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THE ROLE OF MUTAGENICITY IN DETERMINING DRINKING WATER QUALITY

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INTRODUCTION

Many drinking water utilities are considering alternatives to the exclusive use of chlorine for disinfection in order to comply with federal regulations regarding acceptable levels of disinfection by-products (DBP). Current and proposed regulations limit DBP on an individual basis or as a group of related compounds (e.g., trihalomethanes) based on an evaluation of the human health risk and what is technically and economically feasible. Consequently, an evaluation is needed of the risks associated with the use of alternative disinfectants relative to the risks associated with the use of chlorine.

Human epidemiological studies would provide the most relevant information on the risks associated with the use of various drinking water disinfectants. Epidemiological studies have suggested increased risks of cancer in areas with chlorinated waters, but the evidence is inconclusive.[1] Even less epidemiological information is available concerning the potential adverse effects of other disinfectants currently in use or for proposed treatment options.

Another source of information on potential risk would be data from animal studies. Unfortunately, such studies are both time consuming and costly. In addition, due to the variable nature of source waters, multiple studies would likely be needed to evaluate each treatment process. Due to these and other limitations, the routine use of animal studies to evaluate treatment options is impractical.[2]

Short-term in vitro tests for the detection of genotoxic chemicals can be conducted relatively quickly and inexpensively. Consequently, their use in the evaluation of various disinfection processes has been recommended.[2,3,4] Many of these assays are designed to detect mutagens[5], which are substances that cause a permanent change in the genetic material. Such changes in the reproductive cells could be passed on to offspring and potentially

lead to heritable diseases. In non-reproductive cells such damage is thought to be involved in or at least correlated with the processes that lead to cancer.[2] Thus, it seems prudent to try to minimize human exposure to mutagenic compounds.

The Ames Salmonella assay is one of the most commonly used tests for mutagenicity. The advantages of this assay are that it is relatively easy to perform, low in cost, has been well validated, and has an extensive literature base due to its widespread use.[6] Additionally, the underlying genetics of the assay have been well defined.[7] The accuracy of the Ames test for predicting the carcinogenicity of chemicals in rodents has been found to be comparable to that of three other commonly used genotoxicity assays that use mammalian cells.[8]

Over 1000 organic compounds have been identified in drinking water samples and many more have been detected but not identified.[9] However, most of these compounds are present at µg/L levels or less. At these low concentrations most known mutagens would not be detected in the Ames assay.[10] Consequently, it is usually necessary to use some method of concentrating the organic compounds present in drinking water prior to testing for mutagenicity. One of the most popular concentration methods involves the use of Amberlite XAD resins. The major advantage of XAD resins is that they can be used to concentrate the large volumes of water needed for mutagenicity testing in relatively short periods of time.[11]

In the two pilot-scale drinking water plant studies presented here, source waters were treated with a variety of disinfection schemes, incorporating ozone, monochloramine, chlorine dioxide and chlorine. Concentrates of the organic compounds present in the water samples were prepared by XAD resin adsorption/ethyl acetate elution. The concentrates were then tested for mutagenicity in the Ames Salmonella assay in order to compare the relative mutagenic potencies of the water samples following the different methods of disinfection.

METHODS

Sample concentration for mutagenicity testing

The organic compounds present in the water samples were concentrated by adsorption on Amberlite XAD resins (Figure 1). The resins were cleaned by consecutive 24 hour Soxhlet extractions with methanol, ethyl acetate and methanol and stored in methanol. Prior to use, the methanol was replaced by distilled water. The columns contained XAD-8 resin over XAD-2 resin. Immediately prior to passage of the water samples over the columns, the samples were acidified to pH 2 by in-line addition of HCl using a metering pump and a static head mixer. Previous work showed the recovery of mutagenic activity to be much greater from water samples acidified to pH 2 prior to passage over XAD columns than from water samples concentrated at pH 8.[12] The columns were eluted with ethyl acetate. Residual water was removed from the ethyl acetate eluates

by using separatory funnels to drain off the water layers followed by the addition of sodium sulfate. The eluates were then concentrated by rotary vacuum evaporation and dissolved in dimethyl sulfoxide (DMSO) to give 8000-fold concentrates.

