



## **WATER RESOURCES RESEARCH GRANT PROPOSAL**

**Title:** Endocrine Disruptors in Surface Waters: The Occurrence, Distribution, and Fate of Alkylphenol Polyethoxylates and Metabolites in Wastewater Treatment Facilities and Their Impact on Kansas River

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**Project Category:** Research/Educational/Extension Category

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**Abstract**

Wastewater and its constituents are one of the major sources of surface water and groundwater pollution in the State of Kansas. Alkylphenol polyethoxylates, surfactants in domestic and industrial cleaning detergents, are usually discharged into sewers that transport these chemicals to wastewater treatment facilities. During conventional wastewater treatment, the alkylphenol polyethoxylates are aerobically degraded to alkylphenol mono- or di-ethoxylates and alkylphenols, which are more toxic than the parent compounds. Research has shown that alkylphenol mono- or di-ethoxylates and alkylphenols, a class of endocrine disruptors, are estrogenic in fish, birds, and mammals. Recent investigations in Europe have shown significant levels of alkylphenols in rivers and estuaries and attributed their occurrence to domestic and industrial waste discharges. The overall objective of the proposed project is to investigate the occurrence, distribution, and fate of alkylphenol polyethoxylates and metabolites in municipal wastewater treatment facilities and effluent discharges and the impact of these compounds on the

water quality and ecological environment of Kansas River in Kansas. Information generated from this research would provide critical and timely information about the extent of removal of alkylphenol polyethoxylates and their metabolites in Kansas wastewater treatment facilities and the release of those contaminants into the receiving water bodies.

## **Technical Plan**

# **Statement of the Critical State Water Problem**

Wastewater and wastewater constituents are among the major sources of surface water and groundwater pollution in the State of Kansas. The State Water Plan for Kansas envisages a significant increase in recreational opportunities at public lakes and streams by the year 2010. Such activities, however, are directly impacted by the water quality in these surface water bodies. Recent studies have related the occurrence of several hormonally mediated toxic effects in fish and other aquatic organisms to endocrine disrupting chemicals in wastewater discharges. One such class of endocrine disruptors - alkylphenol polyethoxylates- is present in several industrial and household grade detergents and surfactants, and commonly finds its way into municipal wastewater treatment facilities. In the past, very few studies have focussed on evaluating the occurrence, distribution, and fate of alkylphenol polyethoxylates and metabolites in municipal wastewater treatment facilities and effluent discharges in the State of Kansas. Such a study can provide critical and timely information about the extent of removal and release of alkylphenol polyethoxylates and their degradation byproducts in Kansas wastewater treatment facilities and the associated receiving water bodies. Information generated from such a study will help establish novel treatment approaches and best management practices to reduce contamination of public lakes, reservoirs and streams from chemicals that can have potential hormone disrupting effects on the ecosystem.

## **Nature, Scope and Objectives of Proposed Work**

The proposed work will compare the inflow of alkylphenol polyethoxylates into three municipal wastewater treatment plants (WWTPs) and the transformation and distribution of these compounds and their metabolites due to different treatment processes in the 3 plants. These wastewater treatment plants include the Wamego, Manhattan and Northern Topeka facilities (Figure 2). These facilities were selected because they serve a range of size and type of communities along a 30-mile stretch of the Kansas River. The fate of alkylphenol polyethoxylates will be evaluated during various phases of wastewater treatment. Microbiological conversion of the parent compounds and accumulation of biodegradation intermediates such as mono- or diethoxylates and alkylphenols will be studied. Finally, the impact of effluent discharge on the distribution and bioaccumulation of alkylphenol polyethoxylates or metabolites in the water column of the Kansas River will be evaluated.

## The specific objectives of this project are:

**Phase 1** To quantitatively evaluate the occurrence, transformation, and distribution of alkylphenol polyethoxylates and metabolites in various process streams associated with the selected municipal WWTPs.

**Phase 2** To assess the levels and the distribution of alkylphenol polyethoxylates and metabolites in Kansas River

**Phase 3** To investigate the potential for alkylphenol polyethoxylates and metabolites-induced endocrine disruption in fish in Kansas River.

## Related Research

Alkylphenol polyethoxylates find common use as cleaning detergents and as industrial process aids. They are usually used in aqueous solutions and discharged into sewers that transport these chemicals to wastewater treatment facilities. Being nonionic surfactants, these compounds are amphiphilic having a nonpolar hydrocarbon (R-) end and a polar ethoxylate (EO) end (Figure 1A). During conventional wastewater treatment, the alkylphenol polyethoxylates are aerobically degraded to alkylphenol mono- or di-ethoxylates by the sequential "chopping off" of the polar end (Figure 1B). This produces a biodegradation product that is significantly more hydrophobic than the parent chemical. Due to their hydrophobicity, the mono- and di-ethoxylates drop out of solution and tend to associate preferentially with the bacterial biomass or sludge. If the waste activated sludge is processed by anaerobic digestion, the mono- and di-ethoxylates in the sludge are further converted to the recalcitrant, estrogen-like octyl, nonyl or decyl phenols (Figure 1C).

