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EVALUATION OF FOUR CONCENTRATION/EXTRACTION PROCEDURES ON WATERS AND EFFLUENTS COLLECTED FROM THE LOWER GREAT LAKES BASIN FOR USE WITH THE SALMONELLA TYPHIMURIUM SCREENING PROCEDURE FOR MUTAGENS

by

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ABSTRACT

Thirty-three water and effluent samples; fourteen lake, twelve river, and seven effluent, were concentrated/extracted by four procedures; liquid/liquid solvent extraction, XAD resins, flash evaporation, and membrane filtration. The resultant subsamples were tested for mutagenic activity using five strains of Salmonella typhimurium, TA98, TA1538, TA1537, TA100 and TA1535. Three different experimental procedures were followed; with the addition of mammalian microsomes (S-9 mix) to the reaction mixture, without the addition of microsomes, and with preincubation of bacterial cells, microsomes, and sample.

The data indicate that strains TA98, and TA 1538 with microsome addition, were the most sensitive for assessing mutagenic activity in the above samples. Suprisingly, a concentration procedure based on the membrane filter (dissolved in 5 mL DMSO) used to sterilize unconcentrated and flash evaporated samples produced the highest number of positive subsamples. The liquid/liquid extraction procedure produced the next highest number of positive subsamples.

INTRODUCTION

Due to the concern expressed by all levels of government about the presence of mutagenic/carcinogenic chemicals in natural waters and effluents, many laboratories, both governmental and private, have initiated studies on the incidence of mutagens/carcinogens in these waters. Although industrial discharges are primarily responsible for the variety of chemicals found in water, other sources, point and non point, contribute to the burden placed on receiving rivers and lakes. Land leachates containing thousands of chemical compounds and agricultural runoff with its load of organochlorine pesticides some of which are suspected carcinogens contribute to the organic mutagen/carcinogen load of our waters. Furthermore, the practice of wastewater chlorination can result in the formation of a variety of chlorinated compounds, many of which are mutagenic and carcinogenic (Wilkins et al., 1979).

Since these chemicals are usually found in the ppm or ppb range in waters, a variety of concentration/extraction procedures have been used to produce samples which could be tested by one or more of the biological mutagen screening procedures. When dealing with environmental samples the effectiveness of the mutagen screening procedure depends almost entirely on the selectivity and concentration ability of the extraction/concentration procedure used to prepare the sample for testing.

With the great influx of laboratories (over 1000) into mutagen screening activities (Maugh, 1978), and the great variety of extraction/concentration procedures available, there is now a problem of standardization and reproducibility of results.

Several workers, Tahagi et al. (1977) and Nagao et al. (1977), have found that some mutagens such as dimethylnitrosamine and the pyrrolizidine alkaloids cannot be properly detected in the standard Salmonella typhimurium mammalian microsome plate incorporation assay. They have found that by incubating the test chemical with the S-9 mix (microsomes) and bacterial culture for varying periods before adding the soft agar overlay, they could enhance the sensitivity of the Salmonella typhimurium mutagen screening test.

In this study, four concentration/extraction procedures; solvent extraction, passage through XAD resins, flash evaporation, and membrane filtration, were compared for their ability to concentrate and extract potential mutagens from river, lake and effluent samples. In conjunction with the above, three variations of the Salmonella typhimurium mammalian microsome test were assessed in relation to sample and concentration/extraction procedure. The variations assessed were: (a) with the addition of microsomes; (b) without the addition of microsomes; and (c) with the preincubation of Salmonella tester strains, microsomes, and sample for 20 minutes at 37°C prior to the addition of the soft top agar. Details of these studies are presented below.

METHODS

Sample Collection

A total of thirty-three water samples were collected for mutagen content assessment. The sample collection sites are shown in Figure 1 and are listed in Tables 1 and 2. All samples were collected in 20 L aliquots in acid cleaned amber bottles. After collection, the samples were stored at 2 to 4°C until concentration/extraction procedures were completed, usually within seven days of collection.

