Pergamon

PII: S0045-6535(96)00343-8

Chemosphere, Vol. 33, No. 12, pp. 2543–2563, 1996

0045-6535/96 $15.00+0.00

TRANSFORMATION OF N-, O-, AND S- HETEROCYCLES (NOSHs) IN ESTUARINE SEDIMENTS: EFFECTS OF REDOX POTENTIAL AND SEDIMENT PARTICLE SIZE

W. James Catallo
Laboratory for Ecological Chemistry
Pharmacology and Toxicology Program
School of Veterinary Medicine
Louisiana State University
Baton Rouge, LA 70803-7511

(Received in USA 25 April 1996; accepted 25 August 1996)

ABSTRACT- The transformation of 19 N-, O-, and S- heterocycles (NOSHs) was examined in estuarine sediment-water microcosms. The effects of redox potential (Eh) and sediment particle size on compound transformation rates were evaluated, and stable products were identified. Results from stirred, controlled Eh/pH microcosms (CEPMs) showed that most of the NOSHs were significantly transformed under oxidized and reduced conditions over 15 week incubations, and the resulting product distributions were similar. In general, the rates and extent of transformation were greater in oxidized sediments of low surface area vs. those with high particle surface area and reduced redox conditions. Further experiments in sealed, unstirred microcosms also showed that NOSH transformation proceeded more slowly and on fewer compounds in fine vs. coarser grained sediments under oxidized conditions. Unlike the stirred systems, however, NOSH transformation rates were similar or greater under reduced vs. oxidized conditions. Thus, reduced, methanogenic clay of high surface area displayed some of the fastest rates of NOSH transformation. Data from liquid-liquid partitioning experiments suggested that this effect was related to the formation of NOSH complexes with iron and perhaps other redox-active metals in sediments.

Keywords-Heterocycles, estuarine sediments, redox potential, transformation

INTRODUCTION

N-, O-, and S- heterocycles (NOSHs) are found in polar and asphaltic fractions of crude petroleum, produced waters, oil field tars and sludges, shale oils, coal synfuels, and pyrogenic products [1-7]. In single compound assays, a range of N-heterocycles, including quinoline, all C_1-quinolines, alkyl carbazoles, various benzacridine isomers, dibenzo(a,h)acridine, are mutagenic and/or tumorigenic in the Ames/*Salmonella* assay and various mammalian test systems, respectively [1, 6]. In the environment, N-heterocycles from contaminated sediments have been correlated to extract mutagenicity and neoplasia in exposed benthic fish [8, 9]. Further, analyses of coal-fired power plant particulates showed that the "most mutagenic" and "bioactive" fraction contained primarily N-heterocycles and PAH amines [10].

Currently there are few data available on NOSH transformations in marine sediments. Some studies have documented the degradation of selected NOSHs in water, sludges, and model sediments *in vitro* [11-15], but sediment fate data are not well documented for many common NOSHs, and the effects of sediment electrochemical conditions (redox potential or "Eh" [16]) and particle size on NOSH transformations are unknown. Nevertheless, the presence of extended series of NOSH compounds in riverine and marine water and sediments suggests that their transformation *in situ* is slow [6, 17-20].

The principle objectives of this work were; 1) to document biological and chemical transformations of NOSHs in coarse and fine-grained estuarine sediments under different redox conditions in laboratory microcosms and determine the effects of these variables on NOSH fates and, 2) to identify prominent NOSH transformation products and evaluate their significance to the toxicity of aqueous samples and sediment extracts.

METHODS

Reagents and Stock Solutions

The structures of the NOSH compounds used in the current study are presented in Fig. 1. All were obtained from commercial sources (Aldrich, St. Louis, MO; Sigma, St. Louis; and Pfaltz and Baur, Waterbury, CT). Benzothiazole undergoes a series of chemical reactions at room temperature, and therefore was re-distilled prior to use. A concentrated mixture of the NOSH standards (5000 ppm) was made up in acetone and used to spike the test sediments. Dimethylsulfoxide (DMSO) was used as the extraction solvent and carrier in toxicity assays. Sediment extractions for chemical analysis were conducted using dry, ultrapure dichloromethane (DCM).

Sediment Microcosms

Bulk sediment samples were collected from the Bay St. Louis estuary, MS. The sediments were: a) a fine textured sediment with high clay content typical of quiescent depositional environments and, b) a coarser grained sandy silt found in higher energy systems. Sediment size distributions (% sand, silt, and clay) and organic matter content were determined using standard methods [21]. In what follows, the test sediments will be identified as "clay" and "sand" for convenience.

The sediments were diluted 1:8 (w/w) with estuarine water and sterile artificial seawater (15 g/L salinity). Dried, ground *Spartina alterniflora* plant matter was added to each sediment to a final concentration of 5% (w/w

