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^{13}C-NMR Reactivity Probes for the Environment

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Establishing the general reactivity of any segment of the terrestrial environment can be an important means of characterizing it. The sites capacity for transformation may reflect its environmental health, and knowing this capacity can provide a basis for estimating the rates and nature of the transformations that may occur there. However, no direct means for measuring this reactivity has been available. This work expands upon preliminary efforts to develop reactivity probes for the environment. The ^{13}C-labeled compounds chloroacetic acid (CA), chloroacetamide (CAM), and chloroacetonitrile (CCN) have been synthesized and tested as site reactivity probes (SRPs). The reactivity of activated sludge, a dump site soil, coastal marine water and sediments, and lake water and sediments was assessed by the probes. A simple protocol for employing the SRPs entails incubation with a 2-mL slurry, centrifugation/filtration at a desired time, followed by direct NMR analysis of products. The results indicate a broad capacity for the transformation of xenobiotics in the terrestrial environment, and they underscore the probes' capacity to delineate the nature and approximate rates for these processes. The CA probe is the most sensitive, but CAM and CCN allow an assessment of amide and nitrile hydrolysis by a given site. These probes should help in assessing the effectiveness of bioremediation efforts and also in gauging the effects of other alterations to a site.

Introduction

The chemical transformations wrought by the terrestrial and aquatic environments shape and foster the generation of life in all forms and maintain a balance in the ecosystem that nurtures it. As a part of the great cycle of transformations that describe the chemistry of earth, those that occur in soil, the sea, and lakes constitute a myriad of reactions in the environment that may be involved in the transformation of xenobiotics. But what is the reactivity of these environments? Indeed, what is the reactivity of any segment or fraction of the terrestrial and aquatic environments, and how might they be measured?

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In theory, at least, this problem is approachable. If a substance could be designed such that it was capable of undergoing the basic chemical processes of oxidation, reduction, and substitution, it could be allowed to react with a portion of the environment. The chemistry that it undergoes may be used to read the capacity of the site for transformation and the general nature of the transformations that may occur there. Ideally such a reactivity probe should be able to be employed with small samples and its response analyzed directly with minimal or no sample handling or workup.

Given existing structure−activity relationships, the response of the probe may be used as a guide to the rate of transformation and the potential fate of other substances. A reactivity probe could also be useful in comparing the differences in reactivity at a given site with time or upon storage. The effects of bioremediation efforts, as one example, may be assessed with reactivity probes.

Our strategy for the design of site reactivity probes (SRPs) stems from studies of biodehalogenation processes in which ^{13}C-labeled substrates and NMR analyses of pathways have proven most useful (*1*−*4*). A common intermediate in these processes is chloroacetic acid (CA), and a preliminary account of the efficiency of CA as a reactivity probe for soil has been described (*5*). In this work, we amplify our previous studies and compare the effectiveness of three potential probes to map the reactivity of a range of environmental sites. The sites chosen represent different environmental matrices in which the transformation of xenobiotics may be expected to occur. They include a dump site soil, coastal marine water and sediments, lake water and lake sediments, and activated sludge. The three ^{13}C-labeled probes employed are chloroacetic acid (CA), chloroacetamide (CAM), and chloroacetonitrile (CCN). Each of these substances is transformed by the soil methylotroph, *Methylosinus trichosporium* OB-3b, by either an oxidative or hydrolytic processes (*6*).

Experimental Section

Materials. *[1,2-^{13}C]Chloroacetic acid (CA)* (^{13}C-NMR: CH_2-Cl, δ 45, d; CO_2^-, 177, d) was prepared from [1,2-^{13}C]acetic acid by reaction with sulfuryl chloride and phosphorous pentachloride (*5*). Amide and nitrile were synthesized from the acid via the sequence chloroacetic acid, chloroacetyl chloride, methyl chloroacetate, chloroacetamide, and chloroacetonitrile.

[1,2-^{13}C]Chloroacetamide (CAM). In a small flask equipped with reflux condenser and drying tube, 2.8 g of [1,2-^{13}C]chloroacetic acid (0.030 mol) was refluxed with 3.5 mL (0.050 mol) of thionyl chloride at 76 °C for 4 h. At this time, the ^{13}C-NMR analysis indicated a complete conversion to the acid chloride ($ClCH_2$, δ 49, d; COCl δ 167, d). At room temperature, the flask contents were stirred and treated with 3 mL of methanol in dropwise fashion over a period of 15 min. The mixture was warmed to 55 °C for 7 h. The cooled mixture indicated a complete conversion to the methyl ester ($ClCH_2$, δ 41, d; CO_2Me, δ 168, d). The flask was cooled to 2 °C, and with stirring, 7.0 mL of cooled concentrated ammonium hydroxide was added over a 0.5-h period. The mixture was stirred for 1.5 h at 0−2 °C. The white amide precipitate was vacuum filtered and recrystal-

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lized from hot water, filtered, and air dried. The overall yield of chloroacetamide was 1.8 g (64%). The white needles had melting point of 118.5−119. ^{13}C-NMR: $ClCH_2$, δ 42, d; $CONH_2$, δ 172, d. Note: The more direct reaction of the acid chloride with ammonium acetate in acetone (*7*) yielded only 31% of the amide.