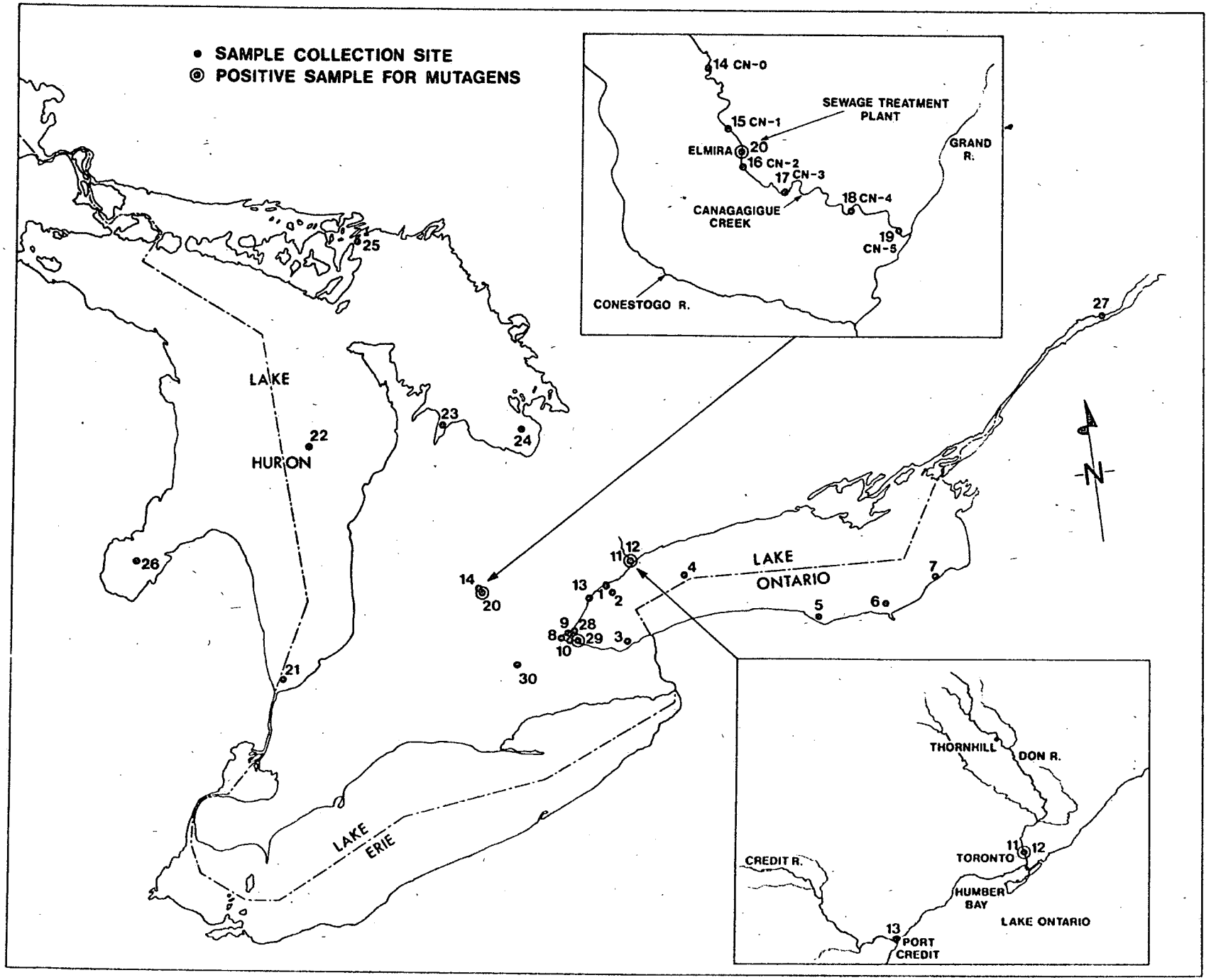
Sample Extraction/Collection

Solvent Extraction

Base/Neutral

Each 10 L sample was extracted in 2 L aliquots. The sample (2L) was poured into a separatory funnel and the pH adjusted to 11 or slightly higher with 6N NaOH. Each aliquot was extracted with 60 mL methylene chloride, three times. The aqueous phases were retained for acid extraction. The combined extracts were dried through a column of anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator at 40°C. The extract was dissolved in DMSO and made up to a final volume of 25 mL in DMSO.

