Environ. Sci. Technol. **1999**, *33,* 737−744

Production and Dechlorination of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Historically-Contaminated Estuarine Sediments

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Polychlorinated dibenzo-*p*-dioxins (PCDD) are ubiquitous and considered to be unreactive to biotic and abiotic transformation processes. Here we demonstrate that sediment-associated 2,3,7,8-substituted dioxin residues in general, and 2,3,7,8-TCDD in particular, are in a state of flux, as they are produced from *peri*-dechlorination of octaCDD, and further laterally dechlorinated to 2-MCDD. This reaction can be stimulated in the presence of organic acids, 2-monobromodibenzo-*p*-dioxin (2-MBDD) and hydrogen, which result in the production of HpCDD, 2,3,7,8-TCDD, and 2-MCDD, respectively. The results indicate that dechlorination of 2,3,7,8-TCDD is not likely a rate-limiting step in the hydrogen-stimulated reaction, which presents a potential strategy to decontaminate dioxin-contaminated environments.

Background

The burden of environmental compartments with PCDD derived from both point- and nonpoint sources is well-documented (*1*−*5*). The various emission and depositional fluxes have resulted in typical total PCDD concentrations of pg/m^3 in the air and ng/kg in urban soils and sediments, of which 2,3,7,8-substituted residues constitute often less than 1% (*5*). The singular exception perhaps to this distribution are environments impacted by chlorophenol production and by sodium 2,4,5-trichlorophenate synthesis (*6*), which tend to generate disproportionately high fractions of 2,3,7,8-TCDD to dominate the dioxin pattern in this impacted environment (*1, 7*).

To evaluate the fate of PCDD in their path from source to sink and to estimate the impact of (bio) degradation processes on the environmental burden, first-level analysis can be performed based on fugacity models (*8*), followed by a second-level analysis based on changes in congener-specific dioxin patterns in source and sink environments (*9*−*13*). Several trends can be derived from these analyses: (i) in topsoil and river sediment environments, the temporal concentrations 2,3,7,8-TCDD have decreased disproportionately to hepta- and octaCDD (*5, 14*); (ii) PCDD patterns in waterways sediments can be discriminated by the relative dominance vs absence of higher chlorinated 2,3,7,8-substituted PCDD residues (*12*); and (iii) source and sink patterns generally are difficult to correlate except for when particular congeners are dominant (*10*−*13*). These trends have been ascribed to a temporal change in dioxin composition generated from the various combustion sources, the multitude of dioxin sources impacting a dioxin pattern in soils or sediments (*1, 12*), the unknown potential for biogenic dioxin production (*15*), and degradation or transformation reactions (*16*).

Dioxin transformation reactions have until now mainly been based on single congener studies using either soil- or sediment-derived pure and mixed cultures or sediments (*17*−*22*). Considering that river sediments, owing to their high organic matter turnover, are predominantly anaerobic and very reduced (*23*), reductive dechlorination is a probable transformation mechanism. Using microbial communities eluted from Lower Passaic River sediment cores, OCDD dechlorination was shown to proceed via two concurrent pathways. Upon removal of a *peri* (1,4,6,9-chlorine) from OCDD, further *peri*-dechlorination is predominant, resulting in the transient formation of 2,3,7,8-TCDD; removal of a lateral (2,3,7,8)-chlorine results in the destabilization of the dioxin molecule (*24, 25*) and production of non-2,3,7,8-substituted tetraCDD isomers (*20, 22*). Further dechlorination of 2,3,7,8- or 1,2,3,4-TCDD isomers to 2-MCDD has been demonstrated in spiked (*19, 21*) and historically contaminated (*20*) environmental systems. During the dechlorination sequence, hepta-, tetra-, tri- and monoCDD congener groups were consistently found to be dominant, with minor contributions of hexa-, penta-, and diCDD homologues (*16, 20, 22*). Dioxin dechlorination is not limited to biotic catalysis, as humic acid- (*22, 26*) and zerovalent- or organometal- (*27*) mediated reductions have been observed as well. Molecular orbital calculations have demonstrated that the thermodynamically most favorable pathway occurred at the most positively charged carbon atom in the ring, which tended to be lateral (2,3,7, or 8 positions) (*25*).

Only recently have methods been developed to analyze for lesser chlorinated congeners in complex environmental matrixes such as sediments (*28*). Intriguing evidence that extensive dechlorination may be occurring naturally was obtained during analysis of a separate sediment core obtained from the same estuary but which was dominated by 2,3,7,8-TCDD (32 ng/kg vs 12 ng/kg of OCDD and 8 ng/kg of 1,2,3,4,6,7,8-HpCDD); 2-MCDD was found at concentrations of 17 ng/kg (*28*). Whereas the rates in natural systems may be exceedingly slow, this study has indicated the potential for stimulation of biogenic formation and further dechlorination of 2,3,7,8-TCDD under anaerobic conditions and suggests that the fate of PCDD in sediment environments may include either activation or detoxification reactions.

In accordance with the above rationale, we hypothesize that dioxins (including the 2,3,7,8-TCDD isomer) can be dechlorinated in contaminated sediments and that their reactivity can be demonstrated by analyzing for changes in the distribution of MCDD to OCDD patterns, relative to the baseline distribution. This communication describes (i) a validation of the dechlorination pathways observed in dioxin-spiked solid-free systems to historically contaminated sediments and (ii) the implications of the dynamics of 2,3,7,8-TCDD production and dechlorination for bioremediation strategies.