[1,2-^{13}C]Chloroacetonitrile (CCN). To a 25-mL three-necked flask equipped with good mechanical stirring, a small distillation head, and a receiver was added 1.3 g (0.014 mol) of [1,2-^{13}C]chloroacetamide. The flask was brought to 130 °C and held there until all of the amide had melted. The receiver was immersed in an ice bath, and a slurry of 1.9 g of phosphorous pentoxide (0.014 mol) in 2 mL of freshly distilled 1,2,4-trichlorobenzene was added all at once. With vigorous stirring, the flask was brought to 140−150 °C for 0.5 h and then rapidly to 175 °C for another 0.5 h. The water white [1,2-^{13}C]chloroacetonitrile liquid, 0.4 g, was obtained in 37% yield. ^{13}C-NMR: $ClCH_2$, δ 25, d; CN, δ 117, d.

The chemical shifts for products have been given elsewhere (*5, 6*) and are indicated in the figures. The ^{13}C−^{13}C coupling constants were ∼2 Hz.

Environmental Samples. *(a) Soils.* Two soil samples were employed. Both were taken from a dump site at UCR (d.S soil) that had been exposed to haloorganics and other pesticides for some time. The d.S-1 sample represents a homogenate of a 2-ft deep core taken from the center of the site. The d.S-2 sample was obtained from the edge of the site. A sample of d.S-1 was held for 1 yr at 5 °C (d.s-1, 1-yr-old) to test any changes in reactivity upon storage.

(b) Marine Coastal Water and Sediments. These sea samples were taken from Shaw's Cove in Laguna Beach, California. Sea water was taken about 100 yards from shore at the surface. In addition, two samples of sediments were collected. The first, Shaw's Cove 1 (SC-1), was a grayish sandy sediment from the top 2 in. of the surface bottom at a depth of 30 ft. The second (SC-2) was a dark, near blackish silty sediment taken at a depth of 40 ft from 3 to 5 in. below the surface bottom. These samples were slurries containing about a two-thirds liquid phase.

(c) Lake Samples and Sediments. These were taken from Lake Perris, Riverside, CA. Lake water (LPW) was a surface sample, and the sediments LP-1 and LP-2 represent an upper sandy sediment (top 1−2 in.) taken at a depth of 20 ft and a lower black silty sand collected at 4−6 in. below the surface bottom at a depth of 12 ft. These slurries were about two-thirds liquid.

(d) Activated Sludge. The sample of activated sludge (AS) was collected from the aeration tank at the Riverside City sewage plant. The blackish slurry contained about one-third solid by volume.

Analyses. For soil, the following protocol was used: 1.0 g of sample was triturated with 2 mL of glass distilled water, and 2 μL of a 1 M ^{13}C probe in dimethylformamide (DMF) was added. The 30-mL volume test tube containing the reactants was capped with a serum cap, and samples were allowed to stand in a rack at room temperature. They were shaken once each day by hand. For anaerobic incubations (see below), the same procedure was employed except that the tube was gassed with hydrogen. At the desired time, approximately 1 mL (or all) of the slurry was centrifuged/filtered. The clear centrifugate (0.5−0.6 mL) was transferred to a serum capped NMR tube, and 100 μL of D_2O/mL of sample was added for the lock. Samples were held at 5 °C until the NMR acquisition could begin. The centrifuge−filtration process required 10 min at 8000 rpm. It was accomplished with a microcentrifuge (Fisher Scientific). Small snap-cap plastic tubes (Eppendorf tubes) containing a 0.45-μm filter were convenient. Note: Unwashed plastic tubes from Rainin Instrument showed resonances at δ 63 and 73 in the filtrate. Tubes from Micron Supplies Incorporated did not.

Two milliliters of seawater and lake water as well as sediment and sludge slurries was incubated without dilution with 2 μL of a 1 M probe in DMF. The slurries were shaken before removing the sample. The clear seawater and lake water samples required no centrifugation or filtration. The sediment slurries were centrifuged and filtered before NMR analysis as described above for soil. For "anaerobic" incubations, unless otherwise stated, the tubes were gassed briefly (30 s) with hydrogen. As the results indicate, the samples were not completely anaerobic. This procedure allowed anaerobic transformations (reductions) to occur without completely suppressing oxidative reactions.

