FINAL REPORT

WHITCHURCH-STOUFFVILLE MUTAGENICITY STUDY

SALMONELLA ASSAY TEST RESULTS ON GROUND WATER EXTRACTS FROM SIX WELLS

November, 1982

Ontario
Ministry of the Environment

The Honourable
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Minister

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Deputy Minister

Ontario

Ministry of the Environment

news release

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TESTS ON STOUFFVILLE WELLS FIND NO TRACES OF MUTAGENS

Laboratory tests on water from wells in the Stouffville area have demonstrated that the water contains no mutagenic activity, the Ontario Ministry of the Environment announced today.

A bacterial test known as the Ames Test was used to check for mutagenic activity. The Ames Test is used only as an indicator test, since a positive response using bacteria would not necessarily indicate a similar response in man. A positive response using the Ames Test is generally confirmed by conducting a second stage battery of confirmatory test. Because the initial Ames results were negative, no additional tests were required in this study.

The tests were carried out by three laboratories selected by a Stouffville citizens' committee and the Environment Ministry. They were the Ontario Research Foundation's mutagenic testing laboratory, the Environment Ministry's biohazards laboratory and the laboratory of Dr. J.E. Cummins at the University of Western Ontario.

The decision to test was made in December, 1981, when the citizens' committee turned over to the Environment Ministry the results of tests on water from one Stouffville area well conducted by Dr. Cummins.

The results obtained by Dr. Cummins were considered inconclusive by Ministry scientists, but data were sufficient to warrant repeating this work.

Because of the proximity of the tested well to a landfill site operated by York Sanitation Co. Ltd., it was inferred that material from the site might be a factor.

In light of these implications, Ministry scientists and the citizens' committee agreed to test this and five other wells in the area. Sampling began January 25 and was concluded March 29.

All three laboratories reported the absence of mutagenic activity in samples from four of the six wells. Included in the four was the well initially tested by Dr. Cummins.

However, the Cummins laboratory reported results normally associated with mutagenic activity in two other wells, which were simultaneously tested and found negative by the Ontario Research Foundation and the Environment Ministry.

To confirm these negative results, and to examine whether seasonal fluctuations in mutagenic activity can occur, the Ministry is retesting selected wells.

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FINAL REPORT

WHITCHURCH-STOUFFVILLE MUTAGENICITY STUDY

Salmonella Assay Test Results on
Ground Water Extracts from Six Wells

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Ministry of the Environment

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FORWORD

The Salmonella assay results presented in this report were obtained from tests conducted at three laboratories, a University of Western Ontario laboratory headed by Dr. Joseph E. Cummins, the Ontario Research Foundation Mutagenicity Testing Laboratory supervised by Mr. Arthur J. Horton and the Ministry of the Environment's Biohazards Laboratory. In addition, one of the concentration cartridges (CUM) was assembled by Dr. J. Cummins while a second cartridge (MOE) was provided by the Pesticides Section of the MOE. All analyses for this study have been funded by the MOE.

SUMMARY

A study utilizing the Salmonella assay for analysis of mutagenic activity, was conducted on ground water extracts from six wells , located in the vicinity of York Sanitation landfill site No. 4, in the Town of Whitchurch-Stouffville. This study was conducted to corroborate a previous detection of mutagenic activity in one of these wells and to determine the presence, if any, of this activity in other wells in the area.

The sampling and testing of each well was conducted in accordance with a defined test protocol prepared by the Ministry of the Environment in conjunction with other members of the study group. A concentration procedure to a maximum of 1000 fold was employed using two types of XAD-2 resin cartridges. Three laboratories, a University of Western Ontario laboratory (CUM) the Ontario Research Foundation Mutagenicity Testing Laboratory (ORF), and the Ministry of the Environment's (MOE) Biohazards Laboratory participated in the testing of replicate samples for mutagenic activity. The results of these tests form the basis of this report.

Results from all three laboratories indicated the absence of mutagenic activity in samples from the Coughlan, Hutchinson, Ministry of Natural Resources and Tranmer wells. These results for the Hutchinson well failed to substantiate the previous report of mutagenic activity in a sample from that well.

Results from the MOE and ORF laboratories indicated the absence of mutagenic activity in samples from the Ballantrae Plaza and Fockler wells. In contrast results from the CUM laboratory indicate an elevated response in one sample from the Ballantrae Plaza well as well as in several concentrated samples from the Fockler well. However, due to inconsistencies in the CUM laboratory data and irregularities in the quality control checks, it is difficult to make definite conclusions as to mutagenic activity in samples from either well. In total the

combined results of the three laboratories' results suggest no mutagenic activity in the Ballantrae Plaza and Fockler wells. The data from the CUM laboratory, however, introduces a doubt about this conclusion and indicates the need for further testing of these two wells.

INTRODUCTION

On December 4, 1981, representatives of the citizens of Stouffville brought to the attention of the Ministry of the Environment (MOE) a report prepared by Dr. J. Cummins of the University of Western Ontario which indicated the possible presence of bacterial mutagenic activity in a well water concentrate. The proximity of this well to a nearby landfill site suggested a possible association between the reported mutagenic activity and material possibly escaping from the landfill. Since the quantities of the original test concentrate were limited, neither a toxicity check nor a confirmation experiment had been performed. However, these unconfirmed results indicated a need for further testing. Ministry of Environment scientists met with the citizens' representatives and on December 8, 1981 mutually agreed to initiate, at the first opportunity, further tests on the well in question. In addition, it was agreed by both parties that other wells in the area should also be tested. With the aid of Stouffville citizens' representatives and representatives of the town, five additional wells were selected.

MOE staff and citizens' representatives selected three laboratories to participate in this study. These laboratories were the Ministry of the Environment's Biohazard laboratory, a University of Western Ontario mutagenic laboratory and the Ontario Research Foundation's Mutagenic Screening Laboratory. Ministry scientists met with each of the investigators to finalize a study protocol prior to the initiation of the study. The sampling and concentration methodology was designed to corroborate the December 4, 1981 finding, and to determine at what concentration up to 1000 fold, if any, well water was capable of inducing mutagenic activity.

On January 5, 1982, MOE officials met with both citizen and town representatives to approve the sampling and testing schedule for the six wells. At that time the citizens' representatives requested that interim results on the first two wells which included the original well in question, be released prior to obtaining

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the results from all wells. The first two wells were sampled on January 25, 1982 and February 8, 1982, respectively. The principal investigators met on the 22nd of February to interpret these results and on March 5, 1982 an interim report was released.

This final report includes mutagenicity test data and conclusions for the six private wells sampled to date.

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OBJECTIVES

The Stouffville Mutagenicity study was initiated to meet the following objectives:

a) to corroborate the report of mutagenic activity in one private well in the Whitchurch-Stouffville area,

b) to test other wells (total of 5 additional wells) in the area for mutagenic activity.

Study Outline

To achieve these objectives a work plan was prepared incorporating the following conditions:

a) mutagenic activity was to be measured using the Salmonella/Mammalian microsomal mutagenicity test (herein called the Salmonella assay or Ames test),

b) this activity was to be determined by the accumulated results of round-robin testing of replicate samples in three laboratories,

c) this testing would be conducted in accordance with a defined protocol,

d) both unconcentrated and concentrated well water samples (to a maximum of 1000 fold) would be tested, and this testing would be conducted at several dose points over a wide dose range,

e) the concentration of organics in water samples would be achieved using duplicate XAD-2 macroreticular resin cartridges,

f) quality control checks of the mutagenicity test and the concentration systems would be included in the study to assess the accuracy of test results,

g) that results generated in the testing would be reported to the Ministry of the Environment, and Ministry scientists would prepare a report incorporating the findings and conclusions of the participating laboratories.

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METHODS

Wells Tested

A total of six wells, from the Whitchurch-Stouffville area, were included in the test program. The selection of these wells was made with input and agreement from the town's consultant, citizens' representatives and staff of the Ministry of the Environment. The location of these wells is shown in Figure 1.

Two private wells at sites adjacent to York Sanitation No. 4 landfill site were selected as 'test' wells. This group included the Hutchinson well which was reported to contain mutagenic activity by Dr. J. Cummins in November, 1981. Two additional wells, whose water quality was considered typical of the area, were chosen as 'background' wells. Furthermore, two 'control' wells, assumed to have localized impacts unrelated to the landfill site, were included in the program. Both 'background' and 'control' wells were considered unaffected by potential contamination by the landfill site, because of the hydrogeological characteristics of the area.

The identity of these wells, their designated code and their classification are as follows:

Code	Well	Classification
BP	Ballantrae Plaza	background well
CO	Coughlan	control well
FO	Fockler	test well
HU	Hutchinson	test well
MN	Ministry of Natural Resources	background well
TR	Tranmer	control well

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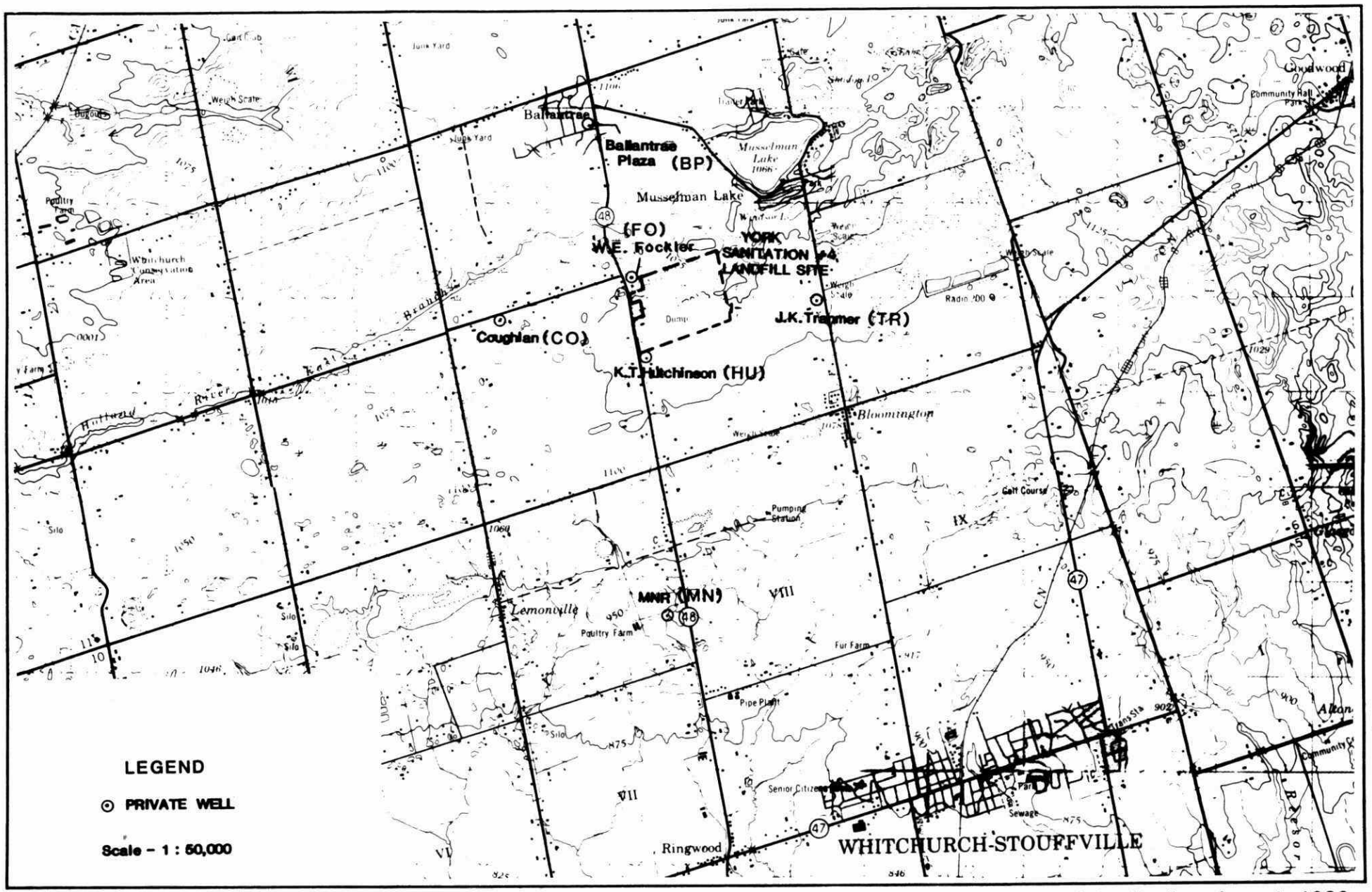
FIGURE 1



Whitchurch - Stouffville Mutagenicity Study - August, 1982

XAD Cartridges

A description of each cartridge type, including a graphic presentation, and the methods by which each cartridge was treated is appended to the protocol (Appendix I).

Collection of Samples

All well samples were collected by MOE laboratory personnel. Collection of samples and concentration of water samples was conducted on site with the aid of a mobile laboratory. Sampling and analysis dates for individual wells are given in the Results Section (Table 1).

Samples Collected

The following samples were taken at each well:

a) Three 1 L unconcentrated water samples

b) nine samples concentrated by the CUM cartridge and including:

   i) three 1 L water concentrates

   ii) three 10 L water concentrates

   iii) three 20 L water concentrates

c) nine samples concentrated by the MOE cartridge including:

   i) three 1 L water concentrates

   ii) three 10 L water concentrates

   iii) three 20 L water concentrates

In addition, at the time of sampling of each well, triplicate cartridge blanks were prepared for each cartridge type. Such cartridge blanks were prepared by passing 1 L of ultrapure distilled water through each cartridge type.

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Distribution of Sample and Elution of Cartridges

A sample set consisted of the following: 1 L of unconcentrated well water; three CUM cartridges charged with 1, 10 or 20 L well water; three MOE cartridges charged with 1, 10 or 20 L well water; one, CUM cartridge blank and one MOE cartridge blank. One sample set was distributed by the MOE to each of the participating laboratories.

The preparation of a filtered unconcentrated water sample, MF (membrane filters) Extract (see definition below) sample and the elution and concentration of the eluate from each cartridge was performed at each laboratory. Preparation of these samples is described in the test protocol (Appendix I).

Each sample set was checked for sterility and toxicity and was then tested for mutagenicity using the Salmonella assay.

Sterility Check

A dose of each sample, at a volume described in the protocol (Appendix I) was mixed with 2.0 mL molten top agar at 42°C. This mixture was overlaid on a Nutrient Agar plate. The inoculated plates were incubated overnight at 37°C. Contaminant bacteria were counted if colonies developed on these plates.

Toxicity Check

A Nutrient Broth culture of Salmonella typhimurium was diluted in Nutrient Broth to a titre of 10^3 to 10^4 cells per mL. A 0.1 mL volume of diluted cell suspension was mixed in 13 x 100 mm test tubes with 2.0 mL molten top agar at 42°C. Tubes of inoculated top agar was overlaid on replicate Nutrient Agar plates. These plates were incubated overnight at 37°C. Colonies developing on these plates were counted and these counts recorded as control bacterial numbers.

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To determine bacterial toxicity, a volume of sample was mixed with top agar and inoculated with the diluted cell suspension. The volume of sample tested (see protocol, Appendix I) was equivalent to the largest volume of sample tested in the Salmonella assay. Top agar containing the cell suspension and the sample were mixed and then overlaid on a Nutrient Agar plate. Colonies developing on these plates were determined in a manner identical to that used for the control plates.