Experimental Methods

Sediment Treatment/Incubations. *(i) Site Description*. A three-meter sediment core was obtained from the lower estuarine Passaic River near the former Diamond Alkali Lister Ave plant (Figure 1) which manufactured sodium 2,4,5-trichlorophenate, a precurser to 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) (*7*). Temporal trends in PCDD and PCDF

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10.1021/es980791t CCC: $18.00
Published on Web 01/20/1999

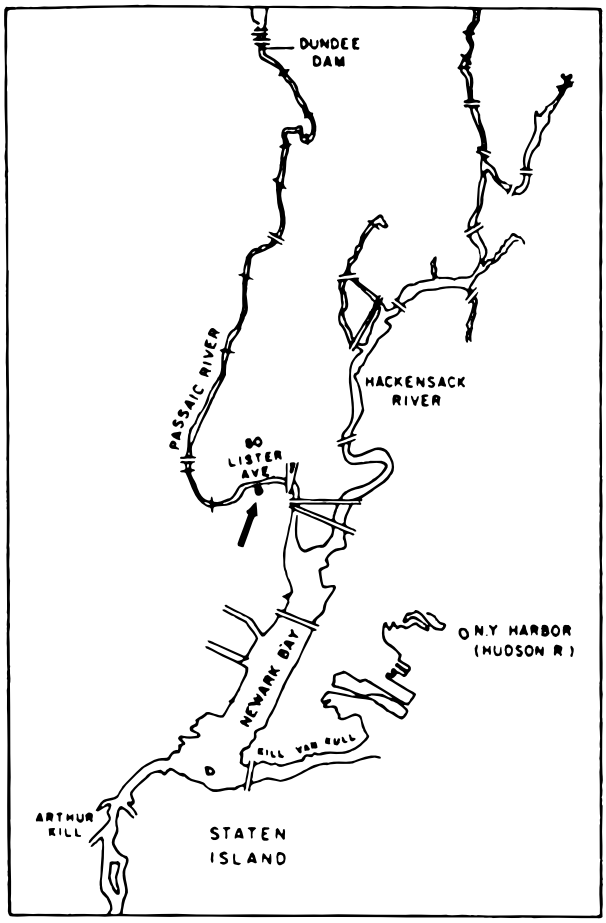

FIGURE 1. Map of the study area from which the sediment core was obtained (modified from ref *7*).

concentration have been described earlier (*7*) and indicated a total estimated deposition of 4−8 kg of 2,3,7,8-TCDD since the 1940s (*7*). The core was divided in 30 cm sections which were capped at both ends, and all sections were shipped on ice to the EWRE dioxin laboratories. Dioxin patterns from this manufacturing process are dominated by the 2,3,7,8-TCDD congener (*7*); however, source-sink correlations are unclear as this urban area is impacted by other point sources and atmospheric deposition resulting in elevated hepta- and octaCDD (*1, 13*). Based on reported sediment dating procedures and historical dioxin deposition rates at this site (*1, 7, 10*), three sections (0.6−1.6 m depth) totalling four kg (wet weight) were selected based on elevated dioxin concentrations (ng/g range) for the microcosm study.

(ii) Experimental Procedures. The sediment cores were transferred into an anaerobic glovebox, extruded from the plexiglass sheething using a Teflon plunger, and homogenized in a glass tray by manual mixing; anaerobic river bottom water collected at the site was used to break up aggregates and to obtain a smooth consistency of the sediment material. The core material was then dispensed in a number of aluminum trays, amended monthly with a 100 mg/L substrate cocktail solution prepared in river bottom water (*20*), covered with plastic wrap, and incubated in the glovebox for a period of 3 months. At the end of this acclimation period, 15 of 200 g (wet weight) subsamples were placed in 250 mL wide-mouthed Mason jars. The sediment was overlaid with 50 mL of river water, resulting in a nitrogen headspace of approximately 50 mL, and the bottles were tightly sealed using Teflon tape.

Triplicate amendments were established as indicated in Table 1. Since all microcosms were sacrificial, the time "zero" (original acclimated sediment) served as the baseline for all treatments. All other microcosms received electron donor cocktail (100 mg/kg) on a monthly basis, in addition to the various treatments. The control was autoclaved for three consecutive days at 120 °C (40 min.). The "organic acid" microcosms served as a baseline for microbial dechlorination activity, whereas the "autoclaved control" was intended to evaluate the abiotic dechlorination potential. Preliminary studies have demonstrated that the autoclaving of sediments as applied does not affect the recovery efficiency of PCDD congeners nor results in transformation reactions. 2-Monobromodioxin (50 ppb) was added at the beginning of the experiment only and served to prime dechlorination activity, as has been observed with polychlorinated biphenyls (PCBs) (*29*). After 3 months, one triplicate set of electron donor-amended microcosms was subjected to the hydrogen treatment, which consisted of a daily replacement of the headspace with pure hydrogen gas for 10 days, and served to stimulate overall anaerobic hydrogenase activity. Hydrogen amendments have been shown to enhance dechlorination of chlorinated solvents in aquifer solids (*30*). All microcosms were manually inverted on a weekly basis during the 3 month incubation, and on a daily basis for the 10-day hydrogen treatment, and sacrificed for dioxin analysis at the end of the incubation period (90 days; 100 days for hydrogen treatment).

TABLE 1. Summary of Triplicate Microcosm Treatments

treatment	amendment
1. baseline	none
2. autoclaved	triple autoclaved, substrate cocktail (100 mg/kg/mo), 2-monobromodibenzo-*p*-dioxin (20 ng/g, single addition), weekly manual mixing
3. organic acid	substrate cocktail (100 mg/kg/mo), weekly manual mixing
4. bromodioxin	substrate cocktail (100 mg/kg/mo), 2-monobromodibenzo-*p*-dioxin (20 ng/g, single addition), weekly manual mixing
5. hydrogen	substrate cocktail (100 mg/kg/mo) for 3 months, then daily evacuation/replenishing of headspace with pure H2 gas and manual mixing for 10 days

