

Report for 2005MO51B: Characterization and Biological Effect Study of Endocrine Disruptors in Indian Creek, Newton, County, Missouri

Publications

- There are no reported publications resulting from this project.

Report Follows

Title: Characterization and Biological Effect Study of Endocrine Disruptors in Indian Creek, Newton County, Missouri

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Research Objectives

Many environmental substances possess estrogenic activity. Studies have shown that exposure to these chemicals can cause abnormality and failure in reproduction. Indian Creek in the Elk River Basin in southwest Missouri has a large aggregate of poultry and turkey farming operations. Animal waste which contains steroid metabolites is applied to crop lands. Corn and soybean croplands use herbicides such as atrazine which has been shown to be estrogenic to wildlife. Up to date, there is no study on potential runoff of these chemicals and their biological impacts in Indian Creek in Newton County, Missouri. The original objective of this project is to identify putative estrogenic chemicals in Indian Creek. Upon discussions with USGS at Rolla, we analyzed samples from their four gauging stations in the Elk River basin in McDonald County. The PI also collected water samples from tributaries of Shoal creek. We did not conduct biological effect studies due to limited resources.

Methods, Procedures and Objectives of Research

Water Sample Collection and Chemical Analyses

Eight liters of grab samples were taken from site with an automatic, time-proportioned sampling device (Isco 3710, Lincoln, NE, USA). Samples were placed in pre-cleaned glass bottles which were immediately transported to the UMR Toxicology Laboratory in an ice-cooled container once sampling concludes. We focused on identifying E2, estrone, estriol, 17 α -ethynylestradiol, atrazine, metolachlor, tebuthiuron, plasticizers, and chemicals commonly found in streams in the USA.

Solid Phase Extraction

As soon as the samples arrive at UMR, organic materials were recovered from the water sample by a solid phase extraction (SPE). A specific amount of surrogate compounds such as d₅-atrazine and d₅-estradiol were added to each water sample to monitor the recovery of analytes through the analysis. Suspended solids in the sample were first removed with 2.7 μ m pore size glass fiber filters. The filtered water sample were pulled through a 1g reversed phase octadecylsilane (C₁₈) SPE cartridge (Alltech Associates, Inc., Deerfield, IL) at a flow rate of 10-15 mL/min by applying a vacuum. Each SPE cartridges used in this study were preconditioned by eluting 10mL each of acetone, methanol, and nanopure water. After the sample was passed through, the SPE cartridge was washed with 6mL of nanopure water and vacuum-dried until all of the water was removed. The dried SPE cartridge was connected in series to a glass column filled with anhydrous granular sodium sulfate. The organic materials in the SPE cartridge were eluted into a graduated test tube by passing 3 mL of acetone twice. The extract was then concentrated to 1mL under gentle stream of nitrogen, and subsequently stored in the freezer until further chemical analysis. For the analytical quality control purpose, a fortified laboratory spike and a blank were be analyzed with each set of water samples.

Derivatization

For the GC/MS analysis, the extract from SPE was be derivatized to improve the stability and sensitivity of the analytes. Fifty μL of Sylon BFT (99:1 mixture of BSTFA + TMCS, Supelco, Bellefonte, PA) was added to the extract after evaporating to dryness. After reacting for 30 min at 60 °C, the derivatized extract was evaporated to dryness under gentle stream of nitrogen and reconstituted with 100 μL of hexane. A specific amount of internal standard was be added to the final sample prior to GC/MS analysis.

GC/MS Analysis

Gas chromatography-mass spectrometry analysis was performed with a Varian Saturn 2000 GC/MS utilizing a DB-5M capillary column (30 m X 0.32 mm i.d. with 0.25 μm film, Agilent Technologies, Wilmington, DE). The GC carrier gas was helium and the following temperature condition was used; initial 1 min held at 120 °C and increased to 190 °C in 8 min, and held at final temperature of 290°C. Injection was splitless with temperature set at 280 °C. Mass spectrometer was operated with electron impact at 70 eV and scan 45-500 amu. The accurate quantitation of low level analytes was achieved via the selected ion monitoring (SIM) mode and MS/MS.

