A STUDY TO IDENTIFY THE SEASONAL VARIATIONS IN WELL-WATER CONTAMINATION AND SURVEY FARM FAMILY HEALTH

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EXECUTIVE SUMMARY

This study is the first stage of an investigation into the seasonality in the quality of well water on farms in Southern Ontario, and the health of farm families drinking water contaminated with bacteria used as indicators of faecal contamination.

A sub-set of the wells investigated in 1991-1992 during the Ontario Farm Groundwater Quality Survey was selected for the study. About 180 wells were screened and the farm families invited to participate in the study. Water quality was measured on up to 188 farms in February and March 1994. The epidemiology associated with water quality was investigated on 157 of the farms.

The aspects of water quality investigated were nitrate, total coliform and *Escherichia coli* bacteria, six pesticides (all herbicides or their breakdown products).

No clear indication was found of seasonality in nitrate concentrations, although a peak was found in March, it appears likely that this was due to a problem in the analysis.

Where present, the concentration of atrazine, metolachlor, metribuzin, and cyanazine in the well water were all smaller in 1994 than in 1992. However, in March 1994 there was a sharp increase in the number of wells in which D-ethyl atrazine was detected, suggesting that the snow melt-water may have leached the material from the soil.

The bacterial contamination showed considerable variation. Based on the results of the Ontario Farm Groundwater Quality Survey, at least half the wells were expected to exceed the Ontario drinking water Objectives. In the event the values were closer to one third. Temperature could well have been an important factor in reducing the level of contamination.

The results from the bacteriological studies confirmed that the plate counts conformed to a Poisson distribution, and therfore the error on the count can be obtained from standard tables. It was also shown that running the tap for a longer period did not have any impact on the quality of the water, so that contamination was unlikely to be due to colonization of the water during the residence time in the pipework. The continuity of contamination by bacterial colonies was found to be relatively short, probably close to two months. However, it appears that many wells remain completely free of bacteria. The reasons for the difference between wells that have some bacteria present, and those that never become infected need further investigation.

Epidemiology of Drinking Contaminated Well Water

The results from the epidemiology study should be treated as extremely preliminary. The average prevalence of diarrhoea was 26%. Early indications suggest that the impact of poor quality water, as defined by the presence of indicators of pathogenic organisms, is not to increase the incidence of acute gastro-intestinal episodes but prolongs the duration of the event. There was an indication of an interaction between bacteria and nitrate in prolonging the number of days that people report having diarrhoea. The results from the full epidemiology study may enable this result to be verified. If verified it would need further study to understand the causes and consequences.

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BACKGROUND

In 1991-92 Agriculture Canada sponsored a major survey of groundwater quality in Ontario "The Ontario Farm Groundwater Quality Survey" (Rudolph and Goss, 1993). The first sampling programme for the survey, sponsored under the Environmental Sustainability Initiative, was designed to evaluate the rural groundwater conditions in Ontario at a provincial scale. This objective was approached through a single sampling of farm wells, and by sampling a limited number of wells specially installed in fields and woodlots. The sampling programme was carried out in winter 1991/92. Confirmation of the results and clarification of certain conclusions was sought in a second sampling carried out in July 1992 under the Land Management Adjustment Program.

Results from the first sampling highlighted the contamination of wells by bacteria commonly associated with faeces. More than 30% of farm wells contained coliform bacteria in excess of the limit of 5 colonies per 100 mL specified in Ontario drinking water objectives (MOE, 1992), and 20% contained faecal coliforms. Nitrate contamination was also found in 13% of wells. Pesticides were not major contaminants, and there was no contamination by petroleum derivatives due to on-farm storage. Some contamination appeared to have its origin in feedlots, and in manure storage systems.

The second sampling showed a 6% increase in the average (geometric mean) concentration of nitrate in wells in the province. The general frequency of contaminants (nitrate and bacteria), showed a small rise. There was a close correlation between the concentration of nitrate in the first round of sampling, and that in the second round, consistent with there being a stable population of contaminated wells. This close correlation did not occur for bacteria, even though the proportion of wells contaminated did not change greatly between sampling occasions. These results highlighted the much more dynamic nature of microbial contamination compared to nitrate contamination.

The study highlighted the need for further investigations into the seasonal variability in the contamination of wells for nitrate, bacteria and pesticides. Furthermore, the long-term trend in the level of contamination needs to be determined for these contaminants.

Underlying any programme on groundwater quality is the assumption that there is a significant health risk associated with drinking contaminated water. There are diseases known to be transmissible by water in Ontario. These include giardiasis (caused by the protozoan parasite Giardia lamblia), campylobacterosis, and salmonellosis (caused by the bacteria Campylobacter and Salmonella,

respectively). All three are intestinal illnesses characterized primarily by diarrhoea (Benenson, 1990). They are also the three most frequently diagnosed reportable diseases in the Province (Ontario Ministry of Health, 1991). Recent studies from Canada and the United States have demonstrated a significant association between giardiasis and the consumption of water from shallow rural wells (Isaac-Renton and Philion, 1992; Chute, Smith and Baron, 1987). To date however, there have been only limited investigations into the health effects of the consumption of microbiologically contaminated well water in Ontario (Chow et al., 1993). Consequently, studies of well water quality need to be augmented with a detailed epidemiological investigation to identify the health risks from the levels of contamination current in groundwater.

This study began an investigation of the seasonality in the quality of well water on farms in Southern Ontario, and the health of farm families drinking water contaminated with bacteria used as indicators of faecal contamination.

METHODOLOGY

1.Selection of Wells

From an analysis of a study of water-related disease in Montreal (Payment *et al*.,1993), it was evident that about 80 wells were required in the test and control groups to establish significance of any differences in health. In each group half were required to have a significant concentration of nitrate to provide background information on any interaction between nitrate concentration and bacterial contamination on health. As samples needed to be submitted to the Ministry of Health within 24 h for bacteriological assay, wells were selected within a radius of 250 km of Guelph. Wells were identified from the Ontario Farm Groundwater Quality Survey that were within an area defined by the maximum distance criterion.

Wells that were contaminated with indicator bacteria (total count of coliform bacteria exceeding 5 colonies per 100 mL, or at least 1 faecal coliform colony per 100 mL) on both previous dates of sampling, and were contaminated with nitrate, were least in number. To ensure a total of 80 wells, this group was augmented with wells that were contaminated with bacteria on one of the two previous dates of sampling, and with wells with an average nitrate concentration between 5 and 10 mg N L^{-1} on the two previous dates of sampling. The group contaminated with bacteria but not nitrate, also needed to be formed from wells that were contaminated with bacteria on one date of sampling, and had some coliform bacteria present on the other date. The average nitrate concentration over the two dates of sampling were less than 5 mg N L^{-1}. The uncontaminated group selected had no previous contamination with bacteria, and the average nitrate concentration did not exceeded 5 mg N L^{-1}. The remaining group were uncontaminated with bacteria but had high nitrate defined by the average concentration on the preceding sampling dates being greater than 5 mg N L^{-1}.

