Appendix C.1

Natural Heritage Methodology

(www.natureserve.org/prodServices/heritagemethodology.jsp)

Products & Services

Natural Heritage Methodology: Supporting Interoperability within the NatureServe Network

The defining characteristic of the NatureServe network is the use of natural heritage methodology. By specifying standard procedures for gathering, organizing, and managing information on biodiversity, natural heritage methodology unites the efforts of hundreds of individuals and dozens of institutions on two continents working to advance the knowledge needed to effectively conserve biodiversity. Over the past quarter-century, natural heritage methodology has evolved to keep pace with the growth in scientific knowledge about the natural world and advances in information technologies. Nevertheless, the underlying continuity of the methodology over time has permitted the network to accumulate knowledge and make available vast amounts of scientifically authoritative data. Natural heritage methodology provides a rigorous set of procedures for identifying, inventorying, and mapping species and ecosystems of conservation concern; for gathering related information on conservation sites and managed areas; and for setting conservation priorities.

Natural heritage methodology has several basic characteristics:

- It is designed to support a decentralized database network that respects the principle of local custodianship of data.
- It supports the collection and management of data at multiple geographic scales, allowing decisions to be made based on detailed local information, yet within a global context.
- It encompasses both spatial and attribute data, but emphasizes the type of fine-scale mapping required to inform on-the-ground decisions.
- It includes multiple quality control and quality assurance steps to ensure that data products have the reliability needed to inform planning and regulatory actions.
- It incorporates explicit estimates of uncertainty and targets additional inventory work to reduce levels of uncertainty.
- It integrates multiple data types, including: species and ecological communities; collections and other forms of observational data; biological and non-biological data.

Because biodiversity encompasses the variety of life at all levels, not just species, natural heritage methodology is designed to deal with both species and ecological communities, referred to collectively as "elements of biodiversity." The NatureServe network has gathered and organized data on over 84,000 such elements of biodiversity, including animals, plants, fungi, and terrestrial and freshwater communities. Scientific names, local and global conservation status, basic biological and ecological characteristics, management requirements, and the location and condition of species populations and community occurrences are among the types of data collected. The information is housed in customized databases that employ sophisticated geographic information systems.

Each part of the network has distinct roles and responsibilities. "Global" (range-wide) information on each element is developed and managed centrally by NatureServe, while detailed local data is developed and managed by member programs. Annual data exchanges between NatureServe and its member programs ensure that up-to-date rangewide data is available to all local databases, and that detailed local data can be shared and aggregated across the network.

At the core of the methodology is the concept of the element occurrence, the spatial representation of a species or ecological community at a specific location. An element occurrence generally delineates a species population or ecological community stand, and represents the geo-referenced biological feature that is of conservation or management interest. Element occurrences are documented by voucher specimens (where appropriate) or other forms of observations. A single element occurrence may be documented by multiple specimens or observations taken from different parts of the same population, or from the same population over multiple years. At present more than one half-million element occurrence records are managed across the network, representing several million observations or specimens.

Basic Steps in Natural Heritage Methodology

In the broadest sense, natural heritage methodology answers three key questions: What species and ecosystems exist in a region (the elements of biodiversity)? How are they doing (their condition and status), and which are priorities for conservation? Where precisely are they found (documenting and mapping element occurrences)?

To answer these questions, natural heritage programs carry out a series of repeated steps. Each time the steps are repeated, the data are refined to give a better picture of biodiversity and of problems and progress in its conservation. The basic steps employed are:

1. Develop a list of the elements of biodiversity in a given jurisdiction, focusing on better-known species groups (e.g., vertebrate animals, vascular plants, butterflies, bivalve molluscs), and on the ecological communities present.
2. Assess the relative risk of extirpation or extinction of the elements to determine conservation status and set initial priorities for detailed inventory and protection.
3. Gather information from all available sources for priority elements, focusing on known locations, possible locations, and ecological and management requirements.
4. Conduct field inventories for these elements and collect data about their location, condition, and conservation needs.
5. Process and manage all the data collected, using standard procedures that will allow compilation and comparison of data across jurisdictional boundaries.
6. Analyze the data with a view toward refining previous conclusions about element rarity and risk, location, management needs, and other issues.
7. Provide access to data and information products to interested parties so that it can be used to guide conservation, management planning, and other natural resource decision-making.

To Learn More

Additional detailed discussion of specific aspects of natural heritage methodology is provided in the About the Data section of our NatureServe Explorer website. For technical documentation of key standards and protocols that are part of natural heritage methodology, see the links below.

- Element Occurrence Data Standard: standards for documenting and mapping species and community element occurrences
- Element Occurrence Specifications for Animals: using functional groups to define criteria for delimiting element occurrences of animals. Appendix A is a list of the separation and Inferred Extent distances for the currently defined functional groups. See Separation Distance and Mapping Guidelines for guidelines on determining suitable separation distances and for mapping animal EOs.
- Element Occurrence Specifications for Plants: A habitat-based strategy for delimiting element occurrences of plants. Download complete document (PDF: 83K). View decision-tree.
- Element Occurrence Rank Specifications: a generic approach that focuses on the probability of persistence for determining element occurrence ranks for species. Also view the Ranking Key.
- NatureServe Biodiversity Data Model: technical architecture and data dictionary for Biotics, NatureServe's core data management system
- FGDC-Compliant Metadata (text file) or FGDC-Compliant Metadata (html file)
- Benchmark Data Content Standards (Word file: 1,666 KB) and Element Global Fields (Excel file: 99 KB): These standards provide guidance to members of the NatureServe network regarding the development and quality control of core data elements. The standards focus on those data that are shared across the network and are necessary for providing regional, national, and international data products and services. Specifically, these standards establish: 1) content goals for element and element occurrence records; 2) spatial data (GIS) standards to facilitate the aggregation of these data; and 3) metadata documentation. Benchmark Data Content Standards also serve as a metric against which to measure the currentness and completeness of NatureServe data.

Appendix C.2

NatureServe Benchmark Data Content Standards

NatureServe Benchmark Data Content Standards

Version 2.0
September 2004

NatureServe
in cooperation with its
Natural Heritage Member Programs

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1. INTRODUCTION

1.1. Purpose

The Benchmark Data Content Standards (BDCS) are intended to provide guidance to all NatureServe member programs. Adherence to these standards will ensure a high level of accuracy, currency and quality to the species data[1] maintained across the network of member programs and NatureServe.

The Benchmark Data Content Standards play an essential role in demonstrating to our partners and clients that the completeness of our core data is measurable and substantial. Adherence to data standards is used by many of our clients as justification to select NatureServe data, because it is a demonstration that our data meet documented quality requirements. The Benchmark Data Content Standards additionally reflect NatureServe's commitment to register its data sets with global data portals such as the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/), so that researchers can discover what we have to offer, determine whether we have data that would be useful to them, and understand how to make data requests. These standards will allow NatureServe to measure and report on the quality of our data, and be used to identify data development and management priorities.

The need to produce regional and global biodiversity data products and services was highlighted at the 1994 Association for Biodiversity Information (ABI) Annual Meeting in Birmingham, Alabama. These data products require data from multiple member programs and are referred to as "multi-jurisdictional data products". The first iteration of Benchmark Data Content Standards was developed and approved by the ABI Data Standards Committee in 1998. This document builds upon those efforts, and will provide the framework for NatureServe staff and member programs to work together in a coordinated way to enhance the usability and relevance of our combined data resources.

These standards establish priorities and network-wide content goals for:

- Element and Element Occurrence records for regional, national and international data products and services;
- Element and Element Occurrence fields for a standardized dataset;
- GIS (spatial data) standards to facilitate the aggregation of these data; and
- Metadata documentation.

In addition, the Benchmark Data Content Standards will allow us to measure the current status of our data, and progress in data improvements.

1.2. Role of Member Programs

By participating in the NatureServe network, each member program is responsible for striving to meet the Benchmark Data Content Standards that apply to locally developed data. This

[1] As of fall 2004, Benchmark Data Content Standards had not been developed for ecological data. When standards for ecological data are complete, they will be added to this document as an addendum.

commitment is formalized in Section III.C.6.b. of the Data Sharing Agreements. To support the whole NatureServe network in meeting these standards, member programs will assist in the following ways, as time and funding permit.

- Help NatureServe staff formulate, prioritize, and review the Benchmark Data Content Standards.
- Assume the lead responsibility for meeting Benchmark Data Content Standards for subnational and Element Occurrence data in its jurisdiction.
- Coordinate with NatureServe staff to assist in meeting Benchmark Data Content Standards for global Element data where possible.
- Collaborate with NatureServe staff where possible to raise funds to support the implementation of these standards.

1.3. Role of NatureServe

As the coordinating body for the network, NatureServe staff are responsible for striving to meet the Benchmark Data Content Standards that apply to data developed and maintained by NatureServe. In addition, NatureServe staff play a key role in supporting the members' efforts to meet the standards as formalized in Section III.B.7.b. of the Data Sharing Agreements in the following ways.

- NatureServe will play the lead role in developing, reviewing, and maintaining Benchmark Data Content Standards documentation, with input from member programs.
- NatureServe staff will assume the lead responsibility for meeting Benchmark Data Content Standards for global data fields, and for national data fields for nations where no national entity has assumed this responsibility.
- NatureServe staff will provide methodological and technical assistance to member programs in meeting Benchmark Data Content Standards.
- NatureServe staff will encourage, and support where possible, member programs wishing to implement recommended Benchmark Data Standards for global Element data.
- NatureServe staff will collaborate with member programs to raise funds to support the implementation of these standards.
- NatureServe staff will measure and report progress on meeting Benchmark Data Content Standards.

1.4. Process for Updating Benchmark Data Content Standards

Periodic updates to the Benchmark Data Content Standards will be done through a small working group that is equally divided between NatureServe and member program staff. The results of any meetings to review and discuss potential changes to the BDCS by this group will be sent to the Section Councils and all member programs for review and comment. The

Benchmark Data Content Standards working group will then evaluate input from all member programs and develop a final update to the Benchmark Data Content Standards.

2. OVERVIEW OF BENCHMARK DATA CONTENT STANDARDS

The Benchmark Data Content Standards document is intended as a resource for the NatureServe network to provide guidance for data development and quality control of a core multi-jurisdictional dataset for use in regional, national and international data products and services.

The BDCS document consists of the following components:

- Elements and Element Fields: Section 3 (Elements) and Section 4 (Element Fields) define subsets of Element records and corresponding fields.
- Element Occurrences: Section 5 (Element Occurrences) and Section 6 (Element Occurrence Fields) define subsets of Element Occurrence records and corresponding fields.
- Spatial Data Aggregation and Documentation: Section 7 (GIS) and Section 8 (Metadata) provide guidance on meeting spatial and metadata standards that are intended to facilitate the creation of an aggregated spatial dataset.
- Measuring Progress: Section 9 gives an overview of how NatureServe will measure our progress towards meeting the Benchmark Data Content Standards.
- Reconciling EO Data: Section 10 provides an outline of how we will begin the process of reconciling EO data across jurisdictional borders.
- Supporting materials: The document concludes with supporting references, resources, and a glossary of terms.

The Element and Element Occurrence fields are specified in Appendix 1, which is provided as an accompanying Excel file. It is recommended that both documents be used together as a single resource.

When using the Benchmark Data Content Standards, the reader may choose to focus on particular sections, depending on their data lead responsibility and individual interests. For example, member programs may wish to concentrate on the Element Subnational and Element Occurrence sections, NatureServe science staff on the Element Global and Element National sections, and GIS staff on the spatial data and metadata sections.

3. ELEMENTS

3.1. Element Record Subsets

For the purposes of the Benchmark Data Content Standards, there are some data fields that apply to all records, regardless of the taxonomic group, conservation status, or protection status. However, there are also data fields that are relevant only to specific subsets of the plant and animal data, especially in Biotics. In addition, it is useful to prioritize the use of a given field for specific sets of records, but not others.

Therefore, it is necessary in this document to distinguish among a limited number of "Element record subsets" to accurately identify the data fields that should be completed for specific subsets of the Element data (see Table 1). Together, these Element record subsets and the priority Element fields (Table 2, below) form the core of our regional, national and international data products and services.

Table 1. Element Record Subsets

Element Record Subset	ANIMALS	PLANTS
1) All Elements	All Element records in the program's database (NatureServe: all records in NatureServe's central database; Member Program: all records contained in their database).	
2) Primary Subset	• For vertebrates (excluding marine fishes), freshwater mussels, butterflies and skippers, crayfishes, tiger beetles, and odonates: All full species (native and non-native) that regularly occur in the U.S. or Canada either as a native or an exotic. • All full species of invertebrates with Rounded Global Rank of GX, GH, G1, G2, or G3 in the following groups: grasshoppers, snails, stoneflies, mayflies, caddisflies, freshwater shrimps, cave obligates (an ecological group), and moths in the taxa Sphingidae, Saturniidae, Notodontidae, Arctiinae, Catacola, and Papaipema. • For species with Federal status: All vertebrate and invertebrate taxa, including infraspecies, that have a status under the U.S. Endangered Species Act; or with a COSEWIC status; or with a Canadian national General Status of 'at risk' or 'may be at risk'; or with other comparable national status.	• For vascular plants, nonvascular plants, and lichens: All taxa, including full species, subspecies and varieties, that regularly occur in the U.S. or Canada either as a native or an exotic, and have a classification status of "Standard" (see Biotics Help). • For species with Federal status: All vascular plant, nonvascular plant, lichen, and organisms traditionally grouped with plants in the NatureServe databases (e.g., fungi) taxa, including subspecies and varieties, that have a status under the U.S. Endangered Species Act; or with a COSEWIC status; or with a Canadian national General Status of 'at risk' or 'may be at risk'; or with other comparable national status.

Element Record Subset	ANIMALS	PLANTS
3) Subnational Protected	• Taxa on official state, provincial, or tribal threatened or endangered lists, or • Taxa with comparable official status at the local level that may afford the taxon some protection or special consideration.	
4) Species At Risk	Taxa within the Primary Subset (2) that have a Rounded Global Rank of GH, G1, G2, or G3; or have a status under the U.S. Endangered Species Act; or with other comparable national status.	
5) Subspecies at Risk & Selected Invertebrates	Vertebrates not in the Primary Subset (2) and with Rounded Global Rank of TH, T1, T2, or T3. Invertebrates not in the Primary Subset (2) and with Rounded Global Rank of GH, TH, G1, T1, G2, T2, G3, or T3.	All taxa in the Primary Subset (2), plus vascular plants with Rounded Global Rank of TH, T1, or T2.
6) Primary Vascular Plant Subset	Not applicable.	All vascular plant taxa included in the Primary Subset (2). (This category is used only for certain subnational fields).

3.2. Taxonomy

NatureServe staff strive to maintain comprehensive, internally consistent, taxonomic and nomenclatural treatments that reflect widely accepted views for many groups of taxa across North America. Maintaining a comprehensive set of "standard" taxa, with associated synonyms and relationships to "nonstandard" taxa, is fundamental in that it allows NatureServe to receive and integrate data from member programs that may be following many different taxonomic and nomenclatural treatments[2], therefore facilitating conservation action.

NatureServe's standard references for vascular plants of North America are the synonymized checklists published by John Kartesz. At this writing, the latest published version is:

> Kartesz, JT. 1999. A synonymized checklist and atlas with biological attributes for the vascular flora of the United States, Canada, and Greenland. First edition. In: Kartesz, JT and CA Meacham. Synthesis of the North American flora [computer program]. Version 1.0. North Carolina Botanical Garden: Chapel Hill, NC.

The Integrated Taxonomic Information System (ITIS) also bases its plant taxonomy on an older version of John Kartesz's via the USDA "PLANTS" database (although "PLANTS" and ITIS are currently not maintained in sync). NatureServe is a member of the ITIS consortium and continues to work with participants to mutually improve the databases of both parties.

[2] Member programs may choose, or be required, to follow alternative taxonomic treatments (for example, to be compatible with species lists used by their parent organization or other agency in their jurisdiction). Taxonomic concepts/circumscriptions that have no equivalent in the standard treatments are handled by creating nonstandard database records. Issues purely of alternative nomenclature (differing names for the same taxonomic concept), including generic placement, are treated as synonyms in NatureServe Central Databases.

Standard References for Nonvascular Plants and Lichens are:

> Anderson LE, Crum HA, Buck WR. 1990. List of the mosses of North America north of Mexico. The Bryologist 93(4):448-499.
>
> Anderson LE. 1990. A checklist of sphagnum in North America north of Mexico. The Bryologist 93(4):500-501.
>
> Esslinger TL, Egan RS. 1995. A sixth checklist of the lichen-forming, lichenicolous, and allied fungi of the continental United States and Canada. The Bryologist 98(4):467-549.
>
> Stotler R, Crandall-Stotler B. 1977. A checklist of the liverworts and hornworts of North America. The Bryologist 80(3):405-428.

For animals, the standard is obtained from a variety of published references. The list of specific major references used by NatureServe's Zoology Department is available online at: http://www.natureserve.org/explorer/classani.htm. Although some references represent the view of a sanctioned group whose opinions are generally followed by researchers with expertise in a given taxonomic group (*e.g.*, the American Ornithologists' Union Check-list of North and Central American Birds), for most animal groups, available taxonomic lists may be regional rather than global, there may not be a single consensus list, there may be multiple lists, and/or any lists may be infrequently updated. For these reasons, and because taxonomy is a dynamic area of investigation, NatureServe scientists strive to continuously review newly published journals and monographs for taxonomic and nomenclatural changes, and they adopt well-founded taxonomic and nomenclatural changes. Hence NatureServe's taxonomy is often more current than available published lists. In all situations, NatureServe attempts to recognize taxa and names that represent accepted consensus opinion among researchers working in a particular group and that are likely to be adopted in subsequent editions of widely used standardized lists.

In addition to widely recognized species, NatureServe Central Databases also include taxa of conservation concern for which a name has not yet been published. Inclusion of these undescribed taxa usually reflects the needs of an individual member program or inclusion of an undescribed species or Evolutionarily Significant Unit on a government conservation list. Such taxa are assigned provisional common and scientific names (*e.g.*, "Comal Springs Salamander, *Eurycea* sp. 8" or "Steelhead – Central California Coast, *Oncorhynchus mykiss* pop. 8").

For additional information about taxonomic groups maintained by NatureServe, please see the following materials on the NatureServe Explorer website:

- Summary of core taxonomic groups: http://www.natureserve.org/explorer/summary.htm
- Standard taxonomic references: http://www.natureserve.org/explorer/class.htm

4. ELEMENT FIELDS

4.1. First and Second Tier Element Fields

The Benchmark Data Content Standards for Element data fields are applicable to particular Element record subsets, as defined above in Table 1, and in addition are prioritized for data development and quality control purposes. This approach, presented below in Table 2, allows the maximum flexibility toward developing a standardized dataset across the network. These fields are the highest priority for filling data gaps where information is available and/or applicable.

The "First Tier Element Fields" are the high priority "core fields" that should be completed and maintained by NatureServe and all member programs for the Element record subsets as specified in Table 2. This standardized dataset will facilitate the production of quality multi-jurisdictional data products and services.

The "Second Tier Element Fields" are the next priority for filling data gaps and conducting quality control procedures. While it is highly desirable to enter data into these fields, fundraising efforts and human resources should initially be directed to completing the First Tier Element Fields when discretion or funding allows.

Definitions in the Biotics Tracker and Mapper Help screens and domain tables, as well as data validation inherent in the Biotics Tracker and Mapper software tools, serve to define acceptable standard data values for each of the fields. Additional details, particularly where the Benchmark Data Content Standards are not enforced by Biotics database constraints, are included in the Element Fields Tables.

4.2. Element Fields Tables

The fields for the Benchmark Data Content Standards First and Second Tier Element fields are specified in Appendix 1, which is provided as the Excel workbook BDCS_2004_ver2.0_fields.xls. This file contains separate tables for Global, National and Subnational fields.

Table 2. Element Fields

Please see the tables in Appendix 1: BDCS_2004_ver2.0_fields.xls

Data Category	Lead Responsibility	Spreadsheet Tab
Element Global Fields	NatureServe	"element_global"
Element National Fields	NatureServe, or national entity as relevant	"element_national"
Element Subnational Fields	Member Programs; each member program is only responsible for records tracked by their subnation.	"element_subnational"

5. ELEMENT OCCURRENCES

5.1. Element Occurrence Priority Taxa

This section establishes priorities for Element Occurrence (EO) data development and quality control at the record (see Table 3) and field levels (Table 4) by NatureServe member program staff for use in multi-jurisdictional data products and services. Given the large number of Element Occurrence records across the network, it is necessary to prioritize among taxa to increase efficiency in addressing information gaps and data quality control. In Table 3 there are three subsets of Element Occurrences listed in descending order of priority for multi-jurisdictional data products. Member program staff are encouraged to focus more resources on addressing data gaps for EOs in the higher priority subsets, with an eye towards meeting data content standards for all three priority subsets over time. Since member programs may have data priorities based on agency requirements or other local needs, the priorities below should be viewed as a recommendation for developing a network-wide standardized dataset for multi-jurisdictional projects and do not preclude other data development. Together, these subsets of Element Occurrences and fields constitute the core of our spatial regional, national, and international data products and services. Using this standard, NatureServe and member programs will be able to identify critical data gaps.

Table 3. Element Occurrence Priority Taxa

EO Priority Subset	Element Occurrences
1) National Protected / Imperiled	• have status under the U.S. Endangered Species Act; or • with a COSEWIC status or Canadian national general status of 'at risk' or 'may be at risk'; or • for other countries, an equivalent national status; or • with Rounded Global Rank of G1 or G2; including any related infraspecific Elements (i.e. G1T1, G2T2, G2T1T2, G2T1, etc.).
2) Subnational Protected	Taxa that are not in EO Priority Subset 1 and are: • on official state, provincial, or tribal threatened or endangered lists; or • with comparable official status at the local level that may afford the taxon some protection or special consideration.
3) Additional Imperiled / Vulnerable	Taxa that are not in EO Priority Subset 1 or 2, and with: • Rounded Global Rank of GH or G3, including any related infraspecific Elements; or • Rounded Global Rank ofT1 or T2 of more common Elements (i.e. G4T2, G4T1T2, G4T1, G5T2, G5T1T2, G5T1, etc.). *NOTE: Partially EO-tracked is an option for portions of this subset. For detailed information, please see the EO Data Standard, Chapter 6 "EO Tracking".*

5.2. Required Element Occurrences

As part of the Benchmark Data Content Standard, any Element that meets the criteria in "EO Priority Subset 1", *and* is currently extant within a subnational jurisdiction with a high degree of distribution confidence should have at least one valid and mappable Element Occurrence for that jurisdiction. Taxa that are falsely reported or potentially occurring within a subnation are excluded from this requirement.

5.3. Selection Criteria for Extant Element Occurrences

Each individual member program has the authority to set its own standards for which Element Occurrences will be delivered to local clients that request only extant EOs. However, for multi-jurisdictional data products and services, a consistent set of selection criteria must be used to identify those EOs that are "likely" to still be present at a given location.

The following criteria will be used to select Element Occurrences for inclusion in multi-jurisdictional projects. The format is:

Biotics_table.Biotics_fieldname (BCD FIELDNAME) = field values used in criteria.

- **Presumed to be Correctly Identified**:

  d_id_confirmed.id_confirmed_cd (IDENT) is not equal to "N" or is null

- **Mappable**:

  Polygon EO Data
  Biotics 4 – Tracker tabular data / Mapper polygon EO data; all other GIS datasets:

  - the tabular EO data has an associated EO polygon record (shape.has_shape_ind = "Y")

  Point EO Data
  BCD or equivalent tabular database:

  - Precision not "unmappable" (eo.precision_bcd (PRECISION) = "S", "M" or "G")
  - EO latitude and longitude coordinates valid and not null

For projects that require ***possibly extant EOs***, the following additional criterion is the default to exclude EO records. This criterion may be changed on a project by project basis depending on the scope of the project and client needs. In these cases, NatureServe will consult with member programs.

Element occurrence rank **extirpated**

(d_basic_eo_rank.basic_eo_rank_cd (EORANK) = "X")

For projects that require ***current and extant EOs***, the following additional criteria are the default to exclude EO records. These criteria may be changed on a project-by-project basis depending on the scope of the project and client needs. In these cases, NatureServe will consult with member programs.

1) Element Occurrence Rank **historical** or **extirpated**
(d_basic_eo_rank.basic_eo_rank_cd (EORANK) = "H", "H?", "X", or "X?"), or
2) EOs where Subnational Rounded Rank is **historical** or **extirpated**
(element_subnational.rounded_s_rank (ROUNDED.SRANK) = "SX" or "SH"), or
3) Element Occurrence Rank "**failed to find**" and the EO Last Observed Date **more than 25 years old**
(d_basic_eo_rank.basic_eo_rank_cd (EORANK) = "F" or "F?"
and eo.last_obs_date (LASTOBS) ≥ current date minus 25 years)

5.4. Conformance to Element Occurrence Specifications

Member programs should develop new Element Occurrences (EOs), and amend existing data as needed, to meet the EO Specifications and EO Rank Specifications developed as part of the revised EO methodology. These specifications are developed and used to achieve consistency in the manner in which occurrences of the same Element are delineated and ranked in different jurisdictions, and to help ensure that EOs are created for data having practical conservation value for the Element.

The process of evaluating existing EO records in the context of the new EO Specifications and EO Rank Specifications will be a multi-year effort. During this period, all EOs regardless of the specifications version will be accepted as meeting Benchmark Data Content Standards. Future versions of the Benchmark Data Content Standards will reevaluate and potentially develop a requirement that EOs meet Specifications.

Member programs will be requested to begin tracking the edition of EO Specifications and EO Rank Specifications that were used when the EO was delineated, mapped and ranked. The NatureServe Methods Group has recommended the addition of fields to Biotics to track this information. These fields were recommended by the EO Working Group but not implemented in Biotics 4. When the EO Specification and EO Rank Specification version tracking fields are developed, they will be added to the Benchmark Data Content Standards as "Tier 1" fields (see below).

6. ELEMENT OCCURRENCE FIELDS

6.1. First and Second Tier Element Occurrence Fields

The Benchmark Data Content Standards for both first and second tier Element Occurrence Fields are applicable to all of the EO Priority Subsets listed above in Table 3. These fields are to be prioritized for filling data gaps where information is available and/or applicable.

The "First Tier Element Occurrence Fields" are the highest priority "core fields" that should be completed and maintained by all NatureServe member programs (Table 4). This standardized dataset will facilitate the production of quality multi-jurisdictional data products and services.

The "Second Tier Element Occurrence Fields" are the next priority for filling data gaps and conducting quality control procedures. While all member programs should strive to meet the Benchmark Data Content Standards for these fields, fundraising efforts and human resources should initially be directed to completing the First Tier Element Occurrence Fields when discretion or funding allows.

For both First and Second Tier EO fields the need for data is urgent. Even in cases where the information in the databases is incomplete or not completely reviewed as meeting standards, it is of sufficient quality that existing data will be made available for inclusion in multi-jurisdictional data products in its current condition, without waiting to fill data gaps. Any multi-jurisdictional data products will clearly state that the data for some fields, especially in the Second Tier, are incomplete. Specific information about known data gaps will be noted where possible in project documentation and/or metadata.

Definitions in the Biotics Tracker and Mapper Help screens, as well as domain tables and data validation inherent in the Biotics Tracker and Mapper software tools, serve to define acceptable standard data values for each of the fields. Additional details, particularly where the Benchmark Data Content Standards are not enforced by Biotics database constraints, are included in the Element Occurrence Fields Table.

6.2. Element Occurrence Fields Table

The fields for the Benchmark Data Content Standards First and Second Tier Element Occurrence fields are specified in Appendix 1, which is provided as the Excel workbook BDCS_2004_ver2.0_fields.xls. Please see the tab "element_occurrence".

Table 4. Element Occurrence Fields (Lead Responsibility: Member Programs)

Please see the EO table in Appendix 1: BDCS_2004_ver2.0_fields.xls

7. GIS (SPATIAL DATA) STANDARDS

These GIS standards are intended to facilitate the aggregation of spatial data for use in multi-jurisdictional data products. NatureServe is developing new processes for managing aggregated spatial data, including work on internet data delivery. As these processes evolve, the spatial data standards will be updated as needed.

7.1. Spatial Data File Format and Projections

Biotics 4 Mapper:

For Biotics users, NatureServe will provide an extension that automatically exports managed layers, both in their original projection and reprojected to facilitate aggregation by NatureServe into a network-wide dataset. For Element Occurrence data, the extension will export species Element Occurrence records for which the member program has the lead responsibility. In addition, NatureServe will provide instructions for QC procedures designed to ensure data integrity and consistency between spatial and tabular data that must be run before the export extension. The extension, QC procedures and instructions will be available on the Biotics support website (http://whiteoak.natureserve.org/hdms/HDMS-DataExchange.shtml) and included with Data Exchange communications.

Two copies of the following shapefiles will be sent to NatureServe during the annual data exchange (one in the original projection and one re-projected):

- 1 EO Polygon Layer,
- 1 EO Point (Centroid) Layer,
- 1 Polygon Source Feature Layer,
- 1 Point Source Feature Layer,
- 1 Line Source Feature Layer,
- 1 Managed Area Layer (if available), and
- 1 Conservation Site Layer (if available).

Non-Biotics 4 Mapper Polygon Data:

For member programs with GIS polygon data that are not using Biotics 4 Mapper, all polygon spatial data will be provided to NatureServe in ArcView shapefile format. In addition, NatureServe will provide a list of attributes to be provided with these shapefiles, including the coordinates of the polygon centroid so NatureServe can create an EO point layer. All shapefiles must be submitted with an appropriate projection (*.prj) file.

The following shapefiles will be sent to NatureServe during the annual data exchange:

- 1 EO Polygon Layer,
- 1 Managed Area Layer (if available), and
- 1 Conservation Site Layer (if available).

No Polygon Data:

If EO, Managed Area, or Site data have not been converted to polygons, the member program will provide only the tabular data (e.g. points with latitude and longitude coordinates) along with datum information (see metadata, Section 7). If relevant (e.g. use UTM), the member programs will convert their latitude and longitude coordinates to the NatureServe standard: Degrees / Minutes / Seconds with leading zeros as appropriate, and the correct final N / S or E / W character. In addition, member programs will provide information indicating preferred distances (e.g. using Precision values) for buffering point EOs in order to generate polygon representations. For additional information please see Table 4 "First and Second Tier Element Occurrence Fields".

The following tabular data will be sent to NatureServe during the annual data exchange:

- 1 EO table,
- 1 Managed Area table (if available), and
- 1 Conservation Site table (if available).

7.2. U.S. Federal Geographic Data Committee (FGDC) Compliant Spatial Metadata

Member programs will provide to NatureServe minimum metadata for spatial data, as specified in Section 7. Where possible, member programs should provide metadata that is in compliance with the U.S. Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (CSDGM), or when available the ISO Metadata Standard. The Canadian Geospatial Data Infrastructure (CGDI) endorses the use of these two metadata content standards. NatureServe will provide support and training, where possible, for creating metadata in compliance with the current FGDC metadata standards.

7.3. Spatial Quality Control

Member programs will ensure that the county, mapsheet (quadrangle), and watershed values for all mappable EOs, as defined above in Section 4, are accurate. Exceptions to this standard will be allowed when the member program is using a reference layer for county, watershed, or mapsheet (quadrangle) that does not conform to the national standard; in the United States these are the national-scale U.S. Geological Survey (USGS) reference layers. Standards for other countries will be documented when agreement is reached with members on the appropriate reference layers. NatureServe will provide appropriate tools and support to accomplish this requirement.

8. METADATA STANDARDS

For all multi-jurisdictional spatial data products, NatureServe is required to deliver metadata that are compliant with the U.S. Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (CSDGM). The Benchmark Metadata Content Standards for Element Occurrence (EO) data are intended to help with this effort. Data Exchange memos will include instructions for the preparation of these metadata. The Metadata Standards section will be updated as appropriate when the ISO Metadata Standard is officially adopted by FGDC as the new metadata standard.

FGDC Metadata Section	**Metadata Information Needed**
Section 2: Data Quality Information, Completeness Report	Taxonomic Completeness *(if your program is using the FGDC Biological Data Profile, this is a separate metadata element in Section 1: Identification Information)*: Information about taxonomic groups that the member program DOES NOT track at the EO level.
	Geographic Completeness: Information about known geographic gaps in the member program EO data set (e.g. military, private, Native American lands). For each gap, the member program will provide the following: a) name of land area missing EO data, b) general location of area, c) approximate size of land area (ha, acres, sq. meters, sq. miles), and d) reason for gap (e.g. inventory gap, data sensitivity).
Section 2: Data Quality Information, Process Step Information describing the following EO processing topics, as relevant. The "Process Step" section can be repeated as many times as needed.	Sub-EOs: If the member program manages sub-EOs, information about tabular data that are not recorded in the principal EO, and any other data that are unique to these sub-EOs.
	Generalized or Sensitive EOs: If the member program generalizes or removes locational information from certain EOs (for reasons of data sensitivity or privacy), information about the process and which EOs are treated that way: • EO "Fuzzing" – description of the "fuzzing" process; • Non-Precise EO (i.e. no GIS or lat / long values provided); • Other ?
	EO Representation/Interpretation**:** If the member program does not conform to the revised EO methodology for the delineation of some or all of its EO representations, a brief description of the methodology used to create EOs (for a complete description of the revised EO methodology, see: http://whiteoak.natureserve.org/eodraft/index.htm). This can include information about which EOs have been mapped with the revised EO methodology, or have been delineated and ranked with the newest EO Specifications. If, for example, the EO(s) represents more than the observed habitat of the Element (and locational uncertainty), information about the methodology used to derive these EOs (e.g. EO includes inferred/extrapolated/predicted ranges or habitat).

FGDC Metadata Section	Metadata Information Needed
	EO Specifications: EO specifications are criteria used for determining whether data constitutes a valid occurrence of the Element, and if so, whether it represents a single EO or multiple occurrences. EO specifications are used to achieve consistency in the manner in which occurrences of the same Element are delineated in different jurisdictions, and to help insure that EOs are created for data having practical conservation value for the Element. Draft EO specifications that are currently under network review have been developed for all vertebrate animal groups, and selected invertebrates and vascular plants. If a standard EO Specification record for an Element exists, provide information for EOs that are delineated using values for the following fields that **ARE NOT** the standard or suggested value (see Biotics 4 Help record for more information): • ***minimum_eo_criteria,*** • ***separation_distance (or provide alt_separation_distance),*** • ***separation_barrier (if applicable),*** • ***mapping_guidance (if applicable), and*** • ***inferred_extent (animals only).***
	Minimum Mapping Unit (MMU): The MMU in Biotics 4 is 12.5 meters in the U.S. (based on mapping from a 1:24K scale map) and 25 meters in Canada (based on mapping from a 1:50K base map). If a MMU other than the standard value is being used, the member program will provide information about their MMU.
	Locational Uncertainty: If the member program does not conform to the revised EO methodology for the delineation of some or all of its EO representations, information about whether locational uncertainty is used to generate EOs and the methodology used to generate EOs.
	Scale: The scale of the base maps that are used to generate EOs. In the case where multiple scales are used, the member program will provide the least accurate scale (e.g. 1:24k when 1:12k and 1:24k maps are used).
Section 3: Spatial Data Organization Information	*NOTE: Software such as an ArcView extension, ArcGIS, or SMMS will automatically capture this information.* Direct Spatial Reference (Point or Vector data). Point / Vector Object Count (number of records in the data set).

FGDC Metadata Section	Metadata Information Needed
Section 4: Spatial Reference Information	*NOTE: Software such as an ArcView extension, ArcGIS, or SMMS will automatically capture this information.* GIS files should be provided with an accompanying projection (*.prj) file that contains the following information (if applicable): • *Projection,* • *Scale,* • *Datum Name,* • *Units,* • *Spheroid (Ellipsoid) Name,* • *Parameters,* • *1st standard parallel,* • *2nd standard parallel,* • *Longitude of central meridian,* • *Latitude of projection origin,* • *False easting, and* • *False northing.*
Section 5: Entity and Attribute Information, Attribute Labels and Definitions	Attribute (field) labels, definitions, and domain values as follows: 1) Subnational Protection Status (SPROT): Values and definitions, including whether or not each status has legal protection within the subnation based on state / province endangered species legislation. 2) Non-Standard Domain Values: Non-standard and/or Customized values for domain tables.

Metadata Resources

- FGDC Metadata Standard - Content Standard for Digital Geospatial Metadata (CSDGM) (version 2.0), FGDC-STD-001-1998: http://www.fgdc.gov and http://www.fgdc.gov/metadata/contstan.html
- Biological Data Profile - Content Standard for Digital Geospatial Metadata, Part 1: Biological Data Profile, FGDC-STD-001.1-1999: http://www.nbii.gov/ and http://www.fgdc.gov/standards/status/sub5_2.html
- U.S. Federal Geographic Data Committee (FGDC) and National Biological Information Infrastructure (NBII) websites recommended by NatureServe metadata trainer: http://whiteoak.natureserve.org/hdms/SupportDoc/metadata_web_resources.htm
- "Template" FGDC metadata record for U.S. and Canadian EO data: http://whiteoak.natureserve.org/HDMS/HDMSDoc/hdms_dx/NatureServe_EO_Metadata_09-2004.html

9. MEASURING PROGRESS IN MEETING BENCHMARK DATA CONTENT STANDARDS

Progress towards meeting the Benchmark Data Content Standards across the NatureServe network will be measured using the record subsets and field priorities specified above: Elements - Tables 1 and 2; Element Occurrences - Tables 3 and 4.

These analyses will be conducted for the entire aggregated dataset, and where relevant for specific geographic or taxonomic subsets. For example, data completeness may be reported separately for nations; for each subnation (i.e., member program); and/or for subsets such as mammals, amphibians and vascular plants.

9.1. Completeness – Elements, Element Occurrences, and Fields

The ultimate goal is to reach 100% completeness for all of the highest priority Element and Element Occurrence records and fields where data are available and/or applicable.

Until this degree of completeness is achieved, the intermediate goal is to increase the completeness of these priority records and fields by 15% per year.

Data completeness will be measured using several interrelated approaches:

- An initial assessment of major data gaps will be conducted by NatureServe using database queries to create reports with the number and percent of fields in different Element and Element Occurrence subsets that have data in the field (i.e. are not null). For example: X percent of the Elements in record subset 4 have data in the Tier 1 field Global Threat Comments (g_threat_com) that is not "null". When the fields to track the edition of EO Specifications and EO Rank Specifications are implemented, they will be analyzed to measure progress towards meeting the updated specifications.

  These quantitative analytical reports will provide a useful overview of the state of the network's data, and will indicate the highest priorities for data development needs across the network and within individual programs.

These quantitative assessments cannot, however, adequately address the quality or accuracy of the data in the fields, nor do they address completeness for the many fields that are designated "where applicable".

- For selected fields, the reports generated from database queries will need to be combined with expert qualitative assessments of data values. This data review will help to identify data that has passed the initial completeness analyses because the field is "not null", but is populated with incorrect information.
- Some fields will require more time-consuming research to verify that all of the "where applicable" information has been correctly captured in the databases. For example, verifying that all Elements that *should* have a national or subnational protection status value actually do have one, or that all Elements that are taxonomic nonstandards have explanatory information in the Classification / Taxonomy Comments (g_classification_com) field.

9.2. Completeness – Taxonomy

Since current and consistent taxonomic standards are the foundation for all other data, evaluations of the degree to which we have met the Benchmark Data Content Standards will include periodic qualitative or quantitative assessment by major group (e.g., family for plants; order or class for animals) of how compliant our standard taxonomy is related to the current scientific consensus for that group (for animals), the most recent Kartesz list (for vascular plants), or stated standard references (for other groups). For animals where there may be no consensus list, or where such a list may be infrequently updated, NatureServe's Zoology Department assesses the taxonomic currentness by keeping track of the degree to which recent journals have been reviewed for possible taxonomic changes.

9.3. Mapping Accuracy

"Priority 1" Element Occurrences (National Protected / Imperiled):

Member programs will ensure that 100% of all mappable EOs, as defined above in Section 4, have been verified as mapping to the correct county, watershed, and/or mapsheet (quadrangle) as determined by the member program's reference layers for county, watershed, and/or mapsheet. NatureServe will provide appropriate tools and support to accomplish this requirement.

9.4. Currentness

Conservation Rank review dates:

Global Rank (g_rank) dates, measured by assessing the Global Rank Review Date (g_rank_review_date), for the Primary Element Subset (2) should be current within the last 5 years.

Alternative Approach: Select a random sample from the data; research the ranks and how often various ranks need to be reviewed. Identify whether or not there is a discontinuity at 5 years, after which it is much more likely that if a highly ranked element (G1-G3) is reviewed the rank will change. Based on this analysis, develop more specific standards for conservation rank quality and currentness.

National and Subnational Protection Statuses:

It is policy that USESA, COSEWIC, Canadian National General Status, Subnational Protection Status, and other national statuses as appropriate are kept up to date. For the national protection status data, NatureServe staff performs periodic quality control to ensure that status values are correct (complete, current, *and* accurate). Metadata should describe data entry standards, data management standards, and procedures for completeness and currency, including how these status values differ between Botany and Zoology.

- USESA: For taxa occurring in the United States, data will be completely accurate within two weeks of posting individual status values in the U.S. Federal Register and within four weeks of publication of any U.S. Fish and Wildlife Service Notice of Review.
- COSEWIC: For taxa occurring in Canada, data will be completely accurate within four weeks of web publication of the annual "Canadian Species At Risk" list.
- Canadian National General Status: For taxa occurring in Canada, data will be completely accurate within eight weeks of web publication and receipt of an electronic copy of any updates to these status values. Data are entered in taxon_national.general_status_com.
- Subnational Protection Status: For taxa occurring on official state, provincial, or tribal threatened or endangered lists, data will be completely accurate within four weeks of listing publication.

9.5. Data Processing

For the highest priority Elements and Element Occurrences, it is recommended that all data from readily available secondary sources be processed as quickly as possible, with an emphasis when possible on the "First Tier" data fields. Secondary sources can include museum specimens (at least those available from institutions within the jurisdiction of the member program), published and unpublished reports.

For G1 taxa and taxa that have additional legal protected status at the national level or within the member program subnation, it is recommended that all available data (including field surveys) be processed when possible into the databases and other files within six months.

Where possible, it is recommended that data for other high priority Elements and Element Occurrences be processed within one year.

Where these recommended timelines cannot be met, the member program should qualify their data accordingly in the metadata as part of the data quality completeness information. Such situations may arise, for example, when numerous species (or numerous EOs of a single species) in a jurisdiction change status, or due to limitations in staffing.

10.RECONCILIATION OF EO DATA BETWEEN MEMBER PROGRAMS

Where Elements occur on or near borders between jurisdictions, it is highly likely that there are duplicate EO records representing the same occurrence being maintained by multiple member programs. In these cases, it may be necessary for two (or more) member programs to reconcile their information, allowing NatureServe to deliver aggregated datasets at regional or national scales that do not contain duplicate EOs along program borders. There are technical, administrative and financial issues to be resolved in order to reconcile data between member programs.

For additional details, please see the Biotics Tracker Help entry "Create and Manage a Multi-jurisdictional EO".

Proposed workflow:

1. NatureServe staff:

   Develop GIS tool to identify potential cross-border duplicate Element Occurrences; run analyses on the aggregated Element Occurrence dataset.

2. NatureServe staff:

   Use the results of these analyses to provide information to the member programs about possible cross-border duplicate EOs. This could potentially be included in regular Data Exchange communications.

3. Member Programs:

   Work collaboratively with neighboring programs to reconcile duplicate EOs and apply the multi-jurisdictional EO methodology to create primary and sub-EO records.

4. Member Programs:

   Provide reconciled cross-border EO data to NatureServe as part of the regular Data Exchange process.

APPENDIX 1: BENCHMARK DATA CONTENT STANDARDS FIRST AND SECOND TIER ELEMENT AND ELEMENT OCCURRENCE FIELDS

The fields for the Benchmark Data Content Standards First and Second Tier Element and Element Occurrence fields are specified in Appendix 1, which is provided as the Excel workbook BDCS_2004_ver2.0_fields.xls. This file contains separate tables for Global, National, Subnational, and Element Occurrence fields.

Please see the tables in: BDCS_2004_ver2.0_fields.xls

Data Category	Lead Responsibility	Spreadsheet Tab
Element Global Fields	NatureServe	"element_global"
Element National Fields	NatureServe, or national entity as relevant	"element_national"
Element Subnational Fields	Member Programs; each member program is only responsible for records tracked by their subnation.	"element_subnational"
Element Occurrence Fields	Member Programs; each member program is only responsible for records tracked by their subnation.	“element_occurrence”

Each table includes the following information:

Column	Definition
Subject Area:	Type of data (such as: classification, identifiers, heritage status, global characterization status, locators, identifiers, description, EO rank, survey information).
ANIMAL Element record subset:	Elements only – Subsets defined in Table 1.
PLANT Element record subset:	Elements only – Subsets defined in Table 1.
Fields 1st or 2nd Tier:	Priority for field data development and quality control.
Biotics 4 Table Name:	Name of the Biotics 4 database table.
Biotics 4 Column Name:	Actual name of the field in the Biotics 4 data model.
Biotics 4 Display Name:	Field name, as displayed in the Biotics 4 software.
BCD Field Name:	Field name in the Biological and Conservation Database (BCD).
Data Entry Comments:	Data entry details, particularly where not enforced by the Biotics 4 software.

Column	**Definition**
Definition:	Field definition, from the Biotics 4 Help documentation.
Biotics 4 Optionality:	Indicates whether or not the field is "nullable" in the Biotics 4 software.

REFERENCES AND RESOURCES

NOTE: Items with highlighting are under development (November 1, 2004).

A. Data Management Guidelines and Resources

- Biotics Help (can be used as a reference independent of the Biotics software): http://whiteoak.natureserve.org/hdms/Biotics-LatestRelease.shtml; click on the link next to "Biotics 4 Help Documents"
- Animal Element Occurrence Specifications (Draft documents): ftp://ftp.natureserve.org/pub/nhp/animalspecs/outgoing/
- Plant Element Occurrence Specifications: https://transfer.natureserve.org/download/longterm/PLANT_EO_%20SPECS/
- EO and EO Rank Specifications version tracking fields:
- U.S. Endangered Species Act (USESA) Status: Data Management Guidelines: See Biotics Help topic "USESA Status" and related fields, and the supplementary document: http://whiteoak.natureserve.org/HDMS/SupportDoc/USESA_data_managment_ver4.1.doc
- National and Subnational distribution fields (origin, regularity, distribution confidence, current presence / absence, population): See Biotics Help topic "Enter Distribution Data for Species Elements"

B. Standard Queries

Standard queries based on the Benchmark Data Content Standards can be found on the Biotics Support website: http://support.natureserve.org/customer/Default.htm; click on "Knowledgebase" – "Sample SQL Queries"

- Element subsets defined in Table 1
- Element fields defined in Table 2
- Element Occurrence subsets defined in Table 3
- "Extant Element Occurrences" as defined in Section 4.3
- Element Occurrence fields defined in Table 4

C. Quality Control (QC) Resources

Quality Control queries can be found on the Biotics Support website: http://support.natureserve.org/customer/Default.htm; click on "Knowledgebase" – "Sample SQL Queries"

- EO latitude / longitude QC query - selects EOs with lat / long values that are not valid according to the NatureServe standard as represented in Biotics

- EO_rank / S_rank QC query - selects EOs where SRANK equals 'SH' or 'SX' and EORANK does not equal 'H' or 'X' or is null
- Checks for "invalid" combinations of data, such as subnational rank of S1 but the origin is exotic in that subnation.

Spatial Quality Control - accuracy of mapping based on county, mapsheet (quadrangle) and watershed reference layers; ArcView tool available on the Biotics Support website: http://whiteoak.natureserve.org/hdms/BioticsDoc/spatial_qc.zip

D. Checklist to assist in the implementation of Benchmark Data Content Standards

Under development

GLOSSARY

BCD: Acronym for the Biological and Conservation Data system developed in 1988 by The Nature Conservancy, Arlington, Virginia, USA. Subsequently replaced by *Biotics*.

Biotics: A customized information management system designed to support the natural heritage methodology used by the NatureServe network. Biotics includes four primary applications, briefly described below. Each application provides a Windows interface and manages data stored within a common Oracle database. The successor to the previous software package, *BCD*, Biotics 4 was released in November 2002.
Tracker: Provides data management capabilities for tabular data.
Mapper: Provides spatial data management capabilities through a custom GIS interface.
Administrator: Provides an interface for managing security, system options, and extensibility.
Exchanger: Provides utilities for data import/export and bi-directional data exchange.

Biotics Help: Help was created by NatureServe to accompany the Biotics data management system. Definitions in Biotics Help serve to define acceptable standard data values for database fields.

Data Exchange: Reconciliation process between NatureServe and member program databases ensuring that up-to-date range-wide data is available to all local databases, and that detailed local data can be shared and aggregated across the network. NatureServe Central Databases are updated with the latest scientific information developed by the member programs at the subnational level, including updated Element Occurrence data. In return, member program databases are updated with the latest scientific information developed at the global scale by NatureServe. The data exchange and reconciliation process is a primary mechanism by which network data standards are upheld, thus helping to ensure a high level of accuracy, currency, and quality to the data.

Data Sharing Agreement: A cooperative agreement between individual member programs and NatureServe, for the purpose of defining the relationship and responsibilities between the member program and NatureServe; defining the terms of use of data shared between the member program and NatureServe; and facilitating the transfer of funds and services, as available, between the member program and NatureServe on projects that promote the interests and missions of both parties.

Element: A biodiversity unit of conservation attention and action for which a heritage Conservation Status Rank is assigned. Elements may be recognized at any taxonomic level (although typically are only recognized at the species level and below for organisms, and the Ecological System, Alliance, and Association levels for communities). Elements may also be recognized for biodiversity units for which there is no systematic hierarchy (e.g., animal assemblages, community Complexes). Elements may be native or exotic at a particular location and collectively represent the full array of biological and ecological diversity for the geographic area covered. Elements may serve

as the targets of heritage inventory. Typically, these targets include native, regularly occurring, vulnerable species (including infraspecific taxa and populations), and exemplary ecological communities.

Element Occurrence: An Element Occurrence (EO) is an area of land and/or water in which a species or natural community is, or was, present. An EO should have practical conservation value for the Element as evidenced by potential continued (or historical) presence and/or regular recurrence at a given location. For species Elements, the EO often corresponds with the local population, but when appropriate may be a portion of a population (e.g., long distance dispersers) or a group of nearby populations (e.g., metapopulation). For community Elements, the EO may represent a stand or patch of a natural community, or a cluster of stands or patches of a natural community. An Element Occurrence record is a data management tool that has both spatial and tabular components including a mappable feature (i.e., an Element Occurrence Representation [EO Rep]) and its supporting database attributes.

GIS: Geographic Information System. A GIS is a computer system capable of capturing, storing, analyzing, and displaying geographically referenced information; that is, data identified according to location. Practitioners also define a GIS as including the procedures, operating personnel, and spatial data that go into the system. (definition from USGS: http://erg.usgs.gov/isb/pubs/gis_poster/)

Member Programs: Local conservation data centers (often called natural heritage programs) located throughout Canada, Latin America, and the U.S. that are all members of NatureServe through: 1) paying dues, 2) adhering to approved data standards and methods, 3) sharing data, and 4) participating in various organizational activities and governing processes.

Metadata: Metadata or "data about data" describe the content, quality, condition, and other characteristics of data. NatureServe creates metadata that is in compliance with the U.S. Federal Geographic Data Committee (FGDC) Content Standard for Digital Geospatial Metadata (http://www.fgdc.gov/metadata/contstan.html), and will follow the new ISO metadata standard when it is formally adopted by FGDC.

Multi-jurisdictional data products: The provision and analysis by NatureServe of biological data at regional, national, and international scales to clients and partners working across jurisdictional borders. These products are developed under the terms of the *Data Sharing Agreements* between NatureServe and the member programs.

Appendix C.3

Wisconsin Natural Heritage Working List

Wisconsin Natural Heritage Working List

The Wisconsin Natural Heritage Working List contains species known or suspected to be rare in the state and natural communities native to Wisconsin. It includes species legally designated as "Endangered" or "Threatened" as well as species in the advisory "Special Concern" category. Most of the species and natural communities on the list are actively tracked and we encourage data submissions on these species. This list is meant to be dynamic–it is updated as often as new information regarding the biological status of species becomes available. The Natural Heritage Program welcomes your input on any aspect of this list. Wisconsin's extirpated species list is at the end. **Changes from the previous list (04/09) are bolded.**

Key

ELCODE: Unique 10 digit code for each element (plant, animal, or natural community).

Scientific Name: Scientific name used by the Wisconsin Natural Heritage Inventory Program.

S: Indicates that the element is a Species of Greatest Conservation Need based on Wisconsin's Wildlife Action Plan (WWAP). For more information see http://dnr.wi.gov/org/land/er/WWAP/.

Common Name: Standard, contrived, or agreed upon common names.

Global Rank: Global element rank. Refer to the Rank Definition Sheet.

State Rank: State element rank. Refer to the Rank Definition Sheet.

US Status: Current federal protection status designated by the Office of Endangered Species, U.S. Fish and Wildlife Service indicating the biological status of a species in Wisconsin. LE = listed endangered; LT = listed threatened; PE = proposed as endangered; **NEP** = nonessential experimental population; C = candidate for future listing; **CH = critical habitat**

State Status: Protection category designated by the Wisconsin DNR. END = Endangered; THR = Threatened; SC = Special Concern.

WDNR and federal regulations regarding Special Concern species range from full protection to no protection. The current categories and their respective level of protection are as follows: SC/P = fully protected; SC/N = no laws regulating use, possession, or harvesting; SC/H = take regulated by establishment of open closed seasons; SC/FL = federally protected as endangered or threatened, but not so designated by WDNR; SC/M = fully protected by federal and state laws under the Migratory Bird Act.

Special Concern species are those species about which some problem of abundance or distribution is suspected but not yet proven. The main purpose of this category is to focus attention on certain species before they become threatened or endangered.

GLOBAL & STATE ELEMENT RANK DEFINITIONS
WISCONSIN NATURAL HERITAGE INVENTORY PROGRAM

GLOBAL ELEMENT RANKS:

G1 Critically imperiled globally because of extreme rarity (5 or fewer occurrences or very few remaining individuals or acres) or because of some factor(s) making it especially vulnerable to extinction.

G2 Imperiled globally because of rarity (6 to 20 occurrences or few remaining individuals or acres) or because of some factor(s) making it very vulnerable to extinction throughout its range.

G3 Either very rare and local throughout its range or found locally (even abundantly at some of its locations) in a restricted range (e.g., a single state or physiographic region), or because of other factor(s) making it vulnerable to extinction throughout its range; typically 21-100 occurrences.

G4 Uncommon but not rare, (although it may be quite rare in parts of its range, especially at the periphery) and usually widespread. Typically >100 occurrences.

G5 Common, widespread, and abundant (although it may be quite rare in parts of its range, especially at the periphery). Not vulnerable in most of its range.

GH Known only from historical occurrence throughout its range, with the expectation that it may be rediscovered.

GNR Not ranked. Replaced G? rank, and some GU ranks.

GU Currently unrankable due to lack of data or substantially conflicting data on status or trends. Possibly in peril range-wide, but status is uncertain.

GX Presumed to be extinct throughout its range (e.g. Passenger pigeon) with virtually no likelihood that it will be rediscovered.

Species with a questionable taxonomic assignment are given a "Q" after the global rank.

Subspecies and varieties are given subranks composed of the letter "T" plus a number or letter. The definition of the second character of the subrank parallels that of the full global rank. (Examples: a rare subspecies of a rare species is ranked G1T1; a rare subspecies of a common species is ranked G5T1.)

STATE ELEMENT RANKS

S1 Critically imperiled in Wisconsin because of extreme rarity, typically 5 or fewer occurrences and/or very few (<1000) remaining individuals or acres, or due to some factor(s) making it especially vulnerable to extirpation from the state.

S2 Imperiled in Wisconsin because of rarity, typically 6 to 20 occurrences and/or few (1000-3000) remaining individuals or acres, or due to some factor(s) making it very vulnerable to extirpation from the state.

S3 Rare or uncommon in Wisconsin, typically 21-100 occurrences and/or 3000-10,000 individuals.

S4 Apparently secure in Wisconsin, usually with >100 occurrences and >10,000 individuals.

S5 Demonstrably secure in Wisconsin and essentially ineradicable under present conditions.

SNA Accidental, non-native, reported, but unconfirmed, or falsely reported.

SH Of historical occurrence in Wisconsin, perhaps having not been verified in the past 20 years, and suspected to be still extant. Naturally, an element would become SH without such a 20-year delay if the only known occurrence were destroyed or if it had been extensively and unsuccessfully looked for.

SNR Not Ranked, a state rank has not yet been assessed.

SU Currently unrankable. Possibly in peril in the state, but status is uncertain due to lack of information or substantially conflicting data on status or trends.

SX Apparently extirpated from the state.

STATE RANKING OF LONG-DISTANCE MIGRANT ANIMALS:

Ranking long distance aerial migrant animals presents special problems relating to the fact that their non-breeding status (rank) may be quite different from their breeding status, if any, in Wisconsin. In other words, the conservation needs of these taxa may vary between seasons. In order to present a less ambiguous picture of a migrant's status, it is necessary to specify whether the rank refers to the breeding (B) or non-breeding (N) status of the taxon in question. (e.g. S2B,S5N).

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
RARE MAMMALS						
AMAJA01030	§ *Canis lupus*	Gray Wolf	G4	**S4**	LE	SC/FL
AMALC01010	***Cervus canadensis***	**Elk**	**G5**	**S2S3**		**SC/P**
AMABA04010	*Cryptotis parva*	Least Shrew	G5	SH		SC/N
AMACC04010	***Eptesicus fuscus***	**Big Brown Bat**	**G5**	**S2S4**		**THR**
AMAFB09020	§ *Glaucomys sabrinus*	Northern Flying Squirrel	G5	**S3**		**SC/P**
AMAJF01010	§ *Martes americana*	American Marten	G5	**S2**		END
AMAFF11140	§ *Microtus ochrogaster*	Prairie Vole	G5	**S2**		SC/N
AMAFF11150	§ *Microtus pinetorum*	Woodland Vole	G5	**S2**		SC/N
AMACC01010	***Myotis lucifugus***	**Little Brown Bat**	**G5**	**S2S4**		**THR**
AMACC01150	§ *Myotis septentrionalis*	Northern Long-eared Bat	**G4**	**S1S3**		**THR**
AMAFH02010	§ *Napaeozapus insignis*	Woodland Jumping Mouse	G5	S2S3		SC/N
AMACC03020	***Perimyotis*** (=*Pipistrellus*) *subflavus*	Eastern Pipistrelle	G5	**S1S3**		**THR**
AMAFF02030	*Reithrodontomys megalotis*	Western Harvest Mouse	G5	**SU**		SC/N
AMABA01150	§ *Sorex palustris*	Water Shrew	G5	**S3**		SC/N
AMAFB05120	§ *Spermophilus franklinii*	Franklin's Ground Squirrel	G5	S2		SC/N
RARE BIRDS						
ABNKC12060	§ *Accipiter gentilis*	Northern Goshawk	G5	S2B,S2N		SC/M
ABPBXA0030	§ *Ammodramus henslowii*	Henslow's Sparrow	G4	**S2S3B**		THR
ABPBXA0040	§ *Ammodramus leconteii*	Le Conte's Sparrow	G4	S2S3B		SC/M
ABPBXA0070	§ *Ammodramus nelsoni*	**Nelson's Sparrow**	G5	S1B		SC/M
ABNGA04040	§ *Ardea alba*	Great Egret	G5	S2B		THR
ABNSB13040	§ *Asio flammeus*	Short-eared Owl	G5	S1B		SC/M
ABNSB13010	*Asio otus*	Long-eared Owl	G5	S2B		SC/M
ABNJB11030	§ *Aythya americana*	Redhead	G5	S2B		SC/M
ABNNF06010	§ *Bartramia longicauda*	Upland Sandpiper	G5	S2B		SC/M
ABNGA01020	§ *Botaurus lentiginosus*	American Bittern	G4	S3B		SC/M
ABNJB18010	*Bucephala clangula*	Common Goldeneye	G5	**S2S3?B**		SC/M
ABNKC19030	§ *Buteo lineatus*	Red-shouldered Hawk	G5	S3S4B,S1N		THR
ABPBJ18100	*Catharus ustulatus*	Swainson's Thrush	G5	S2B		SC/M
ABNNB03070	§ *Charadrius melodus*	Piping Plover	G3	S1	LE	END
ABNNM10020	§ *Chlidonias niger*	Black Tern	G4	S2B		SC/M
ABPBX96010	§ *Chondestes grammacus*	Lark Sparrow	G5	**S3B**		SC/M
ABNTA02020	***Chordeiles minor***	**Common Nighthawk**	**G5**	**S2S3B**		**SC/M**
ABNLC21020	§ *Colinus virginianus*	Northern Bobwhite	G5	**S2S3B**		SC/M
ABPAE32010	§ *Contopus cooperi*	Olive-sided Flycatcher	G4	S2B		SC/M
ABNME01010	§ *Coturnicops noveboracensis*	Yellow Rail	G4	S1B		THR
ABNJB02030	§ *Cygnus buccinator*	Trumpeter Swan	G4	S4B		**SC/M**
ABPBX03240	§ *Dendroica cerulea*	Cerulean Warbler	G4	S2S3B		THR
ABPBX03130	§ *Dendroica dominica*	Yellow-throated Warbler	G5	**S1?B**		END
ABPBX03180	§ *Dendroica kirtlandii*	Kirtland's Warbler	G1	**S1B**	LE	SC/FL
ABNGA06030	§ *Egretta thula*	Snowy Egret	G5	**SNA**		END
ABPAE33020	§ *Empidonax virescens*	Acadian Flycatcher	G5	S3B		THR
ABNLC09010	§ *Falcipennis canadensis*	Spruce Grouse	G5	S1S2B,S1S2N		THR
ABNKD06070	§ *Falco peregrinus*	Peregrine Falcon	G4	S1S2B		END
ABNKC10010	§ *Haliaeetus leucocephalus*	Bald Eagle	G5	**S4B,S4N**		SC/P
ABPBX08010	§ *Helmitheros vermivorus*	Worm-eating Warbler	G5	S1B		END
ABPBX24010	*Icteria virens*	Yellow-breasted Chat	G5	S2B		SC/M
ABNGA02010	*Ixobrychus exilis*	Least Bittern	G5	**S2S3B**		SC/M
ABPBR01030	§ *Lanius ludovicianus*	Loggerhead Shrike	G4	S1B		END
ABNGA13010	§ *Nyctanassa violacea*	Yellow-crowned Night-Heron	G5	S1B		THR
ABNGA11010	*Nycticorax nycticorax*	Black-crowned Night-Heron	G5	S2B		SC/M
ABPBX11020	§ *Oporornis agilis*	Connecticut Warbler	G4	S2S3B		SC/M
ABPBX11010	§ *Oporornis formosus*	Kentucky Warbler	G5	**S1S2?B**		THR
ABNFC01010	*Pelecanus erythrorhynchos*	American White Pelican	**G4**	**S3B**		SC/M
ABNNF20010	§ *Phalaropus tricolor*	Wilson's Phalarope	G5	S1B		SC/M
ABNCA03020	§ *Podiceps grisegena*	Red-necked Grebe	G5	S1B		END
ABPAW01060	§ *Poecile hudsonicus*	Boreal Chickadee	G5	S2S3B		SC/M
ABPAU01010	***Progne subis***	**Purple Martin**	**G5**	**S2S3B**		**SC/M**
ABPBX07010	§ *Protonotaria citrea*	Prothonotary Warbler	G5	S3B		SC/M
ABNME05020	§ *Rallus elegans*	King Rail	G4	S1B		SC/M
ABPBJ05020	***Regulus calendula***	**Ruby-crowned Kinglet**	**G5**	**S2S3B**		**SC/M**
ABPBX10030	§ *Seiurus motacilla*	Louisiana Waterthrush	G5	S3B		SC/M
ABNNM08020	§ *Sterna caspia*	Caspian Tern	G5	S1B,S2N		END
ABNNM08090	§ *Sterna forsteri*	Forster's Tern	G5	S1B		END

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
ABNNM08070	§ *Sterna hirundo*	Common Tern	G5	S1B,S2N		END
ABPBXB2030	§ *Sturnella neglecta*	Western Meadowlark	G5	S2B		SC/M
ABNLC13010	§ *Tympanuchus cupido*	Greater Prairie-Chicken	G4	S1B,S2N		THR
ABNLC13030	§ *Tympanuchus phasianellus*	Sharp-tailed Grouse	G4	S1B,S2N		**SC/H**
ABNSA01010	§ *Tyto alba*	Barn Owl	G5	**SNA**		END
ABPBW01110	§ *Vireo bellii*	Bell's Vireo	G5	S2B		THR
ABPBX16010	§ *Wilsonia citrina*	Hooded Warbler	G5	S2S3B		THR
ABPBXB3010	***Xanthocephalus xanthocephalus***	**Yellow-headed Blackbird**	**G5**	**S3**		**SC/M**
RARE AMPHIBIANS						
AAABC01010	§ *Acris crepitans*	Northern Cricket Frog	G5	S1		END
AAABH01160	§ *Lithobates palustris*	Pickerel Frog	G5	**S3?**		SC/H
RARE REPTILES						
ARAAG01020	§ *Apalone mutica*	Smooth Softshell	G5	S3		SC/H
ARACJ02110	§ *Aspidoscelis sexlineata*	Six-lined Racerunner	G5	**S2S3**		SC/H
ARADB02020	§ *Carphophis vermis*	Western Wormsnake	G5	S1		SC/H
ARADB07010	§ *Coluber constrictor*	North American Racer	G5	S2		SC/P
ARADE02040	§ *Crotalus horridus*	Timber Rattlesnake	G4	S2S3		SC/P
ARADB10013	§ *Diadophis punctatus arnyi*	Prairie Ring-necked Snake	G5T5	**S2S3**		SC/H
ARAAD04010	§ *Emydoidea blandingii*	Blanding's Turtle	G4	**S3S4**		THR
ARAAD02020	§ *Glyptemys insculpta*	Wood Turtle	G4	S2		THR
ARAAD05080	*Graptemys pseudogeographica*	False Map Turtle	G5	**S3?**		SC/H
ARACB02010	§ *Ophisaurus attenuatus*	Slender Glass Lizard	G5	S1		END
ARADB13035	§ *Pantherophis spiloides*	Gray Ratsnake	G5T5	**S3**		SC/P
ARADB26020	§ *Pituophis catenifer*	Gophersnake	G5	S2S3		SC/P
ARACH01100	§ *Plestiodon septentrionalis*	Prairie Skink	G5	S3		SC/H
ARADB27040	§ *Regina septemvittata*	Queensnake	G5	S1		END
ARADE03011	§ *Sistrurus catenatus catenatus*	Eastern Massasauga	**G3G4T3Q**	S1	C	END
ARAAD08020	§ *Terrapene ornata*	Ornate Box Turtle	G5	S1		END
ARADB36020	§ *Thamnophis butleri*	Butler's Gartersnake	G4	**S3S4**		THR
ARADB36090	§ *Thamnophis proximus*	Western Ribbonsnake	G5	S1		END
ARADB36100	*Thamnophis radix*	Plains Gartersnake	G5	**S2?**		SC/H
ARADB36120	§ *Thamnophis sauritus*	Eastern Ribbonsnake	G5	S1		END
RARE FISHES						
AFCAA01020	§ *Acipenser fulvescens*	Lake Sturgeon	G3G4	S3		SC/H
AFCFA01030	§ *Alosa chrysochloris*	Skipjack Herring	G5	S1		END
AFCQC01040	§ ***Ammocrypta clara*** (=*Etheostoma clarum*)	Western Sand Darter	G3	S3		SC/N
AFCEA01010	§ *Anguilla rostrata*	American Eel	G4	S2		SC/N
AFCLB01010	*Aphredoderus sayanus*	Pirate Perch	G5	S3		SC/N
AFCHA01140	§ *Coregonus zenithicus*	Shortjaw Cisco	G3	**S1**		SC/H
AFCQC01010	§ *Crystallaria asprella*	Crystal Darter	G3	S1		END
AFCJC04010	§ *Cycleptus elongatus*	Blue Sucker	G3G4	S2		THR
AFCJB50050	§ *Erimystax x-punctatus*	Gravel Chub	G4	S1		END
AFCJC05020	§ *Erimyzon sucetta*	Lake Chubsucker	G5	S3		SC/N
AFCQC02020	*Etheostoma asprigene*	Mud Darter	G4G5	S3		SC/N
AFCQC02120	§ *Etheostoma chlorosoma*	Bluntnose Darter	G5	S1		END
AFCQC02450	§ *Etheostoma microperca*	Least Darter	G5	S3		SC/N
AFCNB04250	§ *Fundulus dispar*	Starhead Topminnow	G4	S2		END
AFCGA01010	§ *Hiodon alosoides*	Goldeye	G5	S2		END
AFCJC07030	§ *Ictiobus niger*	Black Buffalo	G5	S2		THR
AFCQB11080	§ *Lepomis megalotis*	Longear Sunfish	G5	S2		THR
AFCJB51040	§ *Luxilus chrysocephalus*	Striped Shiner	G5	S1		END
AFCJB52080	§ *Lythrurus umbratilis*	Redfin Shiner	G5	S2		THR
AFCJB53080	§ *Macrhybopsis aestivalis*	Shoal Chub	G5	S2		THR
AFCJB53040	*Macrhybopsis storeriana*	Silver Chub	G5	S3		SC/N
AFCJC10040	§ *Moxostoma carinatum*	River Redhorse	G4	S2		THR
AFCJC10070	§ *Moxostoma duquesnei*	Black Redhorse	G5	S1		END
AFCJC10170	§ *Moxostoma valenciennesi*	Greater Redhorse	G4	S3		THR
AFCJB15010	§ *Notropis amnis*	Pallid Shiner	G4	**S1**		END
AFCJB28080	§ *Notropis anogenus*	Pugnose Shiner	G3	S2		THR
AFCJB28680	§ *Notropis nubilus*	Ozark Minnow	G5	S2		THR
AFCJB28950	*Notropis texanus*	Weed Shiner	G5	S3		SC/N
AFCKA02250	§ *Noturus exilis*	Slender Madtom	G5	S1		END
AFCJB55010	*Opsopoeodus emiliae*	Pugnose Minnow	G5	S3		SC/N
AFCQC04090	§ *Percina evides*	Gilt Darter	G4	**S2S3**		THR
AFCAB01010	§ *Polyodon spathula*	Paddlefish	G4	S2		THR

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
RARE MUSSELS AND CLAMS						
IMBIV02040	*Alasmidonta marginata*	Elktoe	G4	**S3**		SC/P
IMBIV02110	§ *Alasmidonta viridis*	Slippershell Mussel	G4G5	S2		THR
IMBIV04130	§ *Anodonta suborbiculata*	Flat Floater	G5	**S2S3**		SC/P
IMBIV06010	§ *Arcidens confragosus*	Rock Pocketbook	G4	S1S2		THR
IMBIV08010	§ *Cumberlandia monodonta*	Spectacle Case	G3	S1	**PE**	END
IMBIV09010	§ *Cyclonaias tuberculata*	Purple Wartyback	G5	**S2**		END
IMBIV13010	§ *Ellipsaria lineolata*	Butterfly	**G4G5**	S2		END
IMBIV14060	*Elliptio complanata*	Eastern Elliptio	G5	**S2S3**		SC/P
IMBIV14080	§ *Elliptio crassidens*	Elephant Ear	G5	S1		END
IMBIV16190	§ *Epioblasma triquetra*	Snuffbox	G3	S1	**PE**	END
IMBIV17060	§ *Fusconaia ebena*	Ebony Shell	G4G5	S1		END
IMBIV21100	§ *Lampsilis higginsii*	Higgins' Eye	G1	S1	LE	END
IMBIV21240	§ *Lampsilis teres*	Yellow & Slough Sandshells	G5	S1		END
IMBIV29020	*Megalonaias nervosa*	Washboard	G5	S3		SC/P
IMBIV34030	§ *Plethobasus cyphyus*	Bullhead	G3	S1	**PE**	END
IMBIV37070	§ *Potamilus ohiensis*	Pink Papershell	G5	**S3**		SC/P
IMBIV39050	§ *Quadrula fragosa*	Winged Mapleleaf	G1	S1	LE	END
IMBIV39080	§ *Quadrula metanevra*	Monkeyface	G4	S2		THR
IMBIV39090	§ *Quadrula nodulata*	Wartyback	G4	S1S2		THR
IMBIV39120	§ *Quadrula quadrula*	Mapleleaf	G5	**S3**		SC/P
IMBIV41010	§ *Simpsonaias ambigua*	Salamander Mussel	G3	**S2**		THR
IMBIV44010	§ *Tritogonia verrucosa*	Buckhorn	G4G5	S2		THR
IMBIV45020	§ *Truncilla donaciformis*	Fawnsfoot	G5	S1S2		SC/P
IMBIVA4010	§ *Venustaconcha ellipsiformis*	Ellipse	G4	**S3**		THR
IMBIV47060	§ *Villosa iris*	Rainbow Shell	G5Q	S1		END
RARE BUTTERFLIES AND MOTHS						
IILEF2K060	*Acrocercops pnosmodiella*	Marbleseed Leafminer	GNR	**S1S3**		SC/N
IILEYAR030	***Acronicta dolli***	**Doll's Merolonche**	**G3G4**	**S3?**		**SC/N**
IILEP79010	*Atrytonopsis hianna*	Dusted Skipper	G4G5	**S2S3**		SC/N
IILEYAG030	***Bagisara gulnare***	**A Noctuid Moth**	**GU**	**S1S2**		**SC/N**
IILEPJ7140	§ *Boloria chariclea*	Arctic Fritillary	G5	**S3**		SC/N
IILEPH2060	§ *Calephelis muticum*	Swamp Metalmark	G3	S1		END
IILEPE2130	*Callophrys gryneus*	Juniper Hairstreak	G5	S3		SC/N
IILEPE2220	§ *Callophrys irus*	Frosted Elfin	G3	S1		THR
IILEY89730	*Catocala abbreviatella*	Abbreviated Underwing Moth	G4	S3		SC/N
IILEY89520	§ *Catocala semirelicta*	Semirelict Underwing Moth	G5	S2S3		SC/N
IILEY89750	§ *Catocala whitneyi*	Whitney's Underwing Moth	G3G4	S3		SC/N
IILEY9S010	***Cerma cora***	**Owl-eyed Bird Dropping Moth**	**G3G4**	**S3**		**SC/N**
IILEPJ9130	*Chlosyne gorgone*	Gorgone Checker Spot	G5	S3		SC/N
IILEYKP150	§ *Copablepharon michiganensis*	A Noctuid Moth	G1G2	**S1S3**		SC/N
IILEYL2010	*Dichagyris* (*Mesembagrotis*) *reliqua*	A Noctuid Moth	G2G3	S2		SC/N
IILEPF3010	***Erora laeta***	**Early Hairstreak**	**GU**	**S1S3**		**SC/N**
IILEP37140	§ *Erynnis lucilius*	Columbine Dusky Wing	G4	**S2S3**		SC/N
IILEP37100	§ *Erynnis martialis*	Mottled Dusky Wing	G3	S2		SC/N
IILEP37170	§ *Erynnis persius*	Persius Dusky Wing	G5	**S3**		SC/N
IILEY2R260	§ *Grammia phyllira*	Phyllira Tiger Moth	G4	S2		SC/N
IILEX0W020	§ *Hemaris gracilis*	Slender Clearwing	G3G4	**S2S3**		SC/N
IILEW0M053	§ ***Hemileuca nevadensis ssp. 3***	Midwestern Fen Buckmoth	**G5T3T4**	**S3**		**SC/N**
IILEP65100	§ *Hesperia metea*	Cobweb Skipper	G4G5	S2		SC/N
IILEP65050	§ *Hesperia ottoe*	Ottoe Skipper	G3G4	**S1**		SC/N
IILEPG5010	§ *Lycaeides idas*	Northern Blue	G5	S1		END
IILEPG5021	§ *Lycaeides melissa samuelis*	Karner Blue	G5T2	S3	LE	SC/FL
IILEPC1170	§ *Lycaena dione*	Gray Copper	G5	**S2?**		SC/N
IILEP57010	§ *Oarisma powesheik*	Powesheik Skipperling	G2G3	S1		END
IILEPP1040	§ *Oeneis chryxus*	Chryxus Arctic	G5	**S3**		SC/N
IILEYC0450	§ *Papaipema beeriana*	Liatris Borer Moth	G2G3	**S2S3**		SC/N
IILEYC0350	§ *Papaipema silphii*	Silphium Borer Moth	G3G4	**S2S3**		END
IILEPA2020	§ *Pieris virginiensis*	West Virginia White	**G3?**	S3		SC/N
IILEP71010	§ *Problema byssus*	Byssus Skipper	G3G4	**S2S3**		SC/N
IILEYFN010	§ *Psectraglaea carnosa*	Pink Sallow	G3	**S2S4**		SC/N
IILEY39060	§ *Pygarctia spraguei*	Sprague's Pygarctica	G5	S2		SC/N
IILEYMP230	§ *Schinia bina*	Bina Flower Moth	G4	**S2S3**		SC/N
IILEYMP130	§ *Schinia indiana*	Phlox Moth	G2G4	S2S3		END
IILEYMP920	***Schinia lucens***	**Leadplant Flower Moth**	**G4**	**S3**		**SC/N**

