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The biogeochemistry of toluene in coastal seawater: radiotracer experiments in controlled ecosystems

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Abstract. The fate of toluene in coastal seawater was investigated in controlled ecosystems using ^{14}C- and ^{3}H-toluene as tracers. Under winter-like conditions, 80% of the toluene volatilized from the water column in 2 months. Microbial degradation was less important than volatilization and sorption onto particulate matter with resultant loss to the sediments was minor. During summer most of the toluene was degraded by microbes. Nearly 80% of the toluene was converted to CO_2 within 1 week and the label remained in the water column as dissolved CO_2. The experimental results were applied to estimate the removal rates and the residence time of toluene in adjacent Narragansett Bay, Rhode Island. In winter volatilization would dominate the loss of toluene and a residence time of 6 d would be predicted. However, rapid biodegradation in summer would result in a residence time of < 1 d.

Introduction

Toluene is one of the most abundant volatile organic compounds (VOC) in coastal seawater. Concentrations range from several thousands of $ng\,l^{-1}$ in Narragansett Bay near Providence, Rhode Island to tens of $ng\,l^{-1}$ in waters remote from urban source areas (Schwarzenbach et al. 1978; Sauer et al. 1978; Gschwend 1979; Sauer 1980; Gschwend et al. 1980; Sauer 1981; Gschwend et al. 1982; Wakeham et al. 1983a). Toluene's relatively high solubility in seawater (Sutton and Calder 1975; Rossi and Thomas 1981), its relatively high toxicity to marine organisms (Rise et al. 1977; Hutchinson et al. 1979), and its high concentration in many coastal areas makes it important to understand the environmental fate of this and related compounds. Our previous mesocosm experiments (Wakeham et al. 1983b) suggested that volatilization plays a dominant role in removing toluene from seawater under a range of natural conditions, with biodegradation as an additional sink primarily in summer. However these experiments provided limited information about rates and mass balances could not be calculated. In the experiments reported here, the fate of toluene was studied in a marine mesocosm using ^{14}C- and ^{3}H-toluene as tracers. Time-course changes in activities of dissolved ^{14}C-toluene, ^{14}C incorporated into intermediate metabolites, $^{14}CO_2$, and ^{14}C associated with particulate matter were

measured. Microbial activity, ^{3}H-toluene uptake, and ^{14}C-toluene respiration were also examined. With these data, mass balances were calculated which account for most of the ^{14}C-toluene initially added to the ecosystem and allow us to predict the fate and residence time of toluene in Narragansett Bay.

Materials and methods

The MERL (Marine Ecosystems Research Laboratory, University of Rhode Island) mesocosm is a fiberglass tank 5.5 m high and 1.8 m in diameter, containing 13.3 m^3 of Narragansett Bay seawater (Pilson et al. 1977; Santschi 1982). The experiments were run in batch and without sediments. The tank water column was mixed four times a day to simulate tidal turbulence in the bay. Two whole-tank ^{14}C-toluene experiments were conducted.

(1) On 24 March 1982 approximately 250 μCi of [methyl-^{14}C]-toluene (Amersham Corp., 30 mCi $mmol^{-1}$) were added to a tank which had been filled 2 d previously. The spike was prepared by mixing 5 ml of acetone containing the ^{14}C-toluene into 1.5 l of seawater in a 2–1 separatory funnel. This solution was then drained through a Teflon tube into the middle of the water column during mixing. Unlabeled (cold) toluene in the spike gave an initial concentration in the tank of about 2 $\mu g\,l^{-1}$. Difluorodichloromethane (Freon 12, F-12) was added to the water column as a gas exchange tracer (see Bopp et al. 1981 for F-12 addition procedure). After the addition, the tank was mixed for several hours before sampling began. The distribution of the label was followed until 20 May 1982. The purpose of this experiment was to simulate cold-water conditions (hereafter termed winter) so the tank was cooled by heat exchangers; nevertheless water temperatures gradually increased from 2 to 10 °C.

(2) A summer experiment was carried out between 23 August and 19 September 1982, when water temperatures were 18–19 °C. Some 350 μCi of ^{14}C-toluene (30 mCi $mmol^{-1}$), the cold toluene, and F-12 were added as described above to a freshly filled tank.

Water samples were collected through Teflon tubing from 1 m depth during mixing. Samples processed at MERL were analyzed as soon as possible following collection, while samples to be returned to Woods Hole Oceanographic Institution (W.H.O.I.) were collected in glass bottles without headspace, poisoned with $HgCl_2$, and analyzed within a few days. Samples were stored at 0 °C.

We traced the ^{14}C-activity in several compartments of the mesocosm.

(1) *Total-^{14}C.* The total ^{14}C-activity in the tank was determined directly by liquid scintillation counting (LSC) at MERL. Duplicate 2.5–1 samples were collected and two 10 ml subsamples from each were pipetted into liquid scintillation vials containing 10 ml of Aquasol scintillation fluor (New England

Nuclear) and 0.3 ml of phenethylamine. The four samples were counted in a Beckman LS-3150T liquid scintillation counter for 20 min or until 1% counting statistics were achieved. The four values were averaged to give total tank activity (μCi tank^{-1}). Counting efficiencies, which averaged 85%, were determined using the External Standard Channels Ratio method calibrated against a quench curve or by standard additions of a ^{14}C-toluene calibration standard (New England Nuclear). Precision of this procedure was about 5%.

(2) *Volatile (dissolved)-^{14}C-toluene.* To estimate the amount of dissolved, and hence volatile, ^{14}C-toluene in the tank water (as μCi tank^{-1}), we measured the ^{14}C-toluene recovered by closed-loop gas stripping (Grob and Zürcher 1976; Wakeham et al. 1983a) at W.H.O.I. The toluene was sparged from 250–500 ml water samples using headspace air and adsorbed from the gas stream by a trap containing 1.5 mg of activated charcoal. The charcoal was extracted with 5 ml of acetone and the extract counted in Aquasol by LSC in a Beckman LS-100C counter. Counting efficiencies with this solvent and counter were 88%. Duplicates were generally better than ± 15%. Stripping for a second hour yielded only a few percent more ^{14}C, while a second extraction of the charcoal filter recovered < 5% additional ^{14}C.

