Arch. Environ. Contam. Toxicol. 41, 267–273 (2001)
DOI: 10.1007/s002440010248

ARCHIVES OF
Environmental
Contamination
and Toxicology
© 2001 Springer-Verlag New York Inc.

Aerobic and Anaerobic Metabolism of Glutaraldehyde in a River Water–Sediment System

H.-W. Leung

Union Carbide Corporation, 39 Old Ridgebury Road, Danbury, Connecticut 06817, USA

Received: 20 August 2000/Accepted: 3 April 2001

Abstract. Material balance studies of glutaraldehyde in a river water–sediment system demonstrate that glutaraldehyde preferred to remain in the water phase. Glutaraldehyde was metabolized rapidly under both aerobic and anaerobic conditions. The pseudo-first-order half-life of catabolism, based on the loss of glutaraldehyde from the water phase, was 10.6 h aerobically and 7.7 h anaerobically. In contrast, under sterile conditions at pH 5 or 7, no appreciable degradation of glutaraldehyde was observed over a 31-day period. At pH 9, about 30% of the glutaraldehyde degraded over the same period. The major degradate was identified as 3-formyl-6-hydroxy-2-cyclohexene-1-propanal, a cyclicized dimer of glutaraldehyde. The extrapolated half-life of abiotic degradation was 508 days at pH 5, 102 days at pH 7, and 46 days at pH 9. Under aerobic conditions, glutaraldehyde was first biotransformed into the intermediate glutaric acid, which then underwent further metabolism ultimately to carbon dioxide. Metabolism of glutaraldehyde under anaerobic conditions did not proceed ultimately to methane, but terminated with the formation of 1,5-pentanediol via 5-hydroxypentanal as an intermediate.

Glutaraldehyde (1,5-pentanedial, CAS Registry no. 111-30-8) is a biocide used industrially to control the growth of microorganisms, including applications in water treatment, pulp and paper manufacture, and oil production, and as a cross-linking agent in a variety of applications, such as leather tanning, X-ray film developing, and enzyme immobilization (Russell and Hopwood 1976). In commerce, glutaraldehyde is commonly available as a 2%, 25%, or 50% aqueous solution (Cosmetic Ingredient Review 1996). The biocidal activity of glutaraldehyde appears to depend on the presence of free aldehyde groups (Gorman *et al.* 1980). In aqueous solution, glutaraldehyde consists of the cyclic hemiacetal, equally present in the two isomeric forms in equilibrium with free glutaraldehyde (Whipple and Ruta 1974). Approximately 25% of the mixture is present as linear hemihydrate and dihydrate in a ratio of 2:1. The ratio of the components varies with temperature (Whipple and Ruta 1974). Polymerization of glutaraldehyde can occur under both acidic and alkaline conditions. However, the extent of polymerization is time- and temperature-dependent and is more rapid as the pH of the aqueous solution is increased (Gorman *et al.* 1980). Glutaraldehyde can cross-link proteins but retains its antimicrobial activity in the presence of low concentrations of organic matter (Gorman and Scott 1980). Solutions of glutaraldehyde can be inactivated by ammonium compounds (Gorman and Scott 1980) or by reacting with sodium bisulfite (Jordan *et al.* 1996).

The toxicity of glutaraldehyde in humans and animals has been investigated (Beauchamp *et al.* 1992), but its metabolism has not been studied in detail. It was suggested that glutaraldehyde might be oxidized first to a mono- or di-carboxylic acid by aldehyde dehydrogenase (Hjelle and Petersen 1983). Because of the nature of its uses, glutaraldehyde has a great potential to enter surface waters. In addition, release to the aquatic environment may occur during disposal. Despite the potential ecological impact of glutaraldehyde, there was a paucity of environmental fate and effects information available in the published literature. This article reports on studies to better understand the disposition and metabolism of glutaraldehyde in a river water–sediment system under aerobic and anaerobic conditions.

Materials and Methods

Chemicals

Glutaraldehyde (50.9% aqueous solution) was provided by Union Carbide Corporation (Bound Brook, NJ). [1,5-^{14}C-carbonyl]-Glutaraldehyde with a specific activity of 13.6 mCi/mmol was obtained from NEN Research Products (Boston, MA). The radiochemical purity was determined by high-performance liquid chromatography (HPLC) to be 97.8%. [^{14}C]-sodium bicarbonate with a chemical purity of $>$ 99% and a specific activity of 8.3 mCi/mmol was purchased from Sigma Chemical (St. Louis, MO). [1,5-^{14}C]-5-hydroxypentanal and [1,5-^{14}C]-1,5-pentanediol used as analytical reference standards were synthesized by reducing [^{14}C]-glutaraldehyde with sodium borohydride, as follows: 9 μg of $NaBH_4$ was added to 2 ml of aqueous [1,5-^{14}C]-glutaraldehyde solution (10 μg, 1.35 μCi). The reaction was monitored by HPLC. About 41% of the glutaraldehyde was converted to 5-hydroxypentanal in 4 h. Conversion to 1,5-pentanediol was complete after 30 h. ^{14}C-labeled 3-formyl-6-hydroxy-2-cyclohexene-1-propanal was prepared as follows: 1 ml of glutaraldehyde solution containing about 3 mg of [1,5-^{14}C]-glutaraldehyde was added to 99 ml of sterilized 10 mM

