

Appendix A

Fish Collection Standard Operating Procedures

Appendix A.1
**GLFMSP Base Monitoring Program Sample
Collection SOP**

Great Lakes Fish Monitoring and Surveillance Program

Standard Operating Procedure for the Collection of Fish for the Great Lakes Fish Monitoring and Surveillance Program Base Monitoring Program

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Standard Operating Procedure for the Collection of Fish for the Great Lakes Fish Monitoring and Surveillance Program Base Monitoring Program

1.0 Scope and Application

This standard operating procedure (SOP) is used to collect fish for base monitoring in support of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) administered by the U.S. EPA Great Lakes National Program Office (GLNPO). Field sampling teams perform sample collections every year in the late summer to fall. For each field sampling team, a team leader is identified as the primary contact for study implementation and communication with GLNPO. The number and type of fish targeted for collection will be communicated to the field sampling team leader each year. Each field sampling team must have the required permit to collect the required amount of fish.

Adherence to this SOP will ensure that field sampling activities will be performed the same way every time and that they are standardized for all sampling participants.

2.0 Summary of Method

Lake Trout (*Salvelinus namaycush*) are collected in all lakes and Walleye (*Sander vitreus*) are collected in the western basin of Lake Erie at pre-specified sampling sites. Field personnel are responsible for determining the appropriate sampling techniques for each particular sampling site that may include use of gill nets, cage traps, seines, etc. For each target species, 50 individual fish in a specific size range are collected and sent to the homogenization laboratory for processing. Special precautions are taken to prevent contamination of the fish with any foreign materials and ensure the integrity of the sample. Maxillary bones (from lake trout) or dorsal spines (from walleye) are removed from the fish and placed in scale envelopes. Fish are then immediately placed in polyethylene plastic tubes and labeled appropriately. Fish are packaged in large plastic bags in groups of five, frozen as soon as possible after collection, and maintained at -20°C. Samplers store frozen fish at a secure location until directed to ship frozen fish to the appropriate laboratory. Data regarding each individual fish and associated sample location are recorded on standard forms and sent with the fish to the laboratory. If any questions or concerns arise regarding the collection, storage, or shipping procedures, contact Brian Lenell at (312) 353-4891.

3.0 Sampling Equipment

Fish collection methods can be divided into two major categories, active and passive. Each has advantages and disadvantages. Active collection methods involve a wide variety of sampling devices including electrofishing units, seines, trawls, and boat shockers. Passive collection methods employ a wide array of sampling devices, including gill nets, dip nets, trap nets and cage traps. Passive collection devices (e.g., gill nets) must be checked frequently (e.g., at least once every 24 hours) to ensure a limited time lag between fish entrapment and sample preparation. If fish are in nets for over 24 hours, please ensure only live fish are collected.

Careful and thorough planning is necessary to ensure the efficient and effective completion of the field sample collection task. It is the responsibility of each field sampling team to gather and inspect the necessary sampling gear prior to the sampling event and to inspect the sample packaging and shipping supplies received from the laboratory. Additional inspections include verification that:

- collection nets are free of any potential contaminants,
- collection nets are not tarred, and
- ice chests and other sample storage containers have been scrubbed clean with detergent and rinsed with distilled water prior to use (containers provided by the Data Validation and Verification contractor will be pre-washed and rinsed).

4.0 Collection Procedures

Field sampling personnel choose the collection method and appropriate sampling gear to meet the study objectives pertinent to their fish collection effort. Each field sampling team may determine the sampling technique that best fits the situation. Selection of the most appropriate gear for a particular target lake will be at the discretion of the experienced on-site fisheries biologists or collection personnel.

