

Report for 2003MD28B: Fate of Alkylphenolic Compounds in Wastewater Treatment

- Articles in Refereed Scientific Journals:
 - Loyo-Rosales, J. E., Schmitz-Afonso, I., Rice, C. P., and Torrents, A. (2003), “Analysis of octyl- and nonylphenol and their ethoxylates in water and sediments by liquid chromatography- tandem mass spectrometry”. *Anal. Chem.* 75(18), 4811-4817.
 - Loyo-Rosales, J. E., Rosales-Rivera, G. C., Rice, C. P., Torrents, A. (2005), “Linking laboratory experiences to “the real world”: The extraction of octylphenoxyacetic acid from water”. *J. Chem. Educ.* In press.
 - Loyo-Rosales, J. E., Rice, C. P., and Torrents, A., “Determination of octyl- and nonylphenol ethoxylates and carboxylates in environmental samples by isotope dilution and liquid chromatography/tandem mass spectrometry”. Submitted to *J. Chromatogr. A*.

Report Follows

Final project report
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**Fate of alkylphenolic compounds in wastewater treatment plants and a sub-estuary of the
Chesapeake Bay**

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Problem statement and research objectives

A series of recent studies have raised concerns about the presence of persistent organic pollutants in natural waters and the capability of wastewater treatment plants (WWTPs) to remove such chemicals from their effluents. The last decade has also seen an increased interest in pollutants suspected to interfere with the endocrine system, usually known as endocrine disrupters. The hormone-mimicking action of some of these chemicals and their harmful effects were first acknowledged in humans during the late 1940's, but their consequences on wildlife were only recognized decades later (Sonnenschein and Soto 1998). One such compound is 4-nonylphenol (NP), which was found to produce the same effects as estradiol in a line of cancer cells (Soto et al. 1991). NP is a precursor in the synthesis of the nonylphenol ethoxylates (NPnEOs), one class of alkylphenol ethoxylates (APnEOs, where n represents the number of ethoxylate units). APnEOs are nonionic surfactants that have been widely used in industrial processes and as detergents in both industrial and household applications for more than thirty years. After being used, the APnEOs are discharged into wastewater and are treated in WWTPs. During wastewater treatment, APnEOs are subject to microbial degradation processes that

produce different metabolites (NP among them), which are ultimately released into natural waters. Several of these degradation products have been shown to possess estrogenic properties and have been found in WWTP effluents at relatively high concentrations, especially in European systems (Bennie 1999). Although typical concentrations of NP and APnEOs in North American fresh waters are typically below the limit for the onset of endocrine-disruption endpoints, there is still concern for harmful effects in water bodies heavily impacted by WWTP effluent where their concentrations are higher, and for the occurrence of possible additive effects with other estrogenic compounds. Besides, their ubiquity in natural waters has been extensively documented in North America and Europe (Bennie 1999). A recent survey (Kolpin et al. 2002) of organic wastewater contaminants in 139 American streams found NP in 50% of the samples and, together with the nonylphenol mono- and diethoxylates (NP1EO and NP2EO respectively), was one of the pollutants found in the highest concentrations. The toxicity concerns, the ubiquitous presence in water bodies, and the high production volume of these compounds compelled the TSCA Interagency Testing Committee to add NP, 4-octylphenol (OP) and some of their ethoxylates to the US Environmental Protection Agency's Priority Testing List ("Interagency" 2000). The APnEOs have also been signaled as a current pollution issue in the Chesapeake Bay and were highlighted in a recent workshop on emerging contaminants organized by the Scientific and Technical Advisory Committee of the Chesapeake Bay Program on October 18, 2002 at Solomons Island. Currently, little information exists on the presence of these compounds in the State of Maryland or the Chesapeake Bay itself. A recent study by Robert Hale at the Virginia Institute of Marine Science (Hale et al 2000) analyzed water and sediment in 67 separate sites in the Commonwealth of Virginia, and found high levels of NP, not only in wastewater treatment plant effluents, but in other outfalls as well, including stormwater discharges. They observed that some of these outfalls discharge into relatively small streams, where NP could have greater effects on biota due to low dilution factors. Previous work by our group also illustrates the presence of NPnEOs and OPnEOs in WWTP effluents and receiving waters in the area (Loyo-Rosales et al. 2002).

The main objective of the present project is to model the distribution and fate of APnEOs and their degradates in a sub-estuary of the Chesapeake Bay and two WWTPs in the area, and to determine the operating parameters in the plants that control the concentrations of these endocrine-active substances in the estuary.

Results

During the first year of the project, our work was focused in the development of the required analytical methods, and the initial development of the theoretical model.

