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REVIEW

Ecotoxicology of Glutaraldehyde: Review of Environmental Fate and Effects Studies

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Glutaraldehyde is a biocide used in many industrial applications with potential releases to the environment. This review discusses the environmental fate and effects data on this important biocide. Information drawn from this review indicates that glutaraldehyde is acutely toxic to aquatic organisms. Glutaraldehyde is equally toxic to warm water and cold water fish, but is *slightly more toxic to freshwater fish than salt water fish.* The acute toxicity of glutaraldehyde for avian species is comparable to that for mammalian species. The toxicity of glutaraldehyde is not appreciably increased with repeated long-term exposures. Results from environmental partitioning studies indicate that glutaraldehyde tends to remain in the aquatic compartment and has little tendency to bioaccumulate. Aqueous solutions of glutaraldehyde are stable at room temperature under acidic to neutral conditions, and to sunlight, but unstable at elevated temperatures, and under alkaline conditions. Glutaraldehyde is readily biodegradable in the freshwater environment and has the potential to biodegrade in the marine environment. Aquatic metabolism studies suggest that glutaraldehyde, under aerobic conditions, is metabolized to CO_2 via glutaric acid as an intermediate. Under anaerobic conditions, glutaraldehyde is metabolized to 1,5-pentanediol. Pretreatment with sodium bisulfite is the best method for inactivating glutaraldehyde prior to disposal to treatment systems. © 2001 Academic Press

Key Words: **glutaraldehyde; environmental fate and effects; aquatic metabolism; chemical deactivation; risk assessment.**

INTRODUCTION

Glutaraldehyde (1,5-pentanedial, CAS Registry No. 111-30-8) is an industrial biocide used 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. Given the nature of some of these uses, there is a need to understand the impact of glutaraldehyde on the environment, i.e., ecological fate and effects. This review provides a summary of the information on the environmental fate and aquatic toxicity of glutaraldehyde. Glutaraldehyde is usually available commercially as a 50% aqueous solution. However, for the ease of data comparison across different studies, all concentrations of glutaraldehyde in this review have been corrected to 100% active ingredient. Information reviewed includes the acute and chronic toxicity to aquatic organisms, partitioning characteristics relevant for understanding the mobility to and from various environmental compartments, the rates of degradation including hydrolysis and photolysis, and metabolism by aerobic and anaerobic microorganisms. Since glutaraldehyde, because of its biocidal properties, can inhibit microorganisms, this review also discusses the evaluation of chemical deactivation methods to minimize the interference of glutaraldehyde on the performance of biological wastewater treatment units.

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ENVIRONMENTAL EFFECTS

Acute Toxicity

Tables 1 and 2 summarize the results of acute toxicity studies of glutaraldehyde with a variety of freshwater and marine/estuarine organisms. Where the endpoint measured is mortality, the median lethal concentration (LC_{50}) is indicated. For studies in which an endpoint other than death is measured, the median effect concentration (EC_{50}) is indicated. Wherever available, the No Observed Effect Concentration (NOEC) is also given. The LC_{50} from a group of 8 studies in freshwater species centered around 7.7 mg/L, with a range of 3 mg/L in coho salmon to 12 mg/L in the rainbow trout. The acute toxicity of glutaraldehyde to marine/estuarine organisms exhibits greater variation.

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TABLE 1
Acute Toxicity of Glutaraldehyde to Freshwater Species

	Exposure condition	Guideline	LC_{50}[a,b]	NOEC[a]	Reference
Coho salmon (*Oncorhynchus kisutch*)	96 h flowthrough	—	3	—	SFU, 1993
Water flea (*Daphnia magna*)	48 h static	—	5	—	UCC, 1981a
Fathead minnow (*Pimephales promelas*)	96 h satic	—	5.4	2.6	UCC, 1996
Fathead minnow (*Pimephales promelas*)	96 h satic	ASTM	6	4	SLI, 1989
Bluegill sunfish (*Lepomis macrochirus*)	96 h static	—	9.4	2.5	UCC, 1977a
Bluegill sunfish (*Lepomis macrochirus*)	96 h static	—	11	5	UCC, 1978c
Rainbow trout (*Salmo gairdneri*)	96 h static	—	11	8	UCC, 1977b
Rainbow trout (*Salmo gairdneri*)	96 h static	—	12	9	UCC, 1978b

[a]In milligrams of active ingredient (glutaraldehyde) per liter.
[b]The median, arithmetic mean, and geometric mean are 7.7, 7.9, and 7.1 mg/L, respectively.

While the median of 7.1 mg/L is similar to that for freshwater species, the range spans over 4000-fold from 0.11 mg/L in the calanoid copepod to 465 mg/L in the green crab. This vast span of acute toxicity values may be due to the fact that a more diverse range of species was studied, and that not all values were measures of mortality; some were based on sublethal effects such as growth. With the LC_{50} around 7 mg/L, glutaraldehyde is considered toxic to aquatic organisms. There appears to be little difference in toxicity between warm water fish (20–24°C, e.g., fathead minnow and bluegill sunfish) and cold water fish (10–15°C, e.g., rainbow trout and coho salmon). Glutaraldehyde appears to be somewhat more toxic to freshwater fish (e.g., fathead minnow, $LC_{50} = 6$ mg/L) than saltwater fish (sheepshead minnow, $LC_{50} = 32$ mg/L).

The acute toxicity of glutaraldehyde to avian species by dietary feeding (WIL 1978a,b,c) and by gavage (WIL 1978d,e) has been evaluated. Two-week-old bobwhite quails (*Colinus virginianus*) and mallard ducks (*Anas platyrhynchos*) were fed various concentrations of 50% glutaraldehyde-fortified diets for 5 days and then maintained on a normal control diet for an additional 3-day observation period. Ten birds each were randomly assigned to four treatment groups without regard to sex. There was no mortality at any dietary concentration tested. The median lethal concentration of glutaraldehyde in the diet (LC_{50}) was determined to be greater than 5000 ppm (active ingredient, w/w). The NOEC, based on the effect of reduction in body weight gain, was 2320 and 1075 ppm for the bobwhite quails and mallard ducks, respectively. The acute peroral toxicity of glutaraldehyde was studied in mallard ducks. Dosing was achieved by intubation directly into the crop via a stainless-steel catheter. Mortality was measured over an 8-day period. The LD_{50} (95% confidence limits) expressed as the weight of 100% glutaraldehyde/body weight were 408 (314–529) and 466 (354–616) mg/kg for a 25 and 50% glutaraldehyde solution, respectively. These results indicate that the acute oral toxicity of glutaraldehyde for avian species is comparable to that for mammalian species (e.g., rat, LD_{50} of 524–727 mg/kg) (Ballantyne, 1995).