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IILEPJ6040	§ *Speyeria idalia*	Regal Fritillary	G3	S1		END
IILEY7P360	***Zale largera***	**A Noctuid Moth**	**G4**	**S2S3**		**SC/N**
RARE DRAGONFLIES AND DAMSELFLIES						
IIODO14030	§ *Aeshna clepsydra*	Mottled Darner	G4	**S2S3**		SC/N
IIODO14160	§ *Aeshna sitchensis*	Zigzag Darner	G5	S1		SC/N
IIODO14170	§ *Aeshna subarctica*	Subarctic Darner	G5	**S1S2?**		SC/N
IIODO75010	*Archilestes grandis*	Great Spreadwing	G5	**S2S3**		SC/N
IIODO68030	§ *Argia plana*	Highland Dancer	G5	**S2S3**		SC/N
IIODO81070	§ *Arigomphus villosipes*	Unicorn Clubtail	G5	**S2S3**		SC/N
IIODO70020	§ *Coenagrion interrogatum*	Subarctic Bluet	G5	**S1S3**		SC/N
IIODO03020	§ *Cordulegaster diastatops*	Delta-spotted Spiketail	G5	S1		SC/N
IIODO71120	*Enallagma basidens*	Double-striped Bluet	G5	**S2?**		SC/N
IIODO71290	§ *Enallagma clausum*	Alkali Bluet	G5	S1?		SC/N
IIODO71040	§ *Enallagma traviatum*	Slender Bluet	G5	**S1S3**		SC/N
IIODO19010	§ *Epiaeschna heros*	Swamp Darner	G5	**S2S3**		SC/N
IIODO08310	*Gomphus graslinellus*	Pronghorned Clubtail	G5	**S2S3**		SC/N
IIODO66020	§ *Hetaerina titia*	Dark Rubyspot	G5	S1S2		SC/N
IIODO72020	§ *Ischnura kellicotti*	Lilypad Forktail	G5	**S1S2**		SC/N
IIODO45060	§ *Libellula cyanea*	White-spangled Skimmer	G5	**S2**		SC/N
IIODO45090	§ *Libellula incesta*	Slaty Skimmer	G5	**S2S3**		SC/N
IIODO45160	§ *Libellula semifasciata*	Painted Skimmer	G5	**S1S2**		SC/N
IIODO45170	§ *Libellula vibrans*	Great Blue Skimmer	G5	**SU**		SC/N
IIODO26100	§ *Macromia taeniolata*	Royal River Cruiser	G5	**S2S3**		SC/N
IIODO74030	§ *Nehalennia gracilis*	Sphagnum Sprite	G5	S2S3		SC/N
IIODO12020	§ *Ophiogomphus anomalus*	Extra-striped Snaketail	G4	**S2S3**		END
IIODO12090	§ *Ophiogomphus howei*	Pygmy Snaketail	G3	S4		THR
IIODO12200	§ *Ophiogomphus smithi*	Sand Snaketail	G2G3	S2		SC/N
IIODO12180	§ *Ophiogomphus susbehcha*	Saint Croix Snaketail	**G2**	S2		END
IIODO14110	§ *Rhionaeschna mutata*	Spatterdock Darner	G4	S1		THR
IIODO32040	§ *Somatochlora cingulata*	Lake Emerald	G5	**S2S3**		SC/N
IIODO32060	§ *Somatochlora ensigera*	Lemon-faced Emerald	G4	**S2S3**		SC/N
IIODO32080	§ *Somatochlora forcipata*	Forcipate Emerald	G5	**S2S3**		SC/N
IIODO32110	§ *Somatochlora hineana*	Hine's Emerald	G2G3	S1	LE	END
IIODO32130	§ *Somatochlora incurvata*	Warpaint Emerald	G4	**S2S3**		END
IIODO32230	§ *Somatochlora tenebrosa*	Clamp-tipped Emerald	G5	S1S2		SC/N
IIODO34020	§ *Williamsonia lintneri*	Ringed Boghaunter	**G3**	**S3**		**SC/N**
RARE CRUSTACEANS						
ICMAL14310	§ *Procambarus gracilis*	Prairie Crayfish	G5	S2?		SC/N
ICMAL05580	§ *Stygobromus putealis*	Wisconsin Well Amphipod	G2G3	S1		SC/N
RARE BEETLES						
IICOLG6010	§ ***Agabetes acuductus***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL52170	§ ***Agabus aeruginosus***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL52030	§ ***Agabus confusus***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL52230	§ ***Agabus discolor***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL52040	§ ***Agabus gagates***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL52090	§ ***Agabus immaturus***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL52070	§ *Agabus inscriptus*	A Predaceous Diving Beetle	GNR	S2S3		SC/N
IICOL52140	§ ***Agabus leptapsis***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL52080	§ *Agabus wasastjernae*	A Predaceous Diving Beetle	GNR	S2?		SC/N
IICOL02104	§ *Cicindela hirticollis hirticollis*	A Tiger Beetle	G5T4	S2S3		SC/N
IICOL02105	§ *Cicindela hirticollis rhodensis*	Beach-dune Tiger Beetle	G5T4	**S1**		SC/N
IICOL02250	§ *Cicindela lepida*	Little White Tiger Beetle	G3G4	**S1**		SC/N
IICOL02220	§ *Cicindela macra*	A Tiger Beetle	G5	S1S2		SC/N
IICOL02232	§ *Cicindela patruela patruela*	A Tiger Beetle	G3T3	S2		SC/N
IICOLV6020	§ ***Colaspis suggona***	**A Colaspis Leaf Beetle**	**GNR**	**S3**		**SC/N**
IICOLM9040	§ *Collops vicarius*	A Melyrid Beetle	GNR	S1		SC/N
IICOL85010	§ ***Copelatus chevrolati***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL5U070	§ ***Cymbiodyta toddi***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5A040	§ *Dubiraphia robusta*	Robust Dubiraphian Riffle Beetle	G1G3	**S2S3**		SC/N
IICOL76010	§ *Dytiscus alaskanus*	A Predaceous Diving Beetle	GNR	S1S2		SC/N
IICOLM2040	§ *Enochrus perplexus*	A Water Scavenger Beetle	GNR	S2?		SC/N
IICOLS9010	§ *Gyrinus impressicollis*	A Whirlygig Beetle	GNR	S2?		SC/N
IICOL5N080	§ ***Haliplus apostolicus***	**A Crawling Water Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5N050	§ *Haliplus canadensis*	A Crawling Water Beetle	GNR	**S2?**		SC/N
IICOL5N070	§ *Haliplus leopardus*	A Crawling Water Beetle	GNR	**S1S3**		SC/N

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IICOLM3030	§ ***Helophorus latipenis***	**A Water Scavenger Beetle**	**GNR**	**S1S2**		**SC/N**
IICOLM3020	§ ***Helophorus orchymonti***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL55160	§ ***Heterosternuta*** (=*Hydroporus*) *pulcher*	A Predaceous Diving Beetle	GNR	**S2?**		SC/N
IICOL55170	§ ***Heterosternuta* (=*Hydroporus*) *wickhami***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5Q040	§ ***Hydraena angulicollis***	**A Minute Moss Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5Q050	§ ***Hydraena pennsylvanica***	**A Minute Moss Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL77010	§ *Hydrocanthus iricolor*	A Burrowing Water Beetle	GNR	S1		SC/N
IICOL78010	§ *Hydrochara leechi*	A Water Scavenger Beetle	GNR	S1		SC/N
IICOL55190	§ *Hydrocolus persimilis*	A Predaceous Diving Beetle	GNR	**S2S3**		SC/N
IICOL55210	§ ***Hydrocolus rubyae***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL55240	§ ***Hydroporus morio***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL55110	§ *Hydroporus pseudovilis*	A Predaceous Diving Beetle	GNR	**S2S3**		SC/N
IICOL38100	§ ***Hygrotus compar***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL38110	§ ***Hygrotus falli***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL38090	§ ***Hygrotus farctus***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL38070	§ *Hygrotus marklini*	A Predaceous Diving Beetle	GNR	S1S2		SC/N
IICOL38060	§ *Hygrotus sylvanus*	Sylvan Hygrotus Diving Beetle	GU	**S1?**		SC/N
IICOLQ0010	§ ***Ilybius angustior***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL79010	§ ***Ilybius subaeneus***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOLP5020	§ *Laccobius agilis*	A Water Scavenger Beetle	GNR	S2S3		SC/N
IICOLP5010	§ *Laccobius reflexipennis*	A Predaceous Diving Beetle	GNR	S1S2		SC/N
IICOL70020	§ ***Laccophilus undatus***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL6T010	§ *Liodessus cantralli*	Cantrall's Bog Beetle	GNR	**S2S3**		SC/N
IICOL83010	§ *Lioporeus triangularis*	A Predaceous Diving Beetle	GNR	**S2S3**		SC/N
IICOL80010	§ ***Matus ovatus***	**A Predaceous Diving Beetle**	**GNR**	**S1S2**		**SC/N**
IICOL0A010	§ *Megacephala virginica*	Virginia Big-headed Tiger Beetle	G5	**S1S2**		SC/N
IICOL55090	§ ***Neoporus*** (=*Hydroporus*) ***hybridus***	**A Predaceous Diving Beetle**	**GNR**	**S2**		**SC/N**
IICOL5S040	§ ***Ochthebius lineatus***	**A Minute Moss Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL81010	§ *Oreodytes scitulus*	A Predaceous Diving Beetle	GNR	S1		SC/N
IICOL82040	§ *Rhantus sericans*	A Predaceous Diving Beetle	GNR	S2?		SC/N
IICOLYA010	§ ***Saxinis omogera***	**A Leaf Beetle**	**GNR**	**S3**		**SC/N**
IICOL5F060	§ ***Stenelmis antennalis***	**A Riffle Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5F020	§ *Stenelmis douglasensis*	Douglas Stenelmis Riffle Beetle	G1G3	**S1S2**		SC/N
IICOL5F080	§ *Stenelmis fuscata*	A Riffle Beetle	GNR	**S2S3**		SC/N
IICOL5F040	§ *Stenelmis knobeli*	Knobel's Riffle Beetle	G1G3	**S1S2**		END
IICOL5F180	§ ***Stenelmis musgravei***	**A Riffle Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL5F200	§ ***Stenelmis quadrimaculata***	**A Riffle Beetle**	**GNR**	**S2**		**SC/N**
IICOL5F190	§ ***Stenelmis sexlineata***	**A Riffle Beetle**	**GNR**	**S1**		**SC/N**
RARE MAYFLIES						
IIEPH14010	§ *Acanthametropus pecatonica*	Pecatonica River Mayfly	G2G4	S1		END
IIEPH15030	§ ***Ameletus lineatus***	**A Mayfly**	**G5**	**S2?**		**SC/N**
IIEPH09170	§ ***Brachycercus ojibwe***	**Ojibwe Small Square-gilled Mayfly**	**G3**	**S2S3**		**SC/N**
IIEPH17050	§ ***Caenis anceps***	**A Small Square-gilled Mayfly**	**G5**	**S2S3**		**SC/N**
IIEPH17060	§ ***Caenis hilaris***	**A Small Square-gilled Mayfly**	**G5**	**S2S3**		**SC/N**
IIEPH47050	§ ***Cercobrachys fox***	**Fox Small Square-gilled Mayfly**	**G3G4**	**S2S3**		**SC/N**
IIEPH47080	§ ***Cercobrachys lilliei***	**Wisconsin Small Square-gilled Mayfly**	**G2**	**S1S2**		**SC/N**
IIEPH47060	§ ***Cercobrachys winnebago***	**Winnebago Small Square-gilled Mayfly**	**G3G4**	**S1S2**		**SC/N**
IIEPH02010	§ *Dolania americana*	American Sand Burrowing Mayfly	G4	**S1S3**		SC/N
IIEPH50020	§ ***Drunella cornuta***	**A Spiny Crawler Mayfly**	**G5**	**S2S3**		**SC/N**
IIEPH28030	§ ***Eurylophella aestiva***	**A Spiny Crawler Mayfly**	**G5**	**S2S3**		**SC/N**
IIEPH37040	§ ***Hexagenia rigida***	**A Common Burrowing Mayfly**	**G5**	**S2?**		**SC/N**
IIEPH03030	§ *Homoeoneuria ammophila*	A Brush-legged Mayfly	G4	**S2?**		SC/N
IIEPH40160	***Maccaffertium pulchellum***	**A Flat-headed Mayfly**	**G5**	**S2S4**		**SC/N**
IIEPH30010	§ *Macdunnoa persimplex*	A Flat-headed Mayfly	G4	**S1S2**		SC/N
IIEPH31010	§ *Metretopus borealis*	A Cleft-footed Minnow Mayfly	G5	**S1S2**		SC/N
IIEPH72020	§ ***Neoephemera bicolor***	**A Large Square-gilled Mayfly**	**G1G2**	**S1?**		**SC/N**
IIEPH22010	§ *Paracloeodes minutus*	A Small Minnow Mayfly	G5	**S1S2**		SC/N
IIEPH32010	§ *Parameletus chelifer*	A Primitive Minnow Mayfly	G5	**S1S3**		SC/N
IIEPH13020	§ *Pentagenia vittigera*	A Common Burrower Mayfly	G5	**S2S3**		SC/N
IIEPH78010	§ *Plauditus cestus*	A Small Minnow Mayfly	G5	**S2?**		SC/N
IIEPH39200	§ ***Rhithrogena undulata***	**A Flat-headed Mayfly**	**G4Q**	**S2S3**		**SC/N**
IIEPH09050	§ ***Sparbarus lacustris***	**A Small Square-gilled Mayfly**	**G4**	**S1S3**		**SC/N**
IIEPH09080	§ ***Sparbarus maculatus***	**A Small Square-gilled Mayfly**	**G5**	**S2S3**		**SC/N**
IIEPH09040	§ ***Sparbarus nasutus***	**A Small Square-gilled Mayfly**	**G3G4**	**S1S3**		**SC/N**
IIEPH19010	§ *Spinadis simplex*	Wallace's Deepwater Mayfly	G2G4	S1		END
IIEPH75030	§ ***Timpanoga lita***	**A Mayfly**	**G5**	**S1S3**		**SC/N**

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RARE LEAFHOPPERS AND TRUE BUGS						
IIHOM08010	§ *Aflexia rubranura*	Red-tailed Prairie Leafhopper	G2	**S2?**		END
IIHOM50010	§ *Amplicephalus kansiensis*	A Leafhopper	GNR	S1?		SC/N
IIHOM57010	§ ***Aphelonema simplex***	**Piglet Bug**	**GNR**	**S1**		**SC/N**
IIHOM45010	***Attenuipyga platyrhyncha***	**A Leafhopper**	**GNR**	**S2S3**		**SC/N**
IIHOM26010	§ *Attenuipyga vanduzeei*	A Leafhopper	GNR	S1		SC/N
IIHOM48020	*Bruchomorpha extensa*	An Issid Planthopper	GNR	S2S3		SC/N
IIHOM58010	***Ceresa minuta***	**A Treehopper**	**GNR**	**S2S3**		**SC/N**
IIHOM51010	§ ***Cuerna sayi***	**A Leafhopper**	**GNR**	**S1S2**		**SC/N**
IIHEMB5010	§ ***Dasycorixa hybrida***	**A Water Boatman**	**GNR**	**S2**		**SC/N**
IIHOM27010	§ *Destria crocea*	A Leafhopper	GNR	S1		SC/N
IIHOM52010	§ ***Driotura robusta***	**A Leafhopper**	**GNR**	**S1S2**		**SC/N**
IIHOM49010	§ *Fitchiella robertsoni*	An Issid Planthopper	GNR	**S1S2**		SC/N
IIHOM03060	§ *Flexamia prairiana*	A Leafhopper	GNR	S1		SC/N
IIHEM88010	§ *Hebrus buenoi*	A Velvet Waterbug	G4	S1?		SC/N
IIHOM28020	§ *Laevicephalus vannus*	A Leafhopper	GNR	S1?		SC/N
IIHOM02020	§ *Limotettix elegans*	A Leafhopper	GNR	S1?		SC/N
IIHOM02010	§ *Limotettix pseudosphagneticus*	A Leafhopper	GNR	S1?		SC/N
IIHOM53010	§ *Memnonia panzeri*	A Leafhopper	GNR	**S2S3**		SC/N
IIHEM30010	§ ***Microvelia albonotata***	**A Broad-shouldered Water Strider**	**GNR**	**S2**		**SC/N**
IIHOM55010	***Myndus ovatus***	**A Planthopper**	**GNR**	**S1S2**		**SC/N**
IIHEM79010	§ ***Neogerris hesione***	**A Water Strider**	**GNR**	**S1S2**		**SC/N**
IIHEMA4030	§ ***Notonecta borealis***	**A Backswimmer**	**GNR**	**S2S3**		**SC/N**
IIHOM54010	***Paraphlepsius altus***	**A Leafhopper**	**GNR**	**S1?**		**SC/N**
IIHOM20060	§ *Paraphlepsius maculosus*	A Leafhopper	GNR	S1		SC/N
IIHOM29010	§ *Polyamia dilata*	Prairie Leafhopper	GNR	S2		THR
IIHOM17030	§ *Prairiana angustens*	A Leafhopper	GNR	S1S3		SC/N
IIHOM17020	§ *Prairiana cinerea*	A Leafhopper	GNR	S2S3		SC/N
IIHOM17010	§ *Prairiana kansana*	A Leafhopper	GNR	S2?		SC/N
IIHEM04080	§ ***Ramphocorixa acuminata***	**Acuminate Water Boatman**	**G4**	**S1S2**		**SC/N**
IIHOM56010	***Rhynchomitra microrhina***	**A Planthopper**	**GNR**	**S1?**		**SC/N**
IIHEM31010	***Slaterobius quadristriata***	**A Seed Bug**	**GNR**	**S1S2**		**SC/N**
RARE GRASSHOPPERS AND ALLIES						
IIORT96010	§ *Aeropedellus clavatus*	Club-horned Grasshopper	G5	**S2S3**		SC/N
IIORT49050	§ *Arphia conspersa*	Speckled Rangeland Grasshopper	G5	S2		SC/N
IIORT49060	§ *Arphia simplex*	A Grasshopper	G5	**S1S2**		SC/N
IIORT61010	§ *Camnula pellucida*	Clear-winged Grasshopper	G5	**S3**		SC/N
IIORT12030	§ *Chloealtis abdominalis*	Rocky Mountain Sprinkled Locust	G5	S2?		SC/N
IIORT97010	§ *Dichromorpha viridis*	Short-winged Grasshopper	G5	**S2S3**		SC/N
IIORT98010	§ *Eritettix simplex*	Velvet-striped Grasshopper	G5	**S2?**		SC/N
IIORT83020	§ *Hesperotettix speciosus*	A Grasshopper	G5	S1S2		SC/N
IIORT83010	§ *Hesperotettix viridis*	Green-streak Grasshopper	G5	S2?		SC/N
IIORT50010	***Hippiscus ocelote***	**Wrinkled Grasshopper**	**G5**	**SH**		**SC/N**
IIORT01120	§ *Melanoplus bruneri*	Bruner's Spur-throat Grasshopper	G5	S1S2		SC/N
IIORT01150	§ *Melanoplus fasciatus*	Huckleberry Spur-throat Grasshopper	G5	**S2**		SC/N
IIORT01020	*Melanoplus flavidus*	Blue-legged Grasshopper	G4	**S2?**		SC/N
IIORT01470	§ *Melanoplus foedus*	A Spur-throat Grasshopper	G5	**S2?**		SC/N
IIORT01B30	§ *Melanoplus gladstoni*	Gladston's Spur-throat Grasshopper	G5	**S1S2**		SC/N
IIORT01050	§ ***Melanoplus punctulatus***	**Grizzly Spur-throat Grasshopper**	**G4**	**S2?**		**SC/N**
IIORT01N40	§ ***Melanoplus rusticus***	**A Spur-throat Grasshopper**	**G4G5**	**SH**		**SC/N**
IIORT01220	§ *Melanoplus scudderi*	Scudder's Short-winged Grasshopper	G5	S1S2		SC/N
IIORT01060	§ *Melanoplus stonei*	Stone's Locust	G4G5	S1S2		SC/N
IIORT88010	§ *Mermiria bivittata*	Mermiria Grasshopper	G5	**S2?**		SC/N
IIORT79010	***Metaleptea brevicornis***	**Short-horned Grasshopper**	**G5**	**SH**		**SC/N**
IIORT40010	§ *Neoconocephalus lyristes*	Bog Conehead	GNR	S1?		SC/N
IIORT40030	§ *Neoconocephalus robustus*	Crepitating Conehead	GNR	**SH**		SC/N
IIORTF3020	§ *Opeia obscura*	Obscure Grasshopper	G5	S2S3		SC/N
IIORT41010	§ *Orchelimum delicatum*	Delicate Meadow Katydid	GNR	S2?		SC/N
IIORT37010	§ *Orphulella pelidna*	Spotted-winged Grasshopper	G5	S2S3		SC/N
IIORT9T010	§ *Paratylotropidia brunneri*	An Acridid Grasshopper	G4G5	**SH**		SC/N
IIORT05030	§ *Pardalophora haldemanii*	Haldmen's Grasshopper	G5	**SH**		SC/N
IIORT42010	§ *Scudderia fasciata*	Black-striped Katydid	GNR	**S1S2**		SC/N
IIORT64010	§ *Syrbula admirabilis*	Handsome Grasshopper	G5	**S1S2**		SC/N
IIORT65010	§ *Trachyrhachys kiowa*	Ash-brown Grasshopper	G5	S2		SC/N
IIORT36010	§ *Trimerotropis huroniana*	Lake Huron Locust	G2G3	S1		END

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IIORT36050	§ *Trimerotropis maritima*	Seaside Grasshopper	G5	**S2**		SC/N
IIORT36020	§ *Trimerotropis verruculata*	Crackling Forest Grasshopper	G5	**S2?**		SC/N
RARE STONEFLIES						
IIPLE1M010	§ *Attaneuria ruralis*	A Common Stonefly	G4	**S2S3**		SC/N
IIPLE14040	§ ***Haploperla orpha***	**Quadrate Sallfly**	**G4**	**S2S3**		**SC/N**
IIPLE2Q040	***Isogenoides frontalis***	**A Perlodid Stonefly**	**G5**	**S1S2**		**SC/N**
IIPLE2Q070	§ ***Isogenoides olivaceus***	**A Perlodid Stonefly**	**G3**	**S2S3**		**SC/N**
IIPLE24290	***Isoperla lata***	**A Perlodid Stonefly**	**G5**	**S2S3**		**SC/N**
IIPLE1V020	§ ***Perlinella ephyre***	**Vernal Stone**	**G5**	**S2S3**		**SC/N**
IIPLE0R010	§ ***Shipsa rotunda***	**Intrepid Forestfly**	**G5**	**S1S3**		**SC/N**
IIPLE0F010	§ *Zealeuctra narfi*	A Rolled-winged Winter Stonefly	G4	S1		SC/N
RARE CADDISFLIES						
IITRI33090	§ ***Agapetus hessi***	**A Saddle Casemaker Caddisfly**	**G4G5**	**S2S3**		**SC/N**
IITRI30020	§ *Banksiola dossuaria*	A Giant Casemaker Caddisfly	G5	S2S3		SC/N
IITRIA6010	§ ***Beothukus complicatus***	**A Phryganeid Caddisfly**	**G4**	**S1S2**		**SC/N**
IITRI66190	§ *Brachycentrus incanus*	A Caddisfly	G5	**SU**		SC/N
IITRI62050	§ ***Brachycentrus lateralis***	**A Humpless Casemaker Caddisfly**	**G5**	**S1S2**		**SC/N**
IITRI94010	§ *Fabria inornata*	A Giant Casemaker Caddisfly	G4G5	**SU**		SC/N
IITRI01030	§ *Lepidostoma libum*	A Lepidostomatid Caddisfly	G3G4	**S2S3**		SC/N
IITRI01040	§ ***Lepidostoma vernale***	**A Lepidostomatid Caddisfly**	**G5**	**S1S2**		**SC/N**
IITRI41090	§ *Ochrotrichia riesi*	A Purse Casmaker Caddisfly	G3G4	**S1S3**		SC/N
IITRI2G130	§ *Oecetis nocturna*	A Long-horned Casemaker Caddisfly	G5	S1S3		SC/N
IITRI65510	§ ***Psilotreta indecisa***	**A Caddisfly**	**G5**	**S1S2**		**SC/N**
IITRI19300	§ ***Rhyacophila lobifera***	**A Caddisfly**	**G5**	**S1S3**		**SC/N**
IITRI2F210	§ *Triaenodes nox*	A Long-horned Casemaker Caddisfly	G5	**S2S3**		SC/N
IITRI78010	§ ***Wormaldia moesta***	**A Fingernet Caddisfly**	**G5**	**S2S3**		**SC/N**
IITRI78040	***Wormaldia shawnee***	**A Fingernet Caddisfly**	**G4G5**	**S1S3**		**SC/N**
RARE SPIDERS						
ILARAC1010	§ *Paradamoetas fontana*	A Jumping Spider	GNR	S1S2		SC/N
ILARA05030	*Phidippus pius*	A Jumping Spider	GNR	S1S2		SC/N
RARE FLIES - NEW GROUP						
IIDIP7B010	§ ***Pseudodiamesa pertinax***	**A Non-biting Midge**	**GNR**	**S2**		**SC/N**
RARE ANTS, WASPS, AND BEES						
IIHYM24020	³ *Bombus affinis*	Rusty-patched Bumble Bee	**GU**	**S1**		SC/N
IIHYM24360	³ ***Bombus ashtoni***	**A Bumble Bee**	**GU**	**S1?**		**SC/N**
IIHYM24130	³ ***Bombus frigidus***	**A Bumble Bee**	**G4?**	**S1S3**		**SC/N**
IIHYM24260	³ ***Bombus pennsylvanicus***	**A Bumble Bee**	**GU**	**S1S2**		**SC/N**
IIHYM24220	³ *Bombus terricola*	Yellowbanded Bumble Bee	GU	**S1**		SC/N
IIHYM94010	³ ***Epeoloides pilosula***	**A Cuckoo Bee**	**G1**	**SH**		**SC/N**
IIHYM05010	³ ***Macropis ciliata***	**A Mellittid Bee**	**GNR**	**SH**		**SC/N**
RARE AQUATIC AND TERRESTRIAL SNAILS						
IMGAS91020	***Allogona profunda***	**Broad-banded Forestsnail**	**G5**	**S2S3**		**SC/N**
IMGAS11030	§ *Cochlicopa morseana*	Appalachian Pillar	G5	S2		SC/N
IMGAS54090	***Discus patulus***	**Domed Disc**	**G5**	**SU**		**SC/N**
IMGAS15130	§ *Gastrocopta procera*	Wing Snaggletooth	G5	S3		THR
IMGAS73170	§ *Glyphyalinia rhoadsi*	Sculpted Glyph	G5	S2		SC/N
IMGAS73250	§ *Glyphyalinia wheatleyi*	Bright Glyph	G5	S1		SC/N
IMGAS71030	§ *Guppya sterkii*	Brilliant Granule	G5	S2S3		SC/N
IMGAS50190	*Helicodiscus singleyanus*	Smooth Coil	G5	**S2?**		SC/N
IMGAS03010	§ *Hendersonia occulta*	Cherrystone Drop	G4	**S2S3**		THR
IMGAS78210	§ *Paravitrea multidentata*	Dentate Supercoil	G5	S2S3		SC/N
IMGAS21010	§ *Planogyra asteriscus*	Eastern Flat-whorl	G4	S1		SC/N
IMGAS81010	***Striatura exigua***	**Ribbed Striate**	**G5**	**S2S3**		**SC/N**
IMGAS81020	§ *Striatura ferrea*	Black Striate	G5	S2		SC/N
IMGAS24010	§ *Strobilops aeneus*	Bronze Pinecone	G5	S1		SC/N
IMGAS24020	§ *Strobilops affinis*	Eightfold Pinecone	**G4**	S3		SC/N
IMGAS22040	*Vallonia excentrica*	Oval Vallonia	G5	S3		SC/N
IMGAS22060	***Vallonia parvula***	**Trumpet Vallonia**	**G4**	**S2?**		**SC/N**
IMGAS22070	*Vallonia perspectiva*	Thin-lip Vallonia	G4G5	S3		SC/N
IMGASE5110	§ *Valvata winnebagoensis*	Flanged Valvata	G2	SU		SC/N
IMGAS20140	*Vertigo elatior*	Tapered Vertigo	G5	S3		SC/N
IMGAS20380	§ *Vertigo hubrichti*	Midwest Pleistocene Vertigo	G3	S1		END
IMGAS20210	***Vertigo modesta***	**Cross Vertigo**	**G5**	**S1**		**SC/N**
IMGAS20220	§ *Vertigo morsei*	Six-whorl Vertigo	G3	S1		SC/N
IMGAS20230	§ *Vertigo nylanderi*	Deep-throated Vertigo	G3G4	**S1S2**		SC/N

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IMGAS20420	§ *Vertigo paradoxa*	Mystery Vertigo	G4G5Q	S1		SC/N
IMGAS20330	*Vertigo tridentata*	Honey Vertigo	G5	S3		SC/N
IMGAS86040	§ *Vitrina angelicae*	Transparent Vitrine Snail	G5	S1		SC/N
IMGAS85050	§ *Zonitoides limatulus*	Dull Gloss	G4G5	**S1S2**		SC/N
IMGAS23010	§ *Zoogenetes harpa*	Boreal Top	G5	S1		SC/N
RARE PLANTS						
PDACE010C0	*Acer pensylvanicum*	Striped Maple	G5	S1		SC
PDRAN01070	*Aconitum noveboracense*	Northern Wild Monkshood	G3	S2	LT	THR
PDFUM02010	*Adlumia fungosa*	Climbing Fumitory	G4	**S2S3**		SC
PDADO01010	*Adoxa moschatellina*	Musk-root	G5	S2		THR
PDSCR01130	***Agalinis*** (*=Tomanthera*) *auriculata*	Earleaf Foxglove	G3	S1		SC
PDSCR010B0	*Agalinis gattingeri*	Roundstem Foxglove	G4	**S2**		THR
PDSCR010T0	*Agalinis skinneriana*	Pale False Foxglove	G3G4	S2		END
PDLAM03070	*Agastache nepetoides*	Yellow Giant Hyssop	G5	S3		THR
PDROS03060	*Agrimonia parviflora*	Swamp Agrimony	G5	S1S2		SC
PMORC01010	*Amerorchis rotundifolia*	Round-leaved Orchis	G5	**S1S2**		THR
PDRAN04030	*Anemone caroliniana*	Carolina Anemone	G5	S1		END
PDRAN040E1	*Anemone multifida var. hudsoniana*	Early Anemone	G5T5	S1		END
PMORC03010	*Aplectrum hyemale*	Putty Root	G5	S2S3		SC
PDBRA06170	***Arabis missouriensis***	**Missouri Rock-cress**	**G5**	S2		SC
PDBRA061W0	*Arabis shortii*	Short's Rock-cress	G5	**S1S2**		SC
PDCAR0G070	***Arenaria stricta ssp. dawsonensis*** (*=Minuartia dawsonensis*)	Rock Stitchwort	G5	S1		SC
PMPOA0K0C0	*Aristida dichotoma*	Shinners' Three-awned Grass	G5	S1		SC
PDBRA07010	*Armoracia lacustris*	Lake-cress	G4?	S1		END
PDAST0S0H0	*Artemisia dracunculus*	Dragon Wormwood	G5	S2		SC
PDAST0S0L0	*Artemisia frigida*	Prairie Sagebrush	G5	S2		SC
PDASC022A0	*Asclepias lanuginosa*	Woolly Milkweed	G4?	S1		THR
PDASC021D0	*Asclepias ovalifolia*	Dwarf Milkweed	G5?	S3		THR
PDASC021J0	*Asclepias purpurascens*	Purple Milkweed	G5?	S3		END
PDASC021X0	*Asclepias sullivantii*	Prairie Milkweed	G5	S2S3		THR
PPASP02100	*Asplenium pinnatifidum*	Lobed Spleenwort	G4	S1		THR
PPASP021K0	*Asplenium trichomanes*	Maidenhair Spleenwort	G5	S3		SC
PPASP02250	*Asplenium viride*	Green Spleenwort	G4	S1		END
PDASTE80F6	*Aster dumosus var. strictior*	Bushy Aster	G5T4	S1		SC
PDASTE8252	***Aster fragilis var. subdumosus***	**Fragile-stemmed Aster**	**G4G5T3T5Q**	**S1S2**		**SC**
PDASTEB0H0	*Aster furcatus*	Forked Aster	G3	S3		THR
PDAST0T1U0	*Aster longifolius*	Long-leaved Aster	G5	S1		SC
PDASTEK010	***Aster modestus***	**Northwestern Sticky Aster**	**G5**	**S1**		**SC**
PDFAB0F0D0	*Astragalus alpinus*	Alpine Milkvetch	G5	S1		END
PDFAB0F2G0	*Astragalus crassicarpus*	Ground-plum	G5	S2		END
PDFAB0F5U0	*Astragalus neglectus*	Cooper's Milkvetch	G4	S1		END
PDFAB0G0P0	*Baptisia tinctoria*	Yellow Wild-indigo	G5	S1		SC
PDGEN01010	*Bartonia paniculata*	Twining Screwstem	G5	S1		SC
PDSCR09030	*Besseya bullii*	Kitten Tails	G3	S3		THR
PPOPH010W0	*Botrychium campestre*	Prairie Dunewort	G3G4	S1		END
PPOPH01080	*Botrychium lunaria*	Moonwort Grape-fern	G5	S1S2		END
PPOPH010R0	*Botrychium minganense*	Mingan's Moonwort	G4	S2		SC
PPOPH010N0	*Botrychium mormo*	Little Goblin Moonwort	G3	**S1S2**		END
PPOPH010C0	*Botrychium oneidense*	Blunt-lobe Grape-fern	G4Q	S2		SC
PPOPH01130	*Botrychium pallidum*	Pale Moonwort	G3	S1		SC
PPOPH010P0	*Botrychium rugulosum*	Rugulose Grape-fern	G3	S2		SC
PPOPH01140	*Botrychium spathulatum*	Spoon-leaf Moonwort	G3	S1		SC
PDASTD7060	*Cacalia tuberosa*	Prairie Indian-Plantain	G4G5	S3		THR
PDBRA0F020	*Cakile lacustris*	American Sea-rocket	G5	S3		SC
PMPOA17170	*Calamagrostis stricta*	Slim-stem Small Reed Grass	G5	S3		SC
PDLAM08010	*Calamintha arkansana*	Low Calamint	G5	S2		SC
PMPOA18052	*Calamovilfa longifolia var. magna*	Sand Reedgrass	G5T3T5	S2		THR
PDMAL0A080	*Callirhoe triangulata*	Clustered Poppy-mallow	G3	**S2**		SC
PDCLL01030	*Callitriche hermaphroditica*	Autumnal Water-starwort	G5	S2		SC
PDCLL01040	*Callitriche heterophylla*	Large Water-starwort	G5	S1		THR
PMORC0C050	*Calopogon oklahomensis*	Oklahoma Grass-pink	**G3**	SH		SC
PDRAN06020	*Caltha natans*	Floating Marsh-marigold	G5	S1		END
PDONA02040	*Calylophus serrulatus*	Yellow Evening Primrose	G5	S2		SC
PMORC0D010	*Calypso bulbosa*	Fairy Slipper	G5	**S2**		THR
PMLIL0E050	*Camassia scilloides*	Wild Hyacinth	G4G5	S2		END

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PDBRA0K1A0	*Cardamine maxima*	Large Toothwort	G5	S1		SC
PMCYP03130	*Carex artitecta*	Dry Woods Sedge	G5	S1		SC
PMCYP031F0	*Carex backii*	Rocky Mountain Sedge	G4	S1		SC
PMCYP032G0	*Carex capillaris*	Hair-like Sedge	G5	**S1S2**		SC
PMCYP032J0	*Carex careyana*	Carey's Sedge	G4G5	S1		THR
PMCYP03300	*Carex concinna*	Beautiful Sedge	**G5**	S1		THR
PMCYP033B0	*Carex crus-corvi*	Ravenfoot Sedge	G5	S1		END
PMCYP033D0	*Carex cumulata*	Clustered Sedge	G4?	S2		SC
PMCYP033S0	***Carex digitalis***	**Slender Wood Sedge**	**G5**	**S1**		**SC**
PMCYP034F0	*Carex exilis*	Coast Sedge	G5	S1		THR
PMCYP034L0	***Carex festucacea***	**Fescue Sedge**	**G5**	**S2**		**SC**
PMCYP034Y0	*Carex formosa*	Handsome Sedge	G4	S2		THR
PMCYP03520	*Carex garberi*	Elk Sedge	G5	S2		THR
PMCYP035C0	*Carex gracilescens*	Slender Sedge	G5?	SH		SC
PMCYP036Z0	*Carex laevivaginata*	Smooth-sheath Sedge	G5	S1		END
PMCYP037A0	*Carex lenticularis*	Shore Sedge	G5	S2		THR
PMCYP037L1	*Carex livida var. radicaulis*	Livid Sedge	G5T5	S2		SC
PMCYP037P0	*Carex longii*	Greenish-white Sedge	G5	**S1**		SC
PMCYP037T0	*Carex lupuliformis*	False Hop Sedge	G4	**S2**		END
PMCYP039D1	*Carex media*	Intermediate Sedge	G5T5?	S2		END
PMCYP038D0	***Carex merritt-fernaldii***	**Fernald's Sedge**	**G5**	**S3**		**SC**
PMCYP038H0	*Carex michauxiana*	Michaux's Sedge	G5	S2		THR
PMCYP03990	*Carex nigra*	Smooth Black Sedge	G5	S1		SC
PMCYP039F0	*Carex novae-angliae*	New England Sedge	G5	SH		SC
PMCYP03AR0	*Carex platyphylla*	Broad-leaf Sedge	G5	S2		SC
PMCYP03B10	*Carex prasina*	Drooping Sedge	G4	S3		THR
PMCYP03C60	*Carex schweinitzii*	Schweinitz's Sedge	G3G4	S1		END
PMCYP03D00	*Carex straminea*	Straw Sedge	G5	S1		SC
PMCYP03D70	*Carex suberecta*	Prairie Straw Sedge	G4	S1		SC
PMCYP03DD0	*Carex swanii*	Swan Sedge	G5	S1		SC
PMCYP03DE0	*Carex sychnocephala*	Many-headed Sedge	G4	S2		SC
PMCYP03DT0	*Carex torreyi*	Torrey's Sedge	G4	S1		SC
PMPOA19010	*Catabrosa aquatica*	Brook Grass	G5	S1		END
PDAPI0K020	*Chaerophyllum procumbens*	Spreading Chervil	G5	S1		SC
PDEUP0D1Z0	***Chamaesyce*** (*=Euphorbia*) *polygonifolia*	Seaside Spurge	G5?	S2		SC
PDAST2E1C0	*Cirsium hillii*	Hill's Thistle	G3	S3		THR
PDAST2E2A0	*Cirsium pitcheri*	Dune Thistle	G3	S2	LT	THR
PMCOM03052	*Commelina erecta var. deamiana*	Narrow-leaved Dayflower	G5T5	S1		SC
PDFAB160E0	*Crotalaria sagittalis*	Arrow-headed Rattle-box	G5	S1		SC
PDCUS010D0	***Cuscuta coryli***	**Hazel Dodder**	**G5?**	**S1**		**SC**
PDCUS010S0	***Cuscuta glomerata***	**Rope Dodder**	**G5**	**S1**		**SC**
PDCUS01140	***Cuscuta pentagona***	**Field Dodder**	**G5**	**S1**		**SC**
PDCUS01170	***Cuscuta polygonorum***	**Knotweed Dodder**	**G5**	**S1**		**SC**
PMORC0Q020	*Cypripedium arietinum*	Ram's-head Lady's-slipper	G3	S2		THR
PMORC0Q050	*Cypripedium candidum*	Small White Lady's-slipper	G4	S3		THR
PPDRY07040	*Cystopteris laurentiana*	Laurentian Bladder Fern	G3	S2		SC
PDFAB1A1Q0	*Dalea villosa var. villosa*	Silky Prairie-clover	G5	S2		SC
PDSCR0L010	*Dasistoma macrophylla*	Mullein Foxglove	G4	**S1**		SC
PMPOA22050	*Deschampsia cespitosa*	Tufted Hairgrass	G5	**S3**		SC
PDFAB1D090	*Desmodium canescens*	Hoary Tick-trefoil	G5	S1		SC
PMPOA23020	*Diarrhena obovata*	Beak Grass	G4G5	S2		END
PMPOA241Q0	***Dichanthelium*** (*=Panicum*) *wilcoxianum*	Wilcox's Panic Grass	G5	**S1**		SC
PDLYT04010	*Didiplis diandra*	Water-purslane	G5	S1		SC
PDRUB0H071	*Diodia teres var. teres*	Buttonweed	G5T5	S1		SC
PPDRY090C0	*Diplazium pycnocarpon*	Glade Fern	G5	S2		SC
PDPRI03020	*Dodecatheon amethystinum*	Jeweled Shooting Star	G4	**S1S2**		SC
PDBRA11070	*Draba arabisans*	Rock Whitlow-grass	G4	S2		SC
PDBRA112Q0	*Draba lanceolata*	Lanceolate Whitlow-cress	G3G5Q	S1		END
PDDRO02010	*Drosera anglica*	English Sundew	G5	S1		THR
PDDRO02060	*Drosera linearis*	Slenderleaf Sundew	G4	S1		THR
PPDRY0A070	*Dryopteris clintoniana*	Clinton's Woodfern	G5	SH		SC
PPDRY0A0A0	*Dryopteris expansa*	Spreading Woodfern	G5	S2		SC
PPDRY0A0B0	*Dryopteris filix-mas*	Male Fern	G5	S1		SC
PPDRY0A0C0	***Dryopteris fragrans***	Fragrant Fern	**G5**	S3		SC
PDAST38040	*Echinacea pallida*	Pale Purple Coneflower	G4	S3		THR
PMALI02030	*Echinodorus rostratus*	Erect Burhead	G5	SH		SC

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PDAST3A010	*Eclipta prostrata*	Yerba-de-tajo	G5	SH		SC
PDELT02090	*Elatine triandra*	Longstem Water-wort	G5	S1		SC
PMCYP09220	*Eleocharis compressa*	Flat-stemmed Spike-rush	G4	S2		SC
PMCYP090M0	*Eleocharis engelmannii*	Engelmann's Spike-rush	G4G5Q	S1		SC
PMCYP090N0	*Eleocharis equisetoides*	Horsetail Spike-rush	G4	S1		SC
PMCYP091A0	***Eleocharis flavescens var. olivacea*** (*=E. olivacea*)	Capitate Spike-rush	G5	S2		SC
PMCYP092B0	*Eleocharis mamillata*	**Mamillate Spike-rush**	G4?	S1		SC
PMCYP09180	*Eleocharis nitida*	Slender Spike-rush	G4	S2		END
PMCYP091J0	*Eleocharis quadrangulata*	Square-stem Spike-rush	G4	S1		END
PMCYP091K0	*Eleocharis quinqueflora*	Few-flowered Spike-rush	G5	S2		SC
PMCYP091N0	*Eleocharis robbinsii*	Robbins' Spike-rush	G4G5	S3		SC
PMCYP091P0	*Eleocharis rostellata*	Beaked Spike-rush	G5	S2		THR
PMCYP091Z0	*Eleocharis wolfii*	Wolf Spike-rush	G3G4	S1		END
PMPOA2H0S3	*Elymus lanceolatus ssp. psammophilus*	Thickspike	G5T3	S2		THR
PDONA060X0	*Epilobium strictum*	Downy Willow-herb	G5?	S2S3		SC
PPEQU01050	*Equisetum palustre*	Marsh Horsetail	G5	S2		SC
PDAPI0Y010	*Erigenia bulbosa*	Harbinger-of-spring	G5	S1		END
PMCYP0A060	*Eriophorum chamissonis*	Russet Cotton-grass	G5	**S1**		SC
PDEUP0Q0B0	*Euphorbia commutata*	Wood Spurge	G5	SH		SC
PMPOA2V0M0	*Festuca occidentalis*	Western Fescue	G5	S1		THR
PMPOA2V0P0	*Festuca paradoxa*	Cluster Fescue	G5	SH		SC
PMCYP0B0G0	*Fimbristylis puberula*	Hairy Fimbristylis	G5	S1		END
PDOLE040F0	*Fraxinus quadrangulata*	Blue Ash	G5	S1		THR
PMCYP0C040	*Fuirena pumila*	Dwarf Umbrella-sedge	G4	S1		END
PDRUB0N0C0	*Galium brevipes*	Swamp Bedstraw	G4?	**S1**		SC
PDRUB0N1P0	*Galium palustre*	Marsh Bedstraw	G5	S1		SC
PDGEN06020	*Gentiana alba*	Yellow Gentian	G4	**S4**		THR
PDSAN04010	*Geocaulon lividum*	Northern Comandra	G5	S1		END
PDROS0S082	*Geum macrophyllum var. macrophyllum*	Large-leaved Avens	G5T5	S1		SC
PDROS0S081	*Geum macrophyllum var. perincisum*	Large-leaved Avens	G5T5	S2		SC
PDFAB1W020	*Glycyrrhiza lepidota*	Wild Licorice	G5	**S1**		SC
PDAST44092	*Gnaphalium helleri var. micradenium*	Catfoot	G4G5T3?	S1		SC
PDAST440G3	*Gnaphalium obtusifolium var. saxicola*	Cliff Cudweed	G5T2	S2		THR
PDASTDP030	*Gnaphalium sylvaticum*	Woodland Cudweed	G4	S1		SC
PMORC17010	*Goodyera oblongifolia*	Giant Rattlesnake-plantain	G5?	S1		SC
PPDRY0D051	*Gymnocarpium jessoense ssp. parvulum*	Northern Oak Fern	G5T4	S1		SC
PPDRY0D060	*Gymnocarpium robertianum*	Limestone Oak Fern	G5	**S1S2**		SC
PDFAB1X010	*Gymnocladus dioicus*	Kentucky Coffee-tree	G5	S2		SC
PDRUB1T010	*Houstonia caerulea*	**Azure Bluets**	G5	S2		SC
PPLYC020J0	*Huperzia appalachiana*	Appalachian Clubmoss	G4G5	S1		SC
PPLYC02080	*Huperzia porophila*	Rock Clubmoss	G4	S3		SC
PPLYC02070	*Huperzia selago*	Fir Clubmoss	G5	**S1S2**		SC
PDVIO02020	*Hybanthus concolor*	Green Violet	G5	SH		SC
PDHYD08010	*Hydrophyllum appendiculatum*	Great Water-leaf	G5	S2S3		SC
PDCLU031B0	***Hypericum prolificum***	**Shrubby St. John's-wort**	**G5**	**S1**		**SC**
PDCLU031H0	*Hypericum sphaerocarpum*	Round-fruited St. John's-wort	G5	S1S2		THR
PDBRA1H010	***Iodanthus pinnatifidus***	**Purple Rocket**	**G5**	**S1**		**SC**
PMIRI090H0	*Iris lacustris*	Dwarf Lake Iris	G3	S3	LT	THR
PDBER05010	*Jeffersonia diphylla*	Twinleaf	G5	S3		SC
PMJUN011S0	*Juncus marginatus*	Grassleaf Rush	G5	S2		SC
PMJUN012N0	*Juncus stygius*	Moor Rush	G5	S1		END
PMJUN01340	*Juncus vaseyi*	Vasey's Rush	G5?	S3		SC
PDFAB27090	*Lespedeza leptostachya*	Prairie Bush-clover	G3	S2	LT	END
PDFAB27080	*Lespedeza violacea*	Violet Bush-clover	G5	S2		SC
PDFAB270G0	*Lespedeza virginica*	Slender Bush-clover	G5	S2		THR
PDBRA1N110	*Lesquerella ludoviciana*	Silver Bladderpod	G5	S1		THR
PDSOL0E010	*Leucophysalis grandiflora*	Large-flowered Ground-cherry	G4?	S1		SC
PDAST5X0M2	*Liatris punctata var. nebraskana*	Dotted Blazing Star	G5T3T5	S2S3		END
PDAST5X0T0	*Liatris spicata*	Marsh Blazing Star	G5	S3		SC
PMORC1N010	*Listera auriculata*	Auricled Twayblade	G3G4	S1		END
PMORC1N050	*Listera convallarioides*	Broad-leaved Twayblade	G5	S1		THR
PDPLN01010	***Littorella uniflora var. americana***	**American Shoreweed**	G5	S2		SC
PDCPR03450	*Lonicera involucrata*	Fly Honeysuckle	**G5**	S1		END
PPLYC030A0	*Lycopodiella margueritae*	Northern Prostrate Clubmoss	**G1G2**	S1		SC
PMPOA3X0D0	*Melica nitens*	Three-flowered Melic Grass	G5	**S1**		SC
PMPOA3X0F0	*Melica smithii*	Smith's Melic Grass	G4	S1		END

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PDCAR0H020	*Moehringia macrophylla*	Large-leaved Sandwort	**G5**	S1		END
PMPOA481G0	*Muhlenbergia richardsonis*	Soft-leaf Muhly	G5	S1		END
PDBOR0P080	*Myosotis laxa*	Small Forget-me-not	G5	S2		SC
PDAST6P020	***Nothocalais*** (=*Microseris*) *cuspidata*	Prairie False-dandelion	G5	S2		SC
PDNYM04019	*Nuphar advena*	Yellow Water Lily	G5T5	S1		SC
PDNYS01030	*Nyssa sylvatica*	Black Tupelo	G5	**S1**		SC
PDCAC0D0H0	*Opuntia fragilis*	Brittle Prickly-pear	G4G5	S3		THR
PDORO04060	*Orobanche fasciculata*	Clustered Broomrape	G4	S1		THR
PDORO04070	*Orobanche ludoviciana*	Louisiana Broomrape	G5	S1		END
PDORO040F0	*Orobanche uniflora*	One-flowered Broomrape	G5	S3		SC
PDAPI1K010	*Osmorhiza* ***berteroi*** (=*O. chilensis*)	Chilean Sweet Cicely	G5	S3		SC
PDFAB2X041	*Oxytropis campestris var. chartacea*	Fassett's Locoweed	G5T1T2	S1S2	LT	END
PDSAX0P090	*Parnassia palustris*	Marsh Grass-of-Parnassus	G5	**S1S2**		THR
PDSAX0P0A0	*Parnassia parviflora*	Small-flowered Grass-of-Parnassus	G4	S1		END
PDAST6V060	*Parthenium integrifolium*	American Fever-few	G5	**S3S4**		THR
PDFAB5L040	*Pediomelum argophyllum*	Silvery Scurf Pea	G5	S1		SC
PDFAB5L0B0	*Pediomelum esculentum*	Prairie Turnip	G5	S3		SC
PPADI0H020	*Pellaea atropurpurea*	Purple-stem Cliff-brake	G5	S2		SC
PDSCR1L330	*Penstemon hirsutus*	Hairy Beardtongue	G4	S1		SC
PDSCR1L4J0	*Penstemon pallidus*	Pale Beardtongue	G5	S1		SC
PDAST71040	*Petasites sagittatus*	Arrow-leaved Sweet-coltsfoot	G5	S3		THR
PPTHE02020	*Phegopteris hexagonoptera*	Broad Beech Fern	G5	S2		SC
PDPOR080G0	***Phemeranthus*** (=*Talinum*) *rugospermus*	Prairie Fame-flower	G3G4	S3		SC
PDPLM0D0A0	*Phlox bifida*	Cleft Phlox	G5?	S1		SC
PDPLM0D0T2	*Phlox glaberrima ssp. interior*	Smooth Phlox	G5TNR	S2		END
PDLNT01090	*Pinguicula vulgaris*	Common Butterwort	G5	S1		END
PMPOA4J030	***Piptatherum*** (=*Oryzopsis*) *canadense*	Canada Mountain-ricegrass	G5	S1		SC
PDPLN02090	*Plantago cordata*	Heart-leaved Plantain	G4	S1		END
PMORC1Y082	*Platanthera flava var. herbiola*	Pale Green Orchid	G4T4Q	S2		THR
PMORC1Y0A0	*Platanthera hookeri*	**Hooker's Orchid**	G4	**S2**		SC
PMORC1Y0F0	*Platanthera leucophaea*	Prairie White-fringed Orchid	G2G3	S2	LT	END
PDPLT01020	*Platanus occidentalis*	Sycamore	G5	S2		SC
PMPOA4Z1W0	*Poa paludigena*	Bog Bluegrass	G3	S3		THR
PMPOA4Z2F0	*Poa sylvestris*	Woodland Bluegrass	G5	S1		SC
PMPOA4Z2M0	*Poa wolfii*	Wolf's Bluegrass	G4	S1		SC
PDCPP08030	*Polanisia jamesii*	James' Cristatella	G5	SH		SC
PDPLM0E0F4	*Polemonium occidentale ssp. lacustre*	Western Jacob's Ladder	G5?T1Q	S1		END
PDPGL020P0	*Polygala incarnata*	Pink Milkwort	G5	S1		END
PPDRY0R010	*Polystichum acrostichoides*	Christmas Fern	G5	S2		SC
PPDRY0R040	*Polystichum braunii*	Braun's Holly-fern	G5	S3		THR
PDAPI1U010	*Polytaenia nuttallii*	Prairie Parsley	G5	**S2**		THR
PMPOT03030	*Potamogeton bicupulatus*	Snail-seed Pondweed	G4	S2S3		SC
PMPOT03050	*Potamogeton confervoides*	Algae-like Pondweed	G4	S2		THR
PMPOT03070	*Potamogeton diversifolius*	Water-thread Pondweed	G5	S2		SC
PMPOT030F0	*Potamogeton hillii*	Hill's Pondweed	G3	S1		SC
PMPOT030T0	***Potamogeton perfoliatus***	**Clasping-leaf Pondweed**	**G5**	**S1**		**SC**
PMPOT030W0	*Potamogeton pulcher*	Spotted Pondweed	G5	S1		END
PMPOT03140	*Potamogeton vaginatus*	Sheathed Pondweed	G5	**S1**		THR
PMPOT03150	*Potamogeton vaseyi*	Vasey's Pondweed	G4	**S3**		SC
PDAST7K040	*Prenanthes aspera*	Rough Rattlesnake-root	G4?	**S1**		END
PDAST7K080	*Prenanthes crepidinea*	Nodding Rattlesnake-root	G4	S1		END
PDPRI080D0	*Primula mistassinica*	Bird's-eye Primrose	G5	S3		SC
PMCYP0N3A0	*Psilocarya scirpoides*	Long-beaked Baldrush	G4	S2		THR
PDRUT0F020	*Ptelea trifoliata*	Wafer-ash	G5	S2		SC
PDMON07010	*Pterospora andromedea*	Giant Pinedrops	G5	S1		END
PDPYR04060	*Pyrola minor*	Lesser Wintergreen	G5	S1		END
PDFAG053A0	*Quercus muehlenbergii*	Chinquapin Oak	G5	S1S2		SC
PDFAG051P0	*Quercus palustris*	Pin Oak	G5	S1		SC
PDRAN0L0Q0	*Ranunculus cymbalaria*	Seaside Crowfoot	G5	S2		THR
PDRAN0L110	*Ranunculus gmelinii*	Small Yellow Water Crowfoot	G5	S2		END
PDRAN0L1G0	*Ranunculus lapponicus*	Lapland Buttercup	G5	S1		END
PDRHA0C092	*Rhamnus lanceolata ssp. glabrata*	Lanced-leaved Buckthorn	G5T4T5	S1		SC
PDMLS0H0B0	*Rhexia virginica*	Virginia Meadow-beauty	G5	S3		SC
PDERI150G0	*Rhododendron lapponicum*	Lapland Azalea	G5	S1		END
PDANA08010	*Rhus aromatica*	Fragrant Sumac	G5	S1		SC
PMCYP0N0U0	*Rhynchospora fusca*	Brown Beak-rush	G4G5	S2		SC

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PDGRO02180	*Ribes oxyacanthoides*	Canada Gooseberry	G5	S2		THR
PDROS1K7W0	*Rubus uniformis*	Uniform Bramble	G4?Q	SH		SC
PDACA0J080	*Ruellia humilis*	Hairy Wild-petunia	G5	S2		END
PDSAL020U0	*Salix cordata*	Sand Dune Willow	G4	S1		END
PDSAL02260	*Salix pellita*	Satiny Willow	G5	S1		END
PDSAL022B0	*Salix planifolia*	Tea-leaved Willow	G5	S2		THR
PDSAL022N0	*Salix sericea*	Silky Willow	G5	SH		SC
PMCYP0Q060	*Scirpus cespitosus*	Tufted Bulrush	G5	S2		THR
PMCYP0Q0Q0	*Scirpus georgianus*	Georgia Bulrush	G5	**S1**		SC
PMCYP0Q0R0	*Scirpus hallii*	Hall's Bulrush	G2G3	S1		END
PMCYP0Q0T0	*Scirpus heterochaetus*	Slender Bulrush	G5	S1		SC
PMCYP0Q140	*Scirpus pallidus*	Pale Bulrush	G5	SH		SC
PMCYP0Q1J0	*Scirpus torreyi*	Torrey's Bulrush	G5?	S2		SC
PMCYP0R0K0	*Scleria reticularis*	Reticulated Nutrush	G4	S1		END
PMCYP0R0R0	*Scleria triglomerata*	Whip Nutrush	G5	S2S3		SC
PMCYP0R0S0	*Scleria verticillata*	Low Nutrush	G5	S2		SC
PDLAM1U104	***Scutellaria ovata ssp. ovata***	Heart-leaved Skullcap	**G5T5**	S3		SC
PDLAM1U111	*Scutellaria parvula var. parvula*	Small Skullcap	G4T4	S1		END
PPSEL01110	*Selaginella selaginoides*	Low Spike-moss	G5	S1		END
PDAST8H0U0	*Senecio congestus*	Marsh Ragwort	G5	S1		SC
PDAST8H1R0	*Senecio indecorus*	Plains Ragwort	G5	S1		THR
PDAST8H2F0	*Senecio plattensis*	Prairie Ragwort	G5	S3		SC
PDFAB491P0	*Senna marilandica*	Maryland Senna	G5	S1		SC
PDCAR0U120	*Silene nivea*	Snowy Campion	G4?	S2		THR
PDCAR0U220	*Silene virginica*	Fire Pink	G5	S1		END
PMIRI0D030	*Sisyrinchium angustifolium*	Pointed Blue-eyed-grass	G5	S1		SC
PDAST8P081	*Solidago caesia*	Bluestem Goldenrod	G5	S3		END
PDAST8P2U2	*Solidago simplex var. gillmanii*	Dune Goldenrod	G5T3?	S2		THR
PMSPA01070	*Sparganium glomeratum*	Northern Bur-reed	G4?	S2		THR
PMORC2B0J0	*Spiranthes lucida*	Shining Lady's-tresses	G5	S1		SC
PMORC2B0P1	*Spiranthes ovalis var. erostellata*	October Lady's-tresses	G5?T4?	S1		SC
PMLIL1X010	*Streptopus amplexifolius*	White Mandarin	G5	S3		SC
PDFAB3U020	*Strophostyles leiosperma*	Small-flowered Woolly Bean	G5	S2		SC
PDAST92012	*Tanacetum huronense*	Lake Huron Tansy	G5T4T5	S1		END
PDRAN0M0J0	*Thalictrum revolutum*	Waxleaf Meadowrue	G5	S2		SC
PDRAN0M0Q0	*Thalictrum venulosum*	Veined Meadowrue	G5	S1		SC
PDAPI28010	*Thaspium barbinode*	Hairy-jointed Meadow-parsnip	G5	S1		END
PDAPI28033	*Thaspium trifoliatum var. flavum*	Purple Meadow-parsnip	G5T5	S2		SC
PDSAX10010	*Tiarella cordifolia*	Heart-leaved Foam-flower	G5	S1		END
PMLIL1Y035	*Tofieldia glutinosa*	Sticky False-asphodel	G4G5	S2S3		THR
PMJCG02040	*Triglochin palustris*	Slender Bog Arrow-grass	G5	S3		SC
PMLIL200L0	*Trillium nivale*	Snow Trillium	G4	S3		THR
PMORC2F050	*Triphora trianthophora*	Nodding Pogonia	G3G4	S2		SC
PMPOA69080	*Trisetum melicoides*	Purple False Oats	G4	S1		END
PMPOA690C0	*Trisetum spicatum*	Narrow False Oats	G5	S2		THR
PDLNT020K0	*Utricularia resupinata*	Northeastern Bladderwort	G4	S3		SC
PDLNT020M0	*Utricularia subulata*	Zigzag Bladderwort	G5	S1		SC
PDERI18060	*Vaccinium cespitosum*	Dwarf Huckleberry	G5	S2		END
PDERI180T0	*Vaccinium pallidum*	Blue Ridge Blueberry	G5	S1		SC
PDERI18121	*Vaccinium vitis-idaea ssp. minus*	Mountain Cranberry	G5T5	**S1S2**		END
PDVAL030J0	*Valeriana sitchensis ssp. uliginosa*	Marsh Valerian	G4Q	S2		THR
PDVER0N0W0	*Verbena simplex*	Narrow-leaved Vervain	G5	S1		SC
PDCPR07070	*Viburnum edule*	Squashberry	G5	S2		END
PDCPR070E1	*Viburnum nudum var. cassinoides*	Northern Wild-raisin	G5T5	**S1**		SC
PDCPR070J0	*Viburnum prunifolium*	Smooth Black-haw	G5	S2		SC
PDVIO041Z1	*Viola fimbriatula*	Sand Violet	G5T5	S2		END
PDVIO041X0	*Viola rostrata*	Long-spurred Violet	G5	S2S3		SC
PPDRY0U081	*Woodsia oregana ssp. cathcartiana*	Oregon Woodsia	G5T5	S1		SC
PMLIL28032	*Zigadenus elegans var. glaucus*	White Camas	G5T4T5	S2S3		SC
RARE BRYOPHYTES - DRAFT						
NBMUS05010	*Amblyodon dealbatus*	A Moss	G3G5	S1		SC
NBHEP04052	*Anastrophyllum minutum var. minutum*	A Liverwort	G5T5	S1		SC
NBANT01040	*Anthoceros macounii*	A Hornwort	G3G4	S1S2		SC
NBANT01060	*Anthoceros punctatus*	A Hornwort	G5	S1S2		SC
NBMUS0N020	*Aulacomnium androgynum*	A Moss	G5	S2		SC
NBMUS0V010	*Blindia acuta*	A Moss	G5	S1		SC

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NBMUS0Z060	*Brachythecium calcareum*	A Moss	G3G4	S3		SC
NBMUS1B010	*Buxbaumia aphylla*	Bug-on-a-stick	G4G5	S1		SC
NBMUS1F060	*Calliergon richardsonii*	A Moss	G4	S3		SC
NBMUS1F0A0	*Calliergon trifarium*	A Moss	G4	S1		SC
NBHEP0M010	*Calypogeia fissa*	A Liverwort	G5	S1		SC
NBHEP0M060	*Calypogeia sphagnicola*	A Liverwort	G4	S1		SC
NBHEP0P050	*Cephalozia lacinulata*	A Liverwort	G3	S1		SC
NBHEP0Q050	*Cephaloziella divaricata*	A Liverwort	G5	S1		SC
NBHEP0Q0H5	*Cephaloziella rubella var. sullivantii*	A Liverwort	G5T3?Q	S1		SC
NBMUS28010	*Dicranella cerviculata*	A Moss	G5?	S1		SC
NBMUS280A1	*Dicranella schreberiana var. robusta*	A Moss	G5TNR	S1		SC
NBMUS2G020	*Ditrichum flexicaule*	A Moss	G5	S1		SC
NBMUS2J0J0	*Drepanocladus simplicissimus*	A Moss	G1	S1		SC
NBMUS2M070	*Encalypta procera*	Extinguisher Moss	G4G5	S2		SC
NBMUS2N010	*Entodon brevisetus*	A Moss	G4?	S4?		SC
NBMUS2X060	*Fontinalis flaccida*	A Water Moss	G4G5	S1		SC
NBMUS2X0F0	*Fontinalis sphagnifolia*	A Water Moss	G3G5	S2		SC
NBHEP1A0L0	*Frullania selwyniana*	A Liverwort	G2G3	S1		SC
NBMUS2Z010	*Funaria americana*	A Moss	G3?	S3?		SC
NBMUS32120	*Grimmia pulvinata*	A Moss	G4G5	S1?		SC
NBMUS32160	*Grimmia teretinervis*	A Moss	G3G5	S2		SC
NBMUS3U010	*Hyophila involuta*	A Moss	G4G5	S2		SC
NBMUS3X010	*Isopterygiopsis muelleriana*	A Moss	G5	S1		SC
NBMUS97010	*Jaffueliobryum raui*	A Moss	G4?	S2		SC
NBMUS97020	*Jaffueliobryum wrightii*	A Moss	G4G5	S2		SC
NBHEP1P030	*Jungermannia confertissima*	A Liverwort	G5	S1		SC
NBHEP1P040	*Jungermannia crenuliformis*	A Liverwort	G4	S1		SC
NBHEP1P061	*Jungermannia exsertifolia ssp. cordifolia*	A Liverwort	G5?T3T5	S1		SC
NBHEP1P080	*Jungermannia gracillima*	A Liverwort	G5	S1		SC
NBHEP1Q020	*Kurzia setacea*	A Liverwort	G4G5	S1		SC
NBMUS4B020	*Leucodon julaceus*	A Moss	G5	S2		SC
NBHEP1Y030	*Lophozia ascendens*	A Liverwort	G4	S1		SC
NBHEP1Y060	*Lophozia bicrenata*	A Liverwort	G5	S1		SC
NBHEP1Y0M0	*Lophozia longidens*	A Liverwort	G5	S1		SC
NBHEP1Y0X3	*Lophozia ventricosa var. longiflora*	A Liverwort	G5T3T5	S1		SC
NBHEP1Y0X5	*Lophozia ventricosa var. silvicola*	A Liverwort	G5T5	S1		SC
NBHEP20050	*Mannia triandra*	A Liverwort	G3G4	S1		SC
NBMUS4P020	*Micromitrium megalosporum*	A Moss	G4	S2		SC
NBHEP28020	*Moerckia hibernica*	A Liverwort	G4?	S1		SC
NBHEP29010	*Mylia anomala*	A Liverwort	G5	S1		SC
NBANT02020	*Notothylas orbicularis*	A Hornwort	G5	S1S2		SC
NBMUS8D010	*Oxystegus spiralis*	A Moss	G1	S1		SC
NBHEP2F010	*Pallavicinia lyellii*	A Liverwort	G5	S1		SC
NBMUS5E030	*Physcomitrium hookeri*	A Moss	G2G4	S2		SC
NBMUS5E040	*Physcomitrium immersum*	A Moss	G4	S2		SC
NBMUS5K030	*Platydictya minutissima*	A Moss	G3	S3		SC
NBMUS5R060	*Pogonatum urnigerum*	A Moss	G5	S2		SC
NBMUS5S050	*Pohlia carnea*	A Moss	GNR	S2		SC
NBMUS5S0E0	*Pohlia lescuriana*	A Moss	G4?	S2		SC
NBMUS63010	*Pterigynandrum filiforme*	A Moss	G4G5	S1		SC
NBMUS6B010	*Racomitrium aciculare*	A Moss	G5	S1		SC
NBMUS6B070	*Racomitrium heterostichum*	A Moss	G5	S1		SC
NBHEP2Z030	*Riccia beyrichiana*	A Liverwort	G5	S1?		SC
NBHEP2Z080	*Riccia cavernosa*	A Liverwort	G5	S1?		SC
NBHEP2Z0E0	*Riccia frostii*	A Liverwort	G5	S1?		SC
NBHEP2Z0M0	*Riccia sorocarpa*	A Liverwort	G5	S1?		SC
NBHEP2Z0N0	*Riccia sullivantii*	A Liverwort	G4Q	S1?		SC
NBHEP33070	*Scapania carinthiaca*	A Liverwort	G3?	S1		SC
NBHEP330B0	*Scapania cuspiduligera*	A Liverwort	G5	S1		SC
NBHEP330C0	*Scapania degenii*	A Liverwort	G4G5	S1		SC
NBHEP330Y0	*Scapania saxicola*	A Liverwort	G2G4	S1		SC
NBHEP330Z0	*Scapania scandica*	A Liverwort	G5?	S1		SC
NBHEP33160	*Scapania umbrosa*	A Liverwort	G4G5	S1		SC
NBMUS6P010	*Schistostega pennata*	Luminous Moss	G3G4	S1		SC
NBMUS6R010	*Schwetschkeopsis fabronia*	A Moss	G5	S1?		SC
NBMUS6V010	*Scorpidium scorpioides*	A Moss	G4G5	S1		SC