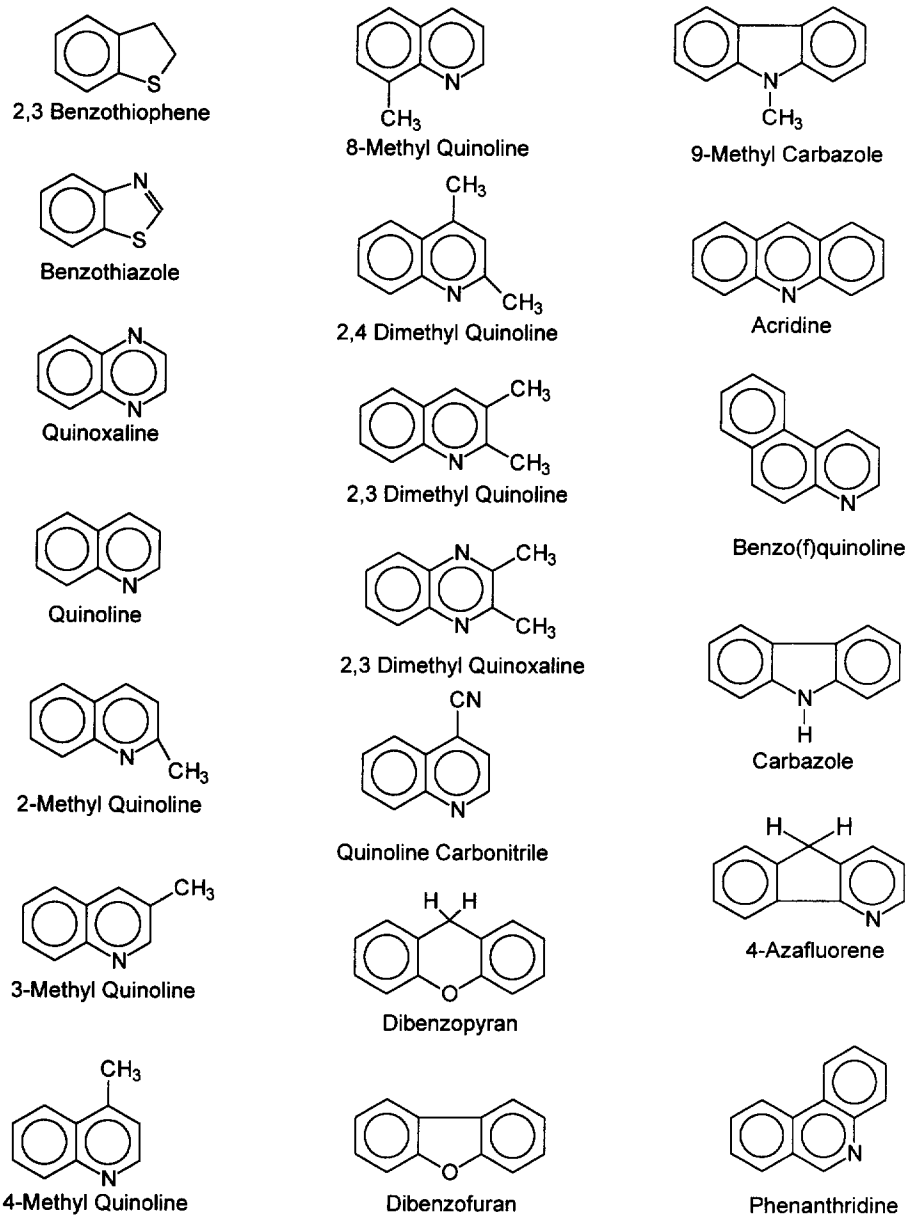


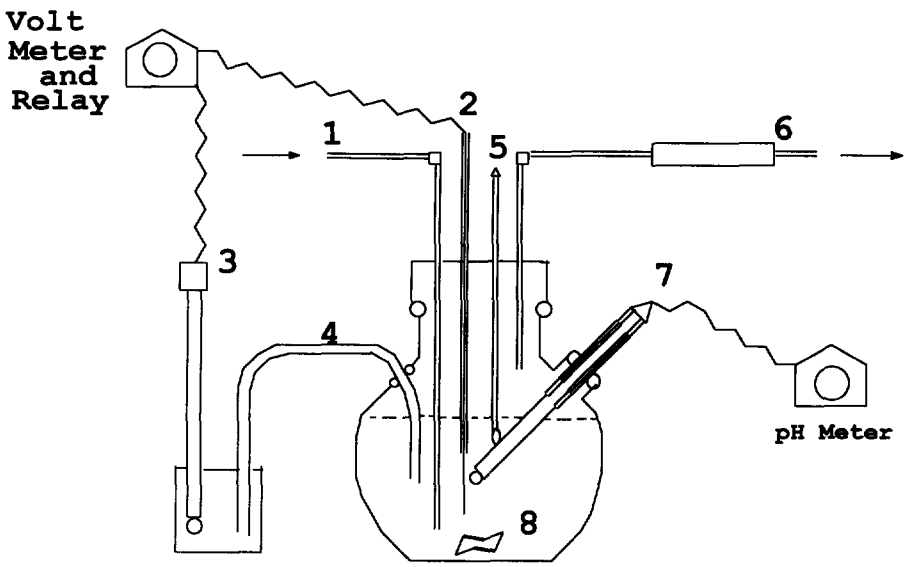
FIGURE 1. N-, O-, and S- Heterocycles (NOSHs) Examined in this Study.

sediment). Preliminary extraction experiments indicated that this organic matter did not significantly affect NOSH recoveries vs. unamended sediments, in agreement with other work [22]. "Killed controls" were identical sediment-water slurries in which biological activity was terminated by extended autoclaving. NOSHs were added aseptically to these systems, which then were sealed and shaken overnight. Randomly selected control chambers were analyzed in their entirety at the beginning and end of each microcosm run to account for NOSH chemical transformations not requiring biological action (*e.g.*, hydrolysis, chemical oxidation).

The sediments were added to controlled Eh/pH microcosms (CEPMs) modified for organic work from prototypes described elsewhere (Fig. 2) [23]. CEPMs were fabricated by a glassblower to be vacuum tight with junctions joined via luer fittings and double seated teflon o-rings. Volatiles were passed through a series of liquid and solid phase (silica bonded octadecyl, Aldrich, St. Louis MO) gas traps [24] and then vented. The sediment-water system in each microcosm was adjusted to pH 7.5 - 8.0 with 1 N acid or base, and the desired Eh ranges were established through constant stirring and intermittent bubbling of air (for oxidized, aerobic conditions) or nitrogen (for reducing, methanogenic conditions).

After desired Eh levels of < -350 mV (strongly reducing) and +150 mV (well oxidized) vs. the saturated calomel electrode (SCE) were established, the systems were equilibrated for seven days. Aliquots of the NOSH mixture (50 ppm of each compound) were added slowly to each sediment followed by mixing for 24 h. The microcosms were maintained for 16 weeks, with sediment sampling occurring at biweekly intervals.

NOSH transformations also were examined under quiescent conditions more closely approximating a natural environment. Oxidized or reduced conditions were established as above, followed by addition of the NOSH compounds with mixing to achieve a homogeneous distribution. The sediments then were added to solvent-clean sealed glass and teflon reactors (c. 40 g wet weight) and covered with sterile artificial seawater. The reduced microcosms were maintained under Ar and N_2 in sealed atmospheric glove bags, while the oxidized systems were left under a normal atmosphere. All microcosms were opened briefly and shaken manually twice per week so that the development of redox gradients in the system could be minimized. Randomized duplicate samples were collected and analyzed for NOSHs and their degradation products at biweekly intervals over a 23 week period. These data were



1. Gas (air or N_2) inlet; 2. Pt working electrode (2 per microcosm); 3. Saturated calomel reference electrode; 4. Agar/KCl salt bridge; 5. Thermometer; 6. Solid phase and liquid volatile organic traps; 7. Combination pH electrode; 8. Teflon stir bar.

FIGURE 2. Schematic Diagram of the Controlled Eh/pH Microcosm (CEPM).

fitted using least squares linear regression, and the equation of the line for each was used to calculate NOSH transformation half-lives.

Extraction and Analysis of NOSHs

Aliquots (10 - 100 mL) of slurry from the CEPMs were liquid-liquid extracted for 24 with DCM followed by drying over anhydrous Na_2SO_4 and volume reduction using Kuderna Danish (K-D) concentrators at 80 °C. Sediments from the unstirred microcosm were prepared by adding the entire contents of randomly selected reactors and duplicates to pre-rinsed soxhlet nitrocellulose extraction thimbles with enough anhydrous Na_2SO_4 to remove water. The sediments were soxhlet extracted for 36h in DCM, followed by K-D volume reduction. The solid and liquid phase gas traps were extracted with DCM followed by chemical drying and volume adjustment. NOSH recoveries were estimated using 3-methylisoquinoline as a surrogate standard. Extracts were analyzed using gas chromatography (Hewlett Packard 5890 GC) with flame ionization detection (FID) or GC-mass spectrometry (Shimadzu QP5000 GC-MS). Aliquots of the samples were dried (100 °C/24 h) and weighed to determine NOSH concentration on a dry weight sediment basis. As the slurries were well-mixed during sampling, the liquid:solids ratios of the subsamples were highly consistent (i.e., coefficient of variation around 15%). Recovery-corrected concentrations for target NOSHs then were used to construct time course transformation profiles. The NOSH concentrations at each time were determined by referencing of extract data (integrals of peaks at appropriate retention times) to external five point calibration curves for each compound. Instrument calibration was checked after every third sample by injection of NOSH standard mixtures at concentrations near the middle of the linear range of the calibration curves (e.g., 50 ppm). Product identification was performed using interpretation of mass spectra (molecular ions, isotopic structures, and logical fragment losses) [25] and chromatographic data. Mass spectra accepted if there was a signal:noise ratio of > 3 for the base peak of interest, and minimal background interference with respect to isotopic clusters and fragments. The NOSH degradation products in the sediment extracts were identified by interpretation of spectra and computer searches of the National Bureau of Standards and Wiley data bases. Reference spectra generated from compounds synthesized in the laboratory, fractionated coal- and petroleum- derived mixtures, and condensates from pyrolysis of petroleum and biogenic mixtures also were consulted during product analysis.