sodium borate buffer, pH 9. A 50 ml subsample of this solution was transferred to an autoclaved flask and placed in an incubator at 35°C. A 50-μl aliquot was analyzed by HPLC after 21 days. The synthesized radiolabeled standard was isolated by a preparatory HPLC procedure that increased the separation between glutaraldehyde and the standard (Similar HPLC system as for the characterization of metabolites described later, except with a slower flow rate of 0.6 ml/min). A number of HPLC runs were performed in which fractions in the retention region of glutaraldehyde and separately of the standard were collected. The first of these was the fraction from 25–30 min. HPLC radiochromatography of this fraction revealed a sample in which glutaraldehyde was enriched, as expected. The next fraction from 30–35 min was found to be exclusively [^{14}C]-3-formyl-6-hydroxy-2-cyclohexene-1-propanal. This fraction was extracted with ethyl acetate, concentrated, and analyzed by direct probe mass spectrometry. All other chemicals used as authentic reference standards for metabolite identification were obtained from Aldrich Chemical (Milwaukee, WI). Oxidizer scintillation cocktail was obtained from R. J. Harvey (Chicago, IL). Safety-Solve scintillation cocktail was obtained from Research Products International (Mount Prospect, IL). All solvents used were HPLC grade and were obtained from Fisher Scientific (Pittsburgh, PA) or EM Science (Gibbstown, NJ).

Source and Microbial Viability of Sediment and Water

The sediment and water were obtained from the Sacramento River Delta at Antioch, Contra Costa County, California. Table 1 shows the physicochemical characteristics of the sediment and water. The aerobic microbial viability of the water and sediment was established throughout the study in potato dextrose agar (fungi), trypticase soy agar (bacteria), and actinomycetes isolation agar (actinomycetes) culture media. Aliquots of water or sediment (1 g) were cultured for 48 h. The water was found to be bacterially active and displayed significant activity of actinomycetes, but no significant fungi colony forming units (CFU) were detected. The sediment showed significant growth of all three types of microorganisms throughout the study. The anaerobic microbial viability of the water and sediment was established by enumerating the CFU of anaerobic microbes. Samples of sediment or water (1 g) were serially diluted with sterile (autoclaved) anaerobic media (Difco, Detroit, MI). Test tubes including controls were incubated inside an anaerobic jar, which was previously rendered anaerobic using the Difco anaerobic conditioning system. Anaerobic indicator was used to verify the absence of O_2. Colonies were counted using a Quebec Darkfield Colony Counter after 3 days of incubation at 35°C. The water and sediment were found to be bacterially active throughout the study. Prior to establishment of the test systems, the intact river water–sediment mixture was allowed to settle. The water was decanted off the sediment and a subsample of sediment was analyzed for entrained water content.

Test System

The test system consisted of 500-ml Erlenmeyer flasks equipped with a ground glass stopper and glass stopcock inlet and outlet tubes, used to remove any volatile metabolites and CO_2 while providing for replacement with fresh air (aerobic metabolism study) or nitrogen (anaerobic metabolism study) on a periodic basis. All flasks were autoclaved (250°F, 15 lb/sq. in.) with a Market Forge Sterimatic prior to the addition of sediment and river water and wrapped in aluminum foil to protect them from light at all times. Each flask contained 9.45 mg/L glutaraldehyde (11.5 μCi radiocarbon), the equivalent of 20 g dry weight of sediment (wet weight = 26.4 g), and 100 ml river water. The final volume of the test system was 106.4 ml, and the flasks were incubated statically at 25°C (Fisher Scientific Low Temperature Incubator Model 307). The dissolved oxygen content was determined using an Orion O_2 electrode (Model 97-08-00) and an Orion Digital Ionizer meter (Model 501) (Orion Research, Boston, MA) throughout the course of the experiment.

For the anaerobic metabolism study, anaerobic conditions were initiated 56 days prior to dosing by displacing the air in the test system with nitrogen for 30 min. This procedure was repeated on consecutive days for a total of six times. Twelve days prior to dosing, 2 g of anhydrous D-glucose was added to each flask in a glove box under a nitrogen atmosphere. After mixing by swirling, the flasks were sealed and returned to the incubator. A day prior to dosing the oxygen content of an extra flask was determined to ascertain anaerobicity.