Figure 1. Sites of Samples Collected for Mutagen Activity Tests



SITES OF SAMPLES COLLECTED FOR MUTAGEN TESTS

TABLE 1. OFF SHORE SAMPLES TESTED FOR MUTAGENIC ACTICITY

Sample No.	Date Collected	Sample Type	Location	Latitude	Longitude
1	Apr 1980	Surface Water	Lake Ontario	43°37'24"	79°27'12"
2	Apr 1980	Surface Water	Lake Ontario	43°35'12"	79°23'42"
3	Apr 1980	Surface Water	Lake Ontario	43°13'30"	79°16'18"
4	Apr 1980	Surface Water	Lake Ontario	43°35'48"	78°48'06"
5	Apr 1980	Surface Water	Lake Ontario	43°16'30"	77°35'30"
6	Apr 1980	Surface Water	Lake Ontario	43°28'36"	76°31'36"
7	Apr 1980	Surface Water	Lake Ontario	43°18'48"	77°00'00"
8	Nov 1980	Surface Water	Hamilton Harbour	43°16'50"	79°52'20"
21	Sept 1980	Surface Water	Lake Huron	43°05'06"	82°24'36"
22	Sept 1980	Surface Water	Lake Huron	44°44'24"	82°03'35"
23	Sept 1980	Surface Water	Georgian Bay	44°42'54"	80°51'42"
24	Sept 1980	Surface Water	Georgian Bay	44°38'38"	80°09'56"
25	Sept 1980	Surface Water	Georgian Bay	45°54'52"	81°35'48"
26	Sept 1980	Surface Water	Saginaw Bay	43°54'35"	83°31'40"

TABLE 2. RIVER AND EFFLUENT SAMPLES TESTED FOR MUTAGENIC ACTIVITY

Sample No.	Date Collected	Sample Type	Location
11	Jul 1980	River Water	Don River upstream from Domtar Paper
12	Jul 1980	River Water	Don River downstream from Domtar Paper
13	Jul 1980	River Water	Credit River above Lakeshore Road Bridge
14	Jul 1980	Creek Water	Canagagique Creek, Elmira
15	Jul 1980	Creek Water	Canagagique Creek, Elmira
16	Jul 1980	Creek Water	Canagagique Creek, Elmira
17a	Jul 1980	Creek Water	Canagagique Creek, Elmira
17b	Jun 1981	Creek Water	Canagagique Creek, Elmira
18	Jul 1980	Creek Water	Canagagique Creek, Elmira
19	Jul 1980	Creek Water	Canagagique Creek, Elmira
20a	Jul 1980	Final Effluent	Elmira Sewage Treatment Plant after chlorination
20b	Jan 1981	Final Effluent	Elmira Sewage Treatment Plant before chlorination
20c	Jan 1981	Final Effluent	Elmira Sewage Treatment Plant after chlorination
27a	Nov 1980	Final Effluent	Cornwall area industry
27b	Nov 1980	Final Effluent	Cornwall area industry
27c	Nov 1980	Final Effluent	Cornwall Sewage Treatment Plant after chlorination
28	Nov 1980	Final Effluent	Burlington Skyway Sewage Treatment Plant after chlorination
29	Dec 1980	Creek Water	Red Hill Creek at mouth
30	Dec 1980	River Water	Grand River at Brantford

Acid (Phenols)

The water from the base/neutral extraction was pH adjusted with H_2SO_4 to 2. Each 2 L aliquot was extracted with 60 mL methylene chloride, three times. The combined solvent phases were dried by passing them through a column of anhydrous sodium sulfate and then evaporated to dryness at 40°C, on a rotary evaporator. The extract was dissolved in DMSO, and made up to a final volume of 25 mL DMSO.

Flash Evaporation

A rotary evaporator with a 45°C waterbath was used to concentrate 600 mL of each water samples to 60 mL for 10X concentration mutagen testing.

Membrane Filtration

A fifty mL aliquot of sample was passed through a 0.20 micron Nalgene filter unit. The sterile filtrate was the 1X sample. The membrane filter was aseptically removed from the Nalgene holder and dissolved in 5 mL DMSO by, just bringing to a boil three times in a microwave oven. The resulting suspension was tested for sterility before being tested for mutagenic activity.

The other procedure followed was to pass the 60 mL of each 10X flash evaporated concentrate through a 0.20 micron Nalgene filter unit. The sterile filtrate was the 10X sample tested for mutagenic activity. The membrane filter was removed from the Nalgene holder, and treated as above. Negative controls for the membrane filter studies 0.2μ Nalgene membrane filters through which some distilled water had been passed. The membrane filters were removed and treated as above.