Stripping may not accurately reflect the fraction of toluene amenable to volatilization processes in the natural environment, since partitioning of toluene from the water into the gas phase may alter the equilibrium between dissolved, sorbed, and biologically accomodated forms. Thus we may actually overestimate the amount of toluene susceptible to volatilization at any given time. Direct measurements of toluene volatilization from the tank gave unrealistic results and we concluded that they were technically unfeasible.

(3) *Non-volatile-^{14}C (polar metabolites).* Dissolved ^{14}C-toluene in the water samples is efficiently recovered by stripping (> 95%). Initial degradation products, such as phenol and catechol (e.g. Gibson 1968), are highly polar and would be inefficiently stripped. To estimate the ^{14}C-activity associated with these non-volatile degradation products, 50 ml of stripped water were extracted in a separatory funnel with 3 x 2 ml of toluene and the ^{14}C determined by LSC (counting efficiency 90%).

(4) *Total-^{14}C recovered by solvent extraction (extractable ^{14}C).* As an independent check on the direct counting at MERL, we extracted 50 ml of tank water with 3 x 2 ml of toluene and counted the extract in Aquasol at W.H.O.I. Reproducibilities of this procedure were within a few percent and extraction of sample with additional toluene did not increase the yield significantly (< 1%).

(5) $^{14}CO_2$. This end-product of microbial degradation of ^{14}C-toluene was measured at MERL by acidifying 500 ml of water to pH 2 with 5 ml of 4N

H_2SO_4, sparging with N_2, passing the effluent gas through a trap containing 100 g of activated granular charcoal, and collecting the CO_2 in an absorber solution (Oxifluor-CO_2, New England Nuclear) for LSC. We checked that no ^{14}C-toluene passed through the charcoal ($< 0.5\%$), and that the $^{14}CO_2$ was quantitatively trapped in the absorber ($> 95\%$ of a $NaH^{14}CO_3$ standard was recovered). Analytical problems resulted in loss of $^{14}CO_2$ data for days 1–13 of the winter experiment.

(6) *Particulate-^{14}C*. The ^{14}C associated with particulate matter was measured by filtering 2–1 of water through glass fiber filters (Gelman A/E, nominal pore size 0.3 μm) and counting the filters in Aquasol by LSC at MERL (counting efficiency 80%). Duplicate filters were dried and suspended particulate carbon concentrations were determined by IR spectrophotometric measurements of CO_2 released by combustion at 950 °C in O_2. Once a week, the particulate matter was size-fractionated to determine if the ^{14}C was partitioned according to particle size. Zooplankton were collected with a 150 μm mesh net, phytoplankton and smaller zooplankton in the size range 10–150 μm were collected by a counter current filtration system (Hinga et al. 1979), and the $< 10\,\mu$m material was collected on glass fiber filters. In all cases, the $< 10\,\mu$m material accounted for $> 73\%$ of the total particulate ^{14}C-activity. Loss of ^{14}C to tank walls or to wall growth was found to be insignificant. This was checked by suspending pieces of wall material in the tank during the experiment and later measuring the accumulated ^{14}C, and by brushing the tank walls at the end of the experiments and collecting the resulting debris for ^{14}C measurements.

(7) *Freon*. Samples for F-12 analysis were collected in duplicate in 125 ml serum bottles, poisoned with $HgCl_2$, capped without headspace with Teflon-lined septa, and stored at 0 °C until the end of each experiment. Then at W.H.O.I. each entire batch of samples was analyzed. Helium was injected through the septum of the serum bottle to displace 2 ml of water. After equilibration overnight at 22 °C, the headspace was analyzed for F-12 by flame ionization gas chromatography (Bopp et al. 1981). Absolute concentrations of F-12 were not calculated; rather detector response (area units) vs. time was plotted to yield the half-life of F-12 in the tank water column.

(8) *Unlabelled toluene*. Concentrations ($\mu g\,l^{-1}$) of the unlabelled toluene in the tank were determined by closed loop gas stripping similar to that used for the volatile-^{14}C measurements. However, after sparging, the charcoal trap was eluted with 15 μl of distilled CS_2 and the toluene analyzed by flame ionization glass capillary gas chromatography as described previously (Wakeham et al. 1983a, b). Recoveries of toluene at the 0.1–2 $\mu g\,l^{-1}$ level were $> 95\%$. We assume that this procedure measures total (labelled + unlabelled)

toluene, although the concentration of ^{14}C-toluene would be small compared to that of the unlabelled toluene.

(9) *Microbial studies.* To investigate the role of bacteria in the disappearance of toluene, we carried out 4-h incubations of tank water with [methyl-^{3}H]-toluene (New England Nuclear) in small vials. The concentration of ^{3}H-toluene in the water was 8–15 nM (0.7–1.4 $\mu g\,l^{-1}$, 0.6 $\mu Ci\,ml^{-1}$). Separate incubations with a ^{3}H-labeled amino acid mixture (New England Nuclear) at a mean concentration of 2.5 μM (0.25 $\mu g\,l^{-1}$, 0.016 $\mu Ci\,ml^{-1}$) gave a relative measure of overall bacterial community metabolism.

All incubations were carried out in the experimental tank in 25 ml Teflon-lined screw cap vials and were terminated by addition of 1.25 ml of concentrated formaldehyde solution. Formaldehyde-killed samples were run as controls. Incubation tubes were returned to the Marine Biological Laboratory (MBL) and samples were filtered through 0.5 μm nylon filters (Rainin, Inc.); the use of nylon filters was found to greatly reduce unwanted adsorption of the unincorporated radioisotope to the filter while retaining bacterial particles. Filters were removed from the filtration apparatus while vacuum was applied to minimize capillary water carried in the filter. The filters were soaked and post-rinsed with at least twice the sample volume of filtered seawater, allowed to air dry for 1–2 hr before addition of Aquasol 2, and left overnight before LSC to allow maximal extraction of the label. The fraction taken-up (f) was calculated as $(dpm_{filter}\text{-}dpm_{blank})/dpm_{added}$. Turnover was calculated as t/f, where t is the incubation time.