Toxicity Evaluation

The toxicity of sediment extracts and water samples from the CEPMs was monitored using the MicroTox assay (Microbics Corp. Carlsbad CA) [26]. Water samples and dilutions thereof were analyzed directly while sediments were extracted in DMSO with ultrasonic treatment (40 min) followed by 0.45 mm nitrocellulose membrane filtration. The author has demonstrated elsewhere that this extraction approach provides recoveries of semivolatile NOSHs and aromatic hydrocarbons that are comparable to Soxhlet extraction with DCM [27]. Aliquots were dried and weighed to determine percent solids in each extract, and results were corrected to a common weight or volume basis. Stepwise protocols for the MicroTox assay are extensive and will not be detailed here. Basically, the assay relies on progressive, toxicity-mediated quenching of bioluminescence in *Photobacterium phosphoreum* by increasing doses of test chemicals [26]. The "EC-50" values (concentration of toxicant required to reduce luminescence by 50% relative to solvent controls) reported in the results were computed from the log-linear regression of dose vs. luminescence intensity over a range of exposure concentrations.

RESULTS AND DISCUSSION

NOSH Fates in Controlled Eh/pH Microcosms (CEPMs)

Based on hydrometric measurements, the test sediments had percent ratios of sand (16 - 2000 μm i.d.):silt (4 - 16 μm):clay (<4 μm) sized particles as follows: "sand": 93.2:4.4:2.4; and "clay": 67.2:20.4:12.4. Using standard conversions [28], approximate total surface areas of the test sediments were: "sand" 192,232 cm^2/g, and "clay" 992,246 cm^2/g. Thus the clay had five-fold greater surface area than the sand.

NOSH concentration-time profiles in the CEPMs are shown in Figures 3 and 4. Data from the killed controls are not presented: NOSH recoveries from these controls consistently were within the range of analytical error (20%) for all compounds except for acridine, benzothiazole, 4-azafluorene, 9-methylcarbazole, and dibenzopyran. These NOSHs underwent chemical transformations and their oxidized products (ketones, phenols) were identified in small amounts in the killed controls and sterile water controls as well as the CEPM extracts. Data shown in Fig. 3 and 4 for these compounds reflect both chemical and biological transformation. Rapid, significant (>20 %) volatilization losses were observed for 2,3-benzothiophene in the sand CEPMs, so no transformation rate data were determined. The remaining 2,3-benzothiophene was oxidized to sulfoxides and sulfones, as determined by product analysis (below). Much smaller

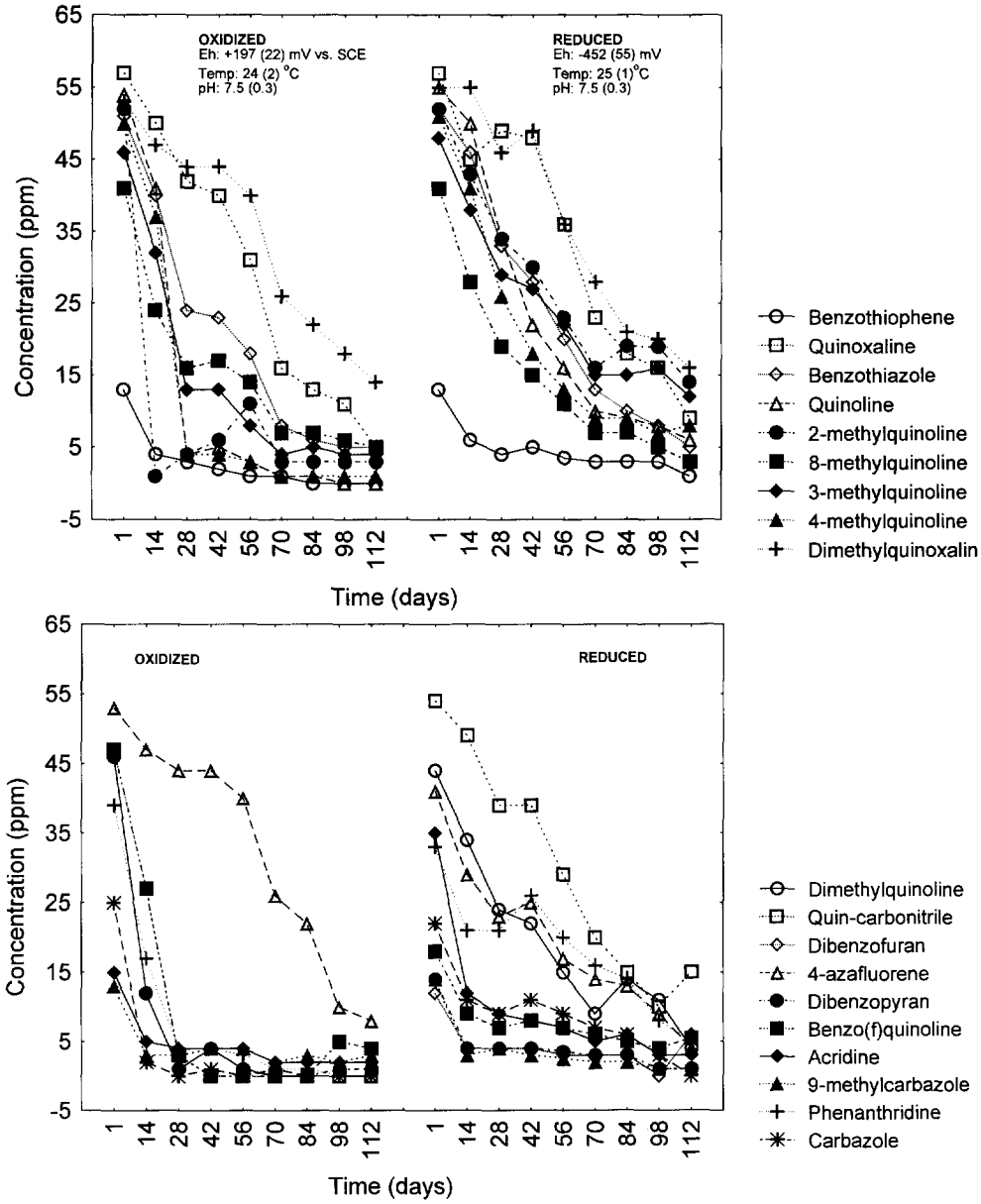


FIGURE 3. Transformation of NOSH Compounds in Oxidized and Reduced Estuarine "Sand". Values for Eh, pH and temperature are means and standard deviations (parentheses) of daily measurments.