TABLE 2
Acute Toxicity of Glutaraldehyde to Marine/Estuarine Species

	Exposure condition	Guideline	LC_{50}[a,b]	NOEC[a]	Reference
Calanoid copepod (*Acartia tonsa*)	48 h static	ISO TC147	0.11	0.029	SPL, 1997b
Mussel (*Mytilus edulis*)	5 days flowthrough	—	0.2[c]	—	SINTEF, 1991
Eastern oyster (*Crassostrea virginica*)	48 h static	—	0.55	—	UCC, 1975
Eastern oyster (*Crassostrea virginica*)	96 h flowthrough	FIFRA 72-3	0.78	0.16	SLI, 1993c
Mysid shrimp (*Mysidopsis bahia*)	96 h flowthrough	FIFRA 72-3	7.1	0.78	SLI, 1993b
Acorn barnacle (*Balanus improvisus*)	10 days static	—	7.5[c]	—	SINTEF, 1991
Sheepshead minnow (*Cyprinodon variegatus*)	96 h static	FIFRA 72-3	32	24	SLI, 1993a
Grass shrimp (*Palaemonetes vulgaris*)	96 h static	—	41	—	UCC, 1975
Green crab (*Carinus maenas*)	96 h static	—	465	—	UCC, 1975

[a]In milligrams of active ingredient (glutaraldehyde) per liter.
[b]The median, arithmetic mean, and geometric mean are 7.1, 61.6, and 4.1 mg/L, respectively.
[c]EC_{50} (growth rate).

TABLE 3
Reproduction Rate[a] of *Daphnia magna* Exposed to Glutaraldehyde for 21 Days

	Glutaraldehyde concentration (mg a.i./L)					
Replicate	0 (control)	0.21	0.53	1.06	2.13	4.25
1	53	6	53	54	42	7
2	38	36	35	45	40	22
3	46	7	47	27	28	11
4	44	35	41	39	26	23
5	44	39	45	17	35	34
6	37	33	46	0	26	2
7	35	34	0	19	46	11
8	41	32	31	36	36	30
9	27	54	35	40	34	31
10	42	31	29	19	54	20
Mean ± SD	41 ± 7	31 ± 14	36 ± 15	30 ± 16	37 ± 9	19 ± 11
% control	100	75.4*	88.9	72.7	90.2	46.9**

[a]Values represent number of live offspring reproduced per adult daphnid.

*$P < 0.05$, **$P < 0.01$. Significantly different from control (Wilcoxon test).

Chronic/Life Cycle/Reproduction Toxicity

The chronic effects of glutaraldehyde have been evaluated in a freshwater invertebrate, in freshwater and marine algae, and in a freshwater fish.

In a chronic toxicity/reproduction test with the freshwater invertebrate conducted according to OECD 202 guideline, the water flea (*Daphnia magna*) was exposed for 21 days to glutaraldehyde semistatically with the test solution renewed every 2–3 days (CCR, 1990). The mortality of adults and the number of young were observed three times per week before renewal of the test media. There was a 10% mortality at 0.21 and 0.53 mg/L, and a 20% mortality at 1.06 mg/L. However, all daphnids exposed to higher concentrations (2.13 and 4.25 mg/L) survived. The lack of a dose-response relationship suggests that the observed mortality was probably not treatment related. The 21-day NOEC based on mortality was therefore greater than 4.25 mg/L. Table 3 presents the reproduction rate of the surviving adults. Reproduction of young started from Day 10 of exposure. At a glutaraldehyde concentration of 4.25 mg/L, a statistically significant inhibition of reproduction was observed. A reduction of reproduction was also observed at the lowest concentration tested (0.21 mg/L). The decrease was attributed to an unexpected low number of offspring born from two daphnids (replicates 1 and 3). Because of the isolated effect and a lack of dose-response relationship, the reduction in reproduction rate at 0.21 mg/L could not be considered to be treatment related. Therefore, the NOEC based on reproduction effects was 2.13 mg a.i./L.

The ability of glutaraldehyde to inhibit the growth of aquatic organisms was studied in two species of freshwater alga and a marine alga. In these studies, glutaraldehyde was incubated with about 10,000 algal cells/mL of nutrient solution with illumination (7000–8000 lux) and constant agitation for 3–5 days. Samples were taken at daily intervals and the cell densities determined by direct counting. Growth inhibition was compared to control based on growth rate and/or biomass. The average maximum growth rate for each culture was calculated from the straight section of the growth curve. Biomass growth was calculated from the area under the growth curve. Table 4 summarizes the results of these studies. The EC_{50} values ranged from 0.17 to 0.97 mg/L, and the NOEC ranged from 0.31 to 0.50 mg/L.

Glutaraldehyde was tested in an early life-stage toxicity study with the fathead minnow (*Pimephales promelas*) conducted according to OECD 210 guideline. In this study (WIL, 1999), two replicates of 40 healthy, newly spawned (less than 1-day-old) embryos were exposed to five test concentrations of glutaraldehyde at 25°C under flow-through conditions. Test concentrations were selected from

TABLE 4
Algal Growth Inhibition Studies with Glutaraldehyde

			EC_{50} (mg a.i./L)			
Alga	Guideline	Exposure (days)	Growth rate[a]	Biomass[b]	NOEC (mg/L)	Reference
Freshwater						
Scenedesmus subspicatus	OECD 201	4	—	0.97	0.31	RCC, 1990b
Selenastrum capricornutum	OECD 201	5	—	0.81	0.50	WIL, 1997
Marine						
Skeletonema costatum	—	3	0.17	—	—	SINTEF, 1991
Skeletonema costatum	ISO 10253	3	0.92	0.61	0.33	SPL, 1997c

[a]Growth rate was calculated from the linear portion of the growth curve.

[b]Biomass growth was calculated from the area under the growth curve.

