



Florida Department of Environmental Protection

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2600 Blair Stone Road
Tallahassee, Florida 32399-2400

Rick Scott
Governor

Carlos Lopez-Cantera
Lt. Governor

Jonathan P. Steverson
Secretary

VIA EMAIL
Read Receipt Requested

December 8, 2016

David L. Evans, Ph.D.
Water & Air Research, Inc.
6821 SW Archer Rd
Gainesville, FL 32608

Re: Alternative Method Approval for Macroinvertebrate Sample Preservation;
OGC No. 16-1424

Dear Dr. Evans

The Aquatic Ecology and Quality Assurance Section (AEQAS) of the Department of Environmental Protection (department) has reviewed the information submitted by Water & Air Research, Inc. (WAR) in support of an approval order for an alternative sample preservation method as required in paragraph 62-160.220(7)(a), Florida Administrative Code (F.A.C.). The basis for the approval, which meets the requirements for alternative method approvals in DEP SOP FA 1000, subparts FA 2210-FA 2230 (DEP 03/01/14), is described below.

Materials Reviewed

This approval of an alternative preservation method is based on the review of the following documents:

1. XWater & Air Research, Inc. Standard Operating Procedure for use of NOTOXhisto® Fixative in Place of Buffered Formalin Solution, September 19, 2016.
2. X"The Comparison of Chemical Preservation Effectiveness in Benthic Macroinvertebrate Samples," Water & Air Research, Inc., poster presentation to the Society for Freshwater Science 61st Annual Meeting. May 19-23, 2013-Jacksonville, FL.

3. d“FDEP Demonstration of NOTOXhisto® as an Alternative Preservative,” Florida Department of Environmental Protection, Biology Program, presentation to the Biocriteria/Q Meeting, March 29-31, 2016.

Scope of Approval

The use of NOTOX, a formalin substitute, was evaluated as an alternative method for sample preservation for the specific intended uses indicated below, according to paragraph 62-160.220(4)(a), F.A.C., and as a project-specific (i.e., site-specific) alternative field procedure, according to criteria described in DEP SOP FA 1000, subparts FA 2210 (subsection 1.4 and section 3), subpart FA 2220 (section 1, including all applicable subsections), and FA 2230 (section 1, including applicable subsections). This approval is only for use of NOTOX for the tissue preservation of benthic macroinvertebrate samples collected using the DEP Standard Operating Procedures (SOPs) for Hester-Dendy (HD) sampling (FS 7400, part 7430), core and dredge sampling (FS 7400, parts FS 7440 and FS 7450), Wetland Condition Index (WCI) (FS 7400, part FS 7470), and Stream Condition Index (SCI) Methods (SCI 1000), performed by WAR, per the WAR SOP for use of NOTOXhisto® Fixative in Place of Buffered Formalin Solution. All other requirements of FS 7400 and SCI 1000 remain in effect with this approval. This approval is only applicable to the following WAR projects:

Coastal Marine, Puerto Rico (Young-modified Van Veen Dredge);
Coastal Marine, Florida (PONAR, Young-modified Van Veen Dredge);
Trout Creek, St. Johns County (SCI);
Withlacoochee River, Hamilton County (SCI, PONAR, Hester-Dendy);
Little Wekiva River, Orange, Seminole Counties (SCI);
Alachua County Streams (SCI);
Escambia County Estuaries, Streams, and Wetlands (SCI, WCI, Hester Dendy, PONAR);
Fenholloway and Econfinia Rivers (PONAR);
Crews Lake, Pasco County (Hester-Dendy);
Crystal River/Kings Bay, Citrus County (core sampling);
Hillsborough County Streams (SCI, HD);
St. Johns River (HD, PONAR);
Suwannee River (HD, SCI); and
South Florida Canals (SCI, HD, Ekman & PONAR Dredges).

Basis for Approval

1. dWAR conducted a blind study comparison in which sixty samples were collected using a variety of collection methods appropriate for the following habitat and sample types: marine and fresh qualitative samples, marine and fresh dredge samples, and fresh SCI samples. A range of both marine and freshwater macroinvertebrate species were preserved in one of the three fixative solutions: NOTOX, a prepared solution of 10% buffered formalin, and 70% ethanol. Samples were stored for differing lengths

of time ranging from 48 hours to two months to address the effectiveness of each preservative over time.

Each organism was given a preservation rating for firmness, dye penetration, completeness, and identifiability typical of the sample type, using a standardized rating matrix. The rating scale was 1-3 where 1 meant that the organism was in poor condition and 3 meant it was in excellent condition. Both the mean (using ANOVA) and the median (using the Mann-Whitney test) were evaluated to compare preservative type (NOTOX, formalin, and ethanol), preservation time (24-48 hours, 7-9 days, 1 month, and 2 months) and preservation criteria (firmness, completeness, and identifiability).