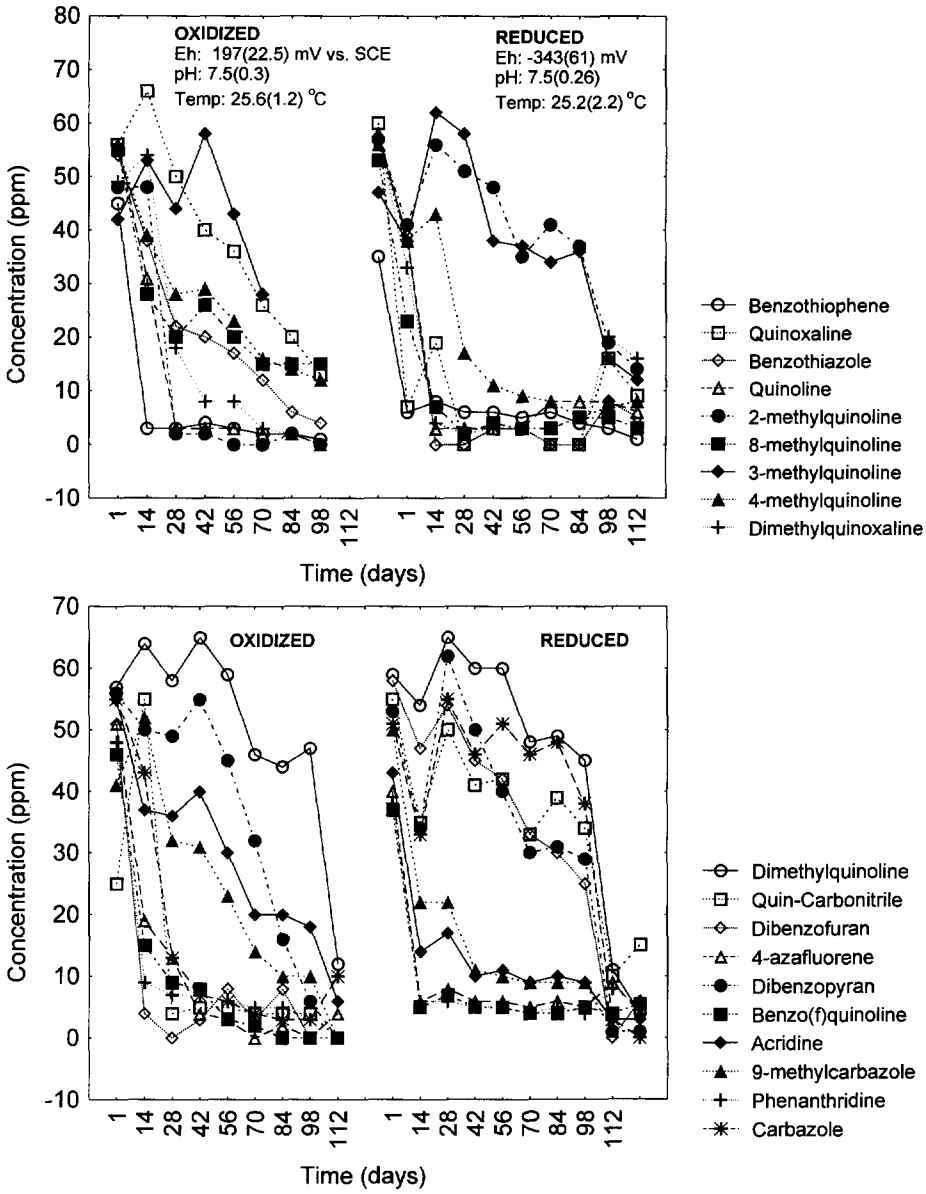


FIGURE 4. Transformation of NOSH Compounds in Oxidized and Reduced Estuarine "Clay". Values for Eh, pH and temperature are means and standard deviations (parentheses) of daily measurments.

(1 - 2%) amounts of benzothiazole and quinoline also were volatilized in the early stages of the runs, but this was constant between microcosms and was not considered significant. Recoveries of acridine, carbazole, 9-methyl carbazole, dibenzopyran and dibenzofuran were consistently poor in the sand microcosms. This was not due to volatilization. Electrochemical studies and product analysis suggested that these and related compounds underwent chemical reactions at potentials achieved in these microcosms that altered their phase partitioning characteristics and limited recoveries [29-31]. These reactions included electrochemical transformation to oxidized products (e.g., ketones), reduction to free radicals followed by dimerization, and protonation of heteroatoms followed by phase transfer as water soluble ion pairs [30]. Interestingly, in the clay microcosms the chemical reactions of these compounds and the concomitant formation of altered products was observed only after extended incubation (e.g., 14 days). This suggested that chemical sequestration of these compounds occurred in the clay, probably at protected surface sites rather than in lattice spaces, where chemical and biochemical reactions can be slow relative to the bulk aqueous phase [11, 14, 17, 29].

It can be seen from Figure 3 that recovery of most NOSHs in the oxidized and reduced sand decreased to low levels (< 1 ppm) over the experimental period. Under oxidized conditions, the most persistent NOSHs were 2,3-dimethylquinoxaline, quinoxaline, and 4-azafluorene. Under reduced conditions the quinoxalines again were persistent, as were 2-methylquinoline, 3-methylquinoline, 8-methylquinoline, and quinoline carbonitrile. There was an apparent redox effect on the transformation rates: with the exception of quinoxaline and 2,3-dimethylquinoxaline, NOSHs including quinoline, 2-methylquinoline, 3-methylquinoline, 4-methylquinoline, 2,3-dimethylquinoline, quinoline carbonitrile and phenanthridine were removed most rapidly from the oxidized vs. the reduced system. Benzothiazole, 8-methylquinoline, dibenzofuran, 4-azafluorene, and carbazole showed similar transformation rates under oxidized and reduced conditions. The recoveries of chemically-reactive NOSHs (e.g., 9-methylcarbazole, acridine, dibenzopyran) were consistently poor in the sand microcosms, with ketones observed as the primary transformation products after 24 h of incubation.

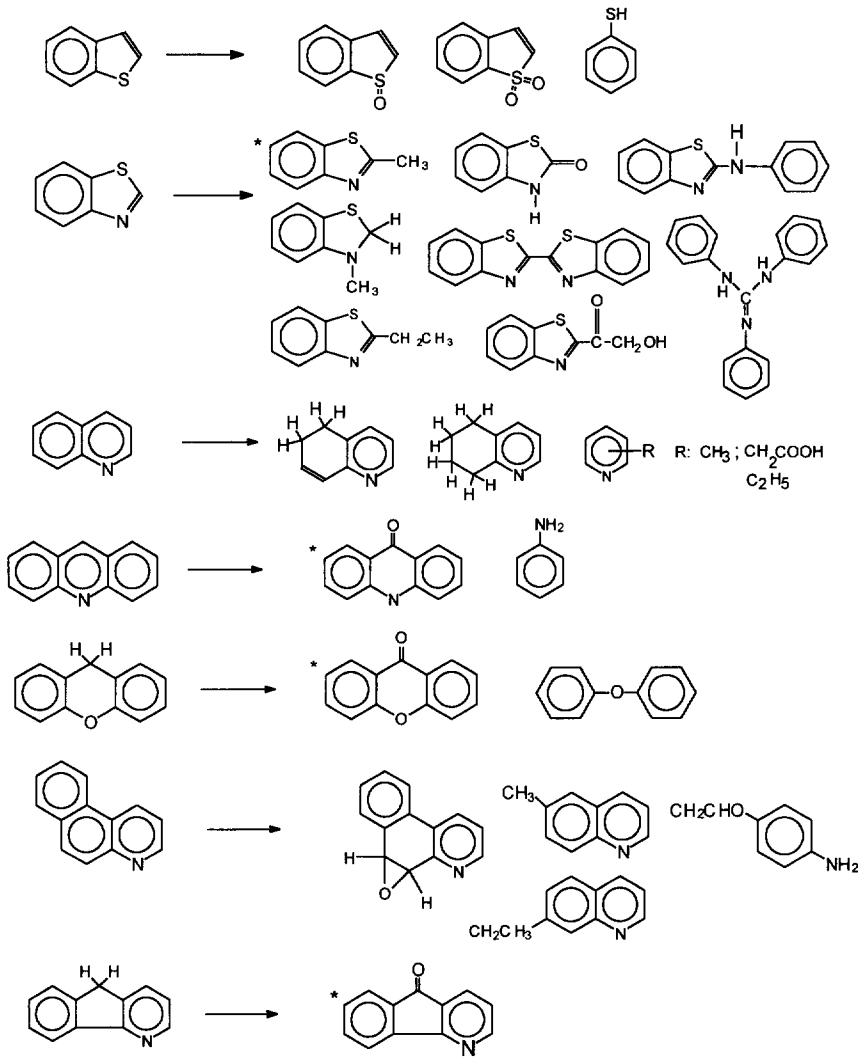
The recoveries of chemically-reactive NOSHs (e.g., 9-methylcarbazole, dibenzopyran) from the clay microcosms were much better than from the sand, and the observed transformation profiles of many target analytes (Fig. 4) were different. Unlike the sand, these NOSHs were stable for significantly longer periods in the clay. Also

unlike the sand, 2,3-benzothiophene was not sparged from the system, but was largely transformed to the sulfoxide or sulfone by 14 days. This suggested that processes limiting recoveries of these NOSHs in the sand (volatilization, chemical reactions) were minimized in the clay, most likely by the five-fold higher particle surface area of the latter relative to the former.