Sample Preparation and Analysis. *(i) Reagents, Columns, and Standards.* Reagent grade potassium hydroxide, sodium hydroxide, silver nitrate, instrument grade mercury metal, chromatographic silica gel 100−200 mesh, and solvents were purchased from Fischer Scientific. Celite 545 was purchased from J. T. Baker, Carbopak C 80/100 mesh from Supelco, and Alumina Basic super 1 50−200 mesh from Alltech. The HRGC column and standard solutions containing all native (^{12}C) PCDD congeners and all ^{13}C-labeled PCDD were custom-synthesized by J&K Environmental (Sydney, Nova Scotia, Canada). The homologue group-specific concentrations of the ^{13}C-labeled PCDD were (in ng/μL) MCDD, 10.5; DCDD, 12.0; TriCDD, 20.4; TCDD, 3.6; PeCDD, 2.0; HxCDD, 0.7; HpCDD, 0.5; OCDD, 0.3. For the ^{12}C-PCDD, the distribution was (ng/μL) MCDD, 3.5; DCDD, 4.5; TriCDD, 10.0; TCDD, 3.3; PeCDD, 4.6; HxCDD, 3.7; HpCDD, 3.0; OCDD, 0.6. The ^{13}C-PCDD mixture was supplemented with ^{13}C-2,3,7,8-TCDD.

(ii) Preparation of Cleanup Reagents. The Cu−Hg amalgam was prepared by adding 82 g of Hg and 20 mL of 30% HNO_3 to 100 g of copper powder (40 mesh, Aldrich), previously rinsed with 20% HNO_3 followed by five rinsings of distilled deionized water. Once the reaction started, 60 mL of water was added, and the mixture was extensively stirred. After settling overnight, the liquid was decanted, and the amalgam rinsed five times with acetone, followed by five rinses with hexane activated silica gel: 33% 1 N NaOH, 30% H_2SO_4, 44%

H_2SO_4; and basic alumina, as described in EPA method 1613 (*31*). Sodium nitrate (10%) and potassium silicate were prepared as described by Lamparski and Nestrick (*32*) and Smith et al. (*33*), respectively. Carbopak 32% was prepared by combining 8 g of Carbopak C with 17 g of Celite 545 and activation at 130 °C.

(iii) Sediment Sample Preparation, Extraction, and Cleanup. Air dried samples (25 g), homogenized with a mortar and pestle, were placed in Soxhlet thimbles each charged with 2 g of preextracted silica gel. After spiking with the required labeled PCDD internal standard and/or isotope dilution standard, a 48 h toluene Soxhlet extraction was implemented.

Ten grams each of Cu−Hg amalgam and 30% H_2SO_4 were added to each extract. The mixtures were stirred for 1 h, filtered through Soxhlet thimbles with 5 × 20 mL hexane rinsings each, and reduced to 3−5 mL via a Rotovap apparatus. Further cleanup was carried out by eluting the conentrate with 600 mL of hexane over layered silica columns consisting of (from bottom to top): 1 g of silica gel, 1 g of 44% H_2SO_4, 4 g of silica gel, 5 g of potassium silicate, 1 g of silica gel, 5 g of 10% $AgNO_3$, 1 g of silica gel, 5 g of 33% 1 N NaOH, 1 g of silica gel, 14 g of 44% H_2SO_4, 6 g of silica gel, 5 g of Na_2SO_4. After concentration to 2 mL, the extracts were applied to columns each consisting of 6 g of activated basic alumina. Elution was carried out with 100 mL of hexane (discarded) followed by 25 mL of 50% CH_2Cl_2 in hexane. In each case, the target fraction was reduced to 1 mL and applied to further cleanup using a column containing 0.55 g carbopak 32% which had been prewashed as described in EPA method 1613 (*31*). The elution program was as follows: fraction 1: application of extract + 2 × 1 mL hexane rinsings; fraction 2: 3 mL hexane; fraction 3: 2 mL of CH_2Cl_2/cyclohexane + 2 mL of CH_2Cl_2/methanol/toluene; fraction 4: 25 mL of toluene in reverse direction. As it was previously found that this cleanup step was highly variable with respect to congener discrimination and overall recoveries (*28*), all fractions were collected and saved. Fractions 3 and 4 were prepared for analysis by volume reduction to 50−100 μL under N_2.

(iv) Instrumental Analysis. The enriched extracts were analyzed on a Hewlett-Packard HP5890/5972A GC-MSD equipped with a 30 m × 0.25 mm id × 0.25 μm ICB-5 column, using split−splitless injection at 280 °C. Temperature program: 100 °C (2 min), 5 °C/min, 230 °C, 10 °C/min, 300 °C (10 min); the interface temperature was 300 °C. The LRMS was operated in EI-SIM mode, monitoring five ions per congener group (three for monoCDD). All PCDD congener groups were monitored. Identification of PCDD required that the following criteria be met: (a) a given peak retention time correspond to one generated by a standard containing all PCDD, (b) the peak is present in mass chromatograms corresponding to at least two signature ions, and (c) the ratios of the peak areas correspond to the expected values based on the natural isotopic abundance of chlorine. Quantitation was based on internal standard calculations using labeled 2,3,7,8-TCDD. One out of each triplicate was also spiked with a labeled mixture consisting of all PCDD congeners in order to aid in the confirmation of peak identification and to provide a basis for comparison of results by using isotope dilution. It was found that with the exception of OCDD, for which the recoveries differed substantially (1 order of magnitude) from the other congeners, there was reasonable consistency between results obtained by internal standard versus isotope dilution. Recovery efficiencies after analyte enrichment were between 50 and 100%, with lower recovery for the lesser chlorinated PCDD predominantly on account of volatilization losses during the Rotovap concentration steps. The detection limit for native dioxins was 0.1 ng on column; ^{13}C-labeled standards were quantifiable at 0.5 ng on column.

