ENVIRONMENT AGENCY

The assessment of taste, odour and related aesthetic problems in drinking waters 1998

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet contains advice and guidance for assessing the likely causes, especially those caused microbially, of taste, odour and related aesthetic problems appearing in drinking waters.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommend methods of sampling and analysis for determining the quality of drinking water, groundwater, river and seawater, waste water and effluents as well as sewage sludges, sediments and biota. In addition, short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated, with results from performance tests reported for most parameters. These methods should be capable of establishing within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated individual results encompassing at least ten degrees of freedom from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors), systematic error (bias), total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available. An indication of the status of the method is shown at the front of the publication on whether or not the method has undergone full performance testing.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets in the series 'Methods for the Examination of Waters and Associated Materials' and their continuous revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage treatment methods and biodegradability
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and the more important parameters and topics is available from HMSO (ISBN 0 11 752669 X).

Every effort is made to avoid errors appearing in the published text. If, however, any are found please notify the Secretary.

Dr D Westwood
Secretary

September 1998

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and any regulations made under the Act, and Control of Substances Hazardous to Health Regulations 1988 (SI 1988/1657). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety, and these should be consulted and be readily accessible to all analysts. Amongst such publications are those produced by the Royal Society of Chemistry, namely 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory' 5th edition, 1992; by Member Societies of the Microbiological Consultative Committee, 'Guidelines for Microbiological Safety', 1986, Portland Press, Colchester; and by the Public Health Laboratory Service 'Safety Precautions, Notes for Guidance'. Another useful publication is produced by the Department of Health entitled 'Good Laboratory Practice'.

The assessment of taste, odour and related aesthetic problems in drinking waters

1 Introduction

Organoleptic problems in drinking waters can be caused by a number of factors. These include: natural products in water used for abstraction; compounds formed during treatment, storage or distribution; and ingress of materials (to distribution systems) that either react with compounds in the water or are themselves responsible for tastes and odours. Many problems related to unacceptable tastes and/or odours experienced by water undertakers and their customers are caused by chemical substances, the majority of which are used in connection with disinfection processes. Taste, odour and related aesthetic problems caused by micro-organisms can be significant, particularly for certain types of water sources. Treated water contains a variety of substances, including bacteria, micro-fungi and yeasts indigenous to its source and the surrounding soil and vegetation. Often, these substances do not necessarily give rise to water quality problems. As well as causing objectionable tastes and odours, the activities of micro-organisms may result in a deterioration of water quality leading to discolouration or other changes. Micro-organisms may also play a role in corrosion processes that occur within water distribution systems. This booklet provides advice and guidance on assessing the most likely microbial causes of taste and/or odour problems. Methods for the detection and identification of certain chemicals are also included. Those for other chemicals and algae are covered elsewhere within this series.

2 Tastes and odours in drinking water

The occurrence of substances which impart undesirable tastes and/or odours in drinking waters is one of the principal causes of complaint by consumers. These substances may be present as a result of man-made or natural processes, and often result from microbial growth and metabolism. The major causes of taste and/or odour problems associated with drinking water supplies are: biological activity in source waters, especially by algae; disinfectants used in water treatment, notably chlorine and derived compounds; and biological activity within distribution systems [1,2]. Tastes and/or odours may also be associated with substances present in construction materials and linings, or by the leaching of industrial chemicals into the supply.

2.1 **Microbially-mediated taste and odour and aesthetic problems**. One of the most commonly reported complaints made by consumers related to microbial activity is the occurrence of earthy or musty tastes and/or odours. These tastes and/or odours are primarily associated with the production of two compounds, namely geosmin and 2-methylisoborneol. These compounds are metabolites produced by a range of micro-organisms, most notably the Actinomycetes (for example *Streptomyces, Nocardia and Microbispora*), the Cyanobacteria (blue-green algae, for example *Oscillatoria, Anabaena and Aphanizomenon*) and a number of species of algae (particularly members of the Chlorophyceae and the Bacillariophyceae). These compounds may be released by organisms which are actively growing or through cell lysis and decomposition, and have very low odour threshold concentrations (typically, 0.015 $\mu g l^{-1}$ for geosmin and 0.02 $\mu g l^{-1}$ for 2-methyl-isoborneol) [3].

A number of other tastes and/or odours can also be associated with the metabolism and decomposition of Actinomycetes and algae. These range from fishy, grassy and woody tastes and/or odours caused by metabolites of sulphur compounds released during decomposition. Sulphur tastes may also be related to microbial reduction of sulphates under anaerobic conditions. Another problem, of an aesthetic nature, is discolouration caused by release of iron compounds resulting from microbial activity in cast-iron mains or iron-rich waters.

Sources of microbially-mediated taste and odour and aesthetic problems can be broadly categorised into four groups:

(i) **Metabolites produced by microbial activity.** As described previously, geosmin and 2-methylisoborneol are produced by members of the Actinomycetes, Cyanobacteria and green algae, and these organisms are the main sources of earthy and musty tastes and/or odours in drinking waters.

These metabolites may be produced in source waters and pass through treatment processes into supply, or may be formed as a result of growth of Actinomycetes within a matrix of micro-organisms (a biofilm) growing on the walls of pipes, or on sediments and other deposits within distribution systems. In addition, the presence of moulds has been associated with taste and/or odour problems [4]. There are limited data on the production of geosmin, 2-methylisoborneol, or other taste and/or odour compounds by fungi isolated from water supplies. Geosmin production has been demonstrated for *Chaetomium globosum* and *Basidiobolus ranarumi* [1]. Other metabolites from Actinomycetes which impart tastes and/or odours are cadin-4-ene-1-ol (woody-earthy odour) and 2-isopropyl-3-methoxypyrazine (musty/mouldy potato odour) [1].

Various odours have been associated with algae, particularly members of the Cyanobacteria. Fishy odours have been related to the production of aldehydes (for example, n-hexanal, n-heptanal and isomers of decadienal) and sulphur-containing compounds. Other substances produced by algae imparting tastes and/or odours are terpenes, aromatic compounds and esters. Fishy odours have also been associated with large populations of some zooplankton (for example, the *rotifer Keratella* and the crustaceans *Cyclops* and *Daphnia*). It is unclear, however, whether the sources of these odours are derived from metabolites produced by these organisms or compounds generated during decomposition of the organisms. Nematodes have been reported to secrete odorous compounds, one of which, when isolated from culture, gives an earthy/musty odour [5]. It has also been reported that some amoebae can cause tastes and/or odours [5]. The significance of these latter sources of taste and/or odour compounds to drinking water supplies remains unassessed.

(ii) **Products from microbial decomposition.** Bacterial decomposition of organic matter can lead to the production of a range of products, including sulphur-containing compounds, many of which impart tastes and/or odours to waters.

Decomposition of algae, particularly following a cyanobacterial bloom in source waters, can result in significant concentrations of mercaptans, dimethyl sulphide and polysulphides, and other volatile sulphur compounds which impart fishy, swampy or septic odours. Some of these compounds are also produced by the putrefaction of proteinaceous material within distribution systems. Species of *Pseudomonas*, *Flavobacterium* and *Aeromonas* have been reported in the production of dimethyl polysulphides resulting in swampy odours [6]. These bacteria have been shown to be capable of producing a number of volatile sulphur compounds, particularly dimethyl disulphide [1]. The mould, *Penicillium caeseicolum*, is also capable of producing dimethyl disulphide [1]. Other odorous compounds are indole and skatole (from tryptophan), several amines (for example, putrescine, cadaverine and ß-phenylethylamine) and fatty acids (for example butyric, propionic and stearic acids).

(iii) **Hydrogen sulphide from reduction of sulphates.** The presence of a microbial population capable of reducing sulphate to hydrogen sulphide can result in a distinctive rotten-egg odour being associated with the water. Under anaerobic conditions, sulphate-reducing bacteria (for example, *Desulfovibrio desulfuricans* and *Desulfotomaculum orientis*) are able to reduce sulphate to sulphite and then to hydrogen sulphide. In distribution systems, other bacteria (for example, species of *Clostridium* and *Bacillus*) may also be involved in the reduction of sulphite to hydrogen sulphide [7]. Clostridia are also capable of degrading proteins containing sulphur thereby releasing hydrogen sulphide. Rotten-egg odour problems are often associated with deep groundwater supplies and corrosion of pipes in systems with very low flow rates.

(iv) **Discolouration due to iron salts.** Microbially-induced corrosion of iron pipes occurs during the reduction of sulphate in the presence of iron resulting in the production of iron(II) sulphide. The subsequent oxidation of this compound to iron(III) oxide (for example, during the growth of iron-precipitating bacteria such as *Gallionella ferruginea*) can result in elevated levels of iron in the water. This may cause discolouration, particularly when clothes are washed in the water or, more dramatically, the production of 'red water'.

The structures of some of these compounds have been determined and are shown in Figure 1.

2.2 **Investigation of microbially-mediated taste and/or odour and aesthetic problems.** When attempting to ascribe a microbiological cause to a taste and/or odour problem, adequate evidence should be gathered to establish the possible relationship. Although a microbial cause may be suspected, it is often very difficult to isolate the causative organisms, particularly when the taste and/or odour problem is identified in a location some distance from the original site of production. The principal steps to be taken to establish the source of microbially-mediated taste and/or odour problems include the following.

(i) The location and definition of those parts of the distribution system affected by the problem. This may include a qualitative survey to confirm the characteristics of the tastes and/or odours reported by consumers. An assessment of the location and timing of consumer complaints (taking into account likely patterns of water flow) may also be useful in helping to determine whether the cause is microbially-mediated.

(ii) If, on the basis of any preliminary assessment, it is considered likely that the problem is microbiological in origin, then the types of organisms that may be involved can be selected for investigation. For example, Figure 2 can be used to identify possible causes. The isolation and, where feasible, enumeration of the suspected organisms can then proceed.

(iii) Confirmation of the identity of the organisms and, where feasible, confirmation that the organisms are capable of producing the compounds associated with the reported tastes and/or odours. Metabolites from the growth of the organisms may also be recovered and analysed by gas chromatography-mass spectrometry.

When investigating microbially-mediated taste and/or odour and aesthetic problems, the major groups of organisms to consider include:

- Heterotrophic colony count bacteria
- Actinomycetes (particularly *Streptomyces* and *Nocardia*)
- Micro-fungi and yeasts
- Sulphate-reducing bacteria (*Desulfovibrio* and *Desulfotomaculum*)
- Sulphite-reducing bacteria (*Clostridium*)
- Iron-precipitating bacteria (*Gallionella* and *Leptothrix*)

The choice of organisms to investigate will depend on the assessment of the type of tastes and/or odours present and the nature of the problem encountered.

3 Assessment of tastes and/or odours in drinking water

The receipt of consumer complaints regarding the quality of tap water are often the first indications of taste and/or odour problems in drinking water. These complaints vary in their nature, drinking waterfrequency and timing, and many may result from intentional changes brought about in the source, treatment or distribution of water supplies. These include:

- the type of source water, for example surface water and groundwater, and the mixing in distribution of waters from different sources;

- the water treatment process, particularly disinfection and amounts of residual disinfectant;

- seasonal and weather-related effects, for example stratification within reservoirs, run-off into reservoirs and rivers following heavy rainfall (particularly after a prolonged,dry period);

- the extent and complexity of the distribution system, residence times, secondary disinfection, materials of construction, etc; and

- the nature of plumbing within individual buildings, contamination from storage tanks or cross-connections.

