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HEALTH ASPECTS OF ORGANICS IN DRINKING WATER (H 4298CD)

Final Report to the Department of the Environment

J K Fawell, R F Lacey,
M Fielding, J W Ridgway,
H Horth, P Wilcox,
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WATER RESEARCH
WRc
ENVIRONMENT

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SUMMARY

In April 1977, the Department of the Environment placed a contract with the Water Research Centre with the overall objective of evaluating the possible health effects of long-term consumption of drinking water derived from sources containing sewage effluent or industrial effluent. This contract ended in March 1981 but was renewed for 3 years finally ending in March 1984. During the second contract, the objective was modified so that the emphasis was changed from a specific investigation of re-use to an assessment of organic compounds in general.

This final contract report summarises the main lines of investigation in each of the four components of the programme of work: epidemiology, mutagenicity, water quality studies and toxicology. The principal findings are summarised and discussed briefly.

The overall conclusion of the original contract concerning the adverse health effects of re-use was that although potentially harmful chemicals are present in water at very low levels, their presence can not be specifically attributed to re-use. Assessment of the toxicology of these compounds led to the conclusion that any risk was probably of a low order. The epidemiological studies of community health also suggested that the effects of re-use were very small. However, the application of short-term screening tests has shown that mutagens will normally be present in drinking water as a result of water treatment chlorination. It has also been shown that much of the organic matter remains unidentified and is consequently of unknown significance to health. Therefore, in terms of the objective of the second phase of the contract, that of investigating the presence of organic compounds in general rather than re-use in particular, the effect of such compounds on health is probably small but remains unquantified.

In order to better assess and quantify these risks to health a number of recommendations for further work are made including: studies of disinfection byproducts; characterisation of non-volatile organics; characterisation of sources of organic pollution of groundwater and contamination in distribution; assessment of the effects of treatment on mutagenic activity and the characterisation of mutagens in water; development of mutagenicity and cytotoxicity assays based on higher cell systems; fundamental research into those aspects of toxicology relating to low doses, long-term exposure and complex mixtures and into the mathematical techniques for extrapolating from high to low doses; epidemiological studies based on individuals rather than geographical areas.

PREFACE

On 1st April 1977, the Department of the Environment placed a contract (Ref. DGR/480/294) with the Water Research Centre to evaluate the possible health effects of the long-term consumption of water from sources containing sewage effluent or industrial effluent. This contract ended on 31st March 1981 but was renewed for three years beginning on 1st April 1981 and ending on 31st March 1984 (Ref. PECD 7/7/015).

This final report summarises the work undertaken between April 1977 and March 1984.

CONTENTS

1. INTRODUCTION

1.1 RE-USE IN THE UNITED KINGDOM

Approximately one third of the water supplies in the United Kingdom are derived from lowland rivers. These rivers are, however, also used for conveying both treated domestic and industrial wastewater to the sea. As these rivers are mostly short and often receive waste from large populations, the proportion of wastewater in the river flow can be large, especially during dry periods. It has been estimated[1] that at least 7 million consumers receive water that, on average, contains over 10% sewage effluent. In the Thames, the Lea and the Great Ouse at points of major abstraction, the proportion of sewage effluent can, at times, be high (Table 1).

Table 1 Sewage effluent as a proportion of total river flow at selected water supply abstraction points (1973 figures)

River	Abstraction point	Effluent as proportion of river flow (%)	
		Average	Maximum*
Great Ouse	Foxcote	1.9	17.7
Great Ouse	Clapham	6.8	52.0
Great Ouse	Offord	12.2	58.3
Lea	New Gauge	16.5	81.4
Thames	Swinford	4.6	33.8
Thames	Sunnymeads	11.9	99.8
Thames	Staines	12.1	101.4
Thames	Surbiton	14.4	141.4

* Using river flow exceeded for 95% of the time

Thus, in the UK, a considerable degree of "indirect" re-use takes place whereby water, abstracted for drinking purposes contains treated wastewater from communities upstream.

These discharges affect the chemical quality of the receiving waters but, at the start of this project, no precise information was available to quantify this. It was possible that there would be an increase of both non-biodegradable compounds and the metabolic products derived from those substances which had been degraded by bacteria.

Although drinking water derived from such sources is considered wholesome, concern about the presence of organic compounds has developed for a series of reasons. Medical research has shown that environmental factors can have an effect on the incidence of certain diseases, including cancers of the gastro-intestinal and urinary tracts, thereby stimulating medical interest in the role of water as a risk factor. There has also been an increasing application to water of advanced analytical techniques which has shown that such waters contain, at very low levels, potentially hazardous organic substances including carcinogens. Concern was heightened by the publication of a study which showed an increase in neoplasms in men drinking water derived from the River Mississippi[2,3]. A meeting, subsequently organised by the World Health Organisation (WHO) to discuss possible health effects of wastewater re-use, concluded that although data on chemical composition of drinking water derived from polluted sources was essential, this had to be complemented by epidemiological and toxicological studies[4].

Therefore, where there is a substantial commitment to using such sources of drinking water, as there is in the UK, these concerns justify careful investigation, as any decision to reduce the use of such sources of drinking water would involve heavy costs. It was with these concerns in mind that the Department of the Environment (DOE) commissioned work at the Water Research Centre (WRc) to investigate the significance to health of organic compounds in drinking water.

1.2 DESIGN OF RESEARCH PROGRAMME

When designing this research programme, WRc took into account research being conducted elsewhere in the world, especially in the United States, the Netherlands, South Africa and Israel. The proposals were discussed with experts from these and other countries in the presence of representatives of the water industry in the UK at a WRc Colloquium[5]. In addition, the proposals were extensively discussed at the DOE/Department of Health and Social Security (DHSS) Joint Committee on the Medical Aspects of Water Quality (MAWQ)[6,7,8].

It was assumed that any adverse health effects of wastewater re-use would arise from the consumption of water containing harmful chemical substances, over a long period of time. The proposed research programme was designed, therefore, to compare situations of varying degrees of re-use to determine:

(a) whether there are significant differences in the health of the respective populations, particularly in relation to cancer and other chronic diseases which can be correlated with the degree of re-use;

(b) the nature and concentration of substances present in drinking water as a result of a re-use;

(c) the relative toxicity of these substances to man.

Importance was attached to the fact that in the proposed programme, the three approaches were to be developed simultaneously thereby providing scope for investigating the problem in a series of contrasting ways.

1.3 CONTRACT AND OBJECTIVES

On the 1st April 1977 the DOE placed a contract (Ref. DGR/480/294) with WRc which ended on 31st March 1981. The overall objective of the programme of work was to evaluate

possible health effects of long-term consumption of drinking water derived from sources containing sewage effluent or industrial effluent.

This contract was renewed for 3 years running from 1st April 1981 to the 31st March 1984 (Ref. PECD 7/7/015). During this second contract the overall objective was modified so that the emphasis was changed from specific investigation of re-use to an assessment of organic compounds in general, the objective being to evaluate possible health effects associated with the presence of organic compounds in drinking water. This change reflected the way in which the study had developed during the first contract.

Throughout both contracts the work fell into three broad categories - epidemiology, toxicology (including mutagenicity) and water quality studies. However, the emphasis given to each of these components varied. Initially epidemiology and water quality studies were major features of the programme but towards the middle of the project the toxicological aspects became of increasing importance, becoming the major component in the last two years.

This report summarises the principal findings of the work undertaken to fulfill both contracts between April 1977 and March 1984. A list of other reports associated with this contract is given in the Appendix.

2. EPIDEMIOLOGICAL STUDIES

2.1 INTRODUCTION

The most direct approach to assessing the possible effects of organic compounds in drinking water on health is by attempting to observe such effects in the human community. This line of research falls under the general heading 'epidemiology', which has been defined as "the study of the distribution and determinants of disease frequency in man"[9].

In relation to organic compounds in water, the health phenomena that have received most attention have been diseases in the cancer group. There have been several epidemiological studies from other countries which have shown associations between cancer mortality and river water as the source of drinking water[10,11,12]. Different studies have focused on different aspects of water supply (type of supply, chlorination practice, trihalomethane concentration), and have examined cancers in different parts of the body (cancer sites) with varying results. Such evidence as was available in 1976 was conflicting and controversial and justified a carefully planned investigation in Britain.

Epidemiological studies may be based on whole communities, on selected sub-groups or on individuals. The principal studies covered by this contract have been of the first type and have been based on contrasting the health statistics of different boroughs or towns served by different water supplies. The methods and results of these studies are outlined in Sections 2.2 and 2.3 below. The research also included a feasibility study for a further research project based on data related to individual people, in a 'case-control' design. Such a case control study has not actually been carried out, but the feasibility has been established and reported to the Department. This is discussed briefly in Section 2.4.

The community studies that were carried out did not involve any procedures requiring qualified clinicians but, at the outset of this work, WRc's experience of epidemiological research was limited. The epidemiological part of this contract was therefore undertaken by placing a subcontract with an established medical research department. This was the Department of Clinical Epidemiology and General Practice (formerly Social Medicine) at the Royal Free Hospital School of Medicine (RFH). The work there was undertaken principally by Dr S A A Beresford and supervised by Professor A G Shaper. The necessary information about water supplies was provided to the RFH by WRc.

2.2 APPROACH

Although we would have liked to investigate possible effects of specific organic compounds in water, suitable analytical data were not obtainable. More general ways of characterising the water supplies had therefore to be adopted. Whilst the initial reason for this work came from concern over the increasing practice of re-using water for potable supplies, not all organic matter in water arises from man's activities. There are many humic and fulvic substances naturally present in surface supplies. Treatment process such as clarification should reduce the levels of such compounds but, on the other hand, chlorination is known to produce chlorinated organic compounds which can be more biologically active than their precursors.

Based therefore upon the results of the previous studies, and upon the data that could be made available, two distinct water hypotheses were proposed:

(a) that the health of a population may be affected by the degree of re-use of its drinking water supplies;

(b) that the health of a population may be affected by the type of water source (groundwater, river, upland catchment).

In relation to these two ways of classifying water supplies two types of health indicator were studied:

(i) mortality or morbidity rates of cancers of the digestive system and urinary tract (which may need long-term exposure to possible causative agents);

(ii) more immediate possible health outcomes, i.e., still-births, infant mortality and fertility rates.

The combination of (a) or (b) with (i) or (ii) thus gave four types of study, concerned with questions that are quite distinct in the sense that the answer to one does not necessarily imply the answers to any of the others. The forms that these studies took, in particular with regard to the sets of communities that were included, were determined principally by the availability of data. An overall plan is shown is Table 2.

The concept of 're-use' was quantified in two different ways in different contexts. For the London boroughs it was possible to characterise the extent of re-use of the water supply to a given borough in terms of proportion of that supply that would have been derived from sewage effluent at a previous stage in the water cycle. This was possible because the quantities of discharge to and flows in the Rivers Thames and Lea were known in sufficient detail. For the water supplies to the larger number of 141 urban areas in Great Britain, this information was, however, not available and it was possible only to formulate a cruder descriptor based on the population density of the catchment areas from which their river abstractions were taken. ('Lowland' in this context means not supplied with water from upland catchments. The reason for this restriction will become clear later when the results are discussed).

The 238 urban areas in Great Britain included all major centres of urban population in England, Scotland and Wales. These were the areas on which data had been collected for an earlier study of cardiovascular disease[13]. The '141 urban areas supplied with lowland water' were the subset whose distributed waters contained less than 10% from upland catchment sources.

The term 'cancer' in Table 2 covers cancers at a number of different body sites. These should be regarded as completely different diseases and a comprehensive aggregation of sites is not biologically meaningful[14]. The analyses undertaken in

Table 2 Summmary of epidemiological studies undertaken - sets of communities on which they were based

FORM OF WATER HYPOTHESIS	HEALTH INDICATOR (i) CANCER Mortality	Morbidity	(ii) OTHER OUTCOMES
(a) RE-USE			
Quantified by % effluent	29 London Boroughs	14 South London Boroughs	27/29 London Boroughs
Quantified by contributing population	141 Urban areas supplied with lowland water	——	——
(b) TYPE OF SOURCE (Upland/river/ groundwater)	238 Urban areas in Great Britain	——	238 Urban areas in Great Britain

these studies concentrated on the particular sites or groups of sites set out in Table 3. Rates of mortality and morbidity were calculated from death and registration data centred on the National Census of 1971. Rates were calculated for males and females separately (except for infants). For reasons of comparability the morbidity study was restricted to a set of boroughs, the 14 in London south of the Thames, that are covered by a single cancer registry.

The statistical methodology of these studies has been described in detail elsewhere[15] but it is important to note two general features. First, we went to great lengths to ensure that comparisons between communities were standardised for age and adjusted for relevant differences in socio-economic conditions. Second, much care was taken over the judgement whether observed differences in health indices, associated with different qualities of water supplies, were greater than could be reasonably attributed to chance fluctuations in mortality or morbidity rates. The method of assessment (significance test) was modified to allow for differences in the sizes of communities being compared and (where possible) the extent of their geographical proximity (spacial correlation).

2.3 RESULTS

Most of the health indicators studied were found not to be associated with the quality of water supplies. Those analyses where significant ($p < 0.05$) associations were found are summarised in Table 4. This table lists the health indicators associated with one or other of the descriptors of water quality, and whether or not the finding emerged from an analysis 'weighted' for borough size.

