Biodegradation and Fish Toxicity of Nonionic Surfactants

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In order to investigate the fate and toxicity to fish of nonionic surfactants in the environment, biodegradation tests of river sediments and river water were carried out. In the biodegradation test of river sediments, residual surfactants in the water phase and sediments were analyzed by the colorimetric cobalt-thiocyanate method (CTAS) and high performance liquid chromatography (HPLC) with a fluorimetric detector. Two kinds of nonionic surfactants, polyoxyethylene nonylphenol ether (C_9APE_9) and polyoxyethylene alcohol ether ($C_{12}AE_7$), were degraded by river sediments under both stirring and standing conditions, detected by CTAS and HPLC measurements. There was little difference between the time-course of both surfactants measured by CTAS and HPLC in the water phase. Although the adsorption of both surfactants on sediments was low, most of both surfactants adsorbed on sediments were biodegraded during the test period. Nonylphenolmonoethoxy acetate (Met 2) and nonylphenolacetate (Met 1) were identified as biodegradation intermediates using preparative HPLC and mass spectrometric analysis.

In the river die-away test of C_9APE_9, the reduction of toxicity to fish of C_9APE_9 was observed in the course of biodegradation (after 10 days). LC_{50} of primary biodegradation intermediates, Met 1 and 2, was almost at the same level as that of C_9APE_9. This result suggests that the reduction of toxicity to fish is due to further biodegradation of C_9APE_9 through Met 1 and 2.

Polyoxyethylene alcohol ether (AE) and polyoxyethylene alkylphenol ether (APE) are widely used as detergents, emulsifiers, solubilizers, wetting agents and dispersing agents in household products and industrial chemicals.

The primary biodegradability of these surfactants has been established by the cobalt-thiocyanate method (CTAS) and Wickbold's bismus chloride method, while their ultimate biodegradability has been investigated using oxygen uptake, CO_2 evolution, total organic carbon measurement and radioisotopic labelling techniques. The Japanese Environmental Agency (JEA) has investigated the existence of nonionic surfactants in Japanese river water and sediments (1978 and 1982) (1). JEA data in 1978 showed that APE levels ranged from 0.0 ppm (80 samples) to 0.13–0.93 ppm (25 samples) for river water and from 0.0 ppm (19 samples) to 2.1–50 ppm (69 samples) for river sediments, while the decrease of both levels was observed in 1982, ranging from 0.0 ppm (29 samples) to 0.015–0.09 ppm (1 sample) for river water and from 0.0 ppm (22 samples) to 2.6–4.9 ppm (8 samples) for river sediments. On the other hand, AE levels reported by JEA in 1982 were less than 0.005 ppm (undetectable: all 30 samples) for river water and ranged from 0.0 ppm (11 samples) to 0.22–1.0 ppm (19 samples) for river sediments. JEA's data suggests that nonionic surfactants are inclined to distribute on river sediments through river water. Nonionic surfactants adsorbed on river sediments are considered to be degraded by microorganisms in river sediments, but little is known about the extent and mechanism of biodegradation by river sediments and the fish toxicity of their biodegradation intermediates.

The purpose of this study was to confirm the biodegradability of nonionic surfactants by river sediments. For C_9APE_9, changes in fish toxicity during biodegradation in river water were further investigated. In this study, high performance liquid chromatography (HPLC) measurements were used to avoid any interference due to components from river sediments. HPLC measurement gave more exact information on the behavior of nonionic surfactants in river sediments and river water.

MATERIALS AND METHODS

Surfactant. Polyoxyethylene lauryl ether, $C_{12}AE_7$, was synthesized by adding about 7 mol of ethylene oxide (EO) to 1 mol of lauryl alcohol in the presence of 0.01 mol NaOH as a catalyst under 3 kg/cm^{2}G of pressure at 150-160 C. The catalyst was removed by adsorbing to SiO_2.MgO. The average number of EO units is calculated as 7 mol by the measurement of OH value of this product.

Polyoxyethylene nonylphenol ether (C_9APE_9), which was derived from tripropylene, was obtained from the Kao Corp. in Japan. This surfactant was a commercial grade product (Emulgen 909 ™) with an average 8.9 mol of EO units.

Synthesis of biodegradation intermediates. Two nonylphenolpolyethoxy carboxylic acids were prepared as model biodegradation intermediates of C_9APE_9. Sodium 4-nonylphenolacetate (Met 1) was obtained by carboxymethylation of sodium alcoholate of 4-nonylphenol with sodium monochloroacetate in toluene, neutralizing the evaporated product with alcoholic NaOH and drying it after removal of unreacted nonylphenol with water/diethylether and subsequent extraction of the acidified product from the water phase with diethylether. Sodium 4-nonylphenolmonoethoxyacetate (Met 2) was prepared by carboxymethylation of 4-nonylphenolmonoethylene glycol ether in the same manner as mentioned above.

