# SAMPLING And ANALYSIS PLAN HUDSON RIVER FISH HEALTH ASSESSMENT PHASE I: FIELD SAMPLING, NECROPSY, HISTOPATHOLOGY, DISEASE, FISH AGE (FIELD VERSION)

# HUDSON RIVER NATURAL RESOURCE DAMAGE ASSESSMENT

# HUDSON RIVER NATURAL RESOURCE TRUSTEES

STATE OF NEW YORK U.S. DEPARTMENT OF COMMERCE U.S. DEPARTMENT OF THE INTERIOR

# FINAL PUBLIC RELEASE VERSION\*

# **O**CTOBER 3, 2001

Available from: U.S. Department of Commerce National Oceanic and Atmospheric Administration Hudson River NRDA, Lead Administrative Trustee Damage Assessment Center, N/ORR31 1305 East-West Highway, Rm 10219 Silver Spring, MD 20910-3281

\*Names of individuals and affiliations have been removed for purposes of litigation







### Sampling and Analysis Plan

### Hudson River Fish Health Assessment

### Phase I: Field Sampling, Necropsy, Histopathology, Disease, Fish Age

### (Field Version)

Prepared for:

Hudson River Trustee Council

Prepared by:

redacted

with assistance from:

redacted

October 3, 2001

# Sampling and Analysis Plan Approval

NOAA Task Order Manager:
Signature:
Date:
Fish Collection Supervisor:
Signature:
Date:
Lead Pathologist:
Signature:
Date:
QA Officer:
Signature:
Date:
Field Team Coordinator:
Signature:
Date:

# **Table of Contents**

Tables Figures Acronyms an	nd Units	vi
Section 1	Introduction	1
Section 2	Sampling Schedule and Personnel Organization	
Section 3	Sampling Locations and Numbers	
Section 4	Fish Collection Procedures	
4.1	Electroshocking Collection Procedures	
4.2	Trap Net Collection Procedures	
4.3	Fish Transport to Processing Crews	11
Section 5	Field Examination and Tissue Collection Procedures	
5.1	Fish Holding	
5.2	Blood Collection and Length and Weight Measurement (Station 1)	
5.3	External Examination (Station 2)	
5.4	Internal Examination (Station 2 — cont.)	
5.5	Tissue Collection	19
5.6	Spines, Scales, and Fillet Collection (Station 3)	
5.7	Equipment and Rinsate Blank Sample Collection	
5.8	Sample Containers, Preservation, and Holding Times	
5.9	Sample Labeling Procedures	
	5.9.1 Sample labels	
	5.9.2 Sample identification code	
5.10	Chain of Custody Procedures	
Section 6	Sample Analysis	
6.1	Histopathological Analysis	
	6.1.1 Inventory	
	6.1.2 Gross trimming	34
	6.1.3 Tissue processing	34
	6.1.4 Embedding	

	6.1.5 Microtomy	35
	6.1.6 Staining	35
	6.1.7 Precheck	35
	6.1.8 Checkout	35
	6.1.9 Pathology	35
6.2	Disease Screen	. 38
6.3	Age Analysis of Scales and Spines	. 38
6.4	Data Analysis	. 38
Section 7	Quality Assurance Plan	. 39
7.1	Project Management	. 39
7.2	QA/QC Samples	41
	7.2.1 Study documentation	42
	7.2.2 Chain of custody procedures	42
	7.2.3 Personnel experience and training	43
7.3	Assessment and Oversight	. 43
7.4	Data Validation and Usability	. 44
Section 8	References	. 45
Appendices		

Α	Site Health and Safety Plan	46
B	Fish Identification	
С	Field Equipment Lists	
D	Hudson River Phase I Fish Health Assessment Field Standard	
	Operating Procedures	
Е	Site Location Maps and GPS Coordinates	117

# Tables

1	Field crew descriptions	3
2	Sample collection goals	7
3	Sample container and preservation requirements	
4	List of possible histopathological conditions in tissues	. 37
5	Field QA/QC samples	

# Figures

1	Approximate sampling locations	5
2	Generalized steps in external examination, internal examination, and sample	
	collection procedures	
3	Generalized diagram of the anatomy of spiny-rayed fish	
4	Liver dissection	
5	Project management structure	46

# Acronyms and Units

#### Acronyms

ASTM BB BGF	American Society for Testing and Materials brown bullhead blue gill fin
BHIA	brain heart infusion agar
CCO	catfish ovary
CHSE	chinook salmon embryo
CPE	cytopathic effect
CPR	cardiopulmonary resuscitation
DC	direct current
DOI	U.S. Department of the Interior
DOJ	U.S. Department of Justice
EPA	U.S. Environmental Protection Agency
EPC	epithelio-papilloma of carp
FHM	fathead minnow
GLP	Good Laboratory Practice
GPS	global positioning system
H&E	hematoxylin and eosin staining (a method of dyeing tissue samples for
	examination under a microscope)
HASP	health and safety plan
HBSS	Hank's balanced salt solution
HPLC	High Performance Liquid Chromatography
HPTs	Histopathology Prevalence Tables
HSO	Health and Safety Officer
IDLH	immediately dangerous to life and health
LC50	concentration which is lethal to 50% of a sample population
MSDSs	material safety data sheets
NIOSH	National Institute for Occupational Safety and Health
NOAA	National Oceanic and Atmospheric Administration
NRDA	natural resource damage assessment
NYSDEC	New York State Department of Environmental Conservation
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PEL	permissible exposure limit
PPE	personal protective equipment

QA	quality assurance
QAP	Quality Assurance Plan
QC	quality control
RI/FS	remedial investigation/feasibility study
SAP	sampling and analysis plan
SOP	standard operating procedure
SPT	Summary Prevalence Table
TLV	threshold limit value
TSA	tryptic soy agar
TWA	time weighted average
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
UTM	Universal Transverse Mercator
WGS 84	World Geodetic System 1984

#### Units

degrees centigrade
centimeter
gram
kilogram
liter
meter
milligram
milliliter
millimeter
microgram
nanogram
ounce
parts per million
volts

# 1. Introduction

This sampling and analysis plan (SAP) describes the field collection and health assessment of Hudson River fish scheduled for fall 2001. Phase I of the study includes the field collection, necropsy assessment, tissue collection, histopathologic examination of tissues, analysis of tissue disease status, and determination of fish age and sex. Phase II is the chemical and biochemical, analyses of tissue residues from fish collected during Phase I. The decision to implement Phase II will be made following completion of Phase I. If Phase II is deemed necessary, a Phase II analysis plan will be developed. This fish health assessment is being conducted for the Hudson River Trustee Council in support of the Hudson River Natural Resource Damage Assessment (NRDA). The Hudson River Trustee Council includes the State of New York, the National Oceanic and Atmospheric Administration (NOAA), and the U.S. Fish and Wildlife Service (USFWS).

The objectives of the Phase I study described here are to:

- Compare the prevalence of toxicopathologic lesions between fish from areas of the Hudson River downstream of polychlorinated biphenyl (PCB) releases from General Electric facilities near Glens Falls, New York (henceforth, the "assessment area") and fish from relatively less contaminated reference areas (Feeder Dam Pool and Oneida Lake).
- Compare bacterial and viral infection prevalence and severity between fish from assessment and from reference areas.
- Collect and archive tissues for Phase II biochemical analysis, chemical residue analysis, and/or chemical metabolite analysis (PAH metabolites) as deemed necessary following evaluation of the results of the Phase I fish health assessment.

This SAP is organized as follows:

- Section 2 lists personnel involved with the field collections and the sampling schedule.
- Section 3 describes sampling locations and numbers, including target fish species.
- Section 4 describes the fish collection procedures.
- Section 5 describes the fish field processing procedures, including external and internal examinations, sample collection, sampling containers, sample preservation, and chain of custody.

- Section 6 describes the Phase I laboratory analyses that will be performed on samples.
- Section 7 describes quality assurance/quality control procedures that will be used in the study.
- Section 8 lists references cited.

In addition, this SAP contains the following appendices:

- Appendix A is the Health and Safety Plan for the field sampling effort.
- Appendix B describes physical characteristics and habitat requirements for each fish species to be sampled.
- Appendix C provides lists of field equipment required for the study.
- Appendix D contains standard operating procedures (SOPs) referenced in the SAP.
- Appendix E includes maps and global positioning system (GPS) coordinates of fish collection locations.

# 2. Sampling Schedule and Personnel Organization

Sampling will be conducted in the fall of 2001. The sampling schedule is subject to change due to inclement weather, equipment problems, scheduling difficulties, or necessary modifications as directed by the Field Team Coordinator.

The field sampling team will be divided into four crews. Two crews will collect the fish and two crews will process the fish and collect tissue samples from the fish. Each of the crews will have an assigned crew chief responsible for execution of the crew's work pursuant to the SAP, as well as for the safety of the crew. Each crew chief will coordinate with the Field Team Coordinator, who has overall responsibility for the execution of the sampling. Table 1 describes the responsibilities of each crew, the number of members for each crew, and the anticipated source of the crewmembers. In addition to the crewmembers identified in Table 1, the Field Team Coordinator will provide oversight of all field activities and provide assistance to crews as necessary.

		Minimum number	
Crew	<b>Responsibility of crew</b>	of crew members	Potential crew source
Fish collection (2 crews)	Collect and tag fish and record information in field notebook	3	NYSDEC, USFWS, NOAA
Fish processing Station 1 (1 crew)	Measure length and weight, take blood sample, record information on fish processing forms	2	USGS, NYSDEC, USFWS, NOAA
Fish processing Station 2 (2 crews)	Conduct external and internal necropsy, collect organ samples, record information on fish processing forms	3	redacted
Fish processing Station 3 (1 crew)	Collect scale, spine, and fillet samples, and record information on fish processing forms	2	NYSDEC, USFWS, NOAA

#### Table 1. Field crew descriptions

The key members of the overall Phase I sampling effort identified to date are as follows:

- Field Team Coordinator: *redacted*.
- Lead pathologist and crew chief for fish processing crew #1: *redacted*.
- Fish collection supervisor and crew chief for fish collection crew #1: *redacted* (NYSDEC).

Additional information on project management structure is provided in Section 7.1.

Members of the field sampling team must read the SAP and the Health and Safety Plan (Appendix A) before conducting fieldwork. In addition, immediately prior to the start of sample collection, the field crews will engage in a "dry run" field exercise in which the entire procedures of each crew are carefully worked through and evaluated. The dry run exercise will be conducted just prior to the start of the sampling program, and will be monitored and evaluated by the Quality Assurance Officer. The dry run exercise will be evaluated and discussed by the Quality Assurance Officer, Field Team Coordinator, lead pathologist, and fish collection supervisor to determine whether any changes to the Sampling and Analysis Plan are required. If any changes are necessary, the changes will be fully documented and justified, and communicated to the entire field crew.

# 3. Sampling Locations and Numbers

Fish health sampling will be performed at two Hudson River assessment areas and two reference locations (Figure 1). The assessment areas are the Thompson Island Pool, which runs from approximately Fort Edward (river mile 195) downstream to the Thompson Island Dam (river mile 189), and the downstream half of the Stillwater Pool, which runs from approximately Lock 5 (river mile 182) downstream to the Stillwater Dam (river mile 168). NYSDEC data indicate that fish in these two areas have historically contained elevated PCB concentrations, and some species from these areas have been shown to have increased prevalence of adverse pathology (Kim et al., 1989; Bowser et al., 1990). The two reference sampling locations are the Hudson River upstream of the Feeder Dam near Fernwood (approximately at river mile 205) and in Oneida Lake near Syracuse, New York. The specific sampling areas within each of these sampling locations will be selected based on available habitat and on the professional judgment of the fish collection crews. Smallmouth bass tend to be located in rockier, less vegetated areas, while yellow perch and brown bullhead may be found in a variety of habitats. Most of the surface area of the selected Hudson River pools are likely to be covered at least once during the sampling.

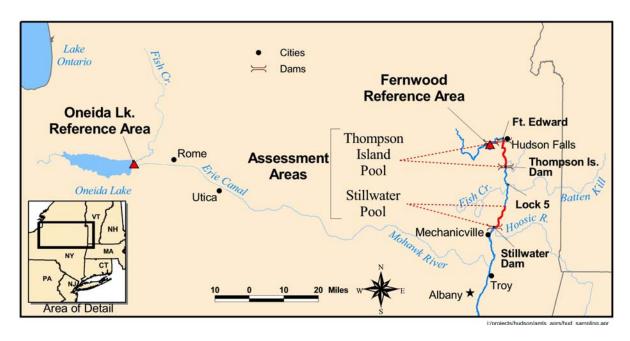


Figure 1. Approximate sampling locations.

Three fish species are targeted in this study: brown bullhead (*Ameiurus nebulosus*), smallmouth bass (*Micropterus dolomieui*), and yellow perch (*Perca flavescens*) (see Appendix B for species descriptions). These three species were selected for study for the following reasons:

- It should be possible to obtain sufficient sample sizes of these species, based on the extensive fish sampling experience of *redacted*, fish collection supervisor.
- Brown bullheads are a potential indicator of PCB-induced injuries in the Hudson River (Kim et al., 1989; Bowser et al., 1990).
- Smallmouth bass are top predators and important game fish, and they have been shown to accumulate relatively high concentrations of PCBs in their tissues in the Hudson River.
- ▶ Yellow perch are relatively abundant in the river, and may be fairly susceptible to PCBinduced injuries given their high sensitivity to dioxin (i.e., relatively low LC50 in juveniles; Monosson, 2000). In addition, yellow perch are in the same family as walleye, which have been the subject of PCB-caused injury studies at other sites (e.g., Barron et al., 2000).

Target sample numbers, fish size (length) criteria, and fish sex criteria are shown in Table 2. The target sample numbers were determined from a power analysis using data from a previous study on the prevalence of foci of alteration and tumors in walleye livers in PCB-contaminated and reference sites (Barron et al., 2000). In the Barron et al. (2000) study, abnormal livers were observed in approximately 30% of fish in the assessment areas, and in approximately 5% of fish from the reference areas. Assuming the same prevalence rates and unequal variance between sample sites, sample sizes of 50 fish from the assessment area and 30 fish from the reference area provide a greater than 90% chance of detecting any real difference between assessment areas and reference areas.

The size criteria are intended to target mature individuals, and are based on information presented in Smith (1985) and professional judgment. The sampling will target a sex ratio of 1:1 for each species at each location, and fish of a given species and sex may be rejected without processing once 30 (of 50) fish at assessment area locations, and 20 (of 30) fish at reference area locations, have been collected and processed for that given species and sex. The sample collection targets listed in Table 2 will be achieved to the extent practically and reasonably possible, with the total sample size for each location and species being the primary objective.

Sampling location	<b>Target species</b>	<b>Target length</b>	Sample size
Stillwater Pool near	Yellow perch	20 cm (~8 in.)	50
Stillwater Dam	Brown bullhead	25 cm (~10 in.)	50
(assessment area)	Smallmouth bass	30 cm (~12 in.)	50
Thompson	Yellow perch	20 cm (~8 in.)	50
Island Pool	Brown bullhead	25 cm (~10 in.)	50
(assessment area)	Smallmouth bass	30 cm (~12 in.)	50
Feeder Dam Pool	Yellow perch	20 cm (~8 in.)	30
(reference area)	Brown bullhead	25 cm (~10 in.)	30
	Smallmouth bass	30 cm (~12 in.)	30
Oneida Lake	Yellow perch	20 cm (~8 in.)	30
(reference area)	Brown bullhead	25 cm (~10 in.)	30
. ,	Smallmouth bass	30 cm (~12 in.)	30

#### Table 2. Sample collection goals

# 4. Fish Collection Procedures

Fish will be collected using electroshocking (described in Section 4.1) and trap netting (described in Section 4.2). Hook and line angling may be used to supplement collections if weather interferes with boats, or if sufficient numbers of species can not be located by electrofishing and trap netting. The fish collected that meet the sampling requirements for species and sizes will be transferred to the fish processing crews according to the procedure described in Section 4.3. Trap netting will be employed initially only at Oneida Lake.

### 4.1 Electroshocking Collection Procedures

#### Equipment

The equipment necessary for the electroshocking collection procedure is listed in Appendix C.

#### Preparation

1. Locate appropriate fish habitat (see Appendix B) within each general sampling location (see Figure 1).

#### **Collection Procedures**

- 1. The electroshocking must be conducted in accordance with the health and safety requirements described in the Health and Safety Plan (Appendix A) and in accordance with applicable collection permits of the State of New York. All members of the electroshocking crew must carefully review the Health and Safety Plan and must be given the opportunity to ask any questions regarding the health and safety requirements before electroshocking begins.
- 2. Begin shocking the selected area using a pulse DC setting and voltage appropriate for the conditions (to be determined by the fish collection supervisor).
- 3. Net any fish that may be longer than 20 cm (approximately 8 in.) in total length. Do not net smaller fish.
- 4. Identify any target species using the species descriptions in Appendix B. If the species is one of the target species for that location (see Table 2), retain and measure the total length of the fish according to SOP 1.

- 5. If the fish falls within the target length for that species (Table 2) and the target sample number for that species has not yet been collected from that sampling location (Table 2), place a Floy tag on the fish according to SOP 2. Place the tagged fish in a live well.
- 6. Transfer fish from the live well to an in-river holding pen or hatchery flow-through tank throughout the sampling day. The maximum holding time in the collection boat live well will be approximately one hour. Fish that cease opercular movement after electrofishing will be disposed of without processing.

#### Documentation

Each electroshocking collection crew will have a dedicated, bound field notebook in which all necessary information will be recorded (see Field Notebooks). The notebook will also include portions of this SAP and all SOPs that are relevant to the electroshocking collection crews.

The general information that will be recorded includes page number, the location being sampled, location number as per assembled maps, and time of the start and end of each electrofishing run, weather, habitat, sampling crew, and recorder's initials (including blank pages). Information specific to each collected fish will be recorded on shore and will include species and Floy tag number. The fate of each Floy tagged fish (used for sample collection, screened out from sample collection, died in holding, etc.) will be documented. All reviewed pages, including blanks, will be underlined and initialed.

A single member of each fish collection crew will be designated as the field recorder. Entries in the field notebooks will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the fish collection crew chief of each fish collection crew, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Field notebooks will be photocopied periodically to lessen loss of data in the event that a field notebook is lost.

### 4.2 Trap Net Collection Procedures

#### Equipment

The equipment necessary for the trap net collection procedure is listed in Appendix C.

#### Preparation

1. Locate appropriate habitat (see Appendix B) within each general sampling location (see Figure 1).

#### **Collection Procedures**

- 1. Place the trap nets on the afternoon or evening before collections are to be initiated at the sample location.
- 2. Place the nets in optimum habitat for each species to be sampled at the sample location. Securely fasten the nets in place and mark the nets so they are easily visible to boaters (including the electroshocking crews.
- 3. Every morning following net placement, the fish transport crew will check each net. Identify and remove any undesirable animal inadvertently captured by the trap net. Turtles, snakes, and large predatory fish (e.g., pike) may be encountered.
- 4. Identify any target species using the species descriptions in Appendix B. If the species is one of the target species for that location (see Table 2), measure the total length of the fish according to SOP 1.
- 5. If the fish falls within the target length for that species (Table 2) and the target sample number for that species has not yet been collected from that sampling location (Table 2 place the fish in the live well for placement and recording of Floy tags and numbers. The maximum holding time in the live well is approximately 1 hour, after which time the fish must be transferred to the in-river holding pen, hatchery flow-through tank, or 50 gallon aerated tank.

#### Documentation

The trap net placement and fish collection details will be recorded in the fish collection crew's field notebook. The notebook will also include portions of this SAP and all SOPs that are relevant to the trap net collection procedure.

The general information that will be recorded (see field notebooks) includes page numbers, the location being sampled, GPS coordinates and time of the trap net placement and re-visit, weather, habitat, sampling crew names and affiliations, and recorder's initials for all pages (including blanks). Information on the status of each net upon arrival to check for fish will also be recorded. Information specific to each collected fish will be recorded once a fish has been Floy tagged, and will include species and Floy tag number. The fate of each Floy tagged fish

(used for sample collection, screened out from sample collection, died in holding, etc.) will be documented.

A single member of the fish collection crew will be designated as the field recorder. Entries in the field notebook will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages and/or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the crew chief, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Field notebooks will be photocopied periodically to lessen loss of data in the event that a field notebook is lost.

### 4.3 Fish Transport to Processing Crews

After collection and Floy tagging, fish will be held in an in-stream holding pen in the river or hatchery flow-through tanks until processing begins. In-stream holding cages (approximately 64 cubic feet, with flow-through netting) will be placed in the ambient river water near the shore. Fish will then be transferred in small groups from the in-stream holding pen to a 50-gallon aerated tank in the fish processing area. Fish will be held in the aerated tank for no more than 4 hours, and aeration will be checked every hour. Fish in hatchery flow-through tanks will be taken for processing, as needed, without an intermediate transfer to an aerated tank. The collection crews will help coordinate timing and effort to ensure that the capabilities of the fish processing team are not overwhelmed. The fate of each fish obtained (transfer to processing crews, died in transit, etc.) will also be recorded.

# 5. Field Examination and Tissue Collection Procedures

This section describes the procedures that will be used by the fish processing crews to conduct external and internal examinations and to collect tissues for chemical, histopathological, or other analyses. This section is organized as follows:

- Section 5.1 describes the procedures for holding the fish until they are processed.
- Section 5.2 describes blood collection and length and weight measurements
- Section 5.3 describes the external examination procedures.
- Section 5.4 describes the internal examination procedures.
- Section 5.5 describes procedures for collecting tissue samples for preservation and shipment to laboratories for additional analysis.
- Section 5.6 describes procedures for collecting spines, scales, and fillet samples.
- Section 5.7 describes procedures for collecting blank samples.
- Section 5.8 describes the requirements for sample containers, preservation, and storage.
- Section 5.9 describes sample-labeling procedures.
- Section 5.10 describes sample chain of custody procedures.

The general steps in the external examination, internal examination, and tissue collection procedures are shown in Figure 2.

#### Equipment

The equipment that is required by the fish processing crews to conduct this work is listed in Appendix C.

#### Documentation

• A project management notebook will track the capture location and holding time for each fish, the tally of fish numbers by sex and species, calibration notes for balances, shipping document numbers, general notes on sites and conditions, and procedural changes.

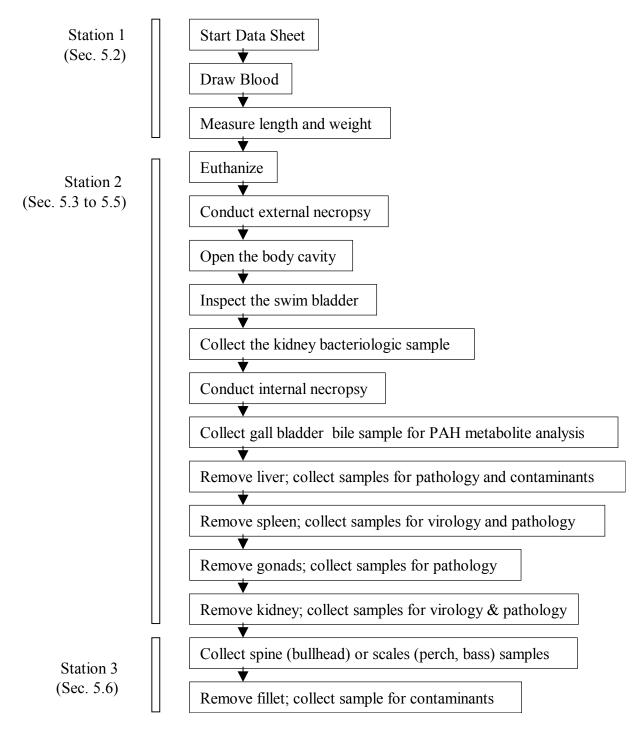


Figure 2. Generalized steps in external examination, internal examination, and sample collection procedures.

Individual Fish Data Sheets (including blank, formatted pages) will accompany each fish through the entire collection process. These data sheets will be collected into notebooks sorted by species and location.

Photographic documentation will be used to record the necropsies. Abnormal tissues designated by the pathologist will be photographed, as will several normal tissues for photographic comparison to abnormal tissues. Photographic documentation will be conducted using a digital camera, and each photograph will be taken against a solid color background sheet with a sample identification label clearly visible in the photograph. The disk will be changed daily and sequentially numbered (month, day, disk number). Disks will be archived as original data.

One member of each fish processing crew will be the designated recorder. Entries on the individual fish data sheets will be made in waterproof ink, and any necessary corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each field recorder will date, initial, and draw a line through any pages and/or entries not filled out. After the completion of each day's field activities, the notes will be reviewed for completeness and accuracy by the field recorder and the crew chief, and any necessary corrections will be made. Any corrections made to data sheets at the end of each day's collection activities will be explained in detail. Individual fish data sheets will be photocopied periodically to lessen loss of data. Copies of relevant data sheets will be provided to laboratories for further analyses (histopathology, aging, disease screen).

### 5.1 Fish Holding

#### Procedure

Fish will be held at the processing station according to the following procedure:

- 1. Place each fish from the fish collection crew into an in-stream holding cage or hatchery flow-through holding tank. Record the species, Floy tag number, and time of transfer into the cooler for each fish.
- 2. Fish will be removed from the holding pen to Station 1 as fish are needed by Station 2.
- 3. After blood has been drawn and the fish weight and length have been determined (see Section 5.2), transfer the fish to a 50 gallon aerated tank. Place fresh water in the aerated tank at least twice per day. Aeration will consist of at least two aeration stones to maintain dissolved oxygen.
- 4. Remove any dead fish from the cooler immediately, and record their Floy tag number and their death. Dispose without further processing.

5. Remove fish for processing in the approximate order that they were collected from the river by keeping group sizes small and processing the entire group before adding fish. Fish will be processed as soon as possible after receiving the fish from the fish collection crews with priority given to the more sensitive species (yellow perch and smallmouth bass). If any fish remain at the end of the processing day, remaining fish may be held overnight in the in-stream holding cage or hatchery flow-through holding tank.

Fish holding information will be documented in a separate field notebook.

