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Iron Porphyrin and Mercaptojuglone Mediated Reduction of Polyhalogenated Methanes and Ethanes in Homogeneous Aqueous Solution

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Reduction rate constants of seventeen polyhalogenated methanes and ethanes were measured in aqueous solutions containing bulk reductants and the electron-transfer mediators iron porphyrin or juglone (5-hydroxy-1,4-naphthoquinone). Rate constants varied 4 orders of magnitude in the case of reaction with iron porphyrin and 7 orders of magnitude in the reaction with mercaptojuglone, an addition product of juglone and hydrogen sulfide. For the iron porphyrin system the results support a reaction mechanism in which one electron is transferred in an outer-sphere process to the polyhalogenated alkane with bond breakage in the transition state. In the juglone system two competing reaction mechanisms are proposed: an outer-sphere electron transfer to the polyhalogenated alkane and an S_N2 reaction at the halogen. The kinetic results are directly applicable to environmental systems containing common reductants such as natural organic matter and reduced iron species, providing a "fingerprint" of reactivity for examination of reactive species and rate-limiting steps in those systems.

Introduction

Because of their widespread use as solvents, aerosol propellants, refrigerants, blowing agents, pesticides, etc., polyhalogenated C_1 and C_2 alkanes (PHAs) represent an important class of environmental pollutants. In reducing environments, as is encountered, for example, at waste disposal sites, PHAs may undergo reductive dehalogenation reactions both by abiotic as well as by microbially catalyzed processes, yielding a variety of products that may or may not be of environmental concern (*1−10*). Presently, there is considerable interest in reductive dehalogenation reactions because of their potential applicability in the treatment of PHA wastes (*11, 12*), as well as in remediation approaches to removing PHAs from contaminated soils and aquifers (*13, 14*). In this context, the reductive transformation of selected PHAs has been investigated by several groups using a variety of model reductants including reduced organic and inorganic sulfur species (*15−19*), biologically relevant transition-metal complexes (predominantly iron (*20−24*) and cobalt species (*25−31*)), and zero-valent metals, in particular, iron metal (*12, 32−35*). Generally, these studies focused on the evaluation of the kinetics and/or product identification of the reduction of a few compounds. Therefore, the available set of kinetic data, as well as the fundamental understanding of the reaction steps that limit the rate of PHA reduction by environmentally relevant reductants, is presently still quite limited. Such data and knowledge are, however, pivotal for a quantitative assessment of the fate of PHAs in natural systems, and, particularly, for the design of optimal remediation schemes. In addition, as has been demonstrated with nitroaromatic compounds, knowledge of the relative reduction rates of a larger number of structurally related compounds with various model reductants may allow one to identify rate-determining steps, and possibly the relevant electron donor(s), for the reaction of the compounds in more complex systems (*36−40*).

In this paper, we present and discuss the reduction rates of a series of polyhalogenated methanes and ethanes by two electron-transfer mediators, an iron porphyrin and a naturally occurring quinone (i.e., juglone). As demonstrated in earlier studies with nitroaromatic compounds (*40*), these mediators, which may transfer electrons from a bulk reductant (i.e., cysteine or hydrogen sulfide, respectively) to an organic pollutant, exhibit reaction characteristics that are representative of other important iron species and organic matter constituents, respectively, in the environment. With respect to the latter case, we have postulated that in the juglone−hydrogen sulfide system, a reactive addition product referred to as mercaptojuglone is formed that, in addition to being a potent reductant, can, in contrast to the iron porphyrin, also be expected to be a strong nucleophile (*19, 41*). This is important for the reaction with PHAs, because these compounds are also susceptible to nucleophilic attack (*18, 42−44*). Possible reaction mechanisms are discussed by comparing results from the two systems with one another and in light of earlier work on electron-transfer reactions. For example, rate constants measured here are compared with rate constants for reaction of PHAs with $Co^{(II)}W_{12}O_{40}^{7-}$, a known outer-sphere one-electron reductant (*45, 46*). Thus, the results of this study provide insight into the reactivities of the PHAs with two environmentally relevant reductants that can be expected to exhibit very different reaction characteristics.

Experimental Section

Chemicals. All chemicals were used as received. The purities were 98% or greater except where indicated. Bromodichloromethane, chlorodibromomethane, 3-chlorofluorobenzene, dibromodichloromethane (95% purity), fluorotribromomethane, fluorotrichloromethane, 2-iodobenzotrifluoride, tetrabromomethane, 1,1-difluorotetrachloroethane, hexachloroethane, pentachloroethane (96% purity), 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, tribromomethane, 1,1,1-trichloroethane, 1,2-diiodoethane, and 1,2-dibromoethane were purchased from Aldrich. Bromotrichloromethane, tetrachloromethane, 1,1,1- and 1,1,2-trichlorotrifluoroethane, 1,1,2,2-tetrabromoethane, 1,2-dibromotetrachloroethane, tetrachloroethene, 2-morpholino-ethanesulfonic acid (MES, pH 6 buffer), sodium dihydrogen phosphate dihydrate (pH

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7), 2-[4-(2-hydroxyethyl)-1-perazinyl]-ethanesulfonic acid (HEPES, pH 7), 4-(2-hydroxyethyl)-piperazine-1-propanesulfonic acid (HEPPS, pH 8), N-[tris(hydroxymethyl) methyl]-3-aminopropanesulfonic acid (TAPS, pH 8.7), 2-(cyclohexylamino)-ethanesulfonic acid (CHES, pH 9.3), glycine (pH 10), L-cysteine, 5-hydroxy-1,4-naphthoquinone (juglone), sodium phosphate, sodium sulfate, potassium superoxide, and sodium sulfide were from Fluka. Methanol (nanograde), chloroform (high purity), pentane (high purity), and hexane (high purity) were obtained from Burdick & Jackson. The iron porphyrin was synthesized (according to ref *47*) from iron(II)chloride and mesotetrakis (*N*-methyl-pyridyl) porphin, which were both purchased from Fluka.

Model Systems. Two different model systems were employed in this work. In one, a water-soluble iron porphyrin (meso-tetrakis (*N*-methylpyridyl) iron porphin) was used as an electron-transfer mediator in the presence of 5 mM cysteine, which served as bulk electron donor. The initial concentration of the iron porphyrin varied between 2 and 50 μM depending on the PHA investigated. If not stated otherwise, the initial PHA concentration was always ~1 μM. The pH was controlled using an appropriate 50 mM pH buffer (see chemicals). For the pH 7 experiments, sodium phosphate (50 mM) was used. From UV−vis spectroscopic measurements carried out on a Hitachi U 2000 spectrophotometer, the iron porphyrin was immediately reduced after addition of cysteine. The other model system consisted of 200 μM juglone as the electron-transfer mediator and 1 mM hydrogen sulfide as the bulk reductant. The pH was controlled with 50 mM HEPES (pH 7) or 61 mM glycine (pH 10) buffer. The initial PHA concentration was always ~1 μM. A detailed description of this model system, particularly with respect to the reactive species, that is, mercaptojuglone, is given elsewhere (*19*).

