Evaluation of *N*-Methylpyrrolidone and Its Oxidative Products Toxicity Utilizing the Microtox Assay

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N-Methylpyrrolidone (NMP) is a cyclic nitrogen-containing organic chemical used to replace more volatile and toxic organic solvents in paint coating and cleaning applications. The Marine Corps Multi-Commodity Maintenance Center was concerned that the high NMP and organic levels in process water would upset treatment processes at the Industrial Process Water Plant (IWP). The NMP contaminated process water was oxidized by a semicontinuous advanced oxidation reactor to reduce the organic concentration. The oxidative byproducts of NMP were identified by GC/MS and tested for their toxicity. A toxicity test, utilizing the Microtox toxicity assay, revealed that methylsuccinimide was the most toxic identifiable product of NMP oxidation. The toxicity of the process water was reduced as methylsuccinimide and was further oxidized to succinimide and other amine products. The results indicate that NMP contaminated process water should be oxidized past the *N*-methylsuccinimide compound prior to standard industrial process water treatment procedures, so as to reduce toxicity concerns associated with NMP contaminated process water.

Introduction

Volatile organic compounds (VOCs) are used in many industrial processes such as lubrication, coating, and cleaning applications. With the passage of the 1990 Clean Air Act Amendments, the Marine Corps Multi-Commodity Maintenance Centers (MC[3]) investigated VOC abatement technologies and reducing VOC content in surface coatings. As part of the investigation into reducing the VOC content of Chemical Agent Resistant Coatings (CARC), the MC[3] and Miles, Inc. formulated a water-based CARC in which water and *N*-methylpyrrolidone (NMP) were the principle solvents (*1*).

A countercurrent wet scrubber at the MC[3] facility was utilized to transfer the NMP vapor to the aqueous phase. The scrubber utilized water as the scrubbing liquor and removed greater than 97% of the NMP from the exhaust air stream at the facility (*1−3*). NMP was the organic compound of highest concentration in the scrubbing process water. Other solvents such as triethylamine, aldehydes, and organic acids were also present at low concentrations in the process water.

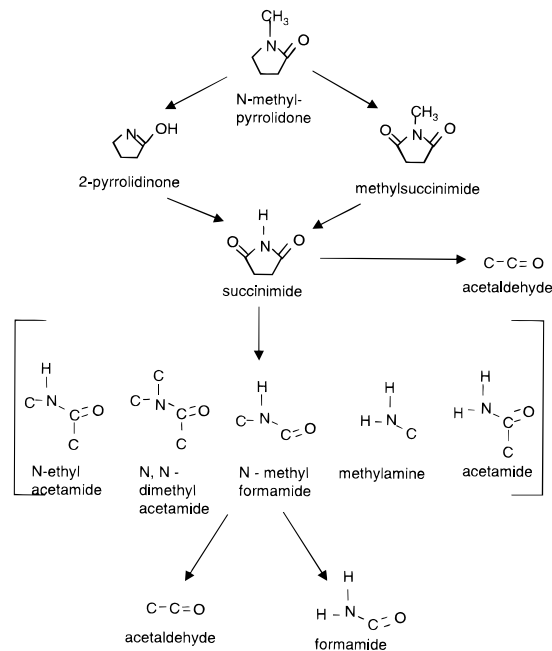* Corresponding author phone: (814)863-9911; fax (814)863-1183; e-mail: bas124@psu.edu.

FIGURE 1. Proposed oxidation pathways of *N*-methylpyrrolidone in a hydrogen peroxide and ozone semicontinuous reactor.

MC[3] was concerned that the high NMP and organic levels of this process water would upset treatment processes at the Industrial Process Water Plant (IWP). An ozone and hydrogen peroxide semicontinuous reactor was used to oxidize NMP in an attempt to reduce the organic loading to the IWP.