The measurement of total substrate metabolism of microbes requires in addition measurement of the amount of substrate respired as well as the substrate metabolized and excreted in forms other than CO_2. While this latter aspect was beyond the scope of our current work, we did measure $^{14}CO_2$ evolution in in-situ incubations of ^{14}C-toluene at summer temperatures in a third experiment (19 September–7 October 1983). A cold toluene spike was added to the mesocosm to give a concentration of 5 $\mu g\,l^{-1}$ at day zero. Over the next 14 d respiration measurements were made using ring-labeled toluene; after two weeks, a parallel incubation comparing mineralization of ring- and methyl-^{14}C-toluenes was conducted. In each measurement, 100 μl of 0.5 $\mu Ci\,ml^{-1}$ of ^{14}C-toluene was added to replicate 25 ml and 125 ml tubes to give final concentrations of 2.3 and 0.46 $\mu g\,l^{-1}$, respectively. After 4 h incubations, alkaline toxin (Cole 1981) was used to preserve samples for analysis of respired ^{14}C while formalin plus NaOH was used to preserve samples for analysis of particulate ^{14}C. One bottle of each size was killed with formalin plus NaOH before addition of ^{14}C-toluene as a control for abiotic uptake and as a control for any $^{14}CO_2$ contained in the ^{14}C-toluene (for example Button (pers. comm.; Button et al 1981a) found 0.07% at the total dpm of ring-labeled ^{14}C-toluene was present as $^{14}CO_2$). Samples were returned to MBL and assayed for total ^{14}C added,

^{14}C retained on particulate matter, and ^{14}C respired. Respired $^{14}CO_2$ was measured by the method of Cole and Likens (1979). After removal of 1 ml for measurement of total ^{14}C and 10 ml for particulate ^{14}C, the remainder of the sample was acidified with 3 ml of 1 N H_2SO_4 and sparged for 4 h with CO_2-free air. Effluent CO_2 was passed through activated charcoal to remove ^{14}C-toluene and then passed through 2 ml of 0.2 N KOH to trap $^{14}CO_2$. The KOH trap contents were assayed by LSC using Aquasol, with a counting effiency of 85%.

During the experiment testing toluene respiration, a measurement of overall microbial activity was obtained by thymidine incorporation (Fuhrman and Azam 1982). Thymidine was added to triplicate 30 m_l centrifuge tubes containing 15 ml of tank water to give a final concentration of 5 nM. After 1 h incubations of the tubes in the mesocosm, 15 ml of ice-cold 10% trichloroacetic acid (TCA) were added and the samples were filtered through 0.45 μm membrane filters. Filters were solubilized in 1 ml of methyl Cellosolve and returned to MBL for LSC. One tube was filled with formalin before thymidine addition as an abiotic control. The increase in microbial biomass was estimated using the factor 2×10^{18} bacterial cells producted per mole of thymidine incorporated (Fuhrman and Azam 1982).

Results and discussion

Winter

During the winter experiment, there was a continual decrease in total ^{14}C-activity, volatile (dissolved)-^{14}C, extractable-^{14}C, cold toluene, and F-12 (Figure 1). Most of the ^{14}C-activity was in the volatile (dissolved) pool at the start of the experiment but both the relative percentage and the absolute amount in this pool decreased over time until most of the activity was in the $^{14}CO_2$ pool at the end (Table 1). The mass balance in Table 1, which is the sum of the various fractions, agrees reasonably well with the total direct counts; they both indicate that most of the ^{14}C was lost from the tank by the end of the experiment. This loss is by volatilization, by biotic transformation to $^{14}CO_2$ and to other non-volatile (polar) organic compounds, and by absorption to particles.

The concentration (or activity) of a volatile substance in the mesocosm is a function of time, volatilization, degradation, and sorption onto sedimenting particles. This relationship may be represented by the equation

$$-dC/dt = k_{obs}C = (k_v + k_d + k_p)C \quad (1)$$

where

C = concentration in bulk water;

k_{obs} = overall rate constant observed for total concentration decrease (d^{-1});

k_v = volatilization rate constant (d^{-1});

k_d = degradation rate constant (d^{-1});

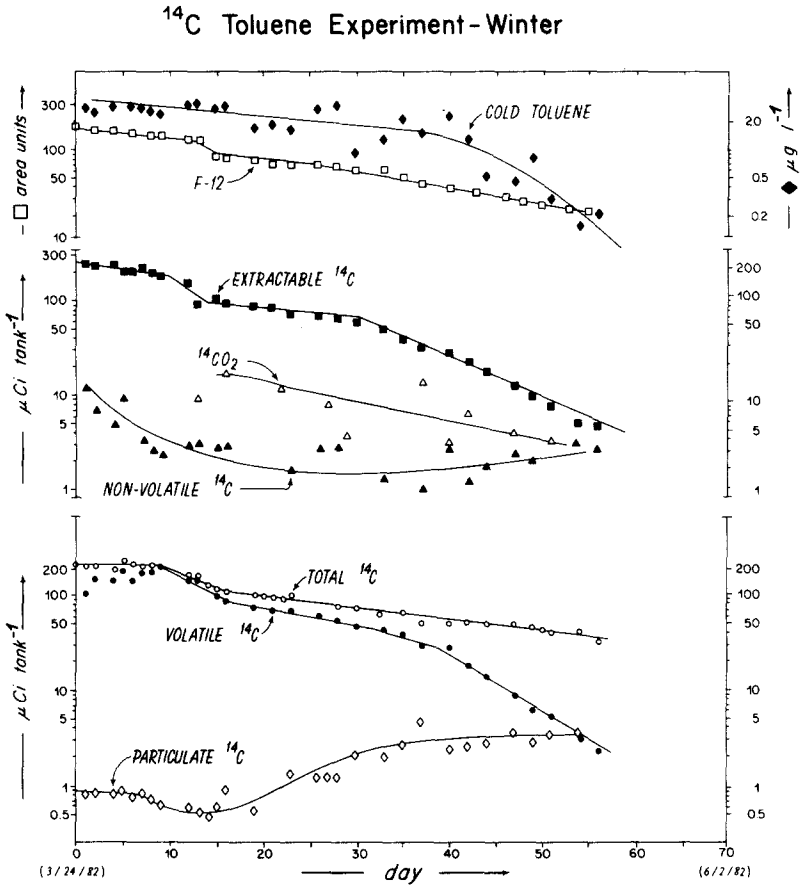

Figure 1. Distributions of ^{14}C-activity in the winter toluene experiment 24 March to 20 May 1982. Straight lines are regression fits to the data while curves are hand drawn and serve only for visual clarity. Total-^{14}C is the activity determined by direct counting.

k_p = rate constant for particle association (d^{-1}).