Pre-labelling reagent for AE. A fluorescent prelabelling reagent for AE, 1-anthroyl nitrile, was synthesized in the manner shown in Figure 1.

River water and river sediments. River sediments for the biodegradation test were obtained twice from the JEA's sampling point (B_2) of the Yahagi River in Kawasaki City on Oct. 18, 1982 and July 25, 1983.

River water for the river die-away test of C_9APE_9 was collected from the Arakawa River, near Horikiri Bridge located 13 stream kilometers above the estuary. In this water, a high concentration of salts was present due to the inflow of sea water. This water also was used for the acclimatization of Japanese killifish (*Oryzias latipse*) for fish toxicity. The quality of this river water is as follows:

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pH 7.0 (at 16.2 C); electric conductivity, 19 mS/cm (at 25 C); water hardness, 3400 mg/l as $CaCO_3$; chloride ion, 6500 mg/l.

Biodegradation test. To confirm whether nonionic surfactants are essentially biodegraded by river sediments, the synthetic test medium (Table 1) described in Japanese Industrial Standard (JIS) K 3363 was used as the test medium in this study instead of river water. After this preliminary study, the river die-away test was carried out for C_9APE_9.

Estimation of biodegradability of nonionic surfactants by river sediments. Biodegradation tests were carried out by adding 20 mg/l of nonionic surfactants ($C_{12}AE_7$ or C_9APE_9) and 3000 mg/l of river sediments to five l of the separate synthetic test media shown in Table 1 under standing or stirring (ca. 60 rpm) conditions. Periodically, 100-ml samples were withdrawn and centrifuged (3000 rpm: 1500 × g, 20 min) to separate the supernatant and sediments. The supernatant was used for the determination of residual nonionic surfactant by the colorimetric method and HPLC. Three to five g of dried sediments, which were separated from test media at 0, 10 and 30 days after inoculation, were extracted with 100 ml of methanol under reflux for 1 hr. After filtration of the extract with No. 5A filter paper, further extraction was repeated twice. The filtered extracts were combined and evaporated to dryness under a reduced pressure. The residues were dissolved in water and diluted to 100 ml for the determination of CTAS and HPLC-nonionic surfactant.

Separation and identification of biodegradation intermediates of C_9APE_9. In order to separate and identify the biodegradation intermediates of C_9APE_9 by river sediments, eluates corresponding to them on HPLC were collected by repeating the HPLC procedure 10 times. The eluates were combined and analyzed by FD- and electron impact-mass spectrometry including methyl esterified intermediates by diazomethane.

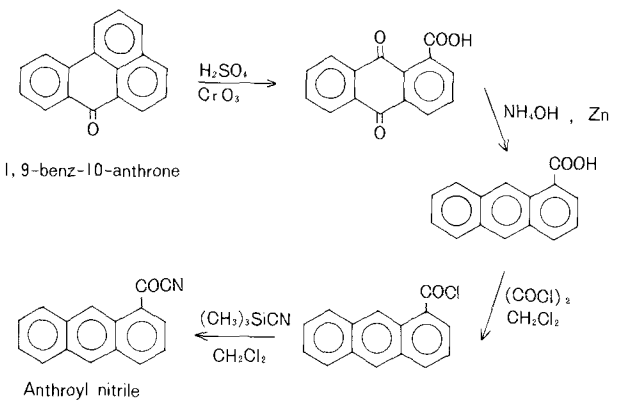

FIG. 1. Schematic diagram for synthesis of 1-anthroylnitrile.

TABLE 1

Composition of Biodegradation Test Medium

Component	Concentration (g/l)
NH_4Cl	3
K_2HPO_4	1
$MgSO_4 \cdot 7H_2O$	0.25
KCl	0.25
$FeSO_4$	0.002
Yeast extract	0.3

River die-away test of C_9APE_9. Twenty mg/l of C_9APE_9 was added to 60 l of river water in a glass tank, which was collected from the Arakawa River, and circulated by pump at a rate of about 2.5 l/min at room temperature (15-22 C). Another tank served as a control receiving no surfactant. Periodically, about two l of the test water were withdrawn individually from each tank for the determination of residual C_9APE_9, TOC, pH and fish toxicity.

Fish toxicity test. Two types of experiments were conducted to evaluate the acute toxicity of intact nonionic surfactants and their intermediates:

- Static toxicity tests to determine the LC_{50} of the intact nonionic surfactants and synthesized model biodegradation intermediates.
- River die-away test to examine the change of fish toxicity of C_9APE_9 during and after biodegradation.

Japanese killifish of 2.0 cm average length and 0.2 g of average weight were obtained from a commercial fish farmer. The fish were acclimatized to either river water (including model river water) or dechlorinated tap water prior to the fish toxicity test.