TABLE 5
Early Life-Stage Toxicity Test of Glutaraldehyde in the Fathead Minnow (*Pimephales promelas*)

Concn (mg a.i./L)	Hatching success[a]	Survival[b]	Length (mm)	Wet weight (mg)	Dry weight (mg)
0	71(89%)	62(87%)	21.1 ± 0.21	68.9 ± 2.97	14.9 ± 0.71
0.29	69(86%)	60(87%)	20.4 ± 0.50	66.5 ± 6.29	13.9 ± 0.71
0.61	71(89%)	57(80%)	21.1 ± 0.78	66.4 ± 5.23	13.5 ± 1.48
1.4	68(85%)	53(78%)	21.0 ± 0.78	65.1 ± 4.38	12.8 ± 1.06
2.9	71(89%)	45(63%)*	19.6 ± 0.21	52.7 ± 3.54	10.2 ± 0.35
5.9	68(85%)	5(7%)*	18.1 ± 0.49	42.6 ± 10.3	7.50 ± 1.84

[a]Number hatched after 4 days per number of embryos. There were 80 embryos at the start of exposure in each group.
[b]Live count on Day 28 posthatch per initial number of larvae.
*Significantly different from controls, $P < 0.05$.

a range-finding study involving groups of 20 embryos exposed under similar conditions for 9 days. The exposure period included a 4-day embryo hatching period, and a 28-day posthatch juvenile growth period. Newly hatched larvae were fed live brine shrimp. To ensure that the feeding rate remained constant, rations were adjusted each week to account for losses due to mortality. Fish were not fed for 2 days before test termination to allow for clearance of the digestive tract before weight measurements were made. Observations were made daily for mortality, clinical signs of toxicity, or abnormal behavior. From these observations, hatching success, time to hatch, and posthatch survival were evaluated. Posthatch growth was evaluated at test termination. Total lengths for each surviving fish and wet and dry weights were measured.

Embryos began hatching on Day 4, and by Day 5 all eggs had hatched or were determined to be nonviable. There were no differences in time-to-hatch or in hatching success (Table 5). Survival in the 2.9 and 5.9 mg/L groups was statistically reduced. There were no differences in growth between the control group and the treatment group at 1.4 mg/L and below. Total length, wet weight, and dry weight in the 2.9 and 5.9 mg/L were reduced, but the data were not analyzed statistically because of a significant effect on survival at these levels. Therefore, the lowest observed effect level (LOEC) in this study was 2.9 mg/L, and the NOEC was 1.4 mg/L.

Chronic toxicity studies with glutaraldehyde in three aquatic species have produced NOECs ranging from 0.31 mg/L (alga) to 4.25 mg/L (*Daphnia magna*). These compare with a range of 2.5–9 and 0.029–24 mg/L from acute toxicity studies in freshwater and marine species, respectively (Tables 1 and 2). Thus, the toxicity of glutaraldehyde does not appear to be significantly increased with repeated exposure. A Maximum Acceptable Toxicant Concentration (MATC) of 2.0 mg/L (geometric mean of the LOEC and NOEC) can be calculated from the early life-stage toxicity study with the fathead minnow (WIL, 1999). According to the EU Technical Guidance Document for the risk assessment of existing substances, with chronic toxicity studies from at least three trophic levels, a Predicted No Effect Concentration (PNEC) of 31 μg/L can be calculated for glutaraldehyde by dividing the lowest NOEC of 0.31 mg/L in the algal study (RCC, 1990b) by a factor of 10.

ENVIRONMENTAL FATE

Environmental Partitioning Characteristics

Air

A recirculating equilibrium still was used to measure the pressure-temperature-liquid composition-vapor composition data on aqueous mixtures of glutaraldehyde between 38 to 85°C (Olson, 1998). Glutaraldehyde was analyzed by gas chromatography equipped with a flame ionization detector. Table 6 presents the partial vapor pressure and equilibrium vapor concentration of aqueous glutaraldehyde solutions. A value of 3.3×10^{-5} L · atm/mol for Henry's constant at 25°C was extrapolated from the regression equation derived from these data. The partition coefficient of glutaraldehyde between air and water at 25°C was calculated to be about 0.00184.

Soil

The adsorption/desorption characteristics of glutaraldehyde to various types of soil have been determined according to FIFRA 163-1 guideline (PTRL, 1994a). Aqueous solutions of [^{14}C] glutaraldehyde in 0.01 M calcium chloride were prepared at concentrations of 0.51, 1.0, 2.5, 5.0, and 10.3 mg/L. The specific activity of the [^{14}C] glutaraldehyde was 13.6 mCi/mmol. The adsorption phase was conducted in Teflon centrifuge tubes containing 30 mL of glutaraldehyde solution and either 10 g of sandy loam, 5 g of silty clay loam, 5 g of silt loam, 20 g of loamy sand, or 20 g of sediment held in a shaking water bath at 25°C. Table 7 gives the physicochemical characteristics of the soils used in this study. After a 24-h incubation, the tubes were

TABLE 6
Partial Vapor Pressure and Equilibrium Vapor Concentration of Aqueous Glutaraldehyde Solutions

Concn of aqueous solution (% by wt)	Partial vapor pressure at 25°C (mm Hg)	Equilibrium vapor concentration at 25°C (ppm)
50	0.157	207
15	0.041	53
2	0.0051	7
0.1	0.00025	0.3

TABLE 7
Physicochemical Characteristics of Soils and Adsorption/Desorption of Glutaraldehyde

Soil type	pH	FC	CEC	% Sand	% Silt	% Clay	% Organic carbon	K	K_{oc}
Sandy loam	6.8	15.1	5.5	67	23	10	1.0	2.06	210
Silty clay loam	5.7	30.0	19.7	16	55	29	0.99	4.94	500
Silt loam	6.7	29.0	16.8	17	62	21	1.42	4.83	340
Loamy sand	5.8	5.1	2.9	83	17	0	0.24	1.10	460
Sediment	8.1	5.5	4.3	93	7	0	0.5	0.59	120

Note. FC, field capacity (g water per 100 g dry soil) at 0.33 bar; CEC, cation exchange capacity (mEq per 100 g dry soil); K, Freundlich adsorption coefficient; and K_{oc}, organic carbon/water partition coefficient.

centrifuged, and supernatants were decanted and immediately radioassayed for the determination of adsorption isotherms. The desorption phase was conducted under the same conditions after 30 mL of fresh 0.01 M $CaCl_2$ was added to the tubes containing the soil pellet. Chemical analysis on the adsorption supernatants was performed by high performance liquid chromatography to determine the stability of [^{14}C] glutaraldehyde under the conditions of the study. The mean material balance of [^{14}C] glutaraldehyde in all soils was 74%. The loss in radioactivity was likely due to biodegradation.

The average Freundlich adsorption coefficient (K) value was 3.23, and the average organic carbon/water partition coefficient (K_{oc}) value was 380 for the four soils (Table 7). The corresponding K and K_{oc} values for sediment were 0.59 and 120. Based on the use of K_{oc} values to predict leaching potential, where K_{oc} values greater than 5000 denote immobility of a chemical in soil, K_{oc} values between 150 and 500 denote moderate mobility in soil, and K_{oc} values of 50 to 150 denote high mobility, glutaraldehyde is predicted to have moderate mobility in each of the four soils and high mobility in sediment.