Data Interpretation. Since all microcosms were sacrificial, and homogenization of the sediment still resulted in heterogeneities with respect to dioxin concentrations, a normalization procedure had to be applied to interpret shifts in congener profiles as the result of the various treatments. Thus, all concentrations were normalized following the assumption that the baseline mole fraction of dioxin congeners represents the best estimate of the relative congener proportions at time zero and that the total moles of PCDD remain constant during the various treatments. These assumptions are reasonable provided that there are no reactions which would either increase or decrease the moles of PCDD, such as dechlorination of MCDD, oxidiation or polymerization reactions, or dioxin synthesis. Moreover, the baseline profile was based on triplicate samples as well as five random subsamples from the intial core. Initial concentrations for each treatment were then calculated by multiplying the baseline mole fraction by a normalizing factor given as the ratio of $\sum PCDD_{final}/\sum PCDD_{baseline}$. The average initial molar concentrations are included in Table 2B. To allow for a comparison of the dioxin distribution in the various treatments, the concentrations are provided as absolute mass (Table 2A) and molar (Table 2B) concentrations, mole percent (Figure 2A), and mole fractions (Figure 2B).

Results and Discussion

Dioxin Distribution in Microcosm Systems. Significant variation in the total PCDD concentration in the sediments (6.7−35.8 ng/g) as well as in the congener patterns was observed among the various treatments. The baseline profile (treatment 1) is dominated by OCDD, followed by a 2:1 ratio of 1,2,3,4,6,7,9- to 1,2,3,4,6,7,8-HpCDD, and 2,3,7,8-TCDD (40% of OCDD) (Figure 2). Trace concentrations of HxCDD were observed as well, but neither PeCDD nor Tri- to MCDD could be detected in these triplicate samples. This pattern is consistent with that reported earlier for the lower Passaic River; however, the concentrations of 2,3,7,8-TCDD are up to 10-fold higher when compared to published data (*1, 7, 10*). This profile has been ascribed to an environment impacted by a multide of urban sources and atmospheric deposition (*1, 10*); the high TCDD concentration may be correlated to a point source of sodium 2,4,5-trichlorophenate production (*7*).

The autoclaved samples (treatment 2) show a decrease in the HpCDD fraction and an increase in the 2,3,7,8-TCDD mole fraction, relative to the baseline profile. Only the absolute concentrations of 1,2,3,4,6,7,9-HpCDD and 2,3,7,8-TCDD increased; those of the 1,2,3,4,6,7,8-HpCDD isomer remained the same. Since the TCDD isomer can only be produced via peri-dechlorination of 1,2,3,4,6,7,8-HpCDD and the OCDD concentration was 2-fold higher when compared to the baseline, it is warranted to evaluate the trends in molar distribution. The molar fraction of 1,2,3,4,6,7,8-HpCDD decreased by approximately 40%, resulting in a 25% increase in 2,3,7,8-TCDD. However, the decrease in 1,2,3,4,6,7,9-HpCDD (30%) was not accompanied by a significant production of non-2,3,7,8-TCDD isomers. It was shown earlier in solid-free systems that during model humic constituent-mediated dechlorination of OCDD, the 2,3,7,8-TCDD fraction produced represented approximately 25% of the total tetraCDD homologue group (*22*). The absence of this trend in the current incubations may be ascribed to analytical interferences, as only the 2,3,7,8-^{13}C-TCDD isomer was quantifiable within this homologue group of the ^{13}C-labeled standard. Only traces of HxCDD or PeCDD were observed, which is consistent with earlier observations (*20, 22*).

Electron donor-amended samples (treatment 3) indicate a molar predominance of HpCDD (42% total, similar to OCDD) and show a divergence from the 2:1 ratio of 1,2,3,4,6,7,9- to 1,2,3,4,6,7,8-HpCDD observed in baseline

TABLE 2.

A. Mass Concentrations and Toxic Equivalents of Major PCDD in Unamended and Treated Passaic River Sediment Systems

	concentration, ng/g						
amendment	OCDD	1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,9-HpCDD	2,3,7,8-TCDD	2-MCDD	∑PCDD	∑TEQ
baseline-1	3.68	0.62	1.44	0.82	0.00	6.56	0.83
baseline-2	3.67	0.74	1.31	0.29	0.00	6.01	0.30
baseline-3	4.26	0.59	0.78	2.01	0.00	7.64	2.02
av	3.87	0.65	1.18	1.04	0.00	6.74	1.05
st dev	0.34	0.08	0.35	0.88	0.00	0.83	0.44
autoclaved-1	6.80	0.76	1.39	1.24	0.00	10.19	1.25
autoclaved-2	8.20	0.78	1.63	1.90	0.00	12.51	1.92
autoclaved-3	5.80	0.43	1.40	3.37	0.00	11.00	3.38
av	6.93	0.66	1.47	2.17	0.00	11.23	2.18
st dev	1.21	0.20	0.14	1.09	0.00	1.18	0.54
organic acid-1	4.86	0.90	0.79	1.69	0.00	8.24	1.70
organic acid-2	13.30	17.40	2.90	2.19	0.00	35.79	2.38
organic acid-3	5.07	5.54	0.93	1.66	0.00	13.20	1.72
av	7.74	7.95	1.54	1.85	0.00	19.08	1.93
st dev	4.81	8.51	1.18	0.30	0.00	14.69	0.19
bromodioxin-1	0.08	0.02	0.04	7.52	0.15	7.81	7.52
bromodioxin-2	3.03	0.66	1.00	2.85	0.00	7.54	2.86
bromodioxin-3	4.23	0.63	1.00	3.00	0.00	8.86	3.01
av	2.45	0.44	0.68	4.46	0.05	8.07	4.46
st dev	2.14	0.36	0.55	2.65	0.09	0.70	1.32
hydrogen-1	6.12	0.85	1.15	1.94	1.47	11.53	1.95
hydrogen-2	40.10	1.74	3.07	3.01	18.60	66.52	3.07
hydrogen-3	13.10	4.69	6.20	2.98	0.68	27.65	3.04
av	19.77	2.43	3.47	2.64	6.92	35.23	2.69
st dev	17.95	2.01	2.55	0.61	10.13	28.27	0.32