Results and Discussions

Table 1 summarized the chemicals found in the USGS gauging station. Atrazine was detected in May 2005 in three of the four USGS gauging stations, ranging between 4865 ng/L water and 599.38 ng/L water. Metolachlor was identified in all four USGS stations, ranging between 5.29 ng/L water to 33.74 ng/L water. The existence of these two chemicals corresponds with the crops seasons.

Several phenols and plasticizers were found in all stations most of the time. They include 4-tert-octyl phenol, 4-octylphenol, nonylphenols, benzyl butyl phthalate, dibutyl phthalate. The concentrations were within the ranges found in other streams in the USA. We are currently compiling information for comparison purpose.

Estrogenic chemicals were identified in the water samples. Estrone, 17 α -estradiol, 17 β -estradiol, 17 α -estradiol, and estriol ranged between 16.37 ng/L water and 63.01 ng/L water. Anti-estrogen, tamoxifen, was found in two stations in May 2005.

Table 2 summarized the chemicals found in the sites we selected in the tributary of Shoal Creek. Atrazine was not found in the two selected site, FR1060 and FR1090, in May, July, and September, 2005. Metolachlor was only found twice at less than 10 ug/L water. Phenols and plasticizers were found less frequent but the concentrations were similar to those found in the USGS gauging stations. Estrone, 17 α -estradiol, 17 β -estradiol, 17 α -estradiol, estriol, and tamoxifen.

The PI is currently requesting USGS to provide other important hydrological data to correlate our water chemistry data.

Education

This project supported two undergraduate students and one master degree student. They have learned a great deal of knowledge in the area of endocrine disruption in the environment. The

master degree student is currently enrolled in the Ph.D. program of Arkansas State University at Jonesborough. The undergraduate student is currently a master degree student at the Department of Biological Sciences.

Perspectives

This is the first attempt to collect water chemistry information using GS/MS in this southwestern area of Missouri. The biological and ecological effects of these chemicals identified were not known and warrant further investigation.

Table 1. Water chemistry data of the USGS gauging stations in the Elk River basin in McDonald County, MO

Compound	GC/MS Parameter		Concentration (ng/L water)								
	Ret. Time (Minute)	Quant Ion (m/z)	8653 May '05	8653 Aug '05	8838 May '05	8838 Aug '05	8885 May '05	8885 Aug '05	9000 May '05	9000 Aug '05	9100 Aug '05
Atrazine	9.55	200	<DL	<DL	48.65	<DL	402.56	<DL	599.38	<DL	<DL
Simazine	9.44	201	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tebuthiuron	6.66	156	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Metolachlor	12.64	162	7.89	<DL	5.29	<DL	33.74	<DL	5.73	<DL	<DL
p,p'-DDE	15.52	318	7.48	<DL	<DL	<DL	1.11	<DL	<DL	<DL	<DL
4-tert-octyl phenol	8.06	207	2.95	<DL	2.66	<DL	<DL	<DL	<DL	<DL	<DL
4-octyl phenol	9.90	207	14.91	139.52	28.35	19.72	13.61	522.76	30.12	<DL	<DL
Nonyl phenol	9.42	207	47.41	97.49	72.81	<DL	44.50	307.33	18.88	<DL	<DL
Benzyl butyl phthalate	18.10	149	56.56	48.52	35.46	<DL	38.17	118.64	29.67	<DL	<DL
Dibutyl phthalate	12.25	149	28.21	32.46	17.30	28.21	35.63	66.79	21.39	37.04	15.32
Bisphenol A	16.35	358	62.37	105.49	52.00	<DL	7.93	220.97	62.62	<DL	<DL
Bioallethrin	14.13	123	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Permethrin	23.64	183	83.80	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Di-p-tolyl sulfone	15.72	246	14.58	<DL	5.04	<DL	8.43	<DL	2.06	<DL	<DL
Diethylstilbestrol	18.48	412	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Estrone	22.38	342	29.31	33.51	27.67	<DL	16.37	63.01	20.49	<DL	<DL
17a-estradiol	22.60	416	20.47	19.95	17.95	<DL	<DL	<DL	15.93	<DL	<DL
17b-estradiol	23.31	416	23.58	24.49	19.18	<DL	<DL	<DL	17.87	<DL	<DL
17a-ethinyl estradiol	24.82	425	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Estriol	26.53	386	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tamoxifen	23.65	368	107.39	<DL	108.28	<DL	<DL	<DL	<DL	<DL	<DL
b-Sitosterol	31.90	357	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Atrazine-d5 ^	9.45	205	-	-	-	-	-	-	-	-	-
Anthracene-d10 *	10.08	188	-	-	-	-	-	-	-	-	-
Cholestane **	25.37	217	-	-	-	-	-	-	-	-	-
Estradiol-d5 ^^	23.25	421	-	-	-	-	-	-	-	-	-