Experimental Procedures. Sample Preparation. Standard assays consisted of pH and redox buffers and a known amount of electron-transfer mediator. The glassware, pH buffer solutions, and distilled water for the preparation of redox buffers were autoclaved at 121 °C for 20 min. The buffers, which were still hot from autoclaving and nonautoclaved methanol (for spike solutions), were made oxygen-free by purging with argon gas (99.999%; used without further purification) for at least 3 h. The solutions were then transferred directly into a glovebox containing a 95/5 vol % mixture of N_2/H_2 and a palladium catalyst. After filling the autoclaved 57-mL serum flasks with pH buffer, redox buffer, and solutions of the electron-transfer mediator, they were sealed with Viton rubber stoppers and aluminum crimp caps. The order of addition for the experiments was the following: (1) buffer, (2) acid, (3) cysteine or sodium sulfide, and (4a) juglone solution (0.02 M juglone in O_2-saturated methanol) or (4b) porphyrin solution (~0.01 M in O_2-free water). The preparation of blank assays followed the same procedure without the addition of electron-transfer mediator solution. All standard assays were then placed in a dark, 25 °C water bath, where they were kept for the kinetic experiments. The samples were allowed to equilibrate in the water bath for at least 12 h before the kinetic experiments were begun.

Spike solutions were prepared in methanol in order to facilitate dissolution of polyhalogenated organic substrate into the aqueous solution. The spike solutions consisted of methanol, 1 mM organic substrate, and a variable amount of internal standard (0.1 or 0.25 M 2-iodobenzotrifluoride or 3-chlorofluorobenzene; note that 2-iodobenzotrifluoride was found to disappear in the solutions and was not used in final determinations of analyte concentration), according to the hexane(pentane)−water extraction ratio for sample workup (see below). For the iron porphyrin experiments, the solutions were prepared in the glovebox with oxygen-free methanol, whereas the solutions containing juglone were prepared with oxygen-saturated methanol. The saturation concentration of oxygen in methanol is 9.81×10^{-3} M (1 bar O_2, 25 °C; ref 48). The final total concentrations of methanol in solutions containing iron porphyrin or juglone were 0.025 and ≤0.5 M, respectively. Methanol concentrations of 0.25 M in the iron porphyrin system resulted in broken kinetics (reaction order 0.6 with respect to CCl_4 disappearance). However, the possible involvement of oxygen in radical chain reactions at concentrations greater than 2 μM in the presence of 0.25 M methanol was not eliminated (*44*, p 79). In the juglone system, the methanol did not appear to influence PHA disappearance kinetics under any conditions but may influence the yield of the mercaptojuglone species. The effect of methanol concentration on mercaptojuglone yield is currently under investigation (*41*).

Kinetic Experiments. A kinetic experiment was initialized by injecting either 57 μL of spike solution through the Viton stopper with a 100-μL glass syringe into samples containing iron porphyrin, or 570 μL with a 1-mL syringe into samples containing juglone. Sample volumes were 250 μL and were compensated with N_2 gas (99.995%, used without further purification) prior to sampling. Transfer into a 3-mL glass vial with screw cap containing an aliquot of hexane (pentane) was followed by extraction for 30 s with a vortex mixer. Hexane−water extraction ratios were typically 2:1, except for bromodichloromethane and hexachloroethane, for which extraction ratios of 5:1 and 10:1, respectively, were used. Extraction efficiencies were greater than 90% for halogenated methanes and greater than 88% for the halogenated ethanes (*44*). The hexane (pentane) was subsequently transferred to autosampler vials containing a small amount of sodium sulfate to dry the hexane (pentane). These vials were sealed with Teflon-lined septa and aluminum crimp-caps. To minimize volatilization losses, the autosampler vials were placed in a tray through which cold water was circulated prior to measurement by gas chromatography. Calibration solutions in hexane were prepared once a week from 0.1 M stock solutions in hexane (pentane), which were re-prepared monthly. Methanol-based stock and spike solutions were stable for one week. Samples were taken over three half-lives, except for fluorotrichloromethane and the blanks, which reacted very slowly and were analyzed over one half-life at most.

The volume of the headspace increased in the serum flasks with each sample taken. Aqueous concentrations were corrected for the amount of solute that partitioned into the headspace according to the following equation:

$$[\mathrm{RX}]_{\mathrm{corr}} = [\mathrm{RX}]_{\mathrm{measured}} + \frac{[\mathrm{RX}]_{\mathrm{measured}} K_{\mathrm{H}} V_{n(\mathrm{gas})}}{V_{n(\mathrm{liquid})} RT} \qquad (1)$$

where $[\mathrm{RX}]_{\mathrm{corr}}$ is the corrected aqueous concentration of a halogenated alkane RX (mol L^{-1}), $[\mathrm{RX}]_{\mathrm{measured}}$ is the measured aqueous concentration of RX (mol L^{-1}), K_{H} is the Henry's law constant of the halogenated alkane (atm L mol^{-1}), $V_{n(\mathrm{gas})} = V_{\mathrm{o(gas)}} + nV_{\mathrm{sample}}$, $V_{n(\mathrm{liquid})} = V_{\mathrm{o(liquid)}} - nV_{\mathrm{sample}}$, R is the ideal gas constant (0.082058 L atm mol^{-1} K^{-1}), n is the sample number, V_{sample} is the sample volume (~250 μL), and T is absolute temperature. An analysis of eq 1 revealed that Henry's law constants of 18 atm L mol^{-1} or lower caused a change of less than 2% in the rate constant as computed below. Compounds having K_{H} values greater than 18 atm L mol^{-1} included fluorotrichloromethane ($K_{\mathrm{H}} = 101$ atm L mol^{-1}; ref *49*) and 1,1,1- and 1,1,2-trichlorotrifluoroethane ($K_{\mathrm{H}} = 88$ atm L mol^{-1}). The Henry's law constants for the trichlorotrifluoroethanes have not been measured, so the values were estimated from the contribution of each C−X bond according to the method of Hine and Mookerjee (*50*).