Assay for mutagenic activity

Mutagenic activity was determined in Salmonella typhimurium using the standard plate method of Maron and Ames.[7] Strain-specific genetic markers were verified for each strain prior to use. Spontaneous and positive control responses and appropriate solvent controls were included with each assay. In assays employing metabolic activation, the methods for preparation of the liver homogenate (S9) from Aroclor 1254-pretreated male, Sprague-Dawley rats and the S9 cofactor mix were as described in Maron and Ames.[7] The S-9 concentration in the S-9 mix was 5% (v/v), and 0.5 ml of S-9 mix was added per plate. The samples were assayed at doses equivalent to 0.05L to 1.6L per plate, using duplicate or triplicate plates per dose. Mutagenic activity was calculated from the initial slopes of the dose-response curves using the method of Bernstein, et al.[13]

Chemical Analyses

Total organic carbon (TOC) concentrations were determined using the persufate-ultraviolet oxidation method and the adsorption-pyrolysis-titrimetric method was used for total organic halide (TOX) analyses.[14]

JEFFERSON PARISH, LOUISIANA, PILOT PLANT STUDY

Ozone (O_3) and monochloramine (NH_2Cl) are among the primary alternatives to chlorine (Cl_2) disinfection being considered for widespread use in the drinking water industry. Although O_3 is an effective disinfectant, its short half-life in water at pH 8,[15] necessitates the use of a secondary disinfectant to ensure a disinfectant residual throughout the distribution system. In the present study, water samples were disinfected with Cl_2 or NH_2Cl alone or following ozonation. These samples were evaluated in the Ames assay in order to compare the relative levels of mutagenic activity present in drinking waters prepared by these different methods of disinfection.

Treatment Process

At a pilot-scale drinking water treatment plant in Jefferson Parish, LA, three studies were conducted in which clarified and sand filtered Mississippi River water was treated with either Cl_2, NH_2Cl, O_3 or was not disinfected (Figure 2). Each treatment stream consisted of a contact chamber followed in series by a sand column and a 55-gallon, stainless-steel drum fitted with a spiral, stainless-steel baffle. The modified drum served as an additional contact chamber. The non-disinfected treatment stream was similar except that the initial contact chamber was omitted. The contact

time in the contact chamber was approximately 30 min. for each of the disinfected streams. The stream treated with O_3 was split after the sand column and post-disinfected with either Cl_2 or NH_2Cl. As a result of stream splitting the flow rate to the two post-disinfected drums was decreased. Therefore, the contact time for the post-disinfected drums was approximately 150-180 min. while the contact time in the drums for the streams treated initially with Cl_2 or NH_2Cl and the non-disinfected stream was approximately 85-100 min. Sufficient Cl_2 or NH_2Cl was added during post-disinfection so that the residual levels of disinfectant in the ozonated samples were approximately equal to those of the water samples disinfected initially with Cl_2 or NH_2Cl. The final residual levels of Cl_2 and NH_2Cl were 0.5 - 1.0 mg/L and 0.8 - 1.5 mg/L respectively.

Sample Collection and Analyses

Samples were collected from each of the five treatment streams in September, 1989, March, 1990 and July, 1990. The water samples were concentrated by adsorption on XAD resins for mutagenicity testing as described in the methods. Columns contained 5L of XAD-8 resin followed by columns containing 5L of XAD-2 resin. Water (1500L) from each treatment stream was passed through the two columns in series at a flow rate of 60L/hr. Following sample collection, each column was filled with sufficient ethyl acetate to provide a standing head. The columns were then agitated to completely wet the resin and allowed to equilibrate for 15 minutes. Each column pair was then eluted serially with 15L of ethyl acetate. The final concentrates were assayed for mutagenicity as described in the methods using Salmonella strains TA100, TA98, TA97 and TA102, with and without metabolic activation.

Results and Discussion

In the Ames assay, genetic damage is indicated by the induction of mutations that cause the histidine-requiring Salmonella tester strains to become histidine independent. Mutation to histidine independence is demonstrated by the growth of bacterial colonies on minimal agar plates. These bacterial colonies are referred to as revertants. A mutagenic response is indicated by a dose-related increase in the number of revertant colonies.

Figure 3 shows the dose-response curves, in strain TA100 (-S9) for each of the five water samples collected in September. It can be seen from the figure that mutagenic activity was detected in all of the water samples, including a very low level in the non-disinfected water. Table 1 shows the mutagenic activities, expressed as revertants per liter equivalent (i.e. the slope of the dose-response curve), for the water samples collected in September under each of the assay conditions used.

In this study, the addition of a metabolic activating system (+S9) resulted in decreased levels of mutagenic activity in all of the tester strains used. Thus the mutagens in the disinfected water samples appear to be direct-acting (do not require metabolic activation). Decreased levels of mutagenic activity in the

presence of S9 have been previously reported for disinfected water samples[16, 17, 18] and for chlorinated aqueous humic acid solutions.[19]

The individual tester strains, TA100, TA98, TA97 and TA102, detect different classes of compounds based on the mechanisms by which they cause mutations. For all of the samples, the highest level of activity was observed in TA100, indicating that many of the compounds present cause mutations by substituting one DNA base for another.

