Metcalf & Eddy

Water Services Division

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Centre for Research into Environment and Health

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guardians of drinking water quality

FUTURE REGULATORY PARAMETERS IMPLICATIONS FOR THE UK

FINAL REPORT FOR PHASE 1

January 2002

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DWI 70/2/145

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1. INTRODUCTION

1.1 Background

The Drinking Water Directive (98/83/EC) contains standards for a range of microbial and chemical parameters, but the number of parameters covered is limited to those that were considered to be of particular importance on public health grounds and for which there were adequate scientific data to support standards. The directive also contains indicator parameter values that are set primarily for monitoring purposes. When the directive was revised in 1998, provision was made to require the European Commission to review the standards at least every 5 years. Commission officials have announced that the first review will take place by the end of 2003. The standards in the directive are largely, but not exclusively, based on the World Health Organisation (WHO) Guidelines for drinking water quality.

There have been, and continue to be, many developments with regard to contaminants in drinking water. Standards and guidelines are developed by national and international regulatory and advisory bodies, including WHO, the United States Environmental Protection Agency (USEPA), other national regulatory agencies such as Health Canada and by committees advising the Commission itself. Indeed WHO are currently in the process of revising their drinking water guidelines for microbial and chemical parameters and introducing a continuous rolling revision of the Guidelines. Not all of these standards and guidelines will be of interest to the European Commission, but it is important for a member state to be aware of developments and the strength of the scientific basis for those developments. In particular it is of great value to understand the potential impact of future regulation and the possible additional treatment and possible restrictions on processes, chemicals and materials that this may entail. There is, for example, the potential for significant cost and process implications from the introduction of more stringent standards for a number of contaminants covered by the directive at present. It would also be important to determine the likely impact of any new parameters that could be introduced, particularly those that are a source of public concern.

The Department needs to know which existing parameters are likely to be more strictly regulated, and for which new parameters standards are likely, both in Europe and elsewhere. The Department contracted Metcalf and Eddy Ltd with their partners, CREH and Owen Hydes OBE, to consider potential future regulatory parameters and their implications for the UK

1.2 Project objectives

The objectives specifically stated by the Department were:

1. To identify parameters which are not currently included in UK drinking water regulations and which might be the subject of future regulations;

2. To identify currently regulated parameters which might be subject to stricter regulation;

3. To review international sources of information on the likely concentrations in drinking water;

4. Where there is reason to suspect that these concentrations are likely to contravene future regulatory requirements, to make recommendations, where necessary, for surveys of drinking water quality to establish the implications for the UK water industry;

5. Where necessary, to develop a UK national position towards regulatory parameters in advance of any proposals by the European Union.

The programme of work was to be divided into two phases to enable an assessment of the requirements for phase 2 that would be derived from the work carried out in phase 1.

1.3 Phase 1 tasks

The programme for phase 1 consisted of four linked tasks that had a logical progression. These tasks were:

1. Identification of potential parameters for future regulation from current and proposed WHO Guidelines for drinking water quality, current and proposed USEPA Standards, current and proposed studies of the European Union and any other important country's standards.

2. Advising on whether there is a defensible scientific basis for the regulatory proposals.

3. Preparation of high, medium and low priority provisional lists of parameters from these potential parameters by assessing all of the available information about their likely or actual presence in drinking water; their likely concentration and toxicity.

4. Identifying information gaps and proposing the future information needs about concentration and toxicology to confirm or modify those provisional priority lists.

The decision to proceed with phase 2 is dependent on the outcome of phase 1 and the nature of the recommendations made as part of the phase 1 programme. This report constitutes the final report relating to phase 1 and includes the recommendations that will be considered as the basis of phase 2.

2. SOURCES OF INFORMATION

2.1 World Health Organisation

The WHO Guidelines for drinking water quality are the most influential source of information and advice with regard to standards for both microbiological and chemical contaminants in drinking water. The publication of the second edition of the Guidelines in 1993 provided a stimulus for the Commission to formally consider member states' requests for a revision of the 1980 Directive. The evaluation of polycyclic aromatic hydrocarbons (PAH) during the preparation for the 1998 addendum to the 1993 Guidelines also lead to the Commission removing fluoranthene from the list of PAH to be considered in the revised directive. WHO embarked on the process of a programme of revision of its guidelines in 1999, with a formal task group meeting in June 2000. This process is now well under way but it is not intended that all parameters will be reviewed at once and there will follow a continuous rolling revision process. This process will fit with the review of the standards in the Directive at intervals of five years, by the Commission.

As on previous occasions, WHO will consider microbial and chemical contaminants separately, but with some overlap. The intention was to consider progress on the preparation of background documents at a meeting in Manila in November 2001. However, following the terrorist attacks in the United States in September 2001, a number of key participants were unable or unwilling to travel to the Philippines and the meeting has been postponed to late spring of 2002. This is unfortunate as it has delayed one of the key meetings for progressing the revision of the Guidelines. The proposed target date of 2003 for formal publication is not likely to be delayed beyond the end of that year, since initial publication will be on the internet. However, this may be too late for the revised guidelines to be taken fully into account in the Commission's review of the Directive's standards in 2003

WHO will be informed by reviews carried out by specialist WHO groups such as the International Programme on Chemical Safety (IPCS) and the Joint Expert Committee on Food Additives and Contaminants (JECFA). These reviews are available for a number of parameters of interest to WHO and reviews for other parameters are still in preparation. The various co-ordinators, and member states preparing background documents, were consulted in the process of this study.

WHO reviews the guideline for an existing parameter when there is new data available on its toxicology, its occurrence in water sources and supplies or its removal in treatment. Parameters for which the guideline value is designated provisional are examined at regular intervals to determine whether there are sufficient new data, or developments in treatment or analysis, to remove the provisional designation. WHO considers developing a guideline for a new parameter when there is some evidence that it occurs in water supplies, that it may be of public health concern and a guideline has been requested by at least one Member State.

2.2 United States Environmental Protection Agency

The USEPA published a draft list of candidate contaminants in 1997 (Federal Register: October 6, 1997 Volume 62, Number 193, Notices, pp. 52193-52219), for consideration under the revised Safe Drinking Water Act (1996). The list was derived from data on occurrence from a range of different sources and on the basis that contaminants might be expected to be present. It provides a basis for prioritising research and selecting new parameters for regulation. This list has been widely circulated and contains both pathogenic microorganisms and chemical contaminants. A significant number of the chemical contaminants were pesticides or pesticide breakdown products. In view of the precautionary standard for pesticides set in the Directive and the levels at which standards were most likely to be set, these were not considered further since they would not affect standards in Europe. Discussions were held between USEPA and various stakeholders, including the American Water Works Research Foundation with a view to collecting data and jointly funding any necessary research. Discussions have been held with senior USEPA personnel with regard to progress on the list and recommendations regarding an initial list of parameters for possible regulation were expected by the end of 2001. This has been delayed by the inter-departmental consultation process but the Federal Register notice is being finalised. Publication is expected by May 2002.

2.3 Other non-European regulatory bodies

There are a small number of other influential regulatory bodies. These may exert their influence by the reputation for scientific and technical excellence or by being involved in the international processes such as WHO or the Organisation for Economic Cooperation and Development (OECD). An example of a regulatory body that falls into both categories is Health Canada. Not only is Health Canada closely involved with the WHO Guidelines as a coordinator and contributor, but it has also been a leader in the development of the use of risk assessment for developing guideline values. Health Canada has recently published proposed guidelines for microcystins (http://www.hcsc.gc.ca/ehp/ehd/bch/water_quality/consult/microcystins.htm) and is currently developing a new guideline for turbidity. It is also in the process of considering a number of disinfection by-products and tri- and tetra-chloroethene. Discussions have been held with Health Canada in order to assess the significance of its proposals, some of which will also influence the preparation of background documents for the WHO Guideline revision.

Japan is also closely involved in the WHO discussions and is currently examining its drinking water standards. Discussions have been held with senior personnel in the Department of Health, who are responsible for drinking water quality, with senior members of the Japanese Water Works Association and with Professor Magara, a leading academic and advisor to the government on drinking water. There are a number of concerns in Japan and it is responsible for preparing some background documents for the WHO Guidelines revision. Information regarding the preparations for these documents has been used in assessing the priority lists ahead of the publication of WHO draft documents.

2.4 European Regulatory Interests

A number of Member States of the European Union have interests in a wide range of specific parameters, some of which are not specifically addressed in the current Directive. Discussions have been held with both regulators and water suppliers in a number of these countries. The concerns of the Member States are often highly influenced by political considerations and these will be particularly important in influencing the European Parliament. The European Parliament will have a much greater role to play in any revisions of the standards in the Directive in view of the change to a co-decision process. The concerns of Members of the European Parliament, therefore, will be of greater importance than in the preparation of the revised Directive in 1998 and may carry greater weight than scientific justification. The concerns of the Parliament with regard to endocrine disrupters are likely to be an important influence on the potential for inclusion of a parameter or parameters relating to endocrine disrupters. This is a continuation of the Parliaments concerns, expressed in 1998 that resulted in the requirement for the Commission to carry out research into endocrine disrupters in drinking water. This contract has been let to the Fraunhofer Institute in Munich who is working with one of the German water laboratories. Contact has been made with this research group. It has prepared and issued a questionnaire that lists a large number of substances that are suspected of possessing endocrine disrupting activity, mostly from the results of *in vitro* assays.

A number of Member States have expressed concerns regarding specific parameters, and these concerns are reflected in the priority lists. Examples of these concerns are Finland and radon and uranium, the Netherlands and *Legionella*, Spain and cyanobacterial toxins and France and aluminium.

A number of potential regulatory parameters have been under discussion in other fora of the EU under the banner of different directives. MTBE was considered under the existing substances directive and there was a relatively low level of concern, except from Denmark, which expressed concern over possible tainting of fish. The approach most favoured was to increase preventive measures, particularly for underground fuel storage points. There was general agreement that MTBE does not pose a risk to health because it would cause significant taste and odour problems at much lower concentrations than any health based standard. MTBE has, therefore been allocated a low priority. However, the occurrence of one high profile incident could change the priority to medium or even high.

3. SCIENTIFIC BASIS FOR NEW PARAMETERS

3.1 Microbial Parameters

There are several issues that require consideration for microbial parameters, not least of which is whether existing standards and indicator parameter values and existing treatment processes will provide adequate protection of public health without the need for introducing additional parameters. The other significant considerations are:

- Is the organism a human pathogen?
- Does the organism act as an indicator of a human pathogen that is not adequately covered by existing indicators?
- Is the organism removed or inactivated by existing or potential future treatment processes
- Is the organism known to occur in drinking water?
- Is drinking water a significant source of exposure to the organism?
- Can the organism be detected by practical methods?
- Will regulating by a drinking water quality standard be of any benefit? For example, it is not appropriate to control *Legionella* in water within premises by the introduction of a standard for drinking water quality. Legionella is an ubiquitous organism and low levels are likely to be present within a water supplier's distribution system despite the low levels of residual disinfectant. These low levels of *Legionella*, once into an appropriate water environment within a building, will multiply. Therefore the best of control is by maintaining and operating water using devices within buildings in a manner that minimises the opportunity for *Legionella* to multiply.

All of these considerations have been taken into account in assessing the scientific basis and justification for introducing a new regulatory parameter. A brief summary of the scientific considerations is included in the prioritisation section (Section 4) and a more extensive evaluation is included as Appendix 3.

3.2 Chemical Parameters

The scientific considerations surrounding chemicals are somewhat different to those of microorganisms. In particular much of the data are derived from experiments in laboratory animals given very high doses and for which there is often considerable uncertainty in extrapolating from the findings in laboratory animals at these high doses to possible effects in humans exposed to much lower doses. These uncertainties are dealt with in a number of ways but can lead to unrealistic values being proposed as standards or guidelines. In some cases the toxicological uncertainties can be resolved by relatively short-term and simple studies, but

increasingly the uncertainties require long-term research and complex research programmes. Such studies and programmes can be prohibitively expensive and may lead to the introduction of politically derived precautionary standards.

The key issues for assessing the scientific basis for standards or guideline values for individual, or groups, of chemicals are:

- Extent of the toxicological database.
- Absorption of the chemical and, if it is inorganic, the chemical speciation.
- The toxicological effect of concern.
- The mechanism of toxicity.
- The availability of epidemiological data and the quality of that data.
- Occurrence in drinking water.
- Availability of appropriate analytical techniques.

There is also an issue with regard to removal in drinking water treatment since many of the more toxic substances are highly lipophilic and will adsorb to particulate matter that can be readily removed by conventional treatment.

All of these considerations have been taken into account and are included, where appropriate, in the section on priorities (Section 5) and are considered in more detail in appendix 4.

4. PRIORITY MICROBIAL PARAMETERS

4.1 Introduction

There is a wide range of microbial parameters that could be considered for regulatory standards and monitoring. Some of these are known to be pathogenic microorganisms for which there are methods of analysis. Many of these (for example, *Campylobacter*) are known to be removed during water treatment and are killed by disinfection. There are others that are less sensitive to disinfection and, with a low infectious dose, may be a serious problem. The viruses fall into this category. Many of the viruses are, however, not culturable and their detection relies on the use of molecular biological techniques. A further group of microorganisms is known to grow in water distribution systems where the conditions permit. The significance of bacteria such as *Aeromonas* and fungi that can produce mycotoxins is unclear.

Although a wide range of microorganisms has been reviewed, it is felt that there is no general scientific basis for the introduction of standards for pathogens. This is because of the move away from heavy reliance of 'end product' monitoring and the move towards an overall management strategy (water safety plan). This strategy covers the raw water source, its treatment and subsequent distribution to the consumer. It is analogous to a HACCP (Hazard Analysis and Critical Control Points) approach for ensuring adequate water safety (Fewtrell and Bartram, 2001; OECD/WHO, 2002).

The information used for this review has been obtained from a detailed literature search including recent peer review papers in journals, books and the Public Health Laboratory Service Communicable Disease Report. Attention has also been given to the views of Regulatory bodies in Europe and further afield. The information gathered has been documented in a paper incorporated into Appendix 3.

The evidence collected relates to drinking water leaving a water treatment facility. There have been outbreaks related to contamination of water in distribution systems either in service reservoirs or in water mains particularly through the repair of damaged infrastructure. There is now evidence emerging from the United States that changes in water pressure alone is sufficient to induce ingress of extraneous material into a water main. Such changes might be short term providing a pulse of contamination that will not be detected by monitoring. Work has been done by Mark LeChevallier for the American Water Works Association Research Foundation using high speed pressure recorders to show that a number of transient negative pressure events can occur. Soil lining the outside of a water main has been shown to contain total coliforms, faecal coliforms and enteric visuses. Pressure drop is of such concern in some states (e.g.Ohio) that if pressure sensors indicate that water pressure has fallen below 20 psi, a boil order is issued (LeChevallier, pers. comm.).

There may also be occasions when monitoring for traditional indicator organisms (such as *E.Coli*, colforms and colony counts) might suggest that more detailed investigation of a treatment and distribution system is necessary. Such an investigation might include testing for organisms for which there are currently no published analytical techniques directed to water.

The DWI might wish to prepare a list of documented methods for such organisms that are not found in the Microbiology of Drinking Water 2001 for example *Yersinia* In addition, recognition of expertise in certain analytical techniques or the identification of microorganisms might be useful. The following sections outline the parameters according to priority rating.

Fewtrell, L. and Bartram, J. (2001) *Water Quality Guidelines, Standards and Health. Assessment of risk and risk management for water-related infectious disease*. IWA Publishing, London. Pp. 424.

OEDC/WHO (2002) *Safer Drinking Water: Improving the Assessment of Microbial Safety*. In Preparation.

4.2 High Priority

Legionella spp.

Legionella has high political profile (because of the recent drinking water standard introduced in the Netherlands) but low scientific justification. Analysis of drinking water samples can clearly demonstrate the presence of low numbers of *Legionella.* Being natural water organisms, small numbers will pass through water treatment and survive disinfection, particularly in association with amoebae. The levels in mains water will be low, insufficient to cause infection in aerosols, and it is difficult to see how they can be eradicated. In addition, the organism may be in a viable but non-culturable state that will not be detected by culture techniques. Prevention in buildings relates to good maintenance and cleaning, the use of appropriate plumbing materials and the application of biocide processes. However, given European interest in the need for a standard, the organism has been given high priority status. Should a standard be proposed for *Legionella*, this seems most likely to be included as a mandatory parameter.

4.3 Medium Priority

Aeromonas spp.

There are a number of documented instances of the isolation of *Aeromonas* spp. from water but no evidence of outbreaks of gastroenteritis. High infective dose in conjunction with the possibility that 'environmental' strains isolated could be non-toxigenic may be an explanation. Given this fact they have been given the status of medium priority. Supply of contaminated water to the food industry or to hospitals may be undesirable. It would seem advisable to determine whether aeromonads isolated from distribution systems are toxigenic. Where routine monitoring detects the growth of large numbers of aeromonads in distribution systems isolates could be checked for enterotoxin production in tissue culture. Alternatively, an assay for a simple virulence marker could be developed.

Bacteriophages

These have been suggested as a surrogate for human enteric viruses, due to similarities in size and behaviour through the water treatment process. Methods of analysis are relatively cheap and easy. It is probable that there may be some pressure from Europe to introduce monitoring for bacteriophages as many continental supplies are not chlorinated. For this reason they are considered to be of medium priority.

Clostridia

Clostridium perfringens (including spores) is included in the indicator parameters for the current Directive. There appears to be a belief in the Commission and some member states that this parameter is a suitable surrogate for *Cryptosporidium* and if this belief persists, there could be a proposal to make it a mandatory standard. There is, however evidence from studies in the UK that demonstrate that such a correlation does not occur.

4.4 Low Priority

The remaining possible microbial parameters, which are reviewed in detail in Appendix 3, have been rated as having low priority. The reasons for the rating vary according to the organism but can be broadly grouped into removal by suitable water treatment (e.g. *Campylobacter*), no suitable method for routine analysis (Calicivirus) or insufficient data (genetically modified organisms).

Campylobacter spp.

There have been a number of outbreaks of *Campylobacter* in the United Kingdom but these have been related to private and not public supplies. Water treatment and disinfection is effective in removing *Campylobacter* from drinking water. *Campylobacter* is more susceptible to chlorine as a disinfectant than is *E. coli.* Outbreaks are likely to occur predominantly from the contamination of ground water where there is no disinfection and private water supplies where there is no treatment. The literature also suggests that faecal coliforms are a good indicator of the presence of *Campylobacter* in drinking water. *Campylobacter* are, therefore, considered to be of low priority.

Escherichia coli O157:H7

Outbreaks in drinking water have occurred where there is no treatment or disinfection or where the latter is ineffective and there has been sewage contamination or contamination by slurry from cattle or sheep. There is no evidence that *E. coli* O157 has any greater resistance to chlorine than other *E. coli*. The maintenance of proper water treatment and disinfection is, therefore, the best protection against waterborne outbreaks. *Escherichia coli O157* has been given low priority status. The organism will be isolated by current Regulatory monitoring for coliforms but grows poorly, if at all, at 44°C and thus will not be included in the E. coli numeration.

Helicobacter pylori

Helicobacter pylori cannot be cultured from water or environmental samples. Molecular and immunological techniques remain the only tool to demonstrate its presence. These techniques cannot be guaranteed to be 100% specific and cross-reactions with other *Helicobacter* spp. remains a possibility. There is no firm evidence that drinking water in developed countries poses a threat of infection. In addition, *H. pylori* is a poor survivor outside the stomach and rapidly develops a viable-but-non-culturable state from which it has not been shown to revert. *Helicobacter pylori* is, therefore considered to be of low priority. Research in America has demonstrated that *H. pylori* is sensitive to chlorine as a disinfectant. Given poor environmental survival and the sensitivity to chlorine, it is unlikely that *H. pylori* is a waterborne problem in the United Kingdom.

Mycobacterium avium complex (MAC)

MAC can clearly be found in raw waters and treatment reduces but does not remove them from finished water. Disinfection is ineffective. The organisms can grow in distributed water, particularly where the temperature exceeds 15^{o}C and there is available organic material. Although a culture medium exists for the isolation of MAC, growth is slow and results may take between 1 – 2 weeks. A recent study by the Public Health Laboratory Service (PHLS), which is not yet published, isolated *Mycobacteria* from 19 of 170 water samples. Only 3 isolates were MAC. In addition, the study failed to isolate *M. paratuberculosis* from any of the waters tested.

Acanthamoeba spp.

Acanthamoeba is distributed worldwide and can survive indefinitely in the cyst form, but there are no documented instances of waterborne outbreaks of infection. Its ubiquitous nature means that immunocompromised individuals will come into contact with it frequently in their daily lives. It can undoubtedly grow in water without any further nutrient being added and will be found wherever water is warm and has been allowed to stagnate (for example in taps and showers). Contact lens wearers should be aware of the risks posed by storing lenses in non-sterile solutions.

Cyclospora cayetanensis

Conventional water treatment should remove this organism although disinfection will have little effect. The numbers of detected cases in the United Kingdom would suggest that the incidence of the organism in sewage and surface waters is low. However, it would be useful to have a draft method available should the need arise and analysts in water laboratories should be familiar with the parasite.

Giardia intestinalis

There is no routine monitoring of potable water supplies for *Giardia* in the United Kingdom, possibly because, unlike *Cryptosporidium*, there has been no good epidemiological evidence of drinking water being implicated in outbreaks. There is, however a substantial reservoir of

infection in the United Kingdom, probably larger than confirmed figures suggest, for which the source of infection is unknown. However, this parasite is considered to be of low priority.

Regulatory monitoring of potable water for *Cryptosporidium* already provides a sample that could be analysed either simultaneously or subsequently, for *Giardia.* It would therefore seem advantageous to perform a combined analysis on at least a proportion of these Regulatory samples to provide documentary evidence that water is not a major route of transmission of the parasite.

Microsporidia spp.

The organisms may be much more widespread in human populations than previously thought, based on seroprevalence. Little is known about the incidence, in water samples, of *Microsporidia* that cause human infections. Environmental detection is hampered by a lack of sensitive and specific tools for isolation and identification. Given the wide distribution of species, without specific tools, confirmed detection of human species in water will be difficult. Limited data would suggest that the incidence of human species in the environment is low and that water treatment and disinfection should remove them. They are, however, much smaller than other waterborne parasites and therefore the efficacy of water treatment may need attention. Recently published information from America would suggest that *Microsporidia* are sensitive to disinfection with chlorine. A four log inactivation by 2 mg/l of chlorine was demonstrated in 6 – 8 minutes at 23^{o}C. In view of the above these organisms are considered to be of low priority.