Under oxidized conditions in the clay microcosms, the most persistent NOSHs were quinoxaline, 3-methylquinoline, 2,3-dimethylquinoline, dibenzopyran, and acridine. Interestingly, 2,3-dimethylquinoxaline was transformed rapidly in the oxidized clay whereas it was more persistent in oxidized and reduced sand. Conversely, dibenzopyran, acridine, and 9-methylcarbazole were persistent in the oxidized clay, but were chemically oxidized in the sand. In the reduced clay, the most stable compounds were 3-methylquinoline, 2-methylquinoline, 2,3-dimethylquinoline, dibenzopyran, carbazole, quinoline carbonitrile, and dibenzofuran. These compounds remained at high levels over most of the incubation, with broad distributions of transformation products observed only after 960 h.

The transformation rates of selected NOSHs in the CEPMs agreed closely with rates reported by other workers [11]. In the cited work, 10 ppm of benzo(f)quinoline was found to be completely degraded in sewage sludge within 48 h. The same amount of degradation in an estuarine water sample took about 35 days. This was very close to the disappearance time of benzo(f)quinoline observed in the stirred microcosms of the current study. There was further agreement between the two studies with respect to quinoline, which degraded in estuarine water in 10 - 15 days vs. 27 - 40 days in this work. These data, and much slower transformation rates measured in the unstirred microcosms (below) indicated that the sediment-water slurries in the CEPMs behaved more like an estuarine water sample than a typical sediment. It would seem that processes resulting from stirring and bubbling of these systems increased the importance of aqueous phase reactions relative to what would be seen in an undisturbed, flooded sediment profile.

The typical concentration vs. time curves observed for most of the NOSHs in the CEPMs were roughly linear or sigmoidal (Fig. 3 and 4). The latter kinetics were characterized by an acclimation time of days to weeks followed by linear decreases in NOSH concentration, and then slower asymptotic approaches to baseline. These kinetics are consistent with those measured for NOSHs in various water samples, and other aromatic hydrocarbons in soils and sediments [11, 14]. During the linear phases of the NOSH degradation profiles, numerous stable products were identified in the extracts (Fig. 5) using GC-MS. These data confirmed that a complex suite of transformation reactions



*Found in killed controls and treatments

FIGURE 5. Examples of NOSH Transformation Products Detected in the CEPM Experiments Using GC-MS.

occurred in the microcosms including, a) chemical and biological oxidation of aromatic rings to phenols, ketones, and epoxides, b) disproportionation, saturation and opening of benzylic rings, c) oxidation of alkyl side chains followed by decarboxylation, d) oxidation of heterocyclic sulfur to sulfoxides and sulfones, e) addition and dimerization reactions and, f) β-oxidation of opened rings and oxidized side chains. Interestingly, the NOSH transformation product distributions were similar in oxidized and reduced systems: many of the chemical and biological transformation products were oxygenated, more polar, and water soluble than the parent NOSH compounds, even in reduced sediments. The 2,3-benzothiophene was an exception: under aerobic conditions, it was oxidized to the sulfone, but thiophenol (C_6H_6S) was a favored endpoint under reduced conditions.

Time Course Toxicity Evaluation of NOSHs in CEPMs

Water and sediment toxicity changes were evaluated using the MicroTox assay at each sampling time point for the NOSH analyses (Fig. 6). It was found that sediments were toxic in this assay even before the addition of NOSHs, and this rendered much of the sediment data uninterpretable: the extract toxicity was unaffected by NOSH addition to the system and changed little throughout the experiments.

On the other hand, water samples from all microcosms except the reduced clay showed toxicity profiles that followed the NOSH degradation profiles. Prior to NOSH addition, the water from these microcosms elicited no toxicity in the assay relative to buffer controls. The toxicity increased sharply upon addition of the NOSHs. Towards the middle of the runs, the water samples showed evidence of detoxification: there consistently was no difference between aqueous samples and controls. Water samples from the reduced clay microcosm, however, were toxic before the NOSHs were added. The most likely explanation for this is dissolved sulfide toxicity. Nevertheless, the water in this microcosm also became relatively detoxified in the latter part of the run.

In general, after an initial period of high toxicity and subsequent nonlinear variations, the aqueous phases tended to become nontoxic towards the end of the runs. The nonlinearities in these profiles suggested that the NOSHs were metabolized and/or chemically weathered to relatively nontoxic products, but that intermediates that entered the aqueous phase were evolved in the process. The presence of polar transformation products in extracts from these time periods supported this interpretation (Fig. 5).