XAD-2 and XAD-7 Resins

Amberlite XAD-2 and XAD-7 are made of macroreticular resins in the form of hard insoluble beads. The XAD-2 resin is based on styrene-divinyl benzene copolymers and has an average area of 330 m_2/g and a nominal pure size of 90 angstroms. The XAD-7 resin is based on acrylate esters and has an average area of 750 m_2/g and pore size of 80 angtroms. Adsorption of organic molecules onto the surface of the resin beads is the basis for the separation of potential mutagens from water samples.

The procedure for XAD-2 and XAD-7 were the same except where noted. The resins were purified by Soxhlet extraction for eight hours with methanol, eight hours with acetonitrile, and eight hours with diethylether. After purification, the resins were stored under methanol.

The resin columns were prepared by packing each glass column with a plug of silanized glass wool followed by purified resin in methanol and topped by a silanized glass wool plug. The columns were washed with methanol followed by XAD-2 purified water to remove all traces of methanol.

Five litres of each sample were passed through an XAD-2 column and 5 L through an XAD-7 column. Prior to sample passage, the 5 L of water to be passed through the XAD-7 column was adjusted with H_2SO_4 to a pH of 1 to 2.

The water samples were passed through the resin columns at a flow rate of 40 to 50 mL/min. After the sample has passed through the column, a separatory funnel was placed under each column (XAD-2 and XAD-7). Ethyl ether was then passed through the columns until two phases were evident in the separatory funnel. The final washing of the XAD-2 resin was with benzene and the XAD-7 resin with petroleum ether. The aqueous layers were discarded and the extracts dried by passing them through anhydrous sodium sulfate.

The extracts were evaporated to dryness in a rotary evaporator, dissolved in a small volume of DMSO, and then made up to 25 mL in DMSO. The DMSO extracts were stored at 4°C in clean sealed glass vials until tested for mutagen content.

Mutagen Screening

The Salmonella typhimurium - microsome procedure for mutagen screening outlined in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments", 1978, was used in this study. Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, were used (a) with the addition of rat liver microsomes (S-9 mix), (b) without the addition of S-9 mix, and (c) with a 20 minute preincubation at 37°C of sample, cells, and S-9 mix before the addition of the molten soft tap agar. Extracts in DMSO were tested at a minimum of three doses 10, 20, and 200 µL with the 10 and 20 µL doses being made up to 200 µL with DMSO. For samples not in DMSO, volumes up to 1 mL were used. Positive and negative controls were done for each experiment. All tests were done in triplicate and all suspected positives were completely repeated for confirmation.

RESULTS AND DISCUSSION

Table 3 is a summary indicating which samples showed mutagenic activity. This table also indicates which concentration/extraction procedures produced the highest number of positive results, as well as which strains of Salmonella typhimurium reacted most frequently with the mutagens. In this study, a positive mutagenic response was one where the number of revertants was at least

double the negative and DMSO controls as well as being statistically significant at the 1.0 percent level,in at least two independent experiments, when possible.

The following nine samples showed mutagenic activity by at least one concentration/extraction procedure: No. 11, Don River, Toronto, upstream from Domtar Paper; No. 12, Don River, Toronto, downstream from Domtar Paper; No. 13, Credit River, immediately above th Lakeshore Road bridge in Port Credit; No. 17b, Canagagigue Creek, downstream from the Elmira Sewage Treatment Plant; No. 20a, Elmira Sewage Treatment Plant, final effluent after chlorination collected July 22, 1980, No. 20b, Elmira Sewage Treatment Plant, final effluent before chlorination; No. 20c, Elmira Sewage Treatment Plant, final effluent after chlorination, collected January 14, 1981; No. 29, Red Hill Creek mouth in Hamilton; and No. 30, Grand River in Brantford.

Table 4 summarizes all positive data for extracted/concentrated samples which had mutagenic activity towards at least one of the Salmonella typhimurium strain combinations used in this study. Typical dose response curves obtained from samples indicating positive mutagen activity are shown in Figures 2a, b, c, and d. In no instance did any filtrates, 1X or 10X, display mutagenic activity, however, with the exception of sample 29, all the samples showed mutagenic activity with either the 1X or 10X membrane filters

Figure 2. Examples of typical dose response curves (a) Sample 20(c) TA1538; (b) Sample 20(c) TA98; (c) Sample 12 TA98 and (d) Sample 20(a) TA1538.