As soon as fish are obtained via active collection methods, or removed from passive collection devices, they are identified to species. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the water bodies designated for collection. Non-target species collected by the field team should be returned to the water. Field sampling personnel should wear clean nitrile gloves (provided by the Data Validation and Verification contractor) to handle fish and sample handling equipment. Individuals of the selected target species are rinsed in ambient water to remove any foreign material from the external surface and placed in clean holding containers (e.g., live well, buckets) to prevent contamination. The buckets are cleaned according to the procedures outlined in Section 3.0 above. Each fish of the selected target species is weighed to the nearest gram and measured to determine total body length (mm). Maximum body length should be measured and is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally). Maxillary bones (lake trout) or dorsal spines (walleye) are removed from the fish and placed in scale envelopes. Note: Measurements must be collected and recorded prior to the removal of maxillary bones or dorsal spines. Detailed sample handling instructions are provided in Section 9.0.

5.0 Species, Size, and Number of Fish

The field sampling teams collect 50 lake trout or walleye in the size range specified in Table 1 at each location cited in Section 6.0, Table 2 (unless otherwise specified by the Data Validation and Verification contractor). If sufficient numbers of fish within the designated size range cannot be obtained by a reasonable sampling effort, it is acceptable to go outside the size range, approximately 5% above or below. However, field sampling teams should try to include similar numbers of fish above and below the designated size range so that the mean size of fish remains near the middle of the range.

Table 1. Species Collected by Lake and Even or Odd Year

Lake	Lake Trout	Walleye
Lake Erie	O	E
Lake Huron	E,O	—
Lake Michigan	E,O	—
Lake Ontario	E,O	—
Lake Superior	E,O	—
Size Range (mm)	600 to 700	400 to 500

E = even year collection
O = odd year collection

Fish are placed singly in polyethylene tubing secured with cable ties, labeled, and then packaged in large plastic bags in groups of five.

All fish collected must meet the following criteria:

- all be of the same species,
- satisfy any legal requirements of harvestable size (or weight), or at least be of consumable size if no legal harvest requirements are in effect,
- be of similar size so that the smallest individual in the group of 50 fish is no less than 75% of the total length of the largest individual, and
- be collected as close to the same time and location as possible, but no more than one week apart.

6.0 Sampling Sites

Grid locations for each station are listed in Table 2. If the specified grid does not contain good fishing grounds or if collection from that grid will cause conflicts with management practices (e.g., excessive impact on native fish versus hatchery produced fish), it is appropriate to collect from grids immediately adjacent to the designated grid. The guiding rule should be that the site sampled represents offshore fishing grounds (i.e., open water populations of fish) and is relatively remote from tributaries or other potential sources of contaminants.

Table 2. Sampling Locations for the Collection of Fish for the GLFMSP

Lake	Grid	Site	Longitude	Latitude
Michigan	2210	Saugatuck	86°25'	42°35'
Michigan	0906	Sturgeon Bay	87°15'	44°45'
Huron	1413	Port Austin	82°45'	44°05'
Huron	0710	Rockport	83°15'	45°15'
Erie	0904	Middle Bass Island	82°55'	41°35'
Erie	0424	Dunkirk	79°35'	42°25'
Ontario	0713	North Hamlin	77°55'	43°25'

Lake	Grid	Site	Longitude	Latitude
Ontario	0623	Oswego	76°15'	43°35'
Superior	1028	Keweenaw Pt.	87°35'	47°25'
Superior	1311	Apostle Islands	90°25'	46°55'

7.0 Sample Integrity and Quality Control

Sample integrity involves preventing loss of target analytes that might be present in the sample and taking precautions to avoid possible introduction of contaminants during handling. The loss of target analytes can be prevented in the field by minimizing the laceration of fish skin. Proper storage of the fish will help to prevent loss of target analytes. All fish collected for this project should be kept in their own fish box separate from other collected fish. The importance of placing the fish in a separate box in order to avoid any contamination from other fish, fuels, or other sources cannot be over-emphasized. Special precautions must be taken by field sampling personnel to prevent contamination of the fish with any foreign materials.

