PRESENCE/ABSENCE OF ESTROGEN IN THE RACCOON RIVER WATERSHED AND DES MOINES WATER WORKS TREATMENT PROCESS

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1. Introduction

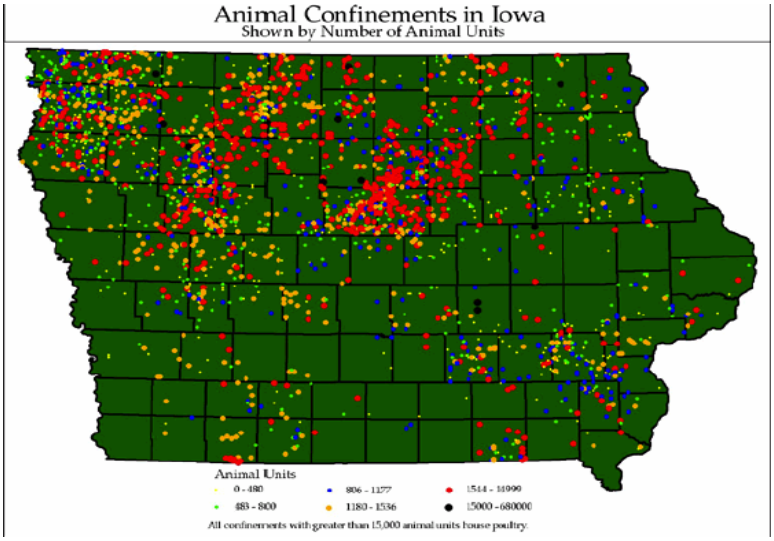
There are three estrogenic hormones, Estrone (E1), 17 Beta-estradiol (E2), and Estriol (E3). Estrone (E1) is the estrogen most commonly found in increased amounts in postmenopausal women. The body derives it from the hormones that are stored in body fat. Estrone may do the same work that estradiol does, but it might be considered weaker in its effects. Estrone is not water soluble.

Estradiol (E2) is the principal estrogen found in all mammalian species during the reproductive years, and is produced by the ovaries. Prescription estradiol may be used for the symptomatic relief of hot flashes, genitourinary symptoms, osteoporosis prophylaxis, psychological well-being and reduction of coronary artery disease. High concentrations of E2 in source waters can result in adverse health effects (kidney impairment, necrosis, and liver damage) on fish. Even concentrations as low as 25ng/L have been found to lead to reproductive impairment and feminization of fish resulting in skewed populations. The main sources of high E2 concentrations to the aquatic environment are sewage treatment waste water and livestock waste (1). Estradiol is not water soluble.

Estriol is the weakest of the three major estrogens. Estriol (E3) is the estrogen that is made in large quantities during pregnancy and has potential protective properties against the production of cancerous cells. Estriol is water soluble. It stands to reason that estrogenic hormones excreted by females may find their way into water supplies by means of wastewater treatment plants.

II. Antibiotics in Livestock

Figure 1: Iowa Concentrated Feeding Operations



In the United States, there are an estimated 376,000 concentrated animal feed operations, CAFOs). Endocrine Disrupting Compounds (EDCs) are known to be used or naturally-produced by the three major categories of CAFOs – cattle, poultry and swine. Cattle CAFOs use growth hormones estrogens (estradiol, estradiol benzoate) in ear implants. Female cattle produce natural estrogens. Poultry CAFOs can contain natural estrogens, estradiol and estrone as well as testrosterone. Swine contain no added growth hormones but do produce natural estrogens and testosterone (2). There is emerging evidence that these EDCs are finding their way into surface waters and sediments via ground water and surface waters from the CAFOs themselves and the biosolids that are be land applied.

III. Discussion

There is speculation that estrogenic hormones may be linked to the escalating incidences of reproductive cancers (testicular, breast and prostate) as well as causing girls to reach puberty earlier than they used to and the feminization of young boys.

Little is known about the transport of EDCs in hydrological systems or their persistence in water supplies. The water supply industry may soon be presented with the issue of water quality with respect to EDCs and should be encouraged to start resource planning now. Des Moines Water Works could potentially be on the forefront by studying the EDCs distribution its watersheds. To our knowledge, the distribution of EDCs in the Raccoon River watershed has yet to be studied. 17 Beta-estradiol (E2) has been chosen for the Des Moines Waterworks study of the Raccoon River Watershed and Des Moines Water Works treatment process, based on the fact that:

1. Estradiol (E2) is the principal estrogen found in all mammalian species during the reproductive years
2. Data indicating cattle are the primary livestock in the Raccoon River watershed
3. 17 Beta-estradiol (E2) is most commonly utilized as a growth hormone for cattle