LC_{50} determination of intact nonionic surfactants and synthesized model biodegradation intermediates (Met 1 and 2) were conducted according to the fish bioassay procedure described in Japanese Industrial Standard (JIS) K0102. LC_{50} of C_9APE_9 was determined in the three kinds of water, from the Arakawa River, model river water and dechlorinated tap water. The quality of river water from the Arakawa was the same as the one used for the river die-away test. Model river water was prepared by adding 19.7 mg of $CaCl_2$, 1.1 mg of K_2SO_4, 8.65 mg of $MgSO_4$ and 25 mg of $NaHCO_3$ to one l of ion-exchanged water. The quality of this water was as follows: pH 7.0; alkali degree, 20 mg/l; water hardness as $CaCO_3$, 25 mg/l. Water hardness and pH of tap water were 50 mg/l and 7.2, respectively.

In each test, the fish were placed at random in groups of 10 in glass beakers containing two l of each concentration of samples. The mass/volume ratio was about one g fish/l of water. After the preliminary test (range-finding), LC_{50} determinations were carried out by observing fish survival in single test solution prepared for each concentration. LC_{50} values were estimated by Doudoroff's graphic interpolation method.

In order to know the change in toxicity to fish in the course of biodegradation of C_9APE_9, about two l of water were withdrawn periodically from the tanks of river die-away test described in biodegradation test method. The determination of exact LC_{50} values of nonionic surfactants during biodegradation was very difficult because the composition of intact surfactants and biodegradation intermediates was changing hourly; only the fish survival rate was determined.

Analytical procedure. The concentration of nonionic surfactants remaining in the test media and river sediments was analyzed using both the colorimetric method and HPLC with a fluorimetric detector. For the colorimetric measurement, the cobalt-thiocyanate method described in Japanese Industrial Standard (JIS) K 3364 was used. Cobalt-thiocyanate reagent was

prepared by dissolving 620 g of reagent grade ammonium thiocyanate and 280 g of reagent grade cobalt nitrate hexahydrate in water and diluting to one l. This reagent was used after two extractions with benzene to lower the blank reading. Fifteen ml of the ammonium cobalt-thiocyanate reagent and 35.5 g of sodium chloride were added to each 100 ml of sample solution and shaken for one min. After this was allowed to stand for 15 min, 25 ml of benzene were added to this solution, then shaken for one min. The peak absorbance of benzene layer at 320 nm was measured. The determination of $C_{12}AE_7$ and C_9APE_9 by HPLC was carried out according to Kudoh's method (2). $C_{12}AE_7$ was measured by reversed phase HPLC after pre-labelling with a fluorescent 1-anthroyl nitrile synthesized in Tochigi Research Laboratory of Kao Corp. (Fig. 1) because of its having no specific ultraviolet active or fluorescent functional group.

The determination of C_9APE_9 was carried out by a fluorescent normal-phase HPLC without derivatization. The conditions for HPLC measurements are described in Table 2.

Mass spectrometry was performed on a Hitachi M-80 with combined field desorption/field ionization/electron impact ion source. Operational conditions of EI-MS and FD-MS were as follows: ion source temperature, 150 C; ionizing electron energy, 70 eV at 100 μA for EI-MS and emitter potential, 2.1 KV; emitter current, increased from 0 to 45 mA at a rate of 2.5 mA/min; chamber potential, 3 KV for FD-MS.

RESULTS AND DISCUSSION

Biodegradation of $C_{12}AE_7$ and C_9APE_9 by river sediments. Biodegradation tests of $C_{12}AE_7$ and C_9APE_9 by river sediments were carried out twice, and reproducible results were obtained. Figure 2 shows that the change of nonionic surfactants remained in the water phase of the test system at the second test. $C_{12}AE_7$ was degraded a little faster than C_9APE_9 under either a standing or a stirring condition. The rate of biodegradation was greater under a stirring than standing condition. Under a stirring condition, more than 98% of $C_{12}AE_7$ and C_9APE_9 disappeared within five days, while 10 days were required for the same extent of biodegradation under a standing condition. There was no difference between the time-course of both surfactants measured by the cobalt-thiocyanate method (CTAS) and HPLC in the water phase. HPLC chromatograms of $C_{12}AE_7$ and C_9APE_9 in the course of biodegradation are shown in Figures 3 and 4, respectively.

On HPLC chromatograms of $C_{12}AE_7$ after five days, disappearance of $C_{12}AEn$ with 0, 1 and 2 mol of EO units was observed, while several new peaks appeared between $C_{12}AE_3$ and $C_{12}APE_6$. C_9APE_9 disappeared within 10 days, as shown in Figure 4, while new peaks appeared at 10-40 min of retention time and remained even 30 days after inoculation. The existence of C_9APEn with 1-3 mol of EO was observed on HPLC chromatograms after 5 and 10 days (Fig. 4). The concentration of these shorter ethoxamers was low (less than 0.4 mg/l). Their existence was detected only when raising the sensitivity of the fluorimetric detector 4-16 times. The concentration of $C_{12}AE_7$ and C_9APE_9 remaining in