*Figure 1. Fate of alkylphenol polyethoxylates in wastewater treatment facilities: (A) parent chemical in raw wastewater; (B) degradation byproducts (alkylphenol mono- and di-ethoxylates) primarily associated with sludge; and (C) recalcitrant and estrogenic alkylphenol in anaerobically digested sludge (R=C<sub>8</sub>H<sub>17</sub>, octyl; R=C<sub>9</sub>H<sub>19</sub>, nonyl; R=C<sub>10</sub>H<sub>21</sub>, decyl). Adapted from Giger et al. (1984)*

Alkylphenol polyethoxylates are notorious for the toxicity, persistence and hormone-mimicking properties of their biodegradation products, specifically alkylphenols. White *et al.* (1994) illustrated that alkylphenols including octyl and nonylphenols were estrogenic in fish, birds, and mammals. Trocme *et al.* (1988) demonstrated bacterial toxicity of alkylphenols by showing declines in CO<sub>2</sub> production and biomass ATP concentration in the presence of nonylphenol. Ahel *et al.* (1993) were able to illustrate the bioaccumulation of nonylphenol and nonylphenol mono- and di-ethoxylates in algae and fish in the surface waters of Glatt Valley, Switzerland.

Brunner *et al.* (1988) and Ahel *et al.* (1994) studied the fluxes of nonyl polyethoxylates, nonyl mono- and di-ethoxylates and nonylphenol during sewage and sludge treatment at several wastewater treatment facilities in Switzerland. The concentrations of nonylphenol

in the secondary effluent and digested sludge at the Zurich-Glatt facility were found to be 2.7 mg/L and 78 mg/kg (dry weight basis), respectively. Approximately 50% of the polyethoxylates in raw sewage were transformed into nonylphenol and accumulated in the digested sludge. Rudel *et al.* (1998) found a total concentration of 30 mg/L for nonyl/octyl phenol and polyethoxylates in groundwater immediately downgradient of septic systems and leach fields in the Cape Cod area of Massachusetts. A recent study reported total alkylphenol levels as high as 80 mg/L in an Italian river and attributed their occurrence to domestic and industrial waste discharges (Davi and Gnudi, 1999). Other researchers have also investigated the occurrence and distribution of alkylphenol compounds in the Great Lakes sediments (Bennett and Metcalfe, 1998), as well as the aquatic environment of rivers and estuaries in England and Wales (Blackburn and Waldock, 1995).

## Methods, Procedures and Facilities

### *Selection of sampling locations*

The occurrence, distribution, and fate of alkylphenol polyethoxylates and metabolites in three wastewater treatment plants (WWTPs) will be investigated. The three wastewater treatment plants selected for this study are located along the Kansas River in Kansas (Figure 2). The effluent from the WWTPs is discharged into the river. The three WWTPs are selected to give a wide range of potential inputs of alkylphenol polyethoxylates and metabolites because of the size of the treatment plants and the variability in the communities they serve.

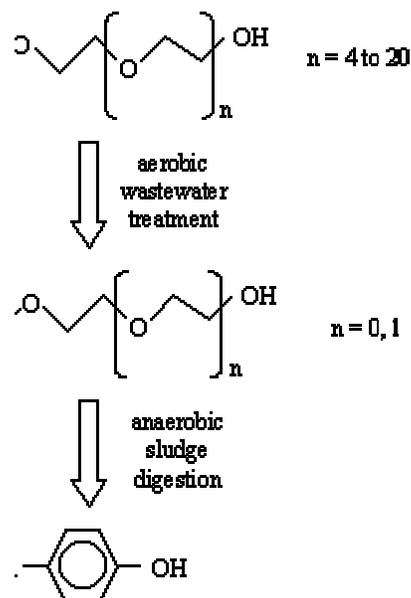


Figure 2. Locations (circle) of the three wastewater treatment plants along the Kansas River.

The Manhattan WWTP (large town) is a 5.5 MGD (million gallons per day) facility with no primary clarifier (Figure 3a). It serves a population of approximately 50,000 with a

few small industries and a major university. The Wamego WWTP (small town) is a 0.75 MGD package treatment plant and serves a population of 3,700 with no major industries (Figure 3b). The Topeka WWTP (city) is a 12 MGD facility with primary and secondary clarifiers (Figure 3c). This facility serves a population of 152,000 with several small and medium industries.

