Carbon and Chlorine Isotope Fractionation During Microbial Degradation of Tetra- and Trichloroethene

Supporting Information

Charline Wiegert,[1] Manolis Mandalakis,[2] Tim Knowles,[3+] Paraskevi N. Polymenakou,[2] Christoph Aeppli,[1°] Jiřina Macháčková,[4] Henry Holmstrand,[1] Richard P. Evershed,[3] Richard D. Pancost[3] and Örjan Gustafsson[1]*

[1]Department of Applied Environmental Science (ITM), Stockholm University, 106 91 Stockholm, Sweden

[2]Hellenic Centre for Marine Research (HCMR), 71003, Heraklion, Crete, Greece

[3]School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom

[4]AECOM CZ s.r.o., Liberec 460 11, Czech Republic

[+]current address: Mass Spec Analytical Ltd., Building 20F, Golf Course Lane, Bristol, BS34 7RP

[°]current address: Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

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S1. Soil sampling: groundwater data

Table S1. Terrain measurements, CEs concentrations and hydrogeochemical parameters at the groundwater wells located at the soil samplings areas.

Soil	Z-32	Z-53	Z-54	Z-65
Borehole Groundwater	N-32	N-53	N-54	N-65
Well depth [m below groundsurface]	2.44	2.78	3	3.37
GWT [m below groundsurface]	0.91	1.33	0.9	0.60
pH	7	6.3	6.3	6.7
Conductivity [µS·cm^{-1}]	374	370	313	364
Temperature [°C]	9.3	8.8	8.5	9.4
Redox potential [mV]	-97	-242	-74	-75
H_2S [ppm]	0.1	0.1	0.3	<d.l.[a]
CH_4	<d.l.[a]	<d.l.[a]	<d.l.[a]	<d.l.[a]
O_2 [mg·L^{-1}]	0.45	0.23	0.92	0.29
CE total [µmol·L^{-1}]	22.48	82.45	95.63	0.02
PCE [µmol·L^{-1}]	8.56	25.27	46.13	0.02
TCE [µmol·L^{-1}]	3.74	28.31	36.15	<0.004
1,2 cis DCE [µmol·L^{-1}]	10.08	26.30	12.17	<0.01
VC [µmol·L^{-1}]	<0.06	2.21	1.05	<0.06
Ethene [µmol·L^{-1}]	<0.18	0.29	<0.18	<0.18
Sum of cations [mg·L^{-1}]	85.8	75	71.3	119
Sum anions [mg·L^{-1}]	239	185	189	290
NH_4^{3+}	0.071	0.176	<0.05	28.4
Cl^- [mg·L^{-1}]	7.84	30.3	12.4	35.3
COD-Mn	0.8	7.3	1.2	4.8
NO_3^{2-} [mg·L^{-1}]	<2	10.7	<2	<2
NO_2^- [mg·L^{-1}]	<0.005	0.0364	0.024	<0.005
F^- [mg·L^{-1}]	<0.2	<0.2	<0.2	<0.2
PO_4^{3-} [mg·L^{-1}]	<0.04	0.065	0.044	<0.04
SO_4^{2-} [mg·L^{-1}]	33.3	48.9	21.2	67.7
HCO_3^- [mg·L^{-1}]	198	95	156	187
Dissolved substances (drying at 105°C) [mg·L^{-1}]	218	222	164	230
Basic neutralisation capacity at pH 8.3 [mg·L^{-1}]	0.229	0.489	0.198	0.404
Acidic neutralisation capacity at pH 4.5 [mg·L^{-1}]	3.24	1.56	2.55	3.07
CO_2 total [mg·L^{-1}]	152.67	90.08	120.89	152.72
CO_2 free [mg·L^{-1}]	10.07	21.52	8.71	17.77
CO_2 agresive [mg·L^{-1}]	0.65	17.89	3.37	7.24
Ca [mg·L^{-1}]	39.2	43.8	54.4	37.9
Fe [mg·L^{-1}]	1.7	4.7	1.1	3.5
K [mg·L^{-1}]	1.7	1.8	2.2	29.0
Mg [mg·L^{-1}]	6.6	4.3	4.8	4.4
Mn [mg·L^{-1}]	0.1	0.6	<d.l.[a]	6.2
Na [mg·L^{-1}]	6.3	19.6	8.7	12.7

