Published in final edited form as:

Environ Sci Technol. 2009 March 1; 43(5): 1487–1492.

Radiolysis Studies on the Destruction of Microcystin-LR in Aqueous Solution by Hydroxyl Radicals

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Abstract

In this study, steady state and time resolved radiolysis methods were used to determine the primary reaction pathways and kinetic parameters for the reactions of hydroxyl radical with microcystin-LR (MC-LR). The fundamental kinetic data is critical for the accurate evaluation of hydroxyl radical based technologies for the destruction of this problematic class of cyanotoxins. The bimolecular rate constant for the reaction of hydroxyl radical with MC-LR is $2.3 (\pm 0.1) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ based on time resolved competition kinetics with SCN^- at low conversions using pulsed radiolysis experiments. The reaction of hydroxyl radical with MC-LR can occur via a number of competing reaction pathways, including addition to the benzene ring and diene and abstraction of aliphatic hydrogen atoms. LC-MS analyses indicate the major products from the reaction of hydroxyl radicals with MC-LR involve addition of hydroxyl radical to the benzene ring and diene moieties of the Adda side chain. Transient absorption spectroscopy monitored between 260–500 nm following pulsed hydroxyl radical generation indicate the formation of a transient species with absorption maxima at 270 and 310 nm. The absorption maxima and lifetime of the transient species are characteristic of hydroxycyclohexadienyl radicals resulting from the addition of hydroxyl radical to the benzene ring. The rate constant for the formation of hydroxycyclohexadienyl radical is $1.0 (\pm 0.1) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ accounting for ~40 % of the primary reaction pathways. Representative rate constants and partitioning of hydroxyl radical reactions were assessed based on the reactivities of surrogate substrates and individual amino acids. Summation of the individual reactivities of hydroxyl radical at the different reactive sites (amino acids) leads to a rate constant of $2.1 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ in good agreement with the rate constant determined in our studies. The relative magnitude of the rate constants for the reactions of hydroxyl radical with the individual amino acids and appropriate surrogates, suggest 60–70 % reactions of hydroxyl radical occur at the benzene and diene functional groups of the Adda moiety.

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Supporting Information Available:

Three figures showing structure of MC-LR and model compounds; LC-MS analysis data for decomposition products. This material is available free of charge via the Internet at <http://pubs.acs.org>

Introduction

The increased occurrence of cyanobacteria (blue-green algae) blooms and the production of associated cyanotoxins present a threat to drinking water sources. Among the most common types of cyanotoxins found in potable water are microcystins (MCs), a large family of cyclic heptapeptides. MCs are strongly hepatotoxic and known tumor promoters. The presence of sub-lethal doses of MCs in drinking water is implicated as one of the key risk factors for an unusually high occurrence of primary liver cancer (1). The acute and chronic toxicity of MCs has increased pressure to ensure their removal from potable water, and WHO published a guideline value for MC-LR of 1 nM in 1998 (2).

A variety of traditional water treatment methods, including coagulation / sedimentation (3), activated carbon adsorption (4) and membrane separation (5), have been attempted for the removal of cyanotoxins, but with limited success. Advanced Oxidation Processes (AOPs) are attractive alternatives to traditional water treatment and have recently received considerable attention for remediation or removal of MCs. AOPs typically involve the generation of hydroxyl radicals as the oxidizing species responsible for the degradation of pollutants / toxic materials. Previous studies have demonstrated TiO_2/UV (6–8), doped $\text{TiO}_2/\text{sunlight}$ (9), Fenton and photo-Fenton (10), $\text{UV}/\text{H}_2\text{O}_2$ (11) and ultrasonic irradiation (12, 13), can effectively destroy cyanotoxins in aqueous solution and hydroxyl radical is responsible for a significant fraction of the observed degradation. An excellent report on the reactivities of ozone and hydroxyl radical with MC-LR recently appeared (14). While the reactivity of ozone is 3–5 orders of magnitude slower than hydroxyl radical towards MC-LR, the authors provide evidence that ozonation may be one of the best treatment options because of the selectivity of ozone, given hydroxyl radicals are readily scavenged by dissolved organic material. Effective methods for treatment of MCs will be highly dependent on water quality. In order to accurately evaluate the application AOPs for treating water contaminated with MCs, it is critical to determine the reactivity of hydroxyl radical, central to all advanced oxidation treatment processes.