# 5.2 Blood Collection and Length and Weight Measurement (Station 1)

#### Procedure

Blood samples will be collected according to the following procedures:

- 1. Put on a fresh pair of nitrile gloves.
- 2. Take a fish from the holding pen or hatchery flow-though tank and measure and record the total length (to the nearest millimeter) and weight (to the nearest gram) of the fish according to SOP 1.
- 3. Draw blood sample from the posterior caudal artery or vein according to Sections 6.1 and 7.0 of Schmitt et al. (1999).
- 4. Record the Floy tag number, species, and site location on an individual fish data sheet.
- 5. Place fish in a holding tank.

# **5.3 External Examination (Station 2)**

The results of the external examination are to be recorded using the field forms, which also serve as a guide for the examination.

#### Preparation

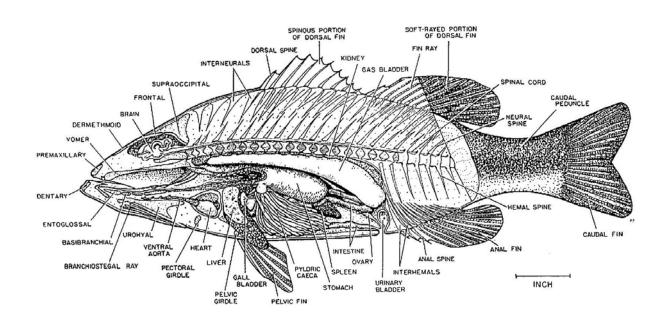
- 1. Prepare a label for each fish with the Floy tag number on it that will be easily visible in photographs of the fish, and label bottles for tissue collections
- 2. Prepare a clean surface for necropsies and tissue collections, and use clean instruments.

#### Procedure

- 1. Put on a fresh pair of nitrile gloves.
- 2. Remove the fish from the holding tank, acquire the corresponding individual fish data sheet and place the fish in a cooler of ice water to subdue the fish.
- 3. Euthanize the fish by cervical dislocation.
- 4. Conduct the external necropsy, using the field form to record the results:
  - a. *Body shape and appearance.* Record any abnormalities such as spinal curvature, swollen abdomen, protruding eyes, scars, lesions, parasites, tumors, or any other abnormalities.
  - b. *Head appearance*. Record any head abnormalities, such as tumors, lesions, scars, or parasites.
  - c. *Eye appearance*. Record fish with protruding or missing eyes, or other eye abnormalities.
  - d. *Fin clips and tags*. Record and describe fin clips and/or tags (other than the Floy tag inserted by the collection crew).
  - e. Operculum. Observe and record the condition of the operculum.
  - f. *Gills*. Record and photograph (if possible) any obvious parasitism or morphological abnormalities, including color and integrity.
  - g. Other. Record and photograph (as appropriate) any other distinct physical anomalies.
- 5. Take photographs of anomalies as determined by the lead pathologist.
- 6. Place the fish on a new sheet of aluminum foil for internal necropsy and tissue collection.

### 5.4 Internal Examination (Station 2 — cont.)

The results of the internal examination are to be recorded using the field forms, which also serve as a guide for the examination. Refer to Figure 3 for a generalized diagram of fish anatomy.



#### Figure 3. Generalized diagram of the anatomy of spiny-rayed fish.

#### Preparation

1. Decontaminate all re-useable sampling equipment according to SOP 4. Place the equipment on a new, dry Kimwipe® or other suitable clean surface.

#### First Procedure: Open the Body Cavity and Collect the Kidney Disease Screen Sample

- 1. Verify sample container labels against fish species and Floy tag number. Verify that all equipment is new (one-time use equipment) or decontaminated and sterilized (re-useable equipment).
- 2. Position the fish with the left side up, ventral side closest to the examiner.
- 3. Wipe the entire left side of the fish with a disposable towel saturated with methanol.
- 4. Using freshly decontaminated and sterilized dissection scissors and forceps, make the following cuts (using care not to puncture any internal organs):
  - a. Cut a small vertical (perpendicular to the backbone) incision approximately 1 cm anterior to the vent at the ventral axis.

- b. Cut from this vertical incision to the base of the operculum along the ventral axis.
- c. Cut from both ends of the ventral axis incision to the lateral line.
- d. Cut along the lateral line and remove the left side of the musculature of the fish to fully expose the internal organs.
- 5. Record any abnormalities on the peritoneal side of the musculature removed from the fish.
- 6. Determine the sex of the fish. If the fish is of the sex still needed, record the sex and proceed. Otherwise, discard the fish and move to the next fish.
- 7. For yellow perch and smallmouth bass, record the condition of the swim bladder before proceeding.
- 8. Collect the kidney disease screen sample as soon as possible after the incision, using the following procedure:
  - a. Using the dull side of a newly sterilized scalpel blade (or a new disposable sterilized scalpel), (as a probe), pull the swim bladder away from the kidney located just posterior, and attached, to the spine.
  - b. Observe, and record any abnormal kidney appearance or structures (lesions, tumors, hemorrhages, discoloration, etc.).
  - c. Cut a small incision in the center of the kidney with the tip of the sterile scalpel blade. Note: The center of the kidney is sampled to avoid tissues to be sampled for histopathology.
  - d. Stab the incision with a new sterile loop to collect the sample.
  - e. Streak the sample onto the labeled BHIA slant and discard the loop.
  - f. Collect a duplicate sample at a frequency of 1 in 20 samples. Use a new sterile loop (see Section 5.7 for duplicate sample labeling procedures).
  - g. Store the sample according to the procedures in Section 5.6.
  - h. Photograph any kidney lesions, as appropriate.

#### redacted

#### Second Procedure: Conduct Internal Necropsy

- 1. The internal necropsy can be performed using the forceps and dull side of the scalpel from the previous procedure.
- 2. Carefully inspect the internal surfaces of the peritoneal cavity for hemorrhages, discoloration, or other abnormalities.
- 3. Carefully inspect the gonads to determine the sex of the fish, and note any abnormalities or unique features.
- 4. Carefully inspect the liver for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.
- 5. Carefully inspect the spleen for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.
- 6. Carefully inspect the gall bladder for any abnormalities, including discoloration and relative amount of fluid.
- 7. Carefully inspect the pyloric caeca for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, hemorrhages, and relative size in relation to the stomach size.
- 8. Carefully inspect the intestine for any abnormalities, including discoloration, abnormal shape, tumors, disfiguring features, and hemorrhages.

### 5.5 Tissue Collection

Note: The procedure for collecting scales or spines for age analysis and fillets for contaminants analysis is described in Section 5.6, and the procedure for collecting a kidney disease screen sample is described in Section 5.4.

This section describes the procedures for collecting fish tissue samples for storage and shipment to laboratories for analysis. All histopathological samples will be placed in labeled cassettes. A single, labeled bottle of fixative will be used for the histopathological samples from each fish. Other tissues will be stored in the containers indicated in Table 3. The following tissues will be collected from each fish:

- gall bladder bile (analysis of PAH metabolites)
- spleen (histopathological, bacteriological, and virological analysis)

- liver (histopathological analysis and contaminants)
- gonad (histopathological analysis)
- head kidney (histopathological analysis)
- trunk kidney (histopathological and bacteriological analysis)
- fillet (contaminant analysis).

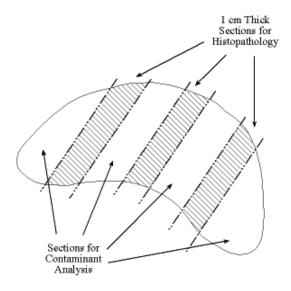
#### Preparation

- 1. Check balance calibration using a standard weight and re-calibrate if necessary.
- 2. Prepare all sample containers and labels. Sample container requirements are described in Section 5.8, and sample labeling procedures are described in Section 5.9.
- 3. Decontaminate all re-useable sampling equipment according to SOP 4. Wrap in clean aluminum foil.

#### Procedures

- 1. Collect gall bladder bile (if sufficient bile is present):
  - a. Use newly decontaminated dissecting equipment to remove the liver by cutting the connective tissue and vasculature that attaches the liver to the viscera and place the liver on a cutting board covered with clean aluminum foil prior to dissection.
  - b. Carefully separate the gall bladder from the liver and grasp the cystic duct with a hemostat. Cut the cystic duct between the hemostat and the liver taking care not to touch the liver with the scissors. Care should also be taken not to spill any bile onto the liver.
  - c. Hold the freed gall bladder over the mouth of the amber vial designated for bile collection.
  - d. With a clean scalpel blade puncture the gallbladder and allow the bile to drip down the tip of the blade into the vial.
  - e. Preserve and store the sample according to the procedures in Section 5.8.
  - f. At a frequency of 1 in 20 samples, a duplicate sample of bile will be collected for analysis. The bile collected from the designated fish will have to be divided into two amber vials that are appropriately labeled.
  - g. Decontaminate the hemostat and scissors before using again.

- 2. Continue to process the liver:
  - a. Remove any non-liver tissue from the surface of the liver and carefully inspect the liver for any lesions or abnormalities.
  - b. Place the liver in an aluminum weigh boat and weigh (to the nearest 0.01 g).
  - c. If there are no gross lesions visible, samples for histopathology will be taken from three different areas of the liver. The samples should not exceed one centimeter in thickness and they should be removed from the right side of the liver, from the center, and from the left side of the liver (see Figure 4). If possible these samples should be identified as to their origin (right, center or left) and placed in cassettes or gauze bags as appropriate to the size of the sample. These samples should be placed in a container with Dietrich's fixative for histopathology (along with other tissues from the same fish for histopathology).



#### Figure 4. Liver dissection.

- d. Place the remainder of the liver in an appropriate sample bottle for contaminant analysis.
- e. If there are gross lesions visible in the liver, sampling will have to be adapted to the site of the gross lesion or lesions. It is difficult to make firm rules for sampling in this case. As a general rule try to sample the same areas as are sampled in the liver with no gross lesions. For example, if a discrete gross lesion is present on the right side of

the liver, sample the gross lesion and then take sections from the left side and center of the liver in addition for consistency. Guidelines for sampling tissue when gross lesion(s) are present are as follows. If there is more than one lesion and the lesions are discrete, sample each lesion taking care to leave an amount of "normal" liver issue around the lesion. Estimate and record the size and location of each lesion along with other discernible features (color, texture, shape). Number the lesions in order with the suffix "EH1," "EH2," etc., after the species identifier and Floy tag number identifier of the sample identification code (see Section 5.7.2). Place each lesion in a separate cassette or gauze pouch as appropriate to size and label that container with the assigned letter. Place these cassettes or pouches in a sample bottle that contains Dietrich's fixative (along with the other tissues from the same fish for histopathology). Any liver that remains after the gross lesions and any appropriate section of "normal" liver are sampled will be placed in the appropriate sample bottle for contaminant analysis.

If the liver has a gross lesion that is not discrete and involves over 50% of the liver, describe the lesion as stated above with the location and size as well as observations of color, texture, and any other unique features. Remove the entire lesion and some adjacent "normal" liver for histopathology if this sampling can be done without compromising the amount of liver needed for contaminant analysis. With a sharp scalpel make incomplete slices into the liver lesion perpendicular to the "normal" liver at one centimeter intervals to assure adequate fixation of the tissue. Place the liver lesion in a sample bottle that contains Dietrich's fixative (along with other tissues from the same fish for histopathology). The remainder of the liver can be divided for placement in the appropriate container for contaminant analysis.

If the liver has a lesion which involves the entire liver diffusely, then sample the liver as described for a liver with no gross lesions. In this case the description of the appearance should be as detailed as possible. If the liver exhibits a variety of features, try to include as many of the different features as possible in the samples. Place each sample in a separate cassette or gauze pouch appropriately labeled as to the location of the sample (right, center, or left). If necessary, take additional samples to include all the various features of the lesion and label them appropriately. Place these specimens in a sample bottle that contains Dietrich's fixative (along with other tissues from the same fish for histopathology). The remainder of the liver can be divided for placement in the appropriate container for contaminant analysis.

- 3. Collect the spleen samples:
  - a. Use newly sterilized dissecting equipment (scalpel, razor blade, forceps), or sterile disposable equipment.

- b. Remove the spleen from the visceral mass using a scalpel and forceps.
- c. Place the spleen on a new piece of weigh paper, blot dry with a Kimwipe®, and weigh (to nearest 0.01 g).
- d. Cut the spleen into two equal sections along the longitudinal axis.
- e. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).
- f. Place one half of the spleen in the 5.5 ml snap-cap tube containing HBSS<sup>1</sup> (for virological analysis).
- g. At a frequency of 1 in 20 samples, collect a duplicate sample for virological analysis by slicing the spleen half in two and placing the two smaller sections in two separate snap-cap tubes (see Section 5.7 for duplicate sample labeling procedures).
- h. Place the other half in an appropriate sample bottle that contains Dietrich's fixative (for histopathological analysis).
- i. Store samples according to the procedures in Section 5.8.
- 4. Collect the gonad samples:
  - a. The dissecting equipment used for the previous procedure can be re-used for this procedure. Wipe the equipment clean before proceeding.
  - b. Remove the gonads from the visceral mass.
  - c. Place the gonads in an aluminum weigh boat and weigh.
  - d. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).
  - e. Place the gonads in a sample bottle with Dietrich's fixative (for histopathological analysis).
  - f. Store the sample according to the procedures in Section 5.6.

<sup>1.</sup> Procedures for preparation of HBSS and buffered formalin preservative solutions are described in SOP 5.

- 5. Collect the head and trunk kidney samples:
  - a. Use newly sterilized dissecting equipment (scalpel, razor blade, forceps), or sterile disposable equipment.
  - b. If possible, remove sufficient trunk kidney for the virology sample using a scalpel and forceps. Otherwise, use head kidney for the virology sample.
  - c. Place the kidney virology sample in the same 5.5 ml snap-cap tube containing HBSS<sup>2</sup> as the spleen virology sample (for virological analysis).
  - d. Remove any gross histopathological lesions and place in a sample bottle that contains Dietrich's fixative (for histopathological analysis).
  - e. Remove 1 cm of the anterior-most portion of the head kidney.
  - f. Place the 1 cm section of head kidney in a sample bottle with Dietrich's fixative (for histopathological analysis).
  - g. Remove 1 cm of the posterior-most portion of the trunk kidney. *Do not remove any tissue that may have been involved with the kidney disease screen sample (Section 5.3).*
  - h. Place the 1 cm section of trunk kidney in a sample bottle with Dietrich's fixative (for histopathological analysis).
  - i. Store the samples according to the procedure in Section 5.6.
  - j. Pass fish on to station 3 with the data sheet and the jar of tissues with fixative.

<sup>2.</sup> Procedures for preparation of HBSS and buffered formalin preservative solutions are described in SOP 5.

### 5.6 Spines, Scales, and Fillet Collection (Station 3)

- 1. Check Floy tag against data sheet for agreement
- 2. Remove the left pectoral fin spine from brown bullheads:
  - a. Apply gentle pressure, then twist the spine, dislocating it from the socket, and complete the operation by tearing the disarticulated spine from the skin. (Try to dislocate the spine, not break it.)
  - b. Place the spine in a properly labeled scale envelope, and store it according to the procedure in Section 5.6.
  - c. For duplicate spine samples, remove the right pectoral fin spine and place in a second scale envelope with the duplicate sample label.
- 3. Remove at least ten scales from yellow perch and smallmouth bass:
  - a. Scrape at least five scales from the skin of the fish just posterior to the longest point of the pectoral fin, below the dorsal line. Place the scales into a properly labeled scale envelope and store it according to the procedure in Section 5.6.
  - b. For duplicate scale samples, add a duplicate sample label to the scale envelope and indicate that a second reading should be taken.
- 4. Collect the fillet sample:
- 5. Use newly decontaminated equipment (scalpel, fillet knife, forceps) for this procedure.
- 6. Turn the fish over so the right side is up.
- 7. Wipe the outside of the fish with a methanol soaked disposable towel.
- 8. For brown bullheads, remove skin from the area to be filleted. For yellow perch and smallmouth bass, remove scales from the area to be filleted.
- 9. Make a cut along the ventral midline of the fish from the vent to the base of the jaw.
- 10. Make a diagonal cut from base of cranium following just behind gill to the ventral side just behind pectoral fin.

- 11. Remove the flesh and ribcage from one-half of the fish by cutting from the cranium along the spine and dorsal rays to the caudal fin. The ribs should remain on the fillet.
- 12. Place fillet in a labeled glass jar.
- 13. Store the samples according to the procedure in Section 5.8.
- 14. Place the Floy tag in the histopathology sample jar and seal the jar with parafilm.
- 15. Stockpile all used chemicals and all used tissues for disposal through NYSDEC at the Hale Creek Laboratory or other state facilities.

# 5.7 Equipment and Rinsate Blank Sample Collection

Field blank samples will be collected at a frequency of once per day or every 20 samples, whichever is more frequent. The following types of blank samples will be collected:

- disease screen equipment blank (kidney bacteriology and spleen/kidney virology)
- contaminant rinsate blank (liver and fillet)
- bottle blank (vials and jars for bile, liver, fillet).

Equipment and rinsate blank samples will be collected using material and procedures similar to those used for actual samples, and will provide information on the possibility that samples are being contaminated in the field. (See also Section 7.2 below.)

Preparation for equipment and rinsate blanks:

- 1. Prepare sample containers and labels for the equipment or rinsate blank samples. Sample container requirements are described in Section 5.8, and sample labeling procedures are described in Section 5.9.
- 2. Decontaminate all re-useable sampling equipment according to SOP 4. Place the clean equipment in clean aluminum foil.

Procedure for equipment and rinsate blanks (every 20th fish):

1. After the excision of tissues (liver and fillets) during sample processing, the instruments used in the excision (forceps, scissors, scalers, fillet knives, etc.) are to be decontaminated following the procedures outlined in SOP 4.

- 2. Collect into an appropriately labeled jar a contaminant rinsate blank by rinsing the cleaned dissecting tools that are used to collect liver or fillet samples with pesticide grade methanol. After rinsing with methanol, one Kimwipe® will be used to swab each tool. This Kimwipe® will be placed in the jar along with the methanol rinsate. This step will be repeated for each tool used during tissue excision with the Kimwipes® and rinsate for all utilized tools collected in a single field blank jar. Separate rinsate blanks will be taken for fillet excision
- 3. Several Kimwipes<sup>®</sup> that have not been used to clean any instruments will be placed in an appropriately labeled sample jar and will be submitted for analysis along with the rinsate blanks.
- 4. Collect a kidney disease screen blank sample by streaking a new sterile loop onto a BHIA slant.
- 5. Store the samples according to the procedure in Section 5.8.

In addition to equipment and rinsate blanks, one bottle from each lot of bottles used for tissue collection will also be reserved as a bottle blank and stored for possible analysis of contaminants.

### 5.8 Sample Containers, Preservation, and Holding Times

Table 3 lists the sample containers, preservative solutions, storage temperature requirements, and holding times for each type of sample being collected. All bottles for PAH metabolites and contaminant samples will be pre-cleaned and a bottle blank will be taken for each new lot of bottles used, or one per day, whichever is more frequent. All sample containers will be packaged to prevent breakage during shipment, including wrapping with bubble wrap, placement in styrofoam or plastic vial holders, placement in taped zip-lock bags, or other techniques to cushion containers and prevent their movement within shipping coolers.

### 5.9 Sample Labeling Procedures

All sample containers will be labeled with a unique numbering system that identifies the fish species, required analysis, and Floy tag number of each fish captured and sampled.

Tissue <sup>a</sup>	Analysis	Container	Preservative solution <sup>b</sup>	Field storage and shipping temperature	Holding time/storage
Blood plasma	,	Cryo-vial	None	Liquid nitrogen (-190°C)	temperature Indefinite/stored over liquid nitrogen (-190°C)
Kidney	Disease screen	BHIA slant culture tube	None	Wet ice	7 days/-20°C
Gall bladder bile	PAH metabolites	4 ml amber-colored screw cap glass vial w/Teflon-lined caps, pre-cleaned	None	Dry ice	2 years/-70°C
Spleen	Histopathology	1 to 2 L Nalgene jar <sup>c</sup>	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
	Virology	5.5 ml snap-cap bullet tube	HBSS	Wet ice	7 days/-20°C
Liver	Histopathology	1 to 2 L Nalgene jar <sup>c</sup>	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
	Contaminants	20 ml vials, with Teflon-lined caps; or larger glass jars with Teflon-lined caps		Dry ice	2 years/-70°C
Gonad	Histopathology	1 to 2 L Nalgene jar <sup>c</sup>	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Head kidney	Histopathology Virology (if insufficient trunk kidney)	1 to 2 L Nalgene jar <sup>c</sup>	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Trunk kidney	Histopathology Virology (if sufficient sample)	1 to 2 L Nalgene jar <sup>c</sup>	Dietrich's fixative	Ambient	3 mos. for embedding, indefinite thereafter/ambient
Scales/spines		Scale envelope	None	Ambient	Indefinite/ambient
Fillet	Contaminants	Glass jars with Teflon-lined caps	None	Dry ice	2 years/-70°C
a Tissues are	listed in the order of	of their collection as specified in Section		2	2

#### Table 3. Sample container and preservation requirements

a. Tissues are listed in the order of their collection, as specified in Sections 5.2 through 56.

b. Procedures for preparation of preservative solutions are described in SOP 5.

c. All histopathological samples will be placed in appropriately sized, labeled Surgipath "cassettes." One labeled bottle will be used for each fish.

### 5.9.1 Sample labels

- Sample labels will be filled out using permanent markers and affixed to the sample containers as follows: For blood plasma samples, label cryovials with the species code and Floy tag number using only cryomarkers.
- For scale envelopes (scales and spine samples), fold over, but do not seal, the scale envelope and affix the completed label to the outside of the envelope.
- For most other sample bottles, jars, and tubes, affix the sample label to the outside of the sample jar and cover it with clear packing tape.

### 5.9.2 Sample identification code

The following sample identification code will be used:

### SP- NUM- AN-C

where:

**SP** = a two-letter code designating the species collected or QC sample type:

- BB = brown bullhead
- SB = smallmouth bass
- YP = yellow perch
- QC = QC samples.

**NUM** = a unique three-digit numerical code that corresponds to the Floy tag number (or the QC sample number, starting with 001). (NUM codes for bottle blanks will begin with 901 and increase sequentially for each bottle blank sample.)

**AN** = a unique two-letter code that designates the analysis to be performed:

- Age analysis:
  - SA = scales or spines for determining the age of the fish
- Blood plasma
  - **BP** = blood plasma for endocrine biomarkers
- Disease screen:
  - **KD** = kidney disease screen sample
  - **SD** = spleen disease screen sample
- Contaminants:
  - **BR** = bile for PAH metabolites
  - **LR** = liver for residues
  - $\mathbf{FR} = \text{fillet for residues}$
  - -- **BB** = bottle blanks
  - **KB** = Kimwipe® blank
- Histopathology:
  - **SH** = spleen for pathology
  - **LH** = liver for pathology
  - **GH** = gonad for pathology
  - **HH** = head kidney for pathology
  - **TH** = trunk kidney for pathology
  - **EH** = gross lesion for pathology.

**C** = a code for additional identification of samples:

- S = single sample only
- **D** = duplicate sample (residue and biochemical analysis, KD samples)
- $\mathbf{R}$  = liver histopathological sample from the right half of the liver
- C = liver histopathological sample from the center of the liver
- $\mathbf{L} =$  liver histopathological sample from the left of the liver
- 1, 2, 3... = the gross lesion number collected from a given fish.

Compact disks with photographs from the digital cameras will also be assigned a five to six digit sample number according to the following :

#### mm-dd-D-#

- mm = one to two digits for the month
- dd = date
- $\square$  D = identifier for photo disc
- # = number for each photo disc.

### 5.10 Chain of Custody Procedures

All samples collected during this study will be maintained under strict chain of custody, which is the documentation of a sample's history from time of collection through sample analysis to final disposal. A chain of custody record will be maintained for each fish starting at the time it is labeled with a Floy tag, and for each tissue sample starting at the time of sample extraction from a fish.

The field recorder of each crew is personally responsible for the care and custody of the samples that are in that crew's possession. A sample is in custody of the field recorder if any of the following occur:

- The sample is in the individual's possession.
- The sample is within view after being in possession.
- The sample is in a locked or sealed container that prevents tampering after being in possession.
- The sample is in a designated secure area.

A chain of custody transfer occurs when the sample's custody is transferred from one crew to another (e.g., from fish collection crew to fish processing crew), or when the samples are shipped to and received by the laboratory or storage facility. Chain of custody transfers that occur in the field (e.g., from the fish collection crew to the fish processing crew) will be documented in the field notebooks of each crew. The date and time of transfer will be recorded in the field notebooks.

When the samples are packed in coolers or other containers for shipment to the laboratory or storage facility, the samples will be accompanied by completed chain of custody records. The chain of custody record will contain the following information:

- project name
- sample identification (unique for each sample)
- date and time of sample collection
- sample matrix (e.g., liver, kidney)
- analysis required for each sample
- name and signature of individual relinquishing custody
- inclusive dates and times of possession for each person
- sample shipping date and mode.

Each shipping container containing samples will be accompanied by an original chain of custody record (in a plastic sealable bag to keep it dry) and be sealed with custody seals after making a copy to keep with the air bill. Custody seals are used to detect unauthorized tampering with samples after sample collection until the time of use or analysis. Signed and dated gummed paper seals may be used for this purpose. The seals will be attached so that they must be broken to open the shipping container.

Coolers or other containers containing samples will be opened at the analytical laboratories or archiving facility only by a person authorized to receive the samples. The containers will first be inspected for integrity of the chain of custody seals or other signs of tampering. The receipt of each sample in the coolers or containers will be verified on the chain of custody forms. The signed chain of custody forms will be photocopied, and the photocopy will be mailed to the sending party. Samples will be stored in a secure area according to procedures documented for each analytical facility.

# 6. Sample Analysis

This section describes the laboratory procedures that will be used in Phase I to analyze the samples. The following types of analyses will be conducted in Phase  $I^3$ :

- histopathological examination of tissues from liver, gonads, spleen, head kidney, trunk kidney, and any gross lesions sampled in the field
- virological and disease screen, of spleen and kidney, respectively
- fish age determination of scales or spines.

