Analysis of Acrolein in Aged Aqueous Media. Comparison of Various Analytical Methods with Bioassays

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The lifetime of acrolein was studied in various buffer systems at pH 5, 7, and 9 at 22 °C. Bioassays indicated that the complete hydrolysis of acrolein occurred at the above pH values within 150, 120–180, and 5–40 h, respectively. The variation in the data for the pH 7 and 9 cases shows a broad range of results when various buffer systems of like pH but unlike composition are compared. Analytical methods involving the conversion of acrolein to a derivative such as 2,4-dinitrophenylhydrazone (DNPH) or 7-hydroxyquinoline, followed by analysis, did not correlate well with the bioassay data. All derivatization methods gave positive, nonzero values for acrolein residuals even though bioassay data were negative. Analytical methods measuring acrolein directly by NMR, direct fluorescence, and differential pulse polarography correlated well with bioassay data over the range of 1 to 4 acrolein half-lives. Regardless of the analytical method chosen, systems containing tris(hydroxymethyl)aminomethane consistently gave poor correlations with bioassay data.

Widespread interest is focused on the toxicity and persistence of chemicals which come in contact with the environment. Acrolein is widely used as an aquatic herbicide in irrigation canals and as a biocide in oilfield waterflood systems (Legator, 1951; Racusen, 1962; Shell Internationale, 1962; and VanOverbeek, 1972). Presently, only acrolein and xylene are registered for use as aquatic, organic herbicides in the United States. It is important that the analytical method used to determine the amount of herbicide (biocide) present in a given system gives meaningful results. Numerous analytical methods are used to determine the amount of acrolein present in aqueous solutions, including techniques involving colorimetry (Bowmer et al., 1974; Schumacher, 1975; Yamate and Matsurmura, 1975), fluorescence (Alarcon, 1968; Suzuki, 1975; Hopkins and Hattrup, 1974), differential pulse polarography (Howe, 1976; Yamate and Matsurmura, 1974), titrimetry (Hall and Stern, 1956; Pressman and Lucas, 1942; and Smith, 1977), and UV spectroscopy (Buswell et al., 1940; Vik, 1973). These methods are both versatile and highly sensitive when applied to the determination of acrolein in freshly prepared aqueous solutions (Brady et al., 1977).

Some experiments describing the loss of acrolein with time in aqueous media by hydrolysis have been reported (Bowmer et al., 1974; Burczyk et al., 1969; Gilbert and Donleavy, 1938; Hall and Stern, 1956; Pressman and Lucas, 1942; Smith, 1977; Yamate and Matsurmura, 1974). These papers describe an initial decay of acrolein to some fraction of the initial concentration within hours or days, followed by slow or negligible changes over periods of months or even years. Available toxicity data (Schumacher, 1975; Smith, 1977), and experience in the field (Magna Corporation, 1970) on the other hand, reveal the decay of acrolein toxicity to biologically inactive levels within a few hours or days, depending on the nature of the system.

These conflicting observations prompted us to examine various analytical methods (e.g., colorimetric, fluorescence, GLC, NMR, polarographic, titrimetric, and UV), and to compare the results obtained from these methods with values obtained from bioassay techniques. Ideally, the data obtained from a chemical analysis should agree with bioassay data. Bioassay techniques are often cumbersome and time-consuming. They are also difficult to reproduce when using field samples. An analytical method that rapidly measures acrolein in aged aqueous media and corresponds to the biological toxicity of the aged acrolein solution would be desirable for quick, accurate, and true residual determinations.

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EXPERIMENTAL SECTION

Reagents. Acrolein was obtained from commercial sources and was distilled under a nitrogen atmosphere, using a 1-m acid washed Vigreux column. The fraction boiling at 52.5–53.5 °C was collected in a receiver initially containing a small amount of hydroquinone. After the distillation, the hydroquinone level was adjusted to 0.3%. The dioxane used in the gas chromatographic method was also distilled before use. The *m*-aminophenol used in the fluorometric method was sublimed before use. All other solvents, reagents, and buffer solutions were obtained from commercial sources and used without further purification.