[a]d.l. = detection limit

S2. Degradation experiments: concentrations analyses

The analysis of PCE and TCE over the course of the incubation was performed as follow: volumes of 50 µL of the culture were mixed to 100 µL of ethyl acetate, containing 25 ppm (0.2 $mmol \cdot L^{-1}$) of isooctane as internal standard, into a polypropylene Eppendorf vial. The vial was shaken for 1 min on a vortex shaker, and allowed to stand for 30 s until phase separation. 1 µL of the organic phase was then injected onto a HP 6890 GCMS. The injection port was kept at 180°C and operated in split mode with a split ratio of 20:1. The analytes were separated on a FS−Supreme−5ms capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) using helium as carrier gas at 35 $cm \cdot s^{-1}$ constant flow rate. The GC oven was ramped from 34 to 36°C at $0.5°C \cdot min^{-1}$, then to 40°C at $3°C \cdot min^{-1}$, and finally to 90°C at $40°C \cdot min^{-1}$, held for 0.5 min. The MS recorded the masses (isooctane: *m/z* 57; TCE: *m/z* 130, 132, 134 and 136*;* PCE: *m/z* 164, 166, 168 and 170) using the positive electron impact ionization (EI+) in the single ion monitoring (SIM) mode.

The transformation of PCE and TCE to DCE and the stability of DCE over the course of time were also verified by a separate biodegradation experiment. A separate culture was inoculated with 10 mL of cell suspension from Z-32 primary culture and amended with a mixture of PCE and TCE to attain an initial concentration of 40 ppm, e.g. 0.24 and 0.30 mmol·L-1 for PCE and TCE respectively. In this experiment, headspace solid-phase microextraction (HS-SPME) coupled with GCMS was used in order to measure the concentrations of PCE, TCE and to perform a semi-quantitative analysis of DCE in the liquid culture (assuming that the response factor of DCE in full-scan mode was similar to that of PCE and TCE). The semi-quantitative analysis of DCE was feasible because HS-SPME is a solvent-free method of analysis. While the concentrations of PCE and TCE remained almost unchanged for the first 10 days, the analysis of

the same culture after 76 days of incubation indicated the complete loss of PCE and TCE and the formation of DCE. The presence of DCE in the culture medium after such a long incubation period provides strong evidence that the degradation of DCE was very slow, if it happens at all, leading to the accumulation of this metabolite in the culture. However, since SPME performance changed over the course of time and replacement of SPME fiber was needed after a limited number of injections, in-vial liquid-liquid microextraction was preferred to HS-SPME during the implementation of the final biodegradation experiments.

S3. Clone Library Construction and Sequence Analysis of the 16S rRNA genes

PCR conditions were designed to minimize bias. Replicate PCR reactions, a low number of amplification cycles and an annealing temperature of 55^{o}C were applied to avoid the preferential amplification of certain sequences that may introduce "artificial" diversity. For each sample, eight replicate PCR reactions of 20 mL were amplified in a MJ Robocycler with initial denaturation at 94^{o}C for 3 min followed by 25 cycles of 1 min at 94^{o}C, 1 min annealing at 55^{o}C, 3 min primer extension at 72^{o}C, and a final extension at 72^{o}C for 7 min. Each tube contained 1–4 ng of target DNA, PCR buffer (10 mM Tris–HCl at pH 9, 50 mM KCl, 0.1% Triton X-100, and 2 mM $MgCl_2$), 100 nM of each primer, 200 mM of each deoxyribonucleotide triphosphate and 0.25 U Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). PCR-negative controls (without DNA template) yielded no products (data not shown), whereas PCR-positive controls (with DNA template known to produce a specific product) were always successful during the experiments. All PCR products were pooled and concentrated in a centrifugal vacuum evaporator (Centrivac, Heraeus Instruments), followed by gel purification using the Qiaquick PCR purification kit (Qiagen, Valencia, CA, USA). The concentration of PCR products generated from the different sediment samples was determined by direct comparison to a Low DNA Mass Ladder (Invitrogen) using 2% agarose gel electrophoresis, ethidium bromide staining, and UV transillumination. For each sample, 5–10 ng of PCR product was cloned into the pCR4-TOPO vector and transformed into One shot TOP10 chemically competent cells of *Escherichia coli* using the TOPO TA Cloning kit (Version M) as recommended by the manufacturer (Invitrogen). At least 100 positive clones from each clone library (selected by blue and white screening) were transferred to 96-well plates and incubated overnight at 37^{o}C in Luria–Bertani medium containing 50 mg kanamycin mL^{-1}. Aliquots of the individual clones were (1) archived at −80^{o}C