Samples will also be collected for potential contaminant analyses in liver, bile, and fillet, and PAH metabolites in bile (Phase II of the study). The purpose of the Phase II analysis will be to evaluate the potential relationship between contaminant concentrations and any tissue abnormalities observed in Phase I. The decision of whether to conduct Phase II analyses, as well as the number and types of analyses if they are to be done, will be made by the Hudson River Trustee Council. If the decision is made to conduct Phase II analyses, Phase II analysis plans will be prepared at that time.

# 6.1 Histopathological Analysis

All samples will be hand delivered to *redacted* to avoid preservative leaks during commercial shipment.

*redacted* will conduct the histopathological analysis of tissue from fish species collected during the survey. Liver, gonad, spleen, head kidney, trunk kidney, and gross lesions will be evaluated in all fish that are collected and necropsied. The histology and histopathological evaluation will be conducted in accordance with the SOPs and protocols of *redacted*, as summarized below. The Quality Assurance Unit of *redacted* will review all of the paperwork for the study from the receipt of specimens to the issuing of the final pathology report to assure that all phases of the operation are conducted in accordance with Good Laboratory Practice (GLP) regulations. In addition, the specific procedures used by *redacted* will be carefully reviewed by an independent histologist before beginning the study to ensure that the specific methods are appropriate for the needs of this study.

<sup>3.</sup> Note that blood plasma samples are to be collected by the USGS concurrent with this study according to the procedures described in Schmitt et al., 1999.

### 6.1.1 Inventory

Wet tissues in fixative from individual fish shipped to *redacted* for histopathology will be inventoried upon arrival. When the inventory is complete, an internal document called the Project Sheet will be prepared to provide directive for the processing of the tissues to slides and the evaluation of the histologic sections by the pathologist. The Project Sheet will be in the form of a protocol and any additional instructions necessary to achieve the required sampling of tissues. Once the Project Sheet is prepared, the study is entered into *redacted*'s computer system such that each animal in the study is assigned a unique *redacted* accession number. An Individual Animal Work Sheet will be prepared that will follow the animal through all phases of slide preparation and slide evaluation. Also, a list of gross lesions and their respective descriptions will be prepared to ensure that these lesions are recognized at gross trimming and during microtomy.

### 6.1.2 Gross trimming

Tissues will be trimmed to a size that is appropriate for adequate processing, no greater than 4 mm in thickness and no longer than 2.5 cm.

Tissues that require decalcification, such as the trunk kidney if it is attached to the vertebral column or skin with scales, will be placed in a decalcifying solution for an appropriate length of time depending upon the size and type of specimen (e.g., skin may require less time for decalcification than the vertebral column). After decalcification the tissue will be trimmed to the appropriate size for processing.

### 6.1.3 Tissue processing

Tissues from fish of the size that will be collected in this project will be processed according to *redacted*'s Tissue Processing Program One in a VIP automatic tissue processor. In this program tissues are dehydrated through a graded series of ethyl alcohol (40 minutes in each of seven alcohol baths), cleared in Clear Rite 3 (60 minutes in each of 2 baths) and infiltrated with paraffin (60 minutes in each of three baths).

### 6.1.4 Embedding

Processed tissues will be embedded in paraffin in a plane of section appropriate to the tissue (e.g., skin would be embedded on edge in order to capture all levels from epidermis to the subcutaneous tissue).

### 6.1.5 Microtomy

The initial phase of microtomy for each tissue block is "facing" the block to remove any artifacts created on the cut surface of the tissue during gross trimming. This is accomplished by cutting through the block until the full face of the tissue is exposed. Care will be taken during this rough trimming phase not to cut through any gross lesions that have been described in the tissue being microtomed.

The final section or sections, as required, will be cut at 4-5 microns and mounted on an appropriate number of slides.

### 6.1.6 Staining

The tissues on slides will be stained with hematoxylin and eosin in an automatic stainer (Hacker linear stainer) and will be coverslipped (Hacker RCM-3660 Robot Coverslipping Machine).

### 6.1.7 Precheck

During the precheck procedure each slide will be evaluated for quality of coverslipping. In addition, the slides and blocks will be properly matched as to tissue and to number, and any discrepancies will be identified and corrected. The precheck technician will compare the slides and blocks for each animal with the information on the Individual Animal Work Sheet to be sure that none are missing.

### 6.1.8 Checkout

During the checkout procedure, slides will be examined and labeled in preparation for evaluation by the *redacted* pathologist. The quality of each slide will be assessed (tissue thickness, presence of sectioning artifacts, poor staining, etc.) and any quality recuts that are necessary will be requested. All tissues required by the Individual Animal Work Sheet will be accounted for by the checkout technician. The slides will be arranged appropriately in boxes and will be delivered to the pathologist who will evaluate them.

### 6.1.9 Pathology

The pathologist will determine the maturity of the gonadal tissue through visual observation. Rate the sexual maturity as follows:

- 1. Males
  - a. Stage 0 undeveloped: little or no spermatogenic activity in germinal epithelium; testicular tissue contained exclusively immature stages of spermatogenesis with no spermatozoa observed.
  - b. Stage 1 Early spermatogenic: mostly thin germinal epithelium with scattered spermatogenic activity; immature stages (spermatocytes to spermatids) predominate, but spermatozoa may also be observed.
  - c. Stage 2 Mid-spermatogenic: germinal epithelia are of moderate thickness; a mix of spermatocytes, spermatids, and spermatozoa are present in roughly equal proportions.
  - d. Stage 3 Late spermatogenic: thick germinal epithelium;
    - i. Stage 3A All stages observed, however, mature sperm predominate; immature spermatogenic stages are still consistently present throughout the testis.
    - ii. Stage 3B All stages observed, however, mature sperm predominate; immature spermatogenic stages are either completely absent or restricted to scattered, small nests of cells.
- 2. Females
  - a. Stage 0 undeveloped: pre-vitellogenic oocytes observed exclusively; oocyte diameter <250 µg; cytoplasm stains basophilic with H&E.
  - b. Stage 1 early development: >90% pre-vitellogenic, remaining oocytes early to mid-vitellogenic; oocytes slightly larger (up to 300 μg); late perinucleolus through cortical alveolar stages.
  - c. Stage 2 mid-development: majority of observed follicles are early and midvitellogenic; oocytes larger, 300-600 µg diameter, and containing peripheral yolk vesicles; globular and uniformly thick chorion; cytoplasm is basophilic, yolk globules eosinophillic.
  - d. Stage 3 late development: majority of developing follicles are late vitellogenic; oocyte diameter is 600-1000 μg; eosinophillic yolk globules distributed throughout the cytoplasm.
  - e. Stage 4 late development/hydrated: majority of developing follicles are late vitellogenic; follicles are much larger (>1,000 μg).

f. Stage 5 — post-ovulatory: spent follicles, remnants of the theca externa and granulose.

The pathologist will examine the tissues (liver, gonads, spleen, head kidney, trunk kidney) from each fish and will dictate any abnormalities found in the tissue(s) into a Lanier Voicewriter 210 recording device. Tapes will be archived at *redacted*. Table 4 presents a list of possible histopathological lesions that may be observed. The taped dictation of the pathologist will be transcribed by a pathology data technician into *redacted*'s computer based pathology data system. The diagnoses made for each tissue from each fish will be printed out on an Individual Animal Record. The tapes recorded by the pathologist will be listened to by a second pathology data technician, and the diagnoses will be checked against the Individual Animal Record to assure that the pathologist's data has been correctly entered into the system. After the diagnoses from all the fishes in the study have been entered into the database, Histopathology Prevalence Tables (HPTs) and Summary Prevalence Tables (SPTs) will be generated and given to the pathologist.

Tissue	Possible histopathology
Liver	Tumors, foci of cellular alteration, glycogen depletion, macrophage aggregates, leukocytes/lymphocytes, megalocytosis/karyomegaly, hemorrhaging, cellular degeneration, altered cell structure
Kidney	Lesions, tumors, hemorrhaging, cell degeneration, altered cell structure including renal corpuscles, tubular epithelial cell necrosis, atrophy, hypertrophy, hyperplasia, edema, macrophage aggregates, inflammation, developing tubules
Gonad	Lesions, tumors, intersex, altered cell structure, inflammation, reduced spermatogenic elements, abnormal spermatozoa, hemorrhaging, abnormal tissue structure, atretic eggs, abnormal yolk development
Spleen	Tumors, macrophage aggregates, abnormalities, cellular necrosis, vascular necrosis

The pathologist will review the data in the tables and will write a narrative summary, which includes the objective(s) of the study, the study design, a summary of the histology methods, results of the histopathologic evaluation of the tissues, and conclusions. The narrative summary and appropriate data tables, including HPTs and SPTs, will be submitted to the sponsor in draft form.

As part of the Quality Assurance/Quality Control procedures, a random subset (10% of all samples) of tissue samples will be examined independently by two other pathologists. The three pathologists will review and discuss the results of their examinations and reach consensus on any differences. If the initial interpretations of the three pathologists are different on more than 10% of the samples examined by all three, then a second pathologist will examine all abnormal tissues, and the two pathologists will reach consensus on any differences in results. If the initial interpretations of the three pathologists are different on 10% or fewer of the samples, the

pathologists will determine whether additional tissue review is necessary, and the type and extent of review if it is deemed necessary.

### 6.2 Disease Screen

The disease screen (bacterial screen of kidney tissue and viral screen of spleen tissue) will be conducted according to the American Fisheries Society Fish Health Section Blue Book (Thoesen, 1994) disease assessment protocols. These protocols are used by the U.S. Fish and Wildlife Service as part of the National Wild Fish Health Survey.

Specifics of the analysis for this study are outlined in SOP 7. The specific analytical procedures of the selected laboratory will be carefully reviewed and evaluated for adherence to the Quality Assurance Plan (QAP) and the Phase I study's objectives.

# 6.3 Age Analysis of Scales and Spines

Scales from all yellow perch and smallmouth bass collected in this study and spines from all brown bullhead will be analyzed to determine the age of each fish. Age analysis will follow standard methods commonly used in fishery science outlined in SOP 6. The specific analytical procedures of the selected laboratory will be carefully reviewed and evaluated for adherence to the QAP and the Phase I study's objectives.

# 6.4 Data Analysis

The data obtained in the Phase I study will be evaluated quantitatively to determine whether fish from the assessment areas within the Hudson River differ in the prevalence and/or severity of the endpoints being examined in the study compared to fish from the reference areas. The conclusions regarding whether differences exist between assessment area and reference area fish will be made based on statistical testing.

The specific statistical procedures that will be used to compare assessment area and reference area fish will depend on the characteristics of the endpoint measurements. Endpoint measurements may be categorical (histopathological scores), frequency based (e.g., presence/ absence of disease), ordinal (e.g., age), or continuous (e.g., weight), with various possible underlying data distributions. For each endpoint, the data type and distributions will be carefully examined by an experienced statistician, who will then make recommendations to the Field Team Coordinator as to the specific statistical procedures that should be used to analyze each endpoint.

# 7. Quality Assurance Plan

This study is being conducted in accordance with the Quality Assurance Plan (QAP) for the Trustee's Hudson River NRDA. As described in the QAP, four general elements of quality assurance/quality control (QA/QC) must be addressed:

- project management
- data generation and acquisition
- assessment and oversight
- data validation and usability.

This section describes the Quality Assurance Plan for the Phase I fish health study, based on these four general elements.

# 7.1 Project Management

The organization of the study team for the Phase I fish health investigation is shown in Figure 5. The study personnel are organized in such a way as to provide clear lines and areas of responsibility and to ensure good communication within the study personnel. Field personnel are first organized by field task, with a separate crew being assigned to each type of task (electroshocking and fish processing). Personnel will remain on the same crews throughout the study (to the extent possible). Each crew has a crew leader, who is responsible for all activities of the crew. All of the fish collection crews are subsequently under the direction and supervision of the fish collection supervisor (*redacted* of NYSDEC), who is responsible for all fish collection activities in the study. Similarly, the two fish processing crews are under the direction of *redacted*, who is the lead pathologist for the study.

The fish collection supervisor and the lead pathologist coordinate with the field team coordinator (*redacted*). The field team coordinator is responsible for resolving any issues raised by the fish collection supervisor or the lead pathologist and will serve as the final decision-maker when issues are not resolved by the crew chiefs, fish collection supervisor, or lead pathologist. The field team coordinator will also coordinate the project teams from each of the analytical laboratories. The field team coordinator reports to the Trustee Council representatives for the Phase I fish health study (*redacted*, U.S. FWS and *redacted*, NOAA), as well as to the Quality Assurance Officer (*redacted*). As described in the QAP, the Quality Assurance Officer will assist the field team coordinator in ensuring that the Phase I fish health study is conducted in accordance with the QAP.

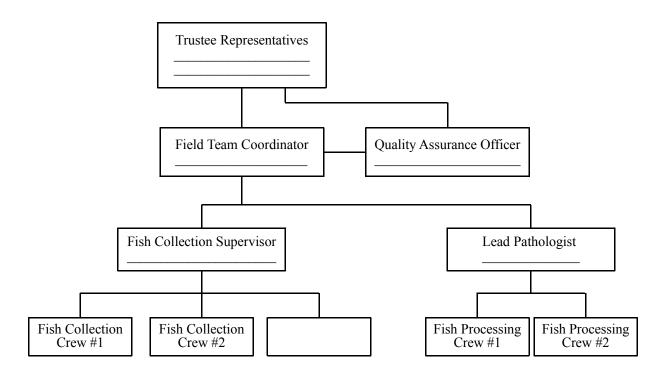


Figure 5. Project management structure.

This SAP for the Phase I health assessment of Hudson River fish was developed to provide detailed and explicit instructions for the field crews to follow in collecting the study data. The SAP has been reviewed and commented on by key parties to the study before the beginning of sample collection. Reliance on a detailed, explicit, and fully reviewed SAP ensures that:

- Study objectives, methods, procedures, and details are completely thought out before sampling.
- Data will be collected in a systematic and consistent way throughout the study.

It is the responsibility of every member of the study team to adhere to the requirements of the SAP. Each field team member is required to sign a statement that they have read the SAP and understand it. In particular, the field crew leaders must make sure that their crews adhere to the SAP.

Nevertheless, the procedures specified in the SAP must be considered somewhat flexible by the field study team. Many events can arise during field data collection that require changes to the procedures being used. In these circumstances, deviations from the SAP will be conducted only after consultation between the field crew chiefs, the fish collection supervisor or the lead pathologist, and the Field Team Coordinator. Any SAP deviations will be carefully documented, including an explanation as to why the deviations are necessary.

# 7.2 QA/QC Samples

QA/QC samples can include samples such as blanks or duplicates that can be used to assess the degree to which the sampling program and analytical measurements meet data quality requirements. QA/QC samples can include those collected in the field (e.g., blanks or duplicates) or those generated within the laboratory as part of analysis (blanks, duplicates, standards).

Table 5 lists the field QA/QC samples that will be collected. Field duplicate samples will be collected for age analysis of fish scales/spines, kidney disease screen, and the spleen virology screen. Duplicate samples will be collected at a frequency of 1 in 20 samples. Equipment blank samples will be collected for the kidney and spleen disease screen analysis at a frequency of 1 per 20 samples or 1 per day, whichever is more frequent. Bottle blanks will be collected one per lot or one per day, whichever is more frequent. One rinsate blank for contaminants analysis will be collected at the end of each sampling day. Procedures for collecting the duplicate, equipment, and rinsate blank samples are included in the specific sampling procedures in Section 5.

Type of analysis	Field duplicates	Equipment blanks	Bottle blanks	Rinsate blanks
Age (scales/spine)	One in 20 samples (two scales from same fish)	Not applicable	Not applicable	Not applicable
Blood Plasma	One in 20 samples	Not applicable	One/day	Not applicable
Disease screen (kidney)	One in 20 samples (two swipes from the same tissue)	Once/day or once/20 samples	Not applicable	Not applicable
PAH metabolites	One in 20 samples	Not applicable	One/lot	Not applicable
Histopathology	Not applicable	Not applicable	Not applicable	Not applicable
Liver contaminants Fillet contaminants		Not applicable Not applicable	One/lot One/lot	One per day One per day

#### Table 5. Field QA/QC samples

redacted

Laboratory QA/QC samples will vary depending on the type of analysis being conducted and will be specified in the laboratory analytical and/or Quality Assurance Plans. Laboratory QA/QC samples may include replicates, blanks, calibration standards, or standard reference materials.

### 7.2.1 Study documentation

All study activities will be documented in bound, waterproof, and paginated notebooks. To the extent possible, information will be recorded on pre-formatted data sheets. The use of pre-formatted data sheets is a QA/QC measure that is designed to:

- ensure that all necessary and relevant information is recorded for each sample and each sampling activity
- serve as a checklist for the field crews to help ensure completeness of the data collection effort
- assist the field crews by making data recording more efficient
- minimize the problem of illegible field notebook entries.

Each field crew will have a single field data recorder who is responsible for documenting all information in the field notebooks or on the forms. Assigning this responsibility to a single person will help ensure that documentation is complete and consistent throughout the sampling event. The field data recorder is also responsible for the care, custody, and disposition of the field notebook.

Field notebook entries will be made in waterproof ink, and corrections will be made with a single line through the error accompanied by the correction date and corrector's initials. Each completed data sheet will be reviewed, corrected (if necessary), and initialed by the field data recorder and the appropriate field crew leader. Following completion of the study, field notebook originals will be stored at the *redacted*.

### 7.2.2 Chain of custody procedures

Strict chain of custody procedures will be used throughout the study. The chain of custody procedures will begin when a sampled fish is Floy tagged, indicating that it may be used in the study. Chain of custody will continue until samples obtained from the fish are analyzed or discarded (or the fish is released before samples are obtained).

### 7.2.3 Personnel experience and training

The field sampling crews will receive explicit instructions in the execution of this SAP. The field crews will be instructed in the field before beginning any sampling, and the instructions will be repeated or refreshed during the sampling period as necessary.

Immediately prior to the start of sample collection, the field crews will engage in a "dry run" field exercise in which the entire procedures of each crew is carefully worked through and evaluated. The dry run exercise will be conducted just prior to the start of the sampling program, and will be monitored and evaluated by the Quality Assurance Officer. The dry run exercise will be evaluated and discussed by the Quality Assurance Officer, Field Team Coordinator, lead pathologist, and fish collection supervisor to determine whether any changes to the Sampling and Analysis Plan are required. If any changes are necessary, the changes will be fully documented and justified, and communicated to the entire field crew.

# 7.3 Assessment and Oversight

The QAP specifies that studies that generate data will be audited to ensure that the projectspecific plans are being properly implemented. Several mechanisms for internal audits of the data generation process will be used in the Phase I fish health assessment. These mechanisms include the following:

- A project management structure that defines clear lines of responsibility and ensures communication between field crews and with the Field Team Coordinator, fish collection supervisor, and lead pathologist. Clear responsibilities and communication can serve as a means of providing internal audits of the sample collection process as it proceeds.
- A requirement that field notebooks be reviewed daily by data recorders and field crew leaders.
- The use of pre-formatted data sheets that serve as a checklist for sampling procedures, thereby helping to ensure that sampling is complete.

The Quality Assurance Officer (*redacted*) or a delegate (*redacted*) will attend, observe, and evaluate the "dry run" field exercise that will be conducted immediately prior to the start of the study, and will provide immediate feedback to the Field Team Coordinator as to whether approaches or procedures should be modified prior to the start of sampling. The sampling will not begin until approval is received from the Quality Assurance Officer or her delegate. The Quality Assurance Officer or her delegate will remain in the field at the start of the sampling effort to ensure that the work is being conducted in accordance with Quality Assurance

requirements for the project. In addition, the Quality Assurance Officer will conduct a field audit of procedures and documentation of the study.

# 7.4 Data Validation and Usability

The SAP for the Phase I fish health assessment has been extensively reviewed for the adequacy of the sampling design and methods. The original filed notebooks will be maintained. Final reports can then be reviewed against the sampling records to ensure that the data presented in the reports represent complete and accurate information.

# 8. References

Barron, M.G., M.J. Anderson, D. Cacela, J. Lipton, S.J. Teh, D.E. Hinton, J.T. Zelikoff, A.L. Dikkeboom, D.E. Tillitt, M. Holey, and N. Denslow. 2000. PCBs, liver lesions, and biomarker responses in adult walleye (*Stizostedium vitreum vitreum*) collected from Green Bay, Wisconsin. *Journal of Great Lakes Research* 26(3):250-271.

Bowser, P.R., D. Martineau, R. Sloan, M. Brown, and C. Carusone. 1990. Prevalence of liver lesions in brown bullheads from a polluted site and a nonpolluted reference site on the Hudson River, New York. *Journal of Aquatic Animal Health* 2:177-181.

Kim, J.C.S., E.S. Chao, M.P. Brown, and R. Sloan. 1989. Pathology of brown bullhead, *Ictalurus nebulosus*, from highly contaminated and relatively clean sections of the Hudson River. *Bulletin of Environmental Contamination and Toxicology* 43:144-150.

Monosson, E. 2000. Reproductive and developmental effects of PCBs in fish: A synthesis of laboratory and field studies. *Reviews in Toxicology* 3:25-75.

Schmitt, C.J., V.S. Blazer, G.M. Dethloff, D.E. Tillitt, T.S. Gross, W.L. Bryant Jr., L.R. DeWeese, S.B. Smith, R.W. Goede, T.M. Bartish, and T.J. Kubiak. 1999. Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants. Information and Technology Report USGS/BRD-1999-0007. U.S. Geological Survey, Biological Resources Division, Columbia, MO.

Smith, C.L. 1985. *The Inland Fishes of New York State*. New York State Department of Environmental Conservation, Albany, NY.

Thoesen, J.C. (ed.). 1995. *Suggested Procedures for the Identification of Certain Finfish and Shellfish Pathogens*. 4th edition. American Fisheries Society, Fish Health Section, Bethesda, MD.

True, K. (ed.). 2000. *National Wild Fish Health Survey Laboratory Procedure Manual*. U.S. Fish and Wildlife Service. First Edition. March.

# A. Site Health and Safety Plan

# A.1 Introduction

This health and safety plan (HASP) was prepared by *redacted* to address health and safety concerns related to the field sampling activities detailed in the sampling and analysis plan. The planned field activities include the collection and tissue sampling of yellow perch, brown bullhead, and smallmouth bass from Stillwater Pool above Stillwater Dam, Thompson Island Pool, Feeder Dam, and Oneida Lake. This study is being conducted in support of the Hudson River Natural Resource Damage Assessment in the Upper Hudson River assessment area upstream from Troy, New York.

This section of the site HASP document defines general applicability and general responsibilities with respect to compliance with health and safety programs.

The purpose of this site HASP is to define the requirements and designate protocols to be followed at the site during investigation activities. Applicability extends to all on-site personnel from *redacted*, NYSDEC, USGS, NOAA, USFWS, DOI, and DOJ.

All personnel on site, contractors and subcontractors included, will be informed of the site emergency response procedures and any potential fire, explosion, health, or safety hazards of the operation. Chemical and physical hazards at the site and planned protective measures are presented in Sections A.3, A.5, and A.11 of this appendix.

This plan must be reviewed and an agreement to comply with the requirements must be signed by all personnel before working at the site.

During development of this plan, consideration was given to current safety standards as defined by EPA/OSHA/NIOSH, health effects and standards for known contaminants, and procedures designed to account for the potential for exposure to unknown substances. Specifically, the following reference sources have been consulted:

- OSHA 29 CFR 1910.120 and EPA 40 CFR 311
- U.S. EPA, OERR ERT Standard Operating Safety
- ▶ NIOSH/OSHA/USCG/EPA Occ. Health and Safety Guidelines.

### redacted

### A.2 Key Personnel

The following personnel and organizations are critical to the planned activities at the site. The organizational structure will be reviewed and updated periodically by the site supervisors.

- redacted
- redacted
- redacted
- redacted
- ▶ redacted
- redacted.

Each crew leader has responsibility for ensuring that the provisions of this HASP are adequate and implemented in the field. In addition, the field team leader has responsibility to coordinate with the crew leaders to help maintain proper safety precautions at all times. Changing field conditions may require decisions to be made concerning adequate protection programs.

## A.3 Task/Operation Safety and Health Risk Analysis

### A.3.1 Historical overview of site

Two capacitor-manufacturing facilities located at Fort Edward (river mile 196) and Hudson Falls (river mile 197) on the Upper Hudson River released large quantities of PCBs to the river between 1947 and 1977. A large fraction of the PCBs discharged before 1973 accumulated behind the Fort Edward dam, located a little over a mile downstream of the Fort Edwards facility. After the deteriorating dam was removed in 1973, subsequent spring floods carried the PCB-contaminated sediments downstream, and many of the PCBs settled in calm areas of the river described as hot spots for their high concentrations of PCBs (TAMS Consultants Inc., 1999).

For a thorough overview of historical information concerning the site, see the following documents:

- Further Site Characterization and Analysis. Data Evaluation and Interpretation Report: Hudson River PCBs Reassessment RI/FS (TAMS Consultants Inc., 1997a).
- Further Site Characterization and Analysis. Low Resolution Sediment Coring Report. Addendum to the Data Evaluation and Interpretation Report: Hudson River PCBs Reassessment RI/FS (TAMS Consultants, 1998).

 PCBs in the Upper Hudson River. Executive Summary (Quantitative Environmental Analysis Inc., 1999).

### A.3.2 Task by task risk analysis

The evaluation of hazards is based on the knowledge of site background presented in Section A.3.1 and anticipated risks posed by the specific operation. The following subsections describe each task/operation and specific associated hazards. In addition, protective measures to be implemented during the tasks/operations are also identified.

### **Planned Activities**

Planned activities at the site include:

- electroshocking and trap netting of fish at Stillwater Pool, Thompson Island Pool, Feeder Dam, and Oneida Lake
- processing fish, including measurements, visual examinations, dissection, and collection of tissue samples.

Field sampling will take place in late summer/fall 2001.

### Hazard Evaluation

Hazards associated with the field sampling activities detailed in the SAP include physical and chemical hazards.