IV. DMWW Estrogen Testing

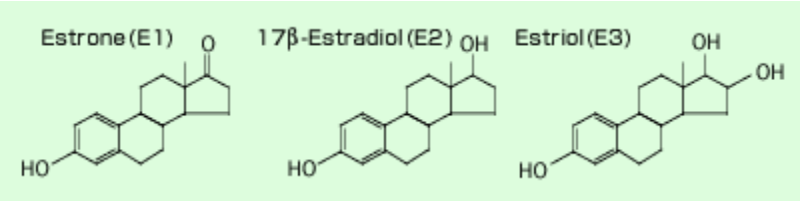
A.. Estrogen Test Kit

The Estrogen (E1/E2/E3) enzyme immunoassay kit licensed from Japan Enviro-Chemicals, Ltd. has been chosen for the use in determination of 17 Beta-estradiol (E2) in this study.

Figure 2: Estrogen (E1/E2/E3) ELISA Kit

B. Method Description

Figure 3: Chemical Structures of Targets



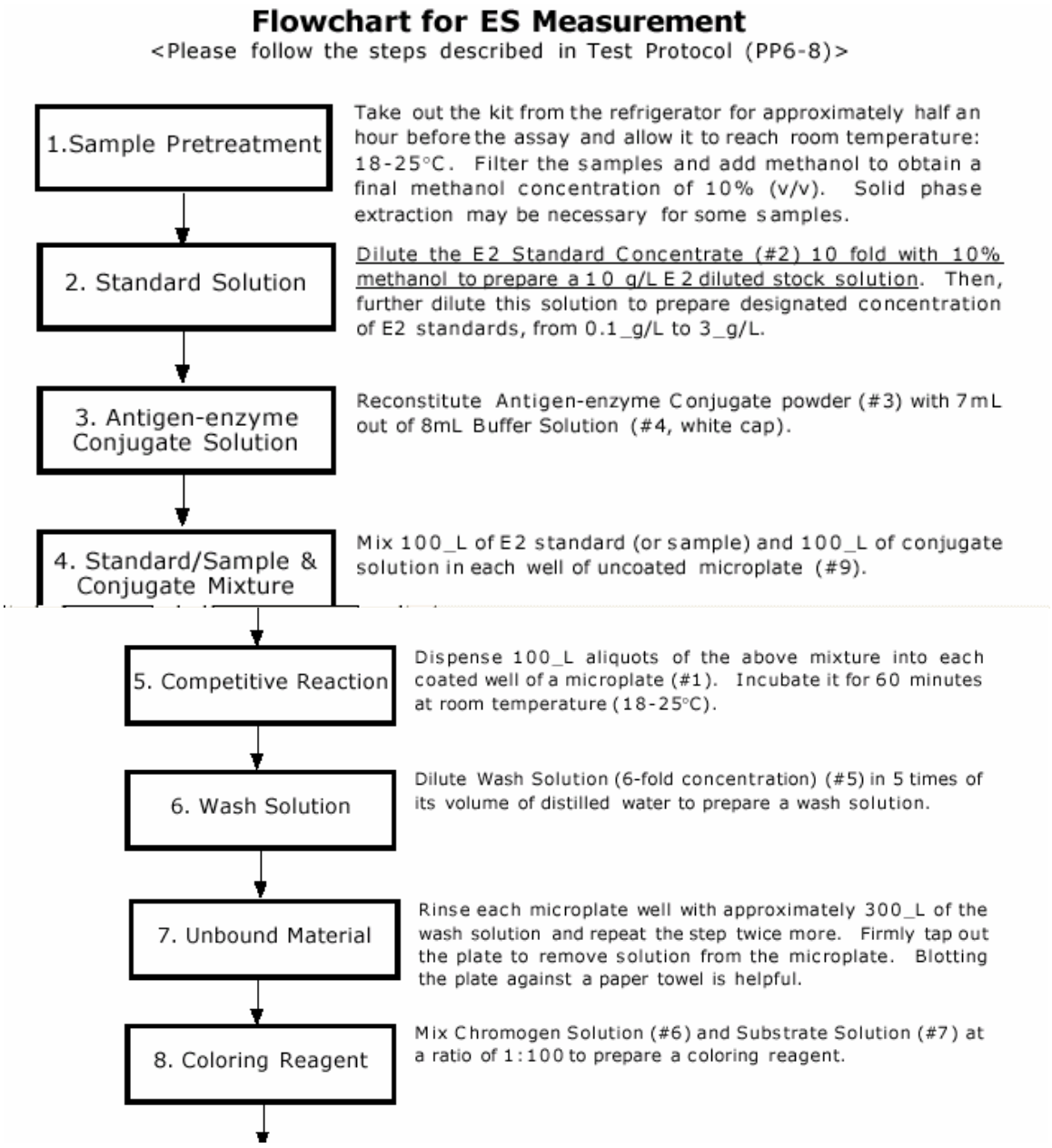
1. ES (Total Estrogen) ELISA test kit

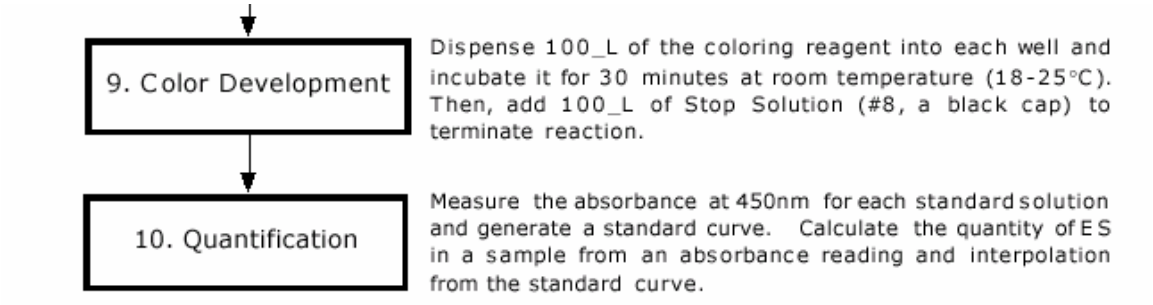
The Total Estrogen (ES) ELISA test kit detects the estrogenic hormones estrone (E1), 17beta-stradiol (E2) and estriol (E3) with similar specificity. This hormone can be found in the blood stream in many different organisms, as well as abundantly in the aquatic environment, such as close to sewage treatment plants.

The analysis is based on a competitive reaction where enzyme-labeled standard E2 competes with free estrogen in the sample for binding to a specific monoclonal antibody immobilized to the surface of the microtiter plate or tube. The amount of labelled E2 bound to the plate is determined by addition of a non-coloured substrate which is converted into a coloured product. The colour intensity is measured at 450 nm and is inversely proportional to the amount of estrogen in the sample. The assay is calibrated using a standard solution of E2 supplied with the kit. The Total Estrogen (ES) ELISA test kit is suitable for analyses of water samples.

The assay is highly sensitive, simple and rapid to perform. The standard curve working range is 0.1-3 µg/L E2. A simple solid phase extraction can be performed on samples with very low concentrations of estrogen.

Figure 4: Analytical Steps





IV. Results

A. 17 Beta-Estradiol (E2) Study of the Raccoon River Watershed and DMWW Treatment Process

1. Overview

For the past six years, the Des Moines Water Works has partnered with Agriculture's Clean Water Alliance to study the Raccoon River Watershed. The purpose of this partnership was to provide a snapshot of where high nitrate levels originated in the watershed, with the idea that remedial efforts could be focused in these areas. This estrogen study utilized these same samples for the determination of the presence-absence of estrogen throughout the Raccoon River watershed.