The volatilization rate constant k_v may further be defined as

$$k_v = K_L/h = D/hz \tag{2}$$

where

K_L = mass transfer coefficient (cm hr^{-1});

h = height of the well mixed water column (cm);

D = molecular diffusion coefficient (cm^2 sec^{-1});

z = stagnant boundary layer film thickness (μm).

Equation 2 represents the stagnant boundary layer model (Lewis and Whitman 1924; Dankwerts 1970; Liss and Slater 1974; Broecker and Peng 1974) which assumes that transfer of a volatile substance across an air-water

Table 1. Mass balances and total counts measured for single days for isotope added as [methyl-^{14}C]-toluene in winter (250 μCi introduced) and summer (350 μCi introduced) mesocosm experiments

	Winter					
	Day 5		Day 13		Day 47	
Form	Tank μCi	%	Tank μCi	%	Tank μCi	%
Volatile	210	(86)*	150	80	10	32
Non-volatile	9	(4)*	3	2	3	10
Particulate	1	(0.4)*	1	0.7	3	10
$^{14}CO_2$	(25)*	(10)*	35	17	15	48
Sum	(245)*	–	190	–	31	–
Total Direct Counts	245	–	170	–	52	–

	Summer							
	Day 2		Day 4		Day 10		Day 18	
Form	Tank μCi	%	Tank μCi	%	Tank μCi	%	Tank μCi	%
Volatile	280	95	155	70	1	0.4	1	0.5
Non-volatile	2	0.7	7	3	10	4	9	4
Particulate	2	0.7	8	4	30	12	10	5
$^{14}CO_2$	12	4	50	23	210	84	200	91
Sum	296	–	220	–	251	–	220	–
Total Direct Counts	330	–	250	–	240	–	230	–

*$^{14}CO_2$ data are not available for the first 13 days of this experiment, but a value of $\approx$ 25 μCi is possible.
*Values calculated assuming 25 μCi $^{14}CO_2$.

boundary is controlled by molecular diffusion across a hypothetical aqueous film of thickness z. The magnitude of z is inversely related to turbulence. Data from the Freon, which is neither bioaccumulated nor biodegradable (Blake and Mergner 1974; Mergner et al. 1975), can thus be used to constrain k_v for toluene. The assumption that the relatively hydrophilic toluene is not removed to any significant degree by sorption, i.e. $k_p = 0$ ($< 0.1\%$ of the toluene in the water column of Narragansett Bay should be associated with suspended particles, Wakeham et al. 1983a), permits calculation of a degradation rate constant as $k_d = k_{obs} - k_v$ where k_{obs} was calculated from the slope of the volatile (dissolved) pool.

The experimental data in Figure 1 show changing slopes; for this reason the experiment was divided into four time-intervals for calculations of volatilization and degradation parameters (Table 2). For F-12, we assumed that $k_{obs} = k_v$. The diffusion coefficient for F-12 in winter was calculated according to a modified Wilke–Chang equation (Wilke and Chang 1955; Hayduk and Laudie 1974) and was 6.2×10^{-1} cm^2 s^{-1} for 6 °C and 30 °/∞ salinity. The height of the MERL water column was 530 cm. The K_L for F-12 and the boundary layer thickness, z, were then calculated (eq. 2). Next, the z and a toluene diffusion coefficient of 4.9×10^{-6} cm^2/sec were used to calculate K_L and k_v for toluene. And finally, k_d for toluene was estimated by difference from k_{obs} for toluene, where k_{obs} was calculated from the slope of the volatile ^{14}C-toluene activity decrease.

Toluene mass transfer coefficients (K_L) and rate constants with respect to volatilization (k_v) thus calculated from the F-12 data vary by roughly an order of magnitude over the course of the experiment (Table 2). The highest volatilization rate for toluene (and F-12) occured between days 12 and 16. This coincided with a winter storm during which wind velocities near MERL averaged 8.5 m s^{-1} compared to about 5.5 m s^{-1} before and after. A significant increase in volatilization is not surprising since exchange rates vary as the square of the wind velocity when winds are greater than about 8 m s^{-1} (Cohen et al. 1978; O'Connor 1983). The half-life ($t_{1/2}$) for toluene in the mesocosm resulting from volatilization during the storm was about 6 days vs 25–35 d during the rest of the experiment. An even greater increase in storm-induced volatilization was observed during a subsequent experiment with ^{14}C-tetrachloroethylene.

The differences between k_v and k_{obs} for toluene and the presence of $^{14}CO_2$ in the tank result from microbial degradation (k_d). During the winter, the estimated k_d were generally less variable than the k_v, but then degradation would not have been expected to show any storm-influenced effect. Relatively low rates of degradation during the first half of the experiment reflect low activities of toluene-degrading microorganisms, probably in part due to low water temperatures (2–4 °C). The apparent degradation rate increased substantially during the second half of the experiment, when a $t_{1/2}$ due to degradation alone was approximately 9 d (vs a $t_{1/2}$ of about 30 d for the

Table 2. Volatilization and degradation results for winter and summer experiments

Interval (days)	Freon			Toluene			
	$k_{obs} = k_v$ (d^{-1})	K_L[a] (cm h^{-1})	z (μm)	k_{obs} (d^{-1})	K_L[b] (cm h^{-1})	k_v (d^{-1})	k_d (d^{-1})
Winter							
0–12	−0.02	0.43	520	−0.05	0.34	−0.02	−0.03
12–16	−0.14	3.1	70	−0.14	2.5	−0.11	−0.03
16–33	−0.02	0.43	520	−0.03	0.34	−0.02	−0.01
33–56	−0.04	0.88	250	−0.11	0.71	−0.03	−0.08
0–56	−0.04	0.88	250	–	0.65	−0.03	–
Summer							
0–4	−0.07	1.5	200	−0.14	1.2	−0.06	−0.08
4–7	−0.03	0.66	470	−1.11	0.53	−0.02	−1.09
7–21	−0.04	0.88	350	−0.04	0.71	−0.03	−0.01
0–21	−0.04	0.88	350	–	0.78	−0.04	–

[a]Freon molecular diffusion coeffients at 30 °/₀₀: 6.2×10^{-6} cm^2 sec^{-1} at 6 °C; 8.7×10^{-6} cm^2 sec^{-1} at 18 °C.
[b]Toluene molecular diffusion coefficients at 30 °/₀₀: 4.9×10^{-6} cm^2 sec^{-1} at 6 °C; 6.9×10^{-6} cm^2 sec^{-1} at 18 °C.