Viruses

Techniques for the detection of viruses in water are generally more complex than those of bacteria. New techniques that encompass part replication in cell culture followed by the use of molecular biology techniques have made detection more sensitive and demonstrated infectivity of virus particles. The caliciviruses can only be detected by molecular biology. Many, although not all, of the virus groups are readily removed by conventional water treatment and disinfection. For these reasons the viruses are considered to be of low priority.

Fungi

Research work shows that fungi producing toxic, as well as odiferous, secondary metabolites can be isolated from drinking water. In addition, such fungi can colonise water systems and produce secondary metabolites. Nothing is known of the existence of most of such metabolites in raw waters at different times of the year or the effect of water treatment on their removal. The identification of fungal genera and species is difficult and few water companies would have the expertise to determine the precise identification of any isolate. In addition, detection and characterisation of secondary metabolites using gas chromatography is not straightforward. The fungi are considered to be of low priority.

Genetically Modified Organisms

This group can cover a wide range of microorganisms and methods for their detection may not be available unless the identity of the organism is known. This group therefore, is considered to be of low priority.

Anthrax

This is one of a group of special organisms for which the concern relates to deliberate introduction into a water supply. Standards and routine monitoring would be inappropriate although the development of reference source of methods for such organisms in drinking water would be of potential value. Whilst ingestion may not present a risk from infection with anthrax in drinking water, inhalation from aerosols generated by showers or running taps could pose the risk of pulmonary anthrax.

5. PRIORITY CHEMICAL PARAMETERS

5.1 Introduction

The debate as to the utility of increasing lists of chemical parameters is continuing on both sides of the Atlantic. WHO is also addressing this question by introducing a more holistic approach to drinking water quality that will enable countries to be more selective with regard to chemical parameters while taking into account the control and quality of raw water sources and the operational and regulatory indicators of treatment performance. The USEPA has selected eight chemical parameters from the candidate contaminant list for initial consideration as to regulatory action that it expects to publish in the Federal Register for comment in the next few weeks. The proposed action will not be for inclusion as standards for all of these parameters but proposals will include the option of producing non-statutory health advisories. Of these eight substances, three are pesticides and two, sodium and sulfate, are primarily associated with taste considerations.

The list of priority substances classified in high, medium and low priority categories are as follows.

5.2 High Priority

Trichloroethene / Tetrachloroethene and other chlorinated solvents.

Both of these substances are commonly found in groundwater sources close to industrial and commercial establishments. It is not clear how much of this contamination is historical and how much is continuing. WHO is reconsidering these substances because the International Agency for Research on Cancer (IARC) has changed the carcinogenicity classification from 2B to 2A based on re-evaluation of the epidemiology. This re-classification is rather controversial but Denmark may well seize on this to press the Commission for more precautionary standards since the previous position and that of some members of the Commission's Scientific Advisory Committee on Toxicology and Ecotoxicology was 1µg/l for both substances. 1,1,1-trichloroethane and dichloromethane are also under consideration by WHO. The USEPA is also in the process of revising its risk assessment for trichloroethene. It has characterised trichloroethene as "highly likely to produce cancer in humans" and is likely to use a mathematical model to estimate additional risk of cancer. In the event of new more stringent guideline values there could be an attempt to group all the chlorinated solvents together under a mandatory precautionary value.

Organic Disinfection By-Products (DBPs) – haloacetic acids.

WHO are reconsidering the DBPs in the revision of the Guidelines and the USEPA has adopted new standards for a range of DBPs under the negotiated rule making. While it seems unlikely that there will be significant pressure to tighten the total THMs standard in the short-term, there could well be calls to introduce a standard for the haloacetic acids as a group parameter or as individual substances, particularly for dichloro- and trichloro- acetic acid,

which can be present in the same concentration range as THMs. Haloacetic acids are considered high priority. The organic disinfection by-products occur wherever chlorination is practised but the concentrations vary according to the precursor concentrations, temperature and process conditions. Data on the THMs are widely available but, apart from the USA, there a few data on the concentrations of any of the other substances that will be present. However it is clear that substances other than the THMs and haloacetic acids, are usually present at concentrations in the region of 1 μg/l or less. However, there is a need for data that indicates how controlling THMs has impacted on the concentrations of the other organic by-products. The one uncertainty regarding the organic DBPs from chlorination as a group remains the possibility of a strong positive in one of the studies on effects on adverse birth outcome. Currently there is a significant increase in environmental pressure group activity in this area in the USA.

Inorganic Disinfection By-Products - chlorite.

The increasing interest in chlorine dioxide is likely to see calls for a regulation of the by-products chlorite and chlorate. The WHO guideline values are not likely to give rise to problems and, as in the UK, control could be by product dosing. The scientific database for chlorite is well developed and therefore chlorite is considered to be high priority. The scientific database for chlorate is still under development and, therefore, the priority for chlorate as a separate value is only considered to be medium.

Endocrine Disrupting Substances.

There is considerable political concern with regard to endocrine disrupters and the Commission has let a contract with the Fraunhofer Institute to carry out a study on drinking water. This is setting out with a long list of substances that have been implicated as possessing endocrine disrupting properties in any way. The main substances found in drinking water are phthalates, although the scientific evidence does not support their role as true endocrine disrupters. This has not stopped them being included in the Water Framework Directive. This area remains very uncertain but it is important that any standards are based on actual occurrence and sound science. WHO has proposed guideline values for diethylhexylphthalate and diethylhexyladipate. A number of the phthalates are commonly found in drinking water at low concentrations but data on other potential endocrine disrupters are more limited. Setting drinking water standards for potential endocrine disrupters is probably not the best way forward. A better way may be to control the discharge of these substances to the water environment and, for those substances that may be derived from constructional materials, to control the use of construction materials.

Radon.

Radon is widespread in Europe and North America, and although it was thought to be lost from drinking water so rapidly that it was entirely an air quality problem, this now does not appear to be quite true. The key issue seems to be how much of a problem is it in public versus private supplies. It is known to be a significant problem in small public and private supplies in Scandinavia and in some private supplies in the UK. An expert group, under the

EURATOM treaty has already produced a draft recommendation on radon and its daughters that could be taken up for use in the Directive. It is also possible that the radon daughters, lead-210 and polonium-210, could be included with a standard for radon, although it seems that the priority for them to be considered separately would be low.

Uranium. Uranium is found in many waters, just as radon, however the concentrations in drinking water in Europe are less well known. Some private supplies in the UK contain concentrations above 2 µg/l, the current provisional WHO guideline value. Concentrations in the USA and Canada appear to vary from less than 1 µg/l to above 20 µg/l, but high concentrations appear to be quite rare. Finland was considering regulation but was persuaded that this was not appropriate at the time. Both the USA and Canada have proposed standards and uranium will be reconsidered by WHO. The science supporting a value has gaps but the proposals from North America would be achievable in the UK.

Aluminium. The French have been under some pressure regarding reports of an association of Alzheimer's disease with aluminium in drinking water, from the use of aluminium salts in drinking water treatment. Canada has been trying to set a health based standard but the data do not enable that to be done in any sensible way and have proposed guidelines based on achievability. The latest French study showed an apparent threshold at 100 µg/l (Rondeau et al 2000) and WHO have indicated the desirability of minimising aluminium concentrations. WHO indicated that 100µg/l should be achievable for larger well-run treatment works. In view of the fact that 100 µg/l should be achievable as an average in Europe, it may be proposed that the current value in the directive be reduced to this while the parameter is retained in the indicator group.

Rondeau, V., Commenges, D., Jacqmin-Gadda, H. and Dartigues, J-F. (2000) Relation between aluminium concentrations in drinking water and Alzheimer's disease: a 8-year follow-up study. American Journal of Epidemiology. 152. 59-66

5.3 Medium Priority

Arsenic.

The USEPA has run into considerable problems with arsenic and the WHO Environmental Health Criteria still remains unpublished. There is considerable controversy over the cancer epidemiology and the applicability of risk models to estimate cancer risk from Taiwan data. There is pressure for a lower value than 10 µg/l, although the difficulties and costs of achieving even 10 µg/l are substantial in many parts of the world. Arsenic is found in many drinking water sources. In most cases the concentrations are below 10 µg/l but many sources are above the 2 to 5 µg/l that have been suggested as regulatory levels. However, following its recent re-evaluation of the risks, costs and benefits USEPA has announced that it will establish a maximum contaminant level of 10 µg/l for drinking water. The environmental pressure group, the National Resource Defense Council, have announced that they will contest this decision.

Nitrate / nitrite.

WHO JECFA proposed a very tight tolerable daily intake (TDI) for nitrate that was not used for the revision of the drinking water guideline value. It has been referred back to JECFA who are now asking for new data. This may be available in time to influence the Commission for the proposed first round of revision of the directive. If not then nitrate is unlikely to be a priority at this time. One key issue for the current directive is that the requirement to take into account the ratio of nitrate and nitrite to their respective standards is derived directly from WHO. The use of the formula is, therefore, based on the WHO guideline values of 50 mg/l for nitrate and 3 mg/l for nitrite. The use of the directive standard for nitrite at the treatment works of 0.1 mg/l or at consumers' taps of 0.5 mg/l for this calculation would not be appropriate as this is a precautionary standard rather than one based on health.

Copper.

WHO are planning to re-examine the guideline value for copper and the document is being prepared by USEPA and Germany. A number of groundwaters in Germany are high in bicarbonate and very aggressive to metal plumbing. The German government has also been under some pressure to tighten the standard for copper in drinking water because of suspicions regarding infantile liver toxicity as a consequence of 'high' copper levels in water. There still is no widespread scientific agreement on this issue, although there is a great deal of scepticism. Much will depend on the German view and what support it receives. Denmark and Sweden may also favour a tighter standard, since the Swedish research indicates that copper in drinking water may be a significant contributor to intake in young children in some areas. The argument is then whether there is a need to change the standard or whether members states with problems of aggressive waters should take action to advise against the use of copper tubing in such circumstances. There are, however, significant new data on the gastrointestinal efforts of copper and these would tend to support the current standard.

Algal Toxins.

There is concern in a number of countries regarding toxins from blue-green algae, particularly the microcystins. France, Spain and Germany are all believed to be considering drinking water standards. WHO take the position that the best way forward is to control growth in water sources and this would not be best dealt with through drinking water standards. The database on the concentrations of algal toxins in final drinking water is very limited although it is clear that in some cases microcystins could be present at concentrations in excess of 1-2 µg/l for varying periods in the summer months. However, microcystin LR and anatoxin a were not observed in a snapshot survey of water supplies in England and Wales (James et al 1994).

James HA, Smith CJ, Sutton A, Cayley, J, Franklin O and Parr W. (1994) Survey of the concentrations of algal toxins in water supplies. DoE report DoE 3761/1.

Turbidity.

Turbidity is an important indicator parameter. It will remain so but there are concerns that the values in the revised directive are not sufficiently stringent, particularly the 1 NTU indicator value for water leaving treatment works. The USEPA and Health Canada have both proposed values of less than 0.5 NTU and this is likely to be raised as an issue for Europe, particularly since turbidity is so important as an operational indicator for the increased risk of microbiological contamination.

Pharmaceuticals.

There has been renewed interest in pharmaceuticals in the media and by the pressure groups. This is associated with re-use of water and although the data do not indicate that there is any real concern for health, pharmaceuticals are likely to the next major group of substances to receive attention in a similar way to endocrine disrupters. Like endocrine disrupters the Environment Agency and other agencies concerned about surface water quality are beginning to assess the possible impact on aquatic life (Ayscough et al 2000). There are only limited data on the concentration of pharmaceuticals in drinking water but concentrations appear to mostly well below 1 µg/l. Most studies have so far concentrated on human pharmaceuticals and there appear to be even fewer data on veterinary pharmaceuticals. The most commonly identified are those used in large doses for extended periods and include anti-hyperlipidaemic drugs such as clofibric acid, non-steroidal anti-inflammatory drugs such as ibuprofen and antibiotics such as erythromycin.

Ayscough NJ, Fawell, JK, Franklin G and Young W. (2000) Review of human pharmaceuticals in the environment. Environment Agency Report TRP 390.

Petroleum Hydrocarbons.

There is always concern about contamination of drinking water by petroleum hydrocarbons. Although the scientific evidence indicate that the primary problems are actually associated with taste and odour it is possible that a value to control petroleum hydrocarbons could be sought as an indicator parameter to replace the total hydrocarbons parameter that was in the previous directive. WHO is preparing a discussion of petroleum hydrocarbons and already has guideline values for the BTEX compounds, these are benzene, toluene, ethylbenzene and xylene. Benzene is included in the current directive but with a value that is ten fold more stringent than the WHO guideline value on the basis of 10^{-6} excess cancer risk.

5.4 Low Priority

Manganese.

WHO and USEPA are considering manganese and it will be one of the parameters from the candidate contaminant list that goes forward. The indications at this time are that the

proposal will be for a health advisory and that any health-based values will be well above the consumer acceptability value currently in use.

Inorganic disinfection by-products – bromate.

Bromate is already regulated but proposals from WHO and USEPA should reduce any pressure to tighten the existing European standard. Bromate would therefore be considered to be of low priority. Chlorate and bromate can be formed in the production and storage of hypochlorite and chlorine but currently only sporadic data appear to be available on their concentrations. The introduction of standards for bromate has resulted in an increased database in North America, Europe and Japan. There have been occasions when bromate has been found as a consequence of past industrial pollution, for example in groundwater below an old industrial site near Hatfield, which has contaminated drinking water. In some countries bromate also appears to be found as a consequence of current waste water discharges.

MTBE

MTBE appears to be much less of a problem in Europe than in the USA. It is accepted in Europe that the issue is taste and odour rather than health and the pressure for a standard will depend more on political pressures than scientific pressures unless a serious incident occurs in the near future.

Strontium

Strontium is being considered by WHO. It is found widely in the environment, including in drinking water and it is associated with calcium. There remain considerable uncertainties over the need for a standard based on health but the fact that this is present at concentrations equivalent to other cations found in hard waters is likely to draw attention if a WHO guideline value is proposed. The USEPA have proposed a health advisory of 4 mg/l but strontium is unlikely to be considered by it in the immediate future.

1.4-Dioxane.

This is an industrial solvent that is quite soluble and has been identified at low concentrations in drinking water in the UK on occasion. It is suspected of carcinogenic activity in laboratory animals but other data are limited. It is listed by WHO for consideration at the request of the Japanese. In the event of it being raised through a revised WHO guideline value this may be a pollution control issue.

PAH.

PAH appear in the Framework Directive in the form of the original six specified PAH from the previous Drinking Water Directive. There is considerable discussion of PAH, including as possible endocrine disrupters. However, there seems little pressure to extend the range of PAH in the revised directive since the issue is not of raw water contamination and is being gradually resolved.

As with the priority list for microorganisms, the position with regard to chemicals could change quite quickly with developments in knowledge and events that result in political pressure. There are a number of substances that are not being considered for drinking water at present, such as chlorinated dibenzodioxins, that are high in the public and political consciousness. There are also a number of other substances that are or have been considered by WHO and the North Americans. These have appeared on various lists but the likelihood of their being considered for drinking water regulation in Europe is very low at present.

6. CONCLUSIONS

The Commission has indicated that the review of the standards in the 1998 revision of the Drinking Water Directive will take place in 2003. There are a number of sources of activity that could influence the Commission's thinking and which are worthy of consideration by the Department. These include WHO, USEPA, Canada and a number of European Member States, which have concerns over specific parameters, on public health grounds or for political reasons. Both microbial and chemical parameters are under consideration but the number of parameters that are likely to receive serious consideration is relatively small.

WHO are proposing a change in approach to ensuring microbial, and to an extent, chemical, safety of drinking water by a more proactive approach based on an adaptation of hazard assessment critical control points (HACCP) that is widely used in the food industry. This approach is being discussed more widely by the water industry and regulators from several parts of the world. Should this approach be more widely adopted, this could see a move to concentrating more on ensuring that pollution control and treatment processes are operating in an optimum fashion rather than relying heavily on post-treatment check monitoring.

There are a number of contaminants that are of potential interest and concern to Europe and the UK. Some of these relate to health concerns, for example arsenic, uranium, cyanobacterial toxins and haloacetic acids. However, others are primarily of political significance, for example *Legionella*, aluminium, endocrine disrupters and pharmaceuticals.

In the case of endocrine disrupters and pharmaceuticals, the database on occurrence and potential health effects at low doses is not well developed. There is a danger that a political precautionary standard could be set which is practically difficult to meet, extremely difficult to measure and which focuses attention away from the most important issue, prevention.

Although there is a substantial list of microbial parameters, only *Legionella* is considered to be of high priority because the Netherlands has already set a standard for this organism and there is political pressure in some other countries.

Three organisms, or groups of organisms, are considered to be medium priority. These are *Aeromonas*, which grows in distribution and plumbing systems, bacteriophage, which has been proposed as a surrogate for viruses, and *Clostridium perfringens*. The scientific basis for setting standards for the first two is limited, particularly for bacteriophage, and if regulation was proposed it is more likely that they would be included among the indicator parameters. It is possible that a proposal will be made to make the indicator parameter value for clostridium perfringens mandatory.

All other organisms are considered to be low priority for a range of scientific and practical reasons.

A significant number of chemical contaminants are under consideration by WHO and other organisations.

Seven substances or groups of substances are considered to be high priority for scientific or political reasons. These are tri- and tetra- chloroethene, haloacetic acids, chlorite, uranium, radon, aluminium and endocrine disrupters, although the last would probably require specification of specific substances.

A further eight substances or groups of substances are considered to be of medium priority. These are arsenic, nitrate/nitrite, copper, algal toxins, turbidity, pharmaceuticals, petroleum hydrocarbons and chlorate

All other substances are considered to be low priority.

The priority rating of an organism or chemical contaminant could change very quickly in the light of significant new scientific data or political pressure, for example following an incident.

7. RECOMMENDATIONS

7.1 Microbiology

It is recommended that the Department investigate whether toxigenic strains of *Aeromonas* can be found frequently in drinking water.

It is recommended that the Department consider investigating the development of a source of reference methods for organisms that are not included in the Microbiology of Drinking Water 2001 and for special organisms such as anthrax, that may be of concern with regard to deliberate introduction into drinking water.

Although *Giardia* is considered to be of low priority at this time it is recommended that the Department should consider investigating the actual occurrence of *Giardia* in drinking water in view of the potential for political pressure. This could be achieved by analysing some of the samples collected for regulatory monitoring for *Cryptosporidium*.

There is increasing interest in Microsporidia and although it is presently considered to be of low priority, it is recommended that the Department consider investigating the susceptibility of this organism to disinfectants such as chlorine.

7.2 Chemical contaminants

There is a potential for a number of chlorinated solvents that can be found in groundwater to be grouped together with a precautionary standard. It is recommended that the Department investigate whether data exist on the occurrence and concentrations of 1,1,1-trichloroethane and dichloromethane in drinking water derived from groundwater sources in the UK.

The haloacetic acids are considered to be of high priority but data on occurrence in UK drinking water is limited. It is recommended that the Department seek more comprehensive data on the concentrations of haloacetic acids, including the brominated compounds, in UK drinking waters following the significant efforts to control THMs.

Uranium is considered to be of high priority but there is uncertainty as to the extent at which it occurs in UK drinking waters at significant concentrations. It is recommended that the Department seek current data on the frequency at which uranium occurs in UK public water supplies at concentrations above 2 µg/l, the current WHO provisional guideline value.

Strontium is expected to be found widely in drinking water but there appears to be no collated data on its occurrence and concentrations in UK drinking waters. It is recommended

that the Department determine what data on the occurrence and concentrations of strontium in UK public water supplies is available.

Pharmaceuticals are considered to be of medium priority because of political and media interest. The EA are considering research in this field and it is recommended that the Department investigate the possibility of extending this research to determine current occurrence of pharmaceuticals in drinking water. In particular it would be helpful to determine whether representatives of those pharmaceuticals most commonly mentioned in the literature are present in drinking water abstracted from sources with a high degree of re-use. These would be anti-hyperlipidaemic drugs, non-steroidal anti-inflammatory drugs and commonly used antibiotics. There have also been reports of β-blockers being found at trace concentrations but these are active at much lower doses.

APPENDIX 1

PRELIMINARY LISTS OF CANDIDATE PARAMETERS

Future Regulatory Parameters. Implications for the UK

Preliminary List of Candidate Parameters

List of Potential Microbiological Parameters

The microbiological parameters are selected from a combination of those listed in the USEPA Candidate Contaminant List and from knowledge of organisms of interest in European countries. WHO are not planning to propose guideline values or monitoring for specific pathogens at this stage but to emphasise a HACCP approach which is more directed at prevention of contamination. How this could or should be introduced into legislation remains uncertain. The specific pathogens of interest are as follows:

Aeromonas hydrophilia	Included in the USEPA candidate list, a probable cause of waterborne GI infection.
Astroviruses	A cause of mild GI infections, principally in infants, which has been associated with a few waterborne outbreaks. Advantages include: it is a genuine enteric pathogen, it is culturable and easily serotyped by PCR.
bacteriophage	Analytically cheap and may be suggested as surrogate for more expensive virus determination, though not human viruses and may not satisfactorily reflect virus survival in the environment.
Caliciviruses	(Norwalk like viruses, NLV) There is much circumstantial evidence that outbreaks, particularly in the USA at private supplies but also other countries (UK, Finland, Ireland), may have been caused by NLVs and it is on the USEPA candidate list. Detection from many matrices is possible by PCR and should be available as routine in ~two years.
Campylobacter	This is a growing and significant cause of GI illness in the UK and elsewhere, which could be important in the absence of chlorination and it is certainly present in private water supplies.
Coxsackie B viruses	Commonly excreted by asymptomatic carriers but *not* a cause of gastroenteritis in the absence of other disease. However, will be present in polluted water supplies. Coxsackie B may be a useful viral indicator if one were felt to be needed. Relatively easy to detect in water samples by cell culture or PCR. In effect, a compromise between an indicator and a ubiquitous potential human/animal pathogen.
Cryptosporidium	Given the current UKWIR work on 'when is it safe to discontinue monitoring' this is likely to be a contentious issue for some time, but probably, no regulatory interest to EU/WHO.
Cyanobacteria	On the USEPA candidate list and has caused problems in 'environmental' waters in the UK. The toxins produced by *cyanobacteria* are considered in the section on chemical parameters.
Echoviruses	Properties similar to Coxsackie B viruses, though more difficult to detect in water samples by cell culture, but is included in the USEPA candidate list.
E. coli O157	Found in UK and USA private water supplies and a known cause of infection. A major public supply outbreak has occurred in Canada.

