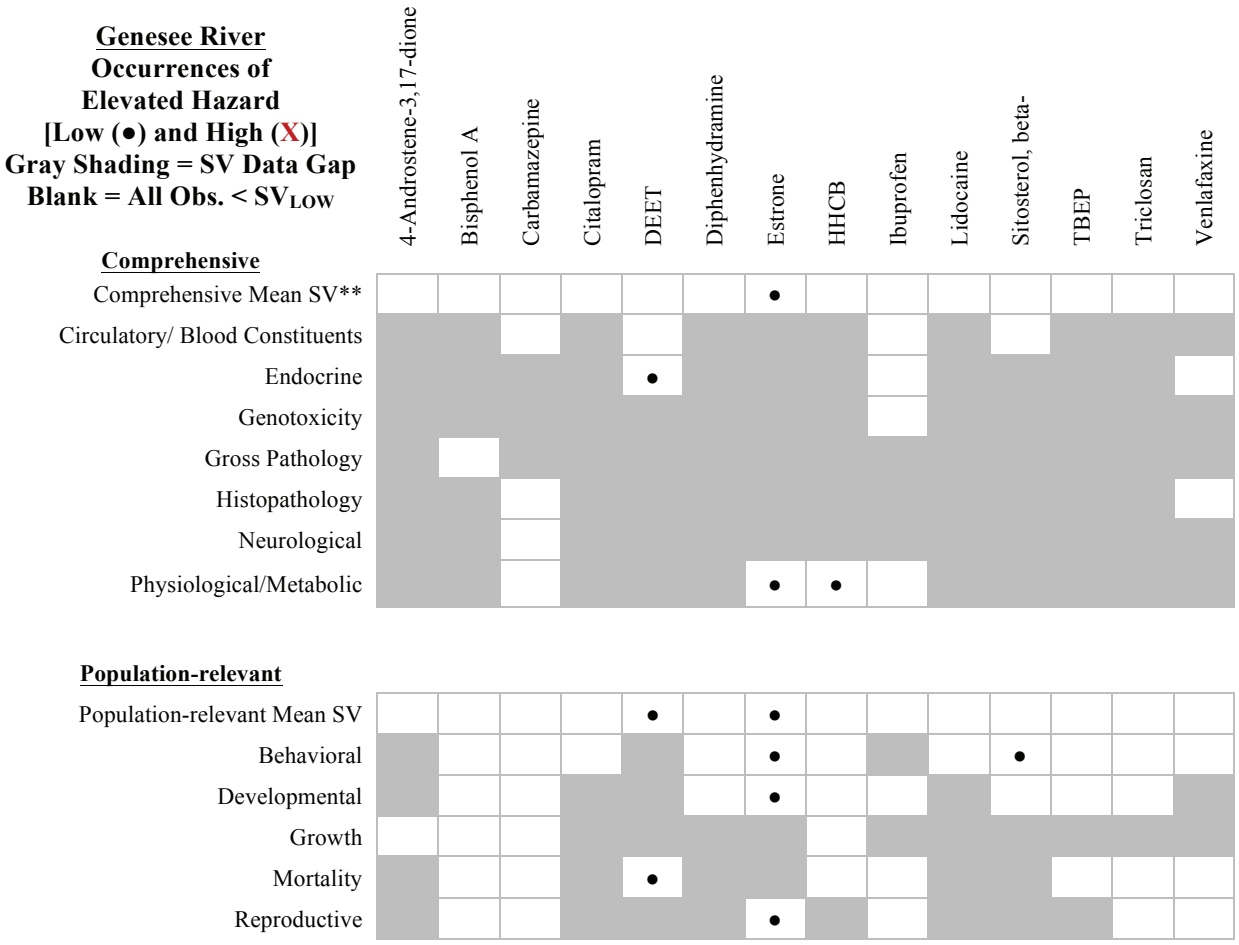
Effect Categories and Emerging Contaminants
There were no exceedances of any SV_{HIGH} value for any effect category at the Genesee River project location (Attachment B; Table B-21); no hazard maps were produced.

SV_{LOW} values were exceeded in at least one sample for the following effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Development, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Endocrine, Physiological/Metabolic

An SV_{LOW} for HHCB was exceeded at each sampling site, suggesting little attenuation of water column concentration from the most likely point source – the WWTP at the uppermost reach of the project location – to the mouth of the river at Lake Ontario. Other exceedances of SV_{LOW} values were less widely distributed for DEET, estrone and β-sitosterol.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-21.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

Exceedances of at least one SV_{LOW} occurred at each sampling site. The uppermost site (GNR-1) had exceedances for four CECs in eight effect categories, strongly indicting unmapped upstream point sources. There was only one SV_{LOW} exceedance at GNR-5; HHCB for physiological/metabolic effects. There were no observations of high hazard at this project location.

Genesee River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. GNR-1 (2)
2. **> GNR-2 (2)**
3. **> GNR -3 (1)**
4. **> GNR -4 (1)**
5. **> GNR -5 (1)**
6. GNR -6 (2)

5.4.21.2 Hazard Rankings Genesee River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Genesee River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Physiological/Metabolic	1.333	1.2-1.4	2
Endocrine	1.100	1.0-1.2	1
Reproductive	1.076	1.0-1.2	1
Behavioral	1.068	1.0-1.2	1
Population-relevant Mean SV	1.049	1.0-1.2	1
Developmental	1.049	1.0-1.2	1
Mortality	1.033	1.0-1.2	1
Comprehensive Mean SV	1.031	1.0-1.2	1
Circulatory/Blood Constituents	1.000	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Genesee River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Estrone	1.417	1.4-1.6	3
DEET	1.150	1.0-1.2	1
HHCB	1.107	1.0-1.2	1
Sitosterol, beta-	1.067	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Ibuprofen	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (bold ">" = site designated as point source influenced)	Genesee River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> GNR-3	1.110	1.0-1.2	1
> GNR-4	1.096	1.0-1.2	1
GNR-1	1.067	1.0-1.2	1
> GNR-2	1.030	1.0-1.2	1
GNR-6	1.024	1.0-1.2	1
> GNR-5	1.014	1.0-1.2	1

5.4.21.3 Breadth of Information Indicating High Hazard at Genesee River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no high hazard observations. However, the evidence suggesting an absence of high hazard is weak. Only one or two surface water samples per site were analyzed for CECs. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was limited due to only two USEPA STORET water quality sampling sites with TSS data located in the lower Genesee River to represent the entire project location (Figure A2-21 in Attachment A2).

Additional potential for false negative findings is due to gaps in the fish ecotoxicity database as discussed above in Section 5.3.2.

5.4.21.4 Genesee River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences in site-specific maximum or median hazard scores between point source influenced and uninfluenced site groups at the Genesee River location. Likewise, no significant differences are observed when all hazard scores for each site - not just maximum or median scores - are included in the statistical analysis[74] (Attachment D). These negative statistical findings may be due to small total sample size in the uninfluenced site group ($n = 3$), an absence of SV_{HIGH} exceedances, and a proportional distribution of SV_{LOW} exceedances between influenced and uninfluenced sites.

A summary of the qualitative comparison is provided in the following chart below. Shown are fractions of samples and of sites at the Genesee River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Low sample numbers limit the strength of evidence in the hazard assessment for this location, with only one or two samples analyzed at each of six sites. Nevertheless, there was a total of 21 exceedances of SV_{LOW} values for DEET, estrone, HHCB and β-sitosterol distributed among the four CEC-influenced sites. At the two "uninfluenced" sites, there was a total of 15 exceedances of SV_{LOW} values for the same four CECs. There were no exceedances of SV_{HIGH} values in either site group.

5.4.21.5 Genesee River Hazard Maps

There were no exceedances of any SV_{HIGH} value at the Genesee River project location; no hazard maps were produced.

Genesee River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	0	0.0 (0/5)	0.0 (0/4)	21	1.0 (5/5)	1.0 (4/4)
Uninfluenced Sites	0	0.0 (0/4)	0.0 (0/2)	15	1.0 (4/4)	1.0 (2/2)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

[74]Cautionary caveats regarding this approach are described in Attachment D.

5.4.22 Irondequoit Bay (NY)

Irondequoit Bay is a natural coastal embayment on the south shore of Lake Ontario just east of Rochester, New York, with a surface area of approximately 6.7 km^2 (2.6 mi^2). Irondequoit Creek, the principal tributary, enters the south end of the bay after flowing northward for approximately 30.6 km (19 mi) from its headwaters in rural agricultural areas, through urban/suburban areas, draining a watershed of 438 km^2 (169 mi^2). The creek received WWTP effluent historically, and is currently stressed by agricultural and urban runoff. All six water sampling sites were designated as 'uninfluenced' by mapped CEC point sources for the purposes of this EHA. Each site was sampled once in April, 2012, for a total of only six surface water samples collected at this project location.

There is limited evidence indicating CEC-related hazard to fish in Irondequoit Bay, even though the exposure dataset was minimal. There were no SV_{HIGH} exceedances in the six Irondequoit Bay samples. However, at least one SV_{LOW} exceedance was observed in each sample, averaging 5.5 per site. There was a total of 33 SV_{LOW} exceedances associated with four CECs and seven effect categories (plus the comprehensive mean SV and population-relevant mean SV). Hazard ranks of CECs and effect categories were very low in the Irondequoit Bay. The absence of CEC-'influenced' sampling sites precluded evaluation of point source-related hazard.

Some Key Points…

Irondequoit Bay (NY)

- **Overall:** Some evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 33 occurrences, involving 100% of sampling events, and 100% of sites
  - *CECs*: DEET, β-Sitosterol, Estrone, Androstenedione
  - *Effect Categories*: 7 of 12
- **Point Source Analysis:** No evaluation ; no CEC-influenced sites
- **Exposure dataset:** Minimal
  - 6 sites
  - 1 sample per site
  - 6 total samples

5.4.22.1 Hazard Brief for Irondequoit Bay

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

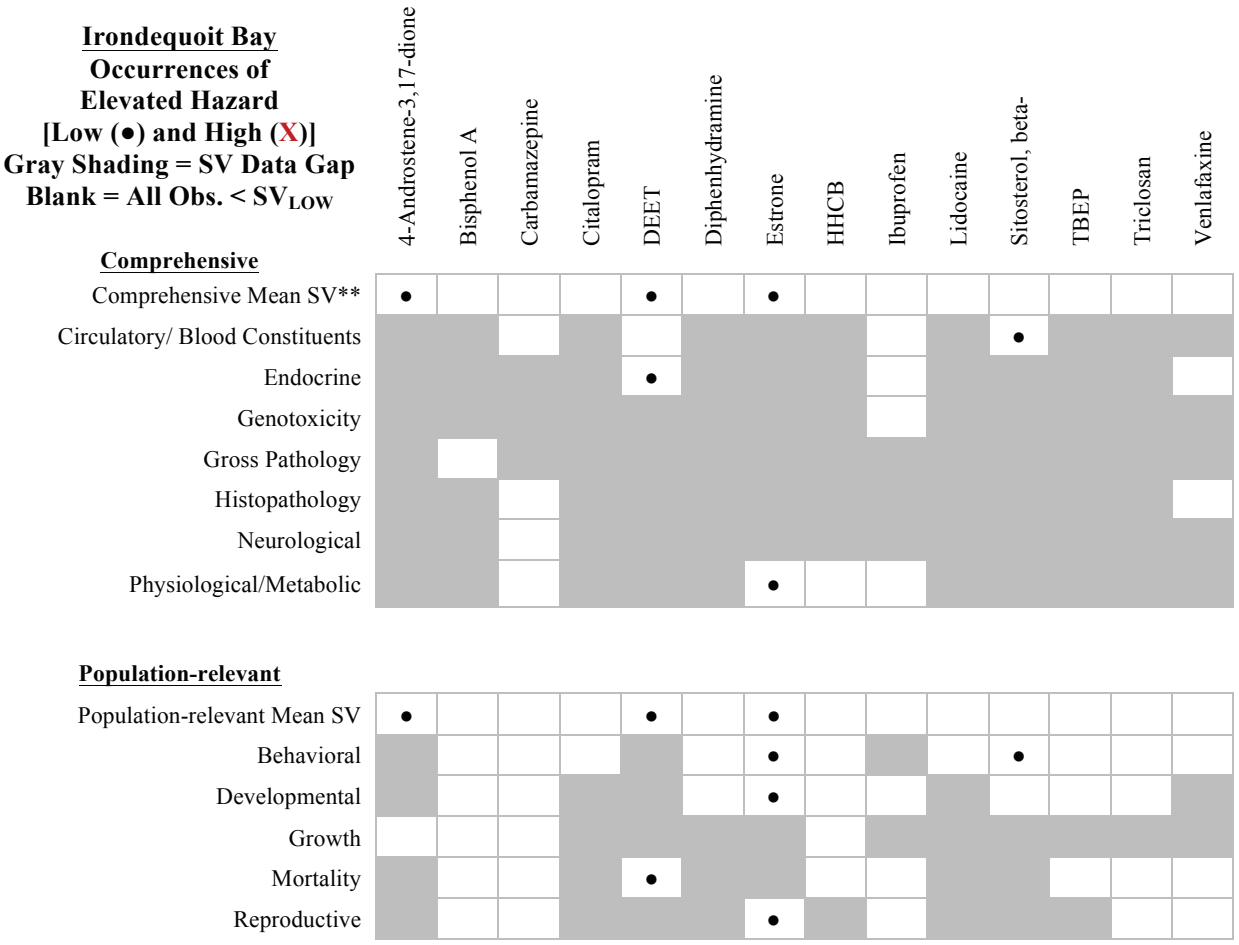
There were no exceedances of any SV_{HIGH} value for any effect category at the Irondequoit Bay project location (Attachment B; Table B-22); no hazard maps were produced.

SV_{LOW} values were exceeded in at least one sample for the following effect categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Development, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine, Physiological/Metabolic

SV_{LOW} exceedances were limited to DEET, β-sitosterol, and the two hormones (estrone and androstenedione).

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-22.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

Sampling Sites

Elevated hazard from DEET and β-sitosterol exposures was distributed among all six sites, but SV_{LOW} exceedances for estrone and androstenedione were restricted to IB-NW-PHRAG and IB05, respectively, suggesting localized, undocumented CEC hormone sources. None of these sites were identified as potentially CEC point source-influenced, and there were no observations of high hazard.

Irondequoit Bay Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site

1. IB_04 (1)
2. IB_05 (1)
3. IB_06 (1)
4. IB_06_REF (1)
5. IB_NW_PHRAG (1)
6. IB_NE_DUNE (1)

5.4.22.2 Hazard Rankings for Irondequoit Bay

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories

Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Irondequoit Bay Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.333	1.2-1.4	2
Mortality	1.125	1.0-1.2	1
Population-relevant Mean SV	1.095	1.0-1.2	1
Behavioral	1.091	1.0-1.2	1
Circulatory/Blood Constituents	1.042	1.0-1.2	1
Physiological/Metabolic	1.042	1.0-1.2	1
Comprehensive Mean SV	1.036	1.0-1.2	1
Reproductive	1.028	1.0-1.2	1
Developmental	1.019	1.0-1.2	1
Genotoxicity	1.000	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants

CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Irondequoit Bay CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.633	1.6-1.8	4
Sitosterol, beta-	1.200	1.0-1.2	1
Estrone	1.167	1.0-1.2	1
4-Androstene-3,17-dione	1.111	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
HHCB	1.000	1.0-1.2	1
Ibuprofen	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

Sampling Sites

Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (No site was designated as point source influenced)	Irondequoit Bay Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
IB_NW_PHRAG	1.122	1.0-1.2	1
IB05	1.073	1.0-1.2	1
IB04	1.061	1.0-1.2	1
IB06	1.061	1.0-1.2	1
IB_NE_DUNE	1.049	1.0-1.2	1
IB06_REF	1.037	1.0-1.2	1

5.4.22.3 Breadth of Information Indicating High Hazard at Irondequoit Bay

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no high hazard observations. However, the evidence suggesting an absence of high hazard is weak. Only one surface water sample per site was analyzed for CECs, and all samples were collected in the spring of 2012 during high flow. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only a single USEPA STORET water quality sampling site with TSS data located in lower Irondequoit Creek – the major tributary to Irondequoit Bay - to represent the entire project location (Figure A2-22 in Attachment A2).

Potential for false negative findings due to gaps in the fish ecotoxicity database is discussed above in Section 5.3.2.

5.4.22.4 Irondequoit Bay Point Source Analysis

All six Irondequoit Bay sampling sites were designated as uninfluenced by point sources, so no influenced-uninfluenced comparisons were possible.

A summary of hazard occurrence at the six "uninfluenced" sites is provided in the chart below. Shown are fractions of samples and of sites at the Irondequoit Bay location that have at least one observation of a SV_{LOW} exceedance, for any CEC and effect category.

Very low sample numbers severely limits the strength of evidence in the hazard assessment for this location, with only one sample analyzed at each of six uninfluenced sites. Nevertheless, there was a total of 33 exceedances of Effect-specific SV_{LOW} values for 4-androstene-3,17-dione, DEET, estrone, and β-sitosterol at the six sampling sites. Only DEET SV_{LOW} values were exceeded at the reference site (IB06-REF). There were no exceedances of SV_{HIGH} values.

5.4.22.5 Irondequoit Bay Hazard Maps

There were no exceedances of any SV_{HIGH} value at the Irondequoit Bay project location; no hazard maps were produced.

Irondequoit Bay Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	NA	NA	NA	NA	NA	NA
Uninfluenced Sites	0	0.0 (0/6)	0.0 (0/6)	33	1.0 (6/6)	1.0 (6/6)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

5.4.23 Oswegatchie River (NY)

The Oswegatchie River flows northward approximately 220 km (137 mi) from its headwaters in remote forests through small municipalities and agricultural areas to its mouth on the St. Lawrence River - draining a 4,120 km^2 (1,592 mi^2) watershed on the Adirondack north slope. The project location is a 60 km reach of the river main stem in the upper watershed, including two CEC-'influenced' sampling sites downstream of mapped small WWTPs, and a relatively remote reference site. Water samples were collected at all five sampling sites in August 2013 and May/August 2014, and at four of the sites (not the downstream most site) in October 2013. Occasional repeated sampling on individual sampling days yielded a total of five to seven sampling events per site.

Information generated in this EHA provides limited evidence that hazards to fish from exposures to CECs are present in the upper Oswegatchie River. Despite a relatively robust exposure dataset, there were no SV_{HIGH} exceedances at this project location and SV_{LOW} exceedances were limited to 87 observations (4%) out of a total of 2,235 location-specific hazard scores generated for the Oswegatchie River location. All effect category and CEC hazard ranks were very low. There was no statistical or qualitative evidence that hazards were higher in downstream proximity to the WWTPs. In fact, observations of slightly elevated hazards at sites initially designated as 'uninfluenced' by CEC point sources suggests there may be additional CEC sources in the system that were not accounted for in this analysis. However, it is possible that if significant ecotoxicity data gaps in the derivation of the current set of SVs were filled, additional and/or greater hazards to fish might be observed.

Some Key Points…

Oswegatchie River (NY)

- **Overall:** Limited evidence of hazards to fish
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 87 occurrences, involving 68% of sampling events, and 100% of sites
  - *CECs*: DEET, β-Sitosterol, Estrone
  - *Effect Categories*: 7 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
- **Exposure dataset:** Substantial
  - 5 sampling sites
  - 5-7 samples per site
  - 31 total samples

5.4.23.1 Hazard Brief for Oswegatchie River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants

There were no exceedances of any SV_{HIGH} value for any effect category at the Oswegatchie River project location (Attachment B; Table B-23); no hazard maps were produced.

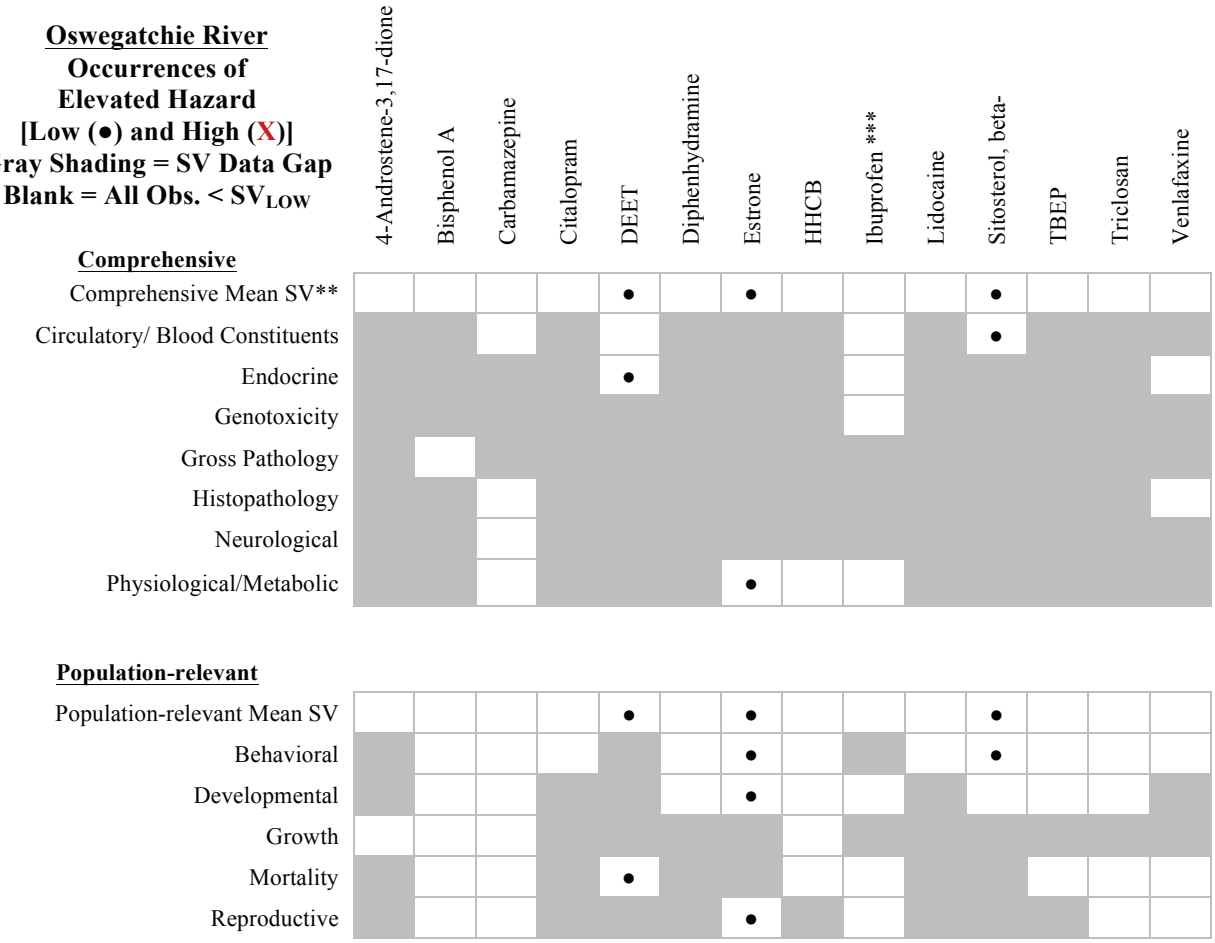
SV_{LOW} values were exceeded in at least one sample for the following Effect Categories:

- Population-relevant: Population-relevant mean SV, Behavioral, Development, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine, Physiological/Metabolic

SV_{LOW} exceedances were observed for three CECs - DEET, β-sitosterol and estrone - with estrone showing potential impacts in four effect categories (plus comprehensive mean SV and population-relevant mean SV).

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-23.

Oswegatchie River
Occurrences of Elevated Hazard [Low (●) and High (X)]
Gray Shading = SV Data Gap
Blank = All Obs. < SV_{LOW}



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

Elevated hazards from DEET and β-sitosterol were distributed among all five sampling sites. Estrone impacts were restricted to OSW-4, a site at the downstream end of a very small impoundment that received inputs from a paper mill until 2011 and historically from a purportedly closed WWTP. Due to this closure, this site had been designated as currently "uninfluenced" by CEC point sources (WWTPs or CSOs) . There were no observations of high hazard at this project location.

Oswegatchie River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source

1. OSW-1 (5)
2. **> OSW-2 (7)**
3. OSW-3 (6)
4. OSW-4 (6)
5. **> OSW-5 (7)**

5.4.23.2 Hazard Rankings for Oswegatchie River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[75]
Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Oswegatchie River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.236	1.2-1.4	2
Circulatory/Blood Constituents	1.161	1.0-1.2	1
Mortality	1.067	1.0-1.2	1
Behavioral	1.047	1.0-1.2	1
Population-relevant Mean SV	1.041	1.0-1.2	1
Comprehensive Mean SV	1.029	1.0-1.2	1
Physiological/Metabolic	1.011	1.0-1.2	1
Reproductive	1.007	1.0-1.2	1
Developmental	1.004	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1
Histopathology	1.000	1.0-1.2	1
Neurological	1.000	1.0-1.2	1

Emerging Contaminants[76]
CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Oswegatchie River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.290	1.2-1.4	2
Sitosterol, beta-	1.262	1.2-1.4	2
Estrone	1.033	1.0-1.2	1
4-Androstene-3,17-dione	1.000	1.0-1.2	1
Bisphenol A	1.000	1.0-1.2	1
Carbamazepine	1.000	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
HHCB	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1
TBEP	1.000	1.0-1.2	1
Triclosan	1.000	1.0-1.2	1
Venlafaxine	1.000	1.0-1.2	1

[75]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

[76]Analytical data for ibuprofen was not included in the exposure database for this project location.

Sampling Sites

Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (bold ">" = site designated as point source influenced)	Oswegatchie River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
OSW-4	1.068	1.0-1.2	1
OSW-1	1.047	1.0-1.2	1
OSW-3	1.041	1.0-1.2	1
> OSW-2	1.025	1.0-1.2	1
> OSW-5	1.022	1.0-1.2	1

5.4.23.3 Breadth of Information Indicating High Hazard at Oswegatchie River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

There were no high hazard observations. The evidence suggesting an absence of high hazard due to the 14 CECs is moderately strong. Between five and seven surface water samples per site were analyzed for CECs, and samples were collected over a two-year period at various times of day and in various seasons, including low-flow periods. However, this finding applies only to the upper half of the river main stem which is point source CEC-influenced by two mapped WWTPs associated with small communities. No river tributaries were sampled and no sampling occurred in the lower river that receives continuous CEC inputs from WWTPs at significantly larger municipalities. Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only a single USEPA STORET water quality sampling site with TSS data located in the lower Oswegatchie River (near its mouth at the St. Lawrence River) to represent the entire project location (Figure A2-23 in Attachment A2).

Further, the potential for false negative findings due to gaps in the fish ecotoxicity database is discussed above in Section 5.3.2.

5.4.23.4 Oswegatchie River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be influenced by CEC discharges from point sources and sites that are expected to be relatively uninfluenced by CEC point sources. We quantitatively evaluated whether site-specific maximum or median hazard scores are elevated at point source CEC-influenced sites versus uninfluenced sites using non-parametric analysis, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4).

CEC	Effect Category	Significant Difference in Hazard Scores Between Groups ($p < 0.1$)	
		Where Maxima Compared	Where Medians Compared
β-Sitosterol	Comprehensive Mean SV	Y	N
	Behavioral	N	Y
	Circulatory/Blood Constituents	N	Y

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and of sites at the Oswegatchie River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Oswegatchie River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	0	0.0 (0/14)	0.0 (0/2)	22	0.5 (7/14)	1.0 (2/2)
Uninfluenced Sites	0	0.0 (0/17)	0.0 (0/3)	65	0.82 (14/17)	1.0 (3/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There were no exceedances of SV_{HIGH} values in either site group. There was a total of 22 exceedances of SV_{LOW} values for DEET and β-sitosterol at the two CEC-influenced sites. However, at the three "uninfluenced" sites, there was a total of 65 exceedances of Effect-specific SV_{LOW} values for DEET, β-sitosterol and estrone. Thirty of these SV_{LOW} exceedances occurred at OSW-4, including six exceedances of estrone.

There was historically a WWTP just upstream of an "uninfluenced" sampling site (OSW-4) at Newton Falls (see Attachment A – Table A2-23) that was purportedly closed prior to water sampling in 2013 and 2014. So for this EHA, OSW-4 was designated a priori as an 'uninfluenced' site. However, the presence of elevated hazard from estrone - only at the OSW-4 site (Attachment B, Tables B-23a and B-23b) - along with numerous other SV exceedances at this site suggest that there was an on-going CEC source at the time of sampling in the near upstream of OSW-4, which is where the "closed" WWTP operated. The higher β-sitosterol-related hazard in the "uninfluenced" group versus the influenced group in the hazard comparison using all sample data (Attachment D) support this hypothesis.

5.4.23.5 Oswegatchie River Hazard Maps

There were no high hazard observations, so no hazard maps were generated.

5.4.24 Raquette River (NY)

The Raquette River flows northward from the Adirondack Mountains to the St. Lawrence River, traversing remote forests, natural and constructed impounded lakes, agricultural lands, numerous small municipalities and their sanitary sewer discharges, and larger municipalities near its mouth. The river is 235 km (146 mi) long, draining a 3,250 km^2 (1,250 mi^2) watershed. The project area was an approximately 90 km (56 mile) reach of the river system in the middle region of the watershed. There were six sampling sites, three of which were designated CEC-'influenced' and three 'uninfluenced' based on downstream proximity to mapped, relatively small municipal WWTPs. A total of between six and eight samples were collected at each site in May and August of 2014 and at five sites in June and July in 2013.

There is substantial evidence for intermittent hazard to fish from aqueous CEC exposures in the middle Raquette River. High hazard (SV_{HIGH} exceedance) was observed a total of five times, involving endocrine hazard from DEET exposure at two sites and physiological/metabolic hazard from estrone exposure at one site. Supporting data indicating hazard included the incidence of SV_{LOW} exceedances - nearly five exceedances per sampling event and nearly 38 exceedances per sampling site. However, the hazard ranks of nearly all CECs and effect categories were assigned the lowest value of '1' based on mean hazard scores at this project location, indicating that most individual hazard scores were very low. In the middle Raquette River, there was little statistical or qualitative evidence that point sources significantly elevated hazard to fish from exposure to the 14 CECs.

The observations of high hazard occurred despite significant data gaps in published ecotoxicity information. Breadth of ecotoxicity information contributing to observations of high hazard was 'moderate' for DEET endocrine SVs and 'sparse' for estrone physiological/metabolic SVs.

Some Key Points…

Raquette River (NY)

- **Overall:** Substantial evidence of hazards to fish
- **High Hazard:**
  - 5 occurrences, involving 11% of sampling events, and 33% of sites
  - *CECs*: DEET, Estrone
  - *Effect Categories*: Endocrine, Physiological/Metabolic
- **Low Hazard:**
  - 227 occurrences, involving 83% of sampling events, and 100% of sites
  - *CECs*: 10 of 14
  - *Effect Categories*: 9 of 12
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources
  Exposure dataset: Substantial
  - 6 sampling sites
  - 6 or 8 samples per site
  - 46 total samples

5.4.24.1 Hazard Brief for Raquette River

This section describes highlights of the hazard characterization for this project location, in terms of hazard associated with effect categories, CECs, and sampling sites.

Effect Categories and Emerging Contaminants
Raquette River effect-specific hazard maps are provided for endocrine and physiological/metabolic effect categories, for which relatively high hazard was observed in at least one sample for at least one CEC (Attachment B; Table B-24):

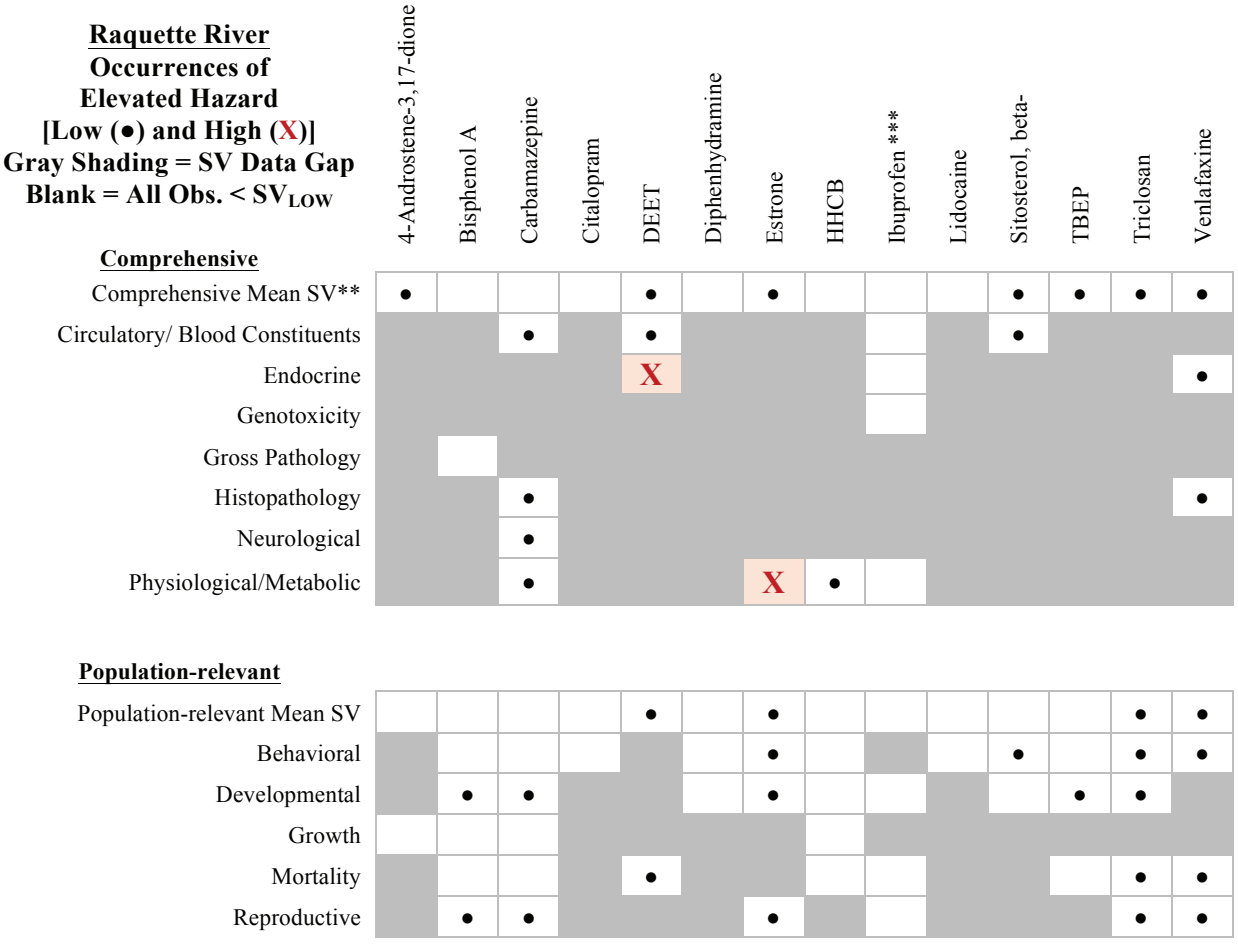
The following additional effect categories showed exceedances of SV_{LOW} values:

- Population-relevant: Population-relevant mean SV, Behavioral, Developmental, Mortality, Reproductive
- Comprehensive: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological

Relatively high hazard was observed only for estrone (physiological/metabolic hazard) and DEET (endocrine

hazard). Concentrations of eight additional CECs exceeded a SV_{LOW} value in at least one sample: androstenedione, bisphenol A, carbamazepine, HHCB, β-sitosterol, TBEP, triclosan and venlafaxine. Emerging contaminants that did not exceed any screening value were citalopram, diphenhydramine and lidocaine. Analytical data for ibuprofen were not available for this project location.

The following chart indicates CECs and effect categories with at least one observation of elevated hazard, based on data presented in Attachment B, Table B-24.



** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA, in order to eliminate confusion with population-relevant SVs for the same effect categories. These comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see introduction to Section 5.4). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** No hazard scores are available for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; this project location was sampled only during 2013-2014.

Sampling Sites

High endocrine hazard was observed at two sites – one site (RAQ-5-RAQ) influenced by WWTP discharge in Raquette Pond, an embayment within the Raquette River system, and one designated uninfluenced site (RAQ-4-PIERCE) in Piercefield Flow - the next downstream impoundment - which is >4 km downstream of the WWTP. The physiological/metabolic SV_{HIGH} was exceeded only at RAQ-5-RAQ. Exceedances of SV_{LOW} values (hazard score = 2) are widespread (Attachment B, Table B-24), with 9 effect categories (plus comprehensive mean SV and population-relevant mean SV) involved at RAQ-5-RAQ and also at the downstream-most site (RAQ-1-NOR) that receives CEC inputs from several point sources. At the reference site (RAQ-6-TUPPER), an 'uninfluenced' site with no mapped upstream point sources, androstenedione, DEET and β-sitosterol exceeded the SV_{LOW} across a total of four effect categories (plus comprehensive mean SV and population-relevant mean SV.) In the chart below, red-highlighted sites had at least one observation of high hazard.

Raquette River Project Location – Surface Water Sampling Sites
(N) = Total Number of sampling events per site
Bold ">" = Identified as Potentially Influenced by mapped CEC Point Source
Red Text = At least one observation of high hazard in at least one sample

1. **> RAQ-1-NOR (8)**
2. **> RAQ-2-WWTP (8)**
3. RAQ-3 (6)
4. RAQ-4-PIERCE (8)
5. **> RAQ-5-RAQ (8)**
6. RAQ-6-TUPPER (8)

5.4.24.2 Hazard Rankings for Raquette River

In this section, we provide rankings of mean hazard among effect categories, among CECs, and among sampling sites as described in the introduction to Section 5.4. In the following charts, we ranked hazard on a scale from 1 to 10 using hazard bins defined in Table 5-3. Elements falling within the same hazard bin were assigned the same hazard rank. We utilized inverse ranking, where a rank of 1 indicated the lowest relative hazard and a rank of 10 indicated the highest possible hazard. It is important to note that higher hazard rank simply indicates that, given the available information, there is a greater expectation that impacts to fish may occur, not that the severity of expected impacts is necessarily greater.

Effect Categories[77]

Effect category ranks based on bins for mean hazard (averaged across CECs and Sites) are as follows (see Table 5-3):

Effect Category	Raquette River Effect-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
Endocrine	1.372	1.2-1.4	2
Physiological/Metabolic	1.125	1.0-1.2	1
Mortality	1.103	1.0-1.2	1
Comprehensive	1.085	1.0-1.2	1
Histopathology	1.083	1.0-1.2	1
Circulatory/Blood Constituents	1.069	1.0-1.2	1
Reproductive	1.068	1.0-1.2	1
Behavioral	1.066	1.0-1.2	1
Neurological	1.063	1.0-1.2	1
Population-relevant Mean SV	1.059	1.0-1.2	1
Population-relevant	1.058	1.0-1.2	1
Comprehensive Mean SV	1.043	1.0-1.2	1
Developmental	1.027	1.0-1.2	1
Gross Pathology	1.000	1.0-1.2	1
Growth	1.000	1.0-1.2	1

[77]Genotoxicity not included because the only CEC with genotoxicity SVs is ibuprofen, and analytical data for ibuprofen was not included in the exposure database for this project location.