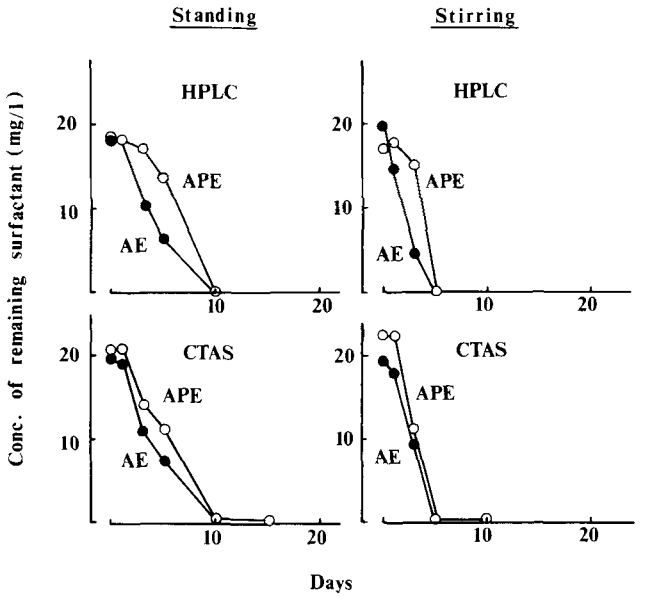

FIG. 2. Time-course of residual $C_{12}AE_7$ and C_9APE_9 in the water phase during biodegradation, detected by CTAS and HPLC. River sediments, 3000 mg/l (The Yahagi River); nonionic surfactant, 20 mg/l.

TABLE 2

Condition of HPLC Analysis of C_9APE_9 and $C_{12}AE_7$

Condition	C_9APE_9	$C_{12}AE_7$
Column	Nucleosil 5CN 5μm 4 mm ø × 150 mm	Hypersil ODS 3 μm 6 mm ø × 150 mm
Eluate	Linear gradient elution; n-hexane was delivered first from the pump and after 60 min was replaced with EtOH/THF (60/40) including 1.5% water	Acetonitrile/H_2O (75/25)
Flow rate	2 ml/min	2 ml/min
Detector	Shimadzu RF-350	Shimadzu RF-530
Ex.	280 nm	395 nm
Em.	310 nm	450 nm

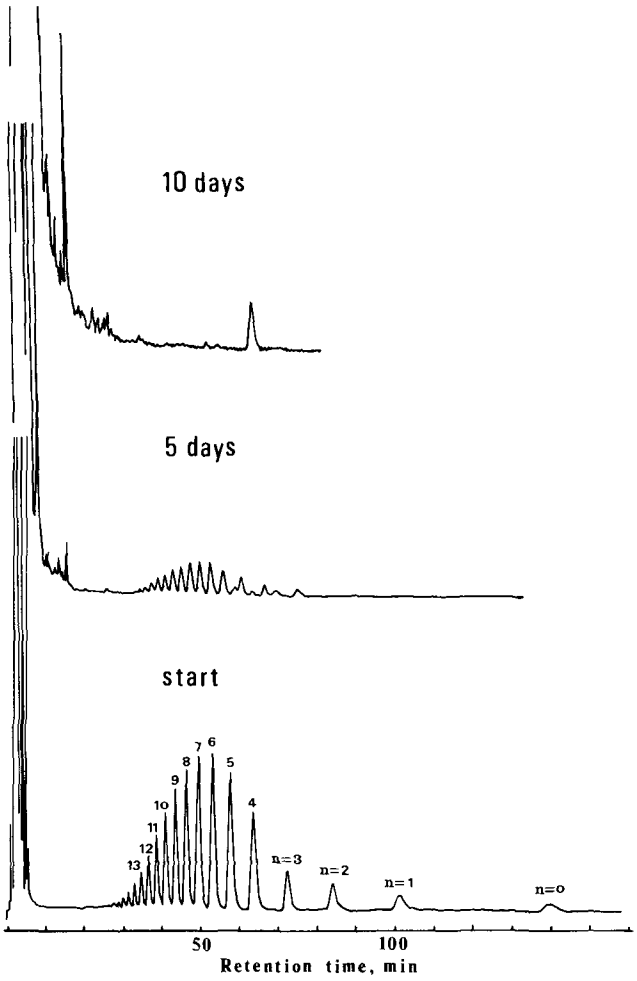

FIG. 3. HPLC chromatograms of $C_{12}Ae_7$ in the water phase during biodegradation by river sediments under a standing condition. River sediments, 3000 mg/l (The Yahagi River); nonionic surfactant, 20 mg/l; sensitivity of HPLC detector, range 1.