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NBMUS6X010	*Seligeria calcarea*	A Moss	G4?	S1		SC
NBMUS6X030	*Seligeria donniana*	A Moss	G4G5	S1		SC
NBMUS6Z1Q0	*Sphagnum andersonianum*	A Peat Moss	G3?	S2		SC
NBMUS6Z240	*Sphagnum nitidum*	A Peat Moss	GNR	S2		SC
NBMUS6Z0X0	*Sphagnum platyphyllum*	A Peat Moss	G5	S1S2		SC
NBMUS6Z0Z0	*Sphagnum pulchrum*	A Peat Moss	G5	S2		SC
NBMUS71010	*Splachnum ampullaceum*	A Moss	G5	S2		SC
RARE LICHENS						
NLLEC6C010	*Ahtiana aurescens*	Eastern Candlewax Lichen	G3G5	SH		SC
NLTEST9030	*Anaptychia setifera*	Hanging Fringed Lichen	G3G4	S1		SC
NLTEST5270	*Bryoria capillaris*	Gray Horsehair Lichen	G4	S1		SC
NLTEST5380	*Bryoria nadvornikiana*	Spiny Gray Horsehair Lichen	GNR	S1		SC
NLTEST5590	*Cetraria arenaria*	Sand Loving Iceland Lichen	G4	S1		SC
NLTEST7090	*Cladonia arbuscula*	Reindeer Lichen	G5	SH		SC
NLT0008300	*Cladonia cornuta*	Bighorn Cladonia	G3G5	S2		SC
NLT0008370	*Cladonia decorticata*	Naked Cladonia	G4G5	S1		SC
NLT0008550	*Cladonia incrassata*	Powder-foot British Soldiers	G5?	S1		SC
NLT0008610	***Cladonia magyarica***	**Magyar Cup Lichen**	**GNR**	**S2**		**SC**
NLTEST7190	*Cladonia stygia*	Black-footed Reindeer Lichen	G5	S1		SC
NLT0008970	*Cladonia sulphurina*	Greater Sulphur Cup	G5?	**SH**		SC
NLTES10080	*Coccocarpia palmicola*	Salted Shell Lichen	G5	S1		SC
NLT0009350	*Collema polycarpon*	Shaly Jelly Lichen	GNR	SH		SC
NLT0010290	*Dirinaria frostii*	Frosty Medallion Lichen	G3G5	SH		SC
NLTEST7510	*Evernia prunastri*	Oakmoss Lichen	G5	SH		SC
NLT0011020	*Fuscopannaria leucophaea*	Rock Shingle Lichen	G5?	**S1**		SC
NLT0011030	*Fuscopannaria leucosticta*	Rimmed Shingle Lichen	G3G5	S1		SC
NLLEC80070	*Heppia lutosa*	Pale Soil Ruby	G3G5	S1		SC
NLT0012480	*Hypogymnia tubulosa*	Powder-headed Tube Lichen	G5?	S1		SC
NLT0016320	*Lempholemma polyanthes*	Many-fruited Membrane Lichen	GNR	S1		SC
NLT0016590	*Leptogium arsenei*	Wrinkled Jellyskin	GNR	S1		SC
NLLEC98430	*Leptogium teretiusculum*	Curly Jellyskin	G4G5Q	S1		SC
NLT0017950	***Melanelia sorediata***	**Powdered Camouflage Lichen**	**GNR**	**S1**		**SC**
NLT0018010	***Melanohalea trabeculata***	**Trabeculate Brown-shield Lichen**	**GNR**	**S1**		**SC**
NLLEC0T010	*Menegazzia terebrata*	Treeflute	G4?	S1		SC
NLT0019520	*Nephroma bellum*	Naked Kidney Lichen	G3G5	S1		SC
NLLEC5Y040	*Parmotrema stuppeum*	Powder-edged Ruffle Lichen	G4G5	SH		SC
NLT0021120	***Peltigera elisabethae***	**Elizabeth's Felt Lichen**	**G5?**	**S1**		**SC**
NLVER00220	***Peltigera extenuata***	**Felt Lichen**	**GNR**	**S1**		**SC**
NLTEST5130	*Peltigera malacea*	Veinless Pelt	G5	S1		SC
NLTEST5230	*Peltigera scabrosa*	Scabby Pelt	G4	S1		SC
NLTES11740	*Physcia tenella*	Fringed Rosette Lichen	G4	S1		SC
NLTES11760	*Physciella melanchra*	Powdery Cryptic Rosette Lichen	G5	S2		SC
NLT0020510	*Protopannaria pezizoides*	Brown-Gray Moss Shingle	G4G5	SH		SC
NLT0023840	*Pseudevernia consocians*	Common Antler Lichen	G3G5	S1		SC
NLLEC3B040	*Pseudocyphellaria crocata*	Yellow Specklebelly	G4?	S1		SC
NLVER00450	***Punctelia caseana***	**Speckleback Lichen**	**G5**	**S2**		**SC**
NLLEC3S100	*Ramalina farinacea*	Dotted Ramalina	G3G5	**SH**		SC
NLT0025470	*Ramalina unifolia*	One Leaf Ramalina	GNR	S1		SC
NLLEC4G020	*Solorina saccata*	Common Chocolate Chip Lichen	G3G5	SH		SC
NLT0028530	*Sticta beauvoisii*	Fringed Moon Lichen	G3G4	S1		SC
NLLEC4W020	*Teloschistes chrysophthalmus*	Gold-eye Lichen	G4G5	**S1**		SC
NLT0030440	*Usnea ceratina*	Warty Beard Lichen	G4G5	S2		SC
NLT0030520	***Usnea diplotypus***	**Beard Lichen**	**GNR**	**S2?**		**SC**
NLLEC5P420	***Usnea longissima***	**Methuselah's Head Lichen**	**G4**	**S1**		**SC**
NLT0030900	*Usnea strigosa*	Bush Beard Lichen	G5?	SH		SC
NLLEC5P730	*Usnea trichodea*	Bony Beard Lichen	G5?	S2		SC
NATURAL COMMUNITIES						
UPLAND FORESTS						
CTFOR040WI	Boreal Forest		G3?	S2		NA
CTFOR044WI	Central Sands Pine-Oak Forest		G3	S3		NA
CTFOR035WI	Hemlock Relict		G2Q	S2		NA
CTFOR041WI	Mesic Cedar Forest		G3?	S1		NA
CTFOR028WI	Mesic Floodplain Terrace		GNR	S2		NA
CTFOR030WI	Northern Dry Forest		G3?	S3		NA
CTFOR032WI	Northern Dry-mesic Forest		G4	S3		NA

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CTFOR034WI	Northern Mesic Forest		G4	S4		NA
CTFOR033WI	Pine Relict		G4	S2		NA
CTFOR012WI	Southern Dry Forest		G4	S3		NA
CTFOR014WI	Southern Dry-mesic Forest		G4	S3		NA
CTFOR016WI	Southern Mesic Forest		G3?	S3		NA
LOWLAND FORESTS						
CPFOR047WI	Black Spruce Swamp		G5	S3?		NA
CPFOR024WI	Floodplain Forest		G3?	S3		NA
CPFOR025WI	Forested Seep		GNR	S2		NA
CPFOR039WI	Hardwood Swamp		G4	S3		NA
CPFOR038WI	Northern Wet Forest		G4	S4		NA
CPFOR036WI	Northern Wet-mesic Forest		G3?	S3S4		NA
CPFOR022WI	Southern Hardwood Swamp		G4?	S2		NA
CPFOR042WI	**Tamarack (Rich) Swamp**		G3	S3		NA
CPFOR046WI	Tamarack (Poor) Swamp		G4	S3		NA
CPFOR037WI	White Pine-Red Maple Swamp		G3G4	S2		NA
SAVANNAS/WOODLANDS						
CTSAV008WI	Cedar Glade		GNR	S4		NA
CTSAV007WI	Great Lakes Barrens		G2	S1		NA
CTSAV002WI	Oak Barrens		G2?	S2		NA
CTSAV004WI	Oak Opening		G1	S1		NA
CTFOR010WI	Oak Woodland		GNR	S1?		NA
CTSAV006WI	Pine Barrens		G2	S2		NA
SHRUB COMMUNITIES						
CPSHR052WI	Alder Thicket		G4	S4		NA
CPSHR053WI	Bog Relict		G3	S3		NA
CPSHR051WI	Muskeg		G4G5	S4		NA
CPSHR054WI	Open Bog		G5	S4		NA
CPSHR050WI	Shrub-carr		G5	S4		NA
UPLAND HERBACEOUS COMMUNITIES						
CTHER080WI	Bracken Grassland		G3	S2		NA
CTHER070WI	Dry Prairie		G3	S3		NA
CTHER072WI	Dry-mesic Prairie		G3	S2		NA
CTHER074WI	Mesic Prairie		G2	S1		NA
CTHER073WI	Sand Prairie		GNR	S2		NA
LOWLAND HERBACEOUS COMMUNITIES						
CPHER065WI	Boreal Rich Fen		G4G5	S2		NA
CPHER064WI	Calcareous Fen		G3	S3		NA
CPHER061WI	Central Poor Fen		G3G4	S3		NA
CPHER066WI	Coastal Plain Marsh		G2?	S1		NA
CPHER056WI	Emergent Marsh		G4	S4		NA
CPHER057WI	Emergent Marsh - Wild Rice		G3G4	S3		NA
CPHER055WI	Floating-leaved Marsh		G4G5	S4		NA
CPHER054WI	Floating-leaved Marsh - American Lotus		G3G4	S3		NA
CPHER068WI	Interdunal Wetland		G2?	S1		NA
CPHER201WI	Lacustrine Mud Flat		GNR	SU		NA
CPHER063WI	Moist Sandy Meadow		GNR	SU		NA
CPHER060WI	Northern Sedge Meadow		G4	S3		NA
CPHER069WI	Poor Fen		G3G4	S3		NA
CPHER200WI	Riverine Mud Flat		GNR	SU		NA
CPHER067WI	Shore Fen		GNR	S2		NA
CPHER062WI	Southern Sedge Meadow		G4?	S3		NA
CPHER058WI	Submergent Marsh		G5	S4		NA
CPHER059WI	Submergent Marsh - Oligotrophic		GNR	S3		NA
CPHER078WI	Wet Prairie		G3	SU		NA
CPHER076WI	Wet-mesic Prairie		G2	S2		NA
GEOLOGICAL FEATURES/PRIMARY COMMUNITIES						
CTGEO085WI	Algific Talus Slope		G2	S1		NA
CTGEO087WI	Alvar		G3	S1		NA
CTGEO086WI	Bedrock Glade		G2	S3		NA
CTGEO097WI	Bedrock Shore		G3G4	S2		NA
CTGEO096WI	**Cave**		**GNR**	**SU**		**NA**
CTGEO095WI	Clay Seepage Bluff		GNR	S2		NA
CTGEO082WI	Dry Cliff		G4G5	S4		NA
CTGEO083WI	Glaciere Talus		G2G3	S2		NA
CTGEO093WI	Great Lakes Alkaline Rockshore		G3	S2		NA

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CTGEO092WI	Great Lakes Beach		G3	S2		NA
CTGEO094WI	Great Lakes Dune		G3	S2		NA
CTGEO091WI	Inland Beach		G4G5	S3		NA
CTGEO084WI	Moist Cliff		GNR	S4		NA
CTGEO099WI	Talus Forest		G4G5	S1		NA
COMMUNITY COMPLEXES						
CCCOM106WI	Great Lakes Estuary		GNR	S2		NA
CCCOM102WI	Great Lakes Ridge and Swale		G3	S2		NA
CCCOM104WI	Patterned Peatland		GNR	S1		NA
LAKES AND PONDS						
CLEPH390WI	Ephemeral Pond		GNRQ	SU		NA
CLDRA340WI	Lake--Deep, Hard, Drainage		GNR	S3		NA
CLSEE342WI	Lake--Deep, Hard, Seepage		GNR	S2		NA
CLDRA344WI	Lake--Deep, Soft, Drainage		GNR	S1		NA
CLSEE346WI	Lake--Deep, Soft, Seepage		GNR	S3		NA
CLSEE347WI	Lake--Deep, Very Soft, Seepage		GNR	S3		NA
CLBOG360WI	Lake--Hard Bog		GNR	S2		NA
CLMER376WI	Lake--Meromictic		GNR	S1		NA
CLDRA348WI	Lake--Shallow, Hard, Drainage		GNR	SU		NA
CLSEE350WI	Lake--Shallow, Hard, Seepage		GNR	SU		NA
CLDRA352WI	Lake--Shallow, Soft, Drainage		GNR	S3		NA
CLSEE354WI	Lake--Shallow, Soft, Seepage		GNR	S4		NA
CLDRA349WI	Lake--Shallow, Very Hard, Drainage (Marl)		GNR	S2		NA
CLBOG362WI	Lake--Soft Bog		GNR	S4		NA
CLSPR375WI	Lake--Spring		GNR	S3		NA
CLUNI380WI	Lake--Unique		GNR	SU		NA
CLRIV374WI	**Riverine Lake/Pond** (formerly Lake--Oxbow)		GNR	SU		NA
CLSPR370WI	Spring Pond		GNR	S3		NA
SPRINGS AND STREAMS						
CRSPR302WI	Springs and Spring Runs, Hard		GNR	S4		NA
CRSPR304WI	Springs and Spring Runs, Soft		GNR	SU		NA
CRSTR310WI	Stream--Fast, Hard, Cold		GNR	S4		NA
CRSTR312WI	Stream--Fast, Hard, Warm		GNR	SU		NA
CRSTR314WI	Stream--Fast, Soft, Cold		GNR	SU		NA
CRSTR316WI	Stream--Fast, Soft, Warm		GNR	SU		NA
CRSTR320WI	Stream--Slow, Hard, Cold		GNR	SU		NA
CRSTR322WI	Stream--Slow, Hard, Warm		GNR	SU		NA
CRSTR324WI	Stream--Slow, Soft, Cold		GNR	SU		NA
CRSTR326WI	Stream--Slow, Soft, Warm		GNR	SU		NA
MISCELLANEOUS ELEMENTS/SELECTED HABITATS						
OBATCOLONY	Bat Hibernaculum		GNR	S3		SC
OWADINGCA1	Bird Rookery		G5	SU		SC
OHERPHIB11	Herp Hibernaculum		GNR	SU		SC
OCHHIEM000	**Hine's Emerald Critical Habitat Area**		**GNR**	**SNR**		**SC**
OMIGLANDC1	Migratory Bird Concentration Site		G3	SU		SC
OMUSSEL000	Mussel Bed		G3	S3?		SC
OCHPIPL000	**Piping Plover Critical Habitat Area**		**GNR**	**SNR**	**CH**	**SC**
CTMIX088WI	Sand Barrens		GNR	SU		NA
OSURRGRASS	Surrogate Grassland		GNR	SNR		NA
ELEMENTS NOT ACTIVELY BEING TRACKED (DATA ARE BEING COLLECTED THOUGH)						
MAMMALS						
AMACC02010	§ *Lasionycteris noctivagans*	Silver-haired Bat	G5	S2S4		SC/N
AMACC05010	§ *Lasiurus borealis*	Eastern Red Bat	G5	S3		SC/N
AMACC05030	§ *Lasiurus cinereus*	Hoary Bat	G5	S3		SC/N
AMAEB03040	§ ***Lepus townsendii***	**White-tailed Jackrabbit**	**G5**	**SNA**		**SC/H**
AMAJH03010	*Lynx canadensis*	Canada Lynx	G5	**SNA**	LT	SC/P
AMAJF02020	***Mustela nivalis***	**Least Weasel**	**G5**	**SU**		**SC/N**
AMACC01100	***Myotis sodalis***	**Indiana Bat**	**G2**	**SNA**		**SC/FL**
AMAFF03040	*Peromyscus maniculatus*	Deer Mouse	G5	**S4**		SC/N
AMAJH04010	***Puma concolor***	**Cougar**	**G5**	**SNA**		**SC/N**
AMABA01180	*Sorex fumeus*	Smoky Shrew	G5	**SNA**		SC/N
BIRDS						
ABNCA04010	***Aechmophorus occidentalis***	**Western Grebe**	**G5**	**S1?B**		**SC/M**
ABPBXA0020	§ *Ammodramus savannarum*	Grasshopper Sparrow	G5	**S3B**		SC/M

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ABNJB10110	***Anas acuta***	**Northern Pintail**	**G5**	**SNA**		**SC/M**
ABNJB10130	§ *Anas discors*	Blue-winged Teal	G5	**S3S4B**		SC/M
ABNJB10040	§ ***Anas rubripes***	**American Black Duck**	**G5**	**S2S3**		**SC/M**
ABNGA04010	*Ardea herodias*	Great Blue Heron	G5	S4B		SC/M
ABNJB11070	§ *Aythya affinis*	Lesser Scaup	G5	S3N		SC/M
ABNJB11020	§ *Aythya valisineria*	Canvasback	G5	**S2N**		SC/M
ABNGA07010	***Bubulcus ibis***	**Cattle Egret**	**G5**	**SNA**		**SC/M**
ABNNF11170	§ *Calidris alpina*	Dunlin	G5	S4N		SC/M
ABNTA07070	§ *Caprimulgus vociferus*	Whip-poor-will	G5	S3B		SC/M
ABPBJ18080	§ *Catharus fuscescens*	Veery	G5	**S3S4B**		SC/M
ABNKC11010	§ *Circus cyaneus*	Northern Harrier	G5	S3B,S2N		SC/M
ABPBY09020	***Coccothraustes vespertinus***	**Evening Grosbeak**	**G5**	**S2S3B**		**SC/M**
ABNRB02020	§ ***Coccyzus americanus***	**Yellow-billed Cuckoo**	**G5**	**S3B**		**SC/M**
ABNRB02010	§ *Coccyzus erythropthalmus*	Black-billed Cuckoo	G5	**S3S4B**		SC/M
ABPBX03050	§ ***Dendroica caerulescens***	**Black-throated Blue Warbler**	**G5**	**S3B**		**SC/M**
ABPBX03040	***Dendroica tigrina***	**Cape May Warbler**	**G5**	**S3B**		**SC/M**
ABPBXA9010	§ *Dolichonyx oryzivorus*	Bobolink	G5	**S3S4B**		SC/M
ABPAE33010	*Empidonax flaviventris*	Yellow-bellied Flycatcher	G5	**S3S4B**		SC/M
ABPAE33070	§ *Empidonax minimus*	Least Flycatcher	G5	S4B		SC/M
ABPAE33040	§ *Empidonax traillii*	Willow Flycatcher	G5	S4B		SC/M
ABPBXB5010	§ *Euphagus carolinus*	Rusty Blackbird	G4	SNA		SC/M
ABNKD06030	*Falco columbarius*	Merlin	G5	S3B,S2N		SC/M
ABNME14020	***Fulica americana***	**American Coot**	**G5**	**S3S4B**		**SC/M**
ABNME13010	***Gallinula chloropus***	**Common Moorhen**	**G5**	**S3**		**SC/M**
ABNBA01030	*Gavia immer*	Common Loon	G5	S3S4B		SC/M
ABNMK01030	§ ***Grus americana***	**Whooping Crane**	**G1**	**SXB**	**NEP**	**SC/FL**
ABNND01010	***Himantopus mexicanus***	**Black-necked Stilt**	**G5**	**SNA**		**SC/M**
ABPBJ19010	§ *Hylocichla mustelina*	Wood Thrush	G5	S4B		SC/M
ABNNM03210	***Larus marinus***	**Great Black-backed Gull**	**G5**	**SNA**		**SC/M**
ABNNF16010	§ *Limnodromus griseus*	Short-billed Dowitcher	G5	S4N		SC/M
ABNNF08040	§ ***Limosa fedoa***	**Marbled Godwit**	**G5**	**S2S3N**		**SC/M**
ABNNF08020	§ ***Limosa haemastica***	**Hudsonian Godwit**	**G4**	**S2S3N**		**SC/M**
ABPBY05010	§ *Loxia curvirostra*	Red Crossbill	G5	S2?B		SC/M
ABPBY05020	***Loxia leucoptera***	**White-winged Crossbill**	**G5**	**SU**		**SC/M**
ABNYF04040	§ *Melanerpes erythrocephalus*	Red-headed Woodpecker	G5	S3B		SC/M
ABNNF07020	§ ***Numenius phaeopus***	**Whimbrel**	**G5**	**S2S3N**		**SC/M**
ABNJB22010	***Oxyura jamaicensis***	**Ruddy Duck**	**G5**	**S2N,S3B**		**SC/M**
ABNKC01010	§ ***Pandion haliaetus***	**Osprey**	**G5**	**S4B**		**SC/M**
ABPAV01010	*Perisoreus canadensis*	Gray Jay	G5	S3B		SC/M
ABNYF07090	§ ***Picoides arcticus***	**Black-backed Woodpecker**	**G5**	**S3B**		**SC/M**
ABNNB02030	§ ***Pluvialis dominica***	**American Golden Plover**	**G5**	**S3N**		**SC/M**
ABNCA03010	§ *Podiceps auritus*	Horned Grebe	G5	S4N		SC/M
ABPBX95010	§ *Pooecetes gramineus*	Vesper Sparrow	G5	**S3S4B**		SC/M
ABNNF19020	§ *Scolopax minor*	American Woodcock	G5	**S3S4B**		SC/M
ABPBX65010	§ ***Spiza americana***	**Dickcissel**	**G5**	**S3B**		**SC/M**
ABPBX94050	§ *Spizella pusilla*	Field Sparrow	G5	**S3S4B**		SC/M
ABNSB12040	***Strix nebulosa***	**Great Gray Owl**	**G5**	**SNA**		**SC/M**
ABPBXB2020	§ *Sturnella magna*	Eastern Meadowlark	G5	**S3S4B**		SC/M
ABPBK06010	§ *Toxostoma rufum*	Brown Thrasher	G5	**S3S4B**		SC/M
ABNNF01070	§ *Tringa solitaria*	Solitary Sandpiper	G5	S4N		SC/M
ABNNF14010	§ *Tryngites subruficollis*	Buff-breasted Sandpiper	G4	S3N		SC/M
ABPBX01030	§ *Vermivora chrysoptera*	Golden-winged Warbler	G4	**S3S4B**		SC/M
ABPBX01020	§ *Vermivora pinus*	Blue-winged Warbler	G5	S4B		SC/M
ABPBW01230	*Vireo philadelphicus*	Philadelphia Vireo	G5	SUB		SC/M
ABPBX16030	§ ***Wilsonia canadensis***	**Canada Warbler**	**G5**	**S3S4B**		**SC/M**
ABPBX16020	*Wilsonia pusilla*	Wilson's Warbler	G5	SUB		SC/M
AMPHIBIANS						
AAAAD08010	§ ***Hemidactylium scutatum***	**Four-toed Salamander**	**G5**	**S3?**		**SC/H**
AAABH01070	***Lithobates catesbeianus***	**American Bullfrog**	**G5**	**S3S4**		**SC/H**
AAABH01170	***Lithobates pipiens***	**Northern Leopard Frog**	**G5**	**S4?**		**SC/H**
AAABH01190	§ ***Lithobates septentrionalis***	**Mink Frog**	**G5**	**S3**		**SC/H**
AAAAE01040	§ ***Necturus maculosus***	**Mudpuppy**	**G5**	**S3S4**		**SC/H**
REPTILES						
ARADB10014	***Diadophis punctatus edwardsii***	**Northern Ring-necked Snake**	**G5T5**	**S3**		**SC/H**
ARADB17020	***Heterodon platirhinos***	**Eastern Hog-nosed Snake**	**G5**	**S3S4**		**SC/H**
ARACH01050	***Plestiodon fasciatus***	**Common Five-lined Skink**	**G5**	**S3S4**		**SC/H**

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FISHES						
AFCJB05010	**§ *Clinostomus elongatus***	**Redside Dace**	**G3G4**	**S3S4**		**SC/N**
AFCHA01070	**§ *Coregonus kiyi***	**Kiyi**	**G3**	**S3S4**		**SC/H**
AFCNB04060	**§ *Fundulus diaphanus***	**Banded Killifish**	**G5**	**S3S4**		**SC/N**
AFCJB28810	***Notropis rubellus***	**Rosyface Shiner**	**G5**	**S3**		**SC/N**
AFCHA03020	***Prosopium coulterii***	**Pygmy Whitefish**	**G5**	**S3S4**		**SC/N**
MUSSELS AND CLAMS						
IMBIV01020	*Actinonaias ligamentina*	Mucket	G5	S4		SC/P
IMBIV22020	*Lasmigona compressa*	Creek Heelsplitter	G5	S3S4		SC/P
IMBIV26020	*Ligumia recta*	Black Sandshell	G5	S3		SC/P
IMBIV35070	***Pleurobema sintoxia***	**Round Pigtoe**	**G4G5**	**S3**		**SC/P**
IMBIV54040	*Pyganodon lacustris*	Lake Floater	GU	SU		SC/P
IMBIV55010	*Utterbackia imbecillis*	Paper Pondshell	G5	S4		SC/P
BUTTERFLIES AND MOTHS						
IILEPJ7020	***Boloria eunomia***	**Bog Fritillary**	**G5**	**S3S4**		**SC/N**
IILEPJ7100	**§ *Boloria freija***	**Freija Fritillary**	**G5**	**S3S4**		**SC/N**
IILEPJ7050	**§ *Boloria frigga***	**Frigga Fritillary**	**G5**	**S3S4**		**SC/N**
IILEPE2230	***Callophrys henrici***	**Henry's Elfin**	**G5**	**S4**		**SC/N**
IILEY89080	**§ *Catocala badia***	**Bay Underwing Moth**	**G4**	**S3S4**		**SC/N**
IILEPF9020	***Cupido* (=*Everes*) *amyntula***	Western Tailed Blue	G5	SU		SC/N
IILEPN8060	**§ *Erebia discoidalis***	**Red-disked Alpine**	**G5**	**S3S4**		**SC/N**
IILEP37150	**§ *Erynnis baptisiae***	**Wild Indigo Dusky Wing**	**G5**	**S3S4**		**SC/N**
IILEU2F150	**§ *Euchlaena milnei***	**A Looper Moth**	**G2G4**	**SU**		**SC/N**
IILEPK4060	***Euphydryas phaeton***	**Baltimore Checkerspot**	**G4**	**S3S4**		**SC/N**
IILEP77090	***Euphyes bimacula***	**Two-spotted Skipper**	**G4**	**S3S4**		**SC/N**
IILEY9A010	**§ *Exyra fax***	**Pitcher Plant Moth**	**G4**	**S3S4**		**SC/N**
IILEYJE050	**§ *Faronta rubripennis***	**Pink-streak**	**G3G4**	**S3?**		**SC/N**
IILEPB9010	***Feniseca tarquinius***	**Harvester**	**G4**	**S4**		**SC/N**
IILEYMM220	***Heliothis borealis***	**Boreal Gem**	**G4**	**S3S4**		**SC/N**
IILEP65030	***Hesperia comma***	**Laurentian Skipper**	**G5**	**S4**		**SC/N**
IILEP65060	***Hesperia leonardus***	**Leonard's Skipper**	**G4**	**S4**		**SC/N**
IILEYFE470	***Lithophane franclemonti***	**Franclemont's Lithophane**	**G2G4**	**S3**		**SC/N**
IILEPG5022	***Lycaeides melissa melissa***	**Melissa Blue**	**G5T5**	**SU**		**SC/N**
IILEPC1120	***Lycaena dorcas***	**Dorcas Copper**	**G5**	**S3S4**		**SC/N**
IILEPC1130	***Lycaena helloides***	**Purplish Copper**	**G5**	**S3S4**		**SC/N**
IILEY45030	**§ *Macrochilo bivittata***	**An Owlet Moth**	**G3G4**	**S3S4**		**SC/N**
IILEPN3020	*Neonympha mitchellii*	Mitchell's Satyr	G1G2	SH	LE	SC/FL
IILEPK3040	***Phyciodes batesii***	**Tawny Crescent Spot**	**G4**	**S3S4**		**SC/N**
IILEP73010	***Poanes massasoit***	**Mulberry Wing**	**G4**	**S4**		**SC/N**
IILEP73070	***Poanes viator***	**Broad-winged Skipper**	**G5**	**S4**		**SC/N**
IILEP66070	***Polites origenes***	**Cross Line Skipper**	**G4G5**	**S3S4**		**SC/N**
IILEP68010	**§ *Pompeius verna***	**Little Glassy Wing**	**G5**	**S3**		**SC/N**
IILEY85030	**§ *Ptichodis bistrigata***	**A Noctuid Moth**	**G3**	**S1S3**		**SC/N**
IILEPD4080	**§ *Satyrium caryaevorum***	**Hickory Hairstreak**	**G4**	**S4**		**SC/N**
IILEPN0010	***Satyrodes eurydice***	**Eyed Brown**	**G4**	**S4**		**SC/N**
DRAGONFLIES AND DAMSELFLIES						
IIODO14060	***Aeshna eremita***	**Lake Darner**	**G5**	**S3**		**SC/N**
IIODO77010	**§ *Amphiagrion saucium***	**Eastern Red Damsel**	**G5**	**S3S4**		**SC/N**
IIODO15030	**§ *Anax longipes***	**Comet Darner**	**G5**	**SNA**		**SC/N**
IIODO81060	**§ *Arigomphus submedianus***	**Jade Clubtail**	**G5**	**S3S4**		**SC/N**
IIODO78010	***Chromagrion conditum***	**Aurora Damselfly**	**G5**	**S3S4**		**SC/N**
IIODO71280	**§ *Enallagma anna***	**River Bluet**	**G5**	**S3S4**		**SC/N**
IIODO71110	***Enallagma aspersum***	**Azure Bluet**	**G5**	**S2S3**		**SC/N**
IIODO71070	**§ *Enallagma vernale***	**Gloyd's Bluet**	**G4**	**S1Q**		**SC/N**
IIODO20020	**§ *Gomphaeschna furcillata***	**Harlequin Darner**	**G5**	**SU**		**SC/N**
IIODO08110	***Gomphurus externus***	**Plains Clubtail**	**G5**	**S3S4**		**SC/N**
IIODO08210	***Gomphurus ventricosus***	**Skillet Clubtail**	**G3**	**S3S4**		**SC/N**
IIODO08290	**§ *Gomphus exilis***	**Lancet Clubtail**	**G5**	**S4**		**SC/N**
IIODO08460	***Gomphus viridifrons***	**Green-faced Clubtail**	**G3G4**	**S4**		**SC/N**
IIODO72140	**§ *Ischnura hastata***	**Citrine Forktail**	**G5**	**SU**		**SC/N**
IIODO72030	**§ *Ischnura posita***	**Fragile Forktail**	**G5**	**S2S3**		**SC/N**
IIODO67060	***Lestes eurinus***	**Amber-winged Spreadwing**	**G4**	**S3S4**		**SC/N**
IIODO50010	**§ *Nannothemis bella***	**Elfin Skimmer**	**G4**	**S3**		**SC/N**
IIODO22010	***Nasiaeschna pentacantha***	**Cyrano Darner**	**G5**	**S3S4**		**SC/N**
IIODO31030	***Neurocordulia molesta***	**Smoky Shadowfly**	**G4**	**S3**		**SC/N**

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
IIODO14100	*Rhionaeschna multicolor*	Blue-eyed Darner	G5	SU		SC/N
IIODO32050	*Somatochlora elongata*	Ski-tailed Emerald	G5	S2S3		SC/N
IIODO32090	*Somatochlora franklini*	Delicate Emerald	G5	**S3**		SC/N
IIODO80010	***Stylurus amnicola***	**Riverine Clubtail**	**G4**	**S3S4**		**SC/N**
IIODO80050	***Stylurus notatus***	**Elusive Clubtail**	**G3**	**S3S4**		**SC/N**
IIODO80070	***Stylurus plagiatus***	**Russet-tipped Clubtail**	**G5**	**S3S4**		**SC/N**
IIODO61050	***Sympetrum danae***	**Black Meadowhawk**	**G5**	**S3**		**SC/N**
IIODO64040	§ ***Tramea carolina***	**Violet-masked Glider**	**G5**	**SU**		**SC/N**
IIODO64060	***Tramea onusta***	**Red-mantled Glider**	**G5**	**S3**		**SC/N**
IIODO34010	***Williamsonia fletcheri***	**Ebony Bog Haunter**	**G4**	**S3S4**		**SC/N**
CRUSTACEANS						
ICCOP14010	§ ***Aglaodiaptomus leptopus***	**A Copepod**	**GNR**	**SNR**		**SC/N**
ICMAL06040	*Crangonyx gracilis*	A Side-swimmer	G4	SU		SC/N
ICMAL06080	§ ***Crangonyx minor***	**Small Stream Crangonyctid**	**G5**	**SNR**		**SC/N**
ICMAL06110	§ *Crangonyx richmondensis*	A Side-swimmer	G5	S3		SC/N
ICBRA19020	***Cyzicus gynecia***	**Feminine Clam Shrimp**	**G2G3Q**	**SU**		**SC/N**
ICCOP14020	§ ***Diaptomus stagnalis***	**A Copepod**	**GNR**	**SNR**		**SC/N**
ICBRA01030	§ *Eubranchipus bundyi*	Knobbedlip Fairy Shrimp	G5	SU		SC/N
ICBRA01090	§ *Eubranchipus ornatus*	Ornate Fairy Shrimp	G3	SU		SC/N
ICBRA01050	§ *Eubranchipus serratus*	Ethologist Fairy Shrimp	G5	SU		SC/N
ICCOP13010	§ ***Limnocalanus macrurus***	**A Common Copepod**	**G5**	**SNR**		**SC/N**
ICMAL03070	§ ***Lirceus lineatus***	**An Aquatic Sow Bug**	**G5**	**SNR**		**SC/N**
ICBRA08010	§ ***Lynceus brachyurus***	**Holarctic Clam Shrimp**	**G5**	**S1S3**		**SC/N**
ICCOP15010	§ ***Onychodiaptomus birgei***	**A Copepod**	**GNR**	**SNR**		**SC/N**
ICMAL11170	*Orconectes propinquus*	Northern Clearwater Crayfish	G5	SU		SC/N
ICMAL18020	§ ***Palaemonetes kadiakensis***	**Mississippi Grass Shrimp**	**G5**	**S3S4**		**SC/N**
ICMAL14230	§ *Procambarus acutus*	White River Crayfish	G5	SNR		SC/N
BEETLES						
IICOLT7020	§ ***Acilius mediatus***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOL52050	§ ***Agabus bicolor***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOL52020	§ *Agabus canadensis*	A Predaceous Diving Beetle	GNR	SU		SC/N
IICOL52060	§ *Agabus confinis*	A Predaceous Diving Beetle	GNR	**S3**		SC/N
IICOL52120	§ ***Agabus disintegratus***	**A Predaceous Diving Beetle**	**GNR**	**S4**		**SC/N**
IICOLM1020	§ ***Berosus aculeatus***	**A Water Scavenger Beetle**	**GNR**	**S4**		**SC/N**
IICOLM1030	§ ***Berosus infuscatus***	**A Water Scavenger Beetle**	**GNR**	**SU**		**SC/N**
IICOLM1040	§ ***Berosus pantherinus***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOLM1080	§ ***Berosus stylifer***	**A Water Scavenger Beetle**	**GNR**	**S3**		**SC/N**
IICOLP6010	§ ***Celina hubbelli***	**A Predaceous Diving Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL02300	§ ***Cicindela longilabris***	**A Tiger Beetle**	**G5**	**S3**		**SC/N**
IICOL02231	§ ***Cicindela patruela huberi***	**A Tiger Beetle**	**G3T3**	**S3S4**		**SC/N**
IICOL02280	§ ***Cicindela purpurea***	**A Tiger Beetle**	**G5**	**S3S4**		**SC/N**
IICOL02320	*Cicindela splendida*	A Tiger Beetle	G5	**S3**		SC/N
IICOL85020	§ *Copelatus glyphicus*	A Predaceous Diving Beetle	GNR	**S3**		SC/N
IICOLQ1010	§ *Crenitis digestus*	A Water Scavenger Beetle	GNR	**S3**		SC/N
IICOL5U020	§ ***Cymbiodyta acuminata***	**A Water Scavenger Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5U030	§ *Cymbiodyta blanchardi*	A Water Scavenger Beetle	GNR	**S2S3**		SC/N
IICOL5U040	§ *Cymbiodyta chamberlaini*	A Water Scavenger Beetle	GNR	**S3**		SC/N
IICOL5U050	***Cymbiodyta minima***	**A Water Scavenger Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5U060	§ *Cymbiodyta semistriatus*	A Water Scavenger Beetle	GNR	**S2S3**		SC/N
IICOL5A050	§ *Dubiraphia bivittata*	A Dubiraphian Riffle Beetle	GNR	S3S4		SC/N
IICOL76020	§ ***Dytiscus carolinus***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL76040	§ ***Dytiscus dauricus***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOLYD010	§ ***Ectopria sp. 2***	**A Water Penny Beetle**	**GNR**	**SU**		**SC/N**
IICOLM2020	§ ***Enochrus consortus***	**A Water Scavenger Beetle**	**GNR**	**S3S4**		**SC/N**
IICOLM2060	§ ***Enochrus diffusus***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOLM2010	§ ***Enochrus hamiltoni***	**A Water Scavenger Beetle**	**GNR**	**S5**		**SC/N**
IICOLM2030	§ ***Enochrus sayi***	**A Water Scavenger Beetle**	**GNR**	**S3S4**		**SC/N**
IICOLP9010	§ *Graphoderus manitobensis*	A Predaceous Diving Beetle	GNR	S3		SC/N
IICOLS9100	§ ***Gyrinus confinis***	**A Whirligig Beetle**	**GNR**	**S4**		**SC/N**
IICOLS9130	§ ***Gyrinus gehringi***	**A Whirligig Beetle**	**GNR**	**SU**		**SC/N**
IICOLS9030	§ ***Gyrinus parcus***	**A Whirligig Beetle**	**GNR**	**SU**		**SC/N**
IICOLS9190	§ ***Gyrinus pectoralis***	**A Whirligig Beetle**	**GNR**	**S3**		**SC/N**
IICOLS9230	§ ***Gyrinus sayi***	**A Whirligig Beetle**	**GNR**	**S3**		**SC/N**
IICOL5N020	§ *Haliplus fasciatus*	A Crawling Water Beetle	GNR	SU		SC/N
IICOL5N090	§ ***Haliplus fulvus***	**A Crawling Water Beetle**	**GNR**	**S3**		**SC/N**

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IICOL5N010	§ ***Haliplus nitens***	**Disjunct Crawling Water Beetle**	**GH**	**SH**		**SC/N**
IICOL5N060	§ ***Haliplus pantherinus***	**A Crawling Water Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5N030	§ *Haliplus tortilipenis*	A Crawling Water Beetle	GNR	SU		SC/N
IICOLG7010	§ *Helocombus bifidus*	A Water Scavenger Beetle	GNR	S3S4		SC/N
IICOLM3040	§ ***Helophorus oblongus***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOLM4020	§ ***Hydrobius melaenum***	**A Water Scavenger Beetle**	**GNR**	**S4**		**SC/N**
IICOL5V030	§ *Hydrochara spangleri*	A Water Scavenger Beetle	GNR	**S3S4**		SC/N
IICOL6Z060	§ ***Hydrochus brevitarsis***	**A Water Scavenger Beetle**	**GNR**	**SU**		**SC/N**
IICOL6Z070	§ ***Hydrochus currani***	**A Water Scavenger Beetle**	**GNR**	**S4**		**SC/N**
IICOL6Z080	§ ***Hydrochus granulatus***	**A Water Scavenger Beetle**	**GNR**	**S2S3**		**SC/N**
IICOL6Z020	§ ***Hydrochus rufipes***	**A Water Scavenger Beetle**	**GNR**	**S3**		**SC/N**
IICOL6Z030	§ ***Hydrochus scabratus***	**A Water Scavenger Beetle**	**GNR**	**SU**		**SC/N**
IICOL6Z040	§ ***Hydrochus setosus***	**A Water Scavenger Beetle**	**GNR**	**SU**		**SC/N**
IICOL6Z050	§ ***Hydrochus subcupreus***	**A Water Scavenger Beetle**	**GNR**	**S4**		**SC/N**
IICOL55180	§ ***Hydrocolus*** (*=Hydroporus*) *stagnalis*	Hydroporus Diving Beetle	GNR	**S3**		SC/N
IICOL55230	§ ***Hydroporus columbianus***	**A Predaceous Diving Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL55150	§ ***Hydroporus dichrous***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOL55220	§ ***Hydroporus obscurus***	**A Predaceous Diving Beetle**	**GNR**	**S4**		**SC/N**
IICOL55260	§ ***Hydroporus tartaricus***	**A Predaceous Diving Beetle**	**GNRQ**	**SU**		**SC/N**
IICOL55130	§ ***Hydroporus vittatus***	**A Predaceous Diving Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL38080	§ ***Hygrotus acaroides***	**A Predaceous Diving Beetle**	**GNR**	**S4S5**		**SC/N**
IICOL38120	§ ***Hygrotus patruelis***	**A Predaceous Diving Beetle**	**GNR**	**S4**		**SC/N**
IICOLQ0030	§ *Ilybius ignarus*	A Predaceous Diving Beetle	GNR	**S3S4**		SC/N
IICOL79020	§ *Ilybius incarinatus*	A Predaceous Diving Beetle	GNR	S3S4		SC/N
IICOLQ0080	§ ***Ilybius picipes***	**A Predaceous Diving Beetle**	**GNR**	**S4S5**		**SC/N**
IICOLQ0040	§ *Ilybius pleuriticus*	A Predaceous Diving Beetle	GNR	**S3**		SC/N
IICOLP5040	§ ***Laccobius minutoides***	**A Water Scavenger Beetle**	**GNR**	**SU**		**SC/N**
IICOLP5030	§ *Laccobius truncatipennis*	A Water Scavenger Beetle	GNR	SU		SC/N
IICOLYH030	§ ***Laccornis deltoides***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOLYH010	§ ***Laccornis latens***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOL6T020	§ ***Liodessus flavicollis***	**A Predaceous Diving Beetle**	**GNR**	**S4**		**SC/N**
IICOLF2010	*Listronotus echinodori*	A Weevil	GNR	SU		SC/N
IICOLF1010	*Lixellus hubbardi*	A Weevil	GNR	SU		SC/N
IICOLD4010	*Longitarsus subrufus*	A Chrysomelid Beetle	GNR	S4		SC/N
IICOLYG010	§ ***Lutrochus laticeps***	**A Minute Marsh-loving Beetle**	**GNR**	**SNR**		**SC/N**
IICOL80020	§ ***Matus bicarinatus***	**A Predaceous Diving Beetle**	**GNR**	**S3S4**		**SC/N**
IICOLD5020	§ ***Microcylloepus pusillus***	**An Elmid Beetle**	**GNR**	**S3**		**SC/N**
IICOLYB010	§ ***Nebrioporus rotundatus***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOLYJ020	§ ***Neoporus superiorus***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOLYJ010	§ ***Neoporus tennetum***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOLQ0070	§ ***Neoscutopterus angustus***	**A Predaceous Diving Beetle**	**GNR**	**S2S3**		**SC/N**
IICOLQ0060	§ ***Neoscutopterus hornii***	**A Predaceous Diving Beetle**	**GNR**	**S3S4**		**SC/N**
IICOLYK010	*Ophraella communa*	A Leaf Beetle	GNR	SU		SC/N
IICOLYE010	***Pachybrachis trinotatus***	**A Leaf Beetle**	**GNR**	**SU**		**SC/N**
IICOL6P020	*Platypsyllus castoris*	Beaver Beetle	GNR	SU		SC/N
IICOL82030	§ ***Rhantus gutticollis***	**A Predaceous Diving Beetle**	**GNR**	**S3**		**SC/N**
IICOL82020	§ *Rhantus sinuatus*	A Predaceous Diving Beetle	GNR	**S4**		SC/N
IICOL71010	§ *Sperchopsis tessellatus*	A Water Scavenger Beetle	GNR	**S3S4**		SC/N
IICOL5F090	§ ***Stenelmis bicarinata***	**A Riffle Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5F160	§ ***Stenelmis cheryl***	**A Riffle Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5F170	§ ***Stenelmis mera***	**A Riffle Beetle**	**GNR**	**S3S4**		**SC/N**
IICOL5F210	§ ***Stenelmis sandersoni***	**A Riffle Beetle**	**GNR**	**S3S4**		**SC/N**
IICOLYF010	§ ***Suphisellus puncticollis***	**A Burrowing Water Beetle**	**GNR**	**SNR**		**SC/N**
IICOLYC010	§ ***Thermonetus basillaris***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOLYC020	§ ***Thermonetus ornaticollis***	**A Predaceous Diving Beetle**	**GNR**	**SNR**		**SC/N**
IICOLQ2010	§ *Tropisternus ellipticus*	A Water Scavenger Beetle	GNR	**S3**		SC/N
IICOL75010	***Xyloryctes jamaicensis***	**Rhinoceros Beetle**	**GNR**	**SU**		**SC/N**
MAYFLIES						
IIEPH61010	§ ***Arthroplea bipunctata***	**A Flat-headed Mayfly**	**G5**	**S3S4**		**SC/N**
IIEPH05010	§ *Baetisca obesa*	An Armored Mayfly	G5	S3S4		SC/N
IIEPH17090	§ ***Caenis diminuta***	**A Small Square-gilled Mayfly**	**G5**	**SU**		**SC/N**
IIEPH17040	§ ***Caenis punctata***	**A Small Square-gilled Mayfly**	**G5**	**S2S4**		**SC/N**
IIEPH17110	§ ***Caenis tardata***	**A Small Square-gilled Mayfly**	**G4**	**SU**		**SC/N**
IIEPH17010	§ ***Caenis youngi***	**A Small Square-gilled Mayfly**	**G4**	**S3S4**		**SC/N**
IIEPH62010	§ ***Callibaetis pallidus***	**A Mayfly**	**G5**	**SU**		**SC/N**
IIEPH62030	§ ***Callibaetis skokianus***	**A Mayfly**	**G4**	**SU**		**SC/N**

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IIEPH34070	§ *Centroptilum album*	A Small Minnow Mayfly	G5	SU		SC/N
IIEPH34120	§ ***Centroptilum conturbatum***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH34040	§ ***Centroptilum triangulifer***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH34050	§ ***Centroptilum victoriae***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH63010	§ ***Diphetor hageni***	**Hagen's Small Minnow Mayfly**	**G5**	**S3S4**		**SC/N**
IIEPH11120	§ ***Ephemerella excrucians***	**A Spiny Crawler Mayfly**	**G5**	**S5**		**SC/N**
IIEPH38050	§ ***Heptagenia pulla***	**A Flat-headed Mayfly**	**G5**	**S3**		**SC/N**
IIEPH37010	§ ***Hexagenia atrocaudata***	**A Common Burrowing Mayfly**	**G5**	**S3S4**		**SC/N**
IIEPH71040	§ ***Leucrocuta maculipennis***	**A Flat-headed Mayfly**	**G5**	**SU**		**SC/N**
IIEPH40180	***Maccaffertium luteum***	**A Flat-headed Mayfly**	**G5**	**SU**		**SC/N**
IIEPH73020	§ ***Nixe inconspicua***	**A Flat-headed Mayfly**	**G5**	**SU**		**SC/N**
IIEPH78030	§ ***Plauditus cingulatus***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH74060	§ ***Procloeon bellum***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH74140	§ ***Procloeon pennulatum***	**A Mayfly**	**G5**	**SU**		**SC/N**
IIEPH74020	§ ***Procloeon rubropictum***	**A Mayfly**	**G5**	**SU**		**SC/N**
IIEPH74030	§ ***Procloeon rufostrigatum***	**A Mayfly**	**G5**	**S3S4**		**SC/N**
IIEPH74160	§ ***Procloeon simplex***	**A Mayfly**	**G5**	**SU**		**SC/N**
IIEPH74320	§ *Procloeon* ***viridoculare*** (=*P. irrubrum*)	A Mayfly	G5	S3		SC/N
IIEPH04020	§ ***Pseudiron centralis***	**A Flat-headed Mayfly**	**G5**	**S3**		**SC/N**
IIEPH82020	§ *Pseudocentroptiloides usa*	A Baetid Mayfly	G4	SU		SC/N
IIEPH48090	§ ***Pseudocloeon dardanum***	**A Small Minnow Mayfly**	**G5**	**SU**		**SC/N**
IIEPH48050	§ ***Pseudocloeon longipalpus***	**A Small Minnow Mayfly**	**G4**	**S3**		**SC/N**
IIEPH39040	§ ***Rhithrogena impersonata***	**A Flat-headed Mayfly**	**G5**	**S3S4**		**SC/N**
IIEPH39140	§ ***Rhithrogena jejuna***	**A Flat-headed Mayfly**	**G5**	**S3**		**SC/N**
IIEPH38100	***Rhithrogena manifesta***	**A Flat-headed Mayfly**	**G5**	**S2S4**		**SC/N**
IIEPH10040	§ ***Serratella serrata***	**A Spiny Crawler Mayfly**	**G5**	**S3**		**SC/N**
IIEPH09020	***Susperatus*** (=*Brachycercus*) *prudens*	A Small Square-gilled Mayfly	G4	S2S4		SC/N
LEAFHOPPERS AND TRUE BUGS						
IIHEMB7010	§ ***Buenoa limnocastoris***	**A Backswimmer**	**GNR**	**S3**		**SC/N**
IIHEMB7020	§ ***Buenoa macrotibialis***	**A Backswimmer**	**GNR**	**S3**		**SC/N**
IIHEM29010	§ *Cenocorixa dakotensis*	A Water Boatman	GNR	SU		SC/N
IIHEM29030	§ ***Cenocorixa utahensis***	**A Water Boatman**	**GNR**	**SU**		**SC/N**
IIHEMB3010	§ ***Corisella edulis***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEMB4010	§ ***Cymatia americana***	**A Water Boatman**	**GNR**	**S2S3**		**SC/N**
IIHEMC0020	§ ***Gerris marginatus***	**A Water Strider**	**GNR**	**S4**		**SC/N**
IIHEM88020	§ ***Hebrus burmeisteri***	**A Velvet Water Bug**	**GNR**	**S3**		**SC/N**
IIHEM45020	§ ***Hesperocorixa interrupta***	**The Interrupted Water Boatman**	**GNR**	**SH**		**SC/N**
IIHEM45030	§ ***Hesperocorixa laevigata***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM45040	§ ***Hesperocorixa lobata***	**A Water Boatman**	**GNR**	**S3S4**		**SC/N**
IIHEM45050	§ ***Hesperocorixa lucida***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM45060	§ ***Hesperocorixa obliqua***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM45010	§ *Hesperocorixa semilucida*	A Water Boatman	GNR	S3		SC/N
IIHEM33020	§ ***Hydrometra martini***	**A Water Measurer**	**G5**	**S4**		**SC/N**
IIHEMB2010	§ ***Lethocerus griseus***	**A Giant Water Bug**	**GNR**	**SU**		**SC/N**
IIHEM30020	§ *Microvelia fontinalis*	A Broad-shouldered Water Strider	GNR	**S3**		SC/N
IIHEM80010	§ *Nepa apiculata*	A Water Scorpion	GNR	**S3S4**		SC/N
IIHOM36010	§ *Paraphilaenus parallelus*	A Spittle Bug	GNR	S2S4		SC/N
IIHEM18020	§ *Pelocoris femorata*	A Creeping Water Bug	GNR	S3S4		SC/N
IIHEM44010	§ ***Ranatra kirkaldyi***	**A Water Scorpion**	**GNR**	**S3**		**SC/N**
IIHEM44040	§ ***Ranatra nigra***	**A Water Scorpion**	**GNR**	**S3S4**		**SC/N**
IIHEM04110	§ ***Sigara dolabra***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM04090	§ ***Sigara macropala***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM04100	§ ***Sigara transfigurata***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM04050	§ ***Sigara variabilis***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
IIHEM32010	§ *Trepobates knighti*	A Water Strider	GNR	SU		SC/N
IIHEM32020	§ ***Trepobates pictus***	**A Water Strider**	**GNR**	**S3**		**SC/N**
IIHEMB6010	§ ***Trichocorixa kanza***	**A Water Boatman**	**GNR**	**S3**		**SC/N**
GRASSHOPPERS AND ALLIES						
IIORT49010	§ ***Arphia xanthoptera***	**Yellow-winged Grasshopper**	**G5**	**S3**		**SC/N**
IIORT51010	§ ***Booneacris glacialis***	**Wingless Mountain Grasshopper**	**G5**	**S3**		**SC/N**
IIORT0A100	§ ***Melanoplus benni***	**A Spur-throat Grasshopper**	**GNR**	**S3**		**SC/N**
IIORT01110	*Melanoplus borealis*	Northern Spur-throat Grasshopper	G5	**S3S4**		SC/N
IIORT01070	***Melanoplus dawsoni***	**Dawson's Spur-throat Grasshopper**	**G5**	**S3?**		**SC/N**
IIORT01190	***Melanoplus islandicus***	**Forest Locust**	**G5**	**S3?**		**SC/N**
IIORT99010	§ ***Phoetaliotes nebrascensis***	**Large-headed Grasshopper**	**G5**	**S3?**		**SC/N**

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
IIORT39010	§ ***Psinidia fenestralis***	**Sand Locust**	**G5**	**S3?**		**SC/N**
IIORT13050	§ *Spharagemon marmorata*	Northern Marbled Locust	G5	S3		SC/N
IIORT56020	§ *Stethophyma gracile*	Northern Sedge Locust	G5	**S3**		SC/N
IIORT56030	§ *Stethophyma lineatum*	Striped Sedge Grasshopper	G5	S3		SC/N
STONEFLIES						
IIPLE1Y030	*Agnetina flavescens*	A Common Stonefly	G5	**S3**		SC/N
IIPLE01060	§ ***Allocapnia frisoni***	**Evansville Snowfly**	**G4**	**SU**		**SC/N**
IIPLE01110	***Allocapnia illinoensis***	**Illinois Snowfly**	**G3**	**SU**		**SC/N**
IIPLE0H040	§ ***Amphinemura linda***	**Lovely Forestfly**	**G4**	**S3S4**		**SC/N**
IIPLE23010	§ ***Clioperla clio***	**Clio Stripetail**	**G5**	**S3S4**		**SC/N**
IIPLE24060	***Isoperla bilineata***	**A Perlodid Stonefly**	**G5**	**S3S4**		**SC/N**
IIPLE24320	***Isoperla marlynia***	**A Perlodid Stonefly**	**G5**	**S3**		**SC/N**
IIPLE24470	***Isoperla richardsoni***	**A Perlodid Stonefly**	**G4**	**S3S4**		**SC/N**
IIPLE0B100	§ ***Leuctra ferruginea***	**Eastern Needlefly**	**G5**	**SU**		**SC/N**
IIPLE08020	§ ***Paracapnia opis***	**Northeastern Snowfly**	**G5**	**SU**		**SC/N**
IIPLE0S060	§ ***Soyedina vallicularia***	**A Nemourid Broad-backed Stonefly**	**G5**	**S1S3**		**SC/N**
CADDISFLIES						
IITRI66680	§ ***Agarodes distinctus***	**A Caddisfly**	**G5**	**S3S4**		**SC/N**
IITRI63680	***Agraylea costello***	**A Caddisfly**	**G3**	**SU**		**SC/N**
IITRI15030	§ *Asynarchus rossi*	A Northern Casemaker Caddisfly	G4G5	SU		SC/N
IITRIA7010	§ ***Hagenella canadensis***	**A Giant Casemaker Caddisfly**	**G5**	**SU**		**SC/N**
IITRI25280	§ ***Hydropsyche arinale***	**A Caddisfly**	**G4G5**	**S3S4**		**SC/N**
IITRI25290	§ *Hydropsyche bidens*	A Common Netspinner Caddisfly	G5	**S3**		SC/N
IITRI25090	§ ***Hydropsyche cuanis***	**A Caddisfly**	**G5**	**S3S4**		**SC/N**
IITRI63590	§ ***Hydropsyche leonardi***	**A Caddisfly**	**G5**	**S3**		**SC/N**
IITRI25150	§ ***Hydropsyche phalerata***	**A Caddisfly**	**G5**	**S4**		**SC/N**
IITRI40360	§ *Hydroptila valhalla*	A Micro Caddisfly	G4	SU		SC/N
IITRI40370	§ *Hydroptila virgata*	A Micro Caddisfly	G5	SU		SC/N
IITRI64110	§ ***Lepidostoma costale***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI01090	§ ***Lepidostoma griseum***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI64190	§ ***Lepidostoma prominens***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI65090	§ ***Limnephilus janus***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI65190	§ ***Limnephilus parvulus***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI65210	§ ***Limnephilus perpusillus***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI65330	§ ***Limnephilus sericeus***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI63850	***Neotrichia falca***	**A Caddisfly**	**G3G4**	**SU**		**SC/N**
IITRI42110	§ *Oxyethira anabola*	A Micro Caddisfly	G4G5	SU		SC/N
IITRI64020	***Oxyethira rossi***	**A Caddisfly**	**G3G4**	**SU**		**SC/N**
IITRI42200	§ ***Oxyethira serrata***	**A Caddisfly**	**G5**	**SU**		**SC/N**
IITRI05230	§ ***Polycentropus glacialis***	**A Caddisfly**	**G3G4**	**SU**		**SC/N**
IITRI05140	§ *Polycentropus weedi*	A Caddisfly	G5	SU		SC/N
IITRI19160	§ ***Rhyacophila vibox***	**A Rhyacophilan Caddisfly**	**G5**	**S4**		**SC/N**
SPIDERS						
ILARA81160	§ ***Araneus groenlandicola***	**An Orb-web Spider**	**GNR**	**SNR**		**SC/N**
ILARAC0010	§ ***Marpissa grata***	**A Jumping Spider**	**GNR**	**SNR**		**SC/N**
ILARA05020	§ ***Phidippus apacheanus***	**A Jumping Spider**	**GNR**	**SNR**		**SC/N**
ILARA93010	§ ***Sassacus papenhoei***	**A Jumping Spider**	**GNR**	**SNR**		**SC/N**
ILARA08040	§ ***Sphodros niger***	**A Purse-web Spider**	**G4G5**	**SNR**		**SC/N**
FLIES						
IIDIP22020	§ ***Blepharicera tenuipes***	**A Net-winged Midge**	**GNR**	**S2S3**		**SC/N**
IIDIP7A010	§ ***Parochlus kiefferi***	**A Midge**	**GNR**	**SU**		**SC/N**
IIDIP7C010	§ ***Phalacrocera replicata***	**A Crane Fly**	**GNR**	**S2S3**		**SC/N**
IIDIP7C020	§ ***Phalacrocera tipulina***	**A Crane Fly**	**GNR**	**S2S3**		**SC/N**
AQUATIC AND TERRESTRIAL SNAILS						
IMGASK9010	***Acella haldemani***	**Spindle Lymnaea**	**G3**	**SU**		**SC/N**
IMGASL0010	***Bulimnaea megasoma***	**Mammoth Lymnaea**	**G4G5**	**SU**		**SC/N**
IMGAS66160	§ ***Catinella exile***	**Pleistocene Catinella**	**G2**	**SU**		**SC/N**
IMGAS66120	§ ***Catinella gelida***	**A Land Snail**	**G1**	**SU**		**SC/N**
IMGAS11020	***Cochlicopa lubricella***	**Thin Pillar**	**G5**	**S3?**		**SC/N**
IMGAS11040	***Cochlicopa nitens***	**Robust Pillar**	**G4**	**SU**		**SC/N**
IMGAS54050	***Discus whitneyi***	**Forest Disc**	**G5**	**S3?**		**SC/N**
IMGAS94050	***Euchemotrema leai***	**Lowland Pillsnail**	**G5**	**S3?**		**SC/N**
IMGAS70060	***Euconulus alderi***	**A Terrestrial Snail**	**G4Q**	**SU**		**SC/N**
IMGAS70050	***Euconulus polygyratus***	**Fat Hive**	**G5**	**S3?**		**SC/N**
IMGASH0010	§ ***Hoyia sheldoni***	**Storm Hydrobe**	**G1**	**SU**		**SC/N**

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
IMGASF4180	***Lyogyrus walkeri***	**Canadian Duskysnail**	**G3G4**	**SU**		**SC/N**
IMGAS64010	***Philomycus carolinianus***	**Carolina Mantleslug**	**G5**	**SU**		**SC/N**
IMGASM0200	§ ***Physella magnalacustris***	**Great Lakes Physa**	**G2Q**	**SU**		**SC/N**
IMGASM0220	§ ***Physella parkeri***	**Broadshoulder Physa**	**G2Q**	**SU**		**SC/N**
IMGASN0180	***Planorbella truncata***	**Druid Rams-horn**	**G3G4**	**SU**		**SC/N**
IMGAS16010	***Pupoides albilabris***	**White-lip Dagger**	**G5**	**S3S4**		**SC/N**
IMGASJ2110	***Somatogyrus depressus***	**Sandbar Pebblesnail**	**G2**	**SU**		**SC/N**
IMGASJ2330	***Somatogyrus tryoni***	**Coldwater Pebblesnail**	**G2G3**	**SU**		**SC/N**
IMGASL5240	***Stagnicola woodruffi***	**Coldwater Pondsnail**	**G1G3**	**SU**		**SC/N**
IMGAS98030	***Stenotrema barbatum***	**Bristled Slitmouth**	**G5**	**S3**		**SC/N**
IMGASE5050	*Valvata perdepressa*	Purplecap Valvata	G3	SU		SC/N
IMGAS20080	§ *Vertigo bollesiana*	Delicate Vertigo	G4	**S3**		SC/N
IMGAS20450	***Vertigo cristata***	**Crested Vertigo**	**G5**	**S3**		**SC/N**
IMGAS20390	§ ***Vertigo occulta***	**Occult Vertigo**	**G2**	**SNA**		**SC/N**
IMGAS20250	***Vertigo ovata***	**Ovate Vertigo**	**G5**	**S3?**		**SC/N**
PLANTS						
PDLYT01050	*Ammannia robusta*	Scarlet Loosestrife	G5	**SU**		SC
PDASTD7040	***Cacalia muehlenbergii***	**Great Indian-plantain**	**G4**	**S3**		**SC**
PMCYP03E90	***Carex vaginata***	**Sheathed Sedge**	**G5**	**S3**		**SC**
PMORC0M030	***Corallorhiza odontorhiza***	**Autumn Coral-root**	**G5**	**S3**		**SC**
PDAST2L0F1	*Coreopsis lanceolata var. lanceolata*	Sand Coreopsis	G5TNR	S2		SC
PDCOR01050	*Cornus drummondii*	Northern Roughleaf Dogwood	G5	S1		SC
PMORC0Q093	***Cypripedium parviflorum var. makasin***	**Northern Yellow Lady's-slipper**	**G5T4T5**	**S4**		**SC**
PMORC0Q0D0	***Cypripedium reginae***	**Showy Lady's-slipper**	**G4**	**S4**		**SC**
PDAST3P1T1	***Eupatorium sessilifolium var. brittonianum***	**Upland Boneset**	**G5T3T5**	**S4**		**SC**
PDRAN0F010	*Hydrastis canadensis*	Golden-seal	G4	S3S4		SC
PDJUG02030	*Juglans cinerea*	Butternut	G4	S3?		SC
PDBOR0L080	***Lithospermum latifolium***	**American Gromwell**	**G4**	**S4**		**SC**
PMLIL1E010	***Medeola virginiana***	**Indian Cucumber-root**	**G5**	**S4**		**SC**
PDMAL0X010	***Napaea dioica***	**Glade Mallow**	**G4**	**S3**		**SC**
PPOPH020F0	***Ophioglossum pusillum***	**Adder's-tongue**	**G5**	**S3**		**SC**
PDARA09010	*Panax quinquefolius*	American Ginseng	G3G4	S4		SC
PMALI04040	***Sagittaria calycina***	**Long-lobe Arrowhead**	**G5**	**S1**		**SC**
PDAST8P1R0	***Solidago sciaphila***	**Shadowy Goldenrod**	**G3G4**	**S4**		**SC**
PGTXA01020	*Taxus canadensis*	Canadian Yew	G5	S4		SC
PDVIO04270	***Viola striata***	**Striped Violet**	**G5**	**S1?**		**SC**
WISCONSIN'S EXTIRPATED SPECIES						
MAMMALS						
AMALE01010	*Bison bison*	American Bison	G4	SX		
AMAJF03010	*Gulo gulo*	Wolverine	G4	SX		
AMAJH04014	***Puma concolor schorgeri***	**Cougar**	G5T3Q	SX		
AMALC04011	*Rangifer tarandus caribou*	Woodland Caribou	G5T4	SX	LE	
BIRDS						
ABNQA05010	*Conuropsis carolinensis*	Carolina Parakeet	GX	SX		
ABNPB05010	*Ectopistes migratorius*	Passenger Pigeon	GX	SX		
ABNKC04010	*Elanoides forficatus*	American Swallow-tailed Kite	G5	SXB		
ABNKD06071	*Falco peregrinus anatum*	American Peregrine Falcon	G4T4	SX		
ABNNF07070	*Numenius americanus*	Long-billed Curlew	G5	SXB		
ABPBG07010	*Thryomanes bewickii*	Bewick's Wren	G5	SXB		END
FISHES						
AFCHA01060	*Coregonus johannae*	Deepwater Cisco	GX	SX		
AFCHA01100	***Coregonus nigripinnis***	**Blackfin Cisco**	**G1Q**	**SX**		
AFCHA01120	*Coregonus reighardi*	Shortnose Cisco	GH	SX		
AFCJC05010	*Erimyzon oblongus*	Creek Chubsucker	G5	SX		
AFCJB28230	*Notropis buchanani*	Ghost Shiner	G5	SX		
AFCJB28310	*Notropis chalybaeus*	Ironcolor Shiner	G4	SX		
MUSSELS AND CLAMS						
IMBIV24020	§ *Leptodea leptodon*	Scaleshell	G1G2	SX	LE	
IMBIV37030	§ *Potamilus capax*	Fat Pocketbook	**G2**	SX	LE	
INSECTS						
IIODO26080	§ ***Macromia pacifica***	**Gilded River Cruiser**	**G4**	**SX**		
IICOL42010	§ *Nicrophorus americanus*	American Burying Beetle	G2G3	SX	LE	END

ELCODE	Scientific Name	Common Name	Global Rank	State Rank	US ESA Status	State Status
PLANTS						
PDASC02150	*Asclepias meadii*	Mead's Milkweed	G2	SX	LT	
PDSCR0H0J0	*Collinsia verna*	Spring Blue-eyed Mary	G5	SX		
PDLAM0C010	***Collinsonia canadensis***	**Canada Horse-balm**	**G5**	**SH**		**END**
PDAPI0P010	***Conioselinum chinense***	**Hemlock Parsley**	**G5**	**SH**		**END**
LICHENS						
NLLEC10020	***Anzia colpodes***	**Black-foam Lichen**	**G3G5**	**SX**		
NLT0008170	*Cladonia acuminata*	Needlepoint Cladonia	G5?	SX		
NLTES10910	*Ephebe lanata*	Rockshag Lichen	G5	SX		
NLT0020800	***Parmotrema chinense***	**Powdered Ruffle Lichen**	**G3G5**	**SX**		
NLT0020940	*Parmotrema perforatum*	Perforated Ruffle Lichen	G3G5	SX		
NLTEST5240	***Peltigera venosa***	**Fan Lichen**	**G4G5**	**SX**		
NLLEC2G260	***Physcia dakotensis***	**Dakota Rosette Lichen**	**G3G5**	**SX**		
NLT0022740	***Physconia subpallida***	**Pale Frost Lichen**	**GNR**	**SX**		
NLTES10620	*Stereocaulon condensatum*	Tiny Foam Lichen	G4	SX		
NLLEC5P050	*Usnea angulata*	Angular Beard Lichen	G3G5	SX		
NLT0030840	*Usnea rubicunda*	Red Beard Lichen	G4G5	SX		

Appendix C.4

Wisconsin Department of Natural Resources

NHI Forested State Lands Bird Surveys — Off-Road Survey Datasheet

NHI Forested State Lands Bird Surveys — Off-Road Survey Datasheet
Wisconsin DNR, P.O. Box 7921, Madison, WI 53707-7921

Observer: _____
Date: _____
Site Name: _____
Point Number: _____
GPS: latitude: _____
longitude: _____
GPS datum: _____
Accuracy (m): _____

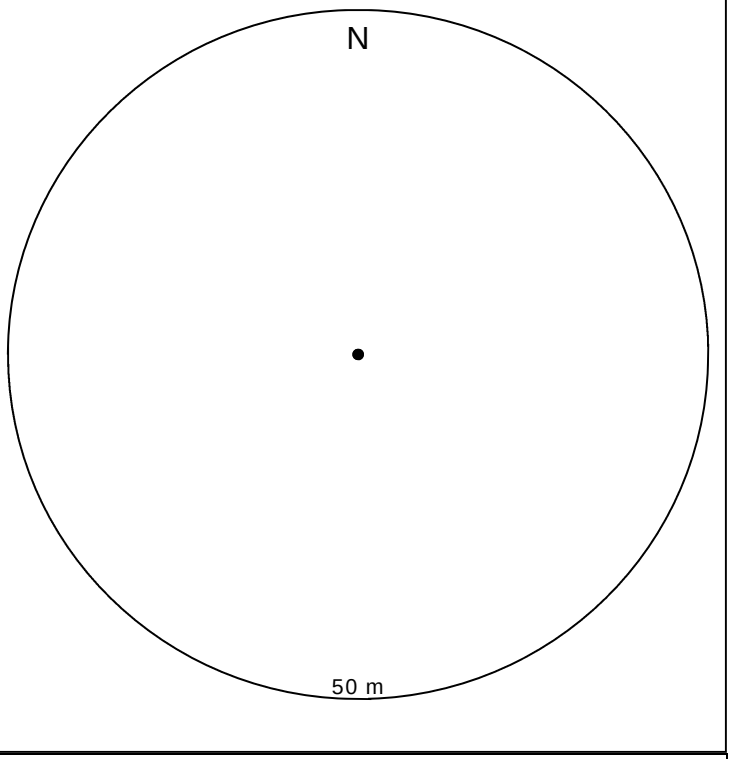
Temp (°F): _____
Sky Code: _____
Wind Code: _____
Start Time: _____
End Time: _____
Total # Species: _____
Total # Birds: _____

Bird Recording Codes

Singing: (OVEN)
Calling: OVEN
Flyover: OVEN →
Observed: OVEN
0-5 min: OVEN[1]
5-10 min: OVEN[2]

Habitat Data:

Natural Community:
Dominant tree sp:
Dominant shrub sp:
Tree size class:
% canopy cover:
% understory cover:
Associated plant spp:
Other features:



Wind Speed Codes

0 No Wind
1 Leaves barely move
2 Leaves rustle, small twigs move
3 Leaves & twigs in constant motion
4 Small branches move
5 Large branches and small trees sway
6 Large branches in constant motion
7 Whole trees in motion

Sky Cover Codes

0 Clear (<10%)
1 Scattered (10-50%)
2 Broken (60-90%)
3 Overcast (>90%)
4 Fog
5 Light mist

Species after count/enroute:

Bird Data Summary:

Code	Species Name	# Ind. 0-5 min.	# Ind. 5-10 min.	Total # ind. 0-10 min.	COMMENTS (breeding evidence, behaviors, etc.)

NHI Forested State Lands Bird Survey – Off-Road Surveys
Datasheet Instructions

1. Fill in observer name. If more than one observer, put leader here and write additional names in space below.
2. Date on which the point was surveyed.
3. For contiguous properties, use the property name (e.g., Wyalusing State Park). For the Lower WI Riverway, use the site name assigned to the individual properties (e.g., LWR-A)
4. If pre-established points are used, then enter the number assigned to the point. If points are determined in field, use the Site Name initials followed by consecutive 3-digit numbers (e.g., LWR-A-001)
5. Enter the lat/long taken at the point. Easting and northing may be entered instead of lat/long, but be sure to record the GPS datum used. Always give the accuracy reading in meters.

6. Abiotic data should be recorded following the 10-minute count period.
7. Do NOT conduct surveys during rain (except light drizzle), heavy fog, or steady winds greater than 10 mph ("3" on the Beaufort Wind Scale).
8. Keys for sky and wind codes are on the front of the datasheet.
9. Start time is the time you begin counting birds. End time is when the 10-minute count period is up.
10. Tallies of species and individuals should be completed after the morning survey has ended.

11. After the 10-minute count period and before leaving for the next station, describe the habitat.
12. Identify WDNR natural community type (if known), list dominant tree and shrub species, describe canopy, shrub, and herbaceous associates, and estimate tree size class (see below), percent canopy cover, and percent understory cover to the best of your ability.
13. Describe other features present (e.g., stream, snags, course woody debris, etc.).
14. Do not spend a lot of time (more than 1-2 minutes) on describing habitat. The most important data to record here is the tree size class estimate and dominant tree or community type as well as other features that may be present.

Tree size class:
Seedling – A usually young tree smaller than a sapling. Trees less than 1 inch dbh.
Sapling – A usually young tree larger than a seedling but smaller than a poletimber tree. Trees ranging from 1 to 5 inches dbh.
Poletimber – A tree of a size between a sapling and a sawtimber tree. Hardwood trees ranging in size from 5 to 11 inches dbh and conifers ranging in size from 5 to 9 inches dbh.
Sawtimber -- Trees with minimum diameter and length and with stem quality suitable for conversion to lumber. Hardwood trees larger than 11 inches dbh and conifers larger than 9 inches dbh.

15. Record the species code for each individual bird in the location where it is first detected using the circle diagram on the datasheet.
16. Record all birds seen and heard within a 50-m radius of the point count station and beyond the 50m radius.
17. Birds flying over the point count station should be recorded separately as "flyovers".
18. Use standard four letter codes for bird names, see http://www.uwgb.edu/birds/wso/.
19. Birds seen or heard at a point station after the point count period has ended should be assigned to that station. Likewise, birds seen enroute to the next station will be recorded on the station from which the observer just left. A clean data sheet will always be used to start a new station.
20. Breeding evidence, bird behaviors, and other items of interest should be noted in the Comments column of the Bird Data Summary table.
21. After the survey period ends, bird species binomial names should be entered in the Bird Data Summary table and numbers should be tallied. (Binomial names are necessary to ensure accurate interpretation of species codes.)

Additional Observers Names and Phone Numbers:

_____

_____

Appendix C.5

NHI Landbird Survey Protocol – Off-Road Surveys

NHI Landbird Survey Protocol – Off-Road Surveys

Placement and Spacing of Bird Point Count Stations within a Site

- Using remote sensing (i.e. aerial photo layer in ArcView), point count stations were placed a minimum distance of 250m apart and were situated to provide maximum coverage of habitat areas within property boundaries. A measured grid pattern was used wherever possible. For patches of irregular shape, slight deviations from the grid pattern were allowed to provide adequate sampling of the area.
- The number of stations per site varied depending on site size.

Field Methods

Assessment of Point Count Stations:

- Point count stations should NOT be sampled if:
  1. Located within a cultivated field such as corn, soybeans, etc.
  2. Located within a pine plantation
  3. Located within a campground or picnic area

- Point count stations will be located using GPS coordinates. Where GPS readings are prohibited by tree cover, a GPS reading will be taken in the nearest canopy gap. A compass and pacing will then be used to find the approximate location of the point relative to the position where the reading was taken.

- For extremely large sites (i.e. sites with more than 10-12 point count stations), sampling may occur over multiple days. Specific instructions on the number of days required and inventory priorities will be provided for each property being surveyed.

Point Count Period and Duration:

- The survey period will begin a half hour before sunrise and end four hours after sunrise.
- The duration of point count surveys at each station will be 10 minutes. Each 10-minute count will be separated into two different segments: from 0 to 5 minutes, and from 5 to 10 minutes.
- Travel time between stations should average from 15 to 20 minutes.
- Recording of birds should begin immediately upon reaching the point count station. Weather conditions, habitat descriptions and other data should be recorded following the 10-minute count.

Weather Conditions and Data:

- Surveys should NOT be conducted during rain or heavy fog: continue through light drizzle.
- Surveys should NOT be conducted in steady winds greater than 10 mph – a "3" on the Beaufort Wind Scale.
- The sky code, wind code, and temperature (°F) should be recorded at each point count station at the end of the bird survey.

Recording Bird Data:

- Record the species code for each individual bird in the location where it is first detected.
- Record all birds seen and heard within a 50-m radius of the point count station and beyond the 50m radius.
- Birds flying over the point count station should be recorded separately as "flyovers".
- Use standard four letter codes for bird names, see http://www.uwgb.edu/birds/wso/.
- Birds seen or heard at a point station after the point count period has ended should be assigned to that station. Likewise, birds seen enroute to the next station will be recorded on the station from which the observer just left. A clean data sheet will always be used to start a new station.

Describing Habitat:

Forests:

- Identify WDNR natural community type (if known), list dominant tree and shrub species, describe canopy, shrub, and herbaceous associates, and estimate tree size class (see below), percent canopy cover and percent understory cover to the best of your ability.
- Describe other features present (e.g., stream, snags, course woody debris, non-native species, etc.).

Grasslands and Savannas:

- Identify WDNR natural community type (if known), list dominant herbaceous species, describe tree, shrub, and herbaceous associates, and estimate percent woody cover and percent cover of stiff-stemmed forbes (e.g., mullein) to the best of your ability.
- Describe other features present (e.g., stream, snags, hedgerows, non-native species, etc.).

Tree size class:
Seedling – A usually young tree smaller than a sapling. Trees less than 1 inch dbh.
Sapling – A usually young tree larger than a seedling but smaller than a poletimber tree. Trees ranging from 1 to 5 inches dbh.
Poletimber – A tree of a size between a sapling and a sawtimber tree. Hardwood trees ranging in size from 5 to 11 inches dbh and conifers ranging in size from 5 to 9 inches dbh.
Sawtimber -- Trees with minimum diameter and length and with stem quality suitable for conversion to lumber. Hardwood trees larger than 11 inches dbh and conifers larger than 9 inches dbh.