B. Molar Concentrations of Major PCDD Congeners in Passaic River Sediment Systems

	concentration, pmol/g					
amendment	OCDD	1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,9-HpCDD	2,3,7,8-TCDD	2-MCDD	∑PCDD
baseline-1	7.93	1.45	3.36	2.55	0.00	15.29
baseline-2	7.91	1.73	3.06	0.90	0.00	13.60
baseline-3	9.18	1.38	1.82	6.24	0.00	18.62
av	8.34	1.52	2.75	3.23	0.00	15.84
st dev	0.73	0.19	0.82	2.74	0.00	2.56
autoclaved-1	14.66	1.78	3.25	3.85	0.00	23.53
autoclaved-2	17.67	1.82	3.81	5.90	0.00	29.20
autoclaved-3	12.50	1.00	3.27	10.47	0.00	27.24
av	14.94	1.53	3.44	6.74	0.00	26.66
st dev	2.60	0.46	0.32	3.39	0.00	2.88
av norm. init. concn	14.04	2.56	4.63	5.44	0	26.67
organic acid-1	10.47	2.10	1.85	5.25	0.00	19.67
organic acid-2	28.66	40.65	6.78	6.80	0.00	82.89
organic acid-3	10.93	12.94	2.17	5.16	0.00	31.20
av	16.69	18.57	3.60	5.73	0.00	44.59
st dev	10.37	19.88	2.76	0.92	0.00	33.67
av norm. init. concn	23.48	4.28	7.74	9.09	0	44.59
bromodioxin-1	0.17	0.05	0.09	23.35	0.69	24.36
bromodioxin-2	6.53	1.54	2.34	8.85	0.00	19.26
bromodioxin-3	9.12	1.47	2.34	9.32	0.00	22.24
av	5.27	1.02	1.59	13.84	0.23	21.95
st dev	4.60	0.84	1.29	8.24	0.40	2.56
av norm. init. concn	11.56	2.11	3.81	4.48	0	21.96
hydrogen-1	13.19	1.99	2.69	6.02	6.74	30.63
hydrogen-2	86.42	4.07	7.17	9.35	85.32	192.33
hydrogen-3	28.23	10.96	14.49	9.25	3.12	66.05
av	42.61	5.67	8.12	8.21	31.73	96.34
st dev	38.68	4.70	5.96	1.89	46.45	85.00
av norm. init. concn	50.73	9.25	16.73	19.65	0	96.36

and autoclaved samples. This stereoselective removal of a *peri*-chlorine from OCDD has resulted in a 2,3,7,9/2,3,7,8-HpCDD ratio of 1:4, which has not been observed in either abiotic or biotic solid-free systems (*20, 22*) and cannot be explained from thermodynamic (*24*) or quantum-mechanical (*25*) calculations. The latter indicate that removal of a *peri*- or a lateral chorine from OCDD is equally likely, based on HOMO−LUMO gap and carbon charge values. Whereas it is possible that this change in distribution may be explained from heterogeneity in dioxins within the core, the 2,3,7,9/2,3,7,8-HpCDD ratio of 2:1 was shown to hold in a large number of cores in this estuary (*1*) and in commercially available OCDD standard solutions where both HpCDD isomers are present as trace contaminants (*20*). The molar fraction of 2,3,7,8-TCDD was similar to that observed in the baseline profile, indicating that the predominant reaction was a single preferential peri-dechlorination step from OCDD to HpCDD.

Bromodioxin-amended samples (treatment 4) showed a predominance of 2,3,7,8-TCDD in the dioxin profile (61%) at the expense of a 50% decrease in both OCDD and 1,2,3,4,6,7,8-HpCDD molar fractions relative to the baseline distribution (Figure 2). This TCDD fraction represents a 4-fold increase in absolute concentrations relative to the baseline