A total of 475 wells was selected in this way, and all the farm families were contacted by letter to seek their agreement for participation in the study. Each household was then contacted by telephone to obtain verbal agreement of participation. Potential participants were questioned about the source of their drinking water, the use of filters and other devices for changing water quality. As a result of this direct contact a final group of 190 wells were selected. Final selection of the participants followed site visits, and the logistics of sampling the well water was the main reason for exclusion from the study list.

2. Data Collection

Well water quality

By prior arrangement each farm was visited twice during the first period of study. The first visit took place between 1 February and 15 February 1994, and was chosen to be representative of winter conditions. The second visit took place between 21 March and 30 March 1994, a period chosen to be representative of conditions during the main spring thaw. A visit was also made between 13 and 29 June 1994, as part of the programme supported by Health Canada. On each occasion separate samples of well water were collected for analysis of bacteria, nitrate and pesticide concentrations.

Pesticide samples were collected in 1-L amber glass bottles, nitrate samples in 20 mL polyethylene vials, and bacteriological samples in a 250 mL plastic, sterile, screw-capped bacteriological bottle that contained a preservative (sodium thiosulphate). Care was taken not to touch the inside of the cap or the neck of bacteriological samples to avoid contamination. Samples were stored in the dark at 5EC. Bacteriological samples were analyzed within 24 hours at the Ministry of Health Palmerston laboratory, and the results mailed to The Centre For Land and Water Stewardship, University of Guelph.

Nitrate-N

The nitrate-nitrogen (nitrate-N) content of the well water was analyzed at the Analytical Services Laboratory, Department of Land Resource Science, University of Guelph. A TRAACS-800 auto-analyzer was used to measure the nitrate-N content colorimetrically after reduction to nitrite, using a copper-cadmium column, and formation of an azo dye using N-1-naphthylethylene diamine. The limit of detection for nitrate was 0.02 mg N L^{-1}.

Bacteria

Bacteriological samples were sent to the Palmerston Laboratory of the Ontario Ministry of Health. All samples were tested for total coliforms and Escherichia coli. Results were reported as colonies per 100 mL.

Total coliforms are oxidase negative, gram negative, non-spore-forming, facultatively anaerobic, rod-shaped bacteria capable of fermenting lactose with the production of acid and gas within 48 h when incubated at 35 ± 0.2EC. When incubated on m-Endo-LES agar, coliform colonies develop a characteristic metallic green sheen imparted by reaction between their metabolic byproducts and components in the agar. The formulation for the agar used for total

coliform is given in Appendix B. Each water sample was drawn through a cellulose-acetate membrane filter having a 0.45 µm pore size. The filter was then placed on an m-Endo-LES agar plate and incubated at 35 ± 0.2EC for 22±2 h.

E. coli were identified following seeding onto an m-FC agar plate in which 4-methyl-umbelliferyl-â-d-glucuronide replaced aniline blue dye and rosolic acid. Colonies which fluoresced under UV light were counted as *E. coli*.

Pesticides (Herbicides)

Each water sample was analyzed for Alachlor, Metolachlor, Atrazine, D-ethyl atrazine, Metribuzin, and Cyanazine

Initially, a 100 mL aliquot of each sample was analyzed using a solid phase extraction technique. Residues of herbicides were detected by capillary gas chromatography using nitrogen selective detectors. Any samples found to be positive or those that showed any remarkable characteristics were repeated using another 100 mL aliquot. Quantification was done using fortified samples run in conjunction with actual samples. The confirmed positive samples were then analyzed by current liquid-liquid extraction procedures. All samples were also investigated by an enzyme linked immunoassay system (ELISA) for the presence or absence of the herbicide atrazine. The test was sensitive and specific for only atrazine and was used to identify samples needing confirmation of the presence of the herbicide.

Reporting Procedures

When a problem level of bacteria, nitrate, or pesticide was detected in a sample, the cooperating landowner was promptly contacted. The landowner was advised to request assistance from the local Board of Health or the Ontario Ministry of the Environment for interpretation and remedial suggestions. An individual water analysis report was mailed to each cooperator.

Health of farm families

Booklets were developed that contained two-week health record sheets for each family member in the study. Sufficient sheets were prepared to cover the twelve month study period. Each booklet gives examples of health events that need to be recorded. Each household was contacted monthly by telephone to obtain information from the forms, and to ensure continued participation in the study.

All records on water quality, modifications to the water supply system, and the geographic location of wells have been entered into a data base, compatible with that established for the Ontario Farm Groundwater Quality Survey. A second data base was prepared to hold the health information.

Statistical Treatment of the Results

The percentage of contaminated wells in each category (eg. well construction type and depth, well age, hydrologic group) was determined arithmetically, and then a 95% confidence interval (C.I.) was calculated for each percentage contamination figure by assuming a binomial distribution. The equation used to calculate a 95% C.I. for percentage of contaminated wells is:

$$95\%\ C.I.\ ' \pm 2\sqrt{\frac{P(100 \& P)}{n}}$$

where:

P = percentage of contaminated wells
n = total number of wells in a category

The confidence intervals were used to help asses whether differences in percentage of wells contaminated were real or due to a chance selection of wells. A difference between two values of percent wells contaminated was considered real (statistically significant) if the values ± their 95% C.I. did not overlap each other.

RESULTS

1. Well depth

The well selection process concentrated on the water quality, and the logistics of collecting samples and epidemiology information. The Ontario Farm Groundwater Quality Survey found that an important facet of water quality was well depth. The selected wells showed a bimodal disribution of depths Fig.1). There were similar numbers of wells in categories less than 100 feet deep, and more than 200 feet deep. There were few wells between 140 and 180 feet deep. Nonetheless, the selected wells showed a satisfactory range of depths.