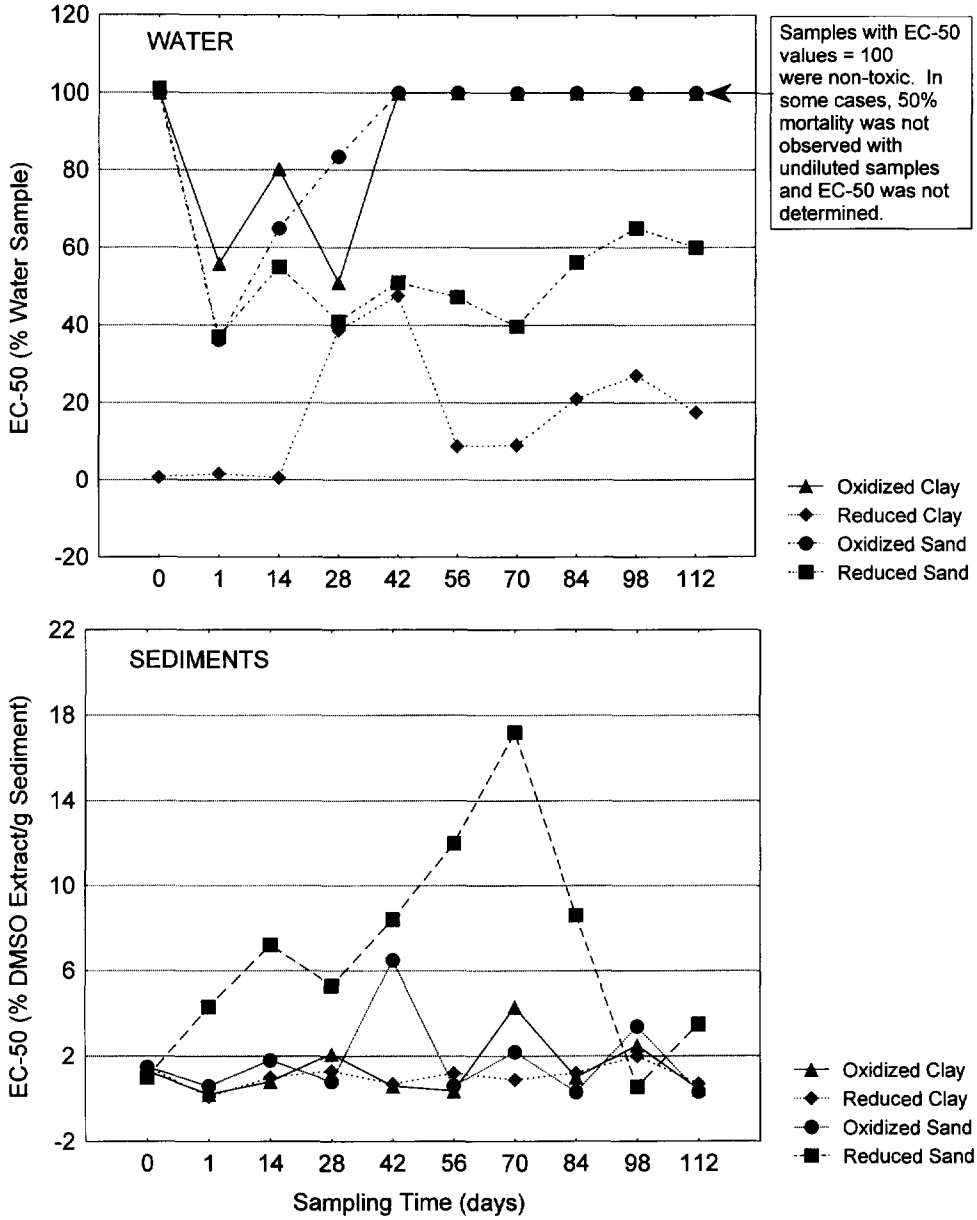


FIGURE 6. Time Course Toxicity Evaluation of Water and Sediment Samples from the CEPMs.

NOSH Fates in the Sealed Batch Vial Microcosms

The results from duplicate batch vial runs of both sediments under oxidized and reduced conditions are presented in Table 1. It is immediately clear from these data that the transformation of most NOSH compounds under

TABLE 1. NOSH Half Lives in the Sealed Batch Vial Microcoms

NOSH Compound	$T_{1/2}$* Oxidized Sand	$T_{1/2}$ Reduced Sand	$T_{1/2}$ Oxidized Clay	$T_{1/2}$ Reduced Clay
2,3-benzothiophene	149	149	521	521
Quinoxaline	95	95	347	69
Benzothiazole	69	80	174	87
Quinoline	nt	87	nt	nt
2-methylquinoline	174	260	nt	95
8-methylquinoline	87	80	nt	80
3-methylquinoline	130	208	nt	61
4-methylquinoline	130	149	nt	95
2,3-dimethylquinoxaline	149	208	nt	260
Quinoline carbonitrile	174	208	nt	95
2,4-dimethylquinoline	65	61	521	95
Dibenzofuran	174	174	nt	nt
4-azafluorene	174	260	nt	260
Dibenzopyran	208	116	nt	nt
Benzo(f)quinoline	130	208	nt	69
Acridine	174	208	nt	347
9-methylcarbazole	149	260	nt	521
Phenanthridine	149	260	nt	65
Carbazole	149	347	nt	260

* Half lives are given in days.
nt = no significant transformation was observed vs. killed controls.

quiescent conditions was minimal relative to the CEPMs. Without the influence of continual stirring and bubbling, the physicochemical and biochemical properties of unstirred systems became anisotropic and heterogeneous, and relative transformation rates decreased significantly. Like the CEPMs, high particle surface area in the oxidized clay corresponded to slower transformation rates than in the oxidized or reduced sand. Unlike the CEPMs, NOSH transformation rates frequently were similar or greater under reduced vs. oxidized conditions. This redox effect was most pronounced in the clay where reduced conditions promoted degradation of virtually all NOSHs vs. the oxidized clay. This redox effect was sufficient to overwhelm the attenuating effects of high surface area, as was observed in the CEPMs. Thus, the reduced clay showed some of the fastest relative transformation rates observed in any treatment in these experiments (e.g., quinoxaline, quinoline carbonitrile, benzo(f)quinoline).

The high relative rates of NOSH transformation in the reduced clay microcosms suggested that complexation reactions with redox-active metals was a factor in NOSH fates [32, 33]. Under reduced conditions, ferric oxyhydroxide colloids and particulates are reduced to divalent, soluble forms. This dissolution corresponds to the removal of chemically active insoluble surface area, and chemicals entrained in or adsorbed to these colloids can be mobilized under these conditions [16]. In order to examine interactions between NOSHs and redox-active iron, mixtures of NOSHs were dissolved in DCM and shaken as biphasic liquid systems with deionized water or deionized water containing ferric ($FeCl_3$ or $Fe(OH)_3$) or ferrous iron ($FeSO_4$). After extended shaking, the phases were separated and the DCM was dried and analyzed using GC (Fig. 7). Results of these experiments indicated that both dissolved and particulate forms of iron could facilitate removal of the NOSHs from the DCM phase into the aqueous phase. Thus, the pure water controls (Fig. 7) show retention of the NOSH compounds in the DCM phase after shaking, while the presence of iron increased the tendency of some NOSHs to partition with the water (reflected in lower NOSH recoveries from the DCM). The effect was much more pronounced with ferric than ferrous iron. This showed that Fe(III) as found in oxidized sediments was capable of complexing many NOSHs on colloidal or particulate phases. Under reduced conditions, reduction of Fe(III) to soluble Fe(II) and subsequent dissolution of these colloids would allow for liberation of NOSHs to the aqueous phase where they would be available to chemical and biochemical reactions. While able to complex some NOSHs (quinoline, 2-methylquinoline, 4-methylquinoline, quinoline carbonitrile) and alter their partitioning with DCM, the effects soluble Fe(II) on the recovery of most compounds was insignificant. These conclusions were supported by inspection of the sediments in the batch vial microcosms: oxidized sediments had reddish-brown surface deposits of iron oxide and oxyhydroxide while the reduced systems had no visible iron depositis. Extraction of the iron deposits from the oxidized sediments confirmed the presense of NOSHs in this matrix.

CONCLUSIONS

Relative to water and microbial cultures, NOSH compounds are degraded slowly and incompletely in sediments under conditions found in estuarine, wetland, and marine ecosystems. Turbulent mixing in high energy areas may increase the availability of sediment-bound NOSHs to chemical and microbiological reactions in the aqueous phase, but