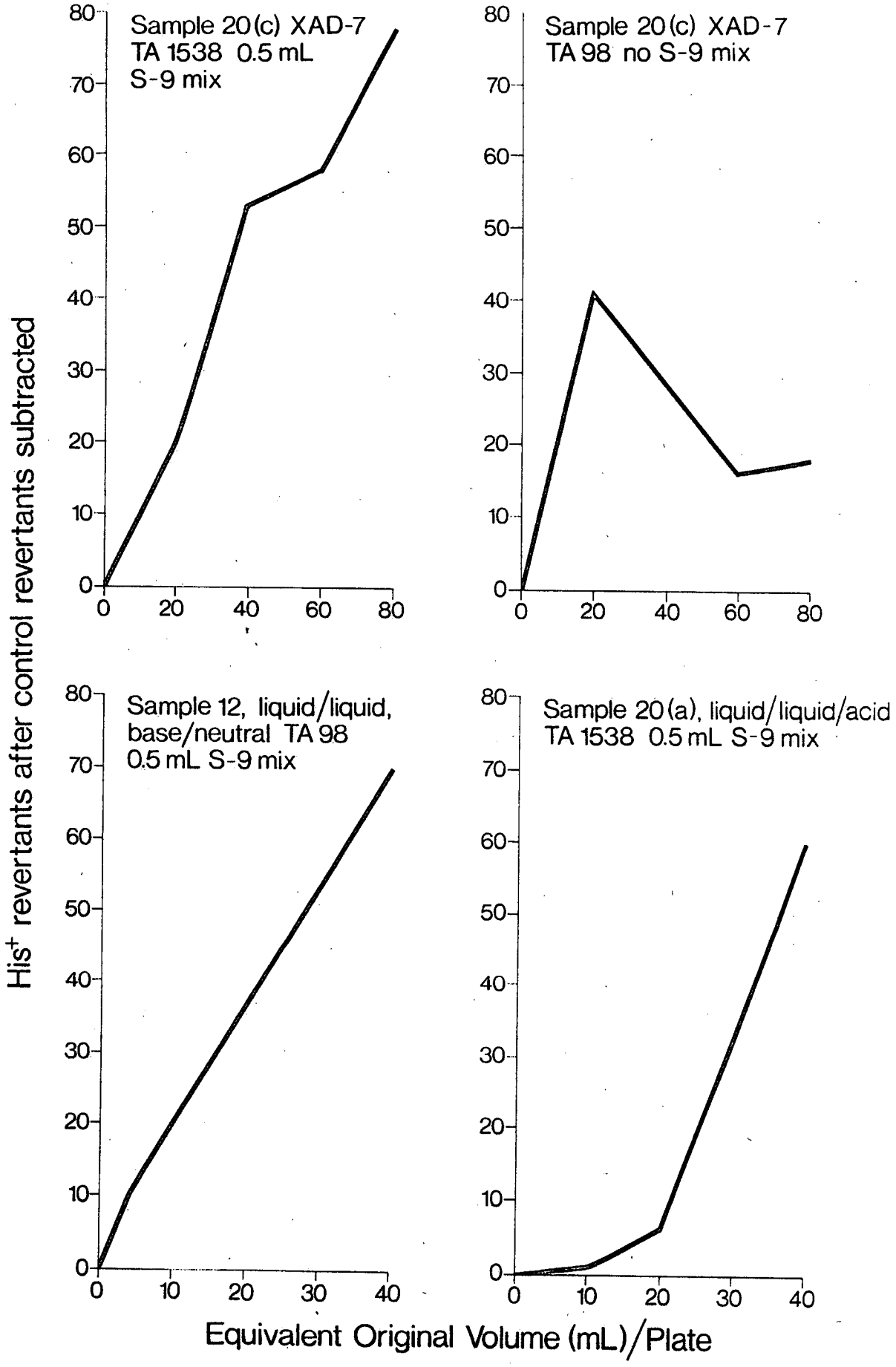

Sample 20(c) XAD-7
TA 1538 0.5 mL
S-9 mix
Sample 20(c) XAD-7
TA 98 no S-9 mix
Sample 12, liquid/liquid,
base/neutral TA 98
0.5 mL S-9 mix
Sample 20(a), liquid/liquid/acid
TA 1538 0.5 mL S-9 mix
His+ revertants after control revertants subtracted
Equivalent Original Volume (mL)/Plate