There are various causes of objectionable tastes and/or odours in drinking waters, only some of which are microbiological in origin. An initial assessment of the problem should be carried out to determine the likelihood of the problem being microbially-related. A key factor may be the description of the taste and/or odour being experienced by consumers. Several of the more common taste and/or odour problems arising from micro-organisms are very specific in their manifestation and require little further information before proceeding to microbiological or chemical analysis; an example of this is the earthy or musty odour of geosmin or 2-methyl-isoborneol from the growth of Actinomycetes.

There may be localised contributing factors affecting the perceived taste and/or odour of the water and the following should be considered where appropriate:

- Anti-splash nozzles, particularly those made from rubber or plastic which are visibly deteriorating. Bacterial and fungal action can give rise to breakdown products from such attachments, which, by themselves or in combination with chlorine in the water, can cause offensive tastes and/or odours.

- Rubber and plastic hoses used to fill drinking water tanks on coaches, caravans, trains, etc may give rise to taste and/or odour problems, as can hoses used to fill drinks-vending machines.

When considering whether a taste and/or odour problem is microbially-related, reference should be made to the descriptions given in Table 2 and Figure 2. If the descriptions provided by consumers are non-specific or contradictory, the analysis of samples for qualitative taste and odour may help to define whether the cause is microbiological. Consideration should be given to the collection of further samples, including those for physical and chemical analysis (which may help to determine the source of the water) and samples from neighbouring properties if appropriate.

4 Aesthetic problems in drinking water

In addition to problems of tastes and/or odours in drinking water, micro-organisms may also be involved in other processes which can give rise to changes in water quality. These include:

- Discoloured water, or staining of appliances, caused by the presence of iron-precipitating bacteria.

- Microbially-induced corrosion of materials and fittings leading to their discolouration and possible failure, the most common form being due to the activity of sulphate-reducing bacteria.

4.1 **Iron-precipitating bacteria.** Iron-precipitating bacteria (iron bacteria) are a diverse group of micro-organisms widely distributed in fresh and marine waters and in soil. They are capable of transforming soluble iron (and occasionally manganese) into an insoluble form that can cause fouling in boreholes, water treatment plants and distribution systems. Iron bacteria convert soluble iron(II) to insoluble iron(III) which is then deposited within or on the exterior of the bacterial cell.

Bacteria that have been associated with fouling and discoloured water include the following.

(i) The sheathed bacteria that produce sheaths of oxidised iron (and occasionally manganese) surrounding the cells. Oxidised iron, or manganese, is deposited outside the sheaths. The most common examples are *Leptothrix* and *Clonothrix*. Another species, *Sphaerotilis*, does not deposit insoluble iron or manganese on the outside of the sheath but may form large flocs.

(ii) Stalked bacteria, such as *Hyphomicrobium*, *Caulobacter* and *Gallionella*, which may have appendages. *Gallionella* form long, spirally-twisted stalks arising from the centre of the cell. *Hyphomicrobium* and *Caulobacter* may oxidise manganese.

(iii) Bacteria of the Genus *Thiobacillus* are capable of oxidising both sulphur and iron(II).

Bacterially-mediated iron precipitation may give rise to taste and/or odour problems, but more usually result in discoloured water and occasionally, frothing. In distribution systems, the presence of iron bacteria may increase disinfectant demand. Biofilms may develop around iron bacteria and accelerate corrosion of susceptible materials.

4.2 **Microbially-induced corrosion.** Sulphur bacteria in water systems are nuisance-organisms that cause severe taste and/or odour problems as well as contributing significantly to corrosion, with subsequent discolouration and failure of materials. Under anaerobic conditions, certain sulphate-reducing bacteria produce sulphide and hydrogen sulphide gas. This may trigger electrolytic corrosion on metal surfaces. Biofilm organisms present in the same location may enhance the corrosion by producing organic acids which are also corrosive.

The main groups of sulphur bacteria include:

- Sulphate-reducing bacteria, for example *Desulfovibrio*, *Desulfotomaculum*;

- Sulphur-reducing bacteria, for example *Desulfuromonas*;

- Sulphur-oxidising bacteria, for example *Thiobacillus* (which may also oxidise iron);

- Colourless sulphur bacteria, for example *Beggiatoa*, *Thiothrix*; and

- Sulphur-oxidising photosynthetic purple and green sulphur bacteria, for example *Chlorobium* and *Chromatium*.

5 Sampling

The investigation of taste and/or odour complaints may require the adoption of modified procedures to those used for routine microbiological examination of drinking water supplies [8] and should be tailored to suit the nature of the complaint. The number and type of samples, and their locations, need to be carefully considered. For routine microbiological sampling, it is essential that the samples taken are representative of the quality of the water supplied to the property. This may often not be the case when investigating taste and/or odour complaints, since there may be particular conditions, such as, for example, the physical state of the tap, which contribute to the cause of the complaint.

Before taking a representative sample of the incoming water, it may be appropriate for example, to take samples without flushing, disinfecting the tap or removing anti-splash nozzles. A number of field or on-site tests may also need to be carried out in order to help identify the possible cause of a taste and/or odour complaint. These may include temperature [9] (before and after flushing), qualitative taste and odour [3], and chlorine residuals [10].

If a taste and/or odour problem has been established which is not related to local conditions but is considered representative of the distribution supply, then sampling may need to be extended. This should then include the raw source water, process samples within the treatment plant, the treated water entering supply, and representative samples from within the distribution system. Procedures for sampling from hydrants and drinks-vending machines, and the transport and storage of samples are described elsewhere in this series [8].
If the problem is associated with algal growth within a raw water reservoir, it may be necessary to undertake depth sampling (for example, in order to advise on an appropriate draw-off point).

5.1 **Distribution system sampling and field tests.** When investigating a complaint of taste and/or odour at a consumer's property, the sampling and field testing regime should be tailored to the specific circumstances of the complaint. Indeed, a sample may not even be required. Figure 2 can be used to ascertain the possible origin of the complaint and the sampling category into which it may fall. These include:

- chlorinous tastes and/or odours;
- metallic, chemical, solvent;
- microbiological.

Microbiological analyses are not, generally, appropriate for situations involving chlorinous tastes and/or odours, and metallic, chemical and solvent tastes and/or odours are not considered here. When taking samples, the procedures used should ensure that samples for microbiological examination are representative and are free from extraneous contamination at the time of collection, and that changes during transportation are minimised or eliminated. For certain parameters, however, for example free chlorine, the concentration may change as a result of the sampling process. For other parameters, such as temperature, values will change as a result of conditions of storage. To ensure that meaningful results are obtained, it is necessary to carry out such determinations at the time of sampling.

For complaints thought to have a microbiological cause, the sequence of sampling should be as follows.

5.1.1 **Water.** Without flushing or disinfecting the tap, or removing any attached fitments or point of use devices, a first-draw microbiological sample should be taken. The analyses carried out on this sample should reflect the type of taste and/or odour reported. Typically, an estimate of the number of heterotrophic bacteria is determined, and if the reported taste and/or odour is described as earthy or musty, analyses for Actinomycetes and micro-fungi are carried out. Coliform organisms are not considered to be a cause of these types of complaints and their examination need not necessarily be included at this stage.

A volume of sample (50-100 ml) should also be collected into a second clean bottle. The bottle should not impart a taste and/or odour to the sample, nor eliminate the offending taste and/or odour from the sample. This sample can be used for determining the on-site temperature [9] and qualitative taste and odour [3], although due consideration should be given to tasting the sample before obtaining a satisfactory microbiological examination report.

Any tap attachments should now be removed and the tap cleaned, disinfected and flushed [8]; a second microbiological sample should then be taken. This sample should undergo the same microbiological analysis identified above.
A comparison of the results should then give some indication as to whether the problem is localised. Consideration should also be given to the analysis of other determinands, including coliform organisms, and a biological examination, including algae.

After adequate flushing, the residual chlorine content of the water should be determined [10].

Samples of water and deposits intended for the analysis of sulphate-reducing bacteria should be taken in sterile containers and filled to the top to exclude air. Sodium thiosulphate should not be added as this may react with sulphides present in the sample. The analysis of pH, redox potential, oxygen concentration, sulphate and sulphide can yield valuable information when investigating the presence of sulphate-reducing bacteria which are able to grow at a redox potential below -100mV in the absence of oxygen. The presence of sulphide suggests conditions suitable for growth of sulphate-reducing bacteria and their possible presence.

The volume of sample required for analysis will depend on the number and nature of micro-organisms under investigation and minimum volumes are shown in Table 1.

5.1.2 **Deposits and sediments.** In certain cases, in addition to water samples, it may be useful to examine sediments and deposits since these may be sources of micro-organisms causing taste and/or odour problems. Various types of deposits may be present in the water supply including slime layers (biofilms), tuberculations and sediments. Special procedures are required for sampling such deposits and the techniques used will depend upon their nature. All equipment (for example swabs, spatulas, scoops, etc) used to collect the deposits, and the containers for transporting the material should be clean and sterile. All material collected should be examined as soon as possible.

Direct access to deposits, for example storage tanks or service reservoirs, may be possible if appropriate parts of the system are drained during the investigation. Deposits on surfaces can be obtained by scraping or swabbing. Firmly adhering deposits can be obtained by scraping with a spatula and transferring the material to a container. Loose deposits can be collected with a scoop and transferred to a suitable container. Swab samples can be taken using cotton-wool swabs (preferably single-pack in a tube). After use, return the swab to its case or place in a suitable container.

Where direct access is not possible, samples of deposits may be obtained by flushing the system from suitable locations such as hydrants. Procedures for disinfecting hydrants before the collection of samples for microbiological examination have already been referred to [8]. To collect a deposit, a nylon bag of suitable mesh size can be attached to the stand-pipe and the flow increased to dislodge any deposits. The deposits retained in the bag should then be transferred to a suitable container. The water which passes through the nylon bag should be allowed to flow into a tank to enable any fine sediment to settle. This material can be collected after the supernatant liquid has been discarded [11].

5.2 **Raw water reservoir sampling.** Tastes and/or odours can be associated with algal growths within impounding or storage reservoirs, or in rivers used as sources of drinking water. Samples should be taken at depths commensurate with the draw-off level.

5.2.1 **Sampling for algae.** If it is intended to count the number of algal cells and/or identify the species, then water samples should be placed in appropriate vessels containing a suitable preservative, such as Lugol's iodine [12]. Alternatively, an indirect assessment of the algal load can be made by an analysis of the chlorophyll content [13]; these samples, however, should not be preserved.

5.2.2 **Sampling for other micro-organisms.** Samples intended for the analysis of micro-organisms other than algae should be placed in a suitable container without further treatment or use of a preservative or neutralising agent.