Stock Solutions. A 250-mL volumetric flask was charged with 12.5 mL of acrolein and diluted with buffer solution producing a 5% (0.749 M) solution. Various standard buffer systems were examined: pH 5, phthalate; pH 7, phosphate, phosphate with commercial yellow dye (obtained from Fisher Chemical Company), and tris(hydroxymethyl)aminomethane (Tris); pH9, borate and Tris. These stock solutions were examined for acrolein by various methods by removing aliquots at various time intervals. The solutions were maintained in the dark at 22 ± 2 °C.

ATP Bioassays. The adenosine triphosphate (ATP) procedure used was identical with that of Littman and SAI. Data for the time kills were obtained by adding 20 μL (for 10 ppm acrolein test) or 100 μL (for 50 ppm acrolein test) of the stock solutions to 100 mL of bacteria-laden water, thereby allowing the bacteria to come in contact with the acrolein samples. This bacteria solution was obtained fresh each day from field sources located in the Water and Oil Department waterflood of the city of Long Beach, Calif. After 2 h (a 2-h time kill), the bacteria solution was filtered using a 0.45-μm Millipore filter system. The filter cake was then added to 5 mL of Tris buffer and 0.5 mL of ATP enzyme (firefly extract, Sigma Chemical Company). The bacteria in these solutions were then counted by using a SAI ATP photometer, Model 1. Appropriate ATP standards were prepared; buffer solutions made with fresh acrolein were used at each analysis interval for comparison with the aged solutions, and buffer solutions without acrolein were added to the bacteria laden water for use as blanks.

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Plate Count Bioassays. The procedure used to determine amounts of aerobic and sulfate-reducing bacteria in the aqueous systems was identical with that used by the American Petroleum Institute. Water from field sources was obtained, and the chloride concentration was measured by titration with $AgNO_3$ using potassium chromate as an indicator. A synthetic brine was then prepared using ASTM brine reagent, with the chloride level adjusted to equal the value obtained from the field water.

The agar medium was prepared by adding 8 g of agar (Difco Laboratories) to 1 L of ASTM synthetic brine solution, followed by gentle heating. After all the agar dissolved, 33 mL of nutrient (sulfate API broth, Difco) was added, and the pH of the solution was adjusted to 7.2–7.6 with 10% NaOH.

In order to measure the effect of the aged acrolein solutions on aerobic bacteria, the following procedure was used. A 2-h time kill at 46–49 °C was run on samples of field water using aliquots which were removed from the acrolein solution. The field water was treated with acrolein from the 5% buffer solutions to provide a mixture containing 10 ppm of acrolein based on the initial concentration of acrolein at time zero. Blanks and controls similar to those used in the ATP bioassay were also prepared. Petri dishes were then charged with 15 mL of the agar medium and 1 mL of the 2-h time kill samples. These agar medium field samples were then diluted several times by serial dilution techniques. The plates were then incubated for 10 days at room temperature. The aerobic colonies were counted on a Brunswick C-110 Bactronic Colony Counter. The resulting number of colonies was then corrected by the appropriate serial dilution factor.

Sulfate-reducing bacteria were measured by adding 1 mL of field water samples containing 10 ppm of the acrolein aliquots, which had undergone the 2-h time kill period, to 30 mL of agar medium in culture tubes and were then serially diluted. Blanks and controls were also prepared. The resulting mixtures were incubated at 30 °C for 2 weeks. The black sulfate reducing colonies were counted visually under strong light, and the number of colonies was corrected by the appropriate serial dilution factor.

Fish Kill Study. Bluegill sunfish having an average length of 1 to 2 in. were obtained from Fish Breeders of Niland, Calif. These fish were maintained in 20-gal aerated aquariums for 7 days prior to use. The aquarium contained water composed of 30 mg/L of calcium sulfate, 30 mg/L of magnesium sulfate, 48 mg/L of sodium bicarbonate, and 2 mg/L of potassium chloride.

