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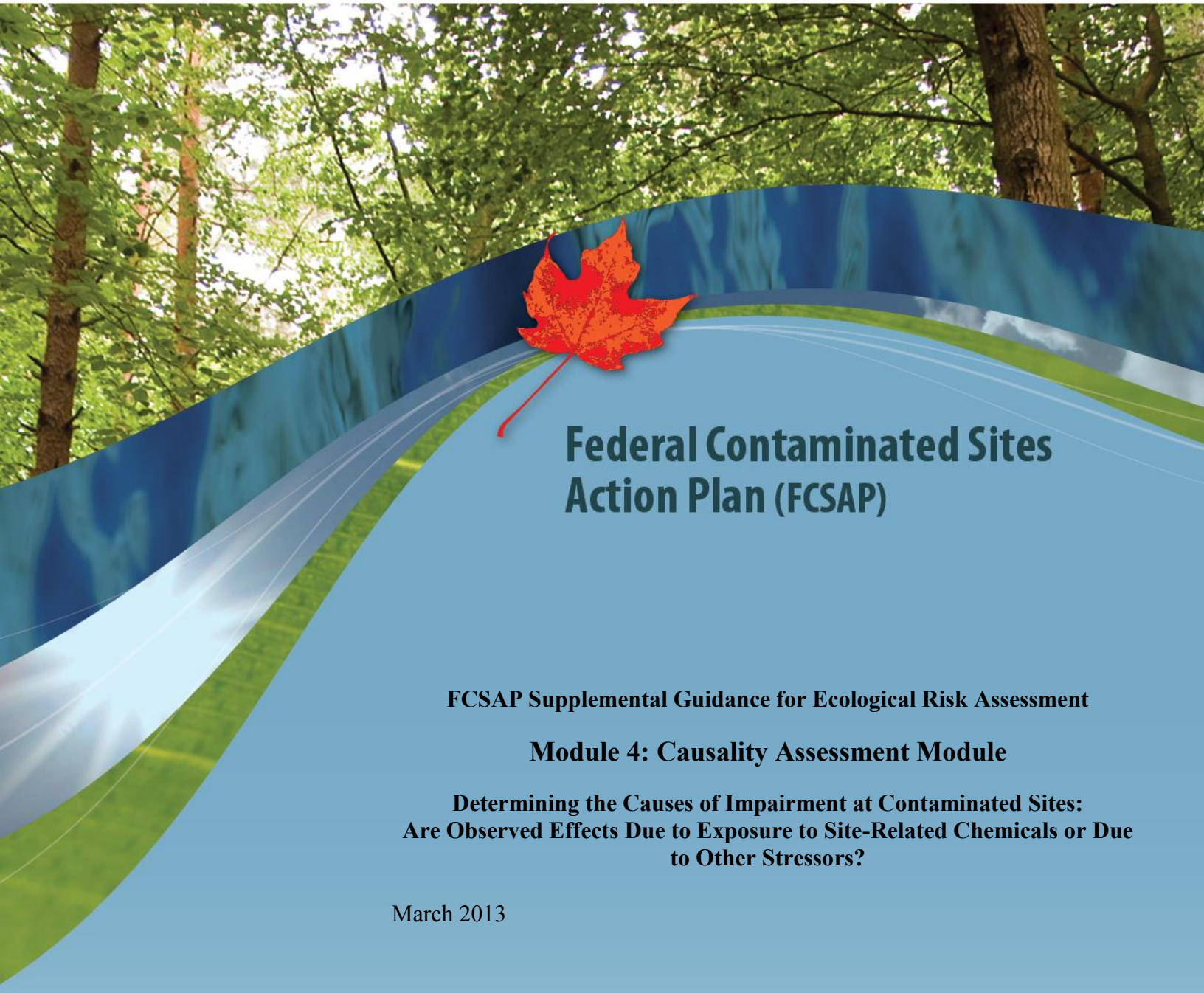

Federal Contaminated Sites Action Plan (FCSAP)

FCSAP Supplemental Guidance for Ecological Risk Assessment

Module 4: Causality Assessment Module

Determining the Causes of Impairment at Contaminated Sites: Are Observed Effects Due to Exposure to Site-Related Chemicals or Due to Other Stressors?

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FCSAP Supplemental Guidance for Ecological Risk Assessment Causality Assessment Module 4 Determining the Causes of Impairment at Contaminated Sites - Are Observed Effects Due to Exposure to Site-Related Chemicals or Due to Other Stressors?

Issued also in French under title:

Lignes directrices supplémentaires du PASCF pour l'évaluation du risque écologique Module 4 sur l'évaluation de causalité déterminer les causes de dégradation des sites contaminés : les effets observés sont-ils dus à l'exposition aux produits chimiques présents sur le site ou sont-ils plutôt causés par d'autres agents de stress?

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1.0 BACKGROUND

The Federal Contaminated Sites Action Plan (FCSAP) was developed to support federal departments, agencies and consolidated crown corporations to reduce the risks to human health and the environment, as well as to reduce the financial liabilities associated with federal contaminated sites. Under FCSAP, ecological risk assessments (ERAs) are commonly used as a site management tool at federal contaminated sites. The FCSAP Ecological Risk Assessment Focus Group has developed guidance for ERA supplemental to the existing CCME guidance (1996, 1997). The FCSAP ERA guidance consists of a comprehensive main ERA guidance document (FCSAP, 2012a) and several specific technical guidance modules (FCSAP, 2010a,b; 2012b).

This document is a technical guidance module on conducting a causality assessment for an ERA. Causality assessment is triggered by observation of ecological impairment and uncertainty as to the cause of the impairment (i.e., which stressors are causing the effect).

> ***Impairment*** is a detrimental effect on the biological integrity of a population, community or ecosystem that prevents attainment of the designated use. Examples of types of impairment are listed in Table 1-1 of this module.

The objective of this module is to provide guidance for evaluating causation – to help differentiate ecological impairment due to chemical stressors from natural variability and from impairment due to other stressors, such as biological or physical stressors. The ultimate objective of causality assessment is to identify factors that can be regulated or remediated in order to improve biological conditions (Suter et al. 2002).

1.1 Causality Assessment in ERA

At least since the 1950's, risk assessors, wildlife managers and ecotoxicologists have explored the technical challenge of determining whether chemical stressors are causative agents of ecological impairment (e.g., Carson, 1962; Fox 1991, Suter et al. 2010, Cormier et al. 2010; Gilbertson, 1997). Fox (1991) coined the term "ecoepidemiology" to mean the study of the ecological effects that are prevalent in certain localities or among certain population groups, communities, and ecosystems and their potential causes." Fox (1991) is perhaps the most influential paper on the topic of causality assessment in applied ecology, due to his use of formal criteria for evaluating causation, as well as the subsequent application of the approach to high profile problems – the decline of fish, birds, and reptiles in the Laurentian Great Lakes.

As discussed by Wickwire and Menzie (2010), formal methods to assess causality evolved to provide risk assessors and risk managers organized frameworks for weighing evidence and decreasing the likelihood of making remediation decisions based on a flawed understanding of the cause(s) of ecological impairment. Progress toward a formal framework to assess causality

for aquatic systems was advanced by the US Environmental Protection Agency (USEPA) and US Fish and Wildlife Service (USFWS) (Durhan et al. 1992, Meyer and Barclay 1990, USEPA 2000a), as well as a series of articles published in the *Journal of Toxicology and Environmental Health* (1991, Volume 33, Issue 4) and *Environmental Toxicology and Chemistry* (2002, Volume 21, Issue 4). USEPA's (2000a) Stressor Identification guidance on assessing the causes of biological impairments in aquatic systems was subsequently issued as a web-based tool called Causal Analysis/Diagnosis Decision Information System (CADDIS; http://www.epa.gov/caddis/). The January 2010 issue of the journal *Human and Ecological Risk Assessment* focused on perspectives and case studies of the application of formal causal analysis procedures to elucidate cause-effect relationships.

1.2 The Importance of Determining Causality

Expensive remediation and litigation decisions are often based on the assumption of causality and as such, it is essential that ERAs attempt to establish cause-and-effect relationships between stressors and responses (Suter et al., 2002; Day et al., 1997). It is important to know the identity of a stressor, so that its effects can be addressed (Day et al. 1997). If causality is not determined or if it is misidentified, expensive remedies may prove ineffective in reducing ecological risk. In addition to wasting time and money, such a mistake can erode public trust and patience, which in turn can make it difficult to reach agreement on remediation, even after causality is correctly determined.

Determining causation is easier said than done as there are many factors that may cause changes in biological conditions. Further complicating the process of determining causality is the fact that stressors do not act on organisms in a vacuum. Interactions between the effects of a natural stressor and a chemical stressor can sometimes result in greater effects than expected from either type of stressor acting alone (Holmstrup et al. 2010).

Despite the importance of determining causality in some circumstances, it is not necessary or even beneficial at every site or for every ERA. When the need for remediation and the cause of impairment are obvious – due to a well understood release, obvious impairment, a single stressor, low cost of cleanup, or other factors – risk managers may conclude that it is most practical to proceed with remediation rather than to further investigate risks and causes. Conversely, when ecological risks are not significant, causality is still a factor, but a causality assessment is likely unnecessary. Examples of scenarios for which an assessment of causality is unlikely to be warranted include: sites lacking any evidence of ecological impairment or significant adverse ecological risks; sites with obvious point source releases, such as leaking underground storage tanks, leaking above ground storage tanks and other petroleum releases; sites with a single or main stressor; and sites with multiple co-located stressors, such that remediation or risk management decisions would not be significantly affected by causality.

Figure 1-1 illustrates the physical, chemical and biological interactions of chemical stressors in an aquatic ecosystem. While this figure shows the stressors acting through the contaminants, stressors can also act on components of the ecosystem directly. Readers are also referred to Rattner and Heath (2003), who provide an overview of interactions between various environmental pollutants and temperature, salinity, water hardness, pH, dissolved oxygen, non-ionizing radiation, photoperiod and season.

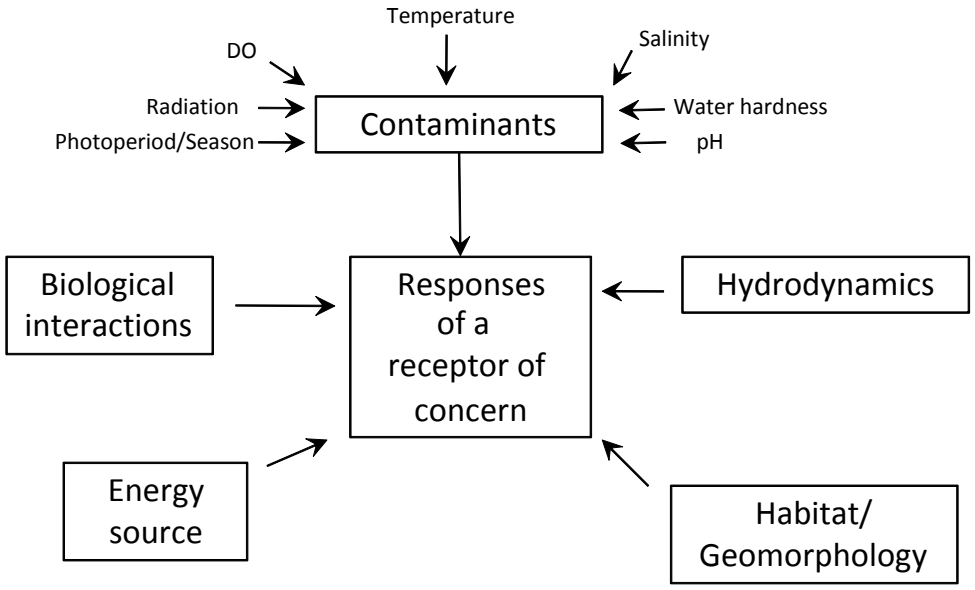

Figure 1-1 Physical and Natural Factor Interactions with Chemical Stressors in an Aquatic Ecosystem
(Modified from Foran, J.A. and Ference, S.A. Eds. *Multiple Stressors in Ecological Risk and Impact Assessment* SETAC Press, Pensacola. FL, 1997)

An array of chronic (e.g., reproductive), lethal or sublethal effects may trigger or indicate the need for a causality assessment. These triggers should be considered in conjunction with the weight of evidence analysis in the ERA to determine whether a causality assessment is required. Some examples of triggers are provided in Table 1-1.

Table 1-1 Examples of Causality Assessment Triggers

• Observed kills of plants, invertebrates, fish or wildlife
• Observed anomalies, such as tumors, lesions, high parasite load of individuals, high prevalence of disease within a certain population, chlorosis
• Observed changes in multiple individual organisms within a population, such as size-at-age, growth, condition, productivity, or survival
• Observed changes in community structure, such as absence, reduction or dominance of a particular taxon
• Adverse responses of ecological indicators, such as the Index of Biotic Integrity (IBI) or the Invertebrate Community Index (ICI)
• Changes in population, ecosystem, or landscape-level endpoints, such as loss of important habitats

In contrast, detection of chemical concentrations in soil, sediment, water, or air that exceed ecotoxicological benchmarks (e.g., CCME soil and water quality guidelines) – if that is the sole line of evidence available – is not sufficient evidence of impairment to warrant proceeding immediately with a causality assessment. Likewise, hazard quotients [HQs] >1 also are not sufficient evidence of impairment to warrant proceeding immediately with a causality assessment (See FCSAP, 2012a for additional details). Chemical concentrations above benchmarks or HQ >1 indicate that further investigation is required in order to determine whether those elevated concentrations or doses are actually causing impairment prior to proceeding with a causality assessment. For aquatic sites, the Framework for Addressing and Managing Aquatic Contaminated Sites under FCSAP (Chapman, 2011), provides further guidance. In addition, federal mining regulations include guidance on investigation of cause related to effects at Canadian mining sites (Environment Canada, 2012).

When designing the studies that will be used to make that determination, risk assessors are encouraged to consider other stressors that may influence any observed impairment. Such stressors might include biological, physical and chemical stressors. These stressors are discussed in further detail in Section 2.

1.3 Scope of Module

This module provides guidance to help risk assessors design and execute ERAs for FCSAP sites that consider cause(s) of impairment, with the overarching goal of ensuring that the ERA supports practical and appropriate risk management decision-making. The framework is scaled to the complexity and size of most FCSAP sites. This framework is based on USEPA's (2000a; CADDIS) Stressor Identification guidance and is consistent with practices recommended by Suter et al., (2010). Certain modifications were made to USEPA (2000a) guidance to extend it to terrestrial sites and to simplify the process so that it is better suited to the small sites that are prevalent under FCSAP. Sites that are very complex – particularly with respect to the number of stressors, range of data, and/or variety of habitats present – may benefit from direct application of USEPA's (2000a) framework. The framework described in this module applies equally well to aquatic and terrestrial sites.

This module focuses on chemical and non-chemical stressors and methods for determining causality – that is, when impairment is observed, what steps should be taken to understand the cause or causes of that impairment?

Different projects will consider questions of causality at different stages, depending on the current state of understanding of the site in question. This module primarily targets work conducted after impairment has been identified. It is assumed here that sufficient sampling and analyses have already been undertaken to establish that: 1) there is the potential for ecological

risk due to chemical stressors; and 2) other stressors (e.g., natural or anthropogenic, and physical, chemical or biological) are present at the site, such that it is not yet possible to conclusively determine that the chemical release is the sole cause of the observed impairment.

Causality assessment is related to but distinct from risk ranking, wherein multiple stressors are ranked with respect to the potential risk they may pose to a receptor, habitat, or ecosystem. Although this module does not address risk ranking, interested readers are referred to Landis and Wiegers (1997), Wiegers et al. (1998), and Obery and Landis (2002) for an introduction to such approaches. This module also does not directly address biological assessment, reference conditions, impairment detection, quality assurance, allocation of responsibility for releases, cost-benefit analysis, net environmental benefit analysis, management actions, or public consultation; however, these activities all interact with causality assessment in important and varied ways.

2.0 GUIDANCE

Causality assessment is comprised of four main steps which include: listing candidate causes; integrating causality into study design and sampling; analyzing data for causality and weighing the evidence and drawing conclusions (See Figure 2-1). An overview of these steps is provided in this section with more details being provided in Sections 2.1 to 2.4.

> ***The complexity of the causality assessment process*** should be scaled to the spatial and temporal scale of the site, as well as to budget and schedule constraints. The entire process may be iterative, as each stage of data analysis may reveal new uncertainties or data gaps.