Appendix C.6

A Trainers Guide to the
“Wisconsin Citizen-Based Acoustic Monitoring Project”

A Trainers Guide to the "Wisconsin Citizen-Based Acoustic Monitoring Project"

- Welcome/Introduction
  - Citizen-based monitoring (CBM) has a long and successful history in Wisconsin. Based on our most recent survey, there are more than 150 volunteer groups in the state which contribute over 300,000 man-hours of labor each year-an estimated value of 20 million dollars annually. The WDNR has since turned to the CBM network to meet growing demands for bat population data and trends. In the United Kingdom using trained citizens to collect long-term bat data has proven to be a cost-effective solution with successful results for gathering large scale inventory and monitoring data. The WDNR is employing a similar approach to collect acoustic bat data.
- Bat Natural History
  - Echolocation
    - Bats produce ultrasonic (above the range of human hearing) echoes to navigate in the dark, and to pursue and capture prey.
    - The detection system we use is capable of detecting and recording these high frequency calls while bats fly through the area.
  - Annual Cycle
    - Wisconsin bats use one of two strategies for dealing with the winter season.
      - Hibernating, or cave bats as they are often called, will use locations which are buffered from freezing temperatures to spend the winter while food resources are limited.
      - Migratory bats, which are usually referred to as foliage roosting, or tree bats, move toward warmer climates during the winter season.
    - April and May is when bats begin to emerge from wintering sites.
    - June and July, females give birth and raise young (pups) while food resources are plentiful.
    - Mating and preparation for the upcoming winter begins in August and September.
- Characteristics of Wisconsin Bats
  - Nocturnal, echolocate, low reproductive rates, some species congregate in a limited number of locations during critical stages of their life (i.e. hibernacula & maternity sites), cryptic coloration.
- Reason for lack of information
  - Nocturnal, fly relatively fast, produce sounds above the range of human hearing, roost in hard to find areas (cracks, rock crevices, cavities) and cryptic coloration.
- Current Threats
  - Natural susceptibility: Low reproductive rates (~1 pup/yr). Certain species gather in large concentrations at critical points of their life (hibernation and maternity sites) leaving the colonies vulnerable to catastrophic events.
  - White-nose Syndrome (WNS): a recently discovered fungal disease that has caused hundreds of thousands of bat deaths in Northeastern and Atlantic states. Causes bats to use up fat reserves needed to hibernate through the winter season. Epicenter is New York (2006), since spread to 11 states total (CT, VT, PA, WV, VA, MA, NH, NY, NJ, MD, TN). Known to affect these WI bats species: MYLU, MYSE, PESU, & EPFU.
  - Wind turbines: large scale wind farms have been known to adversely affect all bat species in Wisconsin. The hardest hit group is the migratory species.
- Detection System
  - SD1 Anabat ultrasound detector, GPS (Global Positioning System), PDA (Personal Data Assistant): Total cost $3000-$3300
    - Setup
      - Rechargeable batteries fully charged for SD1 (AA batteries) and PDA is fully charged

        - PDA should remain on charger until just prior to conducting the survey (leaving the PDA plugged in even when fully charged will not hurt batteries)
    - Sync cable connects SD1 to PDA
    - 3 bolts secure PDA bracket to the SD1
    - Compact Flash (CF) GPS unit is inserted into top of PDA
- Survey Guidelines
    - Bat Survey Protocol
        - ***Daytime*** temperature exceeds 50°F
        - Begin survey 1/2hr after sunset (no earlier)
        - No precipitation during the survey
        - Wind speeds are not to exceed 30 mph
        - Surveys can be conducted from April 1st to September 30th
        - Surveys are to be at least 1 hour in length & may last up to 3hrs past starting time
    - Start / End Times
        - Survey may begin ½ hour after local sunset (no earlier!)
        - The "start time" should reflect the beginning time of the survey (i.e., when all equipment is activated and the surveyor begins walking)
        - Record the "end time" when the survey ends (i.e., when all the equipment is powered down)
    - KEEP MOVING
        - New surveyors tend to stop during bat encounters (especially when bat activity is high), while it is good to record that event we want to stress the importance of continuing to move while surveying.
            - Bat Call: A bat call is an individual sound pulse emitted by a bat. Each call is followed by a period of which provides times for returning echoes. Typically calls are produced at a rate of around 5 -10 calls per second as the bat is commuting and /or searching for insects.
            - Bat Pass: A bat pass is a sequence of individual calls emitted by a bat as it flies within the range of the detector (>1 call separated by less than 1 second). Thus, we need at least 2 calls to record (save) a bat pass; however, additional calls within a pass improve our ability to distinguish species or species groups.
            - Feeding Buzz: A feeding buzz is a rapid increase in call rate as the bat nears an insect target just prior to capture. It is noticeably different form the search phase calls and occurs as call pulses become rapid enough to form a short audible buzz when the bat captures or attempts to capture the insect. Hearing a feeding buzz is an indication that there is a bat foraging in the area.
    - Data Upload
        - Make it clear to all volunteers that after each survey the data must be downloaded to the WDNR website by the regional coordinator or someone who has been trained to upload the data.
        - Everyone should know where to pick up the equipment and where it should be dropped off after each use.
    - Equipment Care
        - Use caution when taking out the expensive bat detection system.
        - Do not leave any monitoring equipment in direct sunlight, in your car overnight, or out during inclement weather (no precipitation of any kind).

Appendix C.7

Wisconsin INVASIVE PLANTS OF THE FUTURE Project

Invasive Plant Report Form

Wisconsin INVASIVE PLANTS OF THE FUTURE Project

Co-sponsored by the Wisconsin State Herbarium and the Wisconsin Department of Natural Resources

Invasive Plant Report Form

Collection information

State _____ **County** _____ **Date collected / observed** _____

Collector name _____

Street address_____**City** _____**State** _____ **Zip** _____

Phone _____ **Email** _____

Characteristics & location

Plant name (Common and/or Latin name) _____

Size & density of infestation. Describe spread and estimate numbers

Habitat description. Describe general habitat type such as forest interior, forest edge, old field, prairie, wetland, lakeshore, crop field, pasture, disturbed ground, urban setting type. Is it public or private land?

Location landmarks. Provide enough details so site can be found again. Note nearby landmarks such as city name, roads, intersections, driveways, lake edges and other natural and cultural features.

Geographic coordinates (Complete one. Pinpoint using www.TopoZone.com)

1. Latitude _____N **Longitude** _____W

2. UTM _____E _____N

3. Township, Range, Section, Part Section _____

Submittal

Mail specimen with its data form to: **Invasive Plants Project, Herbarium, UW-Madison**
430 Lincoln Dr., Madison, WI 53706

Questions? Call **(608) 267-7438** Email: **InvasivePlants@mailplus.wisc.edu**
Website: **http://dnr.wi.gov/invasives/futureplants**

***"Invasive Plants 911"* for Wisconsin**

Based on their invasive behavior in other states and provinces, these six target plants in particular are the ones we are most concerned about. Let us know right away if they have been found in the state.

- Japanese stilt-grass
- Hydrilla
- European frog-bit
- Swallow-wort
- Water chestnut
- Giant hogweed

Notice: Information provided on this form will be used in a statewide volunteer effort to locate, eradicate and monitor selected invasive plants. Your cooperation in reporting these species is much appreciated. Personally identifiable information collected on this form may be provided to requesters as required by Wisconsin's Open Records law [ss. 19.31 - 19.39, Wis. Stats.]. This form is equivalent to DNR form 1700-056.

Version Feb. 2008

Appendix C.8

Wisconsin Natural Heritage Inventory
Rare Plant Field Report

State of Wisconsin
Department of Natural Resources
Bureau of Endangered Resources
PO Box 7921, Madison WI 53707-7921

Wisconsin Natural Heritage Inventory
Rare Plant Field Report
Form 1700-049 (R 9/02)

Notice: Completion of this form is voluntary. Data collected will be used to supplement the Wisconsin Natural Heritage Inventory database. Personal information collected on this form will be used to process your request, and is intended to be used to contact you if DNR staff require additional information; it may also be made available to requesters under Wisconsin's Open Records law [ss. 19.31-19.39, Wis. Stats].

Species Name

Location

Site Name (if known)

County | USGS Quad (if known)

Township N | Range | ☐ E ☐ W | Section | ¼ Section | ¼ / ¼ Section

GPS Coordinates (latitude, longitude) | GPS Position Accuracy – meters | GPS Datum | Date Coordinates Taken

Office Use

Quadcode

Margin | Dot | Ten, Ten

Lat S N | Long N E W

Directions to Site and Location of Plant Population in Relation to Landmarks – Please sketch map or include a map copy to clarify location

Landowner Name (if known)

Note: The Natural Heritage Program can not accept data derived from trespass. Gain landowner's permission before entering private property.

Observation Details

Observation Date | Number of Individuals | (select one) ☐ Stems ☐ Clumps ☐ Clones

Estimate the Percentage of the Population Belonging to the Following Categories:

_____ % in Flower / Bud _____ % in Fruit _____ % Sterile Adults _____ % Seedlings / Juveniles

Area Covered by Observed Population | Do you think you saw the whole population? ☐ Full Extent Known ☐ Full Extent Unknown ☐ Uncertain

Habitat Description – Including associated species, community type, slope, aspect, light level, soil moisture and type as known

Office Use

M.USWIHP*

Observation Details (continued)

Current Management

Describe any Evidence of Disturbance or Threats to the Plant Population – Include evidence of predation, logging, succession, etc., and changes since you last saw the population if this is a return visit

Was a Specimen Taken?	If Yes, Collection Number	Herbarium Name
☐ Yes ☐ No		
Was a Photograph Taken?	If Yes, Storage Location	
☐ Yes ☐ No		

Taxonomic Reference(s) Used:

☐ Memory	☐ *Michigan Flora*	☐ *Spring Flora of Wisconsin*
☐ Gleason & Cronquist	☐ Swink & Wilhelm	☐ *Preliminary Reports*
☐ *Wildflowers & Weeds*	☐ *Gray's Manual*	☐ Comparison with verified herbarium specimen
☐ Peterson Guide	☐ Fassett's *Aquatic Plants*	☐ Other – specify: _____

Observer Information

Name(s)		Telephone Number
E-Mail Address	- OR -	Address
		City / State / ZIP Code

Send completed forms and maps to:

Botanist, Wisconsin Natural Heritage Program
Bureau of Endangered Resources
Department of Natural Resources
PO Box 7921
Madison WI 53707-7921
(608) 266-7012

Office Use
ROW
TRSQ
Prec
Waterbody

Appendix C.9

Wisconsin Natural Heritage Inventory
Natural Community Form

WI Natural Heritage Inventory Natural Community

Office Use Only:
Com_ID_____

EO_ID_____

Observer(s):_____ Date:_____

Surveysite and Managed Area:_____

Community Type:_____

Town Range Section(Quarter/Quarter):_____ County:_____

Community Acreage:_____ Confidence extent: Y N

GPS coordinates or file information (specify format, Degree Minutes and Seconds preferred) *Datum*: NAD27 WGS 84 WTM 83/91

Waypoint #s:_____ Photo #s:_____

Access:_____

_____

Community description:_____

_____

_____

_____

Characteristic spp:_____

_____

Please list the communities' (top 3) dominant species for the appropriate cover class and for each Statrum below:

	% Cover Class code							Dominant Species
Stratum	1	2	3	4	5	6	7	
Canopy	<1	1-5	6-25	26-50	51-75	76-95	96-100	
Subcanopy	<1	1-5	6-25	26-50	51-75	76-95	96-100	
Shrub/Sapling	<1	1-5	6-25	26-50	51-75	76-95	96-100	
Herb	<1	1-5	6-25	26-50	51-75	76-95	96-100	
Bryophyte/Lichen	<1	1-5	6-25	26-50	51-75	76-95	96-100	
Bare Ground	<1	1-5	6-25	26-50	51-75	76-95	96-100	

Invasive spp:_____

_____

Evidence of disturbance/threats: _____

_____

Restoration Potential: _____

_____

_____

Survey/Inventory/Management needs:_____

_____

Rare species, SGCN species, or unusual features present:_____

_____

Aspect	Slope	Hydrologic Regime	Surface Soils
____NW	_____0%	____Inundated (Hydric)	____Sand
____N	____1-10%	____Intermittently Flooded	____Loamy sand
____NE	____10-25%	____Saturated (Wet-mesic)	____Sandy loam
____E	____25-50%	____Moist (Mesic)	____Loam
____S	____50-100%	____Dry-mesic	____Silt loam
____SE	____variable	____Dry (Xeric)	____Silt
____W		____ Variable	____Clay loam
____SW			____Silty clay
____Level			
___ variable			

Form continues on the back. Make any additional comments for any field where you need more room in the addendum space.

Summary Rank of the Community Condition, Context, Quality and Viability

Rank Definition: A- Excellent B – Good C Marginal D - Poor				
EO Condition & Quality (e.g. Compostion, natural and human caused disturbances, exotics & community recovery potential)	A	B	C	D
EO Size	A	B	C	D
EO Context (Quality of biotic & abioitc factors/processes in surrounding landscape)	A	B	C	D
EO Rank (Summary of all factors listed above)	A	B	C	D

Comments_____

_____

Addendum or additional information regarding the site: (e.g. may include BBS, plant or animal species lists, recommendations for additional inventory work etc. ****For addenda, please note before each portion which field you are continuing.)

Appendix C.10

Wisconsin Natural Heritage Inventory
Site Summary Form

WI Natural Heritage Inventory Site Summary Form

Office use only:
Access Site ID_____

Biotics Site ID_____

Observer(s):_____ Date:_____

Surveysite and Managed Area:_____

Land Manager:_____ Contact Info:_____

Town Range Section(Quarter/Quarter):_____

County: _____ Waypoints: _____

Access/Directions_____

_____

Site description:_____

_____

_____

_____

_____

_____

Site Significance:_____

_____

_____

_____

_____

_____

Management condiderations:_____

_____

_____

_____

_____

_____

Land-use of site & surrounding landscape_____

_____

_____

_____

_____

_____

Community type(s):_____

_____

_____

Rare species, SGCN species, or unusual features present:_____

_____

_____

_____

Appendix C.11

Wisconsin Invasive Plants Project

How to Make Voucher Specimens of Plants

Wisconsin Invasive Plants Project

How to Make
VOUCHER SPECIMENS of PLANTS

What is a Voucher?

A ***voucher*** is evidence used to confirm a plant's identity and to prove it was found in a particular location. Even if you are reasonably sure of the name, documented evidence is still needed before a plant can be entered into the permanent records of the State Herbarium. You can submit either:

1) **Physical evidence** (pressed or fresh plant specimens)
2) **Photographic evidence** (showing key parts to confirm identity).

Once received, vouchers are checked by plant experts at the herbarium. For each voucher submitted, a Plant Report Form (or its equivalent) is required that provides details on location, date of collection, habitat, and other information related to the specimen.

This document gives instructions for preparing three types of vouchers:
1. Herbarium vouchers (pressed and dried plant specimens)
2. Photographic vouchers (electronic or film images)
3. Fresh plant vouchers (must be delivered promptly!)

How to Decide?

Regardless of method chosen, it is important to **provide enough evidence** to assure correct identification. The State Herbarium prefers a complete plant specimen that is dried and pressed. This specimen can then be deposited in the collection if needed. However, a fresh or dried sample or photos of key diagnostic parts of the plant (distinctive leaves, flowers or fruit, for example) may be all that are needed to confirm identity.

Positive identification is important, especially if the plant is a new invasive in Wisconsin or new to a county or region of the state. The sooner identification is confirmed that sooner that control work can begin on invasive plant populations. It is also important to be sure that valuable native plants are not mistakenly targeted and eradicated.

To become more familiar with both native and non-native species, check the Herbarium website (www.botany.wisc.edu/wisflora/) and search by either common or scientific name. You can also view herbarium records and see if a species has been collected in your county or area.

1. Herbarium Vouchers (*pressed plant specimens*)

A herbarium voucher specimen is a dried plant sample consisting of pressed leaves, stems, flowers, fruits and/or roots. A key part of the specimen is the written data that shows location, date of collection and other information. Vouchers are valuable because they provide the physical evidence to confirm the presence of plant species at specific locations. They have a variety of uses, such as documenting the occurrence of rare plants or revealing the geographic spread of invasive plants over time. Once received by a herbarium (a plant specimen "library"), vouchers may be mounted, labeled and kept for future reference and research.

For photos and instructions for making and using a full-size (12 x 18 inches) press, see http://www.uwgb.edu/biodiversity/herbarium/voucher02.htm

Making vouchers is easy and fun. Plus, if the specimen is entered into the permanent collection of the Herbarium, the collector's name will be permanently associated with the record. You can become part of botanical history in Wisconsin!

Equipment needed

1. **Plant press** (or equivalent)**, tools, and notebook** (tools may be needed to dig or cut specimens)
2. **Invasive Plant Report Form** (or equivalent record of specimen-related data)

Basic steps for preparing vouchers

1. Collect plant in the field.
2. Record specimen data in a notebook, and later on Plant Report Form
3. Press immediately, or transport temporarily in a plastic bag and press ASAP.
4. Dry completely.
5. Send specimen and completed Report Form to the Wisconsin State Herbarium at UW-Madison. Make sure the sample and its data stay together! Send to address on last page.

PREPARING TO COLLECT

Before collecting, plan how you will transport and preserve your specimens. Know the dimensions of your plant press, or carry a large plastic bag to keep specimens fresh temporarily. Be mindful of hot sunshine, which can cook samples in an enclosed bag. Always carry paper and pencil.

WHAT TO COLLECT

Select one or more healthy plants that are typical of the population. Take samples of the whole plant, if possible, or enough **leaves and stems** to show leaf shape and size, opposite or alternate **branching**, and **buds**. If possible, include **flowers** and **fruits**, which may be needed to confirm a plant's precise identity. For grasses and grass-like plants, try to include roots. For large specimens, bend stems into a V, N or W shape. Thick stems may be cut in half lengthwise. For small plants, collect several and press together. Show upper and lower surfaces of leaves and flowers. Press flowers with the blossom open, and if possible slice one in half lengthwise to show internal structures. Be sure to press the plant before it wilts.

PRESSING PLANTS

If you have one, use a standard-sized (12 x 18 inch) plant press. Herbarium specimens are typically mounted (glued) on standard 11.5 x 16.5 inch sheets of heavy paper. Specimens must not exceed this size (though large plants often are divided up and glued to multiple sheets).

For this invasive plants project, dimensions can be as small as **9 x 12 inches**. This makes it easy to carry the press in a backpack, and specimens can be mailed in large, business-size envelopes.

MAKING A PORTABLE PLANT PRESS

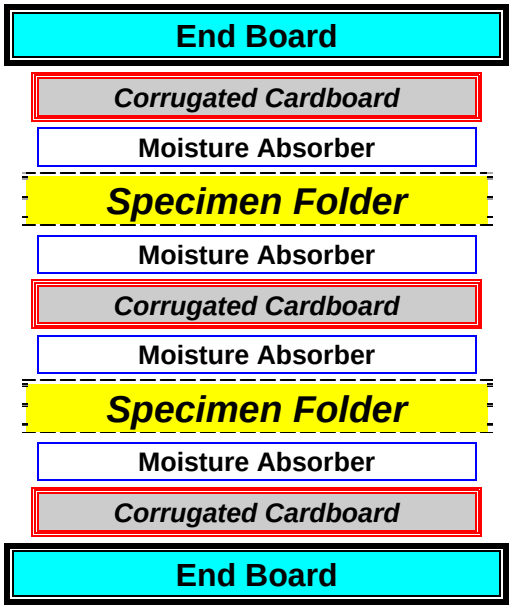
1. End Boards. Prepare two 9 X 12 inch rectangles of a rigid material. Use plywood, masonite, pegboard, the backs of two clipboards, the covers from a 3-ring binder, or even cardboard (several sheets glued together for rigidity). Between the end boards – and cut to the same dimensions -- place alternating layers of corrugated cardboard, moisture-absorbers, and newspaper specimen "folders." See diagram below.

2. Corrugated Cardboard. Cut from corrugated boxes. If possible, cut so the lines of corrugation run across the shortest distance. This will enhance air flow through the press.

3. Moisture Absorber. To wick moisture away from the drying specimens, use sheets of newspaper or paper-toweling. Sheets of thick blotter paper work well, if available.

4. Newspaper Specimen "Folder." Specimens are arranged carefully within a folded piece of newspaper (like placing a document in a file folder). NOTE: Be sure to label each specimen with a post-it note, or some sort of label or code number linking it to its data in your notebook.

Assembling the press. When putting plants in the press, each newspaper specimen folder is sandwiched between moisture-absorbing layers and cardboard. For bulky specimens, extra layers of moisture absorber and cardboard may be needed. Tie the press together tightly with rope, bungee cords, elastic or buckle straps. You may need to adjust tightness as plants dry and flatten out. For thick or succulent plants, add extra layers of cardboard and absorber, or change the folder and absorbing layers at least once. Include 5 to 10 (or more) specimen folders – and surrounding layers – in your press, or as many as you can comfortably carry. More than one specimen folder can go between cardboards, if not too bulky.



Plant Press Assembly
Diagram not to scale

SPECIMEN DATA
For each specimen or photograph, basic information about the occurrence is needed. For all specimens collected, make sure that all documentation stays with, or can be linked to, the sample. Some collectors write data on the newspaper specimen folder or on a sheet enclosed with the sample. Others use a notebook with numbers that correspond to a specific sample.

Use the **Invasive Plant Report Form** to submit information. Or create your own – but make sure it covers the same categories. Start with state, county, location, date collected, and plant name (common or scientific). Provide the collector's name, address, phone and email. Note landmarks and distances, such as city name, roads, intersections, power lines, lake edges and other natural and cultural features. Estimate the size and density of the infestation. Provide a habitat description, such as forest interior, forest edge, old field, prairie, wetland, lakeshore, crop field, pasture, disturbed ground, urban setting type. Tell if found on public or private land. If known, provide name and contact information for the landowner or land manager. Enclose a completed form with each specimen.

Accurate information about **location** is essential. Try to provide **exact geographic coordinates** using a GPS unit, topographic map, or the Wisconsin Gazetteer. If you have access to the internet, you can use http://www.TopoZone.com to find the precise location on a digital topographic map. When you click the cursor on the exact collection site, its coordinates (choose UTM or Latitude/Longitude) are automatically printed in the text above the map. Along with the report form, include a **map** (printed or photocopied) with a colored dot showing the spot.

2. Photographic vouchers *(electronic or film images)*

A photo voucher is an image that shows enough plant characteristics to allow identification by plant experts. The herbarium prefers actual plant specimens which, when properly preserved, can last for centuries. Photographs can be very useful, however. Although they are relatively short-lived media, they offer a quick and clear way to identify and report an invasive plant occurrence. They can also accompany a dried or fresh plant specimen, to help with identification and to show the habits of plants in the field. As with all vouchers, all relevant data must be kept for each plant photographed. Use a notebook to record information (see Specimen Information above) and make sure it remains associated with a specific plant.

WHAT TO PHOTOGRAPH
Try to get close-ups of flowers (from two or more angles), inflorescences, fruits, seed heads, leaves (upper and lower surfaces), branching patterns, buds, roots, and other details. Take photos of the whole plant and of the infestation in the field. Try to include a scale, using objects of known size, such as a pencil, coin, shoe, fingers, or a person. This will not only help confirm identity, but it also shows the extent of infestation, habitat invaded, stage of growth and more.

EQUIPMENT NEEDED
Digital cameras are widely available, and photo files can easily be emailed. With internet technology, images can be "delivered" to the Herbarium within minutes, and plant identity confirmed almost instantaneously. Be sure to use the camera's review function to ensure that the photos are in focus. Film cameras can take excellent images as well, and often with more certainty of clear focus. There is a time delay for film-developing, of course. Prints or slides are equally useful, and can be mailed along with the appropriate plant report form. Pictures can be scanned and sent electronically, too.

3. Fresh plant vouchers *(deliver promptly!)*

It may be easier to mail or hand-deliver fresh plant specimens – especially for high-moisture aquatic plants. But speed is important, so store plants in plastic bags and refrigerate to keep fresh. If mailed, pack the bagged sample in a sturdy box with bubble-wrap or crumpled newspaper for insulation. Avoid sending if delivery could fall on a weekend or holiday. Or bring the plant to the State Herbarium, Room 160 Birge Hall on the UW-Madison campus, during weekday work hours.

WHAT TO COLLECT
Select plants that are typical of the population. Take samples of the whole plant, if possible, or enough **leaves and stems** to show leaf shape and size, opposite or alternate **branching**, and **buds**. If possible, include **flowers and/or fruits**, which may be needed to confirm a plant's precise identity. For grasses and grass-like plants, try to include roots. Use a zip-top bag or tie securely with a twist-tie. Poke a few air holes to allow gases to escape the bag.

WHERE TO SEND? Mail specimen with report form to:

Invasive Plants Project
UW Herbarium – Botany Dept. (Room 160 Birge Hall)
430 Lincoln Dr., Madison, WI 53706
c/o Senior Academic Curator: Ted Cochrane

Phone: 608-262-2792
Website: http://www.botany.wisc.edu/herbarium

Or e-mail photographs to: **InvasivePlants@mailplus.wisc.edu**

Questions? Contact: Invasives of the Future Project Coordinator
Email: InvasivePlants@mailplus.wisc.edu
Phone: (608) 267-7438
or see website http://dnr.wi.gov/invasives/futureplants

Appendix C.12

Ranking Species Occurrences – A Generic Approach
(www.natureserve.org/explorer/eorankguide.htm)

NatureServe *Explorer*

RANKING SPECIES OCCURRENCES - A GENERIC APPROACH

Geoffrey A. Hammerson, Dale Schweitzer, Larry Master, and Jay Cordeiro

January 11, 2008

Introduction

Element occurrence (EO, hereafter simply "occurrence") ranks provide a succinct assessment of the estimated viability (probability of persistence) of occurrences of a given species. They provide an estimation of the likelihood that, if current conditions prevail, a species occurrence will persist for a period of time. Because occurrence ranks are used to represent the relative overall "quality" of an occurrence as it currently exists, they are based solely on criteria that reflect the present status of that occurrence. These criteria can be broadly specified as "rank factors," namely size (including population size and/or occupied area), abiotic and biotic conditions, and landscape context. Future threats should not be used to "downgrade" an occurrence rank, but ongoing events (e.g., successional changes, periodic unfavorable management) that result in inexorable degradation of occurrence quality *should* be considered.

Problems with Previous Occurrence Rank Specifications

NatureServe zoology staff recently reviewed all existing occurrence rank specifications for vertebrates and invertebrates. Many of the rank specifications superficially looked good; they often included quantitative criteria that seemed to appropriately "scientific." However, on closer inspection, these occurrence rank specifications generally turned out to be arbitrary, highly subject to change, of uncertain utility for distinguishing occurrence viability, and impractical or impossible to apply. When viewed as a whole, the occurrence rank specifications lacked a clear and consistent conceptual framework.

One of the major problems involved the use of specific measures of abundance, such as catch or observation rates, to distinguish occurrence viability. Catch rates are known to vary greatly with surveyor, methods, season, or other factors, and so the resulting data may not accurately reflect abundance. Consequently, it is generally unreliable to use such data for determining occurrence ranks. Strict adherence to specified sampling protocols might alleviate this problem, but in the real world such consistency rarely occurs.

Another problem with using quantitative occurrence rank criteria derives from the demographic characteristics of various plant and animal species. In many species, substantial variations in population size occur over periods of multiple years. If we establish precisely defined population criteria (e.g., >1,000 = B, < 1,000 = C) for ranking occurrences, we cannot simply use current population size to rank occurrences because the rank of some occurrences would change through the course of normal annual population fluctuations. Additionally, occurrences may be incorrectly ranked because the year or years sampled represent extreme conditions. One could circumvent this problem by using averages or modal or worst-year conditions, but such information is rarely if ever available. In fact, most of the occurrence rank

specifications that were written in the past often appear to be unusable for most occurrences because the required information does not exist, rarely will be obtained, and sometimes cannot be obtained by known methodologies.

Even if abundance could be determined in a meaningful, repeatable way, we would still lack a secure scientific basis for specifying precisely defined, objective occurrence viability criteria. Conservation biologists have had enormous difficulty in determining or agreeing on quantitative population viability criteria for various taxonomic groups. And long-term population trends from the real world frequently are at odds with theoretical considerations.

Because of these factors, past efforts to establish useful, reliable, and stable occurrence rank criteria were largely unsuccessful.

A Simplified Approach

NatureServe scientists have concluded that elaborate or highly specific quantitative criteria are not required in order to rank species occurrences usefully for conservation purposes. Instead, categorical, qualitatively defined rank guidelines should be sufficient for most occurrence ranking. For a small minority of well-studied species or groups of species it may be possible to develop and employ meaningful, quantitative occurrence rank criteria, and the generic occurrence rank guidelines described in the following section do not preclude the use of more quantitative alternatives. In fact, for some particular species or species groups, the previously existing occurrence rank specifications were modified and retained. Many of these specifications basically offer suggestions as to how to apply the generic concepts to the species or group. When species- or group-specific occurrence rank specifications are available they should be consulted, and the ranker should decide whether these or the generic guidelines (or a combination) work best for the information at hand.

Advantages of Generic Occurrence Rank Guidelines

The generic occurrence rank guidelines for species address the problems mentioned above, and they circumvent additional problems. For example, for some species, the viability of populations of equal size may not be the same in two different regions or even in different habitats of the same region. The qualitative generic criteria deal effectively with species that exhibit substantial ecogeographical variations in demographic characteristics and eliminate the need to write multiple occurrence rank guidelines for single species. By focusing on **probability of persistence**, the criteria should work equally well for occurrences that attempt to represent populations as well as those that are arbitrary conservation units (e.g., occurrences of many migratory birds). Also, the generic criteria allow one to consider all of the variables that affect occurrence viability without having to anticipate them or incorporate them into the occurrence rank criteria. The generic occurrence rank guidelines are much less susceptible to change than are specific quantitative (but arbitrary) criteria. Additionally, the generic occurrence ranking guidelines make it likely that occurrences can be assigned to a rank other than "E," particularly if combination ranks (e.g., AB, AC) are employed.

Most importantly, we believe that the occurrence ranks derived from the generic criteria will be sufficient for conservation prioritization – for identifying a set of target occurrences for conservation action. They should allow users of the ranks to distinguish among occurrences with excellent viability, other robust (good viability) occurrences, occurrences with fair viability, and poor occurrences that have a high risk of extirpation.

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GENERIC GUIDELINES FOR THE APPLICATION OF OCCURRENCE RANKS

Geoffrey A. Hammerson, Dale Schweitzer, Larry Master, and Jay Cordeiro

January 11, 2008

Element occurrence (EO, hereafter simply "occurrence") ranks provide a succinct assessment of the estimated viability (probability of persistence) of occurrences of a given species. They provide an estimation of the likelihood that, if current conditions prevail, a species occurrence will persist for a period of time. Because occurrence ranks are used to represent the relative overall "quality" of an occurrence as it currently exists, they are based solely on criteria that reflect the present status of that occurrence. These criteria can be broadly specified as "rank factors," namely size (including population size and/or occupied area), abiotic and biotic conditions, and landscape context. Future threats should not be used to "downgrade" an occurrence rank, but ongoing events (e.g., successional changes, periodic unfavorable management) that result in inexorable degradation of occurrence quality *should be considered.*

The generic approach to ranking species occurrences focuses on occurrences that are potentially rankable as A, B, C, and D. Many occurrences, such as those based solely on old museum records or on a recent observation with scant data, are not rankable as A, B, C, or D, but they could be ranked as E, H, X, U, F, or NR.

A: Excellent viability. Occurrence exhibits optimal or at least exceptionally favorable characteristics with respect to population size and/or quality and quantity of occupied habitat; and, if current conditions prevail, the occurrence is very likely to persist for the foreseeable future (i.e., at least 20-30 years) in its current condition or better. These occurrences have characteristics (e.g., size, condition, landscape context) that make them relatively invulnerable to extirpation or sustained population declines, even if they have declined somewhat relative to historical levels. For species associated with habitat patches or ephemeral or particularly dynamic habitats, occurrences warranting an A rank generally consist of metapopulations rather than single demes (unless exceptionally large and robust). Occurrences of this rank typically include at least 1,000 mature individuals but may be smaller (100s) or might require larger populations (10,000s), depending on the species and its demographic characteristics. However, occurrences can be ranked A even if population size is not known. For example, for occurrences lacking information on population size, an A rank may be appropriate under the following circumstances: the population is clearly very large but it is not known how large; the area of occupied habitat is exceptionally large; or the occurrence has excellent condition and landscape context and a long history of occurrence persistence. Occurrences with excellent estimated viability are ranked A even if one or more other occurrences have a much larger population size and/or much greater quantity of occupied habitat. In most cases, occurrences ranked A will occupy natural habitats. However, "natural" is an ambiguous concept, and occurrences in "unnatural" conditions (e.g., somewhat modified by human actions) may still be assigned a rank of A if they otherwise meet the criteria.

B: Good viability. Occurrence exhibits favorable characteristics with respect to population size and/or quality and quantity of occupied habitat; and, if current conditions prevail, the occurrence is likely to persist for the foreseeable future (i.e., at least 20-30 years) in its current condition or better. B-ranked occurrences have good estimated viability and, if protected, contribute importantly to maintaining or improving the conservation status of threatened or declining species. For species associated with habitat patches or ephemeral or particularly dynamic habitats, a high-quality occurrence may warrant a B rank if it consists of a single deme rather than a metapopulation (unless the single deme is exceptionally large and robust, in which case an A rank may be appropriate).

C: Fair viability. Occurrence characteristics (size, condition, and landscape context) are non-optimal such that occurrence persistence is uncertain under current conditions, or the occurrence does not meet A or B criteria but may persist for the foreseeable future with appropriate protection or management, or the occurrence is likely to persist but not necessarily maintain current or historical levels of population size or genetic variability. This rank may be applied to relatively low-quality occurrences with respect to size, condition, and/or landscape context if they still appear to have reasonable prospects for persistence for the foreseeable future (at least 20-30 years). Examples include very small non-degraded relict occurrences as well as some remnant occurrences of former landscape-level species such as many extant occurrences of tall-grass prairie insects. These occurrences represent the lower bound of occurrences worthy of protection.

D: Poor viability. If current conditions prevail, occurrence has a high risk of extirpation (because of small population size or area of occupancy, deteriorated habitat, poor conditions for reproduction, ongoing inappropriate management that is unlikely to change, or other factors). Questionably viable occurrences that could be restored to at least fair viability should not be ranked D if restoration is deemed feasible and plausible; in most such cases CD should be used. Very small occurrences that may be vulnerable to deleterious stochastic events may be ranked as follows: If the stochastic event is highly theoretical or of very low probability in the appropriate time frame (e.g., 20-30 years), then a C or CD rank may be appropriate. If a minority of other similar occurrences have disappeared as a result of, say, disease or

inbreeding, then perhaps CD is best. If most of these small occurrences have been extirpated or are disappearing due to such events, then D is probably appropriate. The D rank also applies if the population is so small that there will inevitably be a year (or generation) in the near future in which by chance all adults will be the same gender.

E: Verified extant. Occurrence recently has been verified as still existing, but sufficient information on the factors used to estimate viability of the occurrence has not yet been obtained. Use of the E rank should be reserved for those situations in which the occurrence is thought to be extant, but an A, B, C, D, or combination rank cannot be assigned.

H: Historical. Recent field information verifying the continued existence of the occurrence is lacking. Examples of this rank include occurrences based only on historical collection data, or occurrences that previously were ranked A, B, C, D, or E but that are now, without field survey work, considered to be possibly extirpated due to general habitat loss or degradation of the environment in the area. H may be applied to recently verified occurrences if two or more competent subsequent efforts that should have found the species did not, or if there has been a known major disturbance since the last observation such that continued existence of the occurrence is in doubt (for example, an isolated Lepidoptera occurrence that was sprayed with Dimilin®).

In the absence of known disturbance and with the habitat still extant, H is generally recommended for occurrences that have not been reconfirmed for 20 or more years, but for many short-lived insects a shorter interval may be appropriate, and for unusually stable habitats (like undisturbed caves), or for certain plants whose seeds may persist and remain viable in the soil for decades, a longer interval, up to 40 years, may be used. With very few exceptions, occurrences are to be regarded as H after 40 years without confirmation, even with no effort to locate the species. The time frame for H occurrences is necessarily arbitrary, and the values specified here should be regarded as generally appropriate but somewhat flexible rules. The professional judgment of the assessor should determine when resurveys with negative results have been sufficient in quantity and quality to warrant updating an occurrence rank from F to H or from H to X. Deviations from the suggested time frame should be explained in the EO RANK Comment field.

In some cases, H may indicate occurrences with imprecise locational information such that it may be difficult or impossible to determine whether subsequent observations are of the same occurrence; many of these occurrences may remain H indefinitely. Nevertheless, occurrences with imprecise locational information sometimes may be mapped using an appropriate and reasonable indication of the degree of locational uncertainty.

F: Failed to find. Occurrence has not been found despite a search by an experienced observer at a time and under conditions appropriate for the Element at a location where it was previously reported, but the occurrence still might be confirmed to exist at that location with additional field survey efforts. For occurrences with vague locational information, the search must include areas of appropriate habitat within the range of locational uncertainty.

X: Extirpated. Adequate surveys by one or more experienced observers at times and under conditions appropriate for the species at the occurrence location, or other persuasive evidence, indicate that the species no longer exists there or that the habitat or environment of the occurrence has been destroyed to such an extent that it can no longer support the species.

U: Unrankable. An occurrence rank (including E) cannot be assigned due to lack of sufficient information on the occurrence. As currently defined, this category is not clearly distinguishable from H, and use of U is discouraged until this issue is resolved (perhaps by elimination of the U category). Occurrences that currently cannot be surveyed because of access issues (e.g., a cave entrance has been permanently sealed, or an uncooperative landowner denies access) may be ranked A, B, C, D, E, F, H, or X if the rank is based on recent survey data obtained when access was still possible. Currently inaccessible occurrences that are based only on old (historical) information should be ranked H. Note that access issues often are temporary and may be overcome by negotiation, change in ownership, use of novel survey techniques, or other methods. The U code sometimes has been used to indicate occurrences with "unknown" viability, but such occurrences generally should be coded as H, F, or NR, depending on the circumstances.

NR: Not ranked. An occurrence rank has not been assigned to the occurrence. This category may be used for occurrences that never have been ranked. Additionally, NR may be used for previously ranked occurrences that have been altered to such an extent that the previous rank likely no longer applies but the current appropriate rank is completely unknown. Note that H may be appropriate if there has been a major, presumably detrimental disturbance since the last observation such that continued existence of the occurrence is seriously in doubt (versus unknown).

Dealing with Uncertainty

Note that certain combination ranks (i.e., AB, AC, BC, and CD) are encouraged and should be used to indicate the range of uncertainty regarding the appropriate rank for an occurrence. In fact, due to pervasive limited information about most occurrences, the appropriate rank for most occurrences will be a combination rank. It may be relatively easy to determine an appropriate rank by eliminating clearly inappropriate ranks (e.g., an occurrence is clearly not an A nor a D, so it's BC; or an occurrence appears to be viable and is clearly better than a D, but little else is known, so it's AC). The ranks AD and BD are uninformative regarding conservation value so their use is strongly discouraged; generally E should be used instead.

Attaining Consistency in Occurrence Ranking

Occurrence ranking benefits from multiple opinions and may be accomplished most effectively in an "expert's workshop" setting. Occurrence ranks are best determined by persons who have a good understanding of the population characteristics of the species or who at least have good basic knowledge of the biology and ecology of the group of organisms to which the species belongs. Such knowledge allows the ranker to make a good forecast about the viability of a particular occurrence. The rationale for each rank should be recorded in the EO Rank Comments field in Biotics.

Beyond Occurrence Ranks

For purposes such as monitoring the response of an occurrence to management actions, habitat mitigation, and ecological restoration, occurrence ranks, whether qualitatively or quantitatively defined, will be insufficient. Instead, efforts will need to be made to identify the key ecological attributes (subcomponents of the rank factors) that play an important (driving) function in the viability of the species. For each key ecological attribute, specific indicators or metrics can then be selected that will reflect changes in the viability of a species. For example, "reproductive success" is an indicator of the "reproduction and health" key ecological attribute (a subcomponent of condition), and the specific metric is "number of fledged young."

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HYPOTHETICAL EXAMPLES OF OCCURRENCES WITH SUGGESTED RANKS

Colonial seabird: occurrence consists of 5 adjacent islands, all of which are protected; nesting population recently was estimated at 400,000 pairs, with no evidence of decline compared to estimates made 25 years ago. Suggested rank: A. An occurrence with these highly favorable characteristics is very likely to persist for the foreseeable future (i.e., at least 20-30 years) in its current condition or better.

Pool-breeding ambystomatid salamander of forested landscapes: occurrence is represented by several breeding sites; pools contain dozens to hundreds of egg masses; breeding sites are surrounded by an extensive tract of mature forest. Suggested rank: AB. This occurrence, which likely represents a metapopulation, can be expected to exhibit at least good viability. Further information on occurrence size, condition, and landscape context might allow one to distinguish between A or B.

Pond-breeding odonate: occurrence encompasses a complex of several neighboring wetland patches of various sizes of up to a few hectares; species was first discovered in this location 75 years ago; recent quick surveys yielded multiple adults and exuviae in nearly every patch; wetland and adjacent upland habitat are protected and not subject to pesticide applications. Suggested rank: AB. This occurrence, which likely represents a metapopulation and for which long-term persistence has been documented, can be expected to exhibit at least good viability. Further information on population size, habitat condition, and landscape context might allow one to distinguish between A or B.

Small mammal of arid basins: recent random surveys indicate that the occurrence extends over at least 100 square kilometers; habitat appears to be relatively unaltered, stable, and compatibly managed; population size and density are unknown, but the species is readily detected. Suggested rank: AB. This evidence suggest an at least good probability of continued occurrence persistence in the present condition or better. Nothing here appears to compromise the viability of the occurrence.

Snake of rocky desert mountains: recent 1-day survey found 9 individuals scattered over 2 patches of talus habitat in a single remote mountain range (not readily accessible by road); suitable habitat encompasses several additional adjacent patches totaling a few square kilometers of basically undisturbed

talus. Suggested rank: AB. Snakes in such habitats generally are difficult to observe in quantity, so seeing this many in one day suggests an ample population. Evidence indicates that the habitat is not subject to much disturbance and can be expected to persist in at least good condition for the foreseeable future.

Tadpole shrimp of ephemeral pools: known occurrence consists of a single 1-hectare basin in a landscape altered only by seasonal grazing of livestock, which has occurred over several decades; 25 specimens were collected from the site 20 years ago; last year a single specimen was collected (abundance unknown); the basin was dry this year; unknown whether or not there are any nearby occupied pools. Suggested rank: AC. Tadpole shrimp eggs can persist for years in a dry basin, and these shrimp can be dispersed among basins by birds and other mobile animals. In a particular location, tadpole shrimp may be present for one or more years, then absent for a variable number of years and, when present, they may range from scarce to abundant. Presence of tadpole shrimp in a basin in multiple years over a wide time frame suggests that the basin will continue to support a population (although perhaps irregularly) for the foreseeable future, if current conditions prevail, so the rank--even for this pool alone--is better than D. Further information on population size and landscape context (e.g., presence of nearby occupied pools) might allow one to define the rank more precisely. In particular this pool might or might not prove to be merely a portion of a larger A or B quality metapopulation.

Seed-banking annual plant of river sand bars: isolated relict species; population of many 10,000s of mature individuals occupies much of a 90-km stretch of river corridor; abundance fluctuates greatly from year to year; population appears to be negatively affected by hydrological and sediment transport alterations associated with present dam operations. Suggested rank: BC. The occurrence exhibits good size characteristics but current circumstances indicate that a small or large decline might be occurring. Though the occurrence may persist for the foreseeable future in good or excellent condition, it is also possible that current conditions are resulting in a major decline that could significantly jeopardize occurrence viability. The combination rank reflects this substantial uncertainty.

Pool-breeding amphibian of forested landscapes: occurrence consists of a single, isolated breeding pool and surrounding, mostly intact uplands; 20 percent of the upland area contains rural residential development; single recent survey found several hundred egg masses in the breeding pool. Suggested rank: B. Evidence indicates a substantial population that is, however, confined to a single pool, and the upland habitat has been somewhat degraded, so the occurrence does not meet the criteria for an A rank. On the other hand, an occurrence such as this is likely to persist if current conditions prevail, hence it is not a D occurrence. Given the large number of egg masses, the occurrence has good prospects for persistence and appears to be better than a marginal or C occurrence.

Turtle of freshwater wetlands: population of approximately 80-100 mature individuals, plus various age classes of immatures, occupies most of a 15-hectare wetland; no indication of a major increase or decline but few data are available for trend estimation; wetland is in a wildlife sanctuary and protected from outright destruction. Suggested rank: B. Ideally, A-ranked occurrences should be represented by thousands of adults, but in the case of long-lived species such as turtles, a population of hundreds of adults likely would exhibit excellent viability. This occurrence does not exhibit optimal population size for excellent viability, yet the prospects for long-term persistence without additional protection or management appear to be very good, so the rank should be less than A and higher than C.

Tiger beetle of coastal sand dunes: cursory surveys during the appropriate season consistently yield observations suggesting that a population of at least a few hundred adults (and undetermined numbers of larvae) occupies a suitable habitat patch of 25 hectares in a national park; museum collection records for the area extend back at least 50 years; habitat has natural processes largely intact but is partially encroached on by a small asphalt parking lot that has been present for at least 30 years, and a small portion of the habitat is subject to light foot traffic by humans. Suggested rank: BC. Nonoptimal habitat conditions, and evidence of long-term persistence of a currently significant population, eliminate A and D ranks, respectively. Further information on population size and landscape context (e.g., presence of nearby occupied patches) might allow one to define the rank more precisely.

Small mammal of coastal dunes: occurrence extends along 3 kilometers of protected coastal dunes; surrounding areas are heavily developed (hotels, parking lots, etc.); species was first documented at the site 30 years ago; each of several live-trapping efforts in recent years yielded at least a few individuals per 100 trap-nights. Suggested rank: BC. Limited size and less than highly favorable landscape context eliminate an A rank, and documented persistence over at least 30 years indicate that the occurrence rank is better than D. Further information on population size is needed to determine whether the rank should be B or C.

Perennial herb of calcareous habitat: occurrence restricted to limestone barrens on isolated peninsula of 363 hectares (864 acres) on marine coast; outlier population from main range (~1,600 kilometers away); past quarrying stopped with protection as provincial natural reserve in 1998; harsh environment resulting in short growing season but possible annual seed set and seeds dispersed by wind; no recent surveys for

numbers of plants but observed sporadic distribution throughout peninsula. Suggested rank: BC. Available information indicates that this occurrence does not exhibit optimal or highly favorable size, condition, and landscape context, and it does not suggest an occurrence with a high probability of extirpation, so the A and D ranks can be ruled out. Further information on population size (100s?, 1,000s?) should allow one to define the rank more precisely.

Butterfly associated with a wetland food plant: populations are naturally fragmented throughout the range; species has been known from this site for more than 100 years, and the size and condition of the site appears to have changed little over the past several decades; population occupies a small patch of wetland (stable bog or fen, about 1 hectare); nearest occupied habitat is 50 km away; 5-30 adults seen during recent 2-hour surveys during peak flight season (note that this is the number observed, not the total number of adults present); wetland is on a nature preserve with good prospects for appropriate habitat management. Suggested rank: C. The small size of this occurrence precludes the A and B ranks, while long-term persistence and stable conditions eliminate the D rank.

Passerine bird associated with old field conditions: recent surveys (3-5 years old) found 25-30 singing males during the nesting season; occupied habitat patch is 100 hectares, surrounded by forest and residential development; reforestation is slowly occurring; land is divided among several private owners. Suggested rank: CD. Certainly the size, condition, and landscape context are not optimal or highly favorable, so it is not an A occurrence. If existing conditions (including ongoing reforestation) prevail, the occurrence is likely to undergo habitat degradation and thus not to maintain its current condition, so a rank of B can be eliminated. Appropriate management is at least plausible and probably could maintain this population for the foreseeable future in its current condition or better, so the C rank cannot be eliminated. Because persistence of this occurrence for the foreseeable future depends on appropriate management that may not occur (in which case the occurrence may be extirpated), a rank of CD is warranted.

Long-lived perennial plant of wetlands: occurs only on the edges of wet sphagnum bogs; recent survey found 19 plants, 60% in leaf only, 40% in fruit; 40% seedlings, 20% immature, 40% mature; occupied area is less than 1 hectare; in 2005 there were three main patches of plants, and at least one subsequently was destroyed by bulldozing to expand a cranberry bog; two remaining patches are extant but need follow-up survey. Suggested rank: C or CD. The small size of this occurrence, and its poor condition and landscape context, indicate that A or B ranks are not appropriate. Under current conditions, the probability of persistence seems low, so the occurrence may warrant a rank of D, but evidence of ongoing reproduction suggests that a C rank might be appropriate, especially if only a small proportion of the remaining plants were destroyed and there is a reasonable chance that the remaining habitat can be protected and appropriately managed.

Freshwater mussel of riverine habitat: extensive searching by experienced mussel biologists yields only a few mature individuals and no younger age classes; despite several surveys, no other occupied habitat patches have been found in this river in recent decades. Suggested rank: CD or D. This occurrence might not be completely devoid of viability; some mussels have a long life span, and it is plausible that conditions could change for the better. For example, riverine fish faunas are notoriously dynamic, and arrival of a new host fish could allow a renewal of successful reproduction.

Perennial plant of wooded landscapes: historical records indicate that the species was fairly common in the several hectares encompassed by the occurrence; recent targeted surveys yielded only a few individuals, all browsed by deer; deer population in the area is large and unlikely to decrease. Suggested rank: CD. Clearly not an occurrence with excellent or good estimated viability (too small, poor condition and landscape context), so not an A or B occurrence. Construction of a deer exclosure might prevent total loss of the remaining population, but otherwise the occurrence has a high probability of extirpation in the foreseeable future, so the appropriate rank is CD.

Passerine bird associated with grassland habitat: in recent years 2-3 singing males have been present in May in a 5-hectare hayfield surrounded by residential development and young forest; a few fledglings were observed in July in one of the years. Suggested rank: D. This information substantiates the existence of a legitimate occurrence, but very small size and poor landscape context indicate a high probability of extirpation in the foreseeable future. Feasible management could not do much to improve the prospects for persistence.

Short-lived perennial desert plant, emergence dependent upon spring rains: occurrences and abundance vary widely from year to year in response to variations in precipitation): 78 plants found in intensive survey in 1989, 15 plants observed in brief 1990 survey, 4 plants in 1991 late-season survey, 5 plants observed in 1998 at peak season with fairly intensive surveys after favorable precipitation conditions; site is seasonally grazed by sheep, which decreases the abundance of the plant, and change in management is unlikely. Suggested rank: D. The small size and poor condition of this occurrence immediately rule out A and B ranks. Available data suggest a significant declining trend and high probability of extirpation if current conditions prevail.

Pond-breeding toad: occurrence encompasses a single pond and surrounding uplands; pond is protected but most habitat around the pond has been converted to residential development over the past decade; recent surveys in appropriate season yielded a total of 3 egg masses in the pond. Suggested rank: D. Extremely small size and poor condition and landscape context make it highly unlikely that this occurrence will persist, let alone ever become a viable occurrence.

Remnant grassland butterfly: small habitat scrap of 4 hectares of unmanaged dry native grassland, surrounded by residential development and with a cleared party spot with scraps of burned wood near the middle; absentee owner; neighbors recently started using part of the area for motorcycle recreation; the area mark-release-recapture studies estimated 30, 40, 35 and 50 adults within the past decade; 20 years ago there were three other comparable occupied habitats within 2 kilometers but now none are known within 50 km; populations of this species commonly decline (often in dry years) to as low as 10-20% of the mean during a 10-20 year period; according to old timers and anecdotal literature from several states colonies often suddenly appeared or disappeared; data indicate that immature stages are usually reduced by 90-100% following fires; a huge majority of suitable habitat patches in the state no longer support the species. Suggested rank: D. With a mean of about 39 adults this apparently stable population is expected to fall to around 4-8 adults within 10-20 years from just routine fluctuations and certainly could not recover from two consecutive bad years, and might not from one. Furthermore, fires are nearly inevitable considering current context and uses and are most likely during already bad (dry) years. This occurrence has a high risk of extirpation during the next 20-30 years, and it appears to be implausible that the occurrence will be restored to at least fair viability through appropriate management.

Wetland turtle: Information from 10 years ago established an occurrence in a difficult-to-access wetland; this occurrence was then regarded as a viable occurrence and was ranked BC. Aerial photos taken last year indicate extensive alteration of both the wetland and upland habitat adjacent to the wetland over the past decade. Last year, a reliable person observed and photo-documented a single adult turtle on the site, but other recent information about the population is not available. Suggested rank: E. This information indicates that the rank might be as high as B (if the turtle population has not been negatively affected by the habitat changes) or as low as D (e.g., if the only suitable nesting habitat has been destroyed). An occurrence rank of BD is uninformative for conservation purposes. Because available information is too limited to determine the estimated viability of this occurrence, the occurrence should be categorized only as extant.

Perennial herbaceous plant of upland forests: A botanist collected the species last year and deposited a specimen in an herbarium, but nothing else is known about the occurrence. Suggested rank: E. If we assume the collected specimen was not the last remaining individual, we can consider the occurrence to be extant. Further information is required in order to categorize the occurrence status more precisely.

Wetland turtle: Collection information from 50 years ago established an occurrence in a difficult-to-access wetland; this occurrence was then regarded as a viable occurrence and was ranked B. No further information about the turtle population is available. Recent aerial photos of the area indicate extensive alteration of both the wetland and upland habitat adjacent to the wetland over the past two decades. Suggested rank: H. This occurrence needs to be surveyed in order to verify that it still supports a turtle population and to determine current occurrence viability.

Herbaceous plant of interior, stabilized sand dunes: A university museum includes a specimen collected in the 1940s from a vague location in a dune area, which still exists and is largely intact. Nothing else is known about the occurrence, and the area has not been well surveyed. Suggested rank: H. This information is too old to support an occurrence rank other than H. The occurrence needs to be surveyed in order to verify that it still supports a population and to ascertain occurrence viability.

Wetland orchid: In the 1960s a small population of a wetland orchid was documented on a site on private land that was formerly accessible through landowner permission. Today the site is no longer accessible due to an uncooperative new landowner. Suggested rank: H. Because this occurrence is based only on old information, the appropriate rank is H. The occurrence rank could be updated in the future if access to the site improves.

Cave invertebrate: Surveys 50 years ago found several individuals in a cave pool. Subsequently, the opening used for access to the cave was sealed and likely will remain so indefinitely. No further information on the occurrence is available. Suggested rank: H. Because this occurrence is based only on old information, the appropriate rank is H. In the future, improved remote sensing/survey techniques might allow the rank to be updated. If prior to sealing of the cave entrance the occurrence had been ranked A, B, C, D, or E based on recent surveys, that rank would still apply until the time frame suggested invoking the H rank, unless there is a significant probability that cave sealing might have affected the viability of the occurrence, in which case the appropriate rank would be H.

Rattlesnake that uses communal hibernacula: Museum specimens and a published account from 15 years ago document the existence of a denning population somewhere on remote Snake Mountain. Last year a well-timed, one-day survey failed to find any rattlesnakes, but much suitable, difficult-to-access habitat was not searched. Suggested rank: F. The recent brief survey does not preclude the possibility that the occurrence is still extant, so the appropriate rank is F.

Toad that uses ephemeral bodies of water for breeding: Surveys in the 1970s found a several breeding toads in and around a shallow pool in a prairie landscape then used as cattle pasture. Today the site has been completely replaced by commercial development, and the former breeding site has been filled and covered with asphalt. Recent searches and examination of recent aerial photographs found no evidence of suitable breeding habitat anywhere within a few kilometers of the previous breeding site, and there are no other records of the occurrence of this species in the area. Suggested rank: X. This evidence indicates that it is appropriate to regard the occurrence as extirpated.

Grassland songbird: Surveys in the late 1970s found a few nesting pairs around the margins of a large pasture. Since then, the species has not been detected in that area or in other locations in the vicinity, despite surveys at the appropriate time by expert birders in each of the past three years. Suggested rank: X. This evidence is sufficient to regard the occurrence as extirpated. If the evidence of extirpation were not so conclusive (i.e., there is a reasonable probability that the species is still there but was not detected), then the appropriate rank would be H.

Spring-dwelling fish: Surveys done 5 years ago documented a robust population in a natural spring-fed pool; on this basis the occurrence was ranked B. Examination of aerial photos taken last year and discussions with local residents indicate that the pool has been significantly modified in size and shape, but new fish surveys have not been conducted. Suggested rank: NR. Available information is insufficient to determine whether the changes have been positive, negative, or neutral; at one extreme, the fish may have been eradicated (perhaps in conjunction with the introduction of a predatory species in the modified habitat), or perhaps the changes have benefited the native fish population. Because of the altered conditions, the basis for the previous rank of B no longer applies, and the status of the occurrence needs to be redetermined; meanwhile, the rank should be updated to NR.

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Appendix C.13

Wisconsin Natural Heritage Inventory (NHI)

Recognized Natural Communities – Working Document

Wisconsin Natural Heritage Inventory (NHI)

Recognized Natural Communities – Working Document

Prepared by Eric Epstein, Emmet Judziewicz and Elizabeth Spencer

This document will be periodically updated and expanded. Future editions will include or be linked to additional descriptive information, range maps, and crosswalks to other vegetation classification systems.

Alder Thicket

These wetlands are dominated by thick growths of tall shrubs, especially speckled alder (Alnus incana). Among the common herbaceous species are Canada bluejoint grass (Calamagrostis canadensis), orange jewelweed (Impatiens capensis), several asters (Aster lanceolatus, A. puniceus, and A. umbellatus), boneset (Eupatorium perfoliatum), rough bedstraw (Galium asprellum), marsh fern (Thelypteris palustris), arrow-leaved tearthumb (Polygonum sagittatum), and sensitive fern (Onoclea sensibilis). This type is common and widespread in northern and central Wisconsin, but also occurs in the southern part of the state.

Algific Talus Slope

This rare community of southwestern Wisconsin's Driftless Area consists of steep slopes of fractured limestone (dolomite) rock that retains ice and emits cold air throughout the growing season. The cold microhabitats enable the persistence of northern species and "periglacial relicts" such as northern monkshood (Aconitum noveboracense) and rare terrestrial snails. The woody overstory is often sparse, with scattered small black ash (Fraxinus nigra) and white birch (Betula papyrifera). Mountain maple (Acer spicatum), a northern shrub, may be frequent and extensive beds of bulblet fern (Cystopteris bulbifera) and mosses are characteristic.

Alkaline Clay Bluff (now called Clay Seepage Bluff)

Alvar

This rare community consists of areas of thin discontinuous soil overlying horizontal beds of limestone or dolomite in the vicinity of Great Lakes shorelines. They are characterized by relatively low tree cover and a distinctive biota which includes elements of rock pavement, prairie, savanna and boreal forest communities. Among these are regional endemics, some very rare. This community type is much more common and better-developed in Michigan and Ontario than in Wisconsin. Small coniferous and deciduous trees (cedar, fir, pine, oak, aspen, birch) are scattered among an assemblage of species that can include big bluestem (Andropogon gerardii), little bluestem (Schizachyrium scoparium), Indian-grass (Sorghastrum nutans), and wood lily (Lilium philadelphicum), as well as shoreline plants such as silverweed (Potentilla anserina) and dwarf lake iris (Iris lacustris).

Bedrock Glade

These are xeric, sparsely vegetated non-vertical bedrock exposures with very thin, often discontinuous soils. The rock types vary from quartzite (Baraboo Hills, McCaslin Mountain), to basalt (lower St. Croix River valley), to granite (northeastern Wisconsin). The flora can include prairie, savanna, or barrens components, some at their northern range limits. Trees and shrubs are sparse and may include pines, oaks, and cherries. Xerophytic pteridophytes such as rusty woodsia (Woodsia ilvensis) and rock spikemoss (Selaginella rupestris) are characteristic, as are lichens and mosses.

Bedrock Shore

Wave-splashed bedrock shoreline ledges are best developed on sandstone in the Apostle Islands of Lake Superior. Stunted trees of white cedar (Thuja occidentalis), white birch (Betula papyrifera), showy mountain-ash (Sorbus decora) and green alder (Alnus crispa) are often present in crevices. Common herbs are ticklegrass (Agrostis hyemalis), fireweed (Epilobium angustifolium), and Canada goldenrod (Solidago canadensis), but the flora often includes unusual plants such as bird's-eye primrose (Primula mistassinica), brook lobelia (Lobelia kalmii), and three-toothed cinquefoil (Potentilla tridentata).

Black Spruce Swamp (A split from Curtis' Northern Wet Forest)

An acidic conifer swamp forest characterized by a relatively closed canopy of black spruce (Picea mariana) and an open understory in which Labrador-tea (Ledum groenlandicum) and sphagnum mosses (Sphagnum spp.) are often prominent, along with three-leaved false Solomon's-seal (Smilacina trifolia), creeping snowberry (Gaultheria procumbens), and three-seeded sedge (Carex trisperma). The herbaceous understory is otherwise relatively depauperate. This community is closely related to Open Bogs and Muskegs, and sometimes referred to as Forested Bogs outside of Wisconsin.

Bog Relict

These boggy, acidic, weakly minerotrophic peatlands occur south of the Tension Zone within a matrix of "southern" vegetation. Bog relicts are isolated from the more extensive, better-developed and much more widespread stands of this community found in the northern part of the state. Acidophiles present can include sphagnum mosses (Sphagnum spp), sedges (e.g., few seeded sedge, Carex oligosperma), ericaceous shrubs, and insectivorous herbs. Tamarack (Larix laricina) is usually the most common tree and poison-sumac (Toxicodendron vernix) is often formidably abundant in the understory, especially in the moat (or "lagg") at the upland/wetland interface. Examples in southeastern Wisconsin are all somewhat alkaline and may resemble "shrub-fen" communities described in other states.

Boreal Forest

In Wisconsin, mature stands of this forest community are dominated by white spruce (Picea glauca) and balsam-fir (Abies balsamea), often mixed with white birch (Betula papyrifera), white cedar (Thuja occidentalis), white pine (Pinus strobus), balsam-poplar (Populus balsamifera) and quaking aspen (Populus tremuloides). Mountain-ash (Sorbus spp.) may also be present. Common understory herbs are large-leaved aster (Aster macrophyllus), bluebead lily (Clintonia borealis), Canada mayflower (Maianthemum canadense), wild sarsaparilla (Aralia nudicaulis), and bunchberry (Cornus canadensis). Most Wisconsin stands are associated with the Great Lakes, especially the clay plain of Lake Superior, and the eastern side of the northern Door Peninsula on Lake Michigan. Of potential interest from the perspectives of vegetation classification and restoration, white pine had the highest importance value of any tree in the Lake Superior region, as recorded during the original land survey of the mid-1800's.

Boreal Rich Fen

Neutral to alkaline cold open peatlands of northern Wisconsin through which carbonate-rich groundwater percolates. Sphagnum mosses are absent or of relatively minor importance, as calciphilic species (especially the "brown" mosses) predominate. Dominant/characteristic plants include woolly sedge (Carex lasiocarpa), twig rush (Cladium mariscoides), beaked bladderwort (Utricularia cornuta), rushes (Juncus spp.), and Hudson Bay cotton-grass (Scirpus hudsonianus). Shrubby phases also occur, with bog birch (Betula pumila), sage willow (Salix candida), and speckled alder (Alnus incana) present in significant amounts.

Bracken Grassland

These are open upland areas, in northern Wisconsin on sandy soils, dominated by bracken fern (Pteridium aquilinium), Penn sedge (Carex penyslvanica), Kalm's bromegrass (Bromus kalmii), and Canada bluegrass (Poa compressa). There may be a high cover of low shrubs such as blueberries (Vaccinium angustifolium and V. myrtilloides), sweet fern (Comptonia peregrina), prairie willow (Salix humilis), and hazelnuts (Corylus spp.). Other common herbs include poverty oat-grass (Danthonia spicata), Lindley's aster (Aster ciliolatus), gray goldenrod (Solidago nemoralis), and common strawberry (Fragaria virginiana). Exotics are often frequent. There is disagreement on whether bracken grassland should be considered a "natural community" in Wisconsin and elsewhere in the Upper Great Lakes region.

Calcareous Fen

An open wetland found in southern Wisconsin, often underlain by a calcareous substrate, through which carbonate-rich groundwater percolates. The flora is typically diverse, with many calciphiles. Common species are several sedges (Carex sterilis and C. lanuginosa), marsh fern (Thelypteris palustris), shrubby cinquefoil (Potentilla fruticosa), shrubby St. John's-wort (Hypericum kalmianum), Ohio goldenrod (Solidago ohioensis), grass-of-parnassus (Parnassia glauca), twig-rush (Cladium mariscoides), brook lobelia (Lobelia kalmii), boneset (Eupatorium perfoliatum), swamp thistle (Cirsium muticum), and asters (Aster spp.). Some fens have significant prairie or sedge meadow components, and intergrade with those communities.

Cedar Glade

Dry sandstone, quartzite or dolomite exposures vegetated with dense thickets of red cedar (Juniperus virginiana). Red maple (Acer rubrum), Paper birch (Betula papyrifera) and black and bur oaks (Quercus velutina and Q. macrocarpa) may also be present. This community is usually if not always the result of fire suppression on dry prairies, and in pre-settlement times it may have occurred only where extensive cliffs served as firebreaks. Common herbs include bluestem and grama grasses (Andropogon spp. and Bouteloua spp.), prickly-pear cactus (Opuntia compressa), flowering spurge (Euphorbia corollata), stiff sandwort (Arenaria stricta) and gray goldenrod (Solidago nemoralis).

Central Poor Fen

These open, acidic, low nutrient peatlands occur within the Central Sand Plains of Wisconsin. Central poor fens are floristically depauperate and generally sedge dominated, (Carex oligosperma, C. lasiocarpa, and C. utriculata) Bluejoint grass (Calamagrostis canadensis) is a frequent associate and may co-dominate in some stands. Sphagnum spp. carpets are common but typically lack pronounced hummocks and hollows. Shrubs are present but not dominant, Hard-hack (Spirea tomentosa) is the most consistent in presence, and cover of ericads is generally low. Other characteristic associates include wool grass (Scirpus cyperinus), cotton-grasses (Eriophorum spp.), swamp-candles (Lysimachia terrestris) and Kalm’s St. John’s-wort (Hypericum kalmianum). This community often intergrades with Tamarack (poor) Swamp. Disturbance of this community through mossing may significantly alter community composition, as recolonization by at least some of the vascular plants is very slow. Many plants characteristic of poor fen communities farther north are rare or absent in these central sands peatlands.

Central Sands Pine-Oak Forest

This forest community is associated with the Central Sands ecoregion on dry to dry-mesic sites with acid sandy soils. The dominants are white and red pines (Pinus strobus and P. resinosa), oaks (Quercus alba, Q. rubra, and Q. velutina), and on dry-mesic sites, red maple (Acer rubrum). The understory is typically depauperate consisting primarily of huckleberry (Gaylussacia baccata), early blueberry (Vaccinium angustifolium), bracken fern (Pteridium aquilinium), wood anemone (Anemone quinquefolia) and Penn sedge (Carex pensylvanica). Jack pine (Pinus banksiana) is sometimes co-dominant on the driest sites (jack pine – black / Hills oak dominated stands maybe split out in the future).

Coastal Fen (now called Shore Fen)

Coastal Plain Marsh

Sandy to peaty-mucky lakeshores, pondshores, depressions, and ditches in and around the bed of extinct glacial Lake Wisconsin may harbor assemblages of wetland species including some which are significantly disjunct from their main ranges on the Atlantic Coastal Plain. There is often a well-developed concentric zonation of vegetation. Frequent members of this community are sedges in the genera Cyperus, Eleocharis, Fimbristylis, Hemicarpha, Rhynchospora and Scirpus; rushes (Juncus spp.); milkworts (Polygala cruciata and P. sanguinea), toothcup (Rotala ramosior), meadow-beauty (Rhexia virginica), grass-leaved goldenrod (Euthamia graminifolia), hardhack (Spiraea tomentosa), lance-leaved violet (Viola lanceolata), and yellow-eyed grass (Xyris torta).

Clay Seepage Bluff (formerly called Alkaline Clay Bluff)

Steep, clay bluffs occur along some stretches of the Great Lakes shorelines and less commonly inland on streams draining into Lake Superior and Lake Michigan. Vegetative cover ranges from forested with pines (Pinus resinosa and P. strobus), white cedar (Thuja occidentalis) and white birch (Betula papyrifera), to bare clay with only a few herbs present. Buffaloberry (Sheperdia canadensis) is a characteristic shrub, but more typically, alders (Alnus incana and A. crispa), as well as herbs such as Canada goldenrod (Solidago canadensis) and pearly everlasting (Anaphalis margaritacea) are dominant. Both native and exotic pioneers such as fireweed (Epilobium angustifolium) and Canada thistle (Cirsium arvense) are common, especially on unstable sites. But it is the semi-stabilized “weeping” bluffs that are of the greatest biological interest. Golden sedge (Carex aurea), orchids and calciphilic fen species may colonize such sites, which can be local repositories of rare or otherwise noteworthy species.

Dry Cliff (**Exposed Cliff** of Curtis’ community classification)

These dry vertical bedrock exposures occur on many different rock types, which may influence species composition. Scattered pines, oaks, or shrubs often occur. However, the most characteristic plants are often the ferns, common polypody (Polypodium vulgare) and rusty woodsia (Woodsia ilvensis), along with herbs such as columbine (Aquilegia canadensis), harebell (Campanula rotundifolia), pale corydalis (Corydalis sempervirens), juneberry (Amelanchier spp.), bush-honeysuckle (Diervilla lonicera), and rock spikemoss (Selaginella rupestris).

Dry Prairie

This grassland community occurs on dry, often loess-derived soils, usually on steep south or west facing slopes or at the summits of river bluffs with sandstone or dolomite near the surface. Short to medium-sized prairie grasses: little bluestem (Schizachyrium scoparium), side-oats grama (Bouteloua curtipendula), hairy grama (B. hirsuta), and prairie dropseed (Sporobolus heterolepis), are the dominants in this community. Common shrubs

and forbs include lead plant (Amorpha canescens), silky aster (Aster sericeus), flowering spurge (Euphorbia corollata), purple prairie-clover (Petalostemum purpureum), cylindrical blazing-star (Liatris cylindracea), and gray goldenrod (Solidago nemoralis). Stands on gravelly knolls in the Kettle Moraine region of southeastern Wisconsin and along the St. Croix River on the Minnesota – Wisconsin border may warrant recognition, at least at the subtype level.

Dry-Mesic Prairie

This grassland community occurs on slightly less droughty sites than Dry Prairie and has many of the same grasses, but taller species such as big bluestem (Andropogon gerardii) and Indian-grass (Sorghastrum nutans) dominate. Needle grass (Stipa spartea) may also be present. The herb component is more diverse than in Dry Prairies, including many species that occur in both Dry and Mesic Prairies.

Emergent Aquatic

These open, marsh, lake, riverine and estuarine communities with permanent standing water are dominated by robust emergent macrophytes, in pure stands of single species or in various mixtures. Dominants include cat-tails (Typha spp.), bulrushes (particularly Scirpus acutus, S. fluviatilis, and S. validus), bur-reeds (Sparganium spp.), giant reed (Phragmites australis), pickerel-weed (Pontederia cordata), water-plantains (Alisma spp.), arrowheads (Sagittaria spp.), and the larger species of spikerush such as (Eleocharis smallii).

Emergent Aquatic - Wild Rice

This open community is an emergent macrophyte type, with wild rice (Zizania aquatica or Z. palustris) as the dominant species. The substrate usually consists of poorly-consolidated, semi-organic sediments. Water fertility is low to moderate, and a slow current is present. Wild rice beds have great cultural significance to native peoples, and are important wildlife habitats.

Ephemeral Pond

These ponds are depressions with impeded drainage (usually in forest landscapes), that hold water for a period of time following snowmelt but typically dry out by mid-summer. Common aquatic plants of these habitats include yellow water crowfoot (Ranunculus flabellaris), mermaid weed (Proserpinaca palustris), Canada bluejoint grass (Calamagrostis canadensis), floating manna grass (Glyceria septentrionalis), spotted cowbane (Cicuta maculata), smartweeds (Polygonum spp.), orange jewelweed (Impatiens capensis), and sedges. Ephemeral ponds provide critical breeding habitat for certain invertebrates, as well as for many amphibians such as frogs and salamanders.

Felsenmeer

This rare open primary community consists of steep slopes of quartzite or other metamorphic rock boulders (.25 - 1 meters in diameter) formed by periglacial frost and ice-wedging, and characterized by cool, moist air drainage at or near their base. The vegetation is fairly sparse but may be structurally variable. Lichens especially (Lasallia) are the dominant cover on the boulders. Scattered soil pockets may occur and support scattered white and red pines (Pinus strobus and P. resinosa) often in association with mossy beds of common polypody (Polypodium virginianum) or marginal shield fern (Dryopteris marginalis). The slope base in the zone of cool air drainage is typically shrub dominated, and may include a number of species somewhat disjunct from their more northern ranges, such as squashberry (Viburnum edule), Canada gooseberry (Ribes oxyacanthides). Other frequently occurring shrub or small tree species are Labrador-tea (Ledum groenlandicum), mountain maple (Acer spicatum), mountain ash (Sorbus spp.) and red-berried elder (Sambucus pubens). The vine, purple clematis (Clematis occidentalis) and tree, balsam fir (Abies balsamea) may also be present. This community type has been incompletely surveyed and occurrences should be carefully examined for rare bryophytes, lichens and terrestrial snails.

Floodplain Forest (replaces in part the **Southern Wet** and **Southern Wet-Mesic Forests** of Curtis**)**

This is a lowland hardwood forest community that occurs along large rivers, usually stream order 3 or higher, that flood periodically. The best-development occurs along large rivers in southern Wisconsin, but this community is also found in the north. Canopy dominants may include silver maple (Acer saccharinum), river birch (Betula nigra), green ash (Fraxinus pennsylvanica), hackberry (Celtis occidentalis), swamp white oak (Quercus bicolor), and cottonwood (Populus deltoides). Northern stands are often species poor, but balsam-poplar (Populus balsamifera), bur oak (Quercus macrocarpa), and box elder (Acer negundo) may replace some of the missing "southern" trees. Buttonbush (Cephalanthus occidentalis) is a locally dominant shrub and may form dense thickets on the margins of oxbow lakes, sloughs and ponds within the forest. Nettles (Laportea canadensis and Urtica dioica), sedges, ostrich fern (Matteuccia struthiopteris) and gray-headed coneflower (Rudbeckia laciniata) are important understory herbs, and lianas such as Virginia creepers (Parthenocissus spp.), grapes (Vitis spp.), Canada moonseed (Menispermum canadense), and poison-ivy (Toxicodendron radicans) are often common. Among the striking and characteristic herbs of this community are cardinal flower (Lobelia cardinalis) and green dragon (Arisaema dracontium).