Table 3. Comparison of particle incorporation rates between ^{14}C-methyl toluene tank incubation and ^{3}H-methyl short-term incubation. "f" is fraction taken up (Δparticulate/volatile), T_p is turnover times due to particle uptake

Tank ^{14}C						Short-term ^{3}H		
Interval (days)	Volatile (μCi)	Δ Part. (μCi)	f ($\times 10^{-3}$)	T_p (d)	Turnover rate ($\times 10^{-3}$ d^{-1})	Day	T_p (d)	Turnover rate ($\times 10^{-3}$ d^{-1})
Winter								
0–5	245	1	4.08	1200	0.8	0	4800	0.2
5–45	210	2	9.5	4200	0.2	14	3500	0.3
						49	1500	0.7
Summer								
0–2	330	2	6.06	330	3.0	0	930	1.1
2–4	280	6	21.4	93	11.0	2	840	1.2
4–10	155	22	142	42	24	4	340	2.9
						7	710	1.4
						9	4400	0.2

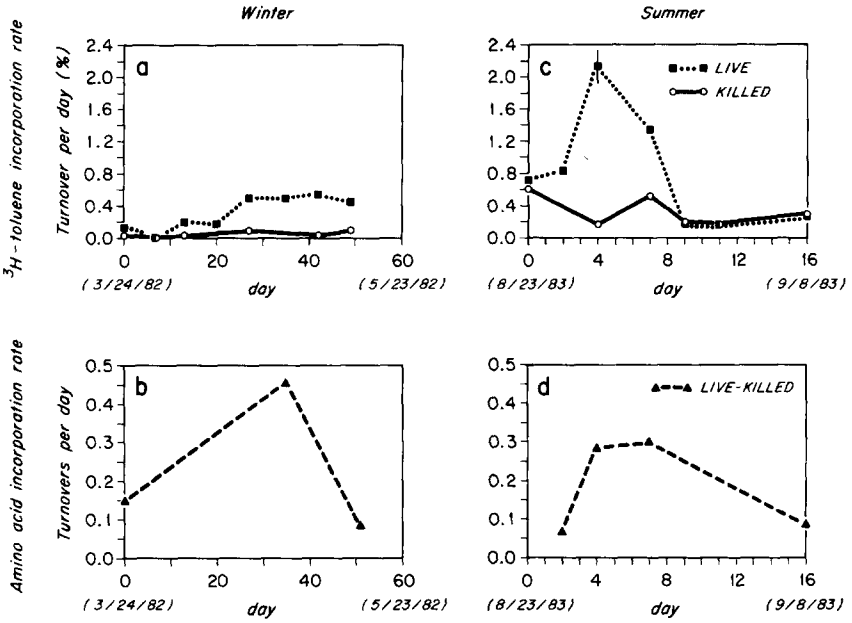

Figure 2. Rates (% turnovers d^{-1}) for ^{3}H-toluene (a, c) and (turnovers d^{-1}) ^{3}H-amino acid (b, d) incorporation (live minus killed) into particulate matter in the winter and summer experiments.

previous 33 d). It was only during this final period that degradation was more important than volatilization. Warmer water temperatures (up to 10°C) during the second half of the experiment would lead to increased microbial activity.

The incubations with ^{3}H-toluene carried out during the winter experiment were designed to assess biotic incorporation onto particulate matter (Table 3 and Figure 2a). This incorporation was quite low but increased over the course of the experiment to a maximum of 0.6% d^{-1} (Figure 2a). In fact incorporation was highest during the entire second half of the experiment when degradation also appeared to be greatest. Turnover rates based on the short-term ^{3}H-toluene incubations are comparable to values calculated from the whole tank ^{14}C-toluene measurements (Table 4). For example, uptake onto particles ranged from 0.02 to 0.08% d^{-1} in the whole-tank measurement and from 0.02 to 0.07% d^{-1} in the short-term incubations. Less than 10% of the uptake occurred in the killed sample, so most of the incorporation was caused by living organisms. Nevertheless turnover times for biotic incorporation were on the order of several years which do not agree with apparent ^{14}C-toluene degradation rates based on the buildup of $^{14}CO_2$. Since some degradation clearly did take place during the winter experiment and since the degrading microorganisms are in the particulate fraction, degradation must occur very rapidly after uptake. The amino acid incorporation (Figure 2b) increased to a peak in the middle of the experiment as the tank water warmed.

Table 4. Short-term ^{14}C-ring toluene incorporation into particulate matter and respiration. Rates calculated as described in the text. Treatment F = formalin preservative, A = alkaline preservative, C = killed control

Date		Resp (dmp 125 ml^{-1})	Particulate (dmp 125 ml^{-1})	Total (dmp 125 ml^{-1})	Particulate turnover rate (day^{-1})	Respired turnover rate (day^{-1})	Total turnover rate (day^{-1})	Respired / Total
	F	800	1400	2200	0.046	0.023	0.069	0.33
9–19	A	660	1030	1690	0.020	0.013	0.033	0.38
	C	490	750	1240				
	F	12400	16600	29000	1.07	0.87	1.94	0.49
9–21	A	11700	10900	22600	0.65	0.82	1.47	0.56
	C	460	1800	2260				
9–23	F	7540	9090	16630	0.58	0.51	1.09	0.47
	C	390	680	1070				
	F	1200	1670	2870	0.067	0.028	0.095	0.42
10–3	A	1030	2670	3900	0.140	0.156	0.016	0.12
	C	810	750	1560				