2. Sample Locations

Figure 5: Raccoon River Watershed Sample Sites

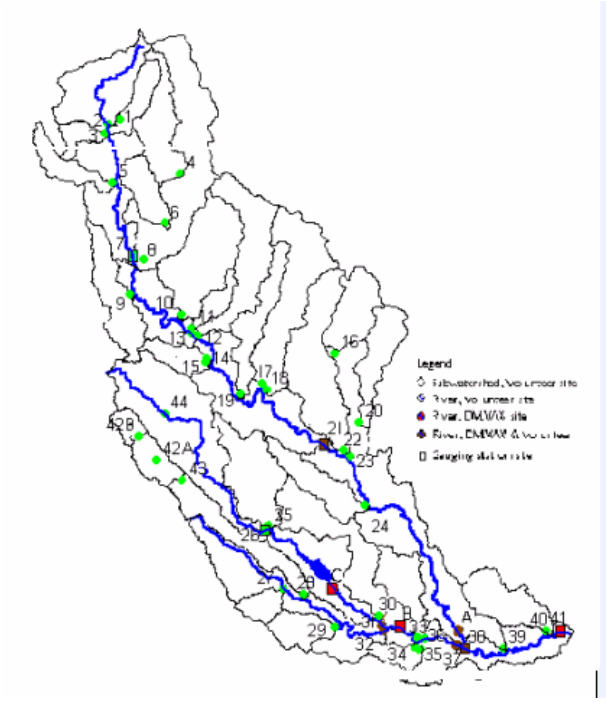


Figure 6: Maffitt Treatment Plant Sample Sites

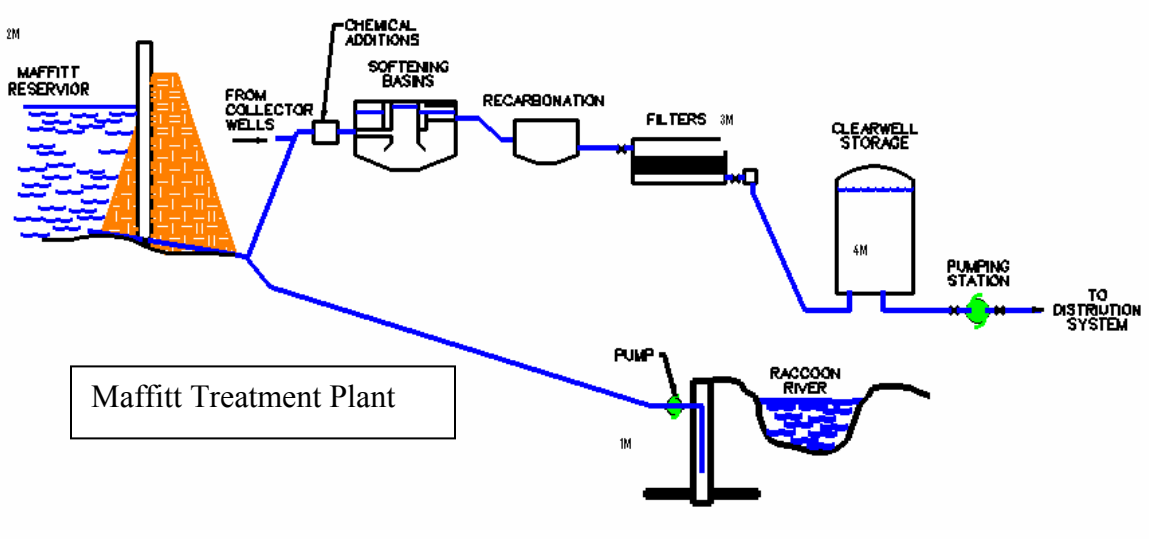
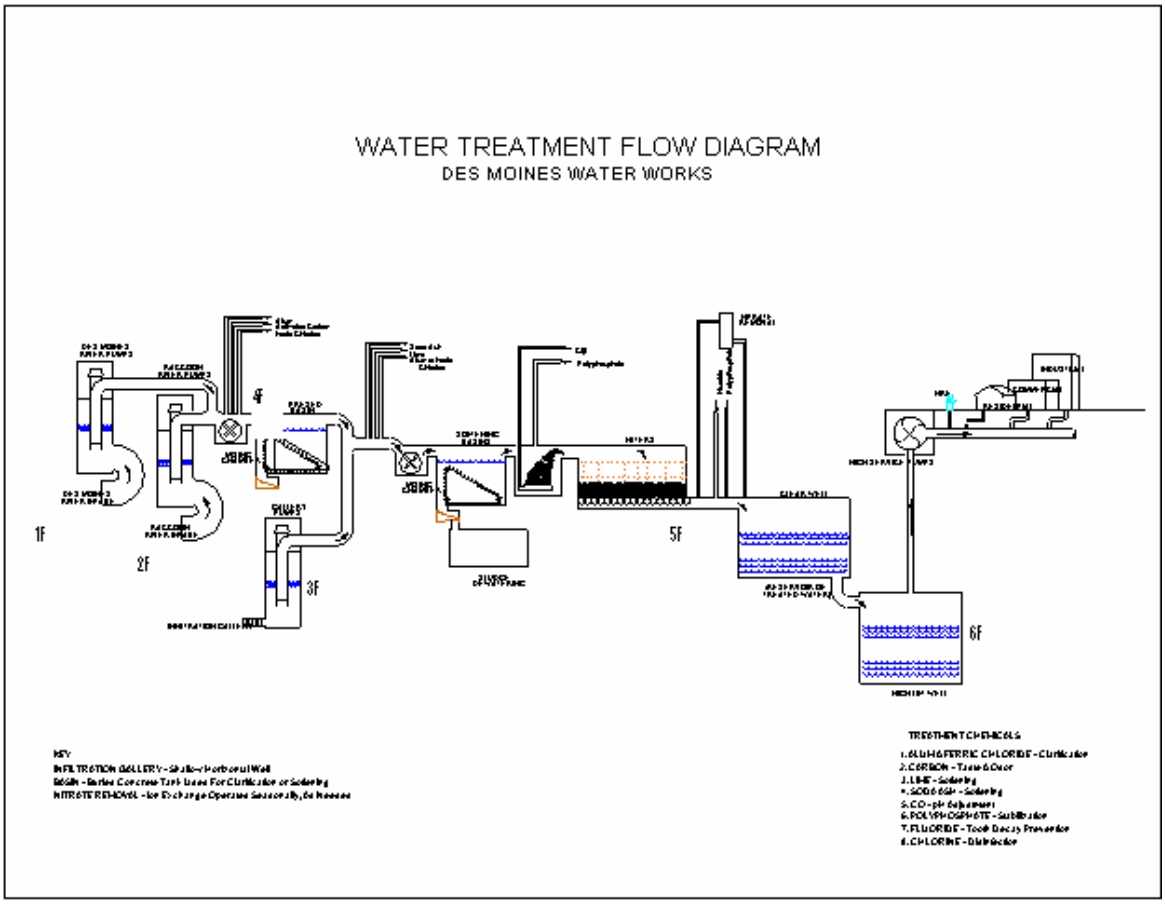