Forested Ridge and Swale (now called **Great Lakes Ridge and Swale**)

Forested Seep

These are shaded seepage areas with active spring discharges in (usually) hardwood forests that may host a number of uncommon to rare species. The overstory dominant is frequently black ash (Fraxinus nigra), but yellow birch (Betula allegheniensis), American elm (Ulmus americana) and many other tree species may be present including conifers such as hemlock (Tsuga canadensis) or white pine (Pinus strobus). Understory species include skunk cabbage (Symplocarpus foetidus), water-pennywort (Hydrocotyle americana), marsh blue violet (Viola cucullata), swamp saxifrage (Saxifraga pennsylvanica), golden saxifrage (Chysosplenium americanum), golden ragwort (Senecio aureus), silvery spleenwort (Athyrium thelypterioides) and the rare sedges (Carex scabrata and C. prasina). Most documented occurrences are in the Driftless Area, or locally along major rivers flanked by steep bluffs.

Great Lakes Alkaline Rockshore

These creviced, wave-splashed, nearly horizontal dolomite ledges are restricted geographically to shoreline exposures along Lake Michigan on the northern Door Peninsula. Depending on lake levels, large expanses of this habitat may be either inundated or exposed during a given year. Common members of this community are the shrubs ninebark (Physocarpus opulifolius), shrubby cinquefoil (Potentilla fruticosa), and the herbs silverweed (Potentilla anserina), goldenrods (especially Solidago hispida), brook lobelia (Lobelia kalmii), gentians (Gentiana spp.), grasses-of Parnassus (Parnassia spp.), Indian paint-brush (Castilleja coccinea), low calamint (Calamintha arkansana) and many other calciphiles. Plants endemic to the Great Lakes shores are significant components of some stands.

Great Lakes Barrens

In Wisconsin, this variant of pine savanna is known from only one sandy site on Lake Superior. The dominant trees in this open stand are wind- and fire-deformed trees, red pines (Pinus resinosa) with white pine (P. strobus) also present. The understory consists of dense growths of lichens with scattered thickets of common juniper (Juniperus communis), early blueberry (Vaccinium angustifolium) and huckleberry (Gaylussacia baccata). Other common plants are hairgrass (Deschampsia flexuosa), ticklegrass (Agrostis hyemalis), false-heather (Hudsonia tomentosa), and bearberry (Arctostaphylos uva–ursi).

Great Lakes Beach

This beach community usually occurs in association with active dune systems. The beaches of the Great Lakes are extremely dynamic features, strongly influenced by water level changes and storm events. They support a suite of very specialized organisms, although unprotected shorelines may be entirely unvegetated. The plant species found in this community include (along Lake Michigan) seaside spurge (Euphorbia polygonifolia) and American sea-rocket (Cakile edentula).

Great Lakes Dune (formerly called Lake Dune)

The dominant plant in these semi-stabilized, open dunes along Great Lakes shorelines, is usually the sand-binding marram grass (Ammophila breviligulata). Frequent associates are common juniper (Juniperus communis), Canada wild-rye (Elymus canadensis), false-heather (Hudsonia tomentosa), beach-pea (Lathyrus japonicus), beach wormwood (Artemisia campestris), sand cherry (Prunus pumila), and various willows (Salix spp.). Two plants endemic to the Great Lakes region, pitcher's thistle (Cirsium pitcheri) and Lake Huron tansy (Tanacetum huronense; possibly now extirpated in Wisconsin), occur in this community along Lake Michigan.

Great Lakes Ridge and Swale (Formerly **Forested Ridge and Swale**)

This is a complex of semi- to fully-stabilized, often forested beach / dune ridges alternating with wet open to forested swales, found on the shores of the Great Lakes but best-developed along Lake Michigan. Both parallel the coast and offer exceptionally complex and diverse habitats for wetland, upland, and Great Lakes shoreline plants. Ridges may support assemblages similar to boreal, northern mesic, or northern dry-mesic forests. Water depth is a controlling factor in the swales, and the vegetation may run the gamut from open (emergent marsh, fen, or sedge meadow), shrub (bog birch, alder), or forested wetlands (often white cedar, black ash are prominent in these).

Hardwood Swamp (this is a split from Curtis' **Northern Wet-Mesic Forest**)

These are northern deciduous forested wetlands that occur along lakes or streams, or in insular basins in poorly drained morainal landscapes. The dominant tree species is black ash (Fraxinus nigra), but in some stands red maple (Acer rubrum), yellow birch (Betula allegheniensis), and (formerly) American elm (Ulmus americana) are also important. The tall shrub speckled alder (Alnus incana) may be locally common. The herbaceous flora is often diverse and may include many of the same species found in Alder Thickets. Typical species are marsh-marigold (Caltha palustris), swamp raspberry (Rubus pubescens), skullcap (Scutellaria galericulata), orange jewelweed (Impatiens capensis), and many sedges (Carex spp.). Soils may be mucks or mucky sands.

Hemlock Relict

These are isolated hemlock (Tsuga canadensis) stands occuring in deep, moist ravines or on cool, north or east facing slopes in southwestern Wisconsin. Associated trees include white pine (Pinus strobus), and yellow birch (Betula allegheniensis). The groundlayer includes herbaceous species with northern affinities such as shining clubmoss (Lycopodium lucidulum), bluebead lily (Clintonia borealis), canada mayflower (Maianthemum canadense), and woodferns (Dryopteris spp). Cambrian sandstone cliffs are usually nearby and often prominent.

Interdunal Wetland

Wind-created hollows that intersect the water table within active dune fields along the Great Lakes. These maybe colonized by wetland plants, including habitat specialists that are of high conservation significance. Common members of this wetland community on Lake Superior are twig-rush (Cladium mariscoides), species of rushes (especially Juncus balticus), pipewort (Eriocaulon septangulare), the sedge (Carex viridula), ladies-tress orchids (Spiranthes sp.) and bladderworts (Utricularia cornuta and U. resupinata).

Inland Beach

The beaches of inland lakes that experience enough water level fluctuation to prevent the development of a stable shoreline forest or other community may, instead support a specialized biota adapted to sandy or gravelly littoral habitats. The shorelines of such lakes (usually seepage lakes) may be subject to fluctuations of as much as several meters over a few years or decades. The alternation of high and low periods maintains populations of the beach specialists over time, including some rare species of unusual geographic affinity such as the Atlantic Coastal Plain of the eastern United States.

Lake Dune (see Great Lakes Dune)

Mesic Cedar Forest

This is a rare upland forest community of mesic sites in northern Wisconsin, characterized by white cedar (Thuja occidentalis) and various associates including hemlock (Tsuga canadensis), white spruce (Abies balsamea), yellow birch (Betula alleghanensis), and white pine (Pinus strobus). The herb layer may contain canada mayflower (Maianthemum canadense), twinflower (Linnaea borealis), clubmosses (Lycopodium spp.), and others. More information is needed on this community type.

Mesic Floodplain Terrace

These are deciduous forests developed on alluvial terraces along rich, infrequently flooding (or flooding only for a very short period) rivers draining into Lake Superior. The dominant trees are usually sugar maple (Acer saccharum), basswood (Tilia americana), and sometimes ashes (Fraxinus spp.). There is a diverse spring ephemeral flora (which in Wisconsin includes many southern species at their northern range limits), but by late spring, these may be overtopped by dense stands of ostrich fern (Matteuccia struthiopteris) and wood-nettle (Laportea canadensis).

Mesic Prairie

This grassland community occurs on rich, moist, well-drained sites. The dominant plant is the tall grass, big bluestem (Andropogon gerardii). The grasses little bluestem (Andropogon scoparius), indian grass (Sorghastrum nutans), porcupine grass (Stipa spartea), prairie dropseed (Sporobolus heterolepis), and tall switchgrass (Panicum virgatum) are also frequent. The forb layer is diverse in the number, size, and physiognomy of the species. Common taxa include the prairie docks (Silphium spp.), lead plant (Amorpha canescens), heath and smooth asters (Aster ericoides and A. laevis), sand coreopsis (Coreopsis palmata), prairie sunflower (Helianthus laetiflorus), rattlesnake-master (Eryngium yuccifolium), flowering spurge (Euphorbia corollata), beebalm (Monarda fistulosa), prairie coneflower (Ratibida pinnata), and spiderwort (Tradescantia ohioensis).

Moist Cliff (**Shaded Cliff** of the Curtis community classification)

This "micro-community" occurs on shaded (by trees or the cliff itself because of aspect), moist to seeping mossy, vertical exposures of various rock types, most commonly sandstone and dolomite. Common species are columbine (Aquilegia canadensis), the fragile ferns (Cystopteris bulbifera and C. fragilis), wood ferns (Dryopteris spp.), rattlesnake-root (Prenanthes alba), and wild sarsaparilla (Aralia nudicaulis). The rare flora of these cliffs vary markedly in different parts of the state; Driftless Area cliffs might have northern monkshood (Aconitum noveboracense), those on Lake Superior, butterwort (Pinguicula vulgaris), or those in Door County, green spleenwort (Asplenium viride).

Moist Sandy Meadow (formerly called Sand Meadow)
This type is included primarily as a placeholder for anomalous herb-dominated assemblages on moist sandy soils in central Wisconsin. Available descriptive information is very limited at this time. Stand size is generally small, seldom, if ever, exceeding more than a few acres. The flora consists of a mixture of plant species typically found in wet prairie, sedge meadow, coastal plain marsh, and pine or oak barrens communities. No one group of associates is clearly dominant. Past human disturbance is evident in some occurrences but native species are prevalent.

Due to a high water table, stands are subject to periodic inundation for short periods of time in the spring and following heavy rain events. This dynamic appears to be at least partially responsible for maintaining the type, but periodic fire, mowing, and browsing may also be important factors.

Muskeg

Muskegs are cold, acidic, sparsely wooded northern peatlands with **composition** similar to the Open Bogs (Sphagnum spp. mosses, Carex spp., and ericaceous shrubs), but with scattered stunted trees of black spruce (Picea mariana) and tamarack (Larix laricina). Plant diversity is typically low, but the community is important for a number of boreal bird and butterfly species, some of which are quite specialized and not found in other communities.

Northern Dry Forest

This forest community occurs on nutrient-poor sites with excessively drained sandy or rocky soils. The primary historic disturbance regime was catastrophic fire at intervals of decades to approximately a century. Dominant trees of mature stands include jack and red pines (Pinus banksiana and P. resinosa) and/or Hill's oak (Quercus ellipsoidalis). Large acreages of this forest type were cut and burned during the catastrophic logging of the late 19th and early 20th century. Much of this land was then colonized by white birch (Betula papyrifera) and/or quaking aspen (Populus tremuloides), or converted **to** pine plantations starting in the 1920s. Common understory shrubs are hazelnuts (Corylus spp.), early blueberry (Vaccinium angustifolium) and brambles (Rubus spp.); common herbs include bracken fern (Pteridium aquilinium), starflower (Trientalis borealis), barren-strawberry (Waldsteinia fragarioides), cow-wheat (Melampyrum lineare), trailing arbutus (Epigaea repens), and members of the shinleaf family (Chimaphila umbellata, Pyrola spp.). Vast acreages of open "barrens" were also planted to pine, or naturally succeeded to densely stocked “dry” forests.

Northern Dry-Mesic Forest

In this forest community, mature stands are dominated by white and red pines (Pinus strobus and P. resinosa), sometimes mixed with red oak (Quercus rubra) and red maple (Acer rubrum). Common understory shrubs are hazelnuts (Corylus spp.), blueberries (Vaccinium angustifolium and V. myrtilloides), wintergreen (Gaultheria procumbens), partridge-berry (Mitchella repens); among the dominant herbs are wild sarsaparilla (Aralia nudicaulis), Canada mayflower (Maianthemum canadense), and cow-wheat (Melampyrum lineare). Stands usually occur on sandy loams, sands or sometimes rocky soils.

Northern Mesic Forest

This forest complex covered the largest acreage of any Wisconsin vegetation type prior to European settlement. Sugar maple (Acer saccharum) is dominant or co-dominant in most stands, while hemlock (Tsuga canadensis) was the second most important species, sometimes occurring in nearly pure stands with white pine (Pinus strobus). Beech (Fagus grandifolia) can be a co-dominant with sugar maple in the counties near Lake Michigan. Other important tree species were yellow birch (Betula allegheniensis), basswood (Tilia americana), and white ash (Fraxinus americana). The groundlayer varies from sparse and species poor (especially in hemlock stands) with woodferns (especially Dryopteris intermedia), bluebead lily (Clintonia borealis), clubmosses (Lycopodium spp.), and Canada mayflower (Maianthemum canadense) prevalent, to lush and species-rich with fine spring ephemeral displays. After old-growth stands were cut, trees such as quaking and bigtoothed aspens (Populus tremuloides and P. grandidentata), white birch (Betula papyrifera), and red maple (Acer rubrum) became and still are important in many second-growth Northern Mesic Forests. Several distinct associations within this complex warrant recognition as communities, and draft abstracts of these are currently undergoing review.

Northern Sedge Meadow

This open wetland community is dominated by sedges and grasses. There are several common subtypes: Tussock meadows, dominated by tussock sedge (Carex stricta) and Canada bluejoint grass (Calamagrostis canadensis); Broad-leaved sedge meadows, dominated by the robust sedges (Carex lacustris and**/or** C. utriculata); and Wire-leaved sedge meadows, dominated by such species as woolly sedge (Carex lasiocarpa) and few-seeded sedge (C. oligosperma). Frequent associates include marsh bluegrass (Poa palustris), manna grasses (Glyceria spp.), panicled aster (Aster lanceolatus), joy-pye-weed (Eupatorium maculatum), and the bulrushes (Scirpus atrovirens and S. cyperinus).

Northern Wet Forest (revised from Curtis, with **Black Spruce** and **Tamarack Swamps** split out)

These weakly minerotrophic conifer swamps, located in the North, are dominated by black spruce (Picea mariana) and tamarack (Larix laricina). Jack pine (Pinus banksiana) may be a significant canopy component in certain parts of the range of this community complex. Understories are composed mostly of sphagnum (Sphagnum spp.) mosses and ericaceous shrubs such as leatherleaf (Chamaedaphne calyculata), Labrador-tea (Ledum groenlandicum), and small cranberry (Vaccinium oxycoccos) and sedges such as (Carex trisperma and C paupercula). The Natural Heritage Inventory has split out two entities, identified (but not strictly defined) by the two dominant species (see **Black Spruce Swamp** and **Tamarack Swamp**).

Northern Wet-Mesic Forest (revised from Curtis, with **Northern Hardwood Swamp** split out)

This forested minerotrophic wetland is dominated by white cedar (Thuja occidentalis), and occurs on rich, neutral to alkaline substrates. Balsam fir (Abies balsamea), black ash (Fraxinus nigra), and spruces (Picea glauca and P. mariana) are among the many potential canopy associates. The understory is rich in sedges (such as Carex disperma and C. trisperma), orchids (e.g., Platanthera obtusata and Listera cordata), and wildflowers such as goldthread (Coptis trifolia), fringed polygala (Polygala pauciflora), and naked miterwort (Mitella nuda), and trailing sub-shrubs such as twinflower (Linnaea borealis) and creeping snowberry (Gaultheria hispidula). A number of rare plants occur more frequently in the cedar swamps than in any other habitat.

Oak Barrens

Black oak (Quercus velutina) is the dominant tree in this fire-adapted savanna community of xeric sites, but other oaks may also be present. Common understory species are lead plant (Amorpha canescens), black-eyed susan (Rudbeckia hirta), round-headed bush clover (Lespedeza capitata), goat's rue (Tephrosia virginiana), june grass (Koeleria cristata), little bluestem (Schizachyrium scoparium), flowering spurge (Euphorbia corollata), frostweed (Helianthemum canadense), false Solomon's-seals (Smilacina racemosa and S. stellata), spiderwort

(Tradescantia ohioensis), and lupine (Lupinus perennis). Distribution of this community is mostly in southwestern, central and west central Wisconsin.

Oak Opening

As defined by Curtis, this is an oak-dominated savanna community in which there is less than 50% tree canopy. Historically, oak openings occurred on wet-mesic to dry sites. The few extant remnants are mostly on drier sites, with the mesic and wet-mesic openings almost totally destroyed by conversion to agricultural or residential uses, and by the encroachment of other woody plants due to fire suppression. Bur, white, and black oaks (Quercus macrocarpa, Q. alba and Q. velutina) are dominant in mature stands as large, open-grown trees with distinctive limb architecture. Shagbark hickory (Carya ovata) is sometimes present. American hazelnut (Corylus americana) is a common shrub, and while the herblayer is similar to those found in oak forests and prairies, with many of the same grasses and forbs present, there are some plants and animals that reach their optimal abundance in the "openings".

Oak Woodland

This "forest" community is structurally intermediate between Oak Openings and Southern Dry Forest. The tree canopy cover is high, but frequent low-intensity fires and possibly (in pre-settlement times) browsing by herbivores such as elk, bison, and deer kept the understory relatively free of shrubs and saplings. Much additional information is needed but it appears that at least some plants (certain legumes, grasses, and composites among them) reached their highest abundance here.

Open Bog

These non-forested bogs are acidic, low nutrient, northern Wisconsin peatlands dominated by Sphagnum spp. mosses that occur in deep layers, often with pronounced hummocks and hollows. Also present are a few narrow-leaved sedge species such as (Carex oligosperma and C. pauciflora), cotton-grasses (Eriophorum spp.), and ericaceous shrubs, especially bog laurel (Kalmia polifolia), leatherleaf (Chamaedaphne calyculata), and small cranberry (Vaccinium oxycoccus). Plant diversity is very low but includes characteristic and distinctive specialists. Trees are absent or achieve very low cover values as this community is closely related to and intergrades with Muskeg. When this community occurs in southern Wisconsin, it is often referred to as a **Bog Relict**.

Patterned Peatland

Very rare in Wisconsin, this wetland type can be characterized as a herb- and shrub-dominated minerotrophic peatland with alternating moss and sedge-dominated peat ridges (strings) and saturated and inundated hollows (flarks). These are oriented parallel to the contours of a slope and perpendicular to the flow of groundwater. Within a patterned peatland the peat "landforms" differ significantly in nutrient availability and pH. The flora may be quite diverse and includes many sedges of bogs and fens, along with ericads, sundews, orchids, arrow-grasses (Triglochin spp.), and calciphilic shrubs such as bog birch (Betula pumila) and shrubby cinquefoil (Potentilla fruticosa).

Pine Barrens

This savanna community is characterized by scattered jack pines (Pinus banksiana), or less commonly red pines (P. resinosa), sometimes mixed with scrubby Hill's and bur oaks (Quercus ellipsoidalis and Q. macrocarpa), interspersed with openings in which shrubs such as hazelnuts, (Corylus spp.) and prairie willow (Salix humilis) and herbs dominate. The flora often contains species characteristic of "heaths" such as blueberries (Vaccinium angustifolium and V. myrtilloides), bearberry (Arctostaphylos uva-ursi), American hazelnut (Corylus americana), sweet fern (Comptonia peregrina), and sand cherry (Prunus pensylvanica). Also present are dry sand prairie species such as june grass (Koeleria macrantha), little bluestem (Schizachyrium scoparium), silky

and sky-blue asters (Aster sericeus and A. azureus), lupine (Lupinus perennis), blazing-stars (Liatris aspera and L. cylindracea), and western sunflower (Helianthus occidentalis). Pines may be infrequent, even absent, in some stands in northern Wisconsin and elsewhere because of past logging, altered fire regimes, and an absence of seed source.

Pine Relict

These isolated stands of white pine (Pinus strobus) and red pine (P. resinosa) or, less commonly, jack pine (P.banksiana), that occur on sandstone outcrops or in thin soils over sandstone in the Driftless Area of southwestern Wisconsin, have historically been referred to as relicts. The understories often contain species with northern affinities such as blueberries (Vaccinium spp.), huckleberry (Gaylussacia baccata), wintergreen (Gaultheria procumbens), pipsissewa (Chimaphila umbellata), and partridge-berry (Mitchella repens), sometimes mixed with herbs typically found in southern Wisconsin's oak forests and prairies.

Poor Fen

This acidic, weakly minerotrophic peatland type is similar to the Open Bog, but can be differentiated by higher pH, nutrient availability, and floristics. Sphagnum (Sphagnum spp.) mosses are common but don't typically occur in deep layers with pronounced hummocks. Floristic diversity is higher than in the Open Bog and may include white beak-rush (Rhynchospora alba), pitcher-plant (Sarracenia purpurea), sundews (Drosera spp.), pod grass (Scheuchzeria palustris), and the pink-flowered orchids (Calopogon tuberosus, Pogonia ophioglossoides and Arethusa bulbosa). Common sedges are (Carex oligosperma, C. limosa, C. lasiocarpa, C. chordorrhiza), and cotton-grasses (Eriphorum spp.).

Sand Barrens

Sand Barrens are herbaceous upland communities that develop on unstable or semi-stabilized alluvial sands along major rivers such the Mississippi and Wisconsin. They are partly or perhaps wholly anthropogenic in origin, occurring on sites historically disturbed by plowing or very heavy grazing. Unvegetated "blow-outs" are characteristic features. Barrens, Dry Prairie and Sand Prairie species such as false-heather (Hudsonia tomentosa), bearberry (Arctostaphylos uva-ursi), sedges (Cyperus filiculmis and C. schweinitzii), sand cress (Arabis lyrata), three-awn grasses (Aristida spp.), rock spikemoss (Selaginella rupestris), and the earthstar fungi (Geaster spp.) are present in this community. Many exotics are present, and rare disturbance dependent species such as fameflower (Talinum rugospermum) occur in some stands.

Sand Meadow (now called Moist Sand Meadow)

Sand Prairie (or Dry Sand Prairie)

This dry grassland community is composed of little bluestem (Schizachyrium scoparium), junegrass (Koeleria macrantha), panic grass (Panicum spp.), and crab grass (Digitaria cognata). Common herbaceous species are western ragweed (Ambrosia psilostachya), the sedges (Carex muhlenbergii and C. pensylvanica), poverty-oat grass (Danthonia spicata), flowering spurge (Euphorbia corollata), frostweed (Helianthemum canadense), common bush-clover (Lespedeza capitata), false-heather (Hudsonia tomentosa), long-bearded hawkweed (Hieracium longipilum), stiff goldenrod (Solidago rigida), horsebalm (Monarda punctata), and spiderwort (Tradescantia ohioensis). At least some stands are Barrens remnants now lacking appreciable woody cover, though extensive stands may have occurred historically on broad level terraces along the Mississippi, Wisconsin, Black, and Chippewa Rivers.

Shore Fen (formerly called Coastal Fen)

This open peatland community occurs primarily along Great Lakes shorelines, especially near the mouths of estuarine streams. Along Lake Superior most stands are separated from the lake waters by a sand spit. The

floating sedge mat is composed mostly of woolly sedge (Carex lasiocarpa); co-dominants are sweet gale (Myrica gale) and bogbean (Menyanthes trifoliata). The following herbs are common in this diverse, circumneutral, nutrient-rich community: twigrush (Cladium mariscoides), marsh horsetail (Equisetum fluviatile), a spikerush (Eleocharis elliptica), intermediate bladderwort (Utricularia intermedia), marsh bellflower (Campanula aparinoides), narrow-leaved willow-herb (Epilobium leptophyllum), water-parsnip (Sium suave), and bog willow (Salix pedicellaris). Coastal fens are distinguished from open bogs and poor fens (which may adjoin them in the same wetland complex) by the lack of Sphagnum spp. mosses, higher pH, and direct hydrologic connection to the Great Lakes. They are distinguished from rich fens by the absence of indicator species such as linear-leaved sundew (Drosera linearis), grass-of-parnassus (Parnassia glauca), false asphodel (Tofiedia glutinosa) and a spikerush (Eleocharis rostellata).

Shrub-Carr

This wetland community is dominated by tall shrubs such as red-osier dogwood (Cornus stolonifera), meadow-sweet (Spiraea alba), and various willows (Salix discolor, S. bebbiana, and S. gracilis). Canada bluejoint grass (Calamagrostis canadensis) is often very common. Associates are similar to those found in Alder Thickets and tussock-type Sedge Meadows. This type is common and widespread in southern Wisconsin but also occurs in the north.

Southern Dry Forest

Oaks are the dominant species in this upland forest community of dry sites. White oak (Quercus alba) and black oak (Quercus velutina) are dominant, often with admixtures of red and bur oaks (Q. rubra and Q. macrocarpa) and black cherry (Prunus serotina). In the well developed shrub layer, brambles (Rubus spp.), gray dogwood (Cornus racemosa), and American hazelnut (Corylus americana) are common. Frequent herbaceous species are wild geranium (Geranium maculatum), false Solomon's-seal (Smilacina racemosa), hog-peanut (Amphicarpaea bracteata), and woodland sunflower (Helianthus strumosus).

Southern Dry-Mesic Forest

Red oak (Quercus rubra) is a common dominant tree of this upland forest community type. White oak (Q. alba), basswood (Tilia americana), sugar and red maples (Acer saccharum and A. rubrum), and white ash (Fraxinus americana) are also important. The herbaceous understory flora is diverse and includes many species listed under Southern Dry Forest plus jack-in-the-pulpit (Arisaema triphyllum), enchanter's-nightshade (Circaea lutetiana), large-flowered bellwort (Uvularia grandiflora), interrupted fern (Osmunda claytoniana), Lady Fern (Athyrium Filix-femina), tick-trefoils (Desmodium glutinosum and D. nudiflorum), and hog peanut (Amphicarpa bracteata) . To the detriment of the oaks, mesophytic tree species are becoming increasingly important under current management practices and fire suppression policies.

Southern Hardwood Swamp (A split from Curtis' **Southern Wet-Mesic Forest**)

This is a deciduous forested wetland community type found in insular basins with seasonally high water tables. It is best developed in glaciated southeastern Wisconsin. The dominant trees are red maple (Acer rubrum), green ash (Fraxinus pennsylvanica), and formerly**,** American elm (Ulmus americana). The exotic reed canary grass (Phalaris arundinacea) is often dominant in the understory. This Natural Heritage Inventory community partly includes the **Southern Wet-Mesic Forest** of the Curtis classification.

Southern Mesic Forest

This upland forest community occurs on rich, well-drained soils. The dominant tree species is sugar maple (Acer saccharum), but basswood (Tilia americana) and (near Lake Michigan) beech (Fagus grandifolia) may be co-dominant. Many other trees are found in these forests, including those of the walnut family (Juglandaceae). The understory is typically open (sometimes brushy with species of gooseberry (Ribes) if there is a past history

of grazing) and supports fine spring ephemeral displays. Characteristic herbs are spring-beauty (Claytonia virginica), trout-lilies (Erythronium spp.), trilliums (Trillium spp.), violets (Viola spp.), bloodroot (Sanguinaria canadensis), blue cohosh (Caulophyllum thalictroides), mayapple (Podophyllum peltatum), and Virginia waterleaf (Hydrophyllum virginianum).

Southern Sedge Meadow

Widespread in southern Wisconsin, this open wetland community is most typically dominated by tussock sedge (Carex stricta) and Canada bluejoint grass (Calamagrostis canadensis). Common associates are water-horehound (Lycopus uniflorus), panicled aster (Aster simplex), blue flag (Iris virginica), Canada goldenrod (Solidago canadensis), spotted joe-pye-weed (Eupatorium maculatum), broad-leaved cat-tail (Typha latifolia), and swamp milkweed (Asclepias incarnata). Reed canary grass (Phalaris arundinacea) may be dominant in grazed and/or ditched stands. Ditched stands can succeed quickly to Shrub-Carr.

Submergent Aquatic

This herbaceous community of aquatic macrophytes occurs in lakes, ponds, and rivers. Submergent macrophytes often occur in deeper water than emergents, but there is considerable overlap. Dominants include various species of pondweeds (Potamogeton spp.) along with waterweed (Elodea canadensis), slender naiad (Najas flexilis), eel-grass (Vallisneria americana), and species of water-milfoil (Myriophyllum) and bladderworts (Utricularia).

Submergent Aquatic – Oligotrophic marsh (formerly called Submergent Aquatic – Oligotrophic)

This herbaceous community of distinctive highly specialized submersed, rosette-forming aquatic macrophytes occurs in clear, deep soft-water lakes in northern Wisconsin. The plants grow at depths ranging from the beach line to several meters. Species in this community include American shore-grass (Littorella americana), pipewort (Eriocaulon septangulare), yellow hedge-hyssop (Gratiola aurea), aquatic lobelia (Lobelia dortmanna), a milfoil (Myriophyllum tenellum), brown-fruit rush (Juncus pelocarpus), and quillworts (Isoetes spp.).

Talus Forest

This description is based on a very limited number of stands examined and should be regarded as preliminary. Talus Forest develops on a substrate of quartzite, sandstone, dolomite, rhyolite, and possibly other rock types. Canopy cover ranges from sparse to moderately dense. Tree dominance is variable, and can include white pine (*Pinus strobus*), red cedar (*Juniperus virginiana*), paper birch (*Betula papyrifera*), northern white cedar (*Thuja occidentalis*), red pine (*Pinus resinosa*) and others. Among the characteristic understory plants noted to date are the shrubs mountain maple (*Acer spicatum*), red-berried elder (*Sambucus pubens*), and bristly sarsaparilla (*Aralia hispida*). Representative herbs include common polypody (*Polypodium vulgare*), wood fern (*Dryopteris marginalis*), walking fern (*Asplenium rhizophyllum*), harebell (*Campanula rotundifolia*), columbine (*Aquilegia canadensis*), fumitory (*Adlumia fungosa*), leafcup (*Polymnia canadensis*),and pale corydalis (*Corydalis sempervirens*). Crustose lichens and various mosses sometimes reach high cover values.

Talus Forest communities often reflect the composition of forests in the surrounding landscape, but include plants and animals that are adapted to take advantage of the rock substrate, microclimatic conditions such as cold air drainage, and groundwater seepage. These habitat specialists, presumably including some of the mosses and lichens, are likely to be the species that are most restricted to such environments and of the greatest conservation concern.

Tamarack (poor) Swamp (formerly called Tamarack Swamp, this is a split from Curtis' **Northern Wet Forest**)

These weakly to moderately minerotrophic conifer swamps are dominated by a broken to closed canopy of tamarack (Larix laricina) and a frequently dense understory of speckled alder (Alnus incana). The understory is more diverse than in Black Spruce Swamps and may include more nutrient-demanding species such as winterberry holly (Ilex verticillata) and black ash (Fraxinus nigra). The bryophytes include many genera other than Sphagnum. Stands with spring seepage sometimes have marsh-marigold (Caltha palustris) and skunk-cabbage (Symplocarpus foetidus) as common understory inhabitats. These seepage stands have been separated out as a distinct type or subtype in some nearby states and provinces.

Tamarack (rich) Swamp (formerly called Tamarack Fen)

This forested wetland community type is a variant of the Tamarack Swamp, but occurs south of the Tension Zone within a matrix of "southern" vegetation types. Poison-sumac (Toxicodendron vernix) is often a dominant understory shrub. Successional stages and processes are not well understood but fire, windthrow, water level fluctuations, and periodic infestations of larch sawfly are among the important dynamic forces influencing this community. Groundwater seepage influences the composition of most if not all stands. Where the substrate is especially springy, skunk cabbage (Symplocarpus foetidus), marsh marigold Caltha palustris), sedges, and a variety of mosses may carpet the forest floor. Drier, more acid stands may support an ericad and sphagnum dominated groundlayer.

Wet Prairie

This is a rather heterogeneous tall grassland community that shares characteristics of prairies, Southern Sedge Meadow, Calcareous Fen and even Emergent Aquatic communities. The Wet Prairie's more wetland- like character can mean that sometimes very few true prairie species are present. Many of the stands assigned to this type by Curtis are currently classified as Wet-Mesic Prairies. The dominant graminoids are Canada bluejoint grass (Calamagrostis canadensis), cordgrass (Spartina pectinata), and prairie muhly (Muhlenbergia glomerata), plus several sedge (Carex) species including lake sedge (C. lacustris), water sedge (C. aquatilis), and woolly sedge (C. lanuginosa). Many of the herb species are shared with Wet-Mesic Prairies, but the following species are often prevalent: New England aster (Aster novae-angliae), swamp thistle (Cirsium muticum), northern bedstraw (Galium boreale), yellow stargrass (Hypoxis hirsuta), cowbane (Oxypolis rigidior), tall meadow-rue (Thalictrum dasycarpum), golden alexander (Zizea aurea), and mountain-mint (Pycnanthemum virginianum).

Wet-Mesic Prairie

This herbaceous grassland community is dominated by tall grasses including big bluestem (Andropogon gerardii), Canada bluejoint grass (Calamagrostis canadensis), cordgrass (Spartina pectinata), and Canada wild-rye (Elymus canadensis). The forb component is diverse and includes azure aster (Aster oolentangiensis), shooting-star (Dodecatheon meadia), sawtooth sunflower (Helianthus grosseseratus), prairie blazing-star (Liatris pycnostachya), prairie phlox (Phlox pilosa), prairie coneflower (Ratibida pinnata), prairie docks (Silphium integrifolium and S. terebinthinaceum), late and stiff goldenrods (Solidago gigantea and S. rigida), and culver's-root (Veronicastrum virginicum).

White Pine - Red Maple Swamp

This swamp community is restricted to the margins of the bed of extinct glacial Lake Wisconsin in the central part of the state. It often occurs along headwaters stream**s** and seepages in gently sloping areas. White pine (Pinus strobus) and red maple (Acer rubrum) are the dominant trees, with other species, including yellow birch (Betula alleghiensis), present in lesser amounts. Common understory shrubs are speckled alder (Alnus incana), winterberry holly (Ilex verticillata), and swamp dewberry (Rubus pubescens); characteristic herbs include skunk cabbage (Symplocarpus foetidus), cinnamon fern (Osmunda cinnamomea), gold thread (Coptis trifolia), and two

disjuncts from the eastern United States, bog fern (Thelypteris simulata) and long sedge (Carex folliculata). Sphagnum and other mosses are common.

Appendix D.1

Sheboygan AOC Mid-Winter Bird Survey data form

Sheboygan AOC Mid Winter Bird Survey

Date: _____

Segment: _____ **Point #** _____ of _____ **Temperature:** _____

Wind: _____

GPS Coords: _____ **Visibility:** _____

Cloud Cover: _____

Start Time: _____ **Stop Time:** _____ **GPS Error:** _____

Species	Number	Species	Number	Species	Number

NOTES:

Appendix D.2

Explanation of changes made to Sheboygan AOC bird survey protocols

Explanation of changes made to Mid-Winter Bird Survey protocols:

Explanation for changes to protocol in regards to extended time allowed for counting and implementation of a dependent dual-observer survey method.

There were/are some potential problems with the original survey design. Further research has led us to conclude that there is an alternative surveying method that can address these issues, and it may be worthwhile changing to this method.

1st - Large numbers of birds, most notably waterfowl in or near the Outer Harbor, are difficult to count accurately within the 5 minute time frame and from long distances. Consequently, the count of their numbers was estimated as accurately as possible and may vary considerably from observer to observer. Also, there can be significant differences in each observer's ability to hear bird calls and to see birds, especially at a distance. Spending a great deal of time looking through binoculars can be helpful for identifying birds, but also limits the field of view and may cause an observer to miss other birds in the surrounding area. In response to these issues, we have allowed for extended time to be taken at each point in order to clarify identifications and to more accurately count large or scattered groups of birds.

2nd – Beginning with the 4th survey, we are differentiating between visual (V) and auditory (A) identifications.

3rd - It is almost impossible for two observers to survey completely independently of one another. Great effort needs to be directed at ignoring the other observer's movements to record a bird or putting up binoculars to focus their attention on a bird. This potential bias can be mitigated by periodically "going through the motions" even when a bird is not spotted or emphasizing that the surveyors always be looking in opposite directions, but it cannot be completely removed. The extreme difficulty in maintaining complete independence during a survey is an acknowledged shortcoming of the independent dual observer survey method. Utilizing a dependent dual observer survey method can address this shortcoming.

A dependent dual observer survey requires a primary observer and a secondary observer. The primary observer's responsibility is to identify all birds by sight and sound, and to dictate them to the secondary observer. The secondary observer's first responsibility is to record the observations of the primary observer. Additionally, the secondary observer records any observations that they make independent of the primary observer. The primary and secondary observers switch roles throughout the day. Studies (Forcey et. al. 2006) have shown that the dependent dual observer method has a higher detection probability, lower standard errors of detections, and fewer logistical constraints than independent dual observer surveys. By working in tandem, supporting one another's observations, and switching roles throughout the survey, many of the issues of bias and missed observations are reduced significantly.

By utilizing the dual observer methodology for point sampling we would maximize our ability to meet our objective: to gather basic information on bird species presence/absence and their relative abundance in the AOC during the winter. According to the study of different methods, specifically independent vs. dependent dual surveys, the dependent point survey maximizes detection of species and relative abundance. This seems to be consistent w/ our stated objectives. It also provides an estimate of detection probabilities between observers that should help meet the QA needs. Our relative small sample size of surveys would likely not be adequate to meet a robust statistical analysis of independent surveyors and the referenced study supports the dependent survey as best - given the other criteria of skilled birders, etc. Approximately half of all the surveys done (4 of 8) will be done as dual-observer surveys. Of those 4, all but the first one will be dependent dual-observer surveys.

The data generated from these dual surveys would allow us to calculate detection probabilities between the two observers. Detection probabilities can be calculated using the computer program DOBSERV and the SURVIV code, both of which are available to download from the USGS web page.

The dependent dual-observer survey methodology is recommended for several reasons. Some of the benefits include higher detection rates, lower standard errors of detection, fewer adverse affects from misidentification of bird species, and fewer adverse affects from differing experience levels between the observers. There is no struggle to maintain true independence between the observers; instead the two observers work in tandem which simplifies the logistical implementation of the survey. Dependent dual-observer surveys should give us as complete a picture as possible of the bird community at each location. Comparing the single-observer surveys to this theoretical "maximum detection rate" should give us an idea of how thorough (or rigorous) the single-observer surveys are.

http://www.jstor.org/stable/4128100?seq=1

Appendix D.3

"Collection and Disposition of Birds and Mammals,"
from WDNR Wildlife Management Operations Handbook

COLLECTION AND DISPOSITION OF BIRDS AND MAMMALS

This section clarifies the authority and responsibility of Department employees, and procedures for the collection and the disposal of wild birds and mammals.

State or federal scientific collector permits granted to Director of the Bureau of Wildlife Management or conservation warden credentials are required to fulfill the official Department duties described below.

Collections of Wild Birds and Mammals Which Are Dead, or Parts of Dead Wild Birds and Mammals

Personnel in their normal course of work may encounter dead wild birds or mammals that should be collected for a number of different purposes. Animals to be collected may fall into one of the following categories: Field station collections (study skins, skull and pelt collections, mounted specimens, educational displays), rare birds and mammals, or diseased wild birds and mammals. See the section below for information and instructions on collecting diseased animals.

This list is not intended to be all-inclusive. The examples are presented to illustrate types of miscellaneous collections of dead wild birds and mammals. Personnel who collect what appear to be rare bird and mammal species are to notify the nearest wildlife biologist for determination of the use and disposition of the specimen.

This section should not be construed to mean that large-scale miscellaneous collections should be made for which there is no separate order or need. Indiscriminate collections of dead birds and mammals which have no particular significance, are not of value.

Collections of Wild Birds and Mammals Which Are Dead or Alive and Obviously Diseased or Suffering

All employees should be on the alert for diseased wild birds and mammals. In the event there is any question on what appears to be an important disease outbreak, the nearest available wildlife biologist or conservation warden should be contacted immediately.

Types of Specimens. The Department's Wildlife Health program does not have the capacity to examine every bird and mammal. Therefore, discretion should be used when submitting animals for necropsy. Specimens to submit include, but are not limited to, sick animals that exhibit abnormal symptoms, such as loss of fear of man, paralysis, or weakness. Species of interest, disease targeted animals, and law enforcement cases should also be submitted to Wildlife Health for examination. If there is uncertainty as to whether a particular animal should be submitted for necropsy, contact Wildlife Health prior to delivery. Wildlife Health should be contacted immediately in the event of a large-scale die-off.

Car-killed animals or gunshot victims should not be submitted to Wildlife Health unless there is some reason to suspect the animals were not healthy, or unless there are law enforcement concerns or specific instructions for car-kill collections.

Care should be taken to prevent exposure to diseases contagious to humans. Live animals should not be handled. Disposable gloves will be worn to pick up carcasses, and carcasses will be double-bagged. In some instances, additional precautions must be taken. Call Wildlife Health program for specific instructions.

General Instructions

1. Handling. Specimens collected or found by Department field personnel which cannot be delivered to the Wildlife Health Specialist, Bureau of Wildlife Management, within 24 hours should be immediately frozen and held in this condition until delivery is possible.

   Whenever personnel handle disease suspect animals, precautions such as wearing disposable gloves and washing with strong soap should be taken. If necessary, call Wildlife Health for additional instructions on decontamination.

   If it is necessary to dispatch an animal, avoid excessive damage to the carcass to keep it suitable for necropsy purposes. See Special Instructions below for rabies suspects.

   If it is possible to transport the specimen from the field, do not perform a field necropsy. If the specimen cannot be transported from the field, then a field examination may be appropriate. Call Wildlife Health for specific instructions.

2. Shipment. All wildlife specimens collected for necropsy may be delivered or shipped by prepaid express to the following:

   DNR, Wildlife Health Specialist
   1350 Femrite Drive
   Monona, WI 53716

   Call Wildlife Health for specific instructions. Do not ship specimens without Wildlife Health contact.

3. Records. Information regarding the actions and conditions of the animal, date found, location information, name and address of person to whom the report should be sent, along with any other pertinent data will be recorded on Diagnostic Specimen Submission, Form 2300-143, and on tag attached to carcass (Specimen Tag, Form 2300-144). Case history is fundamental information, and every effort should be made to include all pertinent data.

   Fill in Form 2300-143 completely (was animal found dead or alive; describe the actions prior to death; injuries noted; respiratory, digestive, or nervous symptoms; abnormal discharge from the nose, eyes, ears, mouth or rectum; abnormal locomotion; or if it has been exposed to possible poisons, etc.). These forms can be e-mailed to Wildlife Health and/or sent along with the carcass. If forms are sent electronically, also be sure to tag the carcass for accurate identification.

Game Farm Mortalities

1. Poynette Game Farm. The Director of the State Game Farm will notify the Wildlife Health Specialist of any disease problem developing at the Game Farm.

2. Private Game Farms. Private game farms will utilize the services of their local veterinarian or Department of Agriculture, Trade and Consumer Protection (DATCP) when disease problems arise. Wildlife Health has assisted private game farms with specialized problems, and upon specific request, this assistance will continue. Call Wildlife Health Program for specific instructions.

Special Instructions (In all cases, wear disposable gloves and take other necessary precautions when handling disease-suspect animals.)

Rabies

Rabies is a contagious viral disease that affects the nervous system. Rabies is almost always fatal once symptoms occur. All mammals, including man, are susceptible to rabies. In Wisconsin, skunks and bats are the most commonly affected animals. Birds and reptiles are not susceptible to naturally acquired rabies, and to not pose a risk of transmitting it to humans.

It is essential that the Department cooperate with public health authorities (including the Department of Healthy and Family Services, DHFS) when there is a rabies risk from wildlife/human contact. The cost of the rabies testing is waived by DHFS in cases of human or domestic animal exposure as defined below.

The Department does not routinely test wild animals for rabies except when human or domestic animal exposure has occurred. The Department and DHFS definition of human exposure from a wild carnivore or bat is:

- a bite
- wet saliva (from a live or dead animal) contact with a person's broken skin or mucous membranes.

The Department and DHFS definition of domestic animal exposure from a wild carnivore or bat is:

- a bite
- salivary contact as described above or a domestic animal scavenging a wildlife carcass
- close association (such as in a barn or other enclosed space) with a highly suspicious wild animal, especially a skunk. (Suspicion is based on abnormal behavior or clinical signs of nervous system disease.)

Rabies is rare in small rodents, rabbits, and opossum. However, bites from large rodents such as woodchucks, beaver, and muskrats should be considered possible exposure.

If you receive a call about an interaction between a person or domestic animal and a wild animal that fits these definitions of exposure, the Department will likely test the wild animal for rabies. The decision to test the wild animal will be made on a case-by-case basis. Please contact Wildlife Health Specialists at (608) 267-6751 or (608) 266-3143 for case approval and submission instructions. It is important to have a complete field history when discussing case submission. Collect the wild animal safely and in such a way that the brain (needed for rabies testing) is not damaged. Make sure to wear disposable gloves when handling the suspect animal.

Additionally, if there has possibly been human exposure, advise the caller to contact their physician AND the local Health Department/DHFS. For answers to questions on rabies issues, call DHFS at (608) 266-2154.

If domestic animal exposure may have occurred, advise the caller to contact the Department of Agriculture, Trade, and Consumer Protection at (608) 255-4888.

Collections of Live, Non-diseased Wild Birds and Mammals Protected by Federal or State Regulations

Live Non-diseased Wild Birds and Mammals Protected by State Regulations or Department of Natural Resources Order, But Not by Federal Regulations.

Periodically it is necessary for Department personnel while carrying out their official duties, to capture, kill or hold healthy wild birds and mammals for research, propagation or educational purposes. Specific assignments provide the authorization for activities of this type. Other activities not covered by project assignment will be approved by the Administrator, Division of Land.

Live Wild Birds and Mammals Protected by Federal Regulations.

Collecting, killing or holding birds and mammals protected by federal law, by Department personnel, for Department purposes such as research, propagation or education, shall be done by permit from the Director of the U.S. Fish and Wildlife Service (USFWS). Personnel are to request permits directly from the USFWS. The applicant will then be supplied with forms that are to be completed and submitted directly to the USFWS, which issues the permit to the individual. The applicant will route copies authorizing the activity to concerned personnel, including the Administrator, Division of Land. When the operation is completed, a full report is to be submitted through channels to the Administrator of the Division of Land.

Bird Banding

Specific project assignments provide the authorization for trapping and banding birds.

Personnel desiring to band migratory birds (all of which are protected by federal laws) shall route requests through channels to the Chief of the Wildlife & Forestry Research Section, Bureau of Integrated Science Services. This person holds the Department's federal master migratory bird banding and salvage permit, and may issue sub-permits to personnel.

Personnel assigned by the Department to band non-migratory birds do not require a state or federal banding permit.

Disposition of Mammals and Birds

When crippled birds protected by federal law are received or picked up by Department personnel and the birds are not needed for Department purposes, the employee receiving such birds shall determine if they can be rehabilitated. If they can, these birds may be turned over to a person holding state and federal wildlife rehabilitation permits. If they cannot be rehabilitated, they shall be immediately dispatched and buried.

This directive is not intended to imply any change in present administrative procedures in the handling of confiscated birds and mammals by law enforcement personnel as prescribed in the Law Enforcement Handbook, 4105. Wild mammals and birds, which are not of further use to the Department, but which may be salvageable, are to be released or turned over to the regional wildlife supervisor. All non-salvageable carcasses will be disposed of by burying or burning.

In no instance will any personal use be made of birds or mammals that have been held or obtained for Department purposes.

General

Any personnel handling wild birds and mammals during the closed seasons, or in any manner which appears contrary to the Natural Resources Laws and Regulations, must be in a position to justify and defend this action by specific authorization, directive or job assignment should the question arise.

Appendix D.4

WDNR Wildlife Health

General guidelines for what to submit to WH for necropsy

1/2011

WDNR Wildlife Health
General guidelines for what to submit to WH for necropsy

The following list of species includes those that Wildlife Health requests for necropsy. Other species or situations will be considered on a case by case basis and could include cases where local DNR staff have specific concerns, etc. Please contact Wildlife Health to discuss species/situations not listed below. Wildlife submitted for necropsy as part of a law enforcement investigation is not restricted by species, but please notify Wildlife Health before delivering carcasses tagged as evidence. Situations of unusual mortality events are also not restricted by species for necropsy submission, but please alert Wildlife Health as soon as possible for coordination of prompt shipping/delivery.

NOTE: New process for necropsy submission forms!!

Necropsy submissions will now be done through the WH database. This process **will replace** the Necropsy Submission e-form 2300-143 which should no longer be used.
Please be sure to add the following link to the WH database necropsy submission page to your "favorites" for easy access.
http://prodoasjava.dnr.wi.gov/whdb/field/home.do.

Your username and password is for this is the same one that you use for time and trips. If you try to use this link and aren't able to get access please contact Erin Larson. Access to this database has been automatically granted to all permanent and LTE Wildlife Biologists and Wildlife Technicians, Wildlife Area and Regional Supervisors, and Regional Ecologists. If you hire a new employee that will be entering necropsy submissions or you are not one of the above positions and need access please contact Erin Larson to have access granted.

Once logged in, the menu will be on the left hand side. To enter a necropsy submission click on the "New Wildlife Necropsy Submission" form and follow through the tabs. It follows the format of the old e-form very closely except that each section is a separate tab. If you have more than one species to submit or animals from more than one location or date you will have to create multiple submissions. Multiple carcasses of the same species from the same date/location/history can be entered into one submission by indicating the numbers. This application will track once your submission is accepted and where it is in the necropsy process. There is also a link available to run searches on your submissions. Since this is a new process this year we anticipate that there will be bugs to work out. Please contact either Erin Larson or Nancy Businga with any questions, problems, or suggestions.

After you enter the data please print out the report created and send it along with the carcass same as you did with the e-form. All carcasses submitted for necropsy should be double-bagged and tagged with a specimen/carcass tag (2300-144) with complete collection information and submitted to the WDNR Wildlife Health Lab (2801 Progress Rd., Madison, WI 53716). Please notify the Wildlife Health Lab Manager-Nancy Businga (608-221-5375) before shipping/delivering carcasses.

Specific necropsy protocols, which include information regarding support of research projects, disposition of carcasses, distribution of necropsy reports, etc., for species listed below, are available on the DNR Intranet Wildlife Health protocols page Wildlife Management Intranet - Wildlife Health Protocols

If a carcass is submitted as a law enforcement case, a signed chain of custody tag must be attached to the carcass and delivery of the carcass to WH coordinated so that someone from WH is available to sign the

chain of custody tag upon delivery. Carcasses arriving without a signed chain of custody tag will not get a forensic necropsy, will not be held after necropsy as evidence, and will be discarded. (See specific Legal Case Necropsy Protocol)

Carcasses that are decomposed (have a strong odor, covered with maggots or carrion beetles, or missing major organs through a large body opening) are not suitable for necropsy and will not be accepted. If carcasses are found to be decomposed upon arrival at Wildlife Health they will be discarded at the discretion of Wildlife Health staff (this does not include carcasses submitted as law enforcement cases).

In addition to the list below, wildlife carcasses should be submitted for necropsy from any unusual disease events (e.g. die-offs of 5 or more animals in the same location over a short time frame) and in support of current targeted disease surveillance programs (e.g. avian influenza, etc.)

BIRDS

Bald and Golden Eagles: only those from any of the following categories:
-all banded eagles
-when death circumstances are considered unusual
-suspected law enforcement cases
-found dead within 5 miles of the lower Wisconsin River from December 1st through March 31st (for ongoing investigations into the "lower WI River Bald Eagle Syndrome")
-found dead within 5 miles of the Great Lakes shoreline from April 1st through August 30th (for contaminants monitoring)
-sick eagles taken to a wildlife rehabilitator that had an unusual clinical course (please contact Wildlife Health for guidance)

Greater Prairie Chickens: all WI Birds found dead (including F1s, etc.), and on a case by case basis for MN birds (i.e. MN birds with evidence of infectious disease, or birds who died during the MN capture/translocation where there is concern about the cause of death)

Hawks, owls, vultures: only hawks, owls, and vultures where there is unusual mortality, special interest of field staff, or in potential legal cases.

Loons and **peregrine falcons**: all

Osprey: opportunistically as submitted due to field staff interest

Trumpeter Swans: only those from any of the following categories:
-all banded/collared swans
-when cause of death is not evident
-suspected law enforcement cases
-part of a cluster of deaths (either geographically and/or temporally)
-swans which die during capture/handling events

Sandhill cranes: any that are easily retrievable and in good post mortem condition (as sentinels for disease surveillance for potential disease risks for whooping cranes)

MAMMALS

Wolves:

-any that are found dead (collared and uncollared) that are not clearly vehicle collisions (see below for vehicle collisions)
-any that die during capture/collaring events

-those removed for depredation control that are pups, have radio-collars, or have signs of disease (including mange)
-vehicle collisions: wolves that are found dead by the side of a road and appear on field examination to have been struck by a vehicle should not be submitted for necropsy unless they are pups, collared, have signs of disease (including mange), or are from counties where it is atypical to find wolves.
-suspected wolf hybrids only at the specific request of the wolf program coordinator

Pine marten: all adult or juvenile pine marten that are found dead, turned in as incidental trapping incidents, or that die during capture and handling events. These carcasses will be coordinated through Jim Woodford at Rhinelander so they can first be checked for subcutaneous PIT tags and have an ear clip DNA sample collected.

Other mustelids (fisher, otter, badger, weasels, mink, etc.): are generally desired for necropsy. Please contact Wildlife Health before submitting other mustelids.

Bats: any groups of dead bats. Bats start to decompose quickly so they should be collected and refrigerated or frozen as soon as possible. If collected very soon after death, refrigerate and contact Wildlife Health for potential overnight shipping on ice.

Bear: contact Wildlife Health; bear may be requested for necropsy on a case by case basis.

Elk: for adult elk- contact Laine Stowell or Matt McKay for a field necropsy; for calves- collect and freeze for transport to Wildlife Health for necropsy.

Deer: please refer to the "Sick Deer Calls Guidance" document available on the DNR Intranet Wildlife Health protocols page Wildlife Management Intranet - Wildlife Health Protocols.

Appendix D.5

WDNR Fish & Wildlife Specimen Submission Diagnostic Tag, Form 2300-144

Department of Natural Resources
FISH AND WILDLIFE SPECIMEN SUBMISSION/DIAGNOSTIC TAG
Form 2300-144 Rev. 8-99

Case Number

Submitter's Ref. Number

☐ Rush ☐ Routine

Date Collected

Submitter

Species

Street or Route

No. Submitted
____ of ____

City, State, Zip Code

County

Telephone Number

Section Town Range

Field History:

Specimen Found Dead
☐ Yes ☐ No

Euthanization Method

Continue on Reverse Side

Appendix D.6

WDNR Wildlife Necropsy Submission/Diagnostic Report, Form 2300-143

State of Wisconsin
Department of Natural Resources

Wildlife Necropsy Submission/Diagnostic Report

Form 2300-143 (R 5/06)

Instructions:

1. Fill out page 1 of this form and place a copy in a bag or envelope separate from the carcass and email a copy to the WDNR Wildlife Health Program at WM Necropsy@dnr.state.wi.us.
2. Fill out Carcass Tag (Form 2300-144) or Warden Seizure Tag (Form 4100-190) and place on carcass.
3. Bag carcass properly for shipment.
4. **Submit to:**

   WDNR Wildlife Health
   2801 Progress Road
   Madison, WI 53716

For guidance on handling and disposition of carcasses, call Wildlife Health at (608) 221-5375.

For Lab Use Only	
WH No.	
Other Lab	Accession No.
Other Lab	Accession No.

DNR Submitter Information ***(Put Citizen Contact Information in"History" below.)***

Last Name	First	MI	Collection Location			
Address			Date Collected			
City	State	ZIP Code	County	Township N	Range E / W	Section
Telephone Number	E-Mail Address		Other Reporting Agent			
Submitter's Reference No.			GPS Location			

Specimen Information

Species	Found Dead ☐ Yes ☐ No	Animal euthanized? ☐ Yes ☐ No	If yes, how?
Date of Onset of Illness (if known)	No. Affected	No. Dead	No. Submitted

Legal Case: ☐ Yes (Chain of Custody attached) ☐ Suspicious Death (Explain below) ☐ No

History

Citizen's name, address, and phone, if relevant:	Last Name	First	MI
Address	City	State	ZIP Code	Telephone Number

General History Information (Explain suspicious death, if box is checked above):

WH Case No.

Wildlife Necropsy Submission/Diagnostic Report

Form 2300-143 (R 5/06)

For Lab Use Only

Radiographs

Date Necropsied	Age	Sex ☐ Male ☐ Female	Prosector	Recorder

Gross Necropsy Findings:	Body condition	PM Condition

Photos Taken	Carcass Disposition

Tests Requested:

Specimen Collected	Bacteriology	Histopathology	Virology	Serology	Toxicology	Other (Specify)	Archive (Specify)
☐ Kidney	☐	☐	☐	☐	☐	☐	☐
☐ Liver	☐	☐	☐	☐	☐	☐	☐
☐ Spleen	☐	☐	☐	☐	☐	☐	☐
☐ Intestine, _____	☐	☐	☐	☐	☐	☐	☐
☐ Heart	☐	☐	☐	☐	☐	☐	☐
☐ Lung	☐	☐	☐	☐	☐	☐	☐
☐ Brain	☐	☐	☐	☐	☐	☐	☐
☐ Muscle, _____	☐	☐	☐	☐	☐	☐	☐
☐ Serum	☐	☐	☐	☐	☐	☐	☐
☐ Blood, Clot	☐	☐	☐	☐	☐	☐	☐
☐ Feces	☐	☐	☐	☐	☐	☐	☐
☐ GI Contents	☐	☐	☐	☐	☐	☐	☐
☐ Swab, Cloacal	☐	☐	☐	☐	☐	☐	☐
☐ Nobuto Strip	☐	☐	☐	☐	☐	☐	☐
☐ Other: _____	☐	☐	☐	☐	☐	☐	☐
☐ Other: _____	☐	☐	☐	☐	☐	☐	☐
☐ Other: _____	☐	☐	☐	☐	☐	☐	☐

Appendix E.1

Sheboygan River AOC Kingfisher Burrow Monitoring data sheet

Sheboygan River AOC Kingfisher Burrow Monitoring

Nest ID: _____ **GPS Location/error:** _____

Date:	Time:	# eggs:	# young:	Notes:

Appendix E.2

Photograph of Kingfisher Burrow Monitoring System


Sentinel ELF Field DVR System
Startup
System Prep.
System Deployment
Manual Shutdown
Sentinel ELF
Field DVR System
12V OK
DVR 5V OK
Camera ON
Motion Detect
Low Battery
Fault
Rec Night
Rec Motion
Custom

Appendix E.3

Sheboygan River AOC Small Mammal Collection data sheet

Sheboygan River AOC Small Mammal Collection

Location ID: _____ **Week #:** _____

GPS Coords: _____

GPS Error: _____ **Date Traps Opened:** _____

Location Description: **Date Traps Closed:** _____

Date	Trap ID - Site #	Foot (mm)	Ear (mm)	Tail (mm)	Weight (g)	Sex	Species	Status
	S1-							
	S2-							
	S3-							
	T1-							
	S1-							
	S2-							
	S3-							
	T1-							
	S1-							
	S2-							
	S3-							
	T1-							
	S1-							
	S2-							
	S3-							
	T1-							

Temp/Precip Information: **Notes:**

Day 1: _____

Day 2: _____

Day 3: _____

Day 4: _____

Appendix E.4

Seeley 1993 study report and subsequent PCB analysis results

September 17, 1993
Andrea Seeley
218 Sims
Stevens Point, WI 54481

SMALL MAMMAL POPULATIONS ALONG PCB CONTAMINATED SECTIONS OF THE SHEBOYGAN RIVER, WISCONSIN

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Abstract: In 1978, the Sheboygan River was discovered to be contaminated by PCBs (Polychlorinated biphenols), volatile organic compounds and heavy metals. Since wildlife along the river are potentially affected by these contaminants, a biomonitoring project was developed to study the effects of PCB contamination on riparian wildlife. As part of this project, small mammals along polluted sections of the river were live trapped to determine species occurrence and abundance. Specimens were collected for contaminant analysis. Deer mice (Peromyscus maniculatus), meadow voles (Microtus pennsylvanicus) and Eastern chipmunks (Tamias striatus) were most frequently captured. Data on abundance and occurrence along with current levels of contaminant loads will allow selective capture of mammals for future biomonitoring. Information on PCB levels in terrestrial mammals will help clarify the effects of PCB's in the food chain.

Results of this study will be used as part of the biomonitoring project of cleanup efforts on the river.

Key Words: Sheboygan River, PCBs, small mammals, biomonitoring, deer mice, *Peromyscus*, voles, *Microtus pennsylvanicus*, chipmunks, *Tamias striatus*, populations

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PCB contamination of fish in the Sheboygan River, Wisconsin was first documented in 1978 (Kleinert et *al*. 1978). Forty samples\of fish contained an average of 155 ppm PCBs on a wet weight basis and some carp (Cyprinus carpio) had hundreds of ppm PCBs. In 1983, bird carcasses contained from 23 to 218 ppm PCBs and brain tissue of one great blue heron contained 220 ppm (Heinz *et al*. 1983). Four out of 5 belted kingfisher (Ceryle alcyon) carcasses had over 180 ppm PCBs. The residues found in the birds were at levels considered harmful to some species tested in the laboratory.

The primary source of the PCBs was the Tecumseh Products Diecasting Plant in Sheboygan Falls (Kleinert et *al*. 1978). Deposits of granular oil absorbent material behind the plant leached PCBs into the adjacent Sheboygan River. The Kohler Co. landfill in Kohler also leached PCBs from solvents and other hazardous wastes deposited there (Wis, Div of Health, 1993). Although cleanup efforts have begun (both the plant and landfill are Superfund sites), the landfill continues to leach PCBs and there are PCBs in the sediments.

A biomonitoring project was designed to assess the effect of contamination on river wildlife. The project proposed population studies and PCB analyses of waterfowl, small mammals and reptiles and amphibians. The information gathered in the project will be used to help determine the success of cleanup efforts on the river. The small mammal study is the first part of the study to be undertaken. The primary objective of this study was to determine what species occurred along the river and their abundance. Control areas were selected upstream of the contaminated area to compare species composition of contaminated to non-contaminated areas. Some specimens were collected for necropsy and to analyze their tissues for toxics.

We would like to thank F. Wedepohl, the River Wildlife Reserve and Sheboygan County for the use of their land. R. Hetzal gave advice on trapping and assisted with trapline establishment. Specimens will be analyzed by S. Hurley and B. Bodenstein.

STUDY AREAS

Samples were taken from 3 sections of the Sheboygan River shoreline near Kohler, Wisconsin (hereafter referred to as "Lodge", "CoA" and "Oxbow") and from 2 control areas upriver from Sheboygan Falls (hereafter referred to as "C1" and "C2") (Fig.1). Vegetation at the study areas consisted of reed canary grass (Phalaris arundinacea), Phlox spp., stinging nettle (Urtica dioica), mayapple (Podophyllum peltatum), birch (Betula spp.) box elder (Acer negundo) and trembling aspen (Populus tremuloides) (Fig. 2-4). The banks of the river were steep and well vegetated

and with occasional mudflats extending into the river. The control areas were grassland with scattered trees Reed canary grass was the dominant ground cover. Clover (Trifolium spp.) were also abundant. Trees consisted of box elder (Acer negundo), willow (Salix spp.) and cottonwood (Populus deltoides). The banks were less steep than the study areas and had few mudflats. The soil type of both control areas was a Matherton silt loam (0-3 percent slope) and that of all study areas was a Bellevue fine sandy loam with a sandy subsoil (U.S. Dept. of Ag. 1973).

The climate consists of hot, humid summers and long, cold winters with average annual precipitation of 59.5 cm (U.S. Dept. of Ag. 1973). Storms are common in all seasons. Weather during the trapping period was wetter than normal. Storms kept the Sheboygan River high for most of June and July. When the river flooded, it rose 1 to 2 m up the bank. Temperatures were normal for summer, with an average of 27 C.

METHODS

The trapping period ran from 31 May 1993 to 20 Aug 1993. Each of the 3 study areas were trapped for 4 weeks as were the 2 control areas. Thirty to 35 small Sherman and Tomahawk live traps were set in pairs or trios on linear transects on one or both sides of the river. Trap stations were 15 m apart and were within 10 m of the river. Peanut butter and oatmeal were used for bait. Because raccoons (Procyon lotor) were interfering with the traplines, Sherman traps were placed inside large

Tomahawk traps. Medium-sized Tomahawk traps were placed at some stations to accomodate squirrels (Sciurus spp.)

Traplines were open Monday night through Thursday night. They were checked from 0800 to 1200 hours Tuesday through Friday. Mammals were marked for recapture with combinations of black hair dye and toe clipping. Several deer mice (Peromyscus maniculatus) and meadow voles (Microtus pennsylvanicus) were kept in captivity to test the longevity of the dye.

After live traps were removed from an area, rat and mouse snap traps were set to obtain data for a second population estimate. Traps were set at 5 or 6 stations where the most animals had been previously caught in live traps. The traps were operated for 1 or 2 weeks depending on trap success.

Traps were also set for mink (Mustela vison) because of their sensitivity to low levels of PCBs (Aulerich and Ringer 1977). Conibear traps were set into holes dug in the river bank and baited with mink lure. Mink sets were located at areas of good mink habitat or areas where mink had been sighted or where there was mink sign.

Specimens that died in the traps were collected for necropsy and PCB analysis on the brain, kidney and liver and, if the animal was one that might be consumed by humans, the carcass. Analysis of whole carcasses will be done by skinning and grinding up the carcass and analyzing the slurry.

RESULTS

Species composition varied between control and study sites (Tables 1 and 2.). Meadow voles and shrews (Sorex cinereous and Blarina brevicauda) were more abundant at the control sites and Eastern chipmunks (Tamias striatus) were found only at the study sites. Deer mice were abundant at all sites. Animals caught occasionally included: cottontail rabbit (Sylvilagus floridanus), longtail weasel (Mustela frenata), fox sparrow (Passerela iliaca), Northern flicker (Colaptes auratus), house wren (Troglodytes aedon) and American toad (Bufo americanus). The Oxbow study site was the most similar in vegetation and species to the control sites.

Relative frequencies per trap night were used as general population indices for all species (Figs. 7-11). Separate frequencies were determined for live and snap trap data. When comparing live and snap trap frequencies, it must be remembered that live traps were run for 544 trap nights and snap traps for 128 and that snap traps were run after live trapping was finished. Species composition and frequency per trap night were similar between C1 and C2 and between Lodge, CoA and Oxbow. An exception was the numbers of meadow voles and masked shrews between C1 and C2 (Figs. 7 and 8). At C1 there were many voles and few shrews; at C2 there were many shrews and few voles.

Lincoln - Peterson estimates (Schemnitz 1980) were calculated for live trapped deer mice, meadow voles and Eastern chipmunks (Table 3). Sample sizes were

too small to calculate population estimates for other species. Subjective evaluation of capture frequencies and population estimates were made and possible reasons for differences were discussed.

One to 3 "trap-happy" animals were encountered at all sites. They did not influence total recaptures unless few animals overall were caught. Deer mice were most commonly conditioned and made up 14% - 26% of the recaptures at a site. Many Eastern chipmunks returned to traps but only one (recaptured 9 times) occured regularly.

Hair dye used as a marker worked well on mice and Eastern chipmunks. The deer mice kept in captivity held their marks for 4 weeks until released. Mice recaptured in snap traps several weeks after live trapping were still marked. Dye did not work on meadow voles or shrews due to their dark fur; toe clipping was satisfactory.

No mink were trapped at any site. Traps were occasionally sprung but no animals were caught. There were past reports of farm bred mink attacking game farm pheasants near Lodge. One set of tracks was found at C1 and questionable tracks were found at other sites. Muskrat tracks were found at C2 and Lodge.

DISCUSSION

Species composition differed between control and study sites. I believe the variation is due to habitat differences. Eastern chipmunks were not found at control sites because are primarily a grassland species. More meadow voles were found at control sites than study sites for the same reason.

Rose (1978) found meadow voles and masked shrews to be the most abundant animals trapped in 4 upland fields at the Horicon Marsh, Dodge County. In St. Croix County, a positive correlation was found between the importance values of non-grass species in fields and the number of small mammals caught (Kjolhaug 1982). Comparing species composition and cover types at the Apostle Islands National Lakeshore, Stowell (1984) found deer mice populations to be high in paper birch (Betula papyrifera) - balsam fir (Abies balsamea) associations and meadow voles to be very abundant in old fields

Habitat differences cannot explain the difference in numbers of meadow voles and masked shrews between C1 and C2. The two sites are identical in vegetation structure and are 1 mile apart and should support similar numbers of meadow voles and masked shrews. The increase in masked shrews at C2 is probably trap related. Shrews eat mice more than vegetable matter (Jackson 1961) and would not be attracted to traps baited with peanut butter until mice had been in them. The fact that masked shrews were not caught at C1 until two weeks into the trap session supports this idea. When the traps were moved to C2, they already smelled like mice and masked and short-tailed shrews were caught through the entire trapping session at C2.

The lack of meadow voles at C2 may also be trap related. Because traps were not cleaned between being moved from C1 to C2, some traps may have failed to work correctly and animals could have eaten the bait without triggering the trap. All trap

stations were used at least once at C1 but only 9 stations out of 15 were used at C2. This may indicate why there were lower numbers of animals overall at C2 (Fig. 8).

Considering the lower number of trap nights for snap traps, (544:128) snap traps caught proportionately similar numbers for all species. Frequencies per trap night may be affected by immigration from animals replacing those removed. Kjolhaug (1982) noticed 3 day cycles in the number of animals trapped ove a 10 day trapping period. He suspected that every 3 days a population would be exhausted and new animals would move in. The same reaction may have occurred here.

The fact that no mink were caught and that only one set of tracks was found in a control area suggests that mink are scarce along the Sheboygan River. Local trappers and residents along the river reported seeing very few mink over the past two decades. Tracks and scat of muskrat (Ondatra zibethica), a mink prey item, were scarce at all sites. Lack of experience in trapping mink may partially explain no mink being caught. Mink may also be limited by PCBs in the river.

MANAGEMENT IMPLICATIONS

This study is a basis for more selective sampling of the small mammal population. Deer mice, meadow voles and Eastern chipmunks would be good species to trap because of their abundance. Deer mice could be used for comparison between areas. Masked shrews are also common and, because insects are part of

their diet, may have more exposure to PCBs. Results of tissue analysis will show which species, if any, are accumulating PCBs. Contaminant analysis was not finished in time for the results to be presented here. I suggest that, a variety of mammal species from the Sheboygan River be sampled in the future, and analyzed for toxics.

For future trapping efforts, I reccommend modifications to the methods in this paper. In trapping shrews, traps should be baited with animal scent such as mouse hair or droppings. Otherwise it will take a few weeks for a trap to attract shrews. Mink traps should be set to exclude raccoons. Snap traps should be set in greater numbers and for a longer time if the only objective is to acquire specimens. Snap traps are more effective when baited with oatmeal and peanut butter rather than just peanut butter.