Giardia	Raised at the inception meeting by DWI, i.e. because *Cryptosporidium* may not be a good surrogate for *Giardia* and if there is a 'hidden problem' in the UK could rise in political importance.
GMOs	This is of political interest (and in the DWI specification), it should therefore be in the review but it would be very difficult to implement as a regulatory parameter at this time. The significance for drinking water remains uncertain in view of the barriers in place against microorganisms but engineered resistance to disinfectants would be of concern.
Helicobacter pylori	Emerging pathogen, included in the USEPA candidate list, with link to serious illness and some evidence (from LDCs) of a public water supply link. Chlorine susceptibility may not be as high as once thought.
Legionella	A ubiquitous pathogen. Netherlands has regulated at 50 cfu/l in drinking water. Interest n France. EU may pick up. Better to control by design, operation and maintenance of water using devices than by drinking water standard
Mycobacterium avium	In particular *mycobacterium paratuberculosis* which has been linked (without evidence) to Crone's disease. Included in the USEPA Candidate list, PHLS work will clarify the UK drinking water risk.
Prion proteins	This is of political interest with questions asked in the UK, France and by the Commission (specified in the DWI specification), it should therefore be considered but it would be very difficult to implement as a regulatory parameter at this time.
Microsporidia	Appears in USEPA contaminant candidate list.
Cyclospora	Appears in USEPA contaminant candidate list.

Lists of Chemical Parameters

The chemical parameters have been derived from knowledge of the revision of the WHO Guidelines, the USEPA Candidate Contaminant List, Health Canada and knowledge of specific interest in Europe.

Please see Table 1 below.

TABLE 1

Chemical	WHO	EPA	Canada	Europe	Notes
Aluminium		x	x	x	EPA and Canada are examining a health based value. France political pressure.
Antimony	x				Existing standard. If an increase in value from WHO may be political pressure to change.
Arsenic	x	x	x	x	NRC in the USA have now said that the risks of cancer at 10 ug/l are about 1 to 3 in 1000.
Barium	x				WHO GV unlikely to change unless it goes up.
Boron	x	x			To be considered in the future by WHO when new data from USA. EPA considering now.
Cadmium	x				Unlikely to change significantly in WHO evaluation.
Copper	x	x	x		New evaluation by WHO as well as others. Acute affects thought to be key.
Fluoride	x			x	Much concern at high levels but unlikely to change as far as Europe is concerned.
Manganese	x	x			We have not seen the new evaluation yet but much will depend on EPA's new data.
Mercury	x				To be considered in future by WHO and could go up a little since based on total mercury.
Nickel	x			x	Under consideration by EU and new data on the reproductive toxicity is being made available.
Nitrate/nitrite	x		x		WHO have asked JECFA to re-examine in light of concerns over their new TDI's.
Sodium		x			Unlikely to change.
Sulphate	x	x			Unlikely to change.
Inorganic Tin	x				Not really an issue.
Uranium	x	x	x	x	Done by EPA and Canada already, WHO provisional value. EU countries interested.
Strontium	x				To be considered in future if sufficient data.
Vanadium	x	x			So far not really considered since insufficient data. EPA producing a new evaluation.
BADGE				x	Interest because of endocrine disruption and use in epoxy resins.
Carbon tetrachloride	x	x	x		Uncertain but unlikely to be reduced GV.
Dichloromethane		x			Widespread chemical on target of some pressure groups. EPA evaluation could be trigger.
Microcystins See above Algal Toxins below			x	x	There is a WHO GV but WHO playing down monitoring and emphasising prevention.
Cyanogen chloride	x	x	x		Disinfection by-product of interest in N. America. Depends how well

					reflected by THMs.
Chemical	**WHO**	**EPA**	**Canada**	**Europe**	**Notes**
Haloacetonitriles	x		x		Unlikely to be included in standards, although already an EPA standard.
MCPA			x		Not likely in view of precautionary standard.
Trichloroethene	x	X	x		Of considerable interest and new evaluations will be important. Danes very anti-TCE.
Tetrachloroethene	x				Same as TCE.
THMs	x	X	x		WHO unlikely to reduce. May make less stringent. See also DBPs in general.
Turbidity		X	x		EPA have a tight standard and Canada are proposing a tight standard.
Chloral Hydrate	x	X	x		Disinfection by-product of interest in N. America. Depends how well reflected by THMs.
Dichlorprop			x		Herbicide unlikely to be of interest.
MTBE	x		x	x	Main issue is odour but some pressure from Denmark. Could be a political parameter.
Acrylamide	x		x		If there are sufficient new data could change.
Chlorine			x		Unlikely to be of interest in Europe.
Nonylphenols			x	x	Of interest because of potential endocrine disrupting activity. Derivatives used in materials.
1,1,1 trichloroethane	x				Widespread use but only considered if significant new data. Interest for groundwater only.
Vinyl chloride	x				Only likely to change if significant new data.
1,1 dichloroethene	x				As for vinyl chloride, common name vinylidene chloride. Not usually found at high levels.
Toluene	x				May change if new data but primary issue is odour.
Xylenes	x				Same as toluene.
PAH	x				Candidate for future revision. Probably soluble PAH.
DEHP & DEHA	x				Future revision only if endocrine disrupting activity deemed to be of significance.
Epichlorohydrin	x				Only likely to change if significant new data.
Hexachlorobutadiene	x	x			Uncertain but there appear to be significant new data. Question then is occurrence in Europe.
Dialkyltins	x				Possible issue for materials where used as stabilizers.
TBTO	x				New data but seem unlikely to result in change of GV.
Petrol Oils	x				To be considered but not for a GV. Guidance as t how to assess risks in incidents.
1,4 dioxane	x				Found in drinking water in several countries. Japan preparing document.

Chemical	WHO	EPA	Canada	Europe	Notes
Munitions	x				Interest raised in relation to Eastern Europe but seems unlikely to require standards as such.
DBPs	x	x	x		See also individual DBPs above. Chlorination DBPs depend on outcome of birth studies.
Bromate	x	x	x	x	Considered by IPCS and EPA's IRIS. Value unlikely to tighten and may relax.
Chlorite and chlorate	x	x	x	x	Wide interest as chlorine replacement. Values likely to be significantly less stringent as EPA.
Iodine	x				Concern over use by travellers and in emergencies.
1,1,2,2 tetrachloroethane		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
1,2,4 trimethylbenzene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
1,1 dichloroethane		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
1,1 dichloropropene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
1,3 dichloropropane		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides

					and wood preservatives.
Chemical	**WHO**	**EPA**	**Canada**	**Europe**	**Notes**
1,3 dichloropropene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
2,4,6 trichlorophenol		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
2,2 dichloropropane		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
2,4 dichlorophenol		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
2,4 dinitrotoluene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
2,6 dinitrotoluene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
o-cresol		x			This chemical is on the USEPA Contaminant Candidate list and was

					chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
Chemical	**WHO**	**EPA**	**Canada**	**Europe**	**Notes**
bromobenzene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
p-isopropyltoluene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
methyl bromide		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
Naphthalene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
nitobenzene		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.

Chemical	WHO	EPA	Canada	Europe	Notes
organotins (See dialkyltins and TBTO above)		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
perchlorate		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives. Concern from contamination at sites where used as rocket propellant.
triazines & degrad. Products		x			This chemical is on the USEPA Contaminant Candidate list and was chosen by a set of criteria that looked at occurrence before looking at health effects. The list includes compounds widely used in industry such as solvents and other volatile organic compounds along with some pesticides, herbicides and wood preservatives.
Pharmaceuticals	x			x	WHO unlikely to specify any individual pharmaceuticals. Primarily a research topic at this time but of interest in Scandinavia, Germany and Holland.
Algal Toxins (See also Microcystins)	x	x	x		WHO and Canada have guidelines for microcystin LR and several research groups in Canada, the USA, Australia and Germany are interested in guidelines for other substances.
Pesticides	x	x	x		Several pesticides are included in the USEPA Candidate List and in the Canadian list. These include a number of breakdown products. WHO are now using JMPR acceptable daily intakes. Seems unlikely in view of EU precautionary limit.

APPENDIX 2

PRELIMINARY PRIORITY LIST

PRELIMINARY PRIORITY LIST.

1. Microorganisms.

Around the world there seems little enthusiasm for introducing standards for specific pathogens, unless there is a political need. However this could lead to interest in a number of organisms. In general, the approach being considered by WHO, of the existing indicators backed by risk assessment of points of contamination and treatment indicators, is the one that is gaining popularity. It is, however, difficult to incorporate into a regulatory structure like the one in Europe and additional standards or indicator parameter values may find favour.

The following are listed in priority order.

Legionella spp. Regulations have already been adopted by the Netherlands and they may wish to press for wider adoption to justify their internal action. Preliminary studies of how *Legionella* gains entry to drinking water have been conducted and it has been found in treated groundwater and surface water in the UK. It would be necessary to determine the importance of drinking water as a source of inoculum for *Legionella* in the systems in buildings that could give rise to infection. It is likely, however, that the most appropriate means of control is through proper design, operation and maintenance of water systems in buildings and water devices rather than through a drinking water standard.

Viruses. Outbreaks of gastrointestinal disease that result from waterborne viruses are well documented. The most likely to be considered are the Caliciviruses (Norwalk like viruses). Analysis would be a challenge, although methods have improved, particularly with the introduction of integrated cell culture RT-PCR. Researchers in South Africa have recently called for a reappraisal of drinking water virus standards.

E. coli O157. There have been cases of waterborne E. coli O157 causing outbreaks in Europe as a consequence of contamination of private supplies. In Canada deaths occurred as a consequence of contamination of a public supply. However, it should be covered by the use of *E. coli* as an indicator.

Giardia sp. There have been two waterborne outbreaks of giardiasis in the UK, one in a public supply and one in a private supply. Epidemiological evidence has never shown other waterborne outbreaks. Giardiasis is known to be a problem in the USA where many small community supplies receive no treatment. Conventional water treatment should remove the parasite. The current Regulatory monitoring for *Cryptosporidium* provides samples for analysis of *Giardia*, which would provide a means of determining the incidence in drinking water in the UK.

Aeromonas spp. Aeromonads will grow in drinking water distribution systems and have been suggested as an indicator for assessing regrowth. What is not clear is whether these 'environmental' strains are pathogenic. There are a number of relatively simple tests available for toxin production and it is necessary to determine whether drinking water isolates are toxigenic before the need for monitoring can be reasonably considered.

Campylobacter sp. Outbreaks of gastroenteritis have been caused by waterborne *Campylobacter* and it has also been associated with the more severe Guillan-Barré syndrome. However, it is more susceptible to disinfection than *E. coli* and so should be covered by the present system of indicators.

BSE. This topic was raised previously and the Commission was decidedly unenthusiastic. The issue is seen as more one for the UK at present but concern may arise in France and Germany. The big problem is the lack of a method for analysis.

Mycobacterium avium complex. These organisms can be found in drinking water but the issue of waterborne disease remains very uncertain. *Mycobacterium paratuberculosis* has been linked, without evidence, to Crohn's disease. USEPA are not covering them in the first list of parameters to go out for comment. The methods of analysis are difficult. The most likely concern relates to people with AIDS. Studies DWI has commissioned will advance knowledge of these organisms and their occurrence in drinking water.

Acanthamoeba. These parasites have come to the fore following highly publicised cases of eye infections in contact lens wearers. The USEPA are going to public comment on this parasite but are most likely to propose a health advisory. The issue for Europe is contamination of water used to wash contact lenses and so the best means of dealing with this would be through the optical profession.

GMOs. At present there is no indication that GMOs would be likely to reach drinking water, or that there is significant pressure to consider them specifically as drinking water contaminants. Any consideration would be purely politically driven at this time. The primary danger would come from anti-GM activity that saw the opportunity to use drinking water as a way of further undermining GM technology.

Cyclospora cayetanensis. Although water may be a vehicle for transmission of *Cyclospora*, there is only one documented outbreak in 12 of 14 soldiers who drank contaminated water. Little is known of the incidence of the parasite in wastewater and surface waters. Oocysts are large, measuring 8 – 10 μm in diameter and can be recognised because of autofluorescence under appropriate ultra-violet light. Given the size of the oocyst, it should be easily removed by conventional water treatment. In addition, since the number of recognised cases in the United Kingdom is low, environmental concentrations are also likely to be low.

Microsporidium. Microsporidia are widespread in the environment and there are currently over 100 genera and more than 1,000 species. The majority of these species infect insects and lower vertebrates, for example, fish. Little is known about the incidence of microsporidia that cause human infections in water samples. Only one waterborne outbreak has been documented. The principal problem with microsporidians is methods for their detection in raw and treated water samples. They are much smaller than *Cryptosporidium* and accurate enumeration of human infecting species relies on species-specific monoclonal antibodies (which the literature considers unreliable) or the use of molecular biology. Better methods for detection are required before serious environmental detection can begin.

Cryptosporidium. The Commission currently seem to believe that *Clostridium perfringens* spores are a suitable surrogate for *Cryptosporidium* in drinking water. The evidence does not indicate that this actually a good surrogate and there may be a change in view. The experience of the UK would be very important in the event that the Commission wishes to consider *Cryptosporidium.*

There are a number of other possible organisms but the current concerns seem to leave these further away than the organisms listed above, however it should be noted that some of these may become more important and that the order of priority in this list could change as knowledge increases and, particularly, with changes in political pressure.

2. Chemical and Radiological Parameters

The debate as to the utility of increasing lists of chemical parameters is continuing on both sides of the Atlantic. WHO are also addressing this question by introducing a more holistic approach to drinking water quality that will enable countries to be more selective with regard to chemical parameters while taking into account treatment indicators and source control. The USEPA have selected eight chemical parameters from the candidate contaminant list for initial consideration as to regulatory action that are expected to be published in the Federal Register for comment in the next few weeks. The proposed action will not be for inclusion as standards for all of these but proposals will include the option of producing non-statutory health advisories. Of these eight substances, three are pesticides and two, sodium and sulphate, are primarily associated with taste considerations.

The preliminary list of priority substances, in order of the probability of action, is as follows.

Arsenic. The USEPA have run into considerable problems with arsenic and the WHO Environmental Health Criteria still remains unpublished. There is considerable controversy over the cancer epidemiology and the applicability of risk models to estimate cancer risk from Taiwan data. There is pressure for a lower value than 10 μg/l, although the difficulties and costs of achieving even this are substantial in many parts of the world.

Trichloroethene/Tetrachloroethene. WHO are reconsidering these substances for which IARC have increased the classification to 2A based on re-evaluation of the epidemiology. This is rather controversial but Denmark may well seize on this to press the Commission for more precautionary standards since their previous position was 1μg/l for both substances. 1,1,1-trichloroethane and dichloromethane are also under consideration by WHO. In the event of new more stringent guideline values there could be an attempt to group all the chlorinated solvents together under a precautionary value.

Organic Disinfection By-Products. WHO are reconsidering the DBPs in the revision of the Guidelines and the USEPA have accepted new standards for a range of DBPs under the negotiated rule making. While it seems unlikely that there will be significant pressure to tighten the total THMs standard, there could well be calls to introduce a standard for the haloacetic acids, particularly dichloro and trichloroacetic acid, which can be present in the same concentration range as THMs.

Inorganic Disinfection By-Products. The increasing interest in chlorine dioxide is likely to see calls for a regulation of the by-products chlorite and chlorate. The WHO guideline values are not likely to give rise to problems and, as in the UK, control could be by product dosing. Bromate is regulated but proposals from WHO and USEPA should reduce any pressure to tighten the existing European standard.

Endocrine Disrupting Substances. There is considerable political concern with regard to endocrine disrupters and the Commission has let a contract with the Fraunhofer Institute to carry out

a study on drinking water. This is setting out with a long list of substances that have been implicated as possessing endocrine disrupting properties in any way. The main substances found in drinking water are phthalates, although the DWI 70/2/145 scientific evidence does not support their role as true endocrine disrupters. This has not stopped them being included in the Water Framework Directive. This area remains very uncertain but it is important that any standards are based on actual occurrence and sound science. WHO have proposed guideline values for diethylhexylphthalate and diethylhexyladipate.

Radon. Radon is widespread in Europe and although it was thought to be lost from drinking water so rapidly that it was entirely an air quality problem, this now does not appear to be quite true. The key issue seems to be how much of a problem is it in public versus private supplies. An expert group, under the EURATOM treaty has already produced a draft recommendation on radon and its daughters that could be taken up for use in the Directive.

Uranium. Uranium is found in many waters, just as radon. The Finns were considering regulation but were persuaded that this was not appropriate at the time. Both the USA and Canada have proposed standards and it will be considered by WHO. The science supporting a value has gaps but the proposals from North America would be achievable in the UK.

Nitrate/nitrite. WHO JECFA proposed a very tight TDI for nitrate which was not used for the revision of the drinking water guideline value. It has been referred back to JECFA who are now asking for new data. This may be available in time to influence the Commission for the proposed round of revision of the directive. If not then nitrate is unlikely to be a priority at this time.

Copper WHO are planning to re-examine the guideline value for copper and the document is being prepared by USEPA and Germany. The German government has been under some pressure to tighten the standard for copper in drinking water because of suspicions regarding infantile liver toxicity as a consequence of 'high' copper levels in water. There still is no widespread scientific agreement on this issue, although there is a great deal of scepticism. Much will depend on the German view and what support it receives.

Algal Toxins. There is concern in a number of countries regarding toxins from blue-green algae, particularly the microcystins. France, Spain and Germany are all believed to be considering drinking water standards. WHO take the position that the best way forward is to control growth in water sources and this would not be best dealt with through drinking water standards.

MTBE. MTBE appears to be much less of a problem in Europe than in the USA. It is accepted in Europe that the issue is taste and odour rather than health and the pressure for a standard will depend more on political pressures than scientific pressures unless a serious incident occurs in the near future.

Aluminium. The French have been under some pressure on aluminium from the use of aluminium salts in drinking water treatment. Canada has been trying to set a health based standard but the data do not enable that to be done in any sensible way. In view of the fact that 100µg/l should be achievable on average it may be proposed that the current value in the directive be reduced to this while the parameter is retained in the indicator group.

Manganese. WHO and USEPA are considering manganese and it will be one of the parameters from the candidate contaminant list that goes forward. The indications at this time are that the

proposal will be for a health advisory and that any health-based values will be well above the consumer acceptability value currently in use.

1.4-Dioxane. This is an industrial solvent that is quite soluble and has been identified at low concentrations in drinking water on occasion. It is suspected of carcinogenic activity in laboratory animals but other data are limited. It is listed by WHO for consideration at the request of the Japanese. In the event of it being raised through a revised WHO guideline value this may be a pollution control issue.

As with the preliminary priority list for microorganisms, the position with regard to chemicals could change quite quickly with changes in knowledge and events that result in political pressure. There are a number of substances that are not being considered for drinking water at present, such as chlorinated dibenzodioxins, that are high in the public and political consciousness. These lists are considered preliminary but will form the basis for the final list.

APPENDIX 3

SCIENTIFIC BASIS FOR MICROBIAL PARAMETERS

A REVIEW OF MICROORGANISMS AND WATERBORNE DISEASE

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CREH

REGULATORY PARAMETERS

MICROBIOLOGICAL PARAMETERS

INTRODUCTION

There is a wide range of microbiological parameters which could be considered for Regulatory monitoring. Some of these are known to be pathogenic micro-organisms for which there are methods of analysis. Many of these, for example, *Campylobacter*, are known to be removed during water treatment and are killed by disinfection. There are others which are less sensitive to disinfection and, with a low infectious dose, may be a serious problem. The viruses fall into this category. Many of the viruses are, however, not culturable and their detection relies on the use of molecular biological techniques. A further group of micro-organisms is known to grow in water distribution systems where the conditions permit. The significance of bacteria such as *Aeromonas* and fungi which can produce mycotoxins is unclear.

Micro-organisms as indicators of the presence of pathogens have been used for over 100 years to ensure that treated water is free from faecal contamination. Such indicators, whilst useful to demonstrate the efficacy of water treatment, will not indicate the range of pathogens nor their numbers in treated water. Waterborne outbreaks due to *Cryptosporidium* have occurred but there is no faecal indicator which correlates with its presence in water, hence there is a need for direct monitoring of treated water for this organism.

The requirement to monitor micro-organisms may be listed under three categories:

a) Those micro-organisms which are used as indicators of faecal contamination. These form the current Regulatory parameters. A review of the current literature and the views of regulatory bodies in other countries will determine whether these are satisfactory or whether there are other parameters which need to be added.

b) Those micro-organisms which might be included in analysis in circumstances where there is evidence from the Regulatory monitoring that they might be present. For example, the detection of *Cryptosporidium* in treated water might require that additional analyses for *Giardia* be included in subsequent samples. The detection of bacterial faecal indicators might require that subsequent samples be analysed for *Campylobacter* or *Escherichia coli* O157. Current methods of analysis should be available either as additions to the current 'blue book' methods or as separate documents available on the Inspectorate web site. These would be available for water companies to access in the event that additional microbiological analyses were required.

c) Those organisms which are known to affect public health under certain circumstances but for which current Regulatory analysis is not required. This would list micro-organisms for which advice could be given to local authorities about their public health significance and their control. For example *Acanthamoeba* is known to cause severe eye infections in wearers of contact lenses. Advice should be available to general practitioners, opticians and the wearers themselves on the nature of the micro-organism and how to prevent infection.

CREH *Analytical* has reviewed the current information concerning bacteria, viruses, protozoa and fungi and the following overview of the organisms is presented below. Reference is given to methods

of analysis where these are available and guidance as to which category of monitoring that they might fall in.

THE BACTERIA

Aeromonas spp.

Aeromonas spp. includes *A. hydrophila, A. caviae, A. sorbria* and *A. salmonicida*. The latter organism is a recognised fish pathogen and will not be considered further in the context of Regulatory monitoring. Aeromonads cause a self-limiting gastroenteritis, wound infections in soft tissue following injuries in aquatic environments or the contamination of wounds with surface water, septicaemia and death. Septicaemia and death are more likely to occur in the elderly and individuals with an immunocompromised condition. Gastroenteritis may take the form of acute profuse watery diarrhoea or a less severe diarrhoea with stomach cramps. Chronic diarrhoea has been recognised particularly in children under 5 years old where prolonged antibiotic treatment has changed gut flora and allowed *Aeromonas* to become established. The incidence of faecal excretion ranges from 0.5% in developed countries to 30 – 50% in countries with poor quality water and sanitation. Principal studies have been conducted on *A. hydrophila*. The bacterium produces a cytotoxic enterotoxin, which can induce apoptosis in human cell culture (Falcon *et al*., 2001).