To evaluate the toxicity, bacterial counts on control plates were averaged and the 95% confidence intervals of this average were calculated. Colonies counted on plates containing sample were related to counts on control plates and expressed in terms of surviving fraction of bacterial cells. Surviving fractions of 0.001 or less were considered to indicate sufficient toxicity which could affect results in the mutagenicity test.

MF Extracts

The MF Extract sample consisted of the particulate fraction, captured from the unconcentrated water, by a 0.2 μ membrane filter. The captured particulates were extracted with dimethylsulphoxide (DMSO) by methods described in the protocol (Appendix I). This sample was tested for mutagenicity at doses of 100 and 200 μL which are equivalent to the particulates contained in 800 and 1600 μL of unconcentrated water.

Cartridge Blanks

The cartridge blanks, X Cu 0 and X M 0, are XAD-2 cartridges through which 1 L of ultrapure distilled water has been passed. A mutagenic analysis of the extract from these cartridges provides a measure of the mutagenic activity as detected in each laboratory for each set of cartridges. These extracts should not elicit mutagenic activity, however, since elution and concentration procedures were conducted by each laboratory, individual differences in these procedures may be reflected in the results with these blanks.

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Mutagenic Testing

The mutagenic activity in each sample was determined in each laboratory by the standard operation procedure (SOP) routinely employed by that laboratory. The SOP for each laboratory is presented in Appendix II.

In general the procedure used for the Salmonella assay differed somewhat among laboratories. One laboratory (CUM) employed a liquid preincubation procedure while two laboratories (ORF and MOE) employed the direct incorporation method. The CUM Laboratory used Vogel-Bonner minimal medium while the MOE and ORF laboratories used a citrate-free minimal medium. Two laboratories (ORF and MOE) utilized Aroclor 1254 induced rat liver S9 product obtained from Intermedico, Willowdale, Ontario, while the CUM laboratory utilized its own S9 reaction product prepared from 3-methylcholanthrene induced female rat liver.

Reporting of Results

All mutagenicity test data and quality control results were reported to the MOE Biohazards laboratory. MOE scientists interpreted and drew final conclusions from these results. The data base from each laboratory was verified by that laboratory and each laboratory reviewed the final report prior to its public release.

Calculation of MUTAR Values

The results reported by each of the three laboratories was corrected for inter- and intralaboratory variability of background mutation frequency for the bacterial tester strains. This correction was achieved by expressing all test results in terms of their mutagenic activity ratio (MUTAR).

The revertant colony counts from an individual test plate were converted to a corresponding MUTAR values using the following formula:

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$$MUTAR = \frac{R - D_{SLT}}{H_{SL}}$$

where

R = Histidine independent colony count on an individual test plate.

D_{SLT} = Spontaneous background revertant counts on replicate control plates, for the corresponding tester strain at that laboratory on the date of analysis. Counts on control plates containing S9 were averaged separately from those control plates to which no S9 had been added.

H_{SL} = Historical spontaneous background revertant mean values for the corresponding tester strain obtained from that laboratory up to that date of analysis. Counts of control plates not containing S9 were averaged separately from those containing S9.

The use of MUTAR values permitted a direct comparison of the results from each of the three laboratories. This is possible because daily inter- and intralaboratory variability in spontaneous background revertant numbers are corrected by the generation of the MUTAR value.

Moreover, the magnitude of the MUTAR value permits an estimation of significance of the response induced by a particular sample. While there is no criterion which permits an absolute classification of an induced response, the objective is to select a MUTAR value which provides the highest probability of correctly distinguishing mutagens from non-mutagens. In this report we have selected three threshold MUTAR values, 0.7 to 1.49 (A), 1.50 to 2.49 (B) and $\geq$ 2.5 (+) based on the following probability table as generated by Dr. B. Commoner.

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Probability Of Correctly Classifying a Presumptive Mutagen

And Non-Mutagen At Various Mutagenic Activity Ratios

MUTAR	0.7	1.5	2.5
Case I.			
Probability of correctly classifying a presumptive mutagen	0.95	0.82	0.81
Case II.			
Probability of correctly classifying a non-mutagen	0.20	0.82	0.95

From Commoner EPA-600/1-76-022

In the tables we have noted MUTAR values as low as 0.7, however, MUTAR values less than 1.5 are considered non-significant because at this level many non-mutagenic compounds could be incorrectly classified as mutagenic (see above table). Values greater than 1.5 are considered significant, however, as noted in the above table, the greatest probability of correctly identifying a non-mutagen as well as providing excellent probability of correctly classifying a mutagen occur at MUTAR values of approximately 2.5. Therefore we have selected a threshold value of 2.5 as one criterion of a positive mutagenic response. (For a listing of all criteria necessary to conclude a sample as mutagenic, the reader is referred to page 12 of this section.)

Expression of Dose as Equivalent Volumes of Unconcentrated Water Sample

The dose (i.e., volume) of a concentrate was converted to a volume equivalent to the unconcentrated original water sample. This conversion permitted a comparison of data for individual doses of concentrate on the basis of equivalent volume (Eq. Vol.).

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In the expression of dose as Eq. Vol. the following assumptions were made:

a) that the XAD-2 resin in each cartridge recovered the majority of potentially mutagenic organic substances from the water sampled,

b) that all cartridges of the same type were equally efficient in concentrating mutagenic compounds,

c) the two cartridge types (i.e. MOE or CUM) were similarly efficient in concentrating these compounds,

d) that the concentration efficiency of the XAD resin remained constant at all volumes (to a maximum of 20 L) of water passed through the cartridge.

e) that the cartridge elution process was equally efficient in all laboratories.

The Eq. Vol. dose was calculated as follows:

a) For unconcentrated water

$$\text{Eq. Vol. (mL)} = \frac{\text{dose unconc. } H_2O \text{ (} \mu\text{L) per plate}}{1000\ \mu\text{L/mL}}$$

b) For concentrated water sample

$$\text{Eq. Vol. (mL)} = \frac{V \times \text{dose (} \mu\text{L) per plate}}{20\ \text{mL} \times 1000\ \mu\text{L/mL}}$$

where V is the volume (mL) of well water passed through the cartridge and the concentrate was made to a volume of 20 mL in DMSO.

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Interpretation of Results (See Appendix I)

A positive mutagenic result was concluded in a well water only if the following criteria were met:

a) Sterility and toxicity quality control criteria were acceptable,

b) Quality control criteria of the Salmonella assay were met,

c) The dose-related increase in MUTAR value on individual concentrated samples were duplicated in a repeat testing of the sample,

d) The MUTAR value at one or more dose points exceeded 2.5 times background,

e) The positive result reported from one laboratory was corroborated by a second laboratory.

Computer Data Entry and Data Retrieval

To facilitate the handling and interpretation of all data (approximately 7500 individual datum) generated in this study, a computerized data entry/data retrieval system was necessary. This system utilized a IBM 3033 computer, using the SAS statistical package to manipulate the data files, analyze and produce the reports.

All pertinent results reported to the MOE were included in the data base and individual results were identified using the following variables:

a) strain,

b) presence (S9) or absence (NO) of metabolic activation,

c) laboratory, i.e., Cummins or University of Western Ontario (CUM), Ministry of the Environment (MOE), Ontario Research Foundation (ORF),

d) date of analysis;

e) coded identification of test well (BP, HU, etc.)

f) sample type,

   i) Sample,

   ii) Background (spontaenous reversion rate) or control,

   iii) Positive standard,

   iv) Blank,

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g) sample identifier,

i) H_2O UNC (filtered unconcentrated water),

ii) MF Ext. (DMSO extract of filter used in gi),

iii) X M V where V = 0, 1, 10 or 20 L water,

iv) X Cu V where V = 0, 1, 10 or 20 L water,

h) dose (dose applied per plate).

The accumulated data were sorted and grouped according to the above categories. In addition, computer generated printouts were obtained for all statistical calculations as well as for the following data summaries:

a) Summary of daily and historical spontaneous (background) mutation frequency - sorted by strain, laboratory and date (Appendix III),

b) Summary of positive standard induced mutation frequency - sorted by positive standard, strain, laboratory and date (Appendix III),

c) Summary of revertant numbers and corresponding MUTAR values induced by cartridge blank extracts and MF extracts - sorted on strain, cartridge identifier, dose, laboratory and date (Appendix IV),

d) A compilation of raw data - sorted by location, strain and laboratory (Appendix V).

e) Summary of mean, minimum and maximum MUTAR values in equivalent volumes of test samples - sorted by location, strain, equivalent volume and laboratory (Appendix VA),

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RESULTS

The location of wells sampled for this study is present in Figure 1. Each well was sampled once during the testing period (January through March 1982). The sampling schedule for each well was as shown in Table 1. Table 1 also contains the date of mutagenicity testing of the respective well samples in each of the three analytical laboratories.

Quality Assurance Testing

An extensive quality assurance program was performed in each laboratory (see Appendix I). The results of such tests were used in the evaluation of the integrity of the data base generated by each laboratory.

Quality Control of Tester Strains

An evaluation of an acceptable response of the tester strains used in the Salmonella assay is based on their spontaneous mutation frequency and on their response to known mutagenic standards.

A. Spontaneous Mutation Rate

Complete presentation of the data can be found in Appendix III A while a synopsis of the data is provided in Table 2. For the most part, the average spontaneous mutation rate (SMF) for each tester strain in each laboratory throughout the study was within the acceptable range as described by de Serres and Shelby (see Table 2 for reference). However in several instances, primarily with the CUM data, the standard deviation was very high indicating that in certain tests there was variability in the behavior of the tester strain. This was further borne out when the range of daily means (calculated from results of 3 to 6 plates) observed during the testing of the wells were examined. In six of eight instances with the CUM laboratory and in one of eight with the MOE laboratory, the range of daily

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TABLE 1 - Schedule of Sampling and Ames Test Analyses of Whitchurch-Stouffville Citizens' Wells

Well Code	Date Sampled	Laboratory Code	Date Analyzed
BP	January 25	CUM	January 29 February 2 February 12
		MOE	February 5 February 21
		ORF	January 29
HU	February 8	CUM	February 14 February 22
		MOE	February 20 February 21
		ORF	February 18
MN	February 22	CUM	March 3 March 10
		MOE	March 4 March 17
		ORF	March 2
FO	March 8	CUM	March 18 April 2 April 22 April 26
		MOE	March 12 March 17
		ORF	April 13
TR	March 22	CUM	April 1 April 5 May 5
		MOE	March 31
		ORF	April 15
CO	March 29	CUM	April 8 April 12
		MOE	April 6
		ORF	April 21

TABLE 2 - Comparison of Spontaneous Background Frequencies Obtained During the Study

Laboratory	Tester Strain							
	TA 1535		TA 1537		TA 98		TA 100	
	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Cummins								
Historical Mean ± STD	43 ± 33	73 ± 81	10 ± 5	29 ± 69	25 ± 14	38 ± 49	178 ± 57	186 ± 71
N	45	44	45	44	45	48	45	48
Range of Daily Means*	18 - 124	16 -237	4 - 18	4 - 268	8 - 58	7 - 186	97 - 335	67 - 356
MOE								
Historical Mean ± STD	21 ± 7	19 ± 8	3 ± 2	4 ± 2	26 ± 12	41 ± 22	147 ± 42	146 ± 40
N	43	35	38	35	43	40	43	40
Range of Daily Means	17 - 27	13 - 23	3 - 5	3 - 7	16 - 49	29 - 102	132 - 181	120 - 182
ORF								
Historical Mean ± STD	28 ± 8	15 ± 4	10 ± 3	9 ± 3	26 ± 8	27 ± 5	180 ± 29	168 ± 22
N	40	40	40	40	40	40	40	40
Range of Daily Means	14 - 38	12 - 21	5 - 12	7 - 13	18 - 45	24 - 30	146 - 206	147 - 187
Optimal Mean	20		7		40		160	
Extremes	5 -50		3 - 25		15 - 75		60 - 200	

B. Ames, J. McCann, E. Yamasaki. Mutat. Res. 31:347-364 (1975).

F. de Serres and M. Shelby. Environmental Mutagenesis 1:87-92 (1979).

* Only include data from days when well samples were tested.

means exceeded extremes compiled by de Serres and Shelby from data of eight other laboratories (Table 2). These extremes are broad relative to that accepted in Dr. B. Ames' laboratory (March 15, 1979), supplement to reference paper - see Table 2.).

The data contained in Table 2 also show a considerable difference among laboratories in the historical means of the spontaneous background frequencies for the tester strains. This interlaboratory variability complicated comparisons of data originating from the different laboratories. To correct for these differences, standardization of the data became necessary. Results were standardized by expressing each data point in terms of a mutagenic activity ratio (MUTAR) value. Since this standardization corrected for spontaneous background mutation frequency, the data from the three laboratories could be compared.

A more thorough appraisal of the individual spontaneous mutation data is given in the discussion of the results for each test well.

B. Positive Control Data

Positive control data as reported by the three testing laboratories during the course of this study is summarized in Tables 3 through 6. A complete listing of this data is given in Appendix III b.

In general, all laboratories demonstrated the detection of mutagenic activity induced by the positive control agents, sodium azide, 2-nitrofluorene or 2-aminoanthracene. However, as expected, considerable intra- and interlaboratory variability was observed in the response of the tester strain to the positive control agents.

Specific interlaboratory differences in positive control results should be noted. In the case of the control agent sodium azide, the CUM laboratory reported a lower number of induced revertants in strains TA 1535 and TA 100 than did the MOE and ORF laboratories (Table 3). The MOE laboratory reported elevated revertants induced by 2AA in strains TA 1535 and TA 100 both in the presence and

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TABLE 3 - Comparison of Induced Mutation Frequencies as Obtained with the Positive Control Agent (Sodium azide (NaN_3- µg/plate)) During this Study

		Non Activated				S9 Activated			
Strain	Laboratory	Historical Mean	STD	n	Range of Daily Means*	Historical Mean	STD	n	Range of Daily Means
TA 100	CUM	480	± 262	30	273-1265	-	-	-	-
	MOE	2113	± 767	22	979-3465	-	-	-	-
	ORF	2181	± 464	19	1701-3083	-	-	-	-
TA 1535	CUM	417	± 328	30	112-1381	-	-	-	-
	MOE	1826	± 671	24	907-3355	-	-	-	-
	ORF	1563	± 481	16	898-2484	-	-	-	-

* Daily means calculated from revertant numbers on two plates per point.

TABLE 4 - Comparison of Induced Mutation Frequencies as Obtained with the Positive Control Agent (2 nitrofluorene (2NF- 2 µg/plate)) During this Study.

		Non-Activated				S9 Activated			
Strain	Laboratory	Historical Mean	STD	n	Range of Daily Means	Historical Mean	STD	n	Range of Daily Means
TA 100	CUM	352	± 242	16	124-1013	-	-	-	-
	MOE	1789	± 833	24	453-3000	-	-	-	-
	ORF	659	± 169	14	422-835	-	-	-	-
TA 98	CUM	334	± 319	26	92-1120	-	-	-	-
	MOE	575	± 446	24	183-1829	-	-	-	-
	ORF	736	± 190	19	600-999	-	-	-	-

TABLE 5 - Comparison of Induced Mutation Frequencies as Obtained with the Positive Control Agent (2 nitrofluorene (2NF- 20 µg/plate)) During this Study.