Figure 4 shows the mutagenicity of the water samples in strain TA100 (-S9) for all three collection times. It is obvious from the figure that the levels of mutagenicity observed for a given treatment varied significantly depending on the time of collection. Seasonal variations in the levels of mutagenic activity in drinking water have often been observed, and are addressed in a review by Noot, et al.[4]

In the present study, the effect of collection time is not consistent for all of the disinfectants used. The samples treated with O_3 + Cl_2 or Cl_2 showed higher levels of mutagenic activity in March and July than in September. Samples disinfected with O_3 + NH_2Cl or NH_2Cl did not show this pattern. In an earlier study, also done at Jefferson Parish, water treated with Cl_2 in July had a lower level of activity compared to samples treated in June or December of the same year. The levels of activity observed for water treated with NH_2Cl were essentially the same for all 3 collection times.[18] Reasons for these inconsistencies are not clear.

In spite of variability between sampling times, the levels of mutagenicity observed following the various disinfection treatments show similar trends within each of the 3 collection times. The levels of mutagenic activity of water samples disinfected with Cl_2 were at least twice that of water treated with NH_2Cl for each of the three collection periods (Figure 4). These results are consistent with previous reports by Cheh et al.[16] and Miller et al.[18] which showed that chlorination produced more mutagenic activity than chloramination.

Figure 4 also shows that, for each sampling time, disinfection with O_3 prior to treatment with either Cl_2 or NH_2Cl resulted in a lower level of mutagenic activity than when either disinfectant was used alone. Kriuthof et al.[20] reported similar results when they treated Rhine River water with O_3 followed by Cl_2 or Cl_2 alone.

EVANSVILLE, INDIANA, PILOT PLANT STUDY

The usefulness of chlorine dioxide (ClO_2) as a pre-disinfectant to control the level of trihalomethanes present in drinking water has been previously demonstrated at the Evansville pilot plant.[21] However, due to concern over the potential toxicity of ClO_2 and its inorganic by-products, chlorite and chlorate,[22] methods for their reduction have been investigated.[23]

Recent studies have shown that a combination of electrochemical generation of ClO_2 and subsequent application of the reducing agent, ferrous chloride ($FeCl_2$) can control the residual levels of ClO_2, chlorite and chlorate.[24] What impact, if any, the incorporation of these treatment processes would have on the level of mutagenic activity present in the finished drinking water was evaluated.

Treatment Process

At a pilot-scale drinking water treatment plant in Evansville, Indiana, three studies were conducted in which raw water from the Ohio River was either treated with liquid ClO_2, gaseous ClO_2, Cl_2 or was not disinfected (Figures 5-7). ClO_2 was produced using an electrochemical generator (Olin Corporation) and chlorination was achieved by addition of a hypochlorous acid solution. Those streams treated with ClO_2 were then clarified by the addition of alum for coagulation followed by settling. In the April study (Figure 5), both streams pre-disinfected with ClO_2 were subsequently treated with the reducing agent, $FeCl_2$, to control chlorite and ClO_2 concentrations. Sufficient Cl_2 was then added to achieve a free Cl_2 residual of approximately 2-3 mg/L. Secondary disinfection was followed by dual media (anthracite and sand) filtration. In the June study (Figure 6), the reducing agent was omitted from the liquid ClO_2 stream. In the August study (Figure 7), NH_2Cl was substituted for Cl_2 as the secondary disinfectant in the stream treated with gaseous ClO_2. NH_2Cl was produced by the addition of Cl_2 followed by ammonia (NH_3) in the treatment process.

Sample Collection and Analyses

Water samples for each study were collected at the end of each treatment stream (#1, 3, 5 and 6) as well as prior to the use of a secondary disinfectant (#2 and 4) in order to evaluate the effects of ClO_2 alone on mutagenicity (Figures 5-7). Total organic carbon (TOC) and total organic halide (TOX) concentrations were determined for each sample. The water samples were concentrated by adsorption on XAD resins for mutagenicity testing as described in the methods. The columns contained 65 ml of each resin, XAD-8 over XAD-2. The flow rate was 200 ml/min. Water samples of 125L were concentrated, except in the August study, when only 50L of each water sample were concentrated. Each column was eluted with 3 bed volumes of ethyl acetate. The 8000-fold concentrates were assayed for mutagenicity as previously described using Salmonella strains TA100, TA98 and TA102, without metabolic activation.

Results and Discussion

Mutagenic activity was detected in all of the water samples, including a low level in the non-disinfected samples. The highest level of activity was observed in strain TA100 for all of the samples in each of the three studies. (Figure 8 and Table 2) These observations are consistent with the results from the Jefferson Parish pilot study previously discussed.

In the April study, the levels of mutagenic activity observed for samples treated with liquid or gaseous ClO_2 alone were essentially the same. The samples in which liquid or gaseous ClO_2 was followed by $FeCl_2$ reduction and chlorination also showed similar levels of mutagenic activity. These data indicate that the method of ClO_2 application did not affect the levels of mutagenicity observed.