This study found little to no difference in the effectiveness of NOTOX as a preservative of macroinvertebrate samples in comparison to formalin. The one-way ANOVA and the Mann-Whitney analyses showed none of the comparisons for preservative type or preservation time versus firmness, identifiability, and completeness were significant. For some samples (Marine and Freshwater Petite PONAR Dredge Samples), firmness was significantly higher for formalin preserved samples compared to ethanol preserved samples. No significant difference was found for comparisons of preservation time. Both NOTOX and formalin preservations produced firm, complete and identifiable specimens more consistently than specimens preserved in ethanol.

Study description and results are contained in the poster by WAR, listed in the Materials Reviewed, above.

2. i The department conducted comparison studies to assess the performance of NOTOX as an alternative preservative to formalin for freshwater benthic macroinvertebrate samples. Benthic macroinvertebrates from a variety of orders, families, genera and/or species were collected from Florida Panhandle streams to compare the performance of formalin and NOTOX preservatives on macroinvertebrate condition in a controlled laboratory experiment. Field-duplicate SCI samples were also collected from two small creeks with adequate habitat to compare the performance of formalin and NOTOX preservatives on macroinvertebrate condition in samples that also contained detritus.

Handpicked samples were evaluated for color, tissue condition, body condition, and identifiability at intervals of 1 week, 1 month, 3 months, and 6 months. Color was characterized by the taxonomists as normal or abnormal, while tissue condition, body condition, and identifiability were assigned ratings. The tissue condition of each specimen was rated 1-disintegrating or brittle, 2-soft, or 3-firm, with 3 being the optimal rating. The body condition of each specimen was rated 1-poor, 2-acceptable, or 3-very good, with 3 being the optimal rating. Identifiability was rated 1-could not

identify to lowest possible taxonomic level, 2-difficult to identify, and 3-easy to identify, with 3 being the optimal rating.

There were no significant noticeable differences in color between the formalin and NOTOX preserved specimens. Color change was observed in decapods after preservation, but this was often observed for both preservatives. The color change was noticeably darker in NOTOX but did not affect the taxonomists' ability to identify the specimen.

For samples in the handpicked laboratory controlled experiment, a one-way ANOVA analysis showed that there were no significant differences between formalin and NOTOX treatments in seven of the ten taxonomic groups identified for tissue condition, body condition, and identifiability at all preservation time intervals. NOTOX-preserved specimens received statistically higher ratings in three taxonomic groups, Diptera, Ephemeroptera, and Plecoptera, at various time intervals.

In the SCI samples, identifiability was high in all taxonomic groups in both preservatives. There were no significant differences in ratings for tissue condition, body condition, and identifiability between the formalin and NOTOX preserved specimen for three taxonomic groups: Acariformes, Decapoda, and Diptera. Unlike the handpicked samples, the formalin ratings were significantly higher than NOTOX for tissue condition for some taxonomic groups (Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera), and for body condition in Trichoptera. However, NOTOX received statistically similar or higher ratings for all taxonomic groups in the identifiability category, which is consistent with the handpicked data.

The final SCI scores for the two preservation treatments at the two creeks were similar. For Mule Creek, the NOTOX SCI score was 76 (laboratory replicates: 74 and 79) and formalin score was 75 (laboratory replicates: 76 and 74). For Yon Creek, the NOTOX SCI score was 73 (laboratory replicates: 67 and 77) and formalin score was 81 (laboratory replicates: 79 and 82). Differences in scores were within the error of the method.

Results of this study were summarized in a presentation by the department's Biology Program, as listed in the Materials Reviewed, and statistical analyses were conducted by the AEQAS. More detailed results are available upon request.

3. The WAR alternative preservation method using NOTOX instead of formalin was evaluated against the approval criteria described in DEP SOP FA 1000, subpart FA 2220, section 1 and all applicable subsections. DEP finds the alternative method of sample preservation to be appropriate for the data quality objectives and data usability requirements for bioassessment data produced from FS 7400 and SCI 1000 methods. The procedures produce sample analysis results that are equivalent to or better than

those generated from samples preserved using DEP SOP procedures, as demonstrated by the studies described above. The alternative methods also meet the data validation requirement for adherence to proper preservation procedures as required by paragraph 62-160.670(1)(b), F.A.C.

Should you have any questions about the approval of this alternative method, please do not hesitate to contact Nia Wellendorf at 850-245-8190 or at Nijole.Wellendorf@dep.state.fl.us

Effective Date and Notice of Rights

This action is final and effective on the date mailed (via e-mail) unless a sufficient petition for an administrative hearing is timely filed under sections 120.569 and 120.57 of the Florida Statutes as provided below. If a sufficient petition for an administrative hearing is timely filed, this agency action becomes only proposed agency action, subject to the result of the administrative review process. Therefore, on the filing of a timely and sufficient petition, this action will not be final and effective until further order of the department. Because an administrative hearing may result in the reversal or substantial modification of this action, the petitioner is advised not to proceed until the deadlines noted below for filing a petition for an administrative hearing or request for an extension of time have expired. Mediation is not available.

A person whose substantial interests are affected by the department's action may petition for an administrative proceeding (hearing) under sections 120.569 and 120.57 of the Florida Statutes. The petition must contain the information set forth below and must be filed (received by the clerk) in the Office of General Counsel of the department at 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida 32399-3000.

