Phase I Procedures for Evaluating the Potential for Effects to Everglades Biota from Discharges from Pilot Testing of Supplemental Technologies

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Introduction

The Everglades Forever Act (Section 373.4592, Florida Statutes) requires the District and the Department to implement a research and monitoring program to optimize the design and operation of the Stormwater Treatment Areas (STAs) and to identify other treatment and management methods and regulatory programs that are superior to STAs in reducing phosphorus loads and concentrations from Everglades Agricultural Area (EAA) runoff.

To identify and evaluate treatment technologies or combinations of technologies, the District retained the services of PEER Consultants/Brown and Caldwell. As a part of the PEER Consultants/Brown and Caldwell evaluation (August, 1996, desktop evaluation), a concern was raised regarding potential adverse impacts the technologies might have on the biota of Everglades Protection Area marshes resulting from changes in water quality in addition to phosphorus concentration reductions. The purpose of this document is to provide a minimum set of guidelines for examining the potential adverse impacts of the effluent waters generated by pilot testing of supplemental technologies for the reduction of phosphorus from EAA runoff on the biota of Everglades Protection Area marshes. These guidelines should be incorporated by project managers as part of the demonstration of each of those supplemental technologies.

Procedure

The determination of the potential effects to biota from the effluents from technologies will be based on a phased approach. Phase I, or the screening phase, will include an evaluation of the effluent on both a physical and chemical-specific basis and a whole effluent basis (algal assays and toxicity testing) to determine potential effects on the biota of Everglades Protection Area marshes. Phase I screening is the primary focus of this document.

Phase II will build on the results from Phase I. Phase II evaluations may include, but are not limited to: additional toxicity testing; examination of micro element deficiencies; description of treatment potential (efficiencies, costs, etc.); and identification of additional relevant studies or analysis. Decisions regarding the efficiency, costs, Phase I toxicity screenings, and the resulting proposed/planned use of the treatment technologies will be used in the determination of whether and how Phase II evaluations should be conducted. For those technologies selected for scale-up or additional study subsequent to Phase I, project managers should contact Department and District staff to develop plans for Phase II evaluations and should incorporate those plans, and the cost for same, into the demonstration of the project.

Phase I Procedures

I. Physico-chemical testing

At a minimum, surface water influent and effluent samples should be analyzed for the physical and chemical parameters expected to potentially influence the biota of Everglades Protection Area marshes and presumed to be present in the effluent (Table 1). Sampling and analysis for such parameters should be incorporated by project research teams into the plans for demonstration of each supplemental technology. Additional parameters may be added to the demonstration if the Department or District has reason to believe the technology will add or remove such parameters. To this end, project research teams must provide information to Department and District staff describing chemical formulation of all chemical additions to be

made during the course of the project and/or Material Safety Data Sheets or certificates of analysis for all the chemicals must be provided.

During Phase I, results of the analysis of effluent waters should be compared to applicable State water quality criteria as defined in Rule 62-302.530, Florida Administrative Code, and to the quality of concurrently collected samples of influent waters. Project Managers should consult with Department and District staff regarding appropriate comparisons. If the effluent waters are statistically different from the influent waters, based on statistical evaluations to be conducted as part of the supplemental technology standard of comparison, further evaluation on the part of the project research team may be needed, including additional sampling, biological testing and a literature survey to determine potential effects of these statistically significant water quality differences.

At a minimum the following parameters should be sampled at frequencies to be determined by the Department and the District for each technology under evaluation. The frequency of sampling will depend on the nature of treatment and will vary from project to project.

Table 1

Nutrients	**Metered Parameters**	**Dissolved Ions**	**Misc. Parameters**	**Metals**
Total Phosphorus (TP)	Dissolved Oxygen	Sulfate	Alkalinity	Dissolved Iron
Total Dissolved Phosphorus (TDP)	Temperature	Silica		Dissolved Aluminum
Soluble Reactive Phosphorus (SRP)	pH	Chloride		
Total Kjeldahl Nitrogen (TKN)	Specific Conductance	Calcium		
Ammonia Nitrogen (NH_3-N)	Turbidity	Magnesium		
Nitrate-Nitrite Nitrogen (NOx-N)	Color	Sodium		
		Potassium		

During Phase I screening, a limited number of samples for analysis of total mercury will be collected by District staff and analyzed by Department staff to investigate potential changes in ultra-trace total mercury concentration from influent to effluent. Project Managers should consult with Department and District staff at the beginning of project initiation to determine a sampling schedule. Since typical methods of sample collection and analysis are not likely to yield useful information regarding mercury, the collection and analysis of such samples by the project research team as part of routine project monitoring is not recommended for Phase I.