6 Methods of microbiological analysis

The following procedures are suitable for the microbiological examination of samples taken for taste and/or odour investigations. The principles, preparation and procedures used for membrane filtration and the most probable number technique have been fully described previously [8] and should be read in conjunction with this booklet.

6.1 **Heterotrophic colony counts.** Samples should be analysed as previously described [8]. The use of an alternative low-nutrient medium such as R2A may prove useful when investigating problems of taste and/or odour.

6.2 **Actinomycetes**

6.2.1 **Introduction.** Members of the Actinomycetes occur ubiquitously in the environment, typically in soil, decomposing organic matter and aquatic habitats. Growth of some of these bacteria in source waters and in drinking water distribution systems can result in the release of substances imparting undesirable tastes and/or odours to the water. Several members of this group, particularly species of *Streptomyces*, are capable of producing geosmin and 2-methyl-isoborneol, which impart characteristic earthy or musty odours.

6.2.2 **Definition.** The Actinomycetes are Gram-positive coccobacillus or rod-shaped, aerobic bacteria of the Order Actinomycetales that form a well developed, branching mycelium.

6.2.3 **Scope**

6.2.3.1	Limitations	Species of the *Streptomyces*, the dominant Actinomycetes associated with odour problems in water, can be isolated. Some species of *Nocardia*, *Micromonospora* and *Microbispora* may also be recovered.
6.2.3.2	Types of sample	Potable water and freshwater. The sample should not contain significant quantities of sediment or particulate matter.
6.2.3.3	Principle	Concentration of the water sample by membrane filtration, followed by culture upon a selective medium and enumeration of characteristic colonies.
6.2.3.4	Time required for analysis	Seven days.
6.2.3.5	Interferences	Several species of micro-fungi are able to grow on the selective medium and, if abundant, may overgrow the plate. For waters where this is a problem, or for previously uncharacterised waters, this may be obviated by the addition of 0.05 gl^{-1} of cycloheximide to the medium prior to autoclaving.

6.2.4 **Procedure.** The medium used for the isolation of Actinomycetes that may be responsible for taste and/or odour problems in water is Actinomycete isolation agar (see 6.2.6). This medium is available commercially. Depending on the number of organisms expected, prepare (making any appropriate dilutions) and filter the sample, or diluted sample. Typically, 100 ml of sample or diluted sample is sufficient. Place the membrane on the surface of the prepared medium. Ensure that the surface of the agar is not too dry, as restricted growth may occur during incubation. Incubate at 22.0 ± 1.0°C for seven days.

Plates should be examined for growth at regular intervals during the incubation period. The final count is made after seven days. Count all compact colonies which are dry, raised and powdery in appearance. Generally, Actinomycete colonies are chalky white in colour, but some may be pink, red, brown, yellow or green. Some slower growing Actinomycetes may exhibit a wrinkled, waxy 'star-shaped' colony within the above incubation time.

6.2.5 **Confirmation of Actinomycetes.** Actinomycete morphology may be confirmed by conducting a Gram-stain on selected isolates. The isolates that possess well developed branching mycelia with long chains of spores on aerial mycelia are often species of *Streptomyces*.

6.2.6 **Actinomycete isolation agar**

Sodium caseinate	2 g
Asparagine	0.1 g
Glycerol	5 g
Sodium propionate	4 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate heptahydrate	0.1 g
Iron(II) sulphate heptahydrate	0.001 g
Agar	15 g
Distilled water	1 litre

Heat to boiling to dissolve all the ingredients. Adjust the pH, if necessary, so that after sterilization the pH is 8.1 ± 0.2. Sterilize by autoclaving at 121 ± 2°C for 15 minutes, allow to cool to about 50°C and distribute into sterile Petri dishes.

6.3 **Micro-fungi and yeasts**

6.3.1 **Introduction.** Micro-fungi are a major group of micro-organisms and occur wherever organic matter is present. They are found in natural (untreated) waters and arise predominantly from decaying vegetation or are washed into water courses from soil. Micro-fungi and yeasts are known to occur in treated water. Some micro-fungi cause undesirable changes in the organoleptic quality of treated water. Bio-transformation of chlorinated compounds, to produce more potent tastes and/or odours, has been demonstrated in certain species. However, little evidence is yet available to establish the nature and significance of micro-fungi in water supplies.

6.3.2 **Definition.** Micro-fungi exhibit mycelial growth and reproduce by spores. The nutritional requirements of micro-fungi and yeasts are such that they grow on simple media. Yeasts exhibit unicellular growth and reproduce by budding or fission.

6.3.3 **Scope**

6.3.3.1	Limitations of the method	Growth of mycelium and spores takes place, which reduces the significance (in terms of a meaningful indication of fungal biomass) of the number of colonies detected.
6.3.3.2	Types of sample	The membrane filtration procedure is applicable only for potable water and natural waters that do not contain appreciable quantities of sediment or particulate matter. The spread or pour plate technique is usually suitable for small volumes of sample, particularly if deposits are present.

6.3.3.3	Principle	For membrane filtration, the fungal propagules retained on the filter are cultured on a selective medium. For spread or pour plate techniques, a dilution series of the sample is prepared and appropriate volumes are processed. The plates are incubated and examined for the presence of micro-fungi and yeasts after an appropriate period of incubation.
6.3.3.4	Time required for the test	Seven days.
6.3.3.5	Interferences	The number of micro-fungi and yeasts may be distorted by excessive handling of cultures during incubation as spores can be released which produce additional colonies on the medium.

6.3.4 **Procedure.** It should be recognised that there is no single medium which is capable of isolating the entire range of micro-fungi and yeasts present in water supplies. The medium, Rose Bengal Chloramphenicol agar has been widely used in the UK to isolate micro-fungi and yeasts from drinking waters, and is available commercially. While Rose Bengal is incorporated into the medium to suppress the growth of rapidly growing micro-fungi, it may prevent growth of other species present. During initial investigations, more than one medium should be used to ensure that a broad range of species is recovered. Details on the selection of suitable media have previously been reported [14].

As the concentration of micro-fungi and yeasts in water is not likely to be known, a range of volumes, from 500 ml to 0.001 ml should be prepared, in duplicate, and processed. Ensure that the surface of the agar is not too dry, as restricted growth may occur during incubation.

The processed samples should be incubated for seven days at 22 ± 1°C and 12 ± 1°C. The plates should be examined daily, as abundant growth of some micro-fungi may obscure the growth of slower growing species. It may be necessary to record and isolate the colonies before the full period of incubation has been completed. Alternatively, it may be possible to remove the rapid growing fungi to enable the slower growing species to emerge. Microscopic examination of the plates may assist the detection of slower growing species which do not form colonies that are readily visible to the naked eye.

Micro-fungi develop as raised colonies which take on a wool-like, cotton-like or powder-like appearance. In contrast, yeasts develop as red colonies and are characterised by a smooth, waxy appearance. Occasionally, aerial mycelium will not be present, as growth may occur in the medium.

6.3.5 **Confirmation of micro-fungi and yeasts.** At present, there is no single source of published information for identifying micro-fungi and yeasts isolated from water. Specialist schemes are available for specific taxonomic groups, but their interpretation can be difficult. Also, the above medium may not allow the production of reproductive structures, which are essential for identification. It may, therefore, be necessary to sub-culture isolates on media more appropriate for sporulation.

Isolates may be submitted for confirmation and a full description and/or photographic record should be maintained, which might assist with future identifications.

6.3.6 **Rose Bengal Chloramphenicol Agar**

Mycological peptone	5.0 g
Glucose	10.0 g
Dipotassium phosphate	1.0 g
Magnesium sulphate	0.5 g
Rose Bengal	0.05 g
Chloramphenicol (0.1g in 5 ml of methanol)	5 ml
Agar	15.5 g
Distilled water	to 1000 ml

Suspend all the ingredients in one litre of distilled water and boil to dissolve. Sterilize the medium by autoclaving at 121 ± 2°C for 15 minutes. Allow to cool to approximately 50°C and distribute into sterile Petri dishes. The final pH should be 7.2 ± 0.2.

Rose Bengal is degraded by light to form toxic derivatives and thus prepared plates should be stored in the dark. Prepared plates may be stored for up to one month at approximately 4°C.

6.4 Sulphate-reducing bacteria

6.4.1 **Introduction.** Sulphate-reducing bacteria are a diverse group of strictly anaerobic micro-organisms distinguished by their ability to reduce sulphate to sulphide. The organisms can be found in many anaerobic environments, in soil, mud, sediment and sewage. However, they occur most commonly where high concentrations of sulphate are present. This group of bacteria is a significant cause of microbially-induced corrosion and taste and/or odour problems in water systems. In addition, major health hazards may arise due to the production of hydrogen sulphide.

6.4.2 **Definition.** The two most common genera are *Desulfovibrio* and *Desulfotomaculum*. *Desulfovibrio* are Gram-negative, non-sporing vibrio rods (2.5-10 µm by 0.5-1.5 µm) which are motile with polar flagellae. *Desulfotomaculum* are Gram-negative rods (3-9 µm by 0.3-1.5 µm) which produce terminal or subterminal spores.

6.4.3 **Scope**

6.4.3.1	Limitations of methods	There are many formulations of media used for enumerating sulphate-reducing bacteria. All include a carbon source (normally lactic acid) and small amounts of yeast extract, inorganic salts and a reducing agent (to equilibrate the medium at a low potential). It has been suggested that the viable count procedures for enumerating sulphate-reducing bacteria may underestimate numbers by a factor of around 1000, compared with *in-situ* sulphate reduction activity measurements [15]. Due to the difficulties in obtaining accurate counts on agar-based plating media, sulphate-reducing bacteria in environmental samples have been enumerated by serial dilution in completely anaerobic liquid media. Variations of the medium used [16] are available commercially. Ensure that a freshwater-based medium is used.

6.4.3.2	Types of sample	Water and deposit samples.
6.4.3.3	Principle	Serial dilution of samples within media bottles.
6.4.3.4	Time required for test	Up to 28 days.
6.4.3.5	Interferences	It should be recognised that the analysis of black deposits and those deposits containing sulphide may make interpretation of the test difficult. Bottles containing black deposits that appear to be positive within a few hours of inoculation should be incubated as normal. Thereafter, a sub-sample should be transferred to a fresh bottle of medium for further incubation to establish the presence of sulphate-reducing bacteria.

6.4.4 **Procedure**

(i) **Water.** For each sample, six bottles of media are required. Using a disposable 1 ml sterile syringe and needle, 1 ml of sample is added to a bottle of medium (through the butyl seal). The syringe and needle are then discarded and the bottle vigorously shaken. Using a fresh syringe and needle, 1 ml of the inoculated liquid is added into the second bottle in the six-bottle dilution series. Mix the contents as before. The procedure is repeated until a series of dilution bottles has been prepared. If a duplicate dilution series is required, 1 ml of the original sample is taken and processed in a similar manner using a further six bottles of media.

(ii) **Deposits.** Remove the cap from the first bottle in the dilution series and add a known amount of the deposit (by weight or volume). The bottle contents should be mixed to disperse the loose deposits. Using a fresh syringe and needle, 1 ml of the inoculated liquid is added into the second bottle in the dilution series. Mix the contents as before. The procedure is repeated until a series of dilution bottles has been prepared. If a duplicate dilution series is required, an identical amount of the original sample is taken and processed in a similar manner.