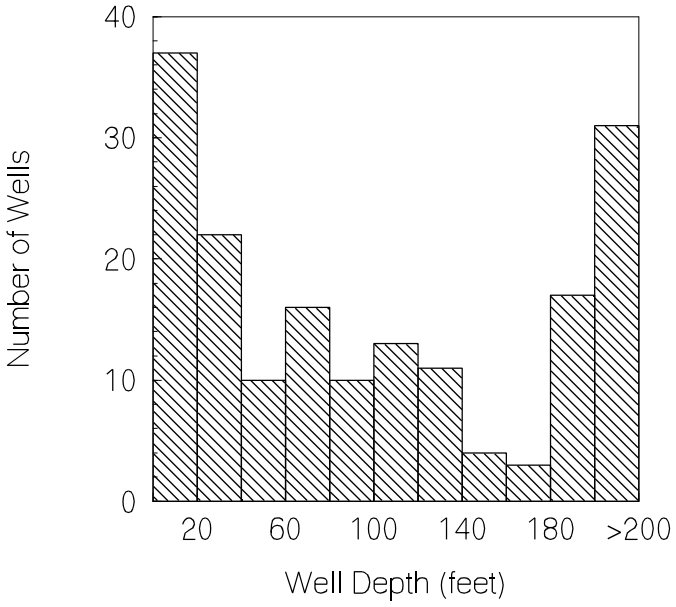

Figure 1. Distribution of wells selected for water quality and epidemiology investigation.

2. Well water quality

Bacterial Contamination

Coliform Bacteria

During the Ontario Farm Groundwater Quality Survey, 85 of the wells selected for preliminary investigations showed the presence of coliform bacteria >5 colonies per 100 mL. Over the period from winter 1991-2 to June 1994, 104 of the 188 wells tested have contained coliform bacteria >5 colonies per 100 mL. However, when wells were sampled in February 1994, only 34 wells (less than half of the wells selected because of previous bacterial contamination) had >5 coliform colonies per 100 mL (Table 1). The number of wells contaminated with coliform bacteria remained constant between February and March, but increased to 57 wells in June 1994. The June value was equivalent to 30.5% of wells sampled being contaminated compared with a value of 42.4% found in the summer of 1992 (Table 2). Of the wells having fewer than five colonies per 100 mL, at least 75% showed no coliform bacteria.

These results indicated that the contamination with coliform bacteria was not continuous. This was investigated further by determining the percentage of wells that were contaminated on each occasion of measurement (Table 3). Nearly 30% of wells were contaminated in both the winter of 1991-2 and in the summer of 1992. About 10% of wells were contaminated in both February and March or in both March and June, but less than 5% were contaminated in all three months. Only 2.9% of wells were contaminated on all five occasions. Although a smaller proportion of wells was contaminated in June 1994 compared with the Summer of 1992, the 12% difference does not of itself explain the marked reduction in continuously contaminated wells. A larger number of wells, 59 (31.4% of wells), showed no colonies of coliform bacteria on any occasion

Escherichia coli contamination

In the winter sampling for the Ontario Farm Groundwater Quality Survey, more of the wells were tested for faecal coliform bacteria than for E. coli (Table 4), but a similar proportion of wells tested showed contamination (Table 5). The proportion of wells showing contamination on the 3 sampling occasions of 1994 was much less than in the winter of 1991-2 and summer of 1992 (Table 5). As found for the coliform bacteria, the continuity of contamination was of relatively short duration, with no well being contaminated on each of the 5 occasions, and less than 1% of all wells (6% of wells contaminated in June) being contaminated on each occasion of 1994 (Table 6).

Table 1. Contamination of 188 test wells with coliform bacteria from the winter of 1991-92 to June 1994. Values are Numbers of wells:

Test	Winter 1991-2	Summer 1992	February 1994	March 1994	June 1994
Zero	84	81	118	115	97
<5	112	99	142	145	130
>5	60	73	34	35	57
Not tested	16	16	12	8	1
Total tested	172	172	176	180	187

Table 2. Contamination of 188 test wells with coliform bacteria from the winter of 1991-92 to June 1994. Values are Percentages of tested wells:

Test	Winter 1991-2	Summer 1992	February 1994	March 1994	June 1994
Zero	48.8	47.1	67.0	63.9	51.9
<5	65.1	57.6	80.7	80.6	69.5
>5	34.9	42.4	19.3	19.4	30.5

Table 3. Continuity of well contamination with coliform bacteria

Occasions	Number of wells contaminated on each occasion	% wells contaminated on each occasion
Winter 1991-2 & Summer 1992	48	27.9
February & March 1994	16	9.1
March & June 1994	20	10.6
February, March & June 1994	8	4.5
Winter & Summer 1992 February, March & June 1994	5	2.9

Table 4. Contamination of 188 test wells with Escherichia coli bacteria from the winter of 1991-92 to June 1994. Values are Numbers of wells:

Test	Winter 1991-2*	Summer 1992*	February 1994	March 1994	June 1994
Zero	30 (136)	95 (69)	168	167	168
1 or more	7 (36)	27 (22)	8	13	19
Not tested	151 (16)	66 (97)	12	8	1
Total tested	37 (172)	122 (91)	176	180	187

* Values in parenthesis are for faecal coliform bacteria measured in the original Ontario Farm Groundwater Quality Survey

Table 5. Contamination of 188 test wells with Escherichia coli bacteria from the winter of 1991-92 to June 1994. Values are Percentages of tested wells:

Test	Winter 1991-2*	Summer 1992*	February 1994	March 1994	June 1994
Zero	81.1 (79.1)	77.9 (75.8)	95.5	92.8	89.8
1 or more	18.9 (20.9)	22.1 (24.2)	4.5	7.2	10.2

* Values in parenthesis are for faecal coliform bacteria measured in the original Ontario Farm Groundwater Quality Survey

Table 6. Continuity of well contamination with Escherichia coli bacteria.

Occasions	Number of wells contaminated on each occasion	% wells contaminated on each occasion
Winter 1991-2 & Summer 1992	4	10.8
February & March 1994	2	1.1
March & June 1994	4	2.1
February, March & June 1994	1	0.6
Winter & Summer 1992 February, March & June 1994	0	0

Reliability of results for contamination of well water by E. coli and coliform bacteria

There is no established protocol for collecting water samples from household taps as required for this study. The samples were usually taken from the tap used by the families for the majority of their drinking water. As it was clear that few wells were continuously contaminated, and that contamination was erratic and relatively ephemeral, the reliability of the sampling procedure was questioned. The protocol for sample collection, and an assessment of the error associated with bacterial counts was therefore investigated on nine selected wells. This required the determination of the variation in bacterial count for samples of contaminated water collected over time. A period of 30 minutes was considered appropriate.