Aeromonads may be isolated from fresh, estuarine and marine waters as well as sewage and sewage sludge. They have also been found in mineral waters and foods such as meats and seafoods. Levels in sewage and surface waters may be higher than those of *E. coli*. They have been isolated from drinking water distribution systems throughout the world. They can grow in surface waters, sand filters and as part of the biofilm in distribution systems. Growth usually occurs in the summer months when the water is warmer. Fernandez *et al.*, (2000) isolated *A. hydrophila* from chlorinated drinking water in the absence of other faecal indicators. The isolate was haemolytic to rabbit erythrocytes and cytotoxic in Vero cell cultures. In a 16 month study, Chauret *et al*., (2001) found counts of aeromonads in source waters of between 10^3 and 10^4 colony forming units (cfu) per 100 ml of water in the summer. The organisms were not found in disinfected water but 7.7% of biofilms were found to contain them.

Legnani *et al*., (1998) examined 7,395 water samples from the Dolomite mountains in Italy and found approximately 22% to be positive with levels ranging from 1 – 240 colony forming units (cfu) per 100 ml. Of the strains isolated, 72.4% were identified as *A. hydrophila*, 14.7% were *A. caviae* and 12.9% *A. sobria*. Incidence correlated with rainfall being highest in the winter and no aftergrowth was found in the distribution system. Alavandi *et al*., (2001) examined a number of isolates from drinking water and children with diarrhoea using randomly amplified polymorphic DNA polymerase chain reaction and whole cell proteins using SDS polyacrylamide gel electrophoresis. The techniques could not differentiate phenotypes and grouped the organisms into clinical and water types. These observations give emphasis to the theory that environmental isolates which can grow in water are different to clinical isolates. Havelaar *et al*., (1992) typed *Aeromonas* strains from drinking water and patients with diarrhoea using biochemistry, serology and cell wall fatty acid methyl ester (FAME) analysis. Given the limited number of isolates, there was little overall similarity between isolates from patients with diarrhoea and drinking water isolates.

In a review article, Gosling, (1997) reviewed the disease range caused by aeromonads and the risk of infection from aeromonad contamination of drinking water. Evidence is presented of different genospecies in human and water environments and that the risk of infection from such strains, although not absent, is small. In addition, examination of strains for virulence factors and determining the reservoir for human pathogens is recommended.

Although there are a number of documented instances of aeromonads in drinking water, there have been no reported outbreaks of waterborne gastroenteritis. High infective dose in conjunction with the possibility that the 'environmental' organisms that colonise water distribution systems may be non-toxigenic may be the explanation. In addition, routine stool examination may not include testing for aeromonads and general levels in distribution systems may be insufficient to cause infection in healthy individuals. A low incidence of gastroenteritis in a population is unlikely to be detected epidemiologically.

The organisms can be isolated on membrane lauryl sulphate broth as part of the routine examination of drinking water in the United Kingdom. Specific media are also available. These include ampicillin dextrin agar (Havelaar *et al*., 1984) and xylose deoxycholate agar (Shread *et al*., 1981). Isolation of the organisms from ground water and disinfected water may be helped by enrichment in alkaline peptone water at pH 8.6 prior to plating on selective media.

In the cumulative reports for gastrointestinal infections for England and Wales, there were a total of 204 reports in 2001 (CDR).

It is currently unclear whether aeromonads which grow in water distribution systems are enterotoxigenic and capable of causing gastroenteritis. Epidemiology of the infection would suggest that waterborne outbreaks do not occur. Aeromonads do grow in water in the absence of other faecal indicators. *Aeromonas* is on the list of organisms of interest in the United States. It would seem advisable to determine whether aeromonads isolated from distribution systems possess enterotoxins either by haemolysis or cytotoxicity. The growth of large numbers of aeromonads in distribution systems is clearly undesirable and where routine monitoring detects them, isolates should be checked for enterotoxin. In addition, measures should be taken to try to eradicate the organisms by increasing chlorine levels. Supply of contaminated water to the food industry or to hospitals may be undesirable.

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Campylobacter spp.

The genus *Campylobacter* contains 16 species and 6 sub species. *Campylobacter jejuni* sub sp. *jejuni*, *C. jejuni* sub sp. *doylei*, *C. coli* and *C. lari* are the most commonly isolated species from human and animal infections (On, 2001). The incubation period is usually 2 – 3 days followed by a 'flu-like' illness, acute abdominal pain and acute diarrhoea. Blood and mucous may also be excreted. The disease is usually self-limiting although the acute nature of the diarrhoea may lead to hospitalisation with dehydration. The infective dose is thought to be low. In developing countries, there is a high rate of asymptomatic carriage with milder symptoms usually seen in young children. This is thought to be due to repeated exposure to the organism and the development of immunity. The bacterium possesses a number of virulence factors including the production of a cholera-like toxin. *Campylobacter* infections comprise the largest number of clinically diagnosed bacterial cases of gastroenteritis in the United Kingdom. The cumulative total for 2,001 is 56,420 reports (CDR). There is a seasonal incidence with an increase in cases during the summer months.

Campylobacter is widely distributed in domestic and wild animals including birds. They have been found in all species of birds examined and are prevalent in poultry. Approximately 80% of all raw chickens sold in the United Kingdom are contaminated with *Campylobacter* (Correy and Atabay, 2001). They are unable to multiply on the surface of chicken meat but the low infective dose means that improperly cooked chicken or poor hygiene in the kitchen will readily lead to infection. Other meats such as liver, beef and lamb may also be contaminated (Frost, 2001). Raw milk has been implicated in a number of outbreaks as has visits to farms. *Campylobacter* can be found in surface waters, sewage and sewage sludge as well as animal faecal material. It is unable to grow in water but numbers between $50 – 10^3$ per litre in surface waters have been quoted (Jones, 2001). *Campylobacter* can survive well in surface waters particularly where there is organic nutrient and the temperature and dissolved oxygen concentrations are low. They have the ability to form coccoid viable-but non-culturable (VNC) forms but it is not clear whether these forms are still infectious.

There have been a number of waterborne outbreaks of *Campylobacter* infection often affecting large numbers of individuals. Between 1998 and 1999, 14 waterborne outbreaks occurred in Finland causing 7,300 cases of illness. The majority of these were associated with undisinfected ground waters contaminated by surface runoff (Miettinen *et al*., 2001). Between 1992 and 1995, 19 outbreaks of disease were reported to the Communicable Disease Surveillance Centre (CDSC) in which ten were associated with public supplies and nine with private supplies (Furtado *et al*., 1998). Although none of the public supplies was contaminated with *Campylobacter*, the majority of the private supplies were including one instance of combined *Campylobacter* and *Cryptosporidium* (Duke *et al*., 1996). A combined waterborne outbreak of *Campylobacter* and *E. coli* O157 occurred in Scotland (Jones and Roworth, 1996) affecting over 700 people. The outbreak was caused by sewage-contaminated stream water getting into the public supply.

There are a number of published methods for the recovery of *Campylobacter* from water. Membrane filtration should use 0.22 μm 47 mm membranes followed by incubation in an enrichment broth with growth supplements and plating onto selective agar (Anon, 1994; Anon, 2000).

The literature would suggest that water treatment and disinfection is effective in removing *Campylobacter* from drinking water. *Campylobacter* is more susceptible to chlorine as a disinfectant than is *E. coli.* Outbreaks are likely to occur predominantly from the contamination of ground water where there is no disinfection and private water supplies where there is no treatment. The literature also suggests that faecal coliforms are a good indicator of the presence of *Campylobacter* in drinking water

(Skjerve and Brennhovd, 1992, Fricker, 1999). Regulatory monitoring of drinking water is unnecessary but where *E. coli* is isolated, examination of the water for *Campylobacter* should be considered.

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Escherichia coli O157:H7

There are a large number of *E. coli* types most of which are commensal in the intestines of humans and warm-blooded animals. The disease causing *E. coli* include the enteropathogenic (EPEC) strains, the enteroinvasive (EIEC) strains, the enterotoxigenic (ETEC) strains, the enteroaggressive (EaggEC) and the enterohaemolytic (EHEC) strains.

The EPEC are predominantly associated with disease in infants, particularly in special care baby units, and here mortality is often high. Typical symptoms include watery diarrhoea, fever and dehydration. EIEC infections cause symptoms similar to infection with *Shigella* characterised by watery diarrhoea, stomach cramps and fever. ETEC is the cause of travellers diarrhoea with watery diarrhoea, stomach cramps and vomiting. EHEC causes diarrhoea which may or may not have blood in the stools (haemorrhagic colitis). Kidney failure through the development of haemolytic uraemic syndrome (HUS) is a risk factor of infection particularly in young children (approximately 5%). The principle EHEC strain is *E. coli* O157:H7, but other strains, for example *E. coli* O111, have been implicated in outbreaks. The infective dose is low (less than 5 organisms) and strains have excellent survival characteristics on surfaces and in acid conditions. The number of identified cases reported in 2001 was 693 (CDR).

Humans are the main reservoir for all pathogenic *E. coli.* The exception is cattle as a primary reservoir for EHEC strains. Contaminated meat products (particularly beef burgers and meat pies), vegetables, raw milk and apple cider have all been implicated in transmission of disease. Farm visits have also caused outbreaks. A number of outbreaks associated with drinking and recreational water have also been reported. EHEC can survive for long periods in bovine faeces (70 days at 5 ^{o}C) and in soil (130 days). It does not require ingress of fresh faecal material but runoff from agricultural land can put significant numbers into surface waters (Chalmers *et al*., 2000).

A waterborne outbreak occurred in Missouri in 1989 following contamination of an unchlorinated municipal supply (Swerdlow *et al*., 1992) with 243 cases and 4 deaths. An outbreak of *E. coli* O157:H7 occurred in Japan in 1990 infecting 174 children in a nursery. A wide range of symptoms were seen including involvement of the central nervous system. Two children died. The drinking water was supplied from a well in the school. The organism was isolated from both the well and the drinking water (Akashi *et al*., 1994). Iaacson *et al*., 1993) describe a large outbreak of haemorrhagic colitis in South Africa and Swaziland caused by *E. coli* O157:H7. Thousands of people were affected. The source was surface water contaminated with cattle carcases and faeces. The outbreak followed heavy rainfall after a prolonged drought. A mixed outbreak of *Campylobacter* and *E. coli* O157:H7 occurred in Grampian in 1990 affecting four people (Dev *et al*., 1991). The outbreak happened during a long, hot summer when the water supply became inadequate and was augmented from two additional reservoirs that had been out of use. One of these reservoirs was fed from a field drain and this was thought to be contaminated with cattle slurry. Water supplied to the house of one patient was found to be heavily contaminated with *E. coli.*

A large outbreak of combined *Campylobacter* and *E. coli* O157 was also reported in Fife (Jones and Roworth, 1996). A number of outbreaks have been associated with recreational waters both in the United Kingdom and overseas.

Enteropathogenic *E. coli* has also been reported to cause outbreaks of waterborne disease. An outbreak in Washington in 1971 was caused by contamination of unchlorinated groundwater. A

larger outbreak occurred in South West Oregon in 1975 affecting over 2,000 people. This was caused by an ETEC (serotype 06) and the water supply was found to be contaminated with sewage.

Enterohaemolytic *E. coli* can be isolated with relative ease from water samples using membrane filtration followed by enrichment in a tryptone soya broth and the use of immunomagnetisable beads to separate target organisms onto selective agar. Rapid identification can be made on the basis of typical colony morphology and latex agglutination. Isolates grow poorly, if at all, at 44 ºC and are unlikely to be cultured on membrane lauryl sulphate broth at this temperature. They are β-glucuronidase negative.

Outbreaks in drinking water have occurred where there is no treatment or disinfection or where the latter is ineffective and there has been sewage contamination. There is no evidence that *E. coli* O157 has any greater resistance to chlorine than other *E. coli* (Rice *et al*., 1999). The maintenance of proper water treatment and disinfection is therefore the best protection against waterborne outbreaks. As with *Campylobacter*, examination for this organism on a routine basis is unnecessary. It might, however, be prudent to include analysis for this organism where faecal contamination has been demonstrated. In a recent survey of private water supplies in the United Kingdom, both *Campylobacter* and *E. coli* O157 were isolated from drinking water (Watkins *et al*., 2001).

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Helicobacter pylori

Helicobacter pylori is present in the stomach of 80% of patients with gastric ulcers and in the duodenum of 90% of patients with duodenal ulcers (Moayyedi and Murphy, 2001). Treatment of patients with non-ulcer dyspepsia for *H. pylori* infection may also resolve symptoms. In addition, *H. pylori* is strongly associated with gastric cancer (Danesh, 1999). The prevalence of *H. pylori* infection in the world is assumed to be approximately 50% with a higher incidence in developing (90%) than developed countries (Engstrand, 2001). The principal reservoir of infection appears to be the human stomach. The routes of transmission in developed countries are thought to be the oral-oral route and the faecal-oral route. The organism has been isolated from saliva and dental plaque and is found within families and institutions rather than as outbreaks (Brown, 2000). There is no evidence of foodborne transmission (van Duynhoven and de Jonge, 2001).

In developing countries, a strong association has been demonstrated between infection and the consumption of drinking water. Children in Peru whose homes had external water sources were three times more likely to have *H. pylori* infections than those with internal water supplies (Klein, *et al*., 1991). However, transmission through water or sewage has not been conclusively proven.

A number of researchers have attempted to demonstrate the genetic material of *H. pylori* in drinking water. Hulten *et al*., (1998), demonstrated *Helicobacter* spp. DNA in 9 of 24 private wells, 3 of 25 municipal tap waters and 3 of 25 wastewater samples in Sweden. They also demonstrated the presence of *H. pylori* DNA in drinking water in Peru (Hulten *et al*., 1996). Hegarty *et al*., (1999) examined surface and shallow groundwater samples for *H. pylori* using immunofluorescence. The majority were found to be positive. Mazari–Hiriart *et al*., (2001), showed the presence of *H. pylori* in 68% of samples from extraction wells, 100% of samples from dams as pre-treated waters, 0% of samples of treated waters and 17 – 20% of wastewater samples. Horiuchi, *et al*., (2001) examined 10 tap waters, 6 well waters, 10 river waters and 10 seawaters using PCR. Only 2 of the 6 well waters were found to be positive. Some of the users of the well had previous *H. pylori* infections. They note that the risk of acquiring infection from contaminated or inadequately treated drinking water still has to be demonstrated. In addition, inadequately treated wastewater used for irrigating crops could provide another waterborne route of infection. *Helicobacter* spp. have also been demonstrated in biofilms in water mains in Scotland by Park *et al*., (2001) using nested PCR.

Johnson *et al*., (1997) have done chlorine disinfection studies on *H. pylori* using laboratory prepared cultures. At pH levels of 6.0, 7.0 and 8.0, a temperature of 5^{o}C and chlorine concentrations of 0.5 mg/l, greater than 3.5 $\log_{10}$ reduction occurred in 80 seconds exposure. The authors concluded that a CT_{99} value of 0.12 mg . min/litre had been demonstrated and that the organisms were chlorine sensitive despite large amounts of particulate material in the test suspensions.

Helicobacter pylori cannot be cultured from water or environmental samples (Engstrand, 2001). Molecular and immunological techniques remain the only tool to demonstrate its presence. These techniques cannot be guaranteed to be 100% specific and cross-reactions with other *Helicobacter* spp. remains a possibility. The literature would suggest that although the waterborne route is possible in developing countries and infection is possible with untreated waters, there is no firm evidence that drinking water in developed countries poses a threat of infection. In addition, *H. pylori* is a poor survivor outside the stomach and rapidly develops a viable-but-non-culturable state from which it has not been shown to revert.

Whilst risks appear minimal, there is no direct cultural technique and molecular and immunological techniques do not have 100% specificity, there is little that can be done with respect to the analysis of water samples for the organism.

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Legionella spp.

Legionella is a ubiquitous environmental organism and can be found in water and soils. There are currently at least 36 different species of *Legionella.* The most common species involved in human infection is *Legionella pneumophila* serogroup 1, causing 95% of all cases. In the United Kingdom, there are approximately 150 cases per year with a mortality of around 10%. Approximately half of these are contracted overseas. The estimated incidence in the United States is between 17,000 – 23,000 cases annually.

The disease caused by *Legionella* is of two types. These are Legionnaires' disease and Pontiac fever. Legionnaires' disease is a severe respiratory illness characterised by pneumonia. The incubation period is 2 – 10 days. Other symptoms may also be evident, including diarrhoea and meningitis. The attack rate is low, between 1 – 6% and underlying factors such as other infections, immunosuppression, males over 50, smoking, alcohol abuse and surgery are recognised as increasing the risk of infection. Pontiac fever has a shorter incubation of between 1 – 2 days and is characterised by a non-pneumonia flu-like illness with an attack rate of 100%. The disease is self-limiting and not fatal.

Legionella are widespread in rivers, streams, ponds and lakes. They are generally present in low numbers, probably because of the low temperature of many aquatic environments and lack of suitable nutrients. Colonisation of artificial environments such as cooling towers where the temperature is higher allows numbers to multiply rapidly. They multiply between the temperatures of 20 – 45^{o}C and are rapidly killed above 60^{o}C. They have been isolated from a wide variety of environments including cooling towers, evaporative condensers, cold and hot water tanks, showers and taps, toilets, misters used for flowers and vegetables and whirlpools to name but a few. They are common colonisers of hospitals and nosocomial infections are not uncommon. Infection is acquired by inhalation of aerosols produced from an environment where sufficient numbers of organisms are growing.

Whilst amplification within an artificial environment provides sufficient numbers for infection, source waters have been examined for the organism. Colbourne *et al*., (1988), examined surface and groundwater for *Legionella pneumophila* serogroup 1 using immunofluorescence and found that it could be detected in 14% of treated groundwater, 11% of treated surface waters and 12% of water in distribution systems in the United Kingdom. In a similar study, Tison and Seidler (1983) examined raw and potable waters for *Legionella* using immunofluorescence. Concentrations in raw waters ranged from 10^4 – 10^5 cells per litre and concentrations in treated waters were 10 – 100 times lower. No *Legionella* were isolated by culture. More recently, Riffard *et al.* (2001) examined groundwater (water and biofilms) for *Legionella.* They found that the organisms could be readily cultured from 10 of 12 water samples and 9 of 12 biofilm samples. PCR was found to be less sensitive. Isolates were identified as *L. dumoffii*, *L. gormanii* or related to *L. rubrilucens.* Hsu *et al*., (1984), isolated 3 different species of *Legionella* from chlorinated public water supplies in the absence of coliforms. Five of 856 samples were positive, 3 of which were *L. pneumophila* serogroup 1.

The sources of the organism gaining access into buildings and cooling towers have been suggested as air, soil particles or supplied mains water. Once within a building, suitable sites can be colonised. The incidence in buildings has been surveyed, for example, Zietz *et al*., (2001) found that of 70 buildings surveyed in Gottingen, 26% of hot water samples were positive for *Legionella*. Leoni *et al*., (2001) studied the swimming pool environment. Two of 48 pool waters were positive for *Legionella* and 27 of 48 samples of hot water from showers were also positive.

Legionella have excellent survival characteristics in the environment over a wide range of pH values and temperatures. Survival is helped by invasion and multiplication within amoebae. Elevated temperatures and the presence of organic materials in water systems such as natural rubber washers and sealing gaskets and stratification and debris in calorifiers assist growth. Control in systems is by cleaning and the application of biocides either in the form of heat or chemicals. Outbreaks have been linked to a number of sources including cooling towers, hot water systems, evaporative condensers, misters and whirlpool baths.

Legionella spp. can be isolated from water samples by membrane filtration followed by elution and culture on charcoal buffered yeast extract agar containing growth and selective supplements. Heavily contaminated samples can be decontaminated by incubating eluates at 50ºC for 30 minutes and incubation in acid at pH 2.2 for 5 minutes. Culture takes a minimum of 3 days and a maximum of 10 days. Once isolated, cultures can be confirmed by immunofluorescent or latex agglutination serology. Their presence can also be demonstrated by molecular techniques using PCR and by immunofluorescence.

Analysis of drinking water samples can clearly demonstrate the presence of low numbers of *Legionella.* Being natural water organisms, small numbers will pass through water treatment and survive disinfection, particularly in association with amoebae. The levels in mains water will be low, insufficient to cause infection in aerosols, and it is difficult to see how they can be eradicated. Prevention in buildings relates to good maintenance and cleaning, the use of appropriate plumbing materials and the application of biocide processes. Regulatory monitoring for *Legionella* in drinking water is not seen as applicable.

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Mycobacterium avium complex

The *Mycobacterium avium* complex (MAC) includes *M. avium* and *M. intracellulare*, which are opportunistic human pathogens that infect the lungs causing symptoms of cough, fatigue and weight loss similar to those of *M. tuberculosis*. Like many opportunistic pathogens, there has been a significant increase in this type of infection in individuals with acquired immunodeficiency syndrome (AIDS) where infection may be life-threatening.

The complex is ubiquitous in the environment being found in soil, water, including wastewater, ground and surface water and drinking water. It can also be found in house dust, animals and poultry. Infection is acquired by inhaling or ingesting contaminated soils, water or other materials. In immunocompromised individuals, ingestion results in gastrointestinal infections with typical symptoms of diarrhoea and vomiting.

A number of studies have looked at distribution systems, particularly in hospitals where the incidence of MAC organisms growing in water systems has resulted in infections. Levels in distribution systems vary from 0.08 cfu to 45,000 cfu per 100 ml. In a recent study, Falkinham *et al*., (2001) examined 8 water distribution systems over an 8 month period. Raw and treated waters were examined along with biofilms and water from distribution systems. *Mycobacteria* were detected in 15% of all samples and the numbers ranged from 10 to 700,000 cfu per litre. Water treatment reduced numbers by 2 – 4 logs. Mycobacterial numbers in treated waters were low but increased by some 25,000 fold in distribution systems. Although *M. intracellulare* was seldom recovered from water, it was present in 6 of 8 biofilm samples with an average count of 600 cfu per cm^2. The numbers correlated with assimilable organic carbon levels and biodegradable organic carbon levels.

Taylor *et al*., (2000), studied the susceptibility of *M. avium* isolates from water samples and patients to chlorine, chlorine dioxide and ozone. They were found to be resistant. The T90 for chlorine required for 3 log inactivation was between 51 and 204.

Organisms belonging to MAC can grow in water without supplementation with nutrient. They can grow between 15 – 45^{o}C, prefer low dissolved oxygen, high organic nutrients and high concentrations of zinc.

