National WWTP effluent screening study

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The Story

DIn 2003 and 2004, EPA ORD's National Exposure Research Laboratory and National Risk Management Research Laboratory collaborated on a study of endocrine disruptors in waste water. Samples were collected of 50 effluents from waste water treatment plants across 10 EPA Regions. Estrogenic activity in water samples was measured using EPA/ORD's molecular diagnostic indicator methods. This effort began EPA/ORD's efforts to study EDCs in the environment at more than a local scale.

Research Goals

Reports of potential wildlife risk from exposure to environmental estrogens emphasize the need to better understand both estrogenic and androgenic presence and persistence in treated wastewater effluents. In addition to wildlife exposure, human exposure should also be examined, especially in situations where estrogenic effluents may return to a drinking water supply. This potential has been examined in rivers and reservoirs in the U.K. where they found reduced estrogenicity downstream from wastewater outfalls and no estrogenicity in reservoirs receiving these waters.

In 2000 NERL and the University of North Texas collaborated in a study where the toxicity and estrogenicity of a final treated municipal effluent was examined. Male fathead minnows were deployed in the effluent for 3 weeks. Vitellogenin (VTG) protein, Gonado-Somatic Index (GSI), Hepato-Somatic Index (HSI) and secondary sexual characteristics were biomarkers used in fish models to assess aqueous estrogenicity. Vitellogenin gene expression was also measured. There was a very good correspondence in the biomarker results and the gene expression results. This study provided evidence that the vitellogenin gene expression assay could be used to assess estrogenic EDC exposures in effluents.

In 2002 and 2003 NERL and NRMRL contacted our 10 Regional Biologists, Regional Science Liaisons, EPA inspectors, State EPA inspectors and Municipal plant operators to voluntarily collect up to 50 effluents and ship them to Cincinnati for male and female fathead minnow exposures. The objective of this study was to determine if adult male or female fathead minnows exposed to a municipal waste water treatment plant (WWTP) effluent elicit a change in vitellogenin gene expression above or below a lab water control.

The goal was to use vitellogenin gene expression results to obtain a rough idea of how common estrogenic and androgenic effluents are in the U.S.

Approach

Contacted Regional Biologists and Regional Science Liaisons – Existing inspections collected 3 gallons from each of 50 municipal effluents from all of the 10 U.S.EPA Regions - Grab or Composite

Selection criteria included type of treatment and demographics of the servicing community Shipped them to Cincinnati overnight for male and female fathead minnow exposures.

Exposure Design

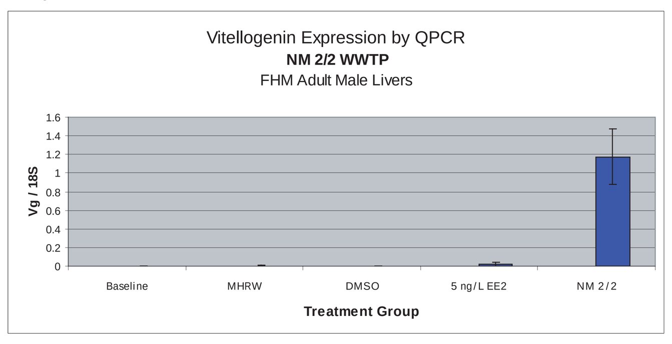
Five male and 5 female fathead minnows were exposed to 2.5L of each effluent for 24-hrs, then analyzed for Vitellogenin Gene Expression.

Each effluent assessed included 3 controls: 1) a lab control water, 2) lab water and DMSO, and 3) a positive control; a synthetic estrogen, Ethynylestradiol (EE2).

2 Liters of effluent were saved and extracted on two separate Solid Phase Extraction C-18 columns; one of the columns was eluted with menthanol and stored at -80 until analysis; the other C-18 was stored at -80 and is awaiting analyses.

Results

Example of Vg Expression in Male Fathead Minnows Exposed To Wastewater Effluents



Example of NO Vg Expression in Male Fathead Minnows Exposed To Wastewater Effluents

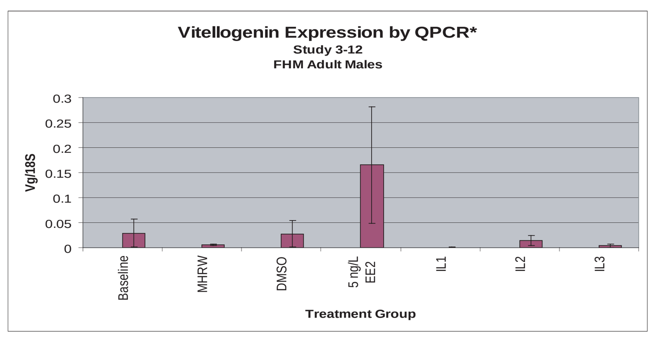
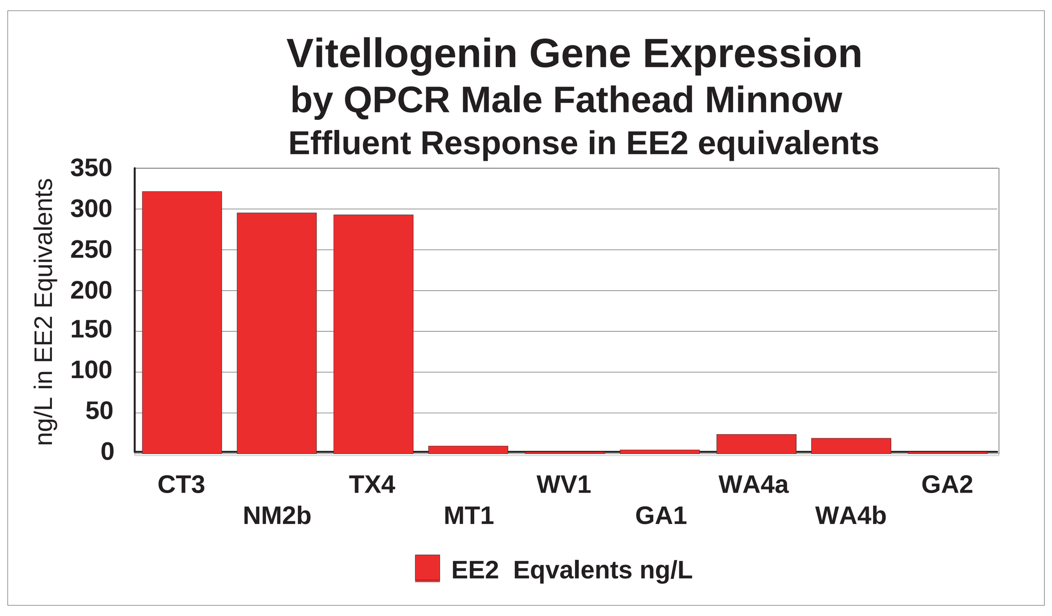