Incubate at a suitable temperature between 20-30°C. Bottles in which the contents turn black within 28 days are considered positive. In many instances, contents will blacken within 14 days. Bacterial numbers are estimated from the highest dilution giving a positive result. For duplicate dilution series, numbers are reported as a range between the highest dilution that shows blackening and the lowest dilution showing no blackening.

6.4.5 **Media**

(i) Modified Postgate's Medium B

Dipotassium hydrogen phosphate (anhydrous)	0.5 g
Ammonium chloride	1.0 g
Sodium sulphate	1.0 g
Calcium chloride hexahydrate	0.1 g
Magnesium sulphate heptahydrate	2.0 g
Sodium lactate solution (60-70%)	5.0 ml in 100 ml of water
Yeast extract	1.0 g
Sodium thioglycollate	0.1 g
Sodium ascorbate	0.1 g
Iron(II) sulphate heptahydrate	0.5 g
Distilled water	to 1000 ml

(ii) API (American Petroleum Industry) Medium

Sodium sulphate	1.0 g
Sodium lactate solution (60-70%)	4.0 ml in 100 ml of water
Yeast extract	1.0 g
Ascorbic acid	0.1 g
Magnesium sulphate heptahydrate	0.2 g
Dipotassium hydrogen phosphate (anhydrous)	0.01 g
Iron(II) ammonium sulphate hexahydrate	0.2 g
Distilled water	to 1000 ml

For the preparation of both media, dissolve all the respective ingredients, with the exception of sodium lactate, in 800 ml aliquots of water; if necessary use gentle heating. The pH should be adjusted to 7.3 ± 0.2 using 1 molar sodium hydroxide solution. To avoid a pH change due to the addition of sodium lactate, a solution of 60-70% sodium lactate should be made up in 100 ml of distilled water and brought to the boil (in order to split dimers). The 100 ml solution is then added to the other media components and the whole made up to 1000 ml with distilled water.

Bottles (of 10 ml capacity) are filled with 9 ml of sulphate-reducing bacteria medium. The bottles are nitrogen-gassed to displace oxygen within the medium, then stoppered and capped using butyl rubber stoppers and metallic caps. The bottles and contents are then autoclaved at 121 ± 2°C for 15 minutes.

6.5 **Sulphite-reducing bacteria.** Samples should be analysed as for sulphite-reducing clostridia as previously described [8].

6.6 **Iron-precipitating bacteria**

6.6.1 **Introduction.** Iron-precipitating bacteria can obtain energy via the oxidation of iron(II) to iron(III). This oxidation results in the precipitation of iron(III) hydroxide, which can impart a red colour to the water. Typically, the source of iron is pipework or the water in supply. Substantial growth of iron-precipitating bacteria can also impart unacceptable odours to drinking water. Bacteria which are commonly associated with iron precipitation problems include species of *Gallionella*, *Leptothrix*, *Crenothrix* and *Siderocapsa*. Other bacteria that have been shown to be capable of non-oxidative precipitation of iron include species of *Klebsiella*, *Enterobacter*, *Serratia*, *Corynebacterium*, *Caulobacter* and *Bacillus*.

6.6.2 **Definition.** Iron-precipitating bacteria may be represented by members from a number of bacterial genera, including sheathed bacteria (for example *Leptothrix*, *Crenothrix* and *Clonothrix*), stalked and budding bacteria (for example *Gallionella* and *Pedomicrobium*) and Gram-negative chemolithotrophic bacteria (for example *Siderocapsa*, *Ochrobium* and *Thiobacillus*).

6.6.3 **Scope**

6.6.3.1	Limitations of the method	Growth and enrichment of relatively fast growing bacteria associated with oxidative and non-oxidative iron-precipitation is supported. Growth, or only limited growth, of sheathed or stalked bacteria is not supported. These bacteria generally require more sophisticated methods for growth [17].

		Microscopical examination (see 6.6.7) of collected growth or sediment will yield valuable information in addition to any attempts to culture iron-precipitating bacteria.
6.6.3.2	Types of sample	All types of water.
6.6.3.3	Principle	An aliquot of sample is added to the growth medium. Growth and precipitation of iron within the incubation period indicates the presence of a significant population of bacteria associated with iron-precipitation in the original sample.
6.6.3.4	Time required for test	Seven days, but the test period can be extended to 30 days for low populations.
6.6.3.5	Interferences	Some non-microbially-induced oxidation of iron may occur during incubation producing a light brown or green colouration.

6.6.4 **Procedure.** The presence or absence of significant populations of bacteria associated with iron precipitation can be determined. The medium used for testing iron-precipitating bacteria is modified W-R medium [18] (see 6.6.6). Variations of this medium are also available commercially. Freshly prepared medium should be used for the test. Prepare double strength modified W-R medium in volumes equal to the intended volume of sample to be tested (typically, 15 - 25 ml) in screw-capped bottles (typically, 50 - 100 ml capacity, depending on volume of sample to be tested). The bottle size chosen should be large enough to ensure a headspace above the sample/medium mixture. Add, for example 15ml of sample to 15ml of medium in a bottle, mix and replace the cap. Incubate at 22 ± 2°C for up to seven days. Examine daily. Incubation may be extended to 30 days if necessary. The presence of iron-precipitating bacteria is typically indicated by growth of a brown scum or pellicle at the surface of the medium, often with a strong yellow or brown colouration of the medium. In some cases, a brown, slimy growth may also occur at the bottom of the bottle, occasionally without surface growth. If bacteria are not present, the medium may slowly auto-oxidise resulting in a clear green colouration. A control sample of medium, inoculated with sterile water should, therefore, be examined with each batch of samples and used for comparison.

Alternatively, prepare a 20-fold strength solution of modified W-R medium [19]. Aliquots of the concentrated medium (0.75 ml) are added to 25 ml screw-capped bottles and aseptically evaporated to dryness at 65°C. The bottles are then capped and stored. For the test, a 15 ml aliquot of sample is added to the bottle and mixed. The bottles are incubated at 22°C as above. If larger sample volumes are to be tested, then the volume of medium and the size of the bottle should be adjusted accordingly (for example, 1.0 ml of medium for 20 ml of sample used in the test, or 1.25 ml of medium for 25 ml of sample used in the test, and so on.)

6.6.5 **Confirmation of iron-precipitating bacteria.** To confirm or identify the presence of bacteria involved in iron-precipitation, conduct a microscopical examination (see 6.6.7) of the growth from positive bottles.

6.6.6 **Modified W-R Medium (single strength)**

Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate heptahydrate	0.5 g
Ammonium nitrate	0.5 g
Calcium chloride dihydrate	50 mg
Iron(II) ammonium citrate	6.0 g
Distilled water	1 litre

Dissolve all the ingredients in the distilled water. Adjust the pH, if necessary, to 6.7 ± 0.1. Distribute in 15 ml volumes into screw-capped bottles of 50 ml capacity. Sterilize by autoclaving at 121 ± 2°C for 15 minutes, allow to cool and tighten bottle caps.

6.6.7 **Microscopical examination.** In many instances, a microscopical examination will be sufficient to identify the organism causing a water quality problem. If the sample is clear and without any obvious turbidity, the sample should be centrifuged or allowed to settle overnight in order to concentrate any cells or filaments prior to examination. If sediment is present, carefully use a Pasteur pipette, or similar, to remove a small amount of deposit from the bottom of the container and place on a microscope slide. Cover with a coverslip. Examine under 400-1000x magnification, ideally under phase contrast. If a conventional light microscope is used, identification may be easier if the sample on the slide is stained beforehand, for example with India ink or lactophenol blue. If the sample contains very little particulate matter, the sample can be filtered through a 0.45 µm nominal pore size membrane filter. The filter is dried, for example by placing the filter (face up) on adsorbent paper, cleared by the addition of immersion oil, and examined at 1000x magnification.

Heavy deposits of iron in the sample may obscure any iron bacteria that are present making identification more difficult. This problem may be overcome if the iron deposits are dissolved in dilute hydrochloric acid, oxalic or citric acid solution. Citric acid will not cause lysis of bacterial cells, but is less effective at removing iron. Place a few drops of 1 molar hydrochloric acid, or a solution of oxalic or citric acid (0.5 - 1% w/v) to one side of the coverslip and draw the solution under the coverslip by placing adsorbent paper to the opposite side. Slimes, flocs or other visible material can also be examined in a similar manner. The structures of several iron bacteria are shown in Figure 3.

7 Determination of taste- and/or odour-causing substances

7.1 **Introduction.** Comprehensive descriptions of the sources of a wide variety of tastes and/or odours in waters have been reported [21] and Table 3 shows some examples of the type of compounds responsible for causing these problems. Reviews covering most aspects of the sort of problems that can be encountered have been published [1]. The use of chemical analysis [22] can be very useful in establishing the cause of a taste and/or odour problem (see also 2.2) and this section describes two such procedures using gas chromatography with mass spectrometric detection. These methods are not intended for routine monitoring purposes of quantitative tastes and odours of drinking waters, as these are dealt with elsewhere [3].

7.2 A method for screening waters using gas chromatography with mass spectrometric detection

7.2.1 Performance characteristics of the method

7.2.1.2	Type of sample	Raw, river and potable waters.
7.2.1.3	Basis of method	The sample is extracted into dichloromethane which is then dried and concentrated by evaporation. An internal standard is then added and the extract analysed using gas chromatography with mass spectrometric detection (GC-MS).
7.2.1.4	Interferences	There are many potential interferences. Compounds of interest may not be extractable into dichloromethane or susceptible to gas chromatography. In addition, compounds may co-elute with other analytes present.

7.2.2 **Principle.** If required, and in order to improve recovery, the pH of the sample can be adjusted to a suitable value appropriate to the compound suspected of being present. Concentrated hydrochloric acid or 5M sodium hydroxide solution may be used. Normally, however, the sample is not pH-adjusted.

The sample is extracted with dichloromethane and the phases allowed to separate. The dichloromethane extract is dried using anhydrous sodium sulphate and evaporated to near dryness in a Kuderna-Danish evaporator. An internal standard is then added to aid quantification. An aliquot of the concentrated extract is then injected into a gas chromatograph fitted with mass spectrometric detection. The mass spectra of peaks of interest are then compared with mass spectra of known compounds.

7.2.3 **Interferences.** In a 'screening' method such as this, where the compounds of interest are generally unknown, it is difficult to list all potential interferences. Many compounds are not susceptible to gas chromatography. Also, compounds of interest may have very low extraction efficiencies and may co-elute with other determinands present. Owing to the very low concentrations of compounds that may be present, contamination of the sample may be a major source of interference; in addition, the compounds of interest may not be able to be detected at these low concentrations. The presence of volatile substances which may be lost during evaporation of the extraction solvent should also be considered.

It is often common practice to add sodium thiosulphate to samples in order to neutralise residual chlorine. The removal of chlorine reduces the formation of chlorinated organic compounds present in the sample. In certain cases, this practice may not be appropriate, for example if a chlorinated phenol is the cause of a particular problem, and it was considered appropriate to increase the concentration and hence improve its detection.