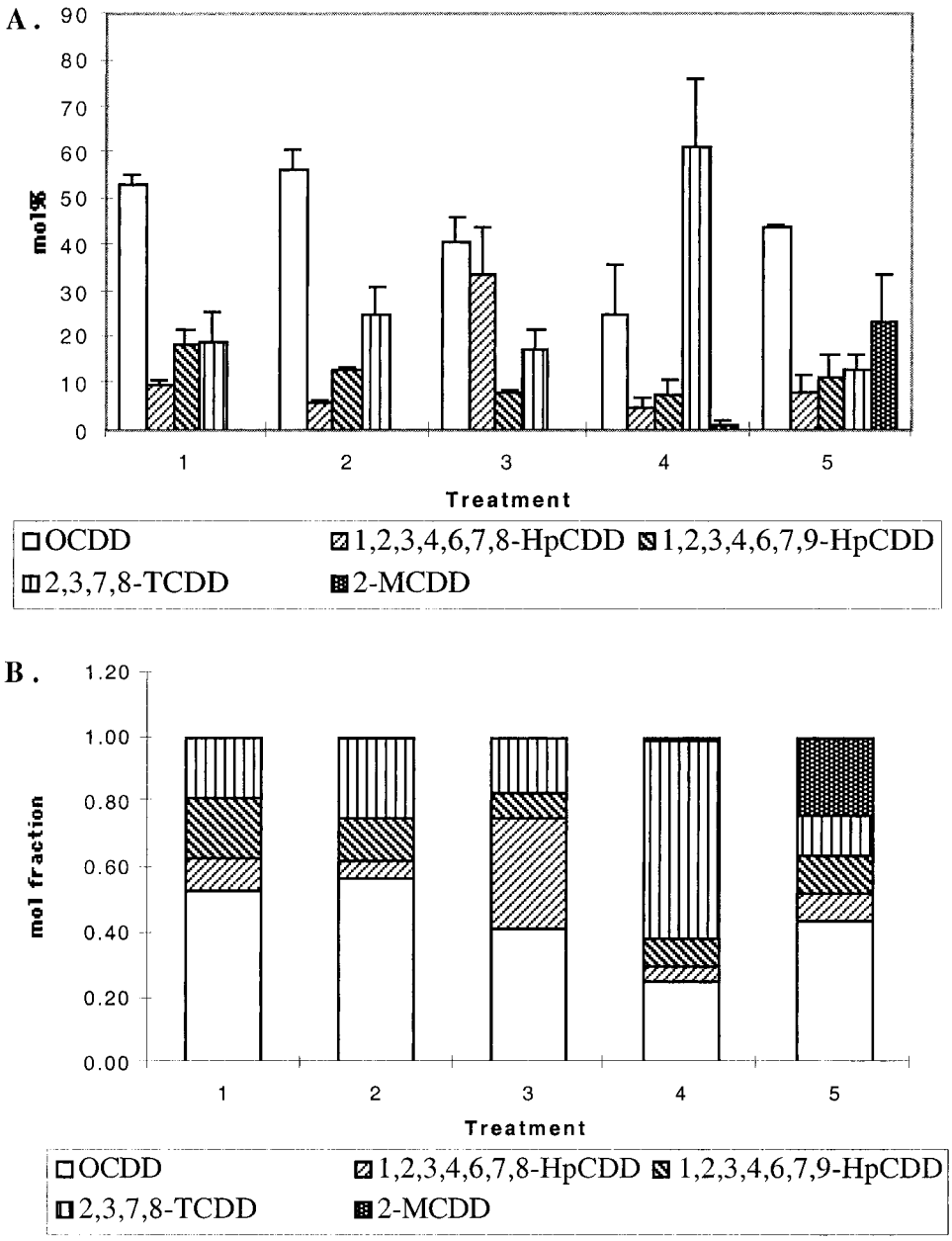

FIGURE 2. Mole percent (A) and mole fraction (B) distribution of dioxin congeners observed during incubation of Passaic River sediments (1. baseline profile; 2. autoclaved control; 3. organic acid amendment; 4. 2-MBrDD amendment; and 5. hydrogen treatment).

and a 2.4-fold increase relative to the organic acid amended samples, despite significant scatter in the data (Table 2). The latter comparison is more meaningful, as the brominated treatments received the electron donor cocktail in addition to 2-bromodioxin. Thus, the molar fraction of OCDD and the 2,3,7,8-substituted HpCDD decreased 1.6- and 7-fold respectively, resulting in a 3.6-fold increase in 2,3,7,8-TCDD and the formation of minor amounts (<0.05 ng/g) of 2-MCDD. The 1,2,3,4,6,7,9-HpCDD congener mole fraction did not change, and the fate of 2-MBDD was not monitored. Amendments of historically contaminated sediments with excess concentrations of brominated analogues to prime reductive dechlorination reactions have been demonstrated to result in extensive dechlorination of commercial PCB mixtures (*29, 34*). The bromobiphenyls were completely dehalogenated as well (*35*). It was hypothesized that the brominated analogue served as an electron acceptor, which may stimulate and support growth of PCB dechlorinators.

The role of 2-MBDD in stimulating extensive *peri*-, rather than lateral dechlorination of higher chlorinated congeners is unclear. Bedard et al. (*29*) reported on predominantly *meta*-dechlorination of Aroclor 1260 in response to priming with 2-bromobiphenyl; they argued that two flanked *ortho*-chlorines are required to initiate *meta*-dechlorination and to sustain priming activity. It is possible that, since the 2,3,7,8-substitution sequence stabilizes the ring with respect to charge on the dioxin carbon skeleton (*25*), *peri*-dechlorination is stimulated.

Hydrogen amendments (treatment five) to the sediment slurry resulted in the accumulation of 23 mol % of 2-MCDD, without significant accumulation of either 1,2,3,4,6,7,8-HpCDD (8.4%) or 2,3,7,8-TCDD (~13%). It should be noted that these sediments contained the highest total PCDD concentrations of all reactor triplicates tested, predominantly on account of the presence of OCDD (Table 1). The observed shift toward lesser chlorinated isomers was surprising considering the short time period of incubation (10 days). When compared to the organic acid system (both treatments received the electron donor cocktail), the OCDD fraction remained the same, but HpCDD and TCDD decreased by

TABLE 3. Dioxin Dechlorination Rates in Spiked and Historical Passaic River Matrices

environmental system	dechlorination rates,[a] pmol/d			
	OCDD	1,2,3,4,6,7,8-HpCDD	2,3,7,8-TCDD	2-MCDD
Solid-Free Systems				
spiked sediment-eluted cells	−38.8 ± 6.2	1.80 ± 0.13	0.14 ± 0.004	0.33 ± 0.02
cells + 3,4-DHBA[b]	−75.3 ± 9.4	2.10 ± 0.17	0.83 ± 0.06	0.43 ± 0.03
3,4-DHBA alone	NC	2.10 ± 0.15	1.23 ± 0.04	ND
nonspiked cells[c]	NA	NA	−0.47 ± 0.29	0.23 ± 0.03
Soil/Sediment Systems				
autoclaved	0.75	−0.28	0.36	0
organic acid	−5.66	3.97	0.08	<0.005
brominated	−5.24	−0.30	2.60	0.06
hydrogen	−6.76	−8.94	−28.59	79.32

[a] The rates were based on 25 g DW of sediments and 5 mL of liquid for the sediment and solid-free systems, respectively. Solid-free systems were spiked with OCDD dissolved in decane to an initial concentration of 11.4 μmol/L (≈5 mg/L). [b] 3,4-DHBA, dihydroxybenzoic acid. [c] Only 2,3,7,8-TCDD was found to have selectively partitioned to microorganisms in sediment cores; ND, not detected; NA, not applicable; NC, not calculated.

76% and 25%, respectively. Figure 3 shows SIM scans of the monoCDD window for both baseline (A) and hydrogen-amended (B) sediment extracts. The bottom profile in each panel shows ^{13}C-labeled 1- and 2-MCDD standards, whereas the top scan indicates the appearance of 2-MCDD in hydrogen treatments.