Organisms can be grown on selective media at 37^{o}C but they are slow growing. Identification of isolates is either by gas chromatography of cell wall lipids or by molecular techniques.

MAC organisms have been found in hospitals and the same strains in AIDS patients. They can be isolated from hot and cold water taps, toilets, sinks and other water sources in hospitals. An epidemiological study of 290 homes of HIV patients found MAC in 0.76% of water samples, 0.25% of food samples, but 55% of soil samples taken from potted plants (LeChevallier, 1999). Khoor *et al*., (2001) report diffuse pulmonary infections in patients in America using a hot tub and Saito *et al*., (2000) report skin infections in 3 patients from a '24 hour bath'.

MAC can clearly be found in raw waters and treatment reduces but does not remove them from finished water. Disinfection is ineffective. The organisms can grow in distribution water, particularly where the temperature exceeds 15^{o}C and there is available organic material. Homes and hospitals can become colonised and immunocompromised individuals are particularly at risk from pulmonary or gastrointestinal infections. Although a culture medium exists for the isolation of MAC, growth is slow and results may take between 1 – 2 weeks. Water companies which decide to test treated water

may isolate the organisms in low numbers. Greater care obviously centres around water distribution systems in hospitals. Control of the organism in hospital water systems can be achieved in hot water systems by keeping the temperature over 60°C. Immunocompromised people should be encouraged to drink only boiled water.

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Pseudomonas aeruginosa and Pseudomonas spp.

Pseudomonads are an ubiquitous group of bacteria found in water soil and on vegetation. They are aerobic bacteria and, even at low temperatures, growth may be rapid. They are capable of utilising a wide range of organic compounds as a carbon and nitrogen source and can grow in low levels of nutrients. Some strains, particularly *Ps. aeruginosa*, *Ps. fluorescens* and *Ps. putida* produce water-soluble yellow, green and red pigments.

Pseudomonas aeruginosa can colonise a wide variety of environments including water distribution systems, cooling towers, in hospitals – sinks, hydrotherapy pools and even disinfectants. It will grow anywhere where there is sufficient organic nutrient. Septicaemia in burns patients and those who are immunocompromised may result in death. It also causes ear and eye infections, epidemic diarrhoea in infants and folliculitis in association with growth in hot tubs and whirlpool baths. It has been found growing in large numbers in imported water-filled toys. The organism can be isolated readily from sewage and from surface waters although numbers will depend on available nutrient and water temperature. Pseudomonads can grow in milk and are responsible for food spoilage, particularly foods stored at low temperatures. They may also be found growing in bottled water.

Pseudomonas aeruginosa may grow in sand or carbon filter beds and in tertiary treatment devices such as reverse osmosis and electrodialysis membranes (Geldrich, 1999). It may also grow in biofilms in water distribution systems and home water treatment devices attached to mains water, for example, water softeners or filters. Poorly maintained dental equipment, whirlpools, hot tubs and even swimming pools can be an additional source of the organism. Infection is acquired by ingestion, coming into contact with the organism in water, particularly during bathing or person-to-person contact in hospitals. *Pseudomonas aeruginosa* is an excellent environmental survivor and has been shown to survive in biofilms in the presence of 50 mg per litre of free chlorine for 7 days (Anderson *et al*., 1990).

There has been one documented outbreak of *Ps. aeruginosa* infection in a newborn nursery where the ground water supply was contaminated with sewage. In a recent study, de Victorica and Galvan, (2001) isolated *Ps. aeruginosa* from 6 of 11 well water samples where coliforms and *E. coli* were absent and 17 of 19 well waters where coliforms were present. The investigation followed an outbreak of gastroenteritis in Mexico where enteropathogenic *E. coli* and *Ps. aeruginosa* were isolated from 5 children who were hospitalised because of the infection.

Because of the widespread nature of the organism and its ability to colonise water air interfaces and biofilms, distribution systems will contain low numbers of the organism. Given that water in distribution systems is seldom static, the temperature and the nutrient levels are usually low and there is a chlorine residual, numbers should not increase significantly. Once inside buildings, however, water storage and change of use may lead to the rapid increase in the organism. It has been known to colonise a domestic property and cause skin irritation through showering.

Pseudomonas aeruginosa can be isolated readily using membrane filtration and *Pseudomonas* agar. Typical blue-green colonies fluoresce under ultra-violet light. Typical confirmation is by demonstrating casein hydrolysis and the production of a yellow-green pigment. The organism will grow on membrane lauryl sulphate broth (MLSB) producing characteristic slate grey colonies with a typical 'musty' pseudomonad smell. Occasionally these non-lactose fermenting colonies can be seen in routine water monitoring and those who read membranes should be aware that they are *Ps. aeruginosa.*

There are no regulations restricting the numbers of *Ps. aeruginosa* in drinking water samples. The European Community restricts its numbers to less than one organism in 250 ml of bottled water where water may be stored, often at ambient temperatures, for long periods. *Pseudomonas aeruginosa* is seldom seen on membranes during routine water analysis. Non-lactose fermenting organisms are sometimes isolated. Records should be kept of the occurrence of such organisms and analysts should be aware of the colony type on MLSB. Isolation of *Ps. aeruginosa* from a treated water or drinking water distribution system is undesirable and should lead to an investigation of the source of the organism and its eradication. Isolation from a consumer tap should also lead to remedial action to remove the organism. The organism does not require Regulatory status but water industry analysts should be aware of the possibility of isolating the organism during routine water monitoring and water companies should take remedial action should it be isolated.

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Salmonella Spp

The genus *Salmonella* belongs in the family Enterobacteriaceae. They can be found as the normal flora of animals and birds and in water, wastewater, soil and on plants. Over 2,000 different serotypes have been identified. Three clinically distinguishable forms of *Salmonella* infections occur in humans. Gastroenteritis usually occurs 18 – 48 after ingestion of the organisms and symptoms include abdominal pain, diarrhoea and fever. Symptoms usually last between 2 – 5 days. Enteric fever is caused by *S. typhi* and *S. paratyphi* A, B and C. Symptoms tend to be more prolonged and, with *S. typhi*, may include damage to the liver, spleen and the respiratory system. The mortality with typhoid fever tends to be higher than with other infections. The third clinical form is septicaemia where organisms invade the blood stream and cause fever, chills and anorexia. Septicaemia may result in focal infections in body organs producing meningitis, endocarditis or pneumonia. Infection may be asymptomatic and these and convalescent cases can spread infection.

Humans and animals form a reservoir of infection for *Salmonella* with the exception of *S. typhi* and *S. para-typhi* in which humans are the only reservoir. Infection is acquired by the faecal oral route or the consumption of infected food, milk or water. The infective dose for *Salmonella* is considered to be relatively high but the organism is capable of growing in a wide variety of foods which might become lightly contaminated. Fermented foods and chocolate have been sources of infection where the infective dose has been shown to be very low. It can be found readily in sewage, surface waters and sewage sludge. In addition, it is carried by birds and roosting gulls have been shown to be a major source of contamination for reservoir water. *Salmonella typhi* has been estimated to cause 17 million cases of infection annually with 600,000 deaths worldwide (Covert, 1999).

Waterborne outbreaks of *Salmonella* have been documented. Between 1971 and 1988 there were 12 documented outbreaks affecting over 2,300 people (Morris, 1997). The majority of outbreaks identified by Morris were related to *S. typhi* which has a lower infective dose than other *Salmonella.* One large outbreak occurred in the United States in 1965 with over 18,000 cases. The water supply was implicated but the source of the *S. typhimurium* was never determined. In another outbreak of *S. typhimurium* in 1993, over 650 people were affected and 7 died. Contamination was traced to a water tower with defective roof vent covers that allowed access to birds. Water treatment, in particular disinfection, is effective in killing *Salmonella. Salmonella typhimurium* is no less susceptible to disinfection than other salmonellae. Taylor *et al*., (2000) report an outbreak of *Salmonella saintpaul* in 28 of 200 construction workers in Rockhampton, Queensland. The source of infection was thought to be frogs or mice contaminating the water. The survival of four *Salmonella* strains in river water microcosms was studied by Santo Domingo *et al*., (2000). Cultural counts reduced to 0.001% of total counts in 31 daysalthough total and direct viable counts suggested the levels were four orders of magnitude higher. Attempts to resuscitate failed unless cell free supernatant from viable cultures was added to the resuscitation step.

Sensitive methods for the detection of *Salmonella* in sewage and water have been available for over 20 years. Pre-enrichment in buffered peptone water is followed by selective enrichment and plating onto selective agars. A variety of different media are available commercially. Confirmation of isolates is by biochemical and serological tests. Conventional tests take around 5 days for a result. Rapid, sensitive methods have been developed by the food industry, including the use of immunomagnetic separation.

Proper control of water treatment and, in particular, disinfection are important in controlling *Salmonella* in treated waters. In addition, the correct maintenance of treated water storage reservoirs and tanks will minimise the risk of water contamination. There have been no documented

waterborne outbreaks of *Salmonella* in the United Kingdom since the infamous typhoid outbreak in 1937. Faecal contamination and lack or failure of disinfection are the risk factors.

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THE INTESTINAL AND OTHER PARASITES

Acanthamoeba spp.

Acanthamoeba spp. are small free-living amoeba which are ubiquitous. They can be found in soil, fresh surface water, brackish water and sea water, sewage and sludge, dust, swimming pools, tap water, bottled water, heating, ventilation and air conditioning units, dialysis machines and dental units, vegetables and mushrooms. There are a number of species which can infect humans including *A. castellanii, A. cuthbertsoni and A. polyphaga.*

They have 2 stages in their life cycle. Trophozoites measure 15 – 45 μm and are characterised by fine, tapering spine-like projections called acanthapodia which protrude from the surface and allow the organisms to undergo slow gliding movements. They feed on bacteria and divide by simple mitosis. Under adverse conditions, they differentiate into a cyst. Cysts are typically wrinkled, double-walled and may be star shaped, polygonal or spherical. They measure 15 – 20 μm in diameter. The trophozoites can be cultured by growing on a non-nutrient agar which has been pre-seeded with *E. coli*. The organism usually grows within 2 – 3 days at 37°C and plates should be examined for trophozoites daily using x 10 magnification. Alternatively the organism can be cultured in 2.5 ml of sterile saline pre-seeded with *Xanthomonas maltophila*. Immunofluorescence may help identification of any isolates.

Acanthamoeba spp. are opportunistic pathogens. In immunocompromised individuals they cause granulomatous amoebic encephalitis (GAE) which is a slowly progressive fatal central nervous system disease. Infection is not usually associated with water. Immunocompromised individuals may also develop skin lesions and pneumonitis. Wearers of contact lenses may develop *Acanthamoeba* keratitis which is a painfull vision-threatening disease of the cornea. If not treated quickly it leads to ulceration and eventually loss of sight. More than 700 cases have been reported worldwide. Cases occur in warm weather and are associated either swimming in lakes or ponds whilst wearing contact lenses or using tap water (more particularly from the bathroom) or home-made, non-sterile saline to store contact lenses. The trophozoites grow in the lens case and are introduced into the eye with the lens.

Although *Acanthamoeba* is distributed worldwide and can survive indefinitely in the cyst form, there are no documented instances of waterborne outbreaks of infection. Its ubiquitous nature means that immunocompromised individuals will come into contact with it frequently in their daily lives. It can undoubtedly grow in water without any further nutrient being added and will be found wherever water is warm and has been allowed to stagnate (for example in taps and showers). Its detection is relatively simple on non-nutrient agar and a method for detection has been published (Anon, 1989). Contact lens wearers should be aware of the risks posed by storing lenses in non-sterile solutions. Regulatory monitoring for *Acanthamoebae* is not indicated but the Government and water companies should be aware that a relatively simple method is available for detection should monitoring be required.

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Cyclospora cayetanensis

Cyclospora were first observed in humans in Papua New Guinea in 1979. They belong to the family Eimeriidae and are coccidians. They are excreted in faeces as unsporulated oocysts and in this state they are not infective to humans. Sporulation takes place in the environment and is temperature dependant. Sporulation takes place maximally between 22 – 30^{o}C in 5 – 11 days. Temperatures outside this range slow down sporulation. Unsporulated and sporulated oocysts measure 8 – 10 μm in diameter. Sporulated oocysts contain 2 ovoid sporocysts each containing 2 sporozoites. Oocysts can be detected in wet mounts of faecal material as non-refractile spheres. They can be stained using a modified Zhiel-Neelsen stain and under ultra-violet illumination (330 – 380 nm) the oocyst wall fluoresces causing them to appear as blue circles. Excitation at a higher wavelength of 450 – 490 nm gives a green appearance. Given their size, oocysts can be concentrated from water samples using the conventional filtration techniques developed for *Cryptosporidium*. Cleaning samples would have to be by ether or sucrose flotation. Detection includes light microscopy using a modified Zhiel-Neelsen stain and autofluorescence.

Sporozoites of *Cyclospora* infect enterocytes of the small intestine. The onset of symptoms following infection is usually abrupt. Symptoms include nausea, anorexia, abdominal cramps and watery diarrhoea alternating with constipation. In immunocompetent individuals, the disease is self-limiting but symptoms can last for up to 7 weeks. In immunocompromised individuals, symptoms can last for up to 4 months. The disease can be treated with trimethoprim-sulphamethoxazole. Humans may be the only reservoir for infection. Given that the oocyst is excreted unsporulated, person to person transmission does not occur.

Epidemiological evidence suggests that water may be a vehicle for transmission of the disease. Oocysts have been found in patients with protracted diarrhoeal disease in many parts of the world. Many cases are linked to travel to developing countries where diarrhoeal disease is more prevalent, however, infections have been reported in many countries there travel is not implicated. One waterborne outbreak has been reported Rabold *et al*., (1994), where 12 of 14 soldiers developed diarrhoea. The chlorinated drinking water, consisting of river water and municipal water was shown to contain *Cyclospora.* The chlorine levels in the water were reported as between 0.3 – 0.8 mg per litre. Coliforms were not detected. A large number of cases were reported in the States in May and June of 1996. These were related to the consumption of raspberries. Further outbreaks were reported in 1997 and these were related to the consumption of raspberries and basil. Infection has also been acquired by swimming in a lake. A report by Cann *et al*., *(*2000), notes that only 44 – 66 laboratory reports of *C. cayetanensis* are made in the United Kingdom each year. A large proportion of these are found to have visited developing countries. In a study by Sherchand *et al*., (1999) 2,123 stool specimens were collected from 3 health care facilities in Nepal between 1995 and 1998. Of these, 632 (29.8%) were positive for *Cyclospora*. Oocysts were identified in sewage, water and green leafy vegetables on four occasions as well as in 2 chickens.

Conventional water treatment should remove this organism although disinfection will have little effect. The numbers of detected cases in the United Kingdom would suggest that the incidence of the organism in sewage and surface waters is low. Conventional sampling of large volumes will concentrate the parasite from water. Cleaning would have to be by sucrose flotation and detection by conventional microscopy using autofluorescence as a means of detection and confirmation. It would be useful to have a draft method available should the need arise and analysts in water laboratories should be familiar with the parasite. The LEAP Scheme should be able to distribute a sample of the

parasite to water company laboratories. The parasite could be obtained from Dr Chalmers of the *Cryptosporidium* reference laboratory. Regulatory monitoring is not indicated.

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Giardia intestinalis (lamblia, duodenalis)

Giardia intestinalis is the most frequently isolated intestinal parasite throughout the world (Marshall *et al*., 1997). Using microscopy, rates of detection vary from 2 – 5% in developed countries and 20-30% in developing countries. Incidence appears to be more common in urban areas than rural areas. The incubation period is usually 1 – 2 weeks but may be up to 75 days. Disease symptoms start with intestinal uneasiness followed by nausea and anorexia. Major symptoms include diarrhoea which is explosive, watery and foul-smelling. Mucous and blood may be in the stool. In addition, foul-smelling flatulence and belching, abdominal cramps, distention, vomiting, and fatigue may also be present. The acute stage lasts for 3 – 4 days. Infection, however, may be asymptomatic and carriers may excrete for years. There are approximately 6,000 cases in the United Kingdom each year. The source of many of those cases is unknown. In 2001 there were 3,5789 reports of infection (CDR).

The environmental stage is the cyst which can survive for long periods in cool dark conditions. It is transmitted by person to person via the faecal oral route, particularly in day care centres and nursing homes. Animals, especially pets, may infect humans. Water both drinking water and recreational water may also be an important vehicle for the transmission of infection. The parasite infects numerous mammals in addition to humans. Dogs and cats, beavers and muskrats as well as cattle and sheep may be infected. In a survey in Canada, Olsen (Pers. comm.), found that 100% of beef cattle were infected. It is unclear whether some of the animal strains infect humans.

Cysts of the parasite can be found readily in sewage (Hirata and Hashimoto, 1997). Concentrations ranged from 130 – 7,900 cysts per litre in crude sewage and 1 – 130 cysts per litre in final effluents. The average removal of *Giardia* cysts by activated sludge was 1.8 $\log_{10.}$ Similar concentrations were found in the Netherlands in settled sewage (Medema *et al.*,1997). Robertson *et al*., (1995) examined six sewage works for cysts in an attempt to correlate concentrations of cysts in crude sewage with cases identified in the community. Cyst levels were found to be higher than cases would predict suggesting either a significant number of asymptomatic cases in the community, failure to diagnose symptomatic cases or substantial inputs from animals. Concentrations ranged from 135 to 43,907 cysts per litre. and surface waters. Cysts have also been detected in ground waters in the United States (Hancock *et al*., 1997) where springs and horizontal wells were found to have the highest contamination and in private water supplies in the United Kingdom (Clapham, 1997; Watkins *et al*., 2001). Cysts are readily inactivated by boiling and freezing. Cysts stored at 8^{o}C have been shown to survive for up to 77 days (Schaefer, 1999). Survival diminishes as the temperature increases. Cysts suspended in tap water die within 14 days. In practice, cysts are not good environmental survivors. Relatively recent water contamination with no treatment and no or minimal disinfection are the best conditions for waterborne outbreaks. *Giardia* cysts are removed by conventional water treatment. Chemical coagulation and rapid gravity filtration has been shown to remove between 1.7 and 3.2 $\log_{10}$ with a mean value of 2.53 $\log_{10}$ (Hashimoto *et* al., 2001). Slow sand filtration can remove at least 2 logs of the parasite. Chemical disinfectants such as chlorine, chlorine dioxide and ozone are known to inactivate *Giardia* cysts. The CT value for 2 log inactivation ranges from 9 – 342 (Schaefer, 1999).

Waterborne outbreaks of giardiasis were a frequent occurrence in the United States. Between 1971 and 1985, 92 (18% of waterborne outbreaks) were due to *Giardia*, resulting in 24,124 cases of infection. Between 1986 and 1994 there were a further 25 outbreaks (Marshall *et al*., 1997). Outbreaks have also been associated with recreational water. Between 1984 and 1994 there were 15 outbreaks resulting in 2,154 cases of infection. A large outbreak occurred in Japan in 1994 affecting 8,705 cases. Inadequate coagulant dosing of polluted source water was suspected as the cause. Swimming pools have also been implicated in outbreaks. Unfiltered and inadequately chlorinated

surface waters and groundwater contaminated by surface water are seen as the principal association with drinking water outbreaks. In addition, many of the outbreaks have occurred in small community systems which have a minimum of 15 service connections and serve customers all year.

There have been 2 documented outbreaks of giardiasis in the United Kingdom. The first was in Bristol in 1985 which affected 108 people (Jephcott *et al.*, 1986). Epidemiological evidence suggested contamination of drinking water following mains repair. A second outbreak in a private water supply in the Worcester area in April of 1992 affected 28 people (Constantine *et al*., 1995). All the cases lived in a small village of 200 inhabitants. The source water was a shallow spring. There was anecdotal evidence that spring water flow increased after heavy rainfall. In addition, animals were allowed to graze in the field where the spring arose. Damaged cysts were also isolated from a consumers tap filter. There was no water treatment at the time of the incident. Significant numbers of cysts were found in private water supplies in the United Kingdom, particularly following heavy rainfall although there was no evidence of disease in the community (Watkins *et al*., 2001).

The incidence of giardiasis is worldwide. Many cases of infection are asymptomatic and go unrecognised, despite excretion of cysts in the faeces. In addition, detection of cysts in infected individuals is difficult as cyst shedding may be intermittent and numbers may be low. Animals, particularly cattle, are known to be carriers and many other species are affected. As is the case with *Cryptosporidium*, water treatment including disinfection is sufficient to remove *Giardia* cysts. Evidence from outbreaks in America would suggest that inadequate water treatment or a lack of water treatment are the major causes of outbreaks there.

Routine monitoring of potable water supplies in the United Kingdom has not been a standard parameter, possibly because, unlike *Cryptosporidium*, there has been no epidemiological evidence of drinking water being implicated in outbreaks. There is, however a substantial reservoir of infection in the United Kingdom, probably larger than confirmed figures suggest, for which the source of infection is unknown.

The cysts can be isolated easily from water by membrane filtration of small volumes (up to 50 litres) and the use of depth filters, for example, the Genera Filta-Max™ for larger volumes of 1,000 litres or more. Immunomagnetic separation beads for cleaning samples and monoclonal antibodies for cyst detection are available commercially. Suspensions of the parasite for quality control purposes are also available commercially (Waterborne Inc. USA).

Regulatory monitoring of potable water for *Cryptosporidium* already provides a sample which could be analysed either simultaneously or subsequently, for *Giardia.* It would therefore seem advantageous to perform a combined analysis on these Regulatory samples to provide documentary evidence that water is not a major route of transmission of the parasite.

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Microsporidia

The microsporidia are a group of obligate intra-cellular parasites which form spores. Their classification is based on size, nuclear arrangement, mode of division and whether the parasite develops within a parasitophorus vacuole or within the cytoplasm of the cell. Microsporidia have a broad host range and there are currently over 100 genera and more than 1,000 species (Smith, 1997; Cali, 1999). The majority of these species infect insects and lower vertebrates, for example, fish.

The microsporidia which infect mammals are Gram-positive, unicellular organisms. The spore ranges in size from 0.5 – 2 μm to 1 – 4 μm in diameter. A number of genera have been identified in humans. These are *Enterocytozoon* of which *E. bieneusi* causes diarrhoea and hepatitis, *Encephalitozoon*, of which *E. intestinalis* causes diarrhoea, *Nosema*, of which *N. connori* causes diarrhoea and may cause disseminated infections, *Pleistophoira* spp. which cause myositis and *Microsporidium* spp. which affect corneal stroma and cause blindness.