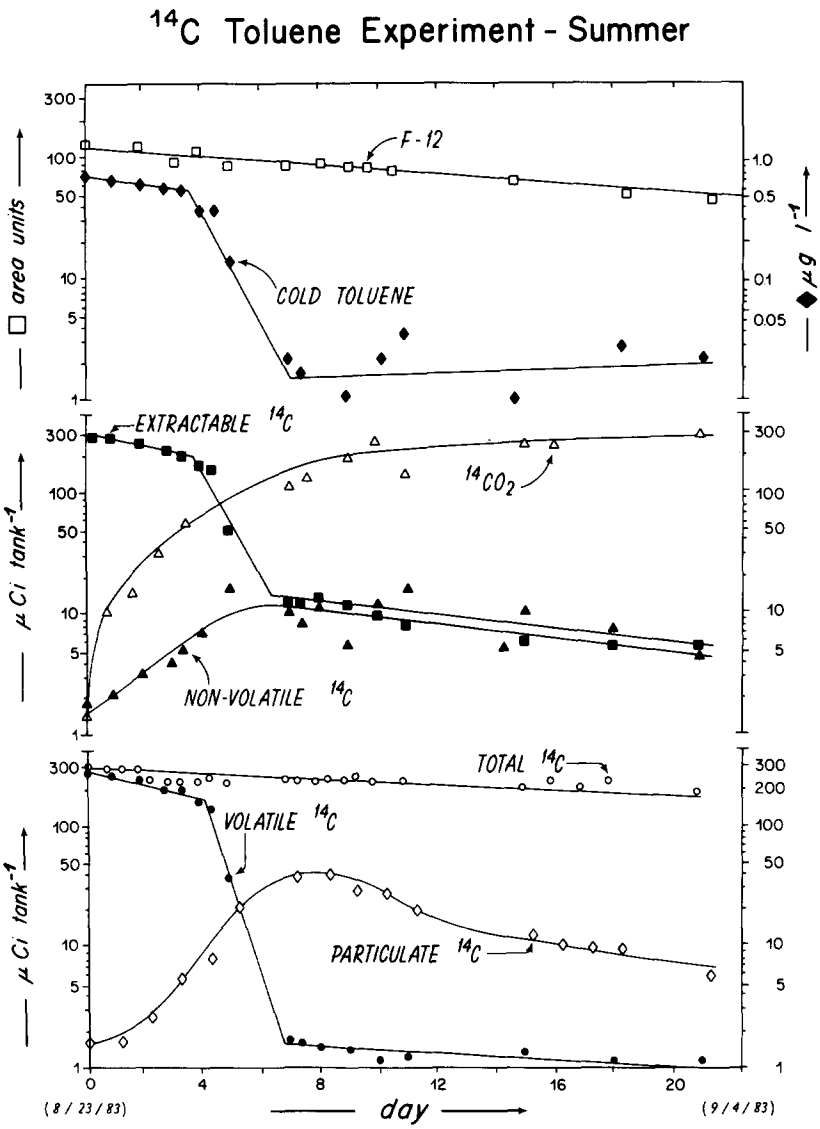

Figure 3. Distributions of ^{14}C-activity in the summer toluene experiment 23 August to 19 September 1982. Total-^{14}C activity was determined by direct counting.

This measurement indicates the activity of heterotrophs in general, however, so it does not tell us if the toluene oxidizers increased.

Summer

The behaviour of toluene during the summer experiment (Figure 3, Table 1) was quite different from that observed in winter. At day 2, most of the

^{14}C-activity was still in the volatile (dissolved) form, there was little $^{14}CO_2$, and ^{14}C associated with particles and in the non-volatile (polar) pool was minimal (Table 1). Over the next 2 d, volatile-^{14}C activities decreased gradually but $^{14}CO_2$-activities increased greatly. Next, the activity of the volatile form (as well as the concentration of cold toluene) dropped more rapidly, reaching near background levels by day 7. The decrease in volatile-^{14}C between days 4 and 7 was accompanied by an increase in particulate-^{14}C and non-volatile-^{14}C. Particulate- and non-volatile-^{14}C activities peaked around days 7–8 and subsequently decreased. By day 10, 84% of the ^{14}C-activity was as $^{14}CO_2$; this continued to increase in relative abundance (up to 93% of total ^{14}C-activity) throughout the remainder of the experiment.

The summer experiment was divided into three time intervals and volatilization rates were calculated using molecular diffusion coefficents for 18 °C (Table 2). The rates were less variable than during the winter experiment since no storms occurred during the summer experiment. For both F-12 and toluene, the mean k_v and K_L for the entire summer experiment (days 0–21) were actually the same as for the entire winter experiment (days 0–56). Evidently decreases in mean turbulence, as evidenced by a larger mean z in summer, were offset by increases in diffusion coefficients.

The k_{obs} for the first two periods were significantly greater than the calculated k_v for toluene. This, coupled with the dramatic increase in $^{14}CO_2$ and the decrease in volatile-^{14}C is convincing evidence of rapid biological degradation of toluene during the first week of the summer experiment. During this period of degradation, particulate-^{14}C remained relatively low and a rate constant for long-term removal to particles may be assumed to be negligible (although later in the experiment particulate-^{14}C did account for up to about 12% of the total ^{14}C-activity). From the very start of this experiment, degradation rates were higher than in winter and degradation apparently was significantly faster in removing toluene from the water than volatilization. Furthermore, the degradation rate increased markedly after a 4-d lag period. Degradation from days 4–7 was some 30 × greater than in winter; the $t_{1/2}$ of biodegradation during this period was 0.6 d. A comparison of rate constants for volatilization and degradation suggests that during this time interval nearly 98% of the toluene removal was via degradation. There was minimal degradation during the last two weeks of the experiment since the toluene levels were so low.

Biotic uptake of ^{3}H-toluene during summer was considerably faster than during winter (Figures 2c and d), even though the amino acid incorporation rate (0.3 turnovers d^{-1}) was similar to that of the winter experiment. This suggests that the microbial population during the summer experiment may have been relatively enriched in active toluene-degrading species. In addition, the ^{3}H-toluene uptake peaked on day 4, which compares well with observed changes in ^{14}C-activity distribution in the mesocosm. As in winter, turnover rates due to biotic uptake of both ^{3}H- and ^{14}C-toluene onto particles were