In the June study, the reducing agent was omitted from the liquid ClO_2 treatment train, however, the two samples treated with ClO_2 followed by chlorination still had similar levels of mutagenic activity. This suggests that the use of $FeCl_2$ did not have a significant effect on the level of mutagenic activity observed.

In each of the three studies, water samples collected after treatment with ClO_2 only, prior to addition of the secondary disinfectant, showed lower levels of mutagenic activity than those samples collected following treatment with a secondary disinfectant, either Cl_2 or NH_2Cl. This indicates that the majority of the mutagenic activity was produced by the secondary disinfectant. This observation is more likely related to the individual disinfectants used rather than the point at which they were added in the treatment train. This is based on the results of a previous study in which river water was treated with either Cl_2, NH_2Cl or ClO_2.[18] In this study, the relative mutagenic potencies of the disinfected water samples were, in order of decreasing activity: $Cl_2 > NH_2Cl > ClO_2$.

In all three studies, the levels of mutagenic activity present in samples treated with liquid or gaseous ClO_2 followed by Cl_2 were essentially the same as samples treated with Cl_2 alone, taking into account the variability in the concentration and assay procedures. Consequently, the results indicate that the treatment processes used in this study, ClO_2 pre-disinfection followed by $FeCl_2$ reduction, had little effect on the levels of mutagenicity observed. The substitution of NH_2Cl for Cl_2 as the secondary disinfectant following gaseous ClO_2 in the August study did, however, appear beneficial. In strain TA100, the level of mutagenicity in the sample treated with NH_2Cl was reduced by more than 50% compared to the levels present in the August samples treated with ClO_2 and Cl_2 or Cl_2 alone. Similar results were observed in strains TA98 and TA102.

The concentrations of TOX present in the Evansville water samples showed a pattern similar to that of the mutagenicity data (Table 3). In each of the studies, those samples treated with ClO_2 alone had low levels of TOX, approximately equal to those of the non-disinfected samples. Samples treated with ClO_2 followed by Cl_2 had TOX concentrations similar to samples treated with Cl_2 alone. The substitution of NH_2Cl for Cl_2 as the secondary disinfectant in the August study resulted in a much lower level of TOX compared to samples in which Cl_2 was used. The concentrations of TOC, by contrast, were similar for all of the water samples collected within a given study. The data thus shows that the pattern observed for

TOX and mutagenicity is not just a function of the amount of organic matter present, but is related to the treatment processes used.

SUMMARY

The results of the Jefferson Parish study presented here showed that treatment with NH_2Cl resulted in a lower level of mutagenic activity than that produced by chlorination. Additionally, disinfection with O_3 prior to treatment with either Cl_2 or NH_2Cl resulted in a lower level of mutagenicity than when either disinfectant was used alone. In the Evansville study, pre-disinfection with ClO_2 followed by reduction with $FeCl_2$ appeared to have little effect on the levels of mutagenicity observed. Most of the mutagenic activity was apparently produced by the secondary disinfectant. As in the Jefferson Parish study, the use of Cl_2 as the secondary disinfectant produced a higher level of mutagenic activity than was produced by chloramination.

In the absence of sufficient human epidemiological and/or animal data, information from genotoxicity tests can assist in determining those drinking water treatment processes which pose the least concern for adverse human health effects. However, the minimization or elimination of mutagenicity is, of course, only one of numerous criteria to be considered in the overall evaluation of drinking water processes. In attempting to minimize the potential risks associated with the use of disinfectants and the subsequent formation of disinfection by-products, one must not lose sight of the necessity of maintaining drinking water that is microbiologically safe as well.

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Table 1

Mutagenicity of Water Samples Disinfected by Alternative Methods at Jefferson Parish Pilot Plant (Sept., 1989)

	Revertants per Liter Equivalent							
	TA100		TA98		TA97		TA102	
Treatment	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Non-Disinfected	138 ± 19	86 ± 10	29 ± 3	35 ± 6	120 ± 13	NS	NS	NS
Cl_2	4104 ± 170	2258 ± 111	623 ± 34	249 ± 19	2239 ± 96	1321 ± 85	2230 ± 201	NS
NH_2Cl	1384 ± 36	715 ± 49	248 ± 19	102 ± 7	1112 ± 24	501 ± 27	NS	NS
$O_3 + Cl_2$	2121 ± 67	849 ± 54	187 ± 14	88 ± 14	1281 ± 119	578 ± 33	939 ± 97	NS
$O_3 + NH_2Cl$	947 ± 34	317 ± 12	80 ± 6	43 ± 6	580 ± 23	232 ± 10	NS	NS

NS: Not significant
S9: Metabolic activation

Table 2

Direct Acting Mutagenicity of Evansville Water Samples Following Alternative Treatments