The nine farm wells were selected on the basis that they were within 150 km of Guelph, Ontario, and that the previous sample showed the presence of coliform bacteria in the range 10 to 70 colonies per 100mL. The counts used were those obtained during the sampling in March 1994. Characteristics for each of the nine wells showed they were either drilled or bored, and the depth to the water table ranged from 5 to 100 ft (Table 7).

Table 7. Characteristics of Drinking Water Well and Farming Practices on nine selected farms.

Well Code Number	Well Date	Well Type	Well Cap	Well Diam. (in.)	Well Cover	Well Depth (ft)	Dist. to water table (ft)
18	1920	drill	yes	6	steel	50	40
202	1965	drill	yes	6	steel	142	100
253	1940	bored	yes	48	brick	30	10
273	1961	bored	yes	48	brick	16	10
706	1930	bored	no	36	brick	35	5
817	1976	bored	yes	3	concrete	50	10
1022	1972	drill	yes	6	steel	95	85
1140	1915	drill	yes	6	steel	45	25
1591	1968	bored	yes	36	concrete	15	10

Wells were sampled in the period from July 4 to July 6, 1994, and analyzed for total coliform and E. coli (Tables 8 & 9. The mean and the variance were computed for the E. coli counts. The two were not significantly different, indicative that the counts showed a Poisson distribution. An assessment of the variability associated with each count of bacteria colonies can therefore be obtained from standard tables, assuming that the Poisson distribution applies.

Table 8. Counts of Coliform bacteria colonies per 100 mL in well water samples collected over a period of 30 min.

Well Code Number	Time of Sample Collection (min)						
	2	2	5	5	10	20	30
18	2	1	1	0	1	*	*
202	6	9	2	5	1	2	6
253	36	36	32	31	47	42	32
273	29	16	15	30	22	40	52
706	>80	>80	>80	51	>80	51	>80
817	1	1	0	0	0	0	0
1022	0	0	0	0	1	1	0
1140	2	4	3	4	5	0	3
1591	72	>80	>80	>80	>80	>80	>80

*Note: Well ran dry within 11 minutes.

On this basis there were no substantial increases or decreases in any of the counts over the 30 min sampling period (Fig. 2).

Table 9. Counts of Esherichia coli colonies per 100mL in well water samples collected over a period of 30 min.

Well Code Number	Time of Sample Collection (min)						
	2	2	5	5	10	20	30
18	2	1	0	0	0	*	*
202	0	0	0	0	0	0	0
253	36	36	32	31	47	42	32
273	5	5	10	7	3	8	5
706	14	13	21	20	23	20	19
817	0	0	0	0	0	0	0
1022	0	0	0	0	0	0	0
1140	2	1	1	0	1	0	0
1591	3	9	3	10	6	19	9

*Note: Well ran dry within 11 minutes.

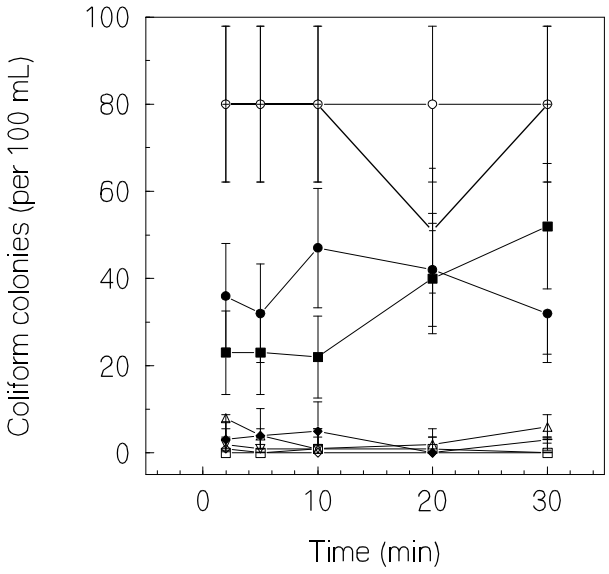

Figure 2. Variation in the total coliform count with time for 9 wells. Bars are ±95% confidence intervals

Relationship with soil hydrological group

In the Ontario Farm Groundwater Quality Survey the contamination of farm wells with bacteria was found to be greater on loamy soils than on coarser-textured materials. The wells in the present study showed a similar trend (Fig. 3). Soils of hydrologic group A are sands and gravels, those of group B are sandy loams, group C soils are silts and silty loams, group D soils are clayey.

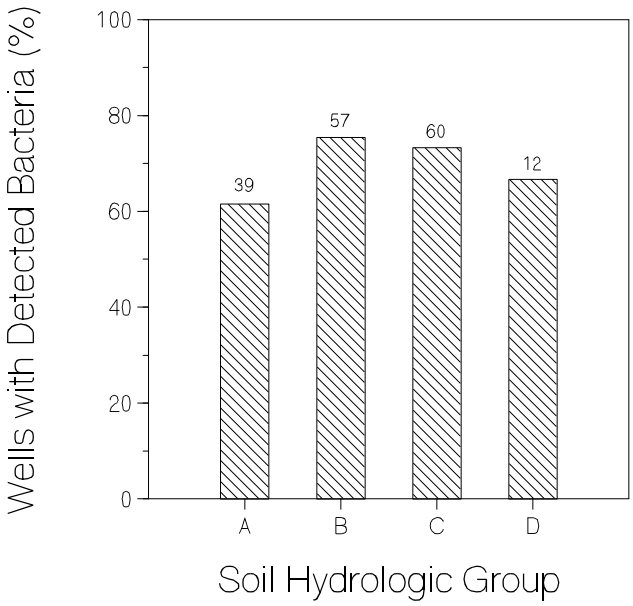

Figure 3. Percentage of wells from soils of different hydrological properties showing the presence of bacteria between January 1992 and March 1994

The general characteristcs of the present selection of wells were considered to be closely similar to those in the earlier study in relation to their bacteriological contamination and the intraction with soil type. The results from the epidemiological study should therefore be readily transferable to similar wells in the Province of Ontario.

Nitrate Contamination

In the preliminary investigations for the present study a number of farms were contacted and their well water tested. During the main study several families had to be eliminated from the epidemiology analysis, but their wells were monitored for water quality.

Water Quality of wells assessed for water quality

The objective this part of the study was to investigate seasonality in the nitrate concentration of farm wells used for drinking water by the family members. A total of 171 wells were monitored for water quality. Based on the results obtained in the Ontario Farm Groundwater Quality Survey, 46 wells exceeded the Ontario drinking water objective for nitrate-N (10 mg N L^{-1}). This represented 27% of the population. This proportion of wells was almost double the proportion found to be contaminated during the original study in 1992. However, as one aim of the present study was to identify the effect on health of any interaction between bacteria and nitrate, half the wells were selected for possible inclusion in the study because the nitrate concentration exceeded 5 mg N L^{-1}. The proportion of wells did not change significantly over the period of the present study (Table 10).