TABLE 3. SUMMARY OF SAMPLES SHOWING MUTAGENIC ACTIVITY BY ONE OR MORE CONCENTRATION/EXTRACTION PROCEDURES

Sample No.	Concentration Procedures						Salmonella Strains and Microsome Treatment											
	Solvent		XAD Resin		Membrane Filter		TA98			TA1537			TA1538			TA1535		
	Acid	Base	2	7	1X	10X	-S9	+S9	INC*	-S9	+S9	INC	-S9	+S9	INC	-S9	+S9	INC
11		+						+			+		+	+				
11					+											+		
11						+								+				
12		+						+			+			+				
12						+								+				
13					+												+	
13						+								+				
17b				+			+						+					
17b					+			+										
20a	+							+						+				
20a		+						+						+				
20a						+								+				
20b	+													+				
20b		+						+						+				
20b			+					+						+	+			
20b					+		+	+						+				
20c	+													+				
20c		+						+						+				
20c			+					+						+	+			
20c				+			+	+						+				
20c					+									+				
29		+						+					+	+				
29			+												+			
30					+									+				
TOTAL	3	6	3	2	6	4	3	12	0	0	2	0	3	19	3	1	1	0

* Salmonella cells + sample + S-9 mix incubated 20 minutes prior to addition of top agar.

TABLE 4. SUMMARY OF SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES AND WITH PREINCUBATION OF MICROSOMES, CELLS AND SAMPLE (INC)

Sample	Concentration Procedure	Equivalent Original Volume (mL)	Tester Strains with Mean of Triplicate Counts											
			TA98			TA1538			TA1537			TA1535		
			-S9	+S9	INC	-S9	+S9	INC	-S9	+S9	INC	-S9	+S9	INC
11	Solvent	80		103		133t	73		180t	58				
	Base	60		124			58			56				
		40		123		20	60		6t	37				
		20		93			35			44				
		8		68		17	34		13	39				
		4		56		16	30		13	32				
Control	Spontaneous			52		20	28		12	18				
Control	DMSO	0.2		49		19	30		14	24				
11	MF 1X	0.9										87		
Control	MF DMSO	0.1										39		
11	MF 10X	9.0					100							
Control	MF DSMO	0.1					25							
12	Solvent	80		120			67			51				
	Base	8		73			39			25				
		4		61			36			32				
Control	Spontaneous			52			28			18				
Control	DMSO	0.2		49			30			24				
12	MF 10X	0.9					98							
Control	MF DMSO	0.1					25							
13	MF 1X	0.9											65	
Control	MF DMSO	0.1											27	
13	MF 10X	0.9					103							
Control	MF DMSO	0.1					25							

t = sample toxic to cells.

TABLE 4 (CONT'D). SUMMARY OF SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES AND WITH PREINCUBATION OF MICROSOMES, CELLS AND SAMPLE (INC)

Sample	Concentration Procedure	Equivalent Original Volume (mL)	Tester Strains with Mean of Triplicate Counts					
			TA98			TA1538		
			-S9	+S9	INC	-S9	+S9	INC
17b	XAD-7	40	84			43		
Control	DMSO	0.2	36			17		
17b	MF 1X	0.9		112				
Control	MF DMSO	0.1		54				
20a	Solvent	80					100	
	Acid	40					36	
		20					31	
		8					37	
		4					36	
Control	Spontaneous						28	
Control	DMSO	0.2					30	
20a	Solvent	80		119				
	Base	40		102				
		20		84				
		8		51				
		4		38				
Control	Spontaneous			57				
Control	DMSO	0.2		52				
20a	MF 10X	9					88	
Control	MF DMSO	0.1					25	
20b	Solvent	80		324			380	
	Base	8		85			96	
		4		73			29	
Control	Spontaneous			58			28	
Control	DMSO	0.2		50			31	

Continued..../

TABLE 4 (CONT'D). SUMMARY OF SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES AND WITH PREINCUBATION OF MICROSOMES, CELLS AND SAMPLE (INC)

Sample	Concentration Procedure	Equivalent Original Volume (mL)	Tester Strains with Mean of Triplicate Counts					
			TA98			TA1538		
			-S9	+S9	INC	-S9	+S9	INC
20b	Solvent	80				77		
	Acid	8				40		
		4				40		
Control	Spontaneous					28		
Control	DMSO	0.2				31		
20b	MF 1X	0.9	79	111		73		
Control	MF DMSO	0.1	25	54		25		
20b	XAD-2	80	81t			71t		
		60	105			89		
		40	85			99	115	
		20	75			84		
Control	DMSO	0.2	40			27	33	
20c	XAD-7	80	144			141t		
		60	101			175		
		40	92			134	220	
		20	68			79		
Control	DMSO	0.2	40			27	33	
20c	Solvent	80	291			337		
	Base	8	94			72		
		4	60			59		
Control	Spontaneous		58			28		
Control	DMSO	0.2	50			31		

t = sample toxic to cells.