Fat

The propensity of glutaraldehyde to partition in fat has been evaluated. The *n*-octanol/water partition coefficient (K_{ow}) at 22°C was determined using a method involving phase separation with a single-stage extraction followed by centrifugation. The respective phases were analyzed by gas chromatography-mass spectrometry, yielding a K_{ow} value of 0.98 or a log K_{ow} value of −0.0088 (UCC, 1981b).

The partition coefficient of glutaraldehyde between *n*-octanol and water was also examined recently following OECD 107 guideline (PTRL, 1996). An aqueous solution of [1,5-^{14}C]glutaraldehyde was agitated with *n*-octanol at 25°C, equilibrated for about an hour, and then centrifuged to achieve phase separation. The separated organic and aqueous phases were radioassayed by direct liquid scintillation counting. An average K_{ow} value of 0.465 or a log K_{ow} value of −0.333 was determined from the ratio of the concentration of radiocarbon in *n*-octanol to that in the corresponding aqueous solution. Chemical analysis by high performance liquid chromatography of the organic and aqueous phases following partition revealed that glutaraldehyde was unstable over time in the *n*-octanol/water test system. The instability may be due to reaction of the glutaraldehyde with an excess of a primary alcohol to form acetals and hemiacetals under slightly acidic conditions (pH 6–7). Since the K_{ow} was calculated based on radiocarbon, it represents both glutaraldehyde and its degradation products. As more degradates were detected in the *n*-octanol phase than the water phase, the K_{ow} value would have been slightly overestimated.

Based on results of the environmental partitioning studies, it can be concluded that there is a very low tendency for glutaraldehyde to enter the atmosphere from the aqueous environment due to its low air to water partition coefficient. In the terrestrial environment, glutaraldehyde exhibits a moderate to high potential to leach from soil. Thus, the principal ecosystem of relevance for glutaraldehyde is the aquatic environment. In addition, the low *n*-octanol/water partition coefficient value indicates that glutaraldehyde is unlikely to bioaccumulate in fatty tissues of aquatic organisms.

Abiotic Degradation

Thermal Stability

A stability study with glutaraldehyde was conducted according to FIFRA 63–13 guideline. A 50-mL sample of filter-sterilized aqueous solution of glutaraldehyde (2 mg a.i./mL) was found to be stable in the dark at 20°C for 28 days. However, at a higher temperature (50°C) some degradation (about 8%) was observed after 14 days (SLI, 1994).

Hydrolysis

The hydrolysis of [1,5-^{14}C]glutaraldehyde was examined according to FIFRA 161-1 guideline in 5-mL aliquots of

sterile aqueous solution (10.1 mg/L containing about 6.88 μCi radiocarbon) at pH 5, 7, and 9 (PTRL, 1992b). The study was conducted in the dark at 25°C. Glutaraldehyde degraded slowly at pH 5 and 7 during the 31 days of the study. The first-order rate constants of hydrolysis ($K_{hydrolysis}$) at pH 5 and 7 were 0.0014 and 0.0068 per day, and the extrapolated half-lives ($t_{1/2}$) were 508 and 102 days, respectively. At pH 9, however, appreciable degradation was observed. The $K_{hydrolysis}$ was 0.015 per day, and the $t_{1/2}$ was 46 days. Only one major degradate was observed and was identified by high performance liquid chromatography/mass spectrometry to be a cyclized dimer of glutaraldehyde, 3-formyl-6-hydroxy-2-cyclohexene-1-propanal (CAS No. 130434-30-9).

Photolysis

The photodegradation of [1,5-^{14}C]glutaraldehyde was examined according to FIFRA 161-2 guideline in sterile aqueous solutions at pH 5 (PTRL, 1992a). The study was conducted by exposing 10-mL aliquots of a 10.4 mg a.i./L glutaraldehyde solution containing about 14.14 μCi radiocarbon to natural sunlight at 25°C for 30 days. The average daily total light energy was 5.68 W·min/cm^2. Glutaraldehyde degraded slowly under these conditions. The first-order rate constant of photolysis ($K_{photolysis}$) was 0.0035 per day and the corresponding $t_{1/2}$ was 196 days.

It can be concluded from the abiotic degradation studies that aqueous solutions of glutaraldehyde are stable at acidic to neutral pH, but unstable at alkaline pH. Solutions of glutaraldehyde are stable at room temperature but reveal some deterioration at elevated temperatures. Glutaraldehyde is stable to sunlight in an aqueous environment.

Biodegradation

Bacterial Inhibition

Glutaraldehyde, as a biocide, can inhibit the metabolism and growth of microbes. Biodegradation, however, relies on the digestion of chemical substances by microbial function. A biocide may exhibit poor biodegradability if the concentration tested is inhibitory to bacteria. Therefore, in order to properly assess the biodegradability of a biocidal material, its effect on bacteria must first be determined.

The effect of glutaraldehyde on sewage bacteria has been evaluated. In one test, the effect was studied by measuring the growth of microorganisms after 16 h of incubation with glutaraldehyde (Alsop *et al.*, 1980). Two sources of microbial seed were tested. One was obtained from a domestic sewage treatment plant and another was prepared from a lyophilized bacterial seed containing a broad spectrum of microorganisms (Polybac Polyseed) available commercially. Bacterial growth was assessed by measuring the turbidity level spectrophotometrically (optical density at 530 nm). The median effective concentration for glutaraldehyde (EC_{50}) was 25 mg/L for the sewage bacteria (UCC, 1988), and for the Polybac Polyseed, the EC_{50} was 17 mg/L and the NOEC was 5 mg/L (UCC, 1994a).

In another test conducted according to OECD 209 guideline, the effect was studied by measuring the respiration of microorganisms after 30 min of incubation with glutaraldehyde. The inoculum was prepared with activated sludge from a domestic wastewater treatment plant. Bacterial respiration was assessed by measuring oxygen consumption. The EC_{50} was greater than 50 mg/L and the NOEC was 16 mg/L (RCC, 1995). Results of this study suggest that concentrations of glutaraldehyde above 16 mg/L can inhibit the metabolic activity of the sewage bacteria following a brief period of contact (half an hour). However, the contact time of glutaraldehyde with sludge bacteria in wastewater treatment facility or in biodegradation test systems is much longer than 30 min, typically spanning many days. Under the longer exposure condition, glutaraldehyde is inhibitory to microbes at a concentration above 5 mg/L (UCC, 1994a).