		Non-Activated				S9 Activated			
Strain	Laboratory	Historical Mean	STD	n	Range of Daily Means	Historical Mean	STD	n	Range of Daily Means
TA 1537	CUM	131	± 223	26	0-796	-	-	-	-
	MOE	35	± 20	19	12-71	-	-	-	-
	ORF	303	± 261	12	148-824	-	-	-	-

TABLE 6 - Comparison of Induced Mutation Frequencies as Obtained with the Positive Control Agent (2 aminoanthracene (2AA- 1 µg/plate)) During this Study

		Non-Activated				S9 Activated			
Strain	Laboratory	Historical Mean	STD	n	Range of Daily Means	Historical Mean	STD	n	Range of Daily Means
TA 100	CUM	177	± 53	24	106-304	1384	± 781	30	321-2687
	MOE	2257	± 936	23	953-3647	1995	± 973	26	790-3471
	ORF	-	-	-	-	4115	± 1290	19	2052-5741
TA 98	CUM	31	± 23	24	0-72	1236	± 717	30	77-2439
	MOE	38	± 50	23	10-90	745	± 663	26	24-2490
	ORF	-	-	-	-	3115	± 1267	19	994-5565
TA 1535	CUM	43	± 29	24	19-89	248	± 371	28	33-1449
	MOE	1814	± 605	23	810-3065	1855	± 792	26	678-3284
	ORF	26	± 2	2	-	243	± 90	12	114-346
TA 1537	CUM	10	± 8	24	0-19	250	± 149	28	28-486
	MOE	3	± 3	21	0-7	37	± 43	21	5-129
	ORF	-	-	-	-	176	± 45	16	86-214

absence of metabolic activation (S9). That laboratory reported detection of elevated revertants induced by this agent in TA 1537 and TA 98 in the presence of S9 only (Table 6). Both the CUM laboratory and the ORF laboratory (personal communications) detected 2AA induced activity in all four strains but only with the presence of S9. In the case of strain TA 1537, the MOE laboratory reported reduced sensitivity to 2AA and 2NF (20 μg) when compared to the CUM and ORF laboratories.

Quality Assurance Testing of XAD Concentration Cartridges and MF Extracts

Part of the results for each well include two sample extracts designated the cartridge blank and the MF extract, each of which provide an additional check on the system. The methodology by which each is generated is described in Appendix I. Although the results obtained from these extracts will be discussed with each test well, it is of interest to appraise these data separately. Appendix IV contains a listing of the results from these samples, and denotes MUTAR values of 0.7 to 1.49 (A), 1.50 to 2.49 (B) and $\geq$ 2.5 (+). However, as discussed previously in the Methods section, only MUTAR values of $\geq$ 1.5 were considered significantly different from background.

A summary of the MUTAR values obtained for MOE and CUM cartridge blank extracts is presented as a frequency distribution in Table 7. In the majority of cases, with both the CUM and MOE cartridges, cartridge blank eluates failed to induce a significant increase in revertant numbers, however when reported the significant MUTAR values were approximately twice as numerous in blanks of the CUM as compared to the MOE cartridge.

The data in Appendix IV were reviewed on a well to well basis to determine those specific cartridge blanks which induced significant MUTAR values. The majority of the significant values (12 of 14 for the CUM cartridge blanks and 5 of 7 for the MOE cartridge blanks) were found in results from the CUM laboratory

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Table 7 - Frequency Distribution of MUTAR Values Induced by Cartridge Blanks for CUM and MOE Cartridges used in this Study: All Strains, All Laboratories

		Number of Determinations			
Cartridge Type	MUTAR Ranking	-	A	B	+
CUM	Non Activated	230	16	6	3
	S9 Activated	244	6	2	3
MOE	Non Activated	232	20	3	0
	S9 Activated	241	10	3	1

Table 8 - Occurrance of Significant MUTAR Values in Eluates of the Cartridge Blanks

				CUM Cartridge		MOE Cartridge	
				MUTAR Rating			
Lab	Well	Strain	S9	1.5 - 2.4	≥ 2.5	1.5 - 2.4	≥ 2.5
CUM	BP			No significant MUTAR value with any strain			
	CO			" "	" "	" "	
	MN			" "	" "	" "	
	FO	98	-	3	0	1	0
			+	1	0	1	0
		35	-	2	2	0	0
			+	0	0	0	0
		37	-	0	0	0	0
			+	1	1	2	0
	HU	98	-	0	0	0	0
			+	0	0	0	1
		37	-	1	0	0	0
			+	0	0	0	0
	TR	35	-	0	0	0	0
			+	0	1	0	0
	Total			8	4	4	1
MOE	CU			No significant MUTAR values with any strain			
	FO			" "	" "	" "	
	MN			" "	" "	" "	
	TR			" "	" "	" "	
	BP	98	-	0	0	1	0
			+	0	0	0	0
	HU	35	-	0	1	0	0
			+	0	0	0	0
		37	-	0	1	0	0
			+	0	0	0	0
	Total			0	2	1	0
ORF	BP			No significant MUTAR values with any strain			
	CO			" "	" "	" "	
	HU			" "	" "	" "	
	MN			" "	" "	" "	
	TR			" "	" "	" "	
	FO	98	-	0	0	1	0
			+	0	0	0	0
	Total			0	0	1	0

(Table 8). Of these the majority were associated with the FO well. A total of 3 significant MUTAR values were shown by the MOE laboratory in these control cartridges for the BP and HU wells (Table 8). Cartridge blanks tested by the ORF laboratory induced only one significant value which was associated with the FO well.

Since the cartridge blank was not expected to induce mutagenic activity, mutagenicity testing of such controls at all prescribed doses should yield negative results. Positive results from any blank would indicate either mutagenic contamination of the eluate attributable to the XAD-2 resin or some degree of variability within the laboratory at that particular time.

An additional check on the system was the MF Extract. Since this sample was prepared from unconcentrated water and since the maximum dose tested (200 µL) was equivalent to a water volume of 1.6 mL, no appreciable mutagenic activity was expected. Thus, a report of a significant mutagenic response induced by these samples would be considered an indication of laboratory variability particularly if the comparable unconcentrated water or 1 L concentrated samples failed to induce similar activities.

A listing of MUTAR values reported for the MF Extract samples is given in Appendix IV and the significant MUTAR values for these samples are summarized in Table 9. The occurrence of significant values, on a laboratory basis, are similar to those observed with the cartridge blanks. In most cases these samples did not induce significant MUTAR values. Such samples, when analyzed by the ORF laboratory, failed to induce a significant response. The MOE laboratory reported five significant MUTAR values, four in samples from the FO well on strain TA 100 plus and minus S9, and one from the HU well on strain TA 1537 minus S9. The single value in the HU well was not duplicated at the higher dose (200 µL) tested on TA 1537 minus S9 or on the two doses tested with TA 1537 plus S9. The significant MUTAR values reported on the FO well MF Extract could not be repeated at the MOE laboratory. In addition, the significant values with TA 100 were not confirmed

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TABLE 9 - MF extract eliciting MUTAR values > 1.5, where B = 1.5 > 2.4 and + = ≥ 2.5

Strain	S9	Laboratory	Dose (µL)	BP	CO	FO	HU	MN	TR
TA 1535	-	CUM	200	.	.	+	.	.	.
		MOE		.	.	.	.	.	.
		ORF		.	.	.	.	.	.
TA 1535	+	CUM	200	.	.	.	.	+	.
		MOE		.	.	.	.	.	.
		ORF		.	.	.	.	.	.
TA 1537	-	CUM	100	.	.	+	.	.	.
			200	B	.	+	B	.	.
		MOE	100	.	.	.	B	.	.
		ORF		.	.	.	.	.	.
TA 1537	+	CUM		.	.	.	.	.	.
		MOE		.	.	.	.	.	.
		ORF		.	.	.	.	.	.
TA 98	-	CUM	100	.	.	*B,+	.	B	.
			200	.	.	.,B	.	+	.
		MOE		.	.	.	.	.	.
		ORF		.	.	.	.	.	.
TA 98	+	CUM	100	.	.	.	.	B	.
		MOE		.	.	.	.	.	.
		ORF		.	.	.	.	.	.
TA 100	-	CUM		.	.	.	.	.	.
		MOE	100	.	.	+	.	.	.
			200	.	.	+	.	.	.
		ORF		.	.	.	.	.	.
TA 100	+	CUM		.	.	.	.	.	.
		MOE	100	.	.	+	.	.	.
			200	.	.	+	.	.	.
		ORF		.	.	.	.	.	.

* indicate values reported on two separate trials

by tests conducted at the other two laboratories, therefore, the elevated MUTAR values observed for this extract are believed to indicate instability in strain TA 100 in the MOE laboratory on that day of analysis.

The majority (12 of 17) of significant MUTAR values induced by MF Extract samples were reported by the CUM laboratory. Of these 6 were associated with the FO well, 4 were reported for the MN well and one was associated with samples from each of the BP and HU wells (Table 9). For reasons discussed previously, it is suspected that these elevated values reflect variability at the CUM laboratory on these analysis dates.

Inter- and Intralaboratory Variability

Daily variations in data for a given sample within a laboratory or between laboratories may make the overall interpretation of the results of a study difficult. In this review of the data base (Appendix V) we have made the assumption that equivalent volumes tested in one laboratory should produce similar responses with a given tester strain. Therefore we have examined the range between the minimum and maximum MUTARs for each equivalent volume tested to obtain some estimation of the variability within a laboratory. Since it has been established that, for this study, the MUTAR significant threshold level is 1.5, we have arbitrarily selected twice that or 3.0 MUTAR units as a variability indicator. That is to say, when the difference between the minimum and maximum MUTAR value observed for a given equivalent volume tested by one laboratory was $\geq$ 3.0, the results were considered suggestive of intralaboratory variability for that sample.

Those MUTAR values whose range for a given equivalent volume was $\geq$ 3.0 are presented in Table 10. Only the ORF results were consistently non-variable. Of the wells tested, data for the FO well was the most variable. With samples from this well, several equivalent volumes tested in the MOE laboratory on strain TA 100 showed significant variability. A review of the raw data reported by

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Table 10 - A Listing by Laboratory of MUTAR Values Whose Range for a Given Equivalent Volume $\geq$ 3.0

Well	Strain	Blank or Eq. Vol.	CUM + S9	CUM - S9	MOE + S9	MOE - S9	ORF + S9	ORF - S9
BP	100							
	98							
		100	7.7	-	-	-	-	-
		150	10.4	-	-	-	-	-
		200	6.2	-	-	-	-	-
	1535							
	1537							
		150.0	6.6	-	-	-	-	-
		200.0	6.1	-	-	-	-	-
CO	100							
	98							
	1535							
	1537							
FO	100	MF Extract	-	-	9.2	11.0	-	-
		0.2			11.3	7.6	-	-
		0.4			11.0	3.5	-	-
		0.8	-	-	6.3	5.8	-	-
		1.2	-		6.1	6.9	-	-
		1.6			6.5	4.7	-	-
		10.0	-	-	6.1	6.7	-	-
		50.0	-	-	7.7	4.6	-	-
		100.0	-	-	9.6	6.1	-	-
		150.0	-	-	7.4	4.2	-	-
		200.0	-	-	8.2	6.3	-	-

Table 10 - cont.

Well	Strain	Blank or	CUM + S9	CUM - S9	MOE + S9	MOE - S9	ORF + S9	ORF - S9
FO	98	MF Extract	-	11.5	-	-	-	-
		0.5	-	6.4	-	-	-	-
		1.0	8.4					
		5.0	4.2	8.5	-	-	-	-
		10.0	5.4	10.6	-	-	-	-
		50.0	5.6	10.8	-	-	-	-
		100.0	6.9	7.4	-	-	-	-
		150.0	7.7	5.6	-	-	-	-
		200.0	9.3	5.9	-	-	-	-
	1535	Cartridge Blank	-	4.2	-	-	-	-
		5.0	-	3.4	-	-	-	-
		50.0	-	3.1	-	-	-	-
		100.0	4.5	-	-	-	-	-
		150.0	3.8	-	-	-	-	-
		200.0	3.5	-	-	-	-	-
	1537	MF Extract	-	72.3	-	-	-	-
		0.5	-	4.1	-	-	-	-
		5.0	-	3.2	-	-	-	-
		10.0	-	3.3	-	-	-	-
		50.0	3.0	4.8	-	-	-	-
		100.0	-	7.3	-	-	-	-
		150.0	-	16.3	-	-	-	-
		200.0	-	7.8	-	-	-	-
HU	100							
	98	Cartridge Blank	17.2	-	-	-	-	-
		5.0	-	22.6	-	-	-	-
		150.0	12.1	-	-	-	-	-
	1535	Cartridge Blank	-	-	-	4.3	-	-
	1537							
MN	100							
	98	Cartridge Blank	5.0	-	-	-	-	-
		MF Extract	3.7	-	-	-	-	-
		5.0	3.7	-	-	-	-	-
		10.0	3.3	-	-	-	-	-
		50.0	3.0	3.5	-	-	-	-
		100.0	3.5	3.9	-	-	-	-
		150	4.4	-	-	-	-	-
		200	3.3	4.6	-	-	-	-

Table 10 - cont.

Well	Strain	Blank or Eq. Vol.	CUM + S9	CUM - S9	MOE + S9	MOE - S9	ORF + S9	ORF - S9
MN	1535							
	1537							
		5.0	4.8	-	-	-	-	-
		10.0	4.6	-	-	-	-	-
		50.0	5.7	-	-	-	-	-
		100.0	5.8	-	-	-	-	-
		150.0	3.8	-	-	-	-	-
		200.0	-	5.1	-	-	-	-
TR	100							
	98							
		50.0	-	5.5	-	-	-	-
		100.0	-	3.5	-	-	-	-
		150.0	-	4.3	-	-	-	-
		200.0	-	4.7	-	-	-	-
	1535							
		5.0	3.4	-	-	-	-	-
		50.0	3.0	-	-	-	-	-
	1537							

the MOE laboratory for this well demonstrated that these eluated responses could not be confirmed in subsequent testing by this laboratory (Appendix V). Thus the response is considered to be due to instability in strain TA 100 on that day. With tester strains TA 98, TA 1535 and TA 1537, the variable data for the FO well was restricted to the CUM laboratory. Similarly this is the case with TA 98 for the HU, MN, TR and BP wells, and with TA 1535 for the TR well.

It should be remembered that the MUTAR value will vary with the spontaneous mutation rate both daily and historically. This may be a source of some variation. Table 2 provides a synopsis of two parameters of the spontaneous mutation frequencies obtained by each laboratory during this study. These parameters are the historical mean, i.e., the average of all the daily means of a tester strain throughout this study and its standard deviation, and the minimum and maximum daily mean obtained during the study. Also provided in Table 2 are the acceptable working ranges for each tester strain.

As with the above results, there was minimal variability in the spontaneous rate obtained at ORF's laboratory. Similarly, the spontaneous mutation rates obtained at the MOE laboratory, except for one test with strain TA 98 plus S9, were fairly consistent. The CUM laboratory produced the least consistent spontaneous mutation data.

Test Well Results

A copy of all raw data including spontaneous and induced (positive control) mutation frequencies is presented in Appendix V. The calculated MUTAR values and summary of significant values are presented separately, and discussed individually as part of the results of each test well.

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BP Well

1. Sterility Check

The results for the sterility check on BP samples demonstrated a single bacterial colony in two concentrates analyzed in the CUM laboratory and in one concentrate analyzed in the MOE laboratory (Table 11). This minimal contamination observed would not affect the results of the mutagenicity test on these samples.