sediments was determined by the cobalt-thiocyanate method and HPLC. CTAS values of extracts from sediments varied in each case and no pattern of change was observed during biodegradation (Table 3). CTAS measurement gave sometimes higher values for control samples than for test samples, e.g., C_9APE_9 at the start under a standing condition and $C_{12}AE_7$ after 30 days under a stirring condition. From the above results, the CTAS method is considered to be unsuitable for the determination of nonionic surfactants in the extracts from sediments. The interference observed in CTAS measurement could be almost totally removed using HPLC (Fig. 5). Although biodegradation intermediates from C_9APE_9 appeared on the same retention time as that of intact C_9APE_9, they will not cause any significant interference for the determination of C_9APE_9 because these intermediates appeared after the disappearance of most C_9APE_9. Such interference was not observed for the determination of $C_{12}AE_7$ by HPLC. HPLC values of $C_{12}AE_7$ and C_9APE_9 in the sediments decreased after 10 days under both conditions. This result suggests that nonionic surfactants are degraded by microorganisms after the transfer to sediments. Material balance is summarized in Table 3. Calculating the distribution of $C_{12}AE_7$ in one l of test medium (including 3 g of sediments) at the start under a standing condition, 18.2 mg and 84.9 μg of $C_{12}AE_7$ were obtained in the water phase and sediments, respectively. A similar result was obtained for C_9APE_9. The result suggests that the transfer of nonionic surfactants to the sediments is very small.

Identification of biodegradation intermediates of C_9APE_9. Figure 6-A shows the EI-MS spectra of biodegradation intermediates of C_9APE_9 collected using preparative HPLC. Each peak observed in Fig. 6A was considered to be attributable to the fragments in Table 4. The peaks at 221, 235 and 249 m/z corresponded to the fragments formed by the addition of each CH_2 unit to 207 m/z shown in Table 4. Similar understanding is

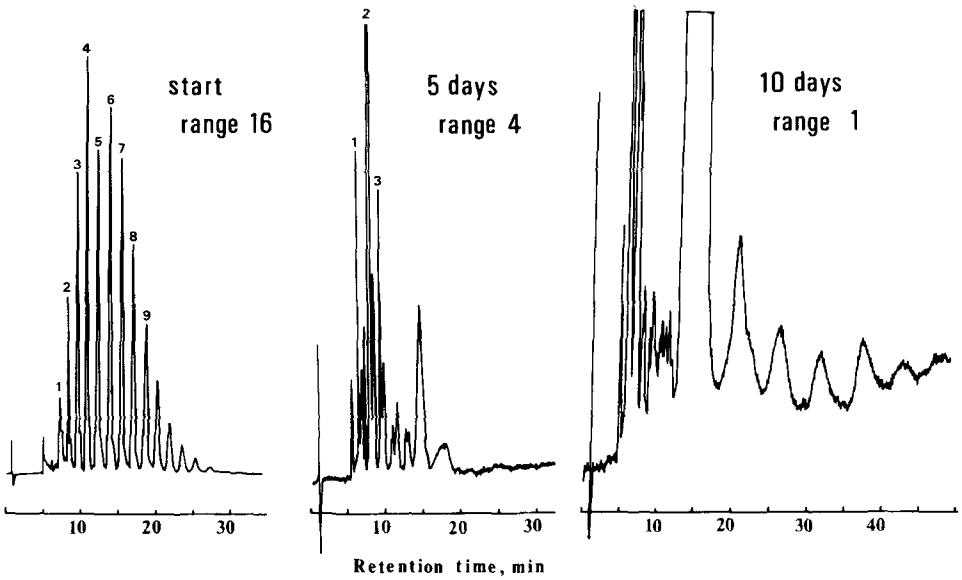

FIG. 4. HPLC chromatograms of C_9APE_9 and their biodegradation intermediates during biodegradation by river sediments under a stirring condition. River sediments, 3000 mg/l (The Yahagi River); nonionic surfactant, 20 mg/l.

TABLE 3

Material Balance of $C_{12}AE_7$ and C_9APE_9 in the Course of Biodegradation[a]

	Water—Standing (mg/l)		Sediments—Standing (μg/g)		Water—Stirring (mg/l)		Sediments—Stirring (μg/g)	
Surfactant	CTAS	HPLC	CTAS	HPLC	CTAS	HPLC	CTAS	HPLC
$C_{12}AE_7$								
0 day	19.6	18.2	5	28.3	19.2	20.0	50	19.3
10 day	0.3	0.22	5	4.1	0.3	0.15	25	1.6
30 day	0.3	0.1	10	1.2	—	—	−25	0
C_9APE_9								
0 day	20.8	18.7	−20	26.3	22.2	17.1	55	33.2
10 day	0.7	0.25	20	7.3	0.2	0.2	110	3.6
30 day	0.6	0.2	37	0.7	—	—	20	0.5

[a]Figures in table are expressed as values subtracted from control.