Step 1 is to identify candidate causes of the impairment, often with the help of stakeholders and risk managers. Because causality assessments described in this guidance are conducted for contaminated sites, chemical exposure is always among the candidate causes identified. Additional candidate causes may include biological or physical stressors, such as invasive species, eutrophication, habitat destruction, flooding, and navigational dredging. At this stage, it is also worth considering whether the impairment may reflect natural variability, rather than an adverse response to a stressor, whether site-related or not.

In Step 2, the risk assessor evaluates the sufficiency of the available data as evidence confirming or refuting the roles of each candidate cause, and develops and implements a study plan to fill any data gaps. Available data may be site-specific, literature-based, local knowledge, *etc*.

In Step 3, evidence across a full range of candidate causes is compared minimizing errors from hypothesis dependence, confirmation bias, hypothesis tenacity, bias toward easy representation and over confidence (Norton et al., 2003). Data are analyzed and information linking the biological impairment to the candidate causes is considered.

> **General Principles for Minimizing Error in Causality Assessments**
>
> 1) Conduct the causal evaluation as a fair and transparent comparison among alternatives
>
> 2) Describe and quantify the conjunction of cause and effect
>
> 3) Consider whether that conjunction between cause and effect is spurious or that a real conjunction was masked (Norton et al. 2003).

In Step 4, after data are collected and analyzed, the evidence is evaluated using a weight-of-evidence approach. This approach is used to draw conclusions regarding which of the candidate causes are expected to have the greatest role in the impairment. It is also possible that the available data collected in the previous step may demonstrate that the observed biological impairment which triggered the causality assessment is not related to the site (e.g., comparison of site to reference data indicates observed impairment is likely due to natural variation). In that event the causality assessment is no longer needed. Finally, the available evidence is integrated to reach a conclusion regarding causality and/or the need for further investigation (implying data were insufficient).

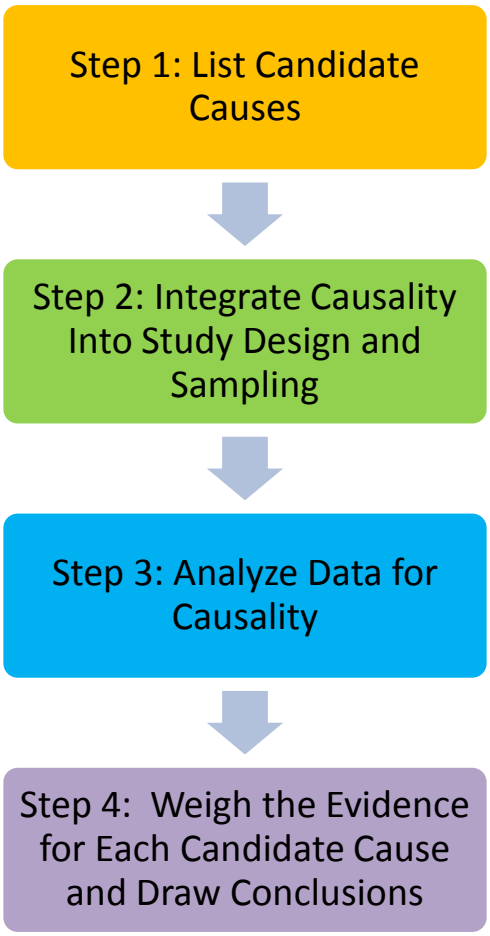

Figure 2-1 Causality Assessment Flowchart

2.1 Listing Candidate Causes

Following the observation of biological impairment at a contaminated site (see examples of triggering events in Table 1-1), the first major step in causality assessment is to list candidate causes (Step 1). Details of the components of this step are provided in the adjacent text box and text below.

Step 1. List Candidate Causes

- Observe and document the biological impairment
- Gather and review information to identify possible candidate causes
    - Gather background information (e.g., site setting, history, surrounding land use)
    - Conduct literature review on biological processes, mechanisms and case studies involving similar impairment
    - Synthesize findings related to background information and scientific literature for distribution before or during session
- Conduct brainstorming session
    - Background discussion (e.g., site history, setting, stressors, biological processes, mechanisms)
    - Brainstorm possible causes and eliminate implausible causes
    - Document outcomes of session

2.1.1 Observe and Document Biological Impairment

Before candidate causes can be effectively explored, specific impairment or adverse effects need to be documented.

In some cases, the observation of adverse biological effects, such as a fish kill or denuded vegetation, may have been the event that led to the site being identified as contaminated. The impairment should be described in terms of its nature, magnitude, and spatial and temporal extent. Ideally, the effect should be quantified as a count (e.g., abundance, diversity, mortality, incidence of malformations or lesions) or as a continuous variable (e.g., length, weight, hatching success, fledging success). Care should be taken to avoid describing multiple effects as a single impairment, as each effect may have a different cause. Documentation of the impairment should include a background statement regarding the data or process that revealed the impairment. It can be quite helpful to map the impairment. Geographic Information Systems (GIS) can be used to map locations, distribution and gradients in the effects observed. Characterization of the geographical extent of impairment is critical. However, GIS has a large demand for data and may have a limited value at sites if data are not sufficient. Identification of candidate causes also may be facilitated by mapping metrics that describe biological effects (e.g., reduced diversity of invertebrates, reduced fish size, reduced eggshell thickness, and reduced small mammal productivity). A discussion of some of the methodologies and tools used to characterize impairment at aquatic and terrestrial sites is provided in the following sections.

2.1.1.1 Considerations Specific to Aquatic Sites

The range of tools available for characterizing impairment at aquatic sites includes those that target organisms that reside within the water column and those that target organisms that reside within or on the sediment. Potentially useful data may come from: chemical analysis of effluents, organisms, ambient waters, and sediments; toxicity tests of effluents, waters, and sediments; necropsies; biotic surveys; caged organisms (e.g., mussels, fish); habitat analyses; hydrologic records; and biomarker analyses. A similar array of data may be obtained from other sites and from laboratory studies. Considerations specific to organisms inhabiting the water column and sediment are discussed in the following subsections.

Evaluation of Receptors Inhabiting the Water Column

Aquatic organisms inhabiting the water column include plankton, aquatic plants, invertebrates and fish. Fish are important from an ecological and human perspective; they are targets of contaminant monitoring programs and are usually selected as one of the receptors of interest in aquatic ERAs [See Alberta Environment (2008) for summary of fish monitoring programs]. Data from fish monitoring programs can be useful for a variety of purposes in causality assessment, such as definition of background conditions, spatial or temporal trends in chemical residues in fish tissue, fitness, and sex ratios.

The sensitivity of fish to physical, biological and chemical stressors has also been extensively studied. For these reasons, this discussion on considerations unique to aquatic organisms primarily focuses on fish. There are numerous factors that make the evaluation of fish unique with respect to causality assessment, ranging from the availability of data from large scale fish monitoring programs, their mobility, often long life spans, variety of study designs, and incidence of fish kills. These and related issues that warrant consideration in causality assessment are discussed below.

Several guidance documents describe the design of robust and scientifically defensible fish sampling programs (e.g., Barbour et al. 1999, Hicks 1999, Stirling 1999, Morgan 2002, USGS 2002, Skinner and Ball 2004, Weiner et al. 2006, Jones and Yunker 2009, USEPA 2000b, 2002, 2008b, http://www.env.gov.bc.ca/fish/methods/toolkits/fishsamp.html). This website from the University of Arizona (http://www.cals.arizona.edu/research/azfwru/scott/scott_overviewProtocols.htm) provides a compilation of fish sampling protocols issued by numerous states and provinces. In addition, CCME is expected to release guidance on sampling biota. The reader is encouraged to consult these and other sources prior to initiating fish sampling programs.

Even when extensive data exist through monitoring programs or other robust field programs, evaluation and diagnosis of causality can be challenging when impairment manifests in chronic sublethal effects. Two inescapable factors that complicate causality assessment in such cases are

that fish are mobile and many species are long lived. As a result, the risk assessor is not always able to objectively judge the attributes of "Time Order" and "Co-occurrence" (See Section 2.1.3). In the case of judging time order, the presence of species with long life spans in the environment may mean that, even after the stressor is removed (i.e., the source is controlled), specimens that were previously exposed continue to inhabit the area and continue to exhibit the same signs of impairment that occurred when the stressor was still present. Recovery in the form of the absence of biological impairment may take at least as long as a generation for the longest lived species. Mobility complicates the evaluation of co-occurrence, such that impairment in fish may not be exactly aligned spatially with the physical location of a stressor. This challenge is particularly evident in flowing waterways, such as rivers and streams, where fish populations are examined upstream of a stressor source, but the fish inhabiting upstream areas are exposed due to their mobility.

Evaluation of impairment and causality may also be complicated by the many different study designs employed to monitor fish. For example, male and female fish may use different habitats in different seasons and may metabolize and accumulate chemical stressors to varying degrees due to their differing life histories. If the sex of fish is not recorded during sampling or if fish samples of both sexes are pooled for other reasons, interpretation of trends over space and time can be confounded. Likewise, if methods of sample preparation (e.g., removal of skin vs. leaving intact, analysis of fillets vs. whole body vs. specific organ) are combined across multiple sampling programs, areas or time periods, observation of trends in exposure can be complicated by variable accumulation of contaminants in muscle, lipids, skin, liver, etc. The literature (e.g., Peterson et al. 2005, Amrhein et al. 1999, Bevelhimer et al. 1997) offers empirical relationships between fillet and whole body concentrations for different species and such algorithms can be used to convert data to a common basis. Targeting a broad range of size classes can improve the accuracy of estimates of dose to anglers and piscivorous wildlife, but can also introduce a great amount of variability and make it difficult to discern temporal or spatial trends that can aid in determining causality. This difficulty arises from the variability in tissue concentrations with age and size of fish (e.g., as many ecological receptors have a preferred prey size). For bioaccumulative chemicals, tissue residue concentrations typically increase with the age and size of a fish. Similarly, chemicals can also concentrate when fish fast (e.g., spawning salmon). However, for rapidly growing species, the dilution of chemical residues that occurs through the growth of fish can be a greater influence than fish age or lipid content. Again, the literature (e.g., Gilmour and Riedel 2000, Farkas et al. 2003, Bhavsar et al. 2008, Gewurtz et al. 2011) and www.fishbase.org offers empirical relationships for size-normalizing chemical concentrations in fish tissue. Targeting the size class can reduce variability and improve comparability of data between years of sampling.

Because different species (as well as different sexes and age classes) often vary in the percent lipids in tissue, evaluation of exposure to lipophilic chemical stressors, such as some PAHs, PCBs, and dioxins and furans can benefit from lipid-normalization of chemical residue levels. Wet weights may also be useful in some cases (e.g., if concentration of the lipophilic contaminant is used for exposure when daily food consumption is based on wet weight).

In contrast with some other receptors, the habitat requirements and the effects of varying habitat attributes on populations and community structure are generally well understood for fish. Hughes (1985) offers a robust methodology for selecting reference streams for estimating effects of metal mining wastes on fish.

Fisheries research is also fairly advanced in the development of field and laboratory methods for evaluating the effects of individual stressors on specific age classes or species. Other advancements in fisheries research include the use of biomarkers, including morphological (e.g., tumor prevalence in flatfish, male / female ratio), biochemical (e.g., measurements of enzyme activities), physiological (e.g., loss of hepatic vitamin A. vitellogenin production in males), and molecular (gene expression) endpoints. For example, Orrego et al. (2005) describe a straightforward laboratory toxicity design intended to determine the likelihood and severity of adverse responses of fish to pulp and paper mill effluent. Swanson et al. (1994) describe a similarly straightforward field study design, focusing on differentiating any effects of bleached-kraft mill effluent from natural stressors (e.g., flooding, low flow conditions, habitat) on fish growth, reproduction, age structure and community structure.

Despite the long and robust history of fisheries research, however, data gaps related to ecotoxicity and interspecies variability in sensitivity to chemical stressors can complicate causality assessment. Although the effects of some chemical stressors, such as mercury and DDT, on different species of fish and under different exposure conditions have been extensively studied (e.g., Drevnick et al. 2008), there may be a paucity of information on the effects of other stressors on different fish species and age classes. Under these circumstances, evaluation of causality will unavoidably introduce uncertainty, as it will be necessary to extrapolate across species, exposure durations, and/or age classes.

Causality assessment is relatively straightforward, however, under one particular type of impairment: fish kills. Fish kills occur when large numbers of fish within a localized area die within a fairly brief period, such that the presence of numerous dead fish is readily apparent. Such fish kills are an overt sign of impairment and they are not uncommon. The need to discern the cause(s) of fish kills has long been recognized by the fisheries community and high quality guidance is available to aid in diagnosing causes in the field (e.g., Meyer and Barclay 1990).

Evaluation of Benthic Organisms

As discussed by Rosenberg et al. (1997), benthic organisms (or benthos) possess several favorable attributes that result in their widespread study at aquatic sites (e.g., large number of species offer a spectrum of responses to environmental stressors, live in sediments so are directly associated with sediments, are sedentary in nature, have short life cycles, etc.).

Evaluation of benthos is further facilitated by the well established sediment quality triad approach (Chapman et al 1991, 1992; Environment Canada and Ontario Ministry of the Environment 2008). The sediment quality triad integrates data from physical and chemical analyses, laboratory exposure to whole (bulk) sediments, and benthic community structure in order to determine effects or impairment.

The sediment quality triad and other weight-of-evidence approaches such as the Canada-Ontario Framework (Environment Canada and Ontario Ministry of the Environment 2008) consider the potential for bioavailability and biomagnification. These approaches offer a fairly standardized set of field, laboratory, and literature-based tools that can be conducted and evaluated in tandem to weigh the overall evidence for or against chemical stressors as the causative agents at contaminated sediment sites. Borgmann et al. (2001) offers an example of an elegant analysis that pairs the sediment quality triad with consideration of the bioavailability of metals in lake sediment in order to help identify the cause of observed impairment.

Another assessment tool is provided by O'Brien et al. (2010) who describe a promising technique that combines rapid bioassessment and field-based microcosms to identify impairment.

The success of correctly assessing the health or degradation of benthic communities depends on how well the responses caused by chemical stressors can be discriminated from responses caused by other environmental factors (Rosenberg et al. 1997, Dunson and Travis 1991, Hughes 1995). Aquatic ERAs are liable to confuse natural variability with environmental degradation because a thorough understanding of the many natural factors that can influence or regulate variability is lacking (Landis et al. 1994). Reynoldson (1984), France (1990), and others have cautioned that benthic communities can respond to seemingly minor changes in substrate particle size, organic content, texture and water quality, as well as to the presence of chemical stressors. Spatial heterogeneity in depositional areas can be high, which necessitates large numbers of samples to distinguish between natural variability and anthropogenic impairment (Rosenberg et al. 1997).

Site-specific variables that may influence the local benthic community include morphology (e.g., gradient, width, depth, substrate type, area, volume), hydrodynamics, temperature, dissolved oxygen, tidal cycles, microhabitats, pH, nutrients, and climate. If their influence on the benthic community is not explicitly recognized and addressed (e.g., during the selection of reference areas), they may confound interpretation of community structure surveys and toxicity tests.

2.1.1.2 Considerations Specific to Terrestrial Sites

It is fairly common to initiate evaluation of a terrestrial site (or terrestrial receptors) through a HQ type analysis, in which exposure (usually expressed as a modeled dose in milligrams chemical per kilogram body weight per day or mg/kg-day) is estimated based on literature-derived input values and equations and then compared to a literature-derived threshold (generally referred to as a toxicity reference value or TRV) or dose-response curve. The FCSAP Supplemental Guidance for Ecological Risk Assessment includes a module on the Selection or Development of Site-specific TRVs that should be consulted for additional information on this topic (FCSAP, 2010b). Ecological risk assessors typically assume that, if exposure is below the TRV (i.e., HQ < 1), then the potential for adverse effects is low and no further evaluation is necessary. However, HQs > 1 indicate only the potential for adverse effects and do not demonstrate actual impairment (Tannenbaum, 2003, 2005).