Microbial Biodegradation

Biodegradation of a chemical substance by microbial action can take place with (aerobic) or without the presence of oxygen (anaerobic). Biodegradation may also be distinguished by the extent to which a chemical substance is transformed. Primary biodegradation occurs when the parent chemical is transformed such that the basic properties of the chemical are lost. Ultimate biodegradation denotes the complete conversion of the organic chemical to single carbon products (carbon dioxide in aerobic biodegradation, and methane in anaerobic biodegradation). Most often biodegradative processes are aerobic, unless in situations where the chemical is buried deep in soil or sediment. A wide range of laboratory tests have been developed to examine the biodegradability of organic chemicals. Information derived from these tests has been used to predict degradation rates and estimates for the reduction in total biomass of a chemical from specific environmental compartments.

Biodegradation tests can be grouped into three major classes: screening tests, inherent biodegradation tests, and simulation tests. The Organization for Economic Cooperation and Development (OECD) has categorized biodegradation testing methods into a sequential or tiered system, beginning with relatively inexpensive fast screening tests and progressing toward more complex testing methods. The first level of biodegradation testing includes the screening test for ready biodegradability (OECD 301 series). These tests are easy to perform and are of short duration. They are designed to evaluate the potential for biodegradation under stringent conditions, often involving the test chemical serving as the sole carbon and energy

TABLE 8
Test Conditions Specified in the OECD 301 Series of Aerobic Ready Biodegradation Tests

Test	301 A DOC die-away	301 B CO_2 evolution	301 C MITI	301 D Closed bottle
Concn. of test material				
mg/L	—	—	100	2–10
mg carbon/L	10–40	10–20	—	—
Concn. of inoculum				
mg/L TSS	≤30	≤30	30	—
CFU/L	10^7–10^8	10^7–10^8	10^7–10^8	10^4–10^6
pH	7.4 ± 0.2	7.4 ± 0.2	7.0	7.4 ± 0.2
Temperature (°C)	22 ± 2	22 ± 2	25 ± 1	22 ± 2
Concn. of mineral in test medium (mmol/L)				
P	3.74	3.74	1.26	0.374
N	0.09	0.09	0.09	0.009
Na	3.74	3.74	0.75	0.374
K	3.12	3.12	0.93	0.312
Mg	0.09	0.09	0.27	0.09
Ca	0.25	0.25	0.74	0.25
Fe	0.0009–0.0018	0.0009–0.0018	0.0027	0.0009–0.0018

Note. CFU/L, colony-forming unit per liter; TSS, total suspended solids.

source, and a rather lean inoculum to test material ratio. The source of inoculum can be derived from naturally occurring bacteria in sewage effluent or activated sludge, but artificial enrichment or preacclimation of the inoculum to the test conditions is not permitted. Table 8 provides the details of the experimental conditions for these screening tests. Results from these tests are usually presented as percentage of dissolved organic carbon (DOC) removal, percentage of CO_2 production, or oxygen consumed as a percentage of theoretical oxygen demand (ThO_2) or chemical oxygen demand (COD). A compound is defined as readily biodegradable by the OECD guidelines if (i) 70% DOC removal, 60% O_2 uptake, or CO_2 evolution is achieved in 28 days, and (ii) this level of biodegradation is reached within a 10-day period after attaining 10% biodegradation. If a ready biodegradation test is passed, the compound is not expected to persist in the environment. However, failure to pass a screening test does not indicate that the material is resistant to biodegradation but may indicate that an acclimation period is required or that the material was toxic to the inoculum. A compound failing the screening tests may then be tested for inherent biodegradability (OECD 302 series). A positive result in an inherent biodegradation test demonstrates that a chemical has the potential to undergo biodegradation given the right circumstances.

Ready Biodegradation

The aerobic biodegradability of glutaraldehyde has been examined with a variety of the screening test methods. Details of the test conditions and the results are summarized in Table 9. Glutaraldehyde exhibits variable biodegradation

TABLE 9
Results of Tests to Assess the Aerobic-Ready Biodegradation of Glutaraldehyde

Source of inoculum	Inoculum concn.	Glutaraldehyde concn. (mg a.i./L)	OECD guideline	Analytical method	% Biodegradation on day 5	10	15	20	28	36	Reference
WWTP Charleston, WV	ND	1.7–16.5	—	O_2	35	66	80	87	—	—	UCC, 1979
		1.7			45	65	87	98	—	—	
WWTP Charleston, WV	ND	0.9–1.7	—	O_2	63	78	86	110[a]	—	—	UCC, 1981a
Polyseed[b]	ND	1.7–3.3	—	O_2	28	57	68	72	—	—	UCC, 1995
Polyseed[b]	ND	2.0	301D	O_2	2	—	56	—	64	—	UCC, 1995
WWTP Sissach, Switzerland	TSS 30 mg/L	50	301C	O_2	13	32	35	—	—	—	RCC, 1990a
				DOC	—	—	80	—	—	—	
WWTP Grasonville, MD	TSS 8.3 mg/L 1.0×10^7 CFU/L	8.3	301B	CO_2	0	24[c]	37[d]	57[e]	60	64	WIL, 1996
				DOC	—	—	—	—	—	80	
WWTP Grasonville, MD	TSS 20.6 mg/L 2.2×10^8 CFU/L	25	301A	DOC	83	74	—	—	—	—	WIL, 2000

Note. CFU/L, colony-forming unit per liter; ND, not determined; TSS, total suspended solids; WWTP, wastewater treatment plant.

[a]A somewhat greater than expected oxygen demand was experienced at the lower test concentrations, probably due to significant nitrification of nutrients in the diluent.

[b]Polybac Polyseed is a commercially available lyophilized bacterial seed source containing a broad spectrum of microorganisms.

[c]Day 11, [d]Day 14, [e]Day 22.

rates depending on the screening test methods. The highest biodegradation rate was observed in the OECD 301A test (83% in 5 days) (WIL, 2000), while the lowest rate was seen in the OECD 301B test (0% in 5 days) (WIL, 1996). It is well known that results of biodegradation tests are quite variable. Many factors contribute to this inconsistency and may include the source, concentration, and acclimation status of the inoculum, the concentration of the test material, and the analytical measurement techniques used. With respect to glutaraldehyde, it is apparent that the results were markedly influenced by the test concentration. A higher biodegradability with a short lag time was observed when the glutaraldehyde concentrations in the test system were low (< 2 mg/L) (UCC, 1979, 1981a) than when the concentrations were high (> 8 mg/L) (RCC, 1990a; WIL, 1996). Since the NOEC for bacterial inhibition is about 5 mg/L (UCC 1994a), the lower biodegradation rates observed in these studies where high concentrations of glutaraldehyde were used (RCC, 1990a; WIL 1996) were likely due to inhibition of the inoculum. Since the DOC die-away test (OECD 301A), the carbon dioxide evolution test (OECD 301B), and the MITI test (OECD 301C) all require high concentrations of test material, the equivalent glutaraldehyde concentrations, 17–67, 17–33, and 100 mg/L, respectively, are inhibitory to microbes. Hence such tests are not optimal for the assessment of glutaraldehyde.