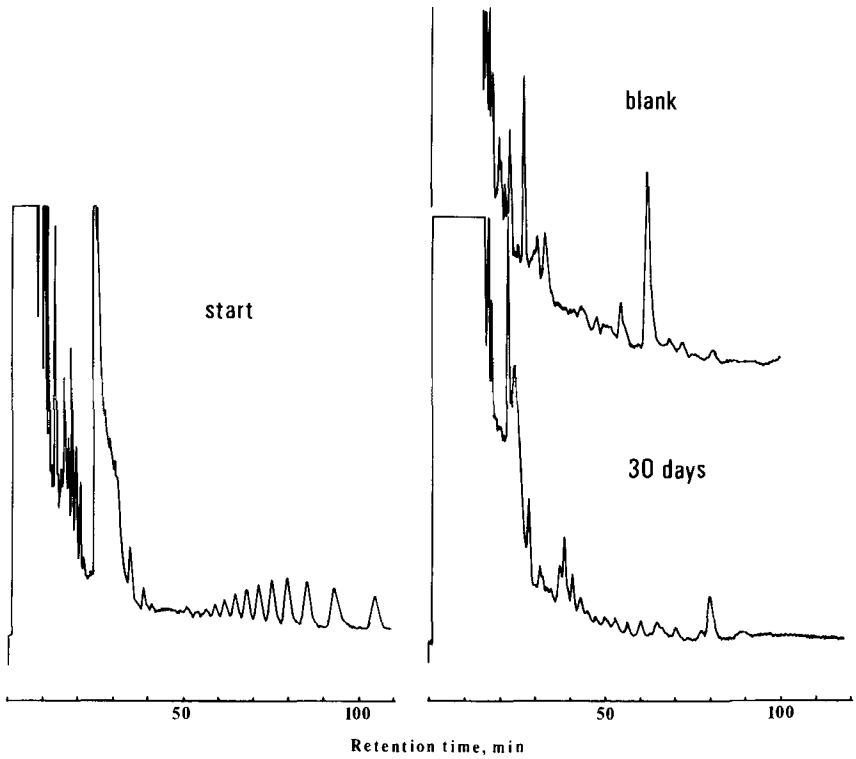

FIG. 5. HPLC chromatograms of the extracts from sediments during biodegradation of $C_{12}AE_7$ under standing condition. River sediments, 3000 mg/l (The Yahagi River); nonionic surfactant, 20 mg/l.

possible for the peaks at 265, 279 and 293 m/z, which are related to 251 m/z shown in Table 4. Each peak observed in the EI-MS spectrum of methyl esterified intermediates was shifted to corresponding ones whose molecules were increased by that of CH_3 group. From the above results, it is inferred that the intermediates are composed of two chemicals whose terminal CH_2OH of polyoxyethylene nonylphenol ether with 1 and 2 mol of EO are oxidized to carboxyl group (Met 1 and 2). To confirm the estimation FD-MS spectral analyses of these intermediates and their methyl esters were carried out. Since the peaks at 278, 322 m/z (Fig. 6C) and 292, 336 m/z (Fig. 6B) corresponded to the above-mentioned nonylphenol polyethoxy carboxylic acids and their methyl esters, respectively, it was concluded that C_9APE_9 was degraded from the hydrophilic group (EO units)shown in Figure 8. The intermediates, Met 1 and Met 2, can be obtained through either an oxidation of terminal alcohol with subsequent hydrolysis of β-position of longer C_9APE_n (I and II) or oxidation of C_9APE_1 and C_9APE_2 (III and IV), which are constituents of intact C_9APE_n.

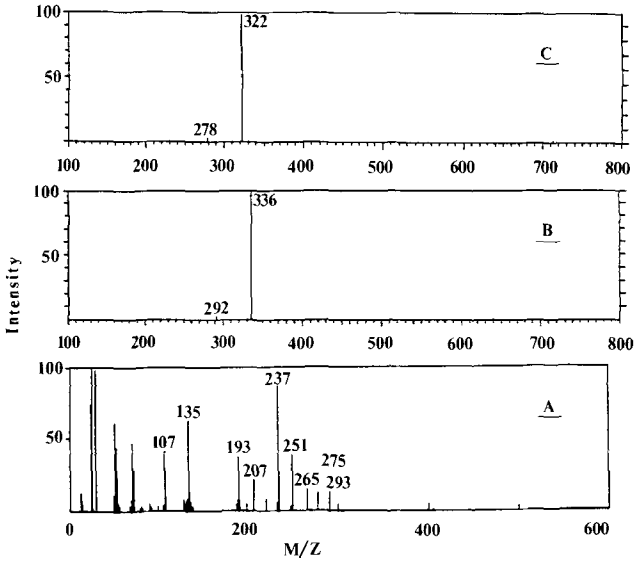

FIG. 6. Mass spectra of biodegradation intermediates. A, EI-Mass spectrum of biodegradation intermediates of C_9APE_9. B, FD-Mass spectrum of the methyl ester of biodegradation intermediates of C_9APE_9. C, FD-Mass spectrum of biodegradation intermediates of C_9APE_9.