2. Toxicity Check

While no toxicity was demonstrated in the unconcentrated samples, all three laboratories observed toxic effects with well water concentrates (Table 12). ORF reported a reduction in bacterial numbers in both cartridge blanks (X Cu 0 and X M 0) while all other concentrates were non-toxic. MOE detected minimal toxicity in two concentrates, the X Cu 10 (at the 200 µL dose) and the X M 20 (at the 100 and 200 µL doses). The results from the CUM laboratory indicated toxicity in most of the concentrates with maximal bacterial toxicity in the X M 0 blank and the X M 1 and X Cu 20 concentrates. However, the toxicity, as measured in all three laboratories was insufficient to reduce cell populations below 1/100 of control numbers, and hence was not expected to affect the results of the mutagenicity test.

3. Performance of Strains

During analysis of the BP samples, the spontaneous mutation frequencies (SMF) for all tester strains were within the acceptable range for the majority of cases (Table 13). Exceptions were noted with both CUM and MOE laboratories. Strain TA 1537, used at the MOE laboratory on February 21, 1982, demonstrated a SMF of approximately 3, the minimal extreme generally accepted for this strain (Table 2). The January 29, 1982 analysis at the CUM laboratory used a TA 1535 strain which in both presence and absence of S9, exceeded the acceptable range of 5-50 and also exceeded their historical SMF for this strain (Table 2). The SMF of strain TA 100 in the absence of S9 used in the CUM laboratory (January 29, 1982)

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TABLE 11

Bacterial Sterility Check of BP Well Samples

Sample[1]	Laboratory ORF[2]	Laboratory CUM[2]	Laboratory MOE[2]
Unconcentrated filtered water	0	0	0
MF Extract	0	0	0
X Cu 0	0	-	0
X Cu 1	0	1	0
X Cu 10	0	0	0
X Cu 20	0	0	0
X M 0	0	-	0
X M 1	0	0	0
X M 10	0	0	0
X M 20	0	1	1

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

TABLE 12

Bacterial Toxicity Check of BP Well Sample - DEGREE OF TOXICITY

Treatment		Laboratory ORF[1]	CUM[1]	MOE[1]
None	Mean ($\bar{x}$)	911	70	1057
	N	5	1	6
	95% C.I.	366	-	75
	Range [2]	0.60 - 1.40	-	0.93 - 1.07
Unconcentrated Water				
800 µL		1.437	-	-
1600 µL		1.665	1.671	0.999
Concentrated Water - 100 µL				
MF Extract		1.310	0.443	1.089
X Cu 0		-	-	-
X Cu 1		1.348	0.286	0.885
X Cu 10		1.212	0.200	0.920
X Cu 20		1.076	0.171	0.965
X M 0		-	-	-
X M 1		1.689	1.014	0.908
X M 10		1.322	0.371	1.067
X M 20		0.957	0.629	0.795
Concentrated Water - 200 µL				
MF Extract		1.470	0.514	0.999
X Cu 0		0.187	0.500	1.033
X Cu 1		0.877	0.214	1.044
X Cu 10		1.170	0.043	0.738
X Cu 20		0.853	< 0.014	1.044
X M 0		0.367	0.014	1.044
X M 1		1.463	0.014	0.984
X M 10		1.348	0.029	1.090
X M 20		0.996	0.114	0.776

[1] Toxicity of test sample relative to control (none treatment) mean. (The control mean has been given an arbitrary value of 1.0).

[2] Range of the relative surviving fraction as generated from the 95% confidence intervals. Values within this range do not differ significantly from the relative mean value of 1.0.

Table 13- Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory

LOCATION--BP

STRAIN	LAB	DATE	NO ACTIVATION					S9 ACTIVATION				
			DAILY	STD	N	HIST.	N	DAILY	STD	N	HIST.	N
TA 1535	CUM	29JAN82	124.0	6.6	3	42.5	13	115.3	6.5	3	43.2	13
		02FEB82	27.3	4.2	3	39.6	16	18.0	7.5	3	38.4	16
	MOE	05FEB82	18.0	2.6	3	21.7	17	12.3	3.2	3	21.9	14
	ORF	29JAN82	30.4	1.7	5	28.1	15	13.4	4.6	5	13.8	15
TA 1537	CUM	29JAN82	12.3	4.0	3	8.7	13	22.0	8.0	3	10.6	13
		02FEB82	4.3	1.5	3	7.9	16	7.3	1.5	3	10.0	16
	MOE	21FEB82	2.5	0.8	6	2.8	20	3.2	1.2	6	3.6	17
	ORF	29JAN82	7.8	3.3	5	10.4	15	8.0	3.9	5	9.1	15
TA 98	CUM	29JAN82	58.0	3.5	3	27.5	13	52.7	7.5	3	26.7	13
		02FEB82	16.3	4.5	3	25.4	16	19.7	4.5	3	25.4	16
		12FEB82						47.8	7.1	4	29.8	20
	MOE	21FEB82	49.3	6.0	6	29.3	20	56.2	17.2	6	38.7	17
	ORF	29JAN82	26.6	3.8	5	26.0	15	28.6	4.0	5	25.8	15
TA 100	CUM	29JAN82	335.7	13.1	3	216.2	13	241.7	10.0	3	187.6	13
		02FEB82	153.0	11.5	3	204.3	16	177.7	8.7	3	185.8	16
		12FEB82						237.8	18.9	4	196.1	20
	MOE	05FEB82	158.3	2.5	3	118.9	17	137.0	9.6	3	117.2	14
	ORF	29JAN82	177.2	17.6	5	183.1	15	161.0	8.0	5	167.6	15

greatly exceeded the acceptable range (Table 13). Similarly that laboratory also reported (January 29, 1982, February 12, 1982) slightly high SMF for strain TA 100 tested in presence of S9. However, since the presence of S9 may result in an increased SMF, these TA 100 counts are considered acceptable.

4. Cartridge Blank

The cartridge blanks (X Cu 0 and X M 0) for the BP well, tested by ORF and CUM laboratories did not induce an increase in revertants (Table 8). When analyzed in the absence of S9 at the MOE laboratory, the X M 0 extracts (at the 100 µL) on TA 1537 and the X Cu 0 and X M 0 extracts (at the 200 µL) on TA 98 induced a slight increase in revertants (Appendix IV). Since the increases were considered non-significant and a characteristic dose-related increase in revertants was not demonstrated by these samples, such increases in revertants are believed to be outlying values and not indicative of a mutagenic response.

5. MF Extract

When the MF extract of the BP well was tested by the MOE and ORF laboratories, MUTAR values less than 0.7 were demonstrated at all doses on all tester strains both with and without S9 (Table 9). In the CUM laboratory, the extract, in the absence of S9, induced an increase in the number of revertants with strains TA 1535 and TA 1537. This extract was not retested by the CUM laboratory hence a duplication of these findings were not provided.

6. Analyses of Well Samples

The results from all laboratories of the mutagenicity testing of samples from the BP well are presented in Table 14. A complete listing of the raw data from which this table was generated is presented in Appendix V - Location BP.

The results for strain TA 1535 ($\pm$ S9), TA 1537 (-S9), TA 98 (-S9) and TA 100 (-S9) indicate that MUTAR values for all samples were not significant. Such results are indicative of negative mutagenic activity.

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TABLE 14 - continued.

WELL - BALLANTRAE PLAZA STRAIN LABORATORY ACTIVATION	TA 1537 MOE NO[1]	TA 1537 MOE S9[2]	TA 1537 ORF NO	TA 1537 ORF S9	TA 98 MOE NO	TA 98 MOE S9	TA 98 ORF NO	TA 98 ORF S9
	CUM	CUM			CUM	CUM		
UNCONC. H_2O	.	.	.	.	.	.	.	.
	.	B						
MF EXTRACT	B	.	.	.	.	.	.	.
			.	.	.	.		
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	. .	. .	. .	. .	. .	. .
			. B	. .	. .	. .		
1 L CONCENTRATE	. B	. .	. .	. .	. .	. .	. .	. .
			. .	. .	. .	. .		
10 L CONCENTRATE	. B	. B	. .	. .	. .	. .	. .	. .
			. .	. .	. .	. .		
20 L CONCENTRATE	. B	. +	. .	. .	. .	. B	. .	. .
	. .		. .	. .	. .	. +		
						+		

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 14 - continued.

WELL - BALLANTRAE PLAZA STRAIN LABORATORY ACTIVATION	TA 1537 MOE								TA 1537 ORF								TA 98 MOE							
	NO[1]		CUM S9[2]		NO		S9		NO		S9		NO		S9		NO		S9		NO		S9	
UNCONC. H_2O	.		.		.		B		.		.		.		.		.		.		.		.	
MF EXTRACT	B		.		.		.		.		.		.		.		.		.		.		.	
CARTRIDGE TYPE	C[3]	M[4]	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
BLANK	.	.	.	.	.	B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1 L CONCENTRATE	.	B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10 L CONCENTRATE	.	B	.	B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
20 L CONCENTRATE	.	B	.	+	.	.	.	.	.	.	.	.	.	.	.	B	.	.	.	.	.	.	.	.
	.	.													.	+								
																+								

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 14 - Summary of Salmonella assay results on samples from the BALLANTRAE PLAZA well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - BALLANTRAE PLAZA STRAIN LABORATORY ACTIVATION	TA 1535 CUM NO[1]	TA 1535 CUM S9[2]	TA 1535 MOE NO	TA 1535 MOE S9	TA 1535 ORF NO	TA 1535 ORF S9	TA 100 CUM NO	TA 100 CUM S9	TA 100 MOE NO	TA 100 MOE S9	TA 100 ORF NO	TA 100 ORF S9
UNCONC. H_2O	.	.	.	.	.	.	.	.	.	.	.	.
MF EXTRACT	.	.	.	.	.	.	.	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
							. B .					

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

The CUM laboratory reported significant MUTAR values for tester strain TA 98 in the presence of S9 in tests with one concentrate from the BP well with the X M 20 cartridge. As indicated below, three separate trials (January 29, February 2 and 12, 1982) at the 100 equivalent volume, two trials (February 2 and 12) at the 150 equivalent volume and at one test (February 2, 1982) at the 200 equivalent volume, exhibited significant data points.

- BP well TA 98 plus S9 data from CUM laboratory -

		January 29		February 2		February 12	
Cartridge	Eq. Vol.	No. Revertants	MUTAR	No. Revertants	MUTAR	No. Revertants	MUTAR
X M 20	10	65	0.46	18	- 0.07		
	50	54	0.05	74	2.14		
	100	95	1.59	196	6.95	100	2.19*
						102	
						99	
						151	
X M 20	150	79	0.99	255	9.27	104	2.49*
						138	
						135	
						111	
X M 20	200	64	0.42	141	4.78		

* Based on an average of 4 plates.

Although the January 29 results show no mutagenic activity, the February 2 data demonstrate a dose-related increase in elevated MUTAR values which were partially confirmed on February 12, and, in isolation, are indicative of mutagenic activity. However, these results were not corroborated in the MOE or ORF laboratory where no MUTAR values greater than 1.5 were observed (Appendix V). Furthermore, these response obtained by the CUM laboratory seem to be restricted to the X M 20 cartridge, as no activity was observed with the

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complementary X Cu 20 cartridge through which the same volume of water was simultaneously passed, or with other cartridges tested at doses which, in theory, were equivalent.

A significant MUTAR value induced by the X M 20 cartridge with TA 1537 plus S9 was also reported. These values originated from tests conducted on January 29 at the CUM laboratory. As indicated in the following table, a repeat testing of this sample on February 2 failed to confirm these results.

- BP Well - TA 1537 plus S9 data from the CUM laboratory -

		January 29		February 2	
Cartridge	Eq. Vol.	No. Revertants	MUTAR	No. Revertants	MUTAR
X M 20	150	86	6.03	3	- 0.43
	200	75	4.99	6	- 0.13

A MUTAR value greater than 1.5 was also reported for one concentrate with TA 100 plus S9 (Table 14). This result originated from the February 2 testing of the X M 20 cartridge at the CUM laboratory. Corroboration of these values were not obtained in either the MOE or ORF laboratories, nor in duplicate tests (January 29, February 21) at the CUM laboratory.

Results of tests by all laboratories at all equivalent volumes on strains TA 1535, TA 1537 and TA 100, with and without S9, yielded negative or unconfirmed mutagenicity test results. Similarly, tests of these volumes on TA 98 minus S9 were negative at all laboratories.

A confirmed mutagenic response was detected in BP samples at the CUM laboratory using strain TA 98 plus S9. This finding was not corroborated by the ORF and MOE laboratories.

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CO Well

1. Sterility

All laboratories reported zero contamination on all plates (Table 15).

2. Toxicity

The results (Table 16) indicate little or no toxicity with either the MOE or CUM tests. Since the latter laboratory did not report counts for control plates, the surviving fraction was compared to the counts for the concentrated water sample.

With similar samples, a 1/10 to 1/100 kill was noted on all plates, execpt the MF extract, in the ORF laboratory. However, the toxicity does not appear to have significantly influenced the test results.

3. Performance of Strains

All tester strains with and without S9 appeared to be functioning normally in all three laboratories (Table 17).

4. Cartridge Blanks

No significant high revertant numbers were noted in any laboratory on any tester strain (Table8).

5. MF Extract

No significant increases in revertant numbers were noted with any tester strain in any laboratory (Table 9).

6. Analysis of Test Samples

The results expressed in terms of MUTAR values (Table 18) as well as the raw data (Appendix V - Location CO) demonstrate that none of the CO well

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TABLE 15

Bacterial Sterility Check of CO Well Samples

Sample[1]	Laboratory ORF[2]	CUM[2]	MOE[2]
Unconcentrated filtered water	0	0	0
MF Extract	0	0	0
X Cu 0	0	0	0
X Cu 1	0	0	0
X Cu 10	0	0	0
X Cu 20	0	0	0
X M 0	0	0	0
X M 1	0	0	0
X M 10	0	0	0
X M 20	0	0	0

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

TABLE 16

Bacterial Toxicity Check of CO Well Sample - DEGREE OF TOXICITY

Treatment		Laboratory ORF[1]	CUM[1]	MOE[1]
None	Mean ($\bar{x}$)	2499	-3	68
	N	5	-	6
	95% C.L.	968	-	41
	Range[2]	0.61 - 1.39	-	0.40 - 1.60
Unconcentrated Water				
800 µL		1.655	-	-
1600 µL		0.066	1.000	0.809
Concentrated Water - 100 µL				
MF Extract		0.843	0.300	-
X Cu 0		-	-	-
X Cu 1		0.196	0.271	-
X Cu 10		0.152	0.629	-
X Cu 20		0.102	0.743	-
X M 0		-	-	-
X M 1		0.053	0.786	-
X M 10		0.050	0.621	-
X M 20		0.026	0.771	-
Concentrated Water - 200 µL				
MF Extract		0.501	0.136	0.412
X Cu 0		0.010	0.629	0.676
X Cu 1		0.139	0.086	0.559
X Cu 10		0.060	0.400	0.221
X Cu 20		0.053	0.486	0.632
X M 0		0.019	0.857	0.897
X M 1		0.072	0.500	0.706
X M 10		0.023	0.293	0.779
X M 20		0.022	0.421	0.324

[1] Toxicity of test sample relative to control (none treatment) mean. (The control mean has been given an arbitrary value of 1.0).

[2] Range of the relative surviving fraction as generated from the 95% confidence intervals. Values within this range do not differ significantly from the relative mean value of 1.0.

[3] No control counts given. Surviving fraction compared to that of unconcentrated water sample.