7.2.4 **Hazards.** Dichloromethane and d_{10} - phenanthrene are toxic and skin contact, inhalation or ingestion should be avoided. Sulphuric acid and sodium hydroxide solutions are extremely corrosive and appropriate protective clothing should be worn when preparing or handling these reagents.

7.2.5 **Reagents.** All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination or confirmation. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide or high pressure liquid chromatography (HPLC) grade solvents and analytical grade materials are normally suitable unless otherwise specified.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by action of light. Reagents should be stored in tightly sealed containers or other suitable vessels and kept in the dark if necessary.

7.2.5.1 **Water.** The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

7.2.5.2 **Dichloromethane.**

7.2.5.3 **Granular anhydrous sodium sulphate.**
Heat at 500 ± 20°C in a muffle furnace for 4.0 ± 0.5 hr. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator. Store in an all-glass container.

7.2.5.4 **Sodium thiosulphate.**

7.2.5.5 **Sodium hydroxide.** (5M solution). To 200 ml of water (7.2.5.1) in a glass beaker, carefully add 40 g of sodium hydroxide, stirring continuously until dissolved. Allow to cool.

7.2.5.6 **Sulphuric acid.** (d_{20} 1.84).

7.2.5.7 **Hydrochloric acid.** (d_{20} 1.18).

7.2.5.8 **Internal standard.** Prepare, for example a suitable solution in dichloromethane of d_{10}-phenanthrene at a concentration of 40 $mg1^{-1}$. This solution should be stored in a refrigerator at approximately 4°C. The internal standard should reflect the taste- and/or odour-causing substances being determined.

7.2.6 **Apparatus.** Apparatus should be free from contamination before use. Glassware should be clean, for example washed in a proprietary detergent solution, rinsed well, heated in a muffle furnace at approximately 400°C for approximately 4 hours and then kept in an oven at approximately 110°C prior to use.

7.2.6.1 **Kuderna-Danish evaporator.** An equivalent system can also be used.

7.2.6.2 **Tapered graduated tubes.** 10 ml capacity.

7.2.6.3 **Micro-Snyder column.**

7.2.6.4 **Steam bath.**

7.2.6.5 **Gas chromatography.** The instrument should be fitted with a mass spectrometric detector and used according to the manufacturer's instructions. It should be fitted with a capillary injector and operated so that all the injected sample reaches the column. An on-column injector has been found to be suitable, with helium carrier gas.

The column oven is temperature programmed as follows:

Initial temperature:	40°C for 2 minutes
Ramp at:	15°C/minute
Final temperature:	270°C for 10 minutes

The injector temperature will depend on the type of injector used.

A DB5 capillary column, 30 metres in length and 0.32 mm internal diameter, operated with a helium back pressure of 4 psi has been found to give good separation. Equivalent products may also be used.

The mass spectrometer may use quadropole, magnetic sector or ion-trap mass separation technology and should be interfaced to the gas chromatograph by a transfer line which is heated to 270˚C. An ion source temperature of 150˚C has been found to be suitable.

The mass spectrometer is operated in electron - impact mode, with m/z 40 to 450 being scanned in 1 second.

7.2.6.6 **Bottle roller.** Suitable for rolling the bottles used for sampling.

7.2.6.7 **Sample bottles.** Clean, glass bottles (2.5 litre capacity) with screw caps fitted with polytetrafluoroethylene (PTFE) liners.

7.2.7 **Sample collection.** Samples are collected by rinsing the bottles with the sample to be collected and then completely filling the bottle so that sample is displaced as the stopper is secured. For treated water, it may be appropriate to add a few crystals of sodium thiosulphate to neutralise any disinfectant that may be present, but see section 7.2.3. Extraction and concentration should take place as soon as possible.

7.2.8 Analytical procedure

Step	Procedure	Notes
7.2.8.1	If required, adjust the pH of the sample with hydrochloric acid (7.2.5.7) or sodium hydroxide (7.2.5.5) (note a). Add 2 litres of sample to a separating funnel.	(a) If a particular compound, or type of compound, is suspected of being present, the pH of the sample may be adjusted to a suitable value in order to improve the extraction efficiency. For example, if a phenolic compound is suspected, the sample may be acidified by the addition of 2 ml of hydrochloric acid. If pH adjustment is to be carried out, it may be necessary to take and analyse duplicate samples, one of which is pH adjusted and the other where the pH is not adjusted. In this way, the risk of loss of any compounds of interest is reduced.
7.2.8.2	Add 100 ml of dichloromethane (7.2.5.2) (note b) to the sample and stopper. Shake well, venting frequently to reduce build up of pressure. Secure the stopper with a clip, place on a bottle roller (7.2.6.6) and roll for approximately one hour. Allow the phases to separate.	(b) The use of dichloromethane as an extraction solvent throughout this procedure is suggested only as a guide. Other water-immiscible solvents may be more suitable depending upon the nature of the suspected problem. For example, the use of methyl tertiary butyl ether may increase the extraction efficiency of compounds which are semi-polar in nature.

7.2.8.3	Pass the dichloromethane extract through a drying column of sodium sulphate (7.2.5.3) into a Kuderna-Danish evaporator (7.2.6.1) fitted with a 10 ml graduated tube (7.2.6.2)	
7.2.8.4	Evaporate the solvent to approximately 5 ml on a steam bath (7.2.6.4), attach a micro-Snyder column (7.2.6.3) and evaporate down to 1 ml on the steam bath.	
7.2.8.5	Add 100 µl of internal standard (7.2.5.8) to the concentrated extract and mix.	
7.2.8.6	Inject a suitable volume of concentrated extract into the gas chromatograph and process any data collected.	

7.2.9 **Calculation and discussion.** Once the data have been collected, the total ion chromatogram can be inspected and mass spectra generated for all peaks of interest. Each mass spectrum can then be compared to mass spectra of known compounds to help, tentatively, identify the compound.

Once each compound has been identified, it can be approximately quantified by comparing its peak area or peak height on the total ion chromatogram with the corresponding response for the internal standard. Using the conditions given in section 7.2.8, the internal standard concentration is equivalent to approximately 2 $\mu g l^{-1}$ in the un-extracted sample and it is assumed that the response of the unknown compound of interest is the same as that of the internal standard. For each compound, the concentration in the un-extracted sample is calculated from:

$$C = \frac{2 \times AC}{AI} \ \mu g l^{-1}$$

where C is the concentration of the compound;
AC is the peak area or height of the compound of interest;
AI is the peak area or height of the internal standard.

Once a compound has been tentatively identified, the pure compound should be used to confirm the analysis. A solution of the standard compound in dichloromethane should give the same retention time as obtained in the sample. The analytical procedures should then be developed and refined, possibly using large-volume injection, to give a more accurate quantification of the concentration of the compound.

7.3 The determination of geosmin and 2-methylisoborneol in raw and treated waters using gas chromatography with mass spectrometric detection

7.3.1 Performance characteristics of the method

7.3.1.1	Substances determined	Geosmin and 2-methylisoborneol.
7.3.1.2	Types of samples	Raw and drinking waters.

7.3.1.3	Range of application	Typically, up to 100 ngl^{-1} for geosmin and 2-methylisoborneol. The upper limit of the range may be extended by diluting the sample. However, exceeding the upper limit may cause an overloading of the mass spectrometer. This may lead to significant differences in the spectral characteristics with consequential non-linearity of signal.
7.3.1.4	Calibration curve	The range of linearity depends upon the mass spectrometer used. The instrument used in these tests gave a linear response for both determinands over the range 0 - 100 ngl^{-1}.
7.3.1.5	Standard deviation	See Tables 4 and 5.
7.3.1.6	Limit of detection	See Table 6.
7.3.1.7	Sensitivity	Dependent upon the mass spectrometer in use. The instrument used for this work gave a response of approximately 100% full scale deflection, with a base-line noise level of 0.25% for 2 ng of 2-methylisoborneol and 0.8 ng of geosmin.
7.3.1.8	Bias	The extraction efficiency is less than 100% and dependent on the sample matrix. See Tables 4 and 5.

7.3.2 **Principle.** The sample is spiked with an internal standard and extracted with dichloromethane. The extract is dried and concentrated by evaporation. The extracted compounds are separated by capillary column using temperature-programmed gas chromatography and quantified by mass spectrometric detection.

7.3.3 **Interferences.** Any substance capable of producing a response on the mass spectrometer at the chosen atomic mass units and showing the same gas chromatographic retention time as the determinand will interfere.

7.3.4 **Hazards.** Dichloromethane is narcotic. Geosmin and 2-methylisoborneol are extremely unpleasant odour-intense compounds possessing musty, earthy odours. Extreme caution should be exercised when preparing stock solutions. Any apparatus coming in contact with the determinands should be placed in a fume cupboard, and the operator protected by suitable safety-ware. The odour may be controlled by weighing geosmin in a syringe which is then flushed with copious amounts of solvent into the graduated flask. Chromic acid is extremely corrosive. Appropriate precautions should be taken.

7.3.5 **Reagents.** All reagents should be of sufficient purity that they do not give rise to interfering peaks during the determination or confirmation. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed. Pesticide or high pressure liquid chromatography (HPLC) grade solvents and analytical grade materials are normally suitable unless otherwise specified.

The water used for blank determinations and preparation of control samples should show negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and/or other materials, particularly plastics, or by degradation caused by action of light. Reagents should be stored in tightly sealed containers or other suitable vessels and kept in the dark if necessary.

7.3.5.1 **Dichloromethane.**

7.3.5.2 **Acetone.**

7.3.5.3 **Granular anhydrous sodium sulphate.** Heat at 500 ± 20°C in a muffle furnace for 4.0 ± 0.5 hr. Cool to about 200°C in the muffle furnace and then to ambient temperature in a desiccator. Store in an all-glass container. Immediately before use it should be rinsed with dichloromethane.

7.3.5.4 **Anti-bumping granules.** Wash with acetone before use.

7.3.5.5 **Methanol.**

7.3.5.6 **Ammonia gas.** High purity from cylinder supply.

7.3.5.7 **Stock surrogate standard solutions.** For example, prepare in methanol, solutions of d_5-geosmin, and d_3-2-methylisoborneol at concentrations of 1000 mgl^{-1}.

7.3.5.8 **Stock internal standard solutions.** For example, prepare in methanol, solutions of 1-chloro-n-octane, 1-chloro-n-decane, and 1-chloro-n-dodecane at concentrations of 1000 mgl^{-1}.

7.3.5.9 **Working surrogate standard solutions.** For example, prepare in methanol, solutions of d_5-geosmin, and d_3-2-methylisoborneol at concentrations of 10 mgl^{-1}.

7.3.5.10 **Working internal standard solutions.** For example, prepare in methanol, solutions of 1-chloro-n-octane, 1-chloro-n-decane, and 1-chloro-n-dodecane at concentrations of 10 mgl^{-1}.