Emerging Contaminants[78]
CEC ranks based on bins for mean hazard (averaged across Effect Categories and Sites) are as follows (see Table 5-3):

CEC	Raquette River CEC-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
DEET	1.438	1.4-1.6	3
Sitosterol, beta-	1.142	1.0-1.2	1
Triclosan	1.063	1.0-1.2	1
Venlafaxine	1.063	1.0-1.2	1
Estrone	1.045	1.0-1.2	1
Carbamazepine	1.038	1.0-1.2	1
HHCB	1.036	1.0-1.2	1
Bisphenol A	1.017	1.0-1.2	1
4-Androstene-3,17-dione	1.014	1.0-1.2	1
TBEP	1.013	1.0-1.2	1
Citalopram	1.000	1.0-1.2	1
Diphenhydramine	1.000	1.0-1.2	1
Lidocaine	1.000	1.0-1.2	1

Sampling Sites
Sampling site ranks based on bins for mean hazard (averaged across Effect Categories and CECs) are as follows (see Table 5-3):

Sampling Site (bold ">" = site designated as point source influenced)	Raquette River Site-Specific Mean Hazard Score (range: 1-3)	Mean Hazard Score Bin (bin range: 1-3, in 0.2 increments)	Mean Hazard Score Bin Rank (rank range: 1-10)
> RAQ-1-NOR	1.127	1.0-1.2	1
> RAQ-5-RAQ	1.113	1.0-1.2	1
RAQ-4-PIERCE	1.065	1.0-1.2	1
RAQ-6-TUPPER	1.043	1.0-1.2	1
> RAQ-2-WWTP	1.029	1.0-1.2	1
RAQ-3	1.025	1.0-1.2	1

5.4.24.3 Breadth of Information Indicating High Hazard at Raquette River

This section provides an assessment of the strength of evidence of available exposure and ecotoxicity information that was incorporated into the hazard characterization.

- *Exposure*: Spatial coverage of the project location was poor, with a total of only six sampling sites clustered in groups of three at both ends of a sixty kilometer stretch of the middle river, leaving a number of point sources unsampled. The lower river was not sampled for CECs in this project. However, the temporal distribution of samples was good, with samples collected at various times of day in four different months over two years (Attachment A, Table A-2). Conversions of total CEC to aqueous CEC (Section 3-5, Attachment A1) required empirical TSS data from the project location. Confidence in the representativeness of the TSS data available for this conversion was minimal due to only a single USEPA STORET water quality sampling site with TSS data located in the middle Raquette River to represent the entire project location (Figure A2-24a in Attachment A2).

- *Ecotoxicity*: The breadth of ecotoxicity information relevant to the high hazard observations in DEET (endocrine effects) and estrone (physiological/metabolic effects) were moderate and sparse, respectively.

[78]Analytical data for ibuprofen was not included in the exposure database for this project location.

5.4.24.4 Raquette River Point Source Analysis

In this section, we provide quantitative and qualitative comparisons of hazard scores, between sites that are suspected to be CEC-'influenced' by discharges from point sources and sites that are expected to be relatively 'uninfluenced' by CEC point sources. We quantitatively evaluated whether hazard is elevated at point source CEC-influenced sites relative to uninfluenced sites using a non-parametric statistical test, for each CEC and effect category evaluated in this EHA (details provided in Section 5.3.4). There were no statistically significant differences in site-specific maximum or median hazard scores between point source influenced and uninfluenced site groups at the Raquette River location, which may be due to reasonably proportional distribution of SV_{HIGH} and SV_{LOW} exceedances between the CEC-influenced and uninfluenced site groups.

However, when all hazard scores for each site in the Raquette River location - not just maximum or median scores - are included in the statistical analysis[79], significant differences between site groups ($p < 0.1$) are observed for reproductive and histopathology effects associated with carbamazepine exposure and for physiological/metabolic effects associated with HHCB exposure (Attachment D).

A summary of the qualitative comparison is provided in the following chart. Shown are fractions of samples and sites at the Raquette River location that have at least one observation of a SV_{HIGH} exceedance, for any CEC and effect category. Also shown are fractions having SV_{LOW} exceedances. These numbers are provided for point source CEC-influenced versus uninfluenced site groups.

Raquette River Site Group	SV_{HIGH} Exceedances *			SV_{LOW} Exceedances *		
	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)	Total Number of Exceedances	Fraction of Sampling Events (N Events with Exceedances/ Total Events)	Fraction of Sampling Sites (N Sites with Exceedances/ Total Sites)
Point Source CEC-Influenced Sites	3	0.13 (3/24)	0.33 (1/3)	153	0.83 (20/24)	1.0 (3/3)
Uninfluenced Sites	2	0.09 (2/22)	0.33 (1/3)	74	0.82 (18/22)	1.0 (3/3)

* Excluding exceedances of SVs in the *comprehensive* behavioral, developmental, growth, mortality and reproductive effect categories.

There were three exceedances of effect-specific SV_{HIGH} values for DEET and estrone at one of the three CEC-influenced sites (RAQ-5-RAQ), which is located in the Raquette Pond impoundment of the river - proximal to the WWTP discharge pipe from the village of Tupper Lake. There were two exceedances of the endocrine SV_{HIGH} for DEET at one "uninfluenced" sampling site (RAQ-4-PIERCE) just downstream of Raquette Pond below the drop structure at the upstream end of Piercefield Flow.

There was a total of 153 exceedances of various effect-specific SV_{LOW} values for 10 of 14 CECs at the three point source CEC-influenced sites. A total of 74 SV_{LOW} exceedances for 4-androstene-3,17-dione, bisphenol A, DEET and β-sitosterol were observed at the three "uninfluenced" sites. These include 25 exceedances for 4-androstene-3,17-dione, DEET and β-sitosterol at the "reference" site (RAQ-6-TUPPER) for the Raquette River project location, at the southern end of Tupper Lake, approximately 10 kilometers upstream of the nearest mapped CEC point source.

The fairly even distribution of SV_{HIGH} and SV_{LOW} exceedances between the site groups suggests there could be non-point and/or unmapped point CEC sources affecting the "uninfluenced" sites.

[79]Cautionary caveats regarding this approach are described in Attachment D.

5.4.24.5 Raquette River Hazard Maps

In this section, we provide hazard maps for effect categories with at least one observation of high hazard, interpreted as described in Section 5.3.3.

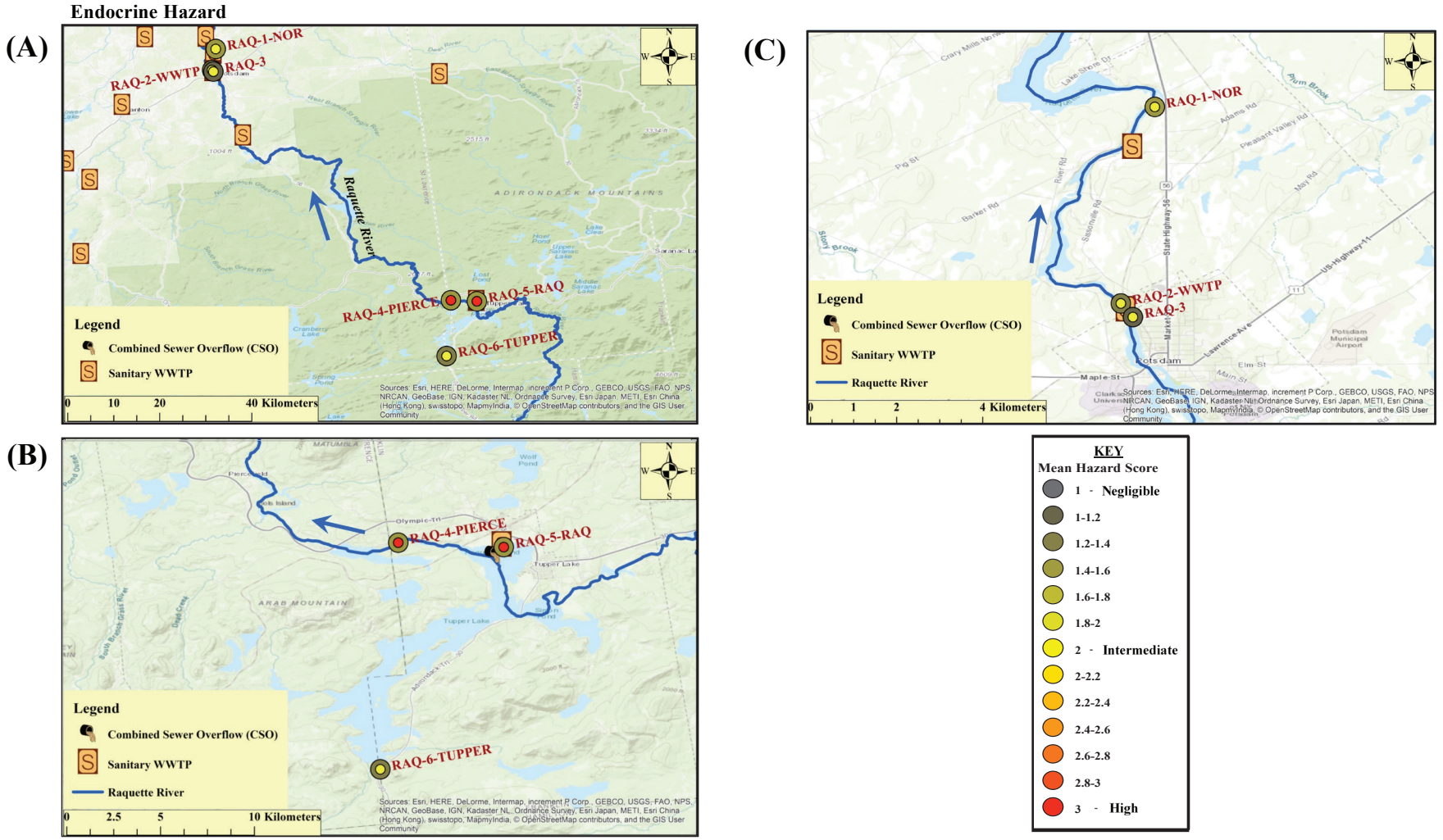


Figure 5-63. Endocrine Hazard Map for Raquette River Watershed. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores.(A) Entire project location; (B) Upper Raquette River; (C) Middle Raquette River. Arrows indicate river flow direction.

Physiological/Metabolic Hazard

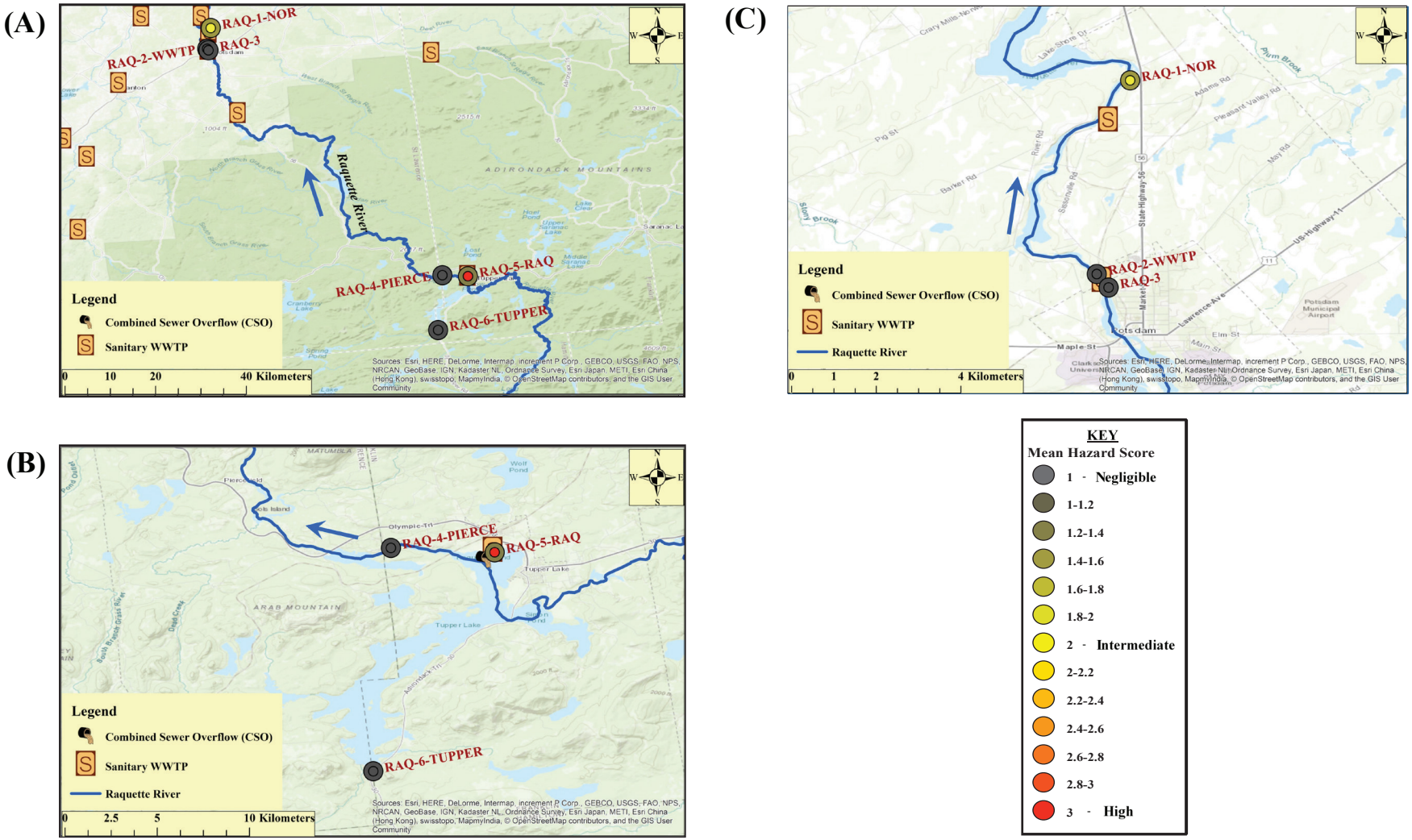


Figure 5-64. Physiological/Metabolic Hazard Map for Raquette River Watershed. Large circles indicate mean hazard scores; smaller dots indicate maximum hazard scores. (A) Entire project location; (B) Upper Raquette River; (C) Lower Raquette River. Arrows indicate river flow direction.

Chapter 6 - Project-Wide Hazard Summary and Synthesis

Are CECs hazardous[80] to fish in the Great Lakes Basin? To address that question, we have conducted a detailed hazard assessment for each of the 24 project locations. We used CEC exposure data on 14 CECs collected at a total of 195 surface water sampling sites across the locations (Figure 3-1), and characterized hazard in each water sample collected at those sites using an array of screening values (Tables 4-1 to 4-3, based on Gefell et al. 2019). For each project location (Section 5.4) we generated: descriptive hazard summaries; SV exceedance tallies; rankings of CECs, effect categories and sampling sites with respect to overall CEC-related hazard; statistical evaluations of point source CEC influence (where possible); strength of evidence evaluations; and effect-specific hazard maps.

We have found that elevated hazard to fish from aqueous CECs is common among the U.S. Great Lakes Basin waterbodies included in this assessment. Despite evaluating hazard for only 14 parent compounds, we found that CEC-related adverse biological effects are plausible at each of the 24 project locations (based on SV_{LOW} exceedances), and that high potential for biological impacts is widespread among the locations (based on SV_{HIGH} exceedances).

While broad patterns were discernable in the project-wide hazard results dataset, universally consistent patterns were not identified. For instance, we observed that:

- the set of effect categories exhibiting hazard varies among project locations,
- diverse CECs may be associated with particular effect-specific hazards, and
- the set of CECs associated with a particular effect-specific hazard often varies between project locations and between sampling sites within project locations.

For most CECs with a non-zero detection frequency at a project location, there was at least one exceedance of an SV_{LOW} or SV_{HIGH} at that location (Table 6-1). Two exceptions were lidocaine and TBEP for which even high percent detections (ranging up to 100%) did not necessarily result in a SV exceedance. Conversely, even very low detection frequencies resulted in observations of elevated hazard in a number of cases – especially where sample size was high (Table 6-1). An implication of this apparent lack of a universally consistent relationship between CEC detectability and observed elevated hazard is that selection of additional CECs for development of SVs should not necessarily be limited by relatively low detection frequencies.

Based on these observations we predict that if this analysis were expanded to include additional CEC SVs with published laboratory assays that report effects in fish at environmentally relevant exposure concentrations – even those less frequently detected than the 14 CECs in this EHA - then additional CEC-specific hazards and greater overall CEC-related hazard would be observed.

Altogether, project-wide observations underscore the importance of assessing hazard using diverse CEC concentration data (chemical analyte list and spatial/temporal distribution of samples) and evaluating concentrations with a variety of effect-specific CEC SVs.

In this chapter we summarize hazard information, identifying project-wide patterns of CEC-related hazard to fish using four lenses:

- *hazard magnitude*, as hazard ranks based on mean hazard scores (Section 6.1),
- *hazard prevalence*, as a project-wide summary of location-specific hazard (Section 6.2),
- *hazard type*, as effect-specific summaries including project-wide hazard maps (Section 6.3), and
- *hazard attribution*, as CEC-specific summaries including project-wide hazard maps (Section 6.4).

[80]We have applied the term "hazard" throughout this EHA to mean that there is potential for biological impacts to fish from aqueous CEC exposures. We have drawn a distinction between "population-relevant" and "comprehensive" SVs and their associated hazards (see explanation in Section 4.2). We have defined "population-relevant" SVs as those that were derived from exposure-effects data directly pertaining to the potential for population-level impacts in fish. Hazard characterization utilizing population-relevant SVs identify population-relevant hazards, which may be of particular interest to natural resource managers and scientists. "Comprehensive" SVs were derived using a superset of exposure-effects data that includes all of the population-relevant information, plus additional data on adverse effects endpoints that are relevant to individual organisms, but were not considered directly pertinent to population-level impacts.

6.1 Hazard Magnitude: Hazard Ranks

In this section, we present compilations of hazard ranks in order to determine whether there are certain effect categories, or CECs, that consistently either dominate hazard or contribute little to overall hazard. That is, we explored whether certain effect categories, or certain CECs, consistently rank high, or low, across project locations.

Project-wide tabulations were assembled from location-specific hazard information provided in Section 5.4, where we ranked simple averages of hazard scores[81] for each project location either by effect category (across CECs), or by CEC (across effect categories), across all sampling sites and events. Inverse ranking was utilized[82] . Within each project location, effect categories and CECs were assigned ranks ranging from 10 (highest hazard rank) to 1 (lowest hazard rank). Ranks were assigned based on mean hazard scores, using hazard score bins (see introduction to Section 5.4), thus ensuring consistent interpretation across project locations. It is important to note that:

- High hazard means that there is potential for biological impacts in fish related to the specific effect endpoints associated with the particular LOAECs used to derive the SV_{HIGH} values that were exceeded,
- Low ranks associated with relatively low mean hazard scores do not preclude intermittent observations of elevated hazard – they only indicate that the average hazard score was low, and
- Patterns in *relative* rankings identified in this section, while informative and sometimes very strongly suggestive, are provisional because there are significant data gaps in the fish ecotoxicity literature for the 14 CECs with SVs.

We compiled hazard ranking information for effect categories and CECs from all of the project locations (Tables 6-2a and 6-2b). To facilitate pattern identification in these tables, we applied a color gradient to hazard ranks with the maximum numeric hazard rank (10) assigned darkest orange and the lowest numeric rank (1) assigned no color. While it may be premature to conclude that observed patterns in hazard rankings are indicative of underlying relative biological effect sensitivity or relative CEC potency, the following analysis nevertheless provides substantial evidence for widespread elevated hazard to fish from aqueous CEC exposures.

6.1.1 Patterns in Hazard Ranks of Effect Categories

Effect categories with relatively high hazard ranks varied between project locations (Table 6-2a). Among population-relevant effect categories, reproductive effects most consistently ranked relatively high across project locations, followed by behavioral and mortality effects, while the growth effect category achieved the lowest hazard rank at all project locations.

Among comprehensive effect categories, endocrine effects ranked relatively high across almost all project locations, followed by histopathology, neurological, and physiological/metabolic. Gross pathology consistently ranked very low. High endocrine hazard was often attributed to DEET and venlafaxine (Table 6-2b); the ubiquity of elevated endocrine hazard is likely related to ubiquitous high DEET hazard (see Section 6.1.2).

Among comprehensive effect categories, prevalence of high hazard among project locations was greatest for endocrine effects, followed by physiological/metabolic and genotoxicity effects (Table 6-4). Prevalence of locations with SV_{LOW} exceedances (Table 6-5) was greatest for endocrine, followed by physiological/metabolic, circulatory/blood constituents, histopathology and neurological effect categories. Among population-relevant effect categories, high hazard occurred in ~25% of project locations for developmental, mortality, and reproductive effect categories (Table 6-4), and SV_{LOW} exceedance was ubiquitous for all population-relevant effect categories except growth (Table 6-5).

North Shore Channel, Little Calumet River, Cuyahoga River and Tinkers Creek consistently ranked relatively high across nearly all effect categories, indicating substantially higher potential for adverse impacts to fish at these project locations as compared to the others (Table 6-2a). The next most affected river systems were the Maumee, Grand/Maple, Milwaukee, Saginaw and Clinton. Effect categories consistently ranked low at the St. Clair River, Detroit River, River Raisin, Ashtabula River, Long Pond, Genesee River, Irondequoit Bay, Oswegatchie River, and Raquette River project locations (Table 6-2a). However, relatively low mean ranks do not preclude the occurrence of intermittent high hazards, which are identified in Section 6.2.

6.1.2 Patterns in Hazard Ranks of CECs

We evaluated whether there are certain CECs that dominate hazard across project locations, or others that frequently have negligible mean hazard scores. Certain project locations had elevated hazard ranks for only a few CECs, and the specific CECs with relatively high hazard ranks varied between these project locations

[81]The rationale for using mean hazard scores rather than median scores in hazard ranking is provided in the introduction to Section 5.4.

[82]A numeric rank of '1' is often used to indicate the greatest or highest among alternatives, and a higher numeric value typically indicates lower rank. However, we employed inverse ranking, where a higher numeric value for rank corresponds with greater hazard. For instance, a hazard rank of '3' indicates greater hazard than a hazard rank of '1'. We did this to simplify interpretation and communication of hazard rank comparisons.

(Table 6-2b).
Ibuprofen and venlafaxine account for all high hazard observations in population-relevant effect categories. High developmental and reproductive hazard is associated only with ibuprofen, while high mortality hazard is associated only with venlafaxine (Table 6-4). No SV_{HIGH} value was exceeded for the behavioral or growth effect categories. Strength of evidence associated with ibuprofen developmental and reproductive SVs is high, based on a 'broad' information base used to derive the SVs, but the breadth of information incorporated into the venlafaxine mortality SVs is 'sparse' (Table 4-5); hence, our confidence in the observed incidence of high reproductive and developmental hazard is higher than that for mortality. Exceedance of population-relevant SV_{LOW} values is pervasive, substantially supporting the plausibility of widespread CEC-related hazard to fish populations and communities. Prevalence of population-relevant SV_{LOW} exceedances is 20 or more project locations in multiple effect categories for DEET and estrone, and between 10 and 20 locations in multiple effect categories for bisphenol A, carbamazepine, citalopram, triclosan and venlafaxine (Table 6-5). The strength of information used to derive these SVs ranged widely (Table 4-5). Exceedance of the mortality SV_{LOW} for DEET occurred in 23/24 locations (Table 6-5), but the corresponding strength of evidence was "limited" (Table 4-5).

DEET stands out among the CECs associated with elevated hazard occurring in comprehensive effect categories. DEET consistently ranked high among the 14 subject CECs across project locations, suggesting that it has the potential to impact aquatic resources regionally (Table 6-2b). However, it is possible the ubiquity of relatively high DEET hazard identified in this EHA is due as much to our sampling schedule (Table 3-1) coinciding with a seasonal DEET loading peak and widespread distribution, as to environmental persistence or relative potency. The environmental half-life of DEET in water is only days to weeks, so it is not considered an environmentally persistent organic pollutant (Weeks et al. 2012). The SV values for DEET are not particularly low compared to the other CECs considered in this EHA; DEET SVs are intermediate values among SVs (Tables 4-2 and 4-3). However, DEET is known to be distributed by atmospheric transport (Ferrey et al. 2018) so elevated concentrations in surface water are not limited to reaches downstream of point sources or areas susceptible to runoff. There are many routes by which DEET enters surface waters, including atmospheric deposition, runoff, incidental direct spray onto surface waters, dissolution from the skin of recreating people, point sources such as WWTPs and CSOs, undocumented point sources, etc. Further, peak DEET use is seasonal and nearly all of the water sampling conducted for this study occurred within the annual usage period for insect repellents (late spring through fall), when the bulk of the annual loading to surface waters likely occurs. Furthermore, with the persistence or spread of serious tick- and mosquito-borne human pathogens, combined with increased efforts to educate the public about these health threats, it is possible that DEET usage and loading to the environment is on the rise.

High hazard (SV_{HIGH} exceedance) attributed to DEET was exclusively associated with the endocrine effect category, occurring at 15 of 24 project locations (Table 6-4). The strength of evidence associated with the endocrine SV_{HIGH} for DEET was classified as "moderate" (Table 4-6), but endocrine effects were evaluated in only one study (Zenobio et al. 2014) in one fish species and effect endpoints are restricted to gene expression for several hormones (from Gefell et al. 2019).

After DEET-related hazard, the next most consistently elevated hazard is associated with venlafaxine, estrone and carbamazepine (Tables 6-2, 6-4), as well as ibuprofen (Table 6-4), all of which are related to medical treatment of humans so WWTPs and CSOs are expected to be principal sources to aquatic environments. For estrone, high physiological/metabolic hazard (SV_{HIGH} exceeded) occurred at 9/24 project locations (Table 6-4), while low hazard (SV_{LOW} exceeded) was observed at nearly every project location for the physiological/metabolic and comprehensive mean SV categories (Table 6-5). Venlafaxine SV_{HIGH} values were exceeded for endocrine, histopathology, and comprehensive mean SV categories at between three and eight project locations out of 24 (Table 6-4). The same three effect categories each had SV_{LOW} exceedances attributable to venlafaxine at >13 locations (Table 6-5).

Among the 14 CECs, lidocaine received the lowest hazard rank at all project locations. Other CECs that consistently scored near the bottom of the relative hazard range across project locations included 4-androstene-3,17-dione, bisphenol A, diphenhydramine, and TBEP. It is important to remember, though, that relatively low overall hazard rank does not preclude intermittent elevated hazard. For example, even though overall ranks for bisphenol A and TBEP were consistently low, developmental and reproductive SV_{LOW} values for bisphenol A were exceeded at half of the project locations, and the developmental SV_{LOW} for TBEP was exceeded at 11/24 locations (Table 6-5). It is also important to note that false negative findings are possible due to many datagaps in SVs and the underlying ecotoxicity information (see Chapter 7). Hazard ranks of other CECs are more widely variable between project locations.

Among project locations, CEC-related hazard most consistently ranked high at the North Shore Channel and Little Calumet River locations, followed by Tinkers Creek, Cuyahoga River, Grand River/Maple River, Maumee River, Saginaw River and Clinton River (Tables 6-2a and 6-2b). These locations are more at risk for negative impacts to fish than the others. The St. Clair River was the only project location with the lowest possible rank for all CECs; the Kewaunee and Oswegatchie Rivers also consistently ranked very low across CECs.

6.2 Hazard Prevalence: Project-wide Summary of Location-Specific Hazards

We gained further insight into the spatial and temporal extent of hazard within and across project locations by inspecting SV_{HIGH} and SV_{LOW} exceedance[83] tallies. We based our tallies of SV exceedances within each project location (Table 6-3) on hazard scores (see Section 5.2). The total number of hazard scores[84] generated at a given project location is determined by the number of sampling sites, the number of samples collected at each sampling site, the set(s) of CECs analyzed in each sample, and the number of applicable SV pairs (see Table 4-1). The total number of hazard scores generated for a single project location ranged from 164 to 7,850 (Table 6-3). The wide range is principally due to highly variable numbers of sampling sites and sampling events among locations. Among all of the hazard scores for a project location, the number of hazard scores equal to '3' is the number of SV_{HIGH} exceedances at that project location. The number of hazard scores equal to '2' is the number of observations of a SV_{LOW} exceedance that did not also exceed the SV_{HIGH}.

For project locations with repeated sampling over time, a high incidence of SV_{HIGH} exceedances (as fraction of total sampling events) suggest a sustained potential for biological impacts in fish. At project locations having several to many sampling sites, a high prevalence of sites with at least one SV_{HIGH} exceedance indicates high potential for widespread biological impacts within project locations.

An SV_{LOW} exceedance provides positive evidence that adverse biological effects in fish are plausible at a site. Thus, a preponderance of sampling events or sites with SV_{LOW} exceedances provide substantial support for the hypothesis that CEC-related effects are occurring at the project location. For example, at Little Lake Butte des Morts there were only three SV_{HIGH} exceedances, but there was a total of 316 SV_{LOW} exceedances with an average of 11 SV_{LOW} exceedances per sample and 63 SV_{LOW} exceedances per sampling site (Table 6-3), strongly indicating that significant CEC-related biological impacts are possible, if not likely, at this location. On the other hand, consistent non-exceedance of SV_{LOW} values at a repeatedly sampled site provides evidence that the 14 CECs considered in this assessment contribute negligibly to chemical hazards to fish at the site – for example, at the Oswegatchie River in New York.

Finally, we used tallies of SV exceedances (Table 6-3) to support the analysis of CEC point source impacts (see Section 5.3.4). Statistical analysis of the association of point sources with CEC-related hazard was not possible within eight of the 24 project locations (Table 5-2). At these locations, all sampling sites were either point source CEC-'influenced' (Milwaukee River, North Shore Channel, Little Calumet River, Swan Creek) or all sites were 'uninfluenced' (Waupaca Chain O'Lakes, Ashtabula River, Long Pond, Irondequoit Bay) – according to the definitions we used for the purpose of this assessment (see Section 5.3.4).

6.2.1 Patterns in SV_{HIGH} Exceedances across Project Locations

The SV_{HIGH} is a CEC aqueous (dissolved) concentration in surface water above which adverse impacts to fish are expected, particularly in contaminant-sensitive fish species (Chapter 4). There was at least one SV_{HIGH} exceedance in 17 of the 24 project locations, indicating a widespread high potential for biological impacts to fish from aqueous exposures to the 14 CECs. Eight of these 17 locations had a greater number of SV_{HIGH} exceedances in CEC-influenced sites than in uninfluenced sites; confidence in these findings is high because of relatively robust numbers of sampling sites and sampling events per site. Four additional locations having at least one SV_{HIGH} exceedance had only sites that we had designated as CEC-influenced, and one location had only uninfluenced sampling sites (Waupaca Chain O'Lakes). There was little difference in the incidence of SV_{HIGH} exceedances between the two site groups in three project locations (Kewaunee River, Grand River/Maple River and Clinton River).

Seven project locations had no SV_{HIGH} exceedances, including three locations with no point source CEC-influenced sampling sites - Ashtabula River, Long Pond and Irondequoit Bay (Table 6-3). However, these same three locations – along with River Raisin - had only one sample collected from each sampling site. It is plausible that the absence of an observed SV_{HIGH} exceedance in these four locations was at least partly due to very low sample size. In the upper Oswegatchie River, however, total numbers of sampling events and sites were relatively substantial (31 and 5, respectively). For the Oswegatchie, the absence of a SV_{HIGH} exceedance is likely an accurate indicator that there is relatively low hazard potential in fish from aqueous exposure to the 14 CECs within the river reach of the project location (Section A2.23 in Attachment A).

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[83]Where the CEC aqueous concentration exceeds a SV_{HIGH} for a particular CEC and effect category, we have high expectation that fish are negatively impacted. However, where the SV_{HIGH} is not exceeded but the corresponding SV_{LOW} is exceeded, we have not excluded the possibility that adverse impacts are occurring or have occurred. This is because neither the SV_{HIGH} nor the SV_{LOW} is likely to be the actual toxicity threshold, if one exists. The actual toxicity threshold falls at an unknown point between the SV_{HIGH} and SV_{LOW} - the concentration range of highest toxicological uncertainty in our hazard scoring system (Figures 4-2a and 4-2b).

[84]Total number of hazard scores for CEC-influenced sites, in all project locations: 25,536
Total number of hazard scores for uninfluenced sites, in all project locations: 14,592
Total number of hazard scores for ALL sites, in all project locations: 40,128

6.2.2 Patterns in SV_{LOW} Exceedances across Project Locations

The SV_{LOW} is a CEC aqueous concentration in water below which negligible adverse impacts are anticipated, even in contaminant-sensitive fish species (Chapter 4). Where a SV_{LOW} for a specific CEC and effect category is exceeded, however, we cannot exclude the possibility that adverse impacts are occurring or have occurred. It stands to reason that, as the number of SV_{LOW} exceedances increases for a given sampling site, project location, or the entire basin project area, our confidence that fish are impacted likewise increases. Multiple SV_{LOW} exceedances occurred in every project location, and at nearly all sampling sites (Table 6-3), meaning that we could not eliminate the possibility that CEC-related adverse biological effects are pervasive in fish of the U.S. Great Lakes basin, considering the 14 CECs in this assessment. At least one SV_{LOW} exceedance was observed at 191 out of 195 sampling sites, and at least one SV_{LOW} was exceeded at 100% of sampled sites within most of the project locations (Table 6-3).

6.2.3 Prevalence of Hazard Associated with Specific CEC-Effect Category Combinations

We evaluated the relative importance of specific CEC-effect category combinations with respect to hazard observations across project locations. In Section 5.4, the hazard brief for each project location included a chart that identified CEC-effect category combinations associated with elevated hazard (either SV_{HIGH} or SV_{LOW} exceedance). We tallied total numbers of project locations with occurrences of high hazard (Table 6-4) or low hazard (Table 6-5) to identify patterns in hazardous CECs-effect category combinations.

More project locations had at least one SV_{HIGH} exceedance for comprehensive effect categories than for population-relevant effect categories (Table 6-4). The greatest number of project locations with high hazard was associated with endocrine hazard in fish from DEET exposure. No project locations had any high hazard occurrences for the following:

- Comprehensive effect categories: Circulatory/Blood Constituents, Gross Pathology, Neurological;
- Population-relevant effect categories: Behavioral, Growth.

In comparison to high hazard, SV_{LOW} exceedances were far more prevalent and more evenly distributed between comprehensive and population-relevant effect categories (Table 6-5). In the assessments of comprehensive effect categories, SV_{LOW} exceedances were observed in 10 or more project locations for all effect categories except genotoxicity and gross pathology, and for all CECs but bisphenol A, ibuprofen, and lidocaine. Only one SV_{LOW} was exceeded at least once at all 24 project locations - the Endocrine SV_{LOW} for DEET – but there were at least 20 out of 24 project locations with exceedances of the following:

- Comprehensive mean SV_{LOW} for DEET and estrone,
- Circulatory/Blood Constituents SV_{LOW} for β-sitosterol,
- Physiologic/Metabolic SV_{LOW} for estrone.

Among population-relevant effect categories, the prevalence of project locations with SV_{LOW} exceedances was 20 or greater for at least one CEC in all effect categories but growth. At least 20 project locations had SV_{LOW} exceedances in all population-relevant effect categories for DEET and estrone, and in the behavioral effect category for β-sitosterol. Among the CECs, only lidocaine had no population-relevant SV_{LOW} exceedances, and the circulatory/blood constituents SV_{LOW} for ibuprofen was not exceeded at any project location.

6.3 Hazard Type: Effect-Specific Hazard Summaries with Project-wide Hazard Maps

This section summarizes hazards associated with specific effect categories, including the association of effect-specific hazards with individual CECs and the relation of hazards to point sources. As we have defined it for this EHA, the strength of the information incorporated into deriving the screening values (see Section 4.3) informs us of the amount of relevant biological information brought to bear. However, the biological importance of SV_{HIGH} exceedances (or, SV_{LOW} exceedances) for a given effect category varies between CECs because the specific effect endpoints that were incorporated into deriving effect-specific SVs vary between CECs.

At both project location and project-wide spatial scales, we describe largely consistent patterns of greater overall hazard magnitude (Table 6-2a) and/or more prevalent hazards (Tables 6-4 and 6-5) for certain comprehensive effect categories (e.g., endocrine and physiological/metabolic) than for population-relevant effect categories. Hazards are less pronounced for other comprehensive effect categories (gross pathology and neurological). In addition, effect endpoints measured at the subcellular, cellular, and tissue/organ levels of biological organization predominate in LOAECs and NOAECs used to derive SVs for "comprehensive" effect categories, while effect endpoints at the organism level predominate in deriving "population-relevant" SVs (Table 6-7).

Greater hazard magnitude was statistically detected in point source CEC-influenced groups compared to uninfluenced groups in five[85] of the 16 project locations where the statistical comparison could be made (Table 6-8). The ecotoxicological dataset supporting SV derivations is broadly more robust for population-relevant effect categories than for comprehensive effect categories (Tables 4-5 and 4-6), but total numbers of significant differences in hazard between CEC-influenced and uninfluenced sites did not appear to differ between the comprehensive and population-relevant groups (Table 6-8).

The array of effect categories and CECs showing significant hazard differences between CEC-influenced and uninfluenced site groups varied between locations. There are few overall patterns, and predicting the occurrence of specific CEC-related hazards is complex. The occurrence of non-significant test results in 11 of 16 locations (Table 6-8) was often attributable to small sample sizes and/or to elevated hazard that was relatively evenly distributed between the site groups, suggesting the presence of CEC sources other than mapped WWTP and CSO point sources. The only effect categories for which no significant elevation in CEC-related hazard was observed in CEC-influenced sites at any project location were gross pathology and growth (Table 6-8).

Some Key Points…

Effect-Specific Hazards

- **Overall:** Project-wide hazard to fish varied widely between effect categories
- **Greatest Evidence of Hazards**: Clear and convincing evidence of widespread and biologically important hazards to fish was observed for Developmental, Reproductive, Physiological/Metabolic
- **Least Evidence of Hazards**: Little evidence of hazards to fish was observed for Growth, Gross Pathology, Neurological
- **Increased hazard downstream of point sources**: Significant evidence of increased downstream hazard at ≥3 project locations for Behavioral, Mortality, Reproductive, Endocrine and Physiological/ Metabolic effects. No evidence for Growth and Gross Pathology effects.

For each effect category, we illustrate the distribution of hazard to fish across 195 sampling sites in the U.S. Great Lakes Basin. Effect-specific maps show maximum hazard score values (across CECs and sampling events) at each of the sampling sites identified in Attachment A2. Site-specific maximum hazard scores are interpreted as follows:

- 3 = high expectation of hazard (red shaded), where a SV_{HIGH} value was exceeded in at least one sample for at least one CEC;
- 2 = low expectation of hazard (yellow shaded), where at least one SV_{LOW} value was exceeded (but no SV_{HIGH} was exceeded) in at least one sample for at least one CEC;
- 1 = negligible expectation of hazard (gray shaded), where no SV_{LOW} was exceeded in any sample for any CEC.

The maps show regional Great Lakes hazard distribution without reference to specific project locations or individual sampling sites, which were treated individually in Section 5.4. Cross-reference Figure 3-1 in Section 3.3 to identify the positions of specific project locations relative to the dots on hazard maps presented in this section. Because distances between sampling sites are small compared with the scale of the maps, in many cases there is considerable overlap of site symbols possibly resulting in obscured site-specific hazards. However, the dots were layered in order to highlight hazard according to the following priority: high, low, negligible. It is important to note that the mapped hazards are *minimal* estimates of the maximum CEC-related hazard to fish, since hazard is likely to have been under-estimated in this EHA (see Chapter 7 – Uncertainty Assessment).

The following subsections describe effect-specific hazards, grouped as population-relevant and comprehensive effect categories.

[85]Although a total of six locations show significant differences between site groups (Table 6-8), in the Oswegatchie River there was significantly lower hazard attributed to β-sitosterol in the CEC-'influenced' group compared to the 'uninfluenced' group, likely due to CEC sources not accounted for in this analysis (see Section 5.4.23).

6.3.1 Behavioral

There were no occurrences of high behavioral[86] hazard (SV_{HIGH} exceedance) (Table 6-4), but behavioral SV_{LOW} exceedances were common among project locations. The prevalence of project locations with at least one behavioral SV_{LOW} exceedance was 10 locations or greater for carbamazepine, citalopram, triclosan and venlafaxine, and greater than 20 locations for estrone and β-sitosterol (Table 6-5). Only lidocaine had no SV_{HIGH} or SV_{LOW} exceedances.