It should be noted that the part of the analysis which investigated the differences between the health ratings of communities served by different types of water source reduced to a contrast between the upland catchments and the rest (there

Table 3 Cancer sites covered in the studies

London mortality study (29 boroughs)

Gastro-intestinal (ICD* 151-154)
Stomach (ICD 151)
Intestinal (ICD 152-154)
All urinary (ICD 188-189)
Bladder (ICD 188)
Oesophageal (ICD 150)
All cancers (ICD 140-209)

South London morbidity study (14 boroughs)

Gastro-intestinal (ICD 151-154)
Stomach (ICD 152-154)
Colo-rectal (ICD 153-154)
Colonic (ICD 153)
Rectal (ICD 154)
All urinary (ICD 188-189)
Oesophageal (ICD 150)

Mortality studies in urban areas in Great Britain (141 or 238 urban areas)

Gastro-intestinal (ICD 151-154)
Stomach (ICD 151)
Intestinal (ICD 188-189)
All urinary (ICD 18-189)
Bladder (ICD 188)
Oesophageal (ICD 150)

* International Classification of Diseases code numbers

being no apparent difference between rivers and groundwater in this part of the study). The health indicators listed against (b) in Table 4 all showed higher rates of illness associated with the upland supplies. (This was the reason why communities receiving more than 10% of their supplies from upland sources were excluded from the analysis of re-use.)

In the parts of the study concerned with the notion of 're-use' it was the waters with higher degrees of re-use that showed the less favourable health statistics. The unre-used water in this part of the study was principally groundwater. The results of analyzing the two different water hypotheses, (a) and (b), thus do not combine to give a consistent picture but have to be regarded as quite separate.

None of the associations listed in Table 4 achieved a high level of statistical significance and so the evidence for real effects is weak. The estimated sizes of the possible effects of water quality were also small in comparison to other risk factors for chronic diseases. For example, the analysis of female stomach cancer incidence in relation to re-use estimated an increase of 11% in the incidence rate for that type of cancer, in changing from zero re-use to the level existing in the River Thames. The other effects listed in Table 4 are associated with differences of similar orders of magnitude. With the still-birth analysis there was an unresolved technical problem, possibly due to our inability to standardise the data well enough in this case, and this could affect the significance of the estimated association with re-use.

The results and the strengths and weaknesses of the methodology of these studies have been discussed at length in a report to the Department, which has been published as a WRc Technical Report[15]. The overall lack of consistency between the results for men and women, and between slightly different methods of analysis (weighted and unweighted), and the rather low

Table 4 Summmary of significant associations

WATER DESCRIPTOR	HEALTH INDICATOR (i) CANCER Mortality	Morbidity	(ii) OTHER OUTCOMES	
(a) RE-USE				
Quantified by % effluent	None	Stomach (women) (unweighted)*	Still-births (unweighted)** (weighted)*	significance perhaps not correctly assessed
Quantified by contributing population	Stomach (men) (weighted)*	-	-	
(b) TYPE OF SOURCE (% Upland)	Gastro-intestinal (women) (unweighted)* Stomach (women) (unweighted)** (weighted)*	-	'Extended perinatal' mortality (unweighted)*	

* $P < 0.05$

** $P < 0.01$

levels of significance reported, are all grounds for caution in interpreting the results of this work. In addition to all of these reservations, the designs of these studies were not of a form that can provide firm ground for inference that any of the associations found were directly causal.

2.4 FEASIBILITY OF FURTHER STUDIES

Although the results of this research and the evidence from other countries are not clear-cut and conclusive, there have been enough positive findings to maintain the original hypotheses and to underline the needs for further research. The limitations of aggregate population studies such as those described here can be overcome only by studies based on individuals. Whilst these also have their difficulties, the possibility of these kinds of study in the UK was therefore explored.

A feasibility study was undertaken of further research based on retrospective case-control methodology, using living cases[16]. Several forms of study were considered and the most promising was designed in outline and costed. Such a study would have to be based on a hospital or group of hospitals serving a large enough area of population, and would entail a greater degree of medical involvement than WRc could provide. The decision whether or not such a study should be undertaken would depend on:

(1) whether the information that it could provide is considered to be worthwhile,

(2) whether a suitable contractor could be found who is able and willing to undertake the project.

It has not been possible to make very clear recommendations on either of these questions. One reason for hesitancy is the lack of specificity of the hypothesis to be investigated. If this could be narrowed to a more specific aspect of water quality

and to a specific disease or medical condition, there would be greater hope of designing a study capable of making a tangible advance, or of determining conclusively that such a requirement could not be met.

3. MUTAGENICITY TESTING OF DRINKING WATER

The specific objectives of this part of the research programme were to determine whether potentially carcinogenic substances are present in drinking water, the nature of such substances and whether their presence is related to the degree of contamination of the water source with sewage effluent or industrial effluent.

There is no practical way in which carcinogens at concentrations likely to be encountered in raw or treated water can be detected in experiments with mammals. Mammalian tests are insensitive and to compensate for this relatively high doses of a substance under examination would normally have to be used. It is possible that these problems could be overcome by concentrating the substances present in drinking waters. It is debatable, however, whether the concentrations of an active agent that could be attained would be high enough to give an unambiguous result in a test with mammals. Nevertheless, such tests could prove useful if active constituents were to be identified and tested independently.

The unsuitability of mammalian tests to investigate this problem was discussed at MAWQ[8], and following these discussions, it was decided to make use of bacterial screening tests for the detection of mutagens in water rather than to test directly for carcinogenicity using animal tests. Much work has been done to demonstrate that a good correlation exists between the ability of chemicals to induce mutation in bacterial assays and the induction of cancer in long-term toxicity tests with animals[17].

A programme of work based on the use of mutagenicity assays containing 3 phases of study was designed:

Phase 1. Preparatory work, development and evaluation of suitable methods.

Phase 2. Survey phase, testing a range of waters, taking account of different types of water source, extent of re-use, seasonal variations and water quality variations in distribution. Systematic fractionation and chemical analysis of positive samples in order to identify potentially hazardous substances.

Phase 3. Follow up studies involving confirmatory tests in higher systems.

The work undertaken in Phase 1 will be outlined in the remainder of this section whilst Phase 2 will be the subject of sections 4 and 5. No work was undertaken, as part of this contract, for Phase 3. This is, however, the subject of a separate DOE funded project entitled "Biological Screening Tests for the Assessment of Drinking Water Quality". This contract (DOE Ref. No. PECD 7/7/94 - 94/83 began in August 1983 and ends in July 1986.

3.1 BACTERIAL MUTAGENICITY SCREENING TESTS

The mutagenicity assay selected for the examination of water samples was the Salmonella/microsome assay developed by Ames and co-workers in the early 1970s. This assay uses a set of strains of Salmonella typhimurium which contain well-characterised mutations in certain genes which code for enzymes involved in the biosynthesis of histidine. As a consequence, these strains cannot produce their own histidine and will only grow if this amino acid is supplied in the growth medium. Very infrequently (i.e. in about 1×10^8 cells/generation) spontaneous reversion of the original mutant site will occur, resulting in the

regeneration of the normal, non-mutated gene. Cells in which such reverse mutations occur will regain the ability to synthesise histidine and can be detected by their ability to grow and form visible colonies on histidine-free medium. Treatment of the Salmonella strains with mutagenic agents will lead to an increase in the frequency of reverse mutations and, hence, an increase in the number of colonies growing on histidine-free medium.

When screening a novel compound for mutagenic activity in this assay, it is necessary to use a number of different tester strains. This is because different strains are reverted by different types of mutagenic agent. For example, Salmonella typhimurium strain TA98 is reverted predominantly by frameshift mutagens, while strain TA100 is most sensitive to mutagens operating via a base-pair substitution mechanism. These terms refer to the molecular events by which the DNA is altered to produce a change in the coding sequence of the gene, i.e. a mutation.

In addition to the histidine mutation, the Salmonella strains carry a number of additional mutations which increase their sensitivity to mutagenic agents. These include:

i) Mutations which give rise to a defective cell wall and thus increase the permeability of the cells to chemical agents;

ii) A mutation in one of the genes involved in error-free DNA excision repair, which increases the likelihood that DNA adducts will be repaired by error-prone pathways and hence lead to more mutations;

iii) The inclusion in some strains of a plasmid, pkM101, which carries 2 mutator genes (muc A and B) which have been implicated in so-called SOS error-prone DNA

repair. The presence of pkM101 in the Salmonella strains has been found to increase their sensitivity to many, but not all, mutagenic compounds.

Another facet of this test is the incorporation of a rat liver homogenate (S9) to simulate metabolism which foreign compounds may undergo in the body. This is important since many compounds are not mutagenic (or carcinogenic) per se, but can be converted to mutagenic metabolites by various cellular enzymes.

The standard Salmonella/microsome assay involves mixing the various components, i.e. bacterial strain, test compound and S9 (if required), with molten salt agar and overlaying onto an agar plate. For testing water samples for mutagenic activity we decided to use a modification of this method known as the fluctuation test. In this test exposure to the test agent is carried out in liquid medium using a large number of replicate cultures (50 or 96). In any of these replicates, growth can occur only if a reverse mutation is induced in one or more of the cells. A positive result is indicated if treatment with the test material leads to an increase in the number of positive cultures (i.e. cultures which grow) compared to a set of untreated control cultures. The fluctuation test is generally more sensitive than the plate incorporation method and can be more easily adapted for testing aqueous samples.

3.2 TESTS WITH UNCONCENTRATED WATER SAMPLES

The original concept of the bacterial mutagenicity screening work was that drinking water samples could be tested without any prior concentration. This approach would avoid the problems of recovery and selectivity (see Section 3.3) introduced by concentration procedures and permit the direct assay of the water as received by the consumer. Furthermore, the fluctuation test, being liquid-based, is well suited to this approach.

The initial stage of the study was sub-contracted by WRc to the Medical Research Council's Cell Mutation Unit at the University of Sussex where the original fluctuation test procedure had been developed. The contract work there was undertaken principally by Dr R Forster and was supervised by Dr M Green and Professor B Bridges.

During the sub-contract, appropriate conditions, media and experimental designs were established, and a number of water samples were assayed. Out of the small number tested at this stage, one water supply was identified which reliably gave positive results in the assay.

The sub-contract to the University of Sussex ended in September 1979 and mutagenicity testing was started at WRc. Further water supplies were examined and other positive samples found.

The results with unconcentrated samples, however, were unsatisfactory as the dose responses obtained with the samples were variable and unpredictable, and the mutagenicity was at the limit of resolution of the test procedure. Positive results were normally detected only with tester strain TA100. No positive results were seen with strain TA98, or in tests with metabolic activation.

It was important that the mutagenicity of unconcentrated drinking water samples be carefully validated. Thus, in conjunction with the survey exercise described below (Section 4.1), ten water supplies chosen to cover a range of water types were tested using an experimental design which included a number of checks and controls for different sources of artifacts. Checks were made for the presence in the sample, of histidine, which interferes with the test, and that positive results were not due to the production of phenotypic revertants, (i.e bacteria which, although growing in the test, had not undergone a genuine

mutation to histidine independence). Both of these checks were in every case negative. Checks were also made for any influence of the samples on the growth of the tester bacteria in the fluctuation test. The final yield of bacteria in the test is limited by the small amount of histidine deliberately added at the start of the test; it being assumed that the bacterial yield will be the same in the control and treated series. If for any reason greater numbers of bacteria are produced in the treated series, then a corresponding increase in the number of spontaneous mutants will be obtained which could be misinterpreted as a positive result.

In this survey, no positive results were obtained with TA98, nor in experiments using metabolic activation. Some apparently positive results were obtained with TA100 - all the lowland surface waters were positive as were two of the upland waters. The groundwater samples were all negative. These findings are similar to those which might be expected in relation to contamination of different water types. However, checks on bacterial yield revealed that growth artifacts had occurred with many of the samples tested. In some cases, the apparent mutagenicity was completely explained by the differences in bacterial numbers. The results have been discussed fully elsewhere[18] but those for TA100 are summarised in Table 5.

These findings would appear to detract from the original simplicity and attractiveness of using unconcentrated samples for screening work, since it would be necessary to monitor bacterial yields in the tests and in many cases it might be difficult to obtain an unequivocal answer as to whether a sample was genuinely mutagenic or not. Even when apparently positive results were obtained with unconcentrated samples, these were only with tester strain TA100 without metabolic activation. In tests with concentrates and extracts (see Sections 4 and 5), however, mutagenicity to TA98 is frequently seen. Thus screening with unconcentrated samples does not appear to be predictive of results

Table 5 Mutagenic activity of unconcentrated drinking waters to Salmonella typhimurium strain TA100 in the absence of metabolic activation

Sample number	Type of water source	Mutagenicity (Significance level)	Response to a dose effect*	Bacterial growth effect@
100	Groundwater	NS	-	-
102		NS	-	-
110	Upland water	NS	-	-
112		0.5	toxic	+
115		0.5	-	-
091	Lowland river	0.001	+	-
103		0.001	+	-
104		0.001	+	+
114		0.001	+	+
116		0.001	+	-

Note: NS = Not significant

Note: * An improvement in the fit of the curve due to "dose" is indicative of mutagenicity.

@ An improvement in the fit of the curve due to "bacteria" is indicative of a bacterial growth artifact.

found using concentrated samples. Furthermore, for the same effort in testing, substantially more data can be produced in tests with concentrates. Taking all these facts together, the testing of unconcentrated samples did not seem to merit further attention and increasing effort was devoted to the development of techniques for concentrating water samples.

3.3 CONCENTRATION OF ORGANIC COMPOUNDS FOR MUTAGENICITY TESTING

Organic compounds are present in drinking waters in very small concentrations (typically 1 µg/l or less). These low levels of organic material are a contributory factor to the problems of unconcentrated samples (see above). Therefore, the organic compounds had to be concentrated, but the introduction of a concentration step presented additional problems that needed to be investigated. When a known compound is to be concentrated, a range of procedures is available from which the most suitable can be selected giving maximum recovery of the compound and minimum interferences. However, since the structures and physico-chemical properties of the mutagens in drinking water are unknown, such optimisation is impossible. Ideally, all the organic material in the water samples should be concentrated without alteration of the composition of the mixture. Unfortunately, there is no guarantee that some of the mutagenic compounds will not be selectively lost using any of the concentration procedures available.