Table 17- Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION--CO

STRAIN	LAB	DATE	NO ACTIVATION DAILY	NO ACTIVATION STD	NO ACTIVATION N	NO ACTIVATION HIST.	NO ACTIVATION N	S9 ACTIVATION DAILY	S9 ACTIVATION STD	S9 ACTIVATION N	S9 ACTIVATION HIST.	S9 ACTIVATION N
TA 1535	CUM	08APR82 12APR82	47.8	17.5	4	41.2	41	58.0	16.4	4	84.2	36
	MOE	06APR82	24.8	3.1	5	21.4	43	18.8	3.4	5	19.4	40
	ORF	21APR82	28.8	7.1	5	28.4	40	13.6	3.2	5	15.0	40
TA 1537	CUM	08APR82 12APR82	17.8	6.8	4	9.7	41	8.5	5.0	4	34.3	36
	MOE	06APR82	4.0	2.2	5	3.2	38	4.0	1.2	5	4.2	35
	ORF	21APR82	11.2	3.7	5	10.0	40	11.0	2.2	5	9.4	40
TA 98	CUM	08APR82 12APR82	29.8	8.2	4	25.4	41	39.5	13.4	4	42.3	40
	MOE	06APR82	18.0	4.0	5	25.8	43	34.8	2.7	5	41.0	40
	ORF	21APR82	20.0	3.5	5	25.7	40	30.0	4.8	5	27.1	40
TA 100	CUM	08APR82 12APR82	196.5	16.8	4	177.0	41	207.5	18.0	4	193.9	40
	MOE	06APR82	131.8	7.2	5	147.5	43	119.6	11.9	5	146.3	40
	ORF	21APR82	146.8	14.7	5	180.1	40	146.8	20.2	5	167.8	40

TABLE 18 - Summary of Salmonella assay results on samples from the COUGHLAN well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - COUGHLAN STRAIN LABORATORY ACTIVATION	TA 1535 CUM NO[1]	TA 1535 CUM S9[2]	TA 1535 MOE NO	TA 1535 MOE S9	TA 1535 ORF NO	TA 1535 ORF S9	TA 100 CUM NO	TA 100 CUM S9	TA 100 MOE NO	TA 100 MOE S9	TA 100 ORF NO	TA 100 ORF S9
UNCONC. H_2O	.	.	.	.	.	.	.	.	.	.	.	.
MF EXTRACT	.	.	.	.	.	.	.	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CARTRIDGE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 18 - continued

WELL - COUGHLAN STRAIN LABORATORY ACTIVATION	TA 1537								TA 98							
	CUM		MOE		ORF		CUM		MOE		ORF					
	NO[1]	S9[2]	NO	S9	NO	S9	NO	S9	NO	S9	NO	S9				
UNCONC. H_2O	.	.	.	.	.	.	.	.	.	.	.	.				
MF EXTRACT	.	.	.	.	.	.	.	.	.	.	.	.				
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .

[1] Without S9 activation; [2] with activation; [3] CUM cartridge; [4] MOE cartridge

samples tested produced revertants significantly in excess of the spontaneous mutation rate. Therefore, these results indicate that the CO well samples were non-mutagenic.

FO Well

1. Sterility

No contamination was noted on any of the ORF laboratory plates. Minimal contamination was observed on one MOE and several CUM laboratory plates. The filtered unconcentrated water sample, tested at the CUM laboratory only, showed 40 contaminant colonies (Table 19). This contamination did not affect the test data, since results for mutagenicity testing of unconcentrated FO water were not reported by the CUM laboratory.

2. Toxicity

A very significant degree of toxicity was reported by the CUM laboratory, while only slight toxicity was noted by ORF laboratory and none was observed in the MOE laboratory (Table 20). The toxicity observed by the CUM laboratory could potentially lead to false positive results.

3. Performance of Strains

(a) TA 1535 - The MOE and ORF laboratories reported no irregularities with TA 1535 (Table 21). However, data from the CUM laboratory on March 18 contained SMF values for TA 1535 and TA 1535 plus S9 which were abnormally high and which make the corresponding test data for that day somewhat suspect. Again on April 22 the SMF was slightly higher than that accepted as the upper limit as reported by de Serres and Shelby (1979) (Table 2).

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TABLE 19

Bacterial Sterility Check of FO Well Samples

Sample[1]	Laboratory ORF[2]	Laboratory CUM[2]	Laboratory MOE[2]
Unconcentrated filtered water	0	40	2
MF Extract	0	0	0
X Cu 0	0	2	0
X Cu 1	0	3	0
X Cu 10	0	0	0
X Cu 20	0	2	0
X M 0	0	0	0
X M 1	0	0	0
X M 10	0	0	0
X M 20	0	2	0

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

Table 21- Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION--FO

STRAIN	LAB	DATE	NO ACTIVATION DAILY	NO ACTIVATION STD	NO ACTIVATION N	NO ACTIVATION HIST.	NO ACTIVATION N	S9 ACTIVATION DAILY	S9 ACTIVATION STD	S9 ACTIVATION N	S9 ACTIVATION HIST.	S9 ACTIVATION N
TA 1535	CUM	18MAR82	100.3	20.7	3	41.5	28	237.3	41.0	3	74.6	28
		02APR82	17.8	15.3	5	40.5	37	.	.	.	.	.
		22APR82	56.8	6.0	4	42.6	45	.	.	.	.	.
		26APR82	.	.	.	.	.	25.0	7.5	4	78.3	40
	MOE	12MAR82	27.2	9.3	5	22.0	28	23.0	7.2	5	20.5	25
		17MAR82	19.4	3.4	5	21.6	33	14.2	2.8	5	19.4	30
	ORF	13APR82	14.2	6.6	5	27.4	30	11.6	4.2	5	15.0	30
TA 1537	CUM	18MAR82	12.0	3.0	3	8.5	28	268.0	103.7	3	40.0	28
		02APR82	6.8	1.9	5	8.8	37	.	.	.	.	.
		22APR82	13.0	1.2	4	10.0	45	.	.	.	.	.
		26APR82	.	.	.	.	.	7.0	1.8	4	31.5	40
	MOE	12MAR82	3.0	2.9	5	2.9	28	6.6	2.7	5	4.2	25
	ORF	13APR82	5.4	1.5	5	9.9	30	7.4	3.2	5	9.2	30
TA 98	CUM	18MAR82	40.0	7.2	3	26.5	28	27.3	10.6	3	44.1	32
		02APR82	7.6	9.4	5	24.9	37	.	.	.	.	.
		22APR82	25.0	4.7	4	25.4	45	.	.	.	.	.
		26APR82	.	.	.	.	.	11.5	3.0	4	39.5	44
	MOE	12MAR82	26.0	4.2	5	27.8	28	32.8	7.3	5	45.2	25
	ORF	13APR82	18.4	4.7	5	27.2	30	24.4	5.0	5	26.3	30
TA 100	CUM	18MAR82	163.0	31.5	3	184.7	28	67.7	11.2	3	171.9	32
		02APR82	97.0	20.8	5	174.9	37	.	.	.	.	.
		22APR82	188.3	12.1	4	178.0	45	.	.	.	.	.
		26APR82	.	.	.	.	.	178.3	28.4	4	192.5	44
	MOE	12MAR82	176.8	29.3	5	142.0	28	182.2	17.6	5	139.0	25
		17MAR82	169.6	23.3	5	146.2	33	180.4	16.9	5	145.9	30
	ORF	13APR82	171.6	50.8	5	181.4	30	186.8	10.1	5	172.1	30

(b) TA 1537 - Except for the March 18 data from the CUM laboratory, where the daily mean for TA 1537 plus S9 was an unacceptable 268 colonies/plate, all laboratories reported SMF data within the normal limits for the strain (Table 21).

(c) TA 98 - The MOE and ORF laboratory data were both within the normal SMF range for the strain (Table 21). However, in the CUM laboratory, the SMF for TA 98 (April 2) and for TA 98 plus S9 (April 26) were below the lower limit for the strain (Table 2).

4. Cartridge Blanks

Numerous values including significantly high MUTAR values were reported for both CUM and MOE cartridge blanks when analyzed by the CUM laboratory. This response was reported with strains TA 1535, TA 1537 plus S9 and TA 98 with and without S9. In addition, the ORF laboratory reported one significant MUTAR value with TA 98 induced by the MOE cartridge blank (Table 8). These unexpected responses with the cartridge blank could be due to either mutagenic contamination for those particular cartridges or to laboratory inconsistencies on those days.

5. MF Extract

In the MOE laboratory abnormally high MUTAR values were observed for both volumes of MF extract tested with strain TA 100 in the presence and absence of S9 (see Table 9). This response, however, was not repeated in subsequent testing by this laboratory. No such irregularities were noted for this strain in the CUM or ORF laboratory. However, MF extracts tested with TA 98, TA 1535 and TA 1537 elicited high MUTAR in the CUM laboratory.

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6. Analysis of Well Samples

With TA 100 and TA 100 plus S9, significant MUTAR values induced by three samples were reported by the MOE laboratory (Table 22). On that day all MF extract, unconcentrated water and X M 20 doses tested with TA 100 induced elevated revertant numbers. However, none of these data were confirmed upon subsequent testing (March 17). The March 12 and 17 data for TA 100 - S9 are presented in the table which follows immediately, however, the TA 100 plus S9 data, which are very similar, are not included.

- FO well TA 100 -S9 data from the MOE laboratory -

Cartridge or Sample	Eq. Vol	March 12 No. Revertants	MUTAR	March 17 No. Revertants	MUTAR
MF Extract (100 µL)	-	1688	10.64	112	- 0.39
(200 µL)	-	1216	7.32	96	- 0.50
H20 UNC[1]	0.2	1232	7.43	140	- 0.20
	0.4	656	3.38	156	- 0.09
	0.8	992	5.74	152	- 0.12
	1.2	1128	6.70	134	- 0.24
	1.6	801	4.40	132	- 0.26
X M 20	10.0	1068	6.28	106	- 0.44
	50.0	832	4.62	163	- 0.05
	100.0	1016	5.91	141	- 0.20
	150.0	746	4.01	142	- 0.19
	200.0	1064	6.25	184	0.10

[1] unconcentrated water

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TABLE 22 - Summary of Salmonella assay results on samples from the FOCKLER well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - FOCKLER STRAIN LABORATORY ACTIVATION	TA 1535												TA 100											
	CUM				MOE				ORF				CUM				MOE				ORF			
	NO[1]		S9[2]		NO		S9		NO		S9		NO		S9		NO		S9		NO		S9	
UNCONC. H_2O					.		.		.		.						+		+		.		.	
					.		.										.		.					
MF EXTRACT	.				.		.		.		.		.		.		+		+		.		.	
	+				.		.						.				.		.					
CARTRIDGE TYPE	C[3]	M[4]	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
BLANK	B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	+	.											.	.										
	.	.											.	.										
			.	.											.	.								
1 L CONCENTRATE	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	+	B											.	.										
	.	.											.	.										
			.	.											.	.								
10 L CONCENTRATE	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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20 L CONCENTRATE	B	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	.
	.	B				.		.					.	.				.		.				
	.	B											.	.										
			.	.											.	.								

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 22 - continued

WELL - FOCKLER STRAIN LABORATORY ACTIVATION	TA 1537 CUM NO[1]	TA 1537 CUM S9[2]	TA 1537 MOE NO	TA 1537 MOE S9	TA 1537 ORF NO	TA 1537 ORF S9	TA 98 CUM NO	TA 98 CUM S9	TA 98 MOE NO	TA 98 MOE S9	TA 98 ORF NO	TA 98 ORF S9
UNCONC. H_2O			.	.	.	.			.	.	.	.
MF EXTRACT	. +	.	.	.	.	.	B +	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. . .	+ B	. .	. .	. .	. .	B B .	. . B	. .	. .	. B	. .
								B B				
1 L CONCENTRATE	. . .	B B	. .	. .	. .	. .	B + +	. . +	B .	. .	. .	. .
									+ +			
10 L CONCENTRATE	. . .	B +	. .	. .	. .	. .	+ . +	+ . +	B .	. .	. .	. .
									+ +			
20 L CONCENTRATE	. + .	+ +	. .	. .	. .	. .	+ . +	+ + +	+ .	. .	. .	. .
									+ +			

[1] Without S9 activation; [2] with S9 activation; 3 CUM cartridge; 4 MOE cartridge

The results with TA 100 at the MOE laboratory on that day were extremely variable as evidenced by the inconsistencies obtained with data for similar equivalent volumes. On the other hand, all the data of the CUM and ORF laboratories with TA 100 were negative and consistent, relative to all the above parameters mentioned. Furthermore, the MOE data on the remaining strains, which include TA 1535, the strain complementary to TA 100, produced completely non-mutagenic and consistent responses (Appendix V - Location FO). Therefore, when all the factors are considered, the high values appear spurious and reflect laboratory inconsistencies on that day.

With strain TA 1535, several samples induced significant elevated MUTAR values (Table 22). Most of the significantly high data were from tests performed on March 18, at the CUM laboratory the day on which their SMF for this strain was unacceptably high (Table 21). Subsequent tests did not produce consistently mutagenic results or confirm the March 18 results (Table 22 and Appendix V - FO). In addition, cartridge blank extracts, particularly those from the CUM cartridge, were observed to produce significant MUTAR values with this strain (Table 8). Moreover, the results from four sets of the same equivalent volume were inconsistent (Table 10). Taken together these indicate that the high values obtained were probably outliers and not truly representative of a mutagenic sample. This conclusion is supported by TA 1535 data from the MOE and ORF laboratory and the TA 1535 plus S9 data from all laboratories (Table 22), for which no significant MUTAR values were observed.

In one of three trials, the CUM laboratory reported significant MUTAR values in four concentrates with strain TA 1537 in the absence of S9 (Table 22). As indicated below, none of these values were corroborated by the previous March 18 testing or the subsequent April 22 testing. In fact, the March 18 and April 22 are confirmatory of negative mutagenic activity.

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- FO Well TA 1537 -S9 Data from CUM Laboratory -

Cartridge	Volume (µL)	March 18 No. Revertants	March 18 MUTAR	April 2 No. Revertants	April 2 MUTAR	April 22 No. Revertants	April 22 MUTAR
X M 1	10	16	0.47	37	3.44	6	- 0.70
	100	11	- 0.12	22	1.73	14	- 0.10
X M 10	10	10	- 0.24	29	2.53	6	- 0.70
X M 20	10	12	0.00	32	2.87	26	1.31
	50	7	- 0.59	42	4.01	14	0.10
	100	8	- 0.47	64	6.51	6	- 0.70
	150	10	- 0.24	139	15.05	7	- 0.60
	200	14	0.24	68	6.97	9	0.40
X Cu 20	100	21	1.06	32	2.87	16	0.30

These data emphasize the importance of confirming positive mutagenic results, as by themselves these results should be considered indicative of mutagenic activity, i.e., significant MUTAR values and a dose-related response.

For TA 1537 in the presence of S9, the CUM laboratory reports an unduplicated set of data in which 8 samples induced significant MUTAR values (Table 22, Appendix V). However, none of these data constitute a mutagenic response for the following reasons: a) there is no good dose-affect response, b) there is considerable variability in the range of MUTAR values for six of the equivalent volumes tested (Table 10), and c) extracts from the cartridges blanks at 4 of 6 doses elicited significant MUTAR values on the day of testing (April 26). When

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the data from these blank cartridge extracts (Appendix IV) are directly compared to the significant MUTAR values in the test sample data (see table below), it can be seen that the cartridge blanks, themselves, account for most of the elevated revertants associated with the test sample. Therefore, it appears that the high revertant numbers obtained by the CUM laboratory with TA 1537 reflect intralaboratory test variability or faulty cartridge, rather than mutagenic samples.