Despite a lack of behavioral SV_{HIGH} exceedances, site-specific maximum behavioral hazard scores were significantly greater ($p < 0.1$) at CEC-influenced sites than at uninfluenced sites attributed to venlafaxine at the Fox River/Green Bay location and to citalopram at the Maumee River (Table 6-8). Median behavioral hazard scores were significantly elevated at CEC-influenced sites for estrone at the Maumee River and citalopram at Tinkers Creek. A significant difference between site groups at the Oswegatchie River was actually due to a lower median hazard score at the 'CEC-influenced' sites compared to 'uninfluenced sites', which may be due to an unidentified CEC source.

The highest rank observed for behavioral hazard among project locations occurred at the North Shore Channel and Little Calumet River, and second highest at Milwaukee River, Maumee River, Cuyahoga River and Tinkers Creek (Table 6-2a). Statistical tests concerning CEC point source impacts were not possible for North Shore Channel, Little Calumet River or Milwaukee River (Table 6-8). Within project locations (Section 5.4), mean behavioral hazard scores consistently achieved low to middle hazard rankings among effect categories.

Some Key Points…

Behavioral Hazard

- **Overall:** Substantial evidence of hazards to fish
- **CECs with Behavioral SVs:** 11 of 14
- **Prevalence of High Hazard**: No occurrences
- **Prevalence of Low Hazard**:
  - *>20 Project* Locations: Estrone, β-Sitosterol
  - *>10 Project Locations*: Carbamazepine, Citalopram, Triclosan, Venlafaxine
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Behavioral SV pairs were available for 11 of the 14 CECs (Table 4-1), but the strength of information used to derive SVs varied between CECs. The breadth ecotoxicity information (Section 4.3) was based on numbers of effect endpoints and fish species used to derive the SVs and varied between CECs; quantity of information for behavioral SVs was 'sparse' for six of the 11 CECs, 'limited' for HHCB and β-sitosterol, and 'moderate' to 'robust' for citalopram, carbamazepine and triclosan (Table 4-5). The biological importance of SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the CEC-specific SVs (see Section 6.4).

[86](Description of Behavioral effects from Attachment 2-1 *in* Gefell et al. 2019):
"*Description:* These endpoints are related to physical movement or orientation in response to chemical (and possibly additional) stimuli. Included are endpoints related to:

- activity patterns, responses to stimuli, or behavioral clinical signs of toxicity in fish exposed individually, and
- altered interactions (e.g., mating displays, resource competition, aggression, or predator-prey interactions) between fish exposed in groups or between an exposed fish and an unexposed challenge organism or simulated organism.

Exposure: Any exposure duration
Life Stages Evaluated: Effects may be evaluated in any life stage.
Population-Relevant Adverse Endpoints: Based on scientific judgement on a case-by-case basis, these include behavioral endpoints related to:

- survival (e.g., startle response time, swimming speed, predator avoidance, or feeding rate), or
- reproduction (e.g., nest defense or spawning behaviors).

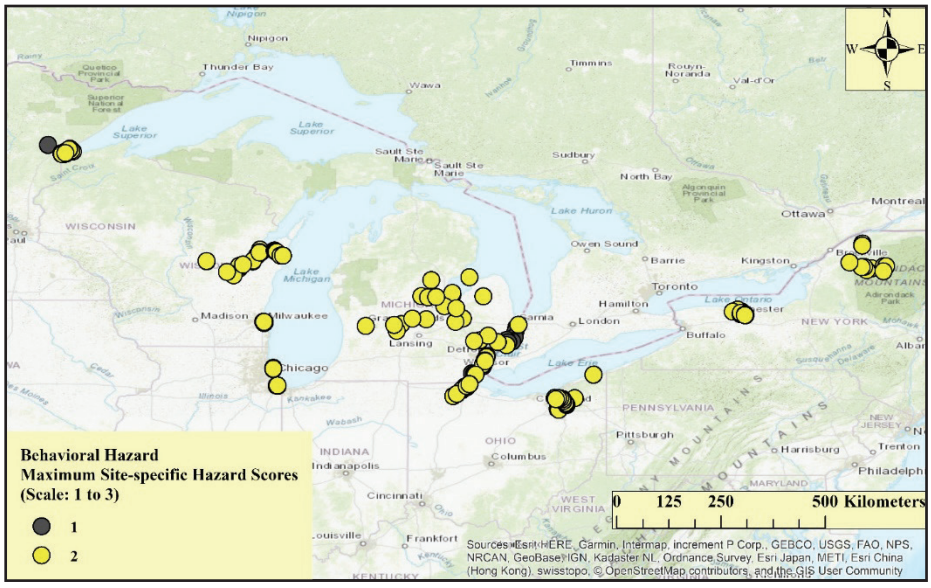
Other Adverse Endpoints: Endpoints not related to survival or reproduction behaviors that could be integrated into a quantitative, mechanistic population model – e.g., righting reflex or light-dark preferences."

The following map illustrates the overall spatial distribution of site-specific *maximum behavioral hazard* to fish (across CECs) in the U.S. Great Lakes Basin. The CECs that had behavioral SV pairs and were included to develop this map were the following: bisphenol A, carbamazepine, citalopram, diphenhydramine, estrone, HHCB, lidocaine, β-sitosterol, TBEP, triclosan, and venlafaxine. Specific behavioral endpoints evaluated in published assays that were included in developing the SVs are listed by CEC in Section 6.4. Each dot represents the position of an individual sampling site.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



6.3.2 Developmental

High developmental[87] hazard (SV_{HIGH} exceedance) was observed at six locations due to ibuprofen exposure (Table 6-4), in spite of the fact that only 13 of the 24 project locations had ibuprofen concentration data with which to generate hazard scores. High developmental hazard was not attributable to any of the other eight CECs with developmental SVs (Table 4-1) even though exposure data was available for all 24 locations for those CECs. Developmental SV_{LOW} exceedances were common among project locations. The prevalence of project locations with at least one developmental SV_{LOW} exceedance was 10 locations or greater for bisphenol A, carbamazepine, TBEP and triclosan, and greater than 20 locations for estrone (Table 6-5). Only β-sitosterol had no exceedance of either the developmental SV_{HIGH} or SV_{LOW}.

Within project locations, developmental mean hazard scores achieved low to middle hazard rankings compared to other effect categories (Table 6-2a). Nevertheless, site-specific developmental hazard scores were statistically significantly greater at CEC-influenced sites than at uninfluenced sites and were attributable to ibuprofen at the St. Louis River/Bay location and to estrone at the Maumee River location (Table 6-8). Among project locations, developmental hazard was ranked highest at the North Shore Channel and second highest at Little Calumet River and Tinkers Creek (Table 6-2a), although statistical tests were not possible for North Shore Channel or Little Calumet River (Table 6-8). Only the Ashtabula River had no exceedance of any developmental SV_{HIGH} or SV_{LOW}.

Some Key Points…

Developmental Hazard

- **Overall:** Clear and convincing evidence of hazards to fish
- **CECs with Developmental SVs:** 9 of 14
- **High Hazard**:
  - *6 Project Locations*: Ibuprofen
- **Low Hazard**:
  - *>20 Project Locations:* Estrone
  - *>10 Project Locations*: Bisphenol A, Carbamazepine, TBEP, Triclosan
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Developmental SV pairs were available for nine CECs, but the strength of information used to derive SVs varied between CECs. The breadth of ecotoxicity information scores was based on numbers of effect endpoints and fish species that were used to derive the SVs (Section 4.3). The quantity of information for developmental SVs was 'moderate' to 'robust' for six CECs, and 'sparse' to 'limited' for only three CECs (Table 4-5). The biological importance of SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the CEC-specific SVs (see Section 6.4), which also vary between CECs even for the same effect category.

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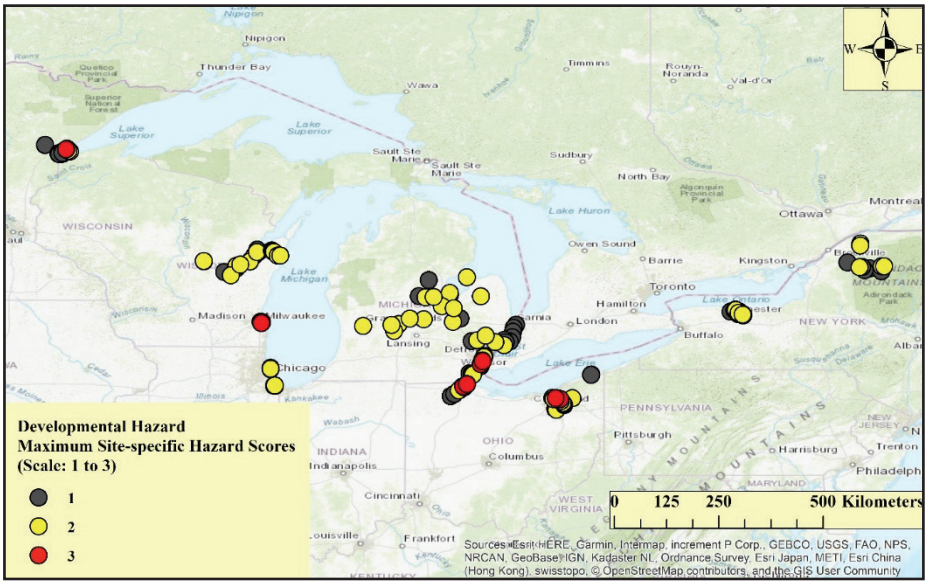
[87](Description of Developmental effects from Attachment 2-1 *in* Gefell et al. 2019):
"*Description:* These effects occur post-fertilization in the exposed generation, or in subsequent generations.
Exposure: Exposure may occur in any life stage and any duration, but at least some of the exposure must occur in early life stages within the same generation being evaluated, and/or any life stage(s) in generation(s) prior to the generation being evaluated within a multigenerational study.
Life Stages Evaluated: For the most part, effects that are observed in early life stages (embryo, larva, fry) are reported under the Developmental Effect Category. Abnormalities in immature and adult fish may also be reported, but only if exposure had occurred during a developmental life stage of the same generation, or in a previous generation. Life cycle tests and early life stage tests typically include developmental endpoints.
Population-relevant Adverse Endpoints: sex ratio, early life stage growth and survival; timing, rate, success of hatch; anatomical deformations or malformations.
Other Adverse Endpoints: occurrence of intersex, metabolic, hormonal, genetic, gross or histological, immunological, circulatory/blood, neurological, and/or physiological abnormalities in early life stages, or in juvenile or adult life stages if exposure had occurred as specified above."

The following map illustrates the overall spatial distribution of site-specific *maximum developmental hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Each dot represents the position of an individual sampling site.The CECs that had developmental SV pairs and were included to develop this map were the following: bisphenol A, carbamazepine, diphenhydramine, estrone, HHCB, ibuprofen, β-sitosterol, TBEP, and triclosan. Specific developmental endpoints evaluated in published assays that were included in developing the SVs are listed by CEC Section 6.4.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



6.3.3 Growth

There was little evidence of a potential for impacts to growth in fish from aqueous exposure to the four CECs that had growth SVs (Table 4-1). There were no growth SV_{HIGH} exceedances (Table 6-4), and SV_{LOW} exceedances were observed only for bisphenol A at five locations and HHCB at two locations (Table 6-5).

Confidence in growth hazard results is relatively low, since the breadth of ecotoxicity data used to derive the SVs was 'sparse' to 'limited' for all four CECs (Table 4-5). Statistical analysis showed no significant differences in site-specific maximum or median growth hazard scores between CEC-influenced sites and uninfluenced sites within project locations (Table 6-8). Within project locations, growth mean hazard consistently achieved the lowest hazard rank among effect categories (Table 6-2a).

The following map illustrates the overall spatial distribution of site-specific *maximum growth hazard* to fish (across CECs) in the U.S. Great Lakes Basin. The CECs that had growth SV pairs and were included to develop this map were the following: 4-androstene-3,17-dione, bisphenol A, carbamazepine, and HHCB. Specific growth endpoints evaluated in published assays that were included in developing the SVs are listed by CEC in Section 6.4. Each dot represents the position of an individual sampling site.

Some Key Points…

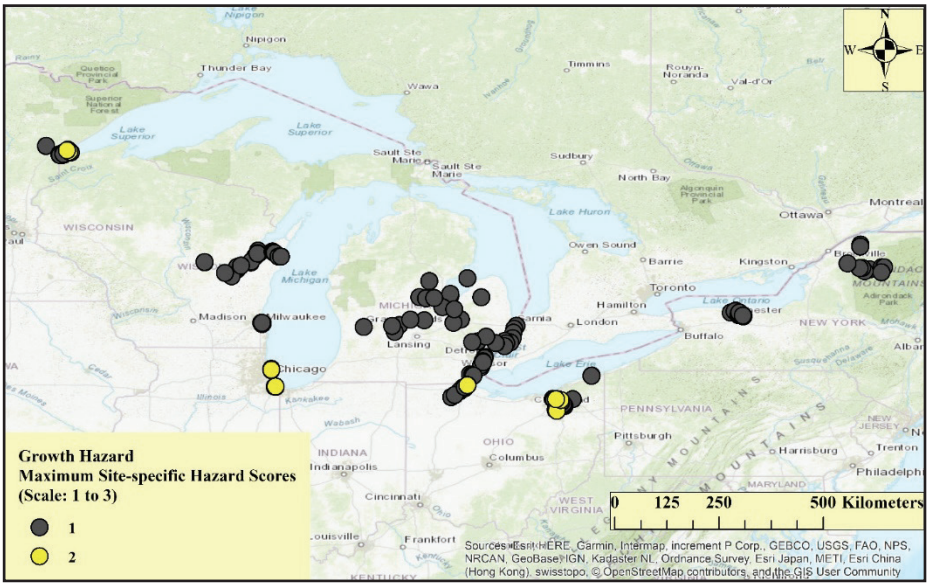
Growth Hazard

- **Overall:** Little evidence of hazards to fish
- **CECs with Growth SVs:** 4-Androstene-3,17-dione, Bisphenol A, Carbamazepine, HHCB
- **High Hazard:** No occurrences
- **Low Hazard:**
  - *5 Project Locations*: Bisphenol A
  - *2 Project Locations*: HHCB
- **Point Source Analysis:** No evidence of elevated hazard downstream of point sources.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



6.3.4 Mortality

The potential for CEC-related mortality hazard appears widespread, but the caveat is that strength of evidence supporting mortality SVs is generally weak. High mortality hazard (SV_{HIGH} exceedance) did occur at six project locations, but was due only to exposure to aqueous venlafaxine (Table 6-4); no mortality SV_{HIGH} was exceeded for the seven other CECs with mortality SVs (Table 4-1). By itself, evidence for mortality due to venlafaxine exposure is relatively weak because the breadth of ecotoxicity information incorporated into the venlafaxine mortality SVs is 'sparse' (Table 4-5).

However, mortality SV_{LOW} exceedances occurred at every project location. At 17 of 24 project locations there were mortality SV_{LOW} exceedances for at least two different CECs per location, and at 11 project locations the mortality SV_{LOW} was exceeded for at least three different CECs per location (Section 5.4). Exceedance of the SV_{LOW} occurred in at least one project location for seven of the eight CECs (Table 6-5)[88] , and at 10 or more locations for DEET, triclosan and venlafaxine. Our results indicate the possibility that mortality due to aqueous DEET exposure could be occurring at all of the 24 locations, while carbamazepine had no mortality SV_{LOW} exceedance (Table 6-5). Breadth of evidence for CEC SVs (Section 4.3) was 'sparse' to 'limited' for mortality SVs in six of the eight CECs, 'moderate' for ibuprofen, and 'robust' only for the bisphenol A mortality SV_{LOW} (Table 4-5).

Since strength of evidence is generally low for mortality SVs, this suggests caution in situations where a single CEC had a mortality SV exceedance, such as mortality SV_{LOW} exceedances attributed exclusively to DEET at eight project locations (Section 5.4). Instead, our degree of confidence in the potential for CEC-related mortality could be based on the *number of CECs* that separately indicate hazard. For instance, the only CEC with

Some Key Points…

Mortality Hazard

- **Overall:** Substantial evidence of hazard to fish
- **CECs with Mortality SVs:** 8 of 14
- **High Hazard**:
  - *6 Project Locations*: Venlafaxine
- **Low Hazard**:
  - *>20 Project Locations*: DEET
  - *>10 Project Locations*: Triclosan, Venlafaxine
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

mortality SV_{HIGH} exceedances is venlafaxine at six project locations (Table 6-4), yet the breadth of evidence for venlafaxine mortality SVs is 'sparse' so if we consider only venlafaxine our confidence in the predictions of high hazard are limited. However, high mortality hazard in fish appears more likely when supported by independent observations of additional CECs with mortality SV_{LOW} exceedances. Confidence in the prediction of mortality hazard was greatest for St. Louis River/Bay, North Shore Channel and Tinkers Creek locations, where the venlafaxine SV_{HIGH} was exceeded, and also where mortality SV_{LOW} values were exceeded for four to five additional CECs (Section 5.4).

At five project locations, mortality mean hazard scores achieved low to middle hazard ranks among effect categories (Table 6-2a), likely because of the predominance of mortality SV_{LOW} exceedances. Site-specific mortality hazard was statistically significantly elevated in CEC-influenced sites as compared to uninfluenced sites attributed to aqueous ibuprofen exposure at the St. Louis River/Bay location and to venlafaxine exposure at Fox River/Green Bay and Tinkers Creek locations (Table 6-8).

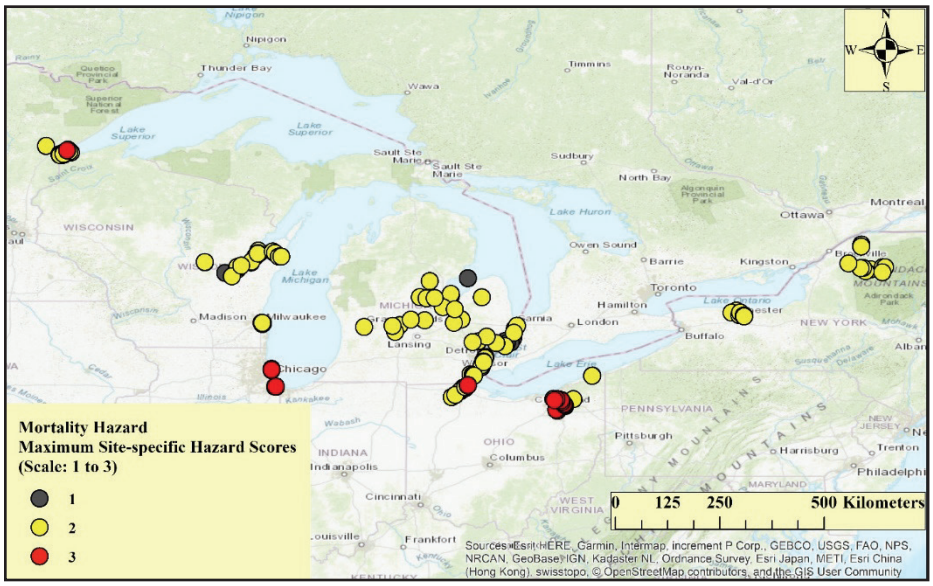
[88] In Table 6-5, tallies of project locations with SV_{LOW} exceedances are inclusive of project locations identified as having SV_{HIGH} exceedances in Section 5.4.

The following map illustrates the overall spatial distribution of site-specific maximum mortality hazard to fish (across CECs) in the U.S. Great Lakes Basin. The CECs that had mortality SV pairs and were included to develop this map were the following: bisphenol A, carbamazepine, DEET, HHCB, ibuprofen, TBEP, triclosan, and venlafaxine. Each dot represents the position of an individual sampling site.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



6.3.5 Reproductive

Elevated reproductive[89] hazard in fish (i.e., at least one exceedance of a reproductive SV_{HIGH} or SV_{LOW} for any CEC) was observed at all project locations except the Ashtabula River. High reproductive hazard (SV_{HIGH} exceedance) attributed to ibuprofen exposure was observed at six project locations (Table 6-4), in spite of the fact that only 13 of the 24 project locations had ibuprofen concentration data with which to generate hazard scores. High reproductive hazard was not attributable to any of the other five CECs with reproductive SVs (Table 4-1) even though exposure data was available for all 24 locations for those CECs. Reproductive hazard was ranked highest among population-relevant effect categories at nine locations (Table 6-2a), although hazard ranks were at the lower end of the range of possible ranks (Table 5-3).

We statistically compared site-specific maximum and median hazard scores in the CEC-influenced site group compared to the uninfluenced site group. For reproductive effects, statistically significantly ($p \leq 0.1$) higher hazard scores in CEC-influenced sites were associated with four different CECs at four separate project locations: ibuprofen at St. Louis River/Bay, venlafaxine at the Fox River/Green Bay location, carbamazepine at Saginaw River and estrone at the Maumee River (Table 6-8). Five CECs had SV_{LOW} exceedances for more than 10 locations (Table 6-5)[90]. These results highlight the importance of assessing effect-specific hazard from as many CECs as possible at each sampling site. Since concentrations of CECs varied independently of each other between project locations, assessing reproductive hazard only due to the most potent of the subject CECs (estrone) would be an overly simplistic approach that could overlook important hazard findings for other CECs.

Some Key Points…

Reproductive Hazard

- **Overall:** Clear and convincing evidence of hazards to fish
- **CECs with Reproductive SVs:** Bisphenol A, Carbamazepine, Estrone, Ibuprofen, Triclosan, Venlafaxine
- **Prevalence of High Hazard**:
  - *6 Project Locations*: Ibuprofen
- **Prevalence of Low Hazard**:
  - *>20 Project Locations*: Estrone
  - *>10 Project Locations*: Bisphenol A, Carbamazepine, Triclosan, Venlafaxine
- **Point Source Analysis:** Significant evidence of elevated hazards downstream of point sources

Reproductive SV pairs were available for six CECs, but the strength of information used to derive SVs varied between CECs. The breadth of ecotoxicity information (Section 4.3) was based on numbers of effect endpoints and fish species used to derive the SVs. The quantity of information for reproductive SVs was 'moderate' to 'robust' for all six SV_{LOW} values and four of the SV_{HIGH} values, and 'sparse' to 'limited' for only the triclosan and venlafaxine SV_{HIGH} (Table 4-5). The biological importance of SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the CEC-specific SVs (see Section 6.4), which also vary between CECs even for the same effect category.

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[89](Description of Reproductive effects from Attachment 2-1 *in* Gefell et al. 2019):
"*Description:* Endpoints are events and effects related to, and influencing the capacity for, successful procreation - up to and including fertilization.
Exposure: Sexually immature (fingerling, juvenile, subadult) or mature (adult) fish, or direct pre-fertilization exposure of gametes; any exposure duration.
Life Stages Evaluated: Effects evaluated in parental (adult) fish, gametes, or embryos, only.
Population-relevant Adverse Endpoints: functional changes in reproductive organs of exposed adults; gametogenesis; production, survival, mobility, size, and viability of gametes; timing, frequency, and success of spawning; fertilization success, GSI.
Other Adverse Endpoints: reproductive effect endpoints that are not readily quantifiable with respect to reduction in the capacity for procreation, such as: external structural change in reproductive organs of exposed adults; reproductive hormone levels; gross pathology or histopathology of reproductive organs; external secondary sex characteristics."

[90]In Table 6-5, tallies of project locations with SV_{LOW} exceedances are inclusive of project locations identified as having SV_{HIGH} exceedances in Section 5.4.

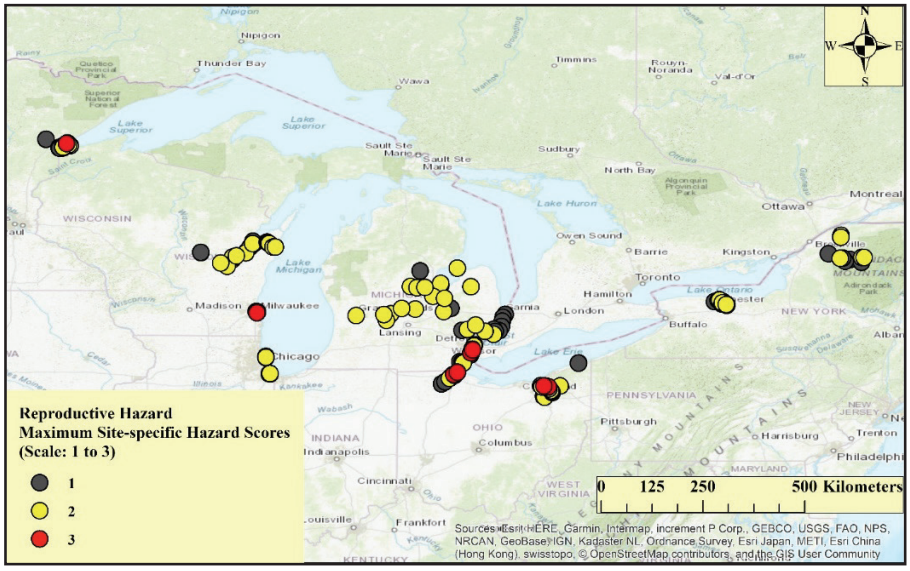
The following map illustrates the overall spatial distribution of site-specific *maximum reproductive hazard* to fish (across CECs) in the U.S. Great Lakes Basin. The CECs that had reproductive SV pairs and were included to develop this map were the following: bisphenol A, carbamazepine, estrone, ibuprofen, triclosan, and venlafaxine.

Specific reproductive endpoints evaluated in published assays that were included in developing the SVs are listed by CEC in Section 6.4. Each dot represents the position of an individual sampling site.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



6.3.6 Circulatory/Blood Constituent

There were no high hazard observations (SV_{HIGH} exceedances) for circulatory/blood constituent[91] effects at any project location (Table 6-4) but low hazard (SV_{LOW} exceedances) was both common and widespread, occurring in >10 project locations for carbamazepine and DEET and in over 20 locations for β-sitosterol (Table 6-5)[92]. The highest circulatory/blood constituents hazard rank based on mean hazard scores was 4 (out of a possible 10) at North Shore Channel and Little Calumet River, followed by Grand River/Maple River and Cuyahoga River each with a hazard rank of 3. Statistically significantly higher hazard attributable to DEET exposure was observed at CEC-influenced sites compared to uninfluenced sites at the St. Louis River/Bay locations and attributable to carbamazepine at the Fox River/Green Bay location (Table 6-8). At the Oswegatchie River project location, significantly lower hazard was observed in the CEC-'influenced' site group because of elevated hazard from β-sitosterol exposure at 'uninfluenced' sites – probably due to a β-sitosterol source not accounted for in the analysis (Section 5.4.23).

Circulatory/blood constituent SV pairs were available for four CECs (carbamazepine, DEET, ibuprofen and β-sitosterol) (Table 4-1), and the strength of information used to derive SVs varied between them. The breadth of ecotoxicity information (Section 4.3) was based on numbers of effect endpoints and fish species used to derive the SVs; quantity of information for circulatory/blood constituent SVs was 'moderate' for DEET and ibuprofen, and 'sparse' for carbamazepine and β-sitosterol (Table 4-6). The biological importance of SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the CEC-specific SVs (see Section 6.4), which also vary between CECs even for the same effect category.

Some Key Points…

Circulatory/ Blood Constituents Hazard

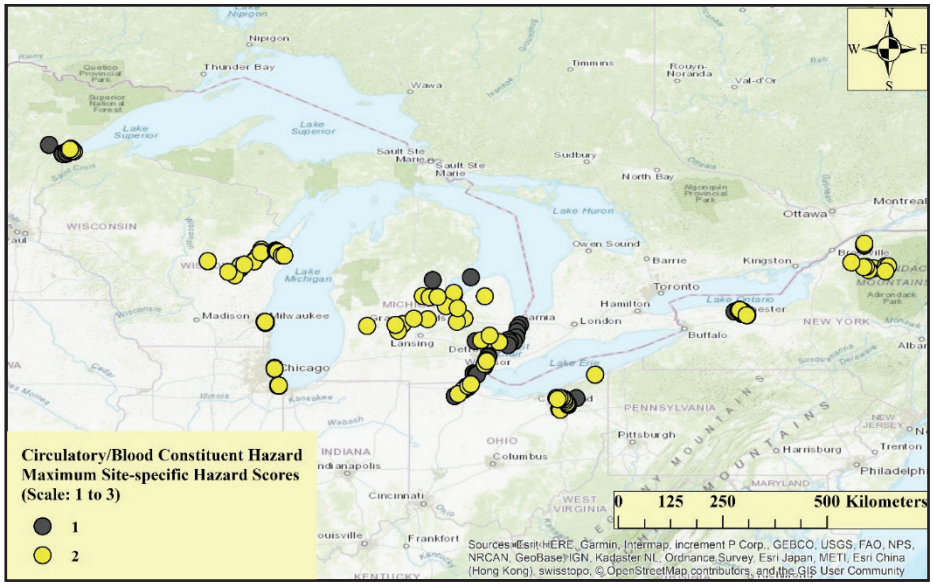
- **Overall:** Substantial evidence of hazard to fish
- **CECs with Circulatory/ Blood Constituents SVs:** Carbamazepine, DEET, Ibuprofen, β-Sitosterol
- **Prevalence of High Hazard**: No occurrences
- **Prevalence of Low Hazard**:
  - *>20 Project Locations*: β-Sitosterol
  - *>10 Project Locations*: Carbamazepine, DEET
- **Point Source Analysis:** Significant evidence of elevated hazards downstream of point sources

The following map illustrates the overall spatial distribution of site-specific *maximum circulatory/ blood constituent hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Specific circulatory/ blood constituent endpoints evaluated in published assays that were included in developing the SVs are listed by CEC in Section 6.4. Each dot represents the position of an individual sampling site.

Meanings of the possible values of mapped maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



[91](Description of Circulatory/Blood Constituent effects from Attachment 2-1 *in* Gefell et al. 2019):
"Exposure: Sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Endpoints include physiological, functional, or anatomical pathology of heart or blood vessels; blood cell formation, bone marrow, lymph nodes, spleen, thymus; hematological parameters, blood, serum or plasma chemistry; and heart enzymes in blood."

6.3.7 Endocrine

Endocrine[93] hazards were greater and more widely distributed than for the other effect categories with elevated hazard (SV_{HIGH} or SV_{LOW} exceedance) observed at every project location, even though endocrine SV pairs were available for only three CECs (DEET, ibuprofen and venlafaxine) (Table 4-1). There were far more observations of high hazard of endocrine effects than for any other effect category. High endocrine hazard (SV_{HIGH} exceedances) associated with DEET exposure was observed at 15 project locations, and high endocrine hazard from venlafaxine occurred at eight locations (Table 6-4). Low hazard (SV_{LOW} exceedances) related to DEET exposure occurred at all 24 project locations and at 14 locations for venlafaxine-related hazard, while low endocrine hazard related to ibuprofen exposure was observed at only four locations (Table 6-5). No high endocrine hazard attributable to ibuprofen was observed.

Endocrine hazard was ranked '8' out of a possible '10' (see hazard ranking in Table 5-3) - the highest hazard rank achieved for any effect category at any project location - at the North Shore Channel and the Little Calumet River (Table 6-2a). The next highest endocrine hazard ranks occurred at the Cuyahoga River, Tinkers Creek and the Grand River/Maple River locations, followed by the St. Louis River/Bay, Waupaca Chain O'Lakes, Milwaukee River, Saginaw River and Clinton River locations. Statistically significantly ($p < 0.1$) higher hazard scores were observed at CEC-influenced sampling sites compared to uninfluenced sites for DEET exposure at the St. Louis River/Bay location and for venlafaxine exposure at the Fox River/Green Bay and Tinkers Creek locations (Table 6-8).

Some Key Points…

Endocrine Hazard

- **Overall:** Substantial evidence of hazard to fish
- **CECs with Endocrine SVs:** DEET, Ibuprofen and Venlafaxine
- **Prevalence of High Hazard**:
  - *15 Project Locations*: DEET
  - *8 Project Locations*: Venlafaxine
- **Prevalence of Low Hazard**:
  - *All Project Locations*: DEET
  - *14 Project Locations*: Venlafaxine
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

The strength of information used to derive endocrine SVs varied between the three CECs. The breadth of ecotoxicity information (Section 4.3) was based on numbers of effect endpoints and fish species used to derive the SVs; quantity of information for endocrine SVs was 'moderate' for DEET and 'sparse' for ibuprofen and venlafaxine (Table 4-6). The biological importance of endocrine SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the SVs (see Section 6.4) that also vary between CECs even for the same effect category.

[92] In Table 6-5, tallies of project locations with SV_{LOW} exceedances are inclusive of project locations identified as having SV_{HIGH} exceedances in Section 5.4.

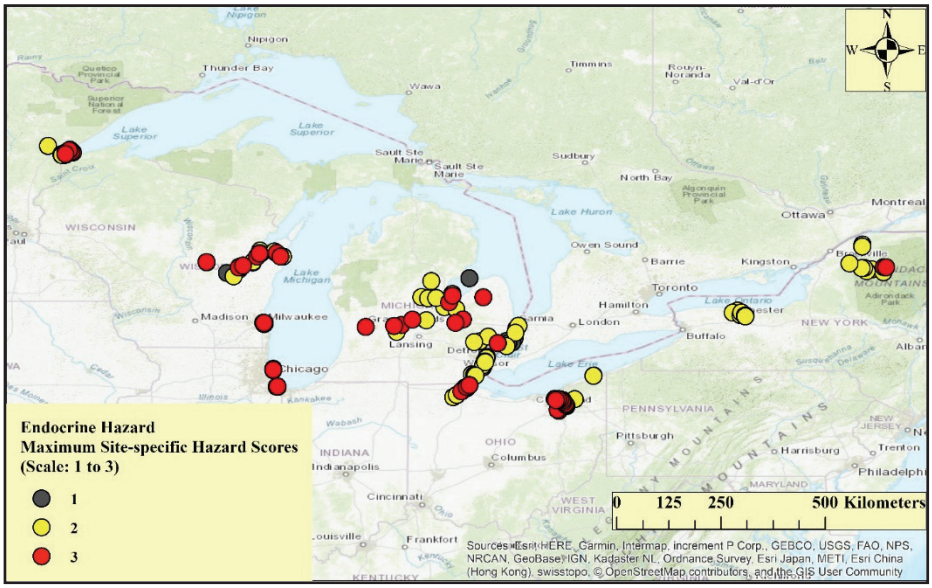
[93](Description of Endocrine effects from Attachment 2-1 *in* Gefell et al. 2019):
"Exposure: sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Endpoints include: measured concentrations of endogenous steroid and non-steroid, endocrine and neuroendocrine hormones, hormone precursors, or hormone metabolites in various tissues; evaluations of gross pathology or histopathology in organs and tissues related to the endocrine and neuroendocrine systems (e.g., hypothalamus, pituitary, thyroid, pancreas, adrenal gland, or pineal gland). Reproductive hormone levels and effects in reproductive organs are not included here; they are included in the Reproductive Effects Category."

This map illustrates the overall spatial distribution of site-specific *maximum endocrine hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Specific endocrine endpoints evaluated in published assays that were included in developing the SVs are listed by CEC in Section 6.4. Each dot represents an individual sampling site.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See the explanation of regional hazard maps in the introduction to Section 6.3.



6.3.8 Genotoxicity

Given several limitations and uncertainties in the exposure and toxicity assessments, we recommend caution in interpreting the observed CEC-related genotoxicity[94] hazard.

High genotoxicity hazard was observed at seven of the 13 project locations (Table 6-4). However, only ibuprofen among the 14 CECs had sufficient genotoxicity information in the literature with which to derive SVs (Table 4-1), and that information was limited to exposure-response data from three tests: COMET test, RAPD-PCR test and percent apoptotic cells (from Gefell et al. 2019). Chemistry data for ibuprofen was available for only 13 of the 24 project locations (see Tables 3-1 and 3-2). Further, the ibuprofen SV_{HIGH} is a lower concentration in water than the detection limit used to determine ibuprofen detect-nondetect status in water. Thus, for ibuprofen-related genotoxicity hazard scoring, all ibuprofen non-detects were assigned a hazard score of '1' and all detects were assigned a score of '3' for high hazard (see uncertainty discussion in Section 7.3); there were no low hazard scores. Despite these limitations, significantly greater ($p < 0.1$) genotoxicity hazard attributable to ibuprofen exposure was observed in the CEC-influenced site group as compared to the uninfluenced site group at the St. Louis River/Bay location (Table 6-8; Section 5.4.1).

Some Key Points…

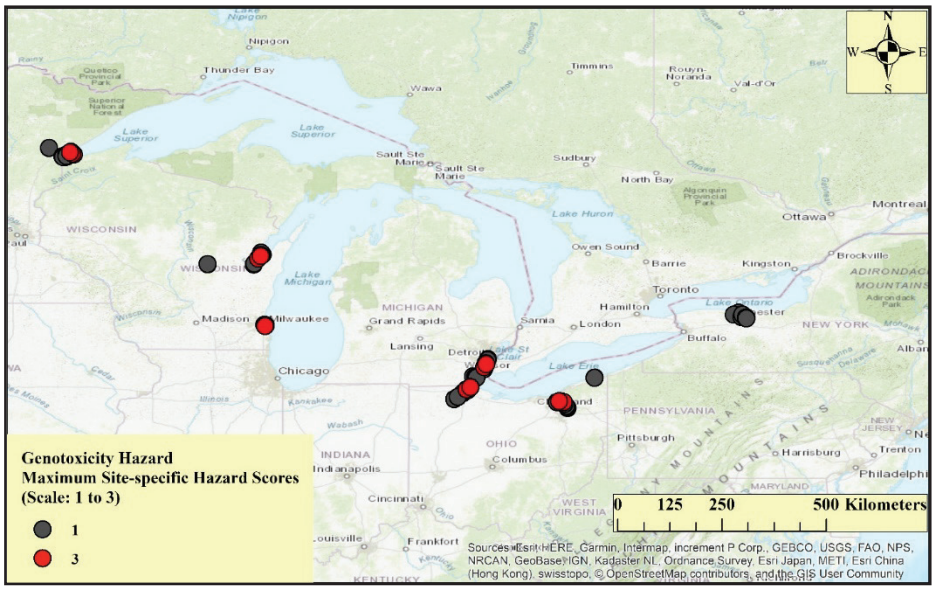
Genotoxicity Hazard

- **Overall:** Some evidence of hazard to fish
- **CECs with Genotoxicity SVs:** Ibuprofen
- **Prevalence of High Hazard**:
  - *7 Project Locations*: Ibuprofen
- **Prevalence of Low Hazard**:
  - *7 Project Locations*: Ibuprofen
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

The following map illustrates the overall spatial distribution of site-specific *maximum genotoxicity hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Each dot represents an individual sampling site. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See the explanation of regional hazard maps in the introduction to Section 6.3.



[94](Description of Genotoxicity effects from Attachment 2-1 *in* Gefell et al. 2019):
"*Exposure:* sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Endpoints include: RNA/DNA ratio; DNA or RNA fragmentation or other damage; nucleic acid synthesis; mutations such as nucleotide substitution; chromosomal damage or aberrations; incomplete or otherwise abnormal cell division, mitosis, or meiosis; micronucleus formation; cell nucleus abnormalities; abnormal DNA synthesis or sequences."

6.3.9 Gross Pathology

No high gross pathology[95] hazard (SV_{HIGH} exceedance) was observed at any project location, and low hazard (SV_{LOW} exceedance) was limited to four project locations (Table 6-5). Gross pathology achieved the lowest possible hazard rank (a rank of 1 on a scale of 1 to 10) in 23 of 24 project locations (Table 6-2a), and there were no statistically significant differences in gross pathology hazard between CEC-influenced and uninfluenced sites at any project location (Table 6-8).

We recommend caution in interpreting the very small number of observed gross pathology hazards, considering the following limitations in the ecotoxicity dataset. Only bisphenol A among the 14 CECs had sufficient published exposure-response information to derive gross pathology SVs (Table 4-1), and the breadth of information was 'sparse' (Table 4-6). Specific effect endpoints incorporated into both SV_{HIGH} and SV_{LOW} derivations was limited to kidney size changes related to histopathologies, and other gross lesion endpoints and body coloration responses that were also incorporated into the SV_{HIGH} derivation (from Gefell et al. 2019). It is conceivable that future application of additional SVs based on targeted fish ecotoxicity assays may reveal additional hazards.