Analytical method development

The analytical methods described in this section have been submitted for publication in the Journal of Chromatography A. As part of this project, we expanded a method previously developed by our group (Loyo-Rosales et al 2003) for the extraction and quantification of nonylphenol (NP), octylphenol (OP) and their ethoxylated derivatives with up to 5 ethoxylate units (APnEO, $n = 1 - 5$) in water, to include NPnEO with $n = 6$ to 16. The main limitation was the lack of adequate analytical standards for these compounds because they are not available individually, only as uncharacterized technical mixtures. We attempted to use Marlophen 810 (Chemische Werke, Hüls, Germany), characterized by Ahel et al (2000), but we discovered that this mixture not only contains the NPnEO, but also the OPnEO, rendering it useless for quantitative purposes. Therefore, we used Surfonic N-95 (Schenectady International, Schenectady, NY), which was characterized by Huntsman Corporation (Austin, TX), and we were able to successfully quantify NPnEO ($n = 6 - 16$). Unfortunately, there are no characterized mixtures of the OPnEOs available; therefore, we are monitoring these analytes only qualitatively.

We also developed an analytical method to quantify the analytes of interest in particulate matter from water based on Soxhlet extraction with methanol and LC/MS/MS analysis. In order to obtain the particulate, one liter of water is vacuum-filtered with two pre-weighed glass microfibre filters (GF/A and GF/F, particle retention 1.6 and 0.7 μm respectively, Whatman Inc., Clifton, NJ; previously baked at 400°C for 4 h to eliminate any possible NP contamination) in a glass filter holder (Millipore Corporation, Billerica, MA). The filtrate is used for water analysis of the APnEO and the filters are allowed to dry overnight in a desiccator under vacuum. Once dry, the filters are weighed again to calculate particulate concentration and then spiked with a ^{13}C -labeled internal standard and Soxhlet-extracted with methanol for 8 hrs. The extracts are then evaporated to approximately 5 mL in a rotary evaporator, transferred to 15-mL glass centrifuge tubes and further reduced to 0.5 mL under a gentle nitrogen stream. After adding 0.5 mL of carbon-free deionized water, the extracts are filtered using an Acrodisc LC 13-mm syringe filter with a 0.2- μm PVDF membrane (Pall Gelman Laboratory, Ann Arbor, MI) into a 2-mL LC vial; the syringe and filter are rinsed with 0.5 mL of a 50:50 methanol/water mixture that is added to the extract. Finally, volume is adjusted to 1.5 mL and the extracts analyzed by LC/MS/MS. Recoveries for this extraction method vary for the different compounds and range from 73 to 100%.

Besides the APnEO, we are also interested in modeling other metabolites, such as their carboxylated derivatives. Due to their ionic nature, these cannot be extracted along with the APnEO, and a separate extraction method was developed for them. In this method, water samples are filtered as described above and part of the filtrate is acidified to pH 2 with HCl, and extracted with dichloromethane (DCM) in a separation funnel. DCM is evaporated and exchanged to approximately 5 mL methanol in a rotary evaporator and treated as above. Recovery was calculated for the three carboxylated metabolites (NP0EC, 93%; NP1EC, 93%; and OP0EC, 94%) for which standards are available. Additionally, we were able to identify carboxylated derivatives with higher molecular weight, and they were monitored qualitatively.

Analysis of the APnEOs in the Back River and WWTPs

During the second year of the project, we conducted a total of eight sampling trips to the WWTPs and the Back River. Blue Plains WWTP was sampled in July and August 2004, and in February and March 2005. Back River WWTP was sampled in September and October 2004, and in February and March 2005. Samples from the Back River were collected by the WWTP personnel in September and October 2004, and in March 2005. All the samples were analyzed for the following compounds: NP, OP, NPnEO (n=1-16), OPnEO (n=1-5) in the dissolved and particulate phases, and NP1EC, NP2EC, and OP1EC in the dissolved phase. Additionally, ancillary measurements were taken, such as temperature, pH, salinity, TOC, DOC, and TSS at every sampling event.