Other, important factors need to be considered before the choice of a method or combination of methods can be made. These include contamination during the concentration procedure, interferences from the sample matrix (which could lead to toxicity or false positives or negatives), alteration of the compounds during the procedure, reproducibility of the method and operational aspects such as sample size, degree of concentration required and speed.

Following extensive investigations, two techniques emerged as the most suitable;

(i) Freeze drying of water samples followed by solvent extraction, (methanol) of the freeze-dried solids produced,

(ii) Adsorption onto XAD-2 macroreticular resin followed by elution of adsorbed substances with organic solvents.

Freeze drying is capable of concentrating a large proportion of the organic material (including non-polar compounds) from drinking water while the XAD method is very selective and removes only a very small fraction of the organic material (relatively non-polar compounds). However, this latter technique was shown to recover a similar amount of mutagenicity from water samples and thus offered high selectivity to mutagens. In addition, the method was more convenient for handling large samples. However, due to its non-selectivity, freeze drying followed by solvent extraction of the residues is a valuable complementary technique, especially for water samples of unknown mutagenic character.

Both methods were thoroughly tested to ensure that false positives or negatives would not occur due to artifacts resulting from the procedure, from impurities in solvents or resin, from effects of residual chlorine in water samples or from the concentration of histidine or other bacterial growth promoting nutrilites from the sample.

XAD adsorption followed by solvent elution was evaluated more extensively with a view to using this technique for routine, large-scale sampling, the investigation of the effects of treatment on mutagenicity and the identification of mutagenic compounds. Although thorough cleaning procedures were applied to the preferred solvent, diethyl ether, prior to use as an XAD eluant, occasional problems of toxicity to the bacteria used in the mutagenicity assay were encountered. Therefore the use of alternative solvents was investigated with the objective of improving the reliability and ease of application of the XAD

concentration procedure. Four different eluants, methanol, acetone, acetonitrile and a hexane/acetone mixture were compared with diethyl ether. The investigation included testing of concentrated solvent blanks, examination of methods of drying the solvent (after elution of the XAD column), assessment of procedural blanks, (including blanks with chlorinated water) and comparison of mutagenicity in extracts of the same treated water. Fluctuation tests and Ames tests were applied in the evaluation of these solvents using strains TA100 and TA98, both with and without activation.

The investigation revealed no major problems with the use of any of the solvents and no single solvent proved to be ideal, although diethyl ether probably remains the most suitable, all-round solvent, especially for the identification of mutagens (see Section 5), where the comparatively high yield of mutagenic compounds and the solvent's compatibility with analytical techniques such as gas chromatography-mass spectrometry (GC-MS) are particularly important. However, for most of the routine sampling for mutagenicity testing, diethyl ether was replaced with acetone, mainly because no extensive purification procedures were required for this solvent and toxicity did not appear to be a problem. Optimum conditions for sampling large volumes of water (10-200 litres) were established, such as sample volume to resin bed volume ratio, optimum flow rates and eluant volumes.

The stability of XAD-diethyl ether extracts (stored frozen or at room temperature) was checked and no significant difference in mutagenic activity was observed over one month. For longer periods there was some loss of activity.

The techniques developed for the concentration of organic matter in water were essential for the detection of mutagens in water but were applicable also to investigations of the identity of mutagens. Both these applications of concentration techniques are described below (Sections 4 and 5 respectively). The

methodology is also applicable to other studies of mutagenic activity, including the use of higher cell systems, and to the study of organics in water generally.

4. SURVEYS OF MUTAGENIC ACTIVITY IN DRINKING WATER

In the latter part of 1980, a series of surveys for mutagenicity in drinking water was begun in which various aspects of water supply practice, relevant to the objectives of the contract, were studied. These aspects included:

(i) the testing of a range of drinking waters to assess the effect of source (e.g. groundwater, upland water and lowland river water). In the case of the samples of treated river water, the sites were chosen to represent varying levels of re-use so that the effect of re-use on mutagenic activity could be investigated;

(ii) the effect of seasonal variations;

(iii) the effect of water treatment, e.g. storage, disinfection, dechlorination.

4.1 EFFECT OF WATER SOURCE AND RE-USE

This survey was designed to test whether a relationship existed between water re-use and the mutagenicity of water samples. Fifteen sites were selected for the survey, including seven from high re-use sources (all lowland rivers) and eight from low re-use sources (three groundwaters, three upland waters and two lowland river sources). These are listed in Table 6.

Samples were tested using three methods:

(i) Unconcentrated - the work described above (Phase 1) on unconcentrated samples was undertaken in conjunction with the survey.

(ii) XAD/diethyl ether extracts - prepared by a large-scale method (150 l samples) were tested in the fluctuation test;

(iii) Freeze-dried/methanol (FD/M) extracts - these were also tested in the fluctuation test.

Drinking water samples were taken before distribution. When samples were taken a number of chemical parameters were also recorded (Table 6) including details of chlorination and the proportion of sewage effluent in the source. Testing was undertaken with tester strains TA98 and TA100 both with and without S9 metabolic activation, using fixed dose levels, based on equivalent volumes of original samples, predetermined for each extraction method. Each assay was repeated at least once on a different day.

To generate the results obtained in the survey required about 360 separate mutagenicity assays [15 (sites) x 3 (types of sample) x 2 (bacterial strains) x 2 (with and without S9) x 2 replicate experments)]. This figure does not include any of the more elaborate tests which were conducted to check for possible artifacts due to the presence of growth promoting nutrilites in the test samples.

Mutagenic activity was found in all lowland and upland waters and in two groundwaters with TA100 in the absence of S9. Eight lowland waters, one upland source and one groundwater were positive with TA98 (Tables 7 and 8). The addition of S9 reduced or abolished the mutagenic activity.

The two methods of concentration produced broadly similar results, though mutagenic activity tended to be lower with the XAD samples, particularly with strain TA98.

Table 6 Water quality at the sites used in the survey of the effect of re-use on mutagenic activity.

Sample number	Type of water source	Reuse (% sewage*)	Total organic carbon (TOC) (mgC/l)	Chlorine dose (mg Cl_2/l)	Chlorine-hours (mg Cl_2/l x contact time in hours
100	Ground/Chalk	0	0.45	0.25	0.02
102	Ground/Lower Greensand	0	0.95	0.13	0.13
113	Ground/Bunter Sandstone	0	0.20	0.13	0.13
110	Upland	0	5.05	8.5	8.5
112	Upland	0	0.80	0.6	0.6
115	Upland	0	1.45	0.5	0.5
114	Lowland river with storage	1.5	3.85	6.5	18.0
116	Lowland river	0.7	1.40	6.5	20.4
108	Lowland river	9.7	1.70	7.0	24.0
109	Lowland river with storage	11.0	2.50	8.5	11.7
111	Lowland river with storage	13.2	1.75	7.0	12.0
091	+Lowland river with storage	12.5	3.70	3.5	9.0
103	Lowland river with storage	7.5	3.40	1.7	1.7
104	Lowland river with storage	24.3	3.60	2.3	2.76
105	Lowland river with storage plus ground (71% from river)	14.7	2.00	2.4	7.2

* % effluent/mean river flow

\+ Not analysed by GC-MS (see Section 6)

Table 7 Mutagenic activity in freeze-dried extracts of treated drinking water

Sample No.	Source	Mutagenic activity in absence of S9	
		TA100	TA98
100	Groundwater	+++	+++
102	"	++	NS
113	"	NS	NS
110	Upland reservoir	++	NS
112	"	+	NS
115	"	+++	++
114	Lowland river	+++	+++
116	"	+++	+++
108	"	+++	+
109	"	+++	TOX
111	"	+++	+++
091	"	+++	+++
103	"	++	+++
104	"	+++	+++
105	"	+++	+++

\+ Significant at 5% level
++ " " 1% "
+++ " " 0.1% "
NS Not significant
TOX Evaluation precluded due to toxicity

Table 8 Mutagenic activity in XAD/solvent extracts of treated drinking water

Sample No.	Source	Mutagenic activity in absence of S9	
		TA100	TA98
100	Groundwater	NT	NT
102	"	NT	NT
113	"	NS	NS
110	Upland reservoir	+++	NS
112	"	NS	+
115	"	+++	NS
114	Lowland river	+++	++
116	"	+++	NS
108	"	+++	NS
109	"	+++	NS
111	"	+	+++
091	"	+++	+++
103	"	+	NS
104	"	+++	++
105	"	NS	+++

\+ Significant at 5% level
++ " " 1% "
+++ " " 0.1% "
NS Not significant
TOX Evaluation precluded due to toxicity
NT Not tested

There were statistically significant correlations between mutagenicity results and some water quality parameters as follows:

(i) TA100 mutagenicity was linearly correlated with chlorine dose and the product of chlorine dose and contact time;

(ii) TA98 mutagenicity correlated non-linearly with chlorine dose but not with the product of chlorine dose and contact time;

(iii) TA98 mutagenicity correlated non-linearly with TOC.

With the exception of (i), none of the above relationships stood up to closer examination of the data. In addition, no clear relationship between mutagenicity and re-use of water emerged when the sites with re-use were examined.

The conclusions of this survey may be summarised as follows:

(a) Mutagenicity was found in all types of drinking waters but the response was generally "stronger" in lowland river waters;

(b) Mutagenicity with TA100 and TA98 appears to be affected by different factors;

(c) Quality of the source water appears to play a more important part in mutagenicity with TA98. There were indications that the character of the TOC is as important as its quantity;

(d) Chlorination increased mutagenicity, especially with strain TA100;

(e) There was also some indication that long-term storage of lowland river derived water was associated with a lowering of mutagenic activity.

As a result of this survey a number of points were identified which required further investigation, namely:

(i) What are the sources of the mutagenic activity detected in treated drinking water? Are the mutagens man-made or of natural origin? Are they present in the raw water or generated during treatment, especially by chlorination?

(ii) If chlorination (and other disinfection processes) lead to the production of mutagens, what steps can be taken to reduce or prevent the formation of mutagens or to remove them once they have been formed.

To answer these questions, a laboratory procedure had to be developed which allowed chlorination to take place under controlled, reproducible conditions. This involved chlorinating the water with a single, fixed dose of 5 mg/l chlorine (using sodium hypochlorite solution) for a contact time of 1h. After this period, the sample was dechlorinated with sodium sulphite to leave a free residual of 0.5 mg/l prior to concentration by freeze drying. This method was used in many of the subsequent experiments in this section.

4.2 EFFECT OF RIVER WATER QUALITY AND CHLORINATION

In an attempt to identify more clearly the source of mutagens or mutagen precursors in raw river water, an experiment was conducted taking samples from a lowland river at three points:

(i) The headwaters;

(ii) Several miles downstream where the river had received no domestic or industrial effluents but was subject to agricultural contamination;

(iii) After an outfall from the sewage works of a large town, the effluent containing both domestic and industrial waste.

Samples were concentrated by XAD adsorption before and after laboratory chlorination and tested for mutagenic activity in TA100 and TA98 with and without S9 activation.

All three raw water samples showed weak but statistically significant activity in strain TA98. Chlorination increased the mutagenic activity of the samples in both strains TA98 and TA100. Although the mutagenic activity was slightly increased after the sewage works outfall, the overall pattern of mutagenicity was the same for all three sites.

To investigate further the interaction between raw water quality, chlorination and mutagenic activity, a series of raw water samples were assayed for activity both before and after chlorination using the standardized laboratory procedure. For this study the samples were concentrated by freeze drying and the residual solids extracted with methanol and exchanged into dimethyl sulphoxide before testing. In this second survey, nine sites were chosen, encompassing a wide range of TOC values and levels of domestic and industrial effluent (Table 9). The sites included five lowland rivers, three upland reservoirs and a chalk groundwater.

Surface derived waters before chlorination showed little evidence of mutagenicity in strain TA100 in the absence of S9 (Table 10). In the presence of S9, however, several sites showed evidence of weak activity in this strain. After chlorination, a clear positive response was observed in TA100 in the absence of

Table 9 Water quality at the sites used in the survey of the interaction between source quality and disinfection

Sample number	Type of water source	TOC mg/l	Re-use (% sewage)*	Boron mg/l
120	Lowland river	4.9	17.5	0.19
121	Lowland river	4.1	17.3	0.45
122	Lowland river	3.6	7.1	0.33
123	Lowland river	1.7	0.6	<0.06
124	Lowland river	2.2	13.2	0.85
125	Upland	6.4	-	0.09
126	Upland	4.0	-	NT
127	Upland	7.0	-	0.09
128	Groundwater chalk	0.4	-	<0.06

* % effluent/mean river flow

NT = Not tested

Table 10 Mutagenic activity of freeze-dried extracts prepared from raw and laboratory chlorinated water samples in *Salmonella typhimurium* TA100

Sample	Slope values (level of significance)			
	Raw water		Chlorinated water	
	TA100 - S9	TA100 + S9	TA100 - S9	TA100 + S9
Lowland rivers				
120	0.04 (NS)	2.95 (+++)	11.75 (+++)	3.49 (+++)
121	0.96 (NS)	1.09 (NS)	17.30 (+++)	1.60 (+++)
122	0.38 (NS)	Toxic	16.17 (+++)	1.42 (+++)
123	0.66 (NS)	6.73 (+++)	7.21 (+++)	1.16 (NS)
124	1.40 (NS)	5.23 (+++)	19.87 (+++)	0.04 (NS)
Upland reservoirs				
125	0.07 (NS)	Toxic	12.25 (+++)	0.28 (NS)
126	< 0 (NS)	Toxic	9.77 (+++)	Toxic
127	< 0 (NS)	Toxic	4.41 (+++)	Toxic
Groundwater				
128	< 0 (NS)	3.19 (+++)	< 0 (NS)	2.17 (+++)

Slope values were derived from dose response plots with each sample in the fluctuation assay.