Sampling of Volatile Metabolites and $^{14}CO_2$

At various sampling times, two test flasks were removed and their headspace were flushed with air (aerobic metabolism study) or nitrogen (anaerobic metabolism study) through a series of traps containing, in order: Two polyurethane foam plugs presoaked with methylene chloride and an ethylene glycol trap for the collection of organic volatiles including [^{14}C]-methane, and two KOH traps for the collection of $^{14}CO_2$. The inlet was connected to a Gelman No. 4210 bacterial vent to prevent introduction of microorganisms. Aliquots of the extracted foam plugs (extracted with methylene chloride) and all liquid traps were radioassayed by direct liquid scintillation counting (LSC) using a Beckman Liquid Scintillation Counter (Models LS 5000CE and LS6001C, Beckman Instruments, Fullerton, CA). The $^{14}CO_2$ trapping efficiency was previously determined by analyzing the recovery of radiocarbon from the acid hydrolysis of 3.6 μg (0.35 μCi) [^{14}C]-$NaHCO_3$. The trapping efficiency was determined to be 100.4%.

Radioassay of Sediment and River Water Samples

Immediately after flushing, river water was separated from the sediment by decanting following centrifugation for 5 min at 2,000 rpm. $^{14}CO_2$ in the water was determined by LSC before and after trapping and precipitation with $BaCl_2$. A 5-g subsample of the sediment was extracted three times with acetonitrile:0.001 N HCl (1:1, v/v). Aliquots (3 × 0.1 ml) were taken from each extract and radioassayed by LSC. The remaining sediment was air-dried. An aliquot (0.1–0.2 g) of the sediment was combusted in a Harvey Biological Sample Oxidizer (Model 0X600, R. J. Harvey) and radioassayed with LSC to determine the residual radiocarbon content.

Characterization of Metabolites

Metabolites were initially identified by HPLC employing a BioRad Aminex HPX-87H ion exclusion column (30 cm × 7.8 mm i.d. × 9 μm particle size) eluting isocratically with 0.01 N H_2SO_4 at a flow rate of 1.0 ml/min (aerobic metabolism study) or 0.8 ml/min (anaerobic metabolism study) and monitoring with a UV detector at 280 nm or a refractive index detector. HPLC radiochromatograms were reproduced from data collected by a Beckman Radioisotope Flow Through Detector or by fraction collection. The output signal from the UV or refractive index detectors was plotted on a Spectra Physics Chrom Jet integrator or a Hewlett Packard 3396 Series II integrator. The ^{14}C signal from the flow-through detector was plotted with an IBM PC and software supplied by Beckman Instruments.

Metabolites other than CO_2 and 3-formyl-6-hydroxy-2-cyclohexene-1-propanal (CAS # 130434-30-9) were confirmed with thin-layer chromatography (TLC). For the aerobic study, one-dimensional TLC

Table 1. Physicochemical characteristics of the river water and sediment

River Water		Sediment	
Alkalinity	86 mg $CaCO_3$/L	Sand	93%
pH	7.7	Silt	7%
Conductivity	810 μmho/cm	Clay	0%
Suspended solids	160 mg/L	Organic matter	0.9%
Hardness	140 mg $CaCO_3$/L	Organic carbon	0.5%
		pH	8.1
		Field capacity at 0.33 bar	0.055 g water/g dry soil

with 20 × 20 cm, 0.25 mm gel thickness, precoated plates containing fluorescent indicator (Merck #5715 Silica Gel 60 F_{254}) in two separate solvent systems were used: toluene:ethyl formate:formic acid (5:7:1, v/v/v) and methanol:ethyl acetate (35:65, v/v). For the anaerobic study, two-dimensional TLC in two separate solvent systems was used: toluene:ethyl formate:formic acid (5:7:1, v/v/v) and chloroform:methanol:acetic acid (7:3:1, v/v/v). After development the plates were visualized under an UV (254 nm) lamp, placed in an iodine tank for about 5 min, and marked. The radiolabeled compound was visualized by radiographic scanning with an Ambis Radioanalytic Imaging System (Mark II detector and AST computer). Carbon dioxide was confirmed as a metabolite by HPLC (retention time = 11.9 min) using $^{14}CO_2$ released from the hydrolysis of [^{14}C]-$NaHCO_3$ by the HPLC eluent (0.01 N H_2SO_4). 3-Formyl-6-hydroxy-2-cyclohexene-1-propanal was confirmed as a metabolite by direct probe mass spectrometry (Hewlett Packard Model 5988A) in the positive ion mode with methane as the reagent gas. Chromatographic methods (HPLC and TLC) were validated with authentic analytical standards (Table 2) achieving the necessary resolution and sensitivity. Products with yields of 0.2% could be reliably quantitated (quantitation limit = 50 dpm, about twice background) and 0.1% yields detected (detection limit = 10 dpm above background).