Continued...../

TABLE 4 (CONT'D). SUMMARY OF SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES AND WITH PREINCUBATION OF MICROSOMES, CELLS AND SAMPLE (INC)

Sample	Concentration Procedure	Equivalent Original Volume (mL)	Tester Strains with Mean of Triplicate Counts					
			TA98			TA1538		
			-S9	+S9	INC	-S9	+S9	INC
20c	Solvent	80					131	
	Acid	8					40	
		4					33	
Control	Spontaneous						28	
Control	DMSO	0.2					31	
20c	MF 1X	0.9				50	63	
Control	MF DMSO	0.1				15	25	
29	Solvent	80	115				100	
	Base	8	52				42	
		4	48				37	
Control	Spontaneous		57				28	
Control	DMSO	0.2	48				30	
29	XAD-2	40						56
Control	DMSO	0.2						24
30	MF 1X	0.9					59	
Control	MF DMSO	0.1					25	

dissolved in 5 mL DMSO. In two samples, Nos. 11 and 13, both membrane filters through which the 1X and 10X samples were filtered, were positive for mutagenic activity. In all instances the control membrane filter dissolved in DMSO yielded negative results.

Several of these samples produced a positive mutagenic effect with the 1X dissolved membrane filter and were in turn negative with the 10X DMSO dissolved membrane filter solution, an indication that the 10X membrane filter solution may have been toxic to the tester strains. The membrane filter experiments could not be repeated nor could dose response relationships be established because 4.5 mL of the original 5 mL dissolved membrane filter solution were used in the initial experiment (Table 4).

Of the nine positive samples, only six were positive by the solvent extraction procedure. All six of these samples had positive base/neutral fractions and only three had positive acid fractions. In all instances, the samples positive by the acid extraction procedure were also positive by the base/neutral procedure, thus the acid extraction procedure did not add any extra sensitivity.

The XAD resin extracts showed mutagenic effects in only four of the nine positive samples, three in the XAD-2 resin extracts, and two in the XAD-7 extracts with one sample, 20c, being positive by both extracts. In no instance was a sample positive by one of the XAD resins and not positive by the membrane filter procedure, however, in one sample (17b), both solvent extracts were negative while a positive result was obtained with a XAD extract.

Based on the samples tested in this study, the DMSO dissolved membrane filter procedure combined with 10X flash evaporated sample, was the most sensitive concentration procedure for mutagenic activity on two counts. One being that this procedure produced the largest number of positive reactions (Table 3), and the second being that the positive reactions were obtained from smaller volumes (0.9 and 9.0 mL) of original sample (Table 4). In contrast, solvent extracts equivalent to 60 to 80 mL original sample and XAD resin extracts equivalent to 40 mL original sample were required to produce a mutagenic effect in one of the Salmonella strains used. Even these volumes are small. It is probable that if doses equivalent to 100, 500 or 1000 mL original sample tested (assuming that these doses would be non toxic), the number of positive samples would increase, especially in those samples where slight increases in revertant rates were noticed. One observation not detailed in this report was the finding that several of the XAD resin extracts were toxic to the tester strains at doses higher than which corresponds to 4 mL of original samples, i.e. sample 23, XAD-2 and XAD-7 with TA98, sample

25, XAD-7 with TA100, sample 27b, XAD-2 with TA100, and sample 20a, XAD-2 with TA1538, TA1535, and TA1537. Other, not so obvious indications of toxic effects, were observed in several instances, and almost entirely in samples concentrated by XAD resin procedures. In these samples, 40 mL sample equivalents were tested and the reversion rates were usually 20 to 30% lower than the control reversion rates, e.g., sample 24, XAD-7, TA98, 41 revertants, control 51 revertants, and sample 24, XAD-2, TA98, 44 revertants, control 66 revertants.