### ***Sample collection***

**Phase 1** The occurrence, transformation, and distribution of alkylphenol polyethoxylates and metabolites in the three selected WWTPs will be investigated. Raw wastewater, primary effluent and sludge, and secondary effluent and sludge will be collected at each treatment plant one day per month for the first year. Figure 3 shows each stage where the samples will be collected following the wastewater treatment processes at each WWTP. Waste samples will be collected every 3 hours during a day.

**Phase 2** The levels and the distribution of alkylphenol polyethoxylates and metabolites in Kansas River will be investigated after the completion of *phase 1*. Water, sediment, and algae samples will be collected at the outlet of each treatment plant and at 4 points across the river at approximately 10 m, 0.5, 1, 2, and 3 miles from the discharge point in the river. Same type of samples will also be collected in the river upstream of Manhattan WWTP and midpoint between each treatment plant. Samples will be collected one day per month for 2 years.

All water samples will be collected without preservative in 1-L pre-rinsed amber glass bottles, stored at 4°C and extracted within a week. One field blank will be prepared for each day of sampling. Sediment samples will be collected with a grab sampler and air-dried at 20°C in a fume hood. Large particles will be removed from dried sediment samples by sieving through 800- $\mu$ m sieve. The dried and sieved sediment samples will be stored in sealed glass vials at room temperature in the dark until analysis. Samples of algae will be collected manually, wrapped in aluminum foil, and kept at -20°C until analysis.

### ***Sample preparation and analysis***

- Extraction

#### Water sample extraction

The pH, ionic strength, and dissolved organic carbon content in each water sample will be measured before extraction of target chemicals. Five  $\mu\text{g L}^{-1}$  of 4-t-butylphenol internal standard will be added to 300 mL water sample and filtered through a solvent rinsed Whatman GF/C filter. Three grams of sodium chloride is added to the solution before the filtration in order to prevent the adhesion of the analytes to the filter paper. The filtrate will be concentrated onto a pre-conditioned 500 mg C18 solid phase extraction minicolumn (Sep-Pak C18, millipore, USA). The 500 mg C18 extraction minicolumn is pre-conditioned by washing it using 5mL ethyl acetate followed by 5 mL 50/50

methanol/distilled water (Blackburn and Waldock, 1995; Marcomini et al., 1987). After concentration the analytes will be eluted with 2.5 mL ethyl acetate followed by 2.5 mL dichloromethane. The combined eluate will be reduced in volume to approximately 0.2 mL under a stream of dry nitrogen at room temperature and stored at -20°C until analysis. *Figure 3. Schematic diagrams of waste water treatment processes at Manhattan WWTP (a), Wamego WWTP (b), and Topeka WWTP (c). The \* sign indicates the stage where the samples will be collected.*

### **Sediment sample extraction**

The extraction of alkylphenol polyethoxylates and metabolites from dried sediment samples will be performed with steam distillation/solvent extraction (Thiele et al., 1997). Twenty five grams of dry sediment sample will be suspended in 1.5 L of water and refluxed for 3 h by using 2 mL of cyclohexane as the extracting solvent through which the distillate percolated. Certain amount of 2,4,6-trimethylphenol will be added to each extract as an internal standard. The enriched extracts will be stored at -20°C until HPLC analysis.

### **Algae sample extraction**

Extraction of alkylphenol polyethoxylates and metabolites from algae will follow the same extraction procedure as that for sediment sample extraction.

- HPLC analysis

Normal- and reversed-phase normal-phase Hewlett Packard 1050 high-performance liquid chromatography (HPLC) will be applied to quantitatively determine concentrations of individual alkylphenol polyethoxylate oligomers (nEO = 3-18) and homologues (C9 and C8), respectively, in samples from waste water, sediment, and the aquatic environment (Ahel and Giger, 1985 a,b). Gas chromatography with mass spectrometer (GC-MS) will be used for verification purpose. In addition, reversed-phase HPLC will be used to determine the total concentration of alkylphenol polyethoxylates.

Prior to the normal-phase HPLC analysis, extracts will be subjected to a clean-up procedure using a column of partially deactivated alumina (1.5% water). The alkylphenol polyethoxylates will be eluted from the alumina column using methanol. The eluate will then be evaporated to dryness and redissolved in exactly 500 µL of a 2-propanol/n-hexane mixture (1:9). For reversed-phase HPLC analysis, the residue will be redissolved in exactly 500 µL of a methanol/water mixture (1:9). Table 1 lists the conditions for HPLC analysis.