- FO Well TA 1537 plus S9 Data from CUM Laboratory -

Cartridge Type	Volume Tested	Test Sample		Cartridge Blank	
		No. of Revertants	MUTAR	No. of Revertants	MUTAR
X M	20	90	2.63	50	1.36
		88	2.57	50	1.36
		85	2.47	50	1.36
X M	100	56	1.55	72	2.06
		91	2.66	72	2.06
X M	200	63	1.78	64	1.81
		98	2.89	64	1.81
X Cu	20	61	1.71	54	1.49
X Cu	100	73	2.09	78	2.75
		72	2.06	78	2.75
X Cu	150	94	2.76	-	-
X Cu	200	83	2.41	92	2.70

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Using tester strain TA 98, with and without S9, numerous significant MUTAR values were observed in data reported by CUM laboratory (Table 22). These results were reported by the CUM laboratory only, as no significant increases in revertant number for comparable well samples tested with this strain were reported by the MOE or ORF laboratory.

A listing of the TA 98 results from the CUM laboratory for those samples producing significant increases in colonies numbers are given in Table 23. Significantly high MUTAR values were present in all concentrates tested. However, certain irregularities appear in this data set and must be addressed. In particular, both X M 0 and X Cu 0 cartridge blanks induced significant increases in revertant levels of TA 98 both with and without S9 (Table 8). This is most outstanding for the April 2 data, where the blank cartridge extracts (listed in the Appendix IV) had an average MUTAR value of 1.29 as compared to 1.31 for the test samples shown in Table 23. In addition, the April 2 data are suspect because of the exceptionally low daily SMF (7.6 revertants/plate) as compared to that laboratory's historical mean SMF (24.9 revertants/plate). Similarly, on April 26 the SMF for TA 98 in the CUM laboratory was below normal extreme for this strain (Table 2). Some of the data can also be accounted for by intralaboratory variability as reflected in the wide range of MUTAR values obtained for many of the equivalent volumes tested (Table 10). In particular, significantly high values with TA 98 plus S9 seen for all X M series cartridges in April 26 did not confirm the March 18 results (Table 23). However, the consistently high MUTAR values on March 18 and April 22 in the absence of S9 would appear to indicate the presence of some mutagenic activity, even considering the lack of a clear dose-related response (Table 23). The lack of clear dose-related responses as well as the uncertainty of the toxic effects reported (Table 20) makes it difficult to accurately appraise these data.

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TABLE 23 - CUM Laboratory FO Well Data Obtained With TA 98. All Data Are Appraised as MUTAR Units

Cartridge	Volume (μL)	DATE TESTED March 18		April 2	April 4	April 26
		- S9	+ S9	- S9	- S9	+ S9
X Cu 1	10	2.42	.33	5.11	3.78	
	20	-	-	-	-	3.73
	100	1.51	1.90	4.06	2.13	3.78
	200	1.59	0.56	3.18	1.77	3.07
X Cu 10	10	2.0	- 0.17	- .22	3.82	
	20	-	-	-	-	4.89
	100	6.76	1.92	0.18	3.63	5.25
	200	6.95	3.46	-0.22	5.40	4.39
X Cu 20	10	7.75	- 0.05	- .30	4.73	
	20	-	-	-	-	2.72
	50	6.23	1.72	- .10	2.05	4.89
	100	5.97	6.62	- .26	2.60	6.76
	150	5.33	6.32	- .3	2.44	7.32
	200	5.63	8.68	- .3	3.59	6.99
X M 1	10	- 0.34	0.22	.86	6.07	
	20					4.72
	100	- 0.42	- 0.37	0.66	4.53	3.83
	200	0.72	- 0.53	0.42	3.55	2.57
X M 10	10	8.12	- 0.21	0.82	7.25	
	20					2.87
	100	3.44	- 0.37	0.90	5.60	4.01
	200	2.76	- .08	1.42	4.65	4.72
X M 20	20	10.32	- .21	2.22	7.76	
	20					2.26
	50	10.69	- 0.23	3.62	5.52	2.97
	100	7.10	- .03	1.62	4.45	2.31
	150	3.25	- .39	2.42	5.32	2.14
	200	4.84	- .57	3.72	4.96	3.83

HU Well

1. Sterility

No contamination was observed on MOE or ORF plates. There were sporadically contaminated plates from the CUM Laboratory, however, only the unconcentrated filtered water and the X M 20 plates exhibited a degree of contaminants which could affect the results of the mutagenic analysis (Table 24).

2. Toxicity

No toxicity was associated with MOE laboratory plates and only minimal, i.e., a 1/10 to 1/100 kill, toxicity was observed on ORF and CUM laboratory plates (Table 25).

3. Performance of Strains

Except for a slightly low SMF for TA 1537 in the MOE laboratory and a slight disparity in SMF between TA 98 and TA 98 plus S9 at the CUM laboratory, the strains all apparently behaved normally in the three laboratories (Table 26).

4. Cartridge Blanks

Results for cartridge blank extracts indicate significant MUTAR values in a few instances, TA 1537 (-S9) and TA (+S9) from the CUM laboratory and TA 1535 (-S9) and TA 1537 (+ S9) from the MOE laboratory (Table 8).

5. MF Extract

The MF extracts produced no significant MUTAR values except for tests with TA 1537 in both the MOE (MF - 100, - S9) and the CUM (MF - 200, - S9) laboratory (Table 9).

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TABLE 24

Bacterial Sterility Check of HU Well Samples

Sample[1]	Laboratory		
	ORF[2]	CUM[2]	MOE[2]
Unconcentrated filtered water	0	7	0
MF Extract	0	0	0
X Cu 0	0	0	0
X Cu 1	0	0	0
X Cu 10	0	0	0
X Cu 20	0	2	0
X M 0	0	1	0
X M 1	0	0	0
X M 10	0	2	0
X M 20	0	20	0

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

TABLE 25

Bacterial Toxicity Check of HU Well Sample - DEGREE OF TOXICITY

Treatment		Laboratory ORF[1]	CUM[1]	MOE[1]
None	Mean ($\bar{x}$)	3761	53	466
	N	5	2	6
	95% C.I.	542	-	41
	Range [2]	0.86 - 1.14	-	0.91 - 1.08
Unconcentrated Water				
800 µL		1.025	-	-
1600 µL		1.332	-	1.030
Concentrated Water - 100 µL				
MF Extract		0.887	1.135	-
X Cu 0		-	-	-
X Cu 1		0.568	1.173	-
X Cu 10		0.595	1.250	-
X Cu 20		0.069	0.885	-
X M 0		-	-	-
X M 1		0.259	0.558	-
X M 10		0.209	1.173	-
X M 20		0.124	0.577	-
Concentrated Water - 200 µL				
MF Extract		0.956	0.346	1.519
X Cu 0		0.021	0.865	1.571
X Cu 1		0.442	0.808	1.777
X Cu 10		0.348	0.519	1.828
X Cu 20		0.062	0.846	1.442
X M 0		0.072	0.115	1.468
X M 1		0.281	0.154	1.828
X M 10		0.164	0.154	1.803
X M 20		0.112	0.654	2.704

[1] Toxicity of test sample relative to control (none treatment) mean. (The control mean has been given an arbitrary value of 1.0).

[2] Range of the relative surviving fraction as generated from the 95% confidence intervals. Values within this range do not differ significantly from the relative mean value of 1.0.

Table 26- Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION--HU

STRAIN	LAB	DATE	NO ACTIVATION					S9 ACTIVATION				
			DAILY	STD	N	HIST.	N	DAILY	STD	N	HIST.	N
TA 1535	CUM	22FEB82	27.3	8.3	3	37.7	19	15.7	5.9	3	34.8	19
	MOE	20FEB82	17.3	1.5	3	21.0	20	12.7	1.5	3	20.3	17
	ORF	18FEB82	38.2	5.5	5	30.6	20	20.6	6.7	5	15.5	20
TA 1537	CUM	14FEB82	6.0	3.6	3	7.6	19	3.7	2.1	3	9.0	19
	MOE	21FEB82	2.5	0.8	6	2.8	20	3.2	1.2	6	3.6	17
	ORF	18FEB82	11.6	2.6	5	10.7	20	7.6	1.8	5	8.7	20
TA 98	CUM	14FEB82	19.3	6.1	3	24.4	19	50.7	10.5	3	32.6	23
	MOE	21FEB82	49.3	6.0	6	29.3	20	56.2	17.2	6	38.7	17
	ORF	18FEB82	44.6	1.8	5	30.6	20	29.4	4.7	5	26.7	20
TA 100	CUM	22FEB82	201.7	4.9	3	203.9	19	144.3	14.4	3	189.4	23
	MOE	20FEB82	175.3	31.3	3	127.3	20	149.3	5.8	3	122.9	17
	ORF	18FEB82	187.0	11.6	5	184.0	20	160.2	9.2	5	165.8	20

6. Analysis of Test Sample

All three laboratories reported negative mutagenic data with strains TA 1535 and TA 100 (Table 27). With TA 98, there are three samples for which significant MUTAR values were observed in the CUM laboratory. However, even though two of these samples produced very high MUTAR values, they are each isolated points, with no confirmation in other laboratories or in complementary cartridges or equivalent volumes.

- HU Well TA 98 plus and minus S9 Data from CUM Laboratory -

Eq. Vol.	Cartridge	Volume	S9	Revertants	MUTAR
5.0	X M 10	10	-	564	22.3
		10	+	46	- 0.14
10.0	X M 1	200	-	117	4.0
		200	+	32	-0.57
150.0	X M 20	150	-	22	0.11
		150	+	439	11.9

There were also significant MUTAR values reported by the MOE with TA 1537 $\pm$ S9 for two concentrates and one cartridge blank. Since a dose-response was not noted for any of these samples (Appendix V - HU well) these results appear to merely reflect laboratory variability.

These results indicate that the HU well water concentrates tested here did not elicit any mutagenic activity in any of the laboratories, thereby are in disagreement with the November 1981 finding. Hence, the original results tabled by Dr. Cummins, which precipitated this follow-up study, were not supported.

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TABLE 27 - Summary of Salmonella assay results on samples from the HUTCHINSON well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - HUTCHINSON STRAIN LABORATORY ACTIVATION	TA 1535								TA 100							
	CUM		MOE		ORF				CUM		MOE		ORF			
	NO[1]	S9[2]	NO	S9	NO	S9			NO	S9	NO	S9	NO	S9		
UNCONC. H_2O	.	.	.	.	.	.			.	.	.	.	.	.		
MF EXTRACT	.	.	.	.	.	.			.	.	.	.	.	.		
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	+ .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 27 - continued

WELL - HUTCHINSON STRAIN LABORATORY ACTIVATION	TA 1537 CUM NO[1]	TA 1537 CUM S9[2]	TA 1537 MOE NO	TA 1537 MOE S9	TA 1537 ORF NO	TA 1537 ORF S9	TA 98 CUM NO	TA 98 CUM S9	TA 98 MOE NO	TA 98 MOE S9	TA 98 ORF NO	TA 98 ORF S9
UNCONC. H_2O	.	.	.	.	.	.	.	.	.	.	.	.
MF EXTRACT	B	.	B	.	.	.	.	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	B .	. .	. .	+ .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CONCENTRATE	. .	. B	. .	. .	. .	. .	. +	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. +	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. B	B .	. .	. .	. .	+ .	. .	. .	. .	. .

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

MN Well

1. Sterility

Very high contamination was noted in the CUM laboratory for the unconcentrated water (Table 28). Other than that only a few inconsequential contaminant colonies were noted between the MOE and CUM laboratory. No contamination was evident on ORF laboratory plates.

2. Toxicity

No toxic effects were noticed by the MOE laboratory and only minimal toxicity was observed at the ORF laboratory. On the other hand, significant toxicity was noted for many of the extracts tested in the CUM laboratory (Table 29).

3. Performance of Strains

In the CUM laboratory, on March 10, there was an unacceptable high SMF for TA 1535 and TA 98 both in the presence of S9 (Table 30). Also at that laboratory, there was an unusually low SMF for TA 98 plus S9 on March 3. On March 4, the MOE laboratory experienced large differences in SMF between TA 98 minus S9 and TA 98 plus S9. In addition, the SMF for TA 1537 in the MOE laboratory on that date was at the lower acceptable limit. The SMF for TA 100 appeared normal for all laboratories (Table 30).

4. Cartridge Blanks

No significantly high MUTAR values were observed with any of the cartridge blank extracts in any of the laboratories (Table 8).

5. MF Extract

Four MF extracts produced significant MUTAR values in the CUM laboratory with strains TA 98 and TA 1535 (Table 9). No such values were noted in either the MOE or ORF laboratories.

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TABLE 28

Bacterial Sterility Check of MN Well Samples

Sample[1]	Laboratory ORF[2]	Laboratory CUM[2]	Laboratory MOE[2]
Unconcentrated filtered water	0	500	0
MF Extract	0	1	0
X Cu 0	0	0	0
X Cu 1	0	0	0
X Cu 10	0	0	0
X Cu 20	0	0	0
X M 0	0	0	1
X M 1	0	-	0
X M 10	0	0	0
X M 20	0	0	1

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

TABLE 29

Bacterial Toxicity Check of MN Well Sample - DEGREE OF TOXICITY

Treatment		Laboratory ORF[1]	CUM[1]	MOE[1]
None	Mean ($\bar{x}$)	874	153	58
	N	5	2	6
	95% C.I.	405	-	11
	Range[2]	0.54 - 1.46	-	0.81 - 1.19
Unconcentrated Water				
800 µL		1.030	-	-
1600 µL		0.873	3.268	1.431
Concentrated Water - 100 µL				
MF Extract		1.189	0.797	-
X Cu 0		-	-	-
X Cu 1		0.801	< 0.007	-
X Cu 10		0.267	0.007	-
X Cu 20		0.216	< 0.007	-
X M 0		-	-	-
X M 1		1.130	-	-
X M 10		1.478	0.183	-
X M 20		0.481	0.281	-
Concentrated Water - 200 µL				
MF Extract		1.112	0.549	1.155
X Cu 0		0.110	< 0.007	0.724
X Cu 1		0.195	< 0.007	0.569
X Cu 10		0.301	< 0.007	0.810
X Cu 20		0.198	< 0.007	0.845
X M 0		0.727	< 0.007	1.103
X M 1		1.190	-	0.862
X M 10		1.167	0.020	0.793
X M 20		0.572	0.026	1.293

[1] Toxicity of test sample relative to control (none treatment) mean. (The control mean has been given an arbitrary value of 1.0).

[2] Range of the relative surviving fraction as generated from the 95% confidence intervals. Values within this range do not differ significantly from the relative mean value of 1.0.