* Internal standard #1

** Internal standard #2

^ Surrogate #1

^^ Surrogate #2

Table 2. Water chemistry in the two sites of the tributary of Shoal Creek in McDonald County, MO

Compound	GC/MS Parameter		Concentration (ng/L water)					
	Ret. Time	Quant Ion	FR1060	FR1060	FR1060	FR1090	FR1090	FR1090
	(Minute)	(m/z)	May '05	July '05	Sept '05	May '05	July '05	Sept '05
Atrazine	9.55	200	<DL	<DL	<DL	<DL	<DL	<DL
Simazine	9.44	201	<DL	<DL	<DL	<DL	<DL	<DL
Tebuthiuron	6.66	156	<DL	<DL	<DL	<DL	<DL	<DL
Metolachlor	12.64	162	<DL	1.87	<DL	<DL	9.86	<DL
p,p'-DDE	15.52	318	<DL	<DL	<DL	<DL	4.10	<DL
4-tert-octyl phenol	8.06	207	<DL	<DL	<DL	<DL	<DL	<DL
4-octyl phenol	9.90	207	<DL	492.02	<DL	<DL	288.24	<DL
Nonyl phenol	9.42	207	<DL	390.91	<DL	<DL	210.11	<DL
Benzyl butyl phthalate	18.10	149	<DL	<DL	<DL	<DL	71.67	<DL
Dibutyl phthalate	12.25	149	9.77	18.83	332.78	58.59	25.23	92.94
Bisphenol A	16.35	358	<DL	<DL	<DL	<DL	<DL	<DL
Bioallethrin	14.13	123	<DL	<DL	<DL	<DL	<DL	<DL
Permethrin	23.64	183	<DL	<DL	<DL	<DL	<DL	<DL
Di-p-tolyl sulfone	15.72	246	<DL	16.26	<DL	104.22	15.52	23.85
Diethylstilbestrol	18.48	412	<DL	<DL	<DL	<DL	<DL	<DL
Estrone	22.38	342	<DL	<DL	<DL	<DL	<DL	<DL
17a-estradiol	22.60	416	<DL	<DL	<DL	<DL	<DL	<DL
17b-estradiol	23.31	416	<DL	<DL	<DL	<DL	<DL	<DL
17a-ethinyl estradiol	24.82	425	<DL	<DL	<DL	<DL	<DL	<DL
Estriol	26.53	386	<DL	<DL	<DL	<DL	<DL	<DL
Tamoxifen	23.65	368	<DL	<DL	<DL	<DL	<DL	<DL
b-sitosterol	31.90	357	<DL	<DL	<DL	<DL	<DL	<DL
Atrazine-d5 ^	9.45	205	-	-	-	-	-	-
Anthracene-d10 *	10.08	188	-	-	-	-	-	-
Cholestane **	25.37	217	-	-	-	-	-	-
Estradiol-d5 ^^	23.25	421	-	-	-	-	-	-

* Internal standard #1

** Internal standard #2

^ Surrogate #1

^^ Surrogate #2