Some Key Points…

Gross Pathology Hazard

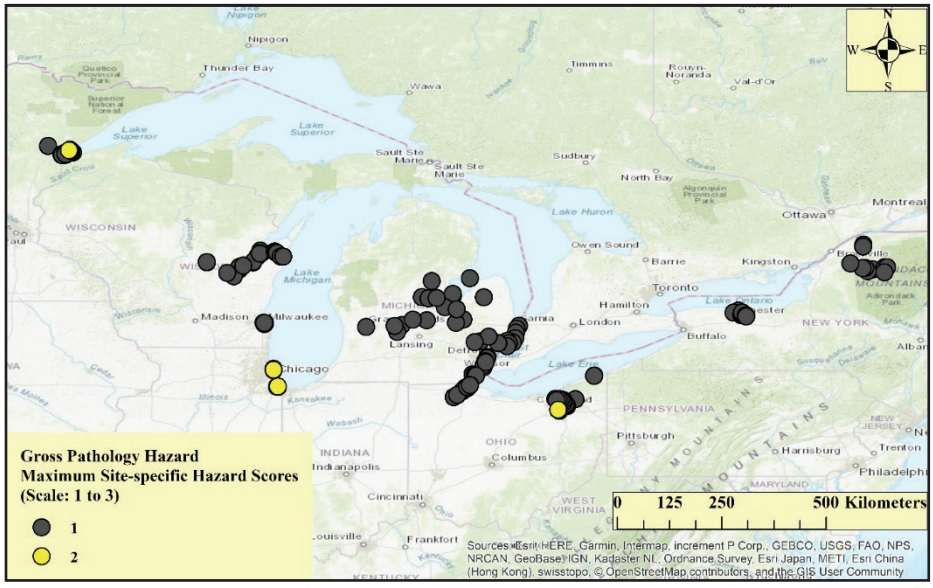
- **Overall:** Little evidence of hazard to fish
- **CECs with Gross Pathology SVs:** Bisphenol A
- **Prevalence of High Hazard**: No occurrences
- **Prevalence of Low Hazard**:
  - *4 Project Locations*: Bisphenol A
- **Point Source Analysis:** No evidence of elevated hazard downstream of point sources

The following map illustrates the overall spatial distribution of site-specific *maximum gross pathology hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Each dot represents an individual sampling site.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See explanation of regional hazard maps in the introduction to Section 6.3.



[95](Description of Gross Pathology effects from Attachment 2-1 *in* Gefell et al. 2019)
"Exposure: sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Endpoints include morphology and external features of the whole fish and organs, including: overall coloration, spotting, and macroscopic lesions; organ size as absolute organ weights or relative weight (e.g., HSI); enzymes in blood indicative of specific organ damage. Nodules, other non-cancer growths, rough surfaces, and other surficial tissue anomalies are included here.
Exclusions:

- Pre-cancerous and cancerous lesions should only be included in the Cancer Effect Category [the Cancer effect category was not evaluated in this EHA because no cancer bioassays were located in fish for the 14 CECs],
- Pathology endpoints for certain organs/tissues that are principally related to other Effect Categories should not be included here, such as Reproductive, Circulatory, Endocrine, or Neurological
- Pathologies in early life stages should be reported under Developmental"

6.3.10 Histopathology

Elevated histopathology[96] hazard was observed at 16 of 24 project locations, even though only carbamazepine and venlafaxine had sufficient histopathology information in the literature with which to develop SVs (Table 4-1). High histopathology hazard (SV_{HIGH} exceedance) due to carbamazepine exposure was observed at the North Shore Channel and Tinkers Creek, and due to venlafaxine at North Shore Channel, Cuyahoga River and Tinkers Creek (Section 5.4). Low hazard (SV_{LOW} exceedance) was widespread with observations at 14 or more project locations for both CECs (Table 6-5). Significantly higher histopathology hazard ($p < 0.1$) was observed in the point source CEC-influenced site group compared to the uninfluenced group attributable to venlafaxine exposure at the Fox River/Green Bay location and to carbamazepine exposure at the Saginaw River project location (Table 6-8).

However, we recommend caution in interpreting these hazard results. Breadth of information - based on numbers of fish species and effect endpoints that were evaluated in the literature used to derive histopathology SVs - was 'sparse' for both CECs (Table 4-6). Specific effect endpoints evaluated in literature studies were limited to adverse histopathologic changes in kidneys, gills and liver for carbamazepine and in kidney, liver and brain for venlafaxine (from Gefell et al. 2019).

Some Key Points…

Histopathology Hazard

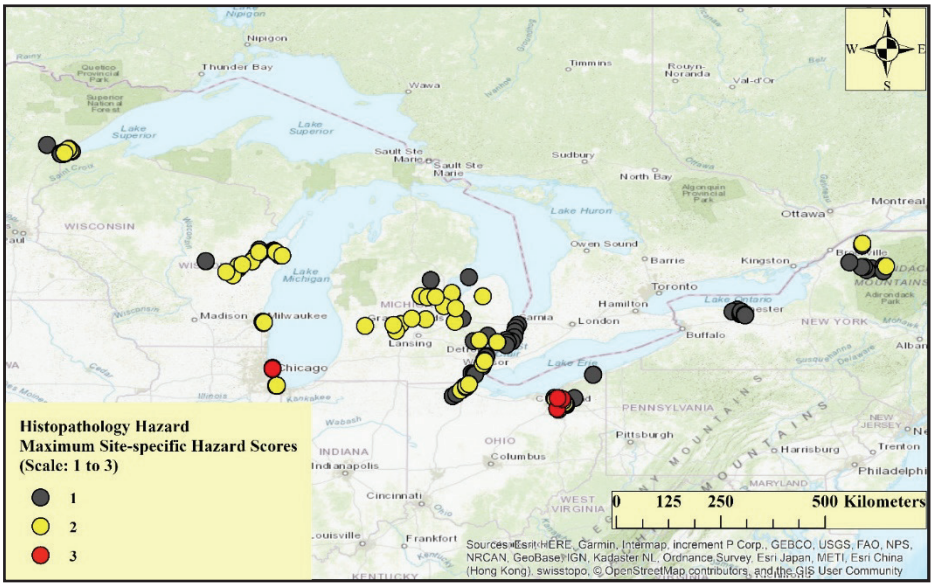
- **Overall:** Substantial evidence of hazard to fish
- **CECs with Histopathology SVs:** Carbamazepine, Venlafaxine
- **Prevalence of High Hazard:**
  - *3 Project Locations*: Venlafaxine
  - *2 Project Location*: Carbamazepine
- **Prevalence of Low Hazard:**
  - *≥ 14 Project Locations*: Carbamazepine, Venlafaxine
- **Point Source Analysis:** Significant evidence of elevated hazards downstream of point sources

The following map illustrates the overall spatial distribution of site-specific *maximum histopathology hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Each dot represents an individual sampling site.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See the explanation of regional hazard maps in the introduction to Section 6.3.



[96](Description of Histopathology effects from Attachment 2-1 *in* Gefell et al. 2019)
"*Exposure:* Sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Endpoints evaluated internally in organs and tissues include microscopic lesions, cellular changes, ultrastructural (subcellular, organelle) changes, etc."

6.3.11 Neurological

There were no observations of high neurological[97] hazard at any project location (Table 6-4), but low neurological hazard (SV_{LOW} exceedance) was observed at 15 project locations (Table 6-5). Neurological effects achieved an intermediate hazard rank ('4' or '5' out of 10 – see explanation in Section 5.4 introduction) at North Shore Channel, Little Calumet River and Tinkers Creek, followed by a rank of '3' at Grand River/Maple River, Maumee River and Cuyahoga River locations (Table 6-2a). Neurological hazard was significantly elevated ($p < 0.1$) in the CEC-influenced site group compared to the uninfluenced site group at the Fox River/Green Bay location, attributable to carbamazepine exposure, but at no other project locations (Table 6-8).

We recommend caution in interpreting these neurological hazard results. Only carbamazepine among the 14 CECs had sufficient ecotoxicity information in the literature to derive neurological SVs (Table 4-1), and the breadth of information was 'sparse' (Table 4-6). Only a single effect endpoint evaluated in the literature - oxidative damage in the brain – was incorporated into neurological SV derivations (from Gefell et al. 2019).

Some Key Points…

Neurological Hazard

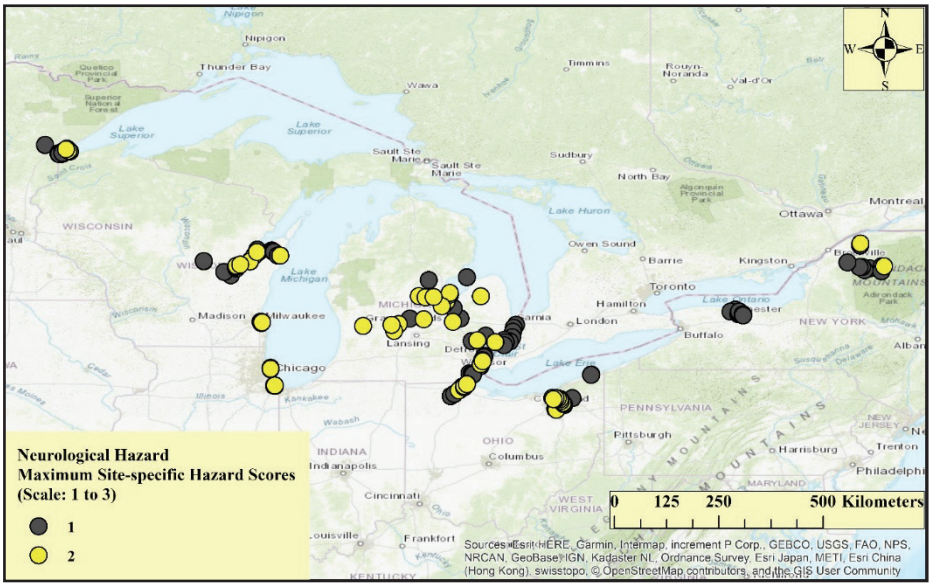
- **Overall:** Little evidence of hazards to fish
- **CECs with Neurological SVs:** Carbamazepine
- **Prevalence of High Hazard:** No occurrences
- **Prevalence of Low Hazard:**
  - *15 Project Locations*: Carbamazepine
- **Point Source Analysis:** Significant evidence elevated hazard downstream of point sources

The following map illustrates the overall spatial distribution of site-specific *maximum neurological hazard* to fish (across CECs) in the U.S. Great Lakes Basin, where each dot represents an individual sampling site.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See the explanation of regional hazard maps in the introduction to Section 6.3.



[97](Description of Neurological effects from Attachment 2-1 *in* Gefell et al. 2019)
"*Exposure:* Sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration.
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Effects are related to neuroanatomy or neurophysiology. Endpoints may include neurotransmitter activity or gene expression, receptor abundance, activation potential, central nervous system (CNS), peripheral nervous system (PNS), locomotor, sensory, mechanosensory, brain, spinal cord, cranial nerve, axon, dendrite, synapse, olfactory, ocular, etc."

6.3.12 Physiological/Metabolic

Elevated physiological/metabolic[98] hazard (SV_{HIGH} or SV_{LOW} exceedances) was widespread, even though applicable SV pairs are limited to four CECs (carbamazepine, estrone, HHCB and ibuprofen) (Table 4-1). High hazard due to estrone exposures was observed at nine project locations, but no SV_{HIGH} exceedances at any project location occurred for the other three CECs that had physiological/metabolic SVs (Table 6-4). At least one SV_{LOW} exceedance was seen at 15 and 18 project locations for carbamazepine and HHCB, respectively, and at 23 locations for estrone (Table 6-5). Hazard ranks for physiological/metabolic effects were intermediate to relatively high ('4', '5', or '6' out of 10 – see explanation in Section 5.4 introduction) compared to the other effect categories at North Shore Channel, Little Calumet River and Cuyahoga River, followed by hazard rank of '3' at the Clinton River and Tinkers Creek locations (Table 6-2a).

No statistical tests comparing hazard levels between CEC-influenced and uninfluenced sites were possible at the North Shore Channel and Little Calumet River, and despite relatively elevated physiological/metabolic hazard ranks, no significant difference in hazard level related to CEC point source proximity was observed at the Cuyahoga River, Clinton River or Tinkers Creek locations (Table 6-8). However, significantly elevated hazard ($p < 0.1$) in point source CEC-influenced sites compared to uninfluenced sites was attributable to carbamazepine and HHCB at the Fox River/Green Bay location, to HHCB at the Saginaw River location, and to estrone and HHCB at the Maumee River location (Table 6-8). Overall, ibuprofen contributed little to observations of elevated physiological/metabolic hazard.

The breadth of ecotoxicity information that was based on numbers of effect endpoints and fish species used to derive the SVs was low and varied only slightly between CECs; quantity of information for SVs was 'limited' for carbamazepine and 'sparse' for estrone, HHCB and ibuprofen (Table 4-6). The biological importance of SV exceedances is related not only to the overall quantity of information incorporated into the SVs, but also to the specific effect endpoints used to derive the CEC-specific SVs (see Section 6.4), which also vary between CECs even for the same effect category.

Some Key Points…

Physiological/Metabolic Hazard

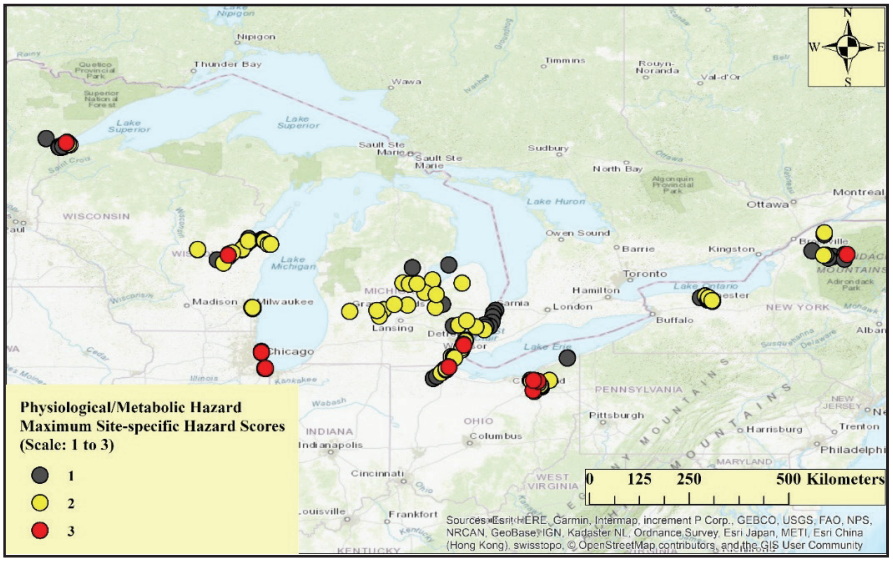
- **Overall:** Clear and convincing evidence of hazard to fish
- **CECs with Physiological/Metabolic SVs:** Carbamazepine, Estrone, HHCB, Ibuprofen
- **Prevalence of High Hazard:**
  - *9 Project Locations*: Estrone
- **Prevalence of Low Hazard:**
  - *>20 Project Locations*: Estrone
  - *>10 Project Locations*: Carbamazepine, HHCB
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

This map illustrates overall spatial distribution of site-specific *maximum physiological/metabolic hazard* to fish (across CECs) in the U.S. Great Lakes Basin. Each dot represents an individual sampling site.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

See the explanation of regional hazard maps in the introduction to Section 6.3.



[98](Description of Physiological/Metabolic effects from Attachment 2-1 *in* Gefell et al. 2019)
"*Exposure:* Sexually immature (fingerling, juvenile) or mature (adult) fish; subchronic or chronic exposure duration.
Life Stages Evaluated: Effects evaluated in exposed sexually immature (fingerling, juvenile) or mature (adult) fish.
Description: Wide variety of effects related to biochemical function, and maintenance of homeostasis (including osmoregulation). Endpoints include: respiration rate, ventilation rate, or oxygen consumption; stress indicators, oxidative stress, or antioxidant abundance or activity; enzyme synthesis, enzyme system induction (e.g., cytochrome P450), or specific enzyme activity in any tissue (except blood); food assimilation efficiency, energy metabolism (e.g., glycolysis); alterations of mRNA transcription or translation; xenobiotic metabolism rates/metabolites; environmental preferences or tolerances (e.g., thermal tolerance)."

6.4 Hazard Attribution: CEC-Specific Hazard Summaries and Project-wide Hazard Maps

This section summarizes ecological hazards to fish associated with individual CECs. For each CEC, we summarized findings regarding hazard incidence and prevalence and the spatial correspondence of hazards to point sources (WWTPS or CSOs). Each CEC-specific hazard summary compiles results that include SV exceedance tallies, hazard rankings, confidence in findings including breadth of ecotoxicity information utilized to derive SVs, and the distribution of CEC-related hazard to fish across 195 sampling sites in the U.S. Great Lakes Basin. We provide CEC-specific maps showing maximum hazard scores (across effect categories) at each of the sampling sites identified in Attachment A2. Mapped CEC-specific maximum hazard scores are interpreted as follows:

- 3 = high expectation of biological impact, where a SV_{HIGH} value was exceeded for at least one effect category;
- 2 = low expectation of biological impact, where at least one SV_{LOW} value was exceeded (but the corresponding SV_{HIGH} was not exceeded) for at least one effect category;
- 1 = negligible expectation of biological impact, where no SV_{LOW} was exceeded for any effect category.

Maps show CEC-specific hazard distribution without reference to specific project locations, which were discussed individually in Section 5.4. Because distances between sampling sites are small compared with the scale of the maps, in many cases there is considerable overlap of site symbols possibly resulting in obscured site-specific hazards. However, the dots were layered in order to highlight hazard according to the following priority: high, low, negligible. It is important to note that the mapped hazards are minimal estimates of the maximum CEC-related hazard to fish, since hazard is likely to have been under-estimated in this EHA (see Chapter 7 – Uncertainty Assessment).

Some Key Points…

CEC-Specific Hazards

- **Overall:** Project-wide hazard to fish varied widely among the 14 CECs
- **Greatest Evidence of Hazards**: Clear and convincing evidence of widespread hazards to fish was observed for DEET, Estrone, Ibuprofen, Venlafaxine
- **Least Evidence of Hazards**: Little or no evidence of hazards to fish was observed for Androstenedione, Lidocaine
- **Elevated hazard downstream of point sources**: Significant evidence of downstream elevated hazard for Carbamazepine, Citalopram, Diphenhydramine, Estrone, HHCB, Ibuprofen and Venlafaxine. No evidence for Lidocaine, β-Sitosterol.

The biological importance of an exceedance or non-exceedance may vary between CECs – even within effect categories - because the specific effect endpoints that were incorporated into deriving effect-specific SVs vary between CECs (see introduction to Section 5.4). Therefore, as an aid to interpreting the biological importance of SV exceedances or non-exceedances, we provide CEC-specific charts that list specific biological effect endpoints (as summarized in Gefell et al. 2019) that were evaluated in the ecotoxicological literature and incorporated into derivations of CEC- and effect-specific SVs.

The following subsections provide CEC-specific summaries of ecological hazard to fish observed in this EHA.

6.4.1 4-Androstene-3,17-dione

Relative to other CECs assessed in this EHA, available evidence is weak that 4-androstene-3,17-dione poses a hazard to fish in the project locations. However, there is considerable uncertainty due to significant data gaps in the published ecotoxicity literature. Few reports were located on androstenedione toxicity in fish; uncertainties could be significantly reduced by targeted fish toxicity assays. However, despite these SV limitations and a low detection frequency, the exceedances of mean comprehensive SV_{LOW} and mean population-relevant SV_{LOW} were fairly widespread (Table 6-5), indicating that hazards to fish are possible from androstenedione exposures and supporting the findings of hazards from other CECs. A re-evaluation of hazard would be prudent if ecotoxicity data gaps are filled – in particular exposure-effect data gaps concerning reproductive endpoints, considering androstenedione's intended therapeutic use.

Some Key Points…

4-Androstene-3,17-dione

- **Overall:** Little evidence of hazards to fish
- **Effect Categories with Androstenedione SVs**: Growth
- **High Hazard:** No occurrences
- **Low Hazard:** No occurrences *
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources

* There were no observations of hazard for growth - the only individual effect category for this CEC. However, low hazard was indicated by exceedances of mean comprehensive SVs and mean population-relevant SVs (Table 6-5).

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	63-05-8
Method Detection Limit (µg/L):	0.0003
$logK_{ow}$:	2.75
Water Solubility (mg/L):	57.2 at 25 deg C
Use:	"Therapeutic androstenedione is a potent androgenic prohormone that is a direct precursor of testosterone and used as a supplement to increase plasma testosterone levels and muscle anabolism…. Androstenedione is a delta-4 19-carbon steroid that is produced not only in the testis, but also in the ovary and the adrenal cortex. Depending on the tissue type, androstenedione can serve as a precursor to testosterone as well as estrone and estradiol. It is the common precursor of male and female sex hormone." (PubChem 2019).
Production/Usage:	No data were located.

4-Androstene-3,17-dione Exposure Data

Number of Samples Analyzed:	524
Percent Detects:	10% (53/524)
Range Detected Concentrations[99]:	0.000317 to 0.00784 µg/L

4-Androstene-3,17-dione Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	3.23	0.00127	sparse
Growth	16.2	0.0254	sparse
Comprehensive Mean SV	0.852	0.000204	sparse

The specific growth effect endpoints evaluated in 4-androstene-3,17-dione fish toxicity assays reported in the literature were standard length and weight gain.

[99] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

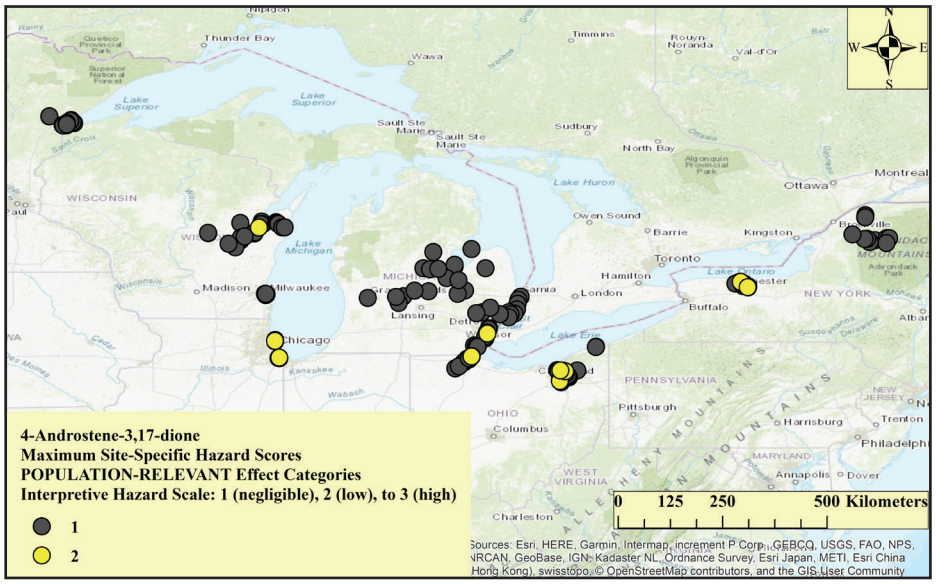
4-Androstene-3,17-dione Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score for 4-androstene-3,17-dione observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

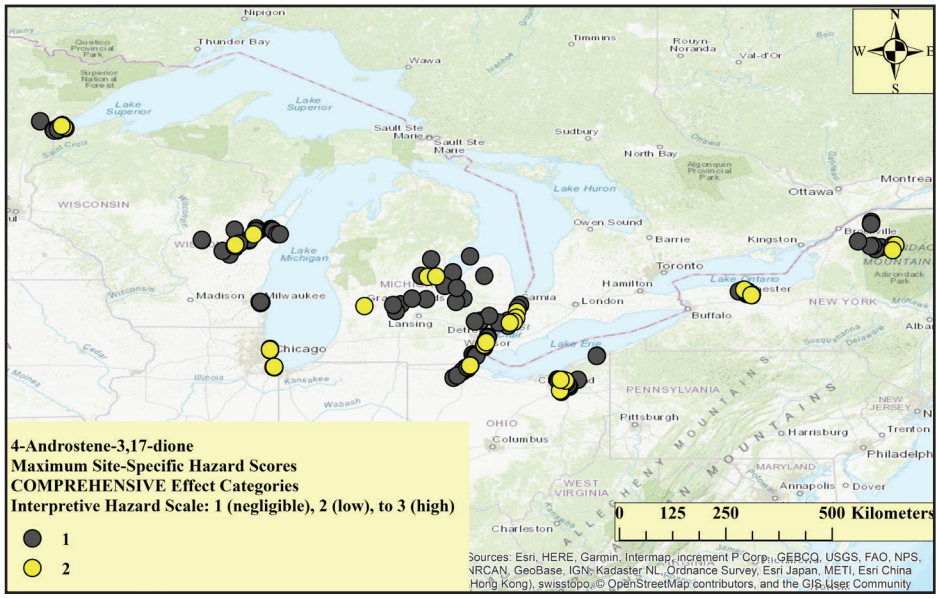
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

4-Androstene-3,17-dione Population-relevant SV pairs: Population-relevant mean SV, Growth



4-Androstene-3,17-dione Comprehensive SV pair: Comprehensive mean SV



6.4.2 Bisphenol A (BPA)

Evidence for hazard to fish from BPA exposure is moderate in this EHA. BPA SVs are available for six effect categories, including all five population-relevant categories. There were 254 BPA SV_{LOW} exceedances involving each of the eight BPA SV_{LOW} values (Table 6-6)[100] , and occurring at up to 11 project locations distributed across the U.S. Great Lakes Basin (Table 6-5). Each of the 73 detected BPA concentrations exceed at least two population-relevant SV_{LOW} values (developmental and reproductive) - and most of these also exceed the mean comprehensive SV_{LOW}. However, no BPA-related high hazard was identified and the BPA detection frequency (14%) was relatively low with all non-detects assigned a hazard score of 1 (see Section 5.2), resulting in the lowest hazard rank for BPA in nearly all project locations (Table 6-2b).

The spatial correspondence of BPA-related hazard to fish and CEC point sources was moderate. Quantitatively, BPA-related hazard was not statistically significantly higher in the point source CEC-influenced site group than in the uninfluenced group. However, a qualitative analysis showed that fractions of sampling events and sampling sites with a SV_{LOW} exceedance were about twice as high in CEC point source-influenced sites as in uninfluenced sites (Table 6-6). The number of exceedances per site was more than four times higher in the CEC point source-influenced site group than in the uninfluenced site group.

It is plausible that a full BPA risk assessment using the same exposure dataset might identify significant risk where we did not identify high hazard. While there were no BPA SV_{HIGH} exceedances in this hazard assessment, effect-specific SV_{HIGH} values are geometric means of effect-specific SV_{HIGH} point estimates (see Gefell et al. 2019) which for BPA ranged (depending on the specific effect endpoint), from 0.404 to 2,436 μg/L for developmental effects, and from 0.17 to 2,020 μg/L for reproductive effects. The lower ends of these ranges of SV_{HIGH} point estimates fall below the high end of the range of detected concentrations (0.013 to 4.32 μg/L), suggesting a potential for BPA-related elevated risk to fish populations.

Some Key Points…

Bisphenol A (BPA)

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with BPA SVs**: Behavioral, Developmental, Growth, Mortality, Reproductive, Gross Pathology
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 254 occurrences, involving 14% of sampling events and 25% of sites
  - *Effect Categories:* 6 of 6
  - *Project Locations*: 12 of 24
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	80-05-7
Method Detection Limit (µg/L):	0.22
Lab Reporting Level (µg/L):	0.04
$\log K_{ow}$:	3.32
Water Solubility (mg/L):	120 at 25 deg C
Use:	PubChem (2019) indicates BPA's principal end uses are as an additive to polycarbonate and epoxy resins. BPA is a plasticizer in household items and food packaging.
Production/Usage:	PubChem (2019) lists BPA as a USEPA High Production Volume Chemical (HPVC). The USEPA Chemical Data Reporting system reports that U.S. production and use was 2.25 billion pounds in 2011, and 1 to 5 billion pounds for each year from 2012-2015 (PubChem 2019). Listed as an Organization for Economic Co-operation and Development (OECD) HPVC in 2004 (OECD 2004).

Bisphenol A Exposure Data

Number of Samples Analyzed:	524
Percent Detects:	14% (71/524)
Range Detected Concentrations[101]	0.013 to 4.32 µg/L

[100]Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in the individual effect categories

[101]These are aqueous concentrations (μg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	177	0.062	Robust
Behavioral	606	3.82	Sparse
Developmental	88.3	0.00064	Robust
Growth	259	0.13	Sparse
Mortality	680	1.65	Limited/Robust
Reproductive	18.3	0.0018	Robust
Comprehensive Mean SV	118	0.032	Broad
Gross Pathology	49.2	0.27	Sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each SV pair for bisphenol A (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	mating behavior	NA
Developmental	Cellular	oxidative stress, apoptosis, DNA damage, neuron axonal growth, histopathology in gonads, kidney, heart, liver, and thyroid	NA
	Tissue/Organ	organ development, edema, hemorrhage	NA
	Organism	hatching rates, hatch timing, survival (parental, embryo, larval), spinal curvature, other structural anomalies, yolk sac edema, heart inflammation, heart rate, embryo and larval length and weight, sex ratio, fecundity and egg fertilization in subsequent generations, development rate	NA
Growth	Organism	length and weight	NA
Mortality	Organism	mortality rate	NA
Reproductive	Cellular	sperm motility and velocity, gonad histopathology, sperm count, sperm length, sperm total mass, sperm density, % viable sperm	NA
	Tissue/Organ	Intersex, GSI, ovulation timing, testis morphology, spermatogenesis, oogenesis	NA
	Organism	reproductive hormone levels, fertility, eggs per female, hatchability, number spawns per breeding pair	NA
Gross Pathology	Tissue/Organ	kidney size changes related to histopathologies	other gross lesion endpoints and body coloration also incorporated into SV_{HIGH} derivation

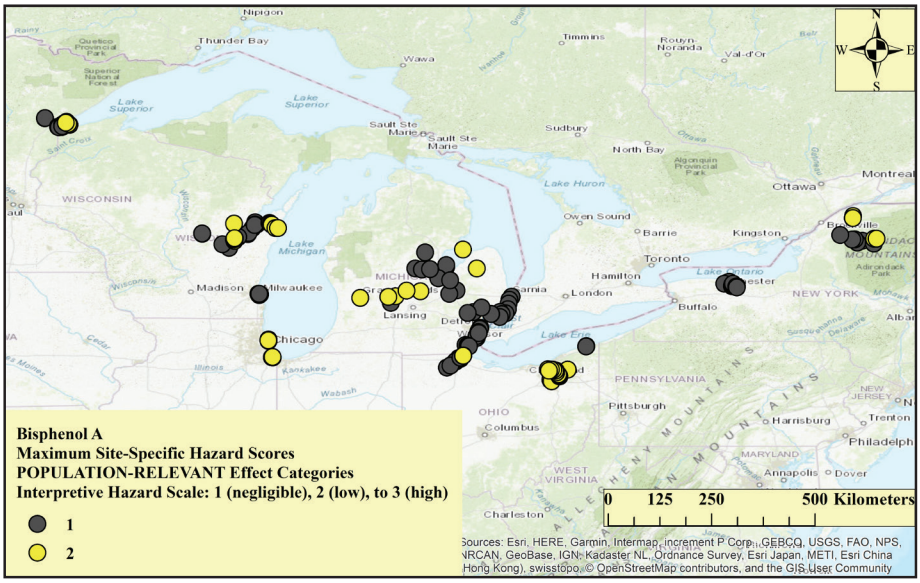
Bisphenol A Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for bisphenol A observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

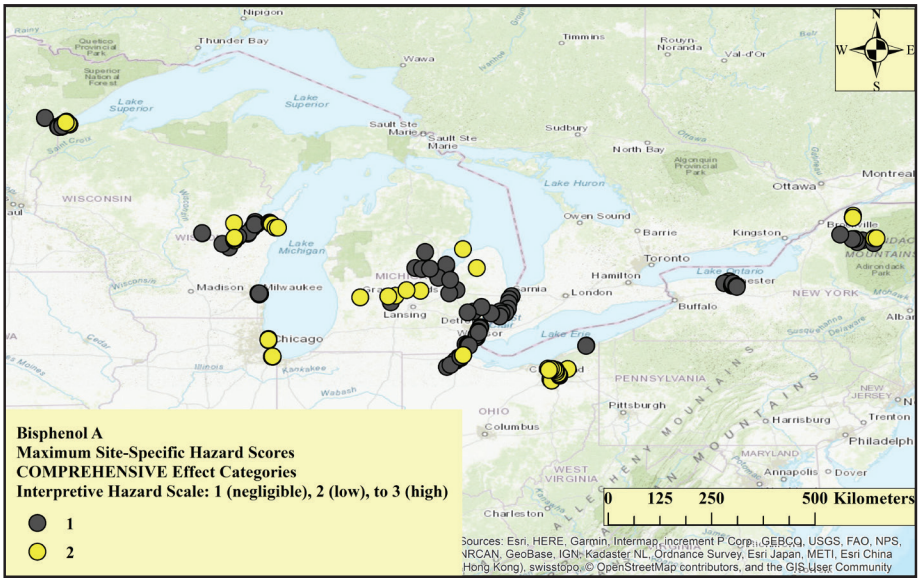
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Bisphenol A Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, Growth, Mortality, and Reproductive



Bisphenol A Comprehensive SV pairs: Comprehensive mean SV, Gross Pathology



6.4.3 Carbamazepine

This EHA provides substantial evidence that aqueous carbamazepine is hazardous to fish, and that hazards are higher in downstream proximity to WWTPs and/or CSOs than at upstream or distant points. A total of 11 carbamazepine SV pairs are available - nine for individual effect categories, plus comprehensive mean SV and population-relevant mean SV. There was a total of 1,315 SV_{LOW} exceedances in 197 samples with detected carbamazepine – 22.8% of the all hazard scores generated for carbamazepine (Table 6-6)[102]. SV_{LOW} exceedances involved nine effect categories and were distributed across 10 or more project locations for seven of those effect categories (Table 6-5)[103] . Possible population-relevant hazards are indicated by developmental and reproductive SV_{LOW} exceedances in at least 14 project locations, and there were six exceedances of the histopathology SV_{HIGH} distributed between two project locations. The highest detected concentration was 75% greater than the histopathology SV_{HIGH}. In addition, carbamazepine exhibited intermediate CEC-specific hazard ranks in nine of 24 project locations (Table 6-2b).

The spatial correspondence of carbamazepine-related hazard to CEC point sources was moderately strong. Quantitatively, maximum hazard scores were statistically significantly higher in the point source CEC-influenced site group than in the uninfluenced groups at the Fox River/Green Bay project location for three effect categories - circulatory/blood constituents, neurological, and physiological/metabolic (Table 6-8). At the Saginaw River, median hazard scores were significantly greater at CEC-influenced sites for reproductive and histopathology effects (Table 6-8). Qualitatively, fractions of sampling events and sampling sites with a SV_{LOW} exceedance, and the numbers of exceedances per event and exceedances per site, were all approximately twice as high in CEC point source-influenced sites than in uninfluenced sites (Table 6-6). In addition, all six SV_{HIGH} exceedances occurred at CEC-influenced sites.

Some Key Points…

Carbamazepine

- **Overall:** Substantial evidence of hazards to fish
- **Effect Categories with Carbamazepine SVs**: 9 of 12 (all 5 population-relevant categories)
- **High Hazard:**
  - 6 occurrences, involving 1% of sampling events, and 2% of sites
  - *Effect Categories:* Histopathology
  - *Project Locations:* 2 of 24
- **Low Hazard:**
  - 1,315 occurrences, involving 37% of sampling events, and 38% of sites
  - *Effect Categories:* 7 of 7
  - *Project Locations:* 16 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	298-46-4
Method Detection Limit (µg/L):	0.00084
$logK_{ow}$:	2.45
Water Solubility (mg/L):	18 at 25 deg C
Use:	"Carbamazepine is a tricyclic compound chemically related to tricyclic antidepressants (TCA) with anticonvulsant and analgesic properties. Carbamazepine exerts its anticonvulsant activity by reducing polysynaptic responses and blocking post-tetanic potentiation. Its analgesic activity is not understood; however, carbamazepine is commonly used to treat pain associated with trigeminal neuralgia." (PubChem 2019)
Production/Usage:	2004 OECD High Production Volume Chemical – "…those chemicals which are produced at levels greater than 1,000 tonnes per year in at least one member country/region." (OECD 2004).
Transformation:	At least 30 metabolites have been found in humans, five of which have been detected in wastewater samples (CCME 2018).

Carbamazepine Exposure Data

Number of Samples Analyzed:	524
Percent Detects:	38% (197/524)
Range Detected Concentrations[104]:	0.00031 to 0.333 µg/L

[102] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in the individual effect categories

[103] Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances

[104] These were measured aqueous concentrations (µg/L) for 2013 and 2014, but calculated aqueous concentrations for 2010-2012, estimated from total concentrations as described in Section 3.5 and Attachment A.

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	299	0.079	Robust/Broad
Behavioral	413	0.025	Broad
Developmental	707	0.0073	Broad
Growth	719	2.29	Sparse/Limited
Mortality	1330	5.66	Sparse/Limited
Reproductive	8.52	0.0013	Moderate
Comprehensive Mean SV	139	0.0087	Broad
Circulatory/Blood Constituents	436	0.0026	Sparse
Histopathology	0.19	0.00051	Sparse
Neurological	436	0.0026	Sparse
Physiological/Metabolic	306	0.0026	Sparse/Limited

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for carbamazepine (from Gefell et al. 2019).