All microsporidian spores have a polar tube which is coiled within the spore and is extruded under appropriate conditions such as pH or the presence of specific ions. The power of the extrusion permits the tubule to penetrate the host cell. Sporoplasm then emerges through the tubule and into the host cell and intracellular multiplication of the parasite occurs (merogony) producing meronts. Meronts develop into sporonts (sporogony) and these grow into sporoblasts. After the last division, sporoblasts undergo metamorphosis and become spores. All the stages of the life cycle can take place in one cell. *Enterocytozoon bieneusi* replicates in intimate contact with the host cell cytoplasm, other *Encephalitozoon* spp. develop within a parasitophorous vacuole and *Pleistophora* develop within a sporophorous vacuole. Spores are shed in faeces, urine or other body fluids and they are very resistant to environmental factors.

Microsporidians cause opportunistic infections resulting in a variety of disease symptoms. The infective dose and incubation period are unknown. Organisms that infect the intestinal tract cause diarrhoea and wasting. Some species infect the cornea causing blindness. Within these two syndromes are species which may become disseminated causing liver, kidney and skeletal muscle damage. Confirmation of infection is by the demonstration of spores in the urine, faeces, cerebro-spinal or other body fluids and, for eye infections, corneal scrapings. With the spread of AIDS, microsporidians were identified as a frequent cause of AIDS related diarrhoea.

Given the large host range and number of species, microsporidian spores are common in the environment. Gastrointestinal infection is acquired by the faecal oral route, through contaminated food or water whereas eye infection is acquired by touching the eye with contaminated hands. Some microsporidians infect animals (including companion animals) and birds as well as humans, for example *E. cuniculi*.

Cotte *et al*., (1999), describe a waterborne outbreak of microsporidia affecting 200 people in 1995. Factors associated with infection were HIV infection, male homosexuality, low CD4 count and diarrhoea. Contamination of lake water was suspected as the cause. The research work also examined the faeces of 1,454 patients for microsporidia. Three hundred and thirty eight people were identified as infected of which 261 were HIV positive, 16 were transplant patients and 61 were HIV negative. Ferreira *et al.* (2001) examined the faeces of AIDS patients for microsporidial infection. The incidence was found to be between 14 and 51% with *E. intestinalis* comprising 71% of infections and *E bieneusi* 29%. Seroprevalence of microsporidia antibodies was examined by Van Gool *et al*.,

(1997). Less than 8% of blood donors had antibodies to *E. intestinalis* and 5% of pregnant French women were seropositive.

In a review of microsporidia, Mota *et al*., (2000) suggests that spores are removed by conventional water treatment and killed by disinfection. Fournier *et al*., (2000) examined surface water samples (300 – 600 litres) for microsporidia over a twelve month period using light microscopy and nested PCR using primers against *E. bieneusi*. Microscopy proved unreliable but PCR gave a positive response in 16 of 25 samples. Only one sample however was confirmed as *E. bieneusi*. The authors suggest low incidence in water and therefore low risk of waterborne transmission. Water samples from household tanks in Egypt were examined for a variety of parasites including microsporidia (Khalifa, *et al*., 2001). Microsporidia were found in 3% of samples, compared with *Giardia* (56%), *Cryptosporidium* (50%), *Blastocystis* (12%) and *Cyclospora* (9%). Ozonated water (1 mg per litre) for 9 minutes contact was found to destroy the infectivity of microsporidia suspensions inoculated into animals.

Disinfection studies have been done by Wolk *et al*. (2000) using chlorine. Spore infectivity was demonstrated in a rabbit kidney cell culture system. Spores were exposed to chlorine concentrations of 0, 1, 2, 5 and 10 mg/l at pH 7.0 and a temperature of 25 ^{o}C. Times of exposure ranged from 0 – 80 minutes. A 3 log reduction was observed at a chlorine concentration of 2 mg/l in 16 minutes. More recently two other species of *Microsporidium* have been investigated (Marshall pers.comm.) following the methods described by Wolk *et al*., (2000). *Encephalitozoon cuniculi* and *E. hellem* in addition to *E. intestinalis* showed for log inactivation with 2 mg/l of chlorine in 6 – 8 minutes at 23 ^{o}C.

Methods for the detection of microsporidia in faecal and water samples include phase contrast or differential interference contrast microscopy, stains including periodic acid-Schiff which reveals a small granule at the anterior end of the spore or calcifluor white M2R, Geimsa or Warthin-Starry silver stain for fixed material. Monoclonal antibodies have also been developed but their species specificity has been questioned. Molecular techniques using PCR have also been used.

Little is known about the incidence of microsporidia that cause human infections in water samples. The organisms may be much more widespread than has been thought, based on seroprevalence. Environmental detection is clearly hampered by sensitive and specific tools for isolation and identification. Given the wide distribution of species, without specific tools, confirmed detection of human species will be difficult. Limited data would suggest that the incidence of human species in the environment is low and that water treatment and disinfection are effective in removing them. They are, however, much smaller than other waterborne parasites and therefore the efficacy of water treatment may need attention.

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Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular coccidian parasite. It is usually transmitted by ingestion of infective tissues from animals, through the placenta or by the consumption of food or water contaminated with infective cat faeces. Cats excrete non-infective oocysts in their faeces. These develop 8 sporozoites within 2 sporocysts within 1 or more days, depending on environmental conditions. The oocysts are 10 – 12 μm in diameter and are able to survive in environmental conditions for long periods.

Infection follows the ingestion of faecally contaminated food or water. Sporozoites are released and multiply in the intestinal cells forming tachyzoites (rapidly multiplying forms). Tachyzoites migrate throughout the body in blood and lymph and encyst in the brain, skeletal and cardiac muscle and liver. Cysts which are approximately 70 μm in size contain slowly multiplying bradyzoites. These cysts are ingested by a second host whereupon the bradyzoites are converted back to tachyzoites which multiply and eventually encyst in tissues. Ingestion of these cysts by cats results in completion of the life cycle and excretion of oocysts.

Toxoplasmosis in generally an asymptomatic infection in adults but can cause mental retardation, loss of vision and hearing and death in congenitally infected children. It causes serious illness and death in immunosuppressed people and AIDS patients where encephalitis is the predominant feature. *Toxoplasma gondii* is a major cause of abortion in sheep and goats and can cause abortion in infected pregnant women. The only reservoir for the infection is in cats. *Toxoplasma gondii* infection is higher in feral cats than domestic cats because they hunt birds and small mammals. The parasite infects virtually all warm-blooded animals and is one of the most widespread infections of human beings (Dubey, 1999).

The parasite oocyst can be concentrated from water by methods used to detect *Cryptosporidium parvum*. Oocysts are detected by light microscopy. Unfortunately other coccidian oocysts can be confused with *T. gondii* and therefore infection of mice and subsequent demonstration of infection is important. Oocysts are resistant to disinfection with chlorine and formalin but susceptible to gamma irradiation. Waterborne outbreaks of toxoplasmosis have been documented. An outbreak in British Columbia was related epidemiologically to a water reservoir (Bell *et al.*, 1995) and a second outbreak occurred in soldiers in Panama who drank pond water.

The incidence of *T. gondii* in surface waters in the United Kingdom is not known but will probably be low. Given the large size of the oocyst, it should be readily removed from water by conventional treatment.

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THE VIRUSES

Viruses differ from bacteria in that they cannot multiply outside living cells and therefore the presence in water of a human enteric virus of whatever sort will always indicate some kind of faecal contamination. Techniques for the detection of viruses in water are generally more complex than those for bacteria though with the increasing use of molecular biological techniques they need be no more complex and take no longer than the techniques employed for *Cryptosporidium* detection. Grabow *et al*., (2001) used cell culture RT-PCR to detect viruses in 23% of 413 drinking water samples and 73% of 224 raw water samples. Enteroviruses were detected in 17% of drinking water samples, adenovirus in 4% and hepatitis A virus in 3%. Astrovirus and rotavirus were detected in raw water samples. All the samples received conventional treatment and disinfection and none were positive for faecal indicators. Amplification of viral nucleic acid in tissue culture before the application of molecular techniques makes this type of analysis much more sensitive.

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Adenoviruses

The adenoviruses (AdV) belong to the family Adenoviridae. They are hexagonal viruses with a DNA genome. They are stable between pH 6.0 and 9.5 and at 4ºC for up to 70 days. They are inactivated at temperatures above 56ºC and by chlorine at 0.1 mg per litre. The group includes human and animal types. There are 49 human adenovirus serotypes in six sub-groups designated A – F, all of which are found in faeces and are indistinguishable by electron microscopy. Types 1, 2, 5 and 6 cause respiratory infections particularly in children. Types 3 and 7 cause pharyngo-conjunctivitis associated with swimming pools in young adults and children during the summer. Adenoviruses type 40 and 41 are an important cause of gastroenteritis, second only to rotavirus particularly in children under 12 months of age. The incidence of infection is not seasonal. The number of reported cases occurring in the United Kingdom in 2001 was 256 (CDR).

All serotypes may be shed in faeces so may be present in sewage and therefore impact on water. Several adenovirus types, particularly sub-groups B, C, D and E and serotypes 1 to 7 and 15 have been isolated from sewage, surface waters, groundwater and recreational bathing waters. Spread of adenovirus 40 and 41 in drinking water and recreational water has been postulated but to date there are no reports of waterborne outbreaks related to drinking water. Morris (1997), reported 6 outbreaks related to swimming pools and recreational waters. These were due to serotypes 3, 4 and 7 and not the group F strains. Dionisio, *et al*., (1997), describe a chronic adenovirus infection in an AIDS patient lasting 13 months. The most common symptoms were diarrhoea, vomiting, abdominal cramps and low-grade fever.

The incidence of adenovirus in young children in developing countries has been studied. Jarecki-Khan *et al*. (1993), examined 4,409 stool specimens from children less than 5 years old. One hundred and twenty five (2.8%) samples were positive of which 51 were adenovirus type 40 and 74 were adenovirus type 41. Cruz *et al*., (1990), examined 458 samples of faeces from children with diarrhoea in Guatemala. Over 22% of rural children excreted adenovirus types 40 and 41 compared with just over 10% excreting rotavirus. Nine children had asymptomatic infections and dual infections with adenovirus and rotavirus were found in 8 children. Like other gastrointestinal infections, the incidence of adenovirus in developing countries may be much higher than in developed countries. Faecal samples from outbreaks of gastroenteritis in day care centres in Houston were screened for adenovirus by Van *et al*., (1992). A total of 4,402 stools from 91 outbreaks were tested. The virus was detected in 10 outbreaks. Of 249 children in these outbreaks, 94 (38%) were positive. In 51, the infection was symptomatic and in 43 it was asymptomatic. Duration of excretion had a mean of 3.9 days with excretion occurring from 7 days before diarrhoea to 11 days after diarrhoea had stopped.

The incubation period following infection is between 1 – 3 days, followed by watery diarrhoea and sometimes vomiting. Infection is acquired via the faecal oral route but the virus can gain access through inhalation and passage to the pharynx. Shedding of the virus in faeces can persist for months or even years because of low level infections in the intestines, tonsils and adenoids (Enriquez, 1999), though the quantity of virus shed will be lower. Humans do not appear to be infected with animal strains.

Adenovirus types 40 and 41 have been grown only in CaCo-2 and Graham 293 cell cultures with the production of cytopathic effect, but growth of type 40 is poor and is unlikely to be useful for routine monitoring purposes. PCR and gene probe techniques have been used to detect adenovirus in the environment. Chapron, *et al*., (2000) collected data on the incidence of adenovirus types 40 and 41 in surface waters using growth in BGM cells followed by PCR on the culture (integrated cell culture-

PCR, ICC-PCR). Fourteen of 29 samples were positive for adenovirus types 40 and 41 and 11 of these samples were determined to be infectious. Adenovirus types 40 and 41 have been shown to have better survival characteristics than hepatitis A virus and polio 1 virus in tap water and sea water.

There is no evidence in the literature that the enteropathogenic strains of adenovirus have caused outbreaks of waterborne disease. They can be found in sewage and surface waters but should be readily removed by treatment and disinfection. There is evidence of a much higher incidence of adenovirus in young children in developing countries and here water may have a role in transmission.

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Astrovirus

Astroviruses is the only genus in the family Astroviridae. They are 27 – 32 nm in diameter and are small spherical positive-stranded RNA viruses with a characteristic five or six pointed star-shaped surface. Seven serotypes of astrovirus have been described (Lee and Kurtz, 1994) and a new type 8 has recently been described (Monroe *et al*., 2001).

Astroviruses cause gastroenteritis, predominantly diarrhoea, mainly in children under 5 years old though they have been reported in adults. The incubation period is 3 – 4 days and symptoms are typically mild diarrhoea and (rarely) vomiting. Dehydration may also occur. The duration of illness is characteristically 2 – 3 days but may extend to between 7 – 14 days. Virus shedding lasts at least during the diarrhoea. A seroepidemiological study in England found that over 70% of individuals acquire antibodies to astrovirus by the time they are 3 – 4 years old. However, resistance to one serotype may not confer resistance to all types. In a large-scale study in Thailand, astroviruses were detected in 8.6% of individuals who were ill. The contribution of astrovirus to virus-associated diarrhoea is between 5 – 10% (Pinto *et al*., 2001) There are no reports of human astroviruses infecting animals and the reservoir for infection is thought to be human. Spread is mainly through the faecal oral route and infection occurs early in life. The number of reported cases occurring in the United Kingdom in 2001 was 116 (CDR).

A large foodborne outbreak occurred in Osaka, Japan involving over 4,700 people (Oishi *et al*., 1994). Few reports exist on the incidence of astroviruses in the aquatic environment. Detection of astrovirus in environmental samples has been described by Marx *et* al. (1995); and by Pintó *et al*., (1996) demonstrated the occurrence of infectious astrovirus in water from an area where a concurrent gastroenteritis outbreak was reported. Abad *et al*., (1997) studied the survival of astroviruses in different water types. The survival characteristics found in these studies were comparable to those determined for rotavirus and enteric adenoviruses, though astrovirus decay was more pronounced at higher temperatures.

Taylor *et al*., (2001) examined river and dam waters in South Africa for astrovirus. Virus was present in 11 (21.6%) river water and 3 (5.9%) dam water samples. Yokoi *et al*., (2001) were able to detect astrovirus RNA in samples of sewage, chlorinated sewage, seawater and oysters. Samples collected from January to June were positive but samples from July to December were negative. Peak positive samples were obtained in March. Chapron, *et al*., (2000), reported on virus surveillance as part of surface water analysis for the information collection rule. Eight of 29 samples were positive for infectious astrovirus. However analysis of sewage and environmental samples by Egglestone *et al*., (1999) found no evidence of astrovirus. In this analysis, cell culture was not used prior to PCR and the evidence would suggest that using cell culture makes detection more sensitive.

Although there is substantial exposure to astroviruses in early life, there is no evidence that this is through water. Astroviruses should be effectively removed by water treatment and disinfection and Regulatory monitoring is not required.

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Calicivirus

Human enteric caliciviruses are positive-sense RNA viruses classified into two groups (Jiang *et al*., 1993; Lambden *et al*., 1993) the *Norwalk-like viruses* (NLVs, formally termed SRSVs in the United Kingdom) which are further divided into genogroups I and II (Pringle, 1998; Anon, 2000). Strains are named after the place of first isolation, hence Norwalk, Southampton and Desert Shield (genogroup I) and Mexico, Hawaii and Bristol (genogroup II). Clinical data suggests that strains of genogroup II type seem to be the most common in the United Kingdom, though environmental studies have shown genotype I strains to be as common as genotype II strains. The *Sapporo-like viruses* (SLV) formally termed 'classic caliciviruses', are also currently divided into two genogroups (Berke *et al*., 1997; Hale *et al*., 1999; Vinje *et al*., 2000). The Calicivirus group also includes hepatitis E virus (HEV).

Disease in affected individuals varies according to the strain of virus; SLVs cause gastroenteritis, mainly in children under five years old. The illness has an incubation period of 48 – 72 hours and usually lasts about 4 days, during which time viruses are excreted in the faeces. Around 80% of children in the 6 to 12 year age group possess antibody, suggesting that exposure to SLVs is common. No environmental studies on caliciviruses have been reported. However, since large numbers of viruses are excreted in faeces, their presence in sewage can be inferred. Contact with infected individuals is the only proven source, although shellfish (cockles, mussels and oysters) have been incriminated indirectly suggesting that contaminated water may be a vehicle of transmission. The evidence for this is equivocal.

NLVs have an incubation period of 24 – 48 hours, but it can be as short as 10 hours. Symptoms typically last 12 – 48 hours. Vomiting (sometimes projectile) is the principal symptom in children but diarrhoea is more typical in adults. The virus is shed both in vomit and faeces and faecal shedding may last a week after diarrhoea has stopped. The virus is highly infectious. Infection is common in adults and in the United States around 40% of adult gastroenteritis is caused by NLVs. Additional symptoms may include abdominal pain, low fever, headache, and nausea. In temperate climates it is typically a disease of the winter months hence its common name 'winter vomiting disease'.

Waterborne outbreaks attributed to NLVs are numerous. An outbreak probably due to NLV occurred in Bramham in Yorkshire in 1980 (Short, 1988). Sewage contamination of a borehole supply with concomitant failure of chlorination was the cause. An outbreak in Finland in 1998 produced between 1,700 and 3,000 cases (Kukkula *et al.*, 1999) and again inadequate chlorination is thought to have contributed to the survival of the virus in the water.

An outbreak in 1994 in South Wales was reported by Brugha *et al*., (1999). The outbreak related to a bakery. Epidemiological evidence implicated drinking water as the source for the staff at the bakery and eating custard slices for over 100 cases in the community. The custard for the slices was made up from powder and cold water. Outbreaks have also been reported via contaminated ice, stored water on cruise ships borehole water and contaminated recreational water. Recreational exposure from swimming and canoeing has also been described (e.g. Gray *et al*., 1997). Many outbreaks are due to NLVs in shellfish grown in polluted water.

Caliciviruses cannot be cultured and molecular techniques using reverse transcription PCR have to be used for detection. There is little information about survival in the environment or the efficacy of water treatment. Early studies on the detection of NLVs in seeded water samples (Woolfaardt *et al*., 1995) have recently led to reports of naturally occurring NLVs in water, Wyn-Jones *et al*., (2000) who

further detected NLVs in a variety of matrices including sewage, effluent and river water. Keswick *et al*., (1985) described human infectivity studies in the inactivation of Norwalk by chlorine. Their conclusion was that 3.75 mg per litre of chlorine inactivated poliovirus, rotavirus and bacteriophage but failed to inactivate Norwalk. However, as most waterborne outbreaks of disease arise from polluted water with unknown concentrations of chlorine, the real contributions of the resistance of NLVs to chlorine and the protective effects from organic matter in the water are undetermined. The literature would suggest that sewage contamination of surface or groundwater and subsequent consumption without their being any treatment or disinfection is the most likely cause of waterborne outbreaks of disease. In a recent review document, Schaub and Oshiro (2000) note that caliciviruses are on the United States EPA 'contaminant candidate list' for regulatory consideration in drinking water. However, because of the difficulty in culture and establishing infectivity, it may be some time before any definitive studies enable us to establish the efficacy of water treatment and disinfection in removing these viruses from raw waters.

HEV causes acute hepatitis, being endemic in parts of Africa, most of Asia, the Middle East and central America. Imported cases have been reported in Europe and North America. It has an incubation period of between 5 – 6 weeks. Children usually develop sub-clinical symptoms whilst adults between 15 and 40 develop jaundice which can last for many weeks. Hepatitis E virus appears to be responsible for the majority of hepatitis cases that occur in Asia. The mortality rate is low (0.1 – 4%) but in pregnant women can be as high as 20%. The virus is more sensitive than other caliciviruses, though it will pass through the stomach to cause infection. HEV has a higher autumn incidence in temperate zones and during the rain season in tropical zones. Unlike other caliciviruses, HEV can have reservoirs in mammals including pigs (Clayson *et al*., 1995).

The majority of HEV outbreaks have occurred in India, Pakistan and Somalia (Morris 1997). The largest outbreak occurred in Delhi in 1955/56 due to sewage contamination of a water supply. Estimates are of over 97,000 cases with 90 deaths. An outbreak in Somalia in 1988 caused over 2,000 cases with nearly as many deaths suggesting that different strains of the virus may have varying virulence.

RT-PCR has been used to detect HEV directly from processed water and sewage samples,

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Enteroviruses

The enteroviruses belong to the family Picornaviridae and are small, non-enveloped, icosahedral particles measuring 25 – 30 nm in size. The genome is positive-stranded RNA. Four main groups are recognised:

- Poliovirus (serotypes 1-3)
- Coxsackievirus A (serotypes 1 – 22, A24)
- Coxsackievirus B (serotypes 1 – 6)
- Echovirus (serotypes 1 – 9, 11 – 27, 29 – 33).

Poliomyelitis, which was a major disease problem in the mid 20th century has been eradicated by vaccination from the western hemisphere and the World Health Organisation has a programme to eradicate the virus from the world by 2004. In areas where the disease has been eradicated, any poliovirus isolated from environmental sources will therefore be oral polio vaccine (OPV) derived from human faecal material. As eradication progresses it will remain necessary to monitor for this virus for an as yet-to-be defined period on public health grounds since live strains of OPV can revert to the neurovirulent form and tracking such revertants will be important in the event of an outbreak. In addition, there is a necessity to monitor the decline of OPV shed in the community.

Coxsackievirus A strains do not grow well in tissue culture so less is known of their environmental occurrence. The three other groups grow readily in cell cultures of primate origin and have been widely studied. Enteroviruses have been used as the principal parameter for monitoring viral pollution of recreational waters following the implementation of the European Union Bathing Water Directive in 1976. This is because, although they cause relatively little waterborne disease, they are robust, easier to concentrate from samples than other viruses and most grow in cell culture with readily identifiable cytopathic effects.

Coxsackie and echoviruses cause a wide range of symptoms in humans. Most infections result in mild or asymptomatic illness, mainly in children. However, symptoms such as meningitis, myocarditis and respiratory illness occur in infants and young children and they are the most common cause of aseptic viral meningitis in developed countries.

The incubation period for enteroviruses varies considerably though it is usually less than 5 days. Respiratory infections usually have an incubation period of 2 – 3 days. Infected individuals often excrete the virus several days before symptoms appear and often for several weeks after symptoms have resolved. The incidence of infection is greater in young children and lower socioeconomic groups. Humans are the only reservoir for human enteroviruses. Most infections are transmitted by the faecal oral route or the respiratory route.