		Revertants per Liter Equivalent	
Treatment	April 1992	TA98	TA102
Non-disinfected		33 ± 7	NS
Liquid ClO_2		35 ± 4	NS
Liquid ClO_2 + $FeCl_2$ + Cl_2 + DM		163 ± 8	559 ± 54
Gaseous ClO_2		33 ± 4	68 ± 11
Gaseous ClO_2 + $FeCl_2$ + Cl_2 + DM		146 ± 8	478 ± 27
Cl_2		236 ± 8	660 ± 54
Treatment	June 1992		
Non-disinfected		37 ± 4	NS
Liquid ClO_2		87 ± 12	98 ± 12
Liquid ClO_2 + Cl_2 + DM		299 ± 13	1397 ± 220
Gaseous ClO_2		66 ± 8	125 ± 23
Gaseous ClO_2 + $FeCl_2$ + Cl_2 + DM		244 ± 13	1170 ± 113
Cl_2		441 ± 16	1750 ± 118
Treatment	August 1992		
Non-disinfected		94 ± 9	NS
Liquid ClO_2		106 ± 9	104 ± 12
Liquid ClO_2 + Cl_2 + DM		298 ± 13	1273 ± 138
Gaseous ClO_2		107 ± 10	137 ± 15
Gaseous ClO_2 + $FeCl_2$ + Cl_2 + NH_3 + DM		173 ± 7	322 ± 50
Cl_2		386 ± 14	1054 ± 119

NS: Not significant

DM: Dual media (anthracite and sand)

Table 3

Total Organic Carbon (TOC) and Total Organic Halide (TOX) Concentrations of Evansville Water Samples Following Alternative Treatments

Treatment	April 1992	TOC (mg/L)	TOX (mg/L)
Non-disinfected		1.5	0.02
Liquid ClO_2		1.6	0.05
Liquid ClO_2 + $FeCl_3$ + Cl_2 + DM		1.5	0.11
Gaseous ClO_2		1.5	0.05
Gaseous ClO_2 + $FeCl_3$ + Cl_2 + DM		1.3	0.10
Cl_2		1.5	0.11
Treatment	**June 1992**		
Non-disinfected		1.8	0.01
Liquid ClO_2		1.7	0.03
Liquid ClO_2 + Cl_2 + DM		1.7	0.20
Gaseous ClO_2		1.6	0.05
Gaseous ClO_2 + $FeCl_3$ + Cl_2 + DM		1.4	0.19
Cl_2		1.7	0.23
Treatment	**August 1992**		
Non-disinfected		2.0	0.05
Liquid ClO_2		2.0	0.03
Liquid ClO_2 + Cl_2 + DM		2.1	0.19
Gaseous ClO_2		2.0	0.05
Gaseous ClO_2 + $FeCl_3$ + Cl_2 + NH_3 + DM		1.9	0.05
Cl_2		2.0	0.19

DM: Dual media (anthracite and sand)