Another problem unique to glutaraldehyde is its reaction with ammonium ions in the test medium to yield a product that is known to be more difficult to biodegrade (UCC, 1995). This is unavoidable since the test medium for the screening biodegradation tests must provide a source of metabolizable nitrogen in the form of ammonium ions for proper microbial growth. The impact of this can be minimized by using a high concentration of glutaraldehyde to ensure that the contribution of the glutaraldehyde-ammonium reaction product to overall biodegradability measurement is minimal. This, however, must be carefully balanced with the need to keep the glutaraldehyde concentration below the level that is inhibitory to microbes. Among the OECD 301 series of ready biodegradation tests (Table 8), the closed bottle test (OECD 301D), with a recommended ammonium concentration of 0.009 mmol/L in the test medium and a test material concentration of 2–10 mg/L (0.02–0.1 mmol glutaraldehyde/L), appears to strike the best balance and is the most optimal test method for glutaraldehyde.

Glutaraldehyde can also interact with proteinaceus material in the microbes. Glutaraldehyde bound to the biomass, while technically not metabolized, is considered biodegraded for the purpose of the screening tests. For test methods such as the OECD 301A test, which monitors the disappearance of dissolved organic carbon, the overall measure of biodegradability is the combined contribution from microbial catabolism and loss of the test material through binding to the biomass. It is in recognition of this that the pass criterion for the OECD 301A test is 70%, 10% higher than the usual 60% for test methods based on the measurement of O_2 consumption (OECD 301D) or CO_2 evolution (OECD 301B). In the biodegradation tests conducted with glutaraldehyde, the fact that the percentage of DOC disappearance (80%) exceeded that of O_2 consumption (35%) (RCC, 1990a) or CO_2 evolution (64%) (WIL, 1996) is consistent with glutaraldehyde removal by sorption to the biomass.

Taking the totality of the biodegradability data in Table 9 into perspective, it is apparent that, regardless of the test methods with their potential problems discussed above, glutaraldehyde has demonstrated a moderate to high rate of aerobic biodegradation. The OECD 301A test (WIL 2000), despite the inoptimal test concentration used, provided the data that fulfilled the OECD criteria to classify glutaraldehyde as readily biodegradable.

Seawater Biodegradation

To assess the potential for biodegradation of chemicals which might find their way into the marine environment, the OECD has developed a seawater biodegradation test (OECD 306). This is based on the OECD 301 series of screening tests for the freshwater environment, but differs in one significant aspect. Unlike the OECD 301 tests which add inoculum in the form of sewage effluent or activated sludge to the test medium, the OECD 306 test uses natural seawater as both the test medium and the sole source of microorganisms. Hence, the seawater biodegradation test is not considered a test for ready biodegradability since no additional microorganisms are added beyond those already present in the seawater. Neither does the test simulate the marine environment since nutrients are added and the concentration of test substance is very much higher than would be present in the sea. The pass criterion for the OECD 306 test is >60% ThO_2 or >70% DOC removal in 28 days. Test materials that meet this criterion are considered to have a potential for biodegradation in the marine environment. However, a negative result does not preclude such a potential but indicates that further study is necessary.

The biodegradability of glutaraldehyde in seawater has been tested (Table 10). In the first test (SPL, 1997a) 52% ThO_2 was attained in 28 days, which fell slightly short of the pass criterion. Two plausible causes might explain this low rate of biodegradability. First, given that the test medium contained 0.009 mmol/L ammonium ions available for reaction, the concentrations of glutaraldehyde tested (1.5 mg a.i./L or 0.015 mmol/L) might have been too low. Second, the seawater might not have contained a sufficient concentration of microorganisms. To minimize the potential error due to ammonium reaction, a second OECD 306 test (ASI 2000) was conducted with a two-fold higher concentration

TABLE 10
Results of Tests to Assess the Aerobic Biodegradation of Glutaraldehyde in Seawater Using the Closed Bottle Test (OECD 306 Guideline)

Source of inoculum	Microbial concentration (CFU/L)	Glutaraldehyde concentration (mg a.i./L)	% Biodegradation[a] on day					Reference
			5	9	15	21	28	
Coastal seawater Skegness, England	ND	1.5	26[b]	48	49	53	52	SPL, 1997a
Coastal seawater Point Pleasant, NJ	1.3×10^6	3.0	0	67[c]	65	71[d]	73	ASI, 2000

[a]Quantified by measuring the consumption of oxygen.
[b]Day 6, [c]Day 11, [d]Day 19.

of glutaraldehyde (3 mg/L) than the first test. In addition, the microbial concentration was assayed to assure that the seawater used in the test contained adequate microorganisms (1.3×10^6 CFU/L). When glutaraldehyde was tested under these conditions a high rate of biodegradation was achieved, meeting the pass criteria by attaining a 73% ThO_2 in 28 days.

Conducting biodegradation studies on glutaraldehyde is technically challenging. The proper test method and the experimental condition, especially the test material concentration, must be carefully selected and optimized within the confines of the testing guidelines, to minimize the dual problems of microbial inhibition and ammonium complexation. The biodegradability of glutaraldehyde was tested with a variety of test methods, which indicated that glutaraldehyde achieved a moderate to high rate of biodegradation, with the DOC die-away test (OECD 301A) meeting the OECD ready biodegradability classification criteria. Glutaraldehyde is considered readily biodegradable in the freshwater environment and has the potential for biodegradation in the marine environment.

Aquatic Metabolism

Aerobic

[1,5-^{14}C]Glutaraldehyde was investigated to determine its metabolic fate in an aerobic sediment-river water system according to FIFRA 162-4 guideline (PTRL, 1993). The sediment and water were obtained from the Sacramento River Delta at Antioch, California. See Table 7 for the physicochemical characteristics of the sediment. The test system consisted of Erlenmeyer flasks each containing 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 at 25°C at a pH of 6.3–7.7, and a dissolved oxygen concentration of 4.9–7.7 ppm. At various sampling times, the headspace of the flasks was flushed with air through a series of traps containing, in order, an ethylene glycol trap for the collection of organic volatiles, and two KOH traps for the collection of $^{14}CO_2$. Immediately after flushing, river water was separated from the sediment by decanting following centrifugation. The sediment was then extracted three times with acetonitrile: 0.001 N HCl (1:1, v/v). The remaining sediment was air-dried and combusted. All radiochemical analyses were performed with liquid scintillation spectrometry. Metabolites were identified by high performance liquid chromatography or thin layer chromatography with analytical reference standards.