Analyses. Gas chromatography (GC) was carried out on either a Carlo Erba HRGC 5160 or a Fisions Instruments GC

8165, both of which were equipped with autosamplers and Cryo 520 CO_2 cooling systems. The injection volume was 1.5 μL. Two types of fused silica columns were used, either a 30-m DB-624 (i.d. 320 μm, 0.25 μm film thickness) or a 30-m DB-5.625 (i.d. 320 μm, 1.8 μm film thickness; both from J&W Scientific). The injector and detector temperatures were 200 and 300 °C, respectively. The carrier gas was H_2 at a flowrate of 1.5 mL min^{-1}. The detector used was an electron capture detector (ECD), Carlo Erba ECD 400, with a ^{63}Ni source. The makeup gas for the ECD was a mixture of 90% Ar and 10% CH_4. Injection was splitless.

Derivation of Rate Constants. All experiments including blanks were performed in 3 or 4 replicates. In general, pseudo-first-order kinetics were observed, which is not surprising since, except for a few cases, the electron-transfer mediator was present in large excess. Thus, pseudo-first-order rate constants, k_{obs}, were obtained from a linear regression of ln [PHA] versus time:

$$\ln[\text{PHA}] = -k_{\text{obs}}t + \ln[\text{PHA}]_{\text{o}} \tag{2}$$

where [PHA] and $[\text{PHA}]_{\text{o}}$ are the concentrations of the polyhalogenated alkanes at times t and 0, respectively. To obtain rate constants reflecting the reaction of the PHA with the electron-transfer mediator alone, observed rate constants from samples containing no mediator, $k_{\text{obs,blank}}$, were subtracted from k_{obs} values obtained in the presence of the mediator:

$$k_{\text{obs}}' = k_{\text{obs}} - k_{\text{obs,blank}} \tag{3}$$

For selected model compounds, k_{obs} increased linearly with iron porphyrin concentrations between 2 and 50 μM (*51*). Therefore, for all compounds, a second-order rate constant, k_{FeP}, was calculated by dividing k_{obs}' by the initial total iron porphyrin concentrations at which k_{obs} had been determined:

$$k_{\text{FeP}} = \frac{k_{\text{obs}}'}{[\text{FeP}]} \tag{4}$$

To determine the rate constants for the disappearance of 1,1,2-trichloro-1,2,2-trifluoroethane, 1,1,1,2-tetrachloroethane and 1,1,1-trichloro-2,2,2-trifluoroethane, and fluortrichloromethane in the juglone system, pH was buffered to 10 in order to speed up the reaction. Assuming similar reaction mechanisms for all of the polyhalogenated ethanes, the following formula was applied to allow comparison of these rate constants with rate constants of the other compounds measured at pH 7:

$$k_{\text{obs,i,pH7}}' = k_{\text{obs,i,pH10}}' \frac{k_{\text{obs,HCE,pH7}}'}{k_{\text{obs,HCE,pH10}}'} \tag{5}$$

This formula should accurately predict a pH correction for substrates which react according to a rate-limiting electron-transfer step and which do not contain acidic protons that can induce elimination reactions. The correction factor from pH 10 to pH 7 turned out to be 0.017 (*19*).

As discussed in detail elsewhere (*19*), k_{obs} did not increase linearly with total juglone concentration, and the exact concentration of the reactive species, that is, mercaptojuglone, could not be determined exactly. Under the conditions of the kinetic experiments with juglone, the estimated concentration of the mercaptojuglone was 4 μM (*19*). An operationally defined second-order rate constant, k_{JUG}, was thus calculated by assuming that the concentration of the reactive species was 4 μM.

$$k_{\text{JUG}} = \frac{k_{\text{obs,pH7}}'}{4 \times 10^{-6}\ \text{M}} \tag{6}$$

Note that k_{JUG} can, therefore, only be used to evaluate the relative reactivities of the various PHAs.

Determination of Activation Energies. Activation energies were determined by measuring the rate constants for a given compound at constant temperature (25−65 °C) in the presence and absence of the mediator. A regression of the natural logarithm of k_{obs}' plotted versus T^{-1} (K^{-1}), gave a slope which, when multiplied by the ideal gas constant R, resulted in the reported activation energies, E_{a}.

Results and Discussion

Second-order rate constants for the disappearance of polyhalogenated methanes and ethanes in the iron porpyhrin- and juglone-mediated systems are presented in Tables 1 and 2. The reaction rate constants in the juglone system span a range of 7 orders of magnitude, whereas in the iron porphyrin system the rate constants span a range of 4 orders of magnitude only. A plot of log k_{JUG} versus log k_{FeP} (Figure 1) shows a slope much greater than 1 for the polyhalogenated methanes and ethanes. Such a slope can be interpreted in the sense that different reaction mechanisms are involved in the transformation of the substrates. This interpretation is further supported by product analyses (*52*). Apparently, the juglone system is more sensitive toward the structure of the substrate than the iron porphyrin system. A closer look at each system is needed in order to get a mechanistic picture of the transformation reactions.

Reaction with the Iron Porphyrin. First-order rate constants for the disappearance of hexachloroethane and tetrachloromethane responded similarly to variations in pH, cysteine concentration, and phosphate concentration (phosphate was used as buffer) in the iron porphyrin system. Variation of these parameters caused less than a factor of 5 difference in the rate constants (Figure 2). The primary transformation product of the hexachloroethane was 90% ± 19% perchloroethylene, and the primary product of the tetrachloromethane transformation was *N*-formylcysteine (*52*). Despite these differing end products, the similarity in the responses to changes in pH and phosphate and cysteine concentrations strongly suggests that the first step in the transformation of the C_1 and the C_2 PHAs is the same in the iron porphyrin system.

A variety of mechanisms have been proposed in the literature for the reduction of PHAs by reduced iron species, including reaction pathways in which the first step is the formation of a PHA−iron complex (*53, 54*). The iron porphyrin system used in this study contains cysteine as bulk reductant. Cysteine also plays a role as an axial ligand of the iron in the iron porphyrin. UV−vis spectra showed a shift of the Soret band ($\lambda_{\text{max}} = 420$ nm) of 23 nm to higher wavelengths when cysteine was added to an oxygen-free iron (III) porphyrin solution (data not shown). Moreover, titrating cysteine into an iron porphyrin solution increased the half-wave potential by 0.04 V/log [cysteine] for cysteine−iron porphyrin ratios of 1−8 (*55*), which implies that one axial position on the iron is occupied by cysteine already at very low cysteine−iron porphyrin ratios (*56*). Because cysteine is present in large excess relative to iron porphyrin (ca. 10^3), two axial cysteine ligands are assumed, and presumably they prevent the PHAs from forming bonds with the iron center of the porphyrin. This thinking is supported by the observation that, in contrast to work reported by other authors (*57*), no shift of the Soret band was observed after addition of a large excess of a PHA (i.e., tetrachloromethane, hexachloroethane) to a porphyrin solution containing the same cysteine−iron porphyrin ratios used in the kinetic measurements (*55*).