Table 1. Estrogens and Androgens in the *12 Positive Effluents (Prelim 2004)

Effluent Sample Source	WA4B	NM2	CT3	MT1	WV1	GA1	MA1	WA4A	TX4	VA3	CT1	WA1
Progesterone	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Testosterone	<MDL	<MDL	3.2	8.8	4.0	2.5	<MDL	<MDL	<MDL	<MDL	1.3	1.2
Dihydrotestosterone	15.2	<MDL	<MDL	<MDL	<MDL	12.6	2.5	<MDL	<MDL	<MDL	<MDL	<MDL
Androstenedione	9.5	40.5	<MDL	1.6	2.6	15.9	<MDL	5.1	5.0	8.5	17.6	7.3
17-b-estradiol (E2)	1.6	2.7	0.8	2.4	2.3	3.9	4.3	2.6	1.3	3.0	2.1	1.7
Estriol (E3)	16.1	77.4	<MDL	1.1	<MDL	23.7	<MDL	23.5	<MDL	<MDL	46.6	<MDL
Estrone (E1)	28.3	108.5	12.4	51.2	36.4	30.4	4.7	26.5	1.8	96.5	28.2	5.2
17-a-ethynylestradiol (EE2)	5.2	16.9	5.5	8.3	5.7	12.2	2.4	1.7	2.7	5.3	4.2	3.7

Table 2. Estrogens and Androgens in the 6 Non Estrogenic and Androgenic Effluents (Prelim 2004)

Effluent Sample Source	OH2B	IL1	IL2	CA5A	HW1	TX3
Progesterone	<MDL	<MDL	9.5	<MDL	<MDL	<MDL
Testosterone	<MDL	<MDL	2.3	1.6	9.3	<MDL
Dihydrotestosterone	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Androstenedione	<MDL	<MDL	8.6	5.0	9.8	3.0
17-b-estradiol(E2)	<MDL	<MDL	1.9	2.9	65.4	<MDL
Estriol (E3)	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Estrone (E1)	<MDL	3.5	4.7	17.1	108.5	8.9
17a-ethynylestradoil (EE2)	<MDL	3.8	4.1	2.7	9.6	2.2



Results of Converting Effluent Gene Expression into EE2 Equivalents
Vg value of effluent ÷ Vg value for 5 ng/L EE2 X 5 = EE2 equivalents in ng/L

Effluent	ng/L
CT3	321.3
NM2	295.5
TX3	294.2
WA4a	22.1
WA4b	9.0
MT1	9.0
GA1	2.9
WV1	0.5
GA2	0.0

Conclusions

Of the 50 effluents tested, 13 (26%) effluents up-regulated Vg expression in male fathead minnows, 2 effluents down-regulated Vg Expression in female fathead minnows, and 2 effluents that were re-sampled and tested, demonstrated up-regulation of Vg in male fathead minnows. Additional assessment of individual operational information of (1) those wastewater plants demonstrating estrogenicity and (2) similar treatment facilities that did not show estrogenicity, needs to take place to determine if certain types of treatment processes and/or operations are more efficient at removing estrogenic EDCs than others. This study demonstrated the utility of using U.S. EPA's male fathead minnow vitellogenin gene expression assay as a tool for screening effluents for estrogenic EDCs. Chemical analyses of effluent samples are currently underway. Relationships among the detected estrogens and androgens, and gene expression results will be examined.

Conclusions

This research study initiated ORD's efforts to monitor EDCs at more than a local scale. Regional scientists and municipal wastewater treatment plant operators helped NERL and NRMRL expand to a more national scale by collecting samples from several treatment plants within 23 States. The feasibility of sample collection, shipment and analysis at this scale was shown. Positive findings are setting the research agenda for both future studies and development of additional indicators. Additional studies are currently being planned to look at relationships between the presence of EDCs in effluents and instream adverse exposures. An EDC Multiyear Plan "Annual Performance Measure" has been generated on the gene expression results and results are being disseminated formally to the ORD Regional Science Liaisons and Biological Advisory Committee biologists.

Future Directions

Repeat effluents that showed positive results in 2003 and measure chemistries for steroids and alkylphenol ethoxylate (APE) – Add influents for chemistry.

Test different exposure regimes at the same facility –grabs, composites, deployments @ 7,14,21 days

Evaluate O&M on those WWTP that were positive and negative for estrogenicity

Conduct a dosing study at the artificial streams facility.

References

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