7.3.5.11 **Calibration mixed standard solutions.** For example, prepare in methanol, solutions of d_5 -geosmin, d_3 -2-methylisoborneol, 1-chloro-n-octane, 1-chloro-n-decane and 1-chloro-n-dodecane, each at concentrations of 1000 $\mu g1^{-1}$, and geosmin and 2-methylisoborneol at a range of concentrations of 0.1, 0.25, 0.4, 0.55, 0.7, 0.85 and 1.0 μgl^{-1}.

7.3.5.12 **Chromic acid.** To 35 ml of a saturated solution of sodium dichromate, carefully add with stirring and cooling, 1000 ml of concentrated sulphuric acid.

7.3.6 **Apparatus**

7.3.6.1 **Sample bottles.** Clean, glass bottles (2.5 litre capacity) with screw caps fitted with polytetrafluoroethylene (PTFE) liners. It will be useful if the bottles are marked at 2 litres.

7.3.6.2 **Shaking machine.** Laboratory type with tray, in which bottles can be shaken in a horizontal plane.

7.3.6.3 **Erlenmeyer flasks.** 500 ml capacity.

7.3.6.4 **Kuderna-Danish evaporator.** An equivalent system may also be used.

7.3.6.5 **Micro-Snyder column.**

7.3.6.6 **Separating funnels** greater than 2 litre capacity.

7.3.6.7 **Buchner funnels.** 500 ml capacity with sintered glass discs.

7.3.6.8 **Graduated centrifuge tubes.** 10 ml, tapered, glass stoppered.

7.3.6.9 **Micro-litre syringes.** Various sizes, for example 25, 10, 5 and 1 µl capacity.

7.3.6.10 **Muffle furnace.** Temperature maximum greater than 500˚C.

7.3.6.11 **Gas chromatograph.** A capillary column instrument fitted with an on-column injector or an injection system capable of operation in the splitless mode and interfaced to a mass spectrometer.

7.3.6.12 **Mass spectrometer.** Any commercially available instrument which can be converted from electron impact to chemical ionisation mode of operation, capable of direct or split interface coupling to a capillary column gas chromatograph and capable of operation in multiple ion detection mode or equivalent.

7.3.7 **Sample storage and preservation.** Samples are collected by rinsing the bottle with some of the sample and then completely filling the bottle so that sample is displaced as the stopper is secured. For treated water, it may be appropriate to add sodium thiosulphate to neutralise any disinfectant present. Extraction and concentration should take place as soon as possible.

7.3.8 Analytical procedure

Step	Procedure	Notes
	Extraction and concentration	
7.3.8.1	Shake the bottle and its contents thoroughly and discard excess sample until 2 litres of sample remain. Add, for example 20 µl of working surrogate standard and internal standard solutions (7.3.5.9 and 7.3.5.10) such that the concentrations are 0.1 µg1^{-1}.	
7.3.8.2	Add 75 ml of dichloromethane (7.3.5.1) to the sample bottle, tightly cap and shake well. Loosen the cap to release pressure, and then, after re-sealing, shake for 30 minutes on a shaking machine.	
7.3.8.3	Transfer the contents of the bottle to a separating funnel and allow the phases to separate before transferring the dichloromethane to an Erlenmeyer flask (note a).	(a) If any emulsion (interfacial cuff) forms leave with the aqueous layer at this stage.
7.3.8.4	Return the aqueous layer to the bottle, add 50 ml of dichloromethane, and shake for 30 minutes on a shaking machine. Repeat step 7.3.8.3, but combine the dichloromethane extracts.	
7.3.8.5	Repeat steps 7.3.8.2 and 7.3.8.3 for a third time, but on this occasion separate any emulsion which may have formed with the dichloromethane layer.	
7.3.8.6	Pass the combined dichloromethane extracts through a column of anhydrous sodium sulphate supported on a sintered glass filter funnel (note b). Leave the filtrate standing over anhydrous sodium sulphate over night. Filter through a sintered glass funnel.	(b) This preliminary drying stage assists when emulsions have formed.
7.3.8.7	Evaporate the solvent to about 5 ml in a Kuderna-Danish apparatus on a steam-bath. Add an anti-bumping granule to the 10 ml centrifuge tube.	
7.3.8.8	Allow the apparatus to cool and connect the centrifuge tube to a micro-Snyder column. Evaporate the solvent to between 0.5 and 1 ml (notes c and d) on a steam bath.	(c) Automated evaporators may be successfully employed at stages 7.3.8.7 and 7.3.8.8 to increase efficiency. (d) The determinands may be lost if the volume is reduced too far.
7.3.8.9	Before analysis, evaporate the extract to approximately 200 µl by gentle evaporation using a stream of nitrogen (note e)	(e) The extracts can be stored in a refrigerator at approximately 4˚C in centrifuge tubes fitted with glass stoppers and sealed with PTFE tape until time of analysis.
	Gas chromatography/mass spectrometry	
7.3.8.10	Set up the gas chromatograph with a temperature programme to maximise separation between C11 and C14 in an n-alkane series (note f).	(f) In this work, the following programme was found to be suitable. 30˚C to 150˚C at 10˚C min^{-1}; 150˚C to 200˚C at 4˚C min^{-1}; 200˚C to 280˚C at 30˚C min^{-1}, hold for 20 minutes.
7.3.8.11	Set up the mass spectrometer at maximum sensitivity in multiple ion monitoring mode. Collect responses at 112 and 114 for geosmin (electron impact mode) and 151 and 154 for 2-methylisoborneol (ammonia gas chemical ionisation mode) (note g).	(g) In this work, all data in the mass range 40 - 200 were collected so that full mass spectral comparisons could be made if required.
7.3.8.12	Inject 2 µl aliquots of each calibration standard and measure the responses (note h). Construct calibration curves of the ratio of responses for mass fragments 112 and 114 for geosmin; and 151 and 154 for 2-methylisoborneol against the corresponding ratio of the amount of sample to internal standard injected (note i).	(h) Peak areas generally gave more consistent results. (i) Most modern mass spectrometers have data management facilities to effect this automatically.
7.3.8.13	Inject 2 µl of the sample extract and locate the geosmin and 2-methylisoborneol peaks by reference to the calibration standard runs or n-chloroalkane internal standards (note j).	(j) Some modern mass spectrometers will effect this automatically by spectral comparison.

7.3.9 **Calculation.** Measure peak areas or heights and calculate the relative response (R_{112} / R_{114} or R_{151} / R_{154}) of determinand to internal standard where:

R_{112} = response at 112 amu for geosmin
R_{114} = response at 114 amu for d_5-geosmin
R_{151} = response at 151 amu for 2-methylisoborneol
R_{154} = response at 154 amu for d_3-2-methylisoborneol

Read off the amount of determinand relative to the amount of internal standard (D/I) from the calibration graph and calculate the amount of determinand in the extract as follows:

$D = R_{112} \times I / R_{114}$ or $D = R_{151} \times I / R_{154}$ (ng)

where I = amount of internal standard added to the sample (ng).

Calculate the concentration (C ngl^{-1}) of determinand in the aqueous sample as follows:

$C = D / V$ (ngl^{-1})

where V = volume of sample in litres.

The mass spectra of geosmin and 2-methylisoborneol are shown in Figure 4.

8 References and further reading

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21. Waggot A, and Bell SM, WRc Report No. 709-S. *An inventory of organic compounds causing taste and odour problems in water*.

22. Preti G *et al*, Analytical Chemistry, Vol 65, No 15, August 1993, pp699-702.

Table 1 Sampling volumes for specific tests

Determinand	Minimum volume of sample required (ml)
Heterotrophic colony count bacteria	5
Actinomycetes	115
Micro-fungi and yeasts	115
Sulphate-reducing bacteria	5
Sulphite-reducing bacteria (Clostridium)	100
Iron-precipitating bacteria	15

Table 2 Sources of tastes and odours in drinking water

Taste or odour description	Source	Compound
Earthy	Actinomycetes, Cyanaobacteria	Geosmin
Musty	Actinomycetes, Cyanaobacteria	2-Methylisoborneol
Mouldy, musty	Actinomycetes	2-Isopropyl-3-methoxypyrazine
Woody, earthy	Actinomycetes	Cadin-4-ene-1-ol
Musty, TCP	Methylation of chlorophenol	Chloroanisole
Cucumber	Green algae	Trans-2 and cis-6-nonadienal
Fruity, fragrant	Ozonation	Aldehydes (C_7 and above)
Decaying	Decaying algae or vegetation	Unknown
Fishy	Green algae, diatoms	n-hexanal; n-heptanal
Cod liver oil	Green algae	Decadienal
Fishy	*Dinobryon* (algae)	Hepta- and decadienals
Malodourous sulphur	Decomposing Cyanobacteria	Mercaptans
Swampy, fishy	*Pseudomonas sp.*	Dimethyl polysulphides (dimethyl trisulphide)
Rotten eggs	Sulphate-reducing bacteria Clostridia	Hydrogen sulphide
Fishy, grassy, septic	Green algae	Unknown
Swampy, swimming pool	Chlorination of amino acids	Aldehydes (low MW)
Cat urine	Chlorine dioxide	Unknown
Medicinal, TCP	Chlorination of phenol	Chlorophenols (2-CP;4-CP; 2,4-DCP; 2,6-DCP;2,4,6-TCP)
Medicinal	Chloramination	Iodinated trihalomethanes
Plastic, burnt plastic	Polyethylene pipes	Phenolic anti-oxidants
Chlorinous	Disinfection of water	Chlorine (free), Monochloramine
Swimming pool	Disinfection of water	Dichloramine

MW = molecular weight; CP = chlorophenol; DCP = dichlorophenol, TCP = trichlorophenol
Adapted from Suffet, Mallevialle & Kawczynski [2]

Table 3 Common taste- and/or odour-causing substances which can be detected by means of chemical analysis

(a) Organic

Compound	Taste and/or odour	LTPRI	MS ions
Butyraldehyde	sweet/rancid	575 (SE)	77,44,43
2-t-Butyl-5-methyl-1-4-benzoquinone	Pencil shavings		178,165,163
Geosmin	Earthy/musty	1384 (SE) 1789 (PG)	112,111,125
Phenylacetaldehyde (Hyacinthin)	Hyacinths/vegetative	91,92,120	
2-Isobutyl-3-methoxypyrazine	Earthy/musty	1160 (SE)	152,137,124
2-Isopropyl-3-methoxypyrazine	Earthy/musty 1076 (SE)	151,124,94 1410 (PG)	
2-Methylisoborneol	Musty/mouldy	1164 (SE) 1562 (PG)	95,107,135
2,4,6-Trichlorophenol	Antiseptic	1328 (SE)	196,198,200
Dimethyl trisulphide	Swampy	948 (SE)	126,111,79
Diethyl phthalate	Plastic	1555 (SE)	149,177,150
2,3,6-Trichloroanisole	Earthy/musty	1341 (SE) 1887 (PG)	195,197,210

(b) Inorganic

Substance	Taste/Odour
Beryllium	Sweet
Iron	Bitter
Copper	Metallic
Zinc	Metallic
Nickel	Sweet
Lead	Sweet

Notes:
(i) LTPRI is the Linear Temperature Programmed Gas Chromatographic Retention Index – in this series [20].
(ii) SE and PG are methyl silicone and polyethylene glycol stationary phases, respectively.
(iii) MS ions refers to the principal mass spectrometric ions produced using electron impact mode.
(iv) Methods for determining inorganic substances are covered by a number of other booklets in this series.