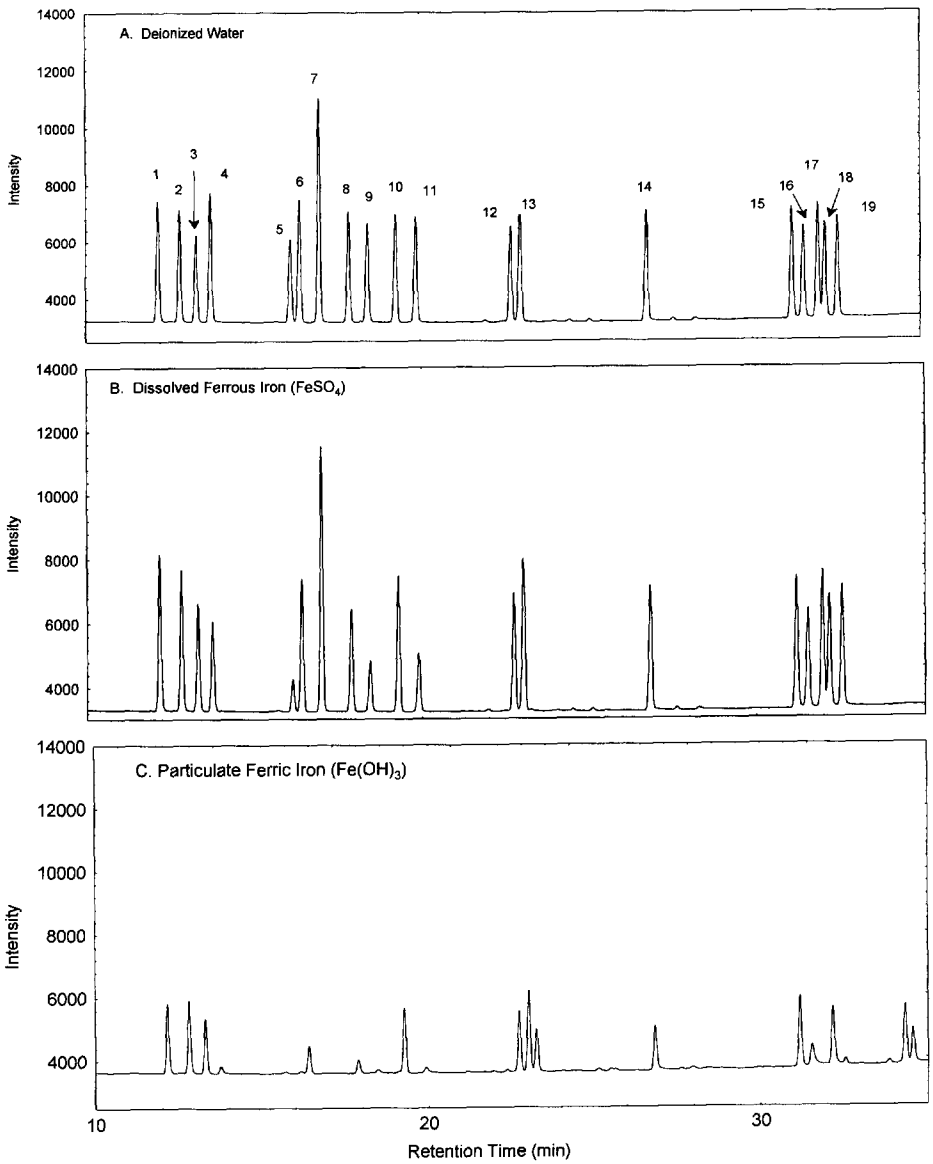


FIGURE 7. Biphasic Liquid-Liquid Partitioning of NOSHs between DCM and Water in the Absence (A) and Presence (B, C) of Dissolved and Particulate Iron. **Compounds:** **1.** Benzothiophene; **2.** Quinoxaline; **3.** Benzothiazole; **4.** Quinoline; **5.** 2-Methylquinoline; **6.** 8-Methylquinoline; **7.** 3-Methylisoquinoline; **8.** 3-Methylquinoline; **9.** 4-Methylquinoline; **10.** 2,3-Dimethyquinoxaline; **11.** Quinoline carbonitrile; **12.** 2,4-Dimethylquinoline; **13.** Dibenzofuran; **14.** 4-Azafluorene; **15.** Dibenzopyran; **16.** Benzo(f)quinoline; **17.** Acridine; **18.** 9-Methylcarbazole; **19.** Phenanthridine

these conditions pertain only a small proportion of the total volume of wetland and coastal systems likely to receive NOSHs from energy and urban/industrial activities.

Under continuously stirred conditions in microcosms, many NOSH compounds will degrade to simpler products under both oxidized and reduced conditions. Some NOSHs such as acridine, benzo(f)quinoline, dibenzopyran, and benzothiazole apparently are transformed to stable products, some of which are toxic and more water soluble than the parent compound. NOSH degradation processes are limited by increased particle surface area and reduced electrochemical conditions (i.e., degradation is fastest in oxidized sediments of large particle size). These observations are consistent with previous work on the effects of Eh and particle size on aromatic hydrocarbon degradation [34, 35].

In quiescent systems not exposed to high energy mixing, NOSH degradation rates also were depressed by decreasing particle size, but are promoted by reducing electrochemical conditions. This supported the idea that most biochemical and chemical transformations of significance to NOSH fates occur in the aqueous phases of the sediments and slurries, rather than at surface sites on particles. Hence, reducing redox conditions promoted liberation of NOSHs from iron colloids and particles into the aqueous phase where chemical and microbial processes proceed at more rapid rates. Other processes that liberate NOSHs from sediment surfaces to the aqueous phase (e.g., protonation to soluble ion pairs) also can be expected to increase the degradation of some NOSHs relative to rates on sediment surfaces. In both cases, the presence of NOSH compounds and their transformation products in the aqueous phase can be expected to correspond to increases in aqueous phase toxicity.

The toxicity data from the stirred microcosms showed general detoxification of the aqueous phases with time. It seemed likely from the bioassays and product analyses that this detoxification was a multi-step process involving intermediates, which sometimes were as (or more) toxic as the starting materials. Many of the NOSH compounds formed stable, polar degradation products that clearly were involved in changes in aqueous toxicity. Analyses of stable products suggested that the pathways of NOSH transformation were diverse, and included a) chemical and biochemical oxidation of rings followed by ring opening and β oxidation of resultant hydrocarbon chains, b) oxidation of alkyl groups to carboxylic acids followed by decarboxylation and c) epoxidation, alkylation, and free radical addition. Benzothiazole demonstrated a series of free radical addition reactions along with degradative oxidation reactions. Surprisingly, the NOSH transformation product spectrum was very similar between oxidized and reduced conditions,

with the rates and relative amounts of products being the main differences between treatments. Chemical reactions including protonation, electrochemical oxidation or reduction followed by transformation, and perhaps catalytic deprotonation of relatively acidic protons (e.g., the proton on the 10 position of acridine) also were indicated with some NOSHs.

Further research on the environmental chemistry on NOSHs is called for, particularly with respect to sorption-desorption chemistry, electrochemical reactions and hydrolysis, metal complexation chemistry, and affiliated changes in bioavailability, transformation rates/pathways, and toxicity. Resolution of these areas would be of interest from a geochemical perspective, and productive with respect to remediation development and risk assessment for NOSH-contaminated sediments (e.g., harbors and creosote dumpsites).

Acknowledgment- The author is indebted to Robert P. Gambrell, Robert S. Murray, Judith Franke (ATCC), Istvan Devai, R. D. DeLaune, Kieth Fessel, and Thomas Junk for support and participation in various parts of this study. Jay Means and David Swenson provided review and critical discussion of this work. Research was conducted under a grant from the US Dept. of Interior/Minerals Management Service, Gulf of Mexico OCS Region, New Orleans LA.

REFERENCES

[1] Warshawsky, D. 1992. Environmental sources, carcinogenicity, mutagenicity, metabolism and DNA binding of nitrogen and sulfur heterocyclic aromatics, *Environ. Carcino. & Ecotox. Revs.* C(10):1-71

[2] Beiko, O. A., N. N. Gerasimova, and T. A. Sagachenko. 1987. Nitrogen bases of industrial west Siberian crude oil, Petrol. Chem. USSR 27(3):200-210.