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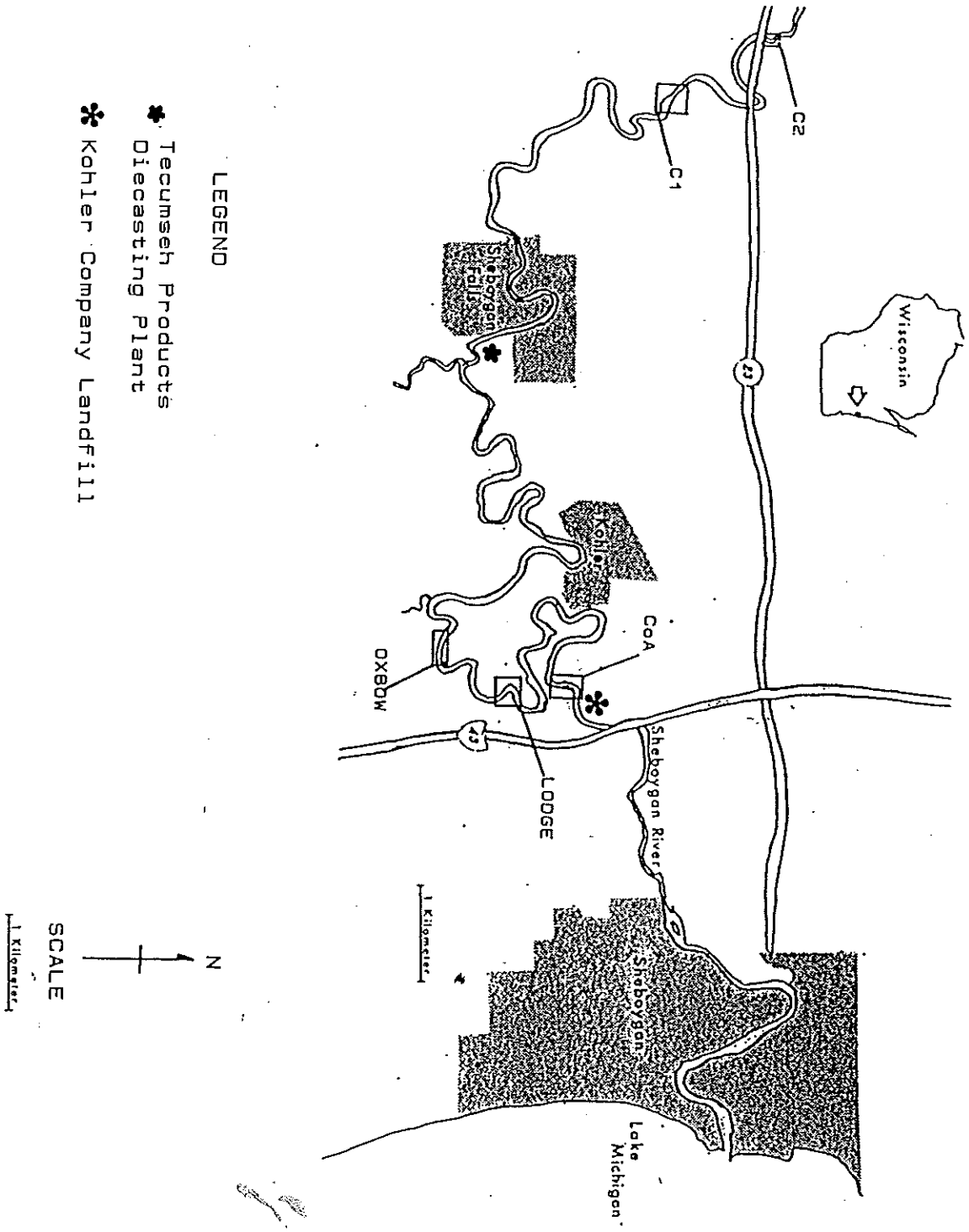
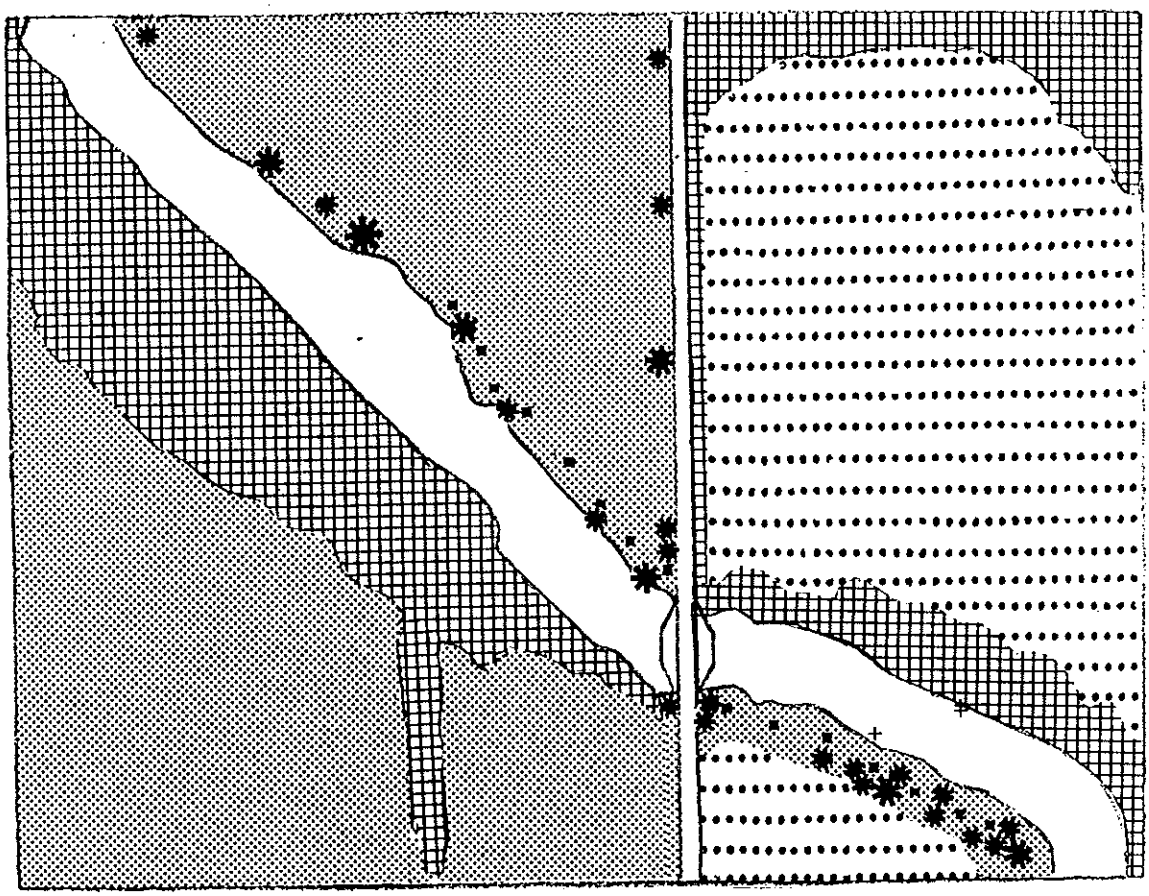


Fig. 1. Sheboygan River area with locations of the control and study areas, Sheboygan County, Wisconsin. 1993.



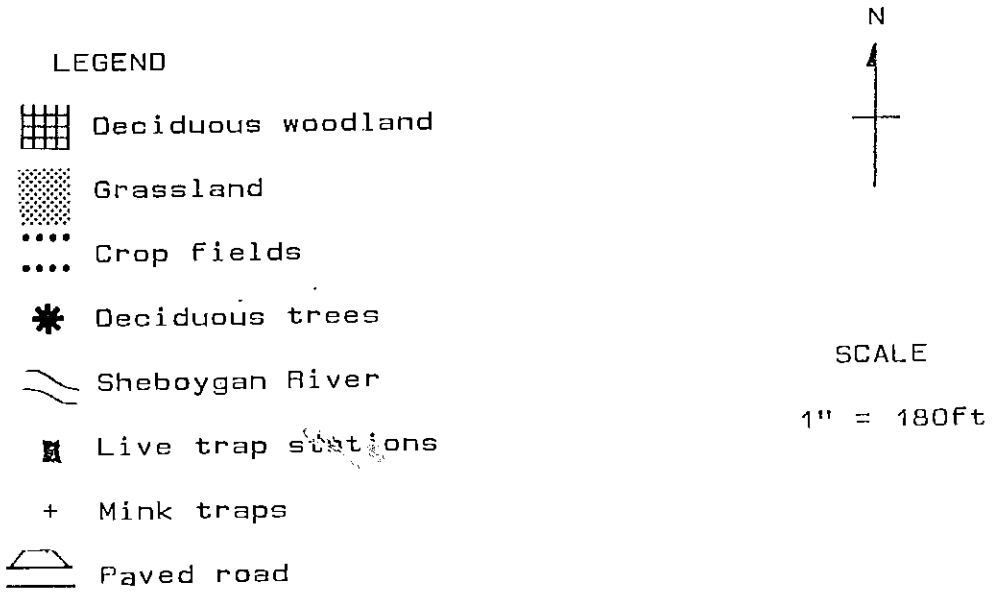
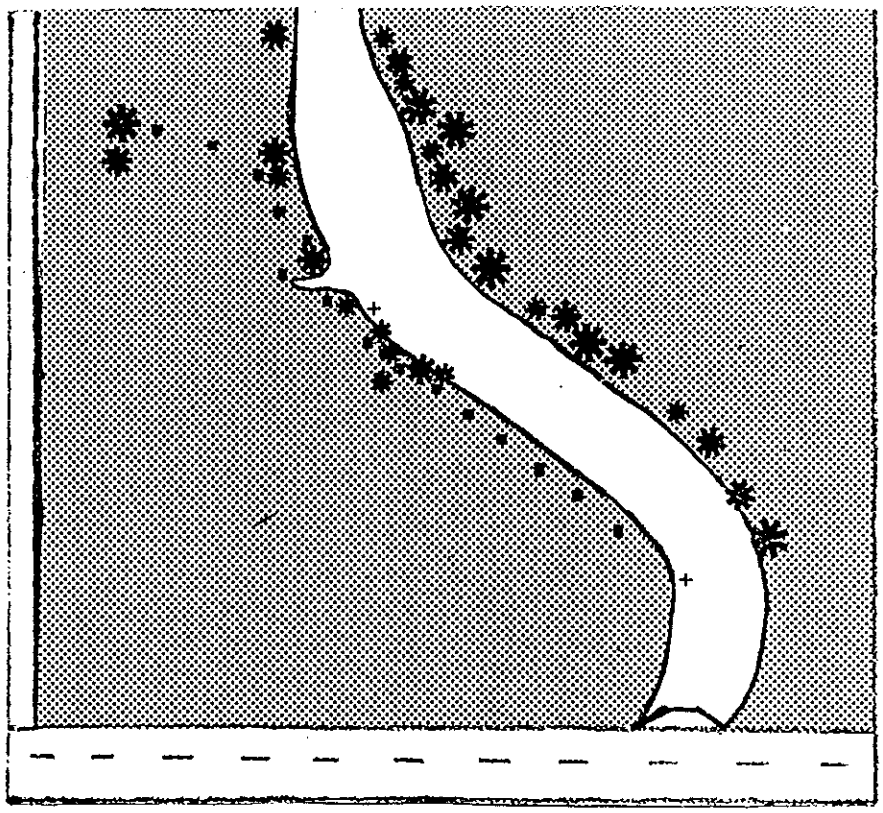


Fig. 2. Cover type map of C1; SW1/4 SW1/4 NW1/4, Sec. 26, NE1/4 SE1/4 NE1/4, Sec. 27, Sheboygan County, Wisconsin. 1993.



LEGEND

Grassland

Deciduous trees

Sheboygan River

Live trap stations

\+ Mink traps

County road

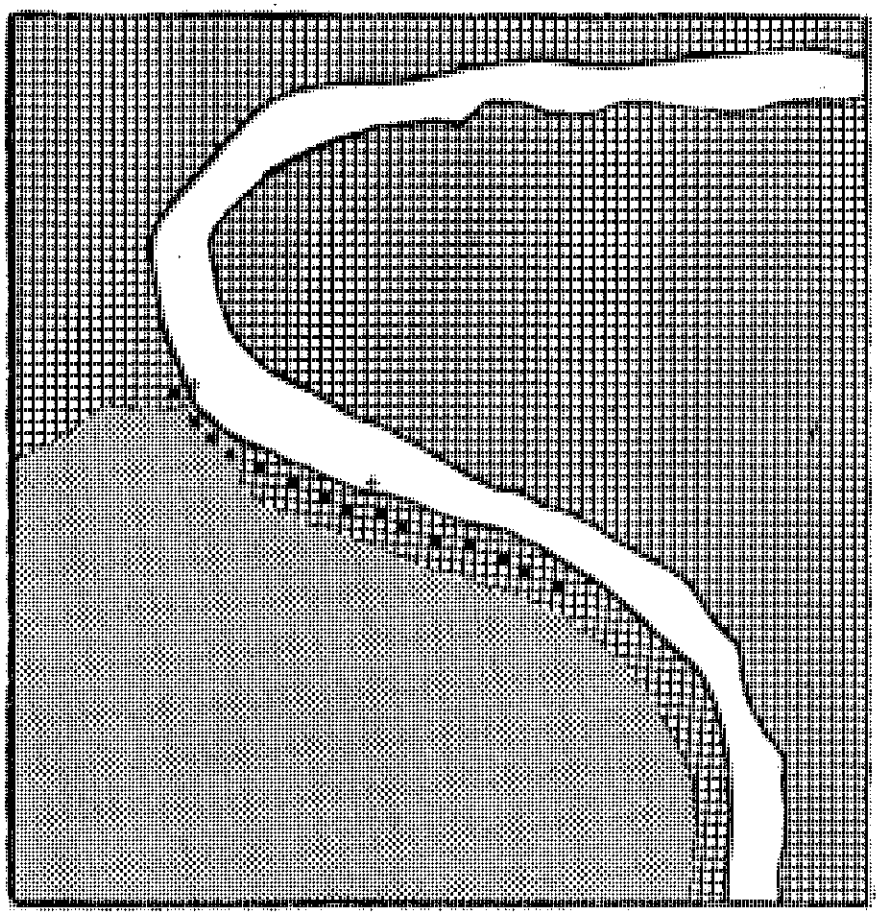
State highway 23

N

SCALE

1" = 180Ft

Fig. 3. Cover type map of C2; SW1/4 NW1/4 SE1/4, Sec. 22, T.15N., R.22E., Sheboygan County, Wisconsin. 1993.



LEGEND

Deciduous woodland

Grassland

Sheboygan River

Live trap stations

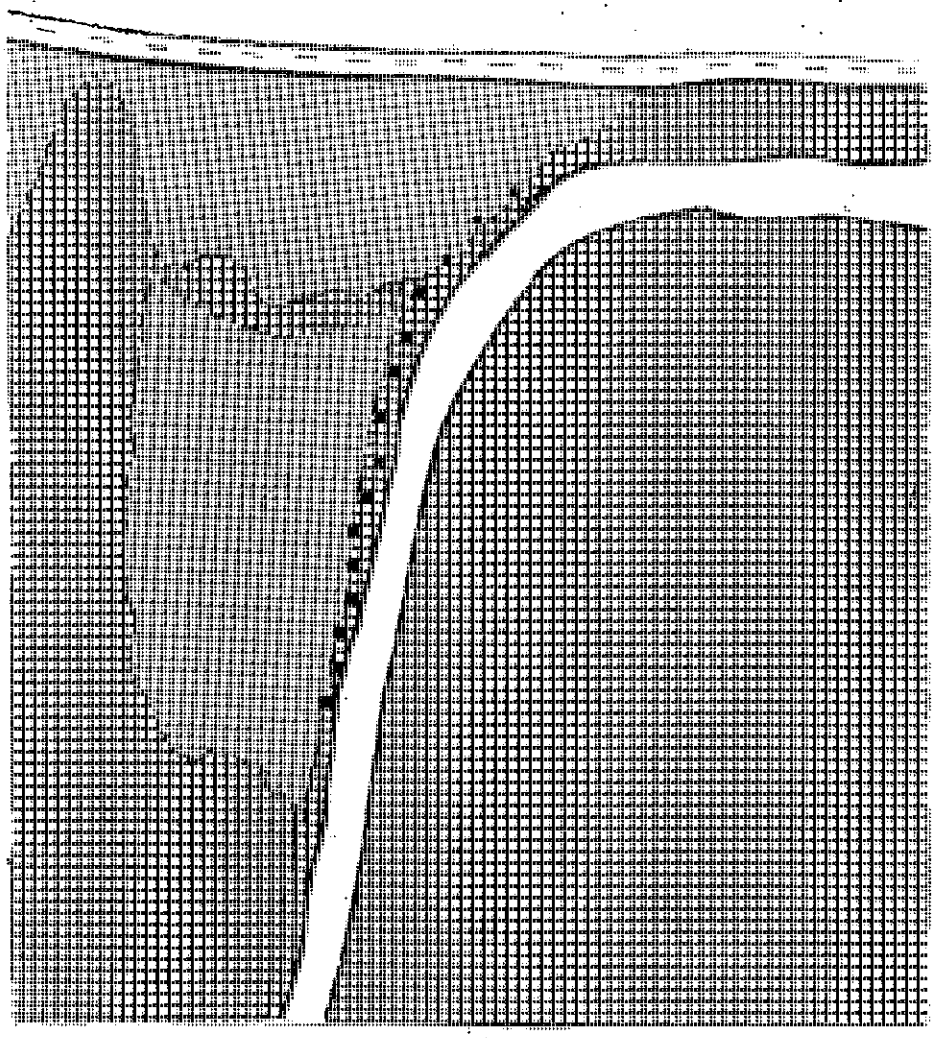
\+ Mink traps

N

SCALE

1" = 229ft

Fig. 4. Cover type map of Lodge; S1/2 S1/2 NE1/4 and the N1/2 N1/2 SE1/4, NW1/4, Sec. 32, Sheboygan County, Wisconsin. 1993.



LEGEND

Deciduous woodland

Grassland

Sheboygan River

County

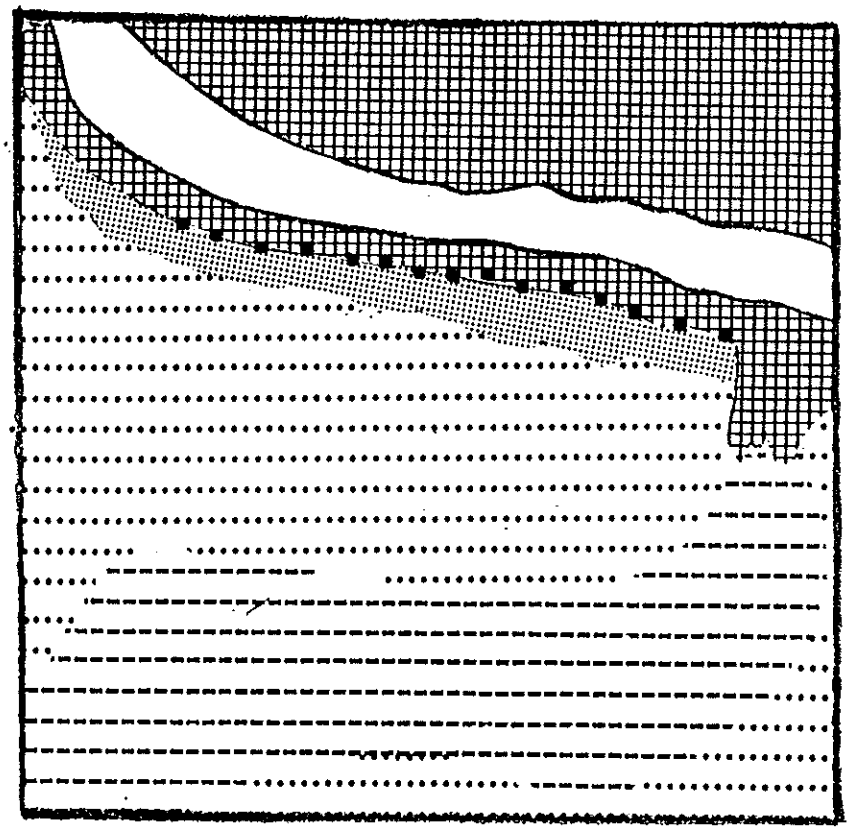
Live trap stations

N

SCALE

1" = 210ft

Fig. 5. Cover type map of CoA; W1/2 SE1/4 SE1/4, Sec. 29, Sheboygan County, Wisconsin. 1993.



LEGEND

Deciduous woodland

Grassland

Wildlife food plot (Sorghum)

Wooded marsh

Sheboygan River

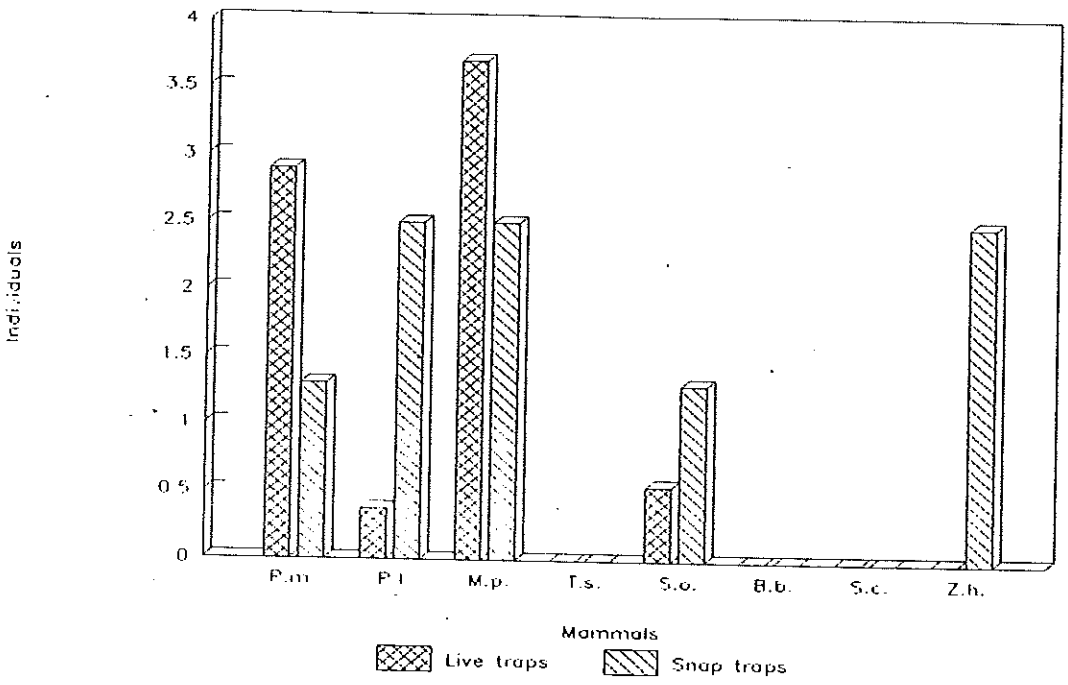
Live trap stations

N

SCALE

1" = 210Ft

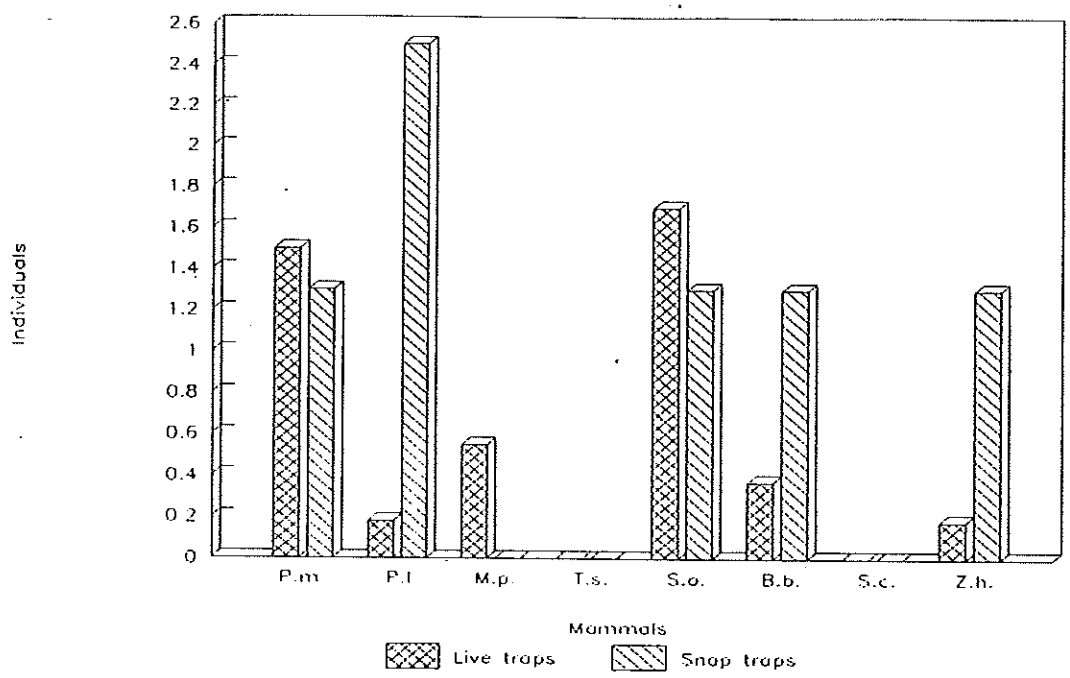
Fig. 6. Cover type map of Oxbow; SE1/4 E1/2 NE1/4 SW1/4 and the S1/2 NW1/4 SE1/4, Sec. 32, Sheboygan County, Wisconsin. 1993.



P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.

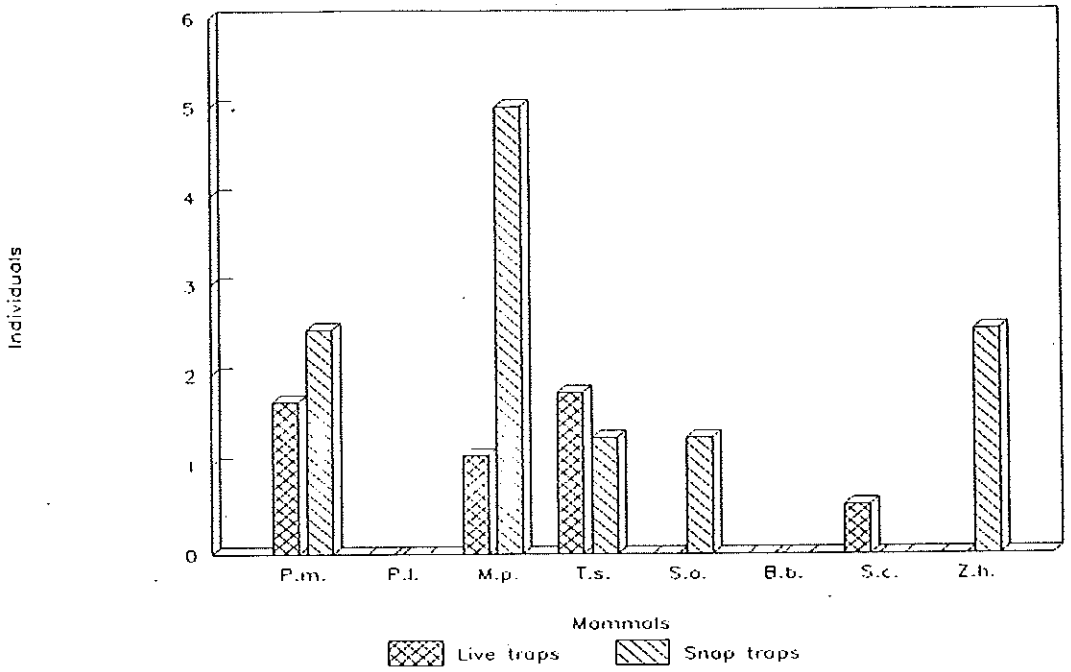
% represents each species'percentage from total individuals of all species at the site.

Figure 7. Small mammals trapped per 100 trap nights at control area C1 along the Sheboygan River in June 1993.



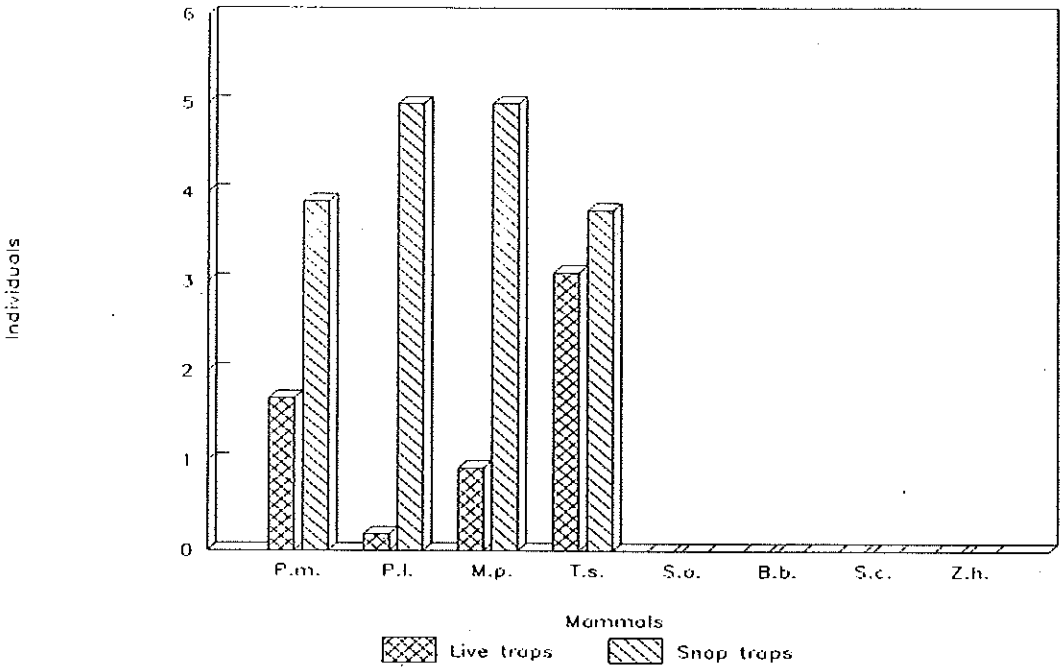
P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.
% represents each species'percentage from total individuals of all species at the site.

Figure 8. Small mammals trapped per 100 trap nights at control area C2 along the Sheboygan River in July 1993.



P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.
% represents each species'percentage from total individuals of all species at the site.

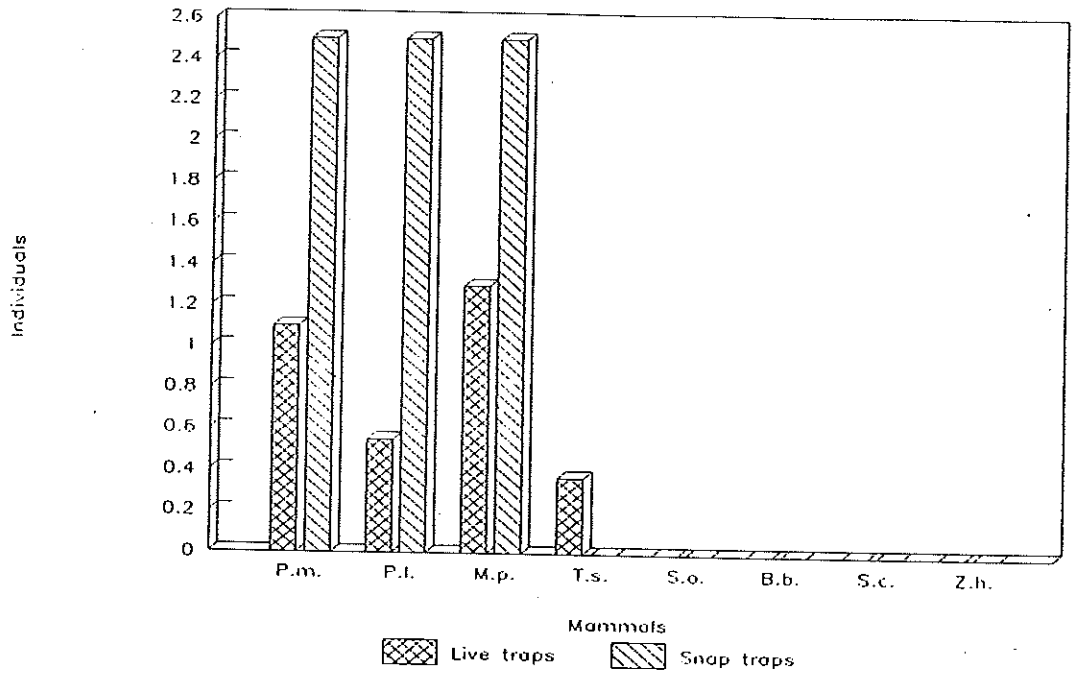
Figure 9. Small mammals trapped per 100 trap nights at study area Lodge along the Sheboygan River in June 1993.



P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.

% represents each species'percentage from total individuals of all species at the site.

Figure 10. Small mammals trapped per 100 trap nights at study area CoA along the Sheboygan River in July 1993.



P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.
% represents each species'percentage from total individuals of all species at the site.

Figure 11. Small mammals trapped per 100 trap nights at study area Oxbow along the Sheboygan River in August 1993.

Table 1. Small mammals live trapped at control and study areas along the Sheboygan River in summer 1993

Area	Mammals[a]								
	P.m.	P.l.	M.p.	T.s.	S.o.	B.b.	S.c.	Z.h.	TOTAL
C1									
first captures	16	2	20	0	3	0	0	0	41
recaptures	15	5	4	0	0	0	0	0	24
%[b]	39	5	49	0	7	0	0	0	100
C2									
first captures	8	1	3	0	9	2	0	1	24
recaptures	15	0	0	0	0	1	0	0	16
%	33	4	13	0	38	8	0	4	100
Lodge									
first captures	9	0	6	10	0	0	2	0	28
recaptures	18	0	3	11	0	0	1	0	33
%	32	0	21	36	0	0	7	0	100
CoA									
first captures	9	1	5	17	0	0	0	0	32
recaptures	21	0	0	26	0	0	0	0	47
%	28	3	16	53	0	0	0	0	100
Oxbow									
first captures	6	3	7	2	0	0	0	0	18
recaptures	22	0	4	3	0	0	0	0	29
%	33	17	39	11	0	0	0	0	100

[a] P.m. = Peromyscus maniculatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; S.c. = Sciurus carolinensis; Z.h. = Zapus hudsonicus.

[b] % represents each species' percentage from total individuals of all species at the site.

Table 2. Small mammals snap trapped at control and study areas along the Sheboygan River in summer 1993

Area	Mammals[a]							
	P.m.	P.l.	M.p.	T.s.	S.o.	B.b.	Z.h.	TOTAL
C1								
first captures	1	2	2	0	1	0	2	8
%[b]	12.5	25	25	0	12.5	0	25	100
C2								
first captures	1	2	0	0	1	1	1	6
%	17	32	0	0	17	17	100	100
Lodge								
first captures	2	0	4	1	1	0	2	10
%	20	0	40	10	10	0	20	100
CoA								
first captures	3	4	4	3	0	0	0	14
%	21	29	29	21	0	0	0	100
Oxbow								
first captures	6	3	7	2	0	0	0	18
recaptures	22	0	4	3	0	0	0	29
%	33	17	39	11	0	0	0	100

[a] P.m. = Peromyscus manicylatus; P.l. = Peromyscus leucopus; M.p. = Microtus pennsylvanicus; T.s. = Tamias striatus; S.o. = Sorex cinereous; B.b = Blarina brevicauda; Z.h. = Zapus hudsonicus.

[b] % represents each species'percentage from total individuals of all species at the site.

Table 3. Lincoln - Peterson estimates for small mammals per hectare at control and study areas along the Sheboygan River in summer 1993

	C1	C2	Lodge	CoA	Oxbow
Peromyscus maniculatus (per hectare)	29.00	6.760	15.15	17.30	
Microtus pennsylvanicus (per hectare)	409.0	*	26.00	*	
Tamias striatus (per hectare)	**	**	7.700	31.90	

* No recaptures; sample size too small
** No chipmunks found

Appendix 1

Specimans sent for contaminant analysis

Area	Mammals[a]						
	P.m.	P.l.	M.p.	S.o.	T.s.	Z.h.	TOTAL
C1	4	2	7	4			17
C2		1		6			7
Lodge	2		6	1	2	2	13
CoA	3	4	4		2		13
Oxbow	1	1	1				3
TOTAL	10	8	18	11	4	2	53

[a] P.m. = (Peromyscus maniculatus); P.l. = (Peromyscus leucopus); M.p. = (Microtus pennsylvanicus); S.o. = (Sorex cinereous); T.s. = (Tamias striatus); Z.h. = (Zapus hudsonicus).

SPECIES	DNR ID	LOCATION	WT	SEX	%FAT	PCB	26	28	52	49	47	44	41	74	70	66	56	84	101	99	97
SOREX	94081	CONTROL 1	4.33	B	4.4	<0.04															
SOREX	94082	CONTROL 1	3.25	B	3.8	<0.04															
BLARINA	94083	CONTROL 1	16		1.9	0.051															
PEROMYSCUS	94084	CONTROL 1	21		3.8	<0.04															
PEROMYSCUS	94085	CONTROL 1	27		4.4	<0.04															
CLETHRINOMYS	94086	RIVER WILDLIFE	46	F	2.5	0.20															
CLETHRINOMYS	94087	RIVER WILDLIFE	46	F	2.5	0.29															
CLETHRINOMYS	94088	RIVER WILDLIFE	22	F	1.3	**		5.6			2.6			17		13	3.6		0.91	2.1	
MICROTUS	94089	RIVER WILDLIFE	51	F	2.1	<0.04															
MICROTUS	94090	RIVER WILDLIFE	28	M	2.3	**		14	1.4	0.87	10			12	6.2	34	8.7		6.1	21	1
MICROTUS	94091	RIVER WILDLIFE	13		1.8	0.33															
PEROMYSCUS	94092	RIVER WILDLIFE	27	F	4.2	**		12	4.9	2.1	34		3.4	12		20	6.2	4.2	5.7	63	
PEROMYSCUS	94093	RIVER WILDLIFE	26	M	4.1	**		24	2.8	0.78	33		2.7	29		24	11		6.8	45	
PEROMYSCUS	94094	RIVER WILDLIFE	18	M	2.4	**		13	1.5		17			16		13	5.7		3.6	24	
PEROMYSCUS	94095	RIVER WILDLIFE	22	M	3.1	**					4.8			3.4		2.8			1.3	9.7	
ZAPUS	94096	RIVER WILDLIFE	13	M	1.6	**					2								0.7	2.3	
ZAPUS	94097	RIVER WILDLIFE	23	F	3.1	0.098															
TAMIAS	94098	RIVER WILDLIFE	88	M	1.3	1.0															
TAMIAS	94099	RIVER WILDLIFE	94	F	1.7	**					2			1.7		4.5				6.9	
MICROTUS	94100	COUNTY A	22	M	2.8	0.072															
MICROTUS	94101	COUNTY A	43	F	4.7	**	0.96	110	8.1	5	50	1.4	11	53	48	140	47		15	32	2.7
PEROMYSCUS	94102	COUNTY A	22	M	0.8	**					6.3			1.4					1.1	19	
PEROMYSCUS	94103	COUNTY A	18	M	3.5	**					1.5			3.1		3.2			1.3	4.8	
PEROMYSCUS	94104	COUNTY A	20	M	2.2	0.50															
TAMIAS	94105	COUNTY A	51	M	1	**		5.4			8.7			36		6.1	11			27	
TAMIAS	94106	COUNTY A	106	F	2.5	**		18	2	1.3	27			30	3.5	35	16		2.5	25	
PEROMYSCUS	94107	CONTROL 2	23	M	2.3	<0.04															
PEROMYSCUS	94108	CONTROL 2	23	F	2.3	<0.04															
PEROMYSCUS	94109	CONTROL 2	22	M	2.6	<0.04															
PEROMYSCUS	94110	CONTROL 2	18	M	4.2	<0.04															
PEROMYSCUS	94111	CONTROL 2	19	M	4.2	<0.04															
MICROTUS	94112	CONTROL 2	35	M	2.5	<0.04															
MICROTUS	94113	CONTROL 2	44	M	2.7	<0.04															
ZAPUS	94115	CONTROL 2	12	B	2.9	<0.04															
SOREX	94116	CONTROL 2	4.33	B	2.5	<0.04															
CLETHRINOMYS	94117	CONTROL 2	15		3.1	<0.04															
CLETHRINOMYS	94118	CONTROL 2	12		3.8	<0.04															
CLETHRINOMYS	94120	CONTROL 2	11		4	<0.04															

87	85	77	149	118	146	132	138	178	182	183	177	172	180	170	201	196	194	206
				51	5.2	1.9	17	1.3	4.1		1.8		7.8	8.1	2.3		1.3	
1.5		4.5	1.6	52	6.7	27	35		3.8		2.4		6.5	9.1				
2.5	8.8	2.7	1.6	33	19	180	140	3.2	23	4.7	1.2	5	46	46	9.2	17	7.8	4.5
2.3	7.1	1.9	1.2	120	22	180	140	4.8	32	5.5	2.6	5.1	59	45	9.7	17	9.2	4.4
1.2	3.8			61	11	92	72	2.5	16	2.8	1.3	2.7	31	23	4.9	8.3	4.7	2.3
				20	6.3	110	71	1.5	26	3.7		3.4	63	42	12	23	9.3	4.7
				18	9.9	110	67	1.4	23	3.1			59	36	3.8	15	6.9	2.5
				10	2.6	28	20		2.4				10	10		3.3	2.4	1.7
7	13	13		97	8.3	16	38		4.4		2.2		8	9.5	2.1		1.1	
	2.4			19	14	160	120	3.6	33	5.1	1.1	5	68	51	14	22	11	5.4
				28	7.7	43	34	1.8	14			2.1	12	9.2	3.3	3.1	1.9	
1.9	4			59	2.3	42	33		1.9				7	7.6			1.1	
2.1	8.7	2.4		37		27	21						5.7	6.5				

From: DNRVAX::PATNOK "Kathy Patnode, WM/4, 608-267-7974" 3-APR-1995
To: PLYMOU::KATSMD
CC:
Subj: RE: Sheb River

Here's the make-shift key to understanding these analysis records:

* and a value = interference in the assay (don't put a lot of emphasis on this number as it may be a combination of contaminants)

** in a metals column= not enough tissue sample was available, so metals assay not run

** in PCB total column = unable to match to commercial PCB pattern. We are finding that for most mammalian livers, metabolism of PCBs leads to a pattern that can't be matched to commmercial mixtures. The result is that the lab can't determine a total PCB value and congeners must be quantified.

columns (26-206) = PCB congener concentrations. Please note:
congener sums are in ng/g and PCB totals are in ug/g. I am trying to decide how to make comparisons between total and sum of congeners, but in this case it is more a matter of having detectable PCBs or not.

Hope this info. helps. If you have any more questions, let me know.

Appendix F.1

Wisconsin State Lab of Hygiene

EHD Metals Method 620.2, Digestion Method for Metals in Tissue by Inductively Coupled Plasma Spectroscopy (ICP)

Wisconsin State Laboratory of Hygiene
Environmental Health Division
EHD Metals Department

EHD METALS METHOD 620.2

Digestion Method for Metals in Tissue by Inductively Coupled Plasma Spectroscopy (ICP)

(Analytical Chemistry, Vol. 59, 1987)

1. Scope and Application

1.1 This method is applicable for the digestion of vegetation and animal tissue to be analyzed by inductively coupled plasma spectroscopy (ICP).

1.2 Samples may be analyzed for the following metals:

Aluminum	Copper	Nickel
Cadmium	Iron	Sodium
Chromium	Lead	Zinc
Cobalt	Molybdenum	

1.3 This procedure may be applicable to other metals

2. Summary of Method

2.1 Portions of well-homogenized tissue are digested in nitric acid with the periodic addition of hydrogen peroxide.

3. Safety and Waste Management

3.1 General safety practices for laboratory operations are outlined in the Chemical Hygiene Plan for the Environmental Health Division. (Ref. 11.3)

3.2 All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations.

3.3 Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide. (Ref. 11.4)

4. Sample Handling and Preservation

4.1 Tissue samples are received by the lab already homogenized and frozen. Some tissues may require further homogenizing using a blender. All samples should be collected in clean polyethylene or glass containers.

4.2 Samples are stored in the freezer in room 118 until ready to thaw and digest.

5. Interference

5.1 Elemental arsenic and selenium and many of its compounds are volatile; therefore, samples may be subject to losses of these metals during sample preparation. Care should be taken during digestion to not allow the samples to go dry.

6. Reagents

6.1 Reagent water, ASTM type I Nitric acid, concentrated, Tracemetal grade
6.2 Nitric acid, concentrated, Tracemetal grade, Fisher Scientific (Cat. # A509SK-212)
6.3 Hydrogen peroxide, 30%, Certified A.C.S., Fisher Scientific (Cat. # H325-4)
6.4 ICP spike solution (ZUWI601-500), VHG Labs, Manchester NH. Stored by the ICP prep area. Element concentrations are listed in EHD METALS Method 400.2, Table 4.
6.5 1000 ppm Stock standards may be used for non-typical spikes. Stored in drawer by the ICP prep area.
6.6 Quality Control Sample(QCS) Cat. # LPC-1-500N SPEX CertiPrep . Stored by the ICP prep area. Element concentrations are listed in EHD METALS Method 400.2, Table 4.
6.7 BCR Reference Material N0327, Mercury and Methyl-mercury in Tuna.

Note: All reagents must be entered in the reagent log (logbook #76, room 118). All stock standards must be entered into the metals stock standard log (logbook #68, room 117). All working standards and matrix modifiers must be entered into the metals working standard log (logbook #69, room 117). Certificates of analysis from venders for all stock standards and/or reagents are kept in a file folder located in room 117.

7. Apparatus

7.1 Glass Test tubes, 50 mL, graduated.
7.2 Technicon BD40 Block Digestor
7.3 Acid repipet dispensers (calibrated quarterly).
7.4 Fume hood
7.5 Assorted motorized and mechanical air displacement pipettes (calibrated quarterly) with appropriate tips.
7.6 Reagent water bottle
7.7 Balance, top loading, weighing to nearest 0.01 g
7.8 Spatulas
7.9 Class A volumetric flasks (various volumes).

8. Quality Control

8.1. Refer to the Environmental Health Division Quality Assurance Manual (11.5) for general information on Quality Control procedures (see section 22.6). Important specifics include:
  8.1.1. Accuracy and precision calculations (see section 22.7).
  8.1.2. Corrective action procedures (including documentation requirements) for instrument problems or analytical problems (see chapter 18 section 22.6.5)
8.2. DLRB (digested laboratory reagent blank) - consisting of reagent water. One must be carried through each sample preparation step for each batch of 20 samples. It is analyzed before any samples and the absolute concentration of each element of interest must be less than the LOD. In the case of failure, take corrective action (8.1). If no obvious errors are detected, it may be rerun once. If it fails again, only those samples with a concentration greater than 20 times the DLRB concentration may be accepted. All other samples must be redigested.

8.3. DLFB (digested laboratory fortified blank) - consisting of reagent water spiked with ICP Spike Solution (6.4). One must be carried through each preparation step for each batch of 20 samples. It is analyzed before any sample and concentration of each analyte of interest must be within 10% of the true value. In the case of failure, take corrective action (8.1). If no obvious errors are detected, it may be rerun once. If it fails again, all samples within that digestion must be reset.

8.4. DQCS (digested quality control sample) - consisting of reagent water spiked with Initial Calibration Verification solution (6.6). Must be carried through each sample preparation step and must be analyzed before any samples. Concentration of each analyte of interest must be within 10% of the true value. In the case of failure, take corrective action (8.1). If no obvious errors are detected, it may be rerun once. If it fails again, all samples within that digestion must be reset.

8.5. DTISS – BCR Tuna (6.7), a control tissue which is processed the same as all samples and must be analyzed after calibration and before any samples. Results must be within the control's published limits for the elements of interest. If there aren't any published limits, results must be within 10 % of the true value. In the case of failure, if no obvious errors are detected, it may be rerun once. If it fails again, all samples within that digestion must be reset.

8.6. Digested matrix spike - A second aliquot of sample is spiked with known concentrations of the elements of interest at a 10% frequency per matrix type and/or element requested. Recovery must be within the limits listed in the QL database in LIMS. In the case of failure, take corrective action (8.1). If no obvious errors are detected, the spike may be rerun once. If it fails again, all samples within that QC group must be reset or qualified.

8.7. Digested Laboratory duplicate - A second aliquot of sample is analyzed for the elements of interest at a 10% frequency per matrix type and/or element requested. Precision of duplicate analyses must be within the QC limits listed in the QL database in LIMS. In the case of failure, take corrective action (8.1). If no obvious errors are detected, the duplicate may be rerun once. If it fails again, all samples within that QC group must be reset or qualified.

9. Procedure

9.1. Remove tissues from the freezer and thaw to near room temperature.

9.2. WEAR SAFETY GLASSES AND GLOVES. As much of the procedure as possible should be carried out in a fume hood.

9.3. Turn on digestion block.

9.4. Create a digestion log at **R:\EHD\ESS(4900)\ESS Inorganic(4910)\METALS\Digestion Log** and record all pertinent information into the spreadsheet, including: tube number, sample numbers, sample bottle letter, matrix duplicates, matrix spikes, spike volume(s), spike code(s), initial volume, final volume, standard codes, reagent codes and hot block temperature. The digestion log is named following the format: D, (type: ITT), fiscal yr letter, date (mmdd). Once the spreadsheet is complete, a printed copy is kept with the digestion.

9.5. Label an empty tube DLRB. Add 5 mL reagent water.

9.6. Label an empty tube DLFB. Add 5 mL reagent water and 1 mL ICP Spike Solution (6.4).

9.7. Label an empty tube DQCS. Add 5 mL reagent water and 1.0 mL ICV solution (6.6).

9.8. Weigh approximately 0.5 g BCR Tuna into a glass tube and record weight in digestion log. Spike with 1.0 mL ICP Spike Solution (6.4).

9.9. Mix sample well and weigh 5.0 to 5.5 g (to nearest 0.01 g) of homogenized sample into a glass tube. Record the weight to the nearest 0.01 g in the digestion log. Sample weight may be 2.5 or 3.5 g if test tubes have graduations for 25 or 35 mL final volumes respectively. Do this for each sample, duplicate and spike in the digestion group.
9.10. Spike designated samples with 1.0 mL ICP spike solution (6.4).
9.11. Add 5.0 mL concentrated HNO_3 (6.2) per 50 mL final volume to all tubes.
9.12. Place the tubes in the digestion block and heat at 95°C. Record temperature in logbook (ESS174). Continue heating until the tissue is mostly dissolved (a layer of fat may remain on top of the liquid). This should take approximately one hour.
9.13. Add 2.5 mL 30% H_2O_2 (6.3) per 50 mL final volume to each tube, mix by vortexing or swirling and continue heating. (It is not necessary to cool tubes to add H_2O_2.)
9.14. Repeat the H_2O_2 additions at half-hour intervals until a total of 10 mL H_2O_2 (6.3) per 50 mL final volume has been added.
9.15. A half-hour after the final H_2O_2 addition, remove the tubes from the digestor and cool to near room temperature.
9.16. Place tubes in walk-in cooler (119C) to cool the tubes to near 4°C.
9.17. Dilute to the pre-determined final volume (50, 35 or 25 mL) with reagent water and mix thoroughly.

10. Reference for Additional Method Requirements

10.1. The following components are described in analysis method EHD METALS Method 400.2: Calibration, Data Management, Definitions and Method Performance.

11. References

11.1. Krynitsky, A.J. Preparation of Biological Tissue for the Determination of Arsenic and Selenium by Graphite Furnace Atomic Absorption Spectrometry. Anal. Chem., Vol. 59, pp 1884-1886. (1987)
11.2. Giesy, J.P. and Wagner, J.G. Frequency Distributions of Trace Metal Concentrations in Five Freshwater Fishes. Trans. Am. Fis. Soc., Vol. 106, No. 4, pp 393-403. (1977)
11.3. Chemical Hygiene Plan for the Environmental Health Division, WI State Laboratory of Hygiene.
11.4. University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety.
11.5. Quality Assurance Procedures and Policies, Wisconsin State Laboratory of Hygiene
11.6. National Environmental Laboratory Accreditation Conference, Constitution, Bylaws, and Standards, United States Environmental Protection Agency, Offices of Research and Development, Washington DC 20460, June 5, 2003.
11.7. ESS INO GENOP 106, Inorganic Sample Receipt
11.8. EHD METALS METHOD 400.2, Inductively Coupled Plasma-Emission Spectrometry

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Reviewed by:____ Roger Schultz_____ Date:_____3/18/2008_____
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Unit:___ EHD Metals_____

Approved by:__ DeWayne Kennedy-Parker_____ Date:_____3/19/2008_____

Title:___ Chemist Supervisor_____

Unit:____ EHD Metals_____

Certification Statements Received from:
Kevin Kaufman
DeWayne Kennedy-Parker
Roger Schultz

Appendix F.2

Wisconsin State Lab of Hygiene

EHD Metals Method 400.2,
Inductively Coupled Plasma-Emission Spectrometry

EHD METALS METHOD 400.2
Inductively Coupled Plasma-Emission Spectrometry
(EPA Method 200.7, SW846-Method 6010B)

1. Scope and Application

1.1 This method is used to determine total, dissolved, or total recoverable elements in drinking waters, surface water, domestic and industrial wastewaters, digested solids, digested animal tissue, TCLP extracts, Wisconsin Occupational Health Laboratory (WOHL) air samples, wipe samples, soils, and bulks using a Perkin Elmer 5300 DV (dual view) Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). This instrument allows for the measurement of emission spectra both radially and axially.

1.2 For a listing of all elements, wavelengths, plasma viewing configurations (radial or axial), LOD's and LOQ's refer to Table 2 at the end of this SOP. For WOHL reporting limits see the end of the appropriate digestion method (15.19, 15.20, 15.21).

1.3 The top standard concentration for all elements can be found in Table 3 and Table 4 at the end of this SOP. If samples have concentrations above the range of the top standard, a high concentration standard, within the linear dynamic range (see LDR section 8.7) may be run to verify linearity. Concentrations up to 90% of the high concentration standard may be accepted. If samples are diluted to within range, the dilution must be ±10% of the original result, or subsequent serial dilutions are required until a ±10% agreement occurs between dilutions.

1.4 This procedure adheres to EPA 200.7 for all undigested samples (odorless, colorless, single phase, free of particulate or suspended matter samples with a turbidity of <1 NTU) and for digested total recoverable water samples. It adheres to SW846-Method 6010B for all digested liquids, TCLPs, tissues, solid waste samples, and WOHL air, wipe, soil, and bulk samples.

2. Summary of Method

2.1 This method describes a technique for the simultaneous multi-element determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Refer to cited reference methods for further information on the ICP technique.

2.2 The only minor deviations from the referenced methods are: the rinse time between samples is less than 60 seconds, but sufficient to remove all memory effects.

2.3 The check standard is made from the same source as the calibration standards; however, the quality control sample (QCS) is a second source. The wavelengths and plasma viewing configurations used on this instrument are listed in the LOD table at the end of this SOP (Table 2).

3. Safety And Waste Management

3.1 General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (15.3).

3.2 All laboratory wastes, excess reagents, and samples are disposed of in a manner that is consistent with applicable rules and regulations

3.3 Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide (15.4).

4. Sampling Handling and Preservation

4.1 All liquid samples received for metals analysis must arrive in approved, clean plastic or glass containers. If not acidified in the field, they are acidified immediately at the laboratory with HNO_3 to 0.5% HNO_3 (pH < 2) and held for a minimum of 16 hours prior to analysis. The holding time for liquid samples is six (6) months. Process samples that require digestion (turbidity >1NTU) by EHD METALS Method 780.3 (15.5).

4.2 Solid samples must arrive in approved, clean plastic or glass containers and are kept cool (4°C) in the walk-in cooler in Room 119C (no chemical preservation is required). There is no holding time for these samples. Process by ESS INO Method 100.1 followed by EHD METALS Method 750.1 (15.6).

4.3 All enforcement samples must arrive with properly filled out Chain-of-Custody forms and stored in the locked walk-in cooler in Room 119C when not being processed or analyzed (see ESS INO GENOP 106, "Inorganic Sample Receipt," (ref. 15.13).

4.4 Tissue samples arrive frozen and are stored in the freezer in Room 118 until they are digested and analyzed. They also have no holding time. Process by EHD METALS Method 620.2 (15.7).

4.5 WOHL samples must arrive on approved media and be processed as described in WOHL Gen Op-013

5. Interferences

5.1 Setting background points can reduce interferences by eliminating the need for some interelement correction factors (IEC's). Instrument software sets background points equidistant from the wavelength peak. To optimize background points, analyze single element standards, examine spectra and set peak and backgrounds points to optimal conditions. A full procedure for determining IECs is described in EHD METALS IOP 500 (15.8).

5.2 The main interferences observed in the ICP-AES technique are spectral in nature and may be corrected for by the use of interelement correction factors (IEC's). The IEC's used for the PE 5300DV may be found in the methods in the WINLAB software. IECs are verified annually or whenever there is significant change in the instrument hardware or analysis conditions. IEC's are validated

daily by reanalyzing each calibration standard. The calibration standards are checked for the other elements at ± the LOQ level. A full procedure for determining IECs is described in EHD METALS IOP 500 (15.8).

5.3 Multi-component spectral fitting (MSF) is another type of interference correction available in the WINLAB software. This type of interference correction is only effective for off- line interferences. **Direct spectral overlap interferences will not be corrected with MSF**. MSF is only a useful correction when one is able to identify the exact interference and emission line. MSF will only be used when the analyst believes that is the best correction for the sample. A full procedure for determining MSF is described in EHD METALS IOP 500 (15.8).

5.4 Physical interferences are corrected by matrix matching calibration standards and/or dilution of the sample. A peristaltic pump on the PE 5300DV also helps reduce these effects.

5.5 Yttrium and Gallium are used as the internal standards to minimize differences in viscosity of all samples analyzed (see section 8.8.10). See EHD METALS IOP 500 (15.8) for specific information on the internal standard.

5.6 Chemical interferences are rare in ICP-AES, but may be corrected by using the method of standard additions (MSA). Normally MSA's are only done for TCLP's that are equal to or greater than 80% of the hazardous limit, as per SW846. MSA's need to contain a minimum of four points. The acceptance criteria are: a correlation coefficient (r) $\geq$0.999, and a slope (m) between 0.4 and 0.6.

5.7 For more information on ICP-AES interferences, refer to the cited methods.

6. Reagents and Standards

6.1 Reagent water, ASTM Type 1 water, U.S. Filter Corp., Lowell, MA.

6.1.1 Calibration blank is made from acidifying reagent water to the same concentration as the standards. The calibration blank is stored in an approved, clean container.

6.2 Nitric acid, concentrated, Fisher Tracemetal Grade

6.3 Hydrochloric acid, concentrated, Fisher Tracemetal Grade

6.4 Chemware PFTE Boiling Stones, Fisher Scientific, (Cat. # 09-191-20)

6.5 Refer to Table 3, Table 4, and Table 5 for the concentrations of standards and controls.

6.5.1 Standards are made from custom standard mixes and/or 1,000 ppm and 10,000 ppm National Institute of Technology (NIST) traceable single element standards obtained from High Purity™, Charleston, SC.

6.5.1.1 Standards are prepared in four or more separate element mixtures based on compatibility. This is critical since the interelement correction (IECs) equations are not active during the calibration process. Consequently, the elements in each standard solution must NOT interfere with each other.

6.5.2 IPC (Instrument Performance Check) is made from custom standard mixes and 1,000 ppm and 10,000 ppm NBS traceable single element standards at ½ the concentration of the calibration standards obtained from High Purity™, Charleston, SC. The IPC and other quality control samples may be prepared in a single standard solution since the IECs are applied when these samples are analyzed.

6.5.3 QCS (second source) obtained from SPEX Certiprep ™. Diluted 25X prior to analysis.

6.5.4 LOQ control obtained from High Purity™, Charleston, SC. Diluted 100X prior to analysis.

6.5.5 Spiking solution (ZWISTATCM#2-500) obtained from VHG™ Labs, Manchester, NH

6.5.6 Internal standard solutions prepared from 10,000 ppm single element yttrium and 10,000 ppm single element gallium. 4 mL gallium and 0.8 mL yttrium diluted to 500 mL in 0.5%HNO_3 (for the #23 ICP), and 10 mL gallium and 4 mL yttrium diluted to 500 mL in 5% HNO_3 / 5% HCl (for the #26 ICP). This solution is added to every standard, control, and sample online using the instrument pump. The internal standard can also be added directly to the samples, standards and controls. This solution is 20 mL 10,000 ppm gallium and 4.0 mL yttrium diluted to 250 mL in 0.5% HNO_3. 70 µL of this internal standard solution is added to 7 mL of every standard, control, and sample analyzed (for the #23 ICP only).

6.5.7 WOHL LOQ obtained from High Purity™, Charleston, SC. Diluted 100X prior to analysis.

6.5.8 Interference Lead control (INT Pb) made from single element standards: final concentrations are aluminum, calcium, iron, and magnesium at 100 ppm, zinc at 50 ppm.

6.5.9 Linear Dynamic Range (LDR) control made from single element standards; see Table 6 at end for elements and concentrations.

6.6 Upon receipt or preparation, all standards and all controls are entered in the appropriate logbooks (stock standard, logbook #68 ICP working standard, logbook #67) and are assigned a traceability code. The code, date prepared, analyst and expiration date is recorded on each standard bottle. The working standards codes are also listed on the cover sheet of each analytical run. The working standards are prepared as needed or at least every 6 months. For a list of standards and controls see Table 3 and Table 4. Single element stocks, custom stock solutions, mixed element control and spiking stocks, working standards and controls, are all stored in the shelves and cabinet drawers near the ICP in room 117. These solutions do not have any special storage requirements.

7. Apparatus

7.1 Perkin Elmer 5300DV inductively coupled plasma optical emission spectrophotometer, (ICP-OES) with dual view capabilities.

7.2 Perkin Elmer AS93plus autosampler.

7.3 FAST autosampler from Elemental Scientific Inc.

7.4 Polyscience recirculator

7.5 Bulk liquid argon gas supplied by AGA, Madison, WI.

7.6 Class A volumetric flasks and pipettes.

7.7 Assorted motorized and mechanical air displacement pipettes (calibrated quarterly) with appropriate tips.

7.8 Test tubes, 16 x 125 mm polypropylene, Environmental Express

7.9 Test tubes, 13 x 100mm polypropylene, Environmental Express

8. Quality Control

8.1 Please refer to the Environmental Health Division Quality Assurance Manual (15.11) for general information on quality control procedures. Important specifics include:

8.1.1 Accuracy and precision calculations (see section 22.7).

8.1.2 Corrective action procedures (including documentation requirements) for instrument problems or analytical problems (see chapter 18 and section 22.6.5).

8.2 Duplicates and spikes must be performed on each group of samples of similar matrix type at a frequency of at least 10% (please note that WOHL air and wipes samples do not have spikes, and only replicates of the digestates are analyzed). Follow the appropriate method for digested samples (15.5, 15.6, 15.7, 15.16, 15.19, 15.20, 15.21). Duplicates and spikes must be within QC limits listed in the QL database in LIMS. If any QC fails, take corrective action (8.1.2). If the QC is exceeded after corrective action and no obvious errors are detected, the entire sample matrix group associated with that QC sample must be reset, or all samples in the QC group must be qualified with a comment as to the specific QC failure. Prepare spikes as follows:

8.2.1 For undigested samples: add 70 µL spiking solution (6.5.5) to 6.93 mL of undigested sample and mix.

8.2.2 For digested samples: add 0.5 mL spiking solution (6.5.5) to the sample aliquot, which is then digested and brought to a final volume of 25 mL.

8.2.3 For WOHL air samples add 1.25 mL spiking solution (6.5.5) to the sample media (MCE or PVC). For WOHL wipes and bulks add 2.5 mL spiking solution (6.5.5) to the sample media (Palintest or Whatman for wipes, Ottawa sand or Lead free paint blank for bulks) and bring to a

final volume of 50 mL.

8.2.4 For WOHL soils (Pb only): add 2 mL of 1,000 ppm Pb standard to the digestate (assumes 0.5 g of sample), process spike through the digestion, and bring to a final volume of 50 mL. If other elements are requested single element spikes may be used, or 1.0 mL spiking solution (6.5.5)

8.2.5 For WOHL bulk or paint chip samples: See sections 9.3.5 and 9.3.6 in EHD METALS METHOD 014. If other elements are requested single element spikes may be used, or 1.0 mL spiking solution (6.5.5)

8.2.6 For solid samples: add 1.0 mL spiking solution (6.5.5) to the digestate solution (assumes 0.5 g of sample), process spike through the digestion, and bring to a final volume of 50 mL.

8.3 A Calibration Blank (CB) must be analyzed immediately following calibration, after every ten samples (or less), and at the end of the run. Each CB must be within zero ± the LOD (ESS samples) or ± one half (½) of the reporting limit (WOHL samples) for each analyte of interest. If a CB fails take corrective action (8.1.2). If the CB fails again after corrective action, only samples that are equal to or greater than 10 times the CB result may be accepted. The laboratory reagent blank (LRB) is equivalent to the CB on all undigested samples. If a digestion is required, a separate LRB will be taken through the entire analytical process and analyzed with each batch of 20 samples. The LRB must meet the same requirements as the CB.

8.4 An Instrument Performance Check (IPC) (6.5.2) must be analyzed immediately following calibration and be within ±5% of true value (for ESS samples) and ± 10% of true value (for WOHL samples). Thereafter, it must be analyzed every ten samples (or less) and at the end of the run and be within ±10% of true value. The concentration values of the IPC are ½ of the concentration values of the calibration standards in Table 3. If a failure occurs, take corrective action (8.1.2). If the IPC fails after corrective action and no obvious errors are observed, all samples from the last acceptable IPC must be reset.

8.5 A Quality Control Sample (QCS) is analyzed daily. As a second source obtained from SPEX Certiprep, this control must be within ±10% of the true values listed in Table 3. Normally 0.28 mL of stock (table 5) is mixed with 6.72mL of blank for undigested, total recoverable, solids, or WOHL analytical runs. These dilutions insure that the analyte concentrations are different from those of the IPC. If this control fails take corrective action (8.1.2). Analysis cannot proceed until after a successful QCS. Corrective action may include re-calibration.

8.5.1 On a quarterly basis, analyze 3 replicates of a USEPA QCS and document this in spreadsheets located in R:/EHD/ESS/ESS INORGANIC/METALS/PE5300 DV documentation. The results must be within ± 5% of the true value. This task is a requirement of EPA method 200.7.

8.6 A Limit of Quantification (LOQ) is analyzed daily. As a second source obtained from High Purity Standards, this control must be within ±30% of the

true values listed in Table 4 (LOQ ESS) for ESS samples, and ±25% of the values (LOQ WOHL) for WOHL samples (except for Pb which must be ±20%). Normally 0.07 mL of stock (table 5) is mixed with 6.93 mL of blank for undigested and total recoverable analytical runs, and 0.14 mL of stock is mixed with 7.86 mL of blank for solid analytical runs. If this control fails take corrective action (8.1.2). Analysis cannot proceed until after a successful QCS. Corrective action may include re-calibration.

8.7 Linear Dynamic Range (LDR) is determined annually. The LDR is determined by analyzing successively higher standard concentrations of an analyte until the observed analyte concentration is no more than 10 % below the known concentration of the standard. LDR concentrations are listed in table 6.

8.7.1 Samples concentrations up to 90% of the LDR may be accepted. Generally, a LDR (single verification standard) will be analyzed daily.

8.7.2 As a general rule if a sample result is above the calibration range, the sample is diluted to be within range and the diluted result compared to the original. The results must agree within 10% Relative Difference (RD) from the original result, or a different dilution is made and analyzed. The second dilution must agree within 10% RD of the first dilution to be acceptable.

8.7.3 If an interfering element concentration exceeds the LDR, samples will be diluted and reanalyzed. The appropriate elevated LOD's will be reported.

8.8 Limit of Detection (LOD) is the concentration at which the element is definitely distinguishable from a blank. LODs for ESS samples are listed in Table 2, Reporting Limits (RL) for WOHL samples are listed at the end of the methods EHD METALS METHODs 001 (airs), 002 (wipes), and 014 (bulks). LODs are verified annually, or when any significant repair work is done on the instrument. Initially LODs are calculated as per EPA 40 CFR part 136, Appendix B. The metals group then determines "common sense" LODs based on blank data, noise levels caused by interfering elements, and analytical experience following the guidelines in ESS INO QA 116 (15.14). Verification is accomplished by analyzing seven replicates of a limit of quantification (LOQ) standard for ESS samples (teflon chips are added for solids). For WOHL samples seven spiked replicates of the reporting limit standard for all media used: air samples; PVC and MCE filters, wipes; Palintest and Whatman, bulks; Ottawa sand is used for soils and Teflon chips are used for bulks. All are run through the entire preparation process. The resulting mean must be within ±20% of the true value. If the result exceeds the 20% limit, a new LOQ standard is prepared and analyzed. If the second LOQ standard also fails, the LOD must be determined by analyzing sequential dilutions of a standard near the original LOQ until it is within the 20% requirement. For more information on LOD protocol see ESS INO QA 116. (15.14) for ESS samples, or AIHA and ELLAP policies for WOHL samples.

8.9 Demonstration of Capability (DOC): EHD METALS QA 115 (15.15) describes the process used in great detail. Four replicates of a standard at approximately 10 times the LOD concentration are analyzed. The mean must be within ±15%

of the true value (bias) and the percent relative standard deviation (%RSD) must be within ≤10% (precision).

8.9.1 For solid DOCs, four replicates of a standard at approximately 10 times the LOD concentration plus acid-washed, Teflon boiling chips are carried through the entire preparation process and analyzed. The Teflon boiling chips are added to simulate the solid matrix. The observed results must be within the precision and bias limits listed in 8.9.

8.9.2 For TCLP DOCs, a reference material is extracted, digested, and analyzed by a single analyst. Analysis results must pass the performance acceptance criteria provided by the manufacturer. If any DOC fails to meet the manufacturer's acceptance criteria, take corrective action. Corrective action may include evaluation of IECs, potential instrument problems, and review of the analyst's technique (extraction, digestion, instrumental analysis, etc). The analyst will be forbidden from doing any TCLP analysis until he/she has successfully performed DOC analyses for the failed parameters as per NELAP rules.

8.10 A logbook is kept by the instrument workstation. Every time the instrument is run, the following items are documented: date, analyst, instrument method used, and comments. Comments may include, but aren't limited to, worklist name, digestion date, and performance issues. Any maintenance or repairs should also be documented in the log.

8.11 Yttrium and gallium are used as the internal standard for all samples (see section 5.5). Based on manufacturer's recommendation, the percent relative standard deviation (%RSD) of the three internal standard replicates must be ≤ 5% and the recovery within 75-125%. If either of these criteria is exceeded, the samples must be re-prepared and reanalyzed. Sample dilution may be needed for the internal standard to pass acceptance criteria.

8.12 For a detailed listing of all Q.C. limits used for various sample matrices, refer to the QL database in LIMS for ESS samples. For WOHL samples the replicate (airs or wipes) or duplicate (soils and bulks) limit is 25% relative standard deviation. Spike recovery limits are 75%-125%. Environmental Lead samples are currently being tracked through QAWRKSHT and will be evaluated after sufficient data has been collected.

8.13 Quality control samples are carried through the digestion process to evaluate digestion performance. These quality control samples are listed in Table 4, and in the QC sections of the appropriate digestion SOP by matrix being analyzed: Total recoverable liquids (15.5), solids (15.6), total liquids and TCLP extracts (15.16), WOHL samples (15.19, 15.20, 15.21), and tissues (15.7).

8.14 Laboratory Fortified Blanks (LFB) are prepared for both undigested and digested sample sets, by mixing 70 µL of the spiking solution (6.5.5) with 6.93 mL of blank solution. Concentration values are listed in Tables 3 & 4. Percent recovery must be within 85-115% before proceeding with analysis. A LFB must be analyzed before any samples and with each subsequent group of 20 samples thereafter. A matrix spike may be analyzed in place of a LFB after the

initial analysis if the acceptance criteria of the matrix spike are equal to or more stringent than that of the LFB (e.g., 85-115%).

8.15 IECs are verified daily by reanalyzing the calibration standards. For each standard, the recovery for elements in that standard should be within 90-110% and all other elements should be within zero ± the LOQ. A full procedure for determining IECs is described in EHD METALS IOP 500 (15.8).

8.16 Dilutions are prepared using calibrated pipettes or class A glass volumetric flasks. Internal standard must be added to all dilutions (100 µL per 10 mL) prior to analysis.

9. Method Calibration

9.1 The viewing height is adjusted both axially and radially using the PE Align View option while aspirating a 1ppm manganese solution.

9.2 On a daily basis the ICP optics are aligned using the Mercury Align option of the Perkin-Elmer (PE) instrument operation software.

9.3 The calibration consists of a calibration blank and a single standard (see Tables 3 and 4 for standard concentrations).

9.4 Three replicate readings are taken for each standard and each sample.

9.5 A rinse time of 45 seconds between samples was determined to be adequate for eliminating memory or carryover effects the majority of the time. Supporting documentation is on file at the instrument workstation. Additional rinse time may be needed for unusual samples.

9.6 Calibration is verified by analyzing the calibration blank, QCS, I-IPC, LOQ, LFB and IEC verification standards (rerun calibration standards) immediately after calibration. If any of these fail to meet the acceptance criteria listed in section 8, corrective action must be taken before proceeding with sample analysis.

9.7 For a more detailed view of the calibration procedure, refer to EHD METALS IOP 500 (15.8) or EHD METALS IOP 501 (15.17).

10. Analysis Procedure

10.1 After the instrument is calibrated, the required QC samples (see 9.6) are analyzed. Provided all are within the defined limits for the elements required, the analysis of the samples begins. Refer to EHD METALS IOP 500 (15.8) or EHD METALS IOP 501 (15.17) for a detailed procedure.

11. Calculations

11.1 The calculations used for PE5300DV sample analysis are done by the software and may be found in detail in the Help section of the software under algorithms. Essentially, the emission signals from a sample are background corrected based on the background points selected. If there are interfering elements selected, the signal is corrected for them based on the IECs. The resulting signal is then

matched to a concentration from the linear regression curve created during calibration (y = mx + b) where m = slope, and b = the y intercept.

11.2 For the instrument to calculate the correct sample concentration in the appropriate units, all pertinent data must be entered, such as prep volume, prep weight and dilutions.

11.3 Precision is measured based on duplicate analyses; one for every 10 samples for each matrix. Accuracy is measured with matrix spiked samples; also one for every 10 samples for each matrix. Calculations may be found in the Environmental Health Division Quality Assurance Manual (15.11).

11.3 Refer to the LIMS manual pages for detailed information on "QAWRKSHT", the LIMS program used to calculate duplicates and spikes.

12. Data Management

12.1. Once a group of ESS samples has been analyzed completely and the QC has been entered into LIMS via QAWRKSHT, the failed QC groups, if any, are returned to "logged-in" status in LIMS for future reanalysis. If the subsequent reanalysis also fails QC, the samples must be reported with a qualifying comment that explains the failure. All acceptable data are electronically transferred to LIMS or manually recorded on a work list. Refer to ESS INO GENOP 102 (15.9) for a detailed description of the data transfer process for the Perkin-Elmer 5300DV ICP.

12.2. For WOHL samples METSAMP2 is used to transfer data to the EINSTEIN database. Refer to EHD METALS IOP 002 METSAMP2 Instructions (15.18) for further information and directions. Once the data has been transferred the analyst must do a validation 1 for each study

12.3. The entire analytical run is passed on to another Metals chemist for QC audit. An analytical run will include: cover sheet, worklist, digestion logbook sheet (if applicable), qawrksht print-out and all raw data. Refer to ESS INO QA 103 (15.10) for detailed information on this procedure.

12.4. For ESS samples, after the QC audit has been completed, the results are downloaded to LIMS or manually entered from a work list. Entering the results in LIMS changes the status to complete. When all analysis have been completed and verified, the sample is released

12.5. For WOHL samples the validation 3 process involves another Metals chemist reviewing the entire analytical run, and the report generated in EINSTEIN. If errors are found the validation 1 must be redone and checked again by a subsequent validation 3. The studies are passed on to a supervisor or designee to do a validation 5, print, and send the report to the client via e-mail or fax.

12.6. The PE5300 DV ICP has the capabilities to "reprocess" data. Once data has been collected, it is saved. Situations where reprocessing may be used include but are not limited to the following:

12.6.1. Incorrect Standard Concentrations entered in method

12.6.2. Incorrect values entered in sample info File

12.6.3. Background points adjusted

12.6.4. IEC factors adjusted

12.6.5. MSF files

12.7. For any reprocessed data, the following criteria must be met:

12.7.1. Original raw data must be included

12.7.2. All changes must be documented, initialed and dated

12.7.3. All blanks, standards, QC controls, and samples must be reprocessed

12.7.4. Must be reviewed by a peer auditor

13. Definitions

13.1 Definitions can be found in EPA Method 200.7 (15.2) section 3.0.

13.2 Definitions can also be found in the QA Manual (15.11), section 19.

14. Method Performance

14.1 Where applicable, the laboratory's initial accuracy and precision data (LOD's and DOC's) were generated in compliance with the reference method and the Inorganic Chemistry Department's standard operating procedures: ESS INO QA 115, "Initial DOC and Annual Continued Proficiency Check Procedures" (15.15), and ESS INO QA 116, "LOD Procedures" (15.14). Supporting data will be retained according to the applicable RDA.

15. References

15.1. Test Methods for Evaluating Solid Waste Physical/Chemical Methods. Third edition, SW-846, USEPA. November 1986, including July 1992, August 1993, September 1994, and January 1995 updates. Method 6010B

15.2. Methods for the Determination of Metals in Environmental Samples. USEPA, 200.7, 1994

15.3. AD SAFETY GENOP 102, "Chemical Hygiene Plan and General Laboratory Safety Plan," State Laboratory of Hygiene, Agriculture Drive Facility, Rev.7, April, 2009.