TABLE 4

Assignment of Fragments in EI-Mass Spectrum of Biodegradation Intermediates

m/z	Structure
251	$+CH_2C(CH_3)_2$—Ph—$OCH_2CH_2OCH_2CH_2COOH$
237	$+C(CH_3)_2$—Ph—$OCH_2CH_2OCH_2CH_2COOH$
207	$+CH_2C(CH_3)_2$—Ph—OCH_2COOH
193	$+C(CH_3)_2$—Ph—OCH_2COOH
135	$+C(CH_3)_2$—Ph—OH
107	$+CH_2$—Ph—OH

Ph = phenylene group.

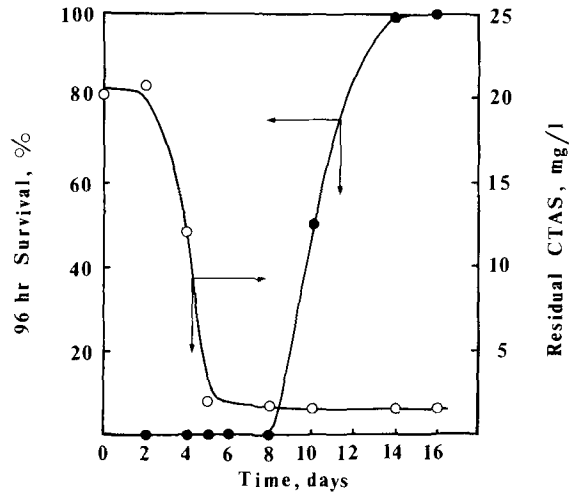

FIG. 7. Change of residual APE and 96-hr survival rate of Japanese killifish during biodegradation of C_9APE_9.

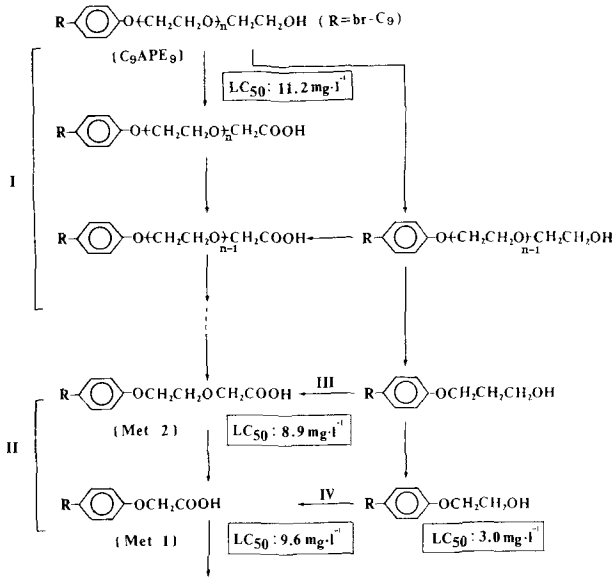

FIG. 8. Proposed pathway for biodegradation of C_9APE_n.

Reinhard et al. (3) reported that alkylphenol polyethoxy carboxylic acids have been detected in biologically treated domestic wastewater. Brüschweiler et al. (4) identified one intermediate from C_9APE_{11} to be Met 2 with GC-MS analysis after derivatization with trimethylsilane. The same intermediate (Met 2) was obtained in this report; therefore, the biodegradation pathway seems to be the same.

Change of fish toxicity C_9APE_9 in river die-away test. Table 5 shows the change of water quality and survival rate (%) of Japanese killifish of test waters taken in the course of the river die-away test of C_9APE_9. Biodegradabilities of C_9APE_9 after 4, 5, 8 and 10-16 days were calculated as 39, 90, 92 and 94%, respectively, by CTAS measurement shown in Table 5. Up to the eighth day, the residual concentration of C_9APE_9 measured by HPLC decreased to the same level of CTAS. Although the concentration of residual CTAS was 1.6 mg/l after 10 days, intact C_9APE_9 could not be found by HPLC. The peaks of intact C_9APE_9 on HPLC after eight days could be found at the eight times sensitivity of the detector compared as that of one and four days after inoculation, but these peaks could not be observed even at the 16 times sensitivity for the sample after 10 days. These results suggest that CTAS measurement may be interfered with by something derived from river water during incubation. Although 96-hr survival rates of killifish were 0% until the eighth day, they increased from the 10th day (50%) and attained 100% after 14 days. The CTAS concentrations of C_9APE_9 after four and five days were 12.2 and 2.1 mg/l, respectively. Since the former is almost the same as LC_{50} of intact C_9APE_9 and the latter corresponds to LC_0 of C_9APE_9, more than half of the fish should be alive. However, the 96-hr survival rates of these samples were 0%.