NS Not significant
Toxic Toxicity of sample to bacteria precluded evaluation of mutagenicity
\+ Significant at 5% level
++ Significant at 1% level
+++ Significant at 0.1% level

S9 with every surface water sample. The level of activity of the chlorinated waters in this strain was generally marginally higher in lowland surface waters compared to upland samples. The presence of rat liver S9 reduced the TA100 activity of the chlorinated surface water in every case. The results obtained with the groundwater were unusual, indicating that the source was contaminated with an indirect-acting base pair substitution mutagen, with both the raw and chlorinated samples showing activity in strain TA100 in the presence of S9.

Although all the water samples showed activity with strain TA98 after chlorination, it was generally weaker than with strain TA100 (Table 11). The effect of S9 on TA98 activity was variable, in some cases increasing activity while in others decreasing the response. Several sites (both lowland and upland) showed evidence of weak activity in TA98 in the presence of S9 before chlorination and one sample (a lowland river) showed clear activity (significant at 0.1% level) in TA98 without S9.

Attempts to statistically correlate the mutagenic activity of the different samples with various parameters of water quality did not reveal any obvious relationships.

The results of this survey indicate that, although pollution of the water source with industrial and domestic effluent may have an effect on the level of mutagenicity, activity can be consistently demonstrated in chlorinated surface water collected from non-polluted sites. This suggests that a major portion of the mutagenic activity in drinking water samples is produced by the chlorination of naturally-occurring compounds in drinking water.

4.3 EFFECT OF SEASONAL VARIATION ON MUTAGENIC ACTIVITY

It is probable that seasonal factors affect mutagenic activity in water from surface sources. This probability was investigated in a survey in which samples of final drinking water

Table 11 Mutagenic activity of freeze-dried extracts prepared from raw and laboratory chlorinated water samples in *Salmonella typhimurium* TA98

Sample	Slope values (level of significance)			
	Raw water		Chlorinated water	
	TA98 - S9	TA98 + S9	TA98 - S9	TA98 + S9
Lowland rivers				
120	1.31 (+)	2.16 (+++)	7.54 (+++)	7.92 (+++)
121	2.03 (NS)	2.38 (++)	10.40 (+++)	7.20 (+++)
122	0.86 (NS)	2.72 (NS)	5.62 (+++)	3.08 (NS)
123	5.50 (+++)	1.45 (NS)	5.57 (+++)	< 0 (NS)
124	0.85 (NS)	6.10 (+++)	5.34 (+++)	5.57 (+++)
Upland reservoirs				
125	0.23 (NS)	3.06 (++)	3.76 (+++)	3.28 (+++)
126	< 0 (NS)	2.63 (++)	0.80 (NS)	4.31 (+++)
127	< 0 (NS)	4.04 (+++)	1.88 (+)	5.45 (+++)
Groundwater				
128	0.38 (NS)	1.38 (NS)	2.09 (+)	2.88 (++)

Slope values were derived from dose response plots with each sample in the fluctuation assay.

NS Not significant
\+ Significant at 5% level
++ Significant at 1% level
+++ Significant at 0.1% level

derived from a lowland river source were taken monthly over a twelve months period. Samples were concentrated by freeze drying and then tested using strains TA98 and TA100 in both the presence and absence of S9. Samples for chemical analysis were taken at the same time. The parameters monitored are listed in Table 12. Although there were variations in mutagenic activity, no clear seasonal pattern emerged nor were there any obvious associations with the analytical data.

4.4 EFFECT OF WATER TREATMENT ON MUTAGENIC ACTIVITY

The information collected so far indicated that the addition of chlorine makes a significant contribution to the mutagenic activity detected in treated drinking water. Consequently, in the final year of the project, some treatment processes were examined in detail to establish if the level of mutagenic activity in the final water could be reduced. Two processes were studied:

(i) Storage;
(ii) Dechlorination.

Throughout these studies, all samples were concentrated by freeze drying.

4.4.1 Storage

A series of experiments was performed in the laboratory in which mutagenic activity was measured following storage of unchlorinated and chlorinated raw water at 4 ^{o}C and ambient temperature (about 20 ^{o}C).

Experiments on the effects of storing raw water samples at 4 ^{o}C prior to chlorination showed no difference in mutagenic activity when stored for up to 8 days. Raw water stored at ambient temperature for 24 hours before chlorination showed no difference in mutagenic activity to TA98 but with TA100 toxicity was observed at the highest dose tested after storage. Similar

Table 12 Chemical and physical parameters of water samples measured as part of the study of seasonal variation

Ammonia	
Nitrate	
Nitrite	
Total organic carbon	
Total adsorbable organic halogen	
Available chlorine	
$CHCl_3$	
$CHCl_2Br$	Total Trihalomethanes
$CHClBr_2$	
pH	
Water temperature	
Chlorophyll-a	

experiments using treatment works final water stored at ambient temperature and 4 ^{o}C indicated that there was a significant reduction in mutagenic activity over an 8-day period.

Investigations were also made on the effect of short-term raw water storage (i.e. bank-side storage) on the mutagenic activity of water samples. Samples were taken from a reservoir inlet and tested both before and after laboratory chlorination. Seven days later (the nominal retention time of the reservoir) further samples were taken from the outlet and again tested before and after laboratory chlorination. The results indicated that storage of raw water for 7 days did not reduce the mutagenic activity of the water after chlorination. Studies were also made on a reservoir with a nominal retention time of 60 days. Spot samples were taken from the reservoir intake and outlet on the same day and tested before and after laboratory chlorination. In this experiment there was evidence of a reduction in mutagenic activity of the stored water after chlorination.

The conclusions of these studies were that short-term, bank-side storage of raw water has little beneficial effects in terms of reducing the potential of the water to generate mutagens on chlorination. Long-term storage (i.e. of several months' duration) may show some benefits in this respect, although further experiments are required to confirm this observation.

4.4.2 Effect of dechlorination

A series of experiments has been conducted to investigate the effect of dechlorinating agents on the mutagenic activity of water samples. Partial dechlorination is frequently used as the final stage of water treatment in the UK. This involves reducing the chlorine levels so that the water is aesthetically more acceptable to the consumer, whilst retaining some disinfection capacity through distribution.

Initial laboratory studies indicated that treatment of chlorinated water samples with dechlorinating agents not only reduced the chlorine residuals, but also reduced the mutagenic activity of the samples. This presumably indicates that the dechlorinating agents are reacting with the organic compounds (produced by chlorination) which are responsible for the mutagenic activity.

The dechlorinating agents which were examined included sulphur dioxide, sodium sulphite, sodium thiosulphate, sodium metabisulphite and biotin. Experiments have been conducted to investigate the effect of partial dechlorination to leave different levels of residual chlorine and total dechlorination. In some experiments dechlorination has been simulated in the laboratory, while for others, samples have been collected from appropriate points at a water treatment works. The results of these experiments are summarised in Table 13. The conclusions from this study are:

(i) Total dechlorination of drinking waters with a variety of dechlorinating agents can substantially reduce the mutagenic activity of the samples in both TA100 and TA98;

(ii) Partial dechlorination had little effect on mutagenic activity unless the free chlorine residual was reduced to below 0.5 mg/l;

(iii) Low-level rechlorination (i.e. to 0.5 mg/l total chlorine) of totally dechlorinated samples restored activity in TA100 but not in TA98;

(iv) Chloramination of totally dechlorinated samples resulted in lower activity than rechlorination

Table 13 Mutagenic activity of concentrated water samples after treatment with various dechlorinating agents

Source of water sample	Dechlorinating agent	Extent of dechlorination	Slope values (with approximate standard errors) TA100	TA98
Chlorine contact tank	None	-	20.43 (2.49)***	8.53 (1.84)***
	Thiosulphate	Total	3.26 (0.92)**	2.56 (2.34)**
Chlorine contact tank	None	-	20.19 (2.99)***	5.11 (1.07)***
	Metabisulphite	Total	8.02 (2.46)***	1.91 (1.03)*
Raw water (Laboratory chlorinated)	None	-	29.50 (1.27)***	NT
	Biotin	Total	5.46 (1.92)***	NT
Raw water (Laboratory chlorinated)	None	-	25.06 (1.59)**	NT
	Sulphite	0.8 mg/l free	21.25 (2.03)***	NT
		Total	3.52 (2.44)NS	NT
		Excess	1.09 (2.86)NS	NT
Chlorine contact tank	None	-	13.5 (1.26)***	NT
	Sulphite	0.5 mg/l free	9.97 (0.83)***	NT
		0.35 mg/l free	5.01 (0.70)***	NT
		Total	3.63 (0.81)***	NT
Chlorine contact tank	None	-	16.81 (1.26)***	7.16 (1.45)***
	Sulphur dioxide	Partial (0.5 mg/l total	4.04 (0.58)***	3.12 (1.10)**
Chlorine contact Tank	None	-	11.78 (1.82)***	5.89 (0/51)***
	Sulphur dioxide	Total	2.71 (0/74)***	0.95 (0.71)NS
Chlorine contact tank	Sulphur dioxide	Total	2.14 (0.76)***	0.99 (1.36)NS
		Total then rechlorinated to 0.5 mg/l total	6.86 (1.15)***	1.64 (1.53)NS
Chlorine contact tank	None	-	14.44 (0.87)***	NT
	Sulphite	Total	4.00 (0.76)***	NT
		Total then chlorinated to 0.44 mg/l total	4.12 (0.68)***	NT

Slope values are derived from dose response plots with each sample in the fluctuation assay.

Superscript on slope values denotes significance of mutagenic response in fluctuation assay.

*** significant at 0.1% level
** significant at 5% level
NS Not significant
NT Not tested

4.5 OVERALL ASSESSMENT OF RESULTS

Concentrated extracts of chlorinated drinking water have been shown to be consistently mutagenic in bacterial assays. This mutagenicity appears to arise primarily from the reaction of chlorine with 'natural' organic compounds in the water, although a contribution from domestic and industrial effluent cannot be ruled out. Occasionally, raw water samples have shown some mutagenic activity in one of the test bacterial strains, usually, Salmonella typhimurium TA 98. The activity in chlorinated water was greater in drinking water derived from lowland rivers than those derived from upland reservoirs. Groundwater showed much lower or no activity.

Studies on storage of water and dechlorination have shown that it may be possible to reduce the mutagenicity of final water without resorting to major changes in treatment practice.

The significance to consumers health of mutagenicity in drinking water concentrates is difficult to assess. The studies described in this report indicate the presence of biologically active compounds in drinking water which are capable of inducing genetic change in bacterial cells. Further investigations, using higher cell systems, are required before an evaluation of the significance of these findings to human health can be made.

5. IDENTIFICATION OF MUTAGENS

The studies described above show that mutagenic activity can be readily detected in samples of drinking water. The significance of such a finding needs to be assessed. One approach is the application of additional assays, for example those involving higher cell systems, to better predict the likely effects in man. However, another approach involves identification of the chemical or chemicals causing the mutagenicity detected in the tests. This approach has been used in other fields, for

example in the identification of the carcinogen benzo(a)pyrene in coal-tar pitch. If the identity of a mutagen is known then several pathways to ascertaining its significance to health are available - for example, appropriate bioassays with the pure substance, examination of available toxicity data and accurate determination of exposure levels. In addition, the source of the substance can be surmised and if necessary located by rapid and specific analyses.

The work has combined mutagenicity assays with trace organic analysis, the latter being highly dependent on the availability of powerful methods of identification. In the early stages of this aspect of the research programme, schemes for identification of mutagens were based upon testing unconcentrated samples of water for mutagenic activity. However, as explained in Section 3.2 such testing proved insensitive and unreliable and consequently, the use of concentration procedures was essential. Preliminary, qualitative analysis of concentrated samples (i.e. extracts) of water which had been shown to contain mutagenic activity showed that they were too complex for effective qualitative analysis and required some form of separation/fractionation.

Thus, the basis of much of the investigations has been chemical/physical and/or chromatographic (high-performance liquid chromatography (HPLC)) fractionation of mutagenic extracts of drinking water followed by retesting of fractions to locate the mutagens; the aim being to separate mutagens from the accompanying complex mixture of non-mutagens normally encountered, prior to attempts at identification. The overall study consisted of four distinct aspects:

(i) Qualitative analysis of mutagenic extracts;

(ii) Solvent partition of mutagenic extracts at different pH combined with HPLC fractionation, prior to qualitative analysis;

(iii) HPLC fractionation of extracts of chlorinated and unchlorinated water in order to detect and characterise the mutagens produced during chlorination of water supplies;

(iv) Chlorination/mutagenicity testing of certain substances which commonly occur in raw waters in order to determine the precursors that react to produce mutagenicity during disinfection (chlorination) of water supplies.

5.1 QUALITATIVE ANALYSIS OF MUTAGENIC EXTRACTS

Extracts of drinking water were subjected to detailed qualitative analysis using GC-MS, mainly in an attempt to identify the mutagens, and generally correlate the presence of specific substances or classes of substances with mutagenicity. Many compounds were identified (see Section 7.1). Some additional substances were detected which have not been identified.

The compounds identified were searched for known mutagens and for substances structurally related to mutagens. The mutagens located in this manner are included in Table 14. Many compounds identified do not appear to have been tested for mutagenicity.

All extracts examined in this way were found to be extremely complex, hence the emphasis placed upon fractionation of extracts to produce simpler fractions prior to application of GC-MS and other techniques.