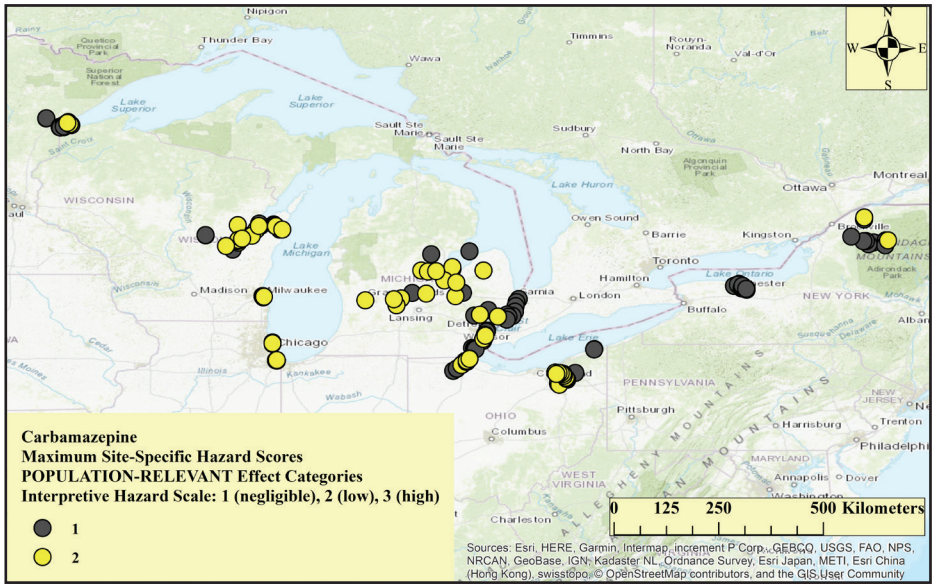
Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	courtship behavior, startle response, light-dark preference, feeding duration, swimming speed, larval activity and swimming velocity	NA
Developmental	Cellular	liver, kidney and gonad histology, sperm morphology and speed in subsequent generations	NA
	Tissue/Organ	pericardial edema, yolk sac edema, heart deformation, hemorrhages, tail length, pigmentation	NA
	Organism	hatch rate, embryo mortality, deformations of spinal cord, head, otolith, and tail, growth, heart rate, dorsal curvature, egg production	hatching success and prevalence of embryos with defects also incorporated into SV_{LOW}
Growth	Organism	condition factor	body length, fork length and weight also incorporated into SV_{LOW}
Mortality	Organism	mortality rate	NA
Reproductive	Cellular	sperm density and gonad histopathology	NA
	Tissue/Organ	GSI, spermatogenesis, oogenesis, gonad morphology	NA
	Organism	embryo production, reproductive hormone levels	NA
Circulatory/Blood Constituents	Tissue/Organ	hematological parameters, leukocyte differential counts, blood plasma chemistry	NA
Histopathology	Cellular	histopathologic endpoints in kidneys, gills and liver	NA
Neurological	Cellular	oxidative damage in the brain	NA
Physiological/Metabolic	Cellular	oxidative stress indicators, enzyme activities related to oxidative stress	NA
	Tissue/Organ	energy metabolism and osmoregulation	NA

Carbamazepine Hazard Distribution

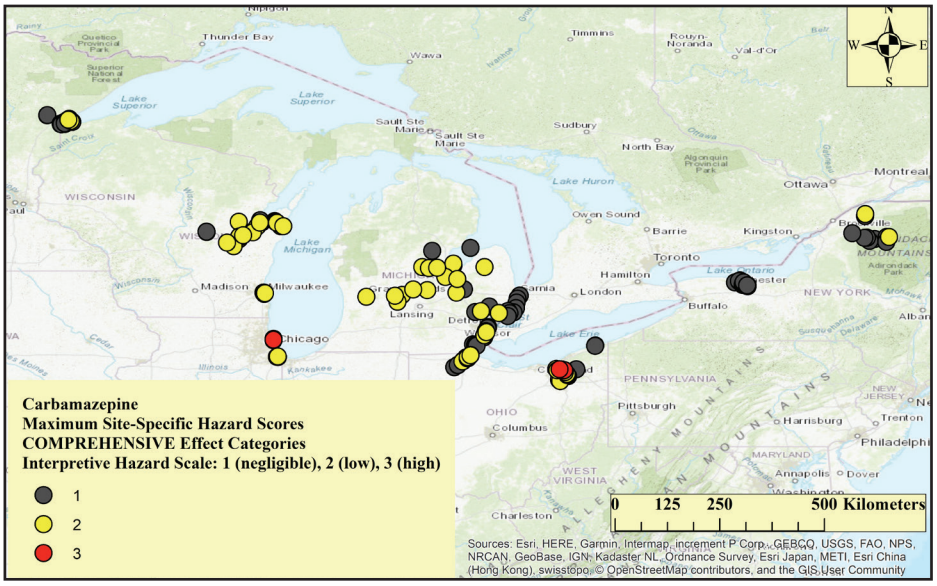
Two hazard maps are provided below. One map illustrates the site-specific maximum hazard score for carbamazepine observed among population-relevant effect categories assessed for this CEC at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Carbamazepine Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, Growth, Mortality, and Reproductive



Carbamazepine Comprehensive SV pairs: Comprehensive mean SV, Circulatory/Blood Constituents, Histopathology, Neurological, Physiological/Metabolic



6.4.4 Citalopram

The hazard score database for citalopram is limited - mostly due to broad gaps in the fish ecotoxicity literature. All three applicable SV pairs (behavioral, mean comprehensive SVs, and mean population-relevant SVs)[105] were based exclusively on behavioral exposure-response metrics; no other effect-specific SVs were available for citalopram. Further, the behavioral SVs themselves were based on a very limited set of effect endpoints (see chart below).

Despite severe fish ecotoxicity data gaps, there are some interesting observations suggesting behavioral hazard in fish from aqueous citalopram exposure. While there were no SV_{HIGH} exceedances for citalopram (Table 6-4), there was a total of 258 SV_{LOW} exceedances observed in 88 samples with detected concentrations – 16.3% of all hazard scores generated for citalopram (Table 6-6)[106] - which were distributed among 12 project locations (Table 6-5)[107]. Citalopram achieved intermediate CEC-specific hazard ranks at seven project locations (Table 6-2b), despite a relatively low overall detection frequency (17%).

Citalopram hazard data support the hypothesis that CEC point sources increase hazard to fish. Site-specific maximum and/or median behavioral hazard scores were statistically significantly ($p < 0.1$) higher in the CEC-influenced group than in the uninfluenced group at the Maumee River and Tinkers Creek project locations (Table 6-8). Site-specific maximum and median hazard scores generated with mean comprehensive SVs and mean population-relevant SVs were statistically significantly higher ($p < 0.1$) at point source CEC-influenced sites than at uninfluenced sites at the Maumee River. Qualitatively, the project-wide database indicates that the percent of sampling sites and sampling events with a SV_{LOW} exceedance in the point source CEC-influenced site group were 29% and 23%, respectively, which were more than three times the percent seen in the uninfluenced group. The number of SV_{LOW} exceedances per site was nearly six times higher in the CEC-influenced site group than in uninfluenced sites (Table 6-6).

Some Key Points…

Citalopram

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with Citalopram SVs**: Behavioral
- **High Hazard** No occurrences
- **Low Hazard:**
  - 258 occurrences, involving 17% of sampling events, and 20% of sites
  - *Effect Categories:* 1 of 1
  - *Project Locations:* 12 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	59729-33-8
Method Detection Limit (µg/L):	0.0013
$logK_{ow}$:	3.5
Water Solubility (mg/mL):	4
Use:	"Citalopram is an antidepressant drug used to treat depression associated with mood disorders. It is also used on occasion in the treatment of body dysmorphic disorder and anxiety; Citalopram belongs to a class of drugs known as selective serotonin reuptake inhibitors (SSRIs)" (PubChem 2019)
Production/Usage:	No data were located.

Citalopram Exposure Data

Number of Samples Analyzed:	524
Percent Detects:	17% (88/524)
Range Detected Concentrations[108]:	0.0003 to 0.106 µg/L

[105]Both sets of citalopram mean SVs were derived from behavioral SVs, only, by applying appropriate database adequacy uncertainty factors (see Gefell et al. 2019). Consideration of all three citalopram SV pairs in statistical analyses evaluating point source impacts would inflate relative importance of behavioral effects in the citalopram hazard assessment, so here we discuss statistical test results for behavioral effects only. On the other hand, tallies of citalopram-related SV exceedances for both CEC-influenced and uninfluenced sites (Table 6-6) include all three SV pairs. We expect that the incidence of exceedances using all three pairs of SVs are proportional to behavioral effects only, so comparisons between the site groups of percent occurrence of exceedances and fractions of sites and events with exceedances are expected to be valid.

[106]Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

[107]Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances.

[108]These were measured aqueous concentrations (µg/L) for 2013 and 2014, but calculated aqueous concentrations for 2010-2012, estimated from total concentrations as described in Section 3.5 and Attachment A.

Citalopram Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	0.24	0.00025	sparse
Behavioral	1.2	0.0013	moderate/broad
Comprehensive Mean SV	0.22	0.0001	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for citalopram (from Gefell et al. 2019).

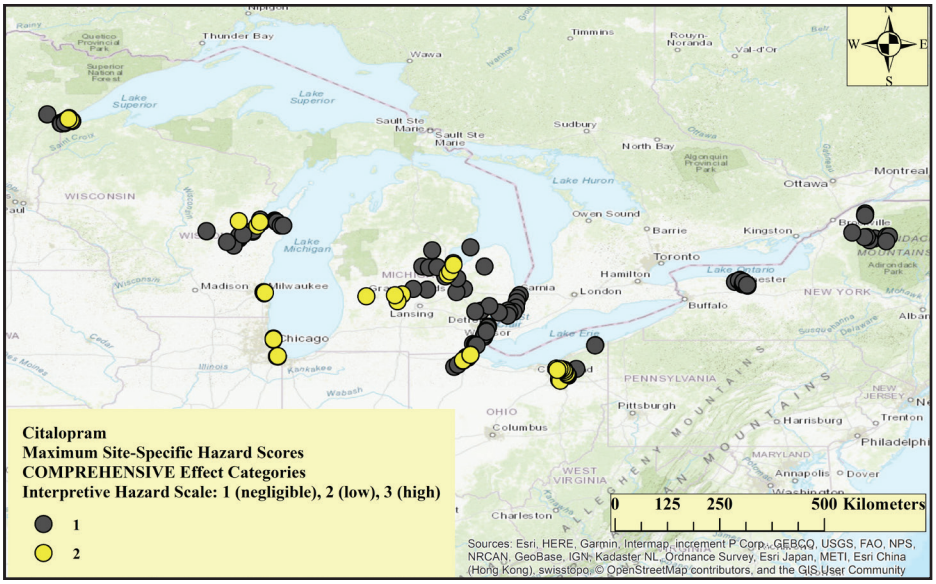
Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	"Freezing" behavior and time remaining frozen, and five additional indicators of stress-related behavior	NA

Citalopram Hazard Distribution

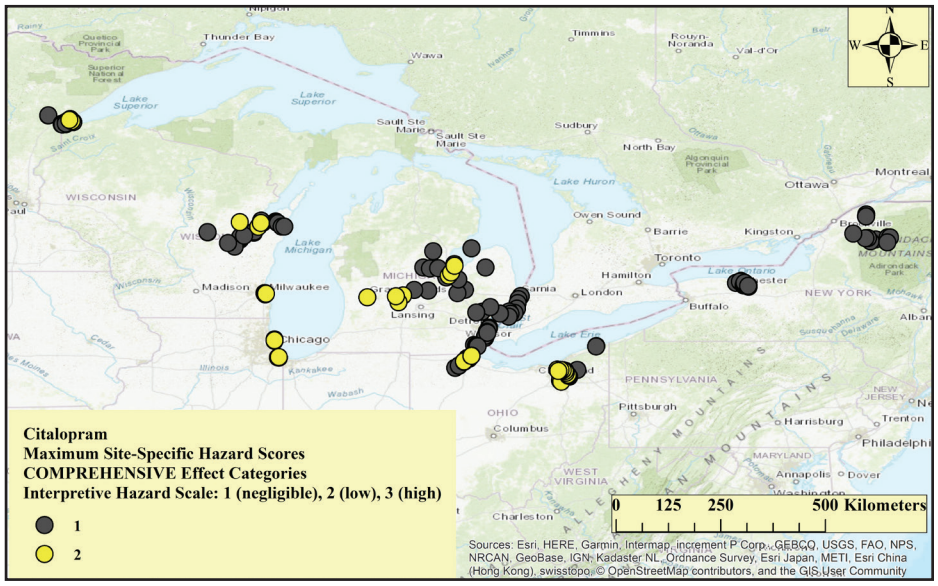
Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for citalopram observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Citalopram Population-relevant SV pairs: Population-relevant mean SV, Behavioral



Citalopram comprehensive SV pairs: Comprehensive mean SV



6.4.5 N,N-diethyl-meta-toluamide (DEET)

Widespread substantial hazard to fish from aqueous DEET exposure is evident. At each project location, DEET consistently ranked at or near the highest overall hazard among the CECs considered in this assessment[109] (Table 6-2b), suggesting it has potential to impact aquatic biota. A SV_{LOW} value was exceeded at 20 or more project locations for each of the following DEET SV pairs (Table 6-5)[110] : mean comprehensive SVs, endocrine, mean population-relevant SVs and mortality. The number of DEET SV_{LOW} exceedances was 1,428 observed among 401 samples with detected concentrations - which is 54.4% of the total number of hazard scores generated for DEET (Table 6-6)[111]. There was an average of over nine SV_{LOW} exceedances per site, and the percent of sampling events and sampling sites with at least one SV_{LOW} exceedance were 76% and 90%, respectively (Table 6-6).

Exceedances of the DEET endocrine SV_{HIGH} value provide supporting evidence of widespread DEET-related hazard to fish. The DEET SV_{HIGH} for endocrine effects was exceeded at 15 separate project locations (Table 6-4) for a total of 107 exceedances (Table 6-6) distributed from the St. Louis River/Bay, MN, to the Raquette River in New York's north country (Section 5.4). The highest detected DEET concentration was more than 60 times higher than the endocrine SV_{HIGH}, which was exceeded in 20% of samples collected and at 29% of sampling sites (Table 6-6). However, the breadth of ecotoxicological information incorporated into deriving the DEET endocrine SV values was moderate (Table 4-6), and relied exclusively on gene expression assays that do not directly measure, but rather imply, expression of functionally or anatomically adverse endocrine effects.

DEET is known to be distributed by atmospheric transport and precipitation (Ferrey et al. 2018) so elevated hazards in surface water are not necessarily limited to reaches downstream of point sources or areas susceptible to runoff events. It appears that observations of elevated DEET-related hazard (SV_{HIGH} and SV_{LOW} exceedances) were so widely distributed that few statistically significant differences ($p < 0.1$) in site-specific maximum or median hazard scores were observed between point source CEC-influenced and uninfluenced site groups, for individual effect categories (Table 6-8). Similarly, inspection of project-wide DEET SV_{LOW} exceedance tallies (Table 6-6) indicates little difference between point source CEC-influenced sites and uninfluenced sites in the following: fraction of sampling events with at least one SV_{LOW} exceedance, fraction of sampling sites with at least one exceedance, number of exceedances per site, or number of exceedances per sampling event. However, there is some limited evidence indicating elevated DEET-related hazard downstream of point sources. Quantitatively, in the St. Louis River/Bay location, circulatory/blood constituents and endocrine median hazard scores attributable to DEET were significantly greater ($p < 0.1$) in the CEC-influenced site group compared to the uninfluenced site group. Qualitatively, the number of endocrine SV_{HIGH} exceedances per sampling site was nearly four times higher in the CEC-influenced site group compared to uninfluenced sites (Table 6-6). Further, the fractions of sampling sites and sampling events with at least one SV_{HIGH} exceedance were, respectively, two and three times higher in the CEC-influenced site group than in the uninfluenced site group (Table 6-6).

Some Key Points…

N,N-diethyl-meta-toluamide (DEET)

- **Overall:** Clear and convincing evidence of hazards to fish
- **Effect Categories with DEET SVs**: Mortality, Circulatory/Blood Constituents, Endocrine
- **High Hazard:**
  - 107 occurrences, involving 20% of sampling events, and 29% of sites
  - *Effect Categories:* Endocrine
  - *Project Locations:* 15 of 24
- **Low Hazard:**
  - 1,428 occurrences, involving 76% of sampling events, and 90% of sites
  - *Effect Categories:* 3 of 3
  - *Project Locations*: 24 of 24
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources

[109] Consistently high ranking for DEET may be due in part to having the highest overall detection rate (76%) with relatively fewer non-detects than the other CECs – non-detects were assigned the lowest hazard score of '1'.

[110] Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances.

[111] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	134-62-3
Method Detection Limit (µg/L):	0.12
$\log K_{ow}$:	2.02
Water Solubility (mg/L):	912 at 25 C (estimated)
Use:	"DEET is a chemical (N,N-diethyl-meta-toluamide) used as the active ingredient in many insect repellent products…. It is now widely used, with approximately 30% of the U. S. population using DEET repellents each year…Approximately 230 products containing DEET are currently registered with the U. S. Environmental Protection Agency (EPA)." (PubChem 2019)
Production/Usage:	Annual DEET usage in the U.S. was 7.7, 6.8 and 9.2 million pounds in 2005, 2009 and 2011, respectively (USEPA 2014b). 2004 OECD High Production Volume Chemical Chemical – "…those chemicals which are produced at levels greater than 1,000 tonnes per year in at least one member country/region." (OECD 2004). We did not locate current production or usage data.

DEET Exposure Data

Number of Samples Analyzed:	525
Percent Detects:	76% (401/525)
Range Detected Concentrations[112]:	0.0039 to 5.07 µg/L

DEET Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	7,100	0.0013	sparse
Mortality	21,300	0.0025	sparse/limited
Comprehensive Mean SV	22	0.024	sparse/broad
Circulatory/Blood Constituents	189	0.13	moderate
Endocrine	0.076	0.00034	moderate

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for DEET (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Mortality	Organism	mortality rate	NA
Circulatory/Blood Constituents	Tissue/Organ	21 hematological and blood chemistry parameters	NA
Endocrine	Cellular	9 endocrine-related gene expressions (in addition to androgen receptor gene expression in ovaries)	NA

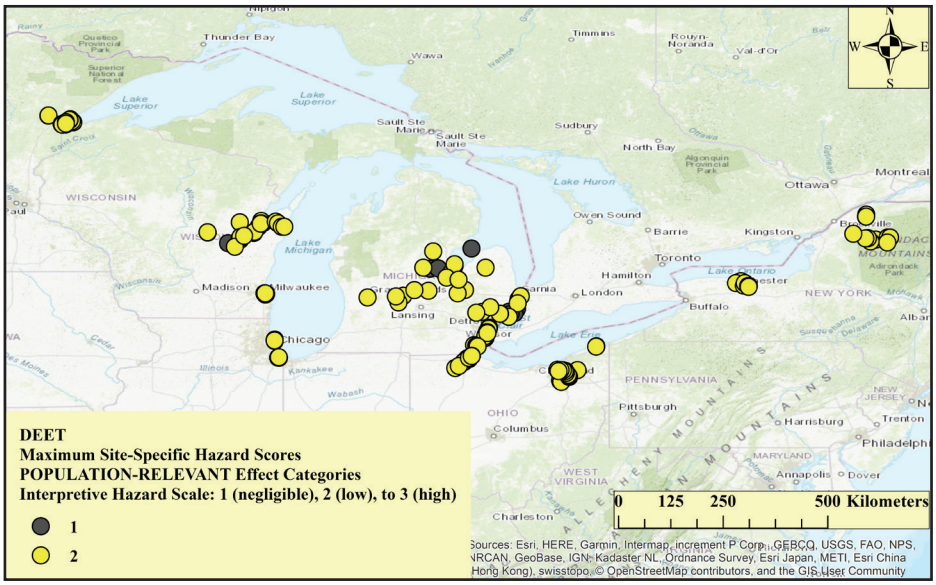
[112]These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

DEET Hazard Distribution

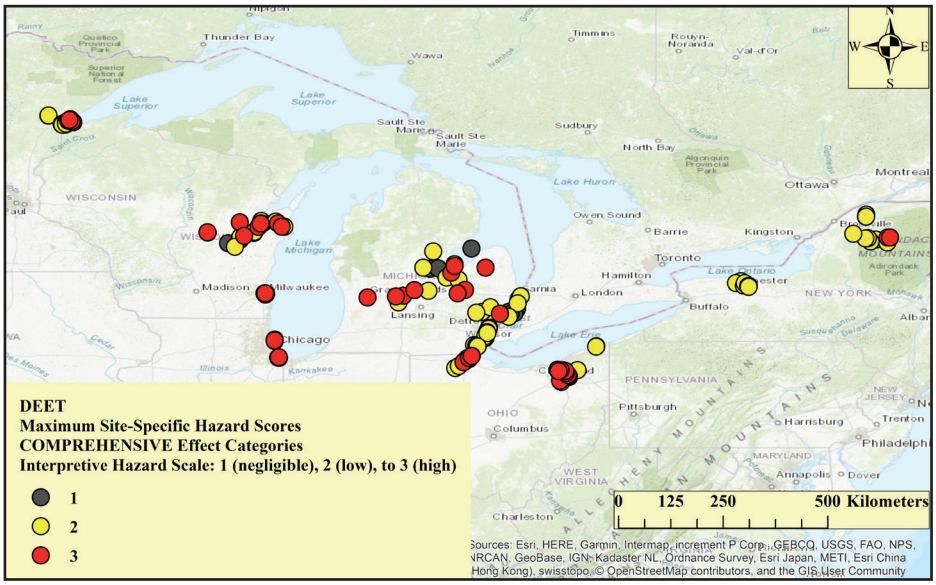
Two hazard maps are provided below. One illustrates the site-specific maximum hazard score for DEET observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

DEET Population-relevant SV pairs: Population-relevant mean SV, Mortality



DEET Comprehensive SV pairs: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine



6.4.6 Diphenhydramine

This EHA provides some evidence that aqueous diphenhydramine poses a significant hazard to fish in Great Lakes Basin waterbodies. There were no SV_{HIGH} exceedances for any of the four diphenhydramine SV pairs: mean comprehensive SVs, mean population-relevant SVs, behavioral or developmental (Table 6-4). However, there were 198 SV_{LOW} exceedances in 89 samples with detected concentrations (9.4% of all hazard scores generated for diphenhydramine) (Table 6-6)[113], with at least one SV_{LOW} exceedance occurring in eight or more project locations for mean comprehensive SV_{LOW}, mean population-relevant SV_{LOW} and the behavioral SV_{LOW} (Table 6-5). These observations of hazard were based on very limited fish ecotoxicity information in the published literature, including just two developmental effects endpoints and only one behavioral endpoint (see below). If future research provides a more robust diphenhydramine ecotoxicity database that fills existing data gaps, then revised and/or additional SVs may indicate greater or additional hazards.

The available evidence indicates that point sources increase diphenhydramine-related hazard to fish. Site-specific maximum and/or median hazard scores generated with mean comprehensive SVs were statistically significantly higher ($p < 0.1$) at point source CEC-influenced sites than at uninfluenced sites in the Maumee River and Tinkers Creek project locations (Table 6-8). Qualitative analysis of project-wide tallies (Table 6-6) indicated that the number of SV_{LOW} exceedances per sampling event was 14 times higher in CEC-influenced sites than in uninfluenced sites, and the number of exceedances per sampling site was 21 times greater in CEC-influenced sites. The fraction of sampling sites with at least one SV_{LOW} exceedance was 25% in the CEC-influenced sites – more than 12 times greater than in uninfluenced sites. Likewise, the fraction of sampling events with a SV_{LOW} exceedance was 24% at CEC-influenced sites, but only 3% at uninfluenced sites (Table 6-6).

Some Key Points…

Diphenhydramine

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with Diphenhydramine SVs**: Behavioral, Developmental
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 198 occurrences, involving 16% of sampling events, and 15% of sites
  - *Effect Categories:* 2 of 2
  - *Project Locations*: 9 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	58-73-1
Method Detection Limit (µg/L):	0.0029
$logK_{ow}$:	3.27
Water Solubility (mg/L):	3060 at 37 deg C
Use:	"Diphenhydramine is a first generation antihistamine and ethanolamine with sedative and anti-allergic properties. … This prevents histamine-induced bronchoconstriction, vasodilation, increased capillary permeability, and GI smooth muscle spasms." (PubChem 2019)
Production/Usage:	No data were located.

Diphenhydramine Exposure Data

Number of Samples Analyzed:	526
Percent Detects:	17% (89/526)
Range Detected Concentrations[114]:	0.007 to 0.45 µg/L

[113] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

[114] These were measured aqueous concentrations (µg/L) for 2013 and 2014, but calculated aqueous concentrations for 2010-2012, estimated from total concentrations as described in Section 3.5 and Attachment A.

Diphenhydramine Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	3.4	0.053	sparse
Behavioral	2.3	0.037	sparse
Developmental	19.8	0.31	sparse
Comprehensive Mean SV	1.3	0.0085	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for diphenhydramine (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	feeding rate	NA
Developmental	Organism	growth, survival	NA

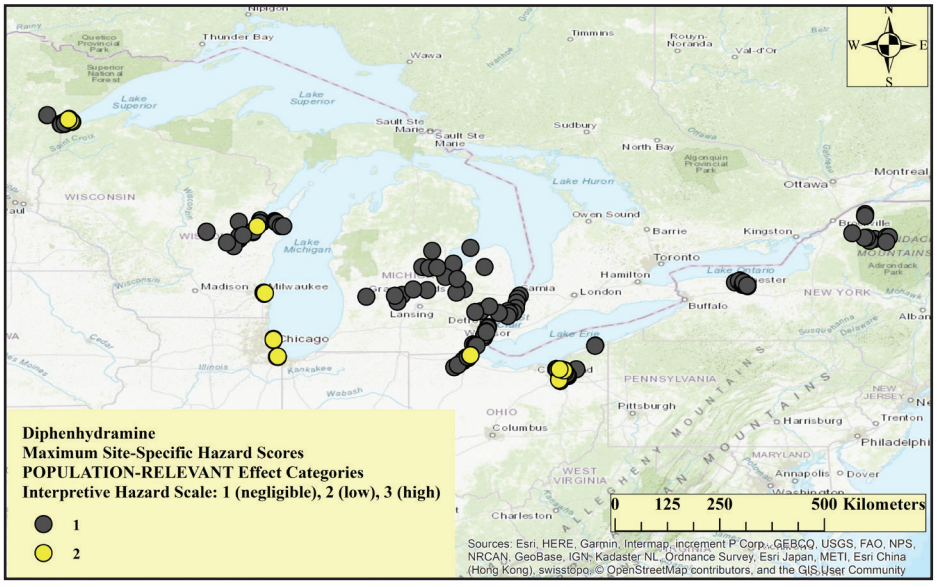
Diphenhydramine Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score for diphenhydramine observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

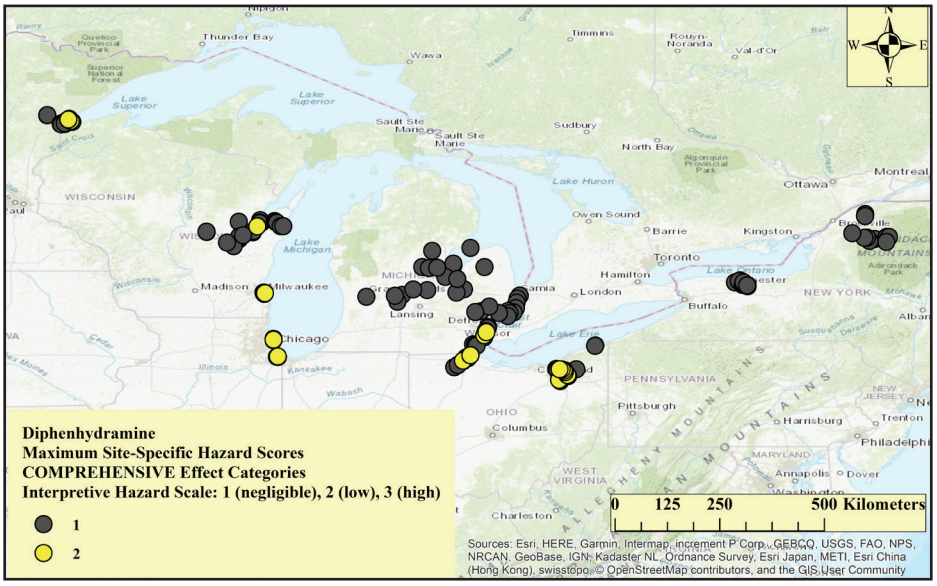
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Diphenhydramine Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental



Diphenhydramine Comprehensive SV pairs: Comprehensive mean SV



6.4.7 Estrone

Evidence generated in this EHA suggests that aqueous estrone is hazardous to fish in U.S. Great Lakes basin waterbodies. There was a total of 755 SV_{LOW} exceedances observed (24% of all hazard scores generated for estrone) involving 74 (38%) of the 195 EHA sampling sites, for an average of 10.2 SV_{LOW} exceedances per site at affected sites and an overall average of 3.9 exceedances/site (Table 6-6)[115] . At least one SV_{LOW} was exceeded at ≥ 21 project locations, for each of six effect categories (Table 6-5)[116] . Limited observations of high hazard support the plausibility of estrone-related hazard to fish. There was a total of 41 exceedances of estrone SV_{HIGH} values (Table 6-6), occurring at four project locations for comprehensive mean SV_{HIGH} exceedances and at nine locations for exceedances of the physiological/metabolic SV_{HIGH} (Table 6-4). The highest detected estrone concentration was almost seven times greater than the physiological/ metabolic SV_{HIGH}.

Some Key Points…

Estrone

- **Overall:** Clear and convincing evidence of hazards to fish
- **Effect Categories with Estrone SVs:** Behavioral, Developmental, Reproductive, Physiological/ Metabolic
- **High Hazard:**
  - 41 occurrences, involving 6% of sampling events, and 9% of sites
  - *Effect Categories:* Physiological/Metabolic
  - *Project Locations:* 9 of 24
- **Low Hazard:**
  - 755 occurrences, involving 25% of sampling events, and 38% of sites
  - *Effect Categories:* 4 of 4
  - *Project Locations*: 23 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

This EHA supports the hypothesis that estrone-related hazard to fish is greater in downstream proximity to point sources than in upstream or distant areas. Quantitatively, Maumee River site-specific median hazard scores were statistically significantly greater ($p < 0.1$) in the CEC-influenced site group than in the uninfluenced site group (Table 6-8). Qualitatively, project-wide numbers of SV_{HIGH} exceedances per sampling event and per sampling site were, respectively, 12 and 17 times greater in the point source CEC-influenced site group than in the uninfluenced group (Table 6-6). Project-wide fractions of events and sites with at least one SV_{HIGH} exceedance were nine and 15 times greater in CEC-influenced sites than in uninfluenced sites. Similarly, fractions of events and sites with SV_{LOW} exceedances were at least twice as high in CEC-influenced sites (Table 6-6).

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	53-16-7
Applied Detection Limit (µg/L):	0.0004
$logK_{ow}$:	3.13
Water Solubility (mg/L):	30 at 25 deg C
Use:	"Estrone is a major mammalian estrogen….Therapeutic estrone is the synthetic form of a naturally occurring estrogen estrone. Estrone diffuses through the cell membrane and binds to and subsequently activates the nuclear estrogen receptor found in the reproductive tract, breast, pituitary, hypothalamus, liver, and bone." (PubChem 2019)
Production/Usage:	No data were located.

Estrone Exposure Data

Number of Samples Analyzed:	524
Percent Detects:	27% (143/524)
Range Detected Concentrations[117]:	0.000062 to 0.014 µg/L

[115] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories

[116] Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances

[117] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

Estrone Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	0.0186	0.000115	limited/broad
Behavioral	0.0202	0.0000318	sparse
Developmental	0.0754	0.0000318	broad
Reproductive	0.0339	0.000102	moderate
Comprehensive Mean SV	0.00665	0.0000144	limited
Physiological/Metabolic	0.00205	0.00000014	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for estrone (from Gefell et al. 2019).

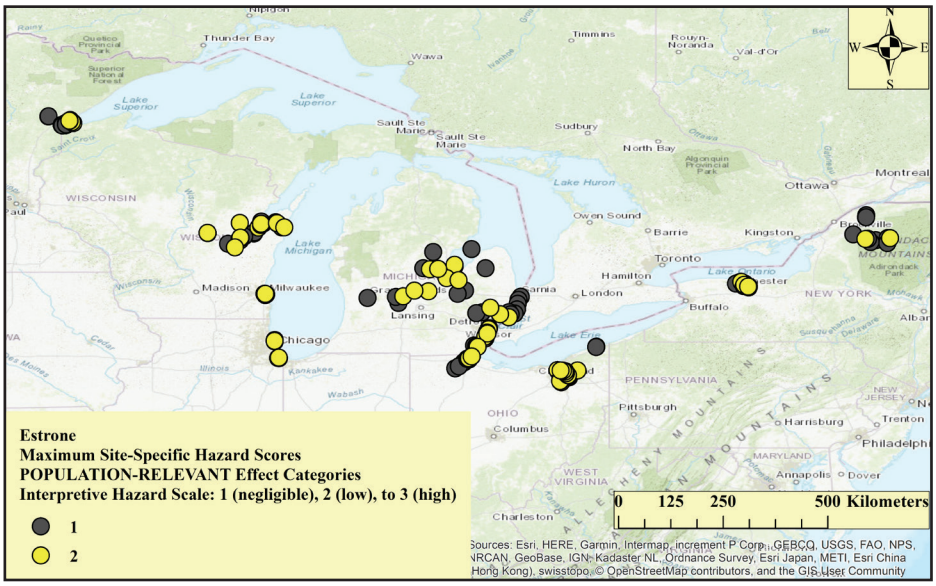
Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	Escape response and response latency, larval behavioral tests	NA
Developmental	Tissue/Organ	GSI, sex reversal in males, intersex, eosinophilia in kidneys and body cavity, HSI	NA
	Organism	survival, hatchability, time to hatch, sex ratio, gross abnormalities, condition factor, growth, egg production and fertility	total wet weight also incorporated into SV_{LOW}
Reproductive	Cellular	gonadal DNA damage	NA
	Tissue/Organ	GSI	NA
	Organism	reproductive hormone levels, spawning frequency, numbers eggs spawned, total eggs produced	NA
Physiological/Metabolic	Organism	basal ventilation	NA

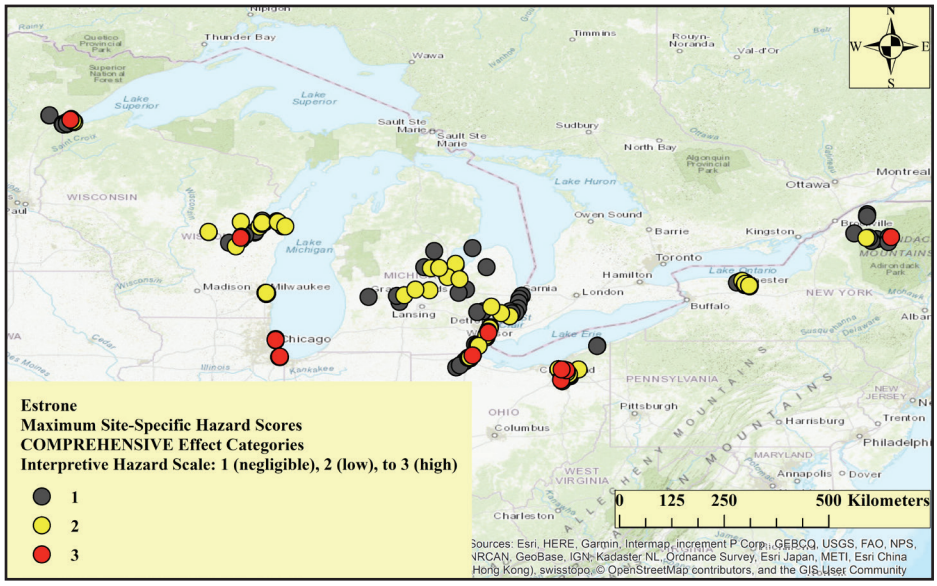
Estrone Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score for estrone observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Estrone Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, and Reproductive




Estrone
Maximum Site-Specific Hazard Scores
COMPREHENSIVE Effect Categories
Interpretive Hazard Scale: 1 (negligible), 2 (low), to 3 (high)
1
2
3
0 125 250 500 Kilometers
Thunder Bay
Wawa
Timmins
Sudbury
North Bay
Ottawa
Montreal
Toronto
Hamilton
London
Kingston
Barrie
Owen Sound
Buffalo
Madison
Milwaukee
Chicago
Lansing
Pittsburgh
Columbus
Harrisburg
Philadelphia
Annapolis
Dover
Trenton
Charleston
WISCONSIN
PENNSYLVANIA
NEW YORK
OHIO
WEST VIRGINIA
MARYLAND
Lake Superior
Lake Michigan
Lake Huron
Lake Ontario
Lake Erie

6.4.8 Hexahydrohexamethylcyclopentabenzopyran (HHCB)

This EHA provides some evidence that HHCB-related hazard to fish is present in waterbodies of the U.S. Great Lakes basin. While there were no SV_{HIGH} exceedances for HHCB, a total of 381 SV_{LOW} exceedances were observed among 214 samples with detected concentrations - 10.4% of all HHCB hazard scores (Table 6-6)[118] . Physiological/metabolic SV_{LOW} and mean comprehensive SV_{LOW} exceedances were observed in 17 and 11 project locations, respectively (Table 6-5), despite limited breadth of relevant fish ecotoxicity information (Table 4-6). For each of five sets of population-relevant SVs, HHCB SV_{LOW} exceedances were limited to four or fewer project locations (Table 6-5). HHCB achieved intermediate CEC-specific hazard ranks, also at four project locations (Table 6-2b).

There is significant evidence that point sources (WWTPs or CSOs) increase HHCB-related hazard to fish. Site-specific median and maximum hazard scores for physiological/metabolic effects were statistically significantly higher in the point source CEC-influenced site group than in uninfluenced sites at three project locations (Table 6-8). Supporting qualitative information included an approximately four-fold greater incidence of SV_{LOW} exceedances (as numbers of exceedances per sampling site or per sampling event) in the point source CEC-influenced site group over that in the uninfluenced site group (Table 6-6). Similarly, prevalence of exceedances - as fractions of sampling sites and sampling events with at least one SV_{LOW} exceedance – was three times greater among CEC-influenced sites than among uninfluenced sites (Table 6-6).

Some Key Points…

HHCB

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with HHCB SVs:** Behavioral, Developmental, Growth, Mortality, Physiological/Metabolic
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 381 occurrences, involving 41% of sampling events, and 45% of sites
  - *Effect Categories:* 5 of 5
  - *Project Locations*: 18 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	1222-05-5
Method Detection Limit (µg/L):	0.11
$logK_{ow}$:	5.9
Water Solubility (mg/L):	1.75 at 25 deg C
Use:	HHCB is a musk fragrance used in air care products, cleaning products, soaps, plastic and rubber products, and personal care products.
Production/Usage:	HHCB has been identified as a High Production Volume chemical by the USEPA Office of Pollution Prevention and Toxics (PubChem 2019). Estimated total usage in the U.S. was 2.8, 3.1, 3.5 and 3.7 million pounds in 2000, 2004, 2008 and 2011, respectively (USEPA 2014c) As of 2001, annual production in the European Union was within the range 1000 to 5000 metric tonnes (2.2 to 11 million pounds) per year (HERA 2004).