Table 11 presents the time course of radiocarbon material balance. During the first 24 h, most of the applied radiocarbon was found in the water phase, with about 10–20% adsorbed in the sediment and no significant volatiles found. During the period of 1 to 30 days, the proportion of aqueous radiocarbon decreased significantly (from 67 to 14%) with a concomitant rise in the evolution of $^{14}CO_2$ to 68% at 30 days, while the proportion of radiocarbon found in the sediment did not change very much.

While this aquatic metabolism study was not technically a biodegradation test, the $^{14}CO_2$ data, however, could offer a supplemental perspective to the standard OECD 301B CO_2 evolution test (WIL, 1996). The glutaraldehyde concentration tested in the aquatic metabolism study was 9.45 mg/L However, even at this concentration, there was significant microbial inhibition, as the bacterial concentration detected in the water after 4 h of incubation (2.5×10^6 CFU/L) had a decrease relative to the predosing measurement (1×10^8 CFU/L). The extent of biodegradation, as measured by the evolution of CO_2 and DOC disappearance, was similar in the two studies, 67.9 and 79.6%, respectively, in 30 days (metabolism study, Table 11) vs 64 and 80%, respectively, in 36 days (biodegradation study, Table 9; WIL, 1996). Similar to the observations in the biodegradation study, the aqueous radiocarbon loss (79.6%) exceeded that of CO_2 formation (67.9%) in the metabolism study, suggesting that some glutaraldehyde was not metabolized but removed by sorption to the biomass. The difference

TABLE 11
Distribution of Radiocarbon from the Aerobic Metabolism of [^{14}C]Glutaraldehyde in a River Water-Sediment System[a]

Sampling time	pH	Dissolved O_2 (ppm)	Microbial concn. in water (CFU/L)	% Dose applied			
				Water	Sediment	CO_2	Total
0 h	7.18	6.36	1.0×10^8	93.6	7.6	—	101.2
4 h	7.34	4.94	2.5×10^6	94.0	8.6	0	102.6
12 h	7.27	5.64	—	84.6	16.7	0.5	101.8
1 day	7.13	6.24	2.9×10^8	67.3	20.4	0.5	88.2
2 days	7.56	6.31	—	49.8	25.3	10.3	85.4
7 days	7.01	6.50	3.3×10^8	36.9	21.9	20.0	78.8
14 days	6.81	7.35	—	18.6	17.1	48.1	83.8
30 days	6.31	6.75	4.5×10^7	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.

(11.7%) was consistent with the percentage of radiocarbon found in the sediment (12.4% at 30 days).

Table 12 provides the proportion of metabolites in the aqueous phase. The major metabolite of glutaraldehyde produced by microbes in an aerobic aquatic system was carbon dioxide, with glutaric acid formed as an intermediate in the water phase. The calculated pseudo-first-order half-life of glutaraldehyde catabolism in water (based on the loss of the parent compound) under aerobic condition was 10.6 h. The radiocarbon in sediment could not be extracted and its identity could not be ascertained.

Anaerobic

The anaerobic metabolism of [1,5-^{14}C]glutaraldehyde was examined according to FIFRA 162-3 guideline with a test system similar to that used to study aerobic metabolism (PTRL, 1994b). Fifty-six days before dosing, anaerobic conditions were established by purging the test system with nitrogen. The dissolved oxygen concentration ranged from 0.1 to 0.6 ppm, and the pH was 4.0–5.3 in this test system. The anaerobic microbial concentration in the water ranged from 2.0×10^6 to 4.7×10^7 CFU/L throughout the course of the study. At various sampling times, the headspace of flasks was flushed with nitrogen through a series of traps to collect organic volatiles and $^{14}CO_2$.

TABLE 12
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 concentration 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.

Table 13 presents the time course of radiocarbon material balance. Most of the applied radiocarbon (> 87%) was found in the aqueous phase throughout the entire course of the study. About 5–9% was adsorbed to the sediment, and no significant organic volatiles were detected. Only a tiny amount (< 0.3%) of $^{14}CO_2$ was formed. Table 14 indicates the proportion of metabolites in the aqueous phase The major metabolites of glutaraldehyde produced by microbes in an anaerobic aquatic system were 1,5-pentanediol with 5-hydroxypentanal formed as an intermediate, and 3-formyl-6-hydroxy-2-cyclohexene-1-propanal, a cyclicized dimer of glutaraldehyde. This latter metabolite was similar to the species found in the abiotic hydrolysis of glutaraldehyde (PTRL, 1992b). The calculated pseudo-first-order half-life of glutaraldehyde catabolism in water (based on the loss of the parent compound) under anaerobic condition was 7.7 h. Extraction of the sediment released about 64% of the adsorbed radiocarbon. The metabolite composition of the extract was similar to that in the corresponding water phase.

The results of the aquatic metabolism studies indicate that glutaraldehyde is rapidly biotransformed by microbes. Under aerobic conditions, metabolism proceeds to complete mineralization, with the formation of glutaric acid as an intermediate and CO_2 as the principal metabolite. This metabolic pattern is similar to that reported in the mammalian system (Karp *et al.*, 1987). Under anaerobic

TABLE 13
Distribution of Radiocarbon from the Anaerobic Metabolism of [^{14}C]Glutaraldehyde in a River Water-Sediment System[a]

Sampling time (day)	pH	Dissolved O_2 (ppm)	Microbial concn. in water (CFU/L)	% Dose applied			
				Water	Sediment	CO_2	Total
0	4.24	0.27	1.9×10^7	91.9	5.7	—	97.6
1	4.25	0.23	2.7×10^6	95.1	6.3	0.1	101.5
3	4.12	0.17	2.0×10^6	89.6	6.1	0.3	96.0
7	4.09	0.29	3.1×10^6	89.0	7.2	0.0	96.2
14	4.45	0.55	—	94.6	7.0	0.1	101.7
30	4.82	0.34	3.7×10^7	87.0	8.3	0.1	95.4
60	4.60	0.29	—	91.6	7.2	0.2	99.0
90	5.13	0.41	4.7×10^7	93.4	7.4	0.3	101.1
123	4.82	0.54	—	91.4	8.4	0.3	100.1
				Overall recovery (mean $\pm$ SD) = 98.7 $\pm$ 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 $\pm$ 0.4°C. Results shown are the average values of two replicate test systems.

conditions, however, only primary biodegradation is observed, with the production of two major metabolites, 1,5-pentanediol and 3-formyl-6-hydroxy-2-cyclohexene-1-propanal.