5.2 SOLVENT PARTITION OF MUTAGENIC EXTRACTS AT DIFFERENT pH COMBINED WITH HPLC

Initially, fractionation of methanol extracts of freeze-dried solids of drinking water was carried out. With the exception of the very volatile compounds, which are normally lost during freeze drying, these extracts contained a wide range of organics due to the non-selectivity of the concentration technique.

Methanol was used because of its suitability for extracting mutagenic compounds from the freeze-dried solids and its compatability with HPLC and with the mutagenicity assay when used in the absence of S9 (methanol being metabolised to toxic products in the presence of S9).

A combination of solvent partition at different pH and separation by HPLC was developed to fractionate the mutagenic methanol extracts. Fractions were assayed in the fluctuation assay using strain TA100 (since this was the most sensitive strain) and analysed qualitatively. The overall aim was to purify the mutagens in the complex mixture and so enable effective qualitative analysis.

Figure 1 illustrates the final fractionation scheme which was developed and shows the fractions obtained and the results of the mutagenicity assays. Initially the pH-stability of the mutagenic compounds was checked, and it was found that a significant proportion of the mutagenic activity was destroyed under alkaline conditions (pH 12.5), while the activity was stable under acidic conditions (pH 1.5). For this reason a plan to isolate bases by extraction at high pH was abandoned. Neutral compounds were isolated by extraction of the aqueous solution with diethyl ether at neutral pH and acids were isolated by extraction at pH 1.5. The resulting fractions, 'neutrals', 'acids' and 'aqueous residue', which were all positive in the fluctuation test, were further separated using HPLC. All fractionation and mutagenicity assays were accompanied by appropriate procedural blanks. Whenever possible, the fractions were tested for mutagenicity together with their parent material and the recombined fractions. This was to check for losses or increases in mutagenic acitivity due to sample handling or chemical alteration during separation and reconcentration, and to check for synergism or antagonism among compounds.

Table 14 Compounds mutagenic to TA100 without activation, which have been identified in drinking water by GC-MS

1-bromobutane
bromochloroacetonitrile
bromochloromethane
bromodichloromethane
bromoethane
bromoform
butan 2,3-dione
1-bromopropane
chloral
chlorodibromomethane
bis(2-chloroethyl)ether
dibromomethane
dichloroacetonitrile
1,2-dichloroethane
dichloropropene
diethylphthalate
dimethylpthalate
iodoethane

As shown in Fig. 1, several mutagenic fractions were obtained which suggests the presence of more than one mutagenic compound. The mutagenic fractions were relatively non-polar (i.e. eluting late in reversed-phase HPLC and eluting early in normal-phase HPLC). All the blanks were satisfactory, i.e. non-mutagenic and non-toxic in the fluctuation assay. There was no evidence of synergism or antagonism among the compounds separated. Some overall losses of the original mutagenicity occurred due to sample handling, particularly as a result of solvent partition and reversed-phase HPLC. Reversed-phase HPLC (which had to be applied to the 'acids' and the 'aqueous residue') presented problems with respect to the reproducibility of HPLC chromatograms and recovery of mutagenicity after separation. Moreover, the fractions obtained by this technique were inherently difficult to analyse qualitatively. Normal-phase separation of the 'neutral' fraction was more promising. However, the mutagenic fractions obtained at the second-stage HPLC fractionation were still complex. Narrower fractions were collected for analysis by field desorption-mass spectrometry (FD-MS). These fractions still contained a large number of compounds. However, no compounds were identified which could account for the mutagenicity in these fractions.

The mutagenic compounds in the HPLC fractions did not appear to be amenable to analysis by GC-MS.

The work revealed that the organic matter in drinking water is so complex that even after considerable separation and fractionation, relatively complex fractions were still obtained. This fact, combined with the indication that numerous mutagens are present led to the following approach, which endeavoured to 'eliminate' organic constituents unrelated to the detected mutagenicity.

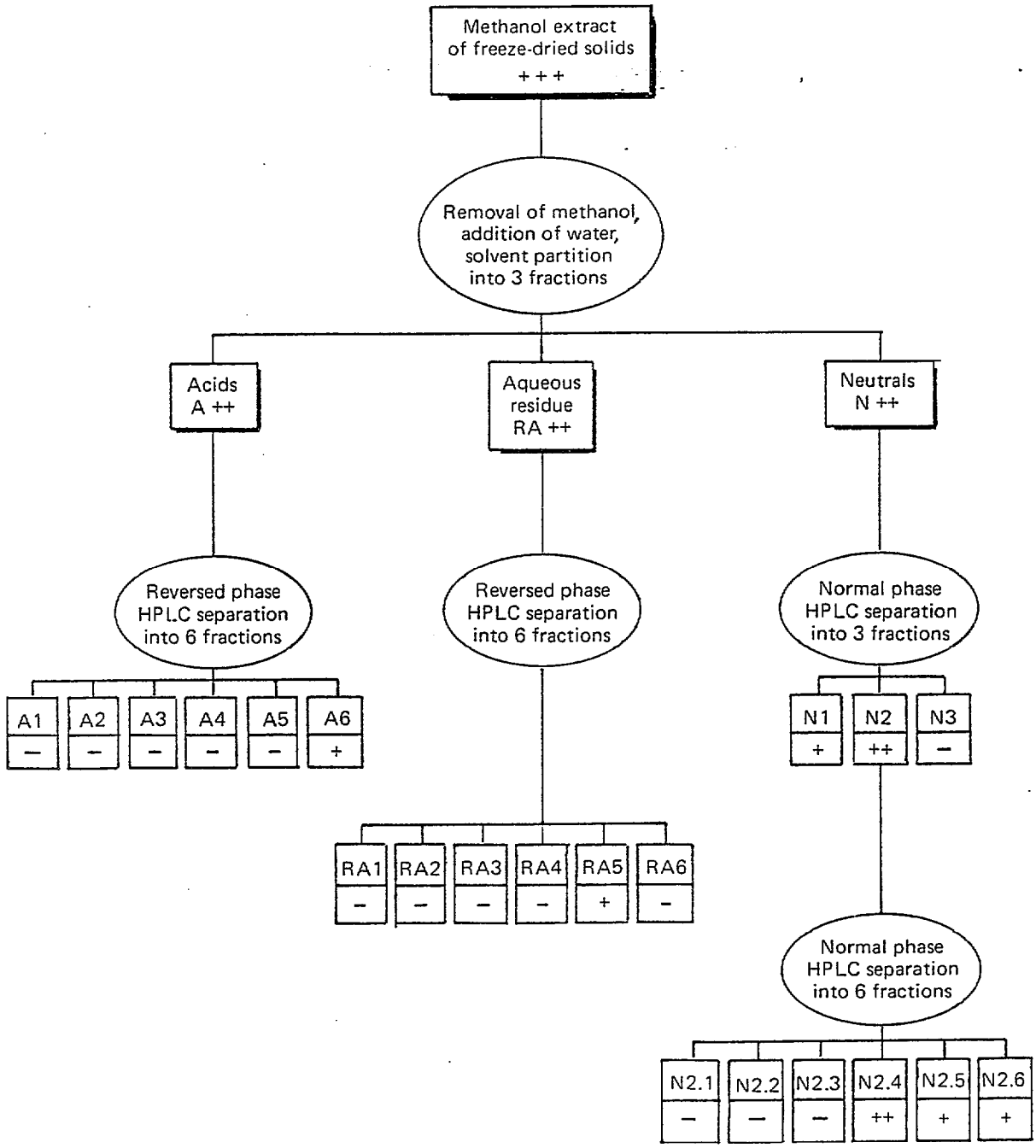

Fig. 1. Fractionation scheme for a methanol extract of the freeze dried solids of an extract of final treated water, indicating the TA100 mutagenicity of the resulting fractions.
+++ mutagenic, slope values > 5.0
++ mutagenic, slope values 0.5 – 5.0
+ mutagenic, slope values < 0.5
– not significantly different from negative controls ($p > 0.5$)
(The slope values were calculated as revertants per litre-equivalent of water per ml incubate.)

5.3 HPLC FRACTIONATION OF EXTRACTS OF CHLORINATED AND UNCHLORINATED WATER

As explained in Section 4, an association between mutagenic activity in strain TA100 and chlorination was encountered. In addition, mutagenicity testing of methanol extracts of freeze-dried solids and XAD/diethyl ether extracts of water sampled before and after final chlorination (at the same water treatment plant), showed a substantial increase in TA100 activity as a result of final chlorination (see also Section 4.2).

Although XAD-adsorption followed by diethyl ether elution is a much more selective concentration technique than freeze-drying followed by methanol extraction, extracts of similar mutagenic potency were obtained. Thus the technique appears to be selective towards concentrating the mutagenic compounds produced by chlorination. In addition, this method allows rapid concentration of large water samples; the large amount of extract obtained facilitates work on fractionation combined with mutagenicity testing and analysis.

For these reasons XAD/diethyl ether extracts of large samples (about 150 l) of water, taken before and after final chlorination, were fractionated by normal-phase HPLC, initially using single-column HPLC, and later coupled-column HPLC for improved separation. In principle, extracts and fractions from the two samples can be compared and compounds unrelated to chlorination eliminated from investigation, thus simplifying the analytical task.

One of the fractionation schemes and the TA100 mutagenicity of the resulting fractions of the final (chlorinated) water are shown in Fig. 2. (Samples of parent material and recombined fractions were always included in the fluctuation tests.)

A number of mutagenic fractions were obtained indicating the presence of several mutagenic compounds. The first-stage fractions of the unchlorinated water were all non-mutagenic and non-toxic. There were some slightly toxic and weakly positive results in the second-stage fractions, but due to the lack of any dose response, these were considered insignificant.

Figure 3 shows the chromatograms of the HPLC fractionation of the XAD/diethyl ether extracts of the final and unchlorinated water samples. The fractions collected are indicated on the figure. The final (chlorinated) and unchlorinated water extracts appear to be similar with respect to UV-absorbing material, except for the range where fractions F2 and F3 were collected. These fractions were selected for further fractionation under modified conditions of elution. Three mutagenic fractions were obtained from the subfractionation of fraction 3 (F3), and two mutagenic fractions from the subfractionation of fraction 2 (F2) of the final water extract (see Fig. 2).

Some overall losses of mutagenic activity were observed as a result of fractionation. Probably these losses were due to sample handling at the various concentration steps, which were required before the fractions could be tested for mutagenicity.

There was no evidence of antagonism among the fractions that were separated, but there was some indication of synergism. This has yet to be confirmed.

The HPLC chromatogram of fraction F2 (Fig. 4) shows a relatively simple fraction with respect to UV-absorbing material. Moreover, a major peak in the mutagenic subfraction (F2.3) of the final (chlorinated) water, which was not present in the corresponding fraction of the unchlorinated water is highlighted. Detailed qualitative (GC-MS and FD-MS) analysis of this UV-peak and of other narrow subfractions collected in the mutagenic range was carried out. The compounds identified were either known to

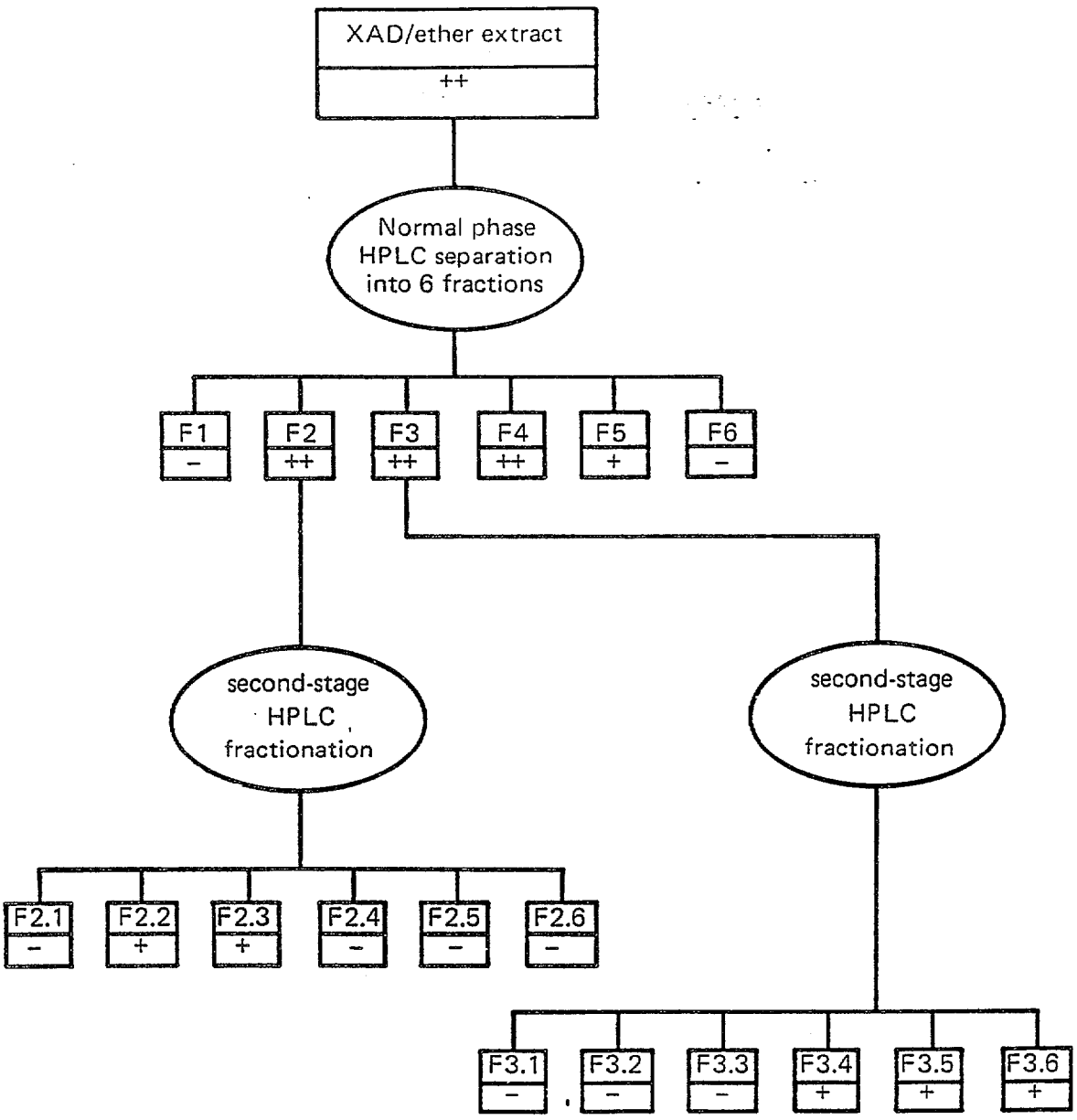

Fig. 2. Fractionation scheme for a XAD/diethyl ether extract of final treated water, indicating the TA100 mutagenicity of the resulting fractions.
++ mutagenic, slope values 0.5 – 5.0
+ mutagenic, slope values < 0.5
– not significantly different from negative controls ($p > 0.5$).
(The slope values were calculated as revertants per litre-equivalent of water per ml incubate).