TABLE 1. Second-Order Rate Constants for the Reaction of the Halogenated Methanes with Iron Porphyrin (k_{FeP}), Mercaptojuglone (k_{JUG}), and $Co^{(II)}W_{12}O_{40}{}^{7-}$ (k_{CoW}) at 20 °C[a]

compound name	formula	k_{FeP}[b] (M^{-1} s^{-1})	k_{JUG}[c] (M^{-1} s^{-1})	k_{CoW}[d] (M^{-1} s^{-1})
chloroform	$CHCl_3$	nmr[e]	nd	
fluorotrichloromethane	$CFCl_3$	$(1.0 \pm 0.1) \times 10^{-1}$	$(1.4 \pm 0.3) \times 10^{-4}$ [f]	
bromodichloromethane	$CHBrCl_2$	$(5.8 \pm 0.9) \times 10^{0}$	$(9.4 \pm 5.3) \times 10^{-2}$	
tetrachloromethane	CCl_4	$(5.9 \pm 0.9) \times 10^{0}$	$(9.5 \pm 0.3) \times 10^{-1}$	2.9×10^{-4} [g]
tribromomethane	$CHBr_3$	$(8.9 \pm 2.1) \times 10^{0}$	$(1.6 \pm 0.2) \times 10^{0}$	6.8×10^{-4} [g]
chlorodibromomethane	$CHBr_2Cl$	$(1.2 \pm 0.3) \times 10^{1}$	$(1.2 \pm 0.7) \times 10^{-1}$	
fluorotribromomethane	$CFBr_3$	$(6.9 \pm 2.8) \times 10^{1}$	$(1.6 \pm 0.5) \times 10^{2}$	
bromotrichloromethane	$CBrCl_3$	$(2.5 \pm 0.6) \times 10^{2}$	$(8.0 \pm 0.1) \times 10^{2}$	1.5×10^{-2}
dibromodichloromethane	CBr_2Cl_2	$(6.1 \pm 2.2) \times 10^{2}$	$(1.6 \pm 1.4) \times 10^{3}$	5.3×10^{-2}

[a] For k_{FeP} and k_{JUG}, the ±95% CI are given. [b] At pH 7.0, 50 mM phosphate buffer, 5 mM cysteine, 25 °C. [c] Apparent second-order rate constant (pH 7.0, 50 mM HEPES buffer, 200 μM juglone, 1 mM Na_2S, 25 °C) calculated by dividing the observed pseudo-first-order rate constant by the estimated mercaptojuglone concentration (4 μM). [d] Reaction with $Co^{(II)}W_{12}O_{40}{}^{7-}$; data from Eberson and Ekström (*46*). [e] nmr = no measurable rate. There was no change in concentration measured in 8 weeks; nd = not determined. [f] Extrapolated from measurements at pH 10.0. [g] Corrected from the value measured at 50 °C to 20 °C by multiplying by the ratio of rate constants measured for CBr_4 by the authors ($k_{CBr_4}(20\ °C)/k_{CBr_4}(50\ °C) = 0.1$).

TABLE 2. Second-Order Rate Constants (±95% CI) for the Reaction of the Halogenated Ethanes with Iron Porphyrin (k_{FeP}) and Mercaptojuglone (k_{JUG}) at 25 °C

compound name	formula	k_{FeP}[a] (M^{-1} s^{-1})	k_{JUG}[b] (M^{-1} s^{-1})
1,1,2,2-tetrachloroethane	$CHCl_2-CHCl_2$	$(1.0 \pm 0.25) \times 10^{-2}$	nd
1,1,1-trichloroethane	CCl_3-CH_3	$(3.2 \pm 0.24) \times 10^{-2}$	nd
1,1,2-trichlorotrifluoroethane	$CCl_2F-CClF_2$	$(3.8 \pm 0.6) \times 10^{-2}$	nmr[c,d]
1,1,1,2-tetrachloroethane	CCl_3-CClH_2	$(1.2 \pm 0.5) \times 10^{0}$	$(2.0 \pm 0.10) \times 10^{-3}$ [c]
1,1,1-trichlorotrifluoroethane	CCl_3-CF_3	$(8.6 \pm 3.3) \times 10^{0}$	$(2.2 \pm 0.30) \times 10^{-2}$ [c]
pentachloroethane	$CHCl_2-CCl_3$	$(9.5 \pm 2.8) \times 10^{0}$	nmr[e]
1,1-difluorotetrachloroethane	$CF_2Cl-CCl_3$	$(1.3 \pm 0.3) \times 10^{1}$	$(4.0 \pm 1.7) \times 10^{-2}$
hexachloroethane	CCl_3-CCl_3	$(4.8 \pm 0.4) \times 10^{1}$	$(5.5 \pm 0.30) \times 10^{-1}$
1,2-diiodoethane	CH_2I-CH_2I	nd	$(6.0 \pm 0.78) \times 10^{1}$

[a] At pH 7.0, 50 mM phosphate buffer, 5 mM cysteine. [b] Apparent second-order rate constant (pH 7.0, 50 mM HEPES buffer, 200 μM juglone, 1 mM Na_2S) calculated by dividing the observed pseudo-first-order rate constant by the estimated mercaptojuglone concentration (4 μM); nd = not determined. [c] Extrapolated from measurements at pH 10.0. [d] nmr = no measurable rate, non-first-order disappearance identical to that observed in 61 mM glycine buffer. [e] Same rate measured in the presence and absence of mediator.

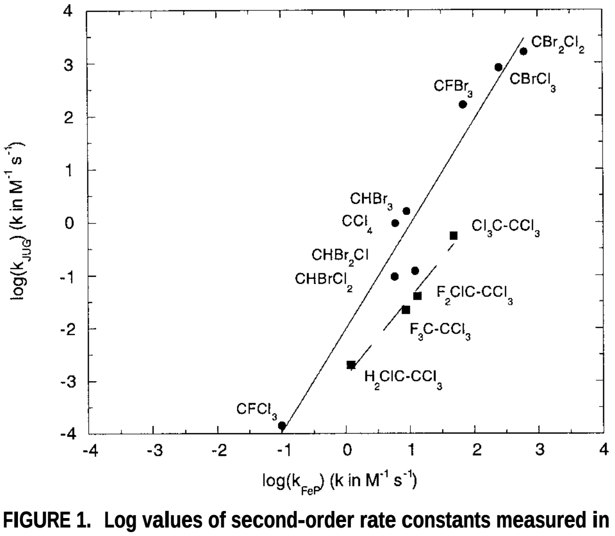
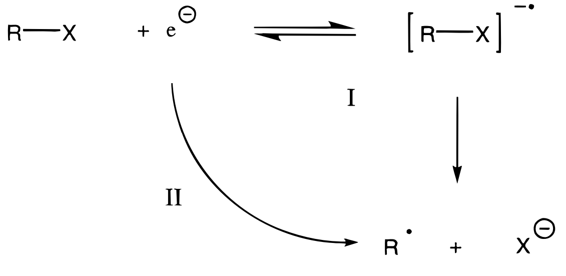


FIGURE 1. Log values of second-order rate constants measured in the iron porphyrin system; log(k_{FeP}) versus log(k_{JUG}) values measured in the mercaptojuglone system for the polyhalogenated methanes and ethanes.