in 7% dimethyl sulfoxide or (2) washed by pelletizing cells in a 30-min centrifugation at 10,000 × g followed by supernatant removal by low-speed centrifugation (500 rpm) of inverted plates. Pelletized cells were resuspended in 30 mL sterile and UV-irradiated MQ-grade water. Cells were lysed by heating at 98°C for 10 min followed by agitation. The lysates were used (1:10 *v/v*) as templates in a PCR amplification of the insert using external (vector) primers M13f-20 (50-GTAAAACGACGGCCAG-30) and M13r (50-CAGGAAACAGCTATGAC-30; Invitrogen) to avoid co-amplification of *E. coli* host-cell DNA. PCR amplification was carried out for 25 cycles as described before and sent to the Macrogen company (Macrogen Europe, Amsterdam, the Netherlands) for sequencing with the primer 27F. A total of 226 sequences were successfully produced on an ABI 3700 sequencer (Applied Biosystems) using the BigDye terminator kit (v3.1 Applied Biosystems). This procedure generated high quality reads of 650-1050 bases. Using Chimera Check software included in the Ribosomal Database Project II (Michigan State University, East Lansing, MI, USA), none of the sequences could be identified as being chimeras. This was further confirmed during the submission of the sequences to the Global Database of Genbank where a quality check is always performed before their public release. All sequences were compared to GenBank entries using BLAST (Basic Local Alignment Search Tool, National Center for Biotechnology Information, Bethesda, MD, USA) in order to obtain preliminary phylogenetic affiliations of the clones.

S4. Clones identification results

Table S2. Clones identified from the PCE degradation experiments at three different time points, corresponding to 100%, 53% and 7.6% remaining fraction of PCE. The names in italics indicate culture strains. The identified operational taxonomic units (OTUs) are classified into five phyla, the *Actinobacteria*, the *Alpha-*, *Beta-* and *Gammaproteobacteria* and *Firmicutes.*