Physical Hazards. Physical hazards associated with this task include:

- possible drowning hazards from working on and near water
- possible slip, trip, and fall hazards
- possible heat and cold stress
- possible burn hazards associated with using liquid nitrogen and dry ice
- possible hazards associated with using scalpels, scissors, forceps, and other sharp objects used to dissect and process the fish
- possible electrocution hazards associated with collecting fish using electroshocking methods

possible skin puncture and skin laceration, and associated infection, from handling fish (from fin spines or from teeth), or from handling other animals caught in trapnets (turtles, snakes, birds, and mammals), or from insect bites and stings.

U.S. Coast Guard approved life preservers will be worn by personnel while on any boat or near the water. Care will be used at all times to avoid slip, trip, and fall hazards, and the field team leader will provide for sufficient work breaks to ensure that the field team members are mentally alert and not physically fatigued.

All personnel who handle liquid nitrogen or dry ice will follow proper handling procedures, including the use of cryo-gloves.

*Chemical Hazards.* Table A.1 lists the chemical compounds of concern for field sampling and sample processing, the advisory levels associated with each compound, and sources and concentrations for the compounds. Table A.2 lists the potential chemical hazards and the first aid procedures required to treat them.

			Agency standa	ırds		
Compound		OSHA	ACGIH	NIOSH	Source or use	Conc.
Dietrich's fixative	Formaldehyde, ethanol, and glacial acetic acid	0.75 ppm (TWA <sup>a</sup> )	0.3 ppm	0.016 ppm recom. TWA <sup>a</sup> ; 0.1 ppm recom. 15 min. ceiling	Tissue preservation	100%
Methanol (methyl alcohol)		N/A	N/A	N/A	Disinfectant/ decontamination	>99% <sup>b</sup>
Polychlorinated biphenyls			$0.5 \text{ mg/m}^3 =$ 500 ng/L for chlorodiphenyl (54% Cl) skin	IDLH <sup>c</sup> not applicable, potential carcinogen	Hudson River surface water	0.02-360 ng/L in the upper Hudson River <sup>d</sup>
Dry ice (froz	zen carbon dioxide)	10,000 ppm (PEL <sup>e</sup> )	5,000 ppm (TLV <sup>f</sup> )	NA	Tissue preservation and storage	100%

# Table A.1. Chemical hazards of concern at the Hudson River assessment area, corresponding standards, sources, and concentrations

a. Time weighted average.

b. Source container concentration.

c. Immediately dangerous to life and health.

d. Source: TAMS Consultants Inc., 1997b.

e. Permissible exposure limit.

f. Threshold limit value.

Compound	Hazard	First aid procedures
Formalde- hyde	Inhalation	Remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
	Skin contact	Flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing. Get emergency medical assistance.
	Eye contact	Flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Get emergency medical assistance.
	Ingestion	If person is conscious, wash out mouth with water. Get immediate medical attention.
Ethanol	Inhalation	Remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
	Skin contact	Wash with soap and copious amounts of water.
	Eye contact	Immediately flush with copious amounts of water for at least 15 minutes.
	Ingestion	If person is conscious, wash out mouth with water. Get immediate medical attention.
Glacial acetic acid	Inhalation	Remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
	Skin contact	Flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing. Get emergency medical assistance.
	Eye contact	Flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Get emergency medical assistance.
	Ingestion	If person is conscious, wash out mouth with water. Get immediate medical attention. Do not induce vomiting.
Methanol	Inhalation	Remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.
	Skin contact	Flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing. Get emergency medical assistance.
	Eye contact	Flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Get emergency medical assistance.
	Ingestion	If person is conscious, immediately give 4 to 8 oz milk or water. Contact a doctor. Do not induce vomiting. Call local poison control for assistance.

Table A.2. Chemical hazards and first aid procedures

Compound	Hazard	First aid procedures
Dry ice	Inhalation	If person is conscious, assist to uncontaminated area and inhale fresh air. If unconscious, remove to uncontaminated area and give mouth-to-mouth resuscitation and supplemental oxygen.
	Skin contact	Remove contaminated clothing. Immediately flush for 15 minutes with soap and water. Get emergency medical assistance.
	Eye contact	Immediately flush with copious amounts of water for at least 15 minutes. Get emergency medical assistance.
	Ingestion	N/A

 Table A.2. Chemical hazards and first aid procedures (cont.)

*Electroshock Hazards.* The following safety requirements will be followed by all personnel involved in collecting fish by electroshocking:

- 1. The fish collection supervisor is responsible for the safety of all crew members, and each field collection crew leader is responsible for the safety of his or her crew.
- 2. A minimum of two properly trained people will conduct every electroshocking effort.
- 3. The crew leader of each boat and at least one additional crew member will have received training in cardiopulmonary resuscitation (CPR) and first aid.
- 4. All electroshocking personnel will be provided with instructions for the standard operating procedures for using electricity for collecting fish safely.
- 5. All electroshocking equipment will be checked before each operation to ensure that they are in working order.
- 6. No field modifications will be made to electroshocking equipment without prior written approval from the manufacturer or a qualified electrical engineer.
- 7. Only dip nets with insulated handles should be used to collect fish.
- 8. All personnel on the boat or near the water will wear flotation devices.
- 9. All personnel on the electroshocking crew will wear linemens' gloves and insulated footwear.
- 10. A labeled first aid kit and fire extinguisher will be on board the boat at all times.

- 11. Multiple (3) foot switch will control the output.
- 12. The generator will be turned off to stop the electrical current during fish transfer operations.
- 13. All electrical connections will be in watertight junctions boxes; all cables will run through electrical conduit or a heavy duty rubber-covered cord recommended for wet locations will be used.
- 14. Electroshocking will NOT be conducted during electrical storms, heavy rain or extreme weather conditions.

### A.4 Personnel Training Requirements

At a minimum all personnel are required to be trained to recognize the hazards associated with the fieldwork, the provisions of this HASP, and responsible personnel.

The topics in Table A.3 will be discussed by a qualified individual at the periodic site briefings.

Training	Frequency	
Chemical hazards (Table A.1)	Study orientation/periodic	
Physical hazards	Study orientation/periodic	
Use of netting equipment	Study orientation	
Electrofishing equipment	Study orientation/periodic	
Fish handling	Study orientation	

Table A.3. Briefing topics and frequency

### A.5 Personal Protective Equipment to Be Used

This section describes the general requirements of the EPA designated Levels of Protection (A-D), and the specific levels of protection required for each task at the site.

### A.5.1 Levels of protection

Personnel must wear personal protective equipment (PPE) when response activities involve known or suspected atmospheric contamination vapors or gases, or when particulates may be generated by site activities, or when direct contact with skin-affecting substances may occur. Full

redacted

face respirators protect lungs, gastrointestinal tract, and eyes against airborne toxicants. Chemical-resistant clothing protects the skin from contact with skin-destructive and absorbable chemicals.

The four levels of protection and their necessary components are as follows:

Level A:	Should be worn when the highest level of respiratory, skin, and eye protection is needed.
Level B:	Should be worn when the highest level of respiratory protection is needed, but a lesser level of skin protection is needed. Level B is the primary level of choice when encountering unknown environments.
Level C:	Should be worn when the criteria for using air-purifying respirators are met, and a lesser level of skin protection is needed.
Level D:	Should be worn only as a work uniform and not in any area with respiratory or skin hazards. It provides minimal protection against chemical hazards.

Modifications of these levels are permitted and routinely employed during site work activities to maximize efficiency. For example, Level C respiratory protection and Level D skin protection may be required for a given task. Likewise, the type of chemical protective ensemble (i.e., material, format) will depend on contaminants and degrees of contact.

The level of protection selected is based on the following:

- type and measured concentration of the chemical substance in the ambient atmosphere and its toxicity
- potential for exposure to substances in air liquids, or other direct contact with material due to work being done
- knowledge of chemicals on site along with properties such as toxicity, route of exposure, and contaminant matrix.

In situations where the type of chemical, concentration, and possibilities of contact are not known, the appropriate level of protection must be selected based on professional experience and judgment until the hazards can be better identified.

### A.5.2 Reassessment of protection program

The level of protection provided by PPE selection may be upgraded or downgraded based on a reassessment necessitated by a change in site conditions or findings of investigations. When a significant change occurs, the hazards should be reassessed. Some indicators of the need for reassessment are:

- beginning a new work phase, such as sampling at a new location
- change in job tasks during a work phase
- change of season/weather
- when temperature extremes or individual medical considerations limit the effectiveness of PPE
- contaminants other than those previously identified are encountered
- change in ambient levels of contaminants
- change in work scope that affects the degree of contact with contaminants.

#### A.5.3 Work mission duration

Before the workers actually begin work in their PPE ensembles, the anticipated duration of the work mission should be established. Several factors limit mission length, including:

- suit/ensemble permeation and penetration rates for chemicals
- ambient temperature and weather conditions (heat, cold stress)
- capacity of personnel to work in PPE.

### A.5.4 Specific levels of protection planned for the site

The following levels of protection will be used during activities described in this SOP:

• Level D.

This decision is based on professional judgment of the hazards associated with low PCB concentrations measured in Hudson River surface water (< 360 ng/L) (TAMS Consultants Inc., 1997b).

Level D PPE will be worn during all sampling activities in the Hudson River and Oneida Lake and will include:

- electrically resistant footwear while on boats and while in the river
- electrically-resistant gloves while electroshocking
- outer garment/coveralls work clothes, flotation device
- chemical resistant gloves during sample preparation
- cut-resistant work gloves while handling live fish with hands
- cryo-gloves for use while handling dry ice.

# A.6 Frequency and Types of Air Monitoring/Sampling

This section explains the general concepts of an air monitoring program and specifies the surveillance activities that will take place during project completion at the site.

No air monitoring is required during this field work, because all work will be conducted well offsite where concentrations are quite low. Therefore, no monitoring activities are planned.

# A.7 Site Control Measures

The following section defines measures and procedures for maintaining site control. Site control is an essential component in the implementation of the site health and safety program.

### A.7.1 Buddy system

During all activities when some conditions present a risk to personnel, the implementation of a buddy system is mandatory. A buddy system requires at least two people who work as a team, each looking out for each other. Teams may also be supplied with marine radios or cell phones so they can communicate with each other and transmit calls for assistance.

### A.7.2 Site communications plan

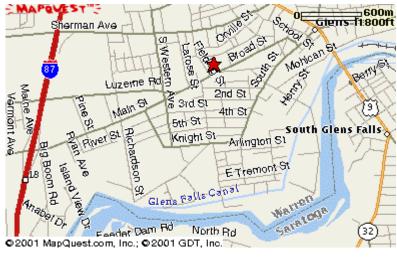
Successful communications between field teams and contact with personnel in the support zone are essential. All teams will be able to communicate using marine radios. The in-person contact communication systems will be used during site activities. Each sampling and processing group will have a cellular telephone (or equivalent) to ensure communication between groups is possible, and to allow each group to contact emergency authorities should the need arise.

### A.7.3 Work zone definition

All work will be conducted in areas of the Upper Hudson River above the Dam at Troy and at Oneida Lake near Syracuse, New York.

### A.7.4 Nearest medical assistance

Figures A.1-A.4 show the route to the nearest medical facility at each sampling location. These facilities can provide emergency care for individuals who may experience an injury or chemical exposure on site. The route to the hospitals should be verified by the Health and Safety Officer, and this route should be familiar to all field personnel.



Irongate Family Practice Assoc. 3 Irongate Ctr. # 2 Glens Falls, NY 12801 (518)793-4409

Figure A.1. Maps depicting locations of medical facilities near Feeder Dam.



Figure A.2. Maps depicting locations of medical facilities near Oneida Lake.

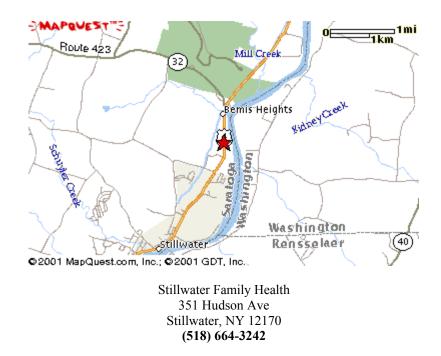


Figure A.3. Maps depicting locations of medical facilities near Stillwater.

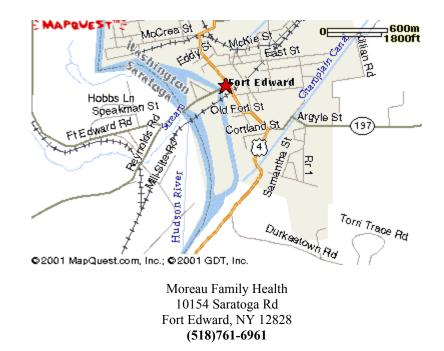


Figure A.4. Maps depicting locations of medical facilities near Thompson Island pool.

### A.8 Decontamination Plan

Decontamination involves the orderly, controlled removal of contaminants. All site personnel should minimize contact with contaminants to minimize the need for extensive decontamination.

#### A.8.1 Levels of decontamination protection required for personnel

The levels of protection required for personnel assisting with decontamination will be Level D.

The Health and Safety Officer (HSO) is responsible for monitoring decontamination procedures and determining their effectiveness.

#### A.8.2 Equipment decontamination

Sampling equipment will be decontaminated according to the procedures in SOP 4 (Appendix D).

### A.8.3 Disposition of decontamination wastes

All equipment and solvents used for decontamination will be decontaminated or disposed of properly. Commercial laundries or cleaning establishments that decontaminate clothing or equipment will be informed of the potentially harmful effects of exposures. Used PPE and other waste will be placed in a trash bag designated for this purpose. At the completion of sampling, the trash will be disposed of at a Class D, nonhazardous landfill.

### A.8.4 Level D decontamination steps

- Step 1: Remove outer garments (i.e., waders). Step 2: Remove gloves.
- Step 3: Wash hands and face.

## A.9 Emergency Response/Contingency Plan

This section describes contingencies and emergency planning procedures to be implemented at the site. This plan is compatible with local, state, and federal disaster and emergency management plans as appropriate.

### A.9.1 Pre-emergency planning

During the site briefings held periodically/daily, all employees will be trained in and reminded of provisions of the emergency response plan, communication systems, and evacuation routes. The plan will be reviewed and revised, if necessary, on a regular basis by the Health and Safety Officer. This will ensure that the plan is adequate and consistent with prevailing site conditions.

### A.9.2 Personnel roles and lines of authority

The designated Health and Safety Officers have primary responsibility for responding to and correcting emergency situations. This includes taking appropriate measure to ensure the safety of site personnel and the public. He is additionally responsible for ensuring that corrective measures have been implemented, appropriate authorities notified, and follow-up reports completed.

Health and Safety Officers: redacted (fish collection); redacted (shore operations)

### A.9.3 Emergency recognition/prevention

Section A.3.2 describes the chemical and physical hazards on site. Personnel will be familiar with techniques of hazard recognition from preassignment training and site specific briefings. The HSO is responsible for ensuring that prevention devices or equipment is available to personnel.

### A.9.4 Evacuation routes/procedures

If an emergency necessitates an evacuation of the site, the following alarm procedures will be implemented:

• The HSO will ensure that a predetermined location is identified off site in case of an emergency, so that all personnel can be accounted for.

### A.9.5 Emergency contact/notification system

Table A.4 provides names and telephone numbers for relevant emergency response/care facilities. Figures A.1 through A.4 show locations of the medical facilities near each of the sampling locations. In the event of a medical emergency, personnel will take direction from the HSO and notify the appropriate emergency organization. In the event of a fire or spill, the field team leader will notify the appropriate local, state, and federal agencies.

#### A.9.6 Emergency medical treatment procedures

All injuries and illnesses must immediately be reported to the field team leader.

Any person being transported to a clinic or hospital for treatment should take with them information on the chemical(s) they may have been exposed to near the site. This information is included in Table A.1.

Any vehicle used to transport contaminated personnel will be treated and cleaned as necessary.

#### A.9.7 Fire or explosion

In the event of a fire or explosion, the local fire department should be summoned immediately. Upon their arrival, the field team leader or designated alternate will advise the fire commander of the location, nature, and identification of the hazardous materials on site.

Organization	Telephone	
Ambulance, local police, state police, fire	911	
Emergency care at Stillwater pool sampling site		
Stillwater Family Health 351 Hudson Ave Stillwater, NY 12170	(518) 664-3242	
Emergency care at Thompson Island pool sampling site		
Moreau Family Health 10154 Saratoga Rd Fort Edward, NY 12828	(518) 761-6961	
Emergency care at Oneida Lake beach sampling site		
East Shore Medical 200 Spencer Ave Sylvan Beach, NY 13157	(315) 761-0507	
Emergency care at Feeder Dam sampling site		
Irongate Family Practice Assoc. 3 Irongate Ctr. # 2 Glens Falls, NY 12801	(518) 793-4409	
Emergency response teams/contacts		
EPA Emergency Response Team	(908) 321-6660	
National Response Center	(800) 424-8802	
Center for Disease Control	(518) 473-8389	
Chemtrec	(800) 424-9555	

#### **Table A.4. Emergency contact personnel**

If it is safe to do so, site personnel may:

- use fire fighting equipment available on site to control or extinguish the fire
- remove or isolate flammable or other hazardous materials that may contribute to the fire.

### **A.10 Confined Space Entry Procedures**

No confined space entries will be conducted as part of this field work.

# A.11 Electrical Hazards

Fish will be collected using electroshocking procedures. Each electroshocking boat will have a foot-activated safety switch that must be pressed to activate the electrical current to the electrodes in the water. If a person loses footing or falls overboard, the electrical current to the electrodes will be stopped. All personnel on board the electroshocking boat will wear electrically insulated footwear. Persons netting fish will wear electrically insulated gloves (5000 V minimum) and use nonconductive nets. All personnel on board the electroshocking boat will receive full orientation on equipment and hazards by the fish collection supervisor.

# A.12 Hazard Communication

To comply with 29 CFR 1910.1200, Hazard Communication, the following written Hazard Communication Program has been established. All employees will be briefed on this program, and have a written copy for review.

### A.12.1 Container labeling

All containers received on site will be inspected to ensure that:

- the container is clearly labeled as to the contents
- appropriate hazard warnings are noted
- hame and address of the manufacturer is listed.

All secondary containers will be labeled either with an extra copy of the original manufacturer's label or with generic labels that have a block for identification and blocks for the hazard warning.

### A.12.2 Material safety data sheets

Copies of material safety data sheets (MSDSs) for all hazardous chemicals known or suspected on site will be maintained in the work area. Copies of relevant MSDSs are attached to the end of this HASP. MSDSs will be available to all personnel for review during each work shift.

### A.12.3 Personnel training and information

Before starting work, each person will attend a health and safety orientation and will receive information and training on the following: (1) an overview of the requirements contained in the Hazard Communication Standard, 29 CFR 1910.1200, (2) chemicals present in their site

operations, (3) location and availability of a written hazard program, (4) physical and health effects of the hazardous chemicals, (5) methods and observation techniques used to determine the presence or release of hazardous chemicals, (6) how to lessen or prevent exposure to these hazardous chemicals through usage of control/work practices and personal protective equipment, (7) emergency procedures to follow if they are exposed to these chemicals, (8) how to read labels and review MSDSs to obtain appropriate hazard information, and (9) location of MSDS file and location of hazardous chemical list.

### References

ACGIH. 1993. Guide to Occupational Exposure Values.

Quantitative Environmental Analysis Inc. 1999. PCBs in the Upper Hudson River. Executive Summary. Prepared for General Electric, Albany, New York. May. Amended July 1999.

TAMS Consultants Inc. 1997a. Further Site Characterization and Analysis: Volume 2C, Book 1 of 3: Data Evaluation and Interpretation Report: Hudson River PCBs Reassessment RI/FS. Phase 2 Report — Review Copy. Prepared by TAMS Consultants, The Cadmus Group, and Gradient Corporation for U.S. EPA Region II and U.S. Army Corps of Engineers, Kansas City District. February.

TAMS Consultants Inc. 1997b. Further Site Characterization and Analysis: Volume 2C, Book 2 of 3: Data Evaluation and Interpretation Report: Hudson River PCBs Reassessment RI/FS. Phase 2 Report — Review Copy. Prepared by TAMS Consultants, The Cadmus Group, and Gradient Corporation for U.S. EPA Region II and U.S. Army Corps of Engineers, Kansas City District. February.

TAMS Consultants Inc. 1998. Further Site Characterization and Analysis: Volume 2C-A, Book 1 of 2: Low Resolution Sediment Coring Report. Addendum to the Data Evaluation and Interpretation Report: Hudson River PCBs Reassessment RI/FS. Phase 2 Report — Review Copy. Prepared by TAMS Consultants, Gradient Corporation and Tetra Tech, Inc. for U.S. EPA Region II and U.S. Army Corps of Engineers, Kansas City District. July.

TAMS Consultants Inc. 1999. Further Site Characterization and Analysis: Volume 2E. Baseline Ecological Risk Assessment: Hudson River PCBs Reassessment RI/FS. Book 1 of 3: Text. Phase 2 Report — Review Copy. Prepared by TAMS Consultants and Menzie-Cura & Associates, Inc. for U.S. EPA Region II and U. S. Army Corps of Engineers, Kansas City District. August.

## **Material Safety Data Sheets**

Riedel-de Haen 3050 Spruce St. St. Louis, MO 63178 USA Tel: 314-289-6000

MATERIAL SAFETY DATA SHEET SECTION 1. - - - - - - - - CHEMICAL IDENTIFICATION- - - - - - - -CATALOG #: 34485 NAME: METHANOL, (PESTANAL) MIN. 99.9% GC SECTION 2. - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - -CAS #: 67-56-1 MF: C-H4-O EC NO: 200-659-6 SYNONYMS ALCOOL METHYLIQUE (FRENCH) \* ALCOOL METILICO (ITALIAN) \* BIELESKI'S SOLUTION \* CARBINOL \* COLONIAL SPIRIT \* COLUMBIAN SPIRIT \* METANOLO (ITALIAN) \* METHANOL (ACGIH) \* METHYL ALCOHOL (DOT:OSHA) \* METHYLOL \* METHYLALKOHOL (GERMAN) \* METHYL HYDRATE \* METHYL HYDROXIDE \* METYLOWY ALKOHOL (POLISH) \* MONOHYDROXYMETHANE \* PYROXYLIC SPIRIT \* RCRA WASTE NUMBER U154 \* WOOD ALCOHOL \* WOOD NAPHTHA \* WOOD SPIRIT \* SECTION 3. - - - - - - - - HAZARDS IDENTIFICATION - - - - - - -LABEL PRECAUTIONARY STATEMENTS FLAMMABLE (USA) HIGHLY FLAMMABLE (EU) TOXIC TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. TOXIC: DANGER OF VERY SERIOUS IRREVERSIBLE EFFECTS THROUGH INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IRRITATING TO EYES AND SKIN. TARGET ORGAN(S): EYES KIDNEYS CAUSES EYE AND SKIN IRRITATION. KEEP CONTAINER TIGHTLY CLOSED. KEEP AWAY FROM SOURCES OF IGNITION - NO SMOKING. TAKE PRECAUTIONARY MEASURES AGAINST STATIC DISCHARGES. AVOID CONTACT WITH SKIN. WEAR SUITABLE PROTECTIVE CLOTHING AND GLOVES. IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). SECTION 4. - - - - - - - - FIRST-AID MEASURES- - - - - - - - -IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL

RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN. IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN. IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN. IF PERSON IS CONSCIOUS AFTER INGESTION OF MATERIAL, IMMEDIATELY GIVE 4 TO 8 OUNCES OF MILK OR WATER. IF SWALLOWED, DO NOT INDUCE VOMITING; CALL A PHYSICIAN IMMEDIATELY. SECTION 5. - - - - - - - FIRE FIGHTING MEASURES - - - - - - -EXTINGUISHING MEDIA WATER SPRAY. CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM. SPECIAL FIREFIGHTING PROCEDURES WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES. UNUSUAL FIRE AND EXPLOSIONS HAZARDS FLAMMABLE LIQUID. EMITS TOXIC FUMES UNDER FIRE CONDITIONS. VAPOR MAY TRAVEL CONSIDERABLE DISTANCE TO SOURCE OF IGNITION AND FLASH BACK. CONTAINER EXPLOSION MAY OCCUR UNDER FIRE CONDITIONS. SECTION 6. - - - - - - - ACCIDENTAL RELEASE MEASURES- - - - -WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES. COVER WITH DRY-LIME, SAND, OR SODA ASH. PLACE IN COVERED CONTAINERS USING NON-SPARKING TOOLS AND TRANSPORT OUTDOORS. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE AREA. SHUT OFF ALL SOURCES OF IGNITION. REFER TO SECTION 8. SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -SAFETY SHOWER AND EYE BATH. USE NONSPARKING TOOLS. USE ONLY IN A CHEMICAL FUME HOOD. WASH CONTAMINATED CLOTHING BEFORE REUSE. WASH THOROUGHLY AFTER HANDLING. NIOSH/MSHA-APPROVED RESPIRATOR. COMPATIBLE CHEMICAL-RESISTANT GLOVES. CHEMICAL SAFETY GOGGLES. KEEP CONTAINER CLOSED. KEEP AWAY FROM HEAT, SPARKS, AND OPEN FLAME. STORE IN A COOL DRY PLACE. DO NOT BREATHE VAPOR. AVOID CONTACT WITH EYES, SKIN AND CLOTHING. AVOID PROLONGED OR REPEATED EXPOSURE. DO NOT USE IF SKIN IS CUT OR SCRATCHED. WASH THOROUGHLY AFTER HANDLING.

SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - -APPEARANCE AND ODOR LIQUID. PHYSICAL PROPERTIES BOILING POINT: 64 **-** 65 C -98 C MELTING POINT: 52F FLASHPOINT 11.11C EXPLOSION LIMITS IN AIR: 36.00 % UPPER LOWER 7.3 % VAPOR PRESSURE: 97.68 MMHG @ 20 C SOLUBILITY: WATER -Z1079 SPECIFIC GRAVITY: 0.791 EVAPORATION RATE: 5.2 1.1 G/L VAPOR DENSITY: FREEZING POINT: -98 C SWISS POISON CLASS: 3 SECTION 10. - - - - - - - - STABILITY AND REACTIVITY - - - - -STABILITY STABLE. INCOMPATIBILITIES ACIDS ACID CHLORIDES ACID ANHYDRIDES OXIDIZING AGENTS ALKALI METALS REDUCING AGENTS HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS CARBON MONOXIDE, CARBON DIOXIDE HAZARDOUS POLYMERIZATION WILL NOT OCCUR. SECTION 11. - - - - - - - TOXICOLOGICAL INFORMATION - - - - - -ACUTE EFFECTS CAUSES SKIN IRRITATION. TOXIC IF ABSORBED THROUGH SKIN. CAUSES EYE IRRITATION. TOXIC IF INHALED. MATERIAL MAY BE IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT. TOXIC IF SWALLOWED. EXPOSURE CAN CAUSE: GASTROINTESTINAL DISTURBANCES MAY CAUSE CONVULSIONS. TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. LD LC CHRONIC EFFECTS

TARGET ORGAN(S): CENTRAL NERVOUS SYSTEM		
RTECS #: PC1400000		
METHANOL		
IRRITATION DATA SKN-RBT 20 MG/24H MOD		107 1000
		-,187,1986
EYE-RBT 40 MG MOD		3/24/1970
EYE-RBT 100 MG/24H MOD	85JCAE	-,187,1986
TOXICITY DATA		100 14 1000
ORL-MAN LDLO:6422 MG/KG		128,14,1983
ORL-HMN LDLO:428 MG/KG		1,74,1974
ORL-HMN LDLO:143 MG/KG		-,382,1969
UNR-MAN LDLO:868 MG/KG		2,73,1970
ORL-RAT LD50:5628 MG/KG		19(11),27,1975
IHL-RAT LC50:64000 PPM/4H		1,74,1974
IPR-RAT LD50:7529 MG/KG		61,321,1985
IVN-RAT LD50:2131 MG/KG		61,321,1985
ORL-MUS LD50:7300 MG/KG		25,271,1982
IPR-MUS LD50:10765 MG/KG		61,321,1985
SCU-MUS LD50:9800 MG/KG		18,185,1971
IVN-MUS LD50:4710 MG/KG		61,321,1985
ORL-MKY LD50:7 GM/KG		3,202,1961
ORL-RBT LD50:14200 MG/KG		48A,105,1970
SKN-RBT LD50:15800 MG/KG		1,74,1974
IPR-RBT LD50:1826 MG/KG		61,321,1985
IVN-RBT LD50:8907 MG/KG		61,321,1985
IPR-GPG LD50:3556 MG/KG		61,321,1985
IPR-HAM LD50:8555 MG/KG	EVHPAZ	61,321,1985
TARGET ORGAN DATA		
SENSE ORGANS AND SPECIAL SENSES (OPTIC NERVE NEUROPATHY)		
SENSE ORGANS AND SPECIAL SENSES (VISUAL FIELD CHANGES)		
BEHAVIORAL (HEADACHE)		
LUNGS, THORAX OR RESPIRATION (DYSPNAE)		
LUNGS, THORAX OR RESPIRATION (OTHER CHANGES)		
GASTROINTESTINAL (NAUSEA OR VOMITING)		
SPECIFIC DEVELOPMENTAL ABNORMALITIES (CENTRAL NERVOUS SYSTEM)		
SPECIFIC DEVELOPMENTAL ABNORMALITIES (MUSCULOSKELETAL SYSTEM)		
ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES		
(RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR		
COMPLETE INFORMATION.		
SECTION 12 ECOLOGICAL INFORMATION		
DATA NOT YET AVAILABLE.		
SECTION 13 DISPOSAL CONSIDERATIONS		
CONTACT A LICENSED PROFESSIONAL WASTE DISPOSAL SERVICE TO DISPOSE OF		
THIS MATERIAL.		
BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND		
SCRUBBER BUT EXERT EXTRA CARE IN IGNITING AS THIS MATERIAL IS HIGHLY		
FLAMMABLE.		
OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.		
SECTION 14 TRANSPORT INFORMATION		

CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION. SECTION 15. - - - - - - REGULATORY INFORMATION - - - - - - - -EUROPEAN INFORMATION EC INDEX NO: 603-001-00-X HIGHLY FLAMMABLE TOXIC R 11 HIGHLY FLAMMABLE. R 23/24/25 TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. R 39/23/24/25 TOXIC: DANGER OF VERY SERIOUS IRREVERSIBLE EFFECTS THROUGH INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. S 45 IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). REVIEWS, STANDARDS, AND REGULATIONS OEL=MAK ACGIH TLV-STEL 250 PPM (SKIN) DTLVS\* TLV/BEI,1999 ACGIH TLV-TWA 200 PPM (SKIN) DTLVS\* TLV/BEI,1999 EPA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION FEREAC 54,7740,1989 MSHA STANDARD-AIR: TWA 200 PPM (260 MG/M3) (SKIN) DTLVS\* 3,155,1971 OSHA PEL (GEN INDU):8H TWA 200 PPM (260 MG/M3) CFRGBR 29,1910.1000,1994 OSHA PEL (CONSTRUC):8H TWA 200 PPM (260 MG/M3) CFRGBR 29,1926.55,1994 OSHA PEL (SHIPYARD):8H TWA 200 PPM (260 MG/M3) CFRGBR 29,1915.1000,1993 OSHA PEL (FED CONT):8H TWA 200 PPM (260 MG/M3) CFRGBR 41,50-204.50,1994 OEL-ARAB REPUBLIC OF EGYPT: TWA 200 PPM (260 MG/M3), SKIN, JAN1993 OEL-AUSTRALIA: TWA 200 PPM (260 MG/M3), STEL 250 PPM, SKIN, JAN1993 OEL-AUSTRIA: MAK 200 PPM (260 MG/M3), SKIN, JAN1999 OEL-BELGIUM: TWA 200 PPM (262 MG/M3), STEL 250 PPM, SKIN, JAN1993 OEL-DENMARK: TWA 200 PPM (260 MG/M3), SKIN, JAN1999 OEL-FINLAND: TWA 200 PPM (260 MG/M3), STEL 250 PPM, SKIN, JAN1999 OEL-FRANCE: VME 200 PPM, VLE 1000 PPM, JAN1999 OEL-HUNGARY: TWA 50 MG/M3, STEL 100 MG/M3, SKIN, JAN1993 OEL-JAPAN: OEL 200 PPM (260 MG/M3), SKIN, JAN1999 OEL-THE NETHERLANDS: MAC-TGG 200 PPM (260 MG/M3), SKIN, JAN1999 OEL-NORWAY: TWA 100 PPM (130 MG/M3), JAN1999 OEL-THE PHILIPPINES: TWA 200 PPM (260 MG/M3), JAN1993 OEL-POLAND: MAC(TWA) 100 MG/M3, MAC(STEL) 300 MG/M3, JAN1999 OEL-RUSSIA: TWA 200 PPM, STEL 5 MG/M3, SKIN, JAN1993 OEL-SWEDEN: NGV 200 PPM (250 MG/M3), KTV 250 PPM (350 MG/M3), SKIN, JAN1999 OEL-THAILAND: TWA 200 PPM (260 MG/M3), JAN1993 OEL-TURKEY: TWA 200 PPM (260 MG/M3), JAN1993

OEL-UNITED KINGDOM: TWA 200 PPM (255 MG/M3), STEL 250 PPM, SKIN, SEP2000 OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV; OEL IN NEW ZEALAND, SINGAPORE, VIETNAM CHECK ACGIH TLV NIOSH REL TO METHANOL-AIR:10H TWA 200 PPM (SK); STEL 250 PPM (SK) NIOSH\* DHHS #92-100,1992 NOHS 1974: HZD 45930; NIS 344; TNF 78840; NOS 203; TNE 737242 NOES 1983: HZD 45930; NIS 373; TNF 101075; NOS 225; TNE 1620617; TFE 388352 EPA GENETOX PROGRAM 1988, NEGATIVE: SHE-CLONAL ASSAY; CELL TRANSFORM.-SA7/SHE EPA GENETOX PROGRAM 1988, NEGATIVE: N CRASSA-ANEUPLOIDY; IN VITRO SCE-NONHUMAN EPA TSCA SECTION 8 (B) CHEMICAL INVENTORY EPA TSCA SECTION 8(D) UNPUBLISHED HEALTH/SAFETY STUDIES EPA TSCA SECTION 8(E) RISK NOTIFICATION, 8EHQ-0892-8989 ON EPA IRIS DATABASE EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, JANUARY 2001 NIOSH ANALYTICAL METHOD, 1994: METHANOL, 2000 NIOSH ANALYTICAL METHOD, 1996: VOLATILE ORGANIC COMPOUND, 2549 U.S. INFORMATION THIS PRODUCT IS SUBJECT TO SARA SECTION 313 REPORTING REQUIREMENTS. SECTION 16. - - - - - - - OTHER INFORMATION- - - - - - - - - -THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT ТΟ BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SIGMA, ALDRICH, FLUKA SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR PACKING SLIP FOR ADDITIONAL TERMS AND CONDITIONS OF SALE. COPYRIGHT 2001 SIGMA-ALDRICH CO. LICENSE GRANTED TO MAKE UNLIMITED PAPER COPIES FOR INTERNAL USE ONLY

Valid 08/2001 - 10/2001 Sigma Chemical Co. P.O. Box 14508 St. Louis, MO 63178 USA Tel: 314-771-5765 MATERIAL SAFETY DATA SHEET SECTION 1. - - - - - - - CHEMICAL IDENTIFICATION- - - - - - - -CATALOG #: F1635 NAME: FORMALDEHYDE (FORMALIN) SECTION 2. - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - -CAS #: 50-00-0 MF: CH2O EC NO: 200-001-8 ADDITIONAL INFORMATION CONTAINS METHYL ALCOHOL, CHEMICAL ABSTRACTS REGISTRY NUMBER 67-56-1. SYNONYMS ALDEHYDE FORMIQUE (FRENCH) \* ALDEHYD MRAVENCI (CZECH) \* ALDEIDE FORMICA (ITALIAN) \* BFV \* FANNOFORM \* FORMALDEHYDE (ACGIH:OSHA) \* FORMALDEHYD (CZECH, POLISH) \* FORMALDEHYDE, GAS \* FORMALIN \* FORMALIN 40 \* FORMALINA (ITALIAN) \* FORMALINE (GERMAN) \* FORMALIN-LOESUNGEN (GERMAN) \* FORMALITH \* FORMIC ALDEHYDE \* FORMOL \* FYDE \* LYSOFORM \* METHALDEHYDE \* METHANAL \* METHYL ALDEHYDE \* METHYLENE OXIDE \* MORBICID \* NCI-C02799 \* OPLOSSINGEN (DUTCH) \* OXOMETHANE \* OXYMETHYLENE \* PARAFORM \* RCRA WASTE NUMBER U122 \* SUPERLYSOFORM \* SECTION 3. - - - - - - - - HAZARDS IDENTIFICATION - - - - - - -LABEL PRECAUTIONARY STATEMENTS TOXIC TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. CAUSES BURNS. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. MAY CAUSE HERITABLE GENETIC DAMAGE. POTENTIAL CANCER HAZARD. CONTAINS FORMALDEHYDE READILY ABSORBED THROUGH SKIN. LACHRYMATOR. COMBUSTIBLE LIQUID. TARGET ORGAN(S): EYES KIDNEYS IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.

WEAR SUITABLE PROTECTIVE CLOTHING, GLOVES AND EYE/FACE PROTECTION. IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). USE ONLY IN WELL VENTILATED AREAS. SECTION 4. - - - - - - - - - FIRST-AID MEASURES- - - - - - -IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN IMMEDIATELY. IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN. IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN. IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEOUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN. SECTION 5. - - - - - - - FIRE FIGHTING MEASURES - - - - - - - -EXTINGUISHING MEDIA CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM. SPECIAL FIREFIGHTING PROCEDURES USE WATER SPRAY TO COOL FIRE-EXPOSED CONTAINERS. WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES. UNUSUAL FIRE AND EXPLOSIONS HAZARDS EMITS TOXIC FUMES UNDER FIRE CONDITIONS. SECTION 6. - - - - - - - ACCIDENTAL RELEASE MEASURES- - - - - - - -WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES. COVER WITH DRY LIME OR SODA ASH, PICK UP, KEEP IN A CLOSED CONTAINER AND HOLD FOR WASTE DISPOSAL. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE AREA. SECTION 7. - - - - - - - - HANDLING AND STORAGE- - - - - - - - -REFER TO SECTION 8. SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -USE ONLY IN A CHEMICAL FUME HOOD. SAFETY SHOWER AND EYE BATH. WASH CONTAMINATED CLOTHING BEFORE REUSE. DISCARD CONTAMINATED SHOES. WASH THOROUGHLY AFTER HANDLING. DO NOT BREATHE VAPOR. DO NOT GET IN EYES, ON SKIN, ON CLOTHING. AVOID PROLONGED OR REPEATED EXPOSURE. NIOSH/MSHA-APPROVED RESPIRATOR. COMPATIBLE CHEMICAL-RESISTANT GLOVES. CHEMICAL SAFETY GOGGLES. FACESHIELD (8-INCH MINIMUM). KEEP TIGHTLY CLOSED. STORE IN A COOL DRY PLACE. SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - -

APPEARANCE AND ODOR LIOUID. PHYSICAL PROPERTIES FLASHPOINT 133F 56.11C EXPLOSION LIMITS IN AIR: 73 % UPPER 7 % LOWER VAPOR PRESSURE: 52 MMHG SPECIFIC GRAVITY: 1.083 VAPOR DENSITY: 1.03 G/L SECTION 10. - - - - - - - - STABILITY AND REACTIVITY - - - - - - -STABILITY STABLE. INCOMPATIBILITIES STRONG OXIDIZING AGENTS HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS CARBON MONOXIDE, CARBON DIOXIDE HAZARDOUS POLYMERIZATION WILL NOT OCCUR. SECTION 11. - - - - - - - TOXICOLOGICAL INFORMATION - - - - - -ACUTE EFFECTS MAY CAUSE ALLERGIC RESPIRATORY AND SKIN REACTIONS. CAUSES BURNS. TOXIC IF ABSORBED THROUGH SKIN. TOXIC IF INHALED. MATERIAL IS EXTREMELY DESTRUCTIVE TO THE TISSUE OF THE MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT. TOXIC IF SWALLOWED. INHALATION MAY RESULT IN SPASM, INFLAMMATION AND EDEMA OF THE LARYNX AND BRONCHI, CHEMICAL PNEUMONITIS AND PULMONARY EDEMA. SYMPTOMS OF EXPOSURE MAY INCLUDE BURNING SENSATION, COUGHING, WHEEZING, LARYNGITIS, SHORTNESS OF BREATH, HEADACHE, NAUSEA AND VOMTTING. MATERIAL IS EXTREMELY DESTRUCTIVE TO TISSUE OF THE MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT, EYES AND SKIN. TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. CHRONIC EFFECTS THIS PRODUCT IS OR CONTAINS A COMPONENT THAT HAS BEEN REPORTED TO BE POSSIBLY CARCINOGENIC BASED ON ITS IARC, ACGIH, NTP OR EPA CLASSIFICATION. TARGET ORGAN(S): EYES KIDNEYS LIVER HEART MAY ALTER GENETIC MATERIAL. POTENTIAL CANCER HAZARD.

```
RTECS #: LP8925000
    FORMALDEHYDE
  IRRITATION DATA
                                                   85DKA8 -,127,1977
    SKN-HMN 150 UG/3D-I MLD
                                                   IAPWAR 4,79,1961
    EYE-HMN 4 PPM/5M
                                                   AIHAAP 44,463,1983
    EYE-HMN 1 PPM/6M NSE MLD
    SKN-RBT 2 MG/24H SEV
                                                   85JCAE -,264,1986
    SKN-RBT 540 MG OPEN MLD
                                                  UCDS** 4/21/1967
    SKN-RBT 50 MG/24H MOD
                                                  TXAPA9 21,369,1972
   EYE-RBT 750 UG/24H SEV
                                                   85JCAE -,264,1986
   EYE-RBT 750 UG SEV
                                                   AJOPAA 29,1363,1946
    EYE-RBT 10 MG SEV
                                                   TXAPA9 55,501,1980
  TOXICITY DATA
                                                   29ZWAE -, 328, 1968
    ORL-WMN LDLO:108 MG/KG
    ORL-WMN LDLO:1 ML/KG
                                                   ICMED9 23,708,1997
    UNR-MAN LDLO:477 MG/KG
                                                   85DCAI 2,73,1970
    ORL-RAT LD50:100 MG/KG
                                                   FCTOD7 26,447,1988
    IHL-RAT LC50:203 MG/M3
                                                   GTPZAB 18(2),55,1974
                                                   APTOA6 6,299,1950
    SCU-RAT LD50:420 MG/KG
    IVN-RAT LD50:87 MG/KG
                                                  AEPPAE 221,166,1954
    ORL-MUS LD50:42 MG/KG
                                                  NTIS** AD-A125-539
                                                  CUTOEX 1,47,1993
    IHL-MUS LC50:454 GM/M3/4H
    SCU-MUS LD50:300 MG/KG
                                                  APTOA6 6,299,1950
                                                   UCDS** 4/21/1967
    SKN-RBT LD50:270 UL/KG
    ORL-GPG LD50:260 MG/KG
                                                   JIHTAB 23,259,1941
  TARGET ORGAN DATA
    SENSE ORGANS AND SPECIAL SENSES (OTHER OLFACTION EFFECTS)
    SENSE ORGANS AND SPECIAL SENSES (OLFACTION TUMORS)
    BEHAVIORAL (SOMNOLENCE)
    BEHAVIORAL (CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD)
    BEHAVIORAL (EXCITEMENT)
    BEHAVIORAL (AGGRESSION)
    LUNGS, THORAX OR RESPIRATION (BRONCHIOLAR CONSTRICTION, INCLUDING
ASTHMA)
    LUNGS, THORAX OR RESPIRATION (ACUTE PULMONARY EDEMA)
    LUNGS, THORAX OR RESPIRATION (RESPIRATORY OBSTRUCTION)
    GASTROINTESTINAL (GASTRITIS)
    GASTROINTESTINAL (ULCERATION OR BLEEDING FROM STOMACH)
    GASTROINTESTINAL (NAUSEA OR VOMITING)
    BLOOD (OTHER CHANGES)
    SKIN AND APPENDAGES (TUMORS)
    PATERNAL EFFECTS (SPERMATOGENESIS)
    PATERNAL EFFECTS (TESTES, EPIDIDYMIS, SPERM DUCT)
    PATERNAL EFFECTS (PROSTATE, SEMINAL VESICLE, COWPER'S, ACCESSORY
GLANDS
    PATERNAL EFFECTS (OTHER EFFECTS ON MALE)
    EFFECTS ON FERTILITY (MALE FERTILITY INDEX)
    EFFECTS ON FERTILITY (POST-IMPLANTATION MORTALITY)
    EFFECTS ON EMBRYO OR FETUS (FETOTOXICITY)
    EFFECTS ON EMBRYO OR FETUS (FETAL DEATH)
```

SPECIFIC DEVELOPMENTAL ABNORMALITIES (CRANIOFACIAL) SPECIFIC DEVELOPMENTAL ABNORMALITIES (MUSCULOSKELETAL SYSTEM) SPECIFIC DEVELOPMENTAL ABNORMALITIES (HEPATOBILIARY SYSTEM) SPECIFIC DEVELOPMENTAL ABNORMALITIES (OTHER DEVELOPMENTAL ABNORMALITIES) EFFECTS ON NEWBORN (GROWTH STATISTICS) EFFECTS ON NEWBORN (BIOCHEMICAL AND METABOLIC) EFFECTS ON NEWBORN (BEHAVIORAL) EFFECTS ON NEWBORN (OTHER POSTNATAL MEASURES OR EFFECTS) TUMORIGENIC (CARCINOGENIC BY RTECS CRITERIA) TUMORIGENIC (EQUIVOCAL TUMORIGENIC AGENT BY RTECS CRITERIA) TUMORIGENIC (TUMORS AT SITE OF APPLICATION) BIOCHEMICAL EFFECTS (EFFECT ON INFLAMMATION OR MEDIATION OF INFLAMMATION) ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION. SECTION 12. - - - - - - - - ECOLOGICAL INFORMATION - - - - - - - - -DATA NOT YET AVAILABLE. SECTION 13. - - - - - - - DISPOSAL CONSIDERATIONS - - - - -CONTACT A LICENSED PROFESSIONAL WASTE DISPOSAL SERVICE TO DISPOSE OF THIS MATERIAL. DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER. OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS. SECTION 14. - - - - - - - - TRANSPORT INFORMATION - - - - - - -CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION. SECTION 15. - - - - - - - REGULATORY INFORMATION - - - - - - -EUROPEAN INFORMATION EC INDEX NO: 605-001-00-5 TOXIC R 23/24/25 TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. R 34 CAUSES BURNS. R 40 POSSIBLE RISK OF IRREVERSIBLE EFFECTS. R 43 MAY CAUSE SENSITIZATION BY SKIN CONTACT. S 26 IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE. S 36/37 WEAR SUITABLE PROTECTIVE CLOTHING AND GLOVES. S 39 WEAR EYE/FACE PROTECTION. S 45 IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). S 51

USE ONLY IN WELL VENTILATED AREAS. TLV AND SOURCE FOR METHYL ALCOHOL - SKIN: ACGIH TLV-TWA: 200 PPM (260 MG/M3); STEL: 250 PPM (310 MG/M3). OSHA PEL: 8 H TWA 200 PPM (260 MG/M3); STEL: 250 PPM (310 MG/M3). REVIEWS, STANDARDS, AND REGULATIONS OEL=MAK ACGIH TLV-SUSPECTED HUMAN CARCINOGEN DTLVS\* TLV/BEI,1999 ACGIH TLV-CL 0.3 PPM DTLVS\* TLV/BEI,1999 IARC CANCER REVIEW: ANIMAL SUFFICIENT EVIDENCE IMEMDT 29,345,1982 IARC CANCER REVIEW: ANIMAL SUFFICIENT EVIDENCEIMEMDT 62,217,1995 IARC CANCER REVIEW: HUMAN LIMITED EVIDENCE IMSUDL 7,211,1987 IARC CANCER REVIEW: HUMAN LIMITED EVIDENCE IMEMDT 62,217,1995 IARC CANCER REVIEW: GROUP 2A IMSUDL 7,211,1987 IARC CANCER REVIEW: GROUP 2A IMEMDT 62,217,1995 EPA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION FEREAC 54,7740,1989 MSHA STANDARD:AIR-CL 2 PPM (3 MG/M3) DTLWS\* 3,19,1973 OSHA PEL (GEN INDU):SEE 1910.1048 CFRGBR 29,1910.1000,1994 OSHA PEL (CONSTRUC):SEE CFR 29,1926.1148 CFRGBR 29,1926.55,1994 OSHA PEL (FED CONT):CL 5 PPM (6 MG/M3) CFRGBR 41,50-204.50,1994 OEL-ARAB REPUBLIC OF EGYPT: TWA 2 PPM (3 MG/M3), JAN1993 OEL-AUSTRALIA: TWA 1 PPM (1.5 MG/M3), STEL 2 PPM, CARCINOGEN, JAN1993 OEL-AUSTRIA: MAK 0.5 PPM (0.6 MG/M3), SUSPECTED CARCINOGEN, JAN1999 OEL-BELGIUM: TWA 1 PPM (1.2 MG/M3), STEL 2 PPM, CARCINOGEN, JAN1993 OEL-DENMARK: TWA 0.3 PPM (0.4 MG/M3), JAN1999 OEL-FRANCE: VME 0.5 PPM, VLE 1 PPM, C3 CARCINOGEN, JAN1999 OEL-GERMANY: MAK 0.5 PPM (0.6 MG/M3), CARCINOGEN, JAN1999 OEL-HUNGARY: STEL 0.6 MG/M3, CARCINOGEN, JAN1993 OEL-JAPAN: OEL 0.5 PPM (0.61 MG/M3), 2A CARCINOGEN, JAN1999 OEL-THE NETHERLANDS: MAC-TGG 1 PPM (1.5 MG/M3), MAC-K 2 PPM (3 MG/M3), JAN1999 OEL-NORWAY: TWA 0.5 PPM (0.6 MG/M3), JAN1999 OEL-THE PHILIPPINES: TWA 5 PPM (6 MG/M3), JAN1993 OEL-POLAND: MAC(TWA) 0.5 MG/M3, MAC(STEL) 1 MG/M3, JAN1999 OEL-RUSSIA: TWA 0.5 PPM, STEL 0.5 MG/M3, SKIN, JAN1993 OEL-SWEDEN: NGV 0.5 PPM (0.6 MG/M3), TGV 1 PPM (1.2 MG/M3), SKIN, JAN1999 OEL-SWITZERLAND: MAK-W 0.5 PPM (0.6 MG/M3), KZG-W 1 PPM (1.2 MG/M3), JAN1999 OEL-THAILAND: TWA 3 PPM, STEL 5 PPM, JAN1993 OEL-TURKEY: TWA 5 PPM (6 MG/M3), JAN1993 OEL-UNITED KINGDOM: TWA 2 PPM (2.5MG/M3), CARCINOGEN, SEP2000 OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV; OEL IN NEW ZEALAND, SINGAPORE, VIETNAM CHECK ACGIH TLV