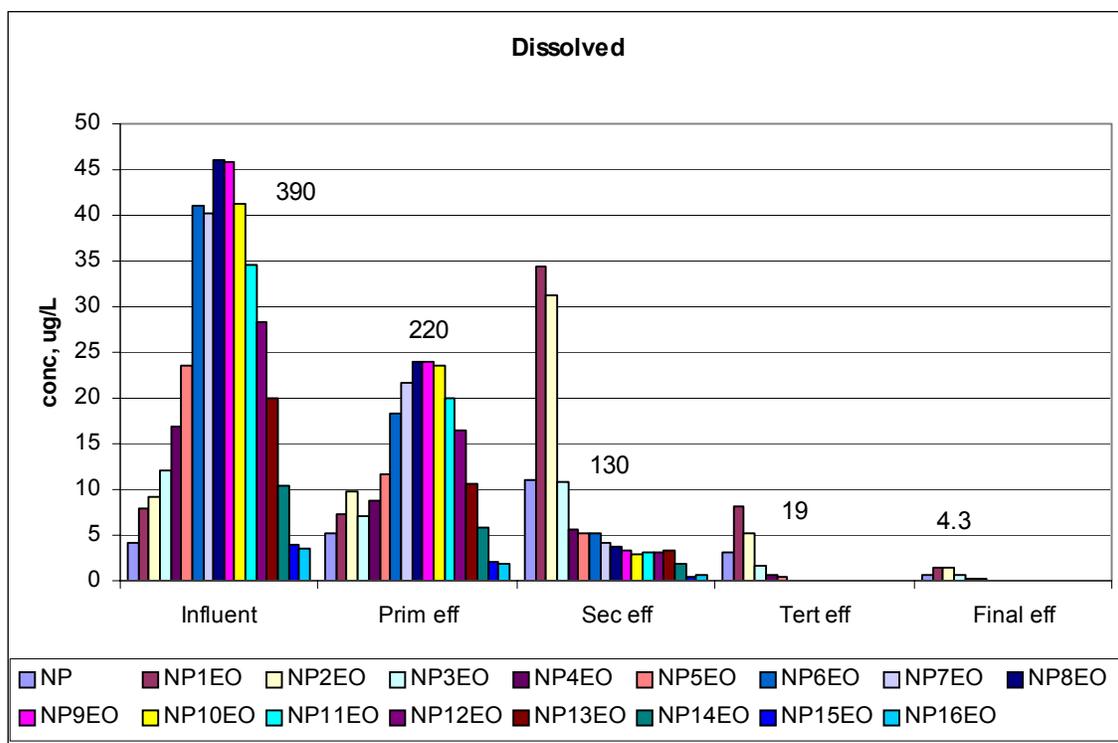


Figure 1. Nonylphenol ethoxylate concentrations in Blue Plains WWTP in the dissolved phase. The numbers above the bars indicate the total concentration ($\mu\text{g/L}$) of NPnEOs (n=0-16) in each treatment stage.

Although all the samples have been processed, we are still analyzing the data. Figures 1 to 3 are an example of the results obtained for Blue Plains WWTP during the first sampling event in July 2004. Figure 1 shows the concentrations of dissolved NPnEOs (n=0-16) along the different treatment stages. Total dissolved NPnEO (n=0-16) concentration was reduced by approximately 99%, from 390 $\mu\text{g/L}$ in the raw wastewater to 4 $\mu\text{g/L}$ in the final effluent. Moreover, the relative composition of the homologue mixture was enriched in the short-chain APnEOs as the treatment progressed. Such phenomenon is in agreement with previous observations that degradation of the APnEOs proceeds by a shortening of the ethoxylate chain (Ahel et al 1994), which results in the formation of the short-chain APnEOs. As a consequence, removal of NP, NP1EO and NP2EO was lower than total NPnEO removal at approximately 85%.

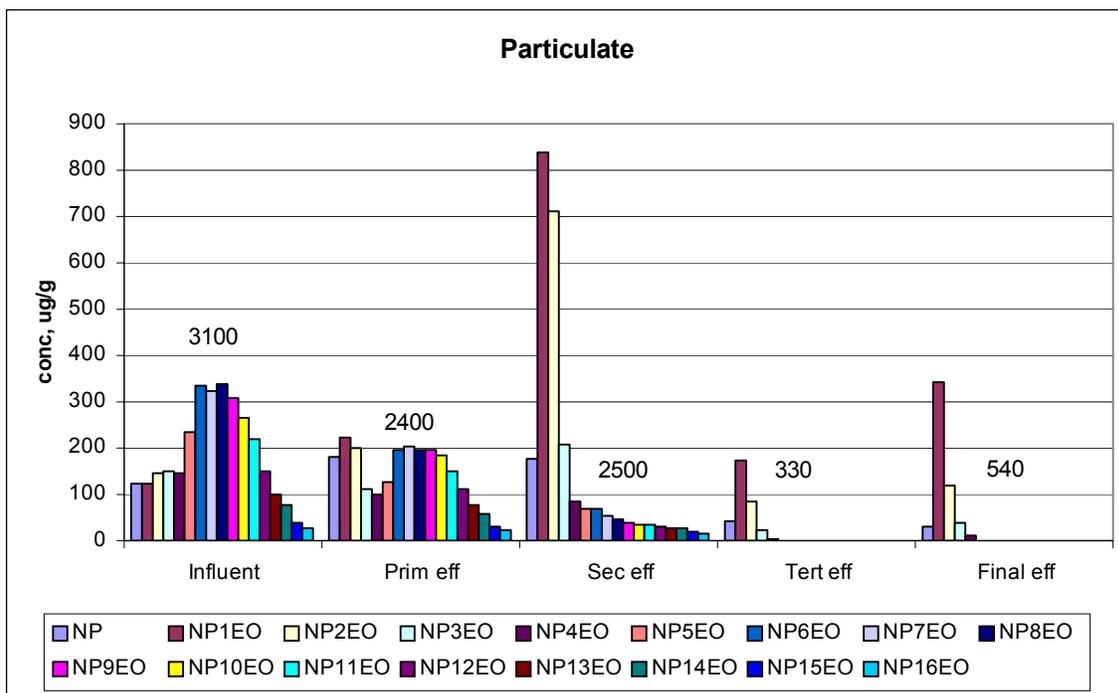


Figure 2. Nonylphenol ethoxylate concentrations in Blue Plains WWTP in particulate. The numbers above the bars indicate the total concentration ($\mu\text{g/g}$) of NPnEOs (n=0-16) in each treatment stage.