Bioassays and/or field studies are critical for verifying the conclusions of HQs. Example field studies used at terrestrial sites include tissue residues in plants, insects, rodents; hair analysis, blood and tissue sampling (for biomarker purposes) and colonization experiments. Other examples include Rodent Sperm Analysis (RSA; Tannenbaum et al. 2003, 2007, Tannenbaum and Thran 2009), nest and nestbox monitoring studies (e.g., Henning et al. 2002, 2003, Custer et al. 1998, 2005), immunosuppression testing (e.g., Grasman et al. 1996), amphibian mesocosm studies (e.g., Boone et al. 2005), mink feeding studies (e.g., Aulerich and Ringer 1977) and mink field studies (e.g., Osowski et al. 1995). The FCSAP Supplemental Guidance for Ecological Risk Assessment module on Toxicity Test Selection and Interpretation (FCSAP, 2010b) should be consulted for additional information on the topic.

Further detail on the spectrum of tools available for evaluating contaminant-effect endpoints in terrestrial vertebrates is provided in Rattner et al.'s (2000) overview of endpoints applicable to individual organisms or higher levels of ecological organization (e.g., biomarkers, bioassays, and bioindicators of contaminant exposure and effects). It is worth noting that many of the study designs discussed by Rattner et al. (2000) are only feasible at sites that are large enough to support a large number of the target organisms, in order to ensure that sample sizes are sufficient to detect a significant difference between exposed and unexposed organisms, if such a difference actually exists. That is, if the small size of a site prevents a field study from having high statistical power then study design limitations may falsely conclude the exposed population is not impaired. Newman (2008) provides guidance related to hypothesis testing that may be helpful to assessors. Risk assessors are encouraged to consult statisticians during study design in order to avoid such problems. Additionally, the cost of such analyses for birds and mammals is substantially higher than it is for aquatic and benthic invertebrates, such that relatively few

FCSAP sites are suspected to have sufficiently serious environmental contamination to warrant such studies.

2.1.2 Gather and Review Information to Identify Possible Candidate Causes

Once the biological impairment is identified and documented, candidate causes are then identified through consideration of the site setting and history, publications that document cause-effect relationships for similar impairments at other sites or under other conditions (generally, as reported in the scientific literature), or knowledge of biological processes or mechanisms. A literature review may be very important in identifying candidate causes for a given effect. Additionally, where data are available for a given site, provincial or federal monitoring programs (e.g., Environment Canada's Ecological Monitoring and Assessment Network (EMAN), the Canadian Aquatic Biomonitoring Network (CABIN), and Environment Canada's Environmental Effects Monitoring (EEM)) can aid in identifying stressors potentially present at the site. Two end products result from this first step: 1) a narrative, tabular, and/or graphical illustration of the specific impairment; and 2) a list of candidate causes, including the basis for including each.

Candidate causes may include physical stressors, chemical stressors and biological stressors. Some of the more common physical, chemical and biological stressors that can cause biological impairment at aquatic or terrestrial sites and that are likely to be present at many contaminated sites are summarized in Table 2-1. This table does not present a comprehensive list of all possible stressors; it does present the most common sources of impairments to biological community composition and function in both terrestrial and aquatic environments. Many of the other stressors have more subtle effects that are manifested over prolonged periods. Therefore, careful selection of reference sites is critical for determining the likely cause of impairments at contaminated sites. For additional details on the use of reference or background conditions, please refer to the FCSAP Ecological Risk Assessment guidance manual (FCSAP, 2012a).

Common indications that a stressor may be a factor at a particular site and the more important effects of those stressors on biological communities are described in Attachment A.

Readers are referred to the following sources for examples of more comprehensive treatment of the subject:

- Environment Canada's *Threats to Sources of Drinking Water and Aquatic Ecosystem Health in Canada* Report (Environment Canada 2001)
- The Canadian Wildlife Service Strategy for Wildlife Conservation (CWS 2000)
- USEPA's (2000a) List of Stressors[1]

[1] http://www.epa.gov/caddis/si_step2_stressorlist_popup.html

Table 2-1 Examples of Potential Stressors, Possible Indicators and Candidate Causes

Stressor Type	Potential Indicators	Example of Potential Candidate Cause
Biological	Decreased diversity, altered community composition	Non-native invasive species
	Dense algal mats/blooms, low dissolved oxygen, fish kills	Nutrient enrichment
	Increased incidence of mortality/deformities, altered species composition	Pathogens [1]
	Altered community composition	Harvesting
Physical	Decreased diversity, altered community composition	Habitat degradation; Hydrologic alteration
	Loss of temperature-sensitive species	Temperature
	Loss of sensitive species, altered community composition	Climate change
Chemical	Increased sediment toxicity related to development and storm events	Stormwater
	Decreased soil fertility, decreased primary production, altered community composition	Acidification
	Decreased fish size; decreased number of small mammals	On-site chemical exposure [2]

1 Caution should be used in ascribing high pathogen frequency at a site as the cause of impairment without also considering the possibility of chemical exposure stressing the receptors and reducing disease resistance.
2 On-site chemical exposure is always an implied candidate cause at a contaminated site.

2.1.3 Conduct Brainstorming Session

After characterizing the impairment and identifying an initial list of possible candidate causes, it is advisable to schedule a brainstorming session. The objective of this session is to finalize the range of possible causes for the impairment and to eliminate implausible causes. This session should include for example, people with specialist knowledge on biological effects assessment and people with local knowledge who know the changes the site has undergone. This may be conducted as a group effort (e.g., at a stakeholder meeting), a team effort (e.g., at a project team meeting), or individually (e.g., by individual risk assessors). Depending on the scope and severity of the impairment, as well as the engagement of the stakeholders, the background material developed may be compiled into a background memorandum or report that is distributed to participants in advance of the session. Alternatively or in addition, the background material may be presented verbally as an early agenda item at the session. In either case, the goal of compiling and presenting background information is to ensure that participants share a common understanding of the site history and setting, as well as the state of scientific knowledge related to possible causes of the type(s) of impairment observed.

Identifying a suite of candidate causes will help guard against the tendency to place undue confidence in any single plausible cause (Norton et al. 2003). Six fundamental characteristics of causation (Cormier et al. 2010) may prove helpful in determining plausibility of candidate causes:

- Time order – the cause (e.g., the stressor and exposure to the stressor) must occur before and / or during the effect.
- Co-occurrence – the cause co-occurs with the receptor in space and time.
- Preceding causation – causes and their effects are the result of a larger web of cause and effect relationships; that is, evidence of the network or pathways that preceded the causal relationship under investigation increases confidence that the causal event actually occurred.
- Sufficiency – the intensity, frequency, and duration of the cause are adequate and the susceptible receptor can exhibit the type and magnitude of the effect.
- Interaction – based on general (e.g. literature-derived) knowledge, the cause is expected to interact with the receptor in a way that induces the effect.
- Alteration – based on general (e.g. literature-derived) knowledge, the receptor is expected to be changed by the interactions with the cause.

Some types of evidence can support more than one characteristic of causation (See USEPA, 2010; http://www.epa.gov/caddis/si_step_characteristics_popup.html) and may come from the specific case under investigation or from other similar cases. Once potential candidate causes

are selected or excluded based on consideration of the characteristics of causation, evidence supporting or refuting each of these types of characteristics will be identified and scored (See Step 4; Section 2.4).

Certain possible causes are clearly implausible or otherwise not applicable and should be excluded from the list of candidate causes (Table 2-2). For example, if the impairment occurred prior to the introduction of the stressor, that stressor is not a plausible candidate cause because it violates the time order characteristic. Similarly, if long-term monitoring indicates that effects continue even after a stressor is substantially or completely removed, it may be appropriate to exclude that stressor from the list of candidate causes[2]. If there is no discernible co-occurrence (in time and/or space) between the stressor and the impairment, that stressor also may be excluded from the list of candidate causes. Except in some cases of effects on mobile species, such as migratory fish, a candidate cause can be dismissed if the effect occurs upstream of the candidate cause or if the cause occurs at reference sites at similar or greater levels. The absence of an exposure-response relationship may also serve as sufficient reason to dismiss a candidate cause (e.g., responses increase with decreasing exposure, or relationships between exposure and response are random)[3]. Table 2-2 illustrates an array of conditions under which candidate causes can be readily eliminated due to implausibility. Caution should be used when eliminating possible causes as there may be some exceptions to the norm (e.g., expecting a negative response in samples upstream of a potential stressor may be incorrect if the stressor is mobilized through groundwater). Similarly, candidate stressors should not be excluded before it has been made certain that they do not contribute to the observed causation (e.g., increased parasite infestation due to immunotoxicity) or are part of an underlying association (e.g., positive correlation between blood hormone concentrations and PCB exposure concentrations could be an artifact of an underlying association of both parameters with body weight).

Among the candidate causes, there may be suites of causes that interact, sometimes additively, sometimes synergistically and sometimes antagonistically. When multiple stressors contribute to an effect, the stressor that makes the greatest contribution is the primary cause. Usually, the primary cause is so dominant that removal of the secondary stressors does not result in a significant or detectable change in the condition. In other cases, two or more stressors may be required in order for the effect to occur. For example, a moderate level of nutrients (i.e., nitrogen, phosphorus) in a water body poses no toxicological threat alone, but if reduced riparian cover (i.e., by land clearing) permits sufficient sunlight to increase algal growth, then eutrophication

[2] Persistent and bioaccumulative chemical stressors can be important exceptions to this guidance, in that they may continue to be present in the tissue of organisms, even after they have been removed from abiotic media. Note also that recovery periods will vary across chemicals and across species and ecosystems. Hence, even after removal of a cause, effects may be observed for some weeks, months, or even years.

[3] However, if the absence of a detectable dose-response relationship is due to data limitations, it would be premature to eliminate that candidate stressor solely due to the absence of a dose-response relationship.

can occur, with a subsequent cascade of effects (USEPA 2000a). Stressors that act together to cause an impairment should be listed as a single scenario.

After identifying candidate causes, the risk assessor closes out Step 1 by documenting the outcomes of the brainstorming session. This includes preparing two work products: 1) a narrative, tabular, and/or graphical illustration of the specific observed impairment; and 2) a list of plausible candidate causes, including the basis for each.

Table 2-2 Application of Common Types of Evidence in Eliminating Alternatives

Type of Evidence	Reason for Rejection	Masking Considerations	Causal Consideration[1]
Associations between measurements of candidate causes and effects: Did the stressor precede the effect in time?	If the effects preceded a candidate cause in time, it cannot be the primary cause.	If the candidate cause is preceded by both the effect and another sufficient cause, its effects may be masked and it should be retained.	Temporality
Associations between measurements of candidate causes and effects: Is there an upstream/downstream conjunction of candidate cause and effect?	If the effect occurs upstream of the candidates cause's source or does not occur regularly downstream (e.g. is distributed spatially independently of a plume, sediment deposition areas, etc.) it cannot be the primary cause.	If the candidate cause is downstream of another sufficient cause, its effects may be masked and it should be retained.	Co-occurrence
Associations between measurements of candidate causes and effects: Is there a reference site/test site conjunction of candidate cause and effect?	If a candidate cause occurs at reference sites and occurs at equal or greater levels, it can be eliminated.	If insufficient data are collected or if reference sites are not similar to the test site, effects may be masked	Co-occurrence
Associations between measurements of candidate causes and effects: Is a decrease in the magnitude or proportion of an effect seen along a decreasing gradient of the stressor?	A constant or increasing level of effect with significantly decreasing exposure would eliminate a cause.	If a decreasing gradient of one sufficient cause coincides with an increasing gradient of second, recovery from the first cause may be obscured	Biological Gradient
Measurements associated with the causal mechanism: Has the stressor co-occurred with, contacted, or entered the receptors(s) showing the effect?	If the candidate cause never contacted or co-occurred with the receptor organisms, the cause may be eliminated. For appropriate stressors, if tissue burdens or other measures of exposure are found not to occur in affected organisms, the cause may be eliminated. For stressors that act through a known chain of events, if a link in the chain can be shown to be missing, the candidate cause can be eliminated.		Complete Exposure Pathway
Association of effects with mitigation or manipulation of causes: Did effects continue when a source or stressor was removed?	If the effect continues even after the stressor is removed, then the candidate cause can be eliminated. This assumes that there is no impediment to recolonization.	The effect may also continue if another sufficient cause is present	Experiment, Temporality Bioaccumulative substances may alter/mask expected recovery

1. Many of the same types of evidence can also be used in the strength of evidence analysis. This column denotes the corresponding causal consideration used there.
Source: USEPA 2000a

2.2 Integrating Causality into Study Design and Sampling

The second major step in causality assessment is to gather evidence of the relationships between the candidate causes and the observed impairment to understand which of the candidate causes is most likely associated with the observed impairment (Step 2).

Step 2. Integrate Causality Into Study Design and Sampling

- Inventory available data and Identify Gaps Relative to Outcomes of Step 1
  - Assemble site-specific data
  - Refer to literature for sources from other cases, and ideas for applicable study designs
- Develop and execute study plan
  - Define optimal study plan
  - Adapt as needed to account for schedule and budget constraints
  - Verify suitability of reference areas
  - Verify that potential confounding factors are addressed
- Confirm compatibility of study design, planned data analysis and modeling with outcomes of Step 1

2.2.1 Inventory Available Data and Identify Data Gaps

Evidence available through previous studies conducted at the site or published in the scientific literature will have been compiled, while other evidence will require new data collection based on data gaps identified. Data gaps are identified following review of available data and possible candidate causes. The identification of data gaps will guide the risk assessor as to what additional data need to be collected.

New data collection necessitates developing and executing a study plan.

2.2.2 Develop and Execute Study Plan

When existing data do not provide sufficient evidence to determine causality, new data must be collected – typically in the form of laboratory bioassays or field studies. It is advisable to document the planned methods in a study plan that details the sampling design, sampling locations, sampling methods, quality assurance and quality control, sample processing and shipping practices, analytical methods, data management practices, and methods for analyzing the resultant data. Recognizing that the underlying purpose for the study or studies is to support causality assessment, the study plan should be developed within a statistical framework, and carefully reviewed with respect to the appropriateness of reference areas, consideration of confounding factors, and documentation of ancillary causes related to all candidate causes (e.g., Benthic habitat at a site is physically altered and chemically contaminated, with chemical contamination

There is no minimum quantity of data required for causality assessment. Existing data may be sufficient to determine the cause of impairment. However, a critical element of causality assessment is the evaluation of whether the data are sufficient to determine causality.

being the principal cause. Restoring physical habitat may have no effect until chemical contamination is removed; habitat alteration is an ancillary cause but is masked by the principal cause). Where applicable, a statistician should be consulted during the study design and prior to the commencement of field work. Statistical tools, such as power analysis, can be considered in the study design phase. The study plan needs to account for schedule and budget constraints of the project while still addressing data gaps.

Comparison of site conditions to reference conditions necessitates careful evaluation of the suitability of the reference areas or conditions employed. Background or baseline levels are independent of a reference site or population and are drawn from common knowledge or historical data (Suter et al. 1999). The local reference approach consists of selecting reference sites and pairing these sites with one or more sites under evaluation. Reference sites are selected based on similarity to the assessment sites in all respects but for the perturbations of interest at the subject site. Examples of guidance on the selection of reference sites for use in ecological risk assessment is offered by Suter (1993), CCME (1996), Munkittrick et al. (1999), Munkittrick and McMaster (2000), Barnthouse et al. (2008), and Environment Canada and Ontario Ministry of the Environment (2008). While multiple reference sites are preferred, it is recognized that not all sites will have the scope and / or budget for this approach. Therefore, in cases where a single reference site is used, its shortcomings should be documented (i.e., potentially influencing factors that differentiate it from the site in question).

Munkittrick et al. (1999) suggest that poorly selected reference sites are the most common criticism of ecological studies and are often used as a scapegoat for avoiding interpretation of differences. Munkittrick and McMaster (2000) note that, because it can be very difficult to pick a good reference site, it is relatively common to use multiple reference sites in order to better characterize variability in reference conditions.

The reference population approach offers the greatest ability to detect a significant, meaningful difference between a reference site and the site in question. In this approach, a population of sites or organisms that is least exposed to the stressors of interest is assumed to exist. That population is surveyed and sampled and the distribution of the relevant measures and derived indices is estimated. Gradient designs, in which sites are established on a gradient of stressors, from low to extremely stressed, allows determination of the biological responses to the stressors (Thompson 1992, Suter 1993).