HHCB Exposure Data

Number of Samples Analyzed:	525
Percent Detects:	41% (214/525)
Range Detected Concentrations[119]:	0.0043 to 2.14 µg/L

[118] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

[119] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A

HHCB Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	60.2	0.91	limited
Behavioral	36.8	0.59	limited
Developmental	56.6	0.87	limited/broad
Growth	79.4	1.16	sparse
Mortality	79.4	1.16	sparse
Comprehensive Mean SV	21.3	0.065	limited
Physiological/Metabolic	28.4	0.0019	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for HHCB (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	equilibrium, respiration, righting reflex, and swimming behavior	NA
Developmental	Tissue/Organ	NA	egg coagulation, tail extension, heartbeat and heart rate, edema were incorporated into SV_{LOW} only
	Organism	survival, growth, balance and swimming behavior, activity level, hatchability	circulation also incorporated into SV_{LOW}
Growth	Organism	growth parameters	NA
Mortality	Organism	mortality rate	NA
Physiological/Metabolic	Cellular	oxidative stress indicators, antioxidant enzyme activity	NA

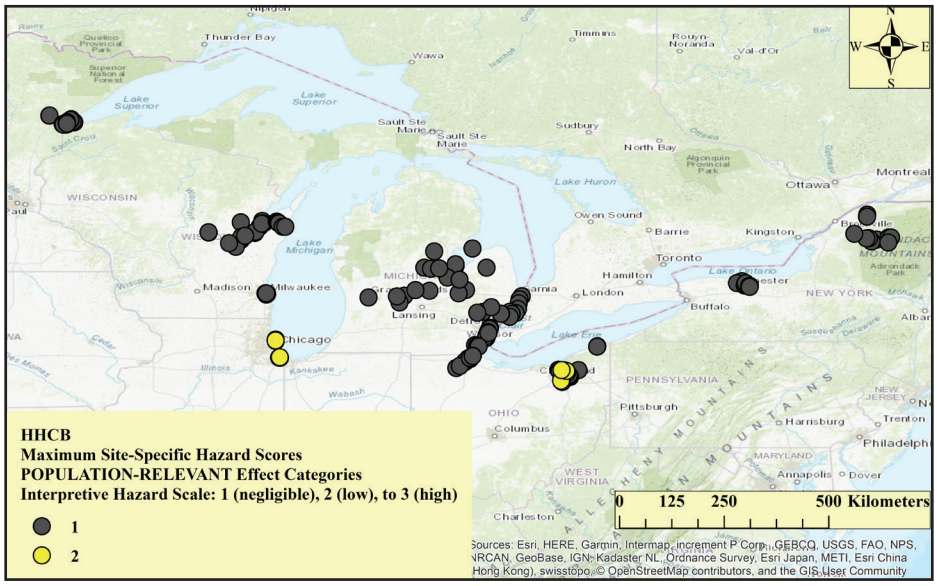
HHCB Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for HHCB observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

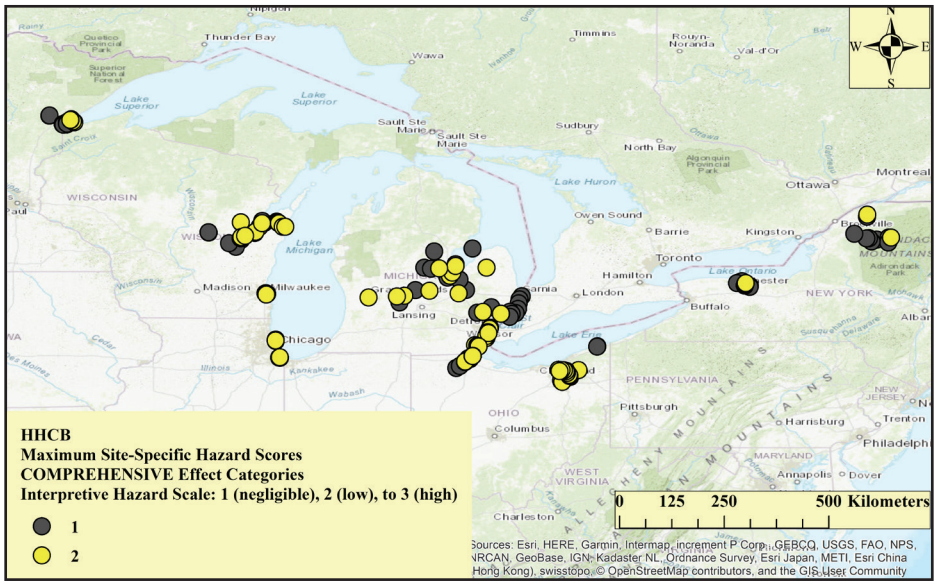
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

HHCB Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, Growth, Mortality



HHCB Comprehensive SV pairs: Comprehensive mean SV, Physiological/Metabolic



6.4.9 Ibuprofen

Aqueous ibuprofen poses a hazard to fish in most of the sampled Great Lakes basin waters in this EHA that were analyzed for ibuprofen[120] . There was a total of 66 exceedances of ibuprofen SV_{HIGH} values (Table 6-6)[121], despite being detected in only 31 samples. High hazard is widely distributed, and is associated with reproductive, developmental and genotoxicity effects that each occurred in six or more out of the 13 project locations where ibuprofen was analyzed (Table 6-4). The highest detected ibuprofen concentration was between two and 1,700 times greater than the range of effect-specific SV_{HIGH} values. In addition, there were 129 SV_{LOW} exceedances in the 31 detected samples, including exceedances at four project locations for endocrine and physiological/metabolic effects, and at seven or more locations for developmental, reproductive, mortality and genotoxicity effects (Table 6-5)[122]. A low overall detection frequency (15.6%), however, contributed to relatively low hazard ranks (based on mean hazard scores) at most project locations compared with other CECs considered in this EHA (Table 6-2b), because all of the non-detects were assigned the lowest hazard score of '1'.

There is substantial evidence that sampling sites with ibuprofen-related elevated hazard spatially correspond with locations of WWTP or CSO point sources. The incidence of high hazard (as numbers of exceedances per sampling site and per sampling event) and the prevalence of high hazard (as the fractions of sites and events with at least one SV_{HIGH} exceedance) are at least four times greater among point source CEC-influenced sites than in uninfluenced sites (Table 6-6). The same pattern was observed in tallies of SV_{LOW} exceedances (Table 6-6). In addition, ibuprofen-related maximum or median hazard scores for each of genotoxicity, developmental, mortality, and reproductive effects were significantly higher in the point source CEC-influenced site group than in the uninfluenced site group in the St. Louis River/Bay project location (Table 6-8).

Some Key Points…

Ibuprofen

- **Overall:** Clear and convincing evidence of hazards to fish
- **Effect Categories with Ibuprofen SVs:** Developmental, Mortality, Reproductive, Circulatory/Blood Constituents, Endocrine, Genotoxicity, Physiological/ Metabolic
- **High Hazard:**
  - 66 occurrences, involving 16% of sampling events, and 18% of sites
  - *Effect Categories:* Genotoxicity, Developmental, and Reproductive
  - *Project Locations:* 7 of 13
- **Low Hazard:**
  - 129 occurrences, involving 16% of sampling events, and 18% of sites
  - *Effect Categories:* 7 of 7
  - *Project Locations*: 7 of 13
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	15687-27-1
Method Detection Limit (µg/L):	0.006
$logK_{ow}$:	3.97
Water Solubility (mg/L):	21 at 25 deg C
Use:	"Ibuprofen is a commonly used nonsteroidal antiinflammatory (NSAID) drug which is available both by prescription and over-the-counter." (PubChem 2019)
Production/Usage:	Ibuprofen total usage in the U.S. during 2010 and 2011 was 4.2 and 3.8 million lbs., respectively (USEPA 2016b). Identified also in 2004 as an OECD High Production Volume Chemical – "…those chemicals which are produced at levels greater than 1,000 tonnes per year in at least one member country/region." (OECD 2004). We did not locate current production or usage data.

Ibuprofen Exposure Data

Number of Samples Analyzed:	199
Percent Detects:	15.6% (31/199)
Range Detected Concentrations[123]:	0.073 to 22.0 µg/L

[120] Ibuprofen concentrations in water are available only for the 12 project locations sampled during 2010-2012, and for Tinkers Creek in 2013, totaling 13 locations.

[121] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

[122] Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances.

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	0.82	0.0042	broad
Developmental	0.27	0.000064	broad
Mortality	57.8	0.00064	moderate
Reproductive	0.285	0.00013	broad
Comprehensive Mean SV	10.5	0.015	broad/robust
Circulatory/Blood Constituents	5,380	14.5	moderate
Endocrine	189	0.51	sparse
Genotoxicity	0.013	0.000057	sparse
Physiological/Metabolic	189	0.51	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for ibuprofen (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Developmental	Cellular	histopathology in gonads, liver, kidneys	NA
	Tissue/Organ	GSI, HSI	NA
	Organism	time to hatch, hatchability, fry survival, total number eggs, fertility, condition index, malformation rates	hatch success and larval body length and weight also incorporated into SV_{LOW}
Mortality	Organism	mortality rate	NA
Reproductive	Cellular	egg diameter	NA
	Tissue/Organ	GSI	NA
	Organism	reproductive hormone levels, time to hatch, hatchability, spawning frequency, number of spawning events, egg production per day, per week, and per spawn, rate of fertilization, total egg production	NA
Circulatory/Blood Constituents	Tissue/Organ	hemoglobin, hematocrit, RBS count, WBC count, mean cellular volume, mean cellular hemoglobin, plasma protein, plasma glucose	NA
Endocrine	Tissue/Organ	plasma cortisol response to seawater exposure	NA
Genotoxicity	Cellular	percent apoptotic cells (plus COMET test, RAPD-PCR test)	NA
Physiological/Metabolic	Tissue/Organ	liver glycogen and liver glucose levels in multiple stressor test	NA

[123] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A. However, in 2010, unfiltered ibuprofen concentrations were reported only for a limited number of samples collected from St. Louis River/Bay, Fox River/Green Bay, and Detroit River – these were used unadjusted. Also, except for Tinkers Creek in 2013, no ibuprofen concentration data were available for project locations sampled during 2013-2014 sampling seasons.

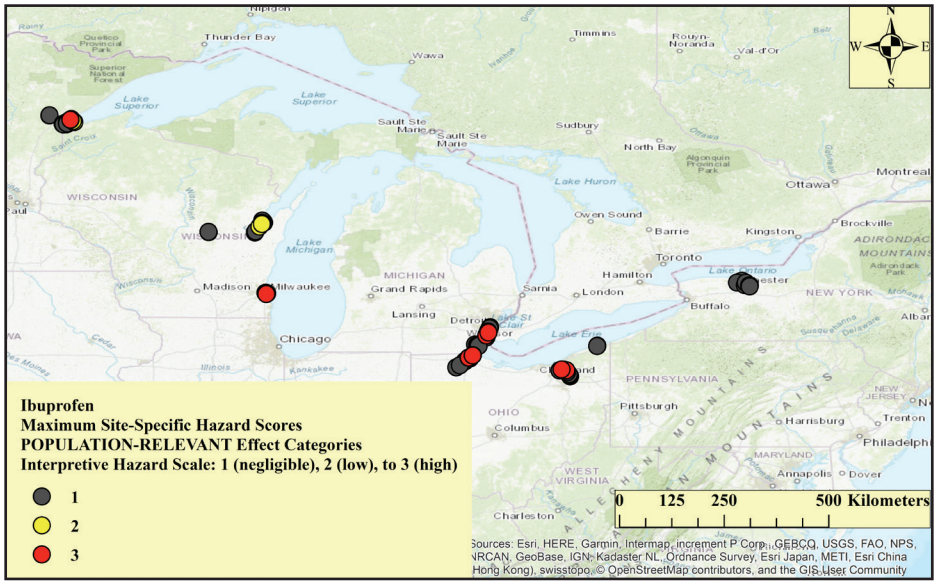
Ibuprofen Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for ibuprofen observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

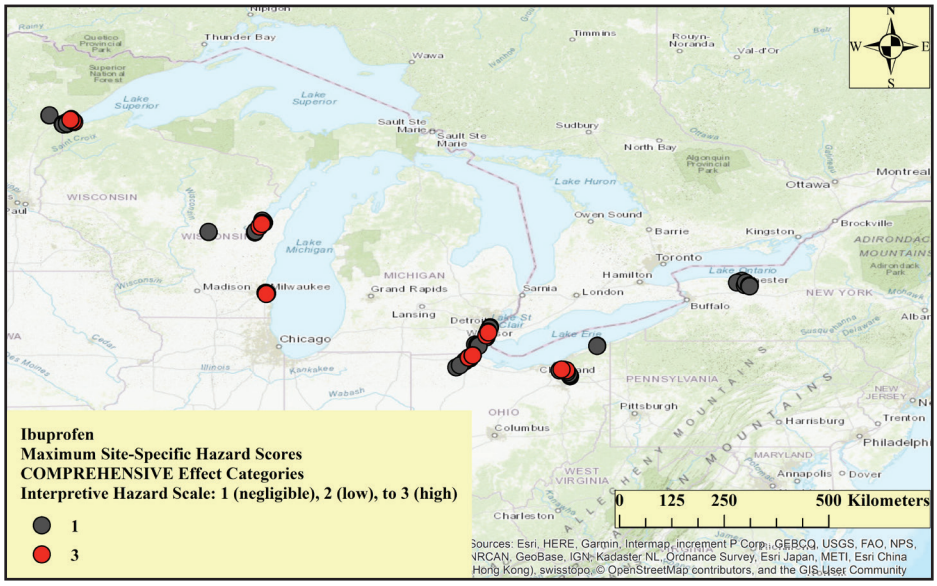
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Ibuprofen Population-relevant SV pairs: Population-relevant mean SV, Developmental, Mortality, and Reproductive



Ibuprofen Comprehensive SV pairs: Comprehensive mean SV, Circulatory/Blood Constituents, Endocrine, Genotoxicity, Physiological/Metabolic



6.4.10 Lidocaine

This hazard assessment has generated no information suggesting that lidocaine is a hazard to fish in the Great Lakes basin. There were no SV_{HIGH} or SV_{LOW} exceedances among the 1,578 lidocaine-related hazard scores generated from 526 water samples collected at 195 sampling sites in this assessment (Table 6-6). However, we caution that, in this case, the absence of identified hazards does not necessarily mean that hazards are absent. These results should be considered provisional, due to the very limited set of lidocaine SVs obtained from sparse ecotoxicity information in the literature and the very limited exposure data in some of the project locations. It is plausible that significant lidocaine-related hazards may be identified if data gaps were to be filled.

Some Key Points…

Lidocaine

- **Overall:** No evidence of hazards to fish
- **Effect Categories with Lidocaine SVs:** Behavioral
- **High Hazard:** No occurrences
- **Low Hazard:** No occurrences
- **Point Source Analysis:** No evaluation; no hazard occurrences

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	137-58-6
Method Detection Limit (µg/L):	0.00305
$logK_{ow}$:	2.26
Water Solubility (mg/L):	410 at 30 deg C
Use:	"Lidocaine is a local anesthetic and cardiac depressant used as an antiarrhythmia agent." (PubChem 2019)
Production/Usage:	No data were located.

Lidocaine Exposure Data

Number of Samples Analyzed:	526
Percent Detects:	41% (218/526)
Range Detected Concentrations[124]:	0.00031 to 2.06 µg/L

Lidocaine Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	949	6	sparse
Behavioral	4,750	30	sparse
Comprehensive Mean SV	890	2.4	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for lidocaine (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	Larval activity levels, light-dark behavior test	NA

[124] These were measured aqueous concentrations (µg/L) for 2013 and 2014, but calculated aqueous concentrations for 2010-2012, estimated from total concentrations as described in Section 3.5 and Attachment A.

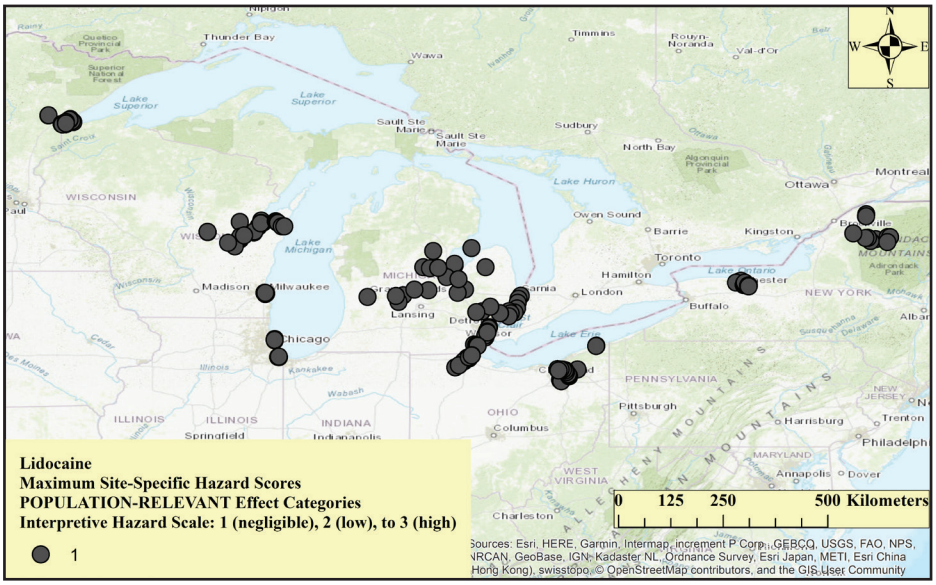
Lidocaine Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for lidocaine observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

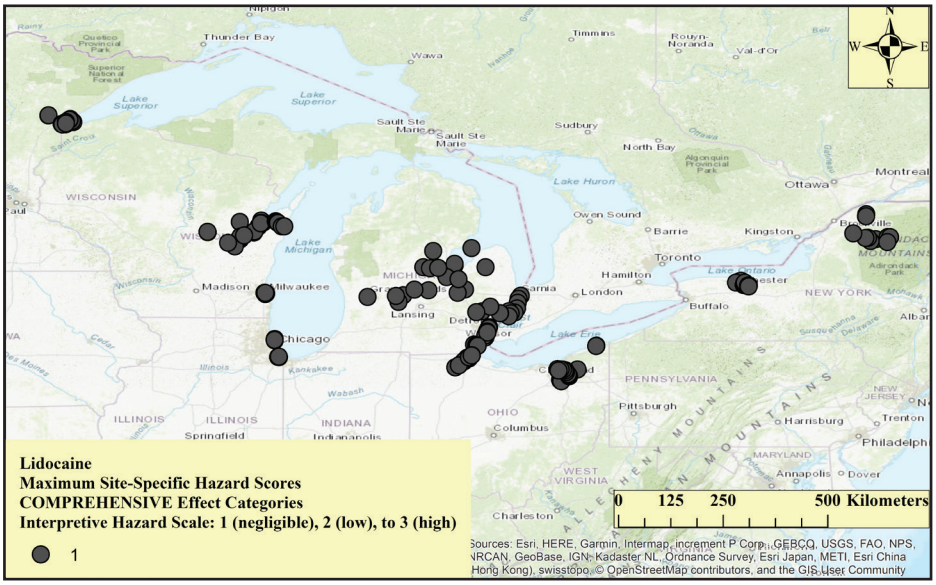
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Lidocaine Population-relevant SV pairs: Population-relevant mean SV, Behavioral



Lidocaine Comprehensive SV pair: Comprehensive mean SV



6.4.11 β-Sitosterol

Evidence provided by this EHA strongly supports the possibility that β-sitosterol poses a widespread hazard to fish in the U.S Great Lakes Basin. While no high hazard (SV_{HIGH} exceedance) was confirmed at any project location (Table 6-4), there was a total of 479 SV_{LOW} exceedances (Table 6-6)[125]. There were circulatory/blood constituent and behavioral SV_{LOW} exceedances at 21 and 23 project locations, respectively (Table 6-5). All 276 detected β-sitosterol concentrations (53% of samples) exceeded the behavioral SV_{LOW}, and 68% of sampling sites had at least one SV_{LOW} exceedance (Table 6-6). There are many data gaps in β-sitosterol SVs (Table 4-1) and limitations in the exposure dataset (such as few sampling sites or very low sample numbers per site) in several project locations, so it is plausible that additional information would indicate further β-sitosterol-related hazards.

Given information limitations, there is no indication that hazard to fish from β-sitosterol exposures is exacerbated by CEC point sources such as WWTPs or CSOs. β-sitosterol is a naturally occurring plant sterol found in fruits, nuts, seeds, and vegetables, and is also a precursor to boldenone, a growth hormone in cattle. There was little difference in the incidence of exceedances (as number per sampling event or sampling site) between CEC-influenced and uninfluenced sites, or in the prevalence of SV_{LOW} exceedances between the site groups (as fractions of sampling events or sampling sites with at least one exceedance) (Table 6-6). There is also no statistically significant ($p < 0.1$) elevation of hazard in sites influenced by CEC point sources (WWTPs or CSOs) compared to sites uninfluenced by CEC point sources (Table 6-8).

Some Key Points…

β-Sitosterol

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with β-Sitosterol SVs:** Behavioral, Developmental, Circulatory/Blood Constituents
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 479 occurrences, involving 53% of sampling events, and 68% of sites
  - *Effect Categories:* 2 of 2
  - *Project Locations*: 23 of 24
- **Point Source Analysis:** No evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	83-46-5
Method Detection Limit (µg/L):	0.11
$logK_{ow}$:	9.65
Water Solubility (mg/L):	"insoluble" in pure water
Use:	"Beta-sitosterol is a main dietary phytosterol found in plants…. Phytosterols are plant sterols found in foods such as oils, nuts and vegetables." (PubChem 2019)
Production/Usage:	No data were located.

β-Sitosterol Exposure Data

Number of Samples Analyzed:	525
Percent Detects:	53% (276/525)
Range Detected Concentrations[126]:	0.0042 to 0.45 µg/L

[125] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories

[126] These are aqueous concentrations (μg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

β-Sitosterol *Surface Water Screening Value Pairs*

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	101	0.17	limited/robust
Behavioral	20.2	0.0032	limited
Developmental	2020	9.5	broad/robust
Comprehensive Mean SV	18.4	0.06	sparse/broad
Circulatory/Blood Constituents	14.2	0.032	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for β-sitosterol (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	Male nest building success, nest size, spontaneous swimming, aggressiveness, female reproductive behavior	NA
Developmental	Organism	time to hatch, egg mortality, hatchability, embryo survival, prevalence of embryo abnormalities, larval/juvenile survival and sex ratio	percent hatch and larval growth also incorporated into SV_{LOW}
Circulatory/Blood Constituents	Tissue/Organ	plasma total cholesterol	NA

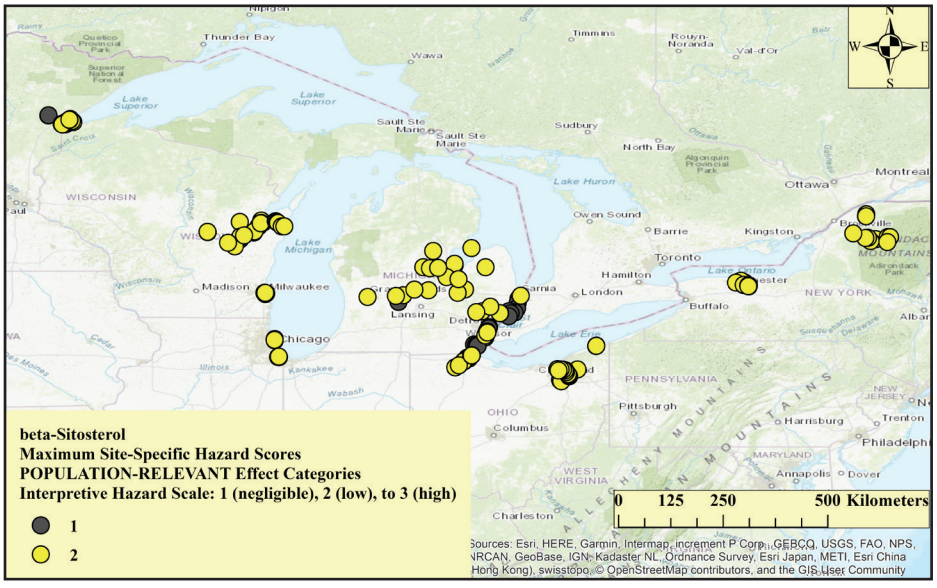
β-Sitosterol *Hazard Distribution*

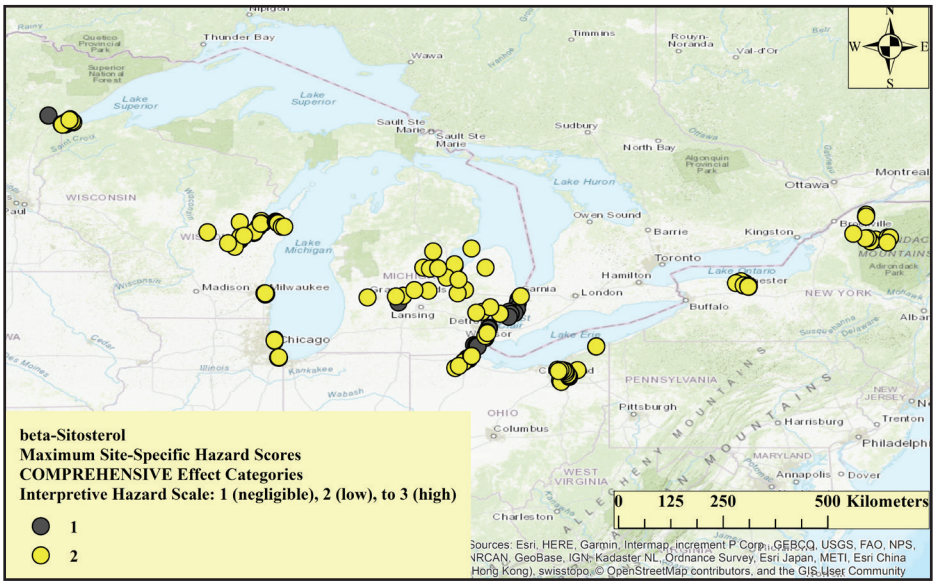
Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for β-sitosterol observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

β-Sitosterol Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental




beta-Sitosterol
Maximum Site-Specific Hazard Scores
COMPREHENSIVE Effect Categories
Interpretive Hazard Scale: 1 (negligible), 2 (low), to 3 (high)
1
2
0 125 250 500 Kilometers
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community
Thunder Bay
Wawa
Timmins
Sudbury
North Bay
Ottawa
Montreal
Toronto
Hamilton
London
Kingston
Barrie
Owen Sound
Buffalo
Chicago
Madison
Milwaukee
Lansing
Pittsburgh
Columbus
Harrisburg
Philadelphia
Trenton
Annapolis
Dover
Charleston
WISCONSIN
PENNSYLVANIA
NEW YORK
OHIO
WEST VIRGINIA
MARYLAND
Lake Superior
Lake Michigan
Lake Huron
Lake Erie
Lake Ontario

6.4.12 Triclosan

There is some evidence generated in this EHA that indicates hazard to fish from aqueous triclosan exposures. There were no observations of high hazard (SV_{HIGH} exceedance), and triclosan SV_{LOW} exceedances occurred in only 12% of sampling events (61/525) and 14% of sampling sites (28/195) (Table 6-6)[127] . A total of 366 SV_{LOW} exceedances comprised only 11.6% of all triclosan-related hazard scores generated in this EHA (Table 6-6). Percent of water samples with detected triclosan concentrations was also 11.6%. This coincidence is because all triclosan detects fall within the concentration ranges bounded by SV_{LOW} and SV_{HIGH} values for each effect category, and are all assigned the hazard score of '2'; the remaining samples were non-detects, so were all assigned the hazard score of '1'. Thus, the two maps of site-specific maximum hazard for population-relevant and comprehensive effect categories are the same (below) and the prevalence of project locations with at least one SV_{LOW} exceedance is 11 for all effect categories with triclosan SVs (Table 6-5).

Available information provides mixed evidence for a triclosan-related hazard with CEC point sources (WWTPS or CSOs). There are no statistically significant ($p < 0.1$) point-source related increases in triclosan hazard in CEC-influenced sites over uninfluenced sites (Table 6-8), but inspection of exceedance tallies provides qualitative evidence that downstream proximity to CEC point sources increases hazard to fish. The incidence of triclosan SV_{LOW} exceedances - as numbers of exceedances per sampling event and per sampling site - was approximately four times greater in CEC-influenced sites than in uninfluenced sites, and the prevalence (as fractions of sampling events and sites with at least one SV_{LOW} exceedance) was also increased in CEC-influenced sites (Table 6-6).

Some Key Points…

Triclosan

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with Triclosan SVs:** Behavioral, Developmental, Mortality, Reproductive
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 366 occurrences, involving 12% of sampling events, and 14% of sites
  - *Effect Categories:* 4 of 4
  - *Project Locations*: 11 of 24
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources

While this assessment considered the potential for biological impacts in fish from exposure to residual parent compound in water, triclosan may transform under aqueous environmental conditions and in traditional wastewater treatment systems to form other toxic compounds - for example, formation of dioxin from triclosan during wastewater chlorination (ECCC 2016).

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	3380-34-5
Method Detection Limit (µg/L):	0.09
$logK_{ow}$:	4.76
Water Solubility (mg/L):	10 at 20 deg C
Use:	"Triclosan is a polychlorophenoxy phenol with antibacterial and antifungal activity. Triclosan is added to toothpastes to prevent gingivitis and has been added to many household products for its topical antibiotic activity." (PubChem 2019)
Production/Usage:	Produced at 1-10 million pounds/year in 1998 (PubChem 2019). Identified in 2004 as an OECD High Production Volume Chemical – "…those chemicals which are produced at levels greater than 1,000 tonnes per year in at least one member country/region." (OECD 2004). We did not locate current production or usage data.
Transformation:	Environmental transformation products include: methyl-triclosan, 2,4,-dichlorophenol, and polychlorinated dibenzo-dioxins (PCDD)s (ECCC 2016).

Triclosan Exposure Data

Number of Samples Analyzed:	525
Percent Detects:	11.6% (61/525)
Range Detected Concentrations[128]:	0.02 to 0.35 µg/L

[127] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories

[128] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	40.8	0.0029	broad/robust
Behavioral	22.8	0.0029	robust
Developmental	73.8	0.0029	moderate/robust
Mortality	40.4	0.0029	sparse/limited
Reproductive	40.8	0.0029	sparse/moderate
Comprehensive Mean SV	12.8	0.0025	limited/broad

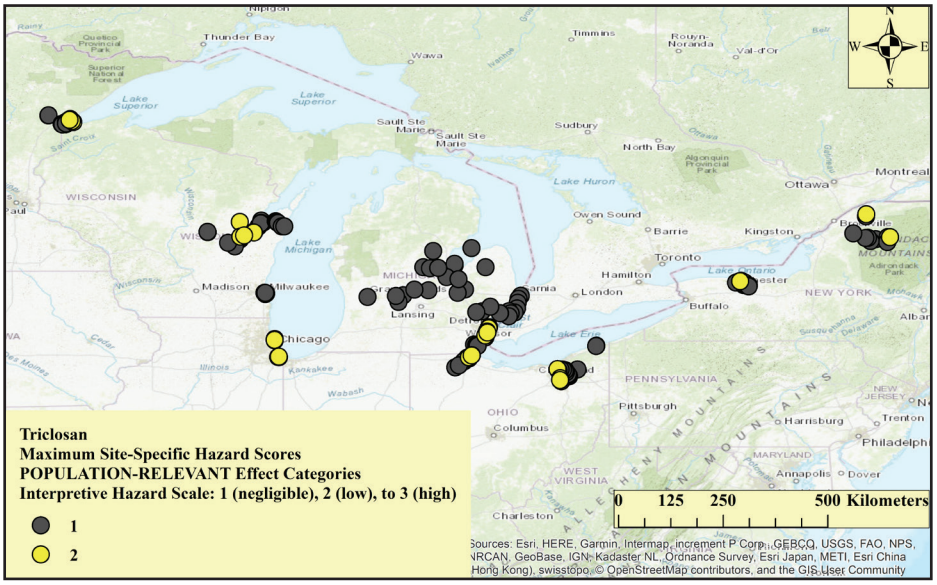
We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for triclosan (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	Swimming speed, spontaneous swimming activity, feeding activity, swim-up, equilibrium, lock-jaw, erratic swimming, quiescence, and opercular movement.	Nest defense, aggression index, and larval escape performance metrics also incorporated into SV_{LOW}
Developmental	Tissue/Organ	NA	otolith formation and pigmentation also incorporated into SV_{LOW}
	Organism	survival, spinal curvature, larval length and weight, percent hatch, time to hatch	hatchability and secondary sex characteristics also incorporated into SV_{LOW}
Mortality	Organism	mortality rate	NA
Reproductive	Cellular	sperm count	gonad histopathology also incorporated into SV_{LOW}
	Tissue/Organ	GSI	NA
	Organism	NA	secondary sex characteristics, fecundity, fertility, hatchability also incorporated into SV_{LOW}

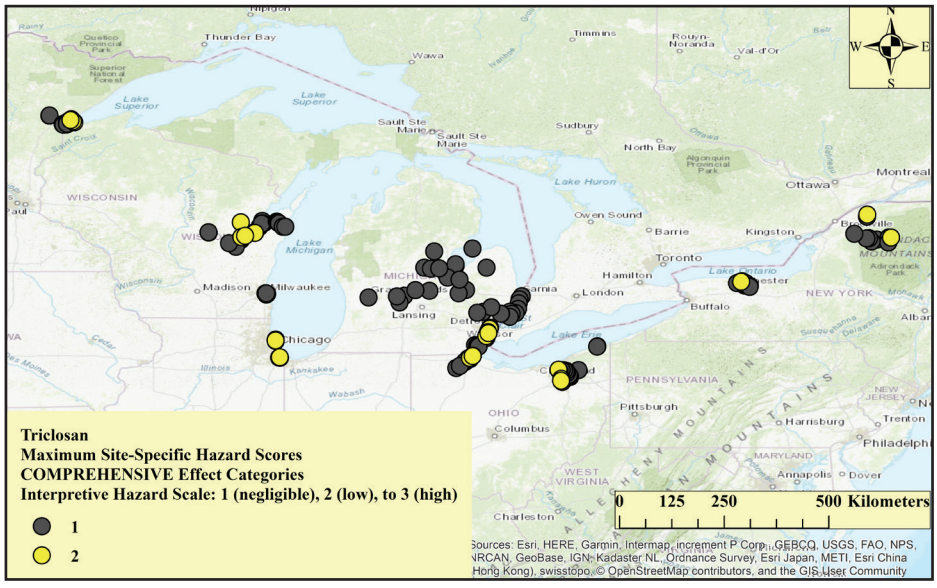
Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for triclosan observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC. Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Triclosan Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, Mortality, and Reproductive



Triclosan Comprehensive SV pairs: Comprehensive mean SV



6.4.13 Tris(2-butoxyethyl)phosphate (TBEP)

This assessment provides some evidence that TBEP is hazardous to fish. While the detection rate was relatively high for TBEP compared to the other CECs, there were no SV_{HIGH} exceedances and only 7% (185/2,625) of TBEP-related hazard scores generated in this EHA indicated exceedance of a SV_{LOW} (Table 6-6)[129]. However, the ecotoxicity information that was used to derive TBEP SVs was 'sparse' to 'limited' for each effect category (Tables 4-5 and 4-6), and several project locations had very limited exposure datasets (Section 5.4). If data gaps were to be filled and TBEP SVs updated or new SVs derived for other effect categories, additional and/or higher hazards may be observed.

Some Key Points…

TBEP

- **Overall:** Some evidence of hazards to fish
- **Effect Categories with TBEP SVs:** Behavioral, Developmental, Mortality
- **High Hazard:** No occurrences
- **Low Hazard:**
  - 185 occurrences, involving 20% of sampling events, and 22% of sites
  - *Effect Categories:* 3 of 3
  - *Project Locations*: 11 of 24
- **Point Source Analysis:** Limited evidence of elevated hazard downstream of point sources

There is mixed evidence supporting the hypothesis that CEC point sources increase hazard to fish in downstream proximity to the source. There was only one statistically significant ($p < 0.1$) difference between site-specific maxima at point source CEC-influenced sites and uninfluenced sites – observed at the Saginaw River location for comprehensive mean SV (Table 6-8). However, project-wide, the incidence of SV_{LOW} exceedances (as exceedances per sampling event and per site) was four or more times higher in the CEC-influenced site group than in the uninfluenced group (Table 6-6). The prevalence of exceedances (as fractions of events and sites with an exceedance) was approximately three times higher in the CEC-influenced site group (Table 6-6).

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	78-51-3
Method Detection Limit (µg/L):	0.05
$logK_{ow}$:	3.75
Water Solubility (mg/L):	1,100 at 25 deg C
Use:	"Phosphate ester flame retardants are human-made chemicals added to consumer and industrial products for the purpose of reducing flammability." (PubChem 2019)
Production/Usage:	Identified in 2004 as an OECD High Production Volume Chemical – "…those chemicals which are produced at levels greater than 1,000 tonnes per year in at least one member country/region." (OECD 2004). USEPA has listed TBEP as a high production volume chemical since 1990, with annual production reported at >1 million pounds/year in 1990, 1994, and 2006 (PubChem 2019) and national aggregate production volumes reported to be between 1 and 10 million pounds/year in 2012 through 2015 (USEPA 2019).

TBEP Exposure Data

Number of Samples Analyzed:	525
Percent Detects:	48% (251/525)
Range Detected Concentrations[130]:	0.045 to 38.7 µg/L

[129] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories.

[130] These are aqueous concentrations (µg/L) that were estimated from total concentrations as described in Section 3.5 and Attachment A.

TBEP Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	511	1.7	sparse/limited
Behavioral	1750	16.5	sparse
Developmental	1620	0.95	limited
Mortality	377	2.4	sparse
Comprehensive Mean SV	267	0.45	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for TBEP (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	equilibrium	NA
Developmental	Organism	embryo heart rate, body weight, survival, and hatch rate	NA
Mortality	Organism	mortality rate	NA

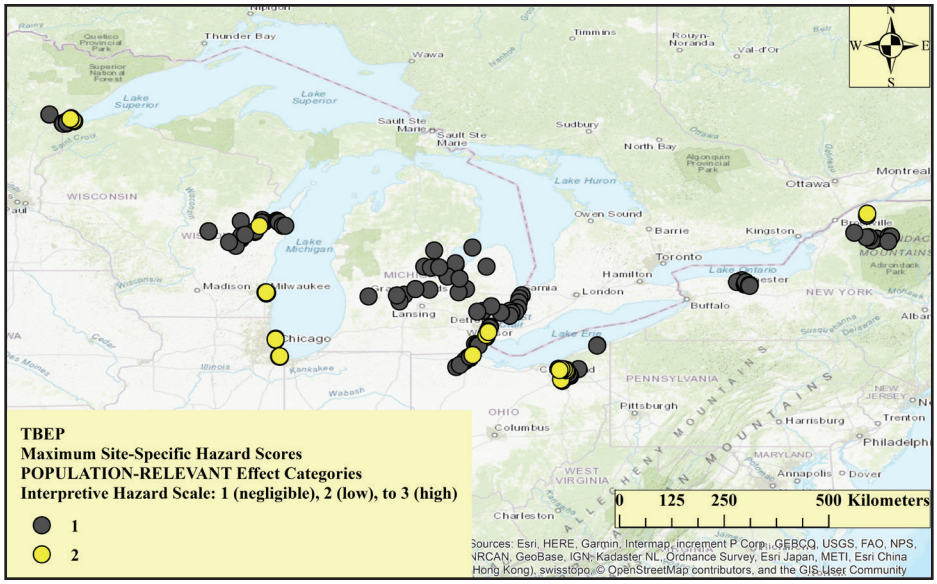
TBEP Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for TBEP observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

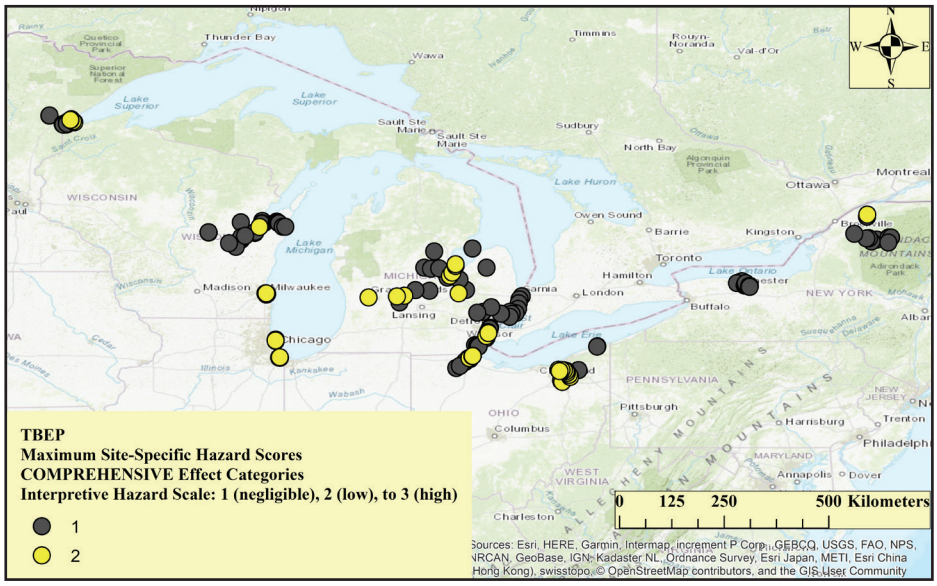
Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

TBEP Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Developmental, Mortality



TBEP Comprehensive SV pairs: Comprehensive mean SV



6.4.14 Venlafaxine

Evidence provided in this EHA strongly indicates that aqueous venlafaxine is hazardous to fish in a number of Great Lakes Basin waterbodies. High hazard (exceedance of SV_{HIGH}) was observed in 4% (149/3,682) of all venlafaxine-related hazard scores generated in this EHA, occurring at 15% of all sampling sites (Table 6-6)[131]. SV_{HIGH} exceedances were distributed among three to eight project locations for mortality, endocrine and/or histopathology effect categories (Table 6-4). At least one SV_{HIGH} exceedance was observed in 17% of water samples analyzed (Table 6-5). The highest detected venlafaxine concentration was 12 times greater than the endocrine SV_{HIGH} and five times greater than the mortality SV_{HIGH}. There was a total of 1,065 venlafaxine SV_{LOW} exceedances – 28.9% (1,065/3,682) of all venlafaxine hazard scores, occurring in 33% of water samples collected in this EHA and involving 32% of sampling sites for an average of 5.5 SV_{LOW} exceedances per site (Table 6-6). SV_{LOW} exceedances were distributed among 13 to 15 out of 24 project locations for endocrine, histopathology, behavioral, mortality and reproductive effect categories (Table 6-5)[132].