N-Methylsuccinimide (methylsuccinimide) and 2-pyrrolidone were the initial oxidative products of NMP identified by GC/MS (*1, 3*). It was found that *N*-methylsuccinimide formed over 2-pyrrolidinone at a ratio of approximately 9:1 under the alkaline conditions (*1*). *N*-Methylsuccinimide and 2-pyrrolidone were stable in the aqueous matrix, and both were further oxidized within the system to form succinimide (2,5-pyrrolidinedione) (Figure 1). This product was also stable within the aqueous matrix. All identified products were confirmed utilizing gas chromatograph/flame ionization detector (GC/FID). Proton nuclear magnetic resonance (PNMR) Chromatography was used to confirm NMP and *N*-methylsuccinimide in the liquid samples (*1*).

The goal of this study was to determine the toxicity of NMP and its oxidative products and to investigate the extent of oxidation required to reduce toxicity. Toxicity values for NMP and its oxidative products were not found in the literature. Formaldehyde was another possible byproduct in the oxidation process. However, formaldehyde was not included in the toxicity study since its toxicity is established in the literature (*4*).

Materials and Methods

AOP Semicontinuous Reactor. The advanced oxidation process (AOP) was simulated utilizing a semicontinuous reactor. The semicontinuous reactor consisted of a 1-L glass reaction flask with a capacity of 700 mL of aqueous solution. The system pH was controlled with a Cole Palmer pH/OPR Controller model number 5656-00. An Oxytech model PXC-20 served as the ozone generator for the experiment. Concentrations of ozone were measured in the inlet and exhaust air streams with a Dasibi model 1008-HC ozone meter. The ozone was diffused into the aqueous phase via

a diffusion stone at a rate of 2.5 L/min. Concentrations of ozone varied between 0 and 1590 ppm.

A stock solution of 35% (w/w) hydrogen peroxide was used to prepare solutions varying from 0 to 16% hydrogen peroxide by weight. The solutions were added to the aqueous phase in the semicontinuous reactor at a flow rate of 0.07 mL/min. The solution was stirred with a $^3/_4$-in. Teflon stirring cross at approximately 300 rpm.

Samples were separated by a Supelco 0.53 mm i.d. × 60 m capillary VOCOL column and quantified with a Hewlett-Packard 5890 GC/FID. NMP oxidation products were analyzed and determined by gas chromatography via a Hewlett-Packard mass selective detector (GC/MS) (1, 5). All samples were quantified using single point calibrations after linearity in the concentration range of interest had been verified. Comparing the spectra generated by the MS detector to standard library spectra allowed identification of compounds in the headspace.

Total organic carbon (TOC) was measured using a DC-80 Dohrmann TOC analyzer. The samples were acidified and sparged with oxygen to remove inorganic carbon before analysis. Samples were quantified using single point calibrations after linearity in the concentration range of interest had been verified using prepared standards.

Microtox Procedure. Tests were performed using the Microtox model 500 instrument following procedures outlined in the literature and the Microtox manual published by AZUR Environmental (6−9). All toxicological tests were performed as described in the Acute Detailed Basic Testing procedure presented by AZUR Environmental.

Microtox utilizes the lyophilized marine microorganism *Vibrio fisheri* (Northern Regional Research Laboratory strain B-11177) known as *Photobacterium phosphoreum,* a bioluminescent marine organism that emits light as a byproduct of metabolic processes. The marine organism is nonindigenous to freshwater systems or to the Western Hemisphere.

Light emissions produced by the organism are directly correlated to energy-production and metabolic rate. The measurement of changes in metabolic rate, and therefore light production, is a valuable endpoint for toxicity tests since changes represent repression or stimulation of the metabolic rate of the organism (10).

The Microtox instrument was used, in part, due to the evidence that Microtox is as sensitive as *Daphnia magna*, rainbow trout (*Oncorhynchus mykiss*), and fathead minnow (*Pimephales promelas*) bioassays for organic chemicals (11, 12). The instrument appears to be well correlated to more traditional aquatic toxicity tests, such as fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), and *Daphnia magna* (12−15). The Microtox instrument has also been used to evaluate the aquatic toxicity of effluents of unknown chemical composition. The U.S. Environmental Protection Agency (U.S. EPA) has supported this instrument for use in determining the aquatic toxicity of treated effluents and process water (12).