Figure 1. Scheme for the Concentration of Water Samples for Mutagenicity Testing

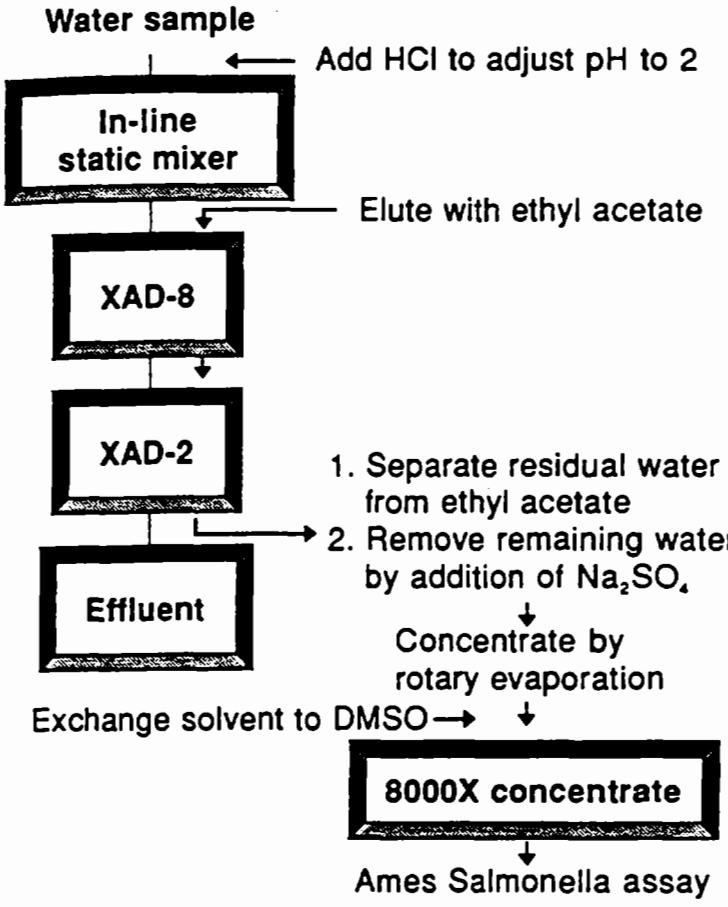


Figure 2. Flow Schematic of Jefferson Parish, LA Pilot Plant

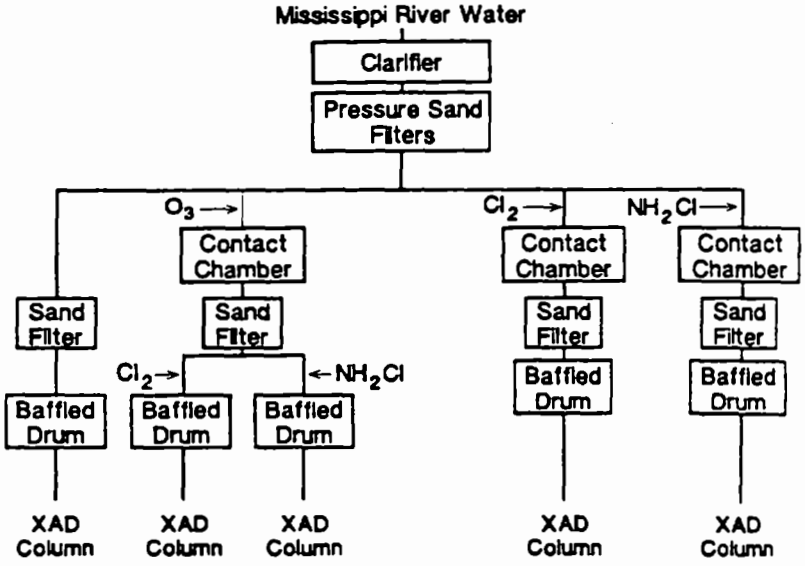


Figure 3. Direct Acting Mutagenicity in Strain TA100 of Water Samples Disinfected by Alternative Methods at Jefferson Parish Pilot Plant (Sept. 1989)

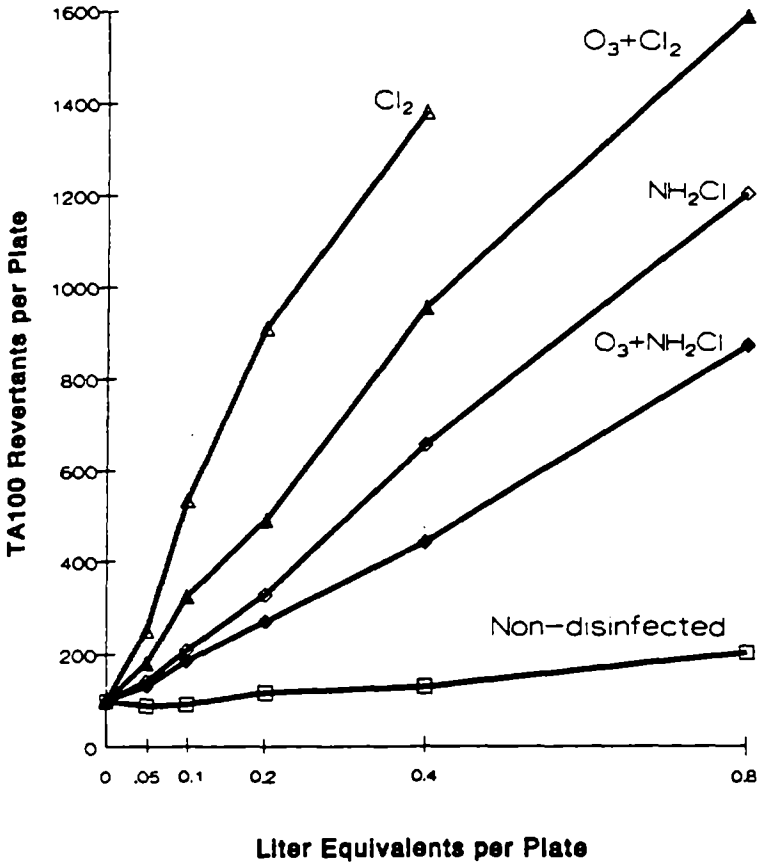


Figure 4. Direct Acting Mutagenicity in Strain TA100 of Water Samples Disinfected by Alternative Methods at Jefferson Parish Pilot Plant