Table 30- Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION--MN

STRAIN	LAB	DATE	NO ACTIVATION DAILY	NO ACTIVATION STD	NO ACTIVATION N	NO ACTIVATION HIST.	NO ACTIVATION N	S9 ACTIVATION DAILY	S9 ACTIVATION STD	S9 ACTIVATION N	S9 ACTIVATION HIST.	S9 ACTIVATION N
TA 1535	CUM	03MAR82	20.7	13.3	3	35.4	22	20.3	3.5	3	32.9	22
		10MAR82						219.7	11.7	3	55.3	25
		15MAR82	28.0	6.2	3	34.5	25					
	MOE	04MAR82	19.3	5.7	3	30.8	23	17.3	2.3	3	19.8	20
	ORF	02MAR82	27.4	4.0	5	30.0	25	16.6	2.1	5	15.7	25
TA 1537	CUM	03MAR82	15.0	3.6	3	8.6	22	26.0	5.3	3	11.3	22
		10MAR82						22.0	3.5	3	12.6	25
		15MAR82	4.3	2.1	3	8.1	25					
	MOE	04MAR82	3.0	1.0	3	2.8	23	3.3	1.5	3	3.5	20
	ORF	02MAR82	11.4	3.4	5	10.8	25	12.8	1.8	5	9.5	25
TA 98	CUM	03MAR82	38.3	11.6	3	26.3	22	6.7	3.1	3	29.6	26
		10MAR82						186.3	124.6	3	45.8	29
		15MAR82	14.0	4.4	3	24.8	25					
	MOE	04MAR82	20.7	3.2	3	28.1	23	102.3	5.0	3	48.3	20
		17MAR82	16.4	5.2	5	26.0	33	29.4	5.1	5	42.5	30
	ORF	02MAR82	22.0	4.9	5	28.9	25	26.4	2.4	5	26.6	25
TA 100	CUM	03MAR82	125.7	23.7	3	193.2	22	115.3	17.0	3	180.8	26
		10MAR82						199.0	8.0	3	182.7	29
		15MAR82	144.0	18.4	3	187.3	25					
	MOE	04MAR82	181.3	4.2	3	134.4	23	158.7	37.2	3	128.3	20
	ORF	02MAR82	180.4	15.8	5	183.3	25	183.0	17.6	5	169.2	25

6. Analysis of Test Sample

The results from all laboratories on strain TA 100 both with and without S9 showed no MUTARs 1.5 and except for one MF extract determination in the CUM laboratory, the TA 1535 results were identical. Results similar to those for TA 100 were obtained in the MOE and ORF laboratories for strains TA 1537 and TA 98, however, data from the CUM laboratory with these latter two strains exhibited several samples inducing significant MUTARs. In particular with TA 1537 ± S9 MUTAR values from three samples exceeded 2.5. However, as noted below, none of these values were confirmed on subsequent tests in the CUM laboratory. Hence these results cannot be classified as mutagenic.

- MN Well Data for TA 1537 ± S9 from CUM Laboratory -

			March 3		March 10	
Cartridge	Eq. Vol.	S9	No. Revertants	MUTAR	No. Revertants	MUTAR
X M 10	5.0	+	63	3.27	20	- 0.16
	50	+	53	2.39	28	0.43
	100	+	74	4.24	21	- 0.08
X M 20	10	+	61	3.09	20	- 0.16
	50	+	71	3.98	24	0.16
	100	+	51	2.21	26	0.32
	150	+	54	2.47	18	- 0.32
X M 20	200	-	50	4.07	3*	- 0.17*

* Test done on March 15

With strain TA 98 in the absence of S9, five of the concentrated samples as well as the unconcentrated water sample induced significant MUTAR values while no significant values were seen with TA 98 plus S9 (Table 31). All these

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TABLE 31 - Summary of Salmonella assay results on samples from the MINISTRY OF NATURAL RESOURCES well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - MINISTRY OF NATURAL RESOURCES STRAIN LABORATORY ACTIVATION	TA 1535												TA 100							
	CUM				MOE				ORF				CUM				MOE				ORF			
	NO[1]		S9[2]		NO		S9		NO		S9		NO		S9		NO		S9		NO		S9	
UNCONC. H_2O	.		.		.		.		.		.		.		.		.		.		.		.	
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	.												.											
MF EXTRACT	.		+		.		.		.		.		.		.		.		.		.		.	
			.												.									
CARTRIDGE TYPE	C[3]	M[4]	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
BLANK	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

TABLE 31 - continued

WELL - MINISTRY OF NATURAL RESOURCES																								
STRAIN	TA 1537												TA 98											
LABORATORY	CUM				MOE				ORF				CUM				MOE				ORF			
ACTIVATION	NO[1]		S9[2]		NO		S9		NO		S9		NO		S9		NO		S9		NO		S9	
UNCONC. H_2O	.		.		.		.		.		.		B		.		.		.		.		.	
	.		.										.		.		.		.					
MF EXTRACT	.		.		.		.		.		.		+		.		.		.		.		.	
			.												.		.		.					
CARTRIDGE TYPE	C[3]	M[4]	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
BLANK	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
			.	.											.	.	.	.	.	.				
	.	.											.	.										
1 L CONCENTRATE	.		.		.	.	.	.	.	.	.	.	B		.		.	.	.	.	.	.	.	.
			.												.		.	.	.	.				
	.												.											
10 L CONCENTRATE	.	.	.	+	.	.	.	.	.	B	.	.	+	+	.	.	.	.	.	.	.	.	.	.
			.	.											.	.	.	.	.	.				
	.	.											.	.										
20 L CONCENTRATE	+	.	.	+	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.
			.	.											.	.	.	.	.	.				
	.	.											.	.										

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

results originated in tests at the CUM laboratory. As with strain TA 1537 none of these values could be corroborated on subsequent testing in the CUM laboratory (see following table).

- MN Well Data for TA 98 -S9 from CUM Laboratory -

		March 3		March 15	
Cartridge	Eq. Vol.	No. Revertants	MUTAR	No. Revertants	MUTAR
Unconc H_2O	0.4	90	1.96	29	0.60
X Cu 1	10	79	1.55	15	0.04
X Cu 10	50	117	2.99	1	- 0.52
	100	109	2.69	0	- 0.56
X Cu 20	50	85	1.77	16	0.08
	10	106	2.57	8	- 0.24
	150	79	1.55	6	- 0.32
	200	96	2.19	4	- 0.40
X M 10	100	124	3.26	20	0.24
X M 20	100	88	1.89	23	0.36
	150	145	4.05	13	0.04
	200	148	4.17	16	0.08

Based on the fact that the data from the CUM laboratory could not be corroborated either by the CUM laboratory itself or by the results from the MOE or ORF laboratory, this well was considered non-mutagenic.

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TR Well

1. Sterility

No contaminant colony growth was observed on any plates in the MOE or ORF laboratory. A couple of inconsequential contaminants were noted in some samples at the CUM laboratory (Table 32).

2. Toxicity

No toxicity was apparent with the MOE data. However, both the ORF and CUM laboratory data show some degree of toxicity with almost all samples. The toxicity was greatest at the high doses tested in the CUM laboratory (Table 33).

3. Performance of Strains

The behavior of strain TA 1535 was normal in the MOE laboratory (Table 34). In the ORF laboratory however, the SMF of TA 1535 differed significantly in the presence and absence of S9. In the CUM laboratory on April 1, the SMF for TA 1535 was slightly above the extremes for this strain, while on April 5, the SMF for TA 1535 plus S9 was unacceptably high (Table 34).

The results with TA 100 followed the general pattern as seen for TA 1535. On April 5, a high SMF was observed in the CUM laboratory, and there was an appreciable difference between the SMF values for TA 100 and TA 100 plus S9 in the ORF laboratory. All daily means with TA 1537 and TA 98 for each laboratory were acceptable (Table 34).

4. Cartridge Blanks

Except for one result in the CUM laboratory, none of the data in any of the laboratories were significant (Table 8). That one exception occurred April 5 with TA 1535 plus S9. Possibly this result can be associated with the high SMF obtained that day for strain TA 1535.

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TR Well

1. Sterility

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TABLE 32

Bacterial Sterility Check of TR Well Samples

Sample[1]	Laboratory ORF[2]	CUM[2]	MOE[2]
Unconcentrated filtered water	0	0	0
MF Extract	0	0	0
X Cu 0	0	1	0
X Cu 1	0	0	0
X Cu 10	0	1	0
X Cu 20	0	0	0
X M 0	0	0	0
X M 1	0	0	0
X M 10	0	0	0
X M 20	0	1	0

[1] With the exception of the filtered unconcentrated water for which a 1600 µl dose was checked, all doses checked were 200 µl.

[2] Number of contaminant bacterial colonies per plate.

TABLE 33

Bacterial Toxicity Check of TR Well Sample - DEGREE OF TOXICITY

Treatment		Laboratory ORF[1]	CUM[1]	MOE[1]
None	Mean ($\bar{x}$)	2499	360	66
	N	5	2	6
	95% C.I.	968	-	21
	Range [2]	0.61 - 1.39	-	0.69 - 1.31
Unconcentrated Water				
800 µL		1.474	-	-
1600 µL		1.478	0.810	0.833
Concentrated Water - 100 µL				
MF Extract		0.972	0.175	-
X Cu 0		-	-	-
X Cu 1		0.756	0.244	-
X Cu 10		0.174	0.531	-
X Cu 20		0.298	0.058	-
X M 0		-	-	-
X M 1		0.223	0.283	-
X M 10		0.235	0.131	-
X M 20		0.084	0.383	-
Concentrated Water - 200 µL				
MF Extract		0.895	0.003	1.030
X Cu 0		0.037	0.356	1.182
X Cu 1		0.372	0.053	1.348
X Cu 10		0.101	< 0.003	1.091
X Cu 20		0.166	< 0.003	0.712
X M 0		0.074	0.081	1.182
X M 1		0.144	0.072	1.091
X M 10		0.087	< 0.003	1.015
X M 20		0.111	0.003	0.788

[1] Toxicity of test sample relative to control (none treatment) mean. (The control mean has been given an arbitrary value of 1.0).

[2] Range of the relative surviving fraction as generated from the 95% confidence intervals. Values within this range do not differ significantly from the relative mean value of 1.0.

Table 34--Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION-- TR

STRAIN	LAB	DATE	NO ACTIVATION DAILY	STD	N	HIST.	N	S9 ACTIVATION DAILY	STD	N	HIST.	N
TA 1535	CUM	01APR82	61.3	11.5	4	44.0	32					
		05APR82						176.0	48.9	4	87.4	32
		03MAY82						18.3	5.4	4	72.8	44
	MOE	31MAR82	17.0	1.6	5	21.0	38	20.0	7.3	5	19.5	35
	ORF	15APR82	34.6	3.3	5	28.4	35	16.0	5.1	5	15.2	35
TA 1537	CUM	01APR82	13.3	5.4	4	9.1	32					
		05APR82						20.0	4.2	4	37.5	32
		03MAY82						6.3	3.0	4	29.2	44
	MOE	31MAR82	4.6	2.1	5	3.1	32	4.6	2.7	5	4.2	30
	ORF	15APR82	9.4	2.5	5	9.9	35	9.0	3.4	5	9.1	35
TA 98	CUM	01APR82	36.0	1.6	4	27.7	32					
		05APR82						31.5	11.2	4	42.7	36
		03MAY82						20.3	5.0	4	37.9	48
	MOE	31MAR82	32.2	6.2	5	26.8	38	38.0	8.4	5	41.9	35
	ORF	15APR82	22.8	1.3	5	26.5	35	29.6	5.8	5	26.7	35
TA 100	CUM	01APR82	203.8	25.0	4	187.1	32					
		05APR82						356.3	52.0	4	192.4	36
		03MAY82						115.3	18.9	4	186.1	48
	MOE	31MAR82	172.4	14.8	5	149.6	38	175.2	4.2	5	150.1	35
	ORF	15APR82	205.8	21.3	5	184.9	35	162.4	23.1	5	170.7	35

Table 34--Summary of Spontaneous Mutation Rate by Well, Strain and Laboratory.

LOCATION-- TR

STRAIN	LAB	DATE	NO ACTIVATION DAILY	STD	N	HIST.	N	S9 ACTIVATION DAILY	STD	N	HIST.	N
TA 1535	CUM	01APR82	61.3	11.5	4	44.0	32					
		05APR82						176.0	48.9	4	87.4	32
		03MAY82						18.3	5.4	4	72.8	44
	MOE	31MAR82	17.0	1.6	5	21.0	38	20.0	7.3	5	19.5	35
	ORF	15APR82	34.6	3.3	5	28.4	35	16.0	5.1	5	15.2	35
TA 1537	CUM	01APR82	13.3	5.4	4	9.1	32					
		05APR82						20.0	4.2	4	37.5	32
		03MAY82						6.3	3.0	4	29.2	44
	MOE	31MAR82	4.6	2.1	5	3.1	33	4.6	2.7	5	4.2	30
	ORF	15APR82	9.4	2.5	5	9.9	35	9.0	3.4	5	9.1	35
TA 98	CUM	01APR82	36.0	1.6	4	27.7	32					
		05APR82						31.5	11.2	4	42.7	36
		03MAY82						20.3	5.0	4	37.9	48
	MOE	31MAR82	32.2	6.2	5	26.8	38	38.0	8.4	5	41.9	35
	ORF	15APR82	22.8	1.3	5	26.5	35	29.6	5.8	5	26.7	35
TA 100	CUM	01APR82	203.8	25.0	4	187.1	32					
		05APR82						356.3	52.0	4	192.4	36
		03MAY82						115.3	18.9	4	186.1	48
	MOE	31MAR82	172.4	14.8	5	149.6	38	175.2	4.2	5	150.1	35
	ORF	15APR82	205.8	21.3	5	184.9	35	162.4	23.1	5	170.7	35

5. MF Extract

No significant results were noted for any of the strains in any of the laboratories (Table 9).

6. Analysis of Test Sample

None of the laboratories reported any potential mutagenic activity on either strains TA 1537 or TA 100 (Table 35). With TA 1535 and TA 98 the MOE and ORF laboratory did not report any significant MUTAR values. With TA 1535 two concentrates inducing significant MUTARs were reported by the CUM laboratory (Table 35). These values were, however, unconfirmed.

With strain TA 98 minus S9, four samples tested in the CUM laboratory indicated the possible presence of mutagenic activity (Table 35). The tests on April 1 from which these data were obtained, were not duplicated. Subsequent complementary tests with this strain in the presence of S9 did not confirm these results. In addition, as indicated below, the data were almost exclusively from the CUM cartridge and were not confirmed by results from complementary MOE cartridges.

- TR Well Data for TA 98 minus S9 from CUM Laboratory -

		Cartridge Type	
Concentration -	Volume	CUM	MOE
X 1	10	3.15	.54
	100	2.35	.80
	200	2.53	- .11
X 10	10	2.96	0.11
	100	3.54	- 0.54
	200	2.82	- 0.72

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TABLE 35 - Summary of Salmonella assay results on samples from the TRANMER well. Each notation (., B or +) represents the maximum MUTAR value obtained for the 2 to 5 doses per sample tested in each trial, with the notation ., B and + representing MUTAR values of < 1.5, 1.5 - 2.4 and ≥ 2.5, respectively. Results from replicate trials of a given sample are presented vertically.

WELL - TRANMER STRAIN LABORATORY ACTIVATION	TA 1535 MOE NO[1]	TA 1535 MOE CUM S9[2]	TA 1535 ORF NO	TA 1535 ORF S9	TA 100 MOE NO	TA 100 MOE CUM S9	TA 100 ORF NO	TA 100 ORF S9
UNCONC. H_2O	.	.	.	.	.	.	.	.
MF EXTRACT	.	.	.	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	+ .	. .	. .	. .	. .	. .	. .
		. .				. .		
1 L CONCENTRATE	. .	. .	. .	. .	. .	. .	. .	. .
		. .				. .		
10 L CONCENTRATE	. B	B .	. .	. .	. .	. .	. .	. .
		. B				. .		
20 L CONCENTRATE	B .	B .	. .	. .	. .	. .	. .	. .
		. .				. .		