The water used in the kill studies was a natural water obtained from the Clear Creek Tunnel, located near the Lewiston Reservoir on the Trinity River in Trinity County, Calif. An analysis of this water was made by the U.S. Bureau of Reclamation located in Sacramento, Calif. The results of this analysis are shown in Table I.

The Trinity River water was placed in 5-gal polyethylene pails, and equilibrated to a laboratory temperature of 22 ± 2 °C for 24 h. These samples were then aerated for 24 h prior to use with aquarium air pumps. No chemicals other than acrolein were added to the water.

Acrolein was serially diluted to 100 ppm using Trinity River water. This solution was stored in a capped amber glass bottle at 22 ± 2 °C.

Each day for 7 days a portion of the aging 100 ppm acrolein solution was added to the contents of a 5-gal pail so that the final solution realized 1.2 ppm acrolein based on the initial 100 ppm acrolein level. A sample of this mixture was removed and analyzed by the aminophenol fluorometric method of Alarcon. Ten bluegill sunfish were then added and observed.

Table I. Chemical Analyses of the Water Sample Collected from the Clear Creek Tunnel

quality parameter	quantity, mg/L
total dissolved solids	134
total hardness	98
calcium	24
magnesium	9
sodium	15
potassium	0.23
bicarbonate	135
sulfate	19
chloride	1
pH	8.1

A blank consisted of aerated Trinity River water and 10 bluegill sunfish; this sample did not contain acrolein. A control experiment consisting of aerated Trinity River water, 10 bluegill sunfish, and 1 ppm fresh acrolein was also examined. A Magna Oxymeter was used to determine the dissolved oxygen content of the bioassay waters to determine the oxygen level of each sample.

Bromination Method. The bromination procedure of Pressman and Lucas was used. An Erlenmeyer flask was filled with 25 mL of cold DI water, 3.5 mL of 0.5 N bromide–bromate solution, 20 mL of 3 N sulfuric acid, and 0.5 mL of the aliquot from the 5% stock acrolein solution to be examined. After 8 min, 10 mL of 10% KI solution was added. After 1 min, 0.4 mL of starch solution was added, and after an additional 1 min, the solution was titrated with 0.1 N sodium thiosulfate solution. Freshly prepared acrolein control solutions and blank buffer solutions were also examined at each time interval.

DNPH Procedure. The procedure used is essentially that developed by Shell and reported by Smith (1977). A portion of the aliquot removed from the 5% stock solution was diluted by a factor of 50 000. Five milliliters of this solution were added to 1 mL of 2,4-dinitrophenylhydrazine reagent. After 5 min, 1 mL of 2 N NaOH was added, and after an additional 1 min period, 3 mL of isopropyl alcohol was added. The resulting 2,4-dinitrophenylhydrazone (DNPH) mixture was analyzed on a Perkin Elmer C-550 UV–vis spectrophotometer, utilizing readings at 480 nm.

Aminophenol Fluorometric Method. The procedure used was that of Alarcon. A portion of the aliquot removed from the 5% stock solution was diluted by a factor of 50 000. Exactly 1 mL of this solution was added to 1 mL of aminophenol reagent and 1 mL of hydroxylamine reagent. The mixture was placed in a water bath at 100 °C for 20 min which produced 7-hydroxyquinoline. After cooling, the samples were analyzed on an Aminco Bowman fluorescence spectrophotometer Model SPE equipped with a xenon lamp. The excitation wavelength used was 372 nm, and the 7-hydroxyquinoline emission peak occurred at 506 nm. These frequencies are different from those used by Alarcon, suggesting that the spectrometer geometry is an important variable to be adjusted accordingly.

GLC Procedure. One milliliter of the aliquot removed from the 5% stock solution was added to 50 μL of dioxane (internal standard). This solution was analyzed by a Varian gas chromatograph, Model 2760, equipped with FID detectors, and a $^1/_8$ in. × 6 ft acid washed Poropak Q column. The following conditions were used: column temperature, 200 °C, injector and detector temperatures, 250 °C, and a nitrogen carrier flow rate of 32 mL/min.