The environmental fate of MCs in natural waters has also attracted considerable attention (15, 16). In surface waters, while biodegradation may be important, abiotic processes, such as photo-transformation and partitioning to sediments may have a greater impact on reducing aqueous concentrations of MCs. Reactive oxygen species, including hydroxyl radicals, can play a critical role in the environmental fate and degradation (17) during solar initiated photooxidation of cyanotoxins in surface waters.

The objective of this study was to determine the kinetic parameters and reaction pathways for the reaction of the hydroxyl radical with MC-LR. Transient spectra observed during the reaction of hydroxyl radical with MC-LR in the time period of 10 – 150 μs after irradiation provide a better understanding of the nature of the intermediate radical species produced. Product studies of the MC-LR degradation using γ -irradiation in aerated solutions were conducted to provide insight into the reaction pathways and mechanisms occurring under hydroxyl radical induced oxidation conditions.

Methods and Materials

Materials

MC-LR was purified from a laboratory culture of *Microcystis aeruginosa* (CCMP299) using the procedure as described previously and the purity was > 98 % confirmed by HPLC (12). Solutions were prepared using water filtered by a Millipore Milli-Q system, which was constantly illuminated by a Xe excimer lamp (172 nm) to keep total organic carbon

concentrations below $13 \mu\text{g L}^{-1}$. All kinetic solutions were sparged with high purity N_2O to remove dissolved oxygen for hydroxyl radical experiments.

Pulse radiolysis and γ -Radiolysis

Electron pulse radiolysis experiments were performed at the Notre Dame Radiation Laboratory with the 8-MeV Titan Beta model TBS-8/16-1S linear accelerator. This irradiation and transient absorption detection system has been described in detail previously (18). Dosimetry (19) was performed using N_2O -saturated, $1.00 \times 10^{-2} \text{ M}$ KSCN solutions at $\lambda = 472 \text{ nm}$, ($G^* \epsilon 5.2 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$) with average doses of 3–5 Gy per 2–3 ns pulse. Throughout this paper, the units of G are $\mu\text{mol J}^{-1}$, and ϵ is in units of $\text{M}^{-1} \text{ cm}^{-1}$. All experimental data were determined by averaging 6 to 9 replicate pulses. The experiments were conducted in static cells with less than 10 % conversion of the starting substrate.

The radiolysis of water is described in Eq 1.

where the numbers in parentheses are the G -values (yields) (20). To study only the reactions of hydroxyl radicals, the solutions were pre-saturated with nitrous oxide (N_2O), which quantitatively converts the hydrated electrons and hydrogen atoms to hydroxyl radicals via the reactions (20):

A Shepherd® 109–86 Cobalt 60 source was used for steady-state γ radiolysis experiments with a dose rate of $0.0151 \text{ kGy min}^{-1}$ as measured by Fricke dosimetry.

LC-MS analysis

The LC-MS system used in the study consisted of an Agilent 1100 HPLC Pump and a Waters LCT Classic Mass Spectrometer with an electrospray ionization source. A sample volume of $10 \mu\text{L}$ was injected onto a Phenomenex Luna C_{18} (2) HPLC column ($2.0 \times 150 \text{ mm}$). The mobile phase was A: 97.8 % H_2O + 2 % CH_3CN + 0.2 % acetic acid and B: 99.8 % CH_3CN + 0.2 % acetic acid. Gradient elution was 2 % of B for 1 min followed by a linear increase to 95 % B at 50 min, and then held constant for an additional 7 min. Mass spectra (m/z 200 – 2000) were obtained in positive ion mode.

Results and Discussion

Transient Absorption Spectroscopy

The reaction of the hydroxyl radical with MC-LR yields a product with the transient absorption spectra shown in Figure 1. The transient lifetime and absorption maxima in the range 300–350 nm are characteristic of hydroxyl radical addition to the benzene ring to form the corresponding hydroxycyclohexadienyl radicals (21, 22). The molar extinction coefficient at 310 nm, $\epsilon_{310} = 2600 \text{ M}^{-1} \text{ cm}^{-1}$, was calculated using a hydroxyl radical initial G -value of 0.59 mol J^{-1} under the experimental conditions, based upon the intraspur scavenging model calculations of LaVerne and Pimblott (23).