An outer-sphere electron transfer from the iron porphyrin to the PHAs is further supported by a linear correlation between the logarithms of the second-order rate constants for the reaction of four polyhalogenated methanes (log k_{FeP}) with $Co^{(II)}W_{12}O_{40}{}^{7-}$ (log k_{CoW7-}; Figure 3; ref. *45*). The $Co^{(II)}W_{12}O_{40}{}^{7-}$ complex is a one-electron reductant that can undergo outer-sphere electron transfer reactions only because the Co(II) ion is centered in an oxo-tungsten covering where bond formation between the substrate and the Co(II) center is absolutely impossible (*46*). The slope of the linear regression through the points is approximately 1, suggesting that in this case the iron porphyrin acts as an outer-sphere reductant.

The kinetics of reduction of the PHAs by iron(II) porphyrin suggest a reaction mechanism which is initiated by an outer-sphere one-electron transfer (eq 7) followed by path I or concomitant with path II dissociation of the weakest carbon−halogen bond.



(7)

Eberson and Eckström (*46*) detected radical intermediates in their solutions. Together with the slope of 1 in Figure 1, the results support a dissociative electron-transfer mechanism (eq 7). One would therefore suppose that if dissociative electron transfer were the rate-limiting step, the activation energy (E_a) of this process would be higher than that of pure electron transfer, because a bond must be broken in the transition state. The E_a of typical outer-sphere electron transfer processes is approximately 40 kJ mol^{-1} (*58*). Activation energies were determined in our systems for two PHAs (CCl_4 and C_2Cl_6; Table 3). As expected, they are higher than 40 kJ mol^{-1} but significantly lower than activation energies for typical S_N2 reactions at carbon (80−120 kJ mol^{-1}; refs *17, 18, 60*).

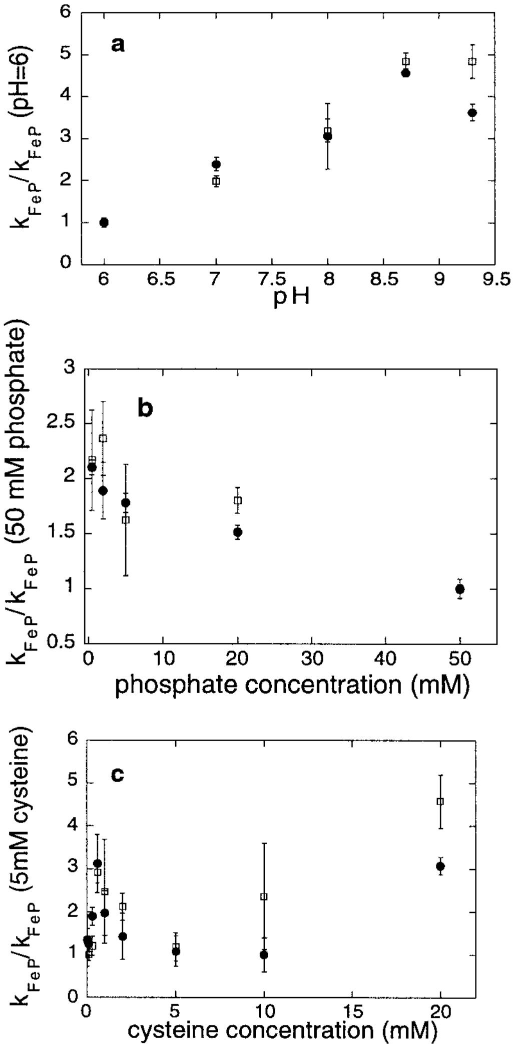


FIGURE 2. Dependence of the second-order rate constants (±95% C.I.) for reaction with CCl_4 (□) and C_2Cl_6 (●) at constant [FeP] = 30 μM on (a) the pH of the solution (normalized to the measured values at pH = 6); (b) the phosphate concentration (normalized to the measured values at [phosphate] = 50 mM); and (c) the cysteine concentration (normalized to the measured values at [cysteine] = 5 mM).

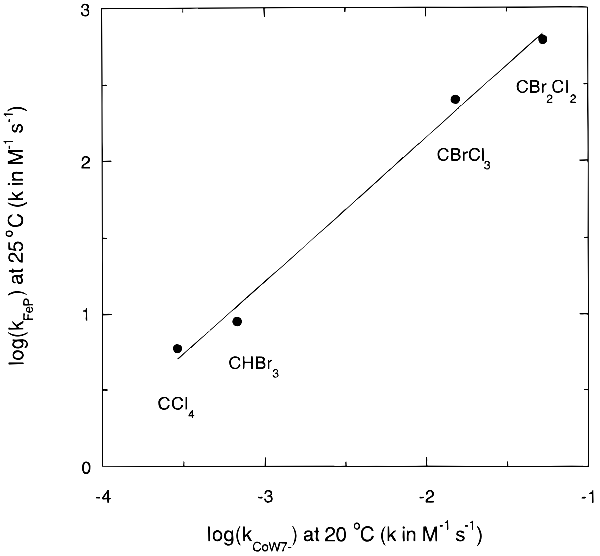


FIGURE 3. The log of the second-order rate constants for reaction with the iron porphyrin increased linearly with the log of the second-order rate constants for reaction with $Co^{(II)}W_{12}O_{40}^{7-}$ reported by Eberson and Ekström (*46*). The equation of the linear regression is $\log(k_{FeP}) = 4.0 + 0.94 \log(k_{CoW7-})$, $r^2 = 0.993$. The slope of nearly 1 indicates that the reaction with the iron porphyrin is outer-sphere, as the cobalt–tungstate complex is strictly an outer-sphere reductant.