The NMR analysis was an overnight acquisition (16 h) on a General Electric QE-300 spectrometer. The partial percent conversions were estimated from relative intensities of the α-carbon resonances except for HCO_3^-. This conversion was estimated from the relative intensities of the HCO_3^- resonances as compared to the C═O or C≡N resonances of the starting probe or product. These latter are tertiary carbons and not subject to the NOE (nuclear Overhauser effect) enchancement attendant upon decoupling the proton resonances with irradiation (*8*). Hence, the corresponding resonances are less intense than those associated with the α-CH_2X (X = Cl, OH) carbons. Based upon the close similarity of authentic spectra of the probes taken under the conditions described, we take the inherent intensities of these α-carbons as the same for the purpose of estimating product distribution. Similarly, and upon the same basis, all tertiary carbons are taken as having the same (lesser) intensity in any given sample. Thus, percent conversions were determined on this basis. In samples where both the tertiary and α-carbons were discernible, relative distributions calculated using either set of carbon intensities were the same (±5). The reproducibility of the distributions obtained from replicate samples for any analysis was within 10% of the values reported. This analysis assumes that all ^{13}C-labeled carbons are equally visible in the centrifugate (no selective adsorption to the environmental matrix) and that the distribution of products observed is the true distribution. In essence, we assume that these highly water-soluble probes and products have large distribution coefficients such that their presence in water is greatly favored at 10^{-3} M. This assumption is buttressed by the observation that the spectra of the probes and ^{13}C-labeled bicarbonate exposed to the approximate 2:1 water/samples, where no reaction was detected, exhibited the same NMR spectrum (following centrifugation/filtration) as authentic standards at that concentration. However, poorly resolved spectra indicated solids in the analyte and/or adsorption to them (this work shown in Figure 3a,b) or perhaps soluble organic matter (*5*). These spectra were greatly refined by the addition of acid. We presume this result, in part, reflects the desorption of anions from the solid matrices.

Chloride analysis was accomplished by direct potentiometry at high ionic strength employing an Orion chloride ion electrode and a Calomel double-jacketed reference electrode as previously described (*9*).

SCHEME 1

(a) reduction

$$ClCH_2X \longrightarrow CH_3X \quad (1)$$

(b) hydrolysis (substitution)

$$ClCH_2X \longrightarrow HOCH_2X \quad (2)$$

(c) oxidation (O insertion)

$$ClCH_2X \longrightarrow (Cl\text{–}CH(OH)\text{–}X) \longrightarrow O{=}CH\text{–}X \longrightarrow CO_2 \quad (3)$$

Results

The general structure of these probes may be formulated as $ClCH_2X$, wherein $X = CO_2H$ (CA), $CONH_2$ (CAM), and CN (CCN). Expected products for reduction, substitution (hydrolysis), and oxidation at the carbon-bearing chlorine are outlined in Scheme 1. However, where $X = CONH_2$ and CN, an additional site for hydrolysis is present in the probe. That is, the amide and nitrile moieties may be more readily hydrolyzed by some sections of the environment than is the C−Cl bond, and this is observed. Thus, subsequent products may derive from CA in some cases. With $X = CO_2H$, the sequence acetic acid, glycolic acid, glyoxylic acid, and CO_2 (eq 4) represents an oxidative

$$CH_3CO_2H \longrightarrow HOCH_2CO_2H \longrightarrow O{=}CHCO_2H \longrightarrow CO_2 \quad (4)$$

cascade to bicarbonate. Some typical spectra are shown in Figures 1−4. Figure 1 illustrates the hydrolysis of CAM to CA and an overall oxidation to bicarbonate by the dump site soil that had been stored at 5 °C for 1 yr. The resonances marked "f" resulted from the filter and was not derived from the sample (see Experimental Section). Figure 2 shows the reduction of CCN to acetonitrile obtained in an anaerobic incubation with the lower silty sediment from Lake Perris. Figure 3a,b shows the partial reduction, hydrolysis, and oxidation of CA by the lower silty sand coastal marine sediment from Shaw's Cove, Laguna Beach. The poorly resolved spectrum in Figure 3a was sharpened by the addition of HCl to the NMR tube. Thus, the HCO_3^- resonance at δ 161 is missing from the acidified sample due to the liberation of CO_2. Note: This sample had been centrifuged, but not filtered, only decanted. The resonances in Figure 3b are shifted due to the acidity. Figure 4 shows both the hydrolysis of the nitrile moiety of CCN to CA and the hydrolysis of the latter (C−Cl) to glycolic acid by activated sludge. In addition, bicarbonate is obtained. The response of each segment of the environment to the probes is summarized in Tables 1−4.