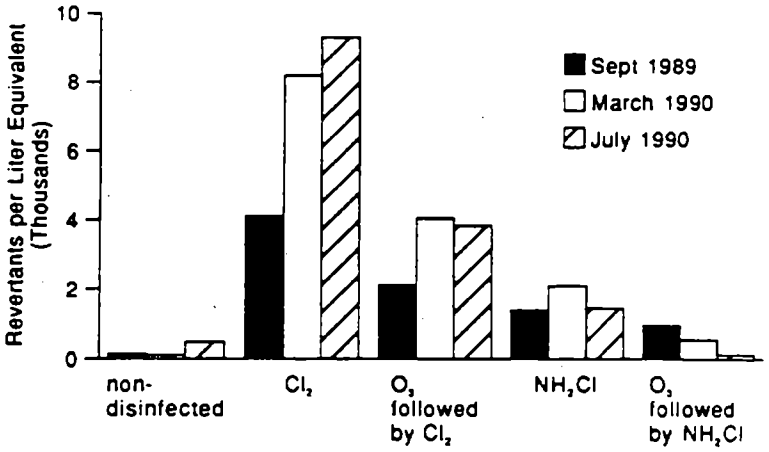


Figure 5. Flow Schematic of Evansville Pilot Plant
April 1992

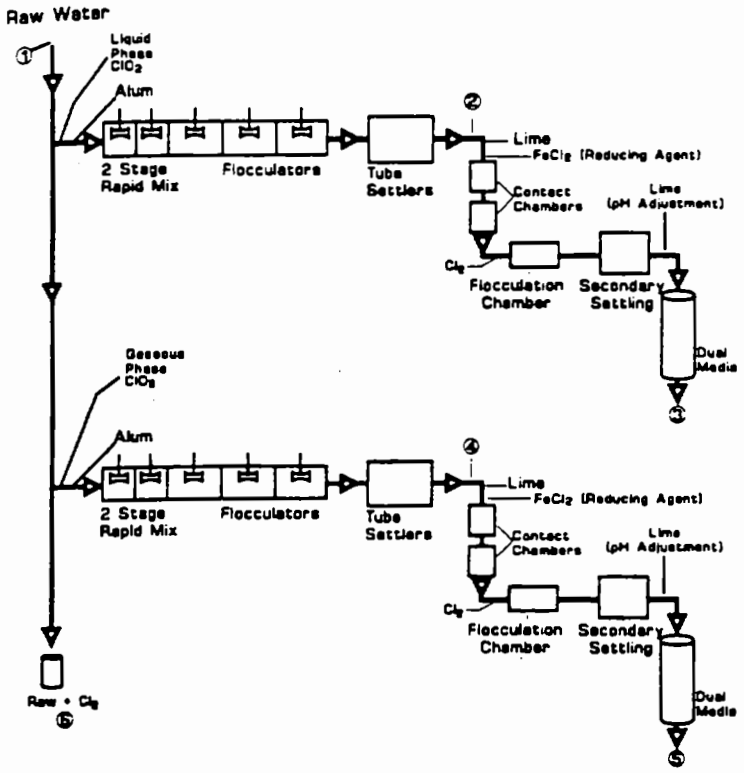


Figure 6. Flow Schematic of Evansville Pilot Plant
June 1992

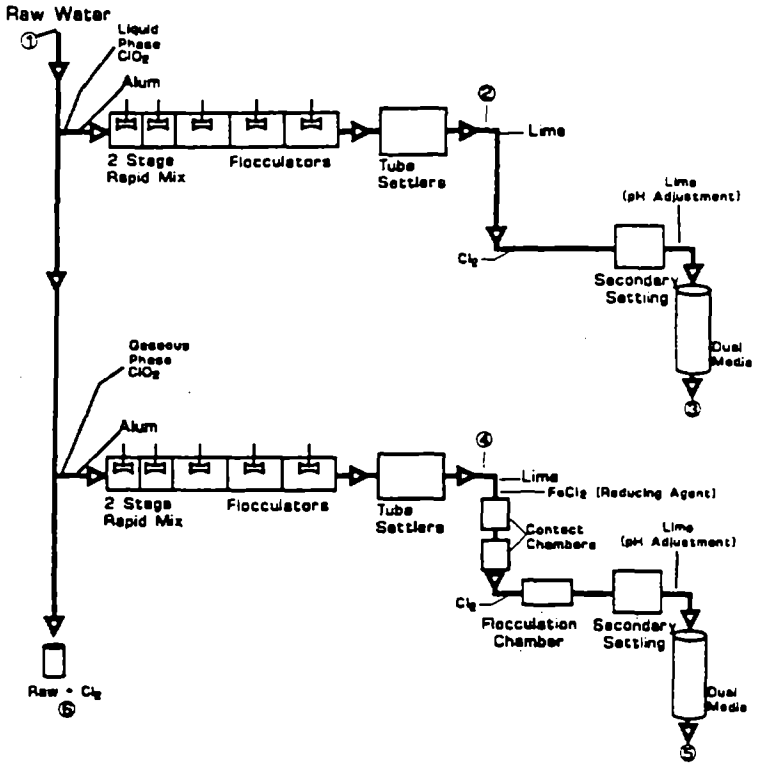