NIOSH REL TO FORMALDEHYDE-AIR:8H CA TWA 0.016 PPM;CL 0.1 PPM/15M NIOSH\* DHHS #92-100,1992 NOHS 1974: HZD M1529; NIS 72; TNF 10311; NOS 67; TNE 66921 NOHS 1974: HZD 33640; NIS 213; TNF 33243; NOS 155; TNE 394660 NOES 1983: HZD M1529; NIS 87; TNF 11309; NOS 69; TNE 207013; TFE 104994 NOES 1983: HZD 33640; NIS 309; TNF 65738; NOS 193; TNE 1329322; TFE 441902 EPA GENETOX PROGRAM 1988, POSITIVE: CARCINOGENICITY-MOUSE/RAT EPA GENETOX PROGRAM 1988, POSITIVE: D MELANOGASTER-RECIPROCAL TRANSLOCATION EPA GENETOX PROGRAM 1988, POSITIVE: N CRASSA-REVERSION; E COLI POLA WITHOUT S9 EPA GENETOX PROGRAM 1988, POSITIVE: D MELANOGASTER SEX-LINKED LETHAL EPA GENETOX PROGRAM 1988, POSITIVE: S CEREVISIAE GENE CONVERSION; S CEREVISIAE-REVERSION EPA GENETOX PROGRAM 1988, INCONCLUSIVE: IN VITRO UDS-HUMAN FIBROBLAST EPA GENETOX PROGRAM 1988, INCONCLUSIVE: CHO GENE MUTATION EPA TSCA SECTION 8(B) CHEMICAL INVENTORY EPA TSCA SECTION 8(D) UNPUBLISHED HEALTH/SAFETY STUDIES ON EPA IRIS DATABASE EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, JANUARY 2001 NIOSH CURRENT INTELLIGENCE BULLETIN 34, APRIL 1981 NIOSH ANALYTICAL METHOD, 1994: ALDEHYDES, SCREENING, 2539 NIOSH ANALYTICAL METHOD, 1994: FORMALDEHYDE BY ON DUST (TEXTILE OR WOOD), 5700 NIOSH ANALYTICAL METHOD, 1994: FORMALDEHYDE BY GC, 2541; BY VIS, 3500 NTP 9TH REPORT ON CARCINOGENS, 2000: REASONABLY ANTICIPATED TO BE HUMAN CARCINOGEN OSHA ANALYTICAL METHOD #ID-102 U.S. INFORMATION THIS PRODUCT IS SUBJECT TO SARA SECTION 313 REPORTING REOUIREMENTS. THIS PRODUCT IS OR CONTAINS CHEMICAL(S) KNOWN TO THE STATE OF CALIFORNIA TO CAUSE CANCER. THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT TΟ BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SIGMA, ALDRICH, FLUKA SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR PACKING SLIP FOR ADDITIONAL TERMS AND CONDITIONS OF SALE. COPYRIGHT 2001 SIGMA-ALDRICH CO. LICENSE GRANTED TO MAKE UNLIMITED PAPER COPIES FOR INTERNAL USE ONLY

Valid 08/2001 - 10/2001 Sigma Chemical Co. P.O. Box 14508 St. Louis, MO 63178 USA Tel: 314-771-5765 MATERIAL SAFETY DATA SHEET SECTION 1. - - - - - - - CHEMICAL IDENTIFICATION- - - - - - - -CATALOG #: A6283 ACETIC ACID, GLACIAL NAME: SECTION 2. - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - -CAS #: 64-19-7 MF: C2H4O2 EC NO: 200-580-7 SYNONYMS ACETIC ACID (ACGIH:OSHA) \* ACETIC ACID, GLACIAL \* ACIDE ACETIQUE (FRENCH) \* ACIDO ACETICO (ITALIAN) \* AZIJNZUUR (DUTCH) \* ESSIGSAEURE (GERMAN) \* ETHANOIC ACID \* ETHYLIC ACID \* GLACIAL ACETIC ACID \* KYSELINA OCTOVA (CZECH) \* METHANECARBOXYLIC ACID \* OCTOWY KWAS (POLISH) \* VINEGAR ACID \* SECTION 3. - - - - - - - - HAZARDS IDENTIFICATION - - - - - - -LABEL PRECAUTIONARY STATEMENTS COMBUSTIBLE (USA) FLAMMABLE (EU) CORROSIVE CAUSES SEVERE BURNS. HARMFUL IN CONTACT WITH SKIN. LACHRYMATOR. TARGET ORGAN(S): TEETH KIDNEYS KEEP AWAY FROM SOURCES OF IGNITION - NO SMOKING. IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE. WEAR SUITABLE PROTECTIVE CLOTHING, GLOVES AND EYE/FACE PROTECTION. SECTION 4. - - - - - - - - FIRST-AID MEASURES- - - - - - - - -IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.

```
DO NOT INDUCE VOMITING.
    IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL
    RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
    IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER
    FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND
    SHOES. CALL A PHYSICIAN.
    IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER
    FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING
    THE EYELIDS WITH FINGERS. CALL A PHYSICIAN.
SECTION 5. - - - - - - - FIRE FIGHTING MEASURES - - - - - - - -
 EXTINGUISHING MEDIA
    CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.
 SPECIAL FIREFIGHTING PROCEDURES
   WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO
    PREVENT CONTACT WITH SKIN AND EYES.
 UNUSUAL FIRE AND EXPLOSIONS HAZARDS
   EMITS TOXIC FUMES UNDER FIRE CONDITIONS.
SECTION 6. - - - - - - - ACCIDENTAL RELEASE MEASURES- - - - - - - -
   WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY
   RUBBER GLOVES.
    COVER WITH DRY LIME OR SODA ASH, PICK UP, KEEP IN A CLOSED CONTAINER
   AND HOLD FOR WASTE DISPOSAL.
   VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.
    EVACUATE AREA.
SECTION 7. - - - - - - - - HANDLING AND STORAGE- - - - - - - - -
    REFER TO SECTION 8.
SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -
   SAFETY SHOWER AND EYE BATH.
   USE ONLY IN A CHEMICAL FUME HOOD.
   WASH CONTAMINATED CLOTHING BEFORE REUSE.
    DISCARD CONTAMINATED SHOES.
   WASH THOROUGHLY AFTER HANDLING.
   DO NOT BREATHE VAPOR.
   DO NOT GET IN EYES, ON SKIN, ON CLOTHING.
   AVOID PROLONGED OR REPEATED EXPOSURE.
   NIOSH/MSHA-APPROVED RESPIRATOR.
    COMPATIBLE CHEMICAL-RESISTANT GLOVES.
    CHEMICAL SAFETY GOGGLES.
    FACESHIELD (8-INCH MINIMUM).
   KEEP TIGHTLY CLOSED.
    STORE IN A COOL DRY PLACE.
SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - -
 APPEARANCE AND ODOR
   LIQUID.
 PHYSICAL PROPERTIES
   BOILING POINT:
                        117 - 118 C
   MELTING POINT:
                      16.2 C
   FLASHPOINT
                            104F
                             40C
   EXPLOSION LIMITS IN AIR:
```

UPPER 19.9 % 4 % LOWER VAPOR PRESSURE: 11.4 MMHG SPECIFIC GRAVITY: 1.049 VAPOR DENSITY: 2.07 G/L SECTION 10. - - - - - - - - STABILITY AND REACTIVITY - - - - -STABILITY STABLE. INCOMPATIBILITIES PROTECT FROM MOISTURE. OXIDIZING AGENTS SOLUBLE CARBONATES AND PHOSPHATES HYDROXIDES OXIDES METALS PEROXIDES PERMANGANATES AMINES ALCOHOLS HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS CARBON MONOXIDE, CARBON DIOXIDE HAZARDOUS POLYMERIZATION WILL NOT OCCUR. SECTION 11. - - - - - - - - TOXICOLOGICAL INFORMATION - - - -ACUTE EFFECTS CAUSES BURNS. HARMFUL IF ABSORBED THROUGH SKIN. MAY BE HARMFUL IF INHALED. MATERIAL IS EXTREMELY DESTRUCTIVE TO THE TISSUE OF THE MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT. MAY BE HARMFUL IF SWALLOWED. MATERIAL IS EXTREMELY DESTRUCTIVE TO TISSUE OF THE MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT, EYES AND SKIN. INHALATION MAY RESULT IN SPASM, INFLAMMATION AND EDEMA OF THE LARYNX AND BRONCHI, CHEMICAL PNEUMONITIS AND PULMONARY EDEMA. SYMPTOMS OF EXPOSURE MAY INCLUDE BURNING SENSATION, COUGHING, WHEEZING, LARYNGITIS, SHORTNESS OF BREATH, HEADACHE, NAUSEA AND VOMITING. INGESTION OR INHALATION OF CONCENTRATED ACETIC ACID CAUSES DAMAGE TO TISSUES OF THE RESPIRATORY AND DIGESTIVE TRACTS. SYMPTOMS INCLUDE: HEMATEMESIS, BLOODY DIARRHEA, EDEMA AND/OR PERFORATION OF THE ESOPHAGUS AND PYLORUS, HEMATURIA, ANURIA, UREMIA, ALBUMINURIA, HEMOLYSIS, CONVULSIONS, BRONCHITIS, PULMONARY EDEMA, PNEUMONIA, CARDIOVASCULAR COLLAPSE, SHOCK AND DEATH. DIRECT CONTACT OR EXPOSURE TO HIGH CONCENTRATIONS OF VAPOR WITH SKIN OR EYES CAN CAUSE: ERYTHEMA, BLISTERS, TISSUE DESTRUCTION WITH SLOW HEALING, SKIN BLACKENING, HYPERKERATOSIS, FISSURES, CORNEAL EROSION,

```
OPACIFICATION, IRITIS, CONJUNCTIVITIS AND POSSIBLE BLINDNESS.
    TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND
    TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.
  CHRONIC EFFECTS
    TARGET ORGAN(S):
    TEETH
    KIDNEYS
  RTECS #: AF1225000
   ACETIC ACID
  IRRITATION DATA
    SKN-HMN 50 MG/24H MLD
                                                  TXAPA9 31,481,1975
                                                 UCDS** 8/7/1963
    SKN-RBT 525 MG OPEN SEV
    SKN-RBT 50 MG/24H MLD
                                                 TXAPA9 31,481,1975
                                                 TXCYAC 23,281,1982
   EYE-RBT 5 MG/30S RINSE MLD
  TOXICITY DATA
    UNR-MAN LDLO:308 MG/KG
                                                  85DCAI 2,73,1970
    ORL-RAT LD50:3310 MG/KG
                                                 DMDJAP 31,276,1959
    IHL-MUS LC50:5620 PPM/1H
                                                 MELAAD 48,559,1957
    IVN-MUS LD50:525 MG/KG
                                                 APTOA6 18,141,1961
                                                 UCDS** 8/7/1963
    SKN-RBT LD50:1060 UL/KG
  TARGET ORGAN DATA
    SENSE ORGANS AND SPECIAL SENSES (OTHER OLFACTION EFFECTS)
    SENSE ORGANS AND SPECIAL SENSES (OTHER EYE EFFECTS)
    BEHAVIORAL (CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD)
    LUNGS, THORAX OR RESPIRATION (OTHER CHANGES)
    GASTROINTESTINAL (CHANGES IN STRUCTURE OR FUNCTION OF ESOPHAGUS)
    GASTROINTESTINAL (ULCERATION OR BLEEDING FROM SMALL INTESTINE)
    GASTROINTESTINAL (ULCERATION OR BLEEDING FROM LARGE INTESTINE)
    EFFECTS ON FERTILITY (MALE FERTILITY INDEX)
    EFFECTS ON NEWBORN (BEHAVIORAL)
    ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES
    (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR
    COMPLETE INFORMATION.
SECTION 12. - - - - - - - ECOLOGICAL INFORMATION - - - - - - - - -
    DATA NOT YET AVAILABLE.
SECTION 13. - - - - - - DISPOSAL CONSIDERATIONS - - - - - - - -
    THIS COMBUSTIBLE MATERIAL MAY BE BURNED IN A CHEMICAL INCINERATOR
    EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.
    OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.
SECTION 14. - - - - - - - TRANSPORT INFORMATION - - - - - - -
    CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION.
SECTION 15. - - - - - - - REGULATORY INFORMATION - - - - - - -
  EUROPEAN INFORMATION
    EC INDEX NO: 607-002-00-6
    FLAMMABLE
    CORROSIVE
   R 10
    FLAMMABLE.
    R 35
    CAUSES SEVERE BURNS.
```

S 23 DO NOT BREATHE VAPOR. S 26 IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE. S 45 IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). REVIEWS, STANDARDS, AND REGULATIONS OEL=MAK ACGIH TLV-STEL 15 PPM DTLVS\* TLV/BEI,1999 ACGIH TLV-TWA 10 PPM DTLVS\* TLV/BEI,1999 EPA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION FEREAC 54,7740,1989 MSHA STANDARD-AIR: TWA 10 PPM (25 MG/M3) DTLVS\* 3,2,1971 OSHA PEL (GEN INDU):8H TWA 10 PPM (25 MG/M3) CFRGBR 29,1910.1000,1994 OSHA PEL (CONSTRUC):8H TWA 10 PPM (25 MG/M3) CFRGBR 29,1926.55,1994 OSHA PEL (SHIPYARD):8H TWA 10 PPM (25 MG/M3) CFRGBR 29,1915.1000,1993 OSHA PEL (FED CONT):8H TWA 10 PPM (25 MG/M3) CFRGBR 41,50-204.50,1994 OEL-AUSTRALIA: TWA 10 PPM (25 MG/M3), STEL 15 PPM, JAN1993 OEL-AUSTRIA: MAK 10 PPM (25 MG/M3), JAN1999 OEL-BELGIUM: TWA 10 PPM (25 MG/M3), STEL 15 PPM, JAN1993 OEL-DENMARK: TWA 10 PPM (25 MG/M3), JAN1999 OEL-FINLAND: TWA 10 PPM (25 MG/M3), STEL 15 PPM (37 MG/M3), SKIN, JAN1993 OEL-FRANCE: VLE 10 PPM (25 MG/M3), JAN1999 OEL-GERMANY: MAK 10 PPM (25 MG/M3), JAN1999 OEL-HUNGARY: TWA 10 MG/M3, STEL 20 MG/M3, JAN1993 OEL-INDIA: TWA 10 PPM (25 MG/M3), STEL 15 PPM (37 MG/M3), JAN1993 OEL-JAPAN: OEL 10 PPM (25 MG/M3), JAN1999 OEL-THE NETHERLANDS: MAC-TGG 10 PPM (25 MG/M3), JAN1999 OEL-NORWAY: TWA 10 PPM (25 MG/M3), JAN1999 OEL-THE PHILIPPINES: TWA 10 PPM (25 MG/M3), JAN1993 OEL-POLAND: MAC(TWA) 5 MG/M3, MAC(STEL) 35 MG/M3, JAN1999 OEL-RUSSIA: TWA 10 PPM, STEL 5 MG/M3, SKIN, JAN1993 OEL-SWEDEN: NGV 5 PPM (13 MG/M3), KTV 10 PPM (25 MG/M3), JAN1999 OEL-SWITZERLAND: MAK-W 10 PPM (25 MG/M3), KZG-W 20 PPM (50 MG/M3), JAN1999 OEL-THAILAND: TWA 10 PPM (25 MG/M3), JAN1993 OEL-TURKEY: TWA 10 PPM (25 MG/M3), JAN1993 OEL-UNITED KINGDOM: TWA 10 PPM (25 MG/M3), STEL 15 PPM (37 MG/M3), SEP2000 OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV; OEL IN NEW ZEALAND, SINGAPORE, VIETNAM CHECK ACGIH TLV NIOSH REL TO ACETIC ACID-AIR: 10H TWA 10 PPM; STEL 15 PPM

NIOSH\* DHHS #92-100,1992 NOHS 1974: HZD 01568; NIS 264; TNF 51469; NOS 150; TNE 486503 NOES 1983: HZD 01568; NIS 266; TNF 49403; NOS 169; TNE 907205; TFE 322123 EPA GENETOX PROGRAM 1988, NEGATIVE: HISTIDINE REVERSION-AMES TEST EPA TSCA SECTION 8(B) CHEMICAL INVENTORY EPA TSCA SECTION 8(D) UNPUBLISHED HEALTH/SAFETY STUDIES EPA TSCA SECTION 8(E) RISK NOTIFICATION, 8EHQ-0892-9237;8EHQ-0892-9238 EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, JANUARY 2001 NIOSH ANALYTICAL METHOD, 1994: ACETIC ACID, 1603 OSHA ANALYTICAL METHOD #ID-118 SECTION 16. - - - - - - - OTHER INFORMATION- - - - - - - - -THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT ΤO BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SIGMA, ALDRICH, FLUKA SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR PACKING SLIP FOR ADDITIONAL TERMS AND CONDITIONS OF SALE. COPYRIGHT 2001 SIGMA-ALDRICH CO. LICENSE GRANTED TO MAKE UNLIMITED PAPER COPIES FOR INTERNAL USE ONLY

Valid 08/2001 - 10/2001 Riedel-de Haen 3050 Spruce St. St. Louis, MO 63178 USA Tel: 314-289-6000 MATERIAL SAFETY DATA SHEET SECTION 1. - - - - - - - CHEMICAL IDENTIFICATION- - - - - -CATALOG #: 32294 ETHANOL 96 VOL.8, ACS REAGENT MIN. 968 NAME: SECTION 2. - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - -CAS #: 64-17-5 MF: C2H6O EC NO: 200-578-6 SYNONYMS ABSOLUTE ETHANOL \* AETHANOL (GERMAN) \* AETHYLALKOHOL (GERMAN) \* ALCOHOL \* ALCOHOL, ANHYDROUS \* ALCOHOL DEHYDRATED \* ALCOOL ETHYLIQUE (FRENCH) \* ALCOOL ETILICO (ITALIAN) \* ALGRAIN \* ALKOHOL (GERMAN) \* ALKOHOLU ETYLOWEGO (POLISH) \* ANHYDROL \* COLOGNE SPIRIT \* ETANOLO (ITALIAN) \* ETHANOL (ACGIH:OSHA) \* ETHYL ALCOHOL (DOT:OSHA) \* ETHYL ALCOHOL ANHYDROUS \* ETHYL HYDRATE \* ETHYL HYDROXIDE \* ETYLOWY ALKOHOL (POLISH) \* FERMENTATION ALCOHOL \* GRAIN ALCOHOL \* JAYSOL \* JAYSOL S \* METHYLCARBINOL \* MOLASSES ALCOHOL \* NCI-C03134 \* POTATO ALCOHOL \* SD ALCOHOL 23-HYDROGEN \* SPIRITS OF WINE \* SPIRT \* TECSOL \* SECTION 3. - - - - - - - - HAZARDS IDENTIFICATION - - - -LABEL PRECAUTIONARY STATEMENTS FLAMMABLE (USA) HIGHLY FLAMMABLE (EU) IRRITANT IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TARGET ORGAN(S): NERVES LIVER KEEP AWAY FROM SOURCES OF IGNITION - NO SMOKING. IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE. WEAR SUITABLE PROTECTIVE CLOTHING. SECTION 4. - - - - - - - - FIRST-AID MEASURES- - - - - - - - -IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN. IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL

RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN. IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER. IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. SECTION 5. - - - - - - - FIRE FIGHTING MEASURES - - - - - - - -EXTINGUISHING MEDIA WATER SPRAY. CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM. SPECIAL FIREFIGHTING PROCEDURES WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES. USE WATER SPRAY TO COOL FIRE-EXPOSED CONTAINERS. UNUSUAL FIRE AND EXPLOSIONS HAZARDS VAPOR MAY TRAVEL CONSIDERABLE DISTANCE TO SOURCE OF IGNITION AND FLASH BACK. CONTAINER EXPLOSION MAY OCCUR UNDER FIRE CONDITIONS. FLAMMABLE LIQUID. EMITS TOXIC FUMES UNDER FIRE CONDITIONS. SECTION 6. - - - - - - ACCIDENTAL RELEASE MEASURES- - - - - - -WEAR RESPIRATOR, CHEMICAL SAFETY GOGGLES, RUBBER BOOTS AND HEAVY RUBBER GLOVES. COVER WITH DRY-LIME, SAND, OR SODA ASH. PLACE IN COVERED CONTAINERS USING NON-SPARKING TOOLS AND TRANSPORT OUTDOORS. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE AREA. SHUT OFF ALL SOURCES OF IGNITION. SECTION 7. - - - - - - - HANDLING AND STORAGE- - - - - - - - -REFER TO SECTION 8. SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -SAFETY SHOWER AND EYE BATH. USE NONSPARKING TOOLS. MECHANICAL EXHAUST REQUIRED. WASH THOROUGHLY AFTER HANDLING. WASH CONTAMINATED CLOTHING BEFORE REUSE. AVOID BREATHING VAPOR. AVOID CONTACT WITH EYES, SKIN AND CLOTHING. AVOID PROLONGED OR REPEATED EXPOSURE. NIOSH/MSHA-APPROVED RESPIRATOR. COMPATIBLE CHEMICAL-RESISTANT GLOVES. CHEMICAL SAFETY GOGGLES. KEEP CONTAINER CLOSED. KEEP AWAY FROM HEAT, SPARKS, AND OPEN FLAME. STORE IN A COOL DRY PLACE. HANDLE AND STORE UNDER NITROGEN. SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - -APPEARANCE AND ODOR CLEAR, COLORLESS LIQUID PHYSICAL PROPERTIES BOILING POINT: 78 C

FLASHPOINT 62F 16.66C SPECIFIC GRAVITY: 0.794 SECTION 10. - - - - - - - - - STABILITY AND REACTIVITY - - - - - - -STABILITY STABLE. INCOMPATIBILITIES PROTECT FROM MOISTURE. ALKALI METALS AMMONIA OXIDIZING AGENTS PEROXIDES HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS CARBON MONOXIDE, CARBON DIOXIDE HAZARDOUS POLYMERIZATION WILL NOT OCCUR. SECTION 11. - - - - - - - TOXICOLOGICAL INFORMATION - - - - - -ACUTE EFFECTS CAUSES SKIN IRRITATION. MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN. CAUSES EYE IRRITATION. MAY BE HARMFUL IF INHALED. MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT. MAY BE HARMFUL IF SWALLOWED. CAN CAUSE CNS DEPRESSION. NARCOTIC EFFECT DAMAGE TO THE HEART TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED. CHRONIC EFFECTS TARGET ORGAN(S): NERVES LIVER HEART THIS PRODUCT IS OR CONTAINS A COMPONENT THAT IS NOT CLASSIFIABLE AS TO ITS CARCINOGENICITY BASED ON ITS IARC, ACGIH, NTP OR EPA CLASSIFICATION. RTECS #: KQ6300000 ETHYL ALCOHOL IRRITATION DATA SKN-RBT 400 MG OPEN MLD UCDS\*\* 7/22/1970 SKN-RBT 20 MG/24H MOD 85JCAE -,189,1986 EYE-RBT 500 MG SEV AJOPAA 29,1363,1946 EYE-RBT 500 MG/24H MLD 85JCAE -,189,1986 EYE-RBT 100 MG/4S RINSE MOD FCTOD7 20,573,1982 TOXICITY DATA ORL-CHD LDLO:2 GM/KG ATXKA8 17,183,1958 ORL-HMN LDLO:1400 MG/KG NPIRI\* 1,44,1974 SCU-INF LDLO:19440 MG/KG AJCPAI 5,466,1935

```
ORL-RAT LD50:7060 MG/KG
                                                TXAPA9 16,718,1970
  IHL-RAT LC50:20000 PPM/10H
                                                NPIRI* 1,44,1974
  IPR-RAT LD50:3600 UG/KG
                                                PHMGBN 2,27,1969
  IVN-RAT LD50:1440 MG/KG
                                                TXAPA9 18,60,1971
                                                TXAPA9 18,60,1971
  IAT-RAT LD50:11 MG/KG
  ORL-MUS LD50:3450 MG/KG
                                                GISAAA 32(3), 31, 1967
  IHL-MUS LC50:39 GM/M3/4H
                                                GTPZAB 26(8),53,1982
  IPR-MUS LD50:528 MG/KG
                                                STRAAA 127,245,1965
  SCU-MUS LD50:8285 MG/KG
                                                FAONAU 48A,99,1970
                                                HBTXAC 1,128,1955
  IVN-MUS LD50:1973 MG/KG
  ORL-RBT LD50:6300 MG/KG
                                                HBTXAC 1,130,1955
                                                EVHPAZ 61,321,1985
  IPR-RBT LD50:963 MG/KG
                                                EVHPAZ 61,321,1985
  IVN-RBT LD50:2374 MG/KG
                                                JIHTAB 23,259,1941
  ORL-GPG LD50:5560 MG/KG
  IPR-GPG LD50:3414 MG/KG
                                               EVHPAZ 61,321,1985
  IPR-HAM LD50:5068 MG/KG
                                               EVHPAZ 61,321,1985
  IPR-MAM LD50:4300 MG/KG
                                                TXAPA9 13,358,1968
TARGET ORGAN DATA
  BEHAVIORAL (SLEEP)
  BEHAVIORAL (CHANGE IN MOTOR ACTIVITY)
  BEHAVIORAL (ATAXIA)
  BEHAVIORAL (ANTIPSYCHOTIC)
  BEHAVIORAL (HEADACHE)
  BEHAVIORAL (CHANGE IN PSYCHOPHYSIOLOGICAL TESTS)
  LUNGS, THORAX OR RESPIRATION (CHRONIC PULMONARY EDEMA OR CONGESTION)
  LUNGS, THORAX OR RESPIRATION (DYSPNAE)
  LUNGS, THORAX OR RESPIRATION (OTHER CHANGES)
  GASTROINTESTINAL (ALTERATION IN GASTRIC SECRETION)
  GASTROINTESTINAL (HYPERMOTILITY, DIARRHEA)
  GASTROINTESTINAL (NAUSEA OR VOMITING)
  GASTROINTESTINAL (OTHER CHANGES)
  LIVER (FATTY LIVER DEGENERATION)
  LIVER (TUMORS)
  ENDOCRINE (CHANGE IN GONADOTROPINS)
  ENDOCRINE (OTHER CHANGES)
  BLOOD (OTHER CHANGES)
  BLOOD (LYMPHOMA INCLUDING HODGKIN'S DISEASE)
  PATERNAL EFFECTS (TESTES, EPIDIDYMIS, SPERM DUCT)
  EFFECTS ON FERTILITY (FEMALE FERTILITY INDEX)
  EFFECTS ON FERTILITY (MALE FERTILITY INDEX)
  EFFECTS ON FERTILITY (POST-IMPLANTATION MORTALITY)
  EFFECTS ON FERTILITY (OTHER MEASURES OF FERTILITY)
  EFFECTS ON EMBRYO OR FETUS (EXTRA EMBRYONIC STRUCTURES)
  EFFECTS ON EMBRYO OR FETUS (CYTOLOGICAL CHANGES)
  EFFECTS ON EMBRYO OR FETUS (FETOTOXICITY)
  EFFECTS ON EMBRYO OR FETUS (FETAL DEATH)
  EFFECTS ON EMBRYO OR FETUS (OTHER EFFECTS TO EMBYRO OR FETUS)
  SPECIFIC DEVELOPMENTAL ABNORMALITIES (EYE, EAR)
  SPECIFIC DEVELOPMENTAL ABNORMALITIES (CRANIOFACIAL)
  SPECIFIC DEVELOPMENTAL ABNORMALITIES (MUSCULOSKELETAL SYSTEM)
```