15.4. University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety.

15.5. EHD METALS Method 780.3, Digestion of Tot. Recoverable Liquids

15.6. EHD METALS Method 750.1, Digestion of Solids for ICP

15.7. EHD METALS Method 620.2, Digestion Method for Metals in Tissue by Inductively Coupled Plasma Spectroscopy

15.8. EHD METALS IOP 500, Instrument Operating Procedure for the Perkin-Elmer 5300DV ICP

15.9. EHD METALS GENOP 102, Data Transfer from Perkin-Elmer 5300DV ICP

15.10. ESS INO QA 103, Q.C. Audits of Analytical Runs for ESS Metals Area

15.11. Wisconsin State Laboratory of Hygiene, Environmental Health Division, *Quality Assurance Manual*.

15.12. National Environmental Laboratory Accreditation Conference, Constitution, Bylaws, and Standards, United States Environmental Protection Agency, Offices of Research and Development, Washington DC 20460, June 5, 2003

15.13. ESS INO GENOP 106, "Inorganic Sample Receipt"

15.14. ESS INO QA 116, "LOD Procedures"

15.15. EHD METALS QA 115, "Initial DOC and Annual Continued Proficiency Check Procedures"

15.16. EHD METALS Method 730.1 Digestion of Total Liquids and TCLP Extracts for ICP

15.17. EHD METALS IOP 501, Instrument Operating Procedure for the Perkin-Elmer 5300DV ICP with FAST autosampler.

15.18. EHD METALS IOP 002, METSAMP2 instructions.

15.19. EHD METALS METHOD 001.1 Analysis of Metal Elements in Air Samples by ICP-OES

15.20. EHD METALS METHOD 002.1 Analysis of Elements in Wipe Samples by ICP-OES

15.21. EHD METALS METHOD 014.1 Analysis of Elements in Bulk Samples by ICP-OES

Version date	**Version #**	**Revised by**	**Changes made**
Nov., 2009	2	D. Kennedy-Parker	Incorporated aspects of WOHL samples into method, and added WOHL digestion methods

Table 1

Acid concentrations for ICP samples

Undigested	0.5% HNO_3
Total Recoverable	2.5% HNO_3 and 5% HCl
Totals digestion	5% HNO_3 and 10% HCl
Solids	10% HNO_3 and 10% HCl
Total Recoverable solids	2% HNO_3 and 2% HCl
Tissue	10% HNO_3
WOHL samples	5% HNO_3 and 5% HCl
WOHL samples for Ag or As only	10% HNO_3

Table 2-Elements, Matrix, Units LOD, LOQ and Viewing Orientation for ICP-OES

Element	Undig and Tot Rec			TCLP Extracts			Solids			Liquids MICRO		
Nominal Wavelength												
	LOD	LOQ	Units	LOD	LOQ	Units	LOD	LOQ	Units	LOD	LOQ	Units
Al 396.153 Axial*	3	10	ug/L							3	10	ug/L
Al 396.153 Radial							1	3	mg/kg			
Sb 206.836 Axial	5	16	ug/L				1	3	mg/kg			
As 188.979 Axial	5	16	ug/L	0.25	0.8	mg/L	1	3	mg/kg			
Ba 233.527 Axial	1	3	ug/L	0.01	0	mg/L						
Ba 233.527 Radial							0.5	1.6	mg/kg			
Be 313.107 Axial	0.5	1.6	ug/L									
Be 313.107 Radial							0.1	0.3	mg/kg			
B 249.677 Axial	10	30	ug/L									
B 249.677 Radial							2	6	mg/kg			
Cd 228.802 Axial	0.5	1.6	ug/L	0.03	0.1	mg/L	0.1	0.3	mg/kg			
Ca 317.993 Radial	0.1	0.3	mg/L				10	32	mg/kg	0.006	0.020	mg/L
Cr 205.560 Axial	1	3	ug/L	0.025	0.1	mg/L	0.5	1.6	mg/kg			
Co 228.616 Axial	1	3	ug/L									
Co 228.616 Radial							0.5	1.6	mg/kg			
Cu 327.393 Axial	2	6	ug/L	0.025	0.1	mg/L	0.5	1.6	mg/kg			
Fe 238.204 Radial	0.1	0.3	mg/L				10	32	mg/kg	0.003	0.010	mg/L
Pb 220.353 Axial	3	10	ug/L	0.15	0.5	mg/L	1	3	mg/kg			
Mg 279.077 Radial	0.1	0.3	mg/L				10	32	mg/kg	0.01	0.04	mg/L
Mn 257.610 Axial	1.0	3.0	ug/L							0.40	1.4	ug/L
Mn 257.610 Radial							0.1	0.3	mg/kg			
Mo 202.031 Axial	3	10	ug/L									
Mo 202.031 Radial							1	3	mg/kg			
Ni 231.604 Axial	1	3	ug/L									
Ni 231.604 Radial							0.5	1.6	mg/kg			
K 766.490 Radial	0.1	0.3	mg/L				10	32	mg/kg			
Se 196.026 Axial	10	30	ug/L				2	6	mg/kg			
Ag 338.289 Axial	2	6	ug/L	0.025	0.1	mg/L						
Ag 338.289 Radial							1	3	mg/kg			
Na 589.592 Radial	0.1	0.3	mg/L				10	32	mg/kg	0.01	0.04	mg/L
Tl 190.801 Axial	5	16	ug/L				1	3	mg/kg			
V 292.402 Axial	1	3	ug/L									
V 292.402 Radial							0.5	1.6	mg/kg			
Zn 206.200 Axial*	1	3	ug/L	0.08	0.3	mg/L	0.5	1.6	mg/kg			
Sr 407.771 Radial	1	3	ug/L				0.5	1.6	mg/kg			
Ti 336.121 Axial	2	6	ug/L									
Ti 336.121 Radial							0.5	1.6	mg/kg			

*Total Recoverable Al LOD = 7.0 ug/L LOQ = 21 ug/L and Zn LOD = 3.0 ug/L LOQ = 9.0 ug/L

Table 3-Standards, Quality Control Samples for Undigested Liquids, Total Recoverable Liquids, and WOHL samples

Standards (mg/L)

STD 1		STD 2		STD 3		STD 4		STD 5	
Ag	0.5	Cr	2.0	Ca	200	Fe	30	As	0.20
Al	5.0	Mn	2.0	Co	2.0	K	30		
B	1.0	Mo	2.0	V	2.0	Mg	100	**WOHL**	**STD 5**
Ba	2.0	Ni	2.0			Na	200	Bi	2.0
Be	0.2	Pb	2.0			Ti	2	Li	2.0
Cd	2.0	Se	2.0					P	2.0
Cu	2.0	Tl	2.0					Sn	2.0
Sb	2.0								
Zn	2.0								
Sr	2.0								

STD 1 2.50 mL of STD 1 High Purity STD diluted to 250 mL

STD 2 2.50 mL of STD 2 High Purity STD diluted to 250 mL

STD 3 2.50 mL of STD 3 High Purity STD diluted to 250 mL

STD 4 2.50 mL of STD 4 High Purity STD diluted to 250 mL

STD 5 0.05mL 1000ppm Arsenic diluted to 250mL

QCS in mg/L

Ag	0.20	Ca	0.80	K	4.0	Pb	0.80
Al	0.80	Cd	0.80	Mg	0.80	Sb	0.80
As	0.80	Co	0.80	Mn	0.80	Se	0.80
B	0.80	Cr	0.80	Mo	0.80	Tl	0.80
Ba	0.80	Cu	0.80	Na	0.80	V	0.80
Be	0.80	Fe	0.80	Ni	0.80	Zn	0.80

QCS 0.40mL SPEX Certiprep ICV stock diluted to 10mL, made fresh daily.

LFB in mg/L

Ag	0.100**	Ca	50.00	K	10.00	Pb	1.00
Al	2.50	Cd	0.20	Mg	30.00	Sb	1.00
As	1.00	Co	0.40	Mn	0.20	Se	1.00
B	0.40	Cr	0.40	Mo	0.40	Tl	2.50
Ba	0.20	Cu	0.50	Na	50.00	V	0.20
Be	0.10	Fe	5.00	Ni	1.00	Zn	1.00

LFB 100µL VHG spike stock diluted to 10mL, ** for Ag LFB use 0.01mL of a 10x dilution of the 1,000ppm, made daily

LOQ (ESS) in mg/L

Ag	0.006	Ca	0.300	K	0.300	Pb	0.009	Ti	0.006
Al	0.009	Cd	0.0015	Mg	0.300	Sb	0.015	Sr	0.003
As	0.015	Co	0.003	Mn	0.003	Se	0.030		
B	0.030	Cr	0.003	Mo	0.009	Tl	0.015		
Ba	0.003	Cu	0.006	Na	0.300	V	0.003		
Be	0.0015	Fe	0.300	Ni	0.003	Zn	0.003		

LOQ 70µL High Purity LOQ stock diluted to 7.0mL, made fresh daily.

LOQ (WOHL) in mg/L

Ag	0.006	Bi	0.090	Fe	0.200	Pb	0.070	V	0.010
Al	0.400	Ca	0.400	Mg	0.200	Sr	0.006	Zn	0.070
As	0.030	Cd	0.010	Mn	0.010	Sb	0.060		
B	0.060	Co	0.010	Mo	0.020	Se	0.050		
Ba	0.006	Cr	0.080	Ni	0.003	Ti	0.020		
Be	0.0005	Cu	0.060	P	0.060	Tl	0.100		

Table 4- Standards, Mixtures and Quality Control Samples for Solids, TCLP, Total Liquids, Total Recoverable Solids and Tissue Samples

Standards (mg/L)

	STD 1		STD 2		STD 3		STD 4
Ag	0.50	As	2.0	Ca	200.0	Fe	200.0
Al	23.0	Cr	20.0	Co	2.0	K	30.0
B	1.0	Mn	2.0	V	2.0	Mg	100.0
Ba	20.0	Mo	2.0			Na	200.0
Be	0.2	Ni	2.0			Ti	2.0
Cd	2.0	Pb	20.0				
Cu	20.0	Se	2.0				
Sb	2.0	Tl	2.0				
Zn	20.0						
Sr	2.0						

STD 1 2.50 mL of STD 1 High Purity STD + 4.5 mL of 1000ppm solutions of Al, Ba, Cu, Zn + diluted to 250 mL

STD 2 2.50 mL of STD 2 High Purity STD + 4.5 mL of 1000ppm solutions of Cr and Pb diluted to 250 mL

STD 3 2.50 mL of STD 3 High Purity STD diluted to 250 mL

STD 4 2.50 mL of STD 4 High Purity STD diluted to 250 mL

QCS in mg/L

Ag	0.20	Ca	0.80	K	4.0	Pb	0.80
Al	0.80	Cd	0.80	Mg	0.80	Sb	0.80
As	0.80	Co	0.80	Mn	0.80	Se	0.80
B	0.80	Cr	0.80	Mo	0.80	Tl	0.80
Ba	0.80	Cu	0.80	Na	0.80	V	0.80
Be	0.80	Fe	0.80	Ni	0.80	Zn	0.80

QCS 0.40mL SPEX Certiprep ICV stock diluted to 10mL, made fresh daily.

LFB in mg/L

Ag	0.10**	Ca	50.00	K	10.00	Pb	1.00
Al	2.50	Cd	0.20	Mg	30.00	Sb	1.00
As	1.00	Co	0.40	Mn	0.20	Se	1.00
B	0.40	Cr	0.40	Mo	0.40	Tl	2.50
Ba	0.20	Cu	0.50	Na	50.00	V	0.20
Be	0.10	Fe	5.00	Ni	1.00	Zn	1.00

LFB 100µL VHG spike stock diluted to 10mL, made fresh daily

** for Ag LFB use 0.01mL of a 10x dilution of the 1,000ppm stock diluted to 10mL

LOQ in mg/L

Ag	0.012	Ca	0.600	K	0.600	Pb	0.018
Al	0.018	Cd	0.003	Mg	0.600	Sb	0.030
As	0.030	Co	0.006	Mn	0.006	Se	0.060
B	0.060	Cr	0.006	Mo	0.018	Tl	0.030
Ba	0.006	Cu	0.012	Na	0.600	V	0.006
Be	0.003	Fe	0.600	Ni	0.006	Zn	0.006
Ti	0.012	Sr	0.006				

LOQ 100µL High Purity LOQ stock diluted to 10mL, made fresh daily.

Table 5-Elements and Concentrations for the Spike Solution and, QCS, LOQ and Soil Check Samples

Element	*QCS (mg/L) stock	LOQ (mg/L) Stock	*Spike Solution (mg/L) stock	For solids use manufacturer's acceptable limits for ERA soil
Al	20	0.9	250	
Sb	20	1.5	100	
As	20	1.5	100	
Ba	20	0.3	20	
Be	20	0.15	10	
B	20	3	40	
Cd	20	0.15	20	
Ca	20	30	5000	
Cr	20	0.3	40	
Co	20	0.3	40	
Cu	20	0.6	50	
Fe	20	30	500	
Pb	20	0.9	100	
Mg	20	30	3000	
Mn	20	0.15	20	
Mo	20	0.9	40	
Ni	20	0.3	100	
K	100	30	1000	
Se	20	3	100	
Ag	5	0.6		
Na	20	30	5000	
Tl	20	1.5	250	
V	20	0.3	20	
Zn	20	0.3	100	

*QCS – Initial Calibration Verification, SICV0100-500B, Spectropure, St. Louis MO
*Spike Solution – Custom Multi #2, ZWISTATCM#2-500, VHG Labs, Manchester NH
*Sol – Metals In Soil, ERA Soil (from past internal blind), Environmental Resource Associates, Arvada CO

Table 6

Linear Dyanmic Range (LDR)

Aluminum	50 ppm
Antimony	10 ppm
Arsenic	10 ppm
Barium	10 ppm
Beryllium	5 ppm
Bismuth	10 ppm
Boron	10 ppm
Cadmium	10 ppm
Chromium	40 ppm
Cobalt	10 ppm
Copper	40 ppm
Iron	500 ppm
Lead	200 ppm
Manganese	20 ppm
Molybdenum	10 ppm
Nickel	40 ppm
Phosphorous	10 ppm
Selenium	10 ppm
Strontium	2.5 ppm
Thallium	10 ppm
Tin	40 ppm
Titanium	10 ppm
Vanadium	10 ppm
Zinc	40 ppm

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Appendix F.3

Wisconsin State Lab of Hygiene

EHD Metals Method 540.4,
Hg in Tissue Samples by Cold Vapor Atomic Absorption

EHD METALS METHOD 540.4

Hg in Tissue Samples by Cold Vapor Atomic Absorption

(Journal of Environmental Science and Health, J.R Sullivan and J.J. Delfino, A 17(2), p. 265-275 (1982))

1. Scope and Application

1.1. This procedure is used for the digestion and subsequent analysis for mercury in tissues. This analysis is sensitive to a LOD of 0.004 µg/g.

2. Summary of Method

2.1. Cold vapor atomic absorption uses the volatile property of elemental mercury absorbing light at the wavelength of 253.7 nm. To release mercury from organic complexes, portions of ground tissue samples are weighed into reflux tubes and digested in a digestion block with a sulfuric-nitric acid mixture and the additional aid of hydrogen peroxide. Potassium permanganate is added to the cooled tubes to ensure complete oxidation and to remove aromatic and nitrogen compounds that could interfere during the photometric measurement. Before analysis, the oxidizing reagents are neutralized. Stannous chloride is added to reduce ionic mercury to the ground state. An argon gas stream carries the resultant volatile elemental mercury into the cell of an atomic absorption spectrophotometer where the absorbance is measured. The absorbance signal is proportional to the amount of mercury in the sample.

3. Safety and Waste Management

3.1. General safety practices for laboratory operations and waste disposal are outlined in the Chemical Hygiene Plan for the Agriculture Drive Facility (16.2).

3.2. All laboratory waste, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations.

3.3. Waste disposal guidelines are described in the University of Wisconsin Laboratory Safety Guide (16.3).

3.4. Mercury is a very toxic element and strong acids are used in the digestion procedure of this SOP. The use of eye protection, gloves and fume hoods is strongly encouraged.

4. Sample Handling and Preservation

4.1. Tissue samples are homogenized and frozen until analysis. Some tissues may require further homogenizing using a blender.

4.2. All homogenized samples should be collected in clean polyethylene or glass containers. Store samples in freezer in Rm. 118 until ready to thaw and digest.

4.3. Refer to ESS INO GENOP 106, “Inorganic Sample Receipt” (16.6) for further details.

5. Interferences

5.1. Potassium permanganate is added to eliminate possible interference from sulfide. Concentrations as high as 20 mg/L of sulfide as NaS do not interfere with the recovery of added inorganic mercury from reagent water (see 16.12).

5.2. Copper has also been reported to interfere; however, copper concentrations as high as 10 mg/L had no effect on recovery of mercury from spiked samples (see 16.12).

5.3. Seawater, brines, and industrial effluent high in chlorides require additional permanganate, as much as 25 mL (see 16.12). During the oxidation step, chlorides are converted to free chlorine, which also absorbs radiation at 253.7 nm. Care must be taken to ensure that free chlorine is absent before the mercury is reduced. This may be accomplished by using an excess of hydroxylamine hydrochloride (25 mL).

5.4. Certain volatile organic materials that absorb at this wavelength may also interfere. A preliminary run without reagents would determine if this type of interference is present.

6. Apparatus and Materials

6.1. "Hot Block" digestion system, Environmental Express (Cat. # SC150)

6.2. 50 mL polypropylene digestion tubes with polyethylene screw caps (Cat. # SC475), received from Environmental Express with certificates of calibration and analysis.

6.3. Digestion tube racks, 18 position

6.4. Motorized and mechanical air displacement pipettes with appropriate tips.

6.5. Acid and reagent repipette dispensers ("repipettors").

6.6. Plastic centrifuge tubes; 15 mL

6.7. Balance, top loading, weighing to the nearest 0.01 g.

7. Reagents

7.1. Reagent water: ASTM type I water.

7.2. Sulfuric acid (H_2SO_4): concentrated, trace metal grade, Fisher Scientific (Cat. # A510SK-212).

7.3. Nitric acid (HNO_3): concentrated, trace metal grade, Fisher Scientific (Cat. # A509SK-212).

7.4. Hydrochloric acid (HCl): concentrated, trace metal grade, Fisher Scientific (Cat. # A508SK-212).

7.5. Potassium permanganate ($KMnO_4$), A.C.S. Reagent, Fisher Scientific, (Cat. # P279-500).

7.6. Sodium chloride (NaCl), Certified A.C.S., Fisher Scientific (Cat. # S271-500).

7.7. Hydroxylamine sulfate $(NH_2OH)_2$ -H_2 SO_4, Certified, Fisher Scientific (Cat. # H331-500).

7.8. Stannous Chloride ($SnCl_2$-$2H_2O$), A.C.S. Reagent, Fisher Scientific, (Cat. # T142-500).

7.9. Potassium Permanganate Solution, 5% w/v: Weigh 100 g of potassium permanganate (7.5) into the reagent bottle, dilute to the line (2 L) with reagent water and mix thoroughly. This solution expires 1 year from preparation.

7.10. Sodium chloride-hydroxylamine sulfate solution: Weigh 240 g of sodium chloride (7.6) and 240 g of hydroxylamine sulfate (7.7) into the reagent bottle, dilute to the line (2 L) with reagent water and mix thoroughly. This solution expires 1 year from preparation.

7.11. Hydrogen Peroxide, 30%, Certified A.C.S., Fisher Scientific (Cat. # H325-4).

7.12. Digestion Acid Solution: Using a clean graduate cylinder, add 400 mL nitric acid (7.3) to the 2.5 L reagent bottle. Place on stir plate and begin gently stirring. Using the same graduate cylinder, **slowly** add 1600 mL sulfuric acid (7.2). Continue stirring until solution is thoroughly mixed. Let cool. Solution is good indefinitely.

7.13. Reductant Solution: Weigh 11 g of stannous chloride (7.8) into the reagent bottle and fill approximately half full with reagent water. Using the calibrated repipettor, add 30 mL HCl (7.4) dilute to the line (1L) with reagent water and mix thoroughly. For longer analytical runs prepare 2 L of solution by adding 22 g of stannous chloride and 60 mL of HCl to the 2 L reagent bottle, dilute to the line with reagent water and mix thoroughly. This solution must be made fresh daily.

7.14. Carrier Solution: Fill the reagent bottle with approximately 500 mL of reagent water. Using the calibrated repipettor, add 30 mL of concentrated HCl (7.4) and dilute to the line (1L) with reagent water. For longer analytical runs prepare 2 L of solution by adding 60 mL of concentrated HCl (7.4) into the 2 L reagent bottle, dilute to the line (2 L) with reagent water and mix thoroughly. This solution must be made fresh daily.

7.15. Stannous Chloride rinse solution: weigh 100 g of stannous chloride (7.8) into the reagent bottle. Add approximately 1 L of reagent water, 100 mL of concentrated HCl (7.4), dilute to the line (2 L) with reagent water and mix thoroughly. This solution expires 1 year from preparation.

Note: All working reagents must be entered into the Metals Reagent Log (logbook #76, stored in Rm. 118) and the reagent code and expiration date recorded on the bottle.

8. Standards

8.1 Mercury Stock Standard, 10 ppm, High Purity Standards (Cat. # 1033-1)

8.2. Mercury Stock Standard, 100 ppm, Environmental Express (Cat. # ICL100-9)

8.3. Working Calibration Standard (WCS), 0.10 ppm. Fill a 100 mL volumetric flask approximately half full with reagent water, add 0.1 mL hydrochloric acid (7.4) and swirl to mix. Add 1.0 mL High Purity stock standard (8.1), fill to volume and mix thoroughly. This standard must be prepared daily.

8.4. Working Quality Control Standard (WQCS), 0.10 ppm. Fill a 100 mL volumetric flask approximately half full with reagent water, add 0.1 mL hydrochloric acid (7.4) and swirl to mix. Add 0.10 mL Environmental Express stock standard (8.2), fill to volume and mix thoroughly. This standard must be prepared daily.

9. Quality Control

9.1. Please refer to the Environmental Health Division Quality Assurance Manual (16.5) for general information on quality control procedures. Important specifics include:

9.1.1. Accuracy and precision calculations.

9.1.2. Corrective action procedures (including documentation requirements) for instrument problems or analytical problems.

9.2. Possible obvious errors for QC failures can include but are not limited to: bad replicate analyses, tubing clogs, autosampler misalignment, system leaks, argon flow irregularities.

9.3. Calibration Blank (CB), run immediately following calibration, after every 10 (or less) analytical samples and at the end of the run. The CB must be within zero ± the LOD or less than 5% of the analytical sample concentration to be acceptable. In the case of failure, if no obvious errors are detected, it may be rerun once. If it fails again, determine the cause of the failure (e.g. contamination, bad calibration intercept, gas flow change); correct the cause of the error (e.g. re-pour the blank, re-standardize, reset gas flow) and rerun. If the CB then passes, rerun all samples from last acceptable CB. If it fails again, the calibration curve must be rerun.

9.4. Instrument Performance Check (IPC), run immediately following calibration, after every 10 (or less) analytical samples and at the end of the run. The Initial IPC (I-IPC) must be within 5% of the true value and all subsequent IPC's must be within 10% of the true value. In the case of failure, if no obvious errors are detected, the IPC may be rerun once. If it fails again, determine the cause of the failure (e.g. contamination, bad calibration, gas flow change), correct the cause of the error (e.g. re-pour the blank, re-standardize, reset gas flow) and rerun.

If the IPC then passes, rerun all samples from last acceptable IPC. If it fails again, the calibration curve must be rerun.

9.5. Lab Reagent Blank (LRB, labeled DLRB on bench records), an aliquot of blank matrix carried through all sample preparation steps. The LRB is analyzed before any samples and the absolute value of its concentration must be less than the LOD. In the case of failure, if no obvious errors are detected, the LRB may be rerun once. If it still fails, only those samples with a concentration greater than 10 times the LRB concentration may be accepted. All other samples must be re-digested.

9.6. Lab Fortified Blank (LFB, labeled DLFB on bench records), a known quantity of Hg spiked into blank matrix and carried through all sample preparation steps. Prepared by adding 1 mL of WCS (8.4) to a reflux tube. The LFB is analyzed before any samples and must be within 10% of the true value. In the case of failure, if no obvious errors are detected, the LFB may be rerun once. If it still fails, all samples within that digestion must be reset.

9.7. Quality Control Sample (QCS, labeled DQCS on bench records), a known quantity of Hg obtained from a different source than the calibration standard, spiked into blank matrix and carried through all sample preparation steps. Prepared by adding 1 mL of WQCS (8.5) a reflux tube. The QCS is analyzed before any samples and must be within 10% of the true value. In the case of failure, if no obvious errors are detected, the QCS may be rerun once. If it still fails, all samples within that digestion must be reset.

9.8. Tissue Control (BCR Tuna), Community Bureau of Reference, Cat.# CRM 463 - a dried and ground tissue sample which is processed through each sample preparation step and must be analyzed before any samples. Result must be within the certified control limits provided by the manufacturer. In the case of failure, if no obvious errors are detected, the control may be rerun once. If it fails again, all samples within that digestion must be reset.

9.9. Matrix spike (MS) - A second aliquot of sample is spiked with a known concentration of mercury. Spikes must be performed on each group of samples of a similar matrix type with a frequency of 10%. Recovery must be within the matrix specific limits listed in the QL database in LIMS. In the case of failure, if no obvious errors are detected, it may be rerun once. If it still fails, all samples within that QC group must be reset.

9.10. Laboratory duplicate (LD) - A second aliquot of sample is analyzed and the precision between the two results is evaluated. Duplicates must be performed on each group of samples of a similar matrix type with a frequency of 10%. The difference between concentrations must be within the matrix specific limits listed in the QL database in LIMS. In the case of failure, if no obvious errors are detected, it may be rerun once. If it still fails, all samples within that QC group must be reset.

9.11. LOD - the concentration at which the result is definitely distinguishable from a blank. Must be verified annually, or after any significant work is done on the instrument. For more information on LOD protocol, see ESS INO QA 116 (16.10).

9.12. Demonstration of Capability (DOC) - an analyst new to the method must successfully complete a DOC before any analyses may be performed. Other analysts must successfully complete a DOC annually to be able to continue to perform the analysis. For more information, see EHD METALS QA 115 (16.9).

9.13. Linear Dynamic Range (LDR) - determined when the instrument is set-up, when a new method is being developed, and annually. The LDR may also be reassessed (based on the judgment of the analyst) if there is a change in analytical performance caused by either new instrument hardware or operating conditions. The instrument is calibrated normally [see 11.7 and EHD METALS IOP 540(16.11)] and standards at continually higher concentrations than the top standard are then analyzed until the percent recovery exceeds 10%.

9.14. High sample concentrations - Although the LDR may be higher than the calibration range, no samples with concentrations higher than the top standard are accepted. Samples must be diluted until their concentration falls within the calibration range. The dilution result must agree with the original (undiluted) result by less than 10% relative difference (R.D.). If the dilution fails to agree with the original result, a second, different dilution is made and must agree with the first dilution result (<10% R.D.). For sample concentrations exceeding the LDR, the analyst may wish to re-digest the sample using a smaller sample aliquot and reanalyze. Prepare dilutions by pipetting appropriate volumes of sample directly into auto-sampler tubes and mixing thoroughly.

9.15. Correlation Coefficient (r value) - must be 0.999 or greater. The calibration algorithm is linear. The standard curve is also printed and checked visually for irregularities.

9.16. The instrument logbook (#71) must be updated with every analysis. Include date, analyst, analysis method, absorbance values for the standard curve, correlation coefficient and any pertinent comments about instrument performance.

10. Method Calibration

10.1. Refer to the Reagents section (7) of this SOP for instructions on preparing the reagents needed for instrument calibration and sample analysis.

10.2. Refer to the Standards section (8) of this SOP for instructions on preparing the working standards needed (8.4, 8.5) for instrument calibration.

10.3. Refer to the Procedure section (11) of this SOP for instructions on preparing calibration standards (11.7).

10.4. Refer to the Instrument Operating Procedure [EHD METALS IOP 540 (16.11)] for instructions on calibrating the instrument.

10.5. Refer to the Quality Control section of this SOP for the requirements to evaluate the calibration curve including correlation coefficient (9.16), CB (9.3), IPC (9.4) and QCS (9.7).

11. Procedure

11.1. Create digestion log spreadsheet, recording all pertinent information including: sample numbers, sample bottle, duplicates, spikes, spike volume, spike code, initial weight, final volume, standard codes, reagent codes and digestion block temperature. The spreadsheet is saved to the directory R:\EHD\ESS(4900)\ESS Inorg(4910)\METALS\Digestion Log. A printed copy accompanies the digestion.

11.2. Use appropriate safety procedures to prepare digestion such as wearing safety glasses and gloves and carrying out the digestion within a fume hood.

11.3. Collect frozen samples and let thaw. Samples may be thawed quicker by placing sample containers in a tray of warm water.

11.4. Place clean digestion tubes in a rack.

11.5. Prepare calibration standards

11.5.1. Label 6 digestion tubes: Cal. Blk., 1, 2 3, 4, and 5. Add approximately 20 mL of reagent water to each tube. Using calibrated adjustable volume pipettes, add the appropriate amount of standard to the respective tubes as follows:

11.5.1.1. Calibration Blank: 0 mL reagent water

11.5.1.2. Std 1 (0.1 µg/L): 0.05 mL WCS (8.4)

11.5.1.3. Std 2 (1.00 µg/L): 0.50 mL WCS (8.4)

11.5.1.4. Std 3 (2.00 µg/L): 1.0 mL WCS (8.4)

11.5.1.5. Std 4 (5.00 µg/L): 2.5 mL WCS (8.4)

11.5.1.6. Std 5 (10.0 µg/L): 5.0 mL WCS (8.4)

11.5.2. Calibration standards do not need to be digested. Add digestion acid (see 11.13) and potassium permanganate (see 11.18) to these tubes, mix, cover with Parafilm® and set aside until instrument analysis is ready to begin.

11.7. Prepare LRB (9.5). Add a small amount of reagent water (approximately 2 mL) into a digestion tube.

11.8. Prepare LFB (9.6). Add a small amount of reagent water (approximately 2 mL) into a digestion tube. Using a calibrated pipette, add 2.5 mL of the 0.10 ppm Hg WCS (8.3) into the tube.

11.9. Prepare QCS (9.7). Add a small amount of reagent water (approximately 2 mL) into a reflux tube. Using a calibrated pipette, add 2.0 mL of the 0.10 ppm Hg WQCS (8.4) into the tube.

11.10. Prepare Tissue Control sample (9.8) - weigh 0.10 - 0.15 g BCR Tuna control into a reflux tube.

11.11. Mix each sample thoroughly to achieve homogeneity and weigh out at least 0.75 g into a digestion tube. Try to get all sample near the bottom of the tube so it is covered by the digestion acid solution.

11.12. Spike the matrix spike tubes with 2.5 mL of the 0.10 ppm Hg WCS (8.3)

11.13. Add 4.0 mL 4:1 digestion acid solution (7.12) to each tube. Make sure any sample clinging to the side of the tube gets coated with the digestion acid solution.

11.14. Cap all tubes.

11.15. Let samples sit in acid overnight until the sample has broken down to a mostly liquid solution.

11.16. Turn on digestion block and let warm to a temperature of 95° C. Record temperature in the digestion temperature notebook (#ESS174).

11.17. Place capped tubes in digestion block for 45 minutes. Remove samples and let cool.

11.18. Remove cap from digestion tube, add two mL reagent water and 0.5 mL hydrogen peroxide, recap and lightly swirl to mix. Repeat for all tubes.

11.19. After reaction has subsided, repeat the hydrogen peroxide addition step three more times. Be sure to cap the tubes and allow the reaction to subside between additions. Reagent water is only added prior to the first addition of hydrogen peroxide.

11.20. Place tubes in digestion block and heat 2 hours at 95°C.

11.21. Remove from block and cool to room temperature.

11.22. Remove cap, add 5 mL potassium permanganate solution (7.9) and recap. If necessary, add additional potassium permanganate crystals until purple color persists.

NOTE: Do not proceed until ready to analyze.

11.19. Remove cap, add 3 mL sodium chloride-hydroxylamine sulfate solution (7.10) and dilute sample with reagent water to the 50 mL mark on the tube. Mix thoroughly, making sure no color persists.

11.19. Set up CVAA instrument for analysis following EHD METALS IOP 540 (16.11).

12. Calculations

12.1. All calculations are done by the instrument software provided the correct information is entered into the sample info file (sample volume, sample weight, dilution factor).

12.2. Since calculations are done by the software, make sure diluted samples are clearly labeled on the bench record as diluted (include size of the dilution).

12.3. The sample concentration calculation is $\frac{calc\ conc \times final\ volume\ digested(liters)}{amount\ digested\ (grams)}$

13. Data Management

13.1. QC results must be entered into the LIMS program "qawrksht". qawrksht is used to evaluate the QC data. Refer to ESS INO QA 114, LIMS Quality Assurance Worksheet Procedures (16.8) for details. A copy of the qawrksht file must be included with the bench record.
13.2. All samples within failed QC groups are returned to "logged in" status.
13.3. Record results and batch numbers on worklist.
13.4. Clearly document corrective action directly on the bench sheet as specified in section 9.1. If there is not enough room on the bench sheet, documentation may also be recorded on the cover sheet or a separate sheet of paper and referenced to the appropriate place in the bench record.
13.5. Pass on entire run (bench record, worklist, qawrksht) to another Metals chemist for QC audit. Refer to EHD METALS QA 103 (16.7) for more information on QC audits.

14. Definitions

14.1. Refer to the reference method (16.1) for any terms not fully defined within this SOP.
14.2. Refer to the QA Manual (16.4) for general definitions.

15. Method Performance

15.1. Where applicable, the laboratory's initial accuracy and precision data (LOD's and DOC's) were generated in compliance with the reference method and the Inorganic Chemistry Dept.'s standard operation procedures: ESS INO QA 115, "Initial DOC and Annual Continued Proficiency Check Procedures" (16.9) and ESS INO QA 116, "LOD Procedures" (16.10). Supporting data will be retained according to the applicable RDA.

16. References

16.1. Sullivan, J.R. and Delfino, J.J., Journal of Environmental Science and Health, A17(2), p265-275 (1982).
16.2. AD Safety GENOP 102, Chemical Hygiene Plan and General Laboratory Safety Plan for the WSLH Agriculture Drive Facility, WI State Laboratory of Hygiene.
16.3. University of Wisconsin—Madison, Chemical & Radiation Protection Office, Safety Department (262-8769), "Laboratory Safety Guide," 2004, http://www.fpm.wisc.edu/safety.
16.4. Quality Assurance Manual, Environmental Health Division, Wisconsin State Laboratory of Hygiene.
16.5. National Environmental Laboratory Accreditation Conference, Constitution, Bylaws, and Standards, United States Environmental Protection Agency, Offices of Research and Development, Washington DC 20460, June 5, 2003.
16.6. ESS INO GENOP 106, Inorganic Sample Receipt
16.7. EHD METALS QA 103, Q.C. Audits of Analytical Runs for ESS Metals Area
16.8. ESS INO QA 114, LIMS Quality Assurance Worksheet Procedures
16.9. EHD METALS QA 115, Initial DOC & Annual Continued Proficiency Check Procedures
16.10. ESS INO QA 116, LOD Procedures
16.11. EHD METALS IOP 540,"Instrument Operating Procedure for the FIMS 100."
16.12. Test Methods for Evaluating Solid Waste--Physical/Chemical Methods, USEPA SW846, 3rd Edition, Method 7471A, Rev. 1, Sept. 1994.

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Appendix F.4

Wisconsin State Lab of Hygiene

ESS Org Method 1410,
Pesticide, PCB and PBDE Residues in Tissue

ESS ORG METHOD 1410

Pesticide, PCB and PBDE Residues in Tissue

Matrix: Tissue Analysis

1. Scope and Application

1.1. This method may be used to analyze fish and wildlife tissue for various chlorinated pesticides, PCBs, and PBDEs. Analysis for PCBs includes: Aroclor mixtures by Megabore capillary column, and individual congeners contained in the "Mullin" mix by capillary/ECD.

1.2. Pesticides and PCBs by Capillary Column Chromatography

Compound	Report Limit (µg/g)	LOQ (µg/g)	LOQ (µg/g)
Polychlorinated biphenyls		0.040	0.13
Dieldrin		0.010	0.033
o,p'-DDE		0.010	0.033
p,p'-DDE		0.010	0.033
o,p'-DDD		0.010	0.033
p,p'-DDD		0.010	0.033
o,p'-DDT		0.020	0.066
p,p'-DDT		0.010	0.033
cis-chlordane		0.010	0.033
trans-chlordane		0.010	0.033
cis-nonachlor		0.010	0.033
trans-nonachlor		0.010	0.033
Aldrin	0.05		
Endrin	0.02		
Hexachlorobenzene	0.01		
alpha-BHC	0.01		
gamma-BHC	0.01		
oxychlordane	0.05		
Methoxychlor	0.05		
Toxaphene	1.0		
Heptachlor epoxide	0.05		

1.3. PCB Congeners by Capillary

Column Chromatography

BZ #(e)	LOD(ng/g)	LOQ(ng/g)	BZ#(e)	LOD(ng/g)	LOQ(ng/g)
#3	16.0	53.0	#77/110	0.80	2.7
#4/10	3.4	11.0	#82	0.60	2.0
#7/9	0.60	2.0	#151	0.70	2.3
#6	1.3	4.3	#135/144	1.0	3.3
#8/5	3.3	11.0	#123/149	0.70	2.3
#19	1.0	3.3	#118	1.0	3.3
#18	1.0	3.3	#146	0.70	2.3
#15/17	1.5	5.0	#132/153/105	1.0	3.3
#24/27	0.60	2.0	#141	0.60	2.0
#16/32	1.7	5.7	#137/176	0.70	2.3
#26	1.0	3.3	#163/138	1.1	3.7
#25	1.0	3.3	#158	3.3	11.0
#28/31	2.0	6.7	#178	1.0	3.3
#33	1.0	3.3	#187/182	0.70	2.3
#53	0.60	2.0	#183	0.70	2.3
#51	0.60	2.0	#128	0.60	2.0
#22	1.5	5.0	#167	0.90	3.0
#45	0.80	2.7	#185	0.50	1.7
#46	0.80	2.7	#174	0.70	2.3
#52	0.90	3.0	#177	0.70	2.3
#49	0.70	2.3	#202/171	0.50	1.7
#47/48	1.2	4.0	#172	1.0	3.3
#44	0.80	2.7	#180	0.80	2.7
#37/42	1.2	4.0	#193	1.0	3.3
#41/71/64	1.2	4.0	#199	0.70	2.3
#40	0.80	2.7	#170/190	0.80	2.7
#63	0.90	3.0	#198	0.80	2.7
#74	0.70	2.3	#201	1.0	3.3
#70/76	0.70	2.3	#203/196	1.7	5.7
#66	1.2	4.0	#208/195	0.60	2.0
#95	0.80	2.7	#207	0.50	1.7
#91	0.90	3.0	#194	0.60	2.0
#56/60	0.90	3.0	#206	0.60	2.0
#92/84	1.7	5.7			
#89	0.70	2.3			
#101	0.70	2.3			
#99	0.60	2.0			
#83	0.60	2.0			
#97	0.50	1.7			
#87	0.70	2.3			
#85	0.80	2.7			
#136	2.0	6.7			

1.4. Poly-Brominated Diphenyl Ethers

PBDE #	LOD (ng/g)	LOQ (ng/g)
#28	2.0	6.7
#47	1.0	3.3
#66	1.0	3.3
#85	1.0	3.3
#99	1.0	3.3
#100	1.0	3.3
#138	1.0	3.3
#153	1.0	3.3
#154	1.0	3.3

2. Summary of Method:

2.1. Tissue samples (fish and wildlife) are homogenized in a blender with dry ice and allowed to stand overnight. Then approximately ten grams is combined with sodium sulfate and extracted with $MeCl_2$. The lipid is removed by gel-permeation chromatography. In addition the extract may require additional clean up or fractionation with silica gel or Florisil. The final extract or extracts are concentrated and injected onto a gas chromatograph equipped with an electron-capture detector. The type of column and GC used varies depending on particular analytes.

2.2. Method Deviations: This section is not applicable to this method.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences

3.2. All laboratory waste, excess reagents and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

4. Sampling Handling and Preservation: Tissue samples are coarsely ground and frozen in glass bottles with aluminum-lined caps.

5. Interferences: Matrix interferences may be caused by contaminants that are co extracted from the sample. Also, note that analytes of interest may not be wholly resolved from one another. In unfamiliar samples, positive identifications will be confirmed.

6. Reagents and Standards:

6.1. Dichloromethane, hexane, acetone, ethyl ether, cyclohexane- pesticide grade.

6.2. Sodium sulfate: ACS granular, 10-60 mesh, stored at 130°C.

6.3. Florisil: PR grade 60-100 mesh, stored at 130°C.

6.4. Silica gel: Fisher, 100-200 mesh activated at 130°C.

6.5. Prepare stock standard solutions by accurately weighing about 10 mg of pure material. Dissolve the material in isooctane and dilute to volume in a 100 ml volumetric flask. If stock is a concentrate, pipet 1.0 ml into 100-ml volumetric flask and dilute with isooctane.

6.6. Transfer the stock standard solution into a Teflon-sealed screw cap amber bottle. Store stock standards in a freezer.

6.7. Stock standard solution must be replaced every year or when signs of degradation or evaporation appear.

6.8. Dry ice

6.9. Ethanol, 95%

7. Apparatus:

7.1. Industrial blender, 1000-ml and 100-ml blender cups

7.2. Florisil and silica gel columns

7.3. Calibrated 15-ml centrifuge tubes

7.4. Syringes: Microliter and Luer-lock (for GPC)

7.5. Nitrogen blow-down apparatus

7.6. Gel-Permeation Chromatograph

7.7. Volumetric flasks - 10, 25, 100 ml

7.8. Megabore column GC/ECD: HP6890N equipped with a PC-based Chemstation integrator

7.9. Capillary column GC/ECD: HP5890 equipped with a PC-based Chemstation integrator or equivalent.

7.10. Beakers: 250 ml, 100 ml

7.11. Balances: Analytical, Top Loading

7.12. Mortar and Pestle

7.13. Glass funnel

7.14. Graduated cylinders: 50, 100, and 250 ml

7.15. Pipettes: volumetric and transfer

7.16. GC autosampler vials and caps

7.17. Spatulas

7.18. Aluminum weighing dishes

8. Quality Control

8.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT".

8.2. Matrix spikes: 1-10 ml of a solution containing spiked parameters (acetone solvent) is added between step 10.1.1 and 10.1.2 of Section 10.1). The spike solution for PCB congeners consists of a mixture of Aroclors 1232, 1248, and 1262 at concentrations of 0.250, 0.180, and 0.180 mg/l, respectively, or higher but at the same ratios.

8.3. For PCB congener and PBDE analysis, surrogate standards are added to all samples to monitor analytical recoveries. The surrogate spike solution consists of PCB congeners #14, #65, and #166 at nominal concentrations of 100, 25, and 25 ng/ml, respectively. From 1 to 5 ml of this solution is added to every sample between steps 10.1.1 and 10.1.2 of Section 10.1. For PBDEs, only the recovery of #166 is used for a quality control measure.

8.4. For each batch of approximately 10 samples, a duplicate sample is analyzed.

8.5. If any of the parameters in Section 8.1, 8.2, and 8.3 exceeds their limits, the samples will be re-analyzed or appropriately flagged.

8.6. For each batch of approximately 10 samples, a method blank will be analyzed. If any analytes of interest are found above their LOD (or report limit), the samples will be re-analyzed or the data appropriately flagged.

8.7. For non-congener PCB and pesticide analysis, the method will be monitored by adding a surrogate. Tetrachloro-m-xylene will be spiked into each sample. Statistical limits are kept and the system will be investigated if the surrogate recovery falls outside those limits.

8.8. All data pertinent to preparation of standards is recorded in the "Preparation of Standards" logbook. Pertinent data is to include date of preparation, origin of parent solution/primary standard, aliquot and dilution information, all weightings and tares, and purity.

8.9. When fresh stock solutions are prepared from primary standards a log sheet is begun noting the origin, purity, date of preparation, and pertinent weightings and dilutions. Subsequent intermediate and working (or spiking) solutions prepared from the stock will also be documented on this same log sheet. Log sheets representing discarded parent (stock) solutions are removed from the active portion of the "Preparation of Standards" and filed by name.

8.10. See Section 10: Procedure for other quality control practices.

9. Method Calibration

9.1. Aroclor Analysis-External Standard: Aroclor analysis is done on the Megabore capillary column using a single point calibration. The response of selected peaks in the sample chromatogram is directly compared to the same peaks in the standard. The response of the sample must be within 30% of the response of the standard for valid calculations, but no greater than 110 percent of the highest standard.

9.2. Pesticide analysis-External Standard: Some pesticides are analyzed on a capillary GC using a calibration curve of at least five points and employing a linear fit. A correlation coefficient of 0.995 (r) or greater is required before analysis can begin.

9.3. PCB Congener analysis-Internal Standard:

9.3.1. The single point PCB calibration standard consists of a dilution of a stock solution of Aroclors 1232, 1248, and 1262 at 183 µg/ml which was supplied by M. Mullin in June 1994. See for Table I congener composition of the stock solution. The diluted standard contains Aroclors 1232, 1248, and 1262 at 0.225, 0.162, and 0.162 µg/ml for 0.549 µg/ml PCB.

9.3.2. Quantitation of congeners #128 and #167 requires the addition of individual standards of these congeners to the calibration mix, at nominal concentrations of 4 ng/ml and 2 ng/ml, respectively. The total concentration of these congeners in the calibration mix must also include the contribution from the Aroclors. This contribution is 0.30 ng/ml of #128 and 0.15 ng/ml of #167. This standard also contains PCB congener #30 at a nominal concentration of 0.014 mg/l (14 ng/ml), and PCB congener #204 at 0.016 mg/l (16 ng/ml) which are used as retention time reference peaks and as internal standards for quantitation. Congeners eluting prior to and including #77/110 use congener #30 as internal standard, those eluting after #77/110 use congener #204 as internal standard. The calibration table contains the concentration in ng/ml of each congener in the mix, including internal standards, as well as surrogates #14, #65, and #166 at nominal concentrations of 32, 7, and 8 ng/ml. See Table III.

9.3.3. A three-level calibration is performed yearly to verify detector response linearity, using the single-point standard and standards at 0.5x and at 2x the single point standard. The RSD of the three response factors for each congener shall be less than 25%. Alternatively, linearity may be demonstrated by a correlation coefficient of at least 0.95.

9.4. PBDE analysis-Internal Standard: Standards are obtained form Canbridge Isotope Labs as concentrates in sealed ampules. A multi-level calibration is used, with at least five points and a range of 1.0 to 20 ng/ml. PCB #204 is used as the internal standard. Quantitation is performed using linear regression. Before a batch of samples is analyzed, the curve is verified with a standard at or near the mid-point. If the recoveries are between 80 – 120%, the curve is considered valid.

10. Procedure:

10.1. Sample Extraction and Clean-up:

10.1.1. Blend tissue with dry ice at high speed to produce a free flowing powder. Rinse the blender jar between samples with ethanol. Let the dry ice sublime overnight in a freezer. Mix 10.0 g of tissue with 60 g of anhydrous sodium sulfate stirring frequently for about 30 minutes.

NOTE: If less than 5 g of tissue is available, blend the entire sample with 30 g sodium sulfate and follow the small sample size procedure (Section 10.1.3).

10.1.2. Pour the tissue and sodium sulfate mixture into a 20 mm ID chromatographic column containing a 2 cm layer of sodium sulfate. Open the stopcock and add 230 ml dichloromethane to the column reservoir. Adjust the elution rate through the column to about 5 ml/minute. Collect the extract in a 250 ml beaker or 250 ml round bottom flask.

10.1.3. Small sample size: If only 1 to 5 g of ground sample is available, use only 30 g sodium sulfate and 130 ml dichloromethane. Generally, fat determination is not done in this case. Concentrate the eluate to less than 5 ml and transfer to hexane, making sure all the dichloromethane has evaporated. DO NOT use a gel permeation clean up. Instead, follow the Florisil and silica gel clean-up steps as in Section 10.2.

10.1.4. After the solvent has completely eluted, concentrate the extract with a gentle stream of filtered nitrogen or rotary evaporator to less than 5 ml. Add 5 ml of cyclohexane and transfer the extract to a 10-ml volumetric flask. Dilute to volume using dichloromethane (ideally, the extract should be 50/50% dichloromethane/cyclohexane).

10.1.5. Take a 2 ml aliquot and transfer it to an aluminum-weighing dish tared to the nearest 0.1 mg using an analytical balance. Evaporate the solvent under a gentle stream of filtered air. Weigh the residue to the nearest 0.1 mg using the analytical balance. Determine the fat content using the following equation:

$$\% \text{ fat} = \frac{(\text{residue} + \text{dish weight} - \text{tare}) \times 100}{\text{sample weight}}$$

10.1.6. Automated GPC is used to separate the PCBs, toxaphene, and other chlorinated pesticides from the bulk of the lipid. A 60-g bed of SX-3 Envirobeads gel resin (Bio Rad) is used with a 1:1 mixture of cyclohexane in dichloromethane solvent system. The resin is packed in a 2.5 cm ID x 48 cm glass column fitted with two adjustable end plungers (Glenco Scientific). The column is placed on an automated low-pressure GPC Autoprep 1000 chromatograph (O.I. Analytical), and solvent is pumped through the column at 5 ml/min. Five milliliters of the sample extract is placed on the GPC column. The GPC eluate is split into two fractions. The first 125 ml is dumped (discarded) as this should contain only the extract lipids. The second fraction of 90 ml is collected in 250-ml Erlenmeyer flasks. The exact volume eluted for each fraction is determined from time settings on the GPC control unit. The times are determined and periodically adjusted by "calibrating" the gel resin column with standards spiked both into solvent and tissue lipids. The GPC eluates are evaporated under a gentle stream of nitrogen to approximately 5 ml before further fractionation.

10.2. Pesticide Fractionation

10.2.1. Florisil and Silica gel column chromatography are employed as pesticide separation techniques prior to EC-GC analysis. The fractionations are required to separate PCBs from as many of the other parameters as possible. This facilitates identification and quantification, and provides adequate sample clean up for capillary column analysis of the pesticides and/or PCB congeners. The Florisil procedure is performed first, followed by silica gel.

10.2.2. Florisil columns are prepared by placing 1 cm of anhydrous sodium sulfate in a 1 cm ID x 30 cm chromatography column (Ace Glass) fitted with a 75 ml reservoir. The column should be previously filled to slightly above the reservoir base with hexane. Eight grams of 60/100 mesh Florisil (Floridin Co.), activated at 130°C for 16 hours, is then added, and topped with another 1-cm layer of

sodium sulfate. Avoid entrapping air bubbles when pouring the column. Adjust the hexane level to within 2 mm of the top layer of sulfate and discard the excess solvent (this also serves as a column wash).

10.2.3. When the hexane reaches the top of the upper sodium sulfate layer, the GPC concentrate is quantitatively transferred to the column and allowed to drain onto the bed of Florisil. The concentrate's container is washed with 5 ml of the elution solvent and added to the column as the original extract has just reached the top layer of sulfate. This also serves to wash down the walls of the column. When the solvent reaches the top of the Florisil, the remaining elution solvent is added, and the eluate is collected for further separation. The volume and makeup of the elution solvent is determined from the Florisil standardization. Currently 50 ml of 94/6 hexane/ethyl-ether is used for the first fraction.

10.2.4. When the ethyl ether/hexane solution reaches the top of the Florisil column, a second elution solvent will be added if dieldrin, endrin, and/or methoxychlor are required. This eluate should be collected in a separate beaker from the first. Currently this elution solvent consists of 100 ml of a 50/50% hexane/ethyl ether mixture. The second fraction when concentrated is ready for GC analysis.

10.2.5. The first fraction of the Florisil column must be concentrated to approximately 5 ml and further fractionated through silica gel to separate PCBs and chlorinated pesticides. Prepare the silica gel by heating at 130°C overnight. Allow to cool. If PBDEs are not requested, deactivate with 3.5% by weight of distilled water. If PBDEs <u>are</u> requested, add 5.0% by weight distilled water. Rotate slowly and occasionally in a glass stoppered flask for an hour to equilibrate. (The percentage of deactivation may change with different manufacturer's lots of silica gel.) Prepare silica gel columns (1 cm ID x 30 cm) by first filling with hexane. Add 1 cm of anhydrous sodium sulfate, 5 g of deactivated silica gel and another 1 cm of sodium sulfate layer and quantitatively add the first Florisil fraction. Start collecting the eluate and elute the PCBs, PBDEs and p,p'-DDE with 50 ml of hexane. If PBDEs are not requested, only add 45 ml of hexane.

10.2.6. When the last of the hexane reaches the top of the sulfate layer, add 60 ml of 25% ethyl ether in hexane to elute the chlorinated pesticides. Again, the volume (and percentage ether) may vary with Silica gel batches.

10.3. Gas Chromatography for select pesticides and PCB Analysis

10.3.1. Column GC Conditions:
- 10.3.1.1. HP6890N GC with µElectron Capture Detector
- 10.3.1.2. Column Conditions:
  - 10.3.1.2.1. Type: 15.0 m megabore capillary, 0.53 mm ID, 1.0 µm film thickness
  - 10.3.1.2.2. Mode: constant flow
  - 10.3.1.2.3. Initial flow: 9.4 ml/min
  - 10.3.1.2.4. Initial pressure: 2.0 psi
- 10.3.1.3. Carrier gas: Hydrogen
- 10.3.1.4. Detector make-up gas: Nitrogen
- 10.3.1.5. Oven Temperature Settings:
  - 10.3.1.5.1. Initial Temp: 85°C for 1.00 min.

10.3.1.5.2. Ramp #1: 16.0°C/min to 165.0°C
10.3.1.5.3. Ramp #2: 4.0°C/min to 275.0°C and hold for 2.0 min.
10.3.1.6. Total Run Time: 35.50 min
10.3.1.7. Injector Settings:
10.3.1.7.1. Mode: splitless
10.3.1.7.2. Temperature: 265°C
10.3.1.7.3. Pressure: 2.00 psi
10.3.1.7.4. Purge flow: 15.0 ml/min
10.3.1.7.5. Purge time: 0.75 min
10.3.1.7.6. Injection Volume: 1.0 µl
10.3.1.7.7. Plunger Speed: fast
10.3.1.8. µECD Detector:
10.3.1.8.1. Temperature: 300°C
10.3.1.8.2. Mode: constant makeup flow
10.3.1.8.3. Makeup flow: 25.0 ml/min
10.3.1.8.4. Makeup Gas Type: Nitrogen

10.3.2. The above conditions are appropriate for Aroclor identification and quantitation; and certain chlorinated pesticides (Silica Gel fraction 1). If PCBs are high, the pesticides in this fraction will be masked and can be analyzed by capillary Column GC as in Section 10.3.2. Hexachlorobenzene, aldrin, heptachlor, and p,p' DDE appear in this fraction. If p,p'-DDE is high relative to PCBs, it can be quantified on the Megabore column by diluting to an appropriate volume. (For PCB congeners and toxic congener analysis, see Section 10.4 and 10.5.)

10.4. The second silica gel fraction contains chlordanes, nonachlors, alpha-BHC, gamma-BHC, p,p-DDT and p,p-DDD, and toxaphene. A capillary column is necessary to separate the individual pesticides. Analysis is done using 60 M DB-1 column, and a 30 M DB-5 confirmation column on a HP6890N GC. Listed below are the operating conditions for SG-2 pesticide analysis.

10.4.1. Oven Temperature Program:
10.4.1.1. Initial temperature: 85°C
10.4.1.2. Initial time: 1.0 min
10.4.1.3. Ramp #1: 15.0°C/min to 165°C and hold for 1.50 min.
10.4.1.4. Ramp #2: 1.5°C/min to 215°C.
10.4.1.5. Ramp #3: 5.0°C/min to 260°C and hold for 2.00 min.
10.4.1.6. Inlet Conditions:

Conditions	**Front Inlet:**	**Back Inlet:**
Mode:	Splitless	Splitless
Temperature:	250°C	250°C
Pressure:	22.50 psi	9.00 psi
Purge flow:	60.0 ml/min	60.0 ml/min
Purge time:	0.75 min	0.75 min
Gas type:	Hydrogen	Hydrogen
Injection volume:	1.0 µl	1.0 µl

10.4.2. Column Conditions:

Conditions	Column 1	Column 2
Type:	DB-1 capillary column: column 60.0M, 0.25mm ID, with 0.25µm film thickness	DB-5 capillary column: 30M, 0.32mm ID, with 0.25 µm film thickness
Mode:	Constant pressure	Constant pressure
Pressure:	22.50 psi	9.00 psi
Initial flow:	2.2 ml/min	3.4 ml/min.

10.4.3. Detector Conditions:

Conditions	Front Detector	Back Detector
Temperature:	300 °C	300 °C
Mode:	Constant makeup flow	Constant makeup flow
Makeup flow:	25.0 ml/min	25.0 ml/min
Makeup gas:	Nitrogen	Nitrogen

10.5. Analyze the second Florisil fraction for dieldrin, endrin and methoxychlor using the GC conditions described below.

10.5.1. HP6890N GC with µECDColumn: 15.0M megabore capillary, 0.45mm I.D., 1.27µm film thickness

10.5.2. Oven temperature settings:
- 10.5.2.1. Initial temperature: 85°C for 1.00 min
- 10.5.2.2. Ramp #1: 15.0°C/min to 210°C
- 10.5.2.3. Ramp #2: 2.0°C/min to 260°C
- 10.5.2.4. Ramp #3: 10.0°C/min to 280°C and hold for 1.50 min

10.5.3. Back injector settings:
- 10.5.3.1. Mode: Splitless
- 10.5.3.2. Temperature: 265°C
- 10.5.3.3. Pressure: 2.00 psi
- 10.5.3.4. Purge flow: 15.0 ml/min
- 10.5.3.5. Purge time: 0.75 min
- 10.5.3.6. Gas type: Hydrogen
- 10.5.3.7. Injection volume: 1.0 µl

10.5.4. Column 2 settings:
- 10.5.4.1. Mode: Constant pressure
- 10.5.4.2. Pressure: 2.00 ml/min

10.5.5. Back detector (µECD):
- 10.5.5.1. Temperature: 300°C
- 10.5.5.2. Mode: Constant makeup flow
- 10.5.5.3. Makeup flow: 35.0 ml/min
- 10.5.5.4. Makeup gas type: Nitrogen

10.6. Results are calculated on a wet weight basis. The following equation is used in determining the results of a given PCB or pesticide.

Concentration = (R. sam/R. std)(V. sam/I. sam)(I. std * C. std/W. sam)

Where: R. sam = sample response
V. sam = sample extract volume (ml)
R. std = std response
I. sam = sample injection volume (µl)
I. std = std injection volume (µl)
C. std = std concentration (mg/l)
W. sam = wet weight of sample (g)

The final results are expressed in µg/g (parts per million).

10.7. Gas Chromatography for PCB Congener and PBDE Analysis by Capillary Column

10.7.1. GC Conditions
10.7.1.1. HP 5890-II Gas Chromatograph
10.7.1.2. Column: 60M DB5 column, 0.25 mm ID, 0.1 µm film
10.7.1.3. Hydrogen carrier gas
10.7.1.4. Electron Capture Detector; 300^{o}C
10.7.1.5. Pressure Programmable Injector; 265^{o}C
10.7.1.6. Initial Pressure 40 PSI, 1.0 min. Hold
10.7.1.7. Programmed from 40 PSI to 20 PSI at 20 PSI/min., then go to constant flow mode for remainder of run
10.7.1.8. Splitless injection; purge on at 0.70 min
10.7.1.9. Injector volume 1 µL

10.7.2. Oven Temperature Profile:
10.7.2.1. Initial Temp 100 ^{o}C, hold for 1.0 min
10.7.2.2. 100^{o}C to 150^{o}C at 3^{o}C/min
10.7.2.3. 150^{o}C to 220^{o}C at 1^{o}C/min
10.7.2.4. 220^{o}C to 280^{o}C at 5^{o}C/min, hold for 3 min

10.7.2.5. Standards: See Section 10.3 and 10.4

10.7.3. Instrument Performance: Response factors are generated from a run of the 0.549 mg/l calibration standard. This standard will be run every 12 hours as a performance standard and evaluated for resolution, reproducibility, and sensitivity. In addition, a PCB standard at either 0.275 mg/l or at 1.1 mg/l (one-half or twice the calibration standard) will be run at a nominal frequency of every other sample batch as a check on system linearity. See TABLE II for concentrations. The calculated concentrations of congeners #26 and #199 (small peaks) shall not differ from their known concentrations by more than 40%, and those of congeners #6, #70/76, #101, #180, and #185 (average and large peaks) shall not differ by more than 20%. If these limits are exceeded, response factors will be regenerated, or the necessary instrument maintenance will be performed.

10.7.4. Samples: All samples are screened by packed column GC-EC. This is to insure that there has been adequate clean up, and that they are diluted or concentrated to

an appropriate volume for injection onto the capillary column. A volume of 5 ml is appropriate for a 5 g sample with PCB congener concentrations near the MDLs. Internal standards are added to the cleaned-up sample extract just prior to capillary column gas chromatography. Twenty-five µl of a standard containing congener #30 at 0.568 mg/l and congener #204 at 0.624 mg/l are added to the entire sample extract or to an exactly known portion of the extract. These results in a MASS of 14.2 ng of congener #30 and 15.6 ng of congener #204 added. The sample size represented by the portion of extract to which internal standards are added must be exactly known.

10.7.5. Calculations

10.7.5.1. Calculations are done by a PC-based HP Chemstation, using the formula for internal standard quantitation:

$$\text{Conc.} = \frac{\text{Response(y)}}{\text{Response(IS)}} \times \frac{\text{RF(y)}}{\text{RF(IS)}} \times \text{Amount(IS)} \times \text{Mult.}$$

Where: y = congener
IS = internal standard
RF = response factor = mass/response
Amount(IS) = mass of internal standard added to the sample
Mult. = multiplier = 1/sample size

10.7.5.2. Response factors are generated from a current run of the calibration standard.

10.7.6. Confirmation of correct PCB and PBDE identification is done on 5% of the samples by retention time agreement on a 60M DB-1 column, using the same standards and GC conditions as given in Section 10.4.1. See Table IV.

11. Calculations: See the above Section 10 for calculations.

12. Data Management: Data is collected using a PC-based HPChemstation. PCB Congener data is also collected in a computer file, reviewed by the analyst, and electronically transferred to the Laboratory's LIMS system. It is then reviewed by peers or the section supervisor before being released. Pesticide, Aroclor and PBDE data is transcribed by the analyst onto the sample worksheet. It is then reviewed (by peers or section supervisor) and manually entered into the Laboratory's LIMS system.

13. Definitions: General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

14. Method Performance: Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". Data generated within the last two years will be located in the filing cabinet in the Department supervisor’s cubicle. Any data older than two years is stored in the Department filing cabinet in the basement.

15. References:

15.1. Ribick, M., Petty, and Stalling, 1982. "Toxaphene Residues in Tissue: Identification, Quantification and Confirmation at Part per Billion Level." Environmental Science and Technology, 16, 310-318.

15.2. Mullin, M.D., PCB Workshop, USEPA Large Lakes Research Station, Grosse Ile, MI, June 1985.

15.3. Ballschmiter, K. and Zell, M., 1980. Fresenius Z. Anal. Chem., 302, 20-31.

15.4. "Florisil Cleanup", EPA Method 3620B, (Revision 2, December, 1996).

15.5. "Silica Gel Cleanup", EPA Method 3630C, (Revision 2, December, 1996).

15.6. "Gel-Permeation Cleanup", EPA Method 3640A,(Revision 1, September, 1994)

15.7. "Quality Assurance Procedures and Policies", The ESS QA Manual.

15.8. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

16. Tables, figures, diagrams, charts, checklists, appendices: See following Pages

Table I
PCB Stock Solution Concentrations -- 183 µg/ml

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Calculated Average

Peak Name	Calc'd Congener Conc'ns µg/ml	Peak Name	Calc'd Congener Conc'ns µg/ml	Peak Name	Calc'd Congener Conc'ns µg/ml
PCB-000	4.1	PCB-056+060(AVE)	3.5	PCB-151	1.7
PCB-001	12.0	PCB-063	0.21	PCB-156	0.06
PCB-003	7.0	PCB-064	1.8	PCB-157+200(AVE)	0.39
PCB-004+010(SUM)	3.4	PCB-066	5.2	PCB-158	0.25
PCB-006	1.9	PCB-070+076(SUM)	3.4	PCB-041+071(AVE)	2.3
PCB-007+009(SUM)	1.2	PCB-074	1.9	PCB-163+138(SUM)	2.7
PCB-008+005(SUM)	14.0	PCB-077	0.23	PCB-167	0.049
PCB-012	0.17	PCB-081	0.16	PCB-170+190(SUM)	1.7
PCB-013	0.097	PCB-082	0.44	PCB-172	0.56
PCB-015+017(SUM)	3.7	PCB-083	0.15	PCB-173	0.038
PCB-016	2.0	PCB-085	0.70	PCB-174	3.2
PCB-018	3.7	PCB-087	1.0	PCB-175	0.20
PCB-019	0.28	PCB-089	0.10	PCB-177	1.7
PCB-021	0.032	PCB-091	0.51	PCB-178	1.1
PCB-022	2.9	PCB-092+084(SUM)	1.8	PCB-180	6.1
PCB-024+027(SUM)	0.26	PCB-095	2.0	PCB-183	1.7
PCB-025	0.32	PCB-099	0.74	PCB-185	0.47
PCB-026	0.72	PCB-100	0.11	PCB-187+182(AVE)	3.6
PCB-029	0.053	PCB-101	1.8	PCB-189	0.040
PCB-031+028(SUM)	9.4	PCB-107	0.13	PCB-191	0.12
PCB-032	1.9	PCB-110	1.9	PCB-193	0.42
PCB-033	3.3	PCB-114+131(SUM)	0.1	PCB-194	1.8
PCB-037	1.2	PCB-118	1.2	PCB-197	0.11
PCB-040	0.94	PCB-119	0.028	PCB-198	0.12
PCB-042	1.4	PCB-123+149(SUM)	2.8	PCB-199	0.43
PCB-043	0.27	PCB-128	0.10	PCB-201	4.2
PCB-044	4.3	PCB-129	0.013	PCB-202+171(AVE)	0.79
PCB-045	0.89	PCB-130	0.075	PCB-203+196(SUM)	4.3
PCB-046	0.40	PCB-132+153+105(SUM)	4.3	PCB-205	0.11
PCB-047	1.0	PCB-134R	0.072	PCB-206	0.68
PCB-048	1.0	PCB-135+144(SUM)	0.89	PCB-207	0.093
PCB-049	2.3	PCB-136	0.75	PCB-208+195(SUM)	0.80
PCB-051	0.18	PCB-137+176(AVE)	0.26	PCB-209	0.012
PCB-052	4.5	PCB-141	1.7		
PCB-053	0.64	PCB-146	0.39		

TABLE II

PCB Congener Check Standard Concentrations - DB-5 Column

Congener name	0.275 ng/ml	0.549 ng/ml	1.1 ng/ml	Congener name	0.275 ng/ml	0.549 ng/ml	1.1 ng/ml
#1	18.00	36.00	72.00	#87	1.50	3.00	6.00
#3	10.50	21.00	42.00	#85	1.05	2.10	4.20
#4/10	5.10	10.20	20.40	#136	1.13	2.25	4.50
#7/9	1.80	3.60	7.20	#77/110	3.20	6.40	12.80
#6	2.85	5.70	11.40	#82	0.66	1.32	2.64
#8/5	21.00	42.00	84.00	#151	2.55	5.10	10.20
#14	15.80	31.60	63.20	#135/144	1.34	2.67	5.34
#19	0.42	0.84	1.68	#123/149	4.20	8.40	16.80
ISTD 1 #30	14.20	14.20	14.20	#118	1.80	3.60	7.20
#18	5.55	11.10	22.20	#146	0.59	1.17	2.34
#15/17	5.55	11.10	22.20	#132/153/105	6.45	12.90	25.80
#24/27	0.39	0.78	1.56	#141	2.55	5.10	10.20
#16/32	5.85	11.70	23.40	#137/176	0.39	0.78	1.56
#26	1.08	2.16	4.32	#163/138	4.05	8.10	16.20
#25	0.48	0.96	1.92	#158	0.38	0.75	1.50
#28/31	14.10	28.20	56.40	#178	1.65	3.30	6.60
#33	4.95	9.90	19.80	#166	4.20	8.40	16.80
#53	0.96	1.92	3.84	#187/182	5.40	10.80	21.60
#51	0.27	0.54	1.08	#183	2.55	5.10	10.20
#22	4.35	8.70	17.40	#128	2.15	4.30	8.60
#45	1.34	2.67	5.34	#167	1.00	2.00	4.00
#46	0.60	1.20	2.40	#185	0.70	1.40	2.80
#52	6.75	13.50	27.00	#174	4.80	9.60	19.20
#49	3.45	6.90	13.80	#177	2.55	5.10	10.20
#47/48	3.00	6.00	12.00	#202/171	1.19	2.37	4.74
#65	3.90	7.80	15.60	#157/200	0.59	1.17	2.34
#44	6.45	12.90	25.80	ISTD 2 #204	15.60	15.60	15.60
#37/42	3.90	7.80	15.60	#172	0.84	1.68	3.36
#41/71/64	6.15	12.30	24.60	#180	9.15	18.30	36.60
#40	1.40	2.80	5.60	#193	0.63	1.26	2.52
#63	0.32	0.63	1.26	#199	0.65	1.30	2.60
#74	2.85	5.70	11.40	#170/190	2.55	5.10	10.20
#70/76	5.10	10.20	20.40	#198	0.18	0.36	0.72
#66	7.80	15.60	31.20	#201	6.50	13.00	26.00
#95	3.00	6.00	12.00	#203/196	6.50	13.00	26.00
#91	0.77	1.53	3.06	#208/195	1.20	2.40	4.80
#56/60	5.25	10.50	21.00	#207	0.14	0.28	0.56
#92/84	2.70	5.40	10.80	#194	2.70	5.40	10.80
#89	0.15	0.30	0.60	#206	1.00	2.00	4.00
#101	2.70	5.40	10.80				
#99	1.11	2.22	4.44				
#83	0.23	0.45	0.90				
#97	0.84	1.68	3.36				

Table III

PCB Congener Calibration Concentrations - DB-5 Column

(0.549 µg/ml Standard)

#	NAME	AMOUNT, ng /ml	#	NAME	AMOUNT, ng/ml
1	#1	3.6000E+01	42	#83	4.5000E-01
2	#3	2.1000E+01	43	#97	1.6800E+00
3	#4/10	1.0200E+01	44	#87	3.0000E+00
4	#7/9	3.6000E+00	45	#85	2.1000E+00
5	#6	5.7000E+00	46	#136	2.2500E+00
6	#8/5	4.2000E+01	47	#77/110	6.4000E+00
7	#14	3.1600E+01	48	#82	1.3200E+00
8	#19	8.4000E-01	49	#151	5.1000E+00
9	ISTD 1#30	1.4200E+01	50	#135/144	2.6700E+00
10	#18	1.1100E+01	51	#123/149	8.4000E+00
11	#15/17	1.1100E+01	52	#118	3.6000E+00
12	#24/27	7.8000E-01	53	#146	1.1700E+00
13	#16/32	1.1700E+01	54	#132/153/105	1.2900E+01
14	#26	2.1600E+00	55	#141	5.1000E+00
15	#25	9.6000E-01	56	#137/176	7.8000E-01
16	#28/31	2.8200E+01	57	#163/138	8.1000E+00
17	#33	9.9000E+00	58	#158	7.5000E-01
18	#53	1.9200E+00	59	#178	3.3000E+00
19	#51	5.4000E-01	60	#166	8.4000E+00
20	#22	8.7000E+00	61	#187/182	1.0800E+01
21	#45	2.6700E+00	62	#183	5.1000E+00
22	#46	1.2000E+00	63	#128	4.3000E+00
23	#52	1.3500E+01	64	#167	1.9000E+00
24	#49	6.9000E+00	65	#185	1.4000E+00
25	#47/48	6.0000E+00	66	#174	9.6000E+00
26	#65	7.8000E+00	67	#177	5.1000E+00
27	#44	1.2900E+01	68	#202/171	2.3700E+00
28	#37/42	7.8000E+00	69	#157/200	1.1700E+00
29	#41/71/64	1.2300E+01	70	ISTD 2#204	1.5600E+01
30	#40	2.8000E+00	71	#172	1.6800E+00
31	#63	6.3000E-01	72	#180	1.8300E+01
32	#74	5.7000E+00	73	#193	1.2600E+00
33	#70/76	1.0200E+01	74	#199	1.3000E+00
34	#66	1.5600E+01	75	#170/190	5.1000E+00
35	#95	6.0000E+00	76	#198	3.6000E-01
36	#91	1.5300E+00	77	#201	1.3000E+01
37	#56/60	1.0500E+01	78	#203/196	1.3000E+01
38	#92/84	5.4000E+00	79	#208/195	2.4000E+00
39	#89	3.0000E-01	80	#207	2.8000E-01
40	#101	5.4000E+00	81	#194	5.4000E+00
41	#99	2.2200E+00	82	#206	2.0000E+00

Table IV

PCB Congener Calibration Concentrations - DB-1 Column

#	NAME	AMOUNT, ng/ml	#	NAME	AMOUNT, ng/ml
1	#1	3.6000E+01	44	#97	1.7000E+00
2	#3	2.1000E+01	45	#87	3.0000E+00
3	#4/10	1.0000E+01	46	#85	2.1000E+00
4	#7/9	3.6000E+00	47	#136	2.3000E+00
5	#6	5.7000E+00	48	#110	5.7000E+00
6	#8/5	4.2000E+01	49	#82	1.3000E+00
7	#14	3.1600E+01	50	#151	5.1000E+00
8	ISTD 1#30	1.4200E+01	51	#135	8.4000E-01
9	#18	1.1000E+01	52	#144	1.8000E+00
10	#15/17	1.1000E+01	53	#123/149/118	1.2000E+01
11	#24/27	7.8000E-01	54	#105/146/132	5.3000E+00
12	#16	6.0000E+00	56	#153	8.1000E+00
13	#32	5.7000E+00	57	#141	5.1000E+00
14	#26	2.2000E+00	58	#130/176	1.0000E+00
15	#25	9.6000E-01	59	#138/163	8.1000E+00
16	#31	1.4000E+01	60	#158	7.5000E-01
17	#28	1.3000E+01	61	#166	8.4000E+00
18	#33/53	1.1800E+01	62	#178	3.3000E+00
20	#22/51	9.2000E+00	64	#182/187/128	1.5000E+01
21	#45	2.7000E+00	65	#183	5.1000E+00
22	#46	1.2000E+00	66	#167	1.9000E+00
23	#52	1.3500E+01	67	#185	1.4000E+00
24	#49	6.9000E+00	68	#174	9.6000E+00
25	#47	3.1000E+00	69	#177	5.1000E+00
26	#48	3.3000E+00	70	#171/156	1.5000E+00
27	#65	7.8000E+00	71	#173/157/202	1.4000E+00
28	#44/37	1.7000E+01	72	#200	1.5000E+00
29	#42	4.2000E+00	73	ISTD 2#204	1.5600E+01
30	#41/71/64	1.2000E+01	74	#172	1.7000E+00
31	#40	2.8000E+00	76	#180	1.8000E+01
32	#63	6.3000E-01	77	#193	1.3000E+00
33	#74	5.7000E+00	78	#199	1.3000E+00
34	#70/76	1.0000E+01	79	#170	3.6000E+00
35	#66	1.6000E+01	80	#190	1.4000E+00
36	#95	6.0000E+00	81	#198	3.6000E-01
37	#91	1.5000E+00	82	#201	1.3000E+01
38	#56/60	1.0000E+01	83	#203/196	1.3000E+01
39	#84	3.9000E+00	84	#195	2.0000E+00
40	#89/92	1.8000E+00	85	#208	3.1000E-01
41	#101	5.4000E+00	86	#207	2.8000E-01
42	#99	2.2000E+00	87	#194	5.4000E+00
43	#83	4.5000E-01	88	#206	2.0000E+00

17. Signatory Page:

17.1. Written by: Dave Rogers — Date: Revision 4: 3/3/2006
Title: Senior Chemist — Revision 5: 12/15/2008
Unit: ESS Organic Chemistry

17.2. Reviewed by: Donna R. Johnsen — Date: Revision 4: 6/30/2006
Title: ESS Organic QC Officer — Revision 5: 1/12/2009
Unit: ESS Organic Chemistry

17.3. Approved by: Steve Geis — Date: Revision 4: 8/10/2006
Title: ESS Organic Supervisor — Revision 5: 1/12/2009
Unit: ESS Organic Chemistry

Appendix F.5

Wisconsin State Lab of Hygiene

ESS Org Method 1440, PCB Analysis in Tissue

ESS ORG METHOD 1440

PCB Analysis in Tissue

Matrix: Tissue

1. Scope and Application

1.1. This method is used for the analysis of PCB Aroclors in tissue samples.

1.2. Note: Often the above Aroclors are analyzed as a mixture (i.e. A sample may exhibit a chromatographic fingerprint resembling two or more PCBs in combination).