Table 10. Variation in the proportion of 171 wells studied that had nitrate concentration exceeding the Ontario Drinking Water Objective value of 10 mg N L^{-1}.

	Sample collection				
	Winter 1991-2	Summer 1992	February 1994	March 1994	June 1994
Contaminated wells (%)	26.9±6.78	29.9±7.00	22.8±6.42	28.7±6.92	25.7±6.68

Over the whole period from the winter sampling of 1991-1992 to June 1994 there was only a small increase in the mean nitrate-N concentration of the wells (Fig. 4). A major peak in the concentration was noted in March 1993. Despite extensive studies of the methodology employed by the analytical laboratory, including internal quality control procedures, it has proved impossible to confirm that this was a real seasonal event. The results of the June sampling would tend to support the conclusion that an error was made in setting-up the TRAACS system when the March samples were analyzed. The samples were frozen immediately after analysis, and on thawing the repeat analysis gave substantially smaller values.

However, a test carried out on samples that were analyzed before and after freezing also gave smaller values after thawing. The time samples were stored in the refrigerator before freezing also affected the magnitude of the reduction in the apparent concentration, so no reasonable correction was available to apply to the March data.

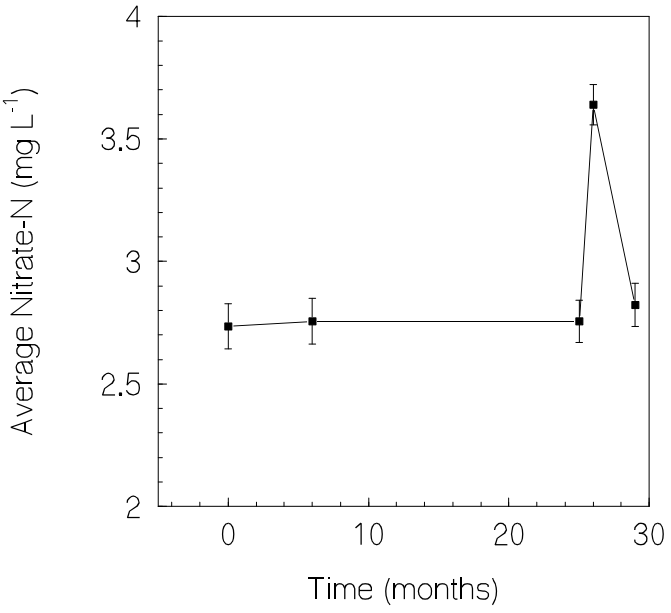

Figure 4. Seasonal variation in nitrate concentration in selected wells. Values are geometrically averaged nitrate concentrations with standard errors.

Relationship between nitrate contamination and land use.

As found in the Ontario Farm Groundwater Quality Survey, nitrate contamination was widely spread among land-use classes identified according to those given in the Agricultural Resources Inventory (Fig. 5). No land-use class was found to be associated with a significantly greater number of contaminated wells than any other class.

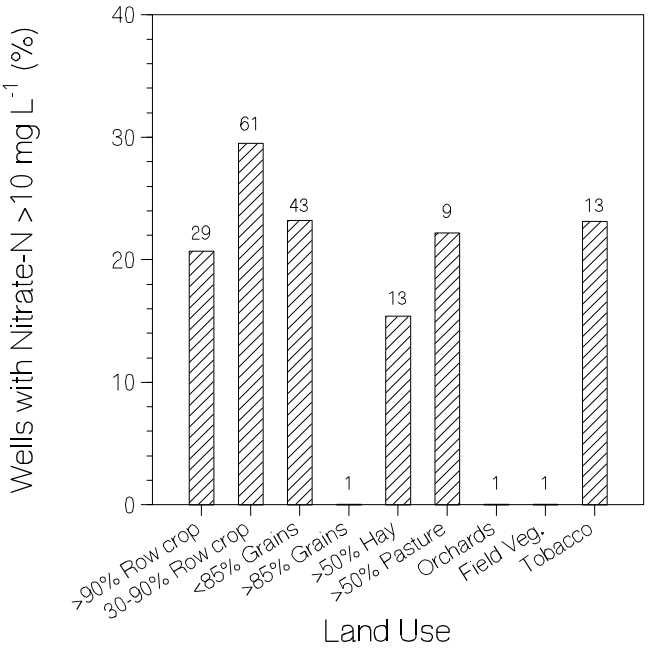

Figure 5. Relationship between nitrate contamination and land use. Bars are percentage of wells contaminated in each land-use class. Numbers of wells in each class are also shown. The average concentration of all five samples were used to identify contaminated wells.

Pesticide Contamination

Pesticides had only been detected during the Ontario Farm Groundwater Quality Survey in 18 of the wells selected for further investigation. Atrazine and its metabolite D-ethyl atrazine were the main contaminants. One well contained metolachlor, another contained cyanazine and one well contained both metalochlor and metribuzin as additional contaminants.

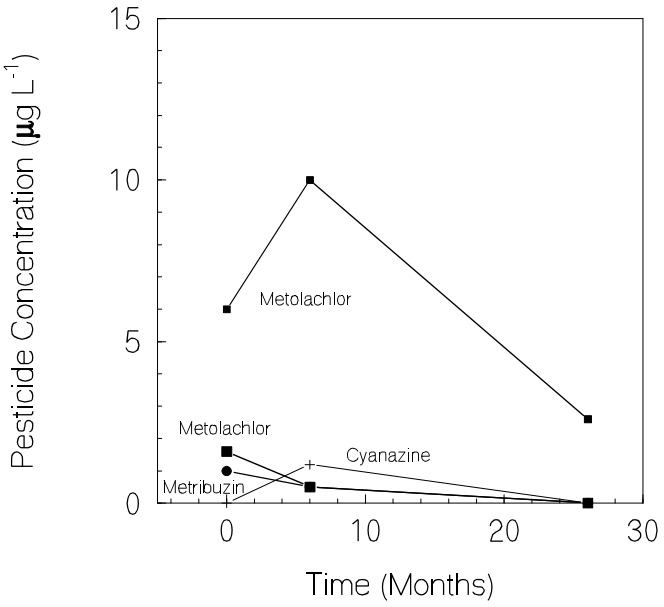

Figure 6. Variation in concentration of infrequent contaminant pesticides from January 1992. Values are for different wells.