SPECIFIC DEVELOPMENTAL ABNORMALITIES (RESPIRATORY SYSTEM) EFFECTS ON NEWBORN (GROWTH STATISTICS) TUMORIGENIC (EQUIVOCAL TUMORIGENIC AGENT BY RTECS CRITERIA) ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION. SECTION 12. - - - - - - - ECOLOGICAL INFORMATION - - - - - - - - -DATA NOT YET AVAILABLE. SECTION 13. - - - - - - - DISPOSAL CONSIDERATIONS - - - - - - -CONTACT A LICENSED PROFESSIONAL WASTE DISPOSAL SERVICE TO DISPOSE OF THIS MATERIAL. BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER BUT EXERT EXTRA CARE IN IGNITING AS THIS MATERIAL IS HIGHLY FLAMMABLE. OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS. SECTION 14. - - - - - - - - TRANSPORT INFORMATION - - - - - - -CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION. SECTION 15. - - - - - - REGULATORY INFORMATION - - - - - - - -EUROPEAN INFORMATION EC INDEX NO: 603-002-00-5 HIGHLY FLAMMABLE IRRITANT R 11 HIGHLY FLAMMABLE. S 7 KEEP CONTAINER TIGHTLY CLOSED. S 16 KEEP AWAY FROM SOURCES OF IGNITION - NO SMOKING. REVIEWS, STANDARDS, AND REGULATIONS OEL=MAK ACGIH TLV-NOT CLASSIFIABLE AS A HUMAN CARCINOGEN DTLVS\* TLV/BEI,1999 ACGIH TLV-TWA 1000 PPM DTLVS\* TLV/BEI,1999 IARC CANCER REVIEW: ANIMAL INADEQUATE EVIDENCE IMEMDT 44,35,1988 EPA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION FEREAC 54,7740,1989 MSHA STANDARD-AIR: TWA 1000 PPM (1900 MG/M3) DTLVS\* 3,103,1971 OSHA PEL (GEN INDU):8H TWA 1000 PPM (1900 MG/M3) CFRGBR 29,1910.1000,1994 OSHA PEL (CONSTRUC):8H TWA 1000 PPM (1900 MG/M3) CFRGBR 29,1926.55,1994 OSHA PEL (SHIPYARD):8H TWA 1000 PPM (1900 MG/M3) CFRGBR 29,1915.1000,1993 OSHA PEL (FED CONT):8H TWA 1000 PPM (1900 MG/M3) CFRGBR 41,50-204.50,1994 OEL-AUSTRALIA: TWA 1000 PPM (1900 MG/M3), JAN1993 OEL-AUSTRIA: MAK 1000 PPM (1900 MG/M3), JAN1999 OEL-BELGIUM: TWA 1000 PPM (1880 MG/M3), JAN1993 OEL-DENMARK: TWA 1000 PPM (1900 MG/M3), JAN1999 OEL-FINLAND: TWA 1000 PPM (1900 MG/M3), STEL 1250 PPM (2400 MG/M3),

JAN1999 OEL-FRANCE: VME 1000 PPM (1900 MG/M3), VLE 5000 PPM, JAN1999 OEL-GERMANY: MAK 1000 PPM (1900 MG/M3), JAN1999 OEL-HUNGARY: TWA 1000 MG/M3, STEL 3000 MG/M3, JAN1993 OEL-THE NETHERLANDS: MAC-TGG 500 PPM (950 MG/M3), JAN1999 OEL-NORWAY: TWA 500 PPM (950 MG/M3), JAN1999 OEL-THE PHILIPPINES: TWA 1000 PPM (1900 MG/M3), JAN1993 OEL-POLAND: MAC(TWA) 1000 MG/M3, MAC(STEL) 3000 MG/M3, JAN1999 OEL-RUSSIA: STEL 1000 MG/M3, JAN1993 OEL-SWEDEN: NGV 500 PPM (1000 MG/M3), KTV 1000PPM (1900 MG/M3), JAN1999 OEL-SWITZERLAND: MAK-W 1000 PPM (1900 MG/M3), JAN1999 OEL-THAILAND: TWA 1000 PPM (1900 MG/M3), JAN1993 OEL-TURKEY: TWA 1000 PPM (1900 MG/M3), JAN1993 OEL-UNITED KINGDOM: TWA 1000 PPM (1950 MG/M3), SEP2000 OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV; OEL IN NEW ZEALAND, SINGAPORE, VIETNAM CHECK ACGIH TLV NIOSH REL TO ETHYL ALCOHOL-AIR: 10H TWA 1000 PPM NIOSH\* DHHS #92-100,1992 NOHS 1974: HZD 31500; NIS 430; TNF 157709; NOS 242; TNE 2088926 NOES 1983: HZD 31500; NIS 334; TNF 86077; NOS 222; TNE 2069125; TFE 1014002 EPA GENETOX PROGRAM 1988, POSITIVE: RODENT DOMINANT LETHAL EPA GENETOX PROGRAM 1988, NEGATIVE: ASPERGILLUS-FORWARD MUTATION; SHE-CLONAL ASSAY EPA GENETOX PROGRAM 1988, NEGATIVE: CELL TRANSFORM.-RLV F344 RAT EMBRYO EPA GENETOX PROGRAM 1988, NEGATIVE: IN VITRO CYTOGENETICS-NONHUMAN; MAMMALIAN MICRONUCLEUS EPA GENETOX PROGRAM 1988, NEGATIVE: N CRASSA-ANEUPLOIDY; HISTIDINE REVERSION-AMES TEST EPA GENETOX PROGRAM 1988, NEGATIVE: IN VITRO SCE-HUMAN LYMPHOCYTES; ΙN VITRO SCE-HUMAN EPA GENETOX PROGRAM 1988, NEGATIVE: IN VITRO SCE-NONHUMAN; SPERM MORPHOLOGY-MOUSE EPA GENETOX PROGRAM 1988, NEGATIVE/LIMITED: CARCINOGENICITY-MOUSE/RAT EPA TSCA SECTION 8(B) CHEMICAL INVENTORY EPA TSCA SECTION 8(D) UNPUBLISHED HEALTH/SAFETY STUDIES EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, JANUARY 2001 NIOSH ANALYTICAL METHOD, 1994: ETHANOL IN BLOOD, 8002 NIOSH ANALYTICAL METHOD, 1994: ALCOHOLS I, 1400 NTP CARCINOGENESIS STUDIES; ON TEST (TWO YEAR STUDIES), OCTOBER 2000 SECTION 16. - - - - - - - - OTHER INFORMATION- - - - - - - - - -THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT ТΟ BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SIGMA, ALDRICH, FLUKA SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE

Valid 08/2001 - 10/2001 Riedel-de Haen 3050 Spruce St. St. Louis, MO 63178 USA Tel: 314-289-6000 MATERIAL SAFETY DATA SHEET SECTION 1. - - - - - - - - CHEMICAL IDENTIFICATION- - - - - -CATALOG #: 35603 NAME: 2,2',4,5,5'-POLYCHLORINATED BIPHENYL (OEKAN AL) PACKAGE WITH 10MG SECTION 2. - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - -CAS #: 37680-73-2 SECTION 3. - - - - - - - HAZARDS IDENTIFICATION - - - - - - - -LABEL PRECAUTIONARY STATEMENTS TOXIC MAY CAUSE CANCER. TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TARGET ORGAN(S): LIVER SKIN DO NOT BREATHE VAPOR. KEEP CONTAINER TIGHTLY CLOSED IN A COOL WELL-VENTILATED PLACE. WEAR SUITABLE PROTECTIVE CLOTHING, GLOVES AND EYE/FACE PROTECTION. IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE). IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN IMMEDIATELY. IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN. IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN. IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN. SECTION 5. - - - - - - - - FIRE FIGHTING MEASURES - - - - - - - -EXTINGUISHING MEDIA

WATER SPRAY. CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM. SPECIAL FIREFIGHTING PROCEDURES WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES. UNUSUAL FIRE AND EXPLOSIONS HAZARDS EMITS TOXIC FUMES UNDER FIRE CONDITIONS. SECTION 6. - - - - - - ACCIDENTAL RELEASE MEASURES- - - - -WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES. ABSORB ON SAND OR VERMICULITE AND PLACE IN CLOSED CONTAINERS FOR DISPOSAL. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE AREA. SECTION 7. - - - - - - - - HANDLING AND STORAGE- - - - - - - - -REFER TO SECTION 8. SECTION 8. - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - -USE ONLY IN A CHEMICAL FUME HOOD. SAFETY SHOWER AND EYE BATH. WASH CONTAMINATED CLOTHING BEFORE REUSE. WASH THOROUGHLY AFTER HANDLING. DO NOT BREATHE VAPOR. DO NOT GET IN EYES, ON SKIN, ON CLOTHING. AVOID PROLONGED OR REPEATED EXPOSURE. NIOSH/MSHA-APPROVED RESPIRATOR. COMPATIBLE CHEMICAL-RESISTANT GLOVES. CHEMICAL SAFETY GOGGLES. KEEP TIGHTLY CLOSED. STORE IN A COOL DRY PLACE. SECTION 9. - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - -DATA NOT AVAILABLE SECTION 10. - - - - - - - - STABILITY AND REACTIVITY - - - - - - - -STABILITY STABLE. INCOMPATIBILITIES STRONG OXIDIZING AGENTS HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS CARBON MONOXIDE, CARBON DIOXIDE HYDROGEN CHLORIDE GAS HAZARDOUS POLYMERIZATION WILL NOT OCCUR. ACUTE EFFECTS CAUSES SKIN IRRITATION. CAUSES EYE IRRITATION. MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT. TOXIC BY INHALATION, INGESTION OR SKIN ABSORPTION. CHRONIC EFFECTS POSSIBLE CARCINOGEN.

```
TARGET ORGAN(S):
   LIVER
    SKIN
SECTION 12. - - - - - - - - ECOLOGICAL INFORMATION - - - - - - - -
    DATA NOT YET AVAILABLE.
SECTION 13. - - - - - - - DISPOSAL CONSIDERATIONS - - - - - -
    DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A
    CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.
    OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.
SECTION 14. - - - - - - - TRANSPORT INFORMATION - - - - - - -
    CONTACT SIGMA CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION.
SECTION 15. - - - - - - - REGULATORY INFORMATION - - - - - - -
 EUROPEAN INFORMATION
   EC INDEX NO: 602--03-9--0
    TOXIC
   R 45
   MAY CAUSE CANCER.
   R 23/24/25
   TOXIC BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.
   R 36/37/38
    IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
    S 23
   DO NOT BREATHE VAPOR.
   S 3/7/9
   KEEP CONTAINER TIGHTLY CLOSED IN A COOL WELL-VENTILATED PLACE.
    S 36/37/39
   WEAR SUITABLE PROTECTIVE CLOTHING, GLOVES AND EYE/FACE
   PROTECTION.
    S 45
    IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE
    IMMEDIATELY (SHOW THE LABEL WHERE POSSIBLE).
SECTION 16. - - - - - - - - OTHER INFORMATION- - - - - - - - - -
    THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT
ТΟ
    BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. SIGMA, ALDRICH,
    FLUKA SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING
    OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE
OR
    PACKING SLIP FOR ADDITIONAL TERMS AND CONDITIONS OF SALE.
    COPYRIGHT 2001 SIGMA-ALDRICH CO.
    LICENSE GRANTED TO MAKE UNLIMITED PAPER COPIES FOR INTERNAL USE ONLY
```

SUNOX -- DRY ICE - CARBON DIOXIDE, TECHNICAL MATERIAL SAFETY DATA SHEET SUNOXNSN: 6830011656174 Manufacturer's CAGE: 6Z872 Part No. Indicator: A Part Number/Trade Name: DRY ICE \_\_\_\_\_ General Information \_\_\_\_\_ Item Name: CARBON DIOXIDE, TECHNICAL Company's Name: SUNOX INC Company's Street: 4236 STATESVILLE RD Company's P. O. Box: 33871 Company's City: CHARLOTTE Company's State: NC Company's Country: US Company's Zip Code: 28233 Company's Emerg Ph #: 704-596-6262 Company's Info Ph #: 704-596-6262 Record No. For Safety Entry: 012 Tot Safety Entries This Stk#: 013 Status: SMJ Date MSDS Prepared: 100CT88 Safety Data Review Date: 07DEC93 MSDS Serial Number: BVBLZ Hazard Characteristic Code: NK \_\_\_\_\_ Ingredients/Identity Information \_\_\_\_\_ Proprietary: NO Ingredient: CARBON DIOXIDE Ingredient Sequence Number: 01 NIOSH (RTECS) Number: FF6400000 CAS Number: 124-38-9 OSHA PEL: 10000PPM; 30000 STEL ACGIH TLV: 5000 PPM; 30000 STEL \_\_\_\_\_ Proprietary: NO Ingredient: MATLS TO AVOID: MAGNESIUM, ALUMINUM, TITANIUM/ZIRCONIUM), THEIR HYDRIDES & MATLS LIKE DIETHYL MAGNESIUM, MOIST (ING 3) Ingredient Sequence Number: 02 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE -----Proprietary: NO Ingredient: ING 2:CESIUM OXIDE/LITHIUM ACETYLIDE W/AMMONIA CAN IGNITE IN CO\*2 ATM. DRY ICE CAN FORM SHOCK SENSITIVE MIX WITH (ING 4) Ingredient Sequence Number: 03 NIOSH (RTECS) Number: 9999992Z

OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 3:SODIUM, POTASSIUM OR SODIUM-POTASSIUM ALLOY. Ingredient Sequence Number: 04 NIOSH (RTECS) Number: 99999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: EFTS OF OVEREXP:COULD RSLT IN FROSTBT/CRYOGENIC (FREEZE) "BURNS." CNTCT W/LIQ/SOLID CAN PRDCE FROSTBT & FREEZE (ING 5) Ingredient Sequence Number: 05 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 5:BURNS. EFTS ARE CHANGE IN COLOR OF SKIN TO GRAY/WHITE POSS FOLLOWED BY BLISTERING. CO\*2 IS MOST POWERFUL (ING 7) Ingredient Sequence Number: 06 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 6:CEREBRAL VASODIALTOR KNOWN. INHAL LG CONC CAUSES RAPID CIRCULATORY INSUFFICIENCY LEADING TO COMA & DEATH. (ING 8) Ingredient Sequence Number: 07 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 7: CHRONIC, HARMFUL EFTS ARE NOT KNOWN FROM RPTD INHAL OF LOW (3-5 MOLAR %) CONC. Ingredient Sequence Number: 08 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO FROSTBT:FLUSH AFFECTED AREAS W/LUKEWARM WATER. DO (ING 10) Ingredient Sequence Number: 09 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_

Proprietary: NO

Ingredient: ING 9:NOT USE HOT WATER. MD SHOULD SEE PROMPTLY IF CRYOGENIC "BURN" HAS RSLTD IN BLISTERING OF DERMAL SURF/DEEP (ING 11) Ingredient Sequence Number: 10 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 10:TISS FREEZING. PROMPT MED ATTN MANDATORY IN ALL CASES OF OVEREXP. RESCUE PERS SHOULD BE EQUIPPED WITH (ING 12) Ingredient Sequence Number: 11 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE ------Proprietary: NO Ingredient: ING 11:NIOSH/MSHA SELF-CONTAINED BREATHING APPARATUS. Ingredient Sequence Number: 12 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: WASTE DISP METH: HVR/AIR. DO NOT PUT DRY ICE IN CLSD CNTNR WHERE EVOLVED GAS CANNOT ESCAPE! REMOVE SCRAP SOLID (ING 14) Ingredient Sequence Number: 13 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: ING 13: ("SNOW" OR "DRY ICE") TO HOOD W/FORCED VENT OR TO A REMOTE OUTSIDE AREA. ALLOW SOLID TO SUBLIME. Ingredient Sequence Number: 14 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: HNDLG/STOR PREC:WHICH OPEN FROM TOP HAVING LOOSE-FITTING LIDS SO CO\*2 VAPOR FROM SUBLIMATION OF SOLID MAY BE (ING 16) Ingredient Sequence Number: 15 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE ------Proprietary: NO Ingredient: ING 15:ALLOWED TO ESCAPE INTO ATM. FOR ADDNL HNDLG & STOR RECOM SEE CGA PAMPHLETS P-1 & G-6. Ingredient Sequence Number: 16

NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Proprietary: NO Ingredient: OTHER PREC:W/MOST PLASTICS & ELASTOMERS. ALSO SEE CGA PAMPHLET G-6.3. Ingredient Sequence Number: 17 NIOSH (RTECS) Number: 9999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE ------Proprietary: NO Ingredient: OTHER PROT EQUIP: TEMP) TO PVNT FREEZE BURNS & FROSTBT IF MORE THAN MOMENTARY CNTCT W/CO\*2 AT LOW TEMP IS POSS. Ingredient Sequence Number: 18 NIOSH (RTECS) Number: 99999992Z OSHA PEL: NOT APPLICABLE ACGIH TLV: NOT APPLICABLE \_\_\_\_\_ Physical/Chemical Characteristics \_\_\_\_\_ Appearance And Odor: WHITE OPAQUE SOLID; COLORLESS, ODORLESS GAS. Boiling Point: -109F,-79C Melting Point: -70F, -57C Vapor Pressure (MM Hg/70 F): 844.7 PSIA Vapor Density (Air=1): 0.1144 Solubility In Water: COEFFICIENT = 0.8704 \_\_\_\_\_ Fire and Explosion Hazard Data \_\_\_\_\_ Flash Point: N/A Lower Explosive Limit: N/A Upper Explosive Limit: N/A Extinguishing Media: NONFLAMMABLE, INERT GAS. Special Fire Fighting Proc: USE NIOSH/MSHA APPRVD SCBA & FULL PROT EQUIP(FP N). USE WATER SPRAY TO COOL FIRE-EXPOS CNTNRS TO PVNT RUPTURE. MATL IS NON-COMBUST. IT CAN BE USED AS (SUPDAT) Unusual Fire And Expl Hazrds: WATER SPRAY IS NOT EFTIVE FOR USE ON FIRES INVOLVING CHEM THAT HAVE THEIR OWN O\*2 SUPPLY (I.E. CELLULOSE NITRATE)/ON FIRES INVOLVING REACTIVE METALS (SUPDAT) \_\_\_\_\_ Reactivity Data \_\_\_\_\_ Stability: YES Cond To Avoid (Stability): DECOMPOSES TO CO & O\*2 WHEN HEATED ABOVE 1700C. WILL REACT W/ALKALINE MATLS TO FORM CARBONATES & BICARBONATES. Materials To Avoid: EXPLO CAN OCCUR WHEN CNTCTS MIX OF SODIUM PEROXIDE W/ ALUMINUM/MAGNESIUM. REACTIVE METALS (SUCH AS ALKALI METALS (ING 2) Hazardous Decomp Products: CARBON MONOXIDE. FORMS CARBONIC ACID IN THE

PRESENCE OF WATER. Hazardous Poly Occur: NO Conditions To Avoid (Poly): NOT RELEVANT \_\_\_\_\_ Health Hazard Data \_\_\_\_\_ LD50-LC50 Mixture: NONE SPECIFIED BY MANUFACTURER. Route Of Entry - Inhalation: YES Route Of Entry - Skin: YES Route Of Entry - Ingestion: NO Health Haz Acute And Chronic: NERVOUS SYS CONTROL OF RESP IS DEPENDENT ON CO\*2 LEVEL BREATHED IN AIR. BY REDUCING O\*2 LEVEL IN AIR, CO\*2 CAN CAUSE SUFFOCATION. SYMP OF OVEREXP INCL HDCH, DIZZ, SHORTNESS OF BREATH, MUSC WEAK, DROW & RINGING IN EARS. INHAL:LOW CONC (3-5 MOLAR %) CAUSE INCR RESP & HDCH. 8-15 MOLAR % CONC CAUSE HDCH, (EFTS OF OVEREXP) Carcinogenicity - NTP: NO Carcinogenicity - IARC: NO Carcinogenicity - OSHA: NO Explanation Carcinogenicity: NOT RELEVANT Signs/Symptoms Of Overexp: HLTH HAZ:NAUS & VOMIT WHICH MAY LEAD TO UNCON IF NOT MOVED TO OPEN AIR/GIVEN 0\*2. HIGH CONC CAUSE RAPID CIRCULATORY INSUFFICIENCY LEADING TO COMA & DEATH. WHEN REFRIGERATED LIQ CO\*2 IS VAPORIZED THRU AN ORIFICE, IT CAN FORM SOLID PARTICLES OF CO\*2 ("SNOW"/"DRY ICE" PWDR). CONTINUOUS DERMAL CNTCT W/COLD SNOW (ING 5) Med Cond Aggravated By Exp: NONE SPECIFIED BY MANUFACTURER. Emergency/First Aid Proc: INGEST:CALL MD IMMED(FP N). EYES:IMMED FLUSH W/POTABLE WATER FOR MIN OF 15 MIN, SEEK ASSISTANCE FROM MD(FP N). INHAL:IF CONSCIOUS, ASSIST TO UNCONTAMD AREA & INHALE FRESH AIR. QUICK REMOVAL FROM AREA IS MOST IMPORTANT. IF UNCON, MOVE TO UNCONTAMD AREA, GIVE MOUTH-TO-MOUTH RESUSCITATION & SUPPLEMENTAL O\*2. ASSURE THAT VOMITED MATL DOES NOT OBSTRUCT AIRWAY BY USE OF POSITIONAL DRAINAGE. (ING 9) \_\_\_\_\_ Precautions for Safe Handling and Use \_\_\_\_\_\_ Steps If Matl Released/Spill: EVACUATE AREA OF MAJOR SPILL/RELS OF CO\*2. NOTIFY SFTY PERS. PROVIDE VENT. CLEAN-UP PERS NEED SPECIAL TRAINING & PROT AGAINST CNTCT W/VERY COLD MATL/EXCESSIVE INHAL OF GASEOUS CO\*2. Neutralizing Agent: NONE SPECIFIED BY MANUFACTURER. Waste Disposal Method: DISP MUST BE I/A/W FED, STATE & LOCAL REGS(FP N). ALLOW GAS TO BLEED OFF AT MOD RATE/SOLID TO SUBLIME TO WELL VENT AREA. INSULATED STOR CNTNR SHOULD BE IN AREA W/ADEQ VENT TO PVNT ACCUM OF CO\*2 VAPS ABOVE TWA. CO\*2 VAP ARE APPROX 1 1/2 TIMES (ING 13) Precautions-Handling/Storing: SOLID CO\*2 GENERALLY DELIVERED IN KRAFT-PAPER-WRAPPED BLOCKS WEIGHING APPROX 50 LBS & APPROX 1/2 CUBIC FT IN VOL. STORE IN INSULATED CNTNRS (ING 15) Other Precautions: DRY CO\*2 CAN BE HNDL W/MOST COMMON STRUCTURAL MATLS. MOIST CO\*2 IS CORR BY ITS FORM OF CARBONIC ACID. FOR APPLICATNS 316,309 & 310 STAINLESS STEELS MAY BE USED AS WELL AS HASTELLOY A, B & C & MONEL. AT NORM TEMP, CO\*2 IS COMPATIBLE (ING 17) \_\_\_\_\_

Control Measures \_\_\_\_\_ Respiratory Protection: PROVIDE NIOSH/MSHA APPRVD SUPPLIED-AIR OR SELF-CONTAINED RESP FOR USE IN NON-ROUTINE/EMER SITUATIONS W/EXPOS ABOVE TLV. FULL FACEPIECE IS REQD FOR CONC > 10%. PROVIDE STANDBY PERSON(S) W/RESCUE EQUIP IF WORK REQD AT > 15% CO\*2 IN AIR. Ventilation: GENERAL AND LOCAL EXHAUST VENTILATION TO MEET TLV REQUIREMENTS. Protective Gloves: LOOSE FITTING, INSULATED GLOVES. Eye Protection: ANSI APPRVD CHEM WORK GOGGLES (SUP DATA) Other Protective Equipment: SFTY SHOES, SOLID CO\*2 HNDLG "TONGS." MAY REQ ADDNL PROT CLTHG (APRON, FSHLD, ETC WHICH ARE RESISTANT TO LOW (ING 18) Work Hygienic Practices: NONE SPECIFIED BY MANUFACTURER. Suppl. Safety & Health Data: FIRE FIGHT PROC:FIRE EXTING AGENT PRIMARILY FOR ITS SMOTHERING EFT (REDUCTION OF O\*2 CONC TO PT WHERE IMMED ATM CANNOT SUPPORT COMBUST). EXPLO HAZ: (SUCH AS POTASSIUM, SODIUM, MAGNESIUM, ALUMINUM, TITANIUM & ZIRCONIUM) OR THEIR HYDRIDES AS THESE MATLS CAN DECOMPOSE CARBON DIOXIDE. EYE PROT: W/FULL LNGTH FCSHIELD (FP N). \_\_\_\_\_ Transportation Data \_\_\_\_\_ Trans Data Review Date: 93337 \_\_\_\_\_ Disposal Data \_\_\_\_\_ \_\_\_\_\_ Label Data Label Required: YES Technical Review Date: 07DEC93 Label Date: 03DEC93 Label Status: G Common Name: DRY ICE Chronic Hazard: NO Signal Word: WARNING! Acute Health Hazard-Moderate: X Contact Hazard-Moderate: X Fire Hazard-None: X Reactivity Hazard-None: X Special Hazard Precautions: ACUTE: NERVOUS SYSTEM CONTROL OF RESP IS DEPENDENT ON CARBON DIOXIDE LEVEL BREATHED IN AIR. BY REDUCING OXYGEN LEVEL IN AIR, CARBON DIOXIDE CAN CAUSE SUFFOCATION. SYMP OF OVEREXP INCLUDE HEADACHE, DIZZINESS, SHORTNESS OF BREATH, MUSCULAR WEAKNESS, DROWSINESS & RINGING IN EARS. INHAL:LOW CONC (3-5 MOLAR %) CAUSE INCR RESP & HEADACHE. 8-15 MOLAR % CONC CAUSE HEADACHE, NAUSEA & VOMIT WHICH MAY LEAD TO UNCONSCIOUSNESS. HIGHER CONC CAUSE RAPID CIRCULATORY INSUFFICIENCY LEADING TO COMA & DEATH. CONTINUOUS DERMAL CONTACT WITH LIQUID/SOLID PARTICLES OF CARBON DIOXIDE COULD RESULT IN FROSTBITE & FREEZE BURNS. CHRONIC: NONE LISTED BY MANUFACTURER. Protect Eye: Y

Protect Skin: Y Protect Respiratory: Y Label Name: SUNOX INC Label Street: 4236 STATESVILLE RD Label P.O. Box: 33871 Label City: CHARLOTTE Label State: NC Label Zip Code: 28233 Label Country: US Label Emergency Number: 704-596-6262

## **B.** Fish Identification

## **B.1** Brown Bullhead (Ameiurus nebulosus)

The brown bullhead is a member of the catfish family (Ictaluridae) that typically ranges from 8 to 14 inches in length at maturity (Smith, 1985). Traits of catfish (family Ictaluridae) include a body that lacks scales, the presence of an adipose fin, four pairs of barbels (whiskers) on the head, and a heavy spine on the leading edge (anterior) of the dorsal and pectoral fins. One pair of barbels are present on the snout, two pairs are present on the chin, and one pair are present on the distal ends of the maxillary. The family Ictaluridae is separated into two or three genera, depending on the reference used. The remaining group of catfish, including several species of madtom and the stonecat is in the genus *Noturus*. There are three species of bullhead in New York, including the yellow bullhead (*Ictalurus natalis*), brown bullhead, and the black bullhead (*Ictalurus melas*).