The results with the dump site soil (Table 1) indicate a capacity for hydrolysis and oxidation. In addition, the results with CA alone indicate that this capacity is diminished upon storage for 1 yr at 5 °C. Thus, in contrast to the complete oxidation to HCO_3^- observed in 2 weeks with the initial sample (d.S-1), no conversion at all was noted in the same period with the 1-yr-old sample. In contrast, the response of CAM and CCN to the 1-yr-old soil and a new fresh sample (d.S-2) was not markedly different. That is, both CAM and CCN were hydrolyzed to CA. Also with CCN, whether the incubation was aerobic or anaerobic did not make much difference. These results suggest that the hydrolysis of the amide and nitrile linkages with this soil may be chemical rather than microbiological in nature. Background rates for these conversions were separately assessed via NMR in phosphate buffer at pH 7.4. In 2 weeks, CAM hydrolyzed to CA to the extent of about 2%. CCN was inert. The background hydrolysis of the C−Cl bond of each

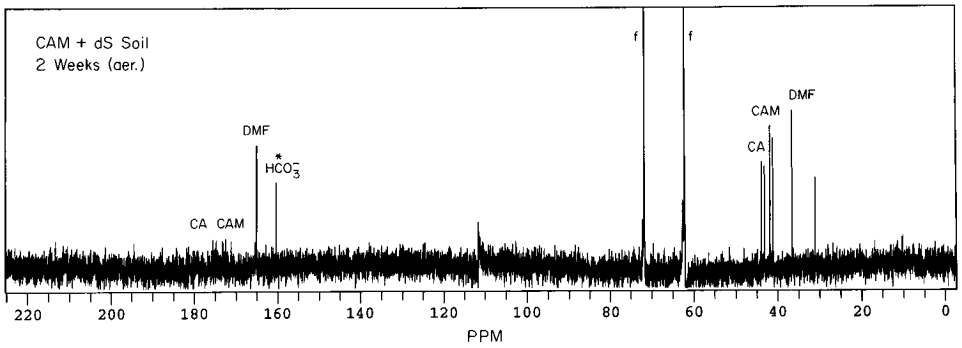


FIGURE 1. Hydrolysis and oxidation of chloroacetamide (CAM) to chloroacetic acid (CA) and bicarbonate by a dump site soil stored at 5 °C for 1 year. Dimethylformamide (DMF) resonances are at δ 32, 37, and 166. The f-labeled resonances are derived from the filter.

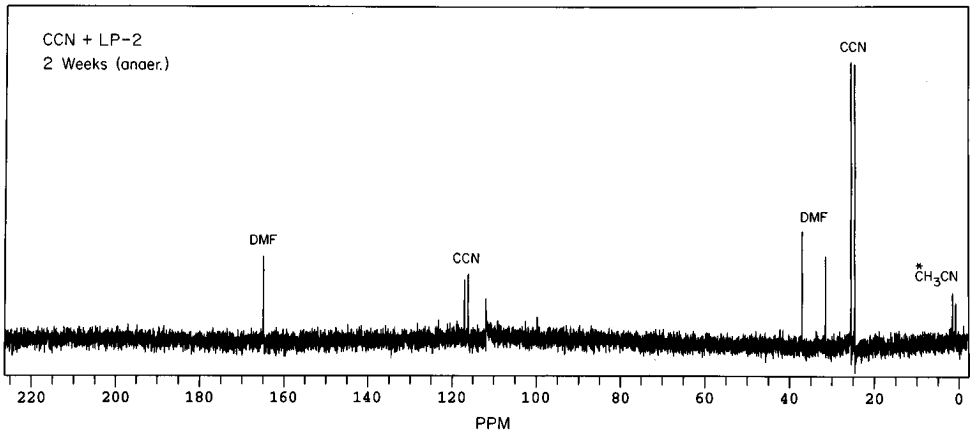


FIGURE 2. Reduction of chloroacetonitrile (CCN) to acetonitrile by the lower black sediment from Lake Perris.