Chemicals used in this study include NMP and its oxidative byproducts: methylsuccinimide, 2-pyrrolidinone, and succinimide. All compounds were of the highest purity grade. Test samples were prepared by diluting the pure compounds with deionized water. Each compound was of high solubility and did not require the addition of a cosolvent (Table 1). Since the prepared solutions are of known concentration, EC50 results were presented in grams per liter (g/L).

Actual process water was obtained from the MC³. All results for the process water are presented as a percentage (%) of response due to the mixed composition of NMP and its oxidation products in the process water. Toxicological test results for the process water are presented as single toxicity values.

Analysis of a phenol standard ensured that the Microtox instrument was working within acceptable limits throughout the test period. The toxicity tests of actual NMP contaminated process water are presented as the 30-min EC50 values. The 30-min test was used due to the fact that the 5- and 15-min EC50 values for the process water showed a larger error (i.e., larger 95% confidence intervals) than those obtained for the 30-min test times. All tests were performed in triplicate.

Statistical Procedure. A p-value was determined to statistically evaluate the threshold curves. The null hypothesis (H_o) for the threshold curve states that the normalized EC50 values at various test concentrations are statistically different. Values of p less than 0.05 cause a rejection of the H_o. This level indicates that the line is statistically different from zero at a 5% significance level. The alternate hypothesis (H_a) states that the normalized EC50 values at various test concentrations are not statistically different. Values of p greater than 0.05 fail a rejection of the H_o and indicate that the slope of the regression line is not statistically different from zero (16). Thus, rejecting the H_o indicates that there is a significant relationship between the contaminant concentration and the observed normalized EC50 value.

Results and Discussion

Toxicity Results. The majority of the results from the toxicological study of NMP and its byproducts are presented as static acute 5-minute EC50 values. The EC50 value is the effective concentration (EC) where 50% of the test organisms are affected after 5 min of contact with the test solution. Results indicate that in this investigation, the 5-minute tests had greater statistical confidence than the 15-minute tests. Consequently, only the results from the 5-minute tests are presented in this paper.

NMP and its oxidative byproducts, N-methylsuccinimide, succinimide, and 2-pyrrolidinone, were tested at various concentrations in order to determine their relative toxicity and the toxicity threshold for each compound (Table 1).

The experimentally determined NMP toxicity had a strong linear relationship ($p = 0.00$, $R^2 = 0.9915$) with the aqueous concentration of NMP (Figure 2). This observation indicates that the Microtox system produced results that were strongly correlated with the concentration of a compound, in this case NMP. The toxicity evaluation of the oxidative byproducts of NMP suggests that the EC50 values of these compounds varied from the parent compound, NMP.

Figure 3 indicates NMP has a toxicity threshold of 0.9 g/L in water. Above this value, an EC50 value of 1.5 ± 0.7 g/L was observed and remained statistically unchanged ($p = 0.770$) at higher concentrations.

The threshold value is defined herein as the concentration above which there is a statistically significant response or effect from the system being studied, in this case, the Microtox reagent. It is the level where the minimum measurable effect occurs (17). Below this value there is no statistically significant response from the system (i.e., there is no statistically observable EC50). The threshold area is shown on an EC50 graph as the point where the response from the system rapidly becomes less toxic, eventually resulting in no measurable toxicity value.

The 2-pyrrolidinone data indicate that this compound has a threshold value of approximately 2.3 g/L. Concentrations above the experimental threshold value showed a toxicity of approximately 5.3 ± 1.0 g/L. This value remained statistically constant with a p-value of 0.344 at all concentrations above the threshold.

N-Methylsuccinimide, the compound of highest formation rate and the compound observed to be the most toxic, had a toxicity threshold value of 0.6 g/L. Above this value the data indicate a statistically constant value of approximately 1.2 ± 0.15 g/L with a 0.110 p-value.