Kinetic Measurements

Measurement of the rate constant for the reaction of hydroxyl radical with a specific functional group can be achieved by monitoring the growth kinetics of a characteristic absorption as a function of substrate concentration using the procedures outlined by Mezyk *et. al* (24). The growth kinetics for the MC-LR hydroxycyclohexadienyl adduct were monitored at 310 nm on the μ sec time scale with initial MC-LR concentrations ranging from 250 to 1000 μ M, see insert in Figure 2. The bimolecular radical rate constant for reaction of hydroxyl radical with the benzene ring in the Adda side chain was determined by fitting exponential curves to the pseudo-first-order growth kinetics and plotting these values as a function of the concentrations of MC-LR (Figure 2). The bimolecular rate constant $1.03 (\pm 0.03) \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ was obtained from the slope of the least squares fit line for the data illustrated in Figure 2. Measurements conducted at 270 nm, yields similar kinetic parameters.

The reaction of hydroxyl radical with the aromatic ring in MC-LR only represents one of the possible reaction sites. Additional reaction pathways, include addition to the diene and hydrogen abstraction of aliphatic hydrogens which have rate constants on the order of 10^9 – $10^{10} \text{ M}^{-1}\text{s}^{-1}$ and $10^8 \text{ M}^{-1}\text{s}^{-1}$ respectively. While hydrogen abstraction is typically slower by one or two orders of magnitude the fact that there are > 50 such reaction sites suggest that such reaction pathways may be significant in the reactions of hydroxyl radical with MC-LR. Direct measurement of the rate constant for hydrogen abstraction is not feasible because the resulting carbon centered radical products absorb only weakly at short wavelengths, below the range of our detection system. With this in mind we decided to measure the overall hydroxyl radical reaction rate constant for all reaction pathways using competition kinetics. The hydroxyl radical rate constants for a variety of amino acids have been measured using thiocyanate –SCN[−] competition kinetics. While thiocyanate anion radical may react directly with cysteine and other sulfur atom containing amino acids, the amino acids contained in MC-LR should not be reactive towards the thiocyanate species generated during the determination of hydroxyl radical rate constants. SCN[−] was employed for competition studies since the hydroxyl radical kinetics are well established and the product has a characteristic absorption at 472 nm which will not overlap with any of the absorbance bands of the MC-LR products.

The competition kinetic experiments were conducted by monitoring the SCN[−] hydroxyl radical product (SCN)₂^{•−} at 472 nm under carefully controlled conditions and low product conversion to minimize the potential interference from by-products. As the MC-LR reaction transient does not absorb at this wavelength, this hydroxyl radical competition can be analyzed to give the following analytical expression: Where

$$\frac{[(\text{SCN})_2^{\bullet-}]_0}{[(\text{SCN})_2^{\bullet-}]} = 1 + \frac{k_{\text{MC-LR}+\text{OH}} [\text{MC-LR}]}{k_{\text{SCN}^{\bullet-}+\text{OH}} [\text{SCN}^{\bullet-}]}$$

Where $[(\text{SCN})_2^{\bullet-}]_0$ is the yield $[(\text{SCN})_2^{\bullet-}]$ measured for the standard SCN[−] solution without MC-LR, and $[(\text{SCN})_2^{\bullet-}]$ is the reduced of this transient in the presence of MC-LR. The concentration ratio of MC-LR/SCN[−] was varied from 0.5 to 5.0. A plot of $[(\text{SCN})_2^{\bullet-}]_0/[(\text{SCN})_2^{\bullet-}]$ (directly proportional to $\text{Abs}_0/\text{Abs}_{\text{SCN}^{\bullet-}}$ against the ratio $[\text{MC-LR}]/[\text{SCN}^{\bullet-}]$ gives a straight line with the slope representing the ratio of rate constants $k_{\text{MC-LR}+\text{OH}}/k_{\text{SCN}^{\bullet-}+\text{OH}}$.

Kinetic data obtained at 472 nm are shown in Figure 3a and, as expected, a decrease in the maximum (SCN)₂^{•−} absorption intensity was observed when increasing amounts of MC-LR were added. The transformed plot shown in Figure 3b gives a weighted linear fit. On the

basis of the established rate constant for hydroxyl radical reaction with SCN^- , $k_{\text{SCN}^-+\text{OH}\cdot} = 1.05 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ the rate constant corresponding to the overall rate constant for the reactions of hydroxyl radical with MC-LR was calculated from the slope is $2.3 (\pm 0.1) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.