TABLE 3. Activation Energies (±95% CI) in the Model Systems at pH 7.0

	E_a (kJ/mol)
tetrachloromethane	
5 mM cysteine, 30 μM iron porphyrin	72 ± 8
5 mM cysteine	86 ± 6
hexachloroethane	
5 mM cysteine, 10 μM iron porphyrin	63 ± 1
5 mM cysteine	100 ± 10
1 mM hydrogen sulfide, 20 μM juglone[a]	52 ± 5
1 mM hydrogen sulfide, 200 μM juglone[a]	46 ± 7
1 mM hydrogen sulfide[a]	75 ± 5

[a] Ref 19.

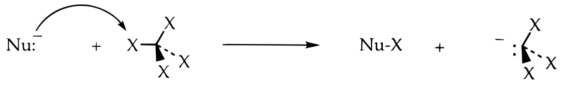
Many investigations (spectrometrical and theoretical) have shown that, in principle, halogenated hydrocarbons can form radical anions which decay to form the products in eq 7 (*61, 62*). For reduction reactions of polyhalogenated alkanes, radical anion intermediates have been observed by electron paramagnetic resonance (*63–67*) albeit under very different conditions (either in the gas phase or in solid solutions at 77 K). In aqueous solution, however, pathway II should be strongly favored relative to reactions in the gas phase due to the solvation energy of the anion X^- that is formed during the reaction. Wentworth et al. (*68*) have published convincing evidence that halogenated aliphatic compounds react preferentially by dissociative electron transfer (eq 7, path II), whereas halogenated aromatic compounds tend to form stable radical anions (eq 7, path I). For more detailed discussions concerning dissociative electron transfer, see references *69* and *70*.

Quantitative structure–reactivity correlations are difficult to make for the reactions investigated here because accurate and precise values for descriptors such as electron affinity and bond dissociation energies of the carbon–halogen bonds that are broken are difficult to obtain. Qualitatively, however, some structure–reactivity considerations can be made. For the iron porphyrin system, the C_1 compounds can be grouped into two categories: those containing bromide as the leaving group and those containing chloride as the leaving group. The rate of C–Br bond cleavage should exceed that of cleavage of a C–Cl bond because the former bonds are weaker than the latter (*71*). Among the C_1 compounds it can then be expected that CCl_4 and CCl_3F are among the slowest compounds for which a rate constant could be determined (the rate of $CHCl_3$ transformation was too slow to be measured). As summarized by Slayden et al. (*72*), increasing the number of fluorines on a carbon atom in a PHA increases the C–F and the C–Cl bond dissociation energies at this carbon atom (α-fluorine effect), but it does not have a systematic effect on the C–H bond dissociation energy. Increasing the number of other halogen atoms on the carbon atom does not systematically increase bond dissociation energies and in some cases causes a slight decrease in bond dissociation energy. Compared to chlorinated methanes, the fluorine atom in CCl_3F increases the C–Cl bond strength and to a lesser extent destabilizes the resulting radical; thus the rate constant of CCl_3F can be expected to be less than that of CCl_4, which is consistent with the experimental data (see Table 1).

Among the C_1 compounds containing a bromine atom, CBr_4 is by far the most reactive compound; its rate was too high to be measured. In the series $k_{CBr_4} \rightarrow k_{CBr_2Cl_2} \rightarrow k_{CBrCl_3}$ the rate constants decrease. This can be rationalized by the assumption that bromine atoms stabilize a radical even more effectively than do chlorine atoms. Substitution of bromine (or chlorine) by a fluorine atom again slows down the rate markedly (cf. k_{CBr_4} vs k_{CBr_3F}). The effect of slowing down the rate is even more pronounced by replacing a bromine (or chlorine) atom by a hydrogen (cf. $k_{CBr_4} \rightarrow k_{CHBr_3}$ and $k_{CCl_4} \rightarrow k_{CHCl_3}$). Due to a lack of nonbonding electrons, hydrogen cannot stabilize a radical by π-bonding. On a qualitative basis, the observed series of rate constants of the C_1 compounds can be quite well rationalized with the arguments presented above.

For the C_2 compounds investigated, the leaving group is a chloride ion in all cases. Most of the compounds bear a CCl_3 group. In the series $CCl_3{-}CCl_3 \rightarrow CHCl_2{-}CCl_3 \rightarrow CH_2Cl{-}CCl_3 \rightarrow CH_3{-}CCl_3$ the rate constants decrease by a factor of 5 in the first two cases. In the last case there is a factor of 40 decrease. The first three compounds are able to form bridged radicals due to electron pairs on the halogen atoms (*59*). The CH_3 group cannot easily form such bridged structures. In the case of $CF_2Cl{-}CFCl_2$, a fluorine atom is placed at the carbon containing the C−Cl bond which breaks, strengthening this bond and causing destabilization of the radical which is formed. Nevertheless, this radical could be stabilized through formation of bridged structures. The destabilizing effect of the fluorine atom at the radical center might be compensated by the bridge stabilization. If a chlorine at the radical center is replaced by hydrogen, the rate is slower than for the fluorinated compound above. This is in complete agreement with the series of radical stabilities discussed for the C_1 compounds.

Reaction with Mercaptojuglone. In contrast to the reaction with the iron(II) porphyrin, a linear correlation of log k_{JUG} versus log k_{CoW7} yields a slope significantly greater than 1 (approximately 1.6, data not shown; "CoW7−" is $Co^{(II)}W_{12}O_{40}{}^{7-}$, a typical outer-sphere one-electron donor (*45*)). This indicates that this mediator does not react primarily via an outer-sphere electron-transfer mechanism. The juglone-mediated system consists of hydrogen sulfide as bulk reductant. The actual electron-transfer mediator is not juglone, however, but rather an addition product of hydrogen sulfide and juglone, most likely 5-hydroxy-2-mercapto-1,4-naphthoquinone (*19, 41*). The thiol group in this species can act as a 1- or a 2-electron donor. Mercaptojuglone acts as a nucleophile at a halogen of the polyhalogenated alkane:



(8)

There is no steric hindrance toward reaction of the polyhalogenated substrates at the reactive center of mercaptojuglone (free thiol group) as in the iron porphyrin system (iron center axially complexed by two cysteine molecules (*55*)). This lack of steric hindrance at mercaptojuglone relative to iron porphyrin may explain its higher sensitivity to structural changes in the PHAs (i.e., 7 vs 4 orders of magnitude difference in rate constants in the juglone versus the iron porphyrin system, respectively). Thus, the two reactive centers in the two systems differ by their electron-transfer properties (1- or 2-electron donor versus 1-electron donor) and by their local accessibility (i.e., the thiol group of mercaptojuglone is accessible whereas the iron center is not accessible).