The published literature offers abundant examples of studies on fish populations and communities that illustrate critical factors in the selection of reference sites (e.g., Gagnon et al., 1995; Schlosser, 1990; Poff and Allan, 1995). Similarly, the Canadian Aquatic Biomonitoring Network (CABIN) may provide references for invertebrates (http://www.ec.gc.ca/rcba-cabin/).

Another important task in study design is to verify that potential confounding factors are addressed. USEPA (2000a) notes that identifying and isolating confounding factors can improve the risk assessor's ability to identify associations between candidate causes and effects. For example, the frequency of hepatic neoplasms in fish is independently correlated with the age structure of the fish population and with the concentration of polycyclic aromatic hydrocarbons (PAHs) in sediment (Baumann et al. 1996). Similarly, bird species diversity typically increases in ecotones (areas on the "edge" of two or more distinct habitat types); thus, unless habitat is carefully mapped, differences in bird species diversity may be erroneously attributed to a chemical stressor, when habitat is a more influential factor. Identification and isolation of confounding factors can be done either as part of study design prior to data collection or after data collection as part of data analysis.

Documenting the broad and ever-growing range of study designs that may be applicable to causality assessment is beyond the scope of this module, in part because conditions at every site are unique and will require case-by-case adaptation.

2.2.3 Confirm Compatibility of Study Design

The final task in Step 2 is to confirm compatibility of study design, planned data analysis and modeling with outcomes of Step 1.

2.2.4 Sources of Evidence Used in Causality Assessment

While specific guidance related to study design is not provided in this document, a brief review of general sources of evidence – observation, manipulation and general knowledge – used in causality assessment.

Evidence for or against a candidate cause is developed by showing an association between the cause and the effect based on analysis of data or other information (Cormier et al. 2010). Cormier et al. (2002) categorize sources of evidence used in causality assessment as observation, manipulation, or general knowledge. **Observation** involves witnessing an attribute of the environment that is associated with the cause, the effect, the interaction, or the causal pathway. This may involve measuring biological, physical, and chemical attributes or noting qualitative attributes. **Manipulation** involves changing the causal agent or affected entity so that the causal relationship also changes. Manipulations may include field and laboratory experiments, such as bioassays. **General knowledge** includes scientific facts and laws that are relevant to the hypothesized causal relationships. Any of these three sources of information becomes evidence for causality when relationships (between the assumed cause and effect) are demonstrated using the following common approaches (Cormier et al. 2010):

- Evidence from a single case or from correlations in a set of paired data describing both the cause and effect;
- Statistical models such as regression to relate data;
- Mechanistic modeling parameterized with data from a cause; and
- Analogy to relate information from another case or from general knowledge to the case of concern.

2.2.4.1 Observation

Observational data are among the strongest lines of evidence in causality assessment. Observational data are typically site-specific, paired empirical data describing both the cause and effect. Such data typically are derived from either laboratory bioassays or field studies and may record associations, such as spatial co-occurrence, spatial gradients, temporal relationships, and temporal gradients (Suter et al. 2002). The strongest associations are often found between stressors and direct effects, such as between nutrient concentrations and algal growth (Suter et al. 2002). Whenever possible, the associations should be quantified.

Design and interpretation of bioassays is thoroughly discussed in the Toxicity Test Selection and Interpretation Module that is part of the FCSAP Supplemental Guidance for Ecological Risk Assessment (FCSAP, 2010b). Although guidance on design of field studies is far more limited, the scientific literature offers numerous examples that may be adapted to the conditions of an individual site.

Field studies typically involve collection of paired data that quantify: 1) gradients of chemical concentrations and/or other measures of various stressors; and 2) measures of impairment, such as reproduction, survival, and community structure.

2.2.4.2 Manipulation

The underlying premise of "manipulation" evidence is that, when effects are diminished after a candidate cause is eliminated or reduced, there is strong evidence of causation. Like observational data, manipulation data also often involves the use of laboratory bioassays and field studies. In this case, however, certain conditions are manipulated by the researcher, who makes paired measurements of stressors and responses, while holding all other influential variables constant. Suter et al. (2002) offer the following theoretical examples of field and laboratory experiments involving manipulation:

1) Manipulation of a source in the field to determine if effects are reduced or eliminated
2) Manipulation of exposure in the field, such as the introduction of previously unexposed organisms or the isolation of organisms from one cause reveals the effects of other causes

3) Laboratory manipulation and testing of site-specific media, such as the extraction of site media into fractions containing different chemical classes prior to toxicity testing.

More specific examples of manipulation experiments include Toxicity Identification Evaluation (TIE), fencing cattle to prevent access to a stream, elimination of an effluent due to plant shutdowns, and caged fish and bivalve studies (Suter 2002). Egg injection studies (e.g. Brunström et al. 1990, Powell et al. 1996, Hoffman et al. 1998) are a manipulation-based study design used to test the relative toxicity of contaminants to bird embryos or the relative sensitivity of different avian species to a given contaminant. Another type of manipulation-based study design used with birds and reptiles involves exchanging eggs between natal nests, in order to differentiate the effects of factors intrinsic to the eggs (e.g. egg size, contaminant level, egg order) from extrinsic factors (e.g. parental quality, ambient temperature) influencing growth and survival of embryos (e.g., Risch and Rohwer 2000). Bursian et al's (2006) mink feeding study involved the manipulation of the composition of the test organisms' diets in order to test specific doses and mixtures using wild caught fish as the primary component of the diet. Brunström's egg injection studies (e.g., Brunström et al., 1990) allow comparison of embryo toxicity across multiple bird species when injected with a known, constant bolus dose. Water effects ratio studies can be used as a tool to determine whether a discharge is toxic when using site receiving water. Hardness and other physical and chemical factors of the receiving water can markedly change the toxicity of a discharge.

2.2.4.3 General Knowledge

While site-specific data, based on either observation or manipulation, can be most compelling in characterizing associations between candidate causes and effects, associations observed from studies conducted on other sites and other conditions can also provide useful supporting information. The objective of considering data from other situations – termed here "general knowledge" – is to determine whether the stressor is present at the study site in sufficient quantity or frequency that a particular effect would be expected, based on information from laboratory tests, field studies, or exposure-response relationships observed elsewhere. Traditional ecological knowledge may also offer an additional line of evidence in support of an association.

Laboratory toxicity tests and other controlled studies from other sites provide the basis for models describing the induction of effects by particular causes (Suter et al. 2002). A simplistic but frequently employed example of such extrapolation is the comparison of chemical concentrations in soil, water, or sediment to thresholds reported in the literature. Measured concentrations also can be applied to concentration-response models in order to estimate the frequency or severity of effects. More complex causal mechanisms, particularly those involving indirect causation, require more complex mechanistic models (Suter et al. 2002).

Several factors influence the quality of evidence when associations are drawn from other sites or conditions. Such evidence, by definition, relies on extrapolations between the conditions tested and reported and conditions of the site at hand. Sample et al.'s (2007) guidelines for selection of ecotoxicity data for use in ERAs can also be helpful in differentiating between extrapolations that offer limited insight into causality and those that are more helpful (e.g., focus on oral exposure for birds and mammals; give preference to studies conducted on most sensitive life stage and studies with most sensitive endpoint; chronic studies preferred over acute, etc.).

In light of these many considerations, the effects of extrapolating from conditions reported in the literature to the case at hand must be carefully considered as part of such assessments.

2.3 Analyzing Data for Causality

Step 3 in causality assessment is analyzing data and involves careful qualitative and/or quantitative consideration of the available information on each candidate cause and its linkage to the impairment. Close collaboration with a statistician is advised.

Step 3. Analyze Data for Causality

- Prepare for analysis
  - Understand stakeholder preferences for viewing and accessing data
  - Create database
  - Identify common set of rules to be applied to all evidence
- Conduct analyses
  - Map spatial trends and graph temporal trends
  - Plot measures of cause and effect as scatter plots
  - Employ appropriate statistical methods
  - Beware of spurious correlations
  - Document analysis methods and results

2.3.1 Prepare for Analysis

A number of preparatory steps should be undertaken before jumping into data analysis including understanding stakeholder preferences for data. Stakeholders will have varying degrees of comfort with different analytical tools. Some may require online access to all data in both raw and interpreted formats. Others may feel greatest confidence in the professional judgment of a panel of experts. Scientists, industry and regulators may prefer statistical and other quantitative tools. Based on the preferences of stakeholders, the most appropriate format for the analytical database can be determined and that database can be constructed. For example, if mapping will be used extensively to visually assess gradients, the database must include geospatial data (i.e., latitude and longitude of observations) and must be linked to GIS. Before initiating analysis, it is advisable to decide upon a common set of rules to be applied across all evidence, to standardize the analysis and to minimize cognitive errors (see Norton et al. 2003).

2.3.2 Conduct Data Analysis

Once data are prepared for analysis, the next step is to analyze the data. Exploratory data analysis is typically the first step in any analysis of data and is particularly important for evaluation of stressor-response relationships in observational data.

Exploratory data analysis probes the data to see if patterns exist. Analysis commonly begins with exploratory mapping and graphing, because understanding how the cause and effect co-occur in time and space can help minimize cognitive errors (see Norton et al., 2003). Analysis of temporal and spatial trends may be descriptive or statistical. Scatter plots of these paired data can reveal associations between the stressor(s) and effects. Of course, evaluation of spatial trends must consider the possibility that organisms may have moved following exposure to the stressor(s); fish birds, mammals, and some invertebrates, reptiles and amphibians can be quite mobile.

Although graphs and regression analysis are often used when only a few variables are involved, if data are available for three or more variables, relationships may be difficult to observe directly. In such cases, a variety of multivariate ordination and classification techniques facilitate visualization of multivariate data (Gentile et al. 1999). Exploratory data analysis can help generate hypotheses that can then be tested using confirmatory statistics, such as Student's t test, ANOVA (analysis of variance), Chi-square tests, association analyses, and multivariate hypothesis testing (Gentile et al. 1999).

As noted by Gentile et al. (1999), multiple regression analysis is a descriptive technique that attempts to incorporate a variety of variables matched with appropriate coefficients (i.e., slopes) and an intercept. Multiple regression can also be used to evaluate the strength of the contribution of each variable as revealed by the coefficients. Gentile et al. (1999) describe this and other multivariate methods for exploring patterns within ecological datasets. Coupled with association analyses, these techniques can also be used to test the hypothesis that a biological variable is related to a specific stressor. There is no single best method that will apply in all cases. Each technique makes different assumptions about the relationships among variables. Ludwig and Reynolds (1988) and Manly (1994) offer introductions to the assumptions, derivations and uses of several multivariate techniques commonly applied for the analysis of ecological communities.

Statistical hypothesis testing should however, be treated with caution. Unless groups are randomly assigned in a way that minimizes the influence of confounding variables, a significant outcome in a hypothesis test may be falsely attributed to a candidate cause. On the other hand, small sample sizes limit one's ability to differentiate groups, potentially leading to mistakenly eliminating a true cause.

Proper statistical design, hypothesis testing, and power analysis are essential to determination of causality. Involvement of an experienced statistician in the planning stages of the data collection, and in data analysis and interpretation is of paramount importance. Spurious correlations should be guarded against. Spurious correlations occur when two events are strongly correlated but the causal relationship is improperly specified (e.g., concluding that crowing roosters cause the sun to rise; Norton et al., 2003). More commonly, these authors note, natural gradients or other stressors that are strongly correlated with the true cause may be confused with it. The converse of a spurious correlation is a masked correlation. Norton et al. (2003) note that masking occurs when evidence for one cause is so strong that it obscures the effects of another sufficient cause.

Although detailed guidance on statistical analysis is beyond the scope of this module, readers are referred to the following examples as sources for detailed treatment of statistical design considerations:

- Skalski (2000) - statistical design of wildlife toxicity studies;

- Chapman et al. (2001) - statistical issues specific to avian toxicity testing; and
- Fairbrother and Bennett (2000) - overview of statistical methods, descriptions of several methods of hypothesis testing, relationship assessment, predicting group membership, structure analysis, and spatially explicit approaches.

Many statistical software packages also provide general background documentation on the choice of appropriate statistical tests.

Norton et al. (2000) provide a case study that employs existing regional monitoring data and correlation analysis to discriminate among multiple stressors in aquatic systems. A case study by Mos et al. (2006) illustrates the use of principal component analysis to identify a biological stressor (pathogens associated with fecal coliform) interacting with chemical-associated immunotoxicity in seals.

As discussed by Fairbrother and Bennett (2000), it is important to recognize that statistical and mathematical manipulations of data return information only about the mathematical relationships among the pieces of input information. They say nothing about the underlying biology or the ecological associations. Therefore, it is of paramount importance that the statistical relationships be placed in an ecological context by asking whether the statistical relationships make sense ecologically and whether they adequately and appropriately explain observed phenomena. It is incumbent upon the risk assessor to effectively communicate the ecological context and meaning of any mathematically derived statistical relationships.

USEPA (2000a) provides guidance for using statistics and statistical hypothesis testing to analyze observations data in causality assessment (See Table 2-3).

The final task in Step 3 of causality assessment is the documentation of the methods and results employed during this data analysis step.

Table 2-3 Using Statistics and Statistical Hypothesis Testing to Analyze Observational Data in Causality Assessment		
Activity	**Application to Observational Data in Causality Assessment**	**Comments**
Using a summary statistics (e.g., mean water or soil concentrations, rates) to summarize measurements	Encouraged	Pay attention to the biological or physical relevance of the summary statistic used. For example, the mean of chemical concentrations over time is often the most relevant (USEPA 1998). As another example, the bankfull flow event is considered to be an important determinant of stream morphology (Rosgen 1996).
Using statistics to determine the probability that two sets or samples are drawn from the same distribution, or that they differ by a prescribed amount (sometimes referred to as bioequivalence testing)	Use Caution	Note that this use is not hypothesis testing in that it does not test a null hypothesis about a treatment (cause). It simply tells you the likelihood that differences are due to sampling variance. Also, the conventional criteria for statistically significant differences are not relevant; the differences must be shown to be biologically significant and the probabilities must be shown to affect the overall strength of evidence. Because the sample sizes are often small relative to variance, the power to detect real differences may be small.
Using the results of statistical hypothesis tests to conclude that a candidate is (or is not) the cause	Wrong	The assumptions of statistical hypothesis testing are violated. In observational studies, replicate treatments cannot be randomly assigned in a way that minimizes the influence of confounding variables. For this reason a significant outcome in a hypothesis test may be falsely attributed to a candidate cause when in fact it is due to another factor.
Using correlations or regression techniques to quantify relationships between variables.	Encouraged	The type of data (continuous, ordinal, or categorical) and the type of relationship (e.g., linear, non linear) will determine the best technique to use.
Using statistics to determine the probability that a relationship is nonrandom, or that the slope of a regression differs from zero.	Use Caution	Note that this analysis indicates only the probability that an apparent relationship is due to sampling variance. It does not test the hypothesis that the relationship is causal. Also the number of samples is likely to be low, so even correlations or models that are not statistically significant can be biologically significant and contribute to the strength of evidence.
Concluding that statistically significantly correlated variables have a causal relationship.	Wrong	Correlation does not indicate causation, and a highly improbable regression model does not indicate that the independent variable caused the relationship. Because stressors often co-vary with each other and with natural environmental attributes, a strong relationship between a candidate cause and biological variable may be due to a factor other than the candidate cause.

Source: USEPA 2000a

2.4 Weighing the Evidence and Drawing Conclusions

Step 4. Weigh the Evidence for Each Candidate Cause and Draw Conclusions

- Review documentation of evidence for each candidate cause
- Characterize evidence related to each candidate cause with respect to attributes of scientific defensibility
- Tabulate findings and verify justification for each characterization
- Integrate findings across attributes in order to draw conclusions regarding the most probable cause(s) or the need for further evaluation. Document conclusions and recommendations

In Step 4 of causality assessment, the risk assessor assembles the available evidence supporting or refuting the linkages between each candidate cause and the observed impairment, and using a weight-of-evidence approach, draws conclusions regarding the most probable cause or the need for further evaluation. A discussion of weight-of-evidence approach is provided in FCSAP (2012a).

2.4.1 Review Evidence for Candidate Causes

The first task in Step 4 is to review the documentation of evidence (both quantitative and qualitative) for each of the candidate causes. Weight-of-evidence analysis organizes all available evidence related to all candidate causes, so that the evidence that supports or refutes each candidate cause can be compared and communicated (Suter et al. 2002).