Due to their affinity for organic matter, the APnEOs tend to accumulate in suspended solids. The shorter the ethoxylate chain, the more hydrophobic the compound (Ahel and Giger 1993).

Therefore, the short-chain APnEOs have a greater affinity for the particulate, as Figure 2 exemplifies. The concentration profile of the homologues in the solid phase is similar to the profile of the dissolved compounds, except in that the short-chain APnEOs were present in higher proportions. In fact, in the raw wastewater and the primary influent, more than 60% of the NP, NP1EO and NP2EO occurred in the particulate phase. This situation, combined to a solids removal during the process of more than 99%, was reflected in a 93% removal of the three compounds when considering both the dissolved and solid phases, in contrast with 85% when considering the dissolved phase only.

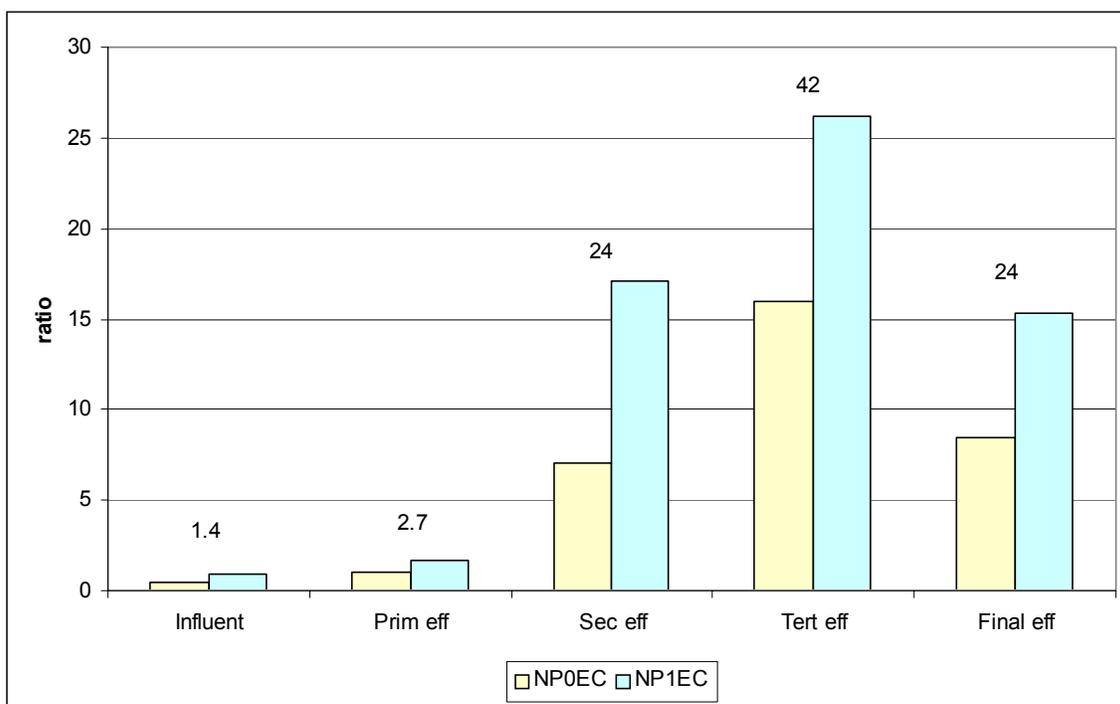


Figure 3. Nonylphenol carboxylate concentrations in Blue Plains WWTP. The numbers above the bars indicate the total concentration (µg/L) of NP0EC and NP1EC in each treatment stage.

In contrast to the APnEOs, the concentration of the APnECs increased along the treatment as can be appreciated in Figure 3. In the final effluent, the amount of APnECs present was approximately six times higher than the amount of ethoxylates, representing 85% of the compounds measured.

We are also investigating the use of the APnEOs as markers of anthropogenic contamination. Although this use has been proposed before, we believe that this concept can be extended beyond using these compounds as wastewater tracers only, but also as an indication of whether the wastewater was treated. As noted above, the initial APnEO mixture degrades in the WWTP to form short-chain APnEOs, NP, OP, and the carboxylated derivatives. In consequence, the ratio of the long-chain APnEOs to the degradation products (i.e. short-chain APnEOs, NP, OP, and APnECs) decreases as degradation occurs.