NMR Method. Proton NMR measurements were made directly on the aliquots removed from the 5% stock solution, using a Varian T-60 A spectrophotometer. The

Table II. Fish Bioassay Study

days aged	ppm of acrolein by fluorescence	results of 10 fish exposure	ppm of dissolved O_2
0	1.2	all fish dead in 0.5 h	8.0
1	0.86	all fish dead in 1 h	7.0
2	0.86	all fish dead in 2 h	8.5
3	0.86	9 fish dead in 3 h	8.5
4	0.82	9 fish dead in 3 h	8.2
5	0.9	all fish dead in 5 h	9.0
6	0.65	all fish survived 5 days	8.0
7	0.8	all fish survived 5 days	8.0

aldehyde signal at 9.44 ppm was recorded and its integral was compared with the integral obtained from a sealed external Me_4Si calibrated capillary.

Direct Fluorescence Procedure. A 1-mL aliquot from the 5% stock solution was diluted to 200 mL with DI water. This solution was analyzed directly on an Amin-co-Bowman fluorescence spectrophotometer. The excitation frequency used was 276 nm, and the observed emission frequency was 370 nm.

Differential Pulse Polarographic Method. Differential pulse polarographic measurements were made using the method of Howe. Exactly 0.5 mL of the aliquot solution was diluted to 500 mL with DI water. One milliliter of this solution was then mixed with 10 mL of phosphate buffer and 100 μL of 10% ethylenediaminetetraacetic acid (EDTA) solution. The resulting sample was analyzed using a Princeton PAR 174-A polarograph equipped with a Houston Omnigraphic 2200 XY-recorder. The polarographic settings used were identical with those of Howe (1976), except that a scan width of −0.75 to −1.50 V was used.

5,6-Dihydro-2*H*-pyran-3-carboxaldehyde. The method of Fischer (1977) was used to prepare 5,6-dihydro-2*H*-pyan-3-carboxaldehyde. A 2-L flask was charged with 426 mL (3 mol) of acrolein, 607 mL of deionized water, 331 g of 85% phosphoric acid, and 150 mL of benzene under a nitrogen atmosphere. This mixture was stirred at reflux for 2 h with vigorous mechanical stirring. The mixture was then cooled, and the benzene layer was removed. The aqueous portion of the mixture was replaced into the 2-L flask, and another 150 mL of benzene was added, and the process was repeated. The combined benzene layers were dried over $MgSO_4$ and distilled to yield 88.2 g (49%) of 5,6-dihydro-2*H*-pyran-3-carboxaldehyde, bp 60–4 °C (3 Torr); IR (neat) 2842, 2730, 1675, 1643, 1236, 1181, 1107, 969, and 699 cm^{-1}; NMR (CCl_4) δ 4.65 (s, 1), 3.43 (m, 1), 2.08 (q, 2), 1.85 (t, 2), and 1.20 ppm (m, 2); UV (H_2O) 229 nm (ε 2260), and 304 nm (ε 48).

RESULTS AND DISCUSSION

In order to compare various analytical methods with each other, several systems were examined. These systems included both those prepared in the laboratory as well as some obtained from natural field sources. The analytical methods chosen for this comparison included those currently being used to measure acrolein concentrations in aqueous media.

Water obtained from the Trinity River project in Northern California was used in a laboratory experiment involving the exposure of bluegill sunfish to various samples of aged aqueous acrolein. The initial acrolein dosage was 1.2 ppm. The data obtained from this experiment are shown in Table II. These data show that while the amount of acrolein as measured over the 7-day period by the aminophenol fluorescence method remained relatively constant and at a high level, the fish survived a 5-day exposure after an acrolein aging period of 5 days. The lethal dosage of acrolein for bluegill sunfish has been determined to be 0.079 ppm for a 24-h exposure at 16 °C (Burdick et al., 1964). After 5 days of aging, the acrolein level should realistically be below 0.08 ppm, not the 0.8 level suggested by the aminophenol fluorescence method. As a precaution, the level of dissolved oxygen was monitored to insure that the fish did not die as a result of oxygen depletion. The Oxymeter reading showed a consistent and adequate supply of dissolved oxygen at all times.