TABLE 1. Experimentally Measured Mean 5-Min EC50 Values Obtained by Microtox Toxicity Assay for *N*-Methylpyrrolidone, *N*-Methylsuccinimide, 2-Pyrrolidinone, and Succinimide[a]

compd	test concn (g/L)	mean EC50 (g/L)	SD norm.	margin of error about normalized mean EC50 ($\pm$)[b]
N-methylpyrrolidone	1000	1.18	0.125	0.142
	100	1.730	0.479	0.542
	10.0	2.100	0.165	0.187
	1.00	1.195	0.228	0.258
	0.90	1.018	—	—
	0.75	NR	—	—
	0.50	NR	—	—
succinimide	100	14.20	0.196	0.560
	50.0	13.72	0.572	2.110
	25.0	14.10	1.870	2.010
	20.0	15.83	2.050	2.07
	15.0	15.9	1.49	—
	12.5	NR	—	—
	5.00	NR	—	—
	4.75	NR	—	—
N-methylsuccinimide	100	0.766	0.121	0.106
	50.0	0.772	—	—
	25.0	0.948	—	—
	10.0	0.757	0.080	0.111
	5.00	1.22	—	—
	2.5	1.24	—	—
	1.75	1.14	—	—
	1.00	1.10	0.071	.098
	0.75	1.23	—	—
	0.60	1.08	0.797	0.980
	0.50	NR	—	—
2-pyrrolidinone	100	3.79	0.326	0.319
	50.0	7.16	0.697	0.788
	25.0	7.49	—	—
	12.5	4.08	—	—
	10.0	6.03	0.317	0.439
	5.00	5.44	0.141	0.196
	2.50	3.25	—	—
	2.25	5.05	1.76	1.74
	2.00	NR	—	—
	1.00	NR	—	—

[a] Abbreviations: g/L, grams per liter; mg/L, milligrams per liter; SD norm., standard deviation of normalized data; NR, no measurable toxicity response; and —, no standard deviations were determined due to single toxicity test. [b] From 95% confidence interval.
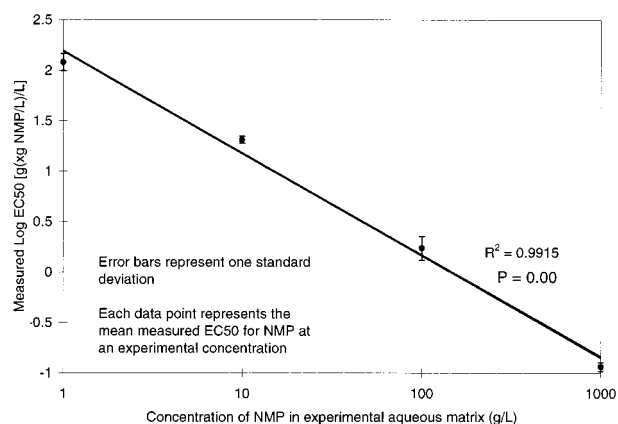

FIGURE 2. Dose-response of *Photobacterium phosphorium* to NMP.

The least toxic byproduct of NMP, succinimide, was observed to have a threshold value of approximately 15.0 g/L. As with the other compounds, the data for succinimide above the threshold concentration show a statistically constant value of 14.0 ± 2.0 g/L ($p = 0.193$).

The EC50 values for all compounds tested were less consistent and more variable as the sample concentration approached the observed threshold value. This observation appears to be a function of the sensitivity of the microbes to the test concentration. At or near the threshold concentration, the system varies between no response and a toxic response. Thus, repeated tests at or near the threshold
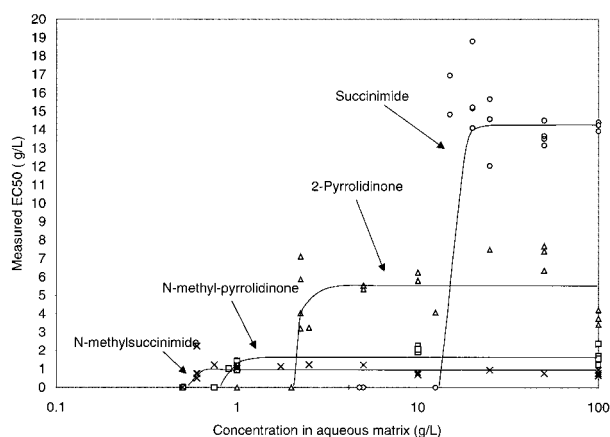