In February and March 1994 atrazine and D-ethyl atrazine were again the main contaminant pesticides. The concentrations of the additional contaminants all declined over time (Fig. 6). However, the pattern of change varied for the different pesticide species (Fig. 7).

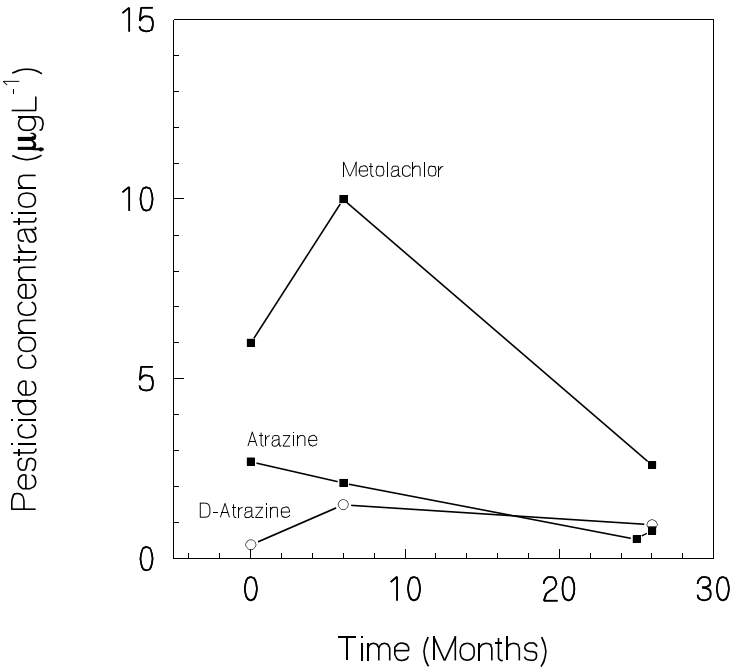

Figure 7. Variation of three contaminant pesticide species in a single well from January 1992.

On average, the concentration of atrazine decreased at a rate of 0.016±0.0065 µg L^{-1} per month over the period from the winter of 1991-2 to March 1994 (Fig. 8). The concentration of D-atrazine tended to decline after the summer of 1992 (Fig. 9).

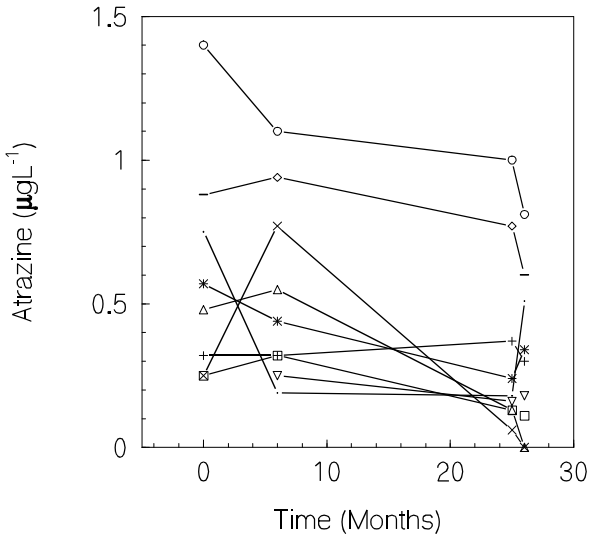

Figure 8. Variation in Atrazine concentration in 9 wells with a measurable concentration in January 1992

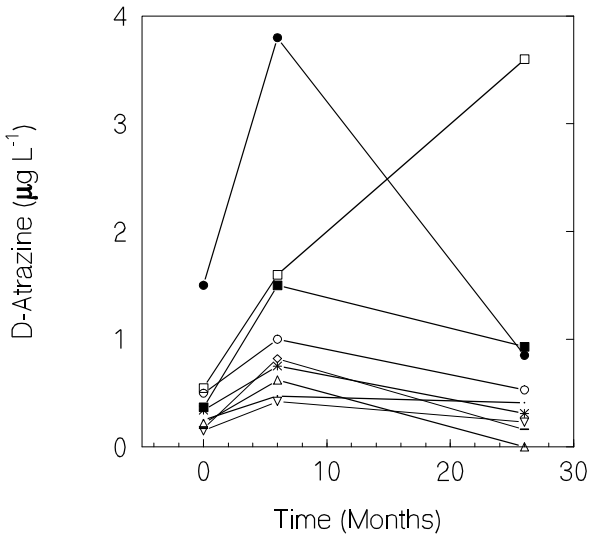

Figure 9. Variation in D-ethyl atrazine concentration in 9 wells with a measurable concentration in January 1992

However, in March 1994 some 16 additional wells contained measureable quantities of D-ethyl atrazine.

Generally there was no correlation between the concentration of atrazine and its breakdown product D-ethyl atrazine (Figs.10 & 11).

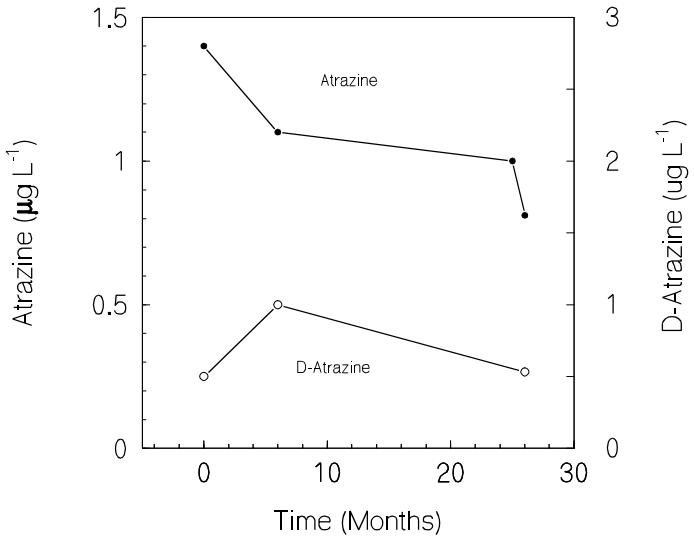

Figure 10. Variation in the concentration of Atrazine and its daughter D-ethyl atrazine in a single well from January 1992.

