

Settlement Agreement Status Report
July 15, 2011
NRDC v. Johnson,
U.S. District Court, Central District of California
No. CV06-4843 PSG (JTLx)

Pursuant to Paragraph 15 of the Settlement Agreement in the above-referenced matter, the United States Environmental Protection Agency (“EPA” or “the Agency”) provides this status report. It includes a description of the actions taken by EPA with respect to its responsibilities under the Settlement Agreement during the preceding six months (January 1, 2011 - June 30, 2011) and a description of the actions EPA intends to take with respect to its responsibilities under the Settlement Agreement during the following six months (July 1, 2011 – December 31, 2011).

Settlement Agreement (SA) ¶ 9(a) Continue to conduct research currently underway to identify genetic sequences that could form the basis of chicken and seagull specific fecal source assays

Actions Taken By EPA from January 1, 2011 to June 30, 2011

EPA completed research to identify genetic sequences for selected chicken and gull assays having the potential to specifically identify avian sources of fecal contamination. EPA completed the analysis of molecular data for use in developing additional gull-specific assays.

Actions Planned By EPA from July 1, 2011 to December 31, 2011

No further action will be taken with regard to subparagraph 9(a).

SA ¶ 9 (b) If EPA determines that it can develop chicken and seagull specific fecal source assays, EPA will evaluate the assays for specificity and sensitivity using a reference collection of fecal samples, as well as environmental water samples with known sources of fecal contamination.

Actions Taken By EPA from January 1, 2011 to June 30, 2011

EPA completed additional studies determining the presence of chicken and gull specific markers used in PCR assays in environmental waters collected in a multi-year study. EPA completed additional studies to determine the detection limit(s) of avian-specific PCR assays against fecal and water DNA extracts. EPA determined it could develop chicken and seagull specific fecal source assays, and EPA evaluated the assays for specificity and sensitivity using a reference collection of fecal samples, as well as environmental water samples with known sources of fecal contamination.

Actions Planned By EPA from July 1, 2011 to December 31, 2011

No further action will be taken with regard to subparagraph 9(b).

SA ¶ 9(c) No later than July 15, 2011, EPA will notify the other Parties in writing whether EPA was able to develop the assays and evaluate the assays as described in subparagraph 9(a). If EPA is able to complete the evaluation by July 15, 2011, the notification will include a brief summary of such evaluation.

Actions Taken By EPA from January 1, 2011 to June 30, 2011

EPA hereby notifies the parties that it was able to develop and evaluate chicken and seagull assays. Summaries describing the development of chicken- and seagull-specific assays and the evaluation of these assays with reference collections of fecal samples and environmental water samples are provided below.

Molecular analyses were used to search for chicken- and gull-specific DNA sequences. Candidate sequences were selected for evaluation based on their affiliation to bacterial groups that are known to be primarily of fecal origin and/or absent in non-targeted hosts. Signature sequences were identified within these short stretches of DNA and PCR-based assays developed to detect these sequences in fecal and water samples.

For chicken-related studies, 25 paired-sequences affiliated to Clostridia, Bacteroidetes, and Bacilli were selected to develop chicken feces-specific PCR assays. Twelve of the assays were determined to be specific for chicken. To determine host-specificity, these assays were tested against fecal DNA extracted from the following animals: cattle, human, goat, sheep, horse, dog, coyote, squirrel, possum, seagull, goose, turkey, pigeon, duck, pig, vulture, raccoon, hedgehog and bobcat, to determine if they were unique to chickens. To understand the sensitivity of the assays, host-distribution was determined for five of the assays using 70 chicken fecal samples collected in DE, WV, OH, GA, and Canada. The host distribution was relatively low, ranging from 6-40% of the chicken fecal samples tested. Additionally, detection limit studies for the three assays showing the highest level of host distribution indicated detection limits ranging from 0.001 to 1 ng per reaction. These assays were then challenged against waters presumed to be impacted with cattle, human, swine, goose, and chicken fecal sources. Positive signals were only detected in chicken contaminated water samples, although the overall detection rates for each of the assays was relatively low (2-3 out of 8 water samples tested).

For the waterfowl assays, gene sequences were analyzed and sequences nearly identical to *Catelliboccus marimammalium* were used to develop conventional PCR and real-time PCR assays. The assays (gull2) were used to test fecal DNA extracts (n=307) from different birds (n = 13) and mammals (n = 26) species. The results showed that both assays were specific to gull fecal DNA and that they were positive for most gull fecal samples (i.e., > 70%) collected from five locations in North America (California, Georgia, Ohio, Wisconsin, and Toronto, Canada). Additionally, all DNA extracts from water samples (n=48) collected from six sites in southern California and the Great Lakes (Lake Michigan, Lake Erie and Lake Ontario) presumed to be impacted with gull feces were positive by the gull assay. Overall, the results indicated that gull2 assays developed by EPA can be used to identify waters impacted with gull feces.

Another gene-based assay (gull3) was recently developed by EPA personnel and its performance compared with the gull2 assay by testing it against additional target (n=232) and non-target feces

(n=477). Host specificity studies showed that both assays cross reacted to some extent with other avian species such as chicken, pelican and Canada geese and on average to 30% of pig fecal samples tested (n=30). Signal intensities were generally lower in non-targeted hosts. Specifically, the gull2 assay was negative for all free range chicken samples while a third of them were positive with the gull3 assay (n=68). The gull2 also tested negative for cattle samples while the gull3 was positive for less than 10% of the cattle samples tested (n=66). No human sources or dog feces were tested in these studies. Of water samples potentially impacted by gull fecal contamination, 86% (294/343) and 73% (251/343) were positive for the gull2 and the gull3 assays, respectively.