Table 4 **Standard deviations**

Sample	Determinand	S_w	S_b	S_t
High level spikes*				
finished water	geosmin	13.1(3)	ns	27.1(3)
	2-methylisoborneol	4.97(3)	ns	7.03(3)
raw water	geosmin	13.8(3)	63.6(3)	65.0(3)
	2-methylisoborneol	3.32(3)	ns	5.80(3)
Low level spikes#				
finished water	geosmin	0.19(3)	0.60	0.63(3)
	2-methylisoborneol	0.50(4)	ns	0.83(4)
raw water	geosmin	0.56(3)	ns	0.94(3)
	2-methylisoborneol	1.53(3)	ns	2.19(3)

Units expressed as ngl^{-1}.

Figures in brackets indicate degrees of freedom.

ns is not significant.

S_w is within-batch standard deviation.

S_b is between-batch standard deviation.

S_t is total standard deviation. The high standard deviation values reflect erratic extraction yields and relative instrumental instability and indicate the importance of employing internal standard calibration procedures.

* geosmin – 100 ngl^{-1}; 2-methylisoborneol – 25 ngl^{-1}.

\# geosmin – 1 ngl^{-1}; 2-methylisoborneol – 5 ngl^{-1}.

Table 5 **Means and standard deviations of 10 water blanks from various sources**

	Geosmin concentration (ngl^{-1})	2-Methylisoborneol concentration (ngl^{-1})
Mean	0.276	0.0715
Standard deviation	0.053	0.01

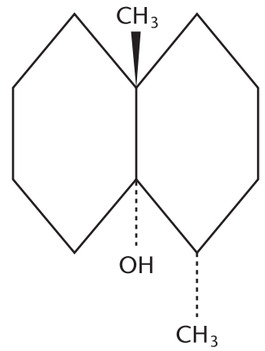
Table 6 **Limits of detection (ngl^{-1})**

Sample	Geosmin	2-Methylisoborneol
finished water	0.25	0.07
raw water	0.97	0.49

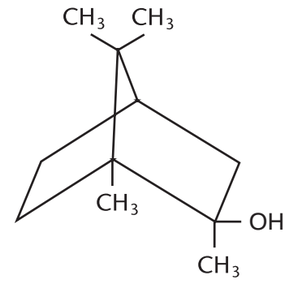
These limits were calculated from the estimate of the standard deviation of finished and raw water blanks (from sources with no measurable concentration of determinand), (4.65 x S_t).

Performance data, including limits of detection, are highly instrument dependent. Variations in the character of mass spectra produced by different types of instrument are also known to occur.

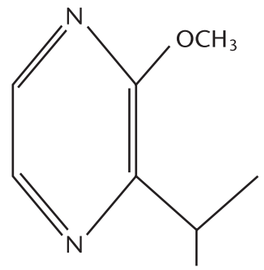
Figure 1 **Structures of various compounds causing taste and/or odour problems**



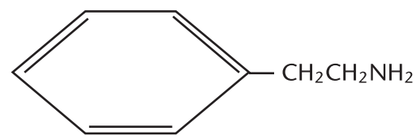
Geosmin



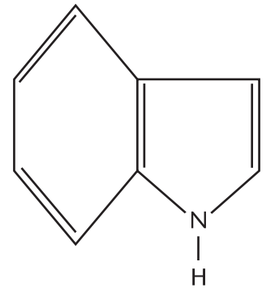
2-Methylisoborneol



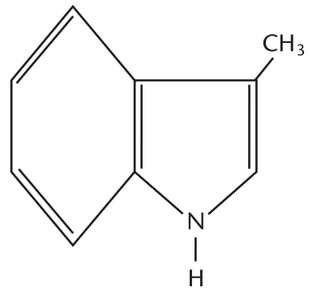
2-Isopropyl-3-methoxypyrazine



ß-Phenylethylamine



Indole



Skatole

$NH_2(CH_2)_4NH_2$
Putrescine

$NH_2(CH_2)_5NH_2$
Cadaverine

Figure 2 Taste and odour wheel

Adapted from Suffet, Mallevialle & Kawczynski [2]

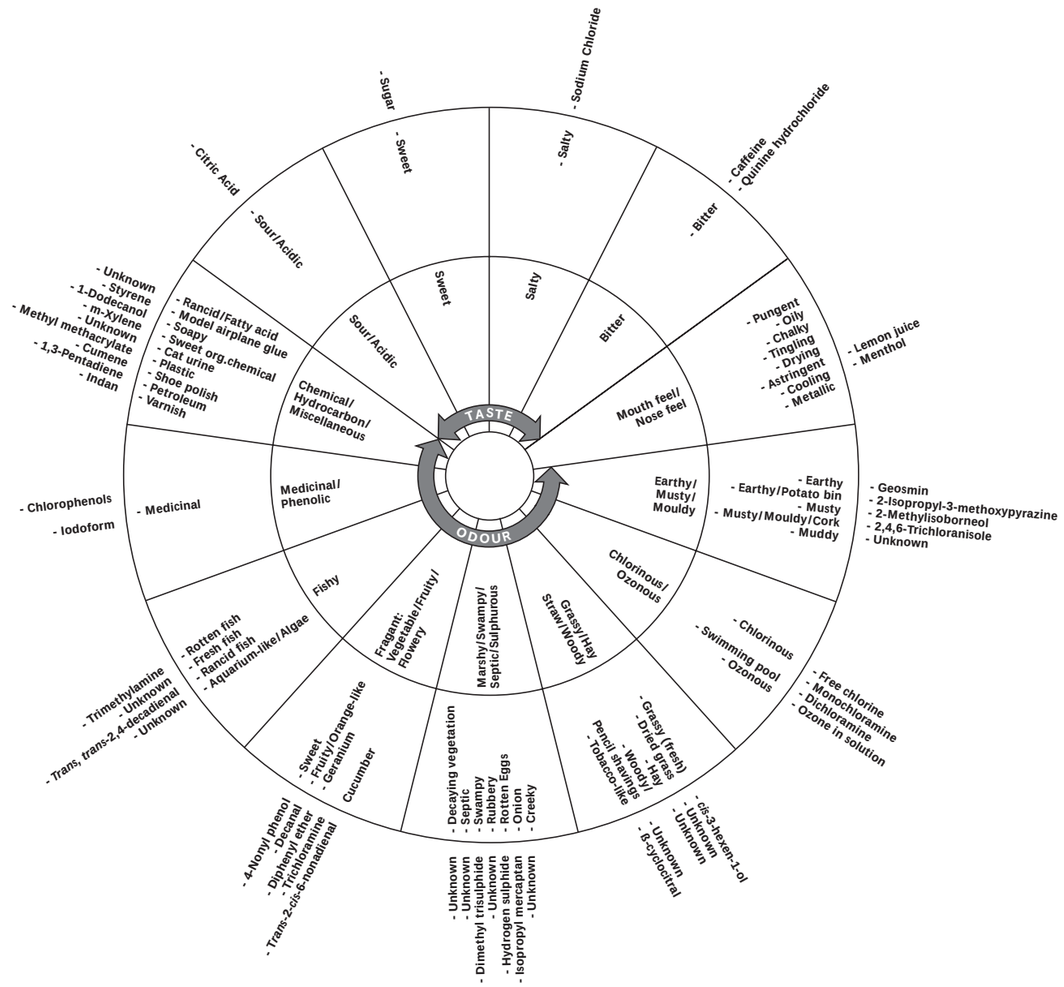
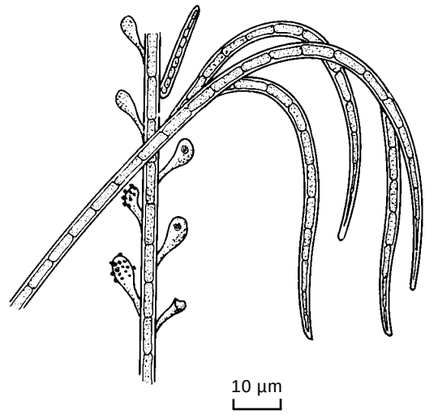
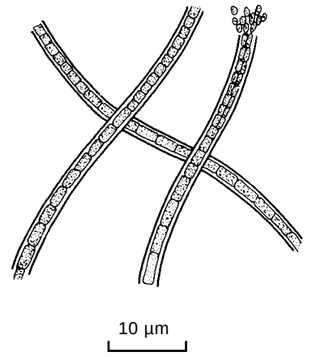


Figure 3 Diagrams of various iron bacteria

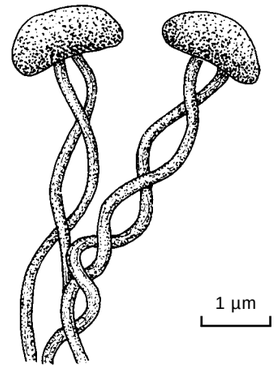
These diagrams are taken from Jones J G, Iron transformations by freshwater bacteria. Advances in Microbial Ecology, 1986, **9**, pp 149 - 185. Ed by K C Marshall, Plenum Press, New York.