Hydrogen amendments have been demonstrated to stimulate reductive dechlorination of chlorinated solvents, resulting in the complete dechlorination to ethene (*30*). No mechanism of activity was inferred. Nevertheless, reports that hydrogenase enzymes in sulfate-reducing bacteria and other anaerobic bacteria can serve as multifunctional metal-ion reductases (*36*) and hydrogen utilization as electron donors for autotrophic halorespiring microorganisms (*37*) indicate a role for hydrogen as a suitable driving force for dechlorination reactions. Our earlier observations showed that nonmethanogenic, nonsporeforming populations were responsible for the production of tri- to monochlorinated dioxins from higher chlorinated congeners under freshwater conditions (*20*). Considering the general reducing properties of hydrogen, it is likely that both biotic and abiotic (quinonic moieties associated with sediment humic matter) may have been responsible for PCDD dechlorination reactions. The apparent correlation between 2,3,7,8-TCDD reduction and 2-MCDD production indicates a predominance of lateral dechlorination reactions, which have been observed earlier in historically-contaminated sediment-derived cell suspensions (*20*).

Implications for Bioremediation and 2,3,7,8-TCDD Dynamics in Sediment Systems. Since 2,3,7,8-TCDD is both produced from dechlorination of higher chlorinated PCDD (activation, upper pathway) and further dechlorinated to 2-monoCDD (detoxification, lower pathway), it is important to recognize the relative kinetics of both pathways. It should be noted that 2-MCDD production is indicative but no absolute proof of 2,3,7,8-TCDD dechlorination, as it can be produced via dechlorination of 1,2,3,4,6,7,9-HpCDD as well. Since dechlorination reactions result in the loss of mass but not in total molar concentration, time-averaged concentrations are given in moles per time. Rates calculated from solid-free abiotic and biotic dioxin dechlorination experiments (*20, 22*) are given for the timepoint at which maximum accumulation of 1,2,3,4,6,7,8-HpCDD, 2,3,7,8-TCDD, and 2-MCDD was observed (Table 3). In the solid free system with historical PCDD, only rates for 2,3,7,8-TCDD disappearance and 2-MCDD production are given, as this isomer had selectively partitioned into the cells (*20*). The data from the sediment incubations were interpreted as [C(treatment) − C(av norm. init.)]*t^{-1} (90 days for treatments 2−4, and 10 days for the hydrogen treatment). Considering only two timepoints were taken into account, not the absolute values but rather the significance of relative magnitude of the rates merit discussion.

Based on the data of nonspiked cells contaminated with aged 2,3,7,8-TCDD, the rate of 2-MCDD production is half that of TCDD dechlorination, indicating that an intermediary dechlorination step is rate-limiting. Indeed, transient buildup of TriCDD was observed during this stoichiometric (>95%) dechlorination reaction. The ratio of 2,3,7,8-TCDD to 2-MCDD production observed in incubations with historically contaminated cells (2:1) is considered to be reflective of the maximum attainable rate of microbial 2,3,7,8-TCDD dechlorination by Passaic River sediment-derived microbial populations. This assumption is reasonable since the aged planar 2,3,7,8-TCDD and cells are most likely at equilibrium after 30 years of sediment contamination.

OctaCDD-spiked sediment eluted (and contaminated) cells exhibit a net production, rather than dechlorination of 2,3,7,8-TCDD on account of dechlorination of higher chlorinated congeners, which masks the trend observed in nonspiked cells. In this system, the rate of 2-MCDD production is twice as high as that of 2,3,7,8-TCDD production and 50% higher than that observed in nonspiked cells. Nevertheless, the production rates of 2-MCDD are not necessarily reflective only of 2,3,7,8-TCDD dechlorination because this TCDD isomer constituted 17% of all TCDDs formed during OCDD dechlorination (*22*). Therefore, the production of 2-MCDD has to be—at least in part—attributed to the dechlorination of non-2,3,7,8-substituted congeners. The combination of model humic constituents (MHC) with cell suspensions does result in higher production rates of both 2,3,7,8-TCDD and 2-MCDD; in these systems, 2,3,7,8-TCDD accounted for up to 50% of all TCDDs observed. Since similar ratios of 2,3,7,8-TCDD to ∑TCDD were observed in MHC-amended reactions alone, at even higher production rates than in cell suspensions, the TCDD isomer will likely accumulate in the presence of MHC as the ratio is 2:1 in favor of 2,3,7,8-TCDD production.

According to this rationale and dechlorination pattern analysis (*16, 20, 22*), the following concepts apply in environmental matrices where both organic matter and cells are present: (i) the upper pathway is strongly enhanced by abiotic reaction mechanisms which influence the relative ratio of 2,3,7,8-TCDD to ∑TCDD, (ii) the lower pathway of 2-MCDD production is microbially mediated, and (iii) deconvolution of 2-MCDD production rates with respect to 2,3,7,8-TCDD dechlorination can only be demonstrated when correlated to production and dechlorination of 2,3,7,8-substituted dioxin residues.