The double functionality of mercaptojuglone (as a 1- and 2-electron-transfer agent) is also supported by the reaction intermediates found. It was assumed that a 2-electron transfer (simultaneously or stepwise) would lead to a carbanion (eq 8) which was either protonated or decayed to a carbene CX_2. Carbenes are known to react with olefins (*73*). 3-Pentenoic acid or 3-methyl-3-butenoic acid was added to the reaction solutions, and cyclopropanes were analyzed as products of carbene reaction with the olefinic acid (*52*). Whereas the iron porphyrin system did not contain any cyclopropanes after complete disappearance of the substrate, cyclopropanes were present in the juglone system.

S_N2 reactions at halogens in PHAs are known to proceed with many kinds of nucleophiles (*74, 75*) and specifically with thiols (*75*−*77*). Although some fraction of the reaction with mercaptojuglone appears to proceed by S_N2 at X, the relative reactivities in that system parallel those in the iron porphyrin system (Figure 1). Together with the knowledge that the iron porphyrin system acts as a 1-electron-transfer reagent, one can conclude that a 1-electron transfer *and* an S_N2 reaction take place.

From these findings one can make the following summarizing conclusions: Iron porphyrin acts as a 1-electron-transfer mediator in an outer-sphere electron transfer. Mercaptojuglone exhibits a double functionality, namely as a 1-electron outer-sphere electron-transfer mediator and second as a nucleophile in S_N2 reaction at X (CHX_3 found as product of CX_4; free $:CX_2$ trapped). The electron-transfer reaction with mercaptojuglone must be outer-sphere rather than inner-sphere because the latter requires reaction with the semiquinone rather than with the completely reduced form, and the completely reduced form is the reactive species at neutral pH (*19*).

The results of this study demonstrate that when considering reductions of polyhalogenated methanes and ethanes (PHAs) by an outer-sphere electron-transfer reaction in homogeneous aqueous solution, relative reaction rates can be qualitatively rationalized through use of thermochemical data. For a quantitative prediction of rate constants, the presently available data for molecular descriptors such as electron affinities and bond dissociation energies are insufficient. Semiempirical and ab initio calculations of such thermochemical properties are in progress in the laboratory of one of the authors (*78*). At present, the reaction rate constants of the compounds with the iron(II) porphyrin (i.e., k_{FeP}, Tables 1 and 2) could be used as references for evaluating the reaction kinetics for the compounds in other systems, as illustrated here with the mercaptojuglone. Based on the limited data available for comparison (*32, 79*−*81*), this approach can describe relative reactivities of PHAs measured in a wide range of aqueous systems from abiotic and homogeneous to abiotic and heterogeneous to biotic systems.

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Literature Cited

(1) Zoro, J. A.; Hunter, J. M.; Eglington, G.; Ware, G. C. *Nature* **1974**, *247*, 235−237.
(2) Bouwer, E. J.; Rittmann, B. E.; McCarty, P. L. *Environ. Sci. Technol.* **1981**, *15*, 596−599.
(3) Bouwer, E. J.; McCarty, P. L. *Appl. Environ. Microbiol.* **1983**, *45*, 223−229.
(4) Criddle, C. S.; McCarty, P. L.; Elliot, M. C.; Barker, J. F. *J. Contam. Hydrol.* **1986**, *1*, 133−142.
(5) Tsukano, Y. *J. Contam. Hydrol.* **1986**, *1*, 47−63.
(6) Vogel, T. M.; Criddle, C. S.; McCarty, P. L. *Environ. Sci. Technol.* **1987**, *21*, 723−736.