2.4.2 Characterize Evidence for Each Candidate Cause

The risk assessor considers all available evidence for each candidate cause relative to pre-defined attributes that together describe scientific defensibility. Several frameworks for weighing evidence are published in the scientific literature (e.g., Menzie et al. 1996, Hull and Swanson 2006, McDonald et al. 2007; SAB, 2010). This particular application employs the following 12 attributes, which include 6 site-specific attributes, 4 attributes based on biological knowledge, and 2 attributes based on multiple lines of evidence (as presented in Suter et al., 2002). Cormier et al. (2010) used a different approach than Suter et al. (2002) where "types of evidence" (here called attributes) was separated into "characteristics of causation" (See Section 2.1.3) and "source of evidence" (Section 2.2.4). Consequently, the attributes discussed in the following sections do not necessarily match with the characteristics of causation and types of evidence presented in Sections 2.1.3 and 2.2.4, respectfully. The approach used by Cormier et al (2010) is compatible with the approach presented in Suter et al. (2002). Either of these frameworks or other applicable frameworks may be selected for use in the causality assessment.

Site-specific Attributes

The six site-specific attributes include co-occurrence, temporality, consistency of association, biological gradient, complete exposure pathway, and experiment. These attributes form the strongest basis for causal inference (Suter et al. 2002).

Co-occurrence – The spatial co-location of the candidate cause and effect. An aquatic example of co-occurrence is that effects occur downstream of a stressor, but not upstream of that stressor. A terrestrial example of co-occurrence is that soil with elevated concentrations of zinc has significantly lower density of living vegetation, compared to soil with background concentrations of zinc (e.g., effect occurs in same location as stressor but does not occur where stressor is absent). This consideration should be interpreted with caution when several sufficient causes are present, particularly if the objective of the analysis is to identify all potential and contributing causes (Suter et al. 2002).

Temporality – A cause must always precede its effects. An aquatic example of temporality is that a baseline monitoring study showed a productive trout population prior to construction of a dam, while the density of trout decreased following dam construction. A terrestrial example of temporality is an observed decrease in live forest stand density following invasion by the mountain pine beetle. As with co-occurrence, this attribute should be applied with caution when several sufficient causes are present, since causes occurring early in the time sequence may mask the effects of causes that occur later (Suter et al. 2002).

Biological gradient – The effect should increase with increasing magnitude or duration of exposure. This classic toxicological requirement that effects must be shown to increase with dose also applies to non-chemical stressors. For example, if coarse sediment grain size causes reduced diversity of benthic invertebrates, then diversity should decline along a gradient of increasing grain size. If dichlorodiphenyltrichloroethane (DDT) reduces egg-shell thickness in birds, then an inverse relationship should be observed between egg-shell thickness and a measure of DDT exposure, such as egg concentration, maternal dose, or prey concentration. Some stressors elicit non-linear (including U-shaped) response. For example, community diversity can increase at low levels of nutrient enrichment, before declining again as enrichment increases. Many metals are beneficial up to a certain threshold, above which they become harmful. Regression and correlation analyses are commonly used to quantify biological gradients; high slopes, large correlation coefficients, increase the weight-of-evidence related to the biological gradient.

Complete exposure pathway – The presence of documented linkages between the source(s) of a stressor, migration pathways, and receptors represent exposure pathways that are a necessary component of cause-effect relationships. If the exposure pathway is incomplete, then the receptor cannot contact the stressor and the response cannot be a result of that stressor. Evidence for a complete exposure pathway must be site-specific and may include data on chemical body

burdens, presence of parasites or pathogens, or biomarkers of exposure. If s stressor does not leave internal evidence of a complete exposure pathway (e.g., siltation), then it may be useful to use measurements showing that the stressor co-occurs in space in time with the receptor (Suter et al., 2002).

Consistency of Association – The effect and candidate cause have been repeatedly observed in different places or times. A consistent association of an effect with a candidate cause is likely to indicate causation. The case for causation is stronger if the number of instances of consistency is greater, if the systems in which consistency is observed are diverse, and if the methods of measurement are diverse (Suter et al. 2002). Consistency can be demonstrated using evidence from the case at hand, or may draw on evidence from many cases. For example, if fish kills repeatedly occur below a particular outfall, there is a consistent association over time of those incidents with a candidate cause. Because egg-shell thinning was observed across a variety of locations and a variety of species exposed to DDT, the consistency of association between DDT exposure and avian eggshell thinning was high.

Experiment – Refers to the manipulation of a cause by eliminating a source or altering exposure (Hill 1965). Examples of experiments of greatest relevance to FCSAP sites include manipulating and testing site media in the laboratory and conducting field experiments by controlling a source. The strongest experimental evidence is site-specific. If evidence from experiments conducted on a similar situation is used, then the relevance to the FCSAP site should be described.

Attributes Based on Biological Knowledge

Attributes based on other situations or biological knowledge include plausibility, analogy, specificity of cause, and predictive performance. These attributes provide corroborative information that can be used to supplement the site-specific observations.

Plausibility – Refers to the degree to which a cause and effect relationship should be expected given known facts. Plausibility may be based on either the mechanism or a known stressor-response relationship. The mechanism relates to the current state of scientific understanding about the biology, physics, and chemistry of the candidate cause, the receiving environment, and the affected organisms and whether it is plausible that the effect resulted from that candidate cause. It is important to distinguish a lack of information concerning a mechanism from evidence that a mechanism is implausible. It is also important to consider whether some indirect mechanism may be responsible. Stressor-response plausibility speaks to the known relationship between the candidate cause and the effect. The comparison of environmental concentrations of chemicals to laboratory-derived concentration-response relationships is a common approach that can provide strong evidence of causality if environmental concentrations exceed a threshold for relevant effects. However, exceedance of water quality criteria or other regulatory values that are intended to be safe levels is not evidence of causation (Suter et al. 2002).

Analogy – Examines whether the hypothesized relationship between cause and effect is similar to any well-established cases (Suter et al. 2002). Hill (1965) used this criterion to refer specifically to similar causes, such as in the case of a new pesticide with structure resembling another pesticide that induces similar effects. As another example, an introduced species may have a similar natural history characteristic to one that was previously introduced and the two may have similar impacts on the ecological system.

Specificity of Cause – Describes the precision with which the occurrence of one variable will predict the occurrence of another (Susser 1986). Specificity of cause is only applicable if the proposed cause is plausible or if it has been consistently associated with the effect, and it addresses cases where relatively few candidate causes are known to elicit the specific type of effect observed. If an effect (e.g., hepatic tumors in fish) observed at a contaminated site has only one or a few known causes (e.g., PAHs), then the occurrence of one of those causes in association with the effect is strong evidence of causation. Lead poisoning in birds represents a terrestrial example of a high specificity of cause, in that cephalic subcutaneous edema and impaction of the upper gastrointestinal tract are highly diagnostic and sensitive effects (Sileo et al. 2001). Specificity can be examined from two perspectives: 1) specificity in the effects of a given causal factor (e.g., certain toxins produce a specific effect); and 2) specificity in the causes of a given effect (e.g., certain outcomes result from a specific stressor).

Predictive Performance – Refers to whether the candidate cause has any initially unobserved properties that were predicted to occur. The ability to make and confirm predictions is one of the hallmarks of a good scientific process. For example, if the proposed cause of a bird kill is aerial drift of an organophosphate insecticide, one could make the specific prediction that cholinesterase levels in birds would be reduced, or the more general prediction that insects would also be killed. Multiple predictions–both positive and negative–would strengthen this criterion.

Attributes based on multiple lines of evidence are consistency of evidence and coherence of evidence. They evaluate the relationships among all available lines of evidence.

Consistency of Evidence – Refers to whether the hypothesized relationship between cause and effect is consistent with all available evidence. Consistency is the ecoepidemiologist's alternative to exact replication as part of the design of experiments. The strength of this criterion increases with the number of lines of evidence (Yerushalmy and Palmer 1959).

Coherence of Evidence – Examines whether a conceptual or mathematical model can explain any apparent inconsistencies among the lines of evidence. Coherence of evidence deals with preconceptions and existing knowledge (Wren 1991). Coherence supports pre-existing theory (Susser 1986) and can be discussed in terms of theoretical fit, biological fit, factual fit, and statistical fit. An example of theoretical fit might occur at a terrestrial site where concentrations of metals in soil are sufficiently high to impair reproduction in small mammals. The observation

that both juvenile and adult small mammals occur at the site might initially appear to contradict the theoretical fit of impaired reproduction due to metals exposure. However if juvenile small mammals are re-colonizing the site from unexposed locations, then the evidence would remain coherent. Another coherent explanation might be that the total metal concentrations are not entirely bioavailable. However, given the possibility that either explanation is wrong, this line of evidence is relatively weak. These hypotheses could, however, lead to experiments or predictions in future iterations of the causal assessment (e.g., testing bioavailability of metals in soils), which could support stronger inferences.

2.4.3 Tabulate Findings and Verify Justification

The weight-of-evidence supporting or refuting each candidate cause, with respect to the attributes listed above is next tabulated, as illustrated in the examples provided in Tables 2-4 and 2-5. See also USEPA's summary table of scores for CADDIS (http://www.epa.gov/caddis/si_step_scores.html) for examples of scores and interpretation.

The attributes related to causality are listed in the left-hand column of Table 2-4, while each row represents the results and rationale for a different type of evidence with respect to a candidate cause. The rows show the appropriate number of +, -, or 0 symbols associated with the strength of evidence for each attribute evaluated for each candidate cause. Supporting narratives should describe how the scores were assigned for each symbol (e.g., +, -, 0) based on the evidence. Scores are based on whether the evidence supports the candidate cause (+), weakens the candidate cause (-) or has no impact / is uncertain (0). The degree to which the evidence supports or weakens the candidate cause will determine the strength of the score and the number of + and - (e.g., if evidence supports the candidate cause only somewhat a score of + could be given, while if the evidence strongly supports the candidate cause as score of +++ could be given). If it is not possible to use the available data to evaluate a type of evidence, then it is scored as "no evidence" or NE (USEPA, 2010). If the type of evidence does not apply for the candidate cause, it is scored "not applicable" or NA. Types of evidence that use site data are based on more than one association and closely link the proximate cause and the effect should be given the highest scores (USEPA, 2010). Other scores may include, for example, "refute" or R (indicating that there is indisputable evidence that the candidate cause is responsible) and "diagnose" or D (indicating a set of symptoms for a particular causal agent or class of agents is, by definition, sufficient evidence of causation, even without the support of other types of evidence)(USEPA, 2010). Scores should not be added, because that would erroneously imply that each attribute is of equal importance. Particular attention should be paid to negative results, which are more likely to be decisive than positive results. Findings should be tabulated for each of the relevant candidate causes.

2.4.4 Integrate Findings and Draw Conclusions

The final step in the weight-of-evidence approach is to integrate findings (as tabulated in previous step) and draw conclusions on the most probable candidate causes (see FCSAP, 2012a for weight-of-evidence approach). Table 2-6, excerpted from Cormier et al. (2010), offers a framework for integrating the quality of evidence of causal characteristics. Once the quality of evidence of causal characteristics is understood, bodies of evidence for alternative candidate causes should be compared, in order to understand which candidate causes are most plausible and important. This is a relative, rather than absolute, comparison. The quality of the body of evidence for all candidate causes may be suboptimal, but some candidate causes may have stronger supporting (or refuting) evidence than others.

Again, the final step in the causality assessment is documentation. In this step, the cause is described, the basis for its determination summarized, and the uncertainties concerning that determination presented (See Tables 2-4 and 2-5). If there are multiple sufficient causes, all are characterized. Only in extreme cases are the effects of the primary cause so severe that the other potential causes remain unidentified. Confidence in the outcome should be expressed through consideration of uncertainty in the data, models, and observations. The final section should clearly state whether the available evidence is sufficient to justify a management decision or action.

Recommendations for additional investigation may be included if the uncertainty in the outcome is too great to support responsible risk management decision-making. No uniform standard for adequacy of proof for causation exists for FCSAP sites. This is not surprising, given the wide ranging and site-specific costs of investigation and remediation, as well as the unique concerns of different stakeholder groups. Hence, decisions regarding whether existing evidence of causality is sufficient to proceed with management actions are necessarily conducted on a case-by-case basis.

Table 2-4 Example Format of Summary Table of Inferred Causation

Consideration	Candidate Cause #1 [1]	
	Result of Evidence for Candidate Cause	**Score [2]**
Case-Specific Considerations		
Co-occurrence	Compatible, Uncertain, Incompatible	+, 0, - - -
Temporality	Compatible, Uncertain, Incompatible	+, 0, - - -
Consistency of Association	Invariant, In many places and times, At background frequencies or many exceptions to the association	++, +, -
Biological Gradient	Strong and monotonic, Weak or other than monotonic, None, Clear association but wrong sign	+++, +, -, - - -
Complete Exposure Pathway	Evidence for all steps, Incomplete evidence, Ambiguous, Some steps missing or implausible	++, +, 0, -
Experiment	Experimental studies: Concordant, Ambiguous, Inconcordant	+++, 0, - - -
Considerations Based on Other Situations or Biological Knowledge		
Plausibility - Mechanism - Stressor-Response [3]	 Actual Evidence, Plausible, Not known, Implausible Quantitatively consistent, Concordant, Ambiguous, Inconcordant	 ++, +, 0, - +++, +, 0, -
Specificity of Cause [4]	Only possible cause, One of a few, One of many	+++, ++, 0
Analogy - Positive - Negative	Analogous cases: Many or few but clear, Few or unclear	 ++, + - -, -
Predictive Performance	Prediction: Confirmed specific or multiple, Confirmed general, Ambiguous, Failed	+++, ++, 0, - - -
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	Evidence: All consistent, Most consistent, Multiple inconsistencies	+++, +, - - -
Coherence of Evidence	Evidence: Inconsistency explained by a credible mechanism, No known explanation	+, 0

1. In cases where there are more than one candidate causes, additional columns can be added or a separate table can be used.
2. In addition to the scores noted, there may be No Evidence (NE) available relevant to the consideration, or the consideration may be Not Applicable (NA) for the particular case. Results and score weights provided in table are examples only and may be defined differently as deemed relevant. Only the result and score weight which applies to the specific consideration is to be included in score cell (e.g., if weight of evidence indicates that candidate cause is considered uncertain the a score of 0 could be placed in score cell; if candidate cause is considered probable then a score of + could be placed in score cell).
3. Stressor-response is not applicable (NA) if the mechanism is clearly implausible.
4. Specificity of cause is not applicable (NA) if either the mechanism is clearly implausible, or if there are many exceptions to the association.
Source: USEPA 2000a; Suter et al., 2002

Table 2-5 Example Table Format for a Summary of the Strength of Evidence of Each of the Candidate Causes

Candidate Casual Cause	**Result of Evidence for Candidate Cause**	**Score**	**Result of Evidence for Candidate Cause**	**Score**	**Result of Evidence for Candidate Cause**	**Score**
	Candidate Cause #1 (e.g., Metal Contamination)		**Candidate Cause #2 (e.g., Habitat Alteration)**		**Candidate Cause #3 (e.g., Nutrient Enrichment)**	
Case-Specific Considerations						
Co-occurrence						
Temporality						
Consistency of Association						
Biological Gradient						
Complete Exposure Pathway						
Experiment						
Considerations Based on Other Situations or Biological Knowledge						
Plausibility - Mechanism - Stressor-Response						
Specificity of Cause						
Analogy - Positive - Negative						
Predictive Performance						
Considerations Based on Multiple Lines of Evidence						
Consistency of Evidence						
Coherence of Evidence						

Table 2-6 Qualities of Pieces of Evidence of Causal Characteristics

Quality of Evidence and Example Descriptors	Evaluation	Explanation
Logical implication - negative - positive	Does the evidence affirm or weaken the argument for a causal relationship?	As Popper (1968) explained, evidence that refutes a hypothesis is stronger than evidence that affirms that hypothesis. Similarly, negative evidence receives more weight than positive evidence.
Directness of cause - proximate cause - sources and intermediate causal connections	How relevant are the measurements to the proximate cause? How relevant are the measurements to the effect of interest?	Evidence that relates to the proximate cause is stronger than evidence that relates to events that led to that cause. This is because events that are more causally remote are less clearly associated with the effect.
Specificity - effect attributable to only one cause - effect attributable to multiple causes	How specific is the effect?	If the effect has only one possible cause, that cause is diagnosed. If the effect is a result of only a few causes, the list of candidate causes is limited, which increases the likelihood for each of the few candidate causes.
Relevance to effect - from the case - from other similar situation	How relevant is the evidence to the case under investigation?	Other things being equal, evidence based on information from the case is stronger than evidence based on other field studies or laboratory experiments, because it is directly relevant.
Nature of the association - quantitative - qualitative	Is the association quantified?	Quantitative relationships between cause and effect are better than mere observations that the cause is demonstrable at the impaired site. Plausibility of association is very weak evidence.
Strength of association - strong relationships and large range - weak relationships and small range	Is the evidence unlikely to be attributable to natural variation (i.e., background variability)?	Strong relationships (e.g., large correlation coefficients, steep slopes) that cover a wide range of exposure levels are better evidence than weaker relationships or relationships that cover a narrow range. Ambiguous or reversed relationships suggest that the entity is not susceptible to the cause or that other factors are involved.
Independence of association - independent - confounded	To what degree have the effects of the causal agent been isolated from other co-occurring factors?	A single cause that can be analyzed alone (such as in laboratory tests) provides evidence that is not confounded or modified by other factors. If many possible causes co-vary, the evidence is weaker, because the effect may have been produced by the other, co-varying factors.
Consistency of information - all consistent - inconsistent	If a piece of evidence is formed from composited information, is it consistent?	If composited pieces of evidence are consistent, the evidence is stronger than if there are inconsistencies.
Quantity of information - many data - few data	Did the study use abundant data?	Many data provide better evidence than few data.
Quality of information - good study - poor study	Is the quality of the study, and therefore the data, reliable and defensible?	Higher quality data results in more reliable information and stronger evidence.