Sources of contamination include the sampling gear, oils and greases on boats, spilled fuel, skin contact, contact with soil or sand, boat motor exhaust, and other foreign materials. All potential sources should be identified prior to and during sample collection, and appropriate measures should be taken to minimize or eliminate them. Examples of preventative measures include the following:

- collection nets should be free of any potential contaminants,
- the use of tarred collection nets is prohibited,
- boats should be positioned so that engine exhaust does not fall on the deck area where samples are being handled,
- ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers originating from the sample control center will be pre-washed and rinsed),
- samples should not be placed directly on dry ice, but should be stored inside plastic tubing first, and
- no PTFE (teflon tape) should come into contact with the sample contents or containers.

The field sampling team leader is responsible for ensuring all sampling equipment is in good working condition and is used properly by field personnel.

8.0 Documentation and Records

Field sampling personnel are required to complete and submit a field recording form and chain-of-custody record at the time that fish are shipped to the homogenization laboratory. Because the sampling effort is a cooperative one involving many different partner agencies and groups, the diligence of the field sampling team in completing the proper records is essential. The field recording form is used to document the sample collection effort and includes specific information regarding each fish specimen including fish sample ID, date of collection, method of collection, estimated collection depth, field collector sample ID, field length, field weight, sex, and fin clip location. The form also is used as a chain-of-custody record to document shipment and handling of all fish from the field personnel to the sample homogenization laboratory. Field sampling teams are provided with *Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program* that provides information on filling out the required form.

The field recording form is designed to capture a unique tracking number for each fish. Specific instructions for assigning the fish sample IDs are included on the form. This tracking number is used by the Data Validation and Verification contractor and the homogenization and analytical laboratories to identify each fish and report results. The fish sample ID includes the following:

- a two-character code for each lake (e.g., LO for Lake Ontario, LM for Lake Michigan),
- the fish species,
- the four digit year of collection,

- the grid number or port code, and
- a sequential number indicating the number of each fish (1-50) from a specific location and year (e.g., F1, F2, F3).

Field team leaders are responsible for reviewing all completed documentation and signing the *Field Recording Form and Chain-of-custody Record* (provided by the Data Validation and Verification contractor).

9.0 Sample Handling, Storage, and Shipping

All fish collected for the GLFMSP should be kept separate from other collected fish in their own fish box. It is very important that all fish collected be placed into a box as soon as possible after they are harvested so as to avoid any contamination from other fish, any oils or fuel, or other sources. Field sampling personnel should wear clean nitrile gloves (provided by the Data Validation and Verification contractor) to handle fish and sample handling equipment. Specific steps for fish collection are provided below, followed by sample handling and shipping instructions.

- 9.1 As soon as fish have been obtained via active or passive collection methods, rinse fish in ambient water to remove any foreign material from the external surface and place fish in holding containers (e.g., buckets, live wells) that have been cleaned according to the specified procedures in Section 3.0. Return any non-target fish or small specimens to the water.
- 9.2 Accurate taxonomic identification is essential in assuring and defining the organisms that have been submitted for analysis. All 50 fish should be of the same species.
- 9.3 Measure each fish to determine total body length. Measure total length of each specimen in millimeters (to the nearest millimeter) from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally). Note: This measurement must be collected and recorded prior to the removal of the maxillary bone or dorsal spine.
- 9.4 Weigh each fish to the nearest gram. Note: This measurement must be collected and recorded prior to the removal of the maxillary bone or dorsal spine.
- 9.5 Prepare a sample label (provided by the Data Validation and Verification contractor) for each fish. If not using labels provided by the Data Validation and Verification contractor, prepare a sample label for each fish that includes all information listed in Table 3. Fill out the *Field Recording Form and Chain-of-custody Record* or wait to fill it out upon completion of sample handling.
- 9.6 Collection of maxillary bones (lake trout) should utilize the following method:
 1. Collect whole maxillary bone from left side of upper jaw.
 2. While removing maxilla, fold the distal end of the maxilla toward tip of snout, then pull proximal end of exposed bone away from snout so that skin/tissue will remain on the snout during removal.
 3. Place in scale envelopes, and label each envelope with the GLNPO fish sample ID (ensure GLNPO fish sample ID on scale envelope matches fish sample ID on labels and field recording form). Allow envelopes to dry for at least 3 nights.
 4. Ship envelopes to the sample homogenization laboratory. Please notify Marian Smith at GDIT before shipping envelopes.