Abiotic Degradation of Glutaraldehyde

The hydrolysis of [^{14}C]-glutaraldehyde in sterile aqueous buffer solutions at pH 5, 7, and 9 was investigated to serve as abiotic controls for the metabolism studies. Aliquots (5 ml) of [^{14}C]-glutaraldehyde solution (50 μg, 6.7 μCi) in either sterile 0.01 M acetate buffer, pH 5, sterile 0.01 M phosphate buffer, pH 7, or sterile 0.01 M borate buffer, pH 9, were applied to sterilized 8-ml Pyrex sample tubes with Teflon screw caps. All glassware used in the experiment was autoclaved (250°F, 15 lb/sq. in.) with a Market Forge Sterimatic. The samples were incubated in the dark at 25°C. Duplicate samples were removed from the incubator at 0, 6, 12, 18, 24, and 31 days. Environmental cultures of the buffer solutions and the day 12 samples revealed no microbial growth, ascertaining the sterility of the solutions. Aliquots (3 × 100 μl) were radioassayed by LSC. Aliquots (50 μl) of all test samples were coinjected with authentic reference standards for analysis by HPLC. Selected samples were analyzed by TLC for confirmation of glutaraldehyde. Plates were developed in two dimensions using the following solvents: methanol:ethyl acetate (35:65, v/v) and toluene:ethyl formate:formic acid (5:7:1, v/v/v).

Results

No appreciable degradation of [^{14}C]-glutaraldehyde was observed in sterile aqueous buffer solutions at pH 5 and 7 over a 31-day period (Table 3). HPLC analysis showed a major peak (> 75% of the applied radiocarbon) consistent with the parent compound glutaraldehyde and several minor peaks each accounting for less than 10% of the applied radiocarbon. The identity of glutaraldehyde was confirmed with TLC. The identity of the minor peaks was unknown, as their HPLC retention times did not match any of the authentic reference standards. At pH 9, appreciable degradation was observed. One major degradation product, a cyclicized dimer of glutaraldehyde, with a yield of 30% after day 31 days, was isolated by HPLC and characterized by direct probe mass spectrometry as 3-formyl-6-hydroxy-2-cyclohexene-1-propanal. The extrapolated first-order half-lives of glutaraldehyde degradation under abiotic conditions were 508 days at pH 5, 102 days at pH 7, and 46 days at pH 9.

The pH of the incubated river water was 7.7 at collection and ranged 6.3–7.6 during the experiment in the aerobic metabolism study (Table 4). Dissolved oxygen concentration was 4.9–7.4 ppm. The microbial activity in the water was 1.0×10^5 CFU at the start of the experiment and showed a marked decrease at 4 h (2.5×10^3 CFU). The microbial activity rebounded to pretreatment level after 1 day. In the initial stages of the experiment (0–4 h) > 90% of the applied radiocarbon was found in the water phase. No significant radioactivity was found in the methylene chloride plugs or in the ethylene glycol trap throughout the study. During the period of 1 to 30 days, the proportion of aqueous radiocarbon decreased from 67% to 14% with a concomitant rise in the evolution of $^{14}CO_2$ to 68% after 30 days, whereas the proportion of radiocarbon found in the sediment decreased slightly from 20% to 12%. Overall material balance based on the recovery of radiocarbon was 93.3 ± 9.8%.

[^{14}C]-glutaraldehyde was the predominant component of the radiocarbon (88.9%) in the aqueous phase at the start of the experiment, but it decreased rapidly to 0.2% after 2 days (Table 5). Based on the time course of the observed glutaraldehyde concentrations, the pseudo-first-order half-life of glutaraldehyde catabolism in this river water–sediment system under aerobic conditions was 10.6 h. The major metabolite of glutaraldehyde produced by microbes in an aerobic aquatic system was carbon dioxide, with glutaric acid formed as an intermediate. Comparison of the HPLC retention times and the TLC profiles of authentic reference standards confirmed glutaric acid as a metabolite. HPLC analyses revealed that CO_2 was the predominant species detected in the aqueous phase after 48 h. The presence of $^{14}CO_2$ in the aqueous phase was confirmed by the quantitative yield of radiocarbon from the precipitate of an aqueous sample treated with barium chloride. The identity of CO_2 as a metabolite was confirmed by comparison of the

Table 2. HPLC retention times of analytical reference standards

Aerobic Metabolism Study		Anaerobic metabolism study	
Compound	Retention Time (min)[a]	Compound	Retention Time (min)[a]
Oxaloacetic acid	5.2	Glutaric acid	10.0
α-Ketoglutaric acid	5.6	Glutaraldehyde	13.2
DL-Malic acid	6.2	3-Formyl-6-hydroxy-2-cyclohexene-1-propanal	16.4
1,3-Acetonedicarboxylic acid	6.7	δ-Valerolactone	16.6
D-α-hydroxyglutaric acid	6.9	1,4-Pentadiene	18.2
Fomic acid	8.7	3,4-Dihydro-2H-pyran	19.0
Glutaric anhydride	9.4	5-Hydroxypentanal	19.4
Glutaric acid	9.5	1,5-Pentanediol	21.6
Succinic semialdehyde	9.5	4-Penten-1-ol	31.3
α-Hydroxyglutaric acid γ-lactone	9.6	Tetrahydropyran	32.0
Glutaraldehyde	10.5	1-Pentanol	45.0
Carbon dioxide	11.9		
δ-Valerolactone	13.2		
1,4-Pentadiene	14.5		
1,5-Pentanediol	17.3		
4-Penten-1-ol	25.0		

[a] HPLC retention times were approximate and subject to variations between runs, especially between columns. However, analytical reference standards were included during all analyses.