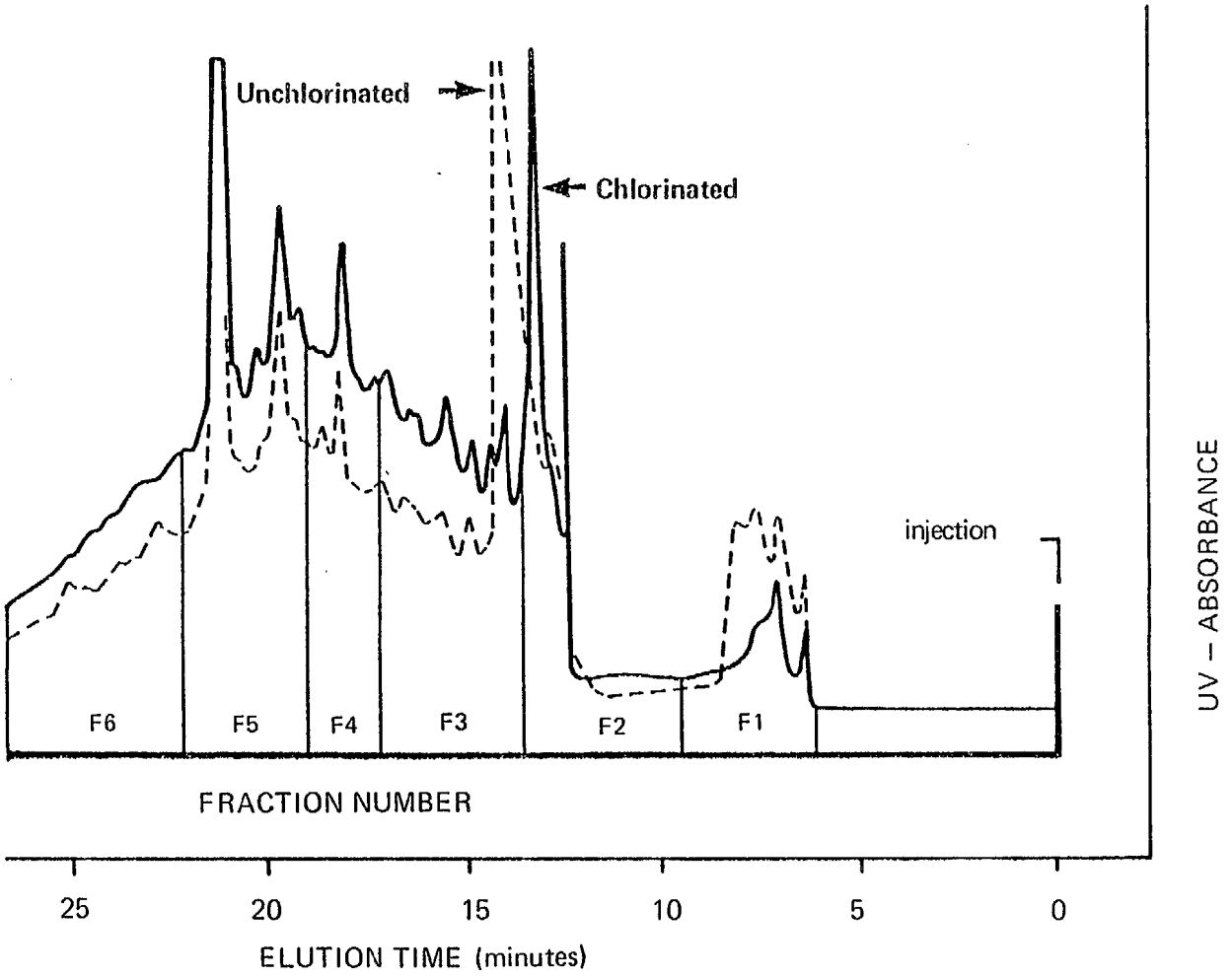

Fig. 3. Normal-phase HPLC chromatograms from XAD/diethyl ether extracts of chlorinated and unchlorinated treated water, indicating the fractions collected.

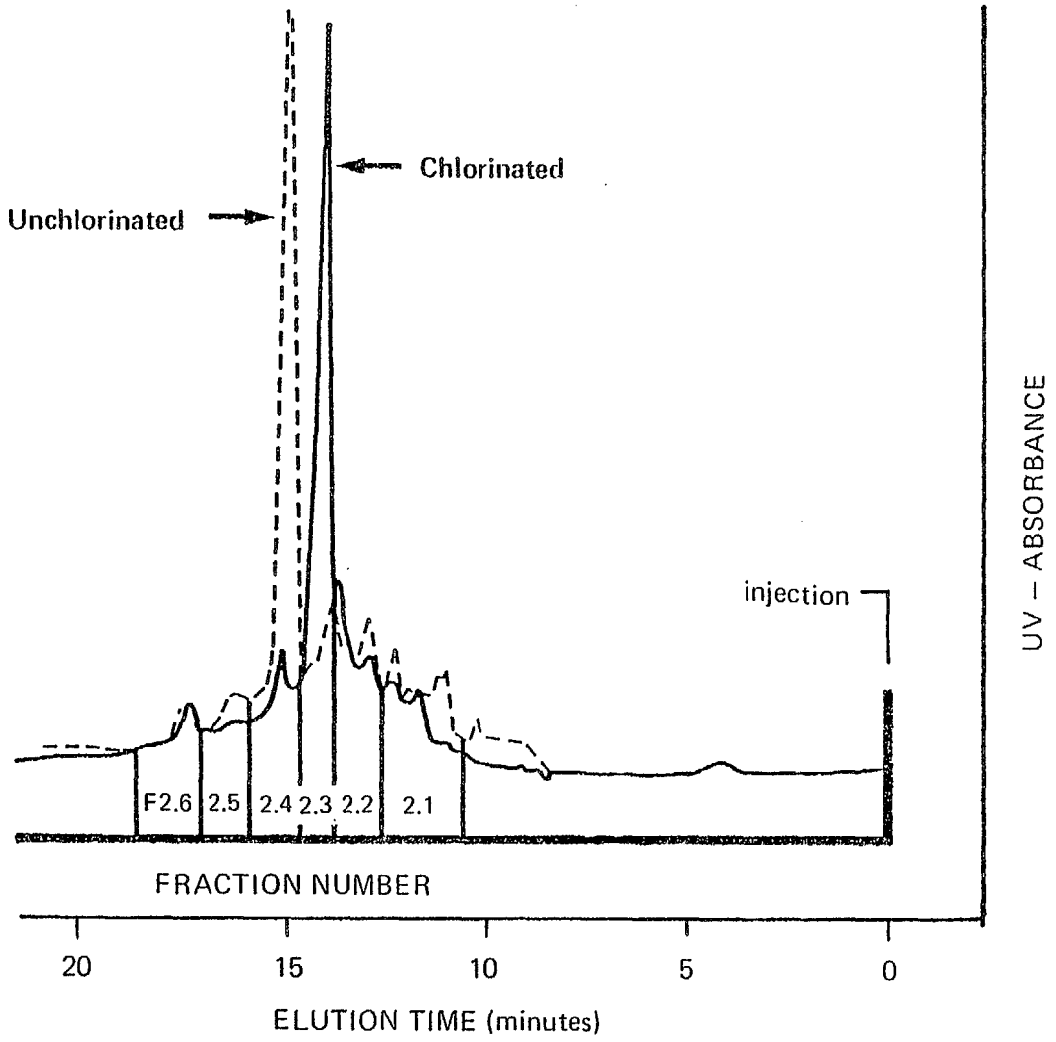

Fig. 4. Normal-phase HPLC chromatograms from second-stage fractionations of fractions F2 of the chlorinated and unchlorinated water extracts, indicating the fractions collected.

be non-mutagenic or were also present in similar amounts in the corresponding non-mutagenic fraction of the unchlorinated water. However, many unidentified compounds were detected at very low levels.

5.4 CHLORINATION OF MODEL COMPOUNDS

The information produced so far in our laboratory suggests that chlorine reacts with commonly-occurring substances, possibly of natural origin, to produce mutagenic products. Work was carried out which involved laboratory chlorination of selected compounds, for example, amino acids, humic acids, purines, pyrimidines, nucleosides and nucleotides. The products of these reactions have been examined for mutagenicity with the same characteristics as that found after water treatment chlorination (e.g. TA100 activity, detectable in the relatively small amount of organic matter recoverable from XAD concentrates and appropriate HPLC behaviour). This work is being pursued further in another DOE funded project entitled "Identification of Mutagens in Potable Water" (DOE Ref. No. PECD 7/7/122-16/84).

The potentially simpler mixtures involved and the ability to produce larger quantities of material for study, combined with knowledge of the precursors, should facilitate identification of mutagens produced by water treatment chlorination.

Four groups of compounds were selected for initial investigation:

(i) Humic acids (from three different sources - as separate solutions);

(ii) Amino acids (21 compounds in one solution);

(iii) Purines and pyrimidines (7 compounds in one solution);

(iv) Nucleosides and nucleotides (13 compounds in one solution).

First, a laboratory chlorination technique was selected which was compatible with the XAD/diethyl ether concentration technique and the mutagenicity assay and produced mutagenicity comparable to that formed during water chlorination. A procedural blank, prepared by chlorinating deionised water, was neither mutagenic nor toxic.

Aqueous solutions containing realistic levels of model compounds were prepared (approximately 3 mg/l TOC), buffered to pH 6.2 and chlorinated using the laboratory technique. The aqueous solutions were extracted by XAD-adsorption and tested for mutagenicity using strain TA100. Corresponding extracts of unchlorinated solutions of the model compounds were also prepared and tested using TA100.

The results of the fluctuation assays indicated that all of the extracts from unchlorinated model compounds and the extracts of chlorinated purines/pyrimidines and chlorinated nucleosides/nucleotides were non-mutagenic in strain TA100. However, the extracts of all three chlorinated humic acids as well as the chlorinated amino acids were mutagenic. (Bacterial growth checks were included in the mutagenicity assays and these indicated that the positive responses were not artifacts caused by enhanced bacterial growth.) The dose-response graphs of the mutagenic extracts are shown in Fig. 5 together with the dose response of an extract of treated water.

The mutagenic response of the chlorinated humic acid and amino acid solutions were of the same order of magnitude as that found in treated water. Thus it is possible that these compounds may be the major precursors of the TA100 mutagenicity which is observed in XAD-diethyl ether extracts of treated water and is a direct result of chlorination in drinking water treatment.

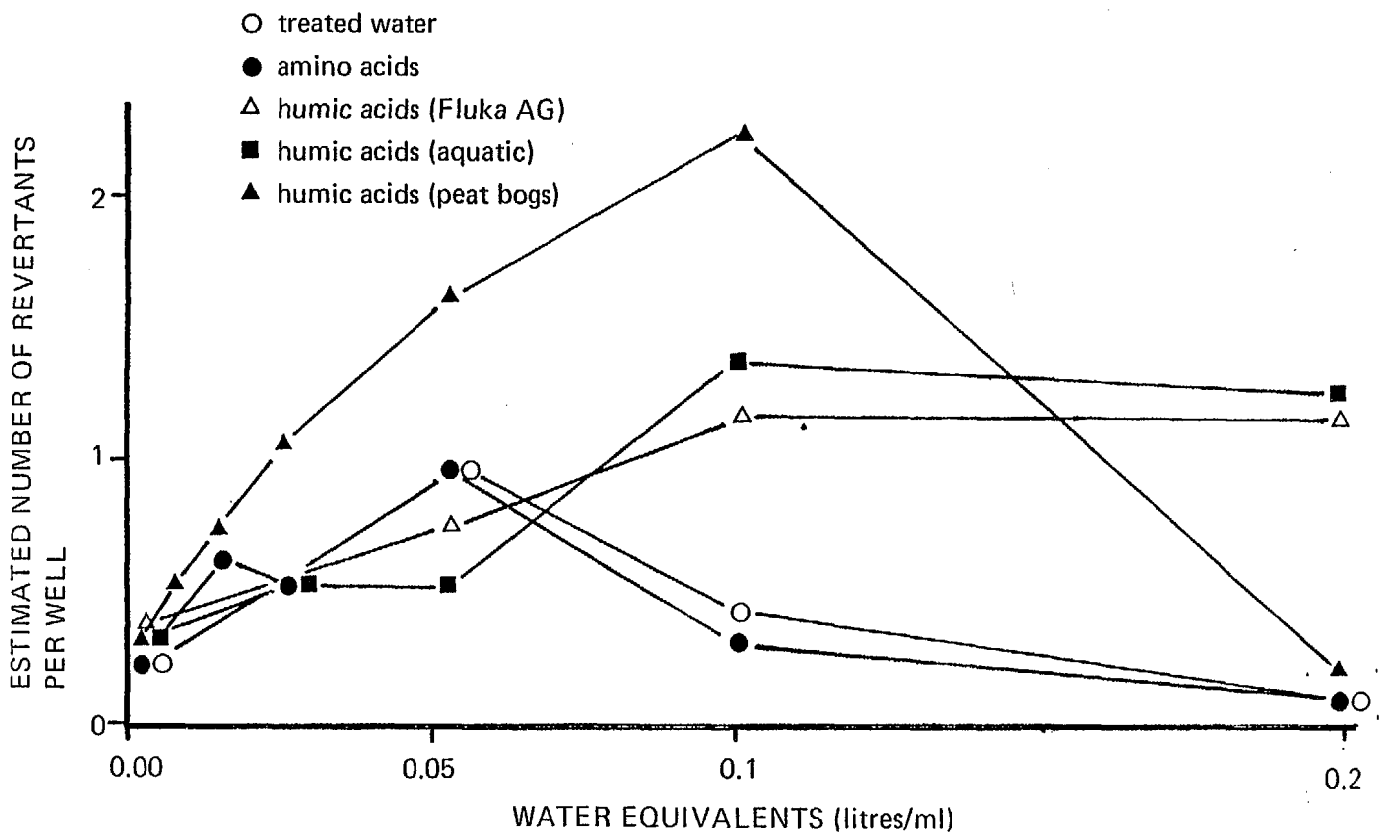

Fig.5. Comparison of TA100 mutagenic activities of XAD/diethyl ether extracts of final treated water and chlorinated model compounds.