Figure 7: Fleur Treatment Plant Sample Sites



2. Results: June 10, 2005

SITE ID	LOCATION	AMOUNT PPB
28	Brushy Creek	<0.05
12	Lake Creek	<0.05
14	Elk Run	<0.05
4A	Big Cedar Creek	<0.05
2a	North Raccoon River	<0.05
WWTP IN	Des Moines Waste Water Treatment Plant Infulent	0.399
WWTP EF	Des Moines Waste Water Treatment Plant Effluent	<0.05
2F	Raccoon River	<0.05
1F	Des Moines River	<0.05
3F	Gallery	<0.05
4F	Fleur PreSed Effluent	<0.05
6F	Fleur Finished Water	<0.05
1M	Maffitt Raw	<0.05
2M	Maffitt Reservoir	<0.05
4M	Maffitt Finished Water	<0.05

3. Discussion

Des Moines Wastewater treatment plant influent contained .399 ppb estradiol. Estradiol in excess of 0.05 ppb was not detected in the remaining samples. After a discussion with Mike Meyer of the USGS, the conclusion was drawn that, except for wastewater influent samples, a magnetic method with lower detection limits needs to be utilized or extractions would be required in order to concentrate the samples. Since only half of the estradiol kit was utilized, the decision was made to extract the next set of samples and repeat the analysis.

B. Extracted 17BETA-ESTRADIOL (E2) STUDY OF RACCOON RIVER WATERSHED AND DMWW TREATMENT PROCESS

1. Overview

The same sites were analyzed as in the previous study except 250mL of sample was extracted through a C18 cartridge following filtration.

2. Extraction procedure

a. Rinse C-18 disk with 5 mL of methanol followed by 10 mL of distilled water at a flow rate not to exceed 20 mL/minute
b. Pour filtrate, filtered sample, through the C18 disk at a flow rate not to exceed 20 mL/minute
c. Rinse C-18 cartridge with 5 mL of distilled water at a flow rate not to exceed 20 mL/minute and the cartridge was allowed to dry

d. Rinse C-18 disk with 5 mL of hexane at a flow rate not to exceed 20 mL/minute
e. Elute analyte from the C-18 disk with 5 ml of dichloromethane at a flow rate not to exceed 3 ml/minute. (Capture)
f. Evaporate the solvent with nitrogen gas or air
g. Add 100% methanol to the residue and stir the mixture with a vortex Terminate the mixing and pour distilled water to adjust the content at 10% methanol v/v – 1 mL methanol + 9 mL distilled water

3. Results 6-23-05

SITE ID		AMOUNT PPB
28	Brushy Creek	<0.002
12	Lake Creek	<0.002
14	Elk Run	0.003
4A	Big Cedar Creek	<0.002
2a	North Raccoon River	<0.002
WWTP IN	Des Moines Waste Water Treatment Plant Influent	0.057
WWTP EF	Des Moines Waste Water Treatment Plant Effluent	0.002
2F	Raccoon River @ Fleur	0.003
1F	Des Moines River @ Fleur	<0.002
3F	Infiltration Gallery	<0.002
4F	Presed Effluent	<0.002
6F	Finished Water @ Fleur	<0.002
1M	Combined Maffitt Raw	0.002
2M	Crystal Lake	0.002
4M	Maffitt Finished	<0.002

4. Extraction notes

a. Brushy Creek sample tube broke before evaporation. 3 mL of solution saved. Therefore question end result.
b. WWTP took 50 min to pass though initial filter – due to high bacteria?
c. Presed effluent sample contained extra water therefore was passed though sodium sulfate to dry.

5. Discussion

Once again, the only significant estrogen detect was the wastewater treatment plant influent. However, 96% of the estrogen detected in this influent appears to be removed during the wastewater treatment process. Thus, a very minimal amount is released back into the watershed. Personal communication with Mike Meyers of indicated that the estrogen amounts detected at the Des Moines wastewater treatment plant were typical.

Trace amounts of estrogen were detected in Elk Run, 0.003 ppb; Crystal Lake, 0.002 ppb; and the Raccoon River, 0.003 ppb. Currently there are limited data

as to the amount of estrogen in watersheds across the United States. Since estrogen has been shown to be an endocrine disruptor and a drinking water standard has yet to be determined, the knowledge of the estrogen amounts in watershed may prove valuable in the near future.