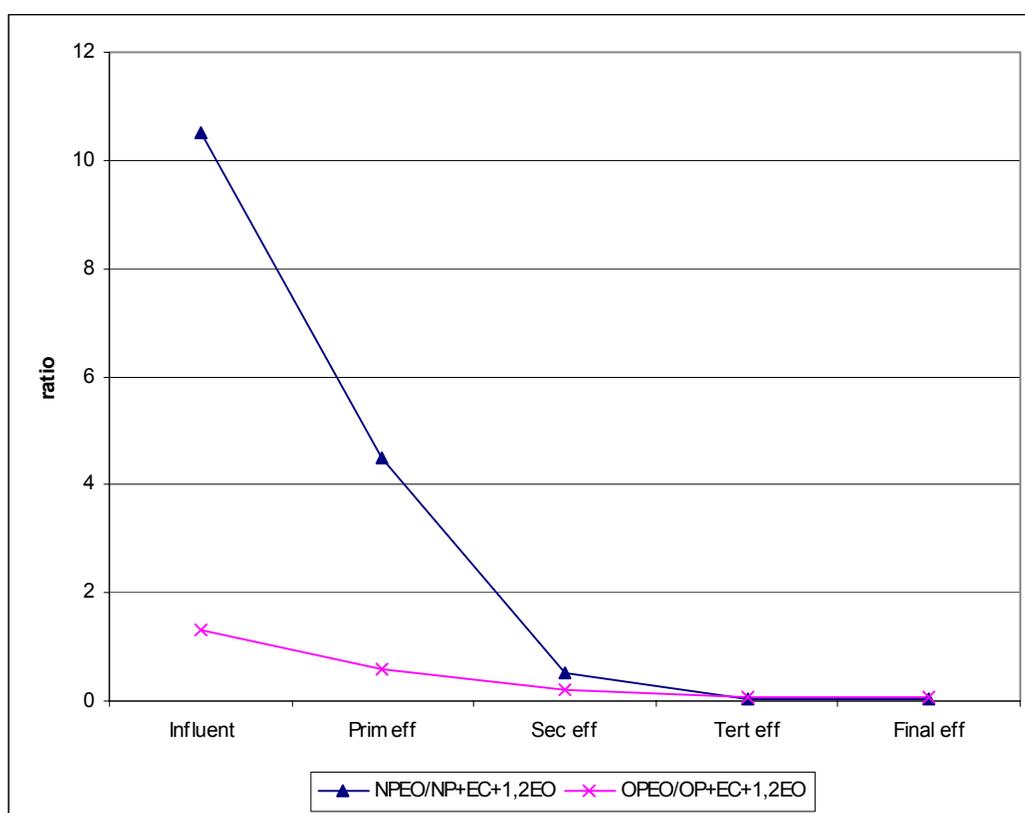


Figure 4. Changes in the ratio of total APEO to degradation products concentrations.

Figure 4 shows the variation in the ratio of the total APnEO concentration to the sum of all the degradation products measured. The ratios range from 11 to 0.04 for the NPnEOs and from 1.3

to 0.05 for the OPnEOs. Therefore, a small ratio indicates a more degraded, or treated, sample. However, this approach would need to be applied carefully because the degradation pathway of the APnEOs in natural waters is reportedly the same as in WWTPs, rendering it difficult to determine if the degradation occurred in the plant or in the stream. This might be especially true in cases when the wastewater source is away from the sampling site, allowing enough residence time for degradation to occur. An additional disadvantage of this approach would be the need to quantify all of the compounds, which makes the determination costly and time-consuming. A possible alternative would be to measure the ratio of individual APnEOs to their respective APnECs, as shown in Figure 5. These ratios change in a very similar way to those obtained using all the metabolites, and only require the quantitation of two compounds.