Collection of dorsal spines (walleye) should utilize the following method:

1. Cut the first three dorsal spines flush at the point of attachment with a pair of side cutters. It is critical that the spines are cut flush with the body for accurate aging.
2. Tear the spines free of the membrane that attached them to adjacent spines.
3. Place spines samples in a scale envelope (pointing downward if possible), and label each envelope with the LGNPO fish sample ID (ensure GLNPO fish sample ID on scale envelope matches fish sample ID on labels and field recording form). Allow envelopes to dry for at least 3 nights.
4. Ship envelopes to the sample homogenization laboratory. Please notify Marian Smith at GDIT before shipping envelopes.

- 9.7 To minimize contamination, samples should not be placed directly on dry ice, but should be placed in polyethylene plastic tubes prior to setting on ice. Samplers are provided with pre-cut polyethylene plastic tubes and rolled-up, un-cut plastic tubing. Once fish are measured and weighed, place each fish separately in pre-cut polyethylene plastic tubes. These pre-cut tubes are fastened with a cable tie on one end. Once fish are in the plastic tubes, fasten the other end of the tubes with the additional provided cable ties. If the fish do not fit in the pre-cut tubing, cut the rolled-up, un-cut plastic tubing to the length of the fish and fasten both ends of the tube with cable ties.
- 9.8 Tape the sample label (prepared in Section 9.5) to the outside of the tube, and secure the label by wrapping tape over the label, around the tube, and back over the label.
- 9.9 Ensure that each individually bagged fish is accurately labeled with the information in Table 3. As an additional measure for ensuring fish are easy to identify at the homogenization lab, write the fish sample ID (or just the fish number (F1, F2, etc.) with a permanent marker directly on the plastic tube wherever there is room).

Table 3. Types of Field Data to be Collected & Recorded on Sample Labels

Data Type	Measurement Units or Allowed Entries
Lake Name	Erie, Huron, Michigan, Ontario, Superior
Collector Identification	Vessel and collector's name
Collection Date	MM/DD/YY
Fish Length	Millimeters (mm), total length
Fish Weight	Grams
Fish Sample ID	First Letters of Lake Name [e.g., LS = Lake Superior], Fish Species, Year Fish Collected, Grid #, Fish Sample ID # [e.g., F1, F2, F3] Example: 10 th lake trout collected from Lake Superior grid # 1028 in year 2013 = LSLakeTrout20131028F10

- 9.10 After five fish are collected that meet the specifications listed in Section 5, combine the individually bagged fish into one large polyethylene bag (it does not matter which five fish are placed into the

large polyethylene bag, the large bag helps when packaging the fish and shipping to the homogenization lab). **Note:** *Please ensure that all sample labels are secured to each individually bagged fish and that labels do not come off when combining fish into large bag.*

- 9.11 Once packaged, samples should be immediately frozen for shipment or placed on dry ice for transport to a processing facility where fish will be immediately frozen. All fish must be kept at $\leq -20^{\circ}\text{C}$, and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least four weeks, or until the GLFMSP Technical Lead has specified a shipment date.
- 9.12 The *Field Recording Form and Chain-of-custody Record* should be completed if it was not already filled out in Section 9.5.
- 9.13 The Data Validation and Verification contractor will arrange for shipping of fish to a lab for processing and homogenization. Follow instructions described in the *Packing and Shipping Instructions for the Great Lake's National Program Office's Fish Monitoring Program* for shipping fish to the location specified by the Data Validation and Verification contractor. These instructions will be provided when the Data Validation and Verification contractor specifies a shipment date and location. As described in these instructions, the field sampling team must include all field and sample handling documentation including the *Field Recording Form and Chain-of-custody Record* (provided by the Data Validation and Verification contractor) with the fish shipment. The field sampling team leader should sign and date the form.
- 9.14 Provide an electronic copy of the *Field Recording Form and Chain-of-custody Record* to the GLFMSP Technical Lead and the Data Validation and Verification contractor. Use overnight delivery services when shipping samples, and only ship Monday through Thursday to ensure samples are not delivered on the weekend.