Issues of potential mercury methylation cannot meaningfully be addressed during Phase I screening, and if applicable, should be addressed as part of longer-term projects or during Phase II screening process. For those technologies selected for longer-term studies or scale-up, project managers should contact Department and District staff to develop plans for addressing this issue, as necessary, and project managers should be prepared to incorporate the costs (potentially large) of this and other Phase II demonstrations judged necessary by Department and District staff into costs for demonstration of the selected projects.

II. Toxicity testing

It is the Department's position that *in-situ* studies would not be feasible during the screening phase of supplemental technologies. The complexity of *in-situ* studies necessitates employing extreme scientific rigor to successfully generate results that would provide assurance that the receiving water would be protected. There are numerous logistical and methodological obstacles to overcome before reliable results may be obtained using *in-situ* tests.

However, it is the Department's experience that laboratory toxicity testing provides very sensitive indications of toxicity, the results of which have proven to be indicative of receiving-water effects. Not finding toxicity in the laboratory testing should be protective of the receiving water, but more elaborate toxicity testing may be required during Phase II. The results of the laboratory toxicity tests will be used in conjunction with information on phosphorus removal performance, cost and other feasibility determinations for a treatment technology in deciding whether additional testing of the technology, and thus Phase II studies, will be warranted. If needed, *in-situ* testing in Phase II will require extensive experimental controls and replication, as well as an understanding of the background variability in order to yield results having sufficient statistical power to provide assurance that the receiving water is being protected.

Thus, as part of the pilot demonstration of supplemental technologies, whole effluent and influent samples should be collected and tested for chronic toxicity in accordance with Department and EPA guidelines (EPA, 1978; EPA, 1991; EPA, 1994). The Department's position that the whole effluent approach should be used in Phase I screening is based on the capabilities of the techniques. Chronic toxicity testing measures sub-lethal effects such as changes in growth or rates of reproduction, as well as organism mortality. These measures are determined during sensitive life stages of the test species. The use of the laboratory test species reduces variability in the test results, thereby enabling the test to detect low-level effects. This results in a more sensitive test compared to those using non-EPA/DEP approved native or non-native test organisms. Mechanisms of toxicity are not species specific, so any toxicity found would indicate the likelihood of effects on receiving water species. Testing with multiple species of different trophic levels (vertebrate, invertebrate, alga) is intended to minimize the risk of 'missing' a type of toxicity. The principal capabilities are (EPA, 1991):

The aggregate toxicity of all constituents in a complex effluent or influent is measured.

Toxicity can be measured even if: 1) any toxic compounds present are commonly not analyzed for in chemical tests; or 2) the substances are toxic at levels below the detection limit of the analytical method.

The bioavailability of the toxic constituents is assessed, and the effects of interactions of constituents are integrated. Additivity, synergism, and antagonism between compounds in an effluent or influent are addressed implicitly by whole effluent toxicity.

The toxicity of the effluent or influent is measured directly for the species tested.

The Department has identified three test species, a vertebrate, an invertebrate, and an alga, as representative species for the toxicity testing for the supplemental technologies to be evaluated. These species, or close relatives, are found in the Everglades. The species were chosen for following reasons:

The background testing and data associated with these species are extensive, providing assurance that the species are sensitive to a wide range of toxic substances.

Quality assurance issues associated with the use of non EPA/DEP approved native species include: (1) assurance that test specimens are all of the same species and life stage; (2) effects of seasonal variations in populations, habitat requirements, and health of the test organisms on test results; and (3) availability of baseline information on the species sensitivity to standard reference toxicants. Studies of sufficient rigor and duration to yield such quality assurance are not within the scope of the Phase I screening, but may be addressed, if Phase II screening is deemed appropriate.