C. Effectiveness of Carbon in the Removal of Estradiol

1. Overview

In this experiment, Estradiol,E2, was spiked into distilled water and into Raccoon River (RR) water. Estradiol was spiked into distilled water to assess possible matrix effects of particulate matter in the Raccoon River. Varying amounts of powdered activated carbon were then added to determine the dose needed to remove estradiol.

2. Jar Test Results 7-20-05

Sample description	mg/l carbon added	mg/l E2 added	mg/l E2 after carbon	% removal
DI + E2 + 0mg/L Carbon (Control)	0	3	2.5	16.7
DI + E2 + 10mg/L Carbon	10	3	0.278	90.7
RR + E2 + 0mg/L Carbon	0	3	2.38	20.7
RR + E2 + .25mg/L Carbon	0.25	3	1.67	44.3
RR + E2 + .5mg/L Carbon	0.5	3	1.95	35.0
RR + E2 + 1mg/L Carbon	1	3	1.06	64.7
RR + E2 + 2mg/L Carbon	2	3	0.904	69.9
RR + E2 + 5mg/L Carbon	5	3	0.47	84.3
RR + E2 + 10mg/L Carbon	10	3	0.296	90.1
RR + E2 + 15mg/L Carbon	15	3	0.138	95.4

Figure 8: Chart

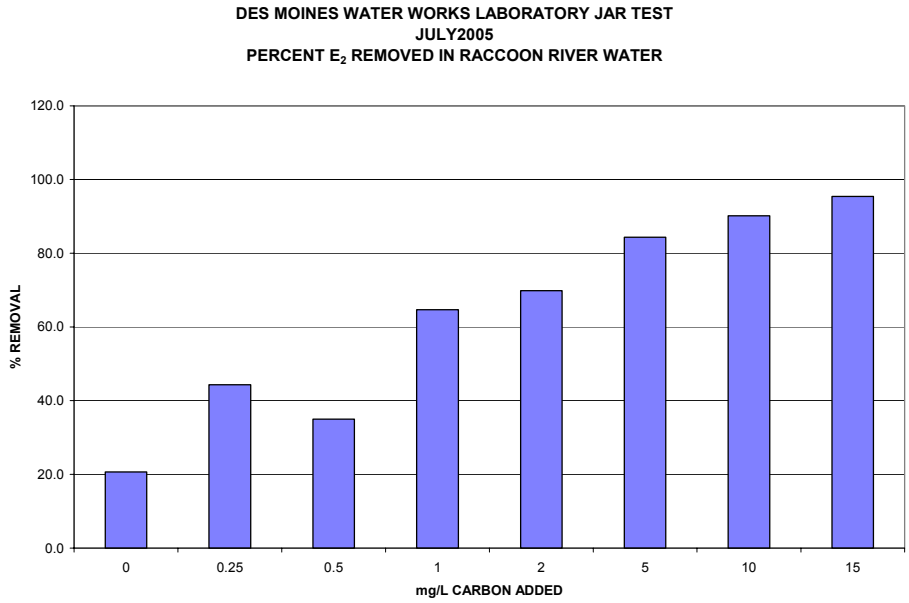
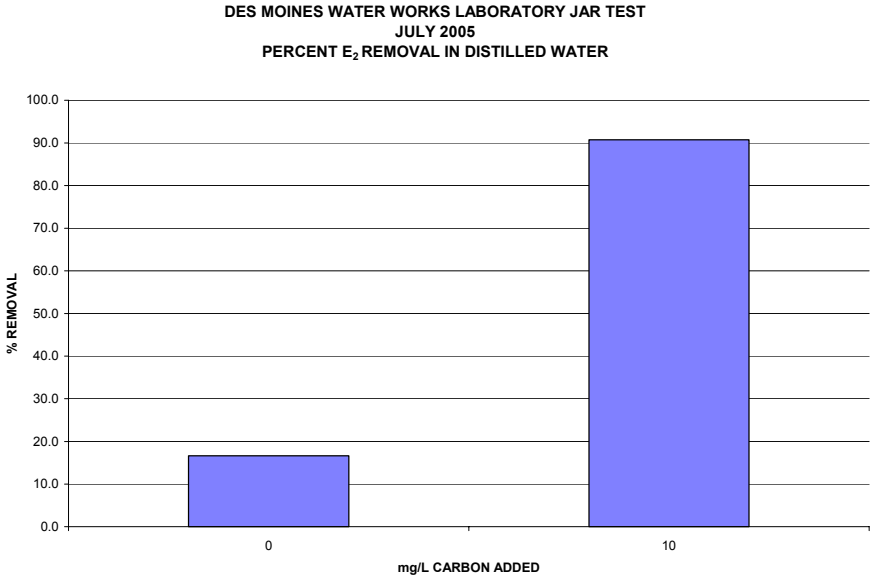


Figure 9: Chart



3. Discussion

This study revealed that if an influx of extradiol was to enter the source water for Des Moines Water works Fleur treatment plant, the plant's average carbon feed of 10mg/L would remove 90% of the estradiol. A 90% removal of estradiol in distilled water spiked with 10mg/L carbon was also seen. This removal efficiency confirms that the particulate matter in the Raccoon River does not interfere with the ability of carbon to remove the spiked estradiol. The addition of carbon to the Maffitt Plant's treatment train would provide the additional

benefit of a cushion in case an influx of emerging contaminates would enter its source water.