In a detailed assessment of the reactivities of ozone and hydroxyl radical toward different cyanotoxins, a bimolecular rate constant of $1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ was previously determined for the reaction of hydroxyl radical with MC-LR (14). The difference between these measured rate constants may be due to variations in experimental conditions and methods of determination. The experimental conditions can have a pronounced effect on reactivity, i.e., the solution pH influences conformation and/or charge impacting the reactivity of MC-LR. Our studies were conducted at natural solution pH and the previous studies were performed under phosphate buffered conditions to a solution pH of 7. In our studies, competitive kinetics with pulsed radiolysis and transient absorption measurements were employed, while the previous studies involved competition kinetics and steady state techniques. In our studies, great care was taken to ensure the level of conversions of MC-LR did not exceed 10 % in the measurements of the rate constant. At higher conversions the reaction products can compete for hydroxyl radical leading to lowering the observed rate constants. The differences between the rate constants determined from our study and those obtained previously are likely due to differences in experimental conditions and/or the possibility of competition for hydroxyl radical between the products of MC-LR and MC-LR during γ irradiation (14).

The reactivity of $\cdot\text{OH}$ with different reaction sites present in MC-LR is expected to vary significantly. The addition of hydroxyl radical to unsaturated hydrocarbon systems, such as the benzene and diene moieties present in the Adda side chain is expected to be the fastest among the competing processes and 10–100 times faster than the abstraction of an aliphatic hydrogen atom. With this in mind the reactivity of hydroxyl radical with MC-LR was modeled based on the individual amino acid moieties. MC-LR contains seven amino acids, hence we considered the hydroxyl radical reactivity of each of the amino acids or appropriate surrogates to estimate the overall hydroxyl radical reactivity of MC-LR. While the individual amino acids contain carboxylic acid functional groups, the corresponding amino acid moieties exist as amides in the MC-LR structure. The reaction pathways of the individual amino acids and amides contained in MC-LR are expected to involve hydrogen abstraction as the preliminary reaction step. The rates of hydrogen abstraction can be influenced by electronic effects, but the amide and carboxylic acid functional groups are both strongly electron withdrawing and thus expected to have analogous inductive effects. In general, inductive effects only occur over short distances and are only expected to influence the reactivity of hydrogens within a few bond lengths of functional group. The hydroxyl radical rate constants for alanine, leucine, arginine and aspartic acid are available in the literature (25, 26). While the rate constant for iso-glutamic acid was not available, the rate constant for glutamic acid (27) should be essentially the same assuming hydrogen abstraction is the predominant reaction pathway (for comparison of the structures see Figure 1S, supporting information). The hydroxyl radical rate constants for acrylic acid and acrylamide are similar at neutral pH (20). Based on this similarity and previous studies of Structure-Activity Relationship (SAR) of hydroxyl radical reaction rates (28), the rate constant for acrylic acid (29) was used for Mdha based on structural similarities (see Figure 1S, supporting information). To estimate the hydroxyl radical reactivity of the Adda amino acid, second-order rate constants for reactions with benzene and butadiene were employed. The rate constants for the individual amino acids and surrogates are summarized in Table 1. Summation of the rate constants for the individual components yields an overall rate constant of $2.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, a slightly lower value ($\sim 10\%$) than observed in our experimental result. While the small difference ($\sim 10\%$) is within experimental error, the

fact that the hydrogen abstraction pathways for the Adda side chain were not included in the summation may also contribute to the difference. We understand estimation of hydroxyl rate constants using summations will have limitations for diffusion-controlled processes and in some cases may lead to an over estimation of the rate constants. For large molecules the reaction rates can be influenced by size (reactive cross section), leading to rate constant at/or above the diffusion controlled limit. Structure-reactivity relationships, steric hindrance and conformational factors may also influence the reactivity of the individual components compared to a large complex system. Regardless of these limitations, it should still be possible to reasonably predict the partitioning of the hydroxyl radical reactions by comparing the individual rate constants for each amino acid site, summarized in table 1.