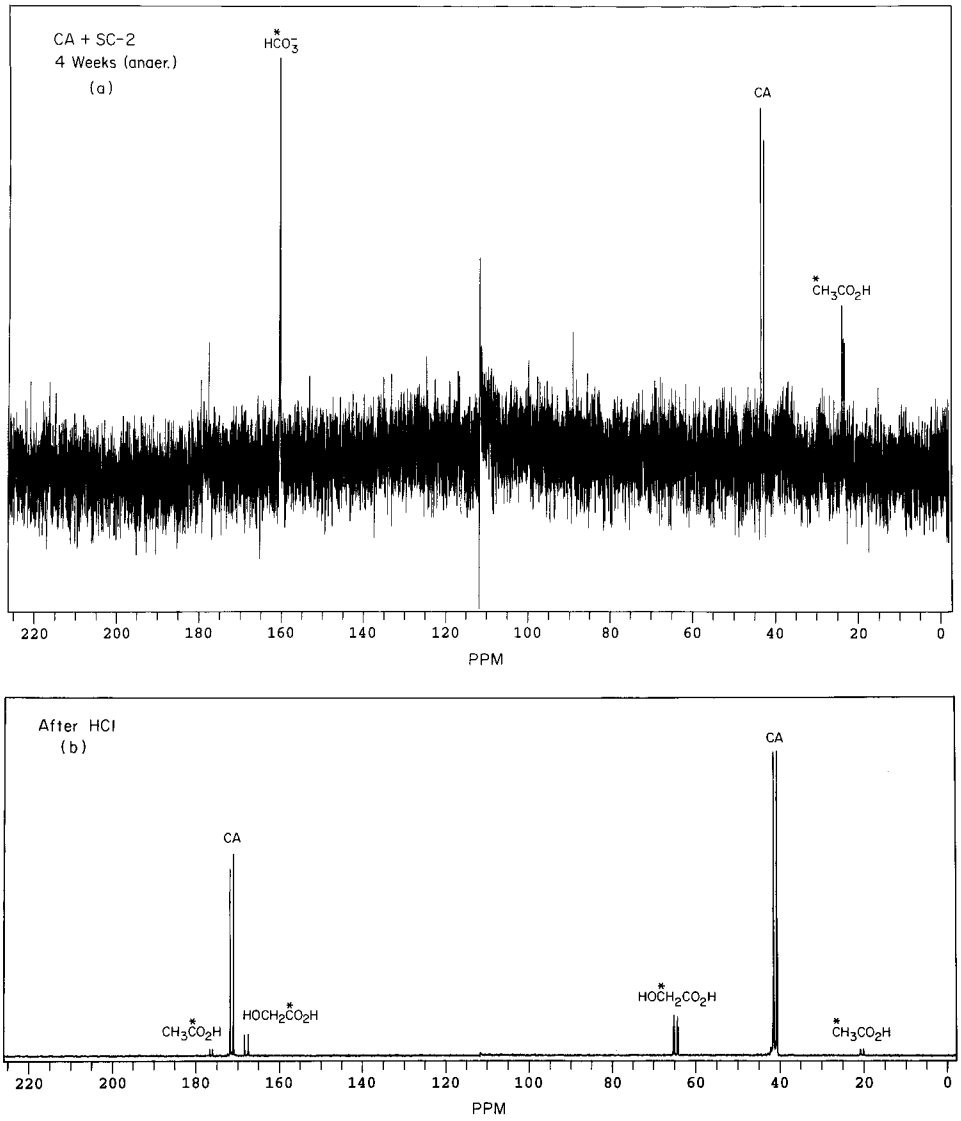


FIGURE 3. (a) Reduction of chloroacetic acid (CA) to acetic acid and the oxidation of CA to bicarbonate by the lower coastal marine sediment from Shaw's Cove. The sample was centrifuged but not filtered. (b) Same sample following the addition of HCl to the NMR tube. The additional hydrolysis product glycolic acid is observed.

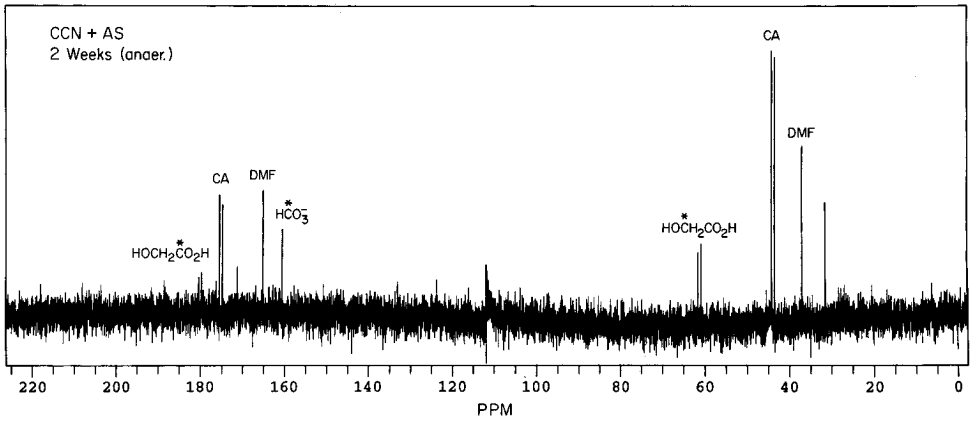


FIGURE 4. Complete hydrolysis of chloroacetonitrile to chloroacetic acid (CA) and the conversion to glycolic acid and bicarbonate by activated sludge.

of the probes was also assessed at 2 weeks and 1, 2, and 4 months at pH 6.0, pH 7.4, and pH 8.0. A plot for the data at pH 8.0 is shown in Figure 5. The C−Cl bond hydrolysis is slow. Thus, only 5% of CCN had hydrolyzed to Cl^- in 4 months. The percent hydrolysis of CA and CAM in 4 months at pH 8.0 was 15 and 27%, respectively. The results at 4 months at pH 7.4 [CCN (1%), CA (15%), CAM (21%)] and at pH 6.0 [CCN (0), CA (2.5%), CAM (3%)] show the probe to be stable over the time period of the environmental incubations. Note at 2 weeks, pH 7.4, the maximum percent hydrolysis of the C−Cl bond was observed with CAM, and it was less than 1%.