Considering that the baseline dioxin patterns were dominated by OCDD, both HpCDD isomers and the 2,3,7,8-TCDD isomer, a correlation between 2-MCDD production

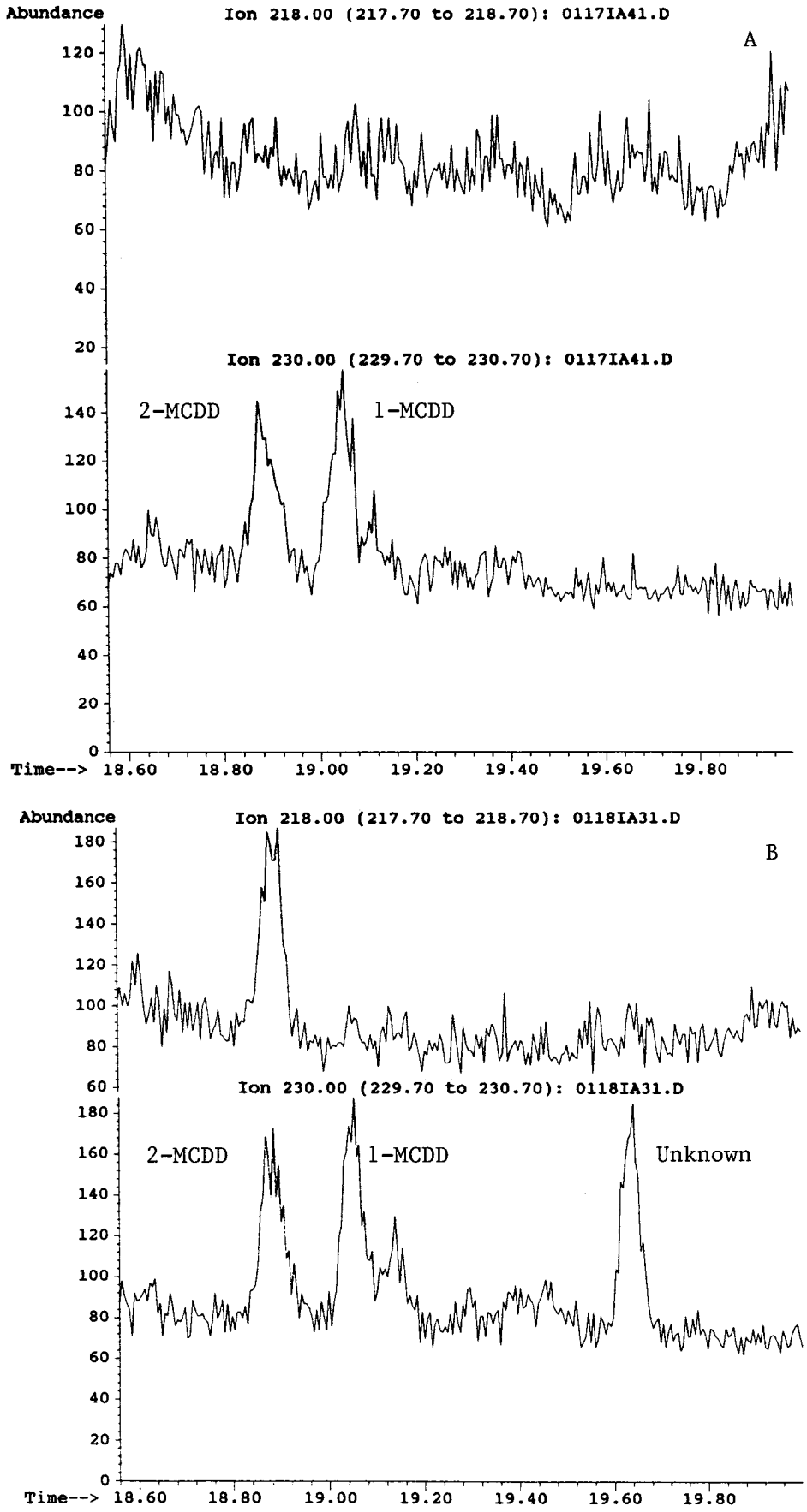

FIGURE 3. SIM chromatograms of the monoCDD window in baseline (A) and hydrogen-amended (B) sediment extracts. Top traces indicate ^{12}C-MCDD and bottom traces show the standard ^{13}C-labeled MCDD.

and the dynamics of the higher chlorinated PCDD congeners is likely significant. Moreover, aside from the production of 1,2,3,4,6,7,9-HpCDD, no other non-2,3,7,8-substituted residues were quantifyable in the sediment extracts, and the predominant reactions were dechlorinations in the peri-positions. The rates of OCDD disappearance relative to product appearance in sediment systems is strongly indicative of a predominance of dechlorination reactions of PCDD transformation in sediments. For example, the rates of appearance of 1,2,3,4,6,7,8-HpCDD, and 2,3,7,8-TCDD are 50−70% of those of OCDD disappearance in the case of organic acid and brominated dioxin treatments. Production rates of HpCDD far exceed those of 2,3,7,8-TCDD in the organic acid and 2-MBDD-amended systems indicating that HpCDD dechlorination may be rate-limiting in these amendments. Contrary, the molar rate of 2-MCDD production in hydrogen-amended treatment is twice as high as that of the combined disappearance rates of OCDD to TCDD, indictating that HpCDD and 2,3,7,8-TCDD are not expected to accumulate in this system. The lack of significant buildup of non-2,3,7,8-substituted dioxins precludes 2-MCDD production via other pathways. As these rates are only indicative of the relative kinetics of production vs dechlorination of 2,3,7,8-TCDD, more detailed multiple timepoint reaction kinetics of dioxin dechlorination are required to elucidate rate-limiting steps in the dechlorination sequence.

Differentiation between microbial and abiotic processes in sediments cannot easily be derived from solid-free systems, since 2,3,7,8-substituted congeners were dominant in the initial dioxin composition and thus *peri*-dechlorination is dominant. However, the production of 2-MCDD in sediments at the expense of a depletion of 2,3,7,8-TCDD, a process which was only observed in the presence of microbial cells, is indicative of a predominance of microbial dechlorination processes in the hydrogen-amended system. The stimulation of *peri*-dechlorination without accumulation of 2,3,7,8-TCDD and further lateral dechlorination to 2-MCDD in the presence of hydrogen gas presents a promising approach for the development of enhanced remediation strategies for dioxin-contaminated sediments.

Acknowledgments

This work was supported in part by EPA Grant R-819605-01 and Office of Naval Research Grant M 00014-96-100595. Because these results were not peer-reviewed by either agency, no endorsement should be inferred.

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Received for review August 3, 1998. Revised manuscript received November 19, 1998. Accepted December 3, 1998.

ES980791T