	Access No.	clones No.	BLAST match (with accession No.)	Match (%)	Taxonomy
100% remaining fraction of PCE	*KC109150*	1	Uncultured ncd2149a03c1 (JF180710)	99	Actinobacteria, Propionibacteriaceae
	KC109147	1	*Agrobacterium sp. SDW052 (AF508209)*	99	Alphaproteobacteria, Rhizobiaceae
	KC109153	1	Uncultured clone E158 (HQ828046)	99	Alphaproteobacteria, Sphingomonadaceae
	KC109156	5	*Sphingomonas rhizogenes strain BW59UT1570 (JF276901)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109145	1	Uncultured Ralstonia sp. IODP_305_1309D_13 (HQ379137)	100	Betaproteobacteria Burkholderiaceae
	KC109157	1	*Ralstonia sp. PH-S1 (JN543508)*	99	Betaproteobacteria, Burkholderaceae
	KC109151	1	Uncultured clone IS-58 (CQ339173)	95	Betaproteobacteria, Gallionellaceae
	KC109152	1	Uncultured clone kab227 (FJ936944)	95	Firmicutes, Clostridiales
	KC109154	1	Uncultured clone ncd2292g09c1 (JF197169)	99	Firmicutes, Clostridiales
	KC109148	***55***	***Clostridium sp. strain DR7 (Y10030)***	***98***	***Firmicutes, Clostridiales***
	KC109155	1	Uncultured clone ASC8 (JF357615)	99	Firmicutes, Lactobacillales, Carnobacteriaceae
	KC109158	1	*Lactobacillus sakei strain kimshi007 (JF781305)*	98	Frmicutes, Lactobacillaceae
	KC109146	1	Uncultured Stenotrophomonas sp. F1jun.8 (GQ416874)	99	Gammaproteobacteria, Xanthomonadaceae
	KC109149	1	Uncultured ncd1466a11c1 (JF126281)	99	unidentified
53% remaining fraction of PCE	*KC109185*	2	*Shingomonas rhizogenes strain RSB-1 (AY962684)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109177	1	*Sphingomonas sp. PPs-1 (FJ605417)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109183	1	*Sphingomonas sp. 070605-23_L09_7 (FJ626798)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109184	1	Uncultured clone E158 (HQ828046)	99	Alphaproteobacteria, Sphingomonadaceae
	KC109178	1	*Ralstonia sp. Tianjin P1 (GU936705)*	99	Betaproteobacteria Burkholderiaceae
	KC109175	3	*Achromobacter sp. Zx6 (FJ463168)*	99	Betaproteobacteria, Alcaligenaceae
	KC109182	1	*Ralstonia pickettii isolate DiSca9 (EF195095)*	99	Betaproteobacteria, Burkholderiaceae
	KC109181	1	*Desulfovibrio idahonensis strain CY2 (AJ582758)*	99	Deltaproteobacteria, Desulfovibrionaceae
	KC109174	***10***	***Desulfitobacteirum aromaticivorans UKTL (EU711071)***	***96***	***Firmicutes, Clostridiales***
	KC109173	***51***	***Clostridium sp. strain DR7 (Y10030)***	***99***	***Firmicutes, Clostridiales***
	KC109176	1	*Lactobacillus sakei strain kimshi007 (JF781305)*	99	Firmicutes, Lactobacillaceae
	KC109180	3	*Leuconostoc carnosum strain NRIC 1722 (NR_040811)*	99	Firmicutes, Lactobacillales
	KC109179	1	*Pelosinus sp. UFO1 (DQ295866)*	97	Firmicutes, Veillonellaceae
	KC109187	1	*Pseudomonas stutzeri strain LYS-86 (GQ402828)*	99	Gammaproteobacteria, Pseudomonadaceae
	KC109188	1	*Pseudomonas sp. BC046 (HQ105014)*	99	Gammaproteobacteria, Pseudomonadaceae
	KC109186	1	Ucultured clone ncd2400h09c1 (JF208943)	99	unidentified
7.6% remaining fraction of PCE	*KC109164*	1	*Propionibacterium acnes 266 (CP002409)*	99	Actinobacteria, Propionibacteriaceae
	KC109163	1	Sphingomonas sp. oral clone AV069 (AF385529)	99	Alphaproteobacteria, Sphingomonadaceae
	KC109167	2	*Shingomonas rhizogenes strain RSB-1 (AY962684)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109169	1	*Sphingomonas sp. PPs-1 (FJ605417)*	99	Alphaproteobacteria, Sphingomonadaceae
	KC109165	4	*Desulfovibrio idahonensis strain CY2 (AJ582758)*	99	Deltaproteobacteria, Desulfovibrionaceae
	KC109171	1	*Geobacter hephaestius (AY737507)*	95	Deltaproteobacteria, Geobacteriaceae
	KC109159	***34***	***Desulfitobacteirum aromaticivorans UKTL (EU711071)***	***92***	***Firmicutes, Clostridiales***
	KC109168	***22***	***Clostridium sp. strain DR7 (Y10030)***	***97***	***Firmicutes, Clostridiales***
	KC109170	2	*Pseudomonas stutzeri (JF970598)*	99	Gammaproteobacteria, Pseudomonadaceae
	KC109172	1	Uncultured clone nbu544d04c1 (GQ032655)	99	unidentified
	KC109162	2	Uncultured spirochete clone ccslm210 (AY133082)	99	unidentified
	KC109166	1	Uncultured clone nbw1140g05c1 (CQ080894)	99	unidentified
	KC109160	1	Uncultured clone 29a08 (EF515483)	98	unidentified
	KC109161	1	Uncultured clone E37 (EU864479)	96	unidentified