FIGURE 3. EC50 values and toxicity thresholds of *N*-methylpyrrolidinone, *N*-methylsuccinimide, 2-pyrrolidone, and succinimide based upon the 5-min Microtox single toxicity test.

concentration vary widely because a portion of the microbes are reacting to the test concentration, while another portion may show no response. This trend of increased variability in results near the threshold value was also noted by Cooney (8).

In addition, the increased variance near the threshold value of a compound prohibits the use of standard statistical confidence intervals. Use of standard statistical methods would decrease the visual spatial variability within the

TABLE 2. Biochemical Oxygen Demand of *N*-Methylpyrrolidone (NMP) and Its Oxidation Products (*1*)

compd	BOD$_5$ (mg/L) (1 g/L sample)	ThOD (mg O$_2$/L) (1 g/L sample)	BOD$_5$/ThOD
N-methylpyrrolidone	728	1.94	0.38
N-methylsuccinimide	96	1.41	0.01
2-pyrrolidone	452	1.69	0.27
succinimide	537	1.13	0.48

threshold graphs and would cause the toxicity threshold to appear as sampling error rather than variability inherent near the threshold value.

Mixed culture biochemical oxygen demand (BOD$_5$) tests correspond with the toxicity evaluation. Tests were performed using 1 g/L of each of NMP, methylsuccinimide, 2-pyrrolidinone, and succinimide at Center Analytic Laboratory, State College, PA (*1*). Results from the analysis are presented in Table 2.

The ratio of BOD$_5$ to the theoretical oxygen demand (ThOD) is another indicator of the biodegradability of the oxidation products. Higher ratios may indicate greater biological growth and lower toxicity.

The BOD$_5$/ThOD tests indicated that succinimide is very biodegradable and is relatively nontoxic. In addition, methylsuccinimide had the lowest BOD$_5$/THOD, indicating that the compound may be less biodegradable or more toxic (*1*). However, other factors, such as enzymatic deficiencies or species adaptation, may also account for these observations. Regardless, results from toxicity tests were consistent with the BOD$_5$/ThOD test results.

N-Methylsuccinimide was the compound of highest toxicity. The parent compound, NMP, was observed to have the second highest toxicity. The compounds 2-pyrrolidinone and succinimide were found to be the least toxic compounds tested in the study.

Toxicity of the Process Water. The process water from MC^3 contained a mixture of common paint solvents. NMP, *N*-methylsuccinimide, succinimide, and formaldehyde accounted for 62−65% of the initial TOC. After 1 h of treatment, NMP and its products dominated the organic composition of the process water, accounting for over 80% of the identified TOC. Triethylamine and methyl isoamyl ketone (MIAK) comprised the remaining identifiable fraction of the wastewater.

The ozone and hydrogen peroxide AOP was shown to oxidize NMP and other organic solvents from the synthetic and actual process water from the Marine Corps Multi-Commodity Maintenance Centers. However *N*-methylsuccinimide, the first product of NMP oxidation under the alkaline conditions of the AOP, increased in concentration. *N*-Methylsuccinimide proved to be the most toxic oxidation product. The parent compound of methylsuccinimide, NMP, was found to be the second most toxic component. Further oxidation resulted in the formation of succinimide, the least toxic of NMP's stable oxidation products. Therefore, partial oxidation of NMP was a concern due to the potential to increase the toxicity of the process water if the oxidation process was not carried to near completion.