D. Estrogen Influence of Wastewater Treatment Plants in the Raccoon River Watershed

1. Discussion

Influent and effluent samples from wastewater treatment plants throughout the Raccoon River watershed were analyzed in addition to samples collected during volunteer monitoring in order to investigate the influence of estrogen in urban communities.

2. Results 7-29-05

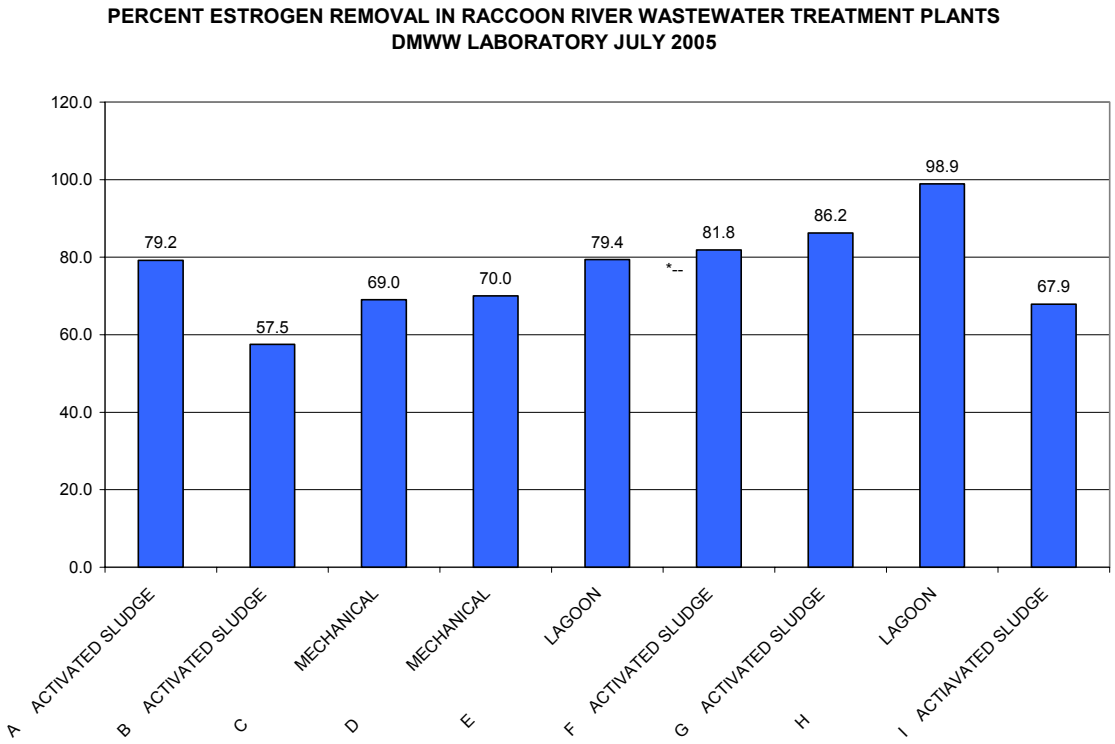
a) Waste Water Treatment Plant Sites

KEY	TYPE OF TREATMENT	INFLUENT PPB ESTROGEN	EFFLUENT PPB ESTROGEN	% REMOVAL
A	ACTIVATED SLUDGE	0.072	0.015	79.2
B	ACTIVATED SLUDGE	0.08	0.034	57.5
C	MECHANICAL	0.071	0.022	69.0
D	MECHANICAL	0.03	0.009	70.0
E	LAGOON	0.034	0.007	79.4
F	ACTIVATED SLUDGE	0.033	0.006	81.8
G	ACTIVATED SLUDGE	0.058	0.008	86.2
H	LAGOON	0.092	0.001	98.9
I	ACTIVATED SLUDGE	0.056	0.018	67.9

b) Volunteer Monitoring Sites

SITE ID	SITE	AMOUNT PPB
28	Brushy Creek	<0.002
12	Lake Creek	<0.002
14	Elk Run	0.003
4A	Big Cedar Creek	<0.002
2a	North Raccoon River	<0.002
2F	Raccoon River @ Fleur	0.003

Figure 10: Chart



3. Discussion

This study indicates urban areas are a potential estrogen source for the Raccoon River watershed. Wastewater treatment does remove significant quantities of estrogen from raw waste. Water treatment plants may want to consider powered activated carbon in their treatment processes in case an influx of estrogen were to enter source waters due to a breach in a wastewater treatment operation or a confinement waste lagoon spill.