At this point it seemed possible that one concerted experiment could be performed in which both analytical procedures and bioassay techniques could be compared and evaluated simultaneously. Stock 5% (0.749 M) solutions of acrolein were prepared in buffered solutions at pH 5, 7, and 9 which were stored at 22 ± 2 °C in the dark. This initial concentration of acrolein was selected in order to facilitate the measurement of acrolein by a variety of different analytical and bioassay methods involving the removal of aliquots from the same solution. The bioassay methods used included adenosine triphosphate (ATP) photometry (SAI Technology Company) and aerobic bacteria plate counts as well as sulfate reducing bacteria culture tubes (Littman, 1975). Some analytical methods involved a derivatization procedure such as the bromide–iodide–thiosulfate titration method (Hall and Stern, 1956; Pressman and Lucas, 1942), the 2,4-dinitrophenylhydrazine (DNPH) colorimetric method (Bowmer et al., 1974; Yamate and Matsurmura, 1975), and the aminophenol fluorescence method (Alarcon, 1968). The other analytical procedures involved the direct measurement of acrolein by UV (Buswell et al., 1940; Yamate and Matsurmura, 1974), GLC (Smith, 1977), NMR, differential pulse polarography (Howe, 1976; Yamate and Matsurmura, 1974), and direct fluorescence.

Various buffered systems were examined. Since different buffer systems are usually commercially available for each desired pH range, the possibility that the presence of different chemical species might produce different results was examined.

Before discussing the results obtained from the bioassay procedures in this experiment, a simplified method for comparing the biological toxicity of each buffered system during the aging process is desirable. This concept utilizes the comparison of the results from the bioassays of fresh and aged solutions on a normalized basis, hereafter referred to as the normalized percent kill. This normalized percent kill is based on the ratio of the killing efficiency of aged acrolein samples to the killing efficiency of freshly prepared solutions, as described by the following equation:

$$\text{normalized \% kill} = \frac{\text{\% kill (aged)} - \text{\% kill blank}}{\text{\% kill (fresh)} - \text{\% kill blank}}(100\%) \quad (1)$$

Numerous living organisms respond to the presence of acrolein (Magna Corporation, 1970; Smith, 1977). As long as the concentration of the acrolein is above a certain value for a particular organism, the organism normally will die. A zero kill should suggest that the acrolein concentration has fallen below this particular fatal limit. Because of this, the normalized percent kill should reflect the amount of acrolein still present in the system, and the bioassay data should serve as a basis for comparing results obtained from the analytical methods.

The ATP bioassay data in Table III suggest that the toxicity levels of the various 5% acrolein-in-various-buffer solutions do eventually reach zero. Several interesting

Table III. Normalized Percent Kill for Various Acrolein-Containing Systems vs. Time

system	method	pH	time, h 0	3	24	48	120	144	240
potassium hydrogen phthalate	ATP[a]	5	100		52	40	11	0	0
potassium dihydrogen phosphate	ATP[a]	7	100		77	61	24	11	0
potassium dihydrogen phosphate with dye	ATP[a]	7	100		69	46	0	0	0
tris(hydroxymethyl)aminomethane	ATP[a]	7	100		75	43	20	8	0
sodium borate	ATP[a]	9	43	26	0				
tris(hydroxymethyl)aminomethane	ATP[a]	9	49	24	0				
potassium hydrogen phthalate	ATP[b]	5	100		92	77	38	20	0
potassium dihydrogen phosphate	ATP[b]	7	100		80	80	28	12	0
potassium dihydrogen phosphate with dye	ATP[b]	7	100		75	71	14	0	0
tris(hydroxymethyl)aminomethane	ATP[b]	7	100		95	75	47	43	0
sodium borate	ATP[b]	9	51	33	0				
tris(hydroxymethyl)aminomethane	ATP[b]	9	50	32	0				
potassium hydrogen phthalate	plate[c]	5	100		84	60	11	2	0
potassium dihydrogen phosphate	plate[c]	7	100		69	30	0		
sodium borate	plate[c]	9	98	10	0				
potassium hydrogen phthalate	tube[d]	5	100		90	80	25	10	0
potassium dihydrogen phosphate	tube[d]	7	100		83	62	5	0	
sodium borate	tube[d]	9	100		25	0			