Chemical Deactivation

As a biocide, glutaraldehyde is selectively toxic to a variety of microorganisms. The NOEC for bacterial inhibition has been determined to be about 5 mg/L (UCC, 1994a). Discharge of glutaraldehyde in excess of this level to a wastewater treatment facility may inhibit the sewage microorganisms and adversely impact the treatment performance. Therefore, it is advisable to dilute the waste glutaraldehyde solutions to below 5 mg/L before discharge. Where dilution is not practical, especially when huge volumes of water are needed to bring the concentration to below 5 mg/L, chemical deactivation to reduce the microbiocidal activity may be a suitable alternative.

Three methods to chemically inactivate glutaraldehyde have been investigated (Jordan *et al.*, 1996). The first one involves raising and maintaining the pH of the glutaraldehyde solution (up to 2% active) to about 12 by the addition of caustic (NaOH or KOH) for 8 h, and then returning to neutrality by the careful addition of an inorganic acid (e.g., HCl) before disposal. The putative chemical deactivation product formed by caustic treatment is the cyclized dimer, 3-formyl-6-hydroxy-2-cyclohexene-1-propanal. The second method involves the complexation of up to 5% glutaraldehyde with excess ammonia (dibasic ammonium phosphate), and the third uses a 2 to 3 molar excess of sodium bisulfite to react with up to 5% glutaraldehyde. Unlike the first two methods, the sodium bisulfite method does not require a long holding period, since the

TABLE 14
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.

TABLE 15
Comparison of Environmental Fate and Effects Data for Treated and Untreated Glutaraldehyde Solutions

	Untreated glutaraldehyde	Caustic	Dibasic ammonium phosphate			Sodium bisulfite	
Molar ratio (per mole glutaraldehyde)		—	0.5	1.25	2.5	2	3
Environmental effects							
Bacteria IC_{50} (mg a.i./L)	17	—	170	540	110	440	230
Daphnia magna LC_{50} (mg a.i./L)	5	4600	47	100	41	109	41
Fathead minnow LC_{50} (mg a.i./L)	5.4	>1000	3.7	1.4	0.8	280	50
Algae EC_{50} (mg a.i./L)	0.81	2.1	—	—	—	—	3.9
Biodegradation at 28 days (%)							
O_2 consumption	64	—	12	3	3	57	34
CO_2 evolution	60	71	—	—	—	83	—

complexation reaction between glutaraldehyde and sodium bisulfite is complete in less than 5 min. To determine the effectiveness of these methods to deactivate glutaraldehyde, relevant environmental fate and effects data of treated glutaraldehyde solutions (UCC, 1990, 1994b,c; 1995, 1996, 1997; WIL 1997) were compared to those of untreated glutaraldehyde solutions (Table 15). Both the caustic and the sodium bisulfite methods were quite effective in reducing the overall toxicity of glutaraldehyde to aquatic organisms. The sodium bisulfite method reduced the toxicity of glutaraldehyde to bacteria by 14- to 26-fold and to aquatic organisms by 6- to 52-fold. The ammonium phosphate method, however, increased the toxicity to fish by 2- to 7-fold relative to untreated glutaraldehyde. In the area of environmental fate, reaction products resulting from the treatment with caustic or sodium bisulfite had comparable biodegradability as untreated glutaraldehyde, while the dibasic ammonium phosphate-glutaraldehyde complex appeared to be less biodegradable.

Results from the chemical deactivation studies demonstrate that pretreatment with caustic or sodium bisulfite is an effective method for reducing the aquatic toxicity of glutaraldehyde. However, the sodium bisulfite deactivation method is preferred for its rapid operation and technical simplicity over the caustic method, which necessitates careful pH adjustments and a long holding period.

CONCLUSION

1. In acute exposures, the median lethal concentration of glutaraldehyde is around 7 mg/L, which would be "toxic to aquatic organisms (R51)" under the EU classification system for aquatic toxicity data. However, glutaraldehyde does not meet the criteria for classification as R51 because it is readily biodegradable according to these same classification criteria. The toxicity to algae ($LC_{50} < 1$ mg/L), however, requires a classification of R50 (very toxic to aquatic organisms). But the European Dangerous Preparations Directive sets a threshold for classification of mixtures of glutaraldehyde at $\geq 25\%$. Hence only those solutions or mixtures containing greater than or equal to 25% a.i. glutaraldehyde will classify as R50.

2. Glutaraldehyde is equally toxic to warm water and cold water fish, but is slightly more toxic to freshwater fish than saltwater fish.

3. Acute toxicity to birds is comparable to that for mammalian species.

4. The toxicity of glutaraldehyde to aquatic organisms is not appreciably increased with repeated long-term exposures. As per the EU guidance for the risk assessment of existing substances, the Predicted No Effect Concentration of glutaraldehyde is 31 μg/L based on the NOECs of three chronic toxicity studies.

5. When glutaraldehyde is introduced into the environment, it is most likely to remain in the aquatic compartment, given its small air/water partition coefficient and soil/water sorption coefficient.

6. The tendency of glutaraldehyde to bioaccumulate is low, based on its high water solubility and low *n*-octanol/water partition coefficient.

7. Glutaraldehyde is stable at room temperature but may degrade at elevated temperatures. It is also stable to sunlight. Aqueous solutions of glutaraldehyde are stable at acidic to neutral conditions, but unstable in an alkaline environment, forming a cyclicized dimer.

8. Glutaraldehyde meets the OECD criteria to be classified as readily biodegradable in the freshwater environment. It also satisfies the OECD criterion to be considered to have the potential for biodegradation in the marine environment.

9. Material balance and metabolism studies in a river water-sediment system demonstrate that just as predicted by the physicochemical partitioning characteristics, glutaraldehyde tends to remain in the water phase. Under aerobic conditions, glutaraldehyde is metabolized to carbon

dioxide via glutaric acid as an intermediate. Under anaerobic conditions, glutaraldehyde is metabolized to 1,5-pentanediol and 3-formyl-6-hydroxy-2-cyclohexene-1-propanal.

10. Pretreatment with sodium bisulfite, in a molar ratio of 2 to 3 parts per part of glutaraldehyde, is the best method to deactivate aqueous solutions glutaraldehyde prior to disposal to treatment systems.

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