TABLE 1

Response of Probes to Fresh and 1-Yr-Old[a] Dump Site Soil from UCR

environment	probe	time incubation (wk)	products	% conversion[b]	conclusion
d.S soil-1 (fresh)	CA	2/anaerobic[e] sterilized	none	0	NR[c,d]
	CA	3/adj to pH 10	$HOCH_2CO_2H$	80	hydrolysis[d]
	CA	0.7/aerobic	HCO_3^-	33	partial oxidation
	CA	2/aerobic	HCO_3^- only	100	complete oxidation[d]
	CA	1/anaerobic[e]	$HOCH_2CO_2H$	8	hydrolysis
d.S soil-1 (1-yr-old)	CA	2/aerobic	none	0	NR
	CAM	2/aerobic	CA	84	amide hydrolysis (15)
			HCO_3^-		oxidation (69)
	CCN	2/aerobic	CA	50	hydrolysis of CN
d.S soil-2 (fresh)	CA	2/aerobic	HCO_3^-	100	oxidation
	CA	2/anaerobic	HCO_3^-	45	oxidation
	CAM	2/aerobic	CA	62	amide hydrolysis (54)
			HCO_3^-		oxidation (8)
	CAM	2/anaerobic[f]	CA	48	amide hydrolysis (42)
			HCO_3^-		oxidation (6)
	CCN	2/aerobic	CA	30	hydrolysis of CN
	CCN	2/anaerobic[f]	CA	30	hydrolysis of CN

[a] Stored at 5 °C for 1 yr. [b] Estimated from ^{13}C-labeled intensities. [c] NR, no reaction, probe recovered. [d] Spectra given in ref 5. [e] Under argon. [f] Under hydrogen.

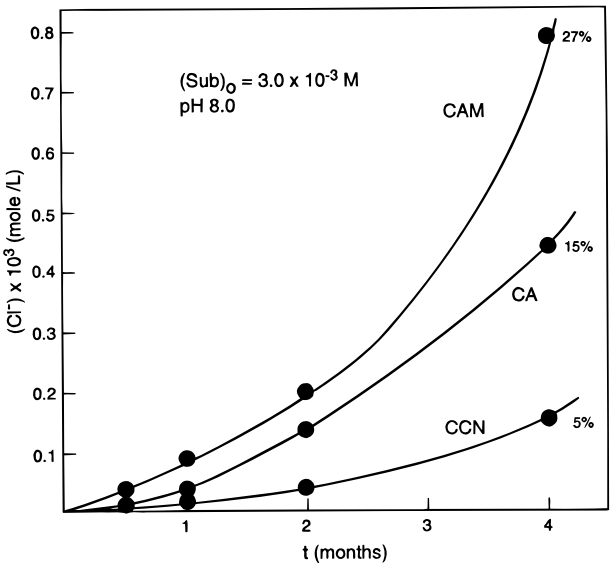


FIGURE 5. Slow chloride ion release from chloroacetonitrile (CCN), chloroacetic acid (CA), and chloroacetamide (CAM) at pH 8.0, phosphate buffer. The percent conversion at 4 mo is indicated.

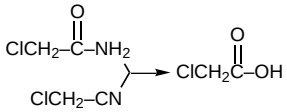
Table 2 summarizes the responses of the probes to the Lake Perris samples upon aerobic and anaerobic (under H_2) incubations. The surface water shows no conversion of CA in 2 weeks (line 1). However, the upper sediment shows a rapid conversion of CA to bicarbonate (line 2), and a sample incubated anaerobically shows no ^{13}C resonance at all at 1 week. CAM is partially oxidized in both aerobic and anaerobic incubations and CCN is inert. The lower sediment exhibits about the same response to CA and CAM except that, in the anaerobic incubations, both the hydrolysis to CA and oxidation are observed. CCN, while not converted aerobically, is reduced to acetonitrile in the anaerobic incubation. Thus, these sediments have the capacity for quick oxidation that may well proceed via initial hydrolysis, and they have reductive capacity as well.

