Online Methods for Evaluating the Safety of Reclaimed Water

Various studies have indicated that unregulated organic contaminants (e.g., pharmaceuticals and personal care products) are present in municipal wastewater effluents and surface waters, as well as ground- and drinking-water sources. The Santa Ana River (SAR) is a wastewater-dominated stream originating from Riverside and San Bernardino Counties. Although concentrations of these compounds are relatively low (nanograms to micrograms per liter), several compounds have been detected at concentrations that could adversely affect endocrine and reproductive systems of fish. The fate of these compounds in the environment and their effects on human health are unclear and not well characterized.

The project team undertook the development of an online, flow-through bioassay to easily judge potential public health impacts. To implement such a system, they used transparent Japanese medaka fish in an investigative mode to develop a standard test platform, evaluate the water quality of shallow groundwater derived from the SAR, and assess risk associated with consuming recycled water.

The Biomonitoring Approach
Bioassays have the potential for providing useful information to managers of utilities supplying water intended for human consumption. Biological tests provide information about effects on biochemical and physiological processes, which, if modified, play a role in the development of adverse health effects. If properly developed and validated, bioassays can supplement chemical-specific water quality parameters by providing information about effects of chemicals that remain unidentified in drinking water, which typically contains trace amounts of a complex mixture of chemicals.

When applying biomonitoring tools to water for purposes of estimating human health risks, one must estimate the relative sensitivity of the bioassay system, and the estimate must consider the sensitivity of humans at the same concentrations. This can be accomplished only by developing a broad experimental database that documents the dose-response relationships of large number of chemicals within the bioassay so that the overall sensitivity and reliability of the test can be assessed.

The Experimental Design
Two stocks of small aquarium fish (orange-red and see-through II Japanese medaka, Orzias latipes) were utilized in three separate nine-, seven-, and four-month exposures. Still Japanese medaka was developed at the University of Nagoya by crossing various color mutants of existing stocks. Because pigment genes are not expressed, the fish are transparent throughout their lifespan. The transparent nature of the body wall makes it possible to view changes in appearance of internal organs, tissues, and body fluids in a

This research developed an online bioassessment platform to help utility managers evaluate the safety of reclaimed water.
noninvasive manner. In future online monitoring, it may be possible to image fish and then place them back in the aquarium to follow alterations over time.

To obtain “chemical-free” negative control water for comparison, SAR groundwater was treated with reverse osmosis and then reconstituted with solutes to allow fish to reside within the water. For a positive control for carcinogenesis, fish larvae were pretreated with the carcinogen diethylnitrosamine and then maintained in negative control water (Exposure I) or SAR groundwater (Exposure II). For Exposure III, granulated active carbon (GAC) was utilized for a negative control, and pulsed exposure to 17β-estradiol was included as a positive control.

Endocrine, reproduction, morphologic, and carcinogenesis endpoints were evaluated as well as daily measurements of survival. Because of the fish’s transparency, the researchers could image living fish without killing them. In right lateral recumbency, they imaged the left side and observed features of eye coloration and morphology; condition of semicircular canals; gill coloration; swim bladder inflation status and horizontal extent; intestine including mural structures and luminal contents; spleen shape, color, and relative vascularization; and heart including the rate of contraction. With the fish maintained in dorsal recumbency, the researchers’ imaged the ventral view and the associated viscera. From the right lateral view (with fish in left lateral recumbency) the following were imaged: gonad (ovary is seen in largest adults, testis is not observed); fat in mesentery as well as in orbit and overheat (indicates general nutritional status); and skin (easily observed). Peritoneal cavity fluid is of interest; when it is clear (normal state), internal organs are easily observed. When it is cloudy, margins of structures are difficult to resolve.

Research Findings

Fish mortality was significantly elevated in negative controls of Exposures I and II when exposure exceeded four months. Death was apparently due to septicemia, which was observed through histological evaluation. No significant effects relative to the negative control were observed in either stock of medaka following Exposures I and II to SAR groundwater.

Assessment of reproduction was limited due to the duration of exposure and negative control issues. Consequently, Exposure III focused specifically on reproduction with modifications to the duration of exposure (four months), maintenance (lower light; dark cycle and temperature), negative control (GAC-treated water; see above), and positive control (17β-estradiol). Histologic evaluations of gonads and measurements of vitellogenin for feminization in fish failed to show significant effects in SAR groundwater relative to GAC. No mortalities were observed in either treatment, indicating that GAC water may serve as a better control for long-term exposures (> four months), which would be necessary for carcinogenic evaluations. Fecundity was not significantly altered, but SAR groundwater appeared to delay hatching of fertilized eggs.

Particularly obvious in the positive control still fish were hemorrhages. These were seen more abundantly over the dorsum of the head. They were observed in 11 of 17 individuals. The eye of one individual in the positive control group also showed hemorrhage.

Next Steps

This project aimed to establish the utility of certain endpoints that, on a theoretical basis, should be addressable in fish. Although the results are promising, it’s necessary to bear in mind that the use of fish for the purpose of online biomonitoring is in the developmental stages. The various endpoints employed will require external validation before fish biomonitoring could be integrated into routine monitoring of the quality of SAR groundwater. The project team also appended a document, “Interim Guidance for Interpretation of Results Obtained in the Biomonitoring Project on the Santa Ana River Watershed” to the report. It illustrates how various endpoints eventually could be incorporated into the decision processes. The interim guidance provides flow diagrams that incorporate estimates of statistical uncertainty and outlines actions based on biomonitoring results.

EXECUTIVE SUMMARY

Online Methods for Evaluating the Safety of Reclaimed Water

PROJECT SUBCOMMITTEE

James Crook, Ph.D., P.E.
Research Council Liaison
Water Reuse Consultant
Bob Arnold, Ph.D.
Research Council Liaison
University of Arizona
Robert K. Bastian
U.S. EPA Office of Wastewater Management
Joseph Gully
Sanitation Districts of Los Angeles County
Bob Hultquist, P.E.
State of California Department of Health Services
William van der Schalie, Ph.D.
U.S. Army Center for Environmental Health Research
Rodney Johnson, Ph.D.
U.S. EPA
Doug Wolf, D.V.M., Ph.D.

CONTRACTOR

Daniel Schlenk, Ph.D.
University of California, Riverside
David E. Hinton, Ph.D.
Duke University
Greg Woodside, PG, CHG
Orange County Water District

The research on which this report is based was funded in part by the U.S. Environmental Protection Agency (U.S. EPA) through Cooperative Agreement No. CR-827345-01 with the Water Environment Research Foundation (WERF). Unless an U.S. EPA logo appears on the cover, this report is a publication of WERF, not U.S. EPA. Funds awarded under the agreement cited above were not used for editorial services, reproduction, printing, or distribution.

Water Environment Research Foundation ■ 635 Slaters Lane, Suite 300 Alexandria ■ VA 22314-1177

01/09