Table 3. Degradation of glutaraldehyde in sterile buffer solution[a]

	pH 5		pH 7		pH 9		
Sample Time	% Parent	% Unknown[b]	% Parent	% Unknown[b]	% Parent	% Unknown[b]	% FHCHP[c]
Day 0	94.5	1.4	97.2	1.9	93.9	1.5	0.3
Day 6	93.3	1.5	96.1	2.6	84.4	2.9	5.2
Day 12	93.1	1.5	91.9	4.3	81.6	1.9	12.7
Day 18	93.4	1.8	92.6	3.8	84.2	1.1	10.7
Day 24	92.4	4.3	88.7	10.7	61.4	8.0	29.5
Day 31	89.6	5.2	76.2	21.6	59.2	6.3	30.9

[a] The test system consisted of sterilized tubes containing 5 ml of [^{14}C]-glutaraldehyde solution (50 μg, 6.7 μCi) in either sterile 0.01 M acetate buffer, pH 5, sterile 0.01 M phosphate buffer, pH 7, or sterile 0.01 M borate buffer, pH 9, incubated in the dark at 25°C. Results shown are the average values of two replicate test systems.
[b] Sum of several low-yield unknown peaks detected by HPLC. No single peak exceeded 10% of the applied radiocarbon.
[c] 3-Formyl-6-hydroxy-2-cyclohexene-1-propanal.

Table 4. Distribution of radiocarbon from the aerobic metabolism of [^{14}C] glutaraldehyde in a river water–sediment system[a]

Sample Time	pH	Dissolved O_2 (ppm)	Microbial Activity[b] in Water	Microbial Activity[b] in Sediment	% Dose Applied: Water	Sediment	CO_2[c]	Total
0 h	7.2	6.4	1.0×10^5	6.5×10^5	93.6	7.6	ND	101.2
4 h	7.3	4.9	2.5×10^3	1.1×10^5	94.0	8.6	0	102.6
12 h	7.3	5.6	ND	ND	84.6	16.7	0.5	101.8
1 day	7.1	6.2	2.9×10^5	2.3×10^5	67.3	20.4	0.5	88.2
2 days	7.6	6.3	ND	ND	49.8	25.3	10.3	85.4
7 days	7.0	6.5	3.3×10^5	2.9×10^6	36.9	21.9	20.0	78.8
14 days	6.8	7.4	ND	ND	18.6	17.1	48.1	83.8
30 days	6.3	6.8	4.5×10^4	4.3×10^6	14.0	12.4	67.9	94.2
					Overall recovery (mean ± SD) = 93.3 ± 9.8%			

[a] The test system contained 1.0055 mg glutaraldehyde (11.5 μCi radiocarbon) and 26.4 g (wet weight) of sediment in 106.4 ml of river water. Incubator temperature averaged 24.7 ± 0.4°C. Results shown are the average values of two replicate test systems.
[b] Colony forming units (CFU) per gram after 48 h of culture.
[c] Values are cumulative totals throughout the study.
ND = not determined.

Table 5. Chemical composition in the water phase from the aerobic metabolism of glutaraldehyde in a river water–sediment system

Sampling Time[a]	% Dose Applied[b]		
	Glutaraldehyde	Glutaric Acid	CO_2/Carbonates
0 h	88.9	0	0
4 h	75.7	12.3	0
12 h	44.7	20.2	13.7
1 day	19.0	10.6	33.8
2 days	0.2	0	51.4
7 days	0	0	35.8

[a] Samplings beyond 7 days were not shown due to serious losses of CO_2 during trapping and precipitation with $BaCl_2$, and low HPLC recoveries. Carbon dioxide was the sole component in the water as determined by HPLC for samplings beyond 7 days.
[b] Results shown are the average values of two replicate test systems.

HPLC retention time of authentic $^{14}CO_2$ released from the acid hydrolysis of [^{14}C]-$NaHCO_3$.

Adsorption of the radiolabel to sediment was evident from the first sampling time (Table 4). The level of radiocarbon in sediment reached a maximum of 25% at 2 days with subsequent decrease to 12% after 30 days. The binding to the sediment was quite strong as repeated extractions with acetonitrile:0.001 N HCl (1:1, v/v) could release only 30–40% of the adsorbed radiocarbon (data not shown). HPLC analyses of the extract revealed multiple minor unidentified products (each constituting less than 1% of the applied dose). Glutaraldehyde was not detected in any significant amount in the extract.