**Table 1 Conditions for HPLC analysis for determination of the concentrations of individual alkylphenol polyethoxylate oligomers and homologues.**

Conditions	Normal-phase HPLC	Reversed-phase HPLC
Analytical column	60x4 mm packed with spherical aminosilica particles of 3 $\mu\text{m}$	100x4 mm packed with irregularly shaped octylsilica particles of 7 $\mu\text{m}$
Mobile phase	A: n-hexane/2-propanol mixture (99:1) B: 2-propanol/water mixture (98:2)	Acetonitril/water mixutre (54:46)
Flow rate	2mL/min, change linearly from 0%B to 95% B over 23 min	1.2 mL/min
Detector	fluorescence detector at 277 nm	Fluorescence detector at 300 nm

***Bioassay study of the potential for alkylphenol polyethoxylates and metabolites-induced endocrine disruption in fish in Kansas River***

Effect of different levels of alkylphenol polyethoxylates and metabolites on the levels of vitellogenin, a biomarker for endocrine disruption in fish, will be studied by exposing 30-day old male and female carp to water containing different amounts of alkylphenol polyethoxylates and metabolites in flow-through monitoring tanks for half year. Every 10 days fish will be collected and weighed. Blood samples will be collected from the caudal sinus into a heparinized 5 mL vacutainer, chilled on wet ice, and centrifuged for 10 minutes at 1000 g. Plasma will be pipetted into 2 mL cryotubes and stored at  $-70^{\circ}\text{C}$  prior to vitellogenin analysis by capture enzyme-linked immunosorbent assay (ELISA) as previously described (Folmar et al., 1996). The sensitivity of ELISA assay used in this study is 1 :g/mL (Goodbred, et al., 1996). Interassay variation will be calculated using internal standards at three points on the standard curve.

Levels of vitellogenin will also be quantified in blood serum of fish from Kansas River. Adult male and female carp (target minimum lengths are 300 mm for males and 375 mm for females) will be collected and weighed at the same sampling sites where water and sediment samples are collected. Blood sample will be collected on site using the same procedures described above. Plasma collected on site will be immediately frozen on dry ice for transportation and stored  $-70^{\circ}\text{C}$  prior to vitellogenin analysis.

### ***Quality assurance/quality control***

All samples will be packed with ice immediately after the collection and transported to the laboratory on the day of the collection. Water samples will be stored at 4°C and extracted within a week. Sediment samples will be air dried at 20°C in a fume hood immediately after they are transported back to the laboratory. The dried and sieved sediment samples will be stored in sealed glass vials at room temperature in the dark until analysis. Samples of algae will be transported at 4°C to the laboratory and kept at -20°C until analysis.

Samples collected, observations, the date and time of preparation and name (s) of the person(s) who is responsible will be regularly recorded for all experiments in a standard bound laboratory notebook with pre-numbered pages. All sample labels and records will be written in indelible ink.

Each collected sample will be divided into 3 sub-samples as 3 replicates. Extraction and HPLC analysis of alkylphenol polyethoxylates will be conducted for each sub-samples (replicates). The relative deviation of HPLC-measured concentrations of 3 sub-samples should be smaller than 10%. An additional sub-sample will be reanalyzed if the deviation exceeds 10%.

External standards and solvent blanks will be analyzed at frequent intervals to assure equipment stability. Standards will be reanalyzed if the relative standard deviation in three determinations exceeds 10%. Data obtained from experiments will be reported in appropriately formatted graphs and tables. Where applicable, analysis of variance (ANOVA) will be used to differentiate treatment effects.

Ongoing progress of the project will be reviewed in regular peer seminars conducted by the two principal investigators. This research will involve one interdisciplinary Master thesis co-advised by Dr. Xia (Dept. of Agronomy) and Dr. Bhandari (Dept. of Civil Engineering) at Kansas State University. The successful completion of this dissertation, dissemination of results at one or two regional/national conference, and acceptance of at least two peer reviewed scientific journal publication, will serve as measures of project success and effective technology transfer.

### **Information transfer plan**

The results from this research will be presented at the Kansas State Water and Environmental Federation Annual Conference and the national conference of the Water Environment Federation both held in 2001. The results will also be presented to and shared with environmental groups, the general public, and the personnel at wastewater treatment plants in Kansas. The results will also be posted on related KSU web-pages

## Anticipated results and expected benefits

This research will help monitor the occurrence and distribution of estrogenic alkylphenol polyethoxylates and metabolites, organic chemicals from human activity, in Kansas water. We expect that significant amount of alkylphenol polyethoxylates and metabolites will be found in raw wastewater due to the wide domestic and industrial use of alkylphenol polyethoxylates as surfactants. This project will provide information about the sources, levels, distribution, and extent of bioaccumulation of alkylphenol polyethoxylates and metabolites in the Kansas River. This project will also provide information about removal of alkylphenol polyethoxylates and their metabolites in representative wastewater treatment facilities in Kansas. Information generated from this project would help establish regulations and novel treatment approaches to prevent and reduce the contamination of alkylphenol polyethoxylates and metabolites in the surface water of Kansas.

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