1.3. List LOD and LOQ:

Compound	LOD	LOQ
PCBs	0.040	0.13

2. Summary of Method:

2.1. Tissue samples (fish and wildlife) are homogenized in a blender with dry ice and allowed to stand overnight. Then approximately ten grams is combined with sodium sulfate and extracted with MeCl2. The lipid is removed by gel-permeation chromatography. In addition, the extract may require additional clean up with silica gel or Florisil. The final extract is concentrated and injected onto a gas chromatograph equipped with a megabore capillary column and micro electron-capture detector (μECD).

2.2. List Regulatory Deviations: This section is not applicable to this method.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences

3.2. All laboratory waste, excess reagents and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

4. Sampling Handling and Preservation:

Grind tissue samples coarsely and freeze in glass containers.

5. Interferences:

Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that analytes of interest may not be completely resolved from one another.

6. Reagents and Standards:

6.1. Methylene chloride, hexanes, isooctane.

6.2. Anhydrous sodium sulfate (10-60 mesh).

6.3. Silica gel, Fisher (100-200 mesh).

6.4. Certified PCB standards from an outside vendor (Ultra Scientific, Supelco, AccuStandard, etc).

6.5. Tetrachloro-m-xylene standard. Neat or certified solution.

6.6. The procedure for "neat" standard preparation follows:

6.6.1. Pre-tare a 100 ml volumetric flask to the nearest 0.1 mg. Introduce 10-50 mg of primary standard to the flask and weigh the flask plus standard to the nearest 0.1 mg.

6.6.2. Determine total standard amount by the following formula:

$$D = \frac{(B-A) * C}{100}$$

Where: A = Flask tare

B = Standard plus tare

C = Percent purity

D = Adjusted standard weight

6.6.3. Dilute with isooctane. Compute the concentration as follows:

$$\text{Standard} = \frac{\text{Adjusted standard weight}}{0.1 \text{ liter}}$$

This is the concentrated stock in mg/l.

6.6.4. Working standards are prepared by serial dilutions using standard volumetric glassware (i.e. pipettes).

7. Apparatus:

7.1. Industrial blender.

7.2. Florisil and silica gel columns

7.3. Calibrated 15-ml centrifuge tubes

7.4. Nitrogen blow-down apparatus

7.5. Hot plate

7.6. Gel-Permeation Chromatograph

7.7. Volumetric flasks: 10, 25, & 100 ml

7.8. Megabore capillary column GC/µECD: HP6890N equipped with a PC-based Chemstation integrator.

7.9. Beakers: 100 & 250 ml

7.10. 2 & 5 ml disposable pipettes

7.11. Transfer pipettes

8. Quality Control

8.1. For general quality control procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT".

8.2. Prepare spiking solutions containing PCB mixtures such that 1-2 ml of solution will spike 10g of tissue at appropriate levels (ca. 1-2 µg /gm recommended). Solutions are prepared in isooctane. Aliquots of spiking solution are introduced to tissue directly after tissue has been mixed with sodium sulfate prior to extraction (between Steps 10.1.1 and 10.1.2 in Section 10.1). Mix thoroughly allowing solvent to evaporate before introducing the tissue/sodium sulfate mixture into the extraction column.

8.3. For each batch of approximately 10 samples, a duplicate sample is analyzed.

8.4. If any of the parameters in Section 8.1, 8.2, and 8.3, exceeds their limits, the samples will be re-analyzed or appropriately flagged.

8.5. For each batch of approximately 10 samples, a method blank will be analyzed. If any analytes of interest are found above their LOD (or report limit), the samples will be re-analyzed or the data appropriately flagged.

8.6. The performance of the method will be monitored by adding a surrogate. Tetrachloro-m-xylene will be spiked into each sample in an Aroclor or Aroclor/pesticide batch. Statistical limits are kept and the system will be investigated if the surrogate recovery falls outside those limits.

8.7. All data pertinent to preparation of standards is recorded in the "Preparation of Standards" logbook. Pertinent data is to include date of preparation, origin of parent solution/primary standard, aliquot, dilution information, all weighing and tare data, purity and unique identifier. Each standard solution is given a unique identifier for traceability purposes.

8.8. When fresh stock solutions are prepared from primary standards a log sheet is begun noting the origin, purity, date of preparation, and pertinent weighing and dilution data. Subsequent intermediate and working (or spiking) solutions prepared from the stock will also be documented on this same log sheet. Each standard generated from a parent source is given a unique identifier for traceability purposes back to the parent source. Log sheets representing discarded parent (stock) solutions are removed from the active portion of the "Preparation of Standards" and filed by name.

9. Method Calibration:

9.1. Aroclor analysis is done on the megabore capillary column using a single point calibration.

9.2. The area response of selected peaks in the sample chromatogram is directly compared to the same peaks in the standard.

9.3. The area of the sample must be within 30% of the area of the standard for valid calculations.

9.4. Note: the sample response will never be >10% of the highest standard run on that analysis day

10. Procedure

10.1. Sample Extraction

10.1.1. Blend tissue with dry ice at high speed to produce a free-flowing powder. Rinse the blender jar between samples with ethanol. Let the dry ice sublime overnight

in a freezer. Then mix 10 g of frozen tissue powder with 60 g anhydrous sodium sulfate stirring frequently for about 30 minutes.

10.1.2. Pour the tissue and sodium sulfate mixture into a 20 mm I.D. chromatographic column initially containing a 2 cm layer of sodium sulfate. Open the stopcock and add 230 ml of methylene chloride to the column reservoir. Adjust the elution rate through the column to about 5 ml/minute. Collect the eluate.

10.1.3. After the solvent has completely eluted, concentrate the extract in a water bath under a gentle stream of nitrogen or rotary evaporator to less than 5 ml. Add 5 ml of cyclohexane and quantitatively transfer to a 10 ml volumetric flask using methylene chloride to rinse beaker and dilute to volume. Take a 2 ml aliquot and transfer to an aluminum-weighing dish tared to the nearest 0.1 mg using an analytical balance. Let the solvent evaporate from the tared weighing dish and weigh the residue to the nearest 0.1 mg using the analytical balance. Determine the fat content using the following equation:

$$\%\text{fat} = \frac{[\text{residue} + \text{beaker weight} - \text{tare}] \times 100}{\text{Sample weight}}$$

10.2. Sample Cleanup:

10.2.1. Autoprep 1000 Chromatograph GPC is used to separate the PCBs and other chlorinated pesticides from the bulk of the lipid. A 60 g bed of S-X3 Envirobeads gel resin (Bio Rad) is used with a 1:1 mixture of cyclohexane/dichloromethane solvent system. The resin is packed in a 2.5 cm I.D. x 48 cm glass column fitted with two adjustable end plungers (Glenco Scientific). The column is placed on an automated low-pressure GPC Autoprep 1000 chromatograph (O.I. Analytical), and solvent is pumped through the column at 5 ml/min. Note: If lipid content is low, GPC may be omitted and Florisil cleanup substituted.

10.2.2. Five milliliters of the sample extract is placed on the GPC column. The GPC eluate is split into two fractions. The first 125 ml is dumped (discarded) as this should contain only the extract lipids. The second fraction of 90 ml is collected in 150-ml Erlenmeyer flask. The exact volume eluted for each fraction is determined from time settings on the GPC control unit. Periodically, the flow rate needs to be checked to determine if the GPC is operating properly. Adjustments are made to the pump, if necessary, and documented in the logbook. The times are determined and periodically adjusted by "calibrating" the gel resin column with standards spiked into solvent. The GPC eluates are evaporated in a water bath, under a gentle stream of nitrogen to approximately 5 ml before further fractionation.

10.2.3. Silica gel chromatography is used as a final cleanup for PCB analysis. It may be repeated for samples which chromatograph poorly following the first treatment.

10.2.4. Prepare silica gel by heating at 130°C overnight and deactivating before use by equilibrating one hour with 3.5% RO water. (The percentage of deactivation may change with different manufacturer's lots of silica gel.) Prepare silica gel columns (1 cm I.D. x 30 cm) by first filling with hexane. Add 1 cm of anhydrous sodium sulfate, 5 g of deactivated silica gel and another 1 cm of sodium sulfate

layer and quantitatively add the GPC eluate. Start collecting the eluate and elute the PCBs with 50 ml hexane. The elution volume may change with different batches of Silica gel and is determined by eluting standards through a column.

10.2.5. Reduce the volume under a gentle stream of nitrogen and transfer to the appropriate volumetric glassware for GC-µECD analysis.

10.3. Gas Chromatography:

10.3.1. Column GC Conditions:

10.3.1.1. HP6890N GC with µElectron-Capture Detector

10.3.1.2. Column: 15.0 m megabore capillary, 0.53mm ID, 1.0µm film thickness

10.3.1.3. Carrier gas: Hydrogen

10.3.1.4. Detector make-up gas: Nitrogen

10.3.1.5. Oven Temperature Settings:
10.3.1.5.1. Initial Temp: 85°C for 1.00 min.
10.3.1.5.2. Ramp #1: 16.0°C/min to 165.0°C
10.3.1.5.3. Ramp #2: 4.0°C/min to 275.0°C and hold for 2.0 min.

10.3.1.6. Total Run Time: 35.50 min

10.3.1.7. Injector Settings:
10.3.1.7.1. Mode: splitless
10.3.1.7.2. Temperature: 265°C
10.3.1.7.3. Pressure: 2.00 psi
10.3.1.7.4. Purge flow: 15.0 ml/min
10.3.1.7.5. Purge time: 0.75 min
10.3.1.7.6. Injection Volume: 1.0 µl
10.3.1.7.7. Plunger Speed: fast

10.3.1.8. Column conditions:
10.3.1.8.1. Mode: constant flow
10.3.1.8.2. Initial flow: 9.4 ml/min
10.3.1.8.3. Initial pressure: 2.0 psi

10.3.1.9. µECD Detector:
10.3.1.9.1. Temperature: 300°C
10.3.1.9.2. Mode: constant makeup flow
10.3.1.9.3. Makeup flow: 25.0 ml/min
10.3.1.9.4. Makeup gas type: Nitrogen

10.4. Analysis:

10.4.1. The first step in PCB quantification is to match the sample chromatogram as closely as possible with that of an Aroclor standard or combination of Aroclor standards. Aroclors 1242, 1248, 1254 and 1260 and combinations thereof are all possible. Sample weathering (loss of some peaks) and the possible presence of co-eluting compounds must be considered. Experience should enable the analyst to identify and match standard Aroclor mixtures to environmental samples from a

qualitative perusal of the sample chromatogram. Complex Aroclor mixtures require careful attention and adjustment as to relative component strengths in order to obtain the best "fingerprint" match with the sample.

10.4.2. Quantification of PCB mixtures is usually done by summing the peak areas of the PCB components present in the sample and comparing that to the sum of the same peaks in the appropriate Aroclor standard, as determined in Section 10.4.1. Peaks in the sample representing an interfering pesticide (e.g. pp'-DDE, or in some cases trans-nonachlor) should of course be avoided.

10.4.3. Aroclor peaks are divided by the standard peaks, multiplied by the standard concentration and multiplied by the extract final volume/sample weight.

10.4.4. µg/gm PCB= $\frac{\text{[Sample area] * [µg/ml PCB] * [Sample volume (ml)]}}{\text{[Standard area] [Sample weight (g)]}}$

11. Calculations: See the above Section 10.4 for calculations

12. Data Management: Data is collected on PC-based Chemstation integrator and transferred to the laboratory worksheet. All data is reviewed (by peers or section supervisors) and then manually entered onto the Laboratory's LIMS system.

13. Definitions: General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

14. Method Performance: Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". Data generated within the last two years will be located in the filing cabinet in the Department hallway. Any data older than two years is stored in the Department filing cabinet in the basement.

15. References:

15.1. Ribick, M., Petty and Stalling, 1982. "Toxaphene Residues in Fish: Identification, Quantification and Confirmation at Part per Billion Level." Environmental Science and Technology, 16, 310-318.

15.2. "Silica Gel Cleanup", EPA Method 3630C, (Revision 2, December, 1996).

15.3. "Gel-Permeation Cleanup", EPA Method 3640A,(Revision 1, September, 1994)

15.4. "Quality Assurance Procedures and Policies", The ESS QA Manual.

15.5. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

16. Tables, figures, diagrams, charts, checklists, appendices: None

17. Signatory Page:

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Appendix F.6

Wisconsin State Lab of Hygiene

ESS Org Method 1480,
Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS

ESS ORG METHOD 1480

Analysis of Perfluorinated Compounds in Fish Tissue by HPLC-MS/MS

Matrix: Tissue

1. Scope and Application

1.1. This is a high performance liquid chromatographic triple quadrupole mass spectrometric (HPLC-MS/MS) method applicable to the determination of perfluorinated compounds in fish tissue.

1.2. The following compounds and reporting limits are listed below for this method:

Analyte	Report limit ng/g
Perfluoro-1-octanesulfonate	0.50
Perfluoro-1-butanesulfonate	0.50
Perflioro-1-hexanesulfonate	0.50
Perfluoro-1-heptanesulfonate	0.50
Perfluoro-1-decanesulfonate	0.50
Perfluoro-n-octanoic acid	0.50
Perfluoro-n-butanoic acid	2.0
Perfluoro-n-pentanoic acid	0.50
Perfluoro-n-hexanoic acid	0.50
Perfluoro-n-heptanoic acid	0.50
Perfluoro-n-nonanoic acid	0.50
Perfluoro-n-decanoic acid	0.50
Perfluoro-n-undodecanoic acid	0.50
Perfluoro-n-dodecanoic acid	0.50
Perfluoro-n-tridecanoic acid	0.50
Perfluoro-n-tetradecanoic acid	0.50
Perfluoro-1-octanesulfonamide	0.50

2. Summary of Method:

2.1. Perfluorinated compounds (PFCs) present in an fish tissue sample are extracted by an ion-pairing liquid/liquid extraction with MTBE, evaporated to dryness under nitrogen gas, reconstituted in 50:50 2 mM ammonium acetate:MeOH and then analyzed by HPLC-MS/MS. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are analyzed using a turbo ion spray triple quadruple mass spectrometer in the negative ionization mode.

2.2. List Regulatory Deviations: This section is not applicable to this method.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences

3.2. All laboratory waste, excess reagents, and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

4. Sampling Handling and Preservation: Samples must be iced or refrigerated at 4°C in transport and frozen as soon as possible away from light from the time of collection until analysis. Perfluorinated compounds have been shown to be stable for several months under these conditions.

5. Interferences: Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference is unknown until further sample analysis is completed.

6. Reagents and Standards:

6.1. Reagents

6.1.1. Methanol, Acetic Acid - HPLC grade

6.1.2. Ammonium Acetate, Reagent grade

6.1.3. Tetrabutylammonium hydrogen sulfate (TBA)

6.1.4. Concentrated sodium hydroxide (NaOH)

6.1.5. Sodium carbonate (Na_2CO_3)

6.1.6. Sodium bicarbonate ($NaHCO_3$)

6.1.7. 18 Mohm water

6.2. Reagent Preparation

6.2.1. 2mM Ammonium Acetate in Water: Add 0.154 g of ammonium acetate to 1 liter of 18 Mohm water

6.2.2. 0.5 M TBA, pH 10: Weigh 169 g TBA into a beaker containing approximately 500 ml 18 Mohm H_2O. Adjust to pH 10 with NaOH solution. Dilute to a final volume of 1,000 ml with 18 Mohm H_2O.

6.2.3. 0.25 M Na_2CO_3/$NaHCO_3$ buffer – Weigh 26.5 g Na_2CO_3 and 21.0 g $NaHCO_3$ into a 1000-ml volumetric flask and dilute with 18 Mohm H_2O.

6.3. Standards

6.3.1. Prepare stock standard solutions by obtaining a known weight of pure material, dissolving the material in methanol and diluting to volume in a volumetric flask.

6.3.2. Stock standard solutions should be stored under refrigerated conditions.

6.3.3. Working calibration standards are extracted matrix spikes.

6.3.4. Stock internal standard solution contains ^{13}C isotopically labeled compounds at a nominal concentration of 50 ng/ml each.

6.3.4.1. Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid

6.3.4.2. Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid

6.3.4.3. Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid

6.3.4.4. Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid

6.3.4.5. Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid

6.3.4.6. Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid

6.3.4.7. Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid

6.3.4.8. Sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate

6.3.4.9. Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate

7. Apparatus:

7.1. Sample bottles: avoid use of Teflon septa/cap liners

7.2. Analytical Balance capable of accurately weighing to the nearest 0.0001 g.

7.3. 15-ml screw capped polypropylene centrifuge tubes, graduated

7.4. Vortex mixer

7.5. Disposable polypropylene luer tip syringes

7.6. 13-mm syringe filters, 0.2 micron nylon

7.7. 2-ml auto-injector vials with aluminum crimp caps (polypropylene septa)

8. HPLC/MS/MS Instrument Conditions

8.1. The HPLC-MS/MS method is performed on one of two (A or B) Applied Biosystems API 4000 triple quadrupole mass spectrometer (Foster City, CA), which is interfaced to an Agilent 1100 HPLC equipped with a degasser, autosampler and column heating compartment.

8.2. Method Name: 20100915_PFC.dam

8.2.1. Synchronization Mode: LC Sync

8.2.2. Auto-Equilibration: Off

8.2.3. Acquisition Duration: 17 minutes 0 seconds

8.2.4. Number of Scans: 937

8.2.5. Period In File: 1

8.2.6. Acquisition Module: Acquisition Method

8.2.7. Software version: Analyst 1.5

8.3. Source height setting-5, Source L/R setting-5

8.4. Agilent 1100 LC Pump Method

8.4.1. Pump Model: Agilent 1100 LC Binary pump

8.4.2. Column: Zorbax Rapid Resolution, 3.5 µm, 30 mm long x 2.1 mm I.D. (Part # 873700-902).

8.4.3. Agilent 1100 LC Pump Method Properties

Minimum Pressure (psi)	0.0
Maximum Pressure (psi)	5801.0
Dead Volume (µl)	40.0
Maximum Flow Ramp (ml/min²)	100.0
Maximum Pressure Ramp (psi/sec)	290.0
Left Compressibility	50.0
Right Compressibility	115.0
Left Dead Volume (µl)	40.0
Right Dead Volume (µl)	40.0

Left Stroke Volume (µl)	-1.0
Right Stroke Volume (µl)	-1.0
Left Solvent	A2
Right Solvent	B2

8.4.4. Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.00	250	70	30
1	0.50	250	20	80
2	9.00	250	20	80
3	9.01	500	10	90
4	11.00	500	10	90
5	11.01	500	70	30
6	14.00	500	70	30
7	14.01	250	70	30
8	17.00	250	70	30

8.5. Agilent 1100 Autosampler Properties

Autosampler Model	Agilent 1100 Autosampler
Syringe Size (µl)	100
Injection Volume (µl)	5.00
Draw Speed (µl/min)	200.0
Eject Speed (µl/min)	200.0
Needle Level (mm)	1.00
Temperature Control	Not used
Wash Vial Number	100
Wash Rack Number	1
Use Custom Injector Program	No

8.6. Agilent 1100 Column Oven Properties

Left Temperature (°C)	30.00
Right Temperature (°C)	30.00
Temperature Tolerance $^{+}$(°C):	1.00
Start Acquisition Tolerance $^{+}$(°C)	0.50
Time Table	(Not Used)
Column Switching Valve	Installed
Position for first sample in the batch	Left
Use same position for all samples in the batch	

8.7. MS/MS Method Properties:

8.7.1. Period 1:

8.7.1.1. Scans in Period: 937

8.7.1.2. Relative Start Time: 0.00 msec

8.7.1.3. Experiments in Period: 1

8.7.2. Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM	No
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0000 msec
MCA:	No
Step Size:	0.00 Da

8.7.3. Parameters:

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE	CXP
PFOS-1	498.90	80.00	25.00	-55.00	-72.00	-5.00
PFOS-2	498.90	99.00	25.00	-55.00	-64.00	-17.00
PFOS-3	498.90	180.00	25.00	-55.00	-50.00	-9.00
PFOA-1	413.10	368.80	25.00	-25.00	-14.00	-1.00
PFOA-2	413.10	168.90	25.00	-25.00	-26.00	-9.00
PFOS IS-1	502.92	80.27	25.00	-100.00	-70.00	-3.00
PFOS IS-2	502.92	98.96	25.00	-100.00	-62.00	-7.00
PFOA IS-1	416.78	372.10	25.00	-35.00	-14.00	-9.00
PFOA IS-2	416.78	169.00	25.00	-35.00	-26.00	-7.00
PFBS	299.00	80.00	25.00	-70.00	-58.00	-5.00
PFHxS	399.05	80.20	25.00	-80.00	-68.00	-1.00
PFHpS	449.00	80.20	25.00	-80.00	-74.00	-5.00
PFDS	599.03	80.10	25.00	-70.00	-86.00	-5.00
PFBA	213.00	169.18	25.00	-25.00	-12.00	-13.00
PFPA	263.15	219.16	25.00	-25.00	-12.00	-11.00
PFHxA	313.05	269.20	25.00	-25.00	-15.00	-15.00
PFHpA	363.10	319.05	25.00	-30.00	-12.00	-7.00
PFNA	463.06	418.82	25.00	-30.00	-14.00	-11.00
PFDA	513.09	468.99	25.00	-35.00	-16.00	-11.00
PFUdA	563.09	519.17	25.00	-40.00	-16.00	-9.00
PFDoA	613.12	569.20	25.00	-35.00	-18.00	-9.00

PFTrDA	663.04	619.23	25.00	-35.00	-16.00	-11.00
PFTeDA	713.09	669.14	25.00	-40.00	-18.00	-11.00
PFBA IS	216.97	171.93	25.00	-40.00	-14.00	-9.00
PFHxA IS	314.98	270.03	25.00	-45.00	-14.00	-7.00
PFHxS IS	403.02	103.18	25.00	-100.00	-52.00	-5.00
PFNA IS	468.00	423.00	25.00	-60.00	-16.00	-5.00
PFDA IS	515.06	469.93	25.00	-60.00	-18.00	-7.00
PFUdA IS	565.05	520.04	25.00	-55.00	-18.00	-15.00
PFDoA IS	615.09	570.19	25.00	-70.00	-18.00	-9.00
FOSA	498.02	78.14	25.00	-80.00	-60.00	-5.00
FOSA IS	506.08	78.15	25.00	-85.00	-60.00	-5.00

8.8. Parameter Table (Period 1 Experiment 1):

CUR:	35.00
GS1:	40.00
GS2:	40.00
IS:	-4500.00
TEM:	400.00
ihe:	ON
CAD:	10.00
EP	-10.00

8.9. Electron Multiplier Settings

Detector Paramenters (Negative):	
CEM	2200.0
DF	200.0

9. Quality Control

9.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure "ESS ORG QA0001 QAWRKSHT" located at O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance (QA)\ESS ORG QA 0001_QAWRKSHT.doc.

9.2. Before any analysis is done, the MS/MS detector should pass a polypropylene glycol (PPG) tune check. A standard containing 300 µM of SCIEX Mixed PPG solution is run. The spectra of should meet the recommended SCIEX operating criteria before samples are run. A tune check should be performed periodically (eg. Quarterly and after PM or other service) See example SCIEX tuning criteria in 9.3 below:

9.3. SCIEX tuning criteria for PPGs in negative Turbo Ion Spray mode (NOTE: cps = counts per second and FWHM = field width at half mass.

Mass	Q1 cps	Mass criteria	Q3 cps	FWHM
934	>/= 2.0e7	0.6 to 0.8 amu	>/= 2.0e7	0.6 to 0.8 amu
2036	>/= 3.0e6	0.6 to 0.8 amu	N/A	N/A

9.4. Minimum quality control (QC) requirements may include initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks and QC samples.

9.5. Laboratory reagent blanks - The analyst must demonstrate that all glassware and reagent interferences are under control. Before a new set of samples is extracted, a laboratory reagent blank (LRB) must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.6. Assessing laboratory performance with laboratory fortified blanks (LFB) – Laboratory fortified blanks spiked over the working range are the calibration standards for this method.

9.7. Assessing analyte recovery with laboratory fortified sample matrix – If sufficient sample is available, the laboratory will add a known concentration to a minimum of 5% of the routine samples or one sample per set, whichever is greater. The spiking concentration should not be less than 2-5 times the background concentration of the sample selected for fortification. Calculate the percent recovery, P, of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.:

$$P = 100 (X - b) / \text{fortifying concentration}$$

9.8. Until the laboratory acquires the appropriate number of LFB data points (20 - 30) for in house statistical limits, the limits specified in the EPA Solid Waste methods manual will be used as guidance which is 70-130%.

9.9. Assessing precision with duplicates: If sufficient sample is available, with each batch of samples, the laboratory should analyze one serum in duplicate. Based upon the analyst's experience, a sample which is expected to contain the analytes above the limit of quantitation should be chosen to duplicate, Until the laboratory acquires the appropriate number of data points (20-30) for in house statistical limits, the limits specified in the EPA Solid Waste methods manual, which are 70-130%, will be used as guidance.

9.10. Identification of Analytes - The retention time window for all compounds is monitored. In addition, each compound that is detected is confirmed by a confirmation ion pair.

10. Method Calibration

10.1. Working Standard Preparation Procedure

10.1.1. Due to matrix effects, volumetric dilutions of stock methanolic standard solutions cannot be used as working calibration standards. Instead, all quantitation is based upon matrix matched extracted standard curves, using the same extraction

protocol that is employed for the samples (Section 11). The matched matrix, ocean perch tissue, is spiked at 6 standard curve levels which are as follows: 0.10, 0.25, 1.0, 2.5, 5.0, 10.0, and 50.0 ng/g.

10.1.2. Inject each extracted calibration standard. The Applied Biosystems Analyst data system is used to prepare an internal standard linear calibration curve for each analyte. An R value of greater than or equal to 0.995 will be used as guidance to verify the acceptability of the curve.

10.2. The working calibration curve must be verified on each working day by the injection of one or more calibration standards at the beginning and end of each analytical run, and after the analysis of 10 samples if 10 or more samples are analyzed in an analysis day. In addition, or minus 20% will be used as guidance for acceptability of the calibration check standard. If the check standard varies by more than 20 percent, then a new calibration curve may need to be generated.

10.3. One calibration check standard, which is a LFB, is extracted with each batch of samples.

11. Sample Preparation Procedure

11.1. A frozen aliquot of 1g of tissue is transferred into a 50 ml polypropylene centrifuge tube. Spike the sample with mass labeled internal standard solution at the same concentration as the standards (ISTD target concentration is nominally 0.5 ng/g).

11.2. Four (4.0) ml Milli-Q water is added to the spiked tissue and the mixture is homogenized using a hand held homogenizer (Tissue Tearor, Biospec Products Inc.).

11.3. Two (2.0) ml of 0.5 M TBA and 3.0 ml of 0.25 M Na_2CO_3/$NaHCO_3$ buffer solutions are added to the homogenate in the tube, and after briefly vortex mixing, 10 ml of MTBE is added. The contents of the tube are vortex mixed again for and then extracted by shaking on a wrist action shaker for approximately 20 minutes.

11.4. The extract is centrifuged at 3000 rpm for at least 10 minutes and the upper MTBE layer is transferred into a 15 ml polypropylene tube. Repeat the MTBE extraction step two additional times with 10 ml of MTBE.

11.5. The extract is then reconstituted using 0.5 ml of MeOH. An additional 0.5 ml of 2 mM ammonium acetate is added to each MeOH extract to achieve a 50:50 solution.

11.6. The reconstituted sample is passed through a 0.2 μm syringe filter into a 2 ml auto-injector vial and a non-PTFE septum crimp cap is affixed.

12. Calculations: Calculations are performed using the Applied Biosystems Analyst software, performing a multilevel calibration, and using a linear fit.

13. Data Management: Data is collected and calculations are made on a PC-based system running SCIEX Analyst Software by the analyst. Perfluorinated analyte data is transcribed onto the sample worksheet, reviewed, and transferred to the Laboratory's LIMS system manually by the analyst (or designee). It is then reviewed by peers or the section supervisor before being released.

14. Definitions: General definitions of other terms that may be used in this method are found in Section 19 of the SLH Quality Assurance Manual.

15. Method Performance: Where applicable the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure "ESS ORG QA0012 LOD and LOQ Determinations". All data is stored with sample date in the year it was run. QC personnel keep a spreadsheet of the MDLs data.

16. References:

16.1. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry". 3M Environmental Laboratory Method Number ETS-8-4.1 (03/01/99).

16.2. Kurunthachalam, K., Franson, J.C., Bowerman, W.W., Hansen, K.J., Jones, P.D., and Giesy, J.P. "Perfluoooctane Sulfonate in Fish-Eating Water Birds Including Bald Eagles and Albatrosses", *Environ. Sci. Technol.* 2001, 35, 3065-3070.

16.3. Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. "Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices", *Environ. Sci. Technol.* 2001, 35, 766-770.

16.4. "Test Methods For Evaluating Solid Waste Physical/Chemical Methods (SW-846) Third Edition,"1996.

16.5. "Methods for the Determination of Organic Compounds in Drinking Water," US EPA/600/4-88/039, 1995.

16.6. "Quality Assurance Procedures and Policies", The ESS QA Manual.

16.7. "Constitution, Bylaws, and Standards", National Environmental Laboratory Accreditation Conference, (July 1999)

17. Tables, figures, diagrams, charts, checklists, appendices: None

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Appendix F.7

Wisconsin State Lab of Hygiene

Quality Control Limits for Sheboygan AOC Pathway to Delisting Project

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
Cd	Tissue	ICP	I220ITT001DTI1	Duplicate, Absolute difference	0 - 1 ug/g	0.03	0.03 ug/g	0.10 ug/g	
Cd	Tissue	ICP	I220ITT001STI1	Spike, % Recovery	all	74 - 117	0.03 ug/g	0.10 ug/g	
Pb	Tissue	ICP	I380ITT001DTI1	Duplicate, Absolute difference	0 - 25 ug/g	0.8	0.8	2.5	
Pb	Tissue	ICP	I380ITT001STI1	Spike, % Recovery	all	75 - 125	0.8	2.5	
Hg	Tissue	AA, cold vapor	I430XTT001DTI1	Duplicate, Absolute difference	0 - 0.13	0.016	0.004 ug/g	0.013 ug/g	
Hg	Tissue	AA, cold vapor	I430XTT001DTI2	Duplicate, % Rel Diff	> 0.13	17.2	0.004 ug/g	0.013 ug/g	
Hg	Tissue	AA, cold vapor	I430XTT001STI1	Spike, % Recovery	all	75.0 - 118.8	0.004 ug/g	0.013 ug/g	
PERCENT FAT	TISSUE	Balance	O1400F1001DTI1	Duplicate, % Rel Diff	all	30	NA	NA	0.50%
Hexachlorobenzene	TISSUE	GC by capillary/ECD	O1410B2001DTI1	Duplicate, % Rel Diff	all	40	NA	NA	0.01 µg/g
Hexachlorobenzene	TISSUE	GC by capillary/ECD	O1410B2001STI1	Spike, % Recovery	all	45-120	NA	NA	0.01 µg/g
Hexachlorobenzene	Method[1]	GC by capillary/ECD	O1410B2001BMX1	Blank: result alone (µg/g)	all	≤0.01	NA	NA	0.01 µg/g
Hexachlorobenzene	Standard	GC by capillary/ECD	O1410B2001CSX1	Check: % Different	all	±25	NA	NA	
HEPTACHLOR EPOXIDE	TISSUE	GC by capillary/ECD	O1410B4001DTI1	Duplicate, % Rel Diff	all	40	NA	NA	0.05 µg/g
HEPTACHLOR EPOXIDE	TISSUE	GC by capillary/ECD	O1410B4001STI1	Spike, % Recovery	all	45-120	NA	NA	0.05 µg/g
HEPTACHLOR EPOXIDE	Method[1]	GC by capillary/ECD	O1410B4001BMX1	Blank: result alone (µg/g)	all	≤0.05	NA	NA	0.05 µg/g
HEPTACHLOR EPOXIDE	Standard	GC by capillary/ECD	O1410B4001CSX1	Check: % Different	all	±25	NA	NA	
OXYCHLORDANE	TISSUE	GC by capillary/ECD	O1410B8001DTI1	Duplicate, % Rel Diff	all	40	NA	NA	0.05 µg/g
OXYCHLORDANE	TISSUE	GC by capillary/ECD	O1410B8001STI1	Spike, % Recovery	all	45-120	NA	NA	0.05 µg/g
OXYCHLORDANE	Method[1]	GC by capillary/ECD	O1410B8001BMX1	Blank: result alone (µg/g)	all	≤0.05	NA	NA	0.05 µg/g
OXYCHLORDANE	Standard	GC by capillary/ECD	O1410B8001CSX1	Check: % Different	all	±25	NA	NA	
#4/10	TISSUE	GC by capillary/ECD	O1410D2002DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	3.4 ng/g	11 ng/g	
#4/10	Lab Matrix[2]	GC by capillary/ECD	O1410D2002SLX1	Spike, % Recovery	all	25-130	3.4 ng/g	11 ng/g	
#4/10	Method[1]	GC by capillary/ECD	O1410D2002BMX1	Blank: result alone (ng/g)	all	≤3.4	3.4 ng/g	11 ng/g	
#7/9	TISSUE	GC by capillary/ECD	O1410D2003DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#7/9	Lab Matrix[2]	GC by capillary/ECD	O1410D2003SLX1	Spike, % Recovery	all	25-130	0.60 ng/g	2.0 ng/g	
#7/9	Method[1]	GC by capillary/ECD	O1410D2003BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#6	TISSUE	GC by capillary/ECD	O1410D2004DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.3 ng/g	4.3 ng/g	
#6	Lab Matrix[2]	GC by capillary/ECD	O1410D2004SLX1	Spike, % Recovery	all	25-130	1.3 ng/g	4.3 ng/g	
#6	Method[1]	GC by capillary/ECD	O1410D2004BMX1	Blank: result alone (ng/g)	all	≤1.3	1.3 ng/g	4.3 ng/g	
#6	Standard	GC by capillary/ECD	O1410D2004CSX1	Check: % Different	all	±20	1.3 ng/g	4.3 ng/g	
#8/5	TISSUE	GC by capillary/ECD	O1410D2005DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	3.3 ng/g	11 ng/g	
#8/5	Lab Matrix[2]	GC by capillary/ECD	O1410D2005SLX1	Spike, % Recovery	all	25-130	3.3 ng/g	11 ng/g	
#8/5	Method[1]	GC by capillary/ECD	O1410D2005BMX1	Blank: result alone (ng/g)	all	≤3.3	3.3 ng/g	11 ng/g	
#19	TISSUE	GC by capillary/ECD	O1410D2006DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#19	Lab Matrix[2]	GC by capillary/ECD	O1410D2006SLX1	Spike, % Recovery	all	25-130	1.0 ng/g	3.3 ng/g	
#19	Method[1]	GC by capillary/ECD	O1410D2006BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#18	TISSUE	GC by capillary/ECD	O1410D2007DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#18	Lab Matrix[2]	GC by capillary/ECD	O1410D2007SLX1	Spike, % Recovery	all	40-130	1.0 ng/g	3.3 ng/g	
#18	Method[1]	GC by capillary/ECD	O1410D2007BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
#15/17	TISSUE	GC by capillary/ECD	O1410D2008DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.5 ng/g	5.0 ng/g	
#15/17	Lab Matrix[2]	GC by capillary/ECD	O1410D2008SLX1	Spike, % Recovery	all	40-130	1.5 ng/g	5.0 ng/g	
#15/17	Method[1]	GC by capillary/ECD	O1410D2008BMX1	Blank: result alone (ng/g)	all	≤1.5	1.5 ng/g	5.0 ng/g	
#24/27	TISSUE	GC by capillary/ECD	O1410D2009DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#24/27	Lab Matrix[2]	GC by capillary/ECD	O1410D2009SLX1	Spike, % Recovery	all	40-130	0.60 ng/g	2.0 ng/g	
#24/27	Method[1]	GC by capillary/ECD	O1410D2009BMX1	Blank: result alone (ng/g)	all	≤0.6	0.60 ng/g	2.0 ng/g	
#16/32	TISSUE	GC by capillary/ECD	O1410D2010DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.7 ng/g	5.7 ng/g	
#16/32	Lab Matrix[2]	GC by capillary/ECD	O1410D2010SLX1	Spike, % Recovery	all	40-130	1.7 ng/g	5.7 ng/g	
#16/32	Method[1]	GC by capillary/ECD	O1410D2010BMX1	Blank: result alone (ng/g)	all	≤1.7	1.7 ng/g	5.7 ng/g	
#26	TISSUE	GC by capillary/ECD	O1410D2011DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#26	Lab Matrix[2]	GC by capillary/ECD	O1410D2011SLX1	Spike, % Recovery	all	40-130	1.0 ng/g	3.3 ng/g	
#26	Method[1]	GC by capillary/ECD	O1410D2011BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#26	Standard	GC by capillary/ECD	O1410D2011CSX1	Check: % Different	all	±30	1.0 ng/g	3.3 ng/g	
#25	TISSUE	GC by capillary/ECD	O1410D2012DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#25	Lab Matrix[2]	GC by capillary/ECD	O1410D2012SLX1	Spike, % Recovery	all	40-130	1.0 ng/g	3.3 ng/g	
#25	Method[1]	GC by capillary/ECD	O1410D2012BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#28/31	TISSUE	GC by capillary/ECD	O1410D2013DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	2.0 ng/g	6.7 ng/g	
#28/31	Lab Matrix[2]	GC by capillary/ECD	O1410D2013SLX1	Spike, % Recovery	all	40-130	2.0 ng/g	6.7 ng/g	
#28/31	Method[1]	GC by capillary/ECD	O1410D2013BMX1	Blank: result alone (ng/g)	all	≤2.0	2.0 ng/g	6.7 ng/g	
#33	TISSUE	GC by capillary/ECD	O1410D2014DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#33	Lab Matrix[2]	GC by capillary/ECD	O1410D2014SLX1	Spike, % Recovery	all	40-130	1.0 ng/g	3.3 ng/g	
#33	Method[1]	GC by capillary/ECD	O1410D2014BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#53	TISSUE	GC by capillary/ECD	O1410D2015DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#53	Lab Matrix[2]	GC by capillary/ECD	O1410D2015SLX1	Spike, % Recovery	all	40-130	0.60 ng/g	2.0 ng/g	
#53	Method[1]	GC by capillary/ECD	O1410D2015BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#51	TISSUE	GC by capillary/ECD	O1410D2016DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#51	Lab Matrix[2]	GC by capillary/ECD	O1410D2016SLX1	Spike, % Recovery	all	40-130	0.60 ng/g	2.0 ng/g	
#51	Method[1]	GC by capillary/ECD	O1410D2016BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#22	TISSUE	GC by capillary/ECD	O1410D2017DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.5 ng/g	5.0 ng/g	
#22	Lab Matrix[2]	GC by capillary/ECD	O1410D2017SLX1	Spike, % Recovery	all	40-130	1.5 ng/g	5.0 ng/g	
#22	Method[1]	GC by capillary/ECD	O1410D2017BMX1	Blank: result alone (ng/g)	all	≤1.5	1.5 ng/g	5.0 ng/g	
#45	TISSUE	GC by capillary/ECD	O1410D2018DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#45	Lab Matrix[2]	GC by capillary/ECD	O1410D2018SLX1	Spike, % Recovery	all	40-130	0.80 ng/g	2.7 ng/g	
#45	Method[1]	GC by capillary/ECD	O1410D2018BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#46	TISSUE	GC by capillary/ECD	O1410D2019DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#46	Lab Matrix[2]	GC by capillary/ECD	O1410D2019SLX1	Spike, % Recovery	all	40-130	0.80 ng/g	2.7 ng/g	
#46	Method[1]	GC by capillary/ECD	O1410D2019BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#52	TISSUE	GC by capillary/ECD	O1410D2020DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.90 ng/g	3.0 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
#52	Lab Matrix[2]	GC by capillary/ECD	O1410D2020SLX1	Spike, % Recovery	all	60-130	0.90 ng/g	3.0 ng/g	
#52	Method[1]	GC by capillary/ECD	O1410D2020BMX1	Blank: result alone (ng/g)	all	≤0.90	0.90 ng/g	3.0 ng/g	
#49	TISSUE	GC by capillary/ECD	O1410D2021DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#49	Lab Matrix[2]	GC by capillary/ECD	O1410D2021SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#49	Method[1]	GC by capillary/ECD	O1410D2021BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#47/48	TISSUE	GC by capillary/ECD	O1410D2022DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.2 ng/g	4.0 ng/g	
#47/48	Lab Matrix[2]	GC by capillary/ECD	O1410D2022SLX1	Spike, % Recovery	all	60-130	1.2 ng/g	4.0 ng/g	
#47/48	Method[1]	GC by capillary/ECD	O1410D2022BMX1	Blank: result alone (ng/g)	all	≤1.2	1.2 ng/g	4.0 ng/g	
#44	TISSUE	GC by capillary/ECD	O1410D2023DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#44	Lab Matrix[2]	GC by capillary/ECD	O1410D2023SLX1	Spike, % Recovery		60-130	0.80 ng/g	2.7 ng/g	
#44	Method[1]	GC by capillary/ECD	O1410D2023BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#37/42	TISSUE	GC by capillary/ECD	O1410D2024DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.2 ng/g	4.0 ng/g	
#37/42	Lab Matrix[2]	GC by capillary/ECD	O1410D2024SLX1	Spike, % Recovery	all	60-130	1.2 ng/g	4.0 ng/g	
#37/42	Method[1]	GC by capillary/ECD	O1410D2024BMX1	Blank: result alone (ng/g)	all	≤1.2	1.2 ng/g	4.0 ng/g	
#41/71/64	TISSUE	GC by capillary/ECD	O1410D2025DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.2 ng/g	4.0 ng/g	
#41/71/64	Lab Matrix[2]	GC by capillary/ECD	O1410D2025SLX1	Spike, % Recovery	all	60-130	1.2 ng/g	4.0 ng/g	
#41/71/64	Method[1]	GC by capillary/ECD	O1410D2025BMX1	Blank: result alone (ng/g)	all	≤1.2	1.2 ng/g	4.0 ng/g	
#40	TISSUE	GC by capillary/ECD	O1410D2026DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#40	Lab Matrix[2]	GC by capillary/ECD	O1410D2026SLX1	Spike, % Recovery	all	60-130	0.80 ng/g	2.7 ng/g	
#40	Method[1]	GC by capillary/ECD	O1410D2026BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#63	TISSUE	GC by capillary/ECD	O1410D2027DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.90 ng/g	3.0 ng/g	
#63	Lab Matrix[2]	GC by capillary/ECD	O1410D2027SLX1	Spike, % Recovery	all	60-130	0.90 ng/g	3.0 ng/g	
#63	Method[1]	GC by capillary/ECD	O1410D2027BMX1	Blank: result alone (ng/g)	all	≤0.90	0.90 ng/g	3.0 ng/g	
#74	TISSUE	GC by capillary/ECD	O1410D2028DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#74	Lab Matrix[2]	GC by capillary/ECD	O1410D2028SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#74	Method[1]	GC by capillary/ECD	O1410D2028BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#70/76	TISSUE	GC by capillary/ECD	O1410D2029DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#70/76	Lab Matrix[2]	GC by capillary/ECD	O1410D2029SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#70/76	Method[1]	GC by capillary/ECD	O1410D2029BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#70/76	Standard	GC by capillary/ECD	O1410D2029CSX1	Check: % Different	all	±20	0.70 ng/g	2.3 ng/g	
#66	TISSUE	GC by capillary/ECD	O1410D2030DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.2 ng/g	4.0 ng/g	
#66	Lab Matrix[2]	GC by capillary/ECD	O1410D2030SLX1	Spike, % Recovery	all	60-130	1.2 ng/g	4.0 ng/g	
#66	Method[1]	GC by capillary/ECD	O1410D2030BMX1	Blank: result alone (ng/g)	all	≤1.2	1.2 ng/g	4.0 ng/g	
#95	TISSUE	GC by capillary/ECD	O1410D2031DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#95	Lab Matrix[2]	GC by capillary/ECD	O1410D2031SLX1	Spike, % Recovery	all	60-130	0.80 ng/g	2.7 ng/g	
#95	Method[1]	GC by capillary/ECD	O1410D2031BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#91	TISSUE	GC by capillary/ECD	O1410D2032DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.90 ng/g	3.0 ng/g	
#91	Lab Matrix[2]	GC by capillary/ECD	O1410D2032SLX1	Spike, % Recovery	all	60-130	0.90 ng/g	3.0 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
#91	Method[1]	GC by capillary/ECD	O1410D2032BMX1	Blank: result alone (ng/g)	all	≤0.90	0.90 ng/g	3.0 ng/g	
#56/60	TISSUE	GC by capillary/ECD	O1410D2033DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.90 ng/g	3.0 ng/g	
#56/60	Lab Matrix[2]	GC by capillary/ECD	O1410D2033SLX1	Spike, % Recovery	all	60-130	0.90 ng/g	3.0 ng/g	
#56/60	Method[1]	GC by capillary/ECD	O1410D2033BMX1	Blank: result alone (ng/g)	all	≤0.90	0.90 ng/g	3.0 ng/g	
#92/84	TISSUE	GC by capillary/ECD	O1410D2034DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.7 ng/g	5.7 ng/g	
#92/84	Lab Matrix[2]	GC by capillary/ECD	O1410D2034SLX1	Spike, % Recovery	all	60-130	1.7 ng/g	5.7 ng/g	
#92/84	Method[1]	GC by capillary/ECD	O1410D2034BMX1	Blank: result alone (ng/g)	all	≤1.7	1.7 ng/g	5.7 ng/g	
#89	TISSUE	GC by capillary/ECD	O1410D2035DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#89	Lab Matrix[2]	GC by capillary/ECD	O1410D2035SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#89	Method[1]	GC by capillary/ECD	O1410D2035BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#101	TISSUE	GC by capillary/ECD	O1410D2036DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#101	Lab Matrix[2]	GC by capillary/ECD	O1410D2036SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#101	Method[1]	GC by capillary/ECD	O1410D2036BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#101	Standard	GC by capillary/ECD	O1410D2036CSX1	Check: % Different	all	±20	0.70 ng/g	2.3 ng/g	
#99	TISSUE	GC by capillary/ECD	O1410D2037DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#99	Lab Matrix[2]	GC by capillary/ECD	O1410D2037SLX1	Spike, % Recovery	all	60-130	0.60 ng/g	2.0 ng/g	
#99	Method[1]	GC by capillary/ECD	O1410D2037BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#83	TISSUE	GC by capillary/ECD	O1410D2038DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#83	Lab Matrix[2]	GC by capillary/ECD	O1410D2038SLX1	Spike, % Recovery	all	60-130	0.60 ng/g	2.0 ng/g	
#83	Method[1]	GC by capillary/ECD	O1410D2038BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#97	TISSUE	GC by capillary/ECD	O1410D2039DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.50 ng/g	1.7 ng/g	
#97	Lab Matrix[2]	GC by capillary/ECD	O1410D2039SLX1	Spike, % Recovery	all	60-130	0.50 ng/g	1.7 ng/g	
#97	Method[1]	GC by capillary/ECD	O1410D2039BMX1	Blank: result alone (ng/g)	all		0.50 ng/g	1.7 ng/g	
#87	TISSUE	GC by capillary/ECD	O1410D2040DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#87	Lab Matrix[2]	GC by capillary/ECD	O1410D2040SLX1	Spike, % Recovery	all	60-130	0.70 ng/g	2.3 ng/g	
#87	Method[1]	GC by capillary/ECD	O1410D2040BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#85	TISSUE	GC by capillary/ECD	O1410D2041DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#85	Lab Matrix[2]	GC by capillary/ECD	O1410D2041SLX1	Spike, % Recovery	all	60-130	0.80 ng/g	2.7 ng/g	
#85	Method[1]	GC by capillary/ECD	O1410D2041BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#136	TISSUE	GC by capillary/ECD	O1410D2042DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	2.0 ng/g	6.7 ng/g	
#136	Lab Matrix[2]	GC by capillary/ECD	O1410D2042SLX1	Spike, % Recovery	all	60-130	2.0 ng/g	6.7 ng/g	
#136	Method[1]	GC by capillary/ECD	O1410D2042BMX1	Blank: result alone (ng/g)	all	≤2.0	2.0 ng/g	6.7 ng/g	
#77/110	TISSUE	GC by capillary/ECD	O1410D2043DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#77/110	Lab Matrix[2]	GC by capillary/ECD	O1410D2043SLX1	Spike, % Recovery	all	60-130	0.80 ng/g	2.7 ng/g	
#77/110	Method[1]	GC by capillary/ECD	O1410D2043BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#82	TISSUE	GC by capillary/ECD	O1410D2044DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#82	Lab Matrix[2]	GC by capillary/ECD	O1410D2044SLX1	Spike, % Recovery	all	60-130	0.60 ng/g	2.0 ng/g	
#82	Method[1]	GC by capillary/ECD	O1410D2044BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)									
Element	**Matrix**	**Instrument**	**Code**	**QC Type**	**Conc. Range**	**Limit**	**LOD**	**LOQ**	**Reporting Limit**
#151	TISSUE	GC by capillary/ECD	O1410D2045DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#151	Lab Matrix[2]	GC by capillary/ECD	O1410D2045SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#151	Method[1]	GC by capillary/ECD	O1410D2045BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#135/144	TISSUE	GC by capillary/ECD	O1410D2046DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#135/144	Lab Matrix[2]	GC by capillary/ECD	O1410D2046SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#135/144	Method[1]	GC by capillary/ECD	O1410D2046BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#123/149	TISSUE	GC by capillary/ECD	O1410D2047DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#123/149	Lab Matrix[2]	GC by capillary/ECD	O1410D2047SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#123/149	Method[1]	GC by capillary/ECD	O1410D2047BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#118	TISSUE	GC by capillary/ECD	O1410D2048DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#118	Lab Matrix[2]	GC by capillary/ECD	O1410D2048SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#118	Method[1]	GC by capillary/ECD	O1410D2048BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#146	TISSUE	GC by capillary/ECD	O1410D2049DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#146	Lab Matrix[2]	GC by capillary/ECD	O1410D2049SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#146	Method[1]	GC by capillary/ECD	O1410D2049BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#132/153/105	TISSUE	GC by capillary/ECD	O1410D2050DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#132/153/105	Lab Matrix[2]	GC by capillary/ECD	O1410D2050SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#132/153/105	Method[1]	GC by capillary/ECD	O1410D2050BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#141	TISSUE	GC by capillary/ECD	O1410D2051DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#141	Lab Matrix[2]	GC by capillary/ECD	O1410D2051SLX1	Spike, % Recovery	all	70-130	0.60 ng/g	2.0 ng/g	
#141	Method[1]	GC by capillary/ECD	O1410D2051BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#137/176	TISSUE	GC by capillary/ECD	O1410D2052DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#137/176	Lab Matrix[2]	GC by capillary/ECD	O1410D2052SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#137/176	Method[1]	GC by capillary/ECD	O1410D2052BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#163/138	TISSUE	GC by capillary/ECD	O1410D2053DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.1 ng/g	3.7 ng/g	
#163/138	Lab Matrix[2]	GC by capillary/ECD	O1410D2053SLX1	Spike, % Recovery	all	70-130	1.1 ng/g	3.7 ng/g	
#163/138	Method[1]	GC by capillary/ECD	O1410D2053BMX1	Blank: result alone (ng/g)	all	≤1.1	1.1 ng/g	3.7 ng/g	
#158	TISSUE	GC by capillary/ECD	O1410D2054DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	3.3 ng/g	11 ng/g	
#158	Lab Matrix[2]	GC by capillary/ECD	O1410D2054SLX1	Spike, % Recovery	all	70-130	3.3 ng/g	11 ng/g	
#158	Method[1]	GC by capillary/ECD	O1410D2054BMX1	Blank: result alone (ng/g)	all	≤3.3	3.3 ng/g	11 ng/g	
#178	TISSUE	GC by capillary/ECD	O1410D2055DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#178	Lab Matrix[2]	GC by capillary/ECD	O1410D2055SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#178	Method[1]	GC by capillary/ECD	O1410D2055BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#187/182	TISSUE	GC by capillary/ECD	O1410D2056DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#187/182	Lab Matrix[2]	GC by capillary/ECD	O1410D2056SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#187/182	Method[1]	GC by capillary/ECD	O1410D2056BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#183	TISSUE	GC by capillary/ECD	O1410D2057DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#183	Lab Matrix[2]	GC by capillary/ECD	O1410D2057SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
#183	Method[1]	GC by capillary/ECD	O1410D2057BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#128	TISSUE	GC by capillary/ECD	O1410D2058DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#128	Lab Matrix[2]	GC by capillary/ECD	O1410D2058SLX1	Spike, % Recovery	all	70-130	0.60 ng/g	2.0 ng/g	
#128	Method[1]	GC by capillary/ECD	O1410D2058BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#167	TISSUE	GC by capillary/ECD	O1410D2059DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.90 ng/g	3.0 ng/g	
#167	Lab Matrix[2]	GC by capillary/ECD	O1410D2059SLX1	Spike, % Recovery	all	70-130	0.90 ng/g	3.0 ng/g	
#167	Method[1]	GC by capillary/ECD	O1410D2059BMX1	Blank: result alone (ng/g)	all	≤0.90	0.90 ng/g	3.0 ng/g	
#185	TISSUE	GC by capillary/ECD	O1410D2060DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.50 ng/g	1.7 ng/g	
#185	Lab Matrix[2]	GC by capillary/ECD	O1410D2060SLX1	Spike, % Recovery	all	70-130	0.50 ng/g	1.7 ng/g	
#185	Method[1]	GC by capillary/ECD	O1410D2060BMX1	Blank: result alone (ng/g)	all	≤0.50	0.50 ng/g	1.7 ng/g	
#185	Standard	GC by capillary/ECD	O1410D2060CSX1	Check: % Different	all	±20	0.50 ng/g	1.7 ng/g	
#174	TISSUE	GC by capillary/ECD	O1410D2061DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#174	Lab Matrix[2]	GC by capillary/ECD	O1410D2061SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#174	Method[1]	GC by capillary/ECD	O1410D2061BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#177	TISSUE	GC by capillary/ECD	O1410D2062DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#177	Lab Matrix[2]	GC by capillary/ECD	O1410D2062SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#177	Method[1]	GC by capillary/ECD	O1410D2062BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#202/171	TISSUE	GC by capillary/ECD	O1410D2063DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.50 ng/g	1.7 ng/g	
#202/171	Lab Matrix[2]	GC by capillary/ECD	O1410D2063SLX1	Spike, % Recovery	all	70-130	0.50 ng/g	1.7 ng/g	
#202/171	Method[1]	GC by capillary/ECD	O1410D2063BMX1	Blank: result alone (ng/g)	all	≤0.50	0.50 ng/g	1.7 ng/g	
#172	TISSUE	GC by capillary/ECD	O1410D2064DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#172	Lab Matrix[2]	GC by capillary/ECD	O1410D2064SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#172	Method[1]	GC by capillary/ECD	O1410D2064BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#180	TISSUE	GC by capillary/ECD	O1410D2065DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#180	Lab Matrix[2]	GC by capillary/ECD	O1410D2065SLX1	Spike, % Recovery	all	70-130	0.80 ng/g	2.7 ng/g	
#180	Method[1]	GC by capillary/ECD	O1410D2065BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#180	Standard	GC by capillary/ECD	O1410D2065CSX1	Check: % Different	all	±20	0.80 ng/g	2.7 ng/g	
#193	TISSUE	GC by capillary/ECD	O1410D2066DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#193	Lab Matrix[2]	GC by capillary/ECD	O1410D2066SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#193	Method[1]	GC by capillary/ECD	O1410D2066BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#199	TISSUE	GC by capillary/ECD	O1410D2067DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.70 ng/g	2.3 ng/g	
#199	Lab Matrix[2]	GC by capillary/ECD	O1410D2067SLX1	Spike, % Recovery	all	70-130	0.70 ng/g	2.3 ng/g	
#199	Method[1]	GC by capillary/ECD	O1410D2067BMX1	Blank: result alone (ng/g)	all	≤0.70	0.70 ng/g	2.3 ng/g	
#199	Standard	GC by capillary/ECD	O1410D2067CSX1	Check: % Different	all	±30	0.70 ng/g	2.3 ng/g	
#170/190	TISSUE	GC by capillary/ECD	O1410D2068DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	
#170/190	Lab Matrix[2]	GC by capillary/ECD	O1410D2068SLX1	Spike, % Recovery	all	70-130	0.80 ng/g	2.7 ng/g	
#170/190	Method[1]	GC by capillary/ECD	O1410D2068BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#198	TISSUE	GC by capillary/ECD	O1410D2069DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.80 ng/g	2.7 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
#198	Lab Matrix[2]	GC by capillary/ECD	O1410D2069SLX1	Spike, % Recovery	all	70-130	0.80 ng/g	2.7 ng/g	
#198	Method[1]	GC by capillary/ECD	O1410D2069BMX1	Blank: result alone (ng/g)	all	≤0.80	0.80 ng/g	2.7 ng/g	
#201	TISSUE	GC by capillary/ECD	O1410D2070DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.0 ng/g	3.3 ng/g	
#201	Lab Matrix[2]	GC by capillary/ECD	O1410D2070SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
#201	Method[1]	GC by capillary/ECD	O1410D2070BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
#203/196	TISSUE	GC by capillary/ECD	O1410D2071DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	1.7 ng/g	5.7 ng/g	
#203/196	Lab Matrix[2]	GC by capillary/ECD	O1410D2071SLX1	Spike, % Recovery	all	70-130	1.7 ng/g	5.7 ng/g	
#203/196	Method[1]	GC by capillary/ECD	O1410D2071BMX1	Blank: result alone (ng/g)	all	≤1.7	1.7 ng/g	5.7 ng/g	
#208/195	TISSUE	GC by capillary/ECD	O1410D2072DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#208/195	Lab Matrix[2]	GC by capillary/ECD	O1410D2072SLX1	Spike, % Recovery	all	70-130	0.60 ng/g	2.0 ng/g	
#208/195	Method[1]	GC by capillary/ECD	O1410D2072BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#207	TISSUE	GC by capillary/ECD	O1410D2073DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.50 ng/g	1.7 ng/g	
#207	Lab Matrix[2]	GC by capillary/ECD	O1410D2073SLX1	Spike, % Recovery	all	70-130	0.50 ng/g	1.7 ng/g	
#207	Method[1]	GC by capillary/ECD	O1410D2073BMX1	Blank: result alone (ng/g)	all	≤0.50	0.50 ng/g	1.7 ng/g	
#194	TISSUE	GC by capillary/ECD	O1410D2074DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#194	Lab Matrix[2]	GC by capillary/ECD	O1410D2074SLX1	Spike, % Recovery	all	70-130	0.60 ng/g	2.0 ng/g	
#194	Method[1]	GC by capillary/ECD	O1410D2074BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
#206	TISSUE	GC by capillary/ECD	O1410D2075DTI1	Duplicate, % Rel Diff	>2 X LOQ	30	0.60 ng/g	2.0 ng/g	
#206	Lab Matrix[2]	GC by capillary/ECD	O1410D2075SLX1	Spike, % Recovery	all	70-130	0.60 ng/g	2.0 ng/g	
#206	Method[1]	GC by capillary/ECD	O1410D2075BMX1	Blank: result alone (ng/g)	all	≤0.60	0.60 ng/g	2.0 ng/g	
PBDE #28	TISSUE	GC by capillary/ECD	O1410D7001DTI1	Duplicate, % Rel Diff	all	30	2.0 ng/g	6.7 ng/g	
PBDE #28	Lab Matrix[2]	GC by capillary/ECD	O1410D7001SLX1	Spike, % Recovery	all	40-140	2.0 ng/g	6.7 ng/g	
PBDE #28	Method[1]	GC by capillary/ECD	O1410D7001BMX1	Blank: result alone (ng/g)	all	≤2.0	2.0 ng/g	6.7 ng/g	
PBDE #28	Standard	GC by capillary/ECD	O1410D7001CSX1	Check: % Different	all	±25	2.0 ng/g	6.7 ng/g	
PBDE #47	TISSUE	GC by capillary/ECD	O1410D7002DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #47	Lab Matrix[2]	GC by capillary/ECD	O1410D7002SLX1	Spike, % Recovery	all	60-130	1.0 ng/g	3.3 ng/g	
PBDE #47	Method[1]	GC by capillary/ECD	O1410D7002BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #47	Standard	GC by capillary/ECD	O1410D7002CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #66	TISSUE	GC by capillary/ECD	O1410D7003DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #66	Lab Matrix[2]	GC by capillary/ECD	O1410D7003SLX1	Spike, % Recovery	all	60-130	1.0 ng/g	3.3 ng/g	
PBDE #66	Method[1]	GC by capillary/ECD	O1410D7003BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #66	Standard	GC by capillary/ECD	O1410D7003CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #100	TISSUE	GC by capillary/ECD	O1410D7004DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #100	Lab Matrix[2]	GC by capillary/ECD	O1410D7004SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
PBDE #100	Method[1]	GC by capillary/ECD	O1410D7004BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #100	Standard	GC by capillary/ECD	O1410D7004CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #99	TISSUE	GC by capillary/ECD	O1410D7005DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #99	Lab Matrix[2]	GC by capillary/ECD	O1410D7005SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
PBDE #99	Method[1]	GC by capillary/ECD	O1410D7005BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #99	Standard	GC by capillary/ECD	O1410D7005CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #85	TISSUE	GC by capillary/ECD	O1410D7006DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #85	Lab Matrix[2]	GC by capillary/ECD	O1410D7006SLX1	Spike, % Recovery	all	40-140	1.0 ng/g	3.3 ng/g	
PBDE #85	Method[1]	GC by capillary/ECD	O1410D7006BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #85	Standard	GC by capillary/ECD	O1410D7006CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #154	TISSUE	GC by capillary/ECD	O1410D7007DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #154	Lab Matrix[2]	GC by capillary/ECD	O1410D7007SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
PBDE #154	Method[1]	GC by capillary/ECD	O1410D7007BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #154	Standard	GC by capillary/ECD	O1410D7007CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #153	TISSUE	GC by capillary/ECD	O1410D7008DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #153	Lab Matrix[2]	GC by capillary/ECD	O1410D7008SLX1	Spike, % Recovery	all	70-130	1.0 ng/g	3.3 ng/g	
PBDE #153	Method[1]	GC by capillary/ECD	O1410D7008BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #153	Standard	GC by capillary/ECD	O1410D7008CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
PBDE #138	TISSUE	GC by capillary/ECD	O1410D7009DTI1	Duplicate, % Rel Diff	all	30	1.0 ng/g	3.3 ng/g	
PBDE #138	Lab Matrix[2]	GC by capillary/ECD	O1410D7009SLX1	Spike, % Recovery	all	60-140	1.0 ng/g	3.3 ng/g	
PBDE #138	Method[1]	GC by capillary/ECD	O1410D7009BMX1	Blank: result alone (ng/g)	all	≤1.0	1.0 ng/g	3.3 ng/g	
PBDE #138	Standard	GC by capillary/ECD	O1410D7009CSX1	Check: % Different	all	±25	1.0 ng/g	3.3 ng/g	
DIELDRIN	TISSUE	GC by capillary/ECD	O1410E9001DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
DIELDRIN	TISSUE	GC by capillary/ECD	O1410E9001STI1	Spike, % Recovery	all	53.8-117	0.01 µg/g	0.33 µg/g	
DIELDRIN	Method[1]	GC by capillary/ECD	O1410E9001BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
DIELDRIN	Standard	GC by capillary/ECD	O1410E9001CSX1	Check: % Different	all	±30	0.01 µg/g	0.33 µg/g	
CIS-CHLORDANE	TISSUE	GC by capillary/ECD	O1410F1001DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
CIS-CHLORDANE	TISSUE	GC by capillary/ECD	O1410F1001STI1	Spike, % Recovery	all	58.2-106	0.01 µg/g	0.33 µg/g	
CIS-CHLORDANE	Method[1]	GC by capillary/ECD	O1410F1001BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
CIS-CHLORDANE	Standard	GC by capillary/ECD	O1410F1001CSX1	Check: % Different	all	±25	0.01 µg/g	0.33 µg/g	
TRANS-CHLORDANE	TISSUE	GC by capillary/ECD	O1410F1002DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
TRANS-CHLORDANE	TISSUE	GC by capillary/ECD	O1410F1002STI1	Spike, % Recovery	all	53.6-111	0.01 µg/g	0.33 µg/g	
TRANS-CHLORDANE	Method[1]	GC by capillary/ECD	O1410F1002BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
TRANS-CHLORDANE	Standard	GC by capillary/ECD	O1410F1002CSX1	Check: % Different	all	±25	0.01 µg/g	0.33 µg/g	
CIS-NONACHLOR	TISSUE	GC by capillary/ECD	O1410F1003DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
CIS-NONACHLOR	TISSUE	GC by capillary/ECD	O1410F1003STI1	Spike, % Recovery	all	49.3-122	0.01 µg/g	0.33 µg/g	
CIS-NONACHLOR	Method[1]	GC by capillary/ECD	O1410F1003BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
CIS-NONACHLOR	Standard	GC by capillary/ECD	O1410F1003CSX1	Check: % Different	all	±25	0.01 µg/g	0.33 µg/g	
TRANS-NONACHLOR	TISSUE	GC by capillary/ECD	O1410F1004DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
TRANS-NONACHLOR	TISSUE	GC by capillary/ECD	O1410F1004STI1	Spike, % Recovery	all	45.3-120	0.01 µg/g	0.33 µg/g	
TRANS-NONACHLOR	Method[1]	GC by capillary/ECD	O1410F1004BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
TRANS-NONACHLOR	Standard	GC by capillary/ECD	O1410F1004CSX1	Check: % Different	all	±25	0.01 µg/g	0.33 µg/g	
P,P'-DDE	TISSUE	GC by capillary/ECD	O1410F2001DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)

Element	Matrix	Instrument	Code	QC Type	Conc. Range	Limit	LOD	LOQ	Reporting Limit
P,P'-DDE	TISSUE	GC by capillary/ECD	O1410F2001STI1	Spike, % Recovery	all	41.3-140	0.01 µg/g	0.33 µg/g	
P,P'-DDE	Method[1]	GC by capillary/ECD	O1410F2001BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
P,P'-DDE	Standard	GC by capillary/ECD	O1410F2001CSX1	Check: % Different	all	±25	0.01 µg/g	0.33 µg/g	
P,P'-DDT	TISSUE	GC by capillary/ECD	O1410F3001DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
P,P'-DDT	TISSUE	GC by capillary/ECD	O1410F3001STI1	Spike, % Recovery	all	56-114	0.01 µg/g	0.33 µg/g	
P,P'-DDT	Method[1]	GC by capillary/ECD	O1410F3001BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
P,P'-DDT	Standard	GC by capillary/ECD	O1410F3001CSX1	Check: % Different	all	±30	0.01 µg/g	0.33 µg/g	
P,P'-DDD	TISSUE	GC by capillary/ECD	O1410F3002DTI1	Duplicate, % Rel Diff	all	40	0.01 µg/g	0.33 µg/g	
P,P'-DDD	TISSUE	GC by capillary/ECD	O1410F3002STI1	Spike, % Recovery	all	41.8-131	0.01 µg/g	0.33 µg/g	
P,P'-DDD	Method[1]	GC by capillary/ECD	O1410F3002BMX1	Blank: result alone (µg/g)	all	≤0.01	0.01 µg/g	0.33 µg/g	
P,P'-DDD	Standard	GC by capillary/ECD	O1410F3002CSX1	Check: % Different	all	±30	0.01 µg/g	0.33 µg/g	
#14 (3,5) - PCB SURROGATE SPIKE	TISSUE	GC by capillary/ECD	O1410B7001STI1	Spike, % Recovery	all	50-120	NA	NA	
#65 (2,3,5,6) - SURROGATE SPIKE	TISSUE	GC by capillary/ECD	O1410B7002STI1	Spike, % Recovery	all	50-120	NA	NA	
#166 (2,3,4,4',5,6) - SURROGATE SPIKE	TISSUE	GC by capillary/ECD	O1410B7003STI1	Spike, % Recovery	all	55-140	NA	NA	
PERFLUORO-1-OCTANESULFONATE	Lab Matrix[2]	HPLC-MS/MS	O1480A1001SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-1-OCTANESULFONATE	Tissue	HPLC-MS/MS	O1480A1001DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-1-OCTANESULFONATE	Method[1]	HPLC-MS/MS	O1480A1001BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-N-OCTANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1002SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-OCTANOIC ACID	Tissue	HPLC-MS/MS	O1480A1002DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-OCTANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1002BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-1-HEXANESULFONATE	Lab Matrix[2]	HPLC-MS/MS	O1480A1003SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-1-HEXANESULFONATE	Tissue	HPLC-MS/MS	O1480A1003DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-1-HEXANESULFONATE	Method[1]	HPLC-MS/MS	O1480A1003BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-N-BUTANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1004SLX1	spike: % Recovery	all	70-130%			2.0 ng/g
PERFLUORO-N-BUTANOIC ACID	Tissue	HPLC-MS/MS	O1480A1004DTI1	Duplicate, % Rel Diff	all	+/-30%			2.0 ng/g
PERFLUORO-N-BUTANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1004BMX1	Blank: result alone	all	0-2.0 ng/g			2.0 ng/g
PERFLUORO-N-HEXANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1005SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-HEXANOIC ACID	Tissue	HPLC-MS/MS	O1480A1005DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-HEXANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1005BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-N-NONANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1006SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-NONANOIC ACID	Tissue	HPLC-MS/MS	O1480A1006DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-NONANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1006BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-N-DECANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1007SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-DECANOIC ACID	Tissue	HPLC-MS/MS	O1480A1007DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-DECANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1007BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g
PERFLUORO-N-UNDODECANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1008SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-UNDODECANOIC ACID	Tissue	HPLC-MS/MS	O1480A1008DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-UNDODECANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1008BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g

Method[1] = Reagent blank; Lab Matrix[2] = clean tissue (example: Ocean perch)									
Element	**Matrix**	**Instrument**	**Code**	**QC Type**	**Conc. Range**	**Limit**	**LOD**	**LOQ**	**Reporting Limit**
PERFLUORO-N-DODECANOIC ACID	Lab Matrix[2]	HPLC-MS/MS	O1480A1009SLX1	spike: % Recovery	all	70-130%			0.5 ng/g
PERFLUORO-N-DODECANOIC ACID	Tissue	HPLC-MS/MS	O1480A1009DTI1	Duplicate, % Rel Diff	all	+/-30%			0.5 ng/g
PERFLUORO-N-DODECANOIC ACID	Method[1]	HPLC-MS/MS	O1480A1009BMX1	Blank: result alone	all	0-0.50 ng/g			0.5 ng/g

Appendix F.8

Wisconsin State Lab of Hygiene

Information and Data Flow for the Organic Chemistry Department

(Figure 8 from Quality Assurance Manual, Revision 8, October 2010)

Figure 8 - Information and Data Flow for the Organic Chemistry Department

1. Samples are received, data is recorded (i.e., temperature), and ID #s assigned

2. Samples are logged into the sample tracking system and delivered to ESS Organic Section.

3. Samples are logged into the sample tracking system to the new storage location

4. Samples are logged into LIMS: ID #s, acct. #s, and analytical & prep tests

5. A worksheet is generated

6. Analytical testing is performed

7. QC Limits are checked - QA worksheets are created and completed
    a. If limits are OK proceed to next step.
    b. If QC fails:
        i. Re-analyze the samples (go back to step 5)
        ii. Qualify results and proceed to next step

8. Results are transferred to LIMS database
    a. Electronically from the instrument
    b. Hand entered by the analyst

9. Senior analyst or supervisor review of data

10. Data is reviewed by Supervisor

11. Results are verified

12. Data is evaluated by a number of LIMS programs

13. Is the data acceptable?
    a. If Yes proceed to next step.
    b. If No check for errors and possible re-analysis

14. Results are released and made available to clients via telecommunications or mailed hardcopy