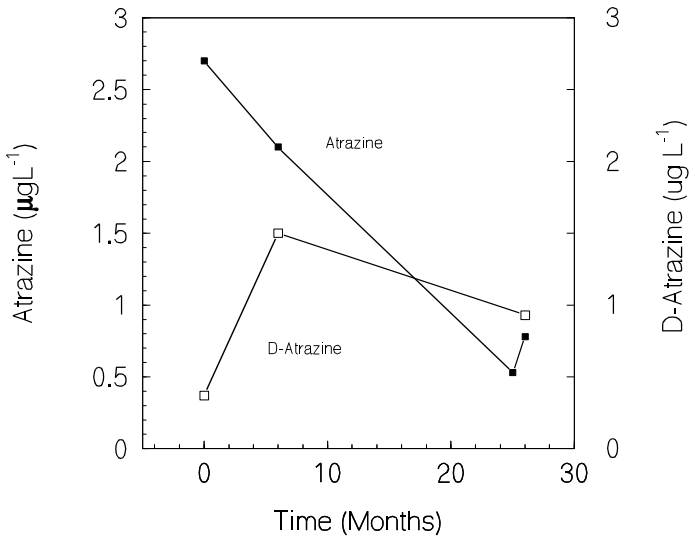

Figure 11. Variation in the concentration of Atrazine and its daughter D-ethyl atrazine in a single well from January 1992.

3. Health of farm families

The health of the farm families has been assessed through an epidemiology approach. The results are based on records collected during February and March 1994, and should therefore be considered as giving only preliminary information.

Full information was available for 157 farms, with an average of 3.55 family members per farm.

The average prevalence of diarrhoea reported during the months of February and March on farms was 26%. There was no significant change from this level of prevelance irrespective of the sub-group of farms investigated.

Diarrhoea was reported by 11% of family members (Table 11). On average, each family member reporting an incidence of diarrhoea recorded 1.26 incidences, each lasted an average of 2.16 days, resulting in 2.72 days of illness. Because the distribution of days sickness was skewed, the data was adjusted geometrically. Using the adjusted data, the average length of sickness reported by sick members was 1.93 days, each episode of diarrhoea lasting 1.53 days.

Using the information on water quality obtained in the original Ontario Farm Groundwater Quality Survey in 1992, there was little evidence that the families using wells contaminated with bacteria had any great differences in incidence of acute gastro-intestinal disease (Table 12a). However, compared with people drinking better quality water, the duration of sickness was slightly greater in family members who became ill on farms with wells that had been contaminated in 1992 (1.8 days compared with 1.3 days per episode).

When the water quality prevailing during the study was used, the duration of sickness was still greater in family members who became ill on farms with wells that were contaminated (2.1 days) compared people drinking better quality water (1.4 days) (Table 12b). When water quality was assessed according to whether any bacteria had been found in the well or not, the difference in the duration of sickness was smaller still (1.7 days for people drinking contaminated water compared with 1.4 days for those drinking bacteria-free water (Table 13).

The wells had also been selected according to the assessment for nitrate contamination. This allowed the interaction between bacterial contamination and nitrate contamination on the incidence of diarrhoea to be investigated (Table 14). There were 50 wells that had no bacteria present in February or March 1994 and contained less than 5mg N L^{-1}, 36 wells that also had no bacteria but had elevated levels of nitrate, 41 wells had some bacteria but little

nitrate, and 30 wells that had bacteria and an elevated nitrate concentration. At nitrate concentrations less than 5 mg N L^{-1} there was little effect of the presence of bacteria, but with elevated levels of nitrate the impact on the duration of diarrhoea was more than 0.5 days. The results suggest an interaction between the two contaminants on the ability of family members to recover from an incidence of diarrhoea.

Table 11. Summary of epidemiology health record information on acute gastro-intestinal episodes.

All farms	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	157	555	61	77	166	118
per farm		3.54	0.39	0.49	1.06	0.75
per family member			0.11	0.14	0.30	0.21
per member reporting sickness				1.26	2.72	1.93
per episode of diarrhoea					2.16	1.53

Table 12a. Relationship between the incidence of diarrhoea and bacteriological contamination of well water.

Well water quality based on results from 1991-1992						
Results for bacterial colonies <MAC	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	80	276	29	41	83	55
per farm		3.45	0.36	0.51	1.04	0.68
per family member			0.11	0.15	0.30	0.20
per member reporting sickness				1.41	2.86	1.88
per episode of diarrhoea					2.02	1.33
Results for bacterial colonies >MAC	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	77	279	32	36	83	63
per farm		3.62	0.42	0.47	1.08	0.82
per family member			0.11	0.13	0.30	0.23
per member reporting sickness				1.13	2.59	1.98
per episode of diarrhoea					2.31	1.76

Table 12b. Relationship between the incidence of diarrhoea and the bacteriological contamination of well water.

Well water quality based on results for February and March 1994						
Results for bacterial colonies <MAC	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	113	390	44	59	110	79
per farm		3.45	0.39	0.52	0.97	0.70
per family member			0.11	0.15	0.28	0.20
per member reporting sickness				1.34	2.50	1.81
per episode of diarrhoea					1.86	1.35
Results for bacterial colonies >MAC	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	44	165	17	18	56	38
per farm		3.75	0.39	0.41	1.27	0.87
per family member			0.10	0.11	0.34	0.23
per member reporting sickness				1.06	3.29	2.26
per episode of diarrhoea					3.11	2.13

Table 13. Relationship between the incidence of diarrhoea and the presence of bacteria in well water.

Well water quality based on results for February and March 1994						
Results for bacterial colonies 0/100mL	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	86	290	34	46	93	65
per farm		3.37	0.40	0.53	1.08	0.76
per family member			0.12	0.16	0.32	0.22
per member reporting sickness				1.35	2.74	1.91
per episode of diarrhoea					2.02	1.41
Results for bacterial colonies >1/100mL	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	71	265	27	31	73	53
per farm		3.73	0.38	0.44	1.03	0.74
per family member			0.10	0.12	0.28	0.20
per member reporting sickness				1.15	2.70	1.95
per episode of diarrhoea					2.35	1.70

Table 14. Interaction between bacterial and nitrate contamination on the incidence of diarrhoea.