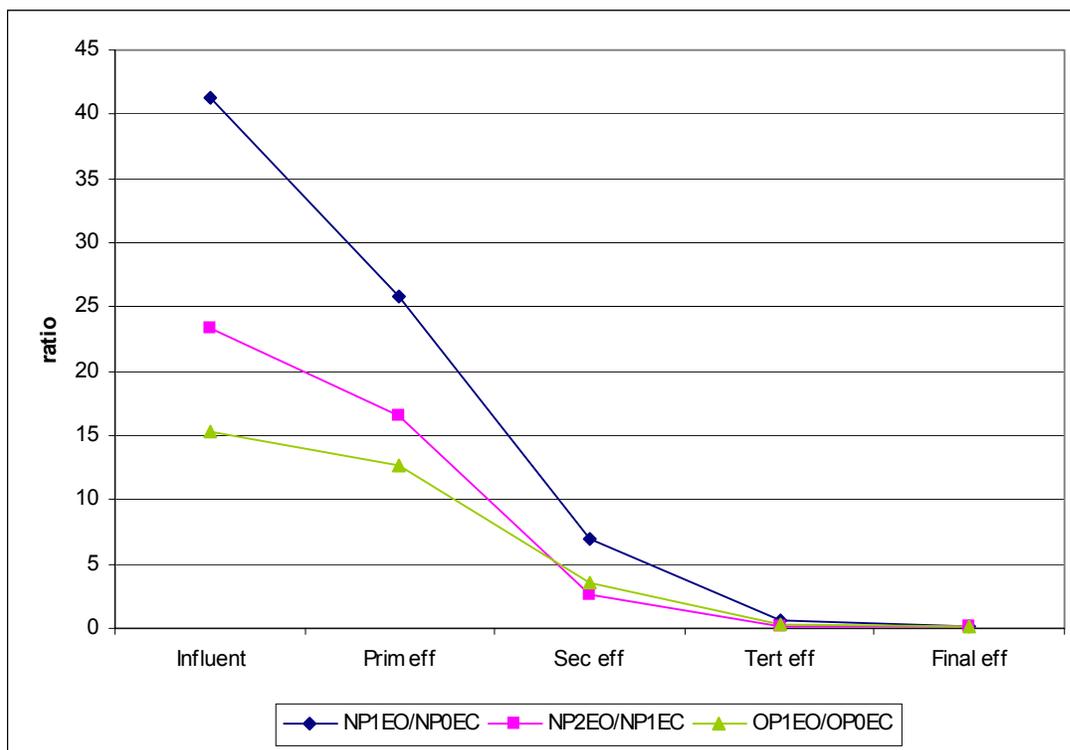


Figure 5. Changes in the ratio of individual APnEOs to their respective APnECs in different wastewater treatment stages.

Theoretical model

For our initial approach, we constructed a model of Back River in a commercial modeling environment (Stella, isee systems, Lebanon, NH). Only NP was considered and the river was divided into four cells (see Figure 6). The first and northernmost cell includes the influent from the two major tributaries, Herring Run and North East Creek and ends before Back River WWTP. The second cell includes the effluent from the WWTP and ends at Muddy Gut. The third cell starts at Muddy Gut and ends at Greenhill Cove. The fourth and last cell runs from Greenhill Cove to the mouth of the river into the Chesapeake Bay. The last two cells receive water only from the preceding cell; no other inputs were considered. This division was based on the location of the influents to the river and specific geographic features; i.e. points where the river turns. Each section of the river was modeled as a well-mixed reactor. The following processes were included in each cell: advection, dispersion, volatilization, photolysis, partition into suspended solids and net deposition into sediments. Advection was modeled as a function of flow rate (Q) and concentration (total concentration of the chemical, including both dissolved and bound species). The flow rate was assumed to be constant and values used were ten times smaller than the actual flow rates to account for tidal flow. Dispersion was modeled as a function of concentration gradients between sections, dispersion coefficient – constant for all sections in the river –, cross sectional area and volume of each section. Volatilization was modeled as a flux out of the water, assuming the concentration of these chemicals is equal to zero in the atmosphere. Photolysis of NP and deposition into sediments were modeled as a first order reaction. Flow diagrams of the model are depicted in Figure 7.

The preliminary results of our model suggest that, after reaching steady state, NP will be present in the water at a concentration of 0.5, 0.7, 0.4 and 0.08 $\mu\text{g/L}$ for cells 1 to 4 respectively. This values are very close to actual concentrations measured in the river in January 2001 (0.3, 0.4, 0.2 and 0.05 $\mu\text{g/L}$ respectively). The steady-state amount of NP in the water represents approximately 10 % of the total amount entering the Back River over a period of 10 days. Photodegradation appears to be the most important removal process for NP in the water (49% of the NP entering the Back River), whereas deposition into sediments is the second most relevant

process (35%), and volatilization losses are minimal (3%). Even with these losses, 13% of the NP would be transported into the Chesapeake Bay. These results are summarized in Figure 8.