Enteroviruses will grow in primate cell tissue culture with cytopathic effect (CPE). Poliovirus and Coxsackie B virus form plaques in monkey kidney cell cultures such as Buffalo Green monkey kidney (BGM), but echovirus vary in this respect and are better detected in liquid culture, or in human rhabdomyosarcoma (RD) cultures. BGM cultures are most usually used for water and environmental samples using either a monolayer plaque assay or suspended cell plaque assay method though liquid culture techniques such as most probable number (MPN) or ID_{50} can be used. Concentration of virus from water samples is usually done by adsorption onto a suitable matrix followed by elution in a beef extract or skimmed milk solution. They can be isolated from sewage with ranges from $10^2 – 10^5$

per litre, surface waters, groundwaters, recreational waters, shellfish and crops irrigated with wastewater. A small number of waterborne outbreaks are listed by Morris, (1997), four of which relate to recreational waters and swimming pools, two to drinking water and one to ice made from drinking water.

The survival of enteroviruses in water is dependent on many factors including temperature, but below 5 ^{o}C they may survive for years. They are readily removed by conventional water treatment and effective disinfection.

The current literature would suggest that enteroviruses are not a waterborne problem in the United Kingdom but they might be much more of a problem in developing countries.

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Hepatitis A Virus

Hepatitis A virus (HAV) is a typical picornavirus, and is generally regarded as an enteric virus due to its mode of transmission, despite its having hepatotropic properties. It is a non-enveloped, icosahedral particle 28 nm in size with a single-stranded RNA genome. Only one serotype of HAV exists containing a number of genotypes. It is generally regarded as amongst the most robust and environmentally resistant enteric viruses.

HAV causes infectious hepatitis. The incubation period ranges from 15 – 50 days with a median of 28 – 30 days and symptoms typically last for 4 weeks with a range from 2 weeks to 6 months. Infection is followed by invasion of the blood stream and localisation in the liver. Typical symptoms include malaise, loss of appetite, dark urine, nausea and vomiting. Jaundice and a tender liver also occur. The virus is shed in the faeces in large numbers beginning two weeks before the onset of symptoms, thus making food handlers a serious hazard in transmitting the disease. It declines during the acute phase of the illness but can continue for weeks. Liver damage is reversible and infection induces life-long immunity. Serum levels of IgM appear during the acute phase of infection and decline as IgG levels rise. Most infections in young children (>90%) are asymptomatic and severity of disease increases with age. Death is rare. Seroprevalence in the United States is about 10% in young children and around 50% in young adults. In developing countries, exposure is much more frequent in young age and the seroprevalence in young adults is around 100%.

The virus is spread via the faecal-oral route; day care centres and overcrowded communities with poor sanitation and hygiene are important settings for HAV transmission. Male homosexual and intravenous drug use communities as well as institutions such as prisons can act as reservoirs of infection. Other sources of exposure include faecally contaminated food and water. Many foodborne outbreaks have been documented. These include salads and sandwiches prepared by infected persons and bivalve molluscan shellfish grown in sewage polluted water and eaten raw or only partially cooked. In 1988, clams taken from water off Shanghai and eaten raw resulted in nearly 300,000 cases of HAV. There are between 20 – 30,000 cases of infection in the United States annually. In a study in 1989, personal contact (26%), attendance at a day care centre (14%), injecting drugs (11%), foreign travel (4%) and food or waterborne infection (3%) were identified as principal sources of infection. Forty two percent of cases had no known risk association (Shapiro *et al.*, 1992).

In temperate regions where HAV is not endemic, it will only be present in the environment when there is an outbreak in the community. HAV is unlikely to be present in a detectable quantity in sewage and surface water in countries of low prevalence. Fogarty *et al.*, (1995) did not detect an increased incidence of clinical hepatitis after sewage polluted drinking water. However, the concentration and detection of HAV in sewage in Germany has been reported by antigen capture RT-PCR (Graff *et al.*, 1993). Antibody was used to bind HAV to a reaction tube that was subsequently assayed by RT-PCR.

A waterborne outbreak was described by De Serres *et al.*, (1999) associated with the consumption of well water contaminated from a cesspool. Patients, the well and the cesspool were all positive for HAV. In addition, HAV was detected in wells up to 60 metres from the cesspool and in the original well 6 months after the initial contamination, in the absence of coliforms. An outbreak of HAV was reported by Bloch *et al.*, (1990) relating to a private well serving a trailer park in north Georgia. Of 18 residents who were serosusceptible, 16 developed HAV. HAV was demonstrated in the water. An outbreak in Meade County in Kentucky in 1982 was related epidemiologically to the consumption of unboiled spring water (Bergeisen *et al.*, 1985). Two waterborne outbreaks from 1991 – 1992 were

reported in the United States. Morris, (1997) found numerous evidence of outbreaks related to drinking water during the last century. Interestingly, one reported outbreak occurred in Kings Lynn in 1895 when 34 cases were associated with the community drinking water supply. There have, however, been no recorded outbreaks of HAV in the United Kingdom in the last century related to public or private drinking water supplies.

HAV is one of the most persistent viruses in the environment. Inactivation rates in faeces, sewage, soil, water and sediments is slow and the virus may persist for years (Sobsey, 1999). HAV is relatively resistant to high temperatures and may survive some pasteurisation conditions. HAV is resistant to desiccation, survives well in soil and from septic tanks, can contaminate groundwater. Most outbreaks described in the States derive from untreated or inadequately treated groundwater that becomes contaminated from deficient on-site sewage treatment and disposal systems.

Conventional water treatment processes can reduce numbers in raw water by up to 99%. Disinfection of treated water with free chlorine, chlorine dioxide, ozone or ultra-violet light can achieve 99.99% inactivation.

Cell culture has been used to demonstrate the presence of HAV in water supplies during investigation of an outbreak. However, environmental HAV grows poorly in cell lines without producing cytopathogenic effect. In addition it can take weeks or months to demonstrate virus replication. RT-PCR has been used to detect virus nucleic acid in water in an outbreak (De Serres *et al*., 1999) and this would be the method of choice.

The literature would suggest that conventional treatment processes are adequate to remove and inactivate HAV. Evidence from the United States suggests that untreated, inadequately treated or chlorination only groundwater is at greatest risk. Although faecal contamination may be evident in these cases at the onset of an outbreak, the virus has excellent survival properties and may survive long after conventional faecal indicators have died out. There is no evidence that HAV is transmitted in the United Kingdom through drinking water and Regulatory monitoring is not required. Identification of a laboratory capable of concentrating HAV from water samples and its detection using RT-PCR would be of value should the need ever arise, particularly in relation to private water supplies.

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Rotavirus

Rotaviruses belong to the family Reoviridae and are double-stranded RNA viruses with a double-layered protein coat. There are seven groups of rotavirus designated A – G. Groups A, B and C are found in humans and animals and the remaining groups are only found in animals. By far the greatest number of human infections are due to group A rotavirus. The nucleic acid is double-stranded RNA and is surrounded by a double-layered protein coat. They are icosahedral in shape and measure 65 – 75 nm in diameter. The genome is divided into 11 segments which allows the potential for genetic reassortment and thus the emergence of novel strains.

Rotaviruses are the leading cause of acute viral gastroenteritis and subsequent death in infants. Outbreaks amongst the elderly and immunocompromised individuals also occur. In the United States, 3.5 million cases and 125 deaths in young children occur annually. In developing countries where sanitation and hygiene are poor, the incidence is much higher. As many as 10% of the infant population may be affected. Rotavirus also affects adults and is thought to be a primary cause of traveller's diarrhoea. Sixteen thousand three hundred and forty five reports of rotavirus infection were diagnosed in the United Kingdom in 2001 (CDR). .

The incubation period is usually less that 48 hours, and the illness may last for 4 – 8 days. Symptoms are typically fever, vomiting and diarrhoea. Fever and respiratory involvement may be seen in children but milder symptoms occur in adults. Dehydration may also be present. The virus is shed in large numbers, up to 10^{10} per gram of faeces, (Abbaszadegan, 1999) during illness but not usually longer than 8 days.

Infection is spread primarily by the faecal-oral route but may also be spread through contaminated food and water. Most infections are seasonal and occur in the winter in temperate climates, but they occur all the year round in tropical climates. The virus is heat sensitive with 99% inactivation at 50^{o}C for 30 minutes. It is stable between the pH ranges 3 – 10 and can survive for many days on vegetables at 4^{o}C or 20^{o}C. The virus has been detected in sewage, surface waters and groundwaters. Kirk and Chadha, (1989) detected rotavirus in groundwater receiving septic tank effluent in the presence of coliforms but with no *E. coli*.

Clinical diagnosis is by the detection of rotavirus particles shed in faeces during infection using ELISA or electron microscopy. However, none of these approaches are practical to detect the level of virus in sewage or water since the filtration method used to concentrate other viruses does not work well with rotavirus and detection is difficult due to the fastidious nature of rotavirus replication in cell culture (Abad, 1998). Molecular methods are beginning to be used for detection (Lodder, 1999) of the virus genome, but it remains difficult to distinguish between complete infectious particles and the more robust incomplete particle found in water.

Rotavirus has also been detected in recreational waters, marine waters and shellfish growing waters. It can remain infectious for up to a week in faeces at room temperature and weeks to months in water, depending on water quality and temperature. The majority of foodborne outbreaks have occurred in Japan and New York State. Large waterborne outbreaks have occurred. The largest recorded outbreak occurred in China in 1982/83 affecting over 18,000 people (Tao *et al*., 1984). Another outbreak in Germany affected 11,600 people when infiltration of river water into groundwater and failure of chlorination was the cause (Walter *et al*., 1982). During waterborne outbreaks, adults as well as children can be affected. In an outbreak in Vail, Colorado, the attack rate in adults was nearly 44%.

Rotavirus are effectively removed by water treatment and disinfection. They are susceptible to disinfection with free chlorine, ozone and ultra-violet light.

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Bacteriophages

Bacteriophages (phages) are viruses which infect and multiply specifically in bacteria. Those which infect bacteria of the coliforms group are called coliphages. Bacteriophages, including coliphages, are excreted by a certain percentage of humans and animals all the time and may therefore be found in sewage effluents and water. Many are structurally similar to enteroviruses and in addition, the survival of some bacteriophages in water and their removal by water treatment and disinfection is similar to that of enteric viruses. For these reasons, they have been proposed as indicators of the presence of enteric viruses (Havelaar *et al*., 1993).

Enteric viruses will only be excreted by infected individuals and their incidence in sewage and surface waters will vary depending on the incidence of disease in the community. Additional confounding factors include the vaccination of populations against viruses and variations in seasonal distribution (Grabow, 2001). In this respect coliphages cannot be an absolute indicator for the presence of enteric viruses. Enteric viruses have been found in treated drinking water which have negative tests for bacteriophages (Morinigo *et al*., 1990). Phages may also carry genetic material which converts harmless bacteria into pathogens. One such example is the shiga toxin-converting phage involved in the pathogenicity of *E. coli* O157:H7 (Muniesa and Jofre, 1998). This may be seen as an additional reason for excluding phages from drinking water.

Coliphages can be divided into two groups both of which can be found in sewage and faecally contaminated water. Somatic coliphages attach to bacteria using receptors on the cell wall. They may infect a number of environmental bacterial species which may not be related to bacterial contamination. In addition, they can replicate in bacteria under environmental conditions and are therefore of limited use as an indicator. F-specific RNA coliphages infect bacteria by absorption to the F or sex pill. They may be found in high numbers in sewage and have been used as an indicator of faecal contamination, the efficacy of water treatment and disinfection and the contamination of groundwaters. Sex pill are only produced at temperatures above 30^{o}C and therefore F-specific coliphages will not replicate in host bacteria under normal environmental conditions.

F-RNA coliphages have been classified into four serogroups designated I – IV and these are selectively excreted by humans or animals. Serogroups I and IV are found exclusively in the faeces of animals. Serogroup III phages are found in human faeces and serogroup II phages have been found in human faeces and 28% of porcine faeces. They have therefore been proposed as a useful indicator to differentiate human from animal faecal contamination (Grabow, 2001).

There is a substantial amount of data on the incidence of phages in the environment. Counts of somatic and F-RNA phages as high as 10^5 per litre have been recorded for lake and river water (Grabow, 2001). They have also been isolated from sea water and used as an indicator of the presence of entertic viruses in shellfish (Grabow *et al*., 1999). Counts of somatic coliphages in sewage are of the order of 10^6 – 10^8 per litre and slaughterhouse waste may contain up to 10^{10} per litre. Counts of F-RNA coliphages in sewage are generally two to five times lower (Havelaar and Hodgeboom, 1984, Havelaar *et al*., 1990). Given the arguments for and against the use of phages as indicators, Grabow, (2001) sees their advantage as models or surrogates in laboratories wishing to compare them with enteric viruses under controlled conditions.

A method has been developed for the detection of F-specific coliphages in water (ISO 1995). It consists of a culture of *Salmonella typhimurium* which has been engineered to incorporate a gene for the production of the F-specific pilus. In addition, the genes coding for pathogenicity have been deleted.

Use of this strain, designated WG49, is designed to minimise infection by somatic coliphages. The strain is grown in a specific growth medium using specific growth conditions and assayed using an agar overlay technique. F-pili are only produced under exponential growth and the method used controlled growth conditions to demonstrate a susceptible host. The host bacterium and sample are inoculated into a semi-solid agar which is overlaid onto a solid agar plate. Phage particles in the overlay replicate in the host bacterium causing plaques or areas of clearing in the overlay. Each assay must incorporate a known F-specific bacteriophage as the host bacterium can loose the F-pilus gene.

Bacteriophages can be plated directly onto agar but the volume which can be asasayed is characteristically one ml. Increasing the size of the Petri-dishes can increase the volume assayed to 10 ml. Grabow and Coubrough, (1986) describe a direct plating technique using 100 ml volumes of test sample. A presence absence test using 500 ml volumes has also been described. Other concentration techniques which have been used are adsorption/elution, glass powder, electropositive filters, glass wool, ultra-filtration, flocculation and hydro-extraction. The advantages and disadvantages of these methods have been reviewed by Grabow, (2001).

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THE FUNGI

A wide range of fungi are present in soil and water. Fungi may also be detected in drinking water. Fungal spores will pass through water treatment systems and are unaffected by chlorine as a disinfectant. Fungal spores may become incorporated into biofilms and as such develop mycelium. Limited organic nutrient and low temperature will restrict growth but significant growth may impart unwanted tastes to water. Fungal spores may also colonise water fittings in buildings and, under suitable temperatures, produce large mats of mycelial growth. Such occurrences are more likely to occur in cold water storage tanks which are not lagged and covered.

Of additional importance is the fact that fungi can produce secondary metabolites. The *Aspergillus* and *Penicillium* spp. produce secondary metabolites when allowed to grow in food. *Aspergillus* spp. produce aflatoxins which are both acutely and chronically toxic to humans. Aflatoxins produce acute liver damage, liver cirrhosis, tumour induction and teratogenesis. In addition they can also cause immunosuppression. Nearly 100 species of *Penicillium* also produce toxins with a wide range of effects. There are a number of other toxigenic molds which can be found in soil, water and on decaying plant materials. Antibiotics are also a secondary metabolite.

Mycotoxins are produced as a result of growth on an abundance of organic material (for example on nuts). Little is known about the ability of fungi to produce toxic metabolites under oligotrophic conditions in water. This is compounded by the fact that species identification is difficult but important. In many circumstances, the taxonomy of fungi is incomplete. In addition strains in the same species can produce different metabolites. Furthermore, strains which are known to produce toxins in food may not produce significant amounts in association with water. A full list of fungi known to produce toxic metabolites has been reviewed by Paterson *et al*. (1997).

Secondary metabolites in drinking water could come from a number of sources. Low levels of mycelial growth may not produce sufficient metabolites to present even a chronic low-level exposure. However, small areas of heavy growth, for example in water tanks, might produce sufficient metabolite to provide chronic exposure. Secondary metabolites may also gain access to water from the environment. Fungi growing on vegetation or grain might produce sufficient metabolite for its access to raw water during rainfall and surface runoff. There is no information on the effect of water treatment on the removal of fungal metabolites. However, to take a similar analogy, actinomycetes can produce earthy tastes in water as a direct growth either in surface waters or more likely the environment around a water source. Subsequent rainfall may wash these metabolites into water and these can be distributed in the treated water.

Patterson *et al*. (1997) studied water systems in the United States for known species which produce secondary metabolites. A wide range of fungi were isolated. In addition, water from a contaminated storage tank was analysed for aflatoxin. On one occasion, B2 at 1.3 μg per litre and on a second occasion B2 at 0.2 μg per litre and G2 at 0.1 μg per litre were found. *Aspergillus flavus* was isolated from the tank. Aflatoxins were not found in the mains water supplying the tank.

The research work shows that fungi producing secondary metabolites can be isolated from drinking water. In addition, such fungi can colonise water systems and produce secondary metabolites. Nothing is known of the existence of such metabolites in raw waters at different times of the year nor the effect of water treatment on their removal. The isolation of fungi from water is relatively simple. Media such as rose bengal chloramphenicol agar and wort agar are available commercially. Such media may be used occasionally on distributed water samples where complaints of musty or earthy

tastes arise. The identification of fungal genera and species is much more difficult and few water companies would have the expertise to determine the precise identification of any isolate. In addition, detection and characterisation of secondary metabolites using gas chromatography may not be straight forward. It would seem useful, however, to determine whether secondary fungal metabolites might be present in raw waters, particularly during summer months and heavy rainfall events. Examination of water samples from distribution systems, particularly consumer premises, during periods of taste complaints might also be useful. Failure to demonstrate the presence of metabolites might not preclude their presence altogether but would provide evidence that under circumstances where metabolites might be present, they could not be found.

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APPENDIX 4

SCIENTIFIC BASIS FOR CHEMICAL PARAMETERS

Scientific Basis for Revision or Introduction of Standards for Chemical Parameters

Introduction

There are several different aspects to the scientific basis for revision of existing standards or introduction of new standards for chemical parameters in drinking water. The primary information relates to occurrence or likely occurrence in drinking water. Should a substance be unlikely to reach drinking water in sufficient quantity to be of concern then introduction of a standard would not be justifiable on scientific grounds. However, it may not be possible to provide sufficient reassurance to consumers should the contaminant be of high public concern and there may be quasi-scientific or political drivers that will over-ride the scientific data.

Should the data on occurrence support the need to develop a standard, the data on toxicity or, in some cases, impact on consumer acceptability are particularly important, since these form the basis for developing a numerical standard. In some circumstances, there may be uncertainties in the data that result in a more stringent standard than might otherwise be necessary if the data were sufficient to reduce those uncertainties. In other circumstances, the data may be inadequate to develop a scientifically based standard but the political concern may be sufficient that a precautionary standard be set. In some cases, such as the current standard for pesticides in Europe, the standard is set as a precautionary political standard but the urgency of the actions to be taken in the light of an exceedence of the standard are informed by scientifically based guidance values.

A third aspect of scientific data relates to the methodology available for the control, removal/reduction by treatment and measurement of the substance. This may inform a political modification of a standard in order to make it practical or may influence the timetable for its introduction, with a series of interim standards, such as those for lead and total THMs in the revised drinking water directive.

The scientific basis for high and medium priority chemical parameters are considered below with some discussion of low priority parameters that could change priority due to political considerations.

Tri and Tetrachlorethene (High Priority)

In the 1993 revision of the WHO Guidelines for Drinking Water Quality, guideline values for both tri and tetrachloroethene were proposed on the basis of a TDI approach. The view of the expert committee was that carcinogenicity in laboratory animals was through a non-genotoxic mechanism and did not justify the use of the linearised multistage model to determine risk. At that time IARC had placed tetrachloroethene in category 2B, possibly carcinogenic to humans, on the basis of animal studies and trichloroethene in category 3, not classifiable as to its carcinogenicity to humans. There are new data on both of these substances and Health Canada will prepare revised background documents for the evaluation. However, IARC have also re-examined both of these compounds and placed them both in category 2A, probably carcinogenic to humans. This was a controversial evaluation because IARC, in its examination of epidemiological studies, drew different conclusions to the authors of the original studies. The key issue is, therefore, the evidence for carcinogenicity in man and the mechanism by which these substances might cause cancer. Currently, it would seem that the balance of evidence would support a non-genotoxic mechanism of action, or at least a mechanism that is highly non-linear that would normally dictate the use of a TDI approach. However, the data are not entirely clear cut and it is probable that the Commission will seek the views of the SCTEE.

The USEPA is also in the process of revising their risk assessment for trichloroethylene and might be expected to take a more conservative stance than WHO did in 1993 and may well decide to use a mathematical model to estimate additional cancer risk..

1,1,1-Trichloroethane and Dichloromethane. (Medium but could be included with tri and tetrachloroethene)

WHO reviewed both of these substances in 1993. The German Environment Ministry is examining the database on 1,1,1-trichloroethane to determine whether there are sufficient data to justify re-evaluation, otherwise it will not be considered at this time. Dichloromethane was included in the USEPA candidate contaminant list but was not one of the parameters to be considered further at this stage. The scientific data do not seem to warrant specific attention but should European countries find traces in groundwater then this could raise the issue from an occurrence standpoint. In that event there could be attempts by some member states to include them in a precautionary standard with tri and tetrachloroethene.

Organic Disinfection By-Products – Haloacetic Acids (High priority all other organic DBPs low priority)

There are many organic by-products of disinfection but most are present at very low concentrations. In the event that total THMs are shown to be inadequate as a surrogate for all chlorination by-products then the haloacetic acids are those substances that can be present at concentrations equivalent to the THMs but which are formed under conditions that do not favour the formation of THMs. The USEPA have set a standard for total haloacetic acids of 60 μg/l under the negotiated rule-making but the scientific basis for this is limited. In the 1993 edition of the WHO Guidelines provisional values were proposed for di and trichloroacetic acid of 50 μg/l and 100 μg/l respectively on the basis of liver toxicity in laboratory animals. However, the provisional designation reflected the limitations in the toxicity database. Since then more data has been generated and in 1998 an IPCS task group considered the data available on chloro and bromoacetic acids. It was concluded that the mechanisms of toxicity were complex but TDIs of 40 μg/kg body weight were proposed for both dichloroacetic acid and trichloroacetic acid. These values would give rise to guideline values of 240 μg/l assuming a 60 kg person drinking 2 ls water per day and allocating only 20% of the TDI to drinking water as in the 1993 Guidelines. Although data on the brominated acetic acids were considered to be very limited, there were good reproductive toxicity data available for dibromoacetic acid and a TDI of 20 μg/kg body weight was proposed on the basis of irreversible effects on the testis. This would give rise to a guideline value of 120 μg/l if applied to an adult, but the developing testis may be more sensitive to these effects and a lower guideline value might be appropriate to take into account increased water intake in relation to body weight in infants and children. This could tighten the guideline value to 20 μg/l. However, it is possible that another group would consider a higher uncertainty factor to be appropriate thus tightening the guideline value even further. The reproductive effects could raise the profile of all haloacetic acids and lead to proposals for a total haloacetic acid standard, similar to that in the United States. There seem to be relatively few data in the public domain on the occurrence in UK waters of the haloacetic acids, particularly the bromoacetic acids following significant moves to reduce the concentrations of THMs.