Other characteristics were investigated in order to examine whether the mutagenic activity produced by chlorination of humic acid was comparable to that observed in treated water. These characteristics included mutagenic activity detected by strains TA100 and TA98, the effect of metabolic activation, the effect of dechlorination and HPLC behaviour. With the exception of the effect of dechlorination, extracts of treated water and chlorinated humic acids were very similar in all these characteristics. Chlorination produced mutagenic activity (as detected by TA100 and TA98) and the activity was reduced in the presence of rat liver S9 fraction. HPLC fractionation of both extracts revealed a highly complex mixture which yielded several mutagenic fractions, indicating the presence of a number of mutagenic compounds. However, while dechlorination appeared to destroy some of the mutagenic activity of the chlorinated water, the activity of the chlorinated humic acid remained unaffected.

These observations suggest that humic acids may be major precursors of mutagenic activity observed in XAD/diethyl ether extracts of treated water. One explanation of the difference in 'dechlorination' behaviour could be the existence of additional precursors, which form mutagens during chlorination that are destroyed by dechlorination.

No clear association was observed between mutagenic potency of the extracts and (i) chlorine consumption of the model compounds and (ii) adsorbable organo-halogen (AOX) in the chlorinated solutions and extracts.

GC-MS analysis of the extracts of unchlorinated and chlorinated model compounds was carried out. Few compounds were detected in the non-mutagenic extracts of chlorinated purines/pyrimidines and nucleosides/nucleotides. Table 15 lists the products of chlorination of humic acids and/or amino acids. Most of these have previously been identified in treated waters. Some of the compounds are mutagenic (see Table 14), but at the

Table 15 Products of chlorination identified by GC-MS in mutagenic extracts of chlorinated humic acids and/or amino acids

benzaldehyde
benzene
benzyl cyanide
bromodichloromethane
carbon tetrachloride
chloroform
chlorohydroxybenzyl cyanide
chlorophenol
dichloroacetonitrile
dichlorophenol
dichloropropene
hexachlorocyclopentadiene
indene
isobutyl nitrile
isopropyl nitrile
p-hydroxybenzyl cyanide
naphthalene
toluene
toluonitrile
1,1,1-trichloroacetone
1,1,1-trichloroethane
1,1,2-trichloroethane
trichlorophenol

levels detected would not appear to account for much of the mutagenic activity. Other compounds have not yet been tested and others have not yet been identified. These products of chlorination require further examination.

5.5 OVERALL ASSESSMENT OF RESULTS

Identification of the mutagenic substances has been hampered by the complexity of the organic mixtures encountered.

The presence of more than one mutagenic compound has been established. The bulk of the mutagenicity appears to be caused by relatively non-volatile substances (i.e. substances not amenable to GC-MS). Identification of non-volatile trace organics is difficult but the application of new mass spectrometric techniques (which are available at WRc), particularly field desorption-mass spectrometry and fast atom bombardment-mass spectrometry, shows promise.

Solvent partition at different pH followed by HPLC fractionation demonstrated that this method can be applied in conjuction with the mutagenicity assay and analytical techniques to separate complex mutagenic extracts into mutagenic and non-mutagenic fractions, but only at neutral or low pH since mutagenicity appeared to be partly destroyed by high pH conditions. Normal-phase HPLC fractionation of the 'neutrals' fraction appeared the most promising pathway, but processing of the large quantities of water sample required is laborious and time-consuming.

Encouraging results were obtained using HPLC fractionation and sub-fractionation of XAD/diethyl ether extracts of chlorinated and unchlorinated water.

To supplement the approach involving comparison of extracts of chlorinated and unchlorinated water, naturally-occurring compounds have been chlorinated in aqueous solution, concentrated using the XAD/diethyl ether method and tested for mutagenicity. Results obtained so far indicate that humic acids and amino acids may be significant precursors of the mutagenic compounds produced during water treatment chlorination. In the work carried out, mutagenic substances were identified, most resulting from chlorination, but in total these do not appear to account for much of the mutagenicity detected.

6. WATER QUALITY STUDIES

6.1 INTRODUCTION

A fundamental element in this programme of research was the identification of the compounds present. A thorough examination of relevant toxicological data could then be undertaken and assessment of the risks to health attempted. Therefore, identification of organic compounds was a major part of this research especially in the years up to 1981 (the end of the first contract).

The nature of organic matter in water, and the limitations of the available analytical techniques had an important bearing on the data produced. As the identities of the determinands were initially unknown, the results are at best only semi-quantitative. Once the identities of the compounds present have been established, better quantitative data can be produced in follow-up studies where the techniques are optimised for specific compounds. Also, the most generally applied technique used for drinking water surveys, GC-MS (which was used for this survey), is only applicable to at most 20% of the total organic matter present. It has therefore to be acknowledged that a full understanding of the nature of organic substances present in drinking water is not presently possible. Research, funded by DOE, into the development of new techniques to extend the range of identifiable organic compounds in water, is being undertaken at WRc (Non-volatiles in Drinking Water (DOE Ref.No. PECD7/7/047)).

Some information on the identities of organic compounds in drinking water has already been reported as an interim contract report[19] and subsequently as a WRc report, TR 159[20]. The report included the results of the examination of 14 treated waters, in which a total of 324 compounds were identified.

In this overall summary report, the results of a further survey of 14 treated waters (14 of the 15 which were subjected to mutagenic testing see Table 6) are included.

6.2 ORGANIC COMPOUNDS FOUND

The data from the two surveys referred to above (28 samples in all) have been combined. A total of 402 compounds were identified, and many (>100) compounds detected which for various reasons could not be identified. The frequency of occurrence data for the identified compounds is given in Table 16.

Table 16 The frequency of occurrence of identified compounds

Frequency	No. of compounds	Frequency	No. of compounds	Frequency	No. of compounds
28x	3	18x	2	8x	10
27x	0	17x	1	7x	15
26x	2	16x	2	6x	12
25x	1	15x	3	5x	23
24x	2	14x	4	4x	26
23x	2	13x	4	3x	36
22x	6	12x	4	2x	53
21x	3	11x	3	1x	166
20x	1	10x	5		
19x	6	9x	7		

Many compounds (41% of the total) were only detected once. This could either indicate uniqueness to a particular site, or presence at a concentration which only exceeded the detection limit at one site. The types of compounds identified are outlined in Table 17 whilst the most frequently occurring compounds (i.e. those present in >50% of the samples examined) are given in Table 18.

Table 17 Types and proportions of identified compounds

Compound class	% of total identifications
Hydrocarbons	35
Halogenated compounds	28
Carboxylic acids and esters	11
Other oxygenated compounds	16
Miscellaneous	10

Of the compounds identified, most occurred at concentrations below 1 µg/l. Those which occurred at higher levels were invariably haloforms and a few fatty acids, and in some groundwaters, trichloroethylene and tetrachloroethylene. A summary of the findings of a specific survey for trichloroethylene, tetrachloroethylene and p-dichlorobenzene in groundwaters[21] is given in Table 19.

Table 18. Most frequently occurring compounds identified (i.e. present in over 50% of compounds examined)

Frequency	Identities
28	bromodichloromethane, chloroform, chlorodibromomethane
26	bromoform, toluene
25	trichloroethylene
24	C_3-alkylbenzene, di-n-butylphthalate
23	C_3-alkylbenzene, p-xylene
22	C_3-alkylbenzene, C_4-alkylbenzene, p-dichlorobenzene, naphthalene, o-xylene, m-xylene
21	benzaldehyde, C_3-alkylbenzene, C_4-alkylbenzene
20	hexene isomer
19	benzene, di-isobutylphthalate, dichloroacetonitrile, 1-methylnaphthalene, 2-methylnaphthalene, tetrachloroethylene
18	C_4-alkylbenzene, diethylphthalate
17	C_3-alkylbenzene
16	C_3-alkylbenzene, bromochloroacetonitrile

Table 18 continued

15	bromochloroiodomethane, dibromoacetonitrile, dichloroiodomethane
14	C_3-alkylbenzene, C_4-alkylbenzene, n-decane, 1,1-diethoxyethane.

Table 19 Summary of levels of chlorinated hydrocarbons found in groundwater

	Trichloro-ethylene	Tetrachloro-ethylene	p-Dichloro-benzene
Average level (ug/l)	10	0.65	<.01
Maximum level found (ug/l)	70	3.2	.08

6.3 SOURCES OF IDENTIFIED COMPOUNDS

The sources of the compounds encountered include those of natural origin; contamination of raw water by industrially - used chemicals (e.g. trichloroethylene solvents in groundwater); agrochemicals (e.g. atrazine in groundwater) and production during disinfection (chlorination). Some of the compounds produced during disinfection are shown in Table 20.

Most of the compounds in Table 20 are formed from humic substances, but others (those marked with *) may also be formed from amino acids or proteinaceous material.

Table 20 Compounds produced during water treatment chlorination

benzaldehyde*
benzyl cyanide*
bromochloroacetonitrile
bromochloroiodomethane
bromochloronitromethane
bromodichloroacetonitrile
bromodichloromethane
chlorohydroxybenzyl cyanide*
dibromoiodomethane
dichloroiodomethane
nitrotrichloromethane
bromodichloronitromethane
bromoform
chloral
chlorodibromoacetonitrile
chlorodibromomethane
chlorodiiodomethane
chloroform*
dibromoacetonitrile
dichloroacetonitrile*
dichloronitromethane

6.4 BOILING EXPERIMENTS

Since most (>90%) water that is drunk is heated or boiled prior to consumption some studies on the effect of boiling on organic compounds present in drinking water were carried out. Initial experiments showed that prolonged (>10 min) boiling in an open vessel removed most of the organics detectable by GC-MS boiling below 150 ^{o}C. Further experiments on specific compounds (chloroform, dibromochloromethane, bromoform, trichloroethylene and tetrachloroethylene) under typical household conditions (automatic kettle) indicated that the levels of these compounds were reduced by about 50%. However, the reproducibility of these latter experiments was poor. Whether this was due to interaction of the compounds of interest with the metal walls of the kettle, or due to irreproducible adsorption onto the calcium carbonate which preciptated when the laboratory tap water (chalk groundwater derived) was boiled, is not known.

From these experiments it seems likely that estimates of the intake of some volatile organics (particularly haloforms) from drinking waters may be too high by a factor of 2.

7. TOXICOLOGICAL ASSESSMENT OF COMPOUNDS IDENTIFIED BY GC-MS

7.1 INTRODUCTION

To obtain a view on the significance to health of the compounds identified in drinking water by GC-MS (see Section 6) it was necessary to collate and evaluate the available toxicological data. A priority listing was made to reflect the need for toxicological examination (Table 21). This was based on the frequency of occurrence in the surveys (Table 17), the estimated concentrations observed and broad aspects of known toxicity such as suspected carcinogenicity.

Table 21 Priority listing of compounds for toxicological evaluation

1. Trihalomethanes, trichloroethylene and tetrachloroethylene
2. Aromatic hydrocarbons
3. Halogenated aromatics
4. Remaining halogenated aliphatics
5. Halogenated acetonitriles and other miscellaneous halogenated compounds and halogenated ethers
6. PAHs
7. Phthalates
8. Benzaldehyde
9. Hydrocarbons, saturated/unsaturated aliphatic
10. Remaining esters of carboxylic acids
11. Hydrocarbons alicyclic and Indan
12. Ketones
13. Miscellaneous compounds
14. Aldehydes
15. Ethers
16. Miscellaneous oxygenated compounds
17. Phenyl derivatives of aliphatic hydrocarbons
18. Acids

7.2 PROBLEMS IN EVALUATING TOXICOLOGY

The toxicity of a compound is its intrinsic capacity to cause injury. The hazard associated with the same compound is the capacity of that compound to cause injury under the circumstances of exposure. In order to study the possible hazard of a compound it is therefore necessary first to evaluate the toxicity. In this several aspects of the biological activity of the compound must be considered:

(i) Acute toxicity;

(ii) Chronic toxicity;

(iii) Reproductive toxicity including teratogenicity, embryotoxicity, fertility and multigeneration studies;

(iv) Mutagenicity;

(v) Carcinogenicity including transplacental carcinogenicity;

(vi) Metabolism and pharmacokinetics;

(vii) Special studies, including behavioural toxicology and immunotoxicity;

(viii) Epidemiology (primarily occupational).