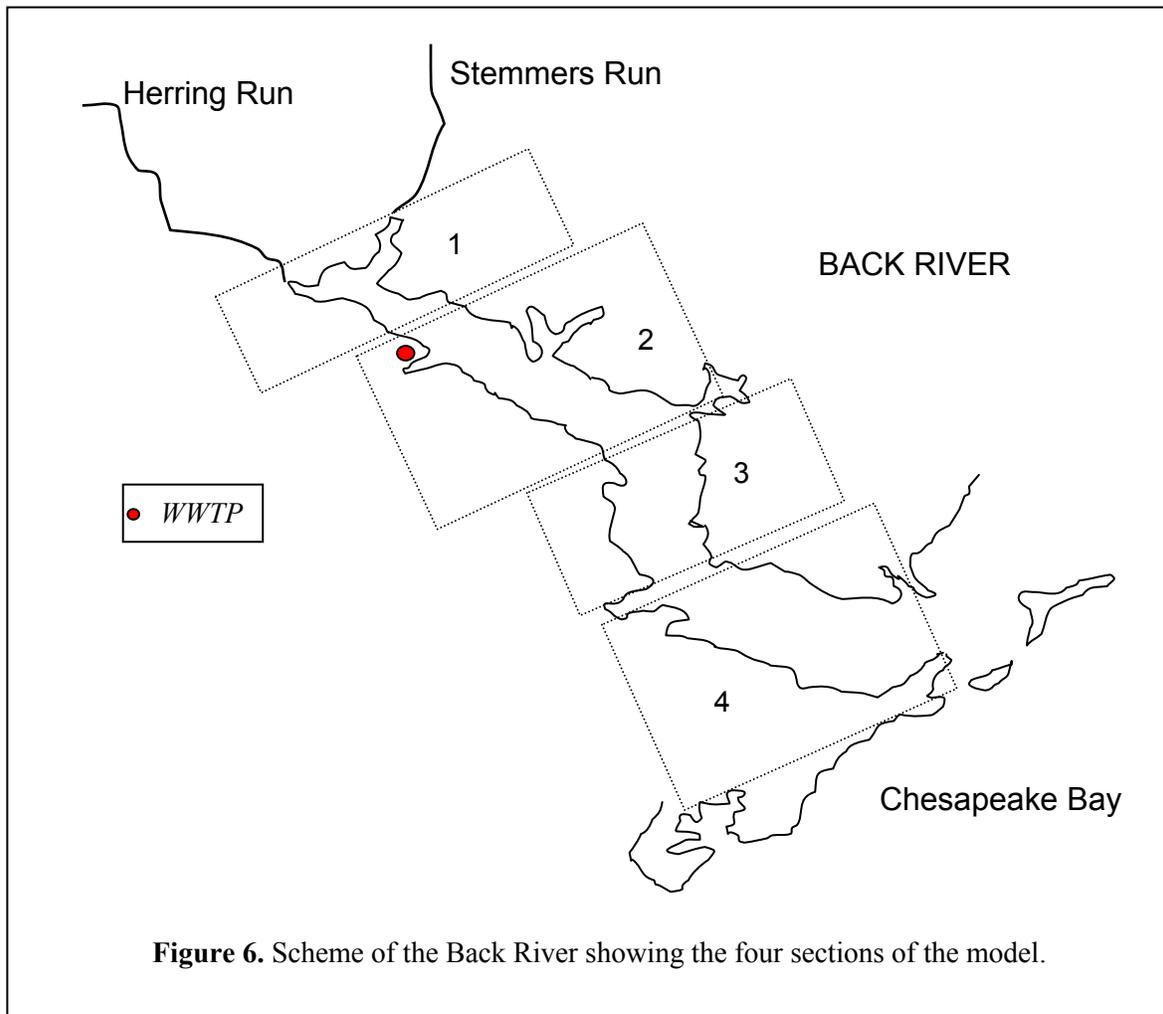


Figure 7. Flow diagram for the Stella model of NP distribution in the Back River.