Well water quality based on results for February and March 1994.						
Results for bacterial colonies 0/100mL & nitrate <5 mg L^{-1}	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	50	173	18	21	43	32
per farm		3.46	0.36	0.42	0.86	0.64
per family member			0.10	0.12	0.25	0.19
per member reporting sickness				1.17	2.39	1.78
per episode of diarrhoea					2.05	1.53
bacterial colonies 0/100mL & nitrate >5 mg L^{-1}	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	36	117	16	25	50	34
per farm		3.25	0.44	0.69	1.39	0.94
per family member			0.14	0.21	0.43	0.29
per member reporting sickness				1.56	3.13	2.12
per episode of diarrhoea					2.00	1.36

Table 14. Cont...

bacterial colonies 1 or more /100mL & nitrate <5 mg L^{-1}	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	41	153	20	20	44	32
per farm		3.73	0.49	0.49	1.07	0.79
per family member			0.13	0.13	0.29	0.21
per member reporting sickness				1.00	2.20	1.62
per episode of diarrhoea					2.20	1.62

bacterial colonies 1 or more/100mL & nitrate >5 mg L^{-1}	Farms	Family members	Family members reporting sickness	Reported episodes of diarrhoea	Days sickness	
					Total	Geometrically adjusted
Number	30	112	7	11	29	21
per farm		3.73	0.23	0.37	0.97	0.70
per family member			0.06	0.10	0.26	0.19
per member reporting sickness					4.14	2.98
per episode of diarrhoea					2.64	1.90

CONCLUSIONS

Seasonal Variation in Water Quality

The primary selection of wells for this study was based on the needs for the epidemiology investigation. Nevertheless, the population of wells covered a range of depths from less than ten feet to more than two hundred feet. Furthermore, the relationships between bacteriological contamination and soil hydrologic group, and nitrate contamination and land-use class in these wells showed similar patterns to those observed in the 1200 wells studied in the Ontario Farm Groundwater Quality Survey as reported by Rudolph and Goss (1993). The proportion of wells exceeding the Ontario Objectives for the quality of drinking water (MOE, 1992), were expected to be greater than reported by Rudolph and Goss (1993), but although this was true for nitrate, the proportion for bacteria was closely similar in the two studies. The sample of wells selected for study therefore appears to have similar characteristics as the main population studied in 1991 to 1992, and suggests that any conclusions drawn in the epidemiology investigation should be applicable to the whole Province.

There was no clear indication of seasonality in the nitrate quality of the well water. The sharp peak reported for March 1994 looks to be the result of an analytical error. The trend in the data is a slight, but not significant increase in the mean.

There was some indication of seasonality in the pesticide quality of the well water, mainly because of the increase in the number of wells with measureable concentrations of D-ethyl atrazine in March. This would appear to be the result of the flushing of soil by snow melt-water. The trend in the data is a decline in the concentration of atrazine, metolachlor, metribuzin and cyanazine.

The bacterial contamination showed considerable variation. Based on the results of the Ontario Farm Groundwater Quality Survey, at least half the wells were expected to exceed the Ontario drinking water Objectives. In the event the values were closer to one third. Temperature could well have been an important factor. During the Ontario Farm Groundwater Quality Survey it was noted that for the winter sampling there was a smaller proprtion of contaminated wells in Northern Ontario than in the south of the Province. For the summer sampling there were no differences. The unusually cold winter of 1993-4 could be the explanation for the smaller frequency of bacteriological contamination reported here.

Epidemiology of Drinking Contaminated Well Water

The results from the epidemiology study should be treated as extremely preliminary. Early indications suggest that the impact of poor quality water, as defined by the presence of indicators of pathogenic organisms, is not to increase the incidence of acute gastro-intestinal episodes but prolongs the duration of the event. The suggestion that there is an interaction between bacteria and nitrate in prolonging the number of days that people report having diarrhoea, needs further study. A mechanism for such an interaction could be the formation of methaemoglobin in the blood by nitrite, which was produced by the reduction of nitrate by the bacteria in the intestinal tract. The presence of methaemoglobin may slow the activity of the immune system.

The study has been planned to continue until February 1995. This report will be superceeded by one prepared at that time.

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APPENDIX

Agar Composition for Bacteriological Plates

Formulation of m-ENDO-LES AGAR

Yeast extract	1.2 g
Casitone or trypticase	3.7 g
Thiopeptone or thiotone	3.7 g
Tryptose	7.5 g
Lactose	9.4 g
Potassium Hydrogen phosphate	3.3 g
Potassium dihydrogen phosphate	1.0 g
Sodium chloride	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulphate	0.05 g
Sodium sulphite	1.6 g
Basic fuchsin	0.8 g
Agar	15.0 g
Distilled water	1000 mL

Reference: A.W.W.A. and A.P.H.A. 1985. Standard Methods for Water. 16th ed.

Formulation of m-FC AGAR

Tryptose	10 g
Proteose Peptone No. 3, Difco	5 g
Yeast Extract	3 g
Lactose	12.5 g
Bile Salts No. 3	1.5 g
Sodium Chloride	5 g
Agar	15 g
Aniline Blue	0.1 g
Distilled water	1000 mL
Final pH 7.4 ± 0.2 at 25EC	
Rosolic acid	150.0 mg
0.2N NaOH	15.0 mL

Reference: Difco Manual. 1984. 10th ed. pp. 351-353.

Pesticide Analysis Methodology

Method 1: solid phase extraction for water samples

Sample Preparation

Well water without particulate matter was transferred directly to a 100 mL. cylinder. If samples had particulate matter, sub-samples were filtered through a glass filter paper on a Buchner funnel using suction.

Method 2: liquid/liquid extraction for water

Approximately 800 mL) of sample water was poured into a 1000 mL separatory funnel,adjusted pH to 9 with ammonium hydroxide (dil 1:2.5). Separation was carried out in 100 mL of chloroform ($CHCl_3$).

The chloroform phase was drained through $CHCl_3$-pre-washed and dried cotton into a 500 mL boiling flask. The extraction was repeated with 100 mL of $CHCl_3$.

The combined $CHCl_3$ extracts were evaporated on a rotary evaporator (50-60EC water bath) almost to dryness, before 10 mL of iso-octane were added and the sample evaporated to dryness. The resulting triazine residues were dissolved in 5 mL of methanol.

Experimental conditions and equipment for analysis

Gas Chromatograph: Hewlett Packard 5890

Detector: OI-Nitrogen

Column: HP-1(Crosslinked Methyl Silicone Gum)
30 m x 0.53 mm
2.65 µm film thickness
Hewlett-Packard

Carrier Gas: He
20 psi
30 cm sec^{-1} linear velocity

Oven Profile:

Temperature 1	170EC
Hold	7 minutes
Ramp Rate 1	30E min^{-1}
Temperature 2	200EC
Hold	0 minute
Ramp Rate 2	5E min^{-1}
Temperature 3	250EC
Hold	6 minutes

Injector Temperature: 250EC

Detector Temperature: 250EC

Injection: 2 µl, split 10:1

Alternate: Perkin Elmer 8500 Gas Chromatographs
Hall Detector (Nitrogen mode) and
Nitrogen/Phosphorous detectors
(Conditions similar as above)

Elisa: Commercial Kit -Agri Diagnostics Associates, Moorestown N.J.
Type: 96 well plate