In the anaerobic metabolism study, the pH ranged from 4.1 to 5.1 in this test system (Table 6). The dissolved oxygen concentration was 0.2–0.6 ppm and was below 0.3 ppm for the first 7 days of the experiment. The anaerobic microbial activity in the water ranged from 2.0×10^3 to 4.7×10^4 CFU throughout the course of the study. The majority of the applied radiocarbon (> 87%) was found in the aqueous phase throughout the entire course of the study. About 5.7–8.4% was adsorbed to the sediment, and no significant organic volatiles were detected in the methylene chloride plugs and ethylene glycol trap. Only a negligible amount (< 0.3%) of $^{14}CO_2$ was formed. Overall material balance based on the recovery of radiocarbon was 98.7 ± 2.5%.

At the start of the experiment (0 h), [^{14}C]-glutaraldehyde was the most significant component (73.1%) of the radiocarbon in the water phase, but its concentration decreased rapidly to 0.1% of the applied dose after 3 days (Table 7). Based on the time course of the observed glutaraldehyde concentrations, the pseudo-first-order half-life of glutaraldehyde catabolism in this river water–sediment system under anaerobic conditions was 7.7 h.

Three metabolites were identified: 5-hydroxypentanal, with a maximum yield of 37% at 1 day and declined to 1.2% by day 30; 1,5-pentanediol, with a maximum yield of 76.1%; and 3-formyl-6-hydroxy-2-cyclohexene-1-propanal, with a maximum yield of 17.8% (Table 7). The latter two metabolites appeared to be stable, as their concentrations did not show any appreciable decrease up to 123 days. The identity of 5-hydroxypentanal and 1,5-pentanediol as metabolites was confirmed by comparison of the HPLC retention times of the prepared radiolabeled reference standards and the TLC chromatographic profiles. 3-formyl-6-hydroxy-2-cyclohexene-1-propanal was established as a metabolite by the match of its HPLC retention times to those of the synthesized radiolabeled reference standard. Confirmation with the TLC method, however, proved to be unsuitable, as it produced poorly resolved chromatographic profiles. Attempts to find a second confirmatory HPLC method were unsuccessful due to inadequate retention (using an amino or a C-18 reverse phase column) and separation (using a silica normal phase column). One reference standard, δ-valerolactone, eluted at similar HPLC retention times (16.4–16.6 min) as 3-formyl-6-hydroxy-2-cyclohexene-1-propanal. However, it could be ruled out as a metabolite candidate because it did not co-chromatograph with the radiolabeled degradate using a second and third analytical HPLC method. Further attempts to find a confirmatory method for this metabolite led to the use of chemical ionization mass spec-

Table 6. Distribution of radiocarbon from the anaerobic metabolism of [^{14}C] glutaraldehyde in a river water–sediment system[a]

Sample Time (day)	pH	Dissolved O_2 (ppm)	Bacterial Activity[b] in		% Dose Applied			
			Water	Sediment	Water	Sediment	CO_2[c]	Total
0	4.2	0.3	1.9×10^4	9.4×10^5	91.9	5.7	—	97.6
1	4.3	0.2	2.7×10^3	1.0×10^4	95.1	6.3	0.1	101.5
3	4.1	0.2	2.0×10^3	1.1×10^5	89.6	6.1	0.3	96.0
7	4.1	0.3	3.1×10^3	1.1×10^5	89.0	7.2	0.0	96.2
14	4.5	0.6	ND	ND	94.6	7.0	0.1	101.7
30	4.8	0.3	3.7×10^4	6.3×10^5	87.0	8.3	0.1	95.4
60	4.6	0.3	ND	ND	91.6	7.2	0.2	99.0
90	5.1	0.4	4.7×10^4	ND	93.4	7.4	0.3	101.1
123	4.8	0.5	ND	ND	91.4	8.4	0.3	100.1
					Overall recovery (mean ± SD) = 98.7 ± 2.5%			

[a] The test system contained 1.0055 mg glutaraldehyde (11.5 μCi radiocarbon) and 26.4 g (wet weight) of sediment in 106.4 ml of river water. Incubator temperature averaged 25.0 ± 0.4°C. Results shown are the average values of two replicate test systems.
[b] Colony forming units (CFU) per g after 3 days of culture.
[c] Values are cumulative totals throughout the study.
ND = not determined.