S5. Stable chlorine isotope analysis: notes on calibration

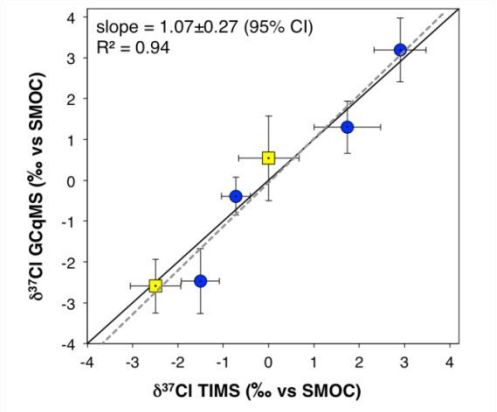

Figure S1. Trueness of $\delta^{37}Cl$ of used analytical method. Shown are PCE (blue circles) and TCE (yellow squares) isotopic standards measured by an off-line reference method (TIMS, x-axis) and the GC/qMS method used for measuring $\delta^{37}Cl$ of PCE and TCE samples described in the main text. The solid line represents the 1:1-line, the dashed line is a liner regression (slope = 1.07±0.27). This figure shows that a one-point calibration produced true results within the average analytical uncertainty of the used GC/qMS method of ±0.6‰ (see main text).

Table S3. Used isotopic standards

	TIMS [‰ vs SMOC]	***n***	**GC/qMS [‰ vs SMOC]**	***n***
PCE0 (Sigma-Aldrich)	-0.27±0.31	5	(a)	
PCE1	2.90±0.58	3	3.19±0.78	10
PCE5	1.74±0.73	3	1.30±0.64	10
PCE PPG	-1.51±0.42	3	-2.47±0.80	8
PCE Merck	-0.72±0.32	2	-0.39±0.46	10
TCE (Sigma-Aldrich)	2.90±0.38	2	(a)	
TCE PPG	-2.49±0.55	3	-2.59±0.66	9
TCE Merck	0.00±0.67	3	0.54±1.04	9

(a) used as isotopic standard in the GCqMS method.

S6. Concentrations of CEs and stable carbon and chlorine isotopic data set

Table S4. Remaining fraction, C and Cl isotope signatures for PCE (left) and TCE (right) degradation experiments, indicated with the precision of the measurements (see the Material and Method section in the main text). The $\delta^{13}C$ values for TCE as a product of PCE degradation in the PCE experiments were also determined. Note that the $\delta^{37}Cl$ signature measured for PCE5 was removed as an outlier (by applying the Cook's distance test),[1,2] and was therefore not used in the ε_{Cl} calculations.

PCE experiments		PCE		TCE	TCE experiments			
Sample	% Remaining Fraction	$\delta^{13}C$ [‰ vs VPDB]	$\delta^{37}Cl$ [‰ vs SMOC]	$\delta^{13}C$ [‰ vs VPDB]	Sample	% Remaining Fraction	$\delta^{13}C$ [‰ vs VPDB]	$\delta^{37}Cl$ [‰ vs SMOC]
Control1	112.9±5.9	-24.0±0.1	1.1±0.9	n.d.	**Control1**	98.9±3.3	-28.1±0.1	3.0±0.5
Control2	100.2±6.6	-25.9±0.7	0.9±0.7	n.d.	**Control2**	99.1±3.9	n.d.	3.6±0.8
PCE1	100.8±5.1	-25.8±0.1	1.1±0.6	n.d.	**TCE1**	97.2±2.7	n.d.	n.d.
PCE2	26.8±1.4	-16.1±0.2	3.8±0.6	-19.6±0.2	**TCE2**	7.2±0.3	2.7±0.2	12.9±0.8
PCE3	3.5±0.2	-7.0±0.7	7.5±0.8	-1.9±0.2	**TCE3**	5.2±0.1	0.3±0.6	15.1±0.5
PCE4	19.4±0.9	-15.5±0.6	2.7±0.5	-17.5±0.6	**TCE4**	74.2±4.3	-23.8±0.6	5.3±0.6
PCE5	13.2±0.7	-13.7±0.8	1.4±0.6	-13.2±0.3	**TCE5**	29.4±1.2	-14.0±0.4	8.6±0.9
PCE6	7.1±0.4	-8.0±0.2	6.4±0.5	-7.9±0.3	**TCE6**	19.8±0.8	-8.5±0.1	10.7±0.4
PCE7	17.3±0.7	-15.6±1.1	5.6±0.9	-14.0±0.2	**TCE7**	13.3±0.3	-5.8±0.6	12.7±0.5
PCE8	71.3±3.3	-21.9±0.1	1.2±0.5	-26.7±0.8	**TCE8**	3.5±0.1	-2.0±0.2	16.6±0.5
PCE9	7.6±0.3	-9.9±0.4	6.6±0.5	-7.5±0.2	**TCE9**	9.4±0.2	-3.6±0.2	14.2±1.0
PCE10	53.1±2.2	-20.9±0.5	2.7±0.4	-25.1±0.6	**TCE10**	57.7±3.0	-20.8±0.1	7.1±0.5
PCE11	37.2±2.2	-17.6±0.3	3.1±0.6	-22.0±0.3	**TCE11**	100.2±3.4	-26.9±0.0	5.2±0.5