Actions Planned By EPA from July 1, 2011 to December 31, 2011

No further action will be taken with regard to subparagraph 9(c)

SA ¶ 11 EPA will convene a stakeholder workshop or other mechanism for stakeholder input during 2009, 2010, and 2011. These workshops will provide an opportunity for Plaintiff, Intervenor, and other interested stakeholders to provide input to EPA and/or bring issues to EPA associated with the development of the new or revised criteria to be issued by October 2012. EPA expects these issues to include issues related to implementation of the new or revised water quality criteria EPA will publish in 2012. The focus of the 2009 and 2010 workshops will be for EPA to update stakeholders on the progress EPA has made in completing studies and framing the issues associated with the development of the new or revised criteria and for Plaintiffs, Intervenor, and other interested stakeholders to comment on EPA's update. The focus of the 2011 workshop will be to provide an opportunity for Plaintiff, Intervenor, and other interested stakeholder to comment on EPA's evaluation, synthesis, summarization and statistical analysis of the studies and development of options for the overall structure and content of the recreational water quality criteria that EPA will publish in 2012.

Actions Taken By EPA from January 1, 2011 to June 30, 2011

On June 14 and 15, 2011, EPA held a stakeholder workshop in New Orleans. EPA presented and sought comments on its evaluation, synthesis, summary, and statistical analysis of the studies and described the general direction of the criteria. Over 40 people attended including representatives from state and local governments, academia, and environmental groups.

Actions Planned By EPA from July 1, 2011 to December 31, 2011

While no further actions will be taken with respect to this obligation, EPA intends to post a summary of the meeting proceedings and presentations on the Recreational Water Quality Criteria Website. EPA also plans to conduct a webinar/webcast of key presentations from the face-to-face meeting held in New Orleans.

SA ¶ 12 EPA will validate and publish a rapid test method for the new or revised criteria by October 15, 2012. Validation of a rapid test method will involve conducting an inter-laboratory study, the purpose of which is to characterize method performance (including recovery and precision) for a rapid method in reference matrices and ambient recreational waters (fresh and marine) in multiple laboratories and to develop quantitative quality

control acceptance criteria. Publication does not include promulgation of an EPA-approved test method in the Code of Federal Regulations.

Actions Taken By EPA from January 1, 2011 – June 30, 2011

EPA continued to conduct the multi-lab validation study of the Enterococcus qPCR test in freshwater in order to develop quantitative quality control acceptance criteria for the test in fresh waters. Reproducibility data was collected.

Actions Planned By EPA from July 1, 2011 to December 31, 2011

EPA plans to continue conducting the multi-lab validation study to characterize method performance and develop quantitative quality control acceptance criteria for Enterococcus qPCR in freshwater. EPA is now in the process of collecting the performance acceptance criteria data.

SA ¶ 14 No later than December 15, 2011, EPA will convene an Experts Scientific Workshop involving both EPA scientists and external scientists for the primary purpose of obtaining input on what future science and research might be conducted to further improve the understanding of potential human health risks from exposure to fecal contamination from avian wildlife and other wildlife in coastal recreational waters. If the experts at the Experts Workshop identify one or more studies that might be conducted to further improve the understanding of the potential health risks from exposure to fecal contamination from avian wildlife or other wildlife, EPA shall evaluate whether the studies should be performed as part of the Agency's review (between 2012 and 2017) of the criteria that EPA will publish in 2012, and EPA shall advise the parties to this Agreement of the results of EPA's evaluation no later than December 15, 2012.

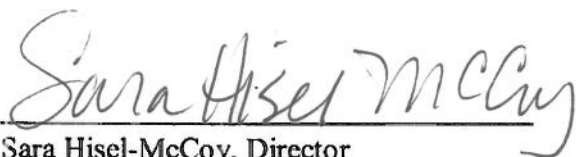
Actions Taken By EPA from January 1, 2011 to June 30, 2011

EPA convened an internal Steering Committee which drafted and reviewed an agenda for the workshop. EPA began a solicitation to identify the contractor to assist EPA planning and running the Experts Scientific Workshop. Three proposals were received and a technical evaluation was performed. The results of this evaluation were submitted to the designated EPA Contracting Officer for processing.

Actions Planned By EPA from July 1, 2011 to December 31, 2011.

The contract to plan and execute the Workshop will be awarded. A timeline for deliverables will be developed. Support documents for the workshop will be identified and/or developed. Appropriate experts will be identified and invited to participate in the workshop. The workshop will be held on or before December 15, 2011.

The undersigned, Sara Hisel-McCoy and Charles I. Noss are, respectively, the Director, Standards and Health Protection Division, Office of Science and Technology and National Program Director for Water Quality, Office of Research and Development. The Office of Science and Technology in EPA's Office of Water and the Office of Research and Development have primary responsibility for discharging EPA's duties under the Settlement Agreement. This report reflects our best current information concerning the actions taken by EPA with respect to its responsibilities under the Settlement Agreement during the preceding six months (January 1, 2011 – June 30, 2011) and a description of the actions EPA intends to take with respect to its responsibilities under the Settlement Agreement during the following six months (July 1, 2011 - December 31, 2011).



Sara Hisel-McCoy, Director
Standards and Health Protection Division
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7/13/11
Date



Charles I. Noss, Sc.D.
National Program Director for Water Quality
Office of Research and Development
U.S. Environmental Protection Agency

July 12, 2011
Date