Results from the toxicological evaluation of NMP contaminated process water under the AOP process are presented in Figure 4. The data demonstrate that the toxicity of the process water increased with time as methylsuccinimide formed under the AOP process. This trend continued until the AOP process oxidized methyl-succinimide to succinimide and other less toxic products. These results are in agreement with the original toxicological evaluations performed on the oxidative byproducts of NMP. It was determined that
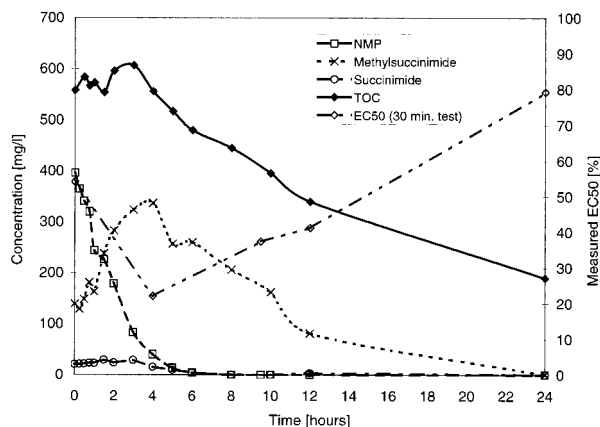

FIGURE 4. NMP destruction, TOC removal, and toxicological analysis of AOP treated process water utilizing the Microtox assay.

methylsuccinimide was the most toxic byproduct of NMP and indeed did dominate the toxicity profile.

Figure 4 shows that between time 0 and 4 h methylsuccinimide steadily increased within the system due to the chemical oxidation of NMP. Based on the initial toxicity evaluations, which showed that methylsuccinimide was the most toxic byproduct of NMP, it is apparent that the formation of methylsuccinimide resulted in the increased toxicity of the process water upon initial chemical oxidation. Once methylsuccinimide was oxidized within the system to form the lesser toxic succinimide, the toxicity of the process water steadily decreased. The system continued to decrease in toxicity as oxidation past succinimide occurred until cessation of the test at hour 24. Therefore, it is recommended that the process water be oxidized beyond the formation of the methylsuccinimide compound to reduce toxicity of the treated process water.

At the termination of the test at hour 24, the toxicity of the process water had decreased (high EC50 value) to a value of 80%. Bulich produced a ranking for process waters of unknown concentration and composition utilizing the Microtox instrument (*7*). According to this ranking, a process water producing an EC50 of <25% would be considered very toxic. EC50 values ranging from 25 to 75% are toxic, while values > 75% are slightly toxic to nontoxic.

According to this ranking the process water system at time 0 was toxic. Once oxidation began, the system quickly fell to the very toxic range. As oxidation continued past the methylsuccinimide compound, the system slowly decreased in toxicity until at hour 24 when the system had reached 80% or a nontoxic level. Thus, chemical oxidation of NMP contaminated process water did reduce the toxicity of the system but only after the methylsuccinimide compound was completely oxidized.

The total organic carbon (TOC) of the system, which is often used as an indicator of water pollution potential, did not reduce to 0.0 mg per L (mg/L) throughout the test period. The TOC values were measurable throughout the entire test period, possibly due to the formation of oxidative byproducts after the succinimide compound. The mass fraction of species accounted for by GC/FID analysis was 105% of the measured TOC at the start of the experiment. The mass fraction of species measured with the GC/FID compared to the TOC decreased steadily over time from the initial 105% to 57% at 4 h, and only 16% after 12 h of treatment. However, based on the toxicity measurements of the process water during the AOP process and the toxicity of the general class of the compounds formed after succinimide, the remaining TOC did not appear to appreciably contribute to the toxicity of the process water.

The results from the study show that the TOC measurement for NMP contaminated process water was not an effective method of measuring the environmental effects of the process water. Specifically, TOC measurement did not correlate with the measured toxicity.

The objective of this study was to investigate the degree of NMP contaminated process water oxidation necessary to reduce toxicity and provide a treatment endpoint based on toxicological evaluation for the AOP process. This was achieved utilizing the Microtox assay and resulted in the determination that methylsuccinimide, the compound of highest formation rate in the AOP, dominated the toxicity profile of the process water. Therefore process water contaminated with NMP that is subjected to chemical oxidation should be monitored for methylsuccinimide prior to disposal.

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