Figure 7. Flow Schematic of Evansville Pilot Plant
August 1992

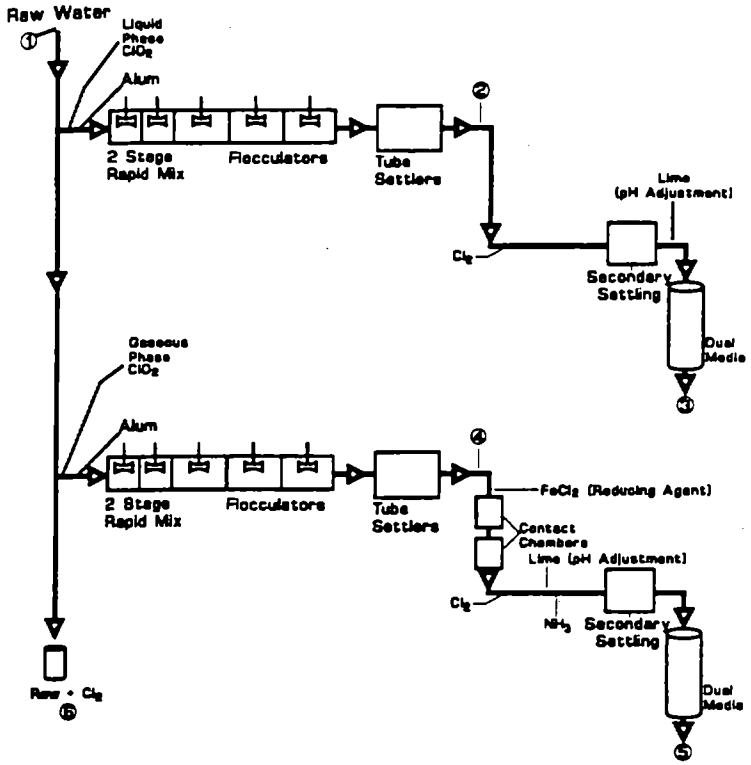
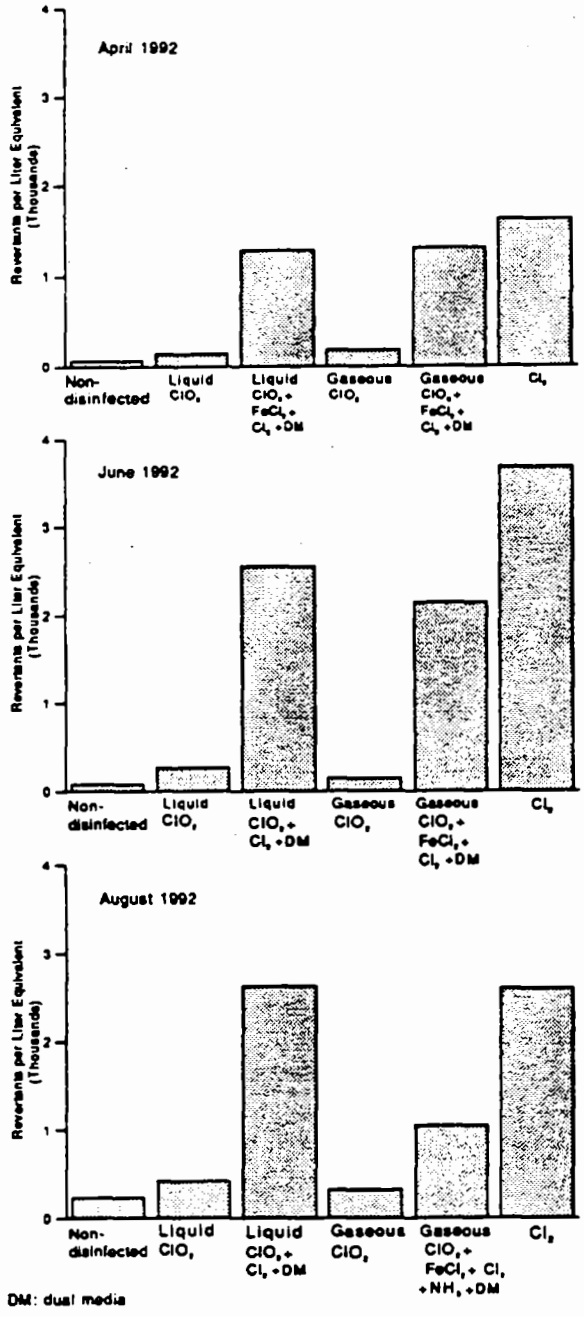


Figure 8. Direct Acting Mutagenicity in Strain TA100 of Evansville Water Samples Following Alternative Treatments



DM: dual media