TABLE 5

Change of Water Quality and Fish Survival Rate of Test Water During Biodegradation of C_9APE_9

Time (day)	Test water							Control					
	Survival (%)			APE (mg/l)		pH	TOC (mg/l)	Survival (%)			APE (mg/l)	pH	TOC (mg/l)
	24 hr	48 hr	96 hr	CTAS	HPLC			24 hr	48 hr	96 hr			
0	0	0	0	20.2	18.6	7.2	31	100	100	100	0.5	7.0	17
2	0	0	0	20.9	14.4	7.9	21	—	—	—	—	7.9	—
4	0	0	0	12.2	8.7	7.9	19	—	—	—	0.5	8.0	7.8
5	0	0	0	2.1	1.7	7.9	12	—	—	—	—	—	—
6	20	0	0	—	—	—	—	—	—	—	—	—	—
8	40	10	0	1.9	1.5	8.0	13	—	—	—	0.5	8.0	9.2
10	80	80	50	1.6	0.1	7.8	9.5	—	—	—	0.5	7.7	7.5
14	100	100	100	1.6	0.1	8.0	16	—	—	—	0.5	8.0	8.2
16	100	100	100	1.6	0.1	7.9	12	100	100	100	0.5	8.0	6.0

TABLE 6

Fish Toxicity (48 hr LC_{50}) of Intact C_9APE_n and Synthetic Biodegradation Intermediates for Japanese Killifish (*Oryzias latipse*)

Sample	Structure	48 hr LC_{50} (mg/l)
C_9APE_n	br-C_9-Ph-O(CH_2CH_2O)$_n$	
	n = 0 (Nonyl phenol)	1.4[b]
	1	3.0[b]
	3.3	2.5[b]
	5.0	3.6
	6.4	5.4
	8.4	11.6
	8.9	11.2
		12.0[c]
		14.0[d]
	13.1	48.0
	16.6	110.0
Synthetic intermediates		
Met 1	br-C_9-Ph-$OCH_2CH_2OCH_2COONa$	8.9
Met 2	br-C_9-Ph-OCH_2COONa	9.6

[a]Ph indicates phenylene group.
[b]As dispersant, Tween 80® was used 2 times as much as water.
[c]Test water: river water from the Arakawa (water hardness, 3400 mg/l; pH, 7.0).
[d]Test water: model river water (water hardness, 25 mg/l; pH, 7.0).
Tests other than [c] and [d] were carried out using tap water after dechlorination with activated carbon (water hardness, 50 mg/l; pH 7.2).

Table 6 shows the LC_{50} values of C_9APE_9 homologues with 0 to 17 mol of EO units and synthesized intermediates, Met 1 and 2. As Gloxhüber and Fischer (5) reported, the fish toxicity of APE_n having shorter EO units is higher than that of longer corresponding ethoxamers. Rudling (6) and Asahara et al. (7) reported the formation of C_9APE_2 as the single major biodegradation intermediate from a br-C_9APE_{10} via shortening of the EO chain. Since the portion of shorter ethoxamers appeared to be increasing in the biodegradation test of C_9APE_9 using river sediments (Fig. 4), the shorter ethoxamers might also be relatively increased until the eighth day in the river die-away test of C_9APE_9. The residual concentration of C_9APE_9 on the eighth day by HPLC was 1.5 ppm in the river die-away test, but the 96-hr survival rate was 0%. This result suggests that the contribution of shorter ethoxamers to fish toxicity during biodegradation is small. On the other hand, the fish toxicity of synthesized biodegradation intermediates, Met 1 and 2, was a little higher than that of intact C_9APE_9. However, the fish toxicity of Met 1, corresponding to the intermediate of C_9APE_1, is about 1/3 that of C_9APE_1 (Table 6). Although a quantitative explanation of the fish toxicity in the course of the river die-away test is not possible because of the lack of data of residual Met 1 and 2, the contribution of Met 1 and 2 is inferred assistance on the synthesis of the fluorescent derivatization reagent.

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REFERENCES

1. Office of Health Studies, Environmental Health Department, Japanese Environmental Agency, *Chemicals in the Environment, Chemical Assessment Annual Report*, 96-102 (1978) and 66-67, 78-79 (1982).
2. Kudoh, Megumi, H. Ozawa, S. Fudano and K. Tsuji, *J. Chromatogr. 287*:337 (1984).
3. Reinhard, M., N. Goodman and K.E. Mortelmans, *Environ. Sci. Technol. 16*:351 (1982).
4. Brüschweiler, H., H. Gamperle and F. Schwager, *Tenside Deterg. 20*:317 (1983).
5. Bloxhüber, C., and W.K. Fischer, *Food Cosmet. Toxicol. 6*:469 (1968).
6. Rudling, O.L., in *Human Safety and Environmental Aspects of Major Surfactants*, Arthur D. Little Inc., Cambridge, Massachusetts, 1977, p. 408.
7. Asahara, S., H. Sekiguchi, N. Hayashi and K. Oba, *Yukagaku 21*:33 (1972).

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