Section S7: Comparison of ε and AKIE values for C and Cl isotopes in different studies on CEs

Table S5. Comparison of of ε and AKIE values of CEs from different studies. The AKIE were compared as the ratio $(AKIE_{Cl}-1)/(AKIE_C-1)$, as described in Elsner and Hunkeler (2008)[3] and applied in Abe et al. (2009).[4]

Compound	Degradation pathway	Type	Experimental conditions	ε_C [‰ vs VPDB] ± 95%CI	n_C	x_C	z_C	$AKIE_C$	ε_{Cl} [‰ vs SMOC] ± 95%CI	n_{Cl}	x_{Cl}	z_{Cl}	$AKIE_{Cl}$	$\varepsilon_{Cl}/\varepsilon_C$	$(AKIE_{Cl}-1)/(AKIE_C-1)$	Reference
PCE	reductive dechlorination	biotic	laboratory	-5.6±0.7	2	2	2	1.0113	-2.0±0.5	4	4	4	1.0081	**0.35±0.11**	**0.71**	This study
	reductive dechlorination	biotic	field											**0.42±0.39**		Wiegert et al. (2012)[5]
	reductive dechlorination	biotic	field											**1.12±0.74**		Wiegert et al. (2012)[5]
TCE	reductive dechlorination	biotic	laboratory	-8.8±0.2	2	1	1	1.0179	-3.5±0.5	3	1	1	1.0106	**0.37±0.11**	**0.59**	This study
	reductive dechlorination	abiotic	laboratory	-13.4±1.7	2	1	1	1.0275	-2.6±0.1	3	1	1	1.0079	**0.19±0.01**	**0.29**	Audí-Miro et al. (2012)[6]
	reductive dechlorination	abiotic	field	-12.40	2	1	1	1.0254	-2.98	3	1	1	1.0090	**0.24**	**0.35**	Lojkasek-Lima et al. (2012)[7]
cDCE	reductive dechlorination	biotic	laboratory	-18.5	2	2	2/1[a]	1.0384	-1.5	2	2	2/1[a]	1.0030	**0.081**	**0.08**	Abe et al.(2009)[4]
	oxidation	biotic	laboratory	-8.5	2	2	1	1.0086	-0.3	2	2	1	1.0003	**0.035**	**0.04**	Abe et al.(2009)[4]
	reductive dechlorination	abiotic	laboratory	-18.7±1.5	2	2	2	1.0387	-6.2±0.8	2	2	2	1.0126	**0.33±0.20**	**0.32**	Audí-Miro et al. (2012)[6]
	reductive dechlorination	biotic	field											**0.48±0.05**		Hunkeler et al (2011)[8]
VC	reductive dechlorination	biotic	laboratory	-25.2	2	1/2[b]	1	1.0531	-1.8	1	1	1	1.0018	**0.071**	**0.03/0.07[a]**	Abe et al.(2009)[4]
	oxidation	biotic	laboratory	-7.2	2	2	1	1.0073	-0.3	1	1	1	1.0003	**0.042**	**0.04**	Abe et al.(2009)[4]

[a] Note that z can be 2 or 1 for the reductive dechlorination of cDCE depending on the mechanism. However the resulting $(AKIE_{Cl}-1)/(AKIE_C-1)$ ratio remains unchanged (see Abe et al.[4] for details about the mechanisms)

[b] Similarly, x_C can be 2or 1 for VC reductive dechlorination, resulting in different $(AKIE_{Cl}-1)/(AKIE_C-1)$ ratios (see Abe et al.[4] for details about the mechanisms)

S8. References

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