[3] Catallo, W. J., D. R. Cleland, and M. E. Bender. 1990a. "Toxicity of nitrogen-containing aromatic compounds (NCACs). Quinoline and 4-azafluorene behavior in an *Escherichia coli* test system: evidence of membrane effects". In: Landis, W. G. and W. H. van der Schalie (eds.), Aquatic Toxicology and Risk Assessment. ASTM Special Technical Publication 1096, American Society for Testing and Materials, Philadelphia, PA, pp. 199 - 221.

[4] Turov, Y. P., N. N. Gerasimova, T. A. Sagachenko, and O. A. Bieko. 1987. Group composition of the low molecular weight nitrogen bases of Samotlor crude oil. Petrol. Chem. USSR 27(1):20-25.

[5] Furlong, E. T. and R Carpenter. 1982. Azaarenes in Puget Sound sediments. Geochim. Cosmochim. Acta 46:1385-1396.

[6] Krone, C. A., D. G. Burrows, D. W. Brown, P. A. Robisch, A. J. Friedman, and D. C. Malins. 1986. Nitrogen-containing aromatic compounds in sediments from a polluted harbor in Puget Sound. Environ. Sci. Technol., 20(11):1144-1150.

[7] Thompkins, B. A. and C. Ho. 1982. Determination of polycyclic aromatic amines in natural and synthetic crudes, Anal. Chem., 54:91-96.

[8] Malins, D. C., M. M. Krahn, M. S. Meyers, L. D. Rhodes, D. W. Brown, C. A. Krone, B. B. McCain, and S. -L. Chan. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole, Carcinogenesis 6(10):1463-1469.

[9] Roubal, W. T. and D. C. Malins. 1985. Free radical derivatives of nitrogen heterocycles in livers of English sole (Parophys vetulis) with hepatic neoplasms and other liver lesions. Aquatic Toxicol. 6:87-103.

[10] EPRI (Electric Power Research Institute). 1985. Inventory of organic emissions from fossil fuel combustion for power generation, EPRI EA-1394-TPS 78-820, 44 pp.

[11] Chou, T. W. and N. Bohonos. 1979. Diauxic and cometabolic phenomena in biodegradation evaluations. In: Microbial Degradation of Pollutants in Marine Environments, EPA-600/9-79-012, pp. 76-88.

[12] Smith, J. H., W. R. Mabey, N. Bohonos, B. R. Holt, S. S. Lee, T. -W. Chou, D. C. Bomberger, and T. Mill (1977,1978). "Environmental pathways of selected chemicals in freshwater systems", Parts I and II, EPA 600/7-77-113 and 600/7-78-074, respectively.

[13] Godsy, E. M., D. F. Goerlitz and D. Grbic-Galic. 1993. Methanogenic degradation kinetics of nitrogen and sulfur containing heterocyclic aromatic compounds in aquifer-derived microcosms, In: Symposium on Bioremediation of Hazardous Wastes: Research Development, and Field Evaluations. EPA/600/R-93/054, pp. 123-128.

[14] Weber, E. J., D. L. Spidle, and K. A. Thorn. 1993. Bioremediation of soils and sediments contaminated with aromatic amines. In: Symposium on Bioremediation of Hazardous Wastes: Research Development and Field Evaluations (Abstracts), EPA/600/R-93/054, pp. 137-138.

[15] Black, J. N. P., R. M. Kauffman, D. Denney, and D. Grbic-Galic. 1993. Biotransformation of indole and quinoline under denitrifying conditions. In: Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations. USEPA Office of Research and Development, EPA/600/R-93/054,pp. 232-233.

[16] Ponnamperumma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

[17] Zitko, V. 1980. The analysis of aquatic sediments for organic compounds. In: Baker, R. A. (ed.), Contaminants and Sediments Volume 2: Analysis, Chemistry and Biology. Ann Arbor Science Publishers, Ann Arbor MI, pp. 89-99.

[18] Wakeham, S. G. 1979. Azaarenes in recent lake sediments. Environ. Sci. Technol. 13(9):1118-1123.

[19] Louter, A. J. H., F. D. Rinkema, R. T. Ghijsen, and U. A. Th. Brinkman. 1994. Rapid identification of benzothiazole in river water with on-line solid-phase extraction-gas chromatography-mass selective detection. Intern. J. Environ. Anal. Chem. 56:49-56.

[20] Lopez-Avila, V. and R. A. Hites. 1980. Organic compounds in an industrial wastewater. Their transport into the sediments. Environ. Sci. Technol. 14(11):1382-90.

[21] Catallo, W. J. and R. P. Gambrell. 1987. The effects of high levels of polycyclic aromatic hydrocarbons on sediment physicochemical properties and benthic organisms in a polluted stream. Chemosphere, 16(5):1053-1063.

[22] Zachara, J. M., C. C. Ainsworth, L. J. Felice, and C. T. Resch. 1986. Quinoline sorption to subsurface materials: role of pH and retention of the organic cation. Environ. Sci. Technol. 20(6):620-626.

[23] Patrick, W. H., B. G. Williams and J. T. Moraghan. 1973. A simple system for controlling redox potential and pH in soil suspensions, Proc. Soil Sci. Soc. America, 37(2):331-2.

[24] Adams, J., E. L. Atlas, and C. S. Giam. 1982. Ultratrace determination of vapor-phase nitrogen heterocyclic bases in ambient air. Environ. Sci. Technol. 54:1515-18.

[25] McLafferty, F. W. 1980. Interpretation of Mass Spectra (3rd Edition), University Science Books, Mill Valley, CA, 303 pp.

[26] Lui, D., and B. J. Dutka (eds). 1988. Toxicity Screening Procedures Using Bacterial Systems, Marcel Dekker, NY, 475 pp.

[27] Catallo, W. J., M. Schlenker, R. P. Gambrell, and B. S. Shane. "Toxic chemicals and metals in sediments from rural and industrialized Louisiana lakes: Recent historical profiles and toxicological significance", Environ. Sci. Technol. 29(6):1436-45.

[28] Millar, C. E., L. M. Turk, and H. D. Foth. 1986. Fundamentals of Soil Science, 4th Edition, John Wiley and Sons, Inc. NY.

[29] Laha, S. and R. G. Luthy. 1990. Oxidation of aniline and other primary aromatic amines by manganese dioxide. Environ. Sci. Technol. 24(3):363-67.

[30] Catallo, W. James. 1994. Fates and Effects of N-, O-, and S- Heterocycles (NOSHs) from Petroleum and Pyrogenic Sources in Marine Sediments. OCS Study MMS 94-0056. U.S. Dept. of the Interior, Minerals Mgmt. Service, Gulf of Mexico OCS Region, New Orleans, La. 72 pp.

[31] Parris, G. E. 1980. Covalent binding of aromatic amines to soil humates. I. Reaction with carbonyls and quinones. Environ. Sci. Technol., 14:1099-1106.

[32] Jorgensen, A. D., J. R. Stetter, and V. C. Stamoudis. 1985. Interactions of aqueous metal ions with organic compounds found in coal gasification: model systems. Environ. Sci. Technol. 19(10):919-924.

[33] Stetter, J. R., V. C. Stamoudis, and A. D. Jorgensen. 1985. Interactions of aqueous metal ions with organic compounds found in coal gasification: process condensates. Environ. Sci. Technol., 19(10):924-928.

[34] DeLaune, R. D., G. A. Hambrick, and W. H. Patrick. 1980. Degradation of hydrocarbons in oxidized and reduced sediments. Mar. Poll. Bull. 11:103-106.

[35] DeLaune, R. D., W. H. Patrick, and M. E. Casselman. 1981. Effect of sediment pH and redox conditions on degradation of benzo(a)pyrene. Mar. Poll. Bull. 12(7):251-253.