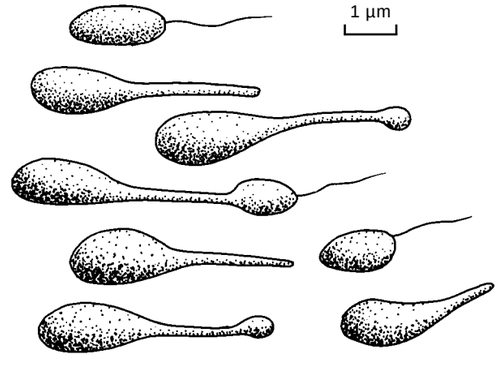
Clonothrix



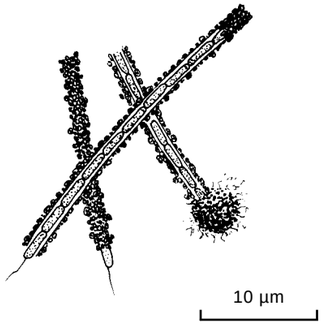
Crenothrix



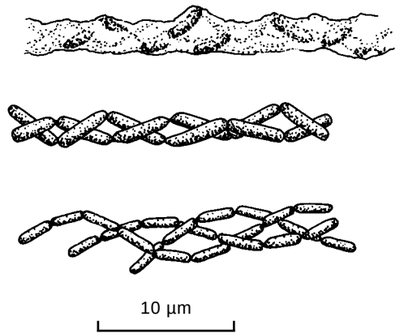
Gallionella



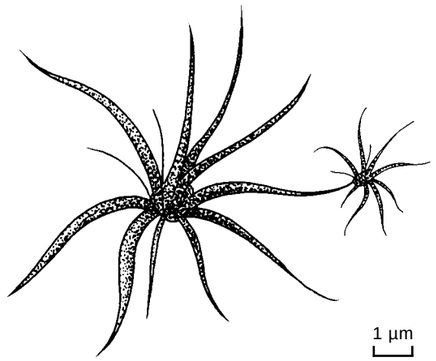
Hyphomicrobium



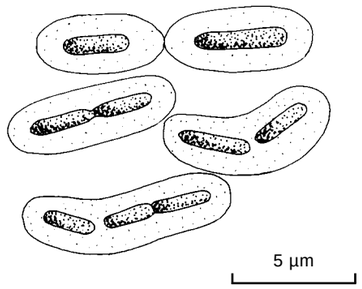
Leptothrix



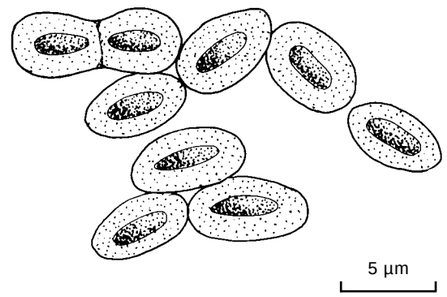
Lieskeella



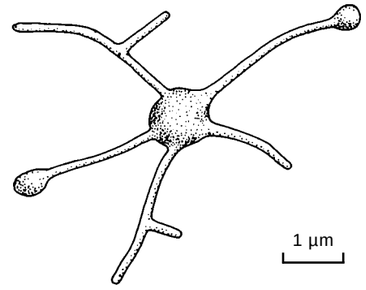
Metallogenium



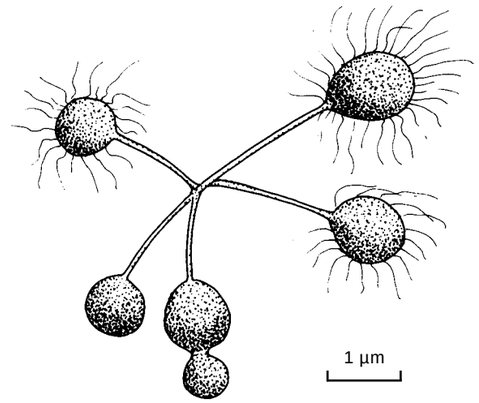
Naumaniella



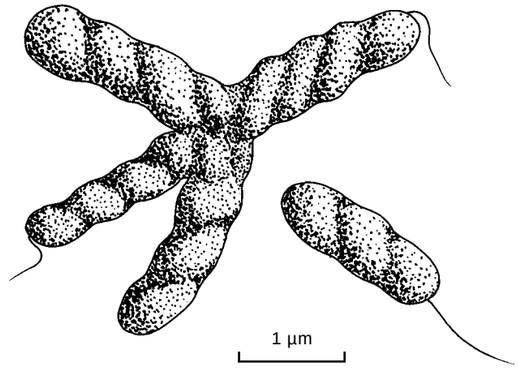
Ochrobium



Pedomicrobium

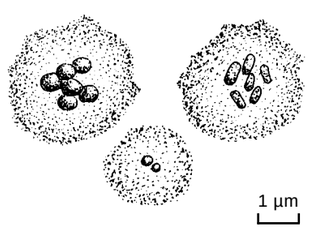


Planctomyces

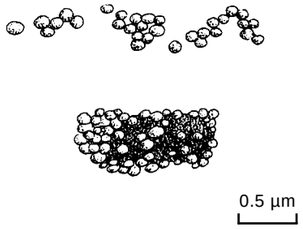


Seliberia

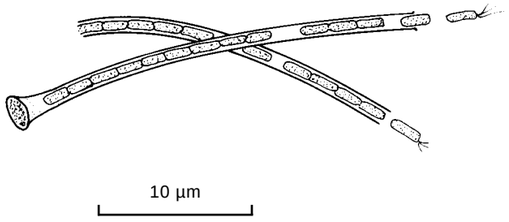
Figure 3 continued



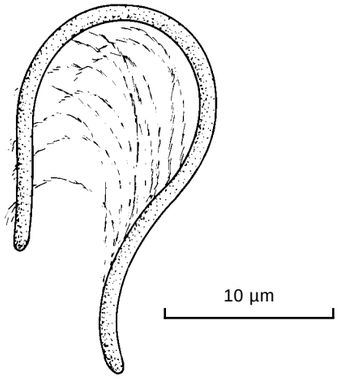
Siderocapsa



Siderococcus

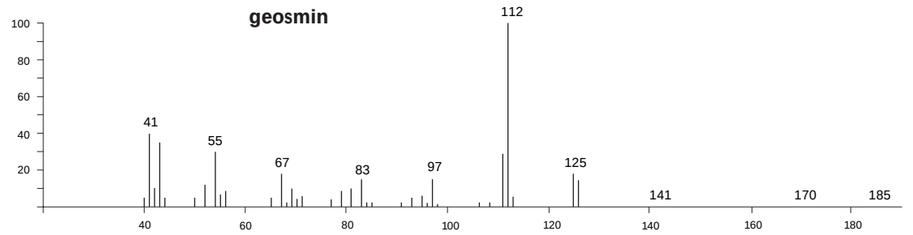


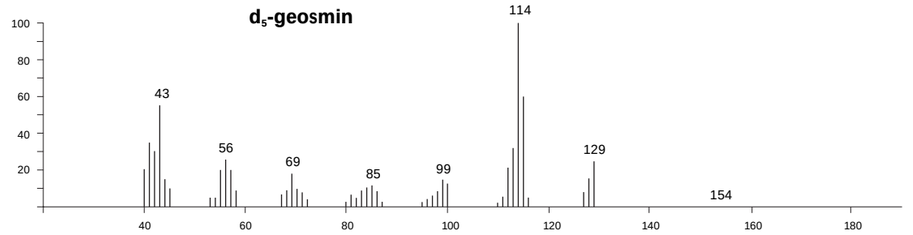
Sphaerotilus

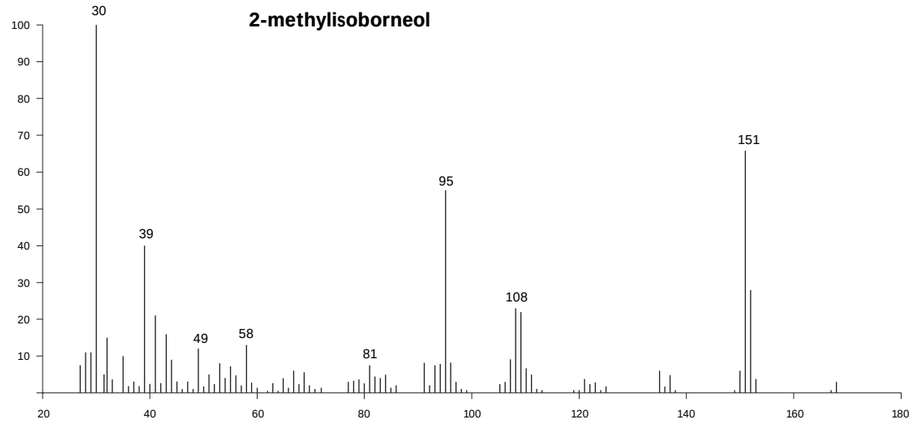


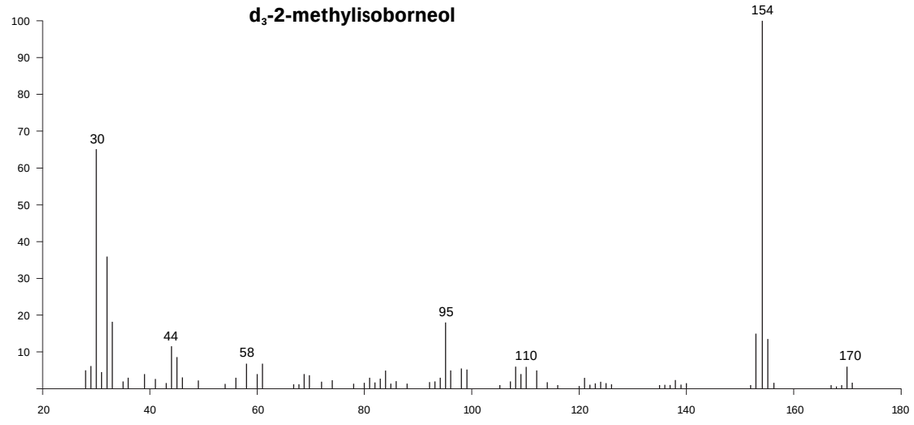
Toxothrix

Figure 4 **Mass spectra of geosmin and 2-methylisoborneol**









Address for correspondence

However well a method is tested, there is always the possibility of discovering a hitherto unknown problem. Users with information on these methods are requested to write to the address below:

The Secretary
Standing Committee of Analysts
Environment Agency
Steel House
11 Tothill Street
London
SW1H 9NF

Environment Agency

Standing Committee of Analysts

Members assisting with this booklet

R Collinge
I Fox
A Godfree
M Halliwell
L Harling-Bowen
D Holt
R Pitchers
L Pittwell
F Reid
D Sartory
G Tarbutt
A Waggott

MANAGEMENT AND CONTACTS:

The Environment Agency delivers a service to its customers, with the emphasis on authority and accountability at the most local level possible. It aims to be cost-effective and efficient and to offer the best service and value for money.

Head Office is responsible for overall policy and relationships with national bodies including Government.

Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol BS32 4UD
Tel: 01454 624 400 Fax: 01454 624 409

Internet World Wide Web www.environment-agency.gov.uk
www.environment-agency.wales.gov.uk

ENVIRONMENT AGENCY REGIONAL OFFICES

ANGLIAN
Kingfisher House
Goldhay Way
Orton Goldhay
Peterborough PE2 5ZR
Tel: 01733 371 811
Fax: 01733 231 840

MIDLANDS
Sapphire East
550 Streetsbrook Road
Solihull B91 1QT
Tel: 0121 711 2324
Fax: 0121 711 5824

NORTH EAST
Rivers House
21 Park Square South
Leeds LS1 2QG
Tel: 0113 244 0191
Fax: 0113 246 1889

NORTH WEST
Richard Fairclough House
Knutsford Road
Warrington WA4 1HG
Tel: 01925 653 999
Fax: 01925 415 961

SOUTHERN
Guildbourne House
Chatsworth Road
Worthing
West Sussex BN11 1LD
Tel: 01903 832 000
Fax: 01903 821 832

SOUTH WEST
Manley House
Kestrel Way
Exeter EX2 7LQ
Tel: 01392 444 000
Fax: 01392 444 238

THAMES
Kings Meadow House
Kings Meadow Road
Reading RG1 8DQ
Tel: 0118 953 5000
Fax: 0118 950 0388

WALES
Rivers House/Plas-yr-Afon
St Mellons Business Park
St Mellons
Cardiff CF3 0LT
Tel: 01222 770 088
Fax: 01222 798 555

For general enquiries please call your local Environment Agency office. If you are unsure who to contact, or which is your local office, please call our general enquiry line.

ENVIRONMENT AGENCY
GENERAL ENQUIRY LINE
0645 333 111

The 24-hour emergency hotline number for reporting all environmental incidents relating to air, land and water.

ENVIRONMENT AGENCY
EMERGENCY HOTLINE
0800 80 70 60

ENVIRONMENT AGENCY