Of the nine samples showing mutagenic activity, seven were shown positive by Salmonella typhimurium indicator strain TA98 and all nine by strain TA1538. Tester strains TA1537 and TA1535 were only positive in the three Toronto River samples and TA1535 indicated a positive mutagenic effect in sample 11, upstream Don River, without the addition of microsomes, an indication of the presence of a primary mutagenic agent not requiring enzymatic activation.

Strain TA98 indicated mutagenic activity in fifteen samples or subsamples (Table 3) only three samples of which were positive without the addition of the S-9 mix (microsomes). In two of these samples, (20 b and 20c, 1X membrane filter) positive results were recorded with and without the addition of microsomes. One sample (17b XAD-7) was mutagenic only when tested by TA98 and TA1538 without the addition of microsomes. This sample (17b) when concentrated by

another procedure (1X membrane filter) produced a mutagenic effect with the routine addition of microsomes to the tester strain TA98. Only two samples, No. 13 (Credit River and No. 30 (Grand River) were not positive when tested by Salmonella typhimurium tester strain TA98.

Strain TA1538 indicated positive mutagenic activity in 25 samples and subsamples of which two, No. 11, upstream Don River, (solvent base/neutral extraction), and No. 20c, Cornwall Sewage Treatment Plant, final effluent after chlorination (1X membrane filter) indicated mutagenic activity with and without the addition of microsomes. Also, only three samples, all positive in strain TA1538, were shown to have mutagenic activity after the preincubation of cells, sample, and S-9 mix indicating the presence of substances requiring this additional contact to produce a mutagenic effect. Conversely, it would also appear that in all other samples showing mutagenic activity by all strains, the microsomal enzymes were able, during the preincubation step, to "detoxify" or inactivate the compounds causing the increased rate of revertant colonies in the tester strains. Curiously, all samples that produced positive results with the preincubation procedure were extracted/concentrated by the XAD-2 resin procedure, and these were the only XAD-2 concentrated samples that displayed mutagenic activity. From these data it may be surmised that the XAD-2 resin was able to extract/concentrate some agent that all of the other extraction/concentration procedures missed.

The data in Tables 3 and 4 suggests that by using Salmonella typhimurium strains TA98 and TA1538 with the routine incorporation of the S-9 mix, it is highly probable that an investigator studying water and effluent samples from within the Great Lakes Basin, would be able to establish which of these samples are mutagenic by the Salmonella typhimurium test. By using only two strains and one concentration procedure with triplicate replicates, this part of the workload and expense could be reduced by over 60% per sample.

All three concentration procedures, liquid/liquid, XAD resins, and membrane filtration/1X and 10X sample concentration, failed to produce a mutagenically active fraction in at least one sample. For example, membrane filtration of 10X concentrated sample missed sample 20a, solvent extraction missed samples 13, 17b, and 30, while XAD resins missed samples 11, 12, 13, 20a, and 30. Thus, there was no single extraction/concentration procedure that was uniquely sensitive to all potential mutagenic agents found in the samples tested. However, if a single concentration procedure had to be chosen based on this study and these data, it would have to be the membrane filtration of 1X sample and 10X flash evaporated sample. The procedure is inexpensive, with $5.00 covering disposables and labour for 1X MF samples, and perhaps $10.00 for the 10X flash evaporated sample which is then membrane filtered. It is possible to make the membrane filter concentration procedure more efficient by filtering larger volumes of water, however, toxicity problems may become more frequent.

The solvent extraction was almost as efficient as the membrane filter procedure. However, it is much more labour and cost intensive with costs varying between $50.00 and $100.00 per sample.

Based on the data presented in Tables 3 and 4, a quick, inexpensive screening procedure for mutagenic activity is available, as part of the battery approach, by using Salmonella typhimurium tester strains TA98 and TA1538 with S-9 addition on membrane filtered 1X and 10X flash evaporated samples. However, it is unrealistic to assume, at this stage of experimentation, that the above would satisfy those investigators who would like to obtain a 99% positive indication of mutagenic activity rather than approximately a 90 to 95% chance. Therefore, the following are recommended as part of the battery approach to screening water and effluent samples for mutagenic activity: solvent extraction of samples for both base/neutral and acid fractions as well as membrane filtration of 1X and 10X flash evaporated samples. These procedures appear to be sufficiently sensitive for most water and effluent samples collected within the lower Great Lakes Basin. The extracts and dissolved membrane filter should be screened by Salmonella typhimurium tester strains TA98 and TA1538 with the addition of S-9 mix.

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