Source: Excerpted verbatim from Cormier et al. 2010

3.0 CASE STUDIES

Aquatic case studies in the peer-reviewed literature which can be used as examples of causality assessments are plentiful. Readers are directed to work by Lowell et al., (2000), Borgman et al. (2001), Culp et al. (2000), Cormier et al. (2002, 2010), Wiseman et al. (2010), Haake et al. (2010), Norton et al. (2000, 2002, 2003), USEPA (2000a, 2007), MDEQ (2005) for examples. Some of the above citations appear together in the January 2010 issue of the journal *Human and Ecological Risk Assessment* (Volume 16, pp 10-148). Still other example case studies for aquatic sites are posted at the CADDIS website (http://cfpub.epa.gov/caddis/examples.cfm?Section=27) and provided in Chapters 6 and 7 of USEPA's (2000a) Stressor Identification guidance.

With respect to terrestrial examples of causality analysis, Cairns et al. (1995) observed that advances toward understanding how contaminants in terrestrial environments affect structural and functional attributes of ecological organization have been nearly non-existent in comparison to our understanding of aquatic ecosystems. Few advances have been made in the intervening 15 years. No suitable Canadian terrestrial case studies were identified, even after consulting with several Canadian risk assessors and conducting a thorough literature search. Perhaps the most detailed treatment of causality assessment for a terrestrial organism can be found in USEPA (2008a). The purpose of USEPA's (2008a) work was to test the utility of CADDIS to determine the cause of effects on a population inhabiting a contaminated terrestrial site. CADDIS was developed to determine the causes of biological impairments in aquatic ecosystems, and prior case studies focused on effects on community metrics in streams. However, the principles and methods of causal analysis should be applicable to all environmental effects, aquatic and terrestrial alike.

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4.1 Additional Resources

Canadian Web Resources

Fisheries and Oceans Canada's (DFO's) Pathways of Effects diagrams describe for a range of activities the type of cause-effect relationships that are known to exist; and the mechanisms by which stressors ultimately lead to effects in the aquatic environment. http://www.dfo-mpo.gc.ca/habitat/what-quoi/pathways-sequences/index-eng.asp

Findlay, D.L., and Kling, H.J. Protocols For Measuring Biodiversity: Phytoplankton in Freshwater, Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6, http://www.eman-rese.ca/eman/ecotools/protocols/freshwater/phytoplankton/intro.html

Martin, J.L. Marine Biodiversity Monitoring, Protocol for Monitoring Phytoplankton, A Report by the Marine Biodiversity Monitoring Committee (Atlantic Maritime Ecological Science Cooperative, Huntsman Marine Science Center) to the Ecological Monitoring and Assessment Network Of Environment Canada, Department of Fisheries & Oceans, Biological Station, St. Andrews New Brunswick, Canada E0G 2X0, http://www.eman-rese.ca/eman/ecotools/protocols/marine/phytoplankton/phyto_marine_e.pdf

Environment Canada's Ecological Monitoring and Assessment Network website (http://www.eman-rese.ca/eman/) is an excellent resource for identify sampling protocols for particular situations, and for identifying experts in various fields of study. It includes a range of monitoring protocols for freshwater, marine, and terrestrial environments.

Paterson, M. Protocols for Measuring Biodiversity: Zooplankton in Fresh Waters. http://www.eman-rese.ca/eman/ecotools/protocols/freshwater/zooplankton/

Anderson, R.S. 1996. Sifting and Berlese protocols. pp. 52-53, in: A.T. Finnamore (editor). The SAGE project, a workshop report on terrestrial arthropod sampling protocols for graminoid ecosystems. Prepared for the Ecological Monitoring Coordinating Office of Environment Canada. EMAN Occasional Paper Series Report 74pp. http://www.cciw.ca/eman

Canadian Aquatic Biomonitoring Network (CABIN) (freshwater). Environment Canada developed CABIN protocols for both wadeable streams and open freshwater. Lab forms and benthic ecology laboratory bench sheets for enumerating organisms are presented. http://cabin.cciw.ca/Main/cabin_online_resources.asp

Environment Canada's Environmental Effects Monitoring (EEM) is used to evaluate the effects of effluents from regulated mills and mines on fish, fish habitat and the use of fisheries resources by humans. Biological monitoring is conducted on fish by comparing adult fish from a study area to adult fish from a reference area. Effects on fish habitat are assessed by comparing benthic invertebrate communities from a study area to those from a reference area. http://www.ec.gc.ca/esee-eem/default.asp?lang=En&n=4CDB9968-1

Sample Sorting and Subsampling Protocols for EEM Benthic Invertebrate Community Surveys. This link contains detailed guidance on benthic sample processing methods and subsampling approaches: http://www.ec.gc.ca/esee-eem/default.asp?lang=En&n=B9DBF4CC-1

Canadian Council on Animal Care, 2003 Guidelines on the Care and Use of Wildlife. This comprehensive guide discusses development of wildlife study objectives and planning, including the requirement for permits, and conduct of the various procedures.
http://www.ccac.ca/en/CCAC_Main.htm

Biological Survey of Canada. 1994. Terrestrial Arthropod Biodiversity: Planning a Study and Recommended Sampling Techniques - A Brief Prepared by the Biological Survey of Canada (Terrestrial Arthropods) – Ottawa, 1994. Reprint edition 2007.
http://www.biology.ualberta.ca/bsc/pdf/planningastudy.pdf

Alberta Biodiversity Monitoring Institute. The Alberta Biodiversity Monitoring Institute includes field protocols for a variety of terrestrial, wetland, and aquatic habitats:
http://www.abmi.ca/abmi/reports/reports.jsp?categoryId=0

British Columbia Ministry of Environment, Lands and Parks. 1998. Inventory Methods for Terrestrial Arthropods, Standards for Components of British Columbia's Biodiversity No. 40, Prepared by Ministry of Environment, Lands and Parks Resources Inventory Branch for the Terrestrial Ecosystems Task Force Resources Inventory Committee, October 19, 1998, Version 2.0 http://www.ilmb.gov.bc.ca/risc/pubs/tebiodiv/terranth/assets/arthropod.pdf

Ontario Benthos Biomonitoring Network Protocol Manual (freshwater). C. Jones, K.M. Somers, B. Craig, and T.B. Reynoldson. 2004. Ontario Benthos Biomonitoring Network Protocol Manual, Version 1.0, May 2004. This manual presents Ontario's approach for assessing aquatic ecosystem condition using a reference condition approach, in which the benthic community at a study area is compared to the benthic community at a reference location: http://www.eman-rese.ca/eman/ecotools/protocols/freshwater/obbn/intro.html

British Columbia Fish Sampling Toolkit. Prepared by the Ministry of Sustainable Resource Management, Aquatic Branch for the Resources Information Standards Committee, the tool kit provides guidance and forms for use in fish sampling programs.
http://www.env.gov.bc.ca/fish/methods/toolkits/fishsamp.html

Biomonitoring Information System for the Yukon. In 1996, Environment Canada (Pacific and Yukon Region) began to combine historical stream biomonitoring data into a common electronic format that could be easily retrievable. As a result of this earlier work the Biomonitoring Information System of the Yukon (BISY) has evolved over the past several years into a repository for aquatic biological information from both published and unpublished sources:
http://www.pyr.ec.gc.ca/bisy/home.asp?lang=_e

Quebec Sustainable Development, Environment and Parks. This website contains guides and protocols for Quebbec rivers and lakes in addition to surface water and sediment quality criteria (in French only). http://www.mddep.gouv.qc.ca/eau/flrivlac/riv-lac-en.htm

American Web Resources

USEPA's Stressor Identification Guidance Document leads users through the Stressor Identification process for determining the causes of stream biological impairment; the process described in CADDIS is a modification of this Stressor Identification process. http://www.epa.gov/waterscience/biocriteria/stressors/stressorid.html

USEPA's biocriteria program provides guidance and technical assistance for state and tribe water quality programs, including a Biological Assessment, Criteria and Indicators Discussion Database. http://www.epa.gov/waterscience/biocriteria/

The "Getting in Step" module of USEPA's Watershed Academy provides information on how to improve outreach and stakeholder involvement within your watershed. http://www.epa.gov/watertrain/gettinginstep/

USEPA's Quality System is used to manage the quality of environmental data collection, generation, and use, by both the USEPA and other organizations. Numerous documents which may be helpful in the causality assessment process are available. http://www.epa.gov/quality/index.html

USEPA's AQUATOX is a simulation model that examines how pollutants (e.g., nutrients, organic compounds) may affect aquatic ecosystems. http://www.epa.gov/ost/models/aquatox/

USEPA's ECOTOX (ECOTOXicology) database provides single chemical toxicity information for aquatic and terrestrial life. ECOTOX is a useful tool for examining impacts of chemicals on the environment. Peer-reviewed literature is the primary source of information encoded in the database. http://cfpub.epa.gov/ecotox/

USEPA's Bioindicators Web site has been developed in partnership with the Office of Water and the Biocriteria Program. The website serves as a repository for a vast amount of information and is focused on education on biological indicators including key concepts, coral reef biocriteria, freshwater fish and invertebrates, aquatic biodiversity, use of statistics and more. The customers of this product are wide-ranging and include federal, state, and local government agencies, academia, non-governmental environmental organizations, secondary schools, and the interested public. http://www.epa.gov/bioindicators

USEPA's Benchmark Dose Software (BMDS) may be used to model stressor response relationships. It contains seventeen (17) different models that are appropriate for the analysis of dichotomous (quantal) data (Gamma, Logistic, Log-Logistic, Multistage, Probit, Log-Probit, Quantal-Linear, Quantal-Quadratic, Weibull and Multistage-Cancer), continuous data (Linear, Polynomial, Power, and Hill) and nested developmental toxicology data (NLogistic, NCTR, and Rai & Van Ryzin). http://www.epa.gov/ncea/bmds.htm

USEPA's Acute-to-Chronic Estimation (ACE) with Time-Concentration-Effect Models software allows prediction of chronic toxicity from acute toxicity datasets. ACE uses linear regression and accelerated life testing to predict no-effect and low-effect concentrations for chronic mortality. http://www.epa.gov/ceampubl/fchain/ace/index.htm

USEPA's web-based Interspecies Correlation Estimation (Web-ICE) estimates the acute toxicity of a chemical to a taxon (i.e., the predicted species, genera, or family) without test data from the known toxicity of the chemical to a species with test data (the surrogate). ICE models are least square regressions between the surrogate species and the predicted taxa to estimate the toxicity of that chemical to the predicted species, genus or family.
http://www.epa.gov/ceampubl/fchain/webice/index.htm

USEPA's Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance. This technical guidance provides an extensive collection of methods and protocols for conducting bioassessments in estuarine and coastal marine waters and the procedures for deriving biocriteria from the results. Several case studies illustrate the bioassessment process and biocriteria derivation procedures.
http://www.epa.gov/waterscience/biocriteria/States/estuaries/estuaries.pdf

USEPA's Environmental Monitoring and Assessment Program: Great River Ecosystems, Field Operations Manual. EPA/620/R-06/002.
http://www.epa.gov/emfjulte/greatriver/EMAPGREFOM.pdf

USEPA's available laboratory analytical methods for the analysis of chemical, physical, and biological components of wastewater and other environmental samples.
http://www.epa.gov/waterscience/methods/.

USEPA's Forum on Environmental Measurements provides a collection of test methods (*i.e.*, "approved procedures for measuring the presence and concentration of physical and chemical pollutants; evaluating properties, such as toxic properties, of chemical substances; or measuring the effects of substances under various conditions").
http://www.epa.gov/osa/fem/methcollectns.htm.

USEPA Region 9 Quality Assurance. Surface Water Field Sampling Procedures. Available at:
www.epa.gov/region09/qa/fieldsamp.html

A collaboration of USEPA and the US Geological Survey (USGS), the National Environmental Methods Index (NEMI) includes a large collection of chemical, microbiological, biological, toxicity, and physical methods at http://www.nemi.gov.

Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as part of the National Water-Quality Assessment Program, US Geological Survey (USGS). 2002. Open-File Report 02-150, Reston, VA. This document presents the protocols used by the USGS to evaluate algal, invertebrate, and fish communities in combination with chemical and physical data to provide an integrated assessment of water quality at local, regional, and national scales:
http://pubs.usgs.gov/of/2002/ofr-02-150/

Illustrated Field Guide for Assessing External and Internal Anomalies in Fish. USGS. 2002. This report presents procedures for documenting external and internal abnormalities as an indication of exposure to physical or chemical stressors. It contains detailed recommendations for field processing, recordkeeping, as well as preservation of tissue samples for histopathological exam: http://www.cerc.usgs.gov/pubs/center/pdfDocs/ITR_2002_0007.pdf

The Interactive Sediment Remedy Assessment Portal, managed by the US Navy Space and Naval Warfare Systems Center in San Diego, California and ENVIRON, is an interactive tool designed to assist in understanding monitoring requirements and tools associated with sediment remediation. The sediment monitoring tools matrix facilitates sediment monitoring program design and optimization. http://www.israp.org/

The US Army Corps of Engineers/US Environmental Protection Agency Environmental Residue-Effects Database (ERED) is a compilation of data, taken from the literature, where biological effects (e.g., reduced survival, growth, etc.) and tissue contaminant concentrations were simultaneously measured in the same organism. http://el.erdc.usace.army.mil/ered/

The National Institute of Standards and Technology (NIST) Engineering Statistics Handbook is an electronic text book providing background on some data analysis methods. URLs for the handbook's home page and cited sections are: http://www.itl.nist.gov/div898/handbook/

A multi-agency partnership, the Integrated Taxonomic Information System (ITIS) is a source for authoritative taxonomic information on plants, animals, fungi, and microbes of North America and the world. http://www.itis.gov/

Ohio Environmental Protection Agency's biocriteria guidance offers methods and protocols for the biological assessment of water bodies.
http://www.epa.ohio.gov/dsw/guidance/guidance.aspx (items G2 through G6)

Other Government and Private Sources

The United Nations Geographic Information Working Group provides a forum for water quality analytical method discussion at http://www.ungiwg.org/openwater

The OZCoasts Australian Online Coastal Information website has a series of questions listed that would be useful in creating a cost efficient sampling program. The web page is titled: How do you design a water quality monitoring program. Available at:
www.ozcoasts.org.au/env_mgmt/mar/info.jsp

NatureServe Explorer is an online database of information on more than 70,000 plants, animals, and ecosystems of the United States and Canada. Explorer includes particularly in-depth coverage for rare and endangered species. http://www.natureserve.org

FishBase is a global information system which provides taxonomic, life history, ecological and economic information for freshwater and marine fish. It was developed at the WorldFish Center in collaboration with the Food and Agriculture Organization of the United Nations and many other partners. Development was supported by the European Commission.
http://www.fishbase.org/