Brown bullhead are olive to black on the dorsal surfaces and pale white or yellow on the ventral surfaces (Smith, 1985). The fins are dark, and the basal third of the anal fin is as dark as the rest of the fin. The barbels are gray to black, although those on the base of the chin may be pale at the base. The dorsal fin spine is strongly serrated. The caudal fin is square (not forked) with rounded corners. The adipose dorsal fin is flag-like (adnexed) and distinctly separate from the caudal fin.

Key distinguishing features separating the three bullhead species from other catfish species include the following:

- Bullhead have a flag-like (adnexed) adipose fin, whereas madtom and stonecat have a keel-like (adnate) adipose dorsal fin (Smith, 1985).
- Bullhead have a round, square, or slightly forked tail, whereas white and channel catfish have a deeply forked tail (Smith, 1985).

The brown bullhead is differentiated from yellow and black bullhead based on the color of chin barbels, color of fin membranes, amount of serration on the dorsal fin spine, and number of anal fin rays.

Brown bullhead are distinguished from yellow bullhead by the following:

Brown bullhead have dark chin barbels, although the base of the chin barbels may be light-colored. Yellow bullhead have white or yellow chin barbels (Scott and Crossman, 1973; Smith, 1985).

- Brown bullhead have dark fin membranes, especially the basal third of the anal fin is as dark as the rest of the fin. Yellow bullhead have light fin membranes, but the margins may be darker (Smith, 1985).
- Brown bullhead have 21 to 24 anal fin rays (including the 2 anterior rudimentary fin rays), whereas yellow bullhead have 24 to 27 anal fin rays (Scott and Crossman, 1973; Smith, 1985).

Brown bullhead are distinguished from black bullhead by:

- Brown bullhead have a strong, definitive servation on the posterior margin of the dorsal fin spine, whereas black bullhead have a nearly smooth dorsal fin spine (Scott and Crossman, 1973; Smith, 1985).
- Brown bullhead have 21 to 24 anal fin rays (including the 2 anterior rudimentary fin rays), whereas black bullhead have 17 to 21 anal fin rays (Scott and Crossman, 1973; Smith, 1985).
- Brown bullhead have 13 to 16 gill rakers, whereas black bullhead have 15 to 24 gill rakers (Smith, 1985).

Brown bullhead spawn in late spring to summer when water temperatures reach 21°C (70°F) (Scott and Crossman, 1973). Nests are built in small depressions or burrow under overhanging banks, under docks, or near any other type of protection. Maturity is reached at age 2 and the usual life span is 6 or 7 years (Smith, 1985). Brown bullhead feed at night mainly on food associated with the bottom sediments. Their diet consists of benthic macroinvertebrates, crustaceans, small fish, and some plant material. Brown bullhead are found in pools and sluggish runs over soft substrates in creeks and rivers; they are also found in lakes and ponds.

## **B.2** Smallmouth Bass (Micropterus dolomieui)

Smallmouth bass are a robust species in the sunfish family (Centrarchidae). Sunfish, including smallmouth and largemouth, have scales and a continuous dorsal fin that comprises an anterior section with numerous spines and a posterior section that has fin rays. The longest fin rays of the dorsal fin are in the middle of this fin ray section. There are three or more anal fin spines (Smith, 1985).

Smallmouth bass are distinguished from other sunfish by several features. The body length is 2 to 3 times the length of the greatest depth of the body. The body is completely scaled, with more than 55 scales along the lateral line (Smith, 1985). The dorsal fin has approximately 10 spines anteriorly and 12 to 15 fin rays posteriorly (Scott and Crossman, 1973). The shortest

dorsal fin spine is more than half as long as the longest dorsal fin spine. The most posterior end of the maxillary, with the mouth closed, extends to the middle of the pupil of the eye. The color is generally greenish bronze to brown, with the dorsal surface darker and the sides lighter, and the ventral surface is greyish or brownish white (Smith, 1985). There are usually 8 to 11 narrow vertical bars below the lateral line and fewer, wider vertical bars above the lateral line. Individuals can reach 9 pounds or more, and 20 inches or more in New York (Smith, 1985).

The black bass (smallmouth and largemouth bass) can be distinguished from other sunfish by the following:

- Black bass have small scales, with more than 55 scales along the lateral line, whereas other sunfish have relatively large scales, with fewer than 53 scales along the lateral line (Smith, 1985).
- ▶ Black bass have a relatively compressed and elongate body with a length that is 3 to 5 times the maximum depth of the body. Other sunfish are more compressed laterally with a length that is 2 to 3 times the maximum depth of the body (Smith, 1985).

Key distinguishing features separating smallmouth bass from largemouth bass are as follows:

- ▶ The posterior end of the maxillary bone (with the mouth closed) of the smallmouth bass reaches to below the middle of the pupil eye, whereas the end of the maxillary of the largemouth bass reaches beyond the posterior border of the eye (Scott and Crossman, 1973; Smith, 1985).
- The shortest dorsal fin spine is more than half as long as the longest dorsal fin spine in smallmouth bass, whereas the shortest dorsal fin spine is less than half as long as the longest fin spine in largemouth bass (Scott and Crossman, 1973; Smith, 1985).
- Smallmouth bass are brown or brassy with a uniform pattern or with one or two series of vertical bars on a lighter background; largemouth bass are greenish with a prominent longitudinal stripe along the middle of the side (Smith, 1985).
- Smallmouth bass have a unified pyloric caeca, whereas largemouth bass have a bifurcated pyloric caeca (Smith, 1985).

The smallmouth bass spawn in the spring when the water temperature reaches 16 to 21°C (60 to 70°F) (Scott and Crossman, 1973). Spawning nests are built in gravel substrate in water 0.6 to 6 meters (2 to 20 feet) deep (Smith, 1985). After the eggs are laid, the male guards the nest until the fry emerge, and then guards the dense school of fry for a short amount of time. Smallmouth bass feed opportunistically on almost any small animal in the water, including insects, crustaceans, tadpoles, frogs, and small fish. Juvenile smallmouth bass feed on zooplankton and

small macroinvertebrates. Smallmouth bass inhabit lakes, ponds, and slow to moderate currents in rivers. They tend to use habitats with clear water and rocky substrate with little vegetation and depositional sediment (Smith, 1985).

## **B.3** Yellow Perch (Perca flavescens)

Similar to walleye, the yellow perch is also a member of the family Percidae (see Section B.4 for characteristics of Percidae). The yellow perch is similar to both walleye and sauger. Yellow perch have uniform-sized teeth arranged in bands, and have 5 to 10 vertical dark bars along the side. The color is yellowish, but darker dorsally and lighter ventrally. The anterior dorsal fin is similar to walleye in that it has a dark patch at the posterior margin. Although the body is scaled, the top of the head lacks scales (Smith, 1985).

Key distinguishing features separating yellow perch from walleye and sauger are as follows:

- Yellow perch have uniform-sized teeth, whereas sauger and walleye have large caniniform teeth (Scott and Crossman, 1973; Smith, 1985).
- Yellow perch have well-defined dark vertical bars, whereas sauger and walleye have irregular dark areas or uniform coloration (Scott and Crossman, 1973; Smith, 1985).
- Yellow perch have 6 to 8 anal fin rays, whereas sauger and walleye have 11 to 13 anal fin rays (Scott and Crossman, 1973; Smith, 1985).

Yellow perch spawn in the spring in water 1.5 to 3 meters (5 to 10 feet) deep when water temperatures reach 7 to 11°C (45 to 52°F). Eggs are laid in sand, gravel, or vegetation and consist of a long gelatinous band. Adults typically reach 25 to 30 cm (10 to 12 inches) and live for 9 years.

Yellow perch feed opportunistically on zooplankton, macroinvertebrates, crayfish, and small fish during the day, and are less active at night. These fish use both lake and river habitats, and are often found in clearer water with vegetation (Smith, 1985).

## References

Scott, W.B. and E.J. Crossman. 1973. Freshwater Fishes of Canada. Canadian Government Publishing Centre, Ministry of Supply and Services Canada, Fisheries Research Board of Canada, Bulletin 184, Ottawa.

Smith, C.L. 1985. *The Inland Fishes of New York State*. New York State Department of Environmental Conservation, Albany, NY.

## C. Field Equipment Lists

## C.1 Equipment for Collecting Fish by Electroshocking

- Sampling and Analysis Plan
- Fish capture field notebook
- Waterproof ink pens
- Camera wand film
- Detailed maps of each sample location
- Hand-held GPS unit (Garmin GPS 12 or GPS 12XL, Garmin International, Lenexa, Kansas)
- Hand-held compass
- Marine radio for communication with other crews
- Cellular phone
- ► 60-ft trap nets
- Floy tag gun and tags
- Meter measuring board
- Electrofishing boat and motor (gas and oil), oars (2), boat hook, anchor, rope
- Boat trailer with working lights
- Generator (check gas, oil, and connections)
- Electroshocking voltage converter
- Electrodes (anode and cathode)
- Foot pedal, deadman switches (one for each person netting)
- Insulated, long-handled dip nets (3)
- Insulated, short-handled dip nets (2)
- Holding tank or live well (aerator or water circulator)
- Electrically insulated footwear (all crew members)
- Electrically insulated gloves (5,000 V minimum)
- Personnel flotation devices (all crew members) and safety toss
- Thermometer
- First aid kit
- Fire extinguisher
- Tool box
- Depth sounder.

## C.2 Equipment for Dissecting and Collecting Samples from Fish

- Sampling and Analysis Plan
- Fish health assessment field notebook
- Waterproof ink pens
- Camera and film
- Marine radio for communication with fish collection crews
- Cellular phones
- First aid kit
- Fire extinguisher
- Bound copy of Appendix B (species descriptions)
- Portable Canopy
- Tables & Stools
- Short-handled dip net (2) (for handling of all live fish, one per crew)
- ▶ 60 to 80 L cooler (2) (fish processing station live well)
- ▶ 20 to 40 L cooler (2) (icewater tank, one for each fish health sampling crew)
- Aerator (2)
- Airstones & tubing
- Water bucket (with liter marks on the inside)
- Meter measuring board with 1 mm divisions (2) (for measuring fish, one per fish health crew)
- Ohaus portable electronic balance and 500 g calibration standard (±20 g accuracy) (2) (for fish wet weight, one balance per fish health crew)
- Ohaus portable electronic balance and 10 g calibration standard (±0.01 g accuracy) (2) (for tissue weights, one balance per fish health crew)
- Weigh paper and boats
- Scale knife (2) (for removing scales, one per fish health crew)
- Wire side cutters (2) (for removing dorsal fin spines, one per fish health crew)
- Polycarbonate cutting boards
- Dissecting scissors (1/fish, may be cleaned; for opening body cavity, cutting intestine and gill)
- Probe (1/fish, may be cleaned; for internal necropsy)
- Medium curved-tip forceps (1/fish, may be cleaned; for handling and manipulating tissues)
- Syringe (5 cc) with 22 G needle (1/fish, for collection of bile)
- Sterile bacterial loop (1/fish, for kidney bacteria sample)
- Sterile scalpel blade (1-#11, 1-#22 per fish, for tissue removal)
- Scalpel handles (2/fish, may be cleaned; one for each blade)
- Surgical steel razor blades (4/fish, for cutting tissues)
- Hemostat clamp (1/fish for scalpel blades)

- ▶ 1 quart heavy duty Ziploc<sup>TM</sup> freezer bags (1/fish for clean dissection tools)
- Pesticide grade methanol (for decontamination of tools)
- ASTM Type II reagent water or distilled water (for decontamination of tools)
- Wash bottles, Teflon, prelabeled for methanol and distilled water (for decontamination)
- Wash basin (1/team, for nondisposable used dissection tools)
- Aluminum foil (for clean tools)
- Disposable nitrile gloves (several sizes, 2 pairs per fish)
- ► Kimwipes® (many boxes)
- Sharps container
- Garbage cans (30 gal with 30-gal heavy duty trash bags)
- Scale envelopes (1/scale or spine sample)
- 4 ml amber vials, with Teflon-lined caps (at least 1/fish for gall bladder bile PAH metabolite samples)
- ▶ 20 ml & 40 ml vials, certified clean, with Teflon-lined caps (at least 1/fish for liver contaminant samples)
- ▶ 125, 250 & 500 ml jars, certified clean (at least 1/fish for fillet contaminant samples)
- 5.5-mL snap-cap bullet tubes prepared with HBSS (Hank's balanced salt solution) (1/fish, for spleen virology sample)
- BHIA slant culture tubes, prepared (1/fish, for kidney bacteriology sample)
- Sterile loops (for kidney bacteriology)
- Surgipath "super cassettes" (at least 8/fish for histopathological samples)
- Gauze bags for tissues
- TBD ml jars (1/fish for histopathological samples)
- Dietrich's fixative (histopathology fixative)
- Marking pens and histology pencils
- Index cards (for fillet sample and scale/spine sample label)
- Prelabeled self adhesive sample labels (for each sample, including QA/QC)
- Clear packing tape (for securing sample labels and sealing shipping containers)
- Small coolers (6) (for bacterial cultures, viral cultures, and scales and spine samples)
- Large hazardous materials coolers (9) (for residue, parasite, histopathology samples)
- Wet ice (for viral samples)
- Dry ice (for residue samples)
- Hazardous materials shipping labels (fixatives, dry ice, flammable materials)
- Chain of custody forms
- Plastic bags (for protecting chain of custody forms)
- Fed Ex airbills, prepared
- Custody seals (for sealing containers for chain of custody)
- Cryo-gloves (for handling dry-ice frozen materials)
- 9 V and D batteries

- Paper towels Parafilm ▶
- ▶
- Flashlight/lanterns/headlamps.

## D. Hudson River Phase I Fish Health Assessment Field Standard Operating Procedures (SOPs)

#### List of SOPs

SOP 1	Measuring fish length and weight
SOP 2	Applying Floy tags to fish
SOP 3	Setting and operating hand-held Global Positioning System (GPS) units
SOP 4	Decontaminating Re-useable Dissection and Sample Collection Tools
SOP 5	Preparing Sample Preservative Solutions
SOP 6	Using Scales and Spines for Estimating Ages of Yellow Perch, Smallmouth Bass, and Brown Bullhead from the Hudson River and Oneida Lake, NY
SOP 7	Detection and Identification of Bacterial and Viral Fish Pathogens from Yellow Perch, Smallmouth Bass, and Brown Bullhead from the Hudson River, NY

## **SOP 1: Measuring Fish Length and Weight**

Before taking measurements of each fish, the measuring board will be inspected to ensure that the board is in good working order. Any visible water will be removed from the balance, and the calibration checked with at least two standard calibration weights. The weight of the calibration weight will be recorded before and after calibration adjustment of the balance.

#### **Measuring Length**

Length will be measured on a measuring board that has a linear scale (mm) with a rigid head piece.

- 1. Place a fish on the measuring board on its right side, with its head facing the recorder's left.
- 2. Hold the head of the fish firmly against the head piece before measuring the fish.
- 3. Measure the total length, fork length, and standard length to the nearest millimeter. Total length is defined as the length from the most anterior part of the fish to the tip of the longest caudal fin ray.

#### **Measuring Weight**

Weight will be measured on a portable scale.

- 1. Calibrate the scale at least two times per sampling day.
- 2. Clean and dry the scale.
- 3. Tare the weight of the weighing tub.
- 4. Wipe any excess water from the fish with a disposable paper towel, and gently place the fish into the weighing tub.
- 5. Record the balance reading to the nearest gram.

redacted

## **SOP 2: Applying Floy Tags to Fish**

Each fish of a target species that is of an acceptable size will be tagged with a unique three-digit identification tag. The procedure for tagging fish is as follows:

- 1. Load the tag into the tagging gun and record the tag number in the field notebook.
- 2. Place the fish into the v-trough holding board in the swimming position with the head facing to your left.
- 3. Hold the fish on both sides just behind the operculum (gill cover) with your left hand.
- 4. Insert the needle of the tagging gun into the musculature approximately 1 cm below and 2 cm behind the most anterior (most forward) portion of the dorsal fin. Angle the needle so the tip will pass the dorsal-ventral midline (i.e., will pass between the bones that produce the dorsal fin spines and rays).
- 5. Compress the trigger of the tagging gun. With the trigger compressed, turn the gun 1/8th turn counterclockwise and gently pull the needle out of the musculature. Keep the trigger compressed while removing the needle.
- 6. Tug on the tag to ensure that the tag will not come out.
- 7. Remove any scales, tissue, or debris from the needle.

# **SOP 3: Operating the Hand-Held Global Positioning System** (GPS) Units

A Garmin (Garmin International, Lenexa, Kansas) GPS 12 or GPS 12XL hand-held GPS unit will be used to identify fish capture locations during the study. The use and operation of the individual unit is provided in the instruction manual provided by the manufacturer. This SOP provides the settings required for consistency of GPS coordinates between units, and for accuracy of identifying locations on maps and for identification of locations during future sampling activities.

The unit allows for GPS locations to be in several different north-south coordinate systems with several different map datum reference protocols. The units will be set up to record UTM (Universal Transverse Mercator) coordinates using the WGS 84 (World Geodetic System 1984) map datum reference points. With this system, each unit refers to one meter east-west or north-south. Under this protocol, coordinates will be in the form of:

13T 0386797

4134887

where:

"13T" refers to the unique global zone of the coordinates.

The top set of numbers (0386797) is the east-west position within the zone.

The bottom set of numbers (4134887) is the north-south position within the zone.

Record the UTM coordinates directly on the data sheet.

# **SOP 4: Decontaminating Re-useable Dissection and Sample Collection Tools**

#### Materials

- Clean potable water.
- HPLC (High Performance Liquid Chromatography)-grade or pesticide-grade methanol.
- Analyte-free distilled water, ASTM (American Society for Testing and Materials) Type-II reagent grade water, or HPLC-grade water.
- Phthalate-free plastic or Teflon containers.
- Alconox soap.

#### Decontamination (for equipment used to obtain samples for contaminant analysis)

- 1. Wash in Alconox soap solution.
- 2. Rinse using clean potable water.
- 3. Rinse thoroughly with methanol.
- 4. Rinse thoroughly with analyte-free water from a phthalate-free plastic or Teflon wash bottle.

#### Storage

Decontaminated tools will be stored in aluminum foil wrapping to protect them from exposure to airborne contamination.

#### redacted

## **SOP 5: Preparing Sample Preservative Solutions**

This SOP describes the preparation of HBSS, which is used to preserve tissue samples for analysis of viral infection.

#### **Preparation of HBSS**

The HBSS must be prepared using a sterile technique in a laminar flow hood, using appropriate sterile glassware and pipettes. The HBSS will be prepared and placed into the sample containers before their shipment to the field.

- 1. Combine and mix the following ingredients in a 500 mL sterile glass bottle:
  - a. 450 mL sterile water
  - b. mL NaHCO<sub>3</sub>
  - c. 10 mL Pen/Strep
  - d. mL Mycostatin ( = Nystatin)
  - e. mL Gentamicin
  - f. 50 mL 10X Hanks solution.
- 2. Dispense 2.7 mL into each sterile snap-cap tube.

### SOP 6: Using Scales and Spines for Estimating Ages of Yellow Perch, Smallmouth Bass, and Brown Bullhead from the Hudson River and Oneida Lake, NY

redacted

#### Introduction

The protocols for preparing scales and spines for use in estimating ages of freshwater fishes taken from North American waters are described below. These protocols are based on standard protocols, professional experience in estimating fish ages, recommendations from the primary literature, and consultation with other experts on age estimation.

#### Methods

#### Age Estimation

Two experienced individuals will estimate ages independently for each fish. The individual estimates will then be compared for consensus. If age estimates for individual readers agree, then that age will be recorded as the final estimated age. Structures from those fish for which there is not consensus will be re-examined by both individuals simultaneously and a consensus age estimate reached. If the readers cannot reach a consensus, then no final age is recorded (the individual age estimates are reported).

#### Scale Preparation

Scales will be cleaned in water or a mild soap solution to remove tissue, mucous, and debris. Permanent impressions of several scales from each fish will be made on clear acetate using a standard scale press. Acetate slides will be uniquely etched with a fish identification number specified by the sample ID on the scale envelope.

Scale impressions will be viewed on a microfiche reader at either 24 or 48 X magnification, depending on the size of the scale and the ability to discern annuli. Generally, higher magnifications will be used for smaller scale impressions. Higher magnification will be used on impressions of large scales when annuli are difficult to discern. Scale annuli will be identified using criteria described in Carlander (1961).

#### Ictalurid Spines

Remaining skin and connective tissue will be removed from the spine. A high-speed rotary cutting tool will be used to cut 1-3 cross-sections through the articulating process, if present. If

the articulating process is not present, then sections will be cut through the shaft. Both sides of each cross-section will then be polished with 600-grain aluminum oxide paper. Cross sections will be viewed under a variable-magnification, dissecting, light microscope using an independent fiber-optic light source with variable light intensity. Magnification and light intensity will be varied as necessary to generate the clearest image.

#### General Considerations: Spines

The field processing crews will make every effort to separate ictalurid spines at the articulating process. Previous research on ictalurids suggests age estimates from cross-sections from the shaft of spines are lower than those from the articulating process because of loss of earlier annuli as the central lumen forms (Turner, 1980).

#### References

Carlander, K. D. 1961. Variations in re-reading walleye scales. *Transactions of the American Fisheries Society* 90:230-231.

Turner, P. R. 1980. Procedures for age determination and growth rate calculations of flathead catfish. *Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies* 34:253-262.

### SOP 7: Detection and Identification of Bacterial and Viral Fish Pathogens from Yellow Perch, Smallmouth Bass, and Brown Bullhead from the Hudson River, NY

redacted

#### Introduction

The protocols for detecting and identifying bacterial and viral fish pathogens from non-salmonid fishes is briefly described below. These protocols are based on standard protocols used by all U.S. Fish and Wildlife Service Fish Health Centers as described in the National Wild Fish Health Survey Laboratory Procedures Manual. The target bacterial pathogens include: *Aeromonas salmonicida, Yersinia ruckeri, Edwardsiella ictaluri,* and *Renibacterium salmoninarum*. Viruses will include channel catfish virus, and Infectious pancreatic necrosis virus.

#### Methods

#### Field Samples

Each fish will be aseptically sampled in the field in the following ways: An inoculum is sampled aseptically from the kidney using a sterile 1 F1 loop. It is streaked onto and individual slant containing brain heart infusion agar (BHIA).

- 1. Kidney and spleen tissues are collected with clean forceps and placed into a culture tube containing HBSS. Both kidney and spleen from a single fish may be placed into each tube.
- 2. Samples are properly labeled, securely packaged, and shipped on ice overnight to the laboratory.

#### Laboratory Processing

Samples received will be assigned an individual Case History Number. Tubes will be externally disinfected and all sample data recorded onto the Case History Sheet. Sample racks will be labeled with the case number and transported to the appropriate lab for analysis.

Tissues for virology will be homogenized and centrifuged for 20 minutes at 4°C. The supernatant is diluted to 1:100 and inoculated onto the following cell lines: brown bullhead (BB), catfish ovary (CCO), blue gill fin (BGF), fathead minnow (FHM, chinook salmon embryo (CHSE) and epithelio-papilloma of carp (EPC). Cells will be examined daily for cytopathic effect (CPE). If no CPE is observed by 14 days, the cultures are discarded and recorded as negative.

Bacterial cultures are incubated at 20°C and checked daily for growth. Tubes containing growth within 7 days are striated onto a petri plate containing tryptic soy agar (TSA). Representative colonies are isolated onto TSA and tested for morphological and biochemical characteristics.

All pathogens identified by the above described techniques are verified by either serological techniques or by the polymerase chain reaction (PCR) technique.

#### References

Thoesen, J. 1994. Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens. American Fisheries Society, Fish Health Section, Bethesda, MD.

National Wild Fish Health Survey, Laboratory Procedures Manual. March 2000. U.S. Fish & Wildlife Service, Division of Fish Hatcheries, Washington, DC.

# E. Site Location Maps and GPS Coordinates