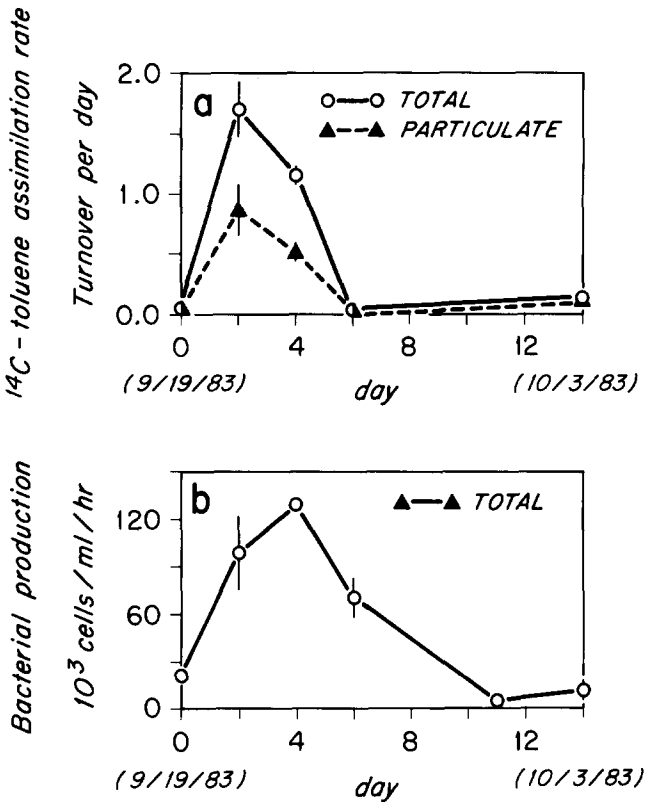

Figure 4. Rates (turnovers d^{-1}) of particulate and total (particulate + respired) turnover of ^{14}C-ring-labeled-toluene (a) and bacterial production (10^3 cells $ml^{-1}\,h^{-1}$) from thymidine incorporation (b) during the third experiment, 19 September to 3 October 1982.

significantly lower than the apparent whole-tank-derived degradation rates (Tables 2 and 4). For example, k_d measured in the tank reached 1 d^{-1} while the biotic uptake was 0.01–0.02 d^{-1}. This discrepancy may have been caused by a peak in uptake (e.g., on day 6) missed by the short-term incubations or may have been caused by degradation due to respiration.

To investigate this further, we measured toluene respiration in a third set of bottle experiments using water taken from a tank spiked with cold toluene on 19 September 1982 (Figure 4). Total turnover (particulate uptake + respired) rates for ring-labeled ^{14}C-toluene peaked 2 d after the experiment began and were many times higher than in previous experiments. The turnover rate for particulate incorporation as measured with ^{14}C- toluene in this experiment (Figure 4a) was nearly 50-fold greater than was measured under similar conditions with ^{3}H-toluene (Figure 2d). Nevertheless the turnover rate for ^{14}C-toluene in Figure 4a peaked at about 1.9 d^{-1} compared to

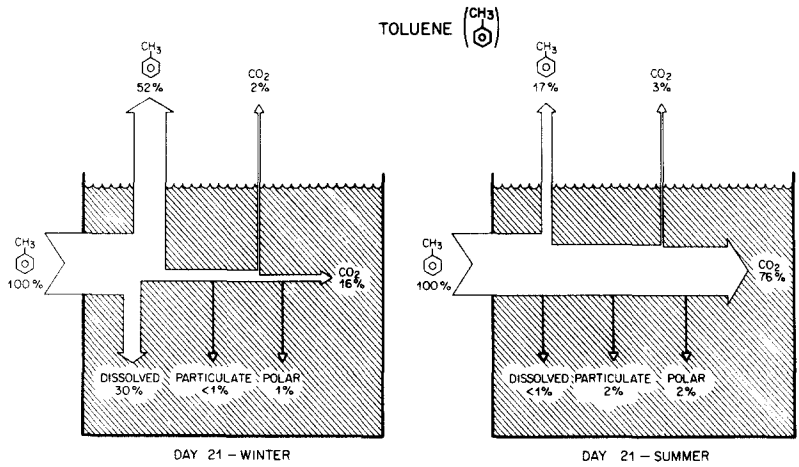

Figure 5. Mass balances for winter and summer ^{14}C-toluene experiments (both calculated to 21 days). Estimated precision for the values is ± 10%.

the 1.1 d^{-1} rate inferred from the previous summer's whole-tank ^{14}C-toluene experiment (Table 2). Unfortunately, we did not have the resources to follow the concentration of the added toluene in this third experiment but did measure the bacterial production with a thymidine incorporation study. Production showed a trend similar to the total turnover but the peak was slightly offset. It is possible that maxima for both ^{14}C-toluene turnover and bacterial production may actually have occurred on day 3. Respiration accounted for up to 50% of the total biotic uptake (Table 3). Short-term experiments showed no significant differences between the rates of respiration of ring- and methyl-^{14}C-toluene (data not shown). This indicates that the results of the whole-tank experiments with methyl-labeled ^{14}C-toluene accurately reflect the respiration of all of the toluene. We do not know whether the increase in microbial activity and toluene uptake and respiration following a lag period of several days is caused by adaptation of the metabolic or transport pathways of the bacteria already present in the tank or if it is caused by growth of a new population of microbes. The only other data of this subject (Button et al. 1981b) suggests that the adaptation of laboratory populations of microbes to toluene will occur at concentrations of 1–5 $\mu g\,l^{-1}$, or about the same concentrations as in the experiments and in upper Narragansett Bay.

Fate of toluene in coastal seawater

At the end of each experiment, a mass balance was calculated to identify the fate of all of the ^{14}C-activity introduced into the tank (Figure 5). Although the winter experiment lasted 56 d, we show a mass balance calculated for 21 d

to facilitate comparison with the shorter summer experiment. Mass balances were calculated as follows. The ^{14}C-activities measured at the end of the experiment in the volatile (dissolved), non-volatile (polar), particulate, and $^{14}CO_2$ pools were divided by the ^{14}C-activity at day zero to obtain the percentage of initial ^{14}C remaining in these pools. The loss of toluene by volatilization was calculated using the rate constants obtained as discussed above. The loss of $^{14}CO_2$ across the air-sea interface was estimated as approximately 0.5% d^{-1} based on other MERL experiments (D. Rudnick pers. comm.). Since the experiments were conducted in batch, no correction for ^{14}C-activity loss due to washout was required.

In a 21-d winter experiment, approximately 52% of the ^{14}C-toluene was lost from the mesocosm by volatilization. Thirty percent remained in the water as volatile (dissolved) ^{14}C-toluene, while 16% of the original activity remained in the tank as dissolved $^{14}CO_2$ and 2% left the tank by gas exchange of $^{14}CO_2$. Negligible amounts of ^{14}C remained in the particulate and non-volatile (polar) pools. The importance of volatilization in the cold-water experiment is further demonstrated by the mass balance calculated for the entire 56 days in which volatilization removed 80% of the initial activity. Only 2% of the original activity remained as dissolved ^{14}C-toluene and 10% as $^{14}CO_2$. Thus we conclude that abiotic removal, primarily volatilization, of toluene dominates in winter.