Animal Diversity Web (ADW) is an online database of animal natural history, distribution, classification, and conservation biology at the University of Michigan. This online reference provides access to thousands of species accounts about individual animal species. It is a large searchable encyclopaedia of the natural history of animals.
http://animaldiversity.ummz.umich.edu/site/index.html

The Birds of North America (BNA) database is a comprehensive reference covering the life histories of North America's breeding birds. Account contents are updated frequently, with contributions from researchers, citizen scientists, and designated reviewers and editors. In addition, BNA Online contains image and video galleries showing plumages, behaviours, habitat, nests and eggs, and more. http://bna.birds.cornell.edu/bna

Guidelines for the Use of Fishes in Field Research
http://www.asih.org/files/fish%20guidelines.doc

Guidelines for the Use of Live Amphibians and Reptiles in Field Research
http://www.asih.org/files/hacc-final.pdf

Recommendations for the Care of Amphibians and Reptiles in Academic Institutions
http://netvet.wustl.edu/species/reptiles/pough.txt

Guidelines for the Use of Wild Birds in Research
www.nmnh.si.edu/BIRDNET/GuideToUse/index.html

The Sediment Management Work Group (SMWG) is an *ad hoc* group of predominantly US industry and government representatives with responsibility for management of sites with contaminated sediments. The SMWG advocates "the use of sound science and risk-based evaluation of contaminated sediment management options." The website provides links to technical papers and workshops. http://www.smwg.org/

SedWeb promotes improvement in contaminated sediment management and research. This website offers an extensive list of links to other resources. http://www.sediments.org/

The Woods Hole Oceanographic Institute, the world's largest private, non-profit ocean research, engineering, and education organization, provides information on technology to sample and study oceans, including photos and descriptions of sensors and samplers that may also be used in lake and pond systems. http://www.whoi.edu/

List of Acronyms

AChE	Acetylcholinesterase
ANOVA	Analysis of Variance
CABIN	Canadian Aquatic Biomonitoring Network
CADDIS	Causal Analysis/Diagnosis Decision Information System
CCME	Canadian Council of Ministers of the Environment
CWS	Canadian Wildlife Service
DDT	Dichlorodiphenyltrichloroethane
DO	Dissolved oxygen
EEM	Environmental Effects Monitoring
EMAN	Ecological Monitoring and Assessment Network
EPT	Ephemeroptera, Plecoptera, and Trichoptera
ERA	Ecological Risk Assessment
FCSAP	Federal Contaminated Sites Action Plan
GIS	Geographic Information System
HQ	Hazard quotient
IBI	Index of Biotic Integrity
ICI	Invertebrate Community Index
mg/kg-day	Milligrams per kilogram body weight per day
NA	Not applicable
NE	No evidence
PAHs	Polycyclic aromatic hydrocarbons
RSA	Rodent sperm analysis
TIE	Toxicity identification evaluation
TRV	Toxicity reference value
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service

Glossary

Agent – A physical, chemical or biological entity that may affect a biotic system positively or negatively. This term is similar to but more general than *stressor*. For example, dissolved oxygen and woody debris are agents; low dissolved oxygen and reduced woody debris may be stressors.

Analogy – A comparison of two things, based on their similarity in one or more respects. In causality assessment, the criterion of an analogy refers specifically to similar causes.

Anthropogenic – Induced by humans.

Associations – Relationships between different types of observations; these relationships become *lines of evidence* supporting or weakening the case for a *candidate cause*.

Bioassay – an evaluation using organisms to measure the effect of a substance, factor, or condition by comparing before and after data.

Bioassessment (biological assessment) – Evaluation of ecosystem condition using biological surveys and other direct measurements of resident biota.

Biocriteria (biological criteria) – Numerical values or narrative expressions describing the *reference* biological condition of aquatic communities inhabiting waters of a given designated aquatic life use. Biocriteria are benchmarks for evaluation and management of water resources.

Biological gradient – A regular increase or decrease in a measured biological attribute with respect to space (e.g., below an outfall), time (e.g., since a flood), or an environmental property (e.g., temperature). Biological gradients are analyzed to generate *stressor-response* relationships based on field data.

Biomarker – A contaminant-induced physiological, biochemical, or histological *response* of an organism.

Body burden – The concentration of a contaminant in a whole organism or a specified organ or tissue.

Candidate cause – A hypothesized cause of an environmental *impairment*, that is sufficiently credible to be analyzed.

Case study – An example illustrating a complete *causal analysis* or a component of the process.

Causal agent – The *agent* that directly induces the *effect* of concern when intensity and duration of exposure are sufficient. This term is similar to, but more neutral than *proximate stressor*.

Causal mechanism – The process by which a *cause* induces an *effect*.

Causal pathway – The sequence of processes and states that causally connect a *source* to *exposure* to a *causal agent*, potentially including release, transport, transformation, and direct effects (if the effect of concern is indirect).

Causal relationship – The relationship between a *cause* and its *effect*.

Causation – The act or fact of causing; the production of an effect by a cause. Causation differs from association (correlation) in that the latter does not imply a mechanistic linkage between observations. An assessment of causation in an ecological risk assessment attempts to distinguish between associations that are coincidental or caused by external factors and associations that are driven by underlying predictable mechanisms.

Cause – 1. That which produces an *effect* (a general definition). 2. A *stressor* or set of stressors that occurs at an intensity, duration, and frequency of exposure sufficient to result in a change in an identified biological characteristic.

Co-occurrence – The spatial or temporal co-location of the *candidate cause* and the *effect*. Synonymous with *spatial/temporal co-occurrence*.

Concentration-response – Relating to the study of how an *exposure* relates mathematically to the observation of biological effects.

Consistency of evidence – The degree to which *types of evidence* in a *strength-of-evidence analysis* are in agreement in either supporting or weakening the case for a *candidate cause*.

Control – As a noun, an aspect of a controlled scientific experiment conducted for the purpose of determining the effect of a single variable of interest on a particular system, used to minimize the unintended influence of other variables on the same system. Negative controls confirm that the procedure is not causing an unrelated effect, and are intended to reduce incidence of false positives. The term control (as a verb) can also be used in experimental design to refer to manipulation of treatments intended to mitigate the confounding effect of external variables.

Correlation – A statistical relationship between two or more variables such that systematic changes in the value of one variable are accompanied by systematic changes in the other.

Diagnosis – A type of *inference* that uses *symptomology* or a set of specific observations to identify a *probable cause*.

Ecoepidemiology – The study of the nature and *causes* of past or ongoing *effects* in ecological systems (also known as environmental epidemiology).

Ecoregion – A geographic area having relatively uniform ecological properties.

Effect – In general, an effect is something that inevitably follows an antecedent (cause or *agent*). A biological effect is the biological result of *exposure* to a *causal agent*. This term is similar to *response*, but emphasizes the *agent* that acts (e.g., the effect of cadmium) rather than *receptor* that responds to it (e.g., the response of trout).

Eutrophication – Enrichment of a waterbody with nutrients, often resulting in high levels of primary production and leading to depletion of dissolved oxygen.

Evidence – 1. Knowledge that changes one's degree of belief in a proposition (a general definition). 2. Results of data analysis concerning *associations* between the *causal agent* and the *effect*, or between *sources* or steps in the causal chain and the *causal agent*.

Experiment – Manipulation of a *candidate cause* through elimination of a *source* or alteration of *exposure*, to evaluate the candidate causal agent's relationship to an *effect*.

Exposure – The co-occurrence or contact of a *stressor* with the biological resource demonstrating *impairment*.

Exposure-response – 1. The relationship between the intensity, frequency, or duration of *exposure* to a *stressor* and the intensity, frequency, or duration of the biological response. 2. A model of that relationship. This term is similar to *concentration-response* and *stressor-response*.

Field studies – Observational or experimental studies carried out in nature.

Hypothesis – A proposed explanation for an observable phenomenon. In experimental design, a hypothesis is set forth and subsequently tested (either singly or along with multiple alternate hypotheses) to determine if the new data support or contradict the hypothesis.

Impairment – A detrimental effect on the biological integrity of a population, community or ecosystem that prevents attainment of the designated use.

Indirect causation – The induction of effects through a series of cause-effect relationships, such that the impaired biological resource may not even be exposed to the initial cause. For example, the direct effect of an herbicide may be reduced algal production, which may indirectly lead to reduced herbivore and predator populations. Compare to *direct causation*.

Inference – The act of reasoning from *evidence*.

Iteration – Repetition of a process; in particular, repetition of the causal analysis process with new data or observations after results of prior stressor identifications were inconclusive.

Life history – Developmental processes and behaviors that sustain and reproduce a species. For example, case formation and net-spinning can be components of the life history of certain caddisflies.

Manipulation of exposure – A *type of evidence* in which human action induces, eliminates, or modifies *exposure* to a *stressor* (e.g., shutting down an effluent source, fencing cattle from a stream, or caging fish in a contaminated lake).

Mechanism – The process by which a system is changed.

Mechanistic plausibility –The ability of a *candidate cause* to realistically induce the observed *effects*, given knowledge of its mode of action.

Pathogens – Organisms capable of inducing disease in susceptible hosts.

Piece of evidence – A specific data analysis or observation that relates to a *type of evidence*. For example, the *type of evidence* 'stressor-response relationships from laboratory studies' may include a chronic value for fathead minnows and an acute *species sensitivity distribution* for freshwater fish as pieces of evidence.

Plausibility – The degree to which a cause-effect relationship would be expected, given known facts.

Pollutant – Any substance introduced into the environment that adversely affects a resource.

Positive evidence – *Evidence* that tends to support the case for a *candidate cause*.

Probable cause – The *cause* that is most likely to be the true cause of an *effect*.

Proximate cause – The *cause* that induces the *effect* through direct *exposure*. Compare to an *indirect cause*.

Proximate stressor – The *stressor* that directly induces the biological *effect* of concern. This is equivalent to *causal agent*, but emphasizes the negative consequences of *exposure*.

Pseudoreplication – The treatment of multiple samples from the same sample unit as replicates for statistical purposes. For example, multiple benthic invertebrate samples taken in a single channelized stream are pseudo-replicates because the stream channel (the hypothesized cause) has not been replicated. True replicates would be taken from multiple channelized streams.

Receptor – Any individual organism, species, population, community, habitat or ecosystem that is potentially exposed to contaminants of potential concern or other stressor.

Reference (condition) – A location or treatment designed to reflect the ambient physical and chemical conditions of a site in the absence of the stressors of concern in the risk assessment. For example, in a study of soil contamination, a reference site should be chosen to depict the climate, substrate, and habitat factors relevant to the site but with no incremental contamination relative to background conditions. In some cases, the term reference may be used in the context of an altered background condition (i.e., where the local conditions surrounding a site are not pristine).

Reference site – A location or waterbody selected for comparison with the impaired location or waterbody being assessed. The type of sites selected and the type of comparative measures used will vary with the purpose of the comparisons. References that lack a *source*, *stressor*, or *impairment* are termed negative or clean references; references that have well-defined and elevated levels of a *stressor* or well-characterized *sources* or *impairments* are referred to as positive or dirty references.

Replicate – 1. One of a set of independent systems that have been randomly assigned a single treatment. 2. The process of generating a set of such systems.

Response – The biological result of an *exposure*. This term is synonymous with *effect*, but emphasizes the *receptor* that responds (e.g., the response of trout) rather than the *agent* that acts upon it (e.g., the effect of cadmium).

Simulation model – Mathematical representation of the entities and processes in a system.

Source – An origination point, area, or entity that releases or emits an *agent* that may be an *indirect cause* or a *proximate cause*.

Spatial gradient – A graded change in the magnitude of some quantity or dimension measured along a transect.

Spatial/temporal co-occurrence – A *type of evidence* that involves observation of two entities or conditions at the same place or time; it is sometimes shortened to *co-occurrence*.

Species sensitivity distribution (SSD) – A cumulative probability distribution of toxicity values for multiple species. For ERA, the chemical concentration that may be used as a hazard level can be extrapolated from an SSD using a specified percentile of the distribution.

Specificity – The degree to which an *effect* is known to result from one or very few possible *causes*, or a *cause* is known to have a distinct *effect*.

Stakeholders – People or organizations with an interest in the outcome of an assessment.

Stream reach – A segment of a stream delimited in some way (e.g., by occurrence of tributaries or effluents).

Stressor – any substance or process that may cause an undesirable response to the health or biological status of an organism..

Stressor-response – 1. The relationship between the intensity, frequency, or duration of *exposure* to a *stressor* and the intensity or frequency of a biological *response*. 2. A model of that relationship. Equivalent to *exposure-response* and *concentration-response*.

Temporal gradient – A graded change in the magnitude of some quantity or dimension measured over time.

Toxicity identification evaluation (TIE) – A tool in which physical/chemical manipulation of a sample is conducted to isolate or change the potency of different groups of toxicants potentially present in a sample. Rather than using a chemical detector to determine whether a change occurred, a biological test, in this case a toxicity test, is used as the "indicator" to determine whether the manipulation changed toxicity.

Type of evidence – A category of relationships that provides a logically distinct way to support, weaken, or refute the case for a *candidate cause*. A type of evidence may contain multiple lines of evidence.

Uncertainty – Uncertainty is a term used in subtly different ways in a number of scientific fields. Generally, it means the lack of perfect knowledge regarding a given parameter, process, or condition. In risk assessment, uncertainty is the state of having limited knowledge where it is impossible to exactly describe an existing state or future outcome. Uncertainties come in many forms, including measurement uncertainty, random variations, conceptual uncertainty, and ignorance.

Variability – Differences among entities or states of an entity attributable to heterogeneity. Variability is an inherent property of nature and may not be reduced by measurement.

Variable, types of – A functional relationship between two variables is expressed by a mathematical formula: Y=f(X). Y denotes the dependent or response variable and X denotes the independent, explanatory, or predictor variable. f denotes a functional relationship between X and Y. When the X variable is the assumed or actual cause of the response, it is referred to as the causal variable. 'f' should be defined as well

Watershed – An area of land from which any released or deposited water flows into the same waterbody. Equivalent to catchment.

Glossary adapted from USEPA 2007. Causal Analysis Diagnosis Decision Information System (CADDIS). http://cfpub.epa.gov/caddis/info_sources.cfm?Section=30&From=A&To=Z. Accessed August 6, 2010.