The greatest uncertainties for the organic DBPs associated with chlorination appear to lie in the current research activity on the association with adverse birth outcomes. Although the data so far do

not yet provide adequate evidence for a causal association, there is a considerable amount of research in progress on this topic (Nieuwenhuijsen et al 2000, Waller et al 2001).

WHO. International Programme on Chemical Safety. Environmental Health Criteria 216. Disinfectants and Disinfection By-products. WHO, Geneva. 2000

Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Elliott, P. and Fawell, J. (2000) Chlorination disinfection by-products in water and their association with adverse reproductive outcomes: a review. Occupational and Environmental Medicine. **57:** 73-85

Waller, K., Swan, S.H., Windham, G.C. and Fenster, L. (2001) Influence of exposure assessment methods on risk estimates in an epidemiological study of total trihalomethane exposure and spontaneous abortion. Journal of Exposure Analysis and Environmental Epidemiology. **11**: 522-531

Inorganic Disinfection By-Products – Bromate, Chlorite and Chlorate (Chlorite high priority, chlorate medium priority, bromate low priority)

Both WHO and USEPA have examined the more recent data on bromate and there is an increasing amount of data to support the view that the dose response for bromate carcinogenicity is highly non-linear. The consequence of this is that these recent evaluations have proposed values that would be above the current European standard of 10 μg/l. In effect this means that any pressure to tighten the standard on health grounds will be significantly reduced. WHO considered bromate in the IPCS task group meeting of 1998 and proposed a TDI of 1 μg/kg body weight based on an uncertainty factor approach. This compares to a risk based value of 0.1 μg/kg bodyweight derived from the use of the linearised multistage model with an excess cancer risk of 10^{-5}. The USEPA have recently published a new assessment of bromate in which they also use a TDI approach and derive a reference dose (their name for a TDI) of 4 μg/kg body weight per day for chronic oral exposure. These values would give a drinking water guideline value in the region of greater than 25 μg/l.

There is a considerable body of new data for chlorite since the WHO evaluation that resulted in a provisional guideline value of 200 μg/l. This has significantly reduced many of the uncertainties associated with chlorite toxicity. The USEPA has taken account of these new data and have proposed an MCL for chlorite of 1000 μg/l with an MCLG of 80 μg . WHO in their 1998 evaluation of disinfectants and disinfection by-products by IPCS also proposed a TDI of 30 μg/kg body weight, the same as that determined by the USEPA in IRIS, although there remain some uncertainties as to the validity of the toxicological endpoint as a consequence of apparent errors in the study report. This would give rise to a guideline value of 750 μg/l assuming an allocation of 80% of the TDI to drinking water, as in the 1993 edition of the Guidelines. It would however, be necessary to ensure that the data have been correctly reported from the industry studies.

The toxicity database on chlorate is not yet complete but studies are under way. The data emerging at this stage indicate that chlorate is less toxic than chlorite and there are also data that indicate that chlorate is unlikely to be carcinogenic at less than toxic concentrations.

There appears to be adequate data to set a standard for chlorite and increasing interest in chlorine dioxide as a disinfectant would be reason to introduce a standard. Such a standard could be met by the UK approach of controlling the applied dose with minimal requirements for monitoring final water.

WHO. International Programme on Chemical Safety. Environmental Health Criteria 216. Disinfectants and Disinfection By-products. WHO, Geneva. 2000

Endocrine Disrupters (High priority)

Many substances have been identified as potential endocrine disrupters and a number have been shown to cause endocrine disruption in aquatic organisms, but very few have been shown to cause endocrine disruption in humans, particularly at the concentrations found in the environment. Currently there are few data that would indicate that drinking water is at risk from contamination with significant concentrations of substances that have been shown to possess endocrine disrupting properties in intact animals. The USEPA endocrine disrupting substances advisory committee (EDSTAC) have estimated that in the region of 87,000 substances need to be tested but little progress has been made at this time. A number of widely used industrial chemicals are suspected of endocrine disrupting activity, including phthalates, alkyl phenols and bisphenol a derivatives. Phthalates and bisphenol a were listed as high priority in a SCTEE document in July 2001. A number of studies are in progress that will help to provide appropriate data with which to carry out a risk assessment. WHO have proposed guideline values for diethylhexyl phthalate and diethylhexyl adipate on the basis of toxicological endpoints that were unrelated to endocrine disruption, but which appear to occur at lower doses than testicular atrophy. However, there is evidence that the mechanism of toxicity resulting in testicular atrophy is not true endocrine disruption. Currently no other authorities appear to be planning to try and set standards or guidelines for these substances on the basis of endocrine disruption.

There have been suggestions that a bioassay should be used as a screening tool but the difficulties and uncertainties associated with this are unlikely to result in their being any significant support for this move. Many of the suspected endocrine disrupters are pesticides for which there is already a precautionary standard and a number of others such as dibenzodioxins are highly lipophilic and do not appear to be found in drinking water. The Commission has let a contract with the Fraunhofer Institute in Germany to make an assessment of endocrine disrupters in drinking water but this study has only recently got underway.

Radon (High priority)

The concern over radon relates to its radioactivity and the fact that it appears to remain in drinking water for longer than was previously thought. That radon is a carcinogen is not in doubt but there may be difficulties in taking total exposure into account in regions where there is significant seepage of radon gas into properties through the ground. However, proposals for standards have already been made in both Europe and North America and there seems to be sufficient scientific evidence that a standard would be beneficial and what that standard might be. However, consideration would need to be given as to how such a standard could be implemented. It is known that there is considerable concern over radon and its daughters in Scandinavia although indoor air is considered to be a much bigger problem than water. There is a draft recommendation for limits for radon and its daughters, ^{210}lead and ^{210}polonium. It is uncertain whether it will be considered necessary to include these in a standard but they are most likely to be incorporated with a standard for radon, rather than be covered by separate standards. It still remains uncertain whether there is a scientific justification for a standard for public water supplies, although it is known that there are significant problems associated with private supplies in some areas of the UK.

Uranium (High priority)

Uranium is of concern because it is a human kidney toxin and standards set to protect against kidney toxicity would be tighter than those set to protect against radioactivity. The evidence that uranium is a kidney toxin to man is quite clear, however the lowest concentration at which this occurs is uncertain. In addition there are potential issues with regard to those with reduced kidney function, including older people, and the period of exposure required to induce clinically significant effects. The uncertainties in the database caused WHO to propose a provisional guideline value of 2 μg/l in 1997. Subsequently Health Canada and USEPA proposed standards of 20 and 30 μg/l respectively based on the same study as WHO but with a larger proportion of the TDI allocated to drinking water. WHO were proposing to await the results of epidemiological studies from Canada but in view of the concern regarding uranium in drinking water there is a proposal to bring the review forward to the current round. This would take account of the USEPA and Canadian evaluations.

Aluminium (High priority)

Although there have been many epidemiological studies on aluminium and adverse neurological effects, particularly Alzheimer's Disease, the data are far from conclusive. WHO considered aluminium through the International Programme on Chemical Safety who concluded, "There is no evidence to support a primary causative role for aluminium in the development of Alzheimer's Disease". In addition they concluded that "there is an inadequate scientific basis for setting a health based standard for aluminium in drinking water".

In France investigators from INSERM in Bordeaux have continued to study populations in that region and have published the latest phase in which they concluded that their study suggested that a concentration of aluminium in drinking water above 0.1 mg/l may be a risk factor of dementia, especially Alzheimer's disease. However, there were a number of serious criticisms, including poor measurement of actual exposure, small numbers in the highest exposure group and no dose response (Rondeau et al 2000).

Recently there have also been a number of studies on aluminium bioavailability that have used ^{26}Al to obtain a much more sensitive measurement of aluminium uptake. These studies in the UK and Australia indicate that the absorption from drinking water in healthy individuals is similar in proportion to the absorption from food. Since intake of aluminium in food is much greater (at least 100 times) than from drinking water, this suggests that less than 1% of absorbed aluminium comes from drinking water Priest et al 1998,Stauber et al 1998).

Health Canada has attempted to develop a health-based standard for aluminium in drinking water but has been unable to do so. Instead they have proposed an operational value of 0.1 mg/l and also concluded that the benefits of using aluminium sulphate in drinking water treatment outweigh the possible small risks to health.

Priest, N.D., Talbot, R.J., Newton, D., Day, J.P., King, S.J. and Fifield, L.K. (1998) Uptake by man of aluminium in a public water supply. Human and Experimental Toxicology **17:** 296-301

Stauber, J.L., Davies, C.M., Adams, M.S. and Buchanan, S.J. (1998) Bioavailability of aluminium in alum-treated drinking water and food. UWRAA Research Report No 202. November 1998

Rondeau, V., Commenges, D., Jacqmin-Gadda, H. and Dartigues, J-F. (2000) Relation between aluminium concentrations in drinking water and Alzheimer's disease: a 8-year follow-up study. American Journal of Epidemiology. 152. 59-66

Arsenic (Medium Priority)

There is currently significant scientific controversy regarding the risks associated with ingested arsenic. It is generally acknowledged that arsenic is a human carcinogen and that it is carcinogenic through ingestion in drinking water. The difficulties arise in two related areas. In the first case there are epidemiological studies that have been published in the peer reviewed literature and which claim to detect various cancers at drinking water concentrations of less than 50 μg/l and in a Finnish one at less than 10 μg/l (Kurttio et al 1999). These studies are mostly quite small and the risks reported are also very small. The results of these studies are open to significant confounding by other sociological and environmental factors and there remains considerable uncertainty as to whether these studies can be concluded to reflect causality or a true outcome under these circumstances.

The second related area is that of extrapolating risks from epidemiological studies to lower exposure concentrations and to other populations. Inorganic arsenic is detoxified in the liver and there is evidence that existing liver damage, nutritional factors and possibly genetic make-up can significantly influence the rate and extent of detoxification. Many of the studies relate to poor populations in tropical countries and the measurement of actual exposure is frequently uncertain. There is also controversy over which tumour types should form the basis of a risk assessment. In addition there is controversy and debate over the shape of the dose response curve and whether linear models of dose response to estimate cancer risks are appropriate. The current debate in the United States regarding the proposed standard of 5 μg/l clearly demonstrates the difficulties. It is doubtful whether science can provide the sort of resolution that may be required but more high quality science would certainly help in resolving the controversies. The USA is currently conducting a debate regarding the risks, benefits and costs of various regulatory concentrations in which there is a danger that the actual risks are being overestimated to justify greater expenditure. The difficulties of removal and of routine measurement would increase significantly as the regulatory level fell below 10 μg/l. EPA have now proposed a standard of 10μg/l following a re-examination of the risks, costs and benefits.

WHO are also re-examining arsenic but it is not predictable at this stage what the outcome will be. In terms of providing assistance to developing countries, WHO are likely to take a particularly cool look at the current data because most of the developing countries would find meeting the current guideline difficult, if not impossible to achieve. The Commission may well seek the views of the Scientific Committee on Toxicity and Ecotoxicity.

Kurttio P, Pukkala E, Kahelin H, Auvinen A and Pekkanen J. (1999) Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. Environmental Health Perspectives **107,** (9): 705-710

Nitrate / Nitrite (Medium priority)

JECFA carried out a review of nitrate and nitrite in 1995. They confirmed the previous ADI of 0-3.7 mg/kg body weight for nitrate ion and established a new ADI of 0-0.06 mg/kg body weight for nitrite. This would give rise to a much lower guideline value if used as the starting point. However, it is not based on methaemoglobinaemia but on effects on the adrenal gland in laboratory animal

experiments. There are a number of questions which remain unanswered, not least of which is the relevance of the rat as a model for man since metabolism of nitrate appears to be significantly different. Man generates and secretes nitrate in saliva and the differences in metabolism between man and laboratory animals needs to be investigated. The WHO expert committee who looked at nitrate/nitrite in 1997 expressed doubts about the JECFA evaluation and nitrate/nitrite have been referred back to JECFA to clarify the uncertainties.

Copper (Medium priority)

WHO changed the basis of the guideline value for copper derived in 1993 in 1997 to an allocation of a proportion of normal daily intake to water. The earlier derivation was based on allocating 10% of the JECFA provisional maximum tolerable daily intake to drinking water. There are two issues surrounding the possible health effects of copper in drinking water. The first is gastrointestinal irritation, which is based on the concentration of copper in the water rather than the dose in relation to body weight. Older data were based on reports of incidents of copper 'toxicity' in man but the details were always uncertain because of the nature of the investigations. More recently there has been a clinical toxicity study in humans carried out in Chile. This study appears to be well conducted and reported a NOEL of about 3 mg/l. The WHO guideline value of 2 mg/l fits well with this value.

The second issue relates to copper induced hepatotoxicity in children. There is a well-established syndrome in children in India in which childhood cirrhosis is related to high copper levels in the body and appears to be related to high copper intakes through food. There may also be a genetic susceptibility associated with this condition. Subsequently, a similar condition has been reported in parts of Germany, but it is unclear what the exact circumstances of this syndrome are and how extensive this is. There have been suggestions that this is due to very high concentrations in drinking water, although this does not correlate very well with gastric irritation, but there have also been suggestions that there is a specific genetic component. At present it remains questionable as to whether the data justify a change in the standard in Europe but there may be a need for guidance as to when the use of copper tubing in domestic plumbing is appropriate.

Algal Toxins (Medium priority microcystins)

Cyanobacteria, or blue-green algae, are normal constituents of the phytoplankton populations of lakes and slow flowing bodies of water. On occasion they can form blooms that consist of millions of cells per ml. Many species produce substances which are extremely toxic to mammals and have caused poisoning of domestic and wild animals in the UK and elsewhere. Over 60% of blooms appear to be toxic. The most common toxins identified in the UK are the microcystins, a family of hepatotoxic peptides that contain a novel amino acid and which vary significantly in toxicity. The most common appears to be microcystin LR but most blooms appear to produce a mixture. Analysis is quite difficult because very few pure standards are available. Reasonably good toxicity data are available for microcystin LR which appears to be one of the most toxic. There is both theoretical and experimental evidence that microcystin LR, and possibly other microcystins, are tumour promoters. Health Canada and WHO have considered guideline values for microcystin LR based on the toxicity data generated in the UK with the joint support of UKWIR and the Department. The value of about 1 μg/l includes a substantial uncertainty factor and was considered to be adequate to take into account the possible risk of tumour promotion. There are issues for analysis with regard to dissolved toxin and toxin that is present inside cells. There is good evidence that activated carbon removes microcystins but simple adsorption requires frequent regeneration. It is probable that biological GAC will remove microcystins since they are readily biodegradable. Ozone rapidly destroys the molecule and chlorine

will remove microcystins after a long contact time in distribution. Previous studies on slow sand filtration have indicated that microcystins are poorly removed but new unpublished data from Finland seems to indicate that there is reasonable removal. A limited survey in England and Wales did not identify microcystin LR or anatoxin a in drinking water but there appear to be no intensive analytical studies on particular supplies derived from vulnerable waters (James et al 1994)
There is a range of other toxins of which the most common in UK/European waters appears to be anatoxin a, which is a neurotoxin. There are data on toxicity, which may be sufficient to develop a guideline value and possibly a standard, but the data are less comprehensive than for microcystin LR. Toxicity data on other toxins are either lacking or studies are still in progress. Some member states are considering setting standards for microcystins based on the WHO provisional guideline value.

James, H.A., Smith, C.J., Sutton, A., Cayley, J., Franklin, O. and Parr, W. (1994) Survey of the concentrations of algal toxins in water supplies. DoE report DoE 3761/1.

Strontium (Medium priority)

Strontium is found in significant quantities in drinking water associated with calcium. There are surprisingly few data but there is a small number of older toxicity studies in laboratory animals. On this basis the USEPA developed a reference dose (RfD) of 0.6 mg/kg body weight per day in 1997 and expressed its confidence in the RfD of medium. The lifetime health advisory value for drinking water is 4 mg/l, which is much lower than the drinking water equivalent level of 20 mg/l. There are gaps in the data but WHO is considering strontium for possible production of a guideline value.

Turbidity (Medium priority)

Turbidity measures all particulate matter in drinking water and is normally measured post treatment. However, turbidity may also be measured immediately post filtration in order to assess the operational efficiency of filters. Since microorganisms are associated with particulate matter, it can be associated with the presence of microbial contaminants. There is no scientific basis for a health-based value for turbidity but microbial contamination is rarely associated with very low turbidities. In addition low turbidity is important in helping to reduce the deposition of particulate matter in distribution. Health Canada have proposed a guideline of 95% of samples less than 0.3 NTU and not to exceed 1.0 NTU at any time where the source water turbidity is equal to or greater than 1.5 NTU.

Petroleum Hydrocarbons (Medium priority)

Petroleum hydrocarbons are a large group of substances of varying molecular weight and water solubility. The smaller molecular weight aromatic components are the most soluble and these are represented by the BTEX compounds, benzene, toluene, ethylbenzene and xylenes, for which there are good toxicological data and for which WHO proposed guideline values in 1993. Petroleum hydrocarbons are frequently detected in drinking water but usually at much lower concentrations than the WHO Guideline values. These substances have relatively low taste and odour thresholds but there is a wide variation between individuals. There does appear to be some synergism between the compounds in terms of odour threshold but the available data are very limited. WHO are preparing a discussion document with regard to spills of petroleum hydrocarbons based on work in the USA but a return to the total hydrocarbons standard from the previous directive seems unlikely on the basis of the science at this time.

Pharmaceuticals (Medium priority)

Pharmaceuticals are excreted by humans and animals. The first data indicating the presence of some pharmaceuticals in drinking water, derived from surface sources with a high level of re-use, was generated in the late 1970s. Since then there have been significant improvements in analytical techniques and recently there has been an upsurge in research interest in this subject. Studies in Europe and some other parts of the world have shown that a number of human pharmaceuticals, primarily those used at high doses for chronic conditions, can be found in surface waters (Halling-Sorenson et al 1998, Ternes 1998). Less is known about the occurrence of veterinary pharmaceuticals. The concentrations are largely less than 1 μg/l and in most cases much less than this. Those that have been identified are present at concentrations that would give an intake many orders of magnitude below the therapeutic dose (Fawell 2000). The database, particularly with regard to final drinking water, is, however, quite limited. The Environment Agency commissioned a review of pharmaceuticals in surface waters and may be considering further research Ayscough et al 2000). Because this subject is of increasing interest to the press and politicians it is important that any decisions are taken on the basis of sound science and increasing the science base with regard to exposure would seem to be appropriate.

Ayscough, N.J., Fawell, J.K., Franklin, G. and Young, W. (2000) Review of human pharmaceuticals in the environment. Environment Agency Report TRP 390.

Fawell, J.K. (2000) Pharmaceuticals in drinking water – issue or scare story. North West Water Water and Health. Issue 29/00. March 2000

Halling-Sorenson, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lutzhoft, H.C. and Jorgenson, S.E. (1998) Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere **36:** 357-393

Ternes, T.A. (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Research **32:** 3254-3260

MTBE (Low priority)

MTBE or methyl tertiarybutyl ether is a fuel oxygenate that has been extensively studied and has gone through the EU existing chemicals risk assessment procedure. The consensus of The EU evaluation, the IARC evaluation and most other health studies is that MTBE is of low toxicity to man and is unlikely to give rise to health risks in man. However, MTBE has a low odour threshold in air and a low taste and odour threshold in drinking water. The USEPA have proposed a health advisory for MTBE on this basis and the California EPA have proposed a secondary standard, a standard based on effects other than health, also on this basis. Unfortunately, setting a standard on this basis is not very scientific because studies in laboratories use trained testers with conditions that are optimised to help sensitivity. Under normal circumstances consumers are not seeking to detect odours and tastes and odours will be to a varying extent be masked by other smells in a domestic or commercial environment. The concentrations that have given rise to complaints in the USA are significantly above the odour and taste thresholds determined in laboratories. However, there are several good quality taste and odour studies available.

Manganese (Low priority)

Manganese has been shown to be likely to cause neurotoxicity at very high concentrations. However, there appears to be little evidence to indicate that this is the case at the concentrations encountered in normal drinking water, even where dirty water problems have been observed. One of the key questions is whether manganese oxides that give rise to dirty water are bioavailable. WHO proposed a provisional health based guideline value on the basis of allocating a proportion of the normal daily intake to water and applying an uncertainty factor of 3 to account for possible increased bioavailability of dissolved manganese in water. The USEPA are currently considering the database on manganese and will determine whether there is sufficient scientific evidence to set a health based MCL.

1,4-Dioxane (Low priority)

1,4-Dioxane is an industrial solvent that is discharged to raw waters in some countries. It has been detected in drinking water at low concentrations in the UK. It is a suspected carcinogen, although there are no adequate epidemiological data to confirm positive results in laboratory animals by the inhalation and oral routes of exposure. There is also some evidence that the mechanism of carcinogenicity may be by tumour promotion, in which case the use of mathematical models to estimate excess risk may not be appropriate. However, based on such an extrapolation by the USEPA, which incorporates a surface area correction, the concentration in drinking water associated with an excess cancer risk of 10^{-6} would be about 3 μg/l.

PAH (Low priority)

PAH are found in drinking water almost entirely due to coal tar linings in water mains. Those of greatest concern are primarily found associated with sediment and particulate matter. The levels associated with the PAH included in the current directive are unlikely to change but there is a question over whether these are the most relevant. Fluoranthene, which is one of the more soluble PAH was specifically excluded because WHO considered it unnecessary to set a guideline value. Although, there are a number of PAH included in the Water Framework Directive, WHO do not propose examining PAH in the current round of revision. They are, however, on the list for consideration under the programme of rolling revision at some future date. The science base is complex and attempts to establish a toxicity equivalents approach to the PAH have not been wholly successful. Good toxicity data are only available on a small number of substances. Since drinking water remains a minor source of exposure to PAH there does not seem to be any significant scientific basis for changing the values in the directive, or adding to the named PAH, at this time.