[a] Use concentration, 10 ppm. [b] Use concentration, 50 ppm. [c] Use concentration, 10 ppm, average number of aerobic colonies per sample 4.5×10^6. [d] Use concentration, 10 ppm. Average number of sulfate colonies per sample 4×10^5.

observations can also be made. For instance, acrolein remains toxic for a longer period of time at pH 5 then at pH 7 or 9. While no significant difference was noted between the two pH 9 cases, the three pH 7 systems displayed different rates in the loss of toxicity of acrolein, with the system containing the commerical yellow dye yielding the most rapid loss in toxicity. The loss of acrolein toxicity in the pH 9 cases was not instantaneous. Finally, the use concentration does seem to have a small, noticeable effect on the rate of loss of acrolein toxicity.

The ATP method has encountered some problems. Measurements by this method are affected by the biomasses present (Melnikov, 1976), suspended solids (Sutcliffe and Orr, 1976), and dissolved materials (Shoaf and Lium, 1976). In this and other work, ATP evaluation of systems treated with acrolein have produced an unexpected result. Bacteria systems treated with quaternary ammonium salts and chlorinated phenols have been found to give large kills approaching 100%. However, known lethal amounts of acrolein do not usually give kills over 75% according to ATP measurements (Magna Corporation, 1970). Since the ATP photometer relies on the amount of fluorescence in the sample, residual amounts of acrolein or by-products may be causing a background fluorescence, which is erroneously registered as a living biomass. This hypothesis was verified by subjecting aqueous acrolein and 5,6-dihydro-2*H*-pyran-3-carboxaldehyde (obtained by the method of Fisher) which did not contain bacteria to the ATP procedure. This did result in readings which could be interpreted as though living biomasses were present.

Plate counts and culture tubes were also subjected to the aging acrolein solutions in order to ascertain the validity of the ATP data. Both aerobic and sulfate-reducing bacteria were examined because of their importance in oilfield water floods and irrigation canals. These results are also shown in Table III. Again, in both cases, the toxicity of acrolein decreases to nontoxic levels and most slowly at pH 5. These rates also appear to be slightly dependent on the use concentration of acrolein relative to the number of bacteria colonies present and the type of colony present.

All of the bioassay data seem to suggest that aged acrolein solutions became biocidally inactive after a finite period of time. For pH 5, this period is about 150 h; for pH 7, the inactive level is reached between 120–180 h, depending on the buffer system; for pH 9, 5–40 h is sufficient to reach zero toxicity. This should reflect on acrolein level below a few parts per million (Magna Corporation, 1970). These rates of loss of biocidal activity should be nearly the same as the rates of decay of acrolein.

It should also be noted that under these buffered conditions, the resulting hydrolysis product mixtures are nontoxic to these bacteria media. The hydrolysis of acrolein has been examined previously (Gilbert and Donleavy, 1938; Hall and Stern, 1956; Pressman and Lucas, 1942; Smith, 1977; and Vik, 1973). While the hydrolysis products were not isolated or characterized, the earlier literature reports that 3-hydroxypropanal is the expected product, while other materials such as 5,6-dihydro-2*H*-pyran-3-carboxaldehyde have also been isolated (Smith, 1977). Bacteria cultures were treated with 50 ppm of 5,6-dihydro-2*H*-pyran-3-carboxaldehyde, and no appreciable effect was observed in their populations by ATP analysis.