In contrast, mineralization of toluene was clearly the dominant process in summer. The production of $^{14}CO_2$ accounted for loss of 79% of ^{14}C-toluene initially introduced; most of the $^{14}CO_2$ remained in the tank water. This accounts for the observation that the total ^{14}C-activity in the tank as obtained by direct counting decreased only very slowly over the course of the experiment (Figure 3); that is, a relatively volatile compound (toluene) was converted into a less volatile form (CO_2). Volatile (dissolved) ^{14}C-toluene at the end of the experiment represented $<$ 1% of the original activity. Final activities in the particulate and non-volatile (polar) forms were small, although both were slightly greater than those observed in the winter experiment.

The MERL mesocosms were designed to simulate physical, biological and chemical processes in estuaries like Narragansett Bay. How then do the ^{14}C-toluene results translate to a quantitative understanding of the fate of toluene in the bay? Previous field measurements of toluene in Narragansett Bay (Wakeham et al. 1983a) showed the bay to be moderately polluted (toluene concentrations reached several $\mu g\,l^{-1}$ at the head of the bay). Strong concentration gradients in summer suggested a biological removal process to be active in addition to volatilization, although volatilization was thought to dominate in winter.

The importance of toluene volatilization in the MERL mesocosm experiments has been discussed above. The physical configuration of the experimental system, a 1.8 m diameter tank with about 20 cm of wall above the

water surface and with discontinuous mixing, means that the measured volatilization rates are probably low compared to at least some natural environments. For example, the thickness of the theoretical stagnant boundary layer in the tank (z) in our experiments was 200–500 μm (excluding the storm period). This range of values is comparable to that obtained in other MERL experiments (200–600 μm; Nixon et al. 1980; Bopp et al. 1981; Wakeham et al. 1983b). But the z-value in MERL is significantly greater than estimates for Narragansett Bay (60–180 μm for wind velocities between 1.8 and 3.3 m/s; Nixon et al. 1980), for San Francisco Bay (100 μm; Hammond et al. 1980), or for the ocean (50 ± 30 μm; Broecker and Peng 1974). Thus, toluene volatilization from Narragansett Bay might be 2–6 × faster than the MERL experiments would predict; in fact, toluene volatilization rates calculated previously for Narragansett Bay by Wakeham et al. (1982a) are about 2–3 × faster. For the oceans an order of magnitude increase might occur. However, as our winter experiment clearly showed, volatilization rates are strongly dependent on wind velocities, and order-of-magnitude variations in volatilization rate may occur over relatively short time scales. In addition to actual wind velocity, the fetch of the water body will also play a major role in controlling wind-driven turbulence, and hence volatilization.

We have assumed that association of toluene with suspended particulate matter is negligible. This would be consistent with a seawater-suspended particulate matter partition coefficient, K_p, of about 14 for toluene in Narragansett Bay ($\log K_p = 1.15$ calculated according to Schwarzenbach and Westall (1981) using an octanol/water partition coefficient of 400 and an organic carbon content for suspended particles of 6%). In the MERL ^{14}C-toluene experiments, $\log K_p$ values of about 1 are predicted based on a suspended particle load of about 8 mg l^{-1} and an average of 2.3% organic carbon for the particles. However, comparing the measured distributions of volatile (dissolved)-^{14}C-toluene and particulate-^{14}C in the experiments gives experimental $\log K_p$ values of 4.3 and 6.4 for winter and summer, respectively. These values are maximum values since they were calculated for the period during which particulate-^{14}C was greatest. We interpret the several orders-of-magnitude difference between predicted and "measured" K_p's as indicative of the importance of active and irreversible biologically-controlled sorption onto or into the particles. Laboratory sorption studies, on which model equations are based, typically begin with washing and/or drying steps in preparing the sorbents. This pre-treatment may remove microbes or inhibit microbial activities, so that the resulting sorption isotherms reflect primarily physico-chemical processes (as they are in fact intended to). However, in the natural environment, biotic uptake may also be important.

The residence time of toluene in Narragansett Bay during winter and summer may be estimated from our MERL ^{14}C-toluene experiments. In this

case, the residence time τ is defined as

$$\tau = 1/k_{\text{total}} = 1/(k_a + k_v + k_d + k_p) \quad (3)$$

where

k_{total} = overall rate constant (d^{-1});

k_a = advective rate constant (d^{-1});

k_v, k_d, and k_p are as defined above.

The hydraulic flushing rate (k_a) in Narragansett Bay is $-0.037\ d^{-1}$ (Kremer and Nixon 1978). For volatilization, the winter and summer rates are assumed to be the same ($-0.03\ d^{-1}$) and are assumed to be about 3 x too low (thus $k_v = -0.10\ d^{-1}$). Water temperatures in the bay in mid-winter may be as low as 0–2 °C for long periods, so a degradation rate constant of $-0.03\ d^{-1}$ should be considered a maximum. A summer degradation rate constant of $-1.0\ d^{-1}$ was used. Removal to particulate matter was again assumed negligible ($k_p = 0$). Thus in winter a k_{total} of $-0.17\ d^{-1}$ and a residence time of 6 d are calculated. Advection would be expected to remove 22% of the toluene while volatilization and degradation would remove 60% and 18%, respectively. On the other hand, a summer-k_{total} of $-1.1\ d^{-1}$ results in a τ of 0.9 d. In this case 88% of the removal would be due to degradation vs. 9% by volatilization and 3% advection.

Summary

Mesocosm experiments using ^{14}C- and ^{3}H-labeled tracer compounds have provided a quantitative picture of the fate of toluene in the coastal environment. Both the persistence and the processes which control the fate vary seasonally. Volatilization is an important process removing toluene from seawater during both summer and winter. Mean volatilization rates may not vary significantly between seasons, although there will be major short-term fluctuations (e.g. during storms) which may in fact be more important than seasonal averages. On the other hand, microbial degradation of toluene is much more rapid in summer and in fact clearly dominates the summer removal processes. The residence time of toluene in a moderately polluted temperate estuary such as upper Narragansett Bay may vary from about a week in winter to as little as a day in summer.

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