1 Without S9 activation; 2 with S9 activation; 3 CUM cartridge; 4 MOE cartridge

TABLE 35 - continued

WELL - TRANMER STRAIN LABORATORY ACTIVATION	TA 1537 CUM NO[1]	TA 1537 CUM S9[2]	TA 1537 MOE NO	TA 1537 MOE S9	TA 1537 ORF NO	TA 1537 ORF S9	TA 98 CUM NO	TA 98 CUM S9	TA 98 MOE NO	TA 98 MOE S9	TA 98 ORF NO	TA 98 ORF S9
UNCONC. H_2O	.	.	.	.	.	.	.	.	.	.	.	.
MF EXTRACT	.	.	.	.	.	.	.	.	.	.	.	.
CARTRIDGE TYPE	C[3] M[4]	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M	C M
BLANK	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
1 L CONCENTRATE	. .	. .	. .	. .	. .	. .	+ .	. .	. .	. .	. .	. .
10 L CONCENTRATE	. .	. .	. .	. .	. .	. .	+ .	. .	. .	. .	. .	. .
20 L CONCENTRATE	. .	. .	. .	. .	. .	. .	+ B	. .	. .	. .	. .	. .

[1] Without S9 activation; [2] with S9 activation; [3] CUM cartridge; [4] MOE cartridge

Concentration - Volume		Cartridge Type CUM	MOE
X 20	10	0.65	2.02
	50	4.99	1.45
	100	2.82	0.51
	150	5.17	0.90
	200	5.21	0.47

Because the experiments with TA 98 (-S9) were not duplicated by the CUM laboratory, it is difficult to interpret the result. However, since direct confirmation is an essential element of a positive mutagenic classification and since there were no confirmation in either complementary cartridge or in the other two laboratories, these data must at present be classified as non-mutagenic.

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DISCUSSION

The results of the Salmonella assay conducted by three laboratories on six sets of well water samples have been collated and this data base was used to assess the mutagenic potential of the samples tested.

Extensive quality assurance checks were introduced into the test protocol and the results of these were helpful in interpreting the data. In addition, a computerized data analysis system was implemented to assess comparability and permitted the standardization in terms of MUTAR values, of the large data base.

The study was designed to introduce some replication into the data base. Because of the number of samples generated and because mutagenic analyses require testing of each sample at several doses, it was impractical to test replicate plates for each sample. However, replication of the analytical results was achieved through a) the comparison of test data independently produced at three laboratories, b) the use of duplicate XAD-2 concentration cartridges, c) the testing of each well at four concentration levels, d) the intralaboratory retesting of all suspect positive samples and 4) pooling of data at each similar equivalent volume of original well water samples.

The interpretations given below to the analytical results from individual wells must be viewed within the framework of both the sampling schedule and analytical procedures used. Conclusions regarding the mutagenic potential of a particular well are based on analysis of a single set of samples. Moreover, the conclusions are based on tests conducted with both unconcentrated and concentrated well water. The highest concentration level tested theoretically was 1000 fold, assuming a 100% efficiency in both the concentration and elution of the XAD-2 resin cartridges.

Concentration of mutagenic substances from water using XAD resin has been demonstrated in the literature. Moreover, based on the November 1981 report by Dr. Cummins, application of XAD-2 concentration system was appropriate

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in this study. However, it must be emphasized that certain organic compounds, particularly volatile organics may not be totally recovered using this concentration procedure.

Analysis of individual samples were conducted by each laboratory at common dose levels outlined in the test protocol. However, the mutagenicity test employed by individual laboratories was somewhat different. Two laboratories (MOE and ORF) employed the plate incorporation version of the test while the CUM laboratory employed a pre-incubation version of this assay. In addition, minor differences existed in media used by the three laboratories. The CUM laboratory used the Vogel-Bonner medium which contains citrate, while the MOE and ORF laboratories used a citrate-free medium. In a comparison of the Vogel-Bonner with a citrate-free medium on 32 compounds, Gocke et. al. (Mutation Research 90: 91, 1981) reported that for at least 5 compounds the citrate-free medium was more sensitive while for one compound it was less sensitive than Vogel-Bonner. Thus, data obtained from the three laboratories are not exactly comparable. Nevertheless, the results from each laboratory were compared assuming a qualitatively similar ability of the two methods to detect mutagenic activity.

Interpretation of Results from Test Wells

The analytical result for samples taken from the CO and HU wells indicate the absence of mutagenic activity. This conclusion was based on the observation that none of the samples from these wells induced a repeatable (confirmed), dose-related increase in revertant numbers for any tester strains in any of the three laboratories. Thus none of the three laboratories in this study was able to corroborate the November 1981 report on the HU well.

Test on samples taken from MN and TR wells have also indicated the absence of mutagenic activity. Results on these wells from both the MOE and ORF laboratories were uniformly negative, however, those from the CUM laboratory contain some variant data. In the CUM laboratory, significant revertant numbers

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were detected on strains TA 98 and TA 1537 with samples taken from the MN well. In addition, that laboratory reported detection of elevated revertants on strain TA 98 with samples from the TR well. However, as discussed in the Results Section, these data were not subsequently confirmed at the CUM laboratory and therefore they were considered to show no mutagenic activity.

A concentrated sample from the BP well and several from the FO well, when analyzed by one (CUM) laboratory were suggestive of mutagenic activity. However, there are problems in accurately interpreting the data from these wells. Firstly, two out of three laboratories reported negative results, thus there is no corroboration of mutagenic activity from the other laboratories and in some cases there was no corroborative evidence even among subsamples within the CUM laboratory. Secondly, the performance of the tester strains for given experiments in the CUM laboratory was inconsistent thereby placing some question as to the credibility of the test data. Thirdly, it is possible that some of the results may be the consequence of toxicity. Fourthly, there are 14 instances with the FO well cartridge blank extracts from the CUM laboratory which show significant MUTAR values. Therefore, although there was a suggestion of mutagenic activity for some concentrates, none of the data can be categorically classified as mutagenic. However, a re-testing of these wells may be warranted.

At present, it is difficult to explain the discrepancy between the results obtained from the CUM laboratory and those obtained by the MOE and ORF laboratories. One possibility is the differences in the S9. The S9 for the CUM laboratory was prepared in that laboratory from 3-methylcholanthrene induced female rats, while S9 for the MOE and ORF laboratories was purchased from Litton Bionetics who prepared it from Arochlor 1254 induced male rats. Another factor contributing to this discrepancy could be associated with differences in the methodology employed. As described in Appendix 2, in the absence of metabolic activation the assay utilized in each of the laboratories was basically the same, although (and only) in the presence of S9 there is a divergence in the techniques.

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The CUM laboratory used a preincubation method in which the cells, test substance and S9 are incubated together as a liquid mixture for about 45 minutes prior to addition of 2 mL top agar for plating. In contrast, the MOE and ORF laboratories use the standard plate incorporation method in which the cells, test substance and S9 are added to 2 mL of top agar and plated immediately, providing a gelatinous or solid matrix in which the reaction proceeds.

A few chemicals (i.e. certain nitrosamines and quinolines) are best detected with the preincubation method, however, with the vast majority of compounds there is little or no qualitative difference in the detection capabilities of either the standard plate or preincubation method. Presently there is no yardstick by which the sensitivity of either method relative to waterborne mutagens can be measured. Based upon a survey of published works, it would appear that the standard plate incorporation method is by far the most widely used in studies on water mutagenicity. Out of some 50 plus publications less than 2% reported using the preincubation method. In particular, the two major Health and Welfare studies on Ontario water used the standard plate incorporation method. This is also true for all groundwater studies of which we are aware. This does not preclude the possibility that the preincubation method is as sensitive or more sensitive in certain instances, as indicated above, it merely reflects the current methodology preferences of the majority of scientists in this field.

The preincubation method theoretically allows for a better interaction between the S9, test substance and cells. As a consequence, the cells are exposed to a higher concentration of test volume. It is possible therefore that the mutagenic activity detected in the samples from the BP and FO wells, with the preincubation method, reflects this slightly greater concentration tested. In particular, the BP data for TA 98 plus S9 agree with the hypothesis that the highest concentration (X M 20 cartridge) when tested with the preincubation method may have surpassed the detectable threshold level, (i.e., that minimal dose of mutagen which will induce a detectable response in a given assay system) for the preincubation method but is

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below that for the standard plate incorporation method. The negative data with TA 98 for the X M 20 cartridge from the MOE and ORF laboratories both with and without S9 as well as that from the CUM laboratory in the absence of S9 are consistent with this hypothesis since the direct incorporation methodology was used in all cases. However, one would have expected the CUM laboratory to detect mutagenic activity with TA 98 plus S9 with the complementary X Cu 20 concentrate, which was, in fact, found to be negative in all laboratories.

It is conceivable that the response obtained by the CUM laboratory for the BP well may reflect mutagenic activity. However, since this response was, at best, marginal, and since there is clearly no corroboration of these findings, additional testing is needed to clarify this uncertainty. It is recommended that in any additional study, higher concentrates should be tested in case the present results indicate threshold values.

Results obtained by the CUM laboratory for the FO wells cannot be attributed solely to difference in methodology. While elevated revertants with TA 98 plus S9 were reported by CUM laboratory, for several FO samples, the majority of significant responses were observed by that laboratory with TA 98 in the absence of S9 in which test conditions should be similar to those employed by the MOE and ORF laboratories. Thus, factors other than those affected by test methodologies must be considered to explain the discrepancies in these results from those of MOE and ORF. In particular, significant revertant levels in cartridge blanks with TA 98 plus and minus S9, as well as for TA 1535 minus S9 and TA 1537 plus S9, would affect the results for concentrated samples from the FO well tested at the CUM laboratory. In addition, that laboratory reported, in one of three trials with TA 98 and in one of two trials with TA 98 plus S9, spontaneous revertant levels significantly below their historical mean and outside the acceptable extremes for this strain. Lastly, in most instances there is no dose-effect response for the FO well concentrates tested on TA 98 minus S9 since the highest MUTAR values were

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reported for the lowest dose tested. Thus, although the results from one laboratory for some FO well subsamples would, in isolation, be considered indicative of a confirmed mutagenic response, the irregularities in the pertinent quality assurance checks lessen the confidence in these findings.

It should be remembered that with the preincubation method there is a concomitant increase in the potential for toxic effects which could lead to false positive data. Therefore, it is hard to completely disregard the possibility that these results may be indicative of a toxic effect. As mentioned in the Results Section, most BP and particularly FO well concentrates demonstrated maximal toxicity when prepared and subsequently tested by the CUM laboratory. Since all toxicity checks were performed using basically the standard plate incorporation method, one would theoretically expect an even greater toxic effect when these concentrations were subsequently tested with the preincubation method. Normally, such toxicity problems are reduced by conducting tests at doses below the toxic level. In this study, although a report on results of a toxicity check was requested prior to initiation of the Salmonella assay, in general no adjustment in dose was made to compensate for the difference in methodology. Since the condition of the lawn (an indication of survival of the tester strain on a given plate) was not reported by the CUM laboratory, we can only assume that either there was no appreciable toxicity or else that the high colony counts observed with some plates reflect merely surviving cells and not true revertants.

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CONCLUSIONS

Samples from the Coughlan, Hutchinson, Ministry of Natural Resources and Tranmer wells, when tested during this study, were found to be non-mutagenic as measured in the Salmonella assay. This conclusion was supported by the results from all three laboratories involved in this study. The findings reported here for the Hutchinson well did not agree with the apparent detection of mutagenic activity as reported in November 1981 by Dr. Cummins. However, the samples for the November report were taken in the Fall of 1981 while in this present study the Hutchinson well was sampled in mid February 1982. Presently, there is no evidence to suggest that the mutagenic potential of ground water would fluctuate as a function of season. However, it should not be overlooked as one possible reason for the discrepancy in the results for this well, although we believe the results from the present study represent an accurate picture of the well water quality at a specific point in time.

The results of the Salmonella assay on samples from two wells, the Ballantrae Plaza and Fockler well, were not so clear-cut. Data obtained from the two laboratories indicate the absence of mutagenic activity in both wells. In contrast, results from the CUM laboratory demonstrated an elevated response with one sample from the BP well and in several samples from the FO well. Because of the uncertainties prevalent in the data base from the CUM laboratory, the lack of corroboration from the other two laboratories, and the necessity for two out of three laboratories to table similar findings, both of these wells must be termed non-mutagenic.

However, the results with TA 98 plus S9 obtained in the CUM laboratory for these two wells should not be ignored. While the responses observed were considered insufficient evidence, by themselves, to conclude mutagenic activity, they indicate the need for further testing of both wells.

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As mentioned previously, some of the discrepancies between the results of the CUM laboratory and those of MOE and ORF could be attributable to the preincubation assay. The preincubation assay, which in this case applies only to those tests involving S9 activation, permits exposure of the strains to an effective concentration of test material 3-4 times that employed in the standard plate incorporation assay. Thus it is conceivable that if a mutagen requiring metabolic activation was present in the sample, it could be detected at a lower concentration with the preincubation assay as opposed to the plate incorporation assay. (This of course does not hold true for situations when S9 activation is not included in the assay.) From data presented in this study, we are unable to compare the two methods in their ability to detect mutagenic activity in water samples, however, a controlled study to evaluate these two procedures is warranted.

The Salmonella assay has been used in this study as a test for mutagenic activity. This assay has been shown to be efficient in detecting agents known to be hazardous to man or animals. However, the detection of mutagenic activity by this test alone does not categorically indicate a hazard. If mutagenic activity is detected in a sample, this finding initiates an alert for further testing. This additional testing would include a screen for mutagenic activity in other biological systems including mammalian cell tissue culture and whole animals. The confirmation of a positive result in the Salmonella assay with similar results in tests using other biological systems is necessary before concluding a potential hazard to man.

The data in this report contains test results on samples taken from six wells, each sampled once during the study period. Conclusions regarding the quality of these wells are made within the limits of this data base. In addition, only limited studies on mutagenic activity in ground water were available in the literature to which we can compare the findings of this report. Based on the test results, this report has concluded the absence of mutagenic activity in water samples from these wells. However, the authors realize the limits of the data base and thus cannot conclude that this activity would be absent if different sampling conditions or sampling times were employed.

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RECOMMENDATIONS

(1) The application of mutagenicity testing to environmental investigations is a relatively new development. Consequently, there is at present a lack of data regarding the presence or absense of mutagenic activity in ground water. A data base of background activity in diverse types of ground water should be established for reference purposes.

(2) Based on a priori criteria for the conclusion of mutagenic activity, set forth in the test protocol, all wells were considered non-mutagenic. However, data from one laboratory indicates that further testing of the Ballantrae Plaza and Fockler wells is warranted. It is recommended that both of these wells be retested using higher concentration levels.

(3) The mutagenicity testing of the Hutchinson well at two different times of the year produced conflicting results. It may be necessary to resample this well at different seasons for a period of 2 to 3 years to resolve whether mutagenic activity exists in this or other ground water sources and, if present, whether the activity varies as a function of season. It is recommended therefore, that seasonal testing of the Hutchinson well be carried out.

(4) There is at present no consensus among scientists concerning the extent to which samples have to be concentrated prior to testing in order to make an absolute statement relative to a sample's mutagenic potential. A standardized test protocol should be established to address this important issue.

(5) The results of this study have, in several cases, indicated variability in quality assurances of the Salmonella assay. Studies of this type should always contain quality assurance checks and participants in these studies must strive to maintain the highest quality in their test systems. However, it should be noted that assays employing biological systems are subject to a degree of variability which is not readily predictable nor readily corrected. These characteristics of the biological assay systems must be considered when reviewing delays or inconsistencies in analytical results.

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