At the Fox River/Green Bay project location, maximum site-specific hazard scores were statistically significantly higher ($p < 0.1$) in the CEC-influenced site group than in the uninfluenced site group for hazard scores generated using the following venlafaxine SV pairs: behavioral, mortality, reproductive, endocrine, histopathology, comprehensive mean SVs, and population-relevant mean SVs (Table 6-8). Additionally, at Tinkers Creek, median site-specific hazard scores were significantly higher for endocrine effects and maximum hazard scores were higher for mortality in the CEC-influenced site group compared to uninfluenced sites (Table 6-8). Qualitatively, the numbers of SV_{HIGH} exceedances per sampling event, and per sampling site, were respectively 8 and 11 times higher in CEC-influenced sites compared to uninfluenced sites, and the prevalence of SV_{HIGH} exceedances (as fractions of events and sites with at least one exceedance) was at least three times higher in CEC-influenced sites (Table 6-6). Similarly, the incidence of SV_{LOW} exceedances (as number per sampling event or site) and prevalence (as fractions of events and sites) of SV_{LOW} exceedances were more than twice as high among CEC-influenced sites than among uninfluenced sites (Table 6-6).

Some Key Points…

Venlafaxine

- **Overall:** Clear and convincing evidence of hazards to fish
- **Effect Categories with Venlafaxine SVs:** Behavioral, Mortality, Reproductive, Endocrine, Histopathology
- **High Hazard:**
  - 149 occurrences, involving 17% of sampling events, and 15% of sites
  - *Effect Categories*: Endocrine, Histopathology, and Mortality
  - *Project Locations*: 7 of 24
- **Low Hazard:**
  - 1,065 occurrences, involving 33% of sampling events, and 32% of sites
  - *Effect Categories:* 5 of 5
  - *Project Locations*: 15 of 24
- **Point Source Analysis:** Significant evidence of elevated hazard downstream of point sources

Chemical Information

The following chemical information is adapted from summaries provided in Gefell et al. (2019), except as otherwise indicated.

CAS Number:	93413-69-5
Method Detection Limit (µg/L):	0.0009
$logK_{ow}$:	3.2
Water Solubility (mg/L):	267 (as the hydrochloride salt)
Use:	"Venlafaxine is a serotonin and norepinephrine reuptake inhibitor widely used as an antidepressant." (PubChem 2019)
Production/Usage:	No data were located.

Venlafaxine Exposure Data

Number of Samples Analyzed:	526
Percent Detects:	33% (176/526)
Range Detected Concentrations[133]:	0.001 to 0.319 µg/L

[131] Incidence tallies in Table 6-6 include exceedances of mean comprehensive SVs and mean population-relevant SVs, not just exceedances in individual effect categories

[132] Tallies of project locations with SV_{LOW} exceedances in Table 6-5 are inclusive of SV_{HIGH} exceedances

[133] These were measured aqueous concentrations (µg/L) for 2013 and 2014, but calculated aqueous concentrations for 2010-2012, estimated from total concentrations as described in Section 3.5 and Attachment A.

Venlafaxine Surface Water Screening Value Pairs

Effect Category	SV_{HIGH} (µg/L)	SV_{LOW} (µg/L)	Breadth of Ecotoxicity Information (Section 4.3)
Population-relevant Mean SV	0.29	0.0026	sparse/broad
Behavioral	0.74	0.0013	sparse
Mortality	0.062	0.00065	sparse
Reproductive	4.04	0.00065	limited/broad
Comprehensive Mean SV	0.16	0.00064	limited
Endocrine	0.025	0.000068	sparse
Histopathology	0.19	0.00051	sparse

We provide the following chart as an additional interpretive tool that lists the specific biological effect endpoint evaluations reported in the ecotoxicological literature and incorporated into derivations of each effect-specific SV pair for venlafaxine (from Gefell et al. 2019).

Effect Category	Level of Ecological Organization	Effect Endpoints Incorporated into Both SV_{HIGH} and SV_{LOW} Derivations	Additional Effect Endpoints
Behavioral	Organism	Larval total escape response, startle response	Response latency also incorporated into SV_{LOW}
Mortality	Organism	mortality rate	NA
Reproductive	Cellular	testis apoptosis, gonad histopathology	NA
	Tissue/Organ	testis morphology, spermatogenesis	GSI also incorporated into SV_{LOW}
	Organism	embryo production, reproductive hormone levels	male secondary sex characteristic scores also incorporated into SV_{LOW}
Endocrine	Tissue/Organ	plasma cortisol in multiple stress test	NA
Histopathology	Cellular	histopathologic endpoints in kidney, liver and brain	NA

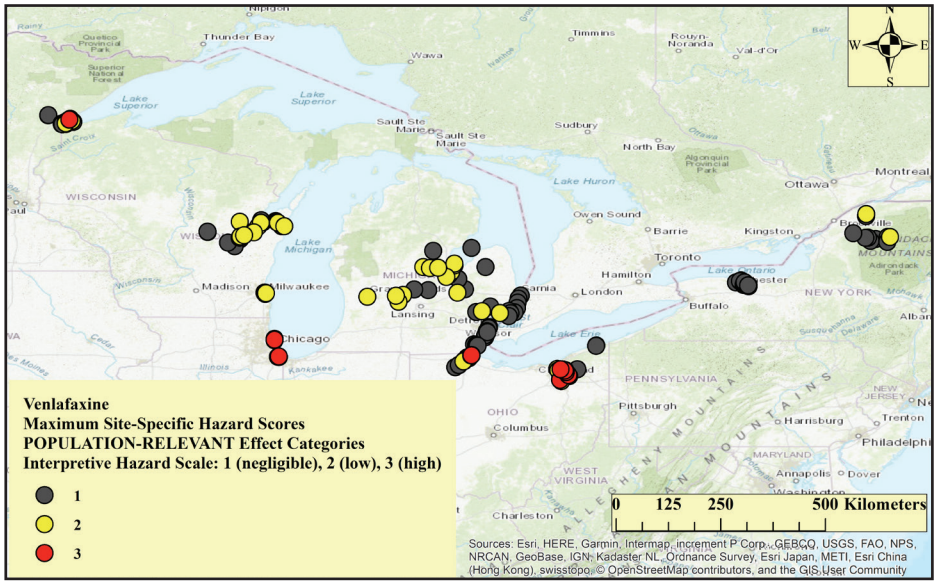
Venlafaxine Hazard Distribution

Two hazard maps are provided below. One illustrates the site-specific maximum hazard score (see Section 5.2) for venlafaxine observed among population-relevant effect categories at the 195 EHA sampling sites across the U.S. Great Lakes Basin; the other illustrates the site-specific maximum hazard score observed among comprehensive effect categories assessed for this CEC.

Meanings of the possible values of maximum hazard score are:

- 1 - negligible hazard (SV_{LOW} not exceeded, including all non-detects);
- 2 - low hazard (SV_{LOW} exceeded, and SV_{HIGH} not exceeded), and
- 3 - high hazard (SV_{HIGH} exceeded).

Venlafaxine Population-relevant SV pairs: Population-relevant mean SV, Behavioral, Mortality, and Reproductive



Venlafaxine Comprehensive SV pairs: Comprehensive mean SV, Endocrine, Histopathology

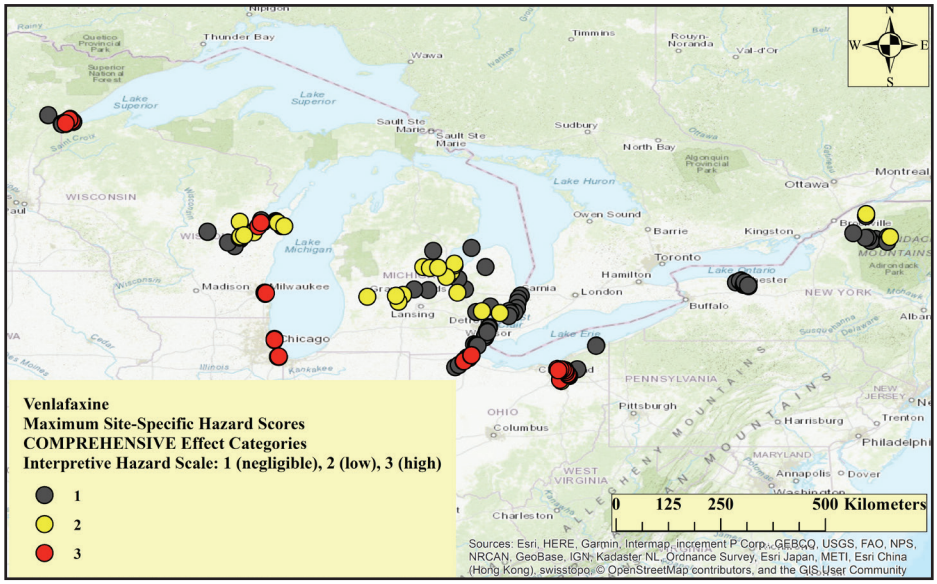


Table 6-1. Detection frequencies (DF = # detects / # samples analyzed) and number of samples analyzed (N) of each CEC, by project location, where detects are identified by a data flag in the analytical chemistry results provided by the USGS and detection is related to method detection limits or reporting limits. Yellow-shaded DF indicates at least one SV_{LOW} was exceeded by at least one detected concentration; red-shaded DF indicates at least one SV_{HIGH} was exceeded by at least one detected concentration.

CEC		St. Louis River/ Bay	Waupaca Chain O'Lakes	LLBDM	Fox River/ Green Bay	Kewaunee River	Milwaukee River	North Shore Channel	Little Calumet River	Grand River/ Maple River	Saginaw River	St Clair River	Clinton River	Detroit River	River Raisin	Swan Creek	Maumee River	Cuyahoga River	Tinkers Creek	Ashtabula River	Long Pond	Genesee River	Irondequoit Bay	Oswegatchie River	Raquette River	TOTALS
4-Androstene-3,17-dione	DF	0.02	0.00	0.29	0.03	0.00	0.00	0.75	0.50	0.06	0.07	0.36	0.00	0.18	0.00	0.00	0.05	0.11	0.24	0.00	0.17	0.00	0.17	0.00	0.04	0.10
	N	98	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	524
Bisphenol A	DF	0.11	0.00	0.21	0.00	0.37	0.00	0.33	0.83	0.29	0.07	0.00	0.00	0.00	0.00	0.00	0.05	0.25	0.33	0.00	0.00	0.00	0.00	0.00	0.13	0.14
	N	98	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	524
Carbamazepine	DF	0.05	0.00	0.68	0.33	0.37	0.25	0.92	1.00	0.71	0.61	0.00	0.50	0.12	0.00	0.00	0.78	0.82	0.91	0.00	0.00	0.00	0.00	0.00	0.13	0.37
	N	98	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	524
Citalopram	DF	0.14	0.00	0.04	0.06	0.00	0.25	0.25	0.25	0.47	0.14	0.00	0.00	0.00	0.00	0.00	0.44	0.39	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.17
	N	98	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	524
DEET	DF	0.90	1.00	0.54	0.78	0.26	1.00	1.00	1.00	0.94	0.71	0.27	1.00	0.59	1.00	1.00	0.78	0.89	0.70	1.00	1.00	0.33	1.00	0.45	0.61	0.75
	N	99	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	44	3	6	9	6	31	46	536
Diphenhydramine	DF	0.18	0.00	0.04	0.03	0.00	0.25	0.67	0.75	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.44	0.36	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.17
	N	99	2	28	37	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	526
Estrone	DF	0.26	0.50	0.54	0.17	0.21	0.38	0.83	0.58	0.18	0.43	0.09	0.40	0.41	0.25	0.42	0.10	0.39	0.39	0.00	0.17	0.33	0.17	0.03	0.04	0.27
	N	98	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	524
HHCB	DF	0.37	0.00	0.32	0.33	0.26	0.25	1.00	1.00	0.35	0.29	0.00	0.50	0.65	1.00	0.16	0.39	0.86	0.68	0.00	0.00	0.44	0.00	0.00	0.26	0.40
	N	99	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	44	3	6	9	6	31	46	536
Ibuprofen[134]	DF	0.20	0.00	NA	0.10	NA	0.13	NA	NA	NA	NA	NA	NA	0.24	0.00	0.43	0.10	NA	0.27	0.00	0.00	0.00	0.00	NA	NA	0.16
	N	71	2	0	20	0	8	0	0	0	0	0	0	17	4	7	41	0	11	3	6	3	6	0	0	199
Lidocaine	DF	0.17	0.00	0.46	0.30	0.37	0.50	0.92	1.00	0.59	0.46	0.00	0.40	0.29	0.25	0.16	0.83	0.79	0.91	0.00	0.00	0.44	0.00	0.16	0.26	0.41
	N	99	2	28	37	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	526

[134] N/A = detection frequency is not applicable; no analytical data for ibuprofen were available for 11 project locations.

Table 6-1 (continued)

CEC		St. Louis River/ Bay	Waupaca Chain O'Lakes	LLBDM	Fox River/ Green Bay	Kewaunee River	Milwaukee River	North Shore Channel	Little Calumet River	Grand River/ Maple River	Saginaw River	St Clair River	Clinton River	Detroit River	River Raisin	Swan Creek	Maumee River	Cuyahoga River	Tinkers Creek	Ashtabula River	Long Pond	Genesee River	Irondequoit Bay	Oswegatchie River	Raquette River	TOTALS
Sitosterol, beta-	DF	0.33	1.00	0.68	0.83	0.58	1.00	0.75	0.75	0.59	0.64	0.09	0.60	0.12	0.00	0.84	0.39	0.68	0.67	0.67	1.00	0.33	0.83	0.45	0.57	0.53
	N	99	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	525
TBEP	DF	0.41	0.00	0.46	0.50	0.00	0.88	1.00	0.92	0.65	0.79	0.09	0.70	0.71	1.00	0.21	0.37	0.82	0.91	0.00	0.00	0.11	0.00	0.00	0.26	0.46
	N	99	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	525
Triclosan	DF	0.17	0.00	0.11	0.06	0.47	0.00	0.75	0.58	0.00	0.00	0.00	0.00	0.35	0.00	0.00	0.15	0.25	0.00	0.00	0.17	0.00	0.00	0.00	0.07	0.12
	N	99	2	28	36	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	525
Venlafaxine	DF	0.31	0.00	0.32	0.16	0.21	0.25	0.92	0.92	0.53	0.57	0.00	0.30	0.00	0.00	0.00	0.46	0.79	0.91	0.00	0.00	0.00	0.00	0.00	0.07	0.33
	N	99	2	28	37	19	8	12	12	17	28	11	10	17	4	19	41	28	33	3	6	9	6	31	46	526

Table 6-2a. Compilation of mean hazard ranks associated with effect categories (range of possible ranks: 1 to 10), reported separately by project location in Section 5.4. Hazard rank values were standardized using hazard binning of effect-specific mean hazard scores (see Table 5-3) in order to facilitate visual inspection for project-wide patterns. Black-shaded cells mean no hazard score were available

Effect Category	St Louis River/ Bay	Waupaca Chain O'Lakes	LLBDM	Fox River/ Green Bay	Kewaunee River	Milwaukee River	North Shore Channel	Little Calumet River	Grand River/ Maple River	Saginaw River	St Clair River	Clinton River	Detroit River	River Raisin	Swan Creek	Maumee River	Cuyahoga River	Tinkers Creek	Ashtabula River	Long Pond	Genesee River	Irondequoit Bay	Oswegatchie River	Raquette River
Comprehensive Mean	1	1	1	1	1	2	4	3	2	1	1	1	1	1	1	2	2	3	1	1	1	1	1	1
Circulatory/Blood Constituents	1	1	2	1	2	2	4	4	3	2	1	2	1	1	1	2	3	2	1	1	1	1	1	1
Endocrine	4	4	3	3	2	4	8	8	5	4	1	4	1	2	3	3	6	5	2	2	1	2	2	2
Genotoxicity	3	1				2							2	1	5	1		2	1	1	1	1		
Gross Pathology	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Histopathology	1	1	3	1	2	2	6	5	4	3	1	2	1	1	1	3	4	5	1	1	1	1	1	1
Neurological	1	1	3	1	1	2	5	5	4	3	1	3	1	1	1	4	4	5	1	1	1	1	1	1
Physiological/Metabolic	1	1	2	1	1	2	6	5	2	2	1	3	2	2	1	2	4	3	1	1	2	1	1	1
Population-relevant Mean	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1
Behavioral	1	1	1	1	1	2	3	3	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1
Developmental	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Growth	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Mortality	1	1	1	1	1	1	3	2	2	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1
Reproductive	1	1	2	1	1	1	4	4	2	2	1	2	1	1	1	2	3	3	1	1	1	1	1	1

Table 6-2b. Compilation of mean hazard ranks associated with CECs (range of possible ranks: 1 to 10), reported separately by project location in Section 5.4. Hazard rank values were standardized using hazard binning of CEC-specific mean hazard scores (see Table 5-3) in order to facilitate visual inspection for project-wide patterns. Black-shaded cells mean no hazard score data were available

CEC	St Louis River/ Bay	Waupaca Chain O'Lakes	LLBDM	Fox River/ Green Bay	Kewaunee River	Milwaukee River	North Shore Channel	Little Calumet River	Grand River/ Maple River	Saginaw River	St Clair River	Clinton River	Detroit River	River Raisin	Swan Creek	Maumee River	Cuyahoga River	Tinkers Creek	Ashtabula River	Long Pond	Genesee River	Irondequoit Bay	Oswegatchie River	Raquette River
4-Androstene-3,17-dione	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Bisphenol A	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Carbamazepine	1	1	2	1	1	1	4	4	2	2	1	2	1	1	1	3	3	4	1	1	1	1	1	1
Citalopram	1	1	1	1	1	2	2	2	3	1	1	1	1	1	1	2	2	4	1	1	1	1	1	1
DEET	4	5	3	4	2	6	5	5	4	3	1	5	2	3	4	4	4	5	3	4	1	4	2	3
Diphenhydramine	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Estrone	2	3	2	1	1	2	6	4	1	2	1	2	3	2	3	1	2	2	1	1	3	1	1	1
HHCB	1	1	1	1	1	1	4	2	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1
Ibuprofen	2	1		1		1							2	1	3	1	1	1	1	1	1	1		
Lidocaine	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sitosterol, beta-	1	2	2	2	2	2	3	2	2	2	1	1	1	1	1	1	2	1	2	2	1	1	2	1
TBEP	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Triclosan	1	1	1	1	1	1	4	3	1	1	1	1	2	1	1	1	2	1	1	1	1	1	1	1
Venlafaxine	2	1	2	1	1	2	6	5	3	3	1	2	1	1	1	3	5	6	1	1	1	1	1	1

Table 6-3. Tallies[135] of SV_{HIGH} and SV_{LOW} exceedances by project location[136]

Great Lake Watershed	State	Project Location	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[137]			SV_{HIGH} Exceedance Occurrence Fractions				SV_{LOW} Exceedance Occurrence Fractions			
							Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
Project-wide			CEC-Influenced	25536	335	110	334	137	50	5298	312	108								
			Uninfluenced	14592	191	85	36	28	19	1568	177	83								
			Totals	40128	526	195	370	165	69	6865	489	191								
Lake Superior	Minnesota/ Wisconsin	St. Louis River/Bay	CEC-Influenced	5315	67	16	85	40	10	1044	64	16	1.27	5.31	0.60	0.63	15.58	65.25	0.96	1.00
			Uninfluenced	2535	32	16	1	1	1	94	28	15	0.03	0.06	0.03	0.06	2.94	5.88	0.88	0.94
			Totals	7850	99	32	86	41	11	1138	92	31	0.87	2.69	0.41	0.34	11.49	35.56	0.93	0.97
Lake Michigan	Wisconsin	Waupaca Chain O'Lakes	CEC-Influenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Uninfluenced	164	2	2	2	2	2	15	2	2	1.00	1.00	1.00	1.00	7.5	7.5	1.00	1.00
			Totals	164	2	2	2	2	2	15	2	2	1.00	1.00	1.00	1.00	7.5	7.5	1.00	1.00
	Wisconsin	Little Lake Butte des Morts	CEC-Influenced	1752	24	4	3	3	2	284	23	4	0.13	0.75	0.13	0.50	11.83	71.00	0.96	1.00
			Uninfluenced	292	4	1	0	0	0	32	4	1	0.00	0.00	0.00	0.00	8.00	32.00	1.00	1.00
			Totals	2044	28	5	3	3	2	316	27	5	0.11	0.60	0.11	0.40	11.29	63.20	1.00	1.00
	Wisconsin	Fox River/ Green Bay	CEC-Influenced	1688	22	8	5	4	3	253	22	8	0.23	0.63	0.18	0.38	11.50	31.63	1.00	1.00
			Uninfluenced	1176	15	7	2	2	2	84	15	7	0.13	0.29	0.13	0.29	5.60	12.00	1.00	1.00
			Totals	2864	37	15	7	6	5	337	37	15	0.19	0.47	0.16	0.33	9.11	22.47	1.00	1.00
	Wisconsin	Kewaunee River	CEC-Influenced	949	13	3	1	1	1	108	11	3	0.08	0.33	0.08	0.33	8.31	36.00	0.85	1.00
			Uninfluenced	438	6	2	1	1	1	20	6	2	0.17	0.50	0.17	0.50	3.33	10.00	1.00	1.00
			Totals	1387	19	5	2	2	2	128	17	5	0.11	0.40	0.11	0.40	6.74	25.60	0.89	1.00
	Wisconsin	Milwaukee River	CEC-Influenced	656	8	4	12	8	4	106	8	4	1.50	3.00	1.00	1.00	13.25	26.50	1.00	1.00
			Uninfluenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Totals	656	8	4	12	8	4	106	8	4	1.50	3.00	1.00	1.00	13.25	26.50	1.00	1.00
	Illinois	North Shore Channel	CEC-Influenced	876	12	3	43	11	3	475	12	3	3.58	14.33	0.92	1.00	39.58	158.33	1.00	1.00
			Uninfluenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Totals	876	12	3	43	11	3	475	12	3	3.58	14.33	0.92	1.00	39.58	158.33	1.00	1.00

[135] Location-specific exceedance tallies in this table are comprised of exceedances of mean comprehensive SVs, exceedances of mean population-relevant SVs, and exceedances in individual effect categories across all CECs.

[136] There were 24 ***project locations*** in this EHA, described in Attachment A2. There were multiple ***sampling sites*** – geographic points where surface water samples were collected – within each project location. We defined a ***sampling event*** as the act of collecting a single physical water sample, which is uniquely identified by a specific date/time at a specific sampling site. The ***total number of exceedances*** of either SV_{HIGH} or SV_{LOW} in a project location is the sum of all SV exceedances across sampling events for that location (Table A-2 in Attachment A1) and across available CEC- and effect-specific SV pairs (Table 4-1).

[137] All SV_{HIGH} exceedances also incidentally exceed the corresponding SV_{LOW}. However, these tallies of SV_{LOW} exceedances are only the cases where hazard score = 2. To avoid double-counting incidence of exceedances, these tallies of SV_{LOW} exceedances do not include cases where the SV_{HIGH} was exceeded.

Table 6-3 (continued)

Great Lake Watershed	State	Project Location	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[137]			SV_{HIGH} Exceedance Occurrence Fractions				SV_{LOW} Exceedance Occurrence Fractions			
							Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
	Illinois	Little Calumet River	CEC-Influenced	876	12	3	25	9	2	432	12	3	2.08	8.33	0.75	0.67	36.00	144.00	1.00	1.00
			Uninfluenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Totals	876	12	3	25	9	2	432	12	3	2.08	8.33	0.75	0.67	36.00	144.00	1.00	1.00
	Michigan	Grand River/ Maple River	CEC-Influenced	438	6	2	2	2	2	94	6	2	0.33	1.00	0.33	1.00	15.67	47.00	1.00	1.00
			Uninfluenced	803	11	4	2	2	2	179	11	4	0.18	0.50	0.18	0.50	16.27	44.75	1.00	1.00
			Totals	1241	17	6	4	4	4	273	17	6	0.24	0.67	0.24	0.67	16.06	45.50	1.00	1.00
Lake Huron	Michigan	Saginaw River	CEC-Influenced	876	12	6	3	3	3	214	12	5	0.25	0.50	0.25	0.50	17.83	35.67	1.00	0.83
			Uninfluenced	1168	16	8	2	2	2	156	15	8	0.13	0.25	0.13	0.25	9.75	19.50	0.94	1.00
			Totals	2044	28	14	5	5	5	370	27	13	0.18	0.36	0.18	0.36	13.21	26.43	0.96	0.93
Lake St. Clair	Michigan	St. Clair River	CEC-Influenced	584	8	5	0	0	0	10	5	4	0.00	0.00	0.00	0.00	1.25	2.00	0.63	0.80
			Uninfluenced	219	3	3	0	0	0	10	2	2	0.00	0.00	0.00	0.00	3.33	3.33	0.67	0.67
			Totals	803	11	8	0	0	0	20	7	6	0.00	0.00	0.00	0.00	1.82	2.50	0.64	0.75
	Michigan	Clinton River	CEC-Influenced	292	4	2	1	1	1	74	4	2	0.25	0.50	0.25	0.50	18.50	37.00	1.00	1.00
			Uninfluenced	438	6	3	1	1	1	65	6	3	0.17	0.33	0.17	0.33	10.83	21.67	1.00	1.00
			Totals	730	10	5	2	2	2	139	10	5	0.20	0.40	0.20	0.40	13.90	27.80	1.00	1.00
Lake Erie	Michigan	Detroit River	CEC-Influenced	738	9	5	6	2	1	96	8	5	0.67	1.20	0.22	0.20	10.67	19.20	0.89	1.00
			Uninfluenced	656	8	5	8	2	1	80	7	5	1.00	1.60	0.25	0.20	10.00	16.00	0.88	1.00
			Totals	1394	17	10	14	4	2	176	15	10	0.82	1.40	0.24	0.20	10.35	17.60	0.88	1.00
	Michigan	River Raisin	CEC-Influenced	164	2	2	0	0	0	14	2	2	0.00	0.00	0.00	0.00	7.00	7.00	1.00	1.00
			Uninfluenced	164	2	2	0	0	0	8	2	2	0.00	0.00	0.00	0.00	4.00	4.00	1.00	1.00
			Totals	328	4	4	0	0	0	22	4	4	0.00	0.00	0.00	0.00	5.50	5.50	1.00	1.00
	Ohio	Swan Creek	CEC-Influenced	1450	19	13	7	3	3	145	19	13	0.37	0.54	0.16	0.23	7.63	11.15	1.00	1.00
			Uninfluenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Totals	1450	19	13	7	3	3	145	19	13	0.37	0.54	0.16	0.23	7.63	11.15	1.00	1.00
	Ohio	Maumee River	CEC-Influenced	2624	32	16	38	16	7	602	31	16	1.19	2.38	0.50	0.44	18.81	37.63	0.97	1.00
			Uninfluenced	738	9	4	2	2	2	120	9	4	0.22	0.50	0.22	0.50	13.33	30.00	1.00	1.00
			Totals	3362	41	20	40	18	9	721	40	20	0.98	2.00	0.44	0.45	17.59	36.05	0.98	1.00

Table 6-3 (continued)

Great Lake Watershed	State	Project Location	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[137]			SV_{HIGH} Exceedance Occurrence Fractions				SV_{LOW} Exceedance Occurrence Fractions			
							Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
	Ohio	Cuyahoga River	CEC-Influenced	1314	18	3	25	8	1	428	17	3	1.39	8.33	0.44	0.33	23.78	142.67	0.94	1.00
			Uninfluenced	730	10	2	8	7	2	272	10	2	0.80	4.00	0.70	1.00	27.20	136.00	1.00	1.00
			Totals	2044	28	5	33	15	3	700	27	5	1.18	6.60	0.54	0.60	25.00	140.00	0.96	1.00
	Ohio	Tinkers Creek	CEC-Influenced	1824	24	6	75	23	6	723	24	6	3.13	12.50	0.96	1.00	30.13	120.50	1.00	1.00
			Uninfluenced	684	9	3	5	4	2	188	9	3	0.56	1.67	0.44	0.67	20.89	62.67	1.00	1.00
			Totals	2508	33	9	80	27	8	911	33	9	2.42	8.89	0.82	0.89	27.61	101.22	1.00	1.00
	Ohio	Ashtabula River	CEC-Influenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Uninfluenced	246	3	3	0	0	0	13	3	3	0.00	0.00	0.00	0.00	4.33	4.33	1.00	1.00
			Totals	246	3	3	0	0	0	13	3	3	0.00	0.00	0.00	0.00	4.33	4.33	1.00	1.00
Lake Ontario	New York	Long Pond	CEC-Influenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Uninfluenced	492	6	6	0	0	0	45	6	6	0.00	0.00	0.00	0.00	7.50	7.50	1.00	1.00
			Totals	492	6	6	0	0	0	45	6	6	0.00	0.00	0.00	0.00	7.50	7.50	1.00	1.00
	New York	Genesee River	CEC-Influenced	374	5	4	0	0	0	21	5	4	0.00	0.00	0.00	0.00	4.20	5.25	1.00	1.00
			Uninfluenced	310	4	2	0	0	0	15	4	2	0.00	0.00	0.00	0.00	3.75	7.50	1.00	1.00
			Totals	684	9	6	0	0	0	36	9	6	0.00	0.00	0.00	0.00	4.00	6.00	1.00	1.00
	New York	Irondequoit Bay	CEC-Influenced	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			Uninfluenced	492	6	6	0	0	0	33	6	6	0.00	0.00	0.00	0.00	5.50	5.50	1.00	1.00
			Totals	492	6	6	0	0	0	33	6	6	0.00	0.00	0.00	0.00	5.50	5.50	1.00	1.00
St. Lawrence River	New York	Oswegatchie River	CEC-Influenced	994	14	2	0	0	0	22	7	2	0.00	0.00	0.00	0.00	1.57	11.00	0.50	1.00
			Uninfluenced	1241	17	3	0	0	0	65	14	3	0.00	0.00	0.00	0.00	3.82	21.67	0.82	1.00
			Totals	2235	31	5	0	0	0	87	21	5	0.00	0.00	0.00	0.00	2.81	17.40	0.68	1.00
	New York	Raquette River	CEC-Influenced	1752	24	3	3	3	1	153	20	3	0.13	1.00	0.13	0.33	6.38	51.00	0.83	1.00
			Uninfluenced	1606	22	3	2	2	1	74	18	3	0.09	0.67	0.09	0.33	3.36	24.67	0.82	1.00
			Totals	3358	46	6	5	5	2	227	38	6	0.11	0.83	0.11	0.33	4.93	37.83	0.83	1.00

Table 6-4. Tally of numbers of project locations having SV_{HIGH} exceedances associated with specific CEC-effect category combinations; 24 project locations were considered in this assessment.

Prevalence* of Project Locations with At Least One SV_{HIGH} Exceedance (Hazard Score = 3)	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**							**4**		**1**					**4**
Circulatory/ Blood Constituents														
Endocrine					**15**									**8**
Genotoxicity									**7**					
Gross Pathology														
Histopathology			**2**											**3**
Neurological														
Physiological/Metabolic							**9**							
Population-relevant														
Population-relevant Mean SV									**2**					**1**
Behavioral														
Developmental									**6**					
Growth														
Mortality														**6**
Reproductive									**6**					

* Gray Shading = SV Data Gap; Blank Cell = All Observations < SV_{HIGH}

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA in order to eliminate confusion with population-relevant SVs for the same effect categories. The comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see Section 4.2). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** Only 13 project locations had hazard scores for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; 11/24 project locations were sampled only during 2013-2014.

Table 6-5. Tally of numbers of project locations having SV_{LOW} exceedances associated with specific CEC-effect category combinations; 24 project locations were considered in this assessment.

Prevalence* of Project Locations with At Least One SV_{LOW} Exceedance (Hazard Score = 2 or 3**)**	4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramine	Estrone	HHCB	Ibuprofen ***	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
Comprehensive														
Comprehensive Mean SV**	**15**	**7**	**13**	**11**	**20**	**9**	**23**	**11**	**7**		**12**	**13**	**10**	**14**
Circulatory/ Blood Constituents			**15**		**13**				**1**		**21**			
Endocrine					**24**				**4**					**14**
Genotoxicity									**7**					
Gross Pathology		**4**												
Histopathology			**15**											**14**
Neurological			**15**											
Physiological/Metabolic			**15**				**23**	**18**	**4**					
Population-relevant														
Population-relevant Mean SV	**9**	**7**	**5**	**11**	**24**	**7**	**23**	**3**	**7**		**4**	**6**	**10**	**14**
Behavioral		**1**	**10**	**11**		**8**	**23**	**4**			**23**	**1**	**10**	**14**
Developmental		**11**	**14**			**2**	**23**	**3**	**7**			**10**	**10**	
Growth		**5**						**2**						
Mortality		**1**			**24**			**2**	**7**			**3**	**10**	**14**
Reproductive		**11**	**15**				**23**		**7**				**10**	**14**

* Gray Shading = SV Data Gap; Blank Cell = All Obs. < SV_{LOW}

** NOTE: Comprehensive effect-specific SVs for behavioral, developmental, growth, mortality, and reproductive effect categories were derived in Gefell et al. (2019), but they were not applied as effect-specific SVs in this EHA in order to eliminate confusion with population-relevant SVs for the same effect categories. The comprehensive effect-specific SVs incorporate information for effect endpoints that were not considered "population-relevant" by our definition (see Section 4.2). However, Gefell et al. (2019) had incorporated them into computing the comprehensive mean SV values used in this EHA.

*** Only 13 project locations had hazard scores for ibuprofen. Analytical data for ibuprofen are available only for project locations that were sampled during 2010-2012; 11/24 project locations were sampled only during 2013-2014.

**** Any exceedance of a SV_{HIGH} also incidentally exceeds the corresponding SV_{LOW}. For this table only, we tallied project locations with either a SV_{LOW} or SV_{HIGH} exceedance. Throughout the rest of this document, SV_{LOW} and SV_{HIGH} exceedances are considered mutually exclusive, where a SV_{LOW} exceedance is counted only where a SV_{HIGH} exceedance does not also occur in the same sample, for the same CEC-effect category combination.

Table 6-6. Tallies[138] of SV_{HIGH} and SV_{LOW} exceedances by CEC.

CEC	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[139]			SV_{HIGH} Exceedance Occurrence Fractions					SV_{LOW} Exceedance Occurrence Fractions				
					Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
4-Androstene-3,17-dione	CEC-Influenced	999	333	110	0	0	0	62	40	22	0	0.00	0.00	0.00	0.00	0.06	0.19	0.56	0.12	0.20
	Uninfluenced	574	191	85	0	0	0	11	8	8	0.000	0.00	0.00	0.00	0.00	0.019	0.06	0.13	0.04	0.09
	Totals	1573	524	195	0	0	0	73	48	30	0.000	0.00	0.00	0.00	0.00	0.046	0.14	0.37	0.09	0.15
Bisphenol A	CEC-Influenced	2664	333	110	0	0	0	217	54	33	0.000	0.00	0.00	0.00	0.00	0.081	0.65	1.97	0.16	0.30
	Uninfluenced	1528	191	85	0	0	0	37	17	15	0.000	0.00	0.00	0.00	0.00	0.024	0.19	0.44	0.09	0.18
	Totals	4192	524	195	0	0	0	254	71	48	0.000	0.00	0.00	0.00	0.00	0.061	0.48	1.30	0.14	0.25
Carbamazepine	CEC-Influenced	3685	335	110	6	6	4	1012	149	53	0.002	0.02	0.05	0.02	0.04	0.275	3.02	9.20	0.44	0.48
	Uninfluenced	2090	190	85	0	0	0	303	45	21	0.000	0.00	0.00	0.00	0.00	0.145	1.59	3.56	0.24	0.25
	Totals	5775	525	195	6	6	4	1315	194	74	0.001	0.01	0.03	0.01	0.02	0.228	2.50	6.74	0.37	0.38
Citalopram	CEC-Influenced	1005	335	110	0	0	0	229	77	32	0.00	0.00	0.00	0.00	0.00	0.227	0.68	2.07	0.23	0.29
	Uninfluenced	573	191	85	0	0	0	30	11	7	0.00	0.00	0.00	0.00	0.00	0.052	0.16	0.35	0.06	0.08
	Totals	1578	526	195	0	0	0	259	88	39	0.00	0.00	0.00	0.00	0.005	0.163	0.49	1.32	0.17	0.20
DEET	CEC-Influenced	1670	334	110	89	89	40	951	261	100	0.053	0.27	0.81	0.27	0.36	0.569	2.85	8.65	0.78	0.91
	Uninfluenced	955	191	85	18	18	16	477	140	76	0.019	0.09	0.21	0.09	0.19	0.499	2.50	5.61	0.73	0.89
	Totals	2625	525	195	107	107	56	1428	401	176	0.041	0.20	0.55	0.20	0.29	0.544	2.72	7.32	0.76	0.90
Diphenhydramine	CEC-Influenced	2008	335	110	0	0	0	191	81	28	0.000	0.00	0.00	0.00	0.00	0.095	0.57	1.74	0.24	0.25
	Uninfluenced	96	191	85	0	0	0	7	5	2	0.000	0.00	0.00	0.00	0.00	0.073	0.04	0.08	0.03	0.02
	Totals	2104	526	195	0	0	0	198	86	30	0.000	0.00	0.00	0.00	0.00	0.094	0.38	1.02	0.16	0.15

[138] CEC-specific exceedance tallies in this table are sums of exceedances of mean comprehensive SVs, exceedances of mean population-relevant SVs, and exceedances in individual effect categories across all project locations.

[139] All SV_{HIGH} exceedances also incidentally exceed the corresponding SV_{LOW}. However, tallies of SV_{LOW} exceedances included in this table are only the cases where hazard score = 2. To avoid double-counting incidence of exceedances, these tallies of SV_{LOW} exceedances do not include cases where the SV_{HIGH} was exceeded.