Table 7. Chemical composition in the water phase from the anaerobic metabolism of glutaraldehyde in a river water–sediment system

Sampling Time (day)	% Dose Applied[a]			
	Glutaraldehyde	5-Hydroxypentanal	1,5-Pentanediol	3-Formyl-6-hydroxy-2-cyclohexene-1-propanal
0	73.1	7.26	0	3.3
1	4.5	37.0	34.8	11.9
3	0.1	8.8	60.7	11.6
7	0	1.9	66.1	12.1
14	0	1.3	76.1	13.0
30	0	1.2	66.2	14.0
60	0	0	73.0	12.8
90	0	0	71.1	17.8
123	0	0	69.6	16.6

[a] Results shown are the average values of two replicate test systems.

trometry. The mass spectrum of this metabolite revealed a complex pattern (Figure 1). Signals above 200 amu were extraneous to the sample and likely arose from the early desorption band in the total ion current, although the possibility that these represented some oligomer species of glutaraldehyde could not be ruled out. The signal at 183 amu was considered to be the quasimolecular ion (MH+) with loss of water to 165 amu. The base peak at 101 amu corresponds to the mass of glutaraldehyde (+1 amu). Tashima *et al.* (1991) described a new oligomer of glutaraldehyde produced by aldol condensation reaction. The proposed structure for the oligomer (Figure 2) has a molecular weight of 182 amu, thus supporting the assignment of 3-formyl-6-hydroxy-2-cyclohexene-1-propanal for the quasimolecular ion at 183 amu. Loss of 18 amu (water) is expected from the hemiacetal of 3-formyl-6-hydroxy-2-cyclohexene-1-propanal. Disproportionation of the bicyclic structure would yield the skeleton of glutaraldehyde (101 amu).

About 5.7–8.5% of the applied [^{14}C]-glutaraldehyde dose was adsorbed to the sediment (Table 6). Extraction of the sediment with acetonitrile:0.001 N HCl (1:1, v/v) released about 64% of the adsorbed radiocarbon. HPLC analysis of the extract revealed similar metabolite composition as that in the corresponding water phase. No glutaraldehyde was detected in the extract.

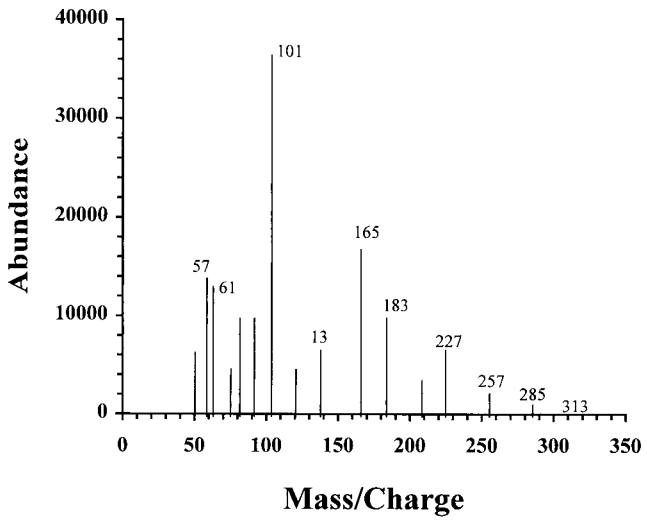

Fig. 1. Metabolite characterization with chemical ionization mass spectrometry

Discussion

Glutaraldehyde, as a biocide, can inhibit the growth and metabolism of microbes. To minimize these effects, a low concentration (9.45 mg/L) was used for the aquatic metabolism studies. However, it is evident that, even at this concentration, there was some microbial inhibition, as the bacterial activity in the water after 4 h of incubation (2.5×10^3 CFU) showed a decrease relative to the predosing measurement (1×10^5 CFU) (Table 4). There was good material balance in the aerobic metabolism study with an overall recovery of 93.3% (Table 4). However, during the period of intermediate samplings (2–14 days) when vigorous production of $^{14}CO_2$ was observed, the material balance was less than quantitative. This was probably due to incomplete recovery of $^{14}CO_2$ generated still in solution and thus not amenable to flow-through collection. This is supported by the observation that in the anaerobic metabolism study where the collection of $^{14}CO_2$ was not an issue, the material balance was almost quantitative throughout the course of the study.

Metabolism of glutaraldehyde was quite rapid under aerobic conditions, with a half-life of 10.6 h based on the disappearance of the parent compound from the water phase. Glutaraldehyde was metabolized ultimately to CO_2, achieving a yield of 68% after 30 days. Based on the experimental results obtained in this study, an aerobic metabolic pathway can be proposed: Glutaraldehyde is first biotransformed into glutaric acid. At this intermediate point, further oxidation can proceed in two ways, either toward α-hydroxyglutaric acid or β-ketoglutaric acid, depending on which of the two carbons, the carbonyl carbon or the C-2 carbon, is activated. Both pathways then proceed toward decarboxylative chain shortening reactions accompanied by the extrusion of $^{14}CO_2$ (Krzeminski *et al.* 1975). As no intermediate metabolite between the production of glutaric acid and CO_2 was detected, neither of the two pathways can be ruled out. This pathway of microbial metabolism, through an acidic intermediate to CO_2, is very similar to