The least reactive amino acids are alanine, glutamic and aspartic acids which account for <0.5 % (0.01/2.1), 1 % (0.023/2.1), <0.5 % (0.0075/2.1) respectively based on the magnitude of the individual hydroxyl radical rate constants. Leucine, arginine and acrylic acid account for 8 % (0.17/2.1), 17 % (0.35/2.1) and 7 % (0.15/2.1) respectively of the expected reaction pathways. The benzene and butadiene moieties for Adda are predicted to account for 67 % (1.4/2.1) of the reaction pathways excluding the possible H-abstraction pathways on the Adda side chain. Clearly these results indicate the reaction of hydroxyl radical at the Adda is the most reactive amino acid in MC-LR.

Transformation pathways for the reaction of MC-LR with •OH

In addition to the reaction kinetics for hydroxyl radical with MC-LR, product studies were carried out. Hydroxyl radicals were generated using ^{60}Co steady-state radiolysis and the resulting products characterized by LC-MS. The experiments were conducted using air-saturated 0.10 mM solutions of MC-LR. In the presence of air, the hydrated electrons and hydrogen atoms produced in the radiolysis react with dissolved oxygen, to produce the superoxide anion radical which is much less reactive than hydroxyl radical. Under such conditions, the chemistry is dominated by the hydroxyl radical reactions. MC-LR was readily degraded during γ -irradiation at an absorbed dose of 1.80 kGy. The degradation of the MC-LR follows exponential decay kinetics, with a half-life of 0.832 kGy.

Analyses by LC-MS at several different irradiation doses revealed a number of decomposition products at detectable levels. Our structural assignments of the breakdown products of MC-LR from γ -irradiation were based on the analysis of the Total Ion Chromatogram (TIC) and the corresponding mass spectra. The masses of the different products were determined from the peaks corresponding to the protonated molecule, $[\text{M} + \text{H}]^+$. For the purpose of this paper, we will refer to the products by molecular weight (MW).

Several products with MW of 1011 were observed, corresponding to the addition of 16 mass units to MC-LR (see Figure 2S, supporting information). This is consistent with hydroxylation of the aromatic ring and such products have been reported for advanced oxidation of MC-LR (13, 30). Addition of the hydroxyl radical to the alkyl substituted benzene ring of the Adda amino acid section can occur at the *ortho*, *para*, *meta* and *ipso* positions. Attack at the *ipso* position is not expected to be an observed reaction pathway, since the radical intermediate cannot re-aromatize by simple loss of a hydrogen atom. An alkyl chain attached to the benzene ring normally directs electrophilic substitution to the *ortho* and *para* positions. Hydroxyl radical is an electrophile but because of its strong radical reactivity formation of *ortho*, *meta*, and *para* products are observed. The structures are illustrated in Figure 4.

The other major products have MW of 1029 corresponding to the addition of 34 mass units (see Figure 3S, supporting information). Analogous products were observed in product

studies of TiO₂ photocatalytic and ultrasonically induced oxidation of MC-LR (6, 13). The products with the MW of 1029 are proposed to be the result of hydroxyl radical attack at the diene leading to the formation of isomeric diols. The addition of hydroxyl radical to the diene will yield allylic radicals, which absorb weakly at wavelengths below 250 nm (31) and thus are difficult to monitor. Subsequent reactions of the allylic radicals can lead to stereoisomeric forms of 1, 2-, 3, 4- and 1, 4-dihydroxylated adducts of MC-LR as shown in Figure 5.

While the reactions of hydroxyl radical with MC-LR involves competing reaction pathways, our product studies and kinetic evaluation clearly indicate reaction Adda amino acid is the major reaction site. The transient absorbance measurements and kinetic studies further indicate hydroxyl radical attacks the benzene ring and diene of Adda side chain as the major degradation pathways accounting for ~ 67 % of the products. Since nodularin and microcystins variants possess the reactive Adda moiety, all these toxins should be readily degraded by hydroxyl radical. The Adda side chain is also critical to the toxicity of these compounds and oxidative degradation has been shown to dramatically reduce or eliminate the biological activity or toxicity of these compounds (32–34).