Table 6-6 (*continued*)

CEC	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[139]			SV_{HIGH} Exceedance Occurrence Fractions					SV_{LOW} Exceedance Occurrence Fractions				
					Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
Estrone	CEC-Influenced	1998	333	110	39	31	16	601	107	53	0.020	0.12	0.35	0.09	0.15	0.301	1.80	5.46	0.32	0.48
	Uninfluenced	1146	191	85	2	2	1	154	26	21	0.002	0.01	0.02	0.01	0.01	0.134	0.81	1.81	0.14	0.25
	Totals	3144	524	195	41	33	17	755	133	74	0.013	0.08	0.21	0.06	0.09	0.240	1.44	3.87	0.25	0.38
HHCB	CEC-Influenced	2338	334	110	0	0	0	331	181	69	0.000	0.00	0.00	0.00	0.00	0.142	0.99	3.01	0.54	0.63
	Uninfluenced	1337	191	85	0	0	0	50	33	18	0.000	0.00	0.00	0.00	0.00	0.037	0.26	0.59	0.17	0.21
	Totals	3675	525	195	0	0	0	381	214	87	0.000	0.00	0.00	0.00	0.00	0.104	0.73	1.95	0.41	0.45
Ibuprofen	CEC-Influenced	1143	127	56	59	28	17	118	28	17	0.052	0.46	1.05	0.22	0.30	0.103	0.93	2.11	0.22	0.30
	Uninfluenced	648	72	47	7	3	2	11	3	2	0.011	0.10	0.15	0.04	0.04	0.017	0.15	0.23	0.04	0.04
	Totals	1791	199	103	66	31	19	129	31	19	0.037	0.33	0.64	0.16	0.18	0.072	0.65	1.25	0.16	0.18
Lidocaine	CEC-Influenced	1005	335	110	0	0	0	0	0	0	0.000	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00
	Uninfluenced	573	191	85	0	0	0	0	0	0	0.000	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00
	Totals	1578	526	195	0	0	0	0	0	0	0.000	0.00	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.00
Sitosterol, beta-	CEC-Influenced	1670	334	110	0	0	0	311	180	76	0.000	0.00	0.00	0.00	0.00	0.186	0.93	2.83	0.54	0.69
	Uninfluenced	955	191	85	0	0	0	168	96	56	0.000	0.00	0.00	0.00	0.00	0.176	0.88	1.98	0.50	0.66
	Totals	2625	525	195	0	0	0	479	276	132	0.000	0.00	0.00	0.00	0.00	0.182	0.91	2.46	0.53	0.68
TBEP	CEC-Influenced	1670	334	110	0	0	0	162	86	33	0.000	0.00	0.00	0.00	0.00	0.097	0.49	1.47	0.26	0.30
	Uninfluenced	955	191	85	0	0	0	23	17	9	0.000	0.00	0.00	0.00	0.00	0.024	0.12	0.27	0.09	0.11
	Totals	2625	525	195	0	0	0	185	103	42	0.000	0.00	0.00	0.00	0.00	0.070	0.35	0.95	0.20	0.22
Triclosan	CEC-Influenced	2004	334	110	0	0	0	324	54	23	0.000	0.00	0.00	0.00	0.00	0.162	0.97	2.95	0.16	0.21
	Uninfluenced	1146	191	85	0	0	0	42	7	5	0.000	0.00	0.00	0.00	0.00	0.037	0.22	0.49	0.04	0.06
	Totals	3150	525	195	0	0	0	366	61	28	0.000	0.00	0.00	0.00	0.00	0.116	0.70	1.88	0.12	0.14

Table 6-6 (*continued*)

CEC	Site Group	Total N Hazard Scores	Total N Sampling Events	Total N Sampling Sites	SV_{HIGH} Exceedance Tallies			SV_{LOW} Exceedance Tallies[139]			SV_{HIGH} Exceedance Occurrence Fractions					SV_{LOW} Exceedance Occurrence Fractions				
					Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Total Number Exceedances	N Events with Exceedance	N Sites with Exceedance	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites	Exceedances / N Hazard Scores	Exceedances per Event	Exceedances per Site	Events with Exceedance / N Events	Sites with Exceedance / N Sites
Venlafaxine	CEC-Influenced	2345	335	110	140	83	25	832	141	48	0.060	0.42	1.27	0.25	0.23	0.355	2.48	7.56	0.42	0.44
	Uninfluenced	1337	191	85	9	9	5	233	35	15	0.007	0.05	0.11	0.05	0.06	0.174	1.22	2.74	0.18	0.18
	Totals	3682	526	195	149	92	30	1065	176	63	0.040	0.28	0.76	0.17	0.15	0.289	2.02	5.46	0.33	0.32

Table 6-7. Level(s) of ecological organization of effect endpoint(s) for the NOAECs and LOAECs incorporated into the 82 CEC SV pairs used in this EHA (from data presented in Gefell et al. 2019). Cell contents identify level(s) of ecological organization: C = cellular effect; T = Tissue/organ effect; O = organism level effect. Blank cells indicate that no SV pair was available.

Effect Category		4-Androstene-3,17-dione	Bisphenol A	Carbamazepine	Citalopram	DEET	Diphenhydramie	Estrone	HHCB	Ibuprofen	Lidocaine	Sitosterol, beta-	TBEP	Triclosan	Venlafaxine
POPULATION-RELEVANT															
Effect-Specific SVs	Behavioral		O	O	O		O	O	O		O	O	O	O	O
	Developmental		C,T,O	C,T,O			O	C,T,O	C,O	C,T,O		C,O	C,O	O	
	Growth	O	O	O					O						
	Mortality		O	O		O			O	O			O	O	O
	Reproductive		C,T,O	C,T,O				C,T,O		C,T,O				T,O	C,T,O
COMPREHENSIVE															
Effect-Specific SVs	Circulatory/ Blood Constituents			T		C,T				C,T		T			
	Endocrine					C				T					T
	Genotoxicity									C					
	Gross Pathology		T												
	Histopathology			C											C
	Neurological			C											
	Physiological/ Metabolic			C,T				O	C	T					

Table 6-8. Statistical test results compiled from individual project location analyses provided in Section 5.4. Cell contents identify specific CECs with a statistically significant difference in hazard scores ($p < 0.1$) between CEC-influenced and uninfluenced sites. Gray shading means no statistical evaluation was possible. Blank cells indicate that no difference between site groups was seen for any CEC.

Great Lakes Basin Project Location (ordered West to East)	Significant Difference ($p < 0.1$) in Median or Maximum Hazard Scores [140]	Comprehensive [141]								Population-relevant					
		Comprehensive Mean SV	Effect-Specific SVs							Population-relevant Mean SV	Effect-Specific SVs				
			Circulatory / Blood Constituents	Endocrine	Genotoxicity	Gross Pathology	Histopathology	Neurological	Physiological/ Metabolic		Behavioral	Developmental	Growth	Mortality	Reproductive
01. St. Louis River/Bay	Y	IB, DT*	DT	DT	IB					IB		IB		IB	IB
02. Waupaca Chain O' Lakes	*na*														
03. Little Lake Butte des Morts	***NS***														
04. Fox River/Green Bay	Y	VE	CA	VE			VE	CA	CA, HH**	VE	VE			VE	VE
05. Kewaunee River	***NS***														
06. Milwaukee River	*na*														
07. North Shore Channel	*na*														
08. Little Calumet River	*na*														
09. Grand River/Maple River	***NS***														
10. Saginaw River	Y	CI**, TB**					CA*		HH**	CI**					CA*
11. St. Clair River	***NS***														
12. Clinton River	***NS***														
13. Detroit River	***NS***														
14. River Raisin	***NS***														
15. Swan Creek	*na*														
16. Maumee River	Y	AN*, CI**, DP**, ES*							ES*, HH**	CI**, ES*	CI**, ES*	ES*			ES*
17. Cuyahoga River	***NS***														
18. Tinkers Creek	Y	DP		VE*							CI*			VE	
19. Ashtabula River	*na*														
20. Long Pond	*na*														
21. Genesee River	***NS***														
22. Irondequoit Bay	*na*														
23. Oswegatchie River[142]	Y	SS	SS*								SS*				
24. Raquette River	***NS***														

[140] 'Y' = at least one statistically significant difference was observed; 'NS' = no significant difference was observed, which may mean that sample sizes were small, or that elevated hazard was too widespread to discern a difference, or that low or negligible hazard was pervasive in both groups; 'na' = no statistical test was possible, due either to insufficient sample sizes or all sites at the location belonged to only one site group.

[141] Cell contents are CECs with statistically significant differences between site groups – abbreviations are the following: 4-androstene-3,17-dione (AN), carbamazepine (CA), citalopram (CI), DEET (DT), diphenhydramine (DP), estrone (ES), HHCB (HH), ibuprofen (IB), β-sitosterol (SS), TBEP (TB), and venlafaxine (VE). No asterisk indicates that only the maximum scores differed; * = only the median hazard scores differed; ** = both maximum and median hazard scores differed.

[142] Only at the Oswegatchie River, statistically significant differences ($p < 0.1$) between site groups is due to higher β-sitosterol-related hazard in "uninfluenced" groups than in "influenced" groups (see Attachment B); which is opposite to the trend expected if mapped point sources increased hazard associated with β-sitosterol exposure. For all of the other statistically significant differences identified in this table, median or maximum (as indicated) hazard scores were greater in the CEC-influenced site group than in the uninfluenced group.

Chapter 7 - Uncertainty Evaluation

This chapter describes uncertainties associated with major components of this CEC EHA. A quantitative treatment of uncertainty was beyond the scope of this EHA, with the notable exceptions of uncertainty associated with estimating aqueous CEC concentrations (see Section 7.2.3) and certain sources of uncertainty in the toxicity assessment that were addressed during derivation of SVs (Gefell et al. 2019): chemical complexity of exposure, inter-species sensitivity, and intra-species sensitivity.

Uncertainties identified in this EHA illustrate competing tendencies to over- and underestimate CEC-related hazards to fish. In our analysis of exposure assessment uncertainties, we observed an 8% hazard overestimation rate in five pharmaceuticals at Tinkers Creek in 2013 attributed to our method of estimating aqueous CEC concentration (Section 7.2.3). On the other hand, most elements of this EHA (problem formulation, toxicity assessment, hazard characterization, and other aspects of the exposure assessment) involve pragmatic limitations or conservative assumptions that lean toward underestimating hazard, as described below. While net bias is unknown, the cumulative effect of an EHA methodology generally skewed to curb identification of hazards likely has resulted in an overall tendency to underestimate hazard.

Future EHAs involving CECs in surface water may benefit from resolving some of the following sources of uncertainty that we identified during the course of our assessment.

7.1 Problem Formulation

Our EHA focused on the potential for direct toxicity to fish of a small set of aqueous parent CECs. This overall approach is likely to have underestimated total CEC hazard to fish populations and to the aquatic community as a whole.

In aquatic systems, there are typically many possible receptor taxa with widely divergent life histories, each of which may be affected directly by chemical exposures (e.g., Hoppe et al. 2012, de Kermoysan et al. 2013). Our focus on hazards to fish excluded explicit consideration of other important aquatic flora and fauna, which may be more sensitive to CECs than fish. Each potential receptor species may also be impacted indirectly via contaminant-caused alterations in the food web, habitat, or other critical aspects of the receptors' aquatic ecosystem (Kidd et al. 2014). There are often numerous chemical contaminants, multiple sources of chemical contamination, and contaminants having varying rates of degradation and bioaccumulation, that partition differently between aquatic media. Parent compounds are likely to have been metabolized or environmentally degraded to an unknown extent (Celiz et al. 2009), so assessing only parent compounds excludes degradates from the assessment that are possibly more hazardous. Further, toxicological interactions between CECs and their derivatives may be antagonistic or synergistic. Each potential receptor population is usually exposed via multiple exposure pathways to a complex mixture of interacting contaminants.

Other environmental factors that may become stressors (e.g., water temperature, suspended sediment, dissolved oxygen, pH, nutritional deficiencies, and predation) are continuously affecting organisms directly. Some of these have been shown to modify contaminant uptake or susceptibility of receptors to effects from chemical exposure, including CEC exposure (e.g., Waring and

Some Key Points…

Uncertainty Evaluation

- **Purpose:** Describe uncertainties associated with major elements of this EHA
- **Overall Summary:** We identified competing tendencies toward over- and underestimating hazard, with an unknown net effect on hazard characterization. However, a tendency to underestimate hazard may predominate due to pragmatic limitations in EHA scope and intentionally conservative assumptions throughout the assessment.
- **Some Sources of Uncertainty:**
  - *Problem Formulation* – life history diversity of Great Lakes basin fish, CEC exposure complexity, non-chemical stressors
  - *Exposure Assessment* – limited exposure routes considered, variability in chemical properties among the 14 CECs, variable timing of sampling, *de facto* sampling schedule may not capture peak CEC concentrations, unaccounted for CEC sources, estimating aqueous CEC concentrations
  - *Toxicity Assessment and Hazard Characterization* – gaps in effect-specific SVs and ecotoxicity literature, hazard score assignment to non-detects, use of NOAECs to derive SV_{LOW} values, level of ecological organization.

Moore 2004, Matson et al. 2008, Park et al. 2011). The magnitude of non-chemical stressors may vary widely, particularly in the gradients upstream to downstream of urban areas, or in temperate zones with large seasonal fluctuations.

7.2 Exposure Assessment

The totality of aqueous chemical exposures cannot be known, since standardized analytical methods do not exist for the vast majority of probable contaminants (and their metabolites and degradation products). Also, in aquatic systems, exposure levels vary spatially and temporally, and uptake varies among receptor organisms and environmental conditions. Depending on the mobility and life history of potential receptors relative to the spatial distribution of toxic contaminants, receptors may be exposed episodically for only a few brief periods, seasonally that primarily affects specific life stages, continuously or repeatedly throughout an individual's lifetime, or over many generations.

7.2.1 Exposure Pathway

In this EHA, hazard was scored exclusively using aqueous concentrations of CECs. Aqueous (dissolved) contaminants enter fish by direct uptake from water via the gills and skin, and via the gut after incidental ingestion of water. However, trophic uptake occurs to varying degrees in fish depending on CEC chemical properties and water quality characteristics (Richmond et al. 2018). Trophic uptake was not addressed in this EHA due to a near absence of lab assays in the peer-reviewed literature that relate ecotoxicological effects to quantified trophic exposures to CECs, so total hazard to fish is likely underestimated. The largest underestimations of trophic-related hazards would occur for CECs that most readily bioaccumulate in, or adhere to, fish forage. CEC-specific log(K_{ow}) values are positively related to bioaccumulation in fish forage and adherence to food particles, but also may be related to adherence to non-food particles. Particularly in eutrophic or turbid systems as compared to clear-water systems, high log(K_{ow}) CECs may preferentially partition to the particulate phase, so hazard to resident fish that consume suspended particles such as organic detritus or phytoplankton may be more influenced by the trophic route of exposure than direct uptake of aqueous CEC. This EHA also did not consider direct absorption or incidental ingestion of sediments by benthic-feeding fish, which contributes to additional underestimation of total hazard. Thus, in eutrophic or turbid water scenarios, underestimation of total hazard may be accentuated using the methods described in this document, which estimate relative hazard exclusively based on aqueous concentrations of CEC.

7.2.2 Measured aqueous CEC in water samples

Aqueous concentrations of pharmaceuticals were measured in surface water samples collected during 2013-2014 (see Table 3-2). For these analyses, filtration occurred at the analytical lab and not in the field upon sampling. During sample shipment, the magnitude of possible re-equilibration between dissolved and particulate phases and its effect on dissolved concentration is unknown. However, the potential for re-equilibration is affected by temperature changes; re-equilibration of sampled water during shipping and handling might alter aqueous concentration from the actual ambient concentration. All water samples were placed on ice at sampling, and shipped on ice, so temperature-induced re-equilibration would have been relatively low for the coldest water samples collected shortly after spring snowmelt or near river headwaters, and relatively high for warmer samples collected from small urban waterbodies in mid to late summer. To the degree that water temperature is positively related to mobilization of CEC from particulate phase to dissolved phase, measured aqueous concentrations in water samples collected from warmer waterbodies were underestimates of the actual aqueous concentrations at the point of sampling. In these cases, CEC-related hazard would be underestimated.

7.2.3 Estimated aqueous CEC from measured total CEC in water samples

Most of the aqueous CEC concentrations scored for hazard in this EHA were estimated from total concentration - they were not measured aqueous concentrations (see Table 3-2). The application of published conversion equations to estimate aqueous CEC concentrations (described in Section 3.5 and Attachment A) introduces an important source of uncertainty into the EHA that could result in over- or underestimation of aqueous concentrations and possible error in corresponding hazard scores.

7.2.3.1 Comparisons of Hazard Scores Developed from Paired Measured and Estimated Aqueous Concentrations of Five Pharmaceuticals in Surface Water

We evaluated the magnitude of error in relative hazard by comparing hazard scores computed from CEC concentrations in 11 replicate samples. We compared hazard scores associated with measured aqueous concentration from filtered samples to an estimated aqueous concentration computed from measured total concentration in the paired sample. There is inherent variability in the CEC concentration dataset, which is comprised of measured concentrations determined for various years, seasons and times of day across many sampling sites in a diverse set of 24 waterbodies. In addition, all of the samples that were filtered at the analytical lab prior to CEC analysis were collected during 2013-2014, but the waterbodies sampled during that period differed from the ones sampled during 2010-2012, when all samples were unfiltered. For these reasons, it would be invalid to compare concentrations between all filtered (aqueous) and all unfiltered (total) samples for individual CECs. Instead, we utilized a limited set of paired pharmaceutical concentration data collected at one of the most heavily CEC-contaminated waterbodies included in this EHA.

Eleven replicate pairs of surface water samples were analyzed for carbamazepine, citalopram, diphenhydramine, lidocaine and venlafaxine, one replicate was analyzed for aqueous (filtered) concentrations and the other replicate for total (unfiltered) concentrations. The paired surface water

samples were collected at each of the nine Tinkers Creek sampling sites on August 15, 2013, and sampling sites TIC-5 and TIC-8 were resampled on August 16, 2013. We considered the data from filtered samples to be measured aqueous concentrations. In the unfiltered replicate samples, we estimated aqueous concentration from measured total concentrations using methods described in Section 3.5 and Attachment A. We generated hazard scores for each of the paired aqueous concentrations, and evaluated differences in aqueous concentration and in relative hazard between paired samples for each of the five CECs.

There was a total of 55 comparisons possible between measured and estimated aqueous CEC concentrations. Six of the comparisons involved non-detects in both filtered and unfiltered replicate samples, five of which were analyzed in the sample collected at TIC-1 – the most upstream sampling site in the creek. Scored hazard did not differ between these paired concentrations because all non-detects were assigned a hazard score of '1'. On two additional occasions at different sampling sites, estimated aqueous concentration was calculated from a detected unfiltered (measured total) concentration, while the paired filtered (measured aqueous) sample was a non-detect, resulting in elevated hazard scores using estimated aqueous concentrations in four of seven possible comparisons. There was a total of 47 comparisons where both filtered and unfiltered concentrations were detected. Among these, there were four occasions when measured aqueous concentration exceeded the estimated, three of which involved lidocaine. There was one occasion when the two detected aqueous concentrations (estimated and measured) were essentially identical, involving venlafaxine.

The remaining 42 comparisons indicate overestimation of aqueous concentration, in which aqueous concentration estimated from detected unfiltered concentration exceeded the detected filtered concentration (Table 7-1). However, in 25 of these 42 cases (60%) principally involving carbamazepine, lidocaine, diphenhydramine and venlafaxine, the estimated aqueous concentration exceeded measured by less than a factor of two. In only three cases (7%) - all involving citalopram - the estimated aqueous concentration exceeded measured by a factor of five or more. The greatest discrepancy involved citalopram at TIC-3, a site immediately downstream of a WWTP, where aqueous concentration estimated from unfiltered concentration was 10.6 times greater than the measured filtered concentration.

The degree of inflation of estimated aqueous concentration over measured concentration varied with CEC (Figure 7-1). The slope of estimated CEC concentration on measured concentration – essentially an overestimation factor – ranged from 1.34 ($r^2 = 0.79$) for lidocaine to 3.19 ($r^2 = 0.66$) for citalopram, with four of the five slopes quantified at less than 1.8.

However, over- or underestimation of aqueous CEC concentration does not automatically translate to a shift in associated hazard score[143]. From the 11 sets of replicate samples described above, which yielded paired filtered and unfiltered CEC concentrations for five CECs, we generated 308 comparisons between effect-specific hazard scores from estimated aqueous concentrations and hazard scores from measured aqueous concentrations. There were 25 occurrences (8.1%) of discrepancies in hazard between measured versus estimated aqueous concentrations. All 25 hazard score discrepancies involved a shift of one unit in hazard score. Twenty-four of the discrepancies (7.8%) indicated a higher hazard associated with estimated aqueous concentration than with measured aqueous concentration – an overestimate of hazard score from '1' to '2', or from '2' to '3'.

Certain effect categories and CECs appear to be associated disproportionately with hazard score overestimations (Table 7-1). The highest percent overestimation was associated with histopathology (23%), mortality (14%), population-relevant mean SVs (11%) and comprehensive mean SVs (11%). The least affected effect categories were behavioral, growth, reproductive, circulatory/blood constituents, neurological, and physiological/metabolic. Among the five pharmaceuticals evaluated, venlafaxine was involved in the greatest number of hazard overestimations, while lidocaine was not associated with any hazard score discrepancies.

7.2.3.2 Uncertainty Associated with Conversion Equation Inputs

Discrepancies between measured and estimated aqueous concentrations and associated hazards, such as those described above, may be due in part to error in regression equation inputs. For example, we applied a conversion equation that requires K_d and TSS data as input values, as described in Section 3.5 and Attachment A. In some cases, the inputs are themselves estimated, so there is potential for an unknown degree of error propagation in estimating aqueous CEC. For instance, for most of the 14 CECs with SVs, no empirical K_{oc} or K_d was located in the literature. In these cases, two published regression equations - applied in sequence - were used to compute K_d estimates from literature $\log(K_{ow})$ values. First, K_{oc} was estimated from $\log(K_{ow})$, and then K_d was computed from the estimated K_{oc}. The use of the output from one regression equation as input to a second regression equation results in error propagation, which inflates uncertainty in the resulting K_d estimate.

Uncertainty also occurs in cases where empirical K_{oc} or K_d values that were measured in waterbodies not sampled in this EHA or in the laboratory were used unadjusted. The relevance of these empirical values obtained from other systems to site-specific conditions of particular Great Lakes CEC surface water samples is uncertain.

Third, K_d for a given CEC can be sensitive to

[143] A hazard score – the metric we used to indicate relative hazard in this EHA – is assigned to a range of CEC concentrations defined by effect-specific SV_{HIGH} and SV_{LOW} values (see Figures 4-2a and 4-2b).

water temperature and possibly other water quality characteristics in aquatic systems (e.g., alkalinity, specific conductance), affecting rates of adsorption to, and desorption from sediment particles (USEPA 1999). Although we made no direct adjustment for water temperature in our computations, in each sampling foray water samples were collected within a few days of each other across sampling sites within project locations, so water temperature may not have varied appreciably between samples collected in a single foray within a project location. Different sets of K_d values were estimated within a few project locations for different sub-waterbodies with presumably different overall water quality profiles – including water temperature (see Attachment A, Table A-4). Further, bias in hazard estimation introduced by omitting temperature as an adjustment variable is relatively constant between sampling sites within a project location, as compared to between project locations. Uncertainty associated with omitting explicit consideration of temperature and other water quality variables was therefore somewhat mitigated in that most of our hazard comparisons were evaluated between sampling sites within project locations (see Section 5.4).

7.2.4 Sampling Timing

Timing of surface water sampling was generally coincidental to field mobilizations for pre- and/or post-spawn fish health surveys in multiple fish species (Table 3-1 in Section 3.4, Table A-2 in Attachment A). Surface water sampling was not designed with the intent to provide exposure data for an EHA. This *de facto* sampling schedule may not have captured seasons of the year or times of day when the highest CEC concentrations - or, the lowest CEC concentrations - tend to occur at a given sampling site. Emerging contaminant concentrations in receiving surface waters may be affected by residential and industrial human behavioral patterns that may result in daily fluctuations in CEC loadings, as well as seasonal and episodic precipitation patterns that affect fluctuations in flow and degree of CEC dilution.

It is possible that concentrations of CECs in receiving waters, particularly downstream of point source inputs, could be related to diurnal human activity patterns. For example, relatively high concentrations of certain CECs or their metabolites may occur downstream of WWTPs that receive wastewater from residential sewersheds during the time window when a large fraction of residents are waking up in the morning. In this scenario, if water samples were collected only during such pulses, then concentrations of certain CECs may be biased high in the exposure dataset, but if samples did not include such pulses, then CEC concentration may be biased low. There also may be a seasonality in surface water concentrations of CECs that came from WWTPs, with relatively high receiving water concentrations during periods when base flow is low and lower concentrations during peak flow periods that dilute the CECs, such as during storm events or spring snowmelt.

The 2010-2014 chemistry dataset was not designed to evaluate associations of CEC concentration with temporal cycles. Choy et al. (2017) reports that diurnal time series data are limited to a few sampling sites in the St. Louis River/Bay and Maumee River project locations in 2012. No discernable temporal patterns in CEC concentration or number of CECs detected in surface water were observed (Choy et al. 2017). Nevertheless, the existence of temporal cycles in CEC concentrations is plausible and may be discernable with a sampling schedule designed to target this question. In this project, surface water sampling schedule was not consistent across all sampling sites; the overall pattern of sampling seasons, individual sampling days, and times of day differed considerably between sites in different project locations. While sites within project locations were sampled on closely similar calendar schedules, with only a few exceptions (Attachment A, Table A-2), there was some variability in time of day at sites sampled within a single project location, with the greatest differences in time of day observed between sites most distant from each other.

Given the plausibility of regular temporal variation in CEC concentrations and the irregularity of water sampling time of day in this project, there is uncertainty in the representativeness of the CEC concentration dataset associated with sampling schedule variability - both across project locations and among sampling sites within project locations.

7.2.5 Potential for CEC Loadings that May Confound Point Source Hazard Analysis

We evaluated the possibility that elevated CEC concentrations in downstream proximity to WWTPs and CSOs result in elevated hazards. However, there are several sources of CECs to surface waters that were not explicitly evaluated in this EHA. These other sources contribute CECs to aquatic systems, and may elevate CEC-related hazard at some sites that were not expected based on orientation to CSOs and WWTPs. Unexpected elevated hazard at certain sampling sites may confound statistical analyses comparing hazard between point source CEC-influenced and uninfluenced sites, where degree of influence is defined by spatial orientation to WWTPs or CSOs (see Section 5.3). Additional possible point sources include, but are not limited to, unmapped or purportedly decommissioned WWTPs or CSOs, paper mills, National Pollution Discharge Elimination System (NPDES) permitted discharges (from facilities such as hospitals, elder care facilities, or pharmaceutical or pesticide manufacturing facilities), and unpermitted rogue pipe discharges. Unevaluated non-point sources include aerial deposition, urban runoff, runoff from agricultural fields amended with WWTP solids or CAFO manure solids or slurry, and seepage from septic ponds, municipal or household leach fields, or CAFO lagoons to groundwater that recharges the subject waterbody.

7.3 Toxicity Assessment and Hazard Characterization

7.3.1 Level of Ecological Organization

Omitting a direct assessment of ecological hazard at higher levels of ecological organization may contribute to underestimation of total CEC-related hazard. This EHA did not incorporate exposure-effect information

for ecotoxicological effect metrics at higher levels of ecological organization, such as population, community, or ecosystem. We found no studies on the 14 CECs that used effect metrics descriptive of higher level ecological structure or function - for example: intrinsic rate of increase or age structure in fish populations; species diversity, species richness, or trophic structure in aquatic communities; or, energy flux or carbon flux in aquatic ecosystems. Effect metrics that describe structure or function in populations, communities and ecosystems are emergent properties that cannot be measured directly at the individual level of ecological organization. All of the ecotoxicity assays that we located in the literature for our 14 target CECs evaluated endpoints that are measured in individual fish. It is possible that modeling could reduce uncertainty related to extrapolating CEC-related hazard based on fish ecotoxicity assays to the potential for adverse impacts at higher levels of ecological organization - such as the fish population or community - but we did not conduct population modeling or apply an adverse outcome pathway analysis in this EHA.

7.3.2 Assigning Hazard Scores to Non-detects

In the exposure dataset, data were identified as non-detects if the concentrations were below the associated detection limit (DL, as either MDL or reporting limit, as appropriate), depending on CEC. We interpreted all non-detects as having negligible hazard, and assigned them a hazard score of '1' (Chapter 5). This simplified approach to characterizing hazard for non-detects likely resulted in false negative findings. These false negatives are related to the magnitudes of DLs relative to screening value concentrations for the same CEC.

In most cases, the DL for a specific CEC is less than both the SV_{HIGH} and SV_{LOW}, so all non-detects plus detects that fall below the SV_{LOW} are interpreted as negligible hazard (Figure 7-2a). However, in a few cases the SV_{LOW} is lower than the DL (Figure 7-2b). For simplicity, and to ensure that we did not overestimate hazard, all samples identified as non-detect were designated negligible hazard and assigned a hazard score of '1'. Actual concentrations are unknown below the DL so there is uncertainty whether the actual concentration of a non-detect falls above or below the SV_{LOW}. In these cases, an unknown fraction of non-detects could have exceeded the SV_{LOW} and otherwise would have been designated low hazard and assigned a hazard score of '2'.

In rare cases, such as with the ibuprofen genotoxicity SV pair, both the SV_{LOW} and SV_{HIGH} fall below the DL (Figure 7-2c). In these cases, the only possible hazard scores were '1' and '3'. All detects were assigned a '3' and designated high hazard. All non-detects were assigned a hazard score of '1' and designated negligible hazard. In these cases, it appears likely that hazard is underestimated. In this circumstance, all non-detect samples (concentrations below the DL) are designated negligible hazard. This includes all samples with unknown actual concentrations that would otherwise (if the DL were sufficiently low) have been designated low hazard, as well as an unknown fraction of non-detects that could have exceeded the SV_{HIGH} and otherwise would have been identified as high hazard.

7.3.3 Limitations of the Ecotoxicity Literature Database

Additional factors were identified that contribute to the overall tendency to underestimate hazard:

- It is possible that greater hazard would be assigned on the basis of CEC exposure-effect relationships that were not included in this EHA:
    - SVs were derived for only 14 CECs among many thousands of parent compounds, metabolites, and environmental degradates,
    - A large number of effect-specific SVs were not developed for the 14 CECs included in this EHA due to data gaps in the literature (see Table 4-1), and
    - Data gaps in the range of possible effect endpoints are inevitable in fish ecotoxicity assays reported in the literature, hence, the set of effect endpoints utilized to develop effect-specific SVs is always incomplete, possibly excluding more sensitive effect endpoints.
- NOAECs from published assays were the basis for deriving SV_{LOW} values, but the NOAECs themselves may be false negative findings. This risk is particularly high in cases where sample sizes in control and/or exposure groups are low, when variance in one or the other group is high, and/or when the threshold p-value used to identify statistically significant differences in treatment response from control response is only slightly exceeded.

7.4 Recommendations for Reducing Uncertainty

We provide the following recommendations for future EHAs that utilize hazard scoring based on CEC SVs as presented in this EHA:

- collect water samples both upstream and downstream of known or suspected CEC sources,
- analyze as many water samples as is feasible at each sampling site, including multiple seasons and times of day,
- filter water samples in the field (first choice) or in the lab prior to analysis (second choice),
- analyze water samples for filtered (aqueous; dissolved) concentrations,
- analyze each water sample for as wide a variety of CEC categories (pharmaceuticals, personal care products, flame retardants, insect repellants, etc.) and as many individual CECs as possible,
- evaluate each water sample for hazard using all available SVs including all effect-specific SVs for each CEC, not only the population-relevant mean SVs and comprehensive mean SVs, and
- expand SV development and usage - to include additional CECs in chemical categories already represented in this EHA, CECs in additional chemical categories, and groups of CECs with common modes of action.

Table 7-1. Summary of comparisons of paired hazard scores determined in 11 replicate surface water samples collected in Tinkers Creek in 2013, for each of five pharmaceuticals - carbamazepine (Ca), citalopram (Ci), diphenhydramine (Di), lidocaine (Li), and venlafaxine (Ve). The hazard score pairs consisted of a score for measured aqueous CEC concentration in one replicate sample and the other score for an estimated aqueous CEC concentration from total concentration measured in the other replicate water sample.

Effect Category		Number of Hazard Score Comparisons	Number of Hazard Overestimations (CECs involved)	Percent (%) Hazard Overestimates
TOTAL		308	24	7.8
Population-relevant	Mean SV	55	6 (Ca, Ci, Ve)	10.9
	Behavioral	55	1 (Ci)	1.8
	Developmental	22	2 (Di)	9.1
	Growth	11	0	0
	Mortality	22	3 (Ve)	13.6
	Reproductive	22	0	0
Comprehensive	Mean SV	55	6 (Ci, Di, Ve)	10.9
	Circulatory/ Blood Constituents	11	0	0
	Endocrine	11	1 (Ve)	9.1
	Genotoxicity	0	No comparison	No comparison
	Gross Pathology	0	No comparison	No comparison
	Histopathology	22	5 (Ca, Ve)	22.7
	Neurological	11	0	0
	Physiological/ Metabolic	11	0	0

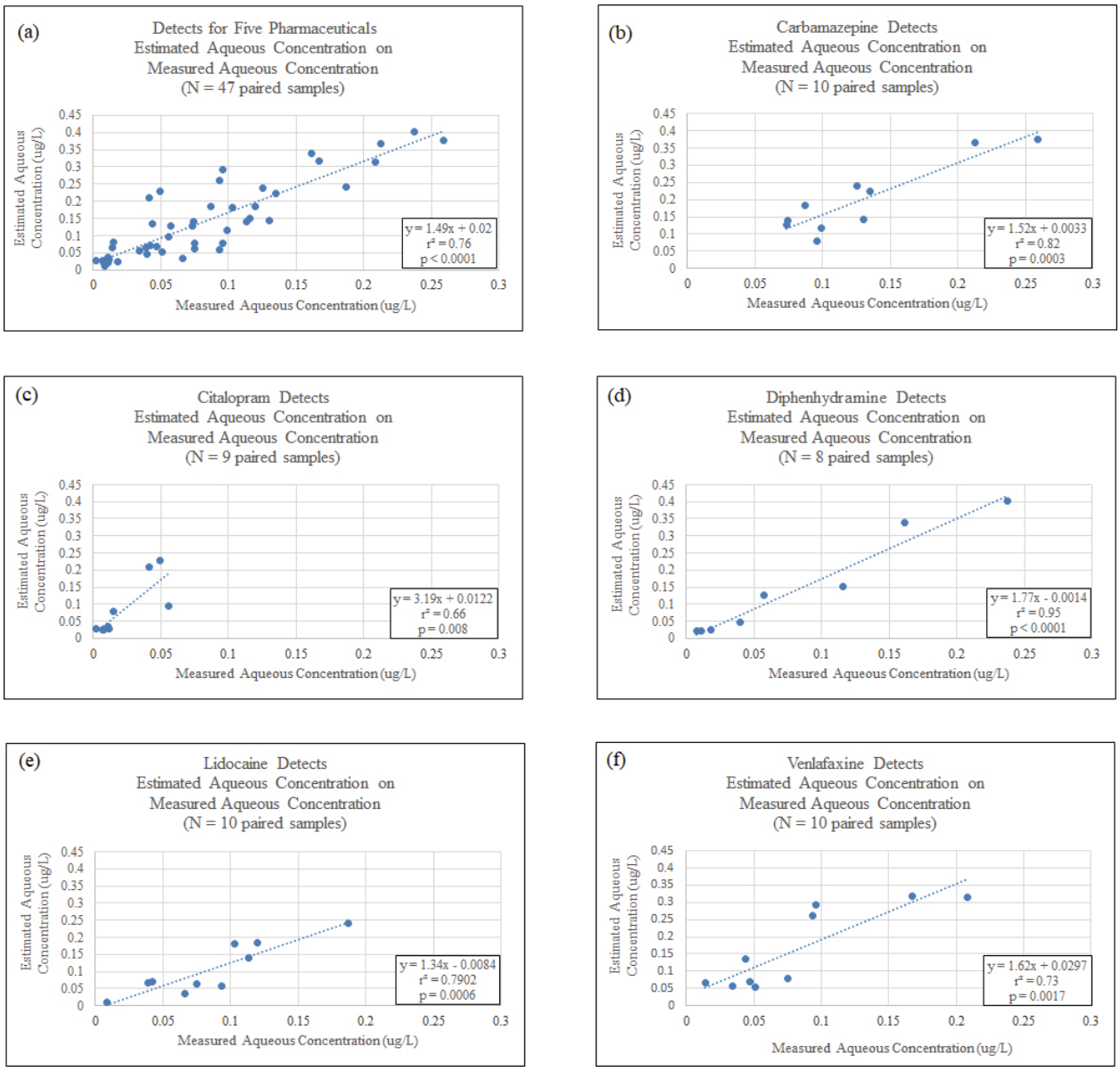


Figure 7-1. Quantitative relationships between measured aqueous concentrations in samples collected in Tinkers Creek in 2013 and aqueous concentrations estimated from measured total concentrations in replicate samples. Regression slopes > 1 indicate CEC-specific potential for overestimating aqueous CEC concentration from total concentration using the estimation methods presented in this EHA.

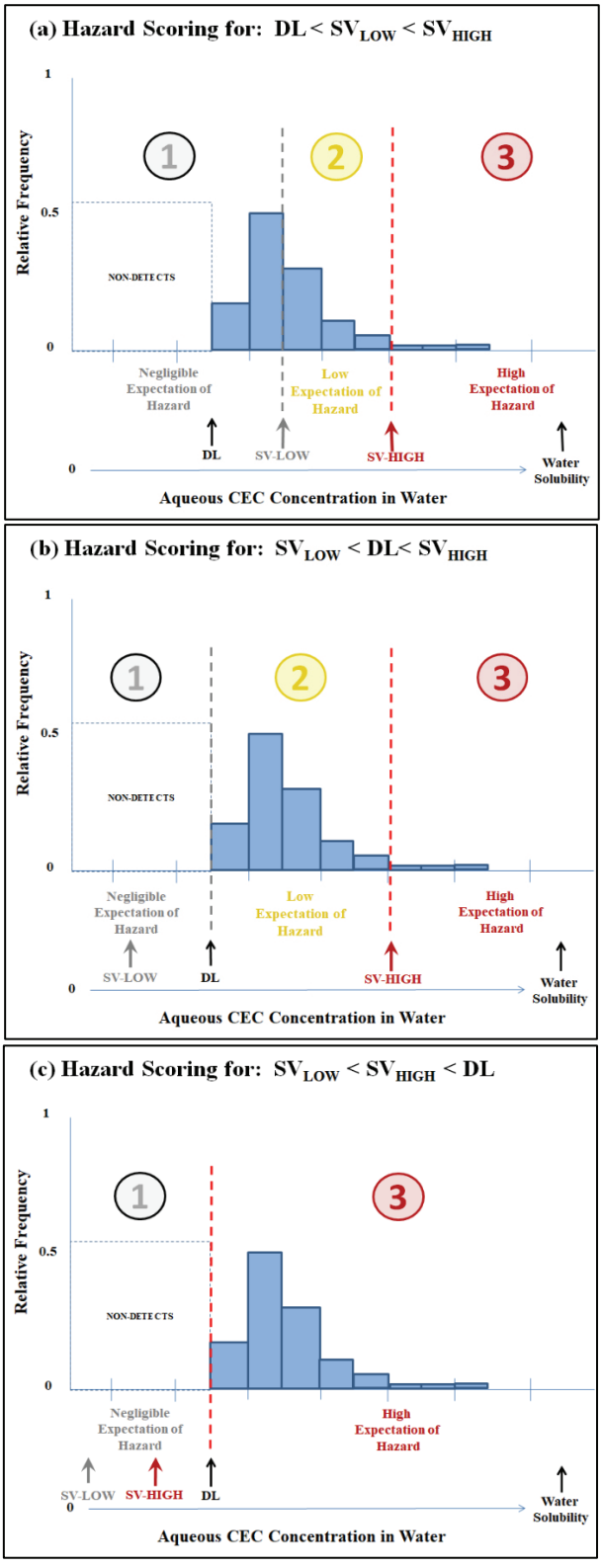


Figure 7-2. Representations of three possible quantitative relationships among SV_{LOW}, SV_{HIGH}, and DL (detection limit, as either method detection limit or reporting limit) and interpretations of hazard corresponding to ranges of aqueous CEC concentration bounded by these values. Numerals within the circles are hazard scores assigned to the defined ranges of CEC concentration. The three hazard scoring scenarios are:

- (a) $DL<SV_{LOW}<SV_{HIGH}$, hazard scores were defined as follows:
  - '1' where CEC is either not detected or concentration < SV_{LOW},
  - '2' where SV_{LOW} < CEC concentration < SV_{HIGH}, and
  - '3' where SV_{HIGH} < CEC concentration
- (b) $SV_{LOW}<DL<SV_{HIGH}$, hazard scores were defined as follows:
  - '1' where CEC is not detected,
  - '2' where CEC concentration is detected and < SV_{HIGH}, and
  - '3' where SV_{HIGH} < CEC concentration
- (c) $SV_{LOW}<SV_{HIGH}<DL$, hazard scores were defined as follows:
  - '1' where CEC is not detected, and
  - '3' where CEC concentration is detected

Literature Cited

(This section includes papers that are cited in Attachments)

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