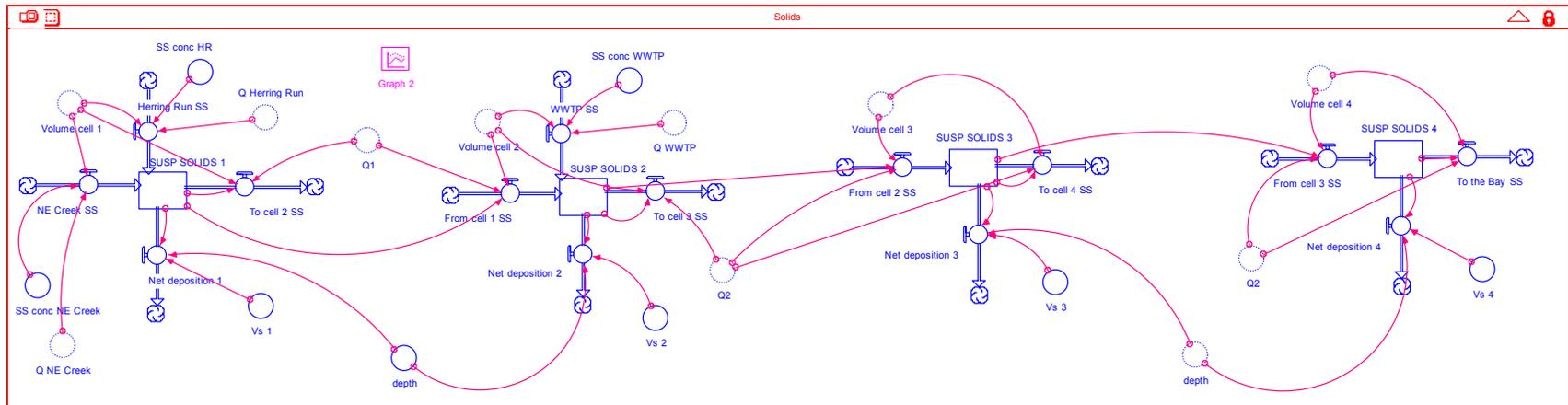
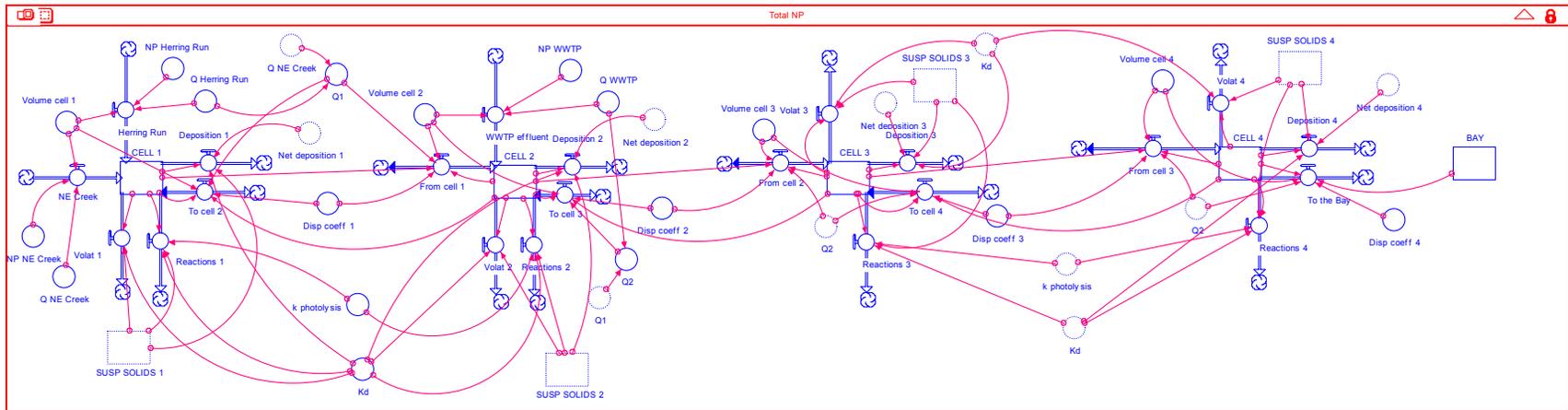
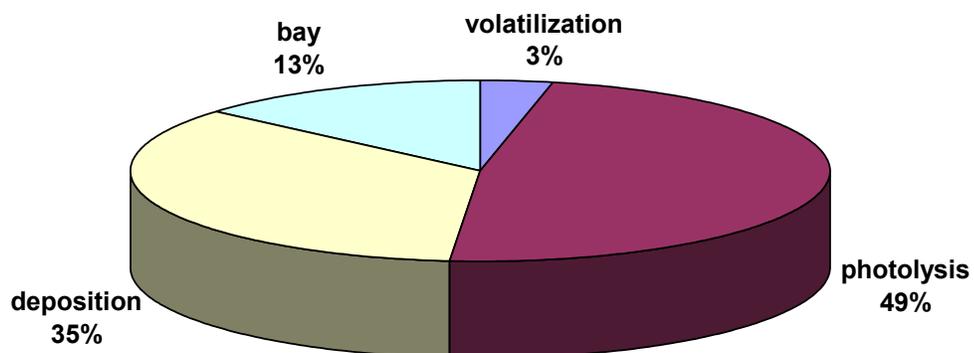


Figure 8. NP distribution in the Back River



Current and future activities

During the extension to our project we will continue the data analysis and model development, including the addition of the APnEOs and APnECs, and a more accurate characterization of the hydrology of the estuary, specifically the tidal flow.

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Papers

Loyo-Rosales, J. E., Rosales-Rivera, G. C., Rice, C. P., Torrents, A. (2005), "Linking laboratory experiences to "the real world": The extraction of octylphenoxyacetic acid from water". *J. Chem. Educ.* In press.

Loyo-Rosales, J. E., Rice, C. P., and Torrents, A., “Determination of octyl- and nonylphenol ethoxylates and carboxylates in environmental samples by isotope dilution and liquid chromatography/tandem mass spectrometry”. Submitted to *J. Chromatogr. A*.

Loyo-Rosales, J. E., Rice, C. P., and Torrents, A., “Fate and distribution of the octyl- and nonylphenol ethoxylates in wastewater treatment plants from the Baltimore-Washington area and the Back River, Maryland”. In preparation for *Environ. Sci. Technol.*

Presentations

Loyo-Rosales, J. E., Rice, C. P., Torrents, A.: Endocrine disruptors in municipal wastewater discharges. Platform presentation. Symposium “Wastewater Treatment Plants and the Chesapeake Bay: Processes and Problems”. Sponsored by the Maryland Water Resources Research Center and the Maryland Sea Grant College. College Park, MD, October 22, 2004.

Loyo-Rosales, J. E., Rice, C. P., Torrents, A.: Alkylphenol ethoxylates in waters from the Baltimore-Washington area. Poster presentation. Maryland Water Policy Conference – What Does the Future Hold? Organized by the Maryland Water Resources Research Center. College Park, MD, October 24, 2003.