It is important that such an evaluation is not just a data gathering exercise. Therefore the available studies must be examined critically. Just as in other branches of science there are good and bad studies and as knowledge increases then studies tend to become more comprehensive whilst earlier studies are viewed more critically. All experimental work has limitations which does not necessarily invalidate the work but does imply that conclusions drawn from the data must be qualified. The conclusions or interpretation should not exceed the power of the experiment. For example when one determines a no effect level this will depend on just how closely one looks for an effect.

7.3 PROBLEMS IN EVALUATING HAZARD

There are many factors which can influence toxicity or the expression of toxicity in animals and man (Table 22). Arguably the most important of these is pharmacokinetics through which the absorption, metabolism and excretion of compounds can be taken into account. The compound must enter the body and be transported to the target organ or tissue in the correct form and quantity to cause damage. However, metabolism of the compound by the appropriate cellular enzymes can occur in a sequence of

Table 22 Factors that influence toxicity

- Absorption, Metabolism, Excretion
- Tolerance
- Reserve Functional Capacity
- Genetic, including Strain or Species
- Sex
- Nutrition
- Disease
- Age
- Special Physiology (e.g. Pregnancy)
- Life Style

metabolic steps, often in the liver. Such metabolism may act in two ways. Firstly the compound may be detoxified and excreted. Alternatively, the compound may be changed to a reactive chemical species which will react with cellular macromolecules and so cause toxicity. Many of the factors which influence toxicity do so by affecting the absorption, metabolism and excretion of toxicants.

When extrapolating from experimental animals to man the confounding effects of these factors must be carefully considered.

Before any evaluation is begun, data must be collected on each compound. Unfortunately, there are very few of the compounds identified for which comprehensive toxicity data exists. There is a particular lack of data on the effects of chronic exposure and for a number of compounds there appears to be no data available.

7.4 RESULTS AND DISCUSSION

Reviews on the toxicity data available on over 300 compounds identified in drinking water have been carried out. In performing this exercise, in excess of 2000 documents were consulted and critically appraised. The compounds are reviewed in 23 separate documents amounting to some 1000 pages of text. These documents have been sent to DOE and are being used by WRc as a source of information when answering enquiries and dealing with pollution incidents.

It is not practicable to generalise about the availability of information on specific compounds. By and large, more data were available on acute toxicity than on chronic toxicity. Compounds such as chloroform, trichloroethylene, tetrachloroethylene and the aromatic hydrocarbons have been well studied. For example, 300 relevant references are quoted for the aromatic hydrocarbons alone. Other compounds such as some of the aliphatic

hydrocarbons and halogenated hydrocarbons have not been well studied and no information exists for some. The assessments were further complicated by the amount of data available on some compounds increasing considerably over the period of the review and necessitating revisions of earlier assessments. This was particularly so for the haloforms.

Though there are a number of known mutagens (Table 23), carcinogens (Table 24) and promoters and a substantial proportion of compounds are known to be toxic at much higher concentrations than those observed in water, there is no evidence at present to suggest that any individual compound is likely to pose a significant hazard to the health of consumers. There are some instances where specific pollution has taken place such as groundwater by some halogenated solvents. In these instances the concentrations are much higher and though it is difficult to identify any substantial risk it is conceivable that a low level effect could accrue from long term exposure to these concentrations. However, the lack of fundamental data on the toxicity of low dose levels make extrapolation from high experimental doses difficult.

The limitations on both basic information on the toxicity of the compounds identified and in the fundamental understanding of some areas of toxicology, prevent definitive statements regarding the health implications of organic micropollutants in drinking water. It must be appreciated that any effects on health which do occur as a result of continuous exposure to low levels of organic compounds in complex mixtures are more likely to be superimposed on the "normal" pattern of disease rather than be novel and, as such, will be all the more difficult to discern.

The groups of compounds of most concern are the halogenated aliphatic hydrocarbons, the halogenated ethers and the miscellaneous halogenated compounds. It is possible that at least in some cases there could be an additive action which would

Table 23 Compounds mutagenic in bacteria

styrene	1,5-dibromopentane
1-methylnaphthalene	trichloroethylene
acenaphthalene	bis(2-chloroethyl)ether
fluoranthene	dichloroacetonitrile
9-methylfluorene	bromochloroacetonitrile
1-methylphenanthrene	chloral
9-methylphenanthrene	trichloronitromethane
2-methylphenanthrene	bromodichloromethane
bromochloromethane	bromoform
dibromomethane	dibromochloromethane
bromoethane	dimethylphthalate
iodoethane	diethylphthalate
1,2-dichloroethane	dibutylphthalate
tetrachloroethane	di-2-ethylhexylphthalate
1-bromopropane	3-butandione
1-bromobutane	butan,2,3-dione
	dichloropropene

Table 24 Known and/or suspected animal/human carcinogens

benzene	bis(2-chloroethyl)ether
styrene	trichloroaniline
tetrachloromethane	clofibrate
1,2-dichloroethane	polychlorinated terphenyls
1,1,1-trichloroethane	2,4,6-trichlorophenol
1,1,2-trichloroethane	chloroform
tetrachloroethane	di-2-ethylhexylphthalate
hexachloroethane	1,4-dioxane
trichloroethylene	carbazole
chlorobenzene	

produce a higher overall effect than any individual compound. The evidence we have to date on the volatile organic micropollutants, taking into account the risk extrapolations for carcinogenesis by the United States Environmental Protection Agency (USEPA) and the work of WHO on organic contaminants in water leading to their Guidelines for Drinking Water Quality, indicates that any risk will be of a very low order. However, it must be emphasised that the volatile compounds account for about 15% of the organic matter present in water.

8. CONCLUSIONS

8.1 ON RE-USE

1. Epidemiological studies have not revealed any strong associations between water re-use and ill-health in communities in the UK. In view of the limitations of these studies, those associations that were found should be viewed with scepticism. Conversely the outcome of this research provides only weak reassurance that re-use is safe.

2. There is no simple relationship between the degree of re-use of a source-water and the range of volatile organic compounds found in the derived drinking water supply.

3. There is no simple relationship between the degree of re-use of a source-water and the potential of the derived drinking water to show mutagenic activity.

4. In view of 2 and 3 the concept of re-use, as such, is of limited help in trying to understand what the effects of the quality of re-used water on health might be.

8.2 ON ORGANICS IN GENERAL

1. Drinking waters derived from a range of water types (groundwater, upland water, lowland waters with various levels

of re-use) were all found to contain complex mixtures of organic compounds.

2. The available methods allowed only the identification of 'volatile' compounds which account for a minor fraction (around 15%) of the total organic matter present in drinking water. Even so, over 400 different compounds have been found.

3. The compounds identified include not only compounds probably attributable to re-use but also many substances produced during water treatment chlorination (e.g. haloforms) and trichloroethylene as a groundwater contaminant.

4. For practical reasons the concentrations of the compounds could not all be accurately determined. Generally concentrations were estimated to be less than 1 µg/l (1 part in 1000 million). Haloforms and trichloroethylene occurred at higher concentrations than this.

5. Toxicological information on many of the identified compounds does not exist.

6. For those compounds for which there is some toxicological data from animal studies, there remain difficulties and uncertainties in applying such data to infer what would be the effect on humans of the very low concentrations found in drinking water. Estimates by other organisations (USEPA and WHO) have indicated that the risks are likely to be of very low order.

7. All of the treated surface waters examined in this project, whether from upland or lowland sources, were active in mutagenicity assays using bacteria. Only a limited number of groundwaters were examined but these appeared to be non-mutagenic or showed only weak activity. It would, however, be unwise to draw conclusions relating to human health risk solely on the basis of bacterial assays.

8. Epidemiological studies which contrasted the health of communities served by different types of water sources did not reveal any strong effects. The possibility of less favourable health statistics being associated with upland supplies should be viewed with reservation until the hypothesis has been refined and investigated further.

8.3 CHLORINATION

1. The mutagenic activity of treated waters appears to be largely associated with chlorination, since the levels of activity were low or undetectable in the corresponding raw waters.

2. Dechlorination can reduce mutagenic activity and the activity is only partially restored if the water is subsequently chloraminated.

3. There was insufficient diversity of chlorination practice in the UK to enable its possible effects to be studied by epidemiology.

8.4 OTHER RELEVANT PROCESSES

1. From rather limited information it is possible to conclude that storage of raw water prior to treatment may reduce the mutagenic activity of the final product. The time scale of this effect would appear to be of months rather than days.

2. The research did not include any investigation of the effects of other treatment processes, alternative disinfectants and the changes in water quality that might occur during distribution.

3. The boiling of drinking water can lead to substantial losses (50%) of volatile organic compounds. As 90% of tap water consumed is boiled or heated before consumption, this would affect the quantity of the volatile compounds that are actually ingested by man.

8.5 OVERALL

The objectives of this project were very general and open-ended. Therefore, unless the task had turned out to be unexpectedly easy, it was unlikely that at the end of this contract the objectives would have been completely met. What has been achieved is the development of a battery of research methods, each of which has been used to assail the objectives from a different angle. The question - of whether organic contaminants in drinking water are of any consequence to human health - still stands. The difficulties that prevent straightforward answers are, however, better understood and it is possible to indicate what the next steps in research in this area should be.

9. RECOMMENDATIONS FOR FURTHER WORK

1. The generation of organic chemicals of potential health risk during water treatment chlorination needs to be fully investigated. This is partially covered by a DOE funded contract entitled "Effects of Disinfection on Organics" (DOE Ref. No. PECD 7/7/137) which recently started. This study includes investigations of disinfectants other than chlorine.

2. The chemical studies should be complemented with short-term screening tests in order to establish the effects of treatment on the formation and removal of mutagens and on the removal of their precursors.

3. Work should continue into the development and application of combined qualitative chemical analysis/bioassay methods to identify mutagens. A DOE funded contract entitled "Identification of Mutagens" (DOE Ref. No. PECD 7/7/122) with this objective is in progress.

   Apart from the specific concern about the formation of organics during disinfection, a number of general recommendations can be

made concerning the need for information on the nature of organic compounds in drinking water.

4. Groundwater needs to be investigated more intensively because of its apparent vulnerability to contamination by organic chemicals. Trichloroethylene and related compounds, together with some pesticides, need special consideration.

5. Studies to date have concentrated on treated water as it leaves the treatment works. Therefore, before human exposure to organics in drinking water can be assessed fully, detailed investigations of the sources of organic contamination within the distribution system and the effects of boiling during food and beverage preparation are needed. A DOE funded study entitled "Effect of Distribution on Organic Contaminants in Potable Water" (DOE Ref. No. PECD 7/7/95) in which some of these factors will be assessed is in progress. A parallel study of the effects of distribution and boiling on mutagenic activity is required.

6. Techniques for the identification of the remaining, unknown, organic matter in drinking water, which comprises over 80% of the organic content, need to be developed and applied. A DOE funded contract entitled "Non-volatile Organics in Water" (DOE Ref. No. PECD 7/7/047) in which such techniques are being developed has been in progress for a number of years.

   Although information on the nature of organic compounds is important, it is essential that information on the significance to health of the low levels of these compounds is also provided.

7. Although bacterial tests have shown mutagens to be present in drinking water, studies in higher cell systems are required before a qualitative hazard to man can be established. There is a need to develop and apply such tests to drinking water. A

DOE funded contract entitled "Biological Screening Tests for the Assessment of Drinking Water Quality" (DOE Ref. No. PECD 7/7/94) in which some preliminary information will be provided, is in progress.

8. Research is needed on the fundamental aspects of toxicology relating to low doses, long-term exposure and complex mixtures. The understanding of the mechanisms of toxicity under such conditions of exposure is particularly important and is a serious deficiency of regulatory toxicology.

9. For many of the numerous compounds which appear in the environment, there is little, if any, knowledge about toxicity and so there are two additional needs. One is the development of short-term in vitro screening tests for cytotoxicity which must be accompanied by fundamental mechanistic studies. The second is for the development of more sophisticated techniques for measuring structure activity relationships in order to develop models for the prediction of toxicity from chemical structure.

10. There is a need for improving the basis of mathematical models for extrapolating from high to low doses and from species to species to assist in interpreting animal experiments in relation to the exposure of man to environmental levels of chemicals. To this end, more fundamental mechanistic studies in toxicology are required to provide information to support the mathematical models.

11. Attempts have been made to assess the effects of this exposure in man using epidemiological techniques. However, the community-based studies that have been undertaken so far in the UK have limitations which can only be overcome by studying individuals. The power, design and cost of such further studies have been investigated and advice provided to the Department. Studies based on case-control methodology are

recommended as the next step in epidemiological research into the relation between specific features of drinking water and the risk of cancers at specific body sites.

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APPENDIX

A total of 42 contract reports, published papers and committee papers have been prepared on various aspects of the contract. A complete list of these, together with a number of documents still in preparation or in press are listed below. A number of the published papers were presented at conferences (marked * in the list).

GENERAL

Apart from this Final Contract Report, two general papers have been published:

1. *PACKHAM, R.F., BERESFORD, S.A.A. and FIELDING, M. Health related studies of organic compounds in relation to re-use in the United Kingdom. Science of Total Environment, 1981, 18, 167 - 186.

2. *RIDGWAY, J.W. Re-use of sewage effluent in the U.K. - an appraisal of health related matters. In, Re-use of Sewage Effluent. London, Thomas Telford Ltd, 1984, pp 181 - 192.

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