(7) Barrio-Lage, G.; Parsons, F. Z.; Nassar, R. S.; Lorenzo, P. A. *Environ. Toxicol. Chem.* **1987**, *6*, 571−578.
(8) Nerger, M.; Mergler-Voelki, R. *Z. Wasser-Abwasser-Forsch.* **1988**, *21*, 16−19.
(9) Lesage, S.; Jackson, R. E.; Priddle, M. W.; Riemann, P. G. *Environ. Sci. Technol.* **1990**, *24*, 559−566.
(10) Klecka, G. M.; Gonsoir, S. J.; Markham, D. A. *Environ. Toxicol. Chem.* **1990**, *9*, 1437−1451.
(11) Boronina, T.; Klabunde, K. J.; Sergeev, G. *Environ. Sci. Technol.* **1995**, *29*, 1511−1517.
(12) Senzaki, T.; Kumagai, Y. *Kogyo Yosui* **1988**, *369*, 19−25.
(13) Semprini, L.; Roberts, P. V.; Hopkins, G. D.; McCarty, P. L. *Ground Water* **1990**, *28*, 715−727.
(14) Semprini, L.; Hopkins, G. D.; McCarthy, P. L.; Roberts, P. V. *Environ. Sci. Technol.* **1992**, *26*, 2454−2461.
(15) Schwarzenbach, R. P.; Giger, W.; Schaffner, C.; Wanner, O. *Environ. Sci. Technol.* **1985**, *19*, 322−327.
(16) Barbash, J. E.; Reinhard, M. In *Biogenic Sulfur in the Environment*; Cooper, W., Ed.; American Chemical Society: Washington, DC, 1989; pp 101−138.
(17) Barbash, J. E.; Reinhard, M. *Environ. Sci. Technol.* **1989**, *23*, 1349−1357.
(18) Roberts, A. L.; Sanborn, P. N.; Gschwend, P. M. *Environ. Sci. Technol.* **1992**, *26*, 2263−2274.
(19) Perlinger, J. A.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1996**, *30*, 3408−3417.
(20) Castro, C. E. *J. Am. Chem. Soc.* **1964**, *86*, 2310−2311.
(21) Nastainczyk, W.; Ullrich, V.; Sies, H. *Biochem. Pharmacol.* **1978**, *27*, 387−392.
(22) Mansuy, D. *Pure Appl. Chem.* **1980**, *52*, 681−690.
(23) Klecka, G. M.; Gonsior, S. J. *Chemosphere* **1984**, *13*, 391−402.
(24) Gantzer, C. J.; Wackett, L. P. *Environ. Sci. Technol.* **1991**, *25*, 715−722.
(25) Wood, J. M.; Kennedy, F. S.; Wolfe, R. S. *Biochemistry* **1968**, *7*, 1707−1713.
(26) Schrauzer, G. N.; Deutsch, E. *J. Am. Chem. Soc.* **1969**, *91*, 3341−3350.
(27) Blaser, H.-U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684−1689.
(28) Connors, T. F.; Arena, J. V.; Rusling, J. F. *J. Phys. Chem.* **1988**, *92*, 2810−2816.
(29) Krone, U. E.; Thauer, R. K.; Hogenkamp, H. P. C. *Biochemistry* **1989**, *28*, 4908−4914.
(30) Marks, T. S.; Allpress, J. D.; Maule, A. *Appl. Environ. Microbiol.* **1989**, *55*, 1258−1261.
(31) Egli, C.; Stromeyer, S.; Cook, A. M.; Leisinger, T. *FEMS Microbiol. Lett.* **1990**, *68*, 207−212.
(32) Gillham, R. W.; O'Hannesin, S. F. *Ground Water* **1994**, *32*, 958−967.
(33) Helland, B. R.; Alvarez, P. J. J.; Schnoor, J. L. *J. Hazard Mater.* **1995**, *41*, 205−216.
(34) Matheson, L. J.; Tratnyek, P. G. *Environ. Sci. Technol.* **1994**, *28*, 2045−2053.
(35) Warren, K. D.; Arnold, R. G.; Bishop, T. L.; Lindholm, L. C.; Betterton, E. A. *J. Hazard Mater.* **1995**, *41*, 217−227.
(36) Glaus, M. A.; Heijman, C. G.; Schwarzenbach, R. P.; Zeyer, J. *Appl. Environ. Microbiol.* **1992**, *58*, 1945−1951.
(37) Dunnivant, F. M.; Schwarzenbach, R. P.; Macalady, D. L. *Environ. Sci. Technol.* **1992**, *26*, 2133−2141.
(38) Heijman, C. G.; Grieder, E.; Holliger, C.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1995**, *29*, 775−783.
(39) Klausen, J. W.; Tröber, S.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1995**, *29*, 2396−2404.
(40) Schwarzenbach, R. P.; Stierli, R.; Lanz, K.; Zeyer, J. *Environ. Sci. Technol.* **1990**, *24*, 1566−1574.
(41) Perlinger, J. A.; Kalluri, V. M.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1998**, manuscript in preparation.
(42) Vogel, T. M.; Reinhard, M. *Environ. Sci. Technol.* **1986**, *20*, 992−997.
(43) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; New York: Wiley-Interscience, 1993.
(44) Perlinger, J. A. Dissertation No. 10892, ETH-Zürich, 1994.
(45) Ekström, M. Dissertation, Lund University, 1988.
(46) Eberson, L.; Ekström, M. *Acta Chem. Scand.* **1988**, *B42*, 113−121.
(47) Schoder, A. Dissertation No. 5491, ETH Zürich, 1975.
(48) Pospisil, J.; Luzny, Z. *Collect. Czech. Chem. Commun.* **1960**, *25*, 589−592.
(49) Ashworth, R. A.; Howe, G. B.; Mullins, M. E.; Rogers, T. N. *J. Hazard Mater.* **1988**, *18*, 25−36.
(50) Hine, J.; Mookerjee, P. K. *J. Org. Chem.* **1975**, *40*, 292−298.
(51) Bürge, I. Diplomarbeit, ETH, 1994.
(52) Buschmann, J.; Angst, W.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1998**, manuscript in preparation.
(53) Wade, R. S.; Castro, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 226−230.
(54) Wade, R. S.; Castro, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 231−234.
(55) Buschmann, J. Dissertation, ETH-Zürich, 1998.
(56) Clark, W. M. *Oxidation−Reduction Potentials of Organic Systems*; Waverly Press: Baltimore, 1960.
(57) Li, S.; Wackett, L. P. *Biochemistry* **1993**, *32*, 9355−9361.
(58) Waisman, E.; Worry, G.; Marcus, R. A. *J. Electroanal. Chem.* **1977**, *82*, 9−28.
(59) Ulstrup, J. *Acta Chem. Scand.* **1973**, *27*, 1067−1072.
(60) Kriegman-King, M. R.; Reinhard, M. *Environ. Sci. Technol.* **1992**, *26*, 2189−2206.
(61) Pritchard, H. O. *Chem. Rev.* **1953**, *53*, 529−563.
(62) Gutsev, G. L.; Ziegler, T. *Can J. Chem.* **1991**, *69*, 993−999.
(63) Bonazzola, L.; Michaut, J.-P.; Roncin., J. *Chem. Phys. Lett.* **1988**, *153*, 52−56.
(64) Bonazzola, L.; Michaut, J.-P.; Roncin., J. *Chem. Phys. Lett.* **1988**, *149*, 316−320.
(65) Brickenstein, E. K.; Khairutdinov, R. F. *Chem. Phys. Lett.* **1985**, *115*, 176−179.
(66) Mishra, S. P.; Symons, M. C. R. *J. Chem. Soc., Chem. Commun.* **1973**, 577−578.
(67) Symons, M. C. R.; Smith, I. G. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1180−1185.
(68) Wentworth, W. E.; George, R.; Keith, H. *J. Chem. Phys.* **1969**, *51*, 1791−1801.
(69) Eberson, L. *Electron-Transfer Reactions in Organic Chemistry*; Springer-Verlag: Berlin, 1987.
(70) Savéant, J.-M. In *Advances in Physical Organic Chemistry*, Bethell, D., Ed.; Academic Press: London, 1990; pp 1−130.
(71) March, J. *Advanced Organic Chemistry*; Wiley-Interscience: New York, 1992.
(72) Slayden, S. W.; Liebman, J. F.; Mallard, W. G. In *Supplement D2: The chemistry of halides, pseudo-halides and azides*; Patai, S., Rappoport, Z., Eds.; Wiley & Sons: New York, 1995; pp 361−402.
(73) Wentrup, C. *Reaktive Zwischenstufen, Radikale, Carbene, Nitrene, gespannte Ringe*; Georg Thieme Verlag: Stuttgart, Germany, 1979.
(74) Zefirov, N. S.; Makhon'kov, D. I. *Chem. Rev.* **1982**, *82*, 615−624.
(75) Li, X.-Y.; Jiang, X.-K.; Pan, H.-Q.; Hu, J.-S.; Fu, W. M. *Pure Appl. Chem.* **1987**, *59*, 1015−1020.
(76) Beadle, C. D.; Bowman, W. R.; Prousek, J. *Tetrahedron Lett.* **1984**, *25*, 4979−4982.
(77) Rico, I.; Cantacuzene, D.; Wakselman, C. *J. Org. Chem.* **1983**, *48*, 1979−1982.
(78) Venkatapathy, R.; Harrison, J. F.; Perlinger, J. A., manuscript in preparation.
(79) Jafvert, C. T.; Wolfe, N. L. *Environ. Toxicol. Chem.* **1987**, *6*, 827−837.
(80) Pecher, K.; Haderlein, S. B.; Schwarzenbach, R. P. *Abstract*, 213th ACS National Meeting of the American Chemical Society, San Fransisco, CA, 1997; Environmental Chemistry Division, pp 185−190.
(81) Castro, C. E.; Wade, R. S.; Belser, N. O. *Biochemistry* **1985**, *24*, 204−210.

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