Implications—The primary requirement for treatment of waters subjected to harmful algal blooms should be to remove intact cells using coagulation, membrane filtration, and/or flotation. Subsequent treatment of the extracellular toxins will be dependent on the water quality and treatment objectives. Our kinetic and mechanistic data provide a quantitative foundation for the initial evaluation of AOP efficiency in removing MC-LR from real-world waters, which can contain high levels of dissolved natural organic matter (NOM) and other hydroxyl radical scavengers. For example, in an aerated water containing 1.0 ppm NOM (82.9 μM NOM assuming 12 g C per mole C), and 10 ppb MC-LR (10 nM) at pH 8.0 and a typical alkalinity of 100 mg L⁻¹ (as CaCO₃, corresponding to ~1.0 mM HCO₃⁻), the hydrated electrons produced will be quantitatively scavenged by dissolved oxygen, and the hydroxyl radical reaction will be partitioned according to: (35, 36)

(20) Based on the relative rates of these three reactions under given conditions, the fraction of hydroxyl radical available to degrade MC-LR (reaction (5)) is calculated to be only 0.89 %. Although these calculated reaction efficiencies are low, they would be expected to remain relatively constant under AOP conditions, and thus provide an estimate for the extent of treatment that would be required to achieve any significant improvement. While the oxidation rates of MC-LR by hydroxyl radical are several orders of magnitude faster than ozone, the selective reactivity of ozone is advantageous for treatment of waters with high levels of NOM (14).

The hydroxyl radical induced degradation of MC-LR occurs predominately via oxidation of the Adda chain, which results in the loss of biological activity. All MC variants and nodularin possess the Adda moiety and thus should be readily destroyed via hydroxyl radical mediated oxidation. The estimation of the hydroxyl radical reactivity towards MC-LR is correctly modeled using the individual reactivities of the specific functional groups and amino acids. The summation of individual kinetic contributions may provide a simple

method for predicting the hydroxyl radical reactivity for different MCs (> 80 variants) and used to effectively determine the partitioning of hydroxyl radical reactions among the different amino acid components. While extension of this type of kinetic evaluation would be useful to assess hydroxyl radical destruction of peptides and proteins, an array of polypeptide substrates must be investigated to determine its general applicability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the reviewers for valuable suggestions and insights. Work performed at the Radiation Laboratory, University of Notre Dame, which is supported by the Office of Basic Energy Sciences, U.S. Department of Energy. This research was funded in part by the U.S. Environmental Protection Agency (RD-83322301). K.E.O. gratefully acknowledges support from the NIH/NIEHS (ARCH S11ESO11181). T. X. is supported by a Presidential Dissertation Fellowship from the University Graduate School at FIU. This is contribution 29 from Urban Water Research Center, University of California, Irvine.

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FIGURE 1. Time-resolved absorption spectra obtained from pulse radiolysis of N_2O saturated solution of MC-LR. The initial concentration of MC-LR is 0.25 mM.

FIGURE 2. Second-order rate constant determination for the reaction of OH radical with MC-LR monitoring growth at 310 nm. The error bars indicate the standard deviation of the mean.

FIGURE 3.

Thiocyanate competition kinetics for determination of hydroxyl radical reaction rate constant with MC-LR. (a). Kinetics of (SCN)•₂ formation at 472 nm for N₂O saturated 100 μM KSCN solution containing 0 (□), 50 (°), 100 (Δ), 250 (▽) and 500 (◇) μM MC-LR at room temperature. (b) Competition kinetics plot for hydroxyl radical reaction with MC-LR using SCN⁻ as a standard. Solid line is a weighted linear fit, with a slope of 2.2 ± 0.1. This gives a second-order rate constant for MC-LR reaction as 2.3 (± 0.1) × 10¹⁰.

FIGURE 4.
Proposed reaction products for hydroxyl radical reaction with benzene group presented in the MC-LR.

FIGURE 5.
Proposed reaction products for hydroxyl radical reaction with diene group presented in the MC-LR.

Table 1

Measured Second-Order Rate Constants for Reaction of Model Compounds with OH.

	•OH rate constants	Partition %[*]	reference
Alanine	1.1×10^8	< 0.5 %	(25)
Glutamic acid	2.3×10^8	1 %	(27)
Leucine	1.7×10^9	8 %	(26)
Arginine	3.5×10^9	17 %	(26)
Aspartic acid	7.5×10^7	< 0.5 %	(26)
Benzene	7.0×10^9	33 %	(20)
Butadiene	7.0×10^9	33 %	(20)
Acrylic acid	1.5×10^9	7 %	(29)
Rate constant based amino acid reactivities	2.1×10^{10}	100 %	

^{*} Based amino acid reactivities to overall bimolecular rate constant