Table 3 portrays the response of the probes to the coastal marine samples from Shaw's Cove. Again the seawater itself was inactive toward CA, but the upper sandy sediment oxidized all three probes to HCO_3^-. With CCN, a hydrolysis of the nitrile moiety is also observed. The lower silty sediment incubated aerobically with CA exhibits a complete oxidation to bicarbonate. Incubated anaerobically, bicarbonate is still produced, but both hydrolysis and reduction products are detectable. CAM is oxidized aerobically, but mainly hydrolyzed to CA in the anaerobic incubation. CCN is both hydrolyzed to CA and oxidized. As with the Lake Perris sediments, these marine sediments exhibit a broad capacity for oxidation that may entail preliminary hydrolysis. The lower sediment is also capable of reduction.

The complete conversion of all of the probes in 2 weeks and less by the activated sludge sample is noted in Table 4. Clearly the sludge shows good capacity for hydrolysis and oxidation. The anaerobic incubations slow down the overall process such that the intermediates (CA, glycolic acid) are detectable.

Discussion

The main point these data establish is that soil, fresh water sediments, marine sediments, and sludge all have a high capacity for the transformation of xenobiotics. The complete oxidation of all three probes to bicarbonate in 2 weeks or less is not uncommon. Clearly this is a multistep process that likely entails the initial hydrolysis of the C−Cl bond. In many cases where the oxidation of CAM and CCN is observed, CA is also a major product. We infer that the general oxidation of CAM and CCN proceeds via an initial hydrolysis of the amide and nitrile moieties to CA (eq 5)



(5)

followed by hydrolysis and oxidation of the latter (eqs 2 and 3, X = CO_2H). However, this is not always true. Thus, CAM is both hydrolyzed and oxidized to bicarbonate by the 1-yr-old dump site soil, and repeated experiments confirm this. It is unlikely though that the CCN probe is directly hydrolyzed at C−Cl or oxidized at $ClCH_2$ because products derived from the corresponding cyanohydrin or mixed halo cyanohydrin (formate, formaldehyde) are not observed as they have been in the metabolism of CCN by

TABLE 2

Response of Probes to Lake Perris Sediments

environment	probe	time incubation (wk)	products	% conversion[a]	conclusion
surface water (LSW)	CA	2/aerobic	none	0	NR[b]
LP-1 (upper sandy sediment)	CA	1/aerobic	HCO_3^-	100	complete oxidation
	CA	1/anaerobic[c]	0[d]	100	all metabolized
	CAM	2/aerobic	HCO_3^-	60	oxidation
	CAM	2/anaerobic[c]	HCO_3^-	30	oxidation
	CCN	2/aerobic	none	0	NR
	CCN	2/anaerobic[c]	none	0	NR
LP-2 (lower silty sediment)	CA	1/aerobic	HCO_3^- only	100	complete oxidation
	CA	2/aerobic	0[d]	100	all metabolized
	CAM	2/aerobic	HCO_3^-	50	oxidation
	CAM	2/anaerobic[c]	CA	38	amide hydrolysis (18)
			HCO_3^-		oxidation (20)
	CCN	2/aerobic	none	0	NR
	CCN	2/anaerobic	CH_3CN	14	reduction

[a] Estimated from ^{13}C-labeled intensities. [b] NR, no reaction, probe recovered. [c] Under H_2. [d] 0, no ^{13}C-labeled resonances detected.

TABLE 3

Response of Probes to Marine Coastal Sediments[a]

environment	probe	time incubation (wk)	products	% conversion[b]	conclusion
surface water (MSW)	CA	5/aerobic	none	0	NR[c]
SC-1 (upper sandy sediment)	CA	2/aerobic	HCO_3^-	50	oxidation
	CA	5/aerobic	HCO_3^- only	100	complete oxidation
	CAM	2/aerobic	HCO_3^- only	100	complete oxidation
	CCN	2/aerobic	CA	55	hydrolysis of CN (11)
			HCO_3^-		oxidation (44)
SC-2 (lower silty sand sediment)	CA	4/aerobic	HCO_3^- only	100	complete oxidation
	CA	4/anaerobic[d]	CH_3CO_2H	57	reducation (1)
			$HOCH_2COH$		hydrolysis (6)
			HCO_3^-		oxidation (50)
	CAM	2/aerobic	HCO_3^- only	100	complete oxidation
	CAM	2/anaerobic[d]	CA	68	amide hydrolysis (25)
			HCO_3^-		oxidation (43)
	CCN	2/aerobic	HCO_3^-	50	oxidation

[a] From Shaw's Cove, Laguna Beach, CA. [b] Estimated from ^{13}C-labeled intensities. [c] NR, no reaction, probe recovered. [d] Under H_2.