E. Follow-Up 17beta-Estradiol (E2) Study of Raccoon River Watershed and DMWW Treatment Process

1. Overview

The same sites were analyzed as in the June study with the addition of samples from the Middle and South Raccoon River, Buttrick and Indian Creek. These additional samples were added due to the detectable amounts of ortho-phosphate, which is an indicator of animal or human waste products. Again, 250mL of sample were extracted through a C18 cartridge following filtration and prior to ELISA analysis.

2. Results 8-09-05

SITE ID	LOCATION	AMOUNT PPB
43	Brushy Creek	<0.002
12	Lake Creek	<0.002
14	Elk Run	0.003
A	North Raccoon River	0.003
44	Middle Raccoon River	0.007
28	South Raccoon River	<0.002
2F	Raccoon River	<0.002
1F	Des Moines River	<0.002
5	Outlet Creek	0.002
9	Indian Creek	0.002
23	Buttrick Creek	0.009
14A	North Raccoon	<0.002
3F	Gallery	<0.002
1M	Maffitt Raw	<0.002
5m	Maffitt Filter Effluent	<0.002
2m	Crystal Lake	<0.002

3. Discussion

The results of this study were similar to the June study. The June study was performed during a rainy period, thus high river flow indicated dilution of possible source water contaminates. The August study was preformed during a dry period, with low river flow providing less dilution water. Concentrations were similar for both low and high flows.

Estrogen was detected on Buttrick Creek and Middle Raccoon South of Carroll. These samples were not analyzed in the June Study. Therefore a comparison between the samples results as to flow and time of year cannot be drawn.

0.007 ppb Estrogen was detected in the Middle Raccoon South of Carroll. Wastewater discharge is suspected to be the primary source of this detect. This suspicion is based on the detection of 0.03 ppb estrogen in waste water

effluent analyzed in this area during the 7-29-05 study. However, Carroll County is also densely populated with livestock. Therefore livestock may have an influence on this estrogen detection.

Most of the effluents from the wastewater treatment in the Buttrick Creek area are from sedimentation lagoons which are discharged biannually in early spring and late fall. Therefore, at this time of year, these wastewater treatment facilities are not suspected to be the source of the estrogen detect in Buttrick Creek.

There is however one wastewater treatment which continuously discharges into Buttrick Creek. This wastewater effluent may possibly influence the estrogen detect in Buttrick Creek. But, due to the fact that the location of this plant is a small rural town with a population of 1,000, one wouldn't expect the town to be the sole source of the estrogen detect. The DNR interactive map indicates an animal feeding operation near Buttrick Creek. Going on the realization that it only takes one animal operation to pollute a creek, one might conclude that the estrogen detect in the Buttrick Creek watershed was probably influenced by the animal operation. There is also the possibility that the estrogen detect may have been influenced by the combination of wastewater effluent and livestock. Without sampling and analyzing the creek downstream from the animal operation and the without analyzing the suspected towns wastewater effluent, one can not accurately draw a conclusion as to the source of the estrogen detect in Buttrick Creek.

V. Conclusions

In conclusion it appears, at this time, the estrogen concentrations observed in this study of the Raccoon River watershed are not significant enough to be considered a threat to human health. Only two of the wastewater plant effluents tested yielded estrogen concentrations near levels shown to cause adverse health effects (kidney impairment, necrosis, liver damage, reproductive impairment and feminization) to fish and aquatic life – 25 ng/L. These finding concurs with research stating that sewage treated waste water is considered to be one of the main sources of estrogen in aquatic environments. It is the opinion of this investigator that water of the Raccoon River Watershed does not appear to present any estrogen-related health concerns to aquatic life or humans at this time.

On the other hand, water treatment plants should have a means in which to remove estrogen in their treatment process in the case sewage due to either livestock or wastewater treatment plants accidentally penetrates the source water serving water treatment plants. This study indicated a dose of 10 mg/L of powdered activated carbon would remove 90% of estrogen at a contaminant level of 3 ppb.

References

(1)Ndubuka, Chinwe, Wicks, Carol M., Kelley, Cheryl A., and Peterson, Eric w., Geological Sciences, Univ of Missouri, 101 Geology Building, Columbia, MO 65211, The Significance of 17 Beta-Estradiol in the Rock Bridge Stream/Spring System, MO

(2)http://www.setac.org/blatimore/files/sessions/c03.html

(3) http://www.health-science.com/what_is_estrogen.html