Attachment A

Stressors, Potential Impacts and Indicators a Stressor May be a Factor at a Particular Site

Table A-1 Stressors, Potential Impacts and Indicators a Stressor May be a Factor at a Particular Site

Stressor / Description	Potential Impacts	Indicators
Biological Stressors		
Invasive non-native (alien) species - Are plants, animals, or micro-organisms purposefully or accidentally introduced in areas outside of their natural current or historical distribution [1] - Occur in both aquatic and terrestrial systems – Organisms naturally colonize new regions via migration and dispersion but human actions have significantly ↑ rate of species introductions	- Predation, parasitism, competition, or changing the ecosystem structure such that the physical habitat becomes less suitable for native species - Adverse economic effects through the loss of desirable species, diminished beneficial uses, or increased maintenance costs on infrastructure present within the site - Commission for Environmental Cooperation (CEC) risk assessment framework for invasive non-native species [2], offers methods for identifying potential risks to the environment, the economy, and human health	- Non-native species typically become invasive as they are well adapted to their new environment and lack natural predators - Invasive vegetation can out-compete native species to form monocultures and animal species can proliferate and quickly dominate ecosystems - Presence of many invasive species, is subtle; introduced pests and diseases can target individual native species - If site impairment is associated with altered community structure, particularly with respect to ↓ diversity or altered species composition, presence of invasive non-native species should be considered a candidate cause of that impairment
Nutrient Enrichment -Includes nitrogen and phosphorus - Nature and severity of impairment differs across ecosystems - In temperate terrestrial sites (e.g., Canada), nitrogen is typically the nutrient that most limits plant growth [3,4] ; in freshwater aquatic systems limiting nutrient is usually phosphorus and in brackish and marine waters is usually nitrogen [3] - Regardless of which nutrient limits growth, potential effects of nutrient enrichment are generally the same	- Initially ↑ productivity, but can ↓ soil fertility over the long term through leaching of critical soil micronutrients (e.g., Ca, K, Mg) [5,4] - ↓ Plant biodiversity in the short-term [6] and affect sensitive species, such as amphibians [7] - Excess can cause excessive aquatic plant growth or nuisance algal blooms that deplete oxygen, adversely affect habitat quality[7] - Depletion of dissolved oxygen in bottom waters (i.e., hypoxia) can result in mortality to benthic organisms and fish or displacement of more mobile species, resulting in “dead zones” [8] - Additional effects of nutrient loading include elevated ammonia and hydrogen sulphide concentrations in sediment, resulting from decay of organic matter under low oxygen conditions	- Nutrient enrichment of terrestrial systems usually a gradual process, therefore indicators are typically subtle and require chemical analysis of soil or leachate (e.g., groundwater, porewater, or surface water) to detect. - Indicators in aquatic systems are far more dramatic and characterized by frequent widespread algal blooms or dense layers of macroalgae in shallow waters and low dissolved oxygen levels, and fish and invertebrate communities may be dominated by pollution-tolerant species - Nutrient enrichment is a potential cause of impairment at contaminated sites characterized by algal blooms, habitat impairment and low dissolved oxygen levels

Pathogens - Can be natural or anthropogenic and play important role in evolution of organisms - Human activities can influence their distribution and virulence - Can be introduced into new environments or spread facilitated by nutrient enrichment or the introduction of non-native species - Are more readily released into aquatic systems via discharge of wastewater, but can impact aquatic and terrestrial systems.	- Can directly impact human health and environment - Substantial waterfowl mortalities throughout Canada attributed to botulism outbreaks [9] - Global decline in amphibians attributed, in part, to viral or fungal pathogens [10, 11] - Organisms affected by other anthropogenic stressors may be more susceptible to infection as a result of pathogens	- Anthropogenically-induced pathogens may be present at sites located downstream of discharges from wastewater treatment plants, urban centers, or agricultural fields [9] - At other sites, natural or anthropogenically-induced pathogens may be a factor affecting local communities - Indications of disease include ↑ mortality within a species or group of closely related species, or evidence of sublethal effects (e.g., deformities or unusual behaviours). - Pathogens are plausible candidate cause at contaminated sites characterized by ↑ incidence of deformities, altered species composition, fish kills or bird kills
Harvesting / Resource Extraction - A broad category of stressors encompassing many activities (e.g., recreational, commercial, or illicit hunting, trapping, and fishing; subsistence foraging; population or ecosystem management) that can have a wide range of effects on terrestrial and aquatic ecosystems - Typically, government policies implemented to establish sustainable harvesting of wildlife [12]; however, even legal harvesting can impart stress on ecological populations and communities, if not managed effectively	- Shifts in population age structure, changes in diversity and community structure, ↓reproduction rates, ↓ population size, and extirpation (local extinction) e.g., removal of a top predator from ecosystem through fishing or hunting can result in a significant ↑ in prey population which, in turn, affects the abundance of food resources for the prey (referred to as trophic cascade) [13] - Species of marine fish that mature slowly, have slower growth rates and lower reproductive outputs tend to be more affected by fishing than those with faster growth and maturation rates [14]	- Indications of stress due to harvesting include ↓ population size, changes in diversity and community structure, fishery collapse and extirpation - While exact relationship between biodiversity and ecosystem function is still scientifically debated [15], experimental studies demonstrated greater diversity is correlated with metrics of ecosystem function such as productivity[16] and stability in response to disturbance [17] - Loss of individual species can have disproportionate effects on ecosystem structure or function (referred to as "keystone" species) [18] - Slight changes in diversity (due to any number of reasons such as hunting, habitat fragmentation, disease, etc.) have the potential to impact all organisms present at the site

Physical Stressors		
Habitat Degradation or Destruction - The most significant stressor impacting organisms globally [19, 20] - A significant stressor for aquatic and terrestrial systems - Closely associated with human development - Includes direct loss of habitat area due to changes in land use and development, and loss of quality or suitability due to changes in specific characteristics (e.g., heavy recreation of terrestrial habitats can lead to soil compaction or ↑soil erosion which could affect community structure), habitat fragmentation or changes resulting from filled wetlands, aquifer level changes, or loss of complexity - Development of previously undeveloped land is most evident - While less apparent, habitat fragmentation also adversely affect many species [36, 37] - Degradation of habitat required for a critical life-stage of a species can ultimately translate to population-level effects for that species - Locally, habitat quality is typically the dominant factor influencing community structure (i.e., the species composition, diversity, and dominance of individual taxa)	- Habitat fragmentation can affect large herbivore and carnivore populations with large home ranges and smaller organisms with small home ranges less capable of moving between habitat patches [21, 22, 23, 24] - Habitat fragmentation may result in genetic isolation which can hinder a local population's ability to adapt to other ecological stressors - Habitat quality is a significant factor impacting aquatic communities [25, 26, 27, 28, 29, 30] - Urban streams are subject to many disparate stressors leading to ↓ biotic richness (# of species present), loss of sensitive species and ↑ dominance by tolerant species (*i.e.*, urban stream syndrome) [31] - Soil erosion significantly ↓soil fertility with resulting ↓ in primary production [32] - Upland soil erosion can result in ↑sediment deposition in receiving waterbodies - ↑sediment deposition can result from forestry, presence / construction of roads, construction, mineral extraction, agriculture, etc. [33, 34] - Sediment deposition can ↓ water clarity, impacting algal communities and loss of suitable sediment substrate for invertebrates / fish [30, 34, 35]	- Many forms of habitat degradation in aquatic systems including channelization of rivers and streams; infilling of ponds, wetlands, etc; navigational dredging of rivers and harbours; bank stabilization; construction of overwater structures; and shoreline development - Development within watershed adversely impacts habitat quality of aquatic systems [38] - Urban and suburban development ↑ the impervious area which alters the pattern of discharge to streams and rivers during storm events - Changes in land use in watershed can alter substrate within the water body by ↑ sediment deposition or removing sources of woody debris that provide organic matter and habitat complexity - Signs of habitat degradation are overt and generally detectable through direct observation and remote sensing (e.g., GIS, aerial photographs) - To differentiate impairment due to habitat degradation from chemical releases, it is critically important that reference areas are well matched to site with respect to habitat quality, type, and fragmentation - Habitat surveys targeting species with known habitat preferences can help determine what habitat exists at a site and what type of habitat is optimal for which species and life stages -Soil erosion likely to be a potential stressor at terrestrial contaminated sites only if there is a history of agriculture or forestry in vicinity of site, or in large areas of exposed soils such as at mine sites - Soil erosion may be a more common stressor in aquatic systems than terrestrial systems

Temperature - Can affect aquatic and terrestrial systems, but most research is on aquatic - Water temperature is an important developmental cue for aquatic and amphibious organisms; even small changes can ↑ susceptibility of some organisms to disease or parasites, delay development, or skew sex ratios (in the case of some reptiles) - Water temperature is affected by changes in climate, discharge of wastewater, dams or other impoundments, and loss of water body shading from the removal of riparian vegetation	- Most prevalent effects of temperature stress (e.g., excess temperature fluctuations) in aquatic systems are changes to aquatic invertebrate, amphibian, and fish community composition - Effects of stream temperature on invertebrate communities include: excluding taxa unable to tolerate the existing temperature ranges of each stream; and determining developmental rates of those taxa that were found in each stream [38] - Stream temperature has been linked to ↑ juvenile mortality, ↑ susceptibility to disease and predation, changes in migration timing, and enhancement of populations of competitors of salmonids [39] - Air and water temperature are important factors in determining habitat suitability and timing for spawning and metamorphosis for amphibians [40, 41]	- Indications in aquatic systems often subtle; most dramatic indication is absence of species known to be sensitive to temperature (e.g., stonefly [42] salmonids [28, 30]) - For clearest indication of temperature stress potential, monitor water temperature on several dates based on timing of sensitive life stages or period when temperatures are near their maximum - Observed surface water temperatures can be compared to local reference and guidelines for water temperature depending on the type of water body and aquatic community (e.g., British Columbia water quality guidelines for temperature) - Should be considered a candidate cause at sites with impaired populations of temperature-sensitive species - Observational water temperature data should be collected at impaired site and ≥ 2 suitable reference areas
Climate Change - Includes many potential biological, physical, and chemical stressors - There is a substantial and growing literature on the potential implications and ecological responses to climate change in a variety of ecosystems [43, 44, 45, 46, 47, 48, 49] - Depending on location, climate change likely to include changes in temperature regime, precipitation patterns leading to changes in stream flow patterns, introduction of new species and pathogens, and acidification	- Potential impacts on forested ecosystems vary greatly depending on region and factors currently constraining ecosystem production - Unusual freeze-thaw events as a result of below average snow cover may predispose tree strands to damage from insects and pathogens [46, 50, 51, 52] - ↑ summer temperatures, ↓ summer precipitation, and warmer minimum winter temperatures have increased potential range of some species [52] - Forest primary production may ↑ under climate change scenarios due to the ↑ temperatures and fertilization from ↑ atmospheric carbon dioxide concentrations [43] - Numerous studies documented earlier leaf-out and longer growing seasons in temperate and boreal forests throughout the world [48]	- Because climate represents long-term patterns in temperature, precipitation, and other meteorological factors, impacts attributed to climate change generally cannot be measured in the short term - Indications of adverse impacts on ecological communities resulting from climate change require long-term data sets - Because climate change is manifested as a variety of specific stressors acting on ecosystems, best indicators for potential impacts are metrics for those individual stressors (e.g., loss of temperature-dependent species)

	- Net effect of all of these factors is likely a shift in the community composition - Loss of wetlands and native freshwater fish, ↑ eutrophication, loss of habitat for coldwater species, ↓ in lake levels, and ↑ sensitivity to acidification are potential impacts to Canadian freshwater systems from climate change [45] - Coastal marine communities composition expected to be significantly affected by changes in temperature, ocean chemistry, circulation patterns, synergistic effects between climate, fishing, and coastal development pressures [49] - Most significant effects expected in polar regions where extent /duration of sea-ice ↓and ↑thawing of permafrost [53] ; significant shifts reported in Arctic ecosystems are well summarized [54]	
Chemical Stressors		
Stormwater Runoff - Generated by runoff from natural landscapes and impervious areas (e.g., paved streets, parking lots, building rooftops) during rain/snow events - Causes hydrological changes and is a source for contaminants originating within watershed. - In urban areas that employ combined sewer overflow systems, extreme precipitation events can trigger overflows that release untreated sewage and stormwater directly to waterbodies. - Can impact terrestrial sites adjacent to areas with extensive impervious surfaces, it is primarily considered a stressor for aquatic	- Chemicals commonly detected in stormwater runoff from urban and suburban areas include metals, petroleum residues (e.g., PAHs), nutrients, and pesticides [55, 56, 57, 58, 59, 60, 61], which may be toxic to aquatic life [60, 61] - Stormwater runoff can be an important stressor for biological communities in streams in urban, suburban, and agricultural areas - Sediment is a significant stressor, impact and indicator of stormwater runoff; leads to ↑ deposition of fines, damages spawning beds, changes benthic community structure, etc. - A significant impact from stormwater runoff is ↑ erosion in aquatic systems	- Stormwater runoff is one of the key factors contributing to urban stream syndrome [31]; adverse impacts to aquatic communities detected in streams in watersheds with only 5% impervious surfaces [62] - Given effects on aquatic communities at very low levels of development, a stream with any level of urban / suburban development in its watershed could be adversely affected by stormwater runoff - Key indicators of impairment are temporal relationships (between storm events & toxicity) and spatial relationships (between stormwater outfalls & toxicity) - Warrants consideration as a candidate cause in urban / suburban aquatic systems that exhibit toxicity that is spatially and/or temporally correlated with extreme precipitation events

sites.		
Acidification - Primarily caused by precipitation of sulfuric and nitric acid, which are secondary pollutants formed from sulfur dioxide and nitrogen oxides and can adversely affect surface water and terrestrial ecosystems [63] - Sensitivity of a system to acidification is dependent on bedrock and surficial geology and influenced by climate and other terrain characteristics (e.g., occurrence of wetlands) [64] - Many factors influence a lake's response to sulfur deposition including: depletion and restoration of base cations in the soil pool, storage and release of acid anions in wetlands and forest soils, dissolved organic carbon inputs from drainage basins, and other stressors related to climatic variation and nitrogen deposition [65] - ↑ in atmospheric carbon dioxide from fossil fuel combustion is expected to lead to gradual ↑ in acidity of coastal marine waters over the next century [66, 67]	- Acidification has been related to ↓ in fish, zooplankton, and macroinvertebrate species richness [65] - Low pH is linked to ↓ fish abundance due to impaired reproduction and mortality in earlier stages of life and to losses of calcium-rich macroinvertebrate taxa that are important sources of food for higher trophic levels [65] - Quality and quantity of food for water-dependent birds can be affected with acidification [65] - In terrestrial ecosystems, adverse effects can include acute toxic interactions of acid with plants, nutrient deficiencies in soils, decreases in the health and biological productivity of forests, and aluminum mobilization [63] - Acid-mobilized aluminum can be toxic to fish in streams and lakes and damage roots and leach nutrients from forest foliage [68, 69]	- ↑ Ratios of sulfate (SO_4^{2-}) to base cations (Ca^{2+} and Mg^{2+}) can be an indicator of acidification in freshwater lakes and streams and their watersheds [69] - Lower benthic fauna diversity has been observed in acidified compared to circumneutral streams, and evidence for ecological damage from acidification can lie in fishery declines [69] - Focusing on assessment of adult game fish, rather than more sensitive juveniles or organisms lower in the food web, can lead to underestimating damages caused by acid rain [69] - Algal species that respond rapidly to acidification (e.g., mallomonadaceans and diatoms) have been used as paleoindicators of changes in lake acidity over time [68, 70] - Aquatic acidification is of concern in southeastern region of Canada due to elevated acid deposition and acid sensitive terrain [64]

Notes:

1. Environment Canada, 2004; 2. CEC, 2009; 3. Vitousek and Howarth, 1991; 4. Aber et al., 1998; 5. Vitousek et al., 1997; 6. Tilman, 1987; 8. Committee on Environment and Natural Resources, 2010; 9. Environment Canada, 2001; 10. Bollinger et al., 1999; 11. Beebee and Griffiths, 2005; 12. Johnston et al., 2000; 13. Pace et al., 1999; 14. Jennings and Kaiser, 1998; 15. Ives and Carpenter, 2007; 16. Tilman, 1996; 17. McCann, 2000; 18. Paine, 1969; 19. United Nations, 1993; 20. CWS, 2000; 21. Proctor et al., 2000; 22. Wayne et al., 2002; 23. Noel et al., 2007; 24. Patterson, 2008; 25. Cormier et al. 2002; 26. Norton et al. 2002; 27. MDEQ 2005; 28. USEPA 2007; 29. Haake et al. 2010; 30. Wiseman et al. 2010; 31. Walsh et al., 2005; 32. Pimentel et al., 1995; 33. Allan, 2004; 34. Owens et al., 2005; 35. Wood and Armitage, 1997; Debinski and Holt, 2000; 37. Fahrig, 2003; 38. Hawkins et al., 1997; 30. USEPA, 2003; 40. Gilbert et al., 1994; 41. Litzgus and Brooks, 2000; 42. Nebeker, 1971; 43. Aber et al., 1995; 44. McCarthy, 2001; 45. Schindler, 2001; 46. Hogg et al., 2002; 47. McLaughlin et al., 2002; 48. Walther et al., 2002; 49. Harley et al., 2006; 50. Carroll et al., 2003; 51. Aukema et al., 2006; 52. Kurz et al., 2008; 53. Chapin et al., 2006; 54. Parmesan, 2006; 55. Christensen and Guinn 1979; 56. MacKenzie and Hunter 1979; 57. Davis et al. 2001; 58. Kayhanian et al. 2003; 59. Councell et al. 2004; 60. Weston et al. 2005; 61. Domagalski et al. 2010; 62. Cufney et al., 2010; 63. USEPA, 1999; 64. Jefferies et al., 2003; 65. Environment Canada, 2001; 66. Caldeira and Wickett, 2003; 67. Orr et al., 2005; 68. Dixit et al., 1992; 69. Schindler, 1998; 70. Hartmann and Steinberg, 1986

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Additional information can be obtained at:

Environment Canada
Inquiry Centre
10 Wellington Street, 23rd Floor
Gatineau QC K1A 0H3
Telephone: 1-800-668-6767 (in Canada only) or 819-997-2800
Fax: 819-994-1412
TTY: 819-994-0736
Email: enviroinfo@ec.gc.ca