TABLE 4

Response of Probes to Activated Sludge

environment	probe	time incubation (wk)	products	% conversion[a]	conclusion
activated sludge (AS)	CA	0.57/aerobic	HCO_3^-	50	oxidation
	CA	2/aerobic	HCO_3^-	100	complete oxidation
	CA	2/anaerobic	$HOCH_2COH$	87	hydrolysis (33)
			HCO_3^-		oxidation (54)
	CAM	0.71/aerobic	CA	100	amide hydrolysis (35)
			HCO_3^-		oxidation (65)
	CAM	2/aerobic	HCO_3^- only	100	oxidation
	CAM	0.86 anaerobic	CA	100	amide hydrolysis (60)
			HCO_3^-		oxidation (40)
	CAM	2/anaerobic	CA	100	amide hydrolysis (40)
			HCO_3^-		oxidation (60)
	CCN	0.71/aerobic	CA	100	CN hydrolysis (65)
			HCO_3^-		oxidation (40)
	CCN	2/aerobic	HCO_3^-	100	oxidation
	CCN	2/anaerobic	CA	100	hydrolysis: amide (55)
			$HOCH_2COH$		C−Cl (15)
			HCO_3^-		oxidation (35)

[a] Estimated from ^{13}C intensities.

a soil methylotroph (*6*). Only the Lake Perris sediments did not hydrolyze CCN to CA. However, the lower sediment (LP-2) was capable of its reduction to acetonitrile. The combined activities of anaerobic and aerobic processes are exemplified by the response of CA to the lower marine sediment from Shaw's Cove (Table 3, line 7) and in the astonishing quick, complete metabolism of CA by Lake Perris sediments (Table 2, lines 3 and 9). In these cases, the HCO_3^- had also been consumed, such that the NMR exhibited no ^{13}C-labeled resonances above background. Thus the probe as well as the DMF had been wholly consumed, and we presume incorporated into many diverse cellular constituents. Methane (δ −2.3) was not detected in the anaerobic incubation.

Based upon the results of this work, we believe that the CA probe is the simplest and gives the most direct assessment of reactivity. In terms of overall sensitivity, CA is more reactive than CAM or CCN. Based upon conversion by the four segments of the environment tested, the order of reactivity is CA > CAM > CCN. For example with LP-1 (Table 2), the percent conversion of the various probes at 2 weeks in aerobic incubations is CA (100), CAM (60), and CCN (0). With LP-2, the conversion ratios are 100:50:0, and with d.S soil (Table 1) they are 100:48:30. On the other hand, the CAM and CCN probes do allow a direct measure of amide and nitrile hydrolysis rates.

In interpreting the responses of CA, it is important to realize that products corresponding to hydrolysis, reduction, or oxidation may not be detected if they are more quickly converted to CO_2 than the initial reaction with CA occurs. This is very clear with activated sludge where in only 4 days (Table 4, line 1) a 50% conversion to bicarbonate occurred, but no intermediates were detected. The anaerobic incubation (line 3) slowed the overall oxidation, however, such that glycolic acid (hydrolysis product) was observed. In such cases, a reduction to acetic acid followed by oxidation (eq 4) may also be missed. This pathway may be expected in mixed aerobic−anaerobic environments (e.g., Table 3, line 7). In short, an observation of HCO_3^- from CA does indicate oxidative capacity at the tested site, but additional processes may be discerned by an anaerobic or partially anaerobic incubation.

Comparing the response of all three probes with each segment of the environment allows the latter to be ranked for general overall reactivity. As an approximation of aerobic transformation capacity, we have simply added the percent conversion of each probe at a 2-week incubation without regard for products. The ranking and sum of the percentage conversion (in parentheses) is as follows: AS (300) > SC-1 (250) > d.S (190−200) > LP-1 and LP-2 (150−160) > d.S-1 yr old (134) ≫ LSW, MSW (0). Assuming SC-2 would completely mineralize CA in 2 weeks as it did CAM, it would rank with SC-1. Using CA alone as the probe, where mineralization is the major occurrence, the ranking would be LP-1, LP-2 ∼ AS > d.S (100) > SC-1, SC-2 (∼60) ≫ d.S-1 yr old, LSW, MSW (0). (The LP-1 and LP-2 samples mineralize CA in less than 1 week.) The similarity in results with CAM and CCN with the fresh or 1-yr-old dump site soil suggest that the hydrolysis of the amide and nitrile moieties is a chemical rather than microbial conversion catalyzed by this soil.

In this work, the probe concentrations varied from ∼100 to 200 ppm, yet in many cases they were mineralized in less than 2 weeks. Though our results are limited, it is obvious that a large diverse capacity for the transformation of xenobiotics exists in the terrestrial environment. Moreover, ^{13}C-NMR site reactivity probes can monitor the nature and assess the speed of these processes in a direct and simple way.

At this time, the predictive value of site reactivity probes is limited by the knowledge of environmental reactivity generally. However, the probes can provide the basis for a reasonable approximation of the nature and rate of transformation of other substances. Thus, for strictly chemical conversions, structure−activity relationships are well developed (*10*). Unfortunately, a correspondingly broad knowledge of defined microbial transformations and their rates is lacking, but it is emerging (*11*).

Acknowledgments

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