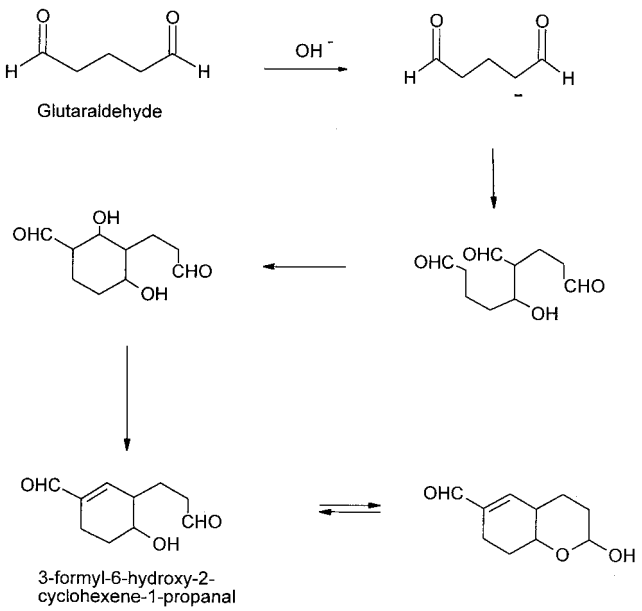

Fig. 2. Proposed pathway for the formation of 3-formyl-6-hydroxy-2-cyclohexene-1-propanal. Conclusive evidence leading to the assignment of this molecular structure was provided by Tahima *et al.* (1991).

that reported for the mammalian system (McKelvey *et al.* 1992).

The metabolism of glutaraldehyde under anaerobic conditions was also rapid ($t_{1/2}$ of 7.7 h). However, the anaerobic metabolism did not proceed ultimately to methane but terminated with the production of 1,5-pentanediol. Based on the experimental results obtained in this study, this anaerobic metabolic pathway is proposed to involve glutaraldehyde being first metabolized to 5-hydroxypentanal as an intermediate, which then undergoes transformation into 1,5-pentanediol. In addition, a minor pathway proceeds by a series of aldol condensation, cyclization, and dehydration reactions, yielding 3-formyl-6-hydroxy-2-cyclohexene-1-propanal as the product, similar to the degradate observed in the abiotic hydrolysis of glutaraldehyde under alkaline conditions (Tashima *et al.* 1991). Although this type of reaction is generally believed to be chemical in nature, the fact that the dimer was detected at earlier time points in the metabolism study (12% after 1 day) than in the hydrolysis study (5% after 6 days), and that sterile solutions of glutaraldehyde were stable under acidic conditions (Table 3), at a pH range of 4.1–5.1 similar to that in the anaerobic metabolism study (Table 6) is suggestive of some catalytic involvement by the microbes.

Acknowledgments. These studies were conducted at the PTRL West, Inc., Richmond, CA, under the direction of Dr. Thomas Esser. I would also like to acknowledge the contribution provided by Ms. Joan Young and Dr. Jonathan Leder.

References

Beauchamp RO, St. Clair MB, Fennell TR, Clarke DO, Morgan KT (1992) A critical review of the toxicology of glutaraldehyde. Crit Rev Toxicol 22:143–174

Cosmetic Ingredient Review (1996) Final report on the safety assessment of glutaral. J Am Coll Toxicol 15:98–139

Gorman SP, Scott EM (1980) The state of glutaraldehyde molecule in relation to its biocidal activity. J Pharm Pharmacol 32:131–132

Gorman SP, Scott EM, Russell AD (1980) A review: antimicrobial activity, uses and mechanism of action of glutaraldehyde. J Appl Bacteriol 48:161–190

Hjelle JJ, Petersen DR (1983) Metabolism of malondialdehyde by rat liver aldehyde dehydrogenase. Toxicol Appl Pharmacol 70:57–66

Jordan SLP, Russo MR, Blessing RL, Theis AB (1996) Inactivation of glutaraldehyde by reaction with sodium bisulfite. J Toxicol Environ Health 47:299–309

Krzeminski SF, Brackett CK, Fisher JD, Spinnler JF (1975) Fate of microbicidal 3-isothiazolone compounds in the environment. Products of degradation. J Agric Food Chem 23:1068–1075

McKelvey JA, Garman RH, Anuszkiewicz CM, Tallant MJ, Ballantyne B (1992) Percutaneous pharmacokinetics and material balance studies with glutaraldehyde. J Toxicol Cutaneous Ocular Toxicol 11:341–367

Russell SD, Hopwood D (1976) The biological uses and importance of glutaraldehyde. Prog Med Chem 13:271–301

Tashima T, Imai M, Kuroda Y, Yagi S, Nakagawa T (1991) Structure of a new oligomer of glutaraldehyde produced by aldol condensation reaction. J Org Chem 56:694–697

Whipple EB, Ruta M (1974) Structure of aqueous glutaraldehyde. J Org Chem 39:1666–1668