Table IV contains data obtained by analytical methods using the derivative concept. These methods rely on the conversion of acrolein into a derivative, followed by analysis for the derivative. These methods include the bromide–iodide–thiosulfate tritration (Hall and Stern, 1956; Pressman and Lucas, 1942), the DNPH (Bowmer et al., 1974; Yamate and Matsurmura, 1975), and the *m*-aminophenol fluorescence (Alarcon, 1968) methods. It is important to note that in direct contrast to the bioassay data none of the buffered systems gave a negative response for acrolein even after a period of 1 month, regardless of the derivatization method used. Examination of the data for any of the pH 7 systems containing tris(hydroxymethyl)aminomethane (Tris) in Table IV relative to the other pH 7 systems shows that the Tris system consistently gave the highest values for acrolein and, consequently, the slowest rates of decay. Also, the data for the pH 7 phosphate systems are nearly identical with the data for the pH 7 phosphate systems containing the yellow dye, which is quite different from the results obtained in the bioassay work. It is also surprising that the DNPH data in Table IV are very similar to that of Bowmer (1976), even though Bowmer used a different DNPH analysis procedure and much lower initial acrolein concentrations.

Each of the derivatization methods gave different results than the bioassay procedures, and they disagreed with each other. The worst agreement appears when the amino-

Table IV. Percentage of Acrolein Remaining in Various Buffer Systems vs. Time, as Measured by Derivatization Methods

system	method	pH	time, h 2	7	24	48	96	144	240	30 days
potassium hydrogen phthalate	titrimetric[a]	5			78	62	40	27	18	3
potassium dihydrogen phosphate	titrimetric[a]	7			66	43	27	20	18	3
tris(hydroxymethyl)aminomethane	titrimetric[a]	7			88	76	68	59	52	8
sodium borate	titrimetric[a]	9	72	52	34	29	28	26	24	10
tris(hydroxymethyl)aminomethane	titrimetric[a]	9	93	74	43	30	28	27	25	12
potassium hydrogen phthalate	colorimetric[b]	5	96		95	90	88	85	78	75
potassium dihydrogen phosphate	colorimetric[b]	7	72		58	55	42	38	29	28
potassium dihydrogen phosphate with dye	colorimetric[b]	7	70		63	64	47	46	30	31
tris(hydroxymethyl)aminomethane	colorimetric[b]	7	78		72	73	72	61	50	36
sodium borate	colorimetric[b]	9	43	24	22	20				16
tris(hydroxymethyl)aminomethane	colorimetric[b]	9	56	35	35	27				18
potassium hydrogen phthalate	fluorometric[c]	5	99		99	99	98	95	90	78
potassium dihydrogen phosphate	fluorometric[c]	7	96		94	92	87	72	64	50
potassium dihydrogen phosphate with dye	fluorometric[c]	7	95		93	92	84	77	63	48
tris(hydroxymethyl)aminomethane	fluorometric[c]	7	97		95	96	95	91	84	68
sodium borate	fluorometric[c]	9	70	63	33	30				7
tris(hydroxymethyl)aminomethane	fluorometric[c]	9	92	85	78	72				10

[a] Iodide-bromide-thiosulfate reagents. [b] DNPH method. [c] *m*-Aminophenol method.

Table V. Percentage of Acrolein Remaining in Various Buffer Systems vs. Time, as Measured by Direct Methods

system	method	pH	time, h 2	24	48	144	30 days
potassium hydrogen phthalate	GLC	5	99	90	88	85	76
potassium dihydrogen phosphate	GLC	7	95	91	87	73	48
potassium dihydrogen phosphate with dye	GLC	7	93	87	83	62	44
tris(hydroxymethyl)aminomethane	GLC	7	99	90	87	79	63
sodium borate	GLC	9	72	28	18		9
tris(hydroxymethyl)aminomethane	GLC	9	90	60	43		10
potassium hydrogen phthalate	NMR	5		72	55	17	8
potassium dihydrogen phosphate	NMR	7		55	37	8	6
potassium dihydrogen phosphate with dye	NMR	7		55	39	8	8
tris(hydroxymethyl)aminomethane	NMR	7		78	60	22	8
sodium borate	NMR	9	60	21	15		12
tris(hydroxymethyl)aminomethane	NMR	9	88	38	22		14
potassium hydrogen phthalate	polarograph[a]	5	98	83	68	20	6
potassium dihydrogen phosphate	polarograph[a]	7	97	67	42	7	1
potassium dihydrogen phosphate with dye	polarograph[a]	7	97	68	43	7	1
tris(hydroxymethyl)aminomethane	polarograph[a]	7	98	84	80	47	5
sodium borate	polarograph[a]	9	62	9	4	4	1
tris(hydroxymethyl)aminomethane	polarograph[a]	9	78	33	11	3	1
potassium hydrogen phthalate	fluorometric[b]	5	97	96	98	94	90
potassium dihydrogen phosphate	fluorometric[b]	7	52	27	1	<0.1	
potassium dihydrogen phosphate with dye	fluorometric[b]	7	52	25	2	<0.1	
tris(hydroxymethyl)aminomethane	fluorometric[b]	7	62	64	41	40	26
sodium borate	fluorometric[b]	9	<0.1	<0.1			
tris(hydroxymethyl)aminomethane	fluorometric[b]	9	4	<0.1	<0.1		