Appendix B

Field Recording Forms and Chain-of-Custody Records

**Great Lakes Fish Monitoring and Surveillance Program
Field Recording Form and Chain-of-custody Record
Base Monitoring Program**

Fish Collector Name:
Affiliation:
Address/Phone:
Contact Name/Phone:

Site/Sample Location Information (Enter the appropriate information for each site/sample location visited.)

Site/Sample Location 1	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 2	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Sampled by: (signature)	Date/Time:	Shipped by: (signature)	Date/Time:
Received by: (signature)	Date/Time:		

Please see *Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program* for further information on filling out and distributing this form.

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

Composite ID	Fish Number	Date of Collection (Month/Day/Year)	Method of Collection	Estimated Collection Depth (m)	Sample ID	Field Length (mm)	Field Weight (g)	Sex (M = male, F= female)	Fin Clips - If none, record none.
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
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	3								
	4								
	5								

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Great Lakes Fish Monitoring and Surveillance Program

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	1								
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	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								

Please see *Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program* for further information on filling out and distributing this form.

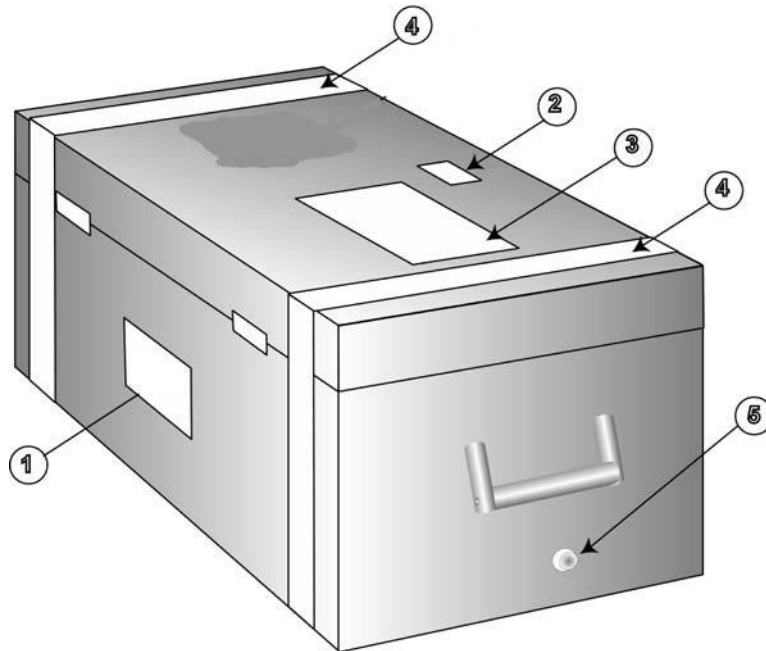
Appendix C

Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program

Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program

These instructions apply to fish collected for the United States Environmental Protection Agency's (U.S. EPA) Great Lakes National Program Office (GLNPO) Great Lakes Fish Monitoring and Surveillance Program (GLFMSP). These fish are being sent to Aquatec Biological Sciences, Inc. (Aquatec) for sample preparation prior to analysis. These instructions supplement the fish collection SOPs provided by the GLFMSP. We are requesting that you complete a chain-of-custody and field recording form, provided when you received your sampling kits, to send to Aquatec with your fish. This will allow tracking of the shipments, identify all fish being shipped to Aquatec, and confirm that fish remained frozen during transport. This is in addition to any data sheets you may be providing. If you received dry ice, please ship samples within 24 hours of receipt. Please follow the instructions detailed below.