Under rule 62-110.106(4), F.A.C., a person whose substantial interests are affected by the department's action may also request an extension of time to file a petition for an administrative hearing. The department may, for good cause shown, grant the request for an extension of time. Requests for extension of time must be filed with the Office of General Counsel of the department at 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida 32399-3000, before the applicable deadline. A timely request for extension of time shall toll the running of the time period for filing a petition until the request is acted upon. If a request is filed late, the department may still grant it upon a motion by the requesting party showing that the failure to file a request for an extension of time before the deadline was the result of excusable neglect. If a timely and sufficient petition for an administrative hearing is filed, other persons whose substantial interests will be affected by the outcome of the administrative process have the right to petition to intervene in the proceeding. Intervention will be permitted only at the discretion of the presiding officer upon the filing of a motion in compliance with rule 28-106.205, F.A.C.

In accordance with rules 28-106.111(2) and 62-110.106(3)(a), F.A.C., petitions for an administrative hearing by the petitioner must be filed within 21 days of receipt of this

written notice. Petitions filed by any persons who were not entitled to written notice under section 120.60(3) of the Florida Statutes must be filed within 21 days of publication of the notice, if published, or within 21 days of receipt of the written notice, whichever occurs first. Under section 120.60(3) of the Florida Statutes, however, any person who has asked the department for notice of agency action may file a petition within 21 days of receipt of such notice, regardless of the date of publication.

The petitioner shall mail a copy of the petition to Water & Air Research, Inc., at the address indicated above at the time of filing. The failure of any person to file a petition for an administrative hearing within the appropriate time period shall constitute a waiver of that person's right to request an administrative determination (hearing) under sections 120.569 and 120.57 of the Florida Statutes.

A petition that disputes the material facts on which the department's action is based must contain the following information: (a) The name and address of each agency affected and each agency's file or identification number, if known; (b) The name, address, and telephone number of the petitioner; the name, address, and telephone number of the petitioner's representative, if any, which shall be the address for service purposes during the course of the proceeding; and an explanation of how the petitioner's substantial interests are or will be affected by the agency determination; (c) A statement of when and how the petitioner received notice of the agency decision; (d) A statement of all disputed issues of material fact. If there are none, the petition must so indicate; (e) A concise statement of the ultimate facts alleged, including the specific facts that the petitioner contends warrant reversal or modification of the agency's proposed action; (f) A statement of the specific rules or statutes that the petitioner contends require reversal or modification of the agency's proposed action, including an explanation of how the alleged facts relate to the specific rules or statutes; and (g) A statement of the relief sought by the petitioner, stating precisely the action that the petitioner wishes the agency to take with respect to the agency's proposed action.

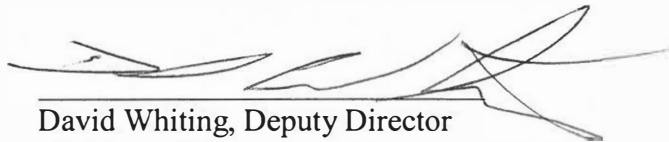
A petition that does not dispute the material facts on which the department's action is based shall state that no such facts are in dispute and otherwise shall contain the same information as set forth above, as required by rule 28-106.301, F.A.C. Under paragraphs 120.569(2)(c) and (d) of the Florida Statutes, a petition for administrative hearing must be dismissed by the agency if the petition does not substantially comply with the above requirements or is untimely filed.

This agency action constitutes an order of the department. Subject to the provisions of paragraph 120.68(7)(a) of the Florida Statutes, which may require a remand for an administrative hearing, the applicant has the right to seek judicial review of the order under section 120.68 of the Florida Statutes, by the filing of a notice of appeal under rule 9.110 of the Florida Rules of Appellate Procedure with the Clerk of the Department in the Office of General Counsel, 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida, 32399-3000; and by filing a copy of the notice of appeal

accompanied by the applicable filing fees with the appropriate district court of appeal. The notice of appeal must be filed within 30 days from the date when the final order is filed with the Clerk of the Department.

Executed in Tallahassee, Florida.

STATE OF FLORIDA DEPARTMENT
OF ENVIRONMENTAL PROTECTION



David Whiting, Deputy Director
Division of Environmental Assessment
and Restoration
2600 Blair Stone Road
Tallahassee, FL 32399
850-245-8191

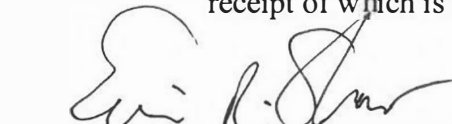
Copies furnished to:
Stacey Cowley, DEP, Office of General Counsel
File

CERTIFICATE OF SERVICE

The undersigned duly designated clerk hereby certifies that this agency action, including all copies, was e-mailed before the close of business on Dec. 8, 2016, to the above listed persons.

FILING AND ACKNOWLEDGMENT

FILED, on this date, pursuant to 120.52(7)
Florida Statutes, with the designated Department Clerk,
receipt of which is hereby acknowledged.


Clerk

12/8/2016
Date