[a] Differential pulse mode. [b] Direct method.

phenol fluorescence data in Table IV are compared with the bioassay data. A possible rationalization for these differences could be that the hydrolysis products interfere with the analyses by derivatization methods. This hypothesis was confirmed when 5,6-dihydro-2*H*-pyran-3-carboxaldehyde gave positive responses when subjected to each of these derivatization methods. These responses behaved as though lesser amounts of acrolein were being analyzed instead of the dihydropyran and therefore demonstrate that none of these methods are specific for acrolein.

The other analytical methods examined were those that actually attempt to measure acrolein, without conversion to derivatives. These direct analytical procedures include UV (Buswell and Dunlop, 1940; Vik, 1973), GLC, NMR, differential pulse polarography (Howe, 1976; Yamate and Matsurmura, 1975), and direct fluorescence. The data obtained from these direct analytical methods are contained in Table V.

Of the five direct measurement techniques investigated, the UV method did not provide useful data. When the 5% stock solutions were analyzed by UV, extraneous peaks appeared during the experiments. These peaks were often more intense than the acrolein peaks and tended to mask the acrolein because the wavelength differences were small. For example, 5,6-dihydro-2*H*-pyran-3-carboxaldehyde produced just such a peak. Reproducible UV data were observed when some solutions similar to these used by Buswell et al. (1940) and Vik (1973) were prepared and analyzed.

While the GC method provided data similar to that collected by derivatization methods, the data obtained by NMR, differential pulse polarograph, and direct fluorescence were very encouraging (Table V). These three techniques provided data that closely matched those obtained by the bioassay techniques. Even though nonzero values for the aged acrolein concentration were recorded, except in certain cases by direct fluorescence, the rates of the dissipation of acrolein for 1 to 4 half-lives were similar within experimental error to the loss in toxicity as recorded by the bioassay methods. As noted with the derivatization methods, the presence of yellow dye in the commercial

buffer solution did not adversely affect the data. Furthermore, the pH 5 phthalate system did not demonstrate a loss of acrolein when measured by the direct fluorescence method (see Table V). All three methods did not provide data from the Tris-containing systems which correlated well with the bioassay work.

In order to compare the results obtained from these above experiments, representative data contained in each of the tables concerning the pH 7 phosphate buffered system can be used. Different values for acrolein were obtained when different analytical methods were used. In this phosphate case, the data obtained by either NMR or differential pulse polarography correlate better with the aerobic plate count bioassay data than do the results of the other analytical methods.

CONCLUSIONS

Acrolein in some aging aqueous systems can be monitored for 1 to 4 half-lives by nuclear magnetic reasonance, direct fluorescence, and differential pulse polarography. Acrolein concentrations of about 7×10^{-2} M (0.5%) can be measured by NMR, while concentrations approaching 3×10^{-4} M (20 ppm) can be measured with direct fluorescence and 3×10^{-7} M (30 ppb) with differential pulse polarography (Brady et al., 1977). The results obtained by using these three analytical methods can parallel the biological toxicity of acrolein in these same systems. At this point, however, it does not appear possible that zero values for most aged acrolein residuals are possible when determined by analytical methods, assuming that biological assays provide a more realistic measure.

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