1. Complete a chain-of-custody and field recording form provided with the sampling kits, for all fish being shipped. All entries must be in black ink and coincide with fish information on the sample identification labels adhered to the samples (as described in GLFMSP fish collection SOPs). In order to simplify entering data onto the form, feel free to use ditto marks as seen on the example form. This form also is available electronically, although it will need to be printed and signed prior to sending.
 - a. For identifying sample location (grid number), please use the List of Grid Numbers and Port Codes for the GLFMSP that you received with your sampler information packet prior to sampling.
 - b. For composite numbers, please assign numbers to each set of five fish being used for a composite. Assign 001 to the five fish being used for one composite, 002 to the next five fish being used for a composite, etc. This is a component of the sample identification numbering scheme that is being requested by the analytical laboratory.
2. Please scan and email the chain-of-custody and field recording forms to the Data Verification and Validation contractor before or immediately after shipping. This is critical for monitoring shipments to ensure they reach Aquatec before the samples thaw.
3. Make a copy of the chain-of-custody and field recording form. Place the original chain-of-custody and field recording form in a waterproof bag. If you have additional forms containing sampling or specimen data, place them in the waterproof bag as well and seal. Place the sealed forms in one of the coolers with the samples.
4. Please bag each fish in its own piece of tubing and seal the other end with a cable tie. Place each set of five fish being used for a composite inside the large composite bag provided in your sampling kit and seal with a cable tie.
5. As soon as each sample is packaged, place it immediately on dry ice for shipment in the cooler. Try to surround all fish with dry ice. If possible, fill the entire cooler with dry ice.
6. If possible, keep all five fish designated for a particular composite in the same cooler for transport.
7. Secure each cooler with packaging tape. Prepare the coolers with the labels and other information as described on page 2. Ship each cooler to Aquatec via FedEx Priority Overnight delivery service. All costs can be charged to the provided FedEx account number.



1. **Class 9 Dangerous Goods Label:** List the amount of dry ice in kg (2 lbs = 1 kg) on each label. Place one label on each long side of the cooler (number 1 on the figure above and in the same position on the opposite side of the cooler). Completely tape over the labels with clear tape.
2. **Perishable Goods Label:** Be sure to completely tape over this label with clear tape.
3. **FedEx Airbill** (please be sure to secure airbill with clear tape): To fully complete the FedEx airbill, please enter the information in the following sections:
 Section 1: Date
 Section 6: Dry Ice weight in kg
4. **Packaging Tape:** Each end of the cooler needs to be wrapped with strapping tape at least three (3) times.
5. **Cooler Drain Hole:** Please make sure the cooler drain hole has been taped so that the drain hole is CLOSED. If the tape has been removed, please tape the plug so that the drain hole remains CLOSED.
6. **Custody Seal** (not pictured in figure above): Tape custody seal over the cooler to help ensure that cooler is unopened during shipping.

NOTE: If you have any questions regarding the packing or shipping of these samples or if you need assistance filling out the paperwork, please contact Marian Landon (703) 461-2351.

Special Instructions:

- Not all FedEx locations will accept shipments containing dry ice. Please be sure to call in advance (800-Go-FedEx) to ensure that your FedEx drop-off location accepts

dry ice. In the event that you cannot locate a station in your area that accepts dry ice, simply call for a pickup (800-Go-FedEx) and explain that you have a shipment containing dry ice. FedEx will gladly pick-up these shipments.

- FedEx Dangerous Goods personnel have given approval for shipment of these packages using the instructions listed above. Failure to follow these instructions could result in the package being “bumped” and therefore, not reaching its destination. If you have any problems with FedEx personnel accepting your package, please contact the Data Verification and Validation contractor immediately.