The three test species to be used for chronic toxicity testing under Phase I screening are:

Cyprinella leedsi (bannerfin shiner)	EPA/600/4-91/002 method 1000.0
Ceriodaphnia dubia (water flea)	EPA/600/4-91/002 method 1002.0
Selenastrum capricornutum (green alga)	EPA/600/4-91/002 method 1003.0

The minimum number of chronic toxicity tests to be conducted during Phase I screening of each technology will be determined by the Department and the District, and will depend on the nature of treatments and duration of the project. Chronic screening toxicity testing should be performed on influent and effluent samples for each treatment technology to be evaluated. Samples for chronic toxicity testing must be collected at the same time that water samples are collected for the determination of all the physical and chemical demonstration parameters for a technology (e.g., Table 1). All tests must be performed in accordance with Department and EPA guidelines.

Screening toxicity tests expose test organisms to full-strength sample and a control water. These tests show the presence of toxicity, but provide little information on the magnitude of that toxicity. Definitive toxicity tests expose the organisms to a series of concentrations of the test water and, while more complex to conduct, provide a measure of the amount of toxicity present. If screening tests identify toxicity of the effluent in excess of that which may be present at the influent of the treatment technology, chronic definitive assays may need to be performed on the effluent sample using laboratory water as a diluent. Department and District staff shall be consulted at that point to determine how best to proceed.

III. Algal Assays

It is the Department's experience that Algal Growth Potential (AGP) assays will help confirm that a given technology is effective in reducing the bioavailable nutrient concentrations of water delivered to the marsh. AGP assays, using *Selenastrum capricornutum*, (EPA-600/9-78-018) should be performed concurrently with toxicity tests. While different algal species have different nutrient requirements, the AGP assays will provide information on any changes in the bioavailable nutrients as a result of the treatment. These assays are being used in other Everglades programs and thereby also provide a means to compare results from different locations and programs. The minimum number of AGP assays to be conducted also will be determined by the Department and District, and will depend on the nature and duration of the project.

NOTE: All sampling protocols are subject to Department evaluation and are subject to modification at the Department's discretion.

Phase II Procedures

Plans for Phase II evaluations will be dependent on the specifics of the technology being tested, and will be determined by the Department and the District for those technologies selected for scaled-up testing. Phase II evaluations may include but are not limited to:

I. Pilot Study Results

Prior to additional testing, the results of the pilot study of the subject technology will be evaluated. These shall include evaluations of technology performance, whether the technology performed as expected, what cost was associated with phosphorus removal, what volume of water could realistically be treated, whether the technology removed anything other than phosphorus, whether the technology added anything to the effluent, and if the technology were to be used full scale, where it would be located in the treatment train.

II. Toxicity Testing

If it is determined that the treatment technology is viable at full scale, additional toxicity analyses may be warranted. These tests may include use of additional test species (including additional indigenous species), additional test methods (e.g., long-term assays, dilutions using Everglades marsh water and/or receiving waters), and/or testing at higher levels of organization (e.g., community-response studies). If the chemical analysis outlined in Phase I identifies that a specific chemical is in excess or deficit, relative to the influent, the most sensitive EPA/DEP approved species to the chemical specific toxicity should be included in any additional toxicity evaluations.

III. Micro Element Deficiency Evaluation

Chemical testing of the effluent should be performed to determine deficiencies of micro elements and other parameters of concern. The results of the test should be compared to the water quality of the receiving Everglades Protection Area marsh waters. If the effluent is statistically different from the receiving marsh waters, further evaluation may follow including additional sampling, biological testing and a literature survey to determine potential effects of these statistically significant water quality differences.

References

EPA, 1991. Technical Support Document for Water Quality-based Toxics Control, EPA/505/2-90-001.

EPA, 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, 3rd Ed. EPA/600/4-91/002.

EPA, 1978. The *Selenastrum Capricornutum* Printz Algal Assay Bottle Test, EPA-600/9-78-018.