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HEALTH POLICY BRIEF

MOLECULAR TECHNOLOGIES FOR SAFE DRINKING WATER: RESULTS FROM THE INTERLAKEN WORKSHOP, SWITZERLAND, 5-8 JULY 1998

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FOREWORD

"Molecular Technologies for Safe Drinking Water" summarises an OECD workshop held in Interlaken, Switzerland, on 5-8 July 1998. The workshop was planned by the OECD Working Party on Biotechnology (WPB) under the chairmanship of Dr. David Harper and hosted by EAWAG (the Swiss Federal Institute for Environmental Science and Technology), whose director is Professor Alexander Zehnder. Responsible for the organisation of the workshop were Dr. Mario Snozzi, with the help of Dr. Thomas Egli of EAWAG, and Dr. Salomon Wald, with the help of Dr. Tadashi Hirakawa and Dr. Elettra Ronchi of the OECD. Active support for this workshop was also provided by the OECD Environment Directorate.

Let no one be deceived by the dry technical tone of this document which, it is true, has been written by, and for technical experts. This document is about a life-and-death issue which concerns millions of people every year, mainly children. It is about an enormous, and probably growing, health hazard and economic burden for many hundreds of millions more.

The workshop was attended by leading international experts, including representatives of the World Health Organization. It examined the threat posed by a growing number of water-borne infectious diseases. It compared traditional methods of detection, still valid in certain cases, with the faster, more specific and comprehensive ones derived from molecular biology, or new "biotechnology". It underlined the urgency for public authorities to consider adopting the newer technologies whenever possible.

There is a background to the Interlaken workshop. In 1993, 400 000 residents of Milwaukee, Wisconsin (United States) fell ill, and more than 100 died, from cryptosporidiosis, a gastrointestinal infection caused by a parasite commonly harboured by cattle. In 1996, the American Academy of Microbiology, under Dr. Rita Colwell, now Director of the National Science Foundation, issued a "Call for Global Action", which recalled the Milwaukee incident, warned of serious consequences from deteriorating water quality, and criticised policy makers' complacency.

Mexico heeded this call, and in October 1996, Mexico's CONACYT (National Council for Science and Technology) hosted an OECD/WPB workshop on "Biotechnology for Water Use and Conservation". This workshop, organised by Dr. Efrain Aceves Piña for CONACYT and Dr. Salomon Wald for the OECD, identified the biological quality of drinking water, its contamination by pathogens and the opportunities afforded by biotechnology, as salient present and future issues. At the end of the Mexico workshop, Switzerland proposed to continue to address this issue, at the Interlaken workshop and beyond, with the ultimate goal of bringing about the policy changes required by public health and made possible by biotechnology. It is perhaps symbolic that Mexico and Switzerland have been working together towards the same goal. The safety of drinking water is a source of concern for countries both big and small, both highly developed and still developing.

The full text of the papers presented at the Interlaken Workshop are available on the EAWAG Web site at: http://www.eawag.ch/publications_e/proceedings/oecd.html.

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THE INTERLAKEN WORKSHOP

Setting the scene

Water, particularly drinking water, is a precious resource and necessary to human health and well-being and socio-economic development. Civilisation first arose in areas where ample water resources existed, and the history of mankind has been intimately related to water management.

An essential component of sustainable development is improving health worldwide. For this, clean water is vital. The workshop consequently brought together, for the first time, two major strands of the OECD's activities in the area of biotechnology, those related to health and to a cleaner environment. Not only will developments in one area have broad relevance for the other, but both must analyse the cost-effectiveness of modern methodologies against traditional ones. A holistic approach is essential because of the disease cycle from water to food, to contact between people, and back to water.

The supply of safe drinking water will become ever more important. The global population is increasing primarily in urban areas of the developing world, and it is just these areas that lack this vital resource. Many populations in developing nations suffer from infectious diseases transmitted via contaminated water. Nearly one-half of the world's population suffers from diseases contracted by drinking water of inadequate quality; and there is a clear relation between the poor quality of drinking water and child mortality. The discharge of increasing quantities of wastewater may in fact result in an overall deterioration of water quality. There are other problems as well, such as the ageing of water treatment infrastructures and the increasing occurrence, or perhaps the increasing recognition and detection, of organisms resistant to conventional disinfection treatments.

While water-borne diseases cannot be eradicated – there are too many infectious agents, reservoirs and asymptomatic infected individuals – these agents can be controlled as long as they can be detected and monitored. Coliforms, the traditional indicators of pathogens, are beginning to fail in some cases by giving misleading information; in tropical climates, for example, coliform bacteria are widely present without correlation to faecal pollution. Protozoa and viruses are more resistant to treatment and therefore may not be detected by traditional indicators. There is thus a need for more appropriate methodologies, both for routine monitoring (from resource water through individual treatment steps to the final product, drinking water) and for investigating disease outbreaks. The Interlaken workshop was opened by Prof. Thomas Zeltner, Director of the Federal Office for Public Health, who greeted participants on behalf of Switzerland's federal government and underlined the growing importance of the workshop's theme for his own office. Prof. Alexander Zehnder, who chaired the workshop, proposed that participants should compare current methods for measuring and monitoring pathogens with novel molecular biological methods, identify the role of each method and evaluate its strengths and weaknesses. The goal should be to stimulate co-ordinated research to optimise these methodologies, to encourage international co-operation to develop and standardise methodology, and to promote the introduction of improved technologies.

Mr. Risaburo Nezu, Director of Science, Technology and Industry at the OECD, and Dr. David Harper, in his capacity as Chair of the OECD Working Party on Biotechnology, presented greetings on behalf of the OECD. Mr. Nezu noted that the workshop would contribute to sustainable development work at the OECD and to improving links with the World Health Organization (WHO), and would be informative for public health and environment agencies. Dr. Harper encouraged delegates to relate different health areas, for example drinking water and diseases such as tuberculosis. Bringing academia, industry, regulators and policy makers together would make it easier to get policy-relevant messages to the right audience.

Prof. P. Payment of Canada spoke on behalf of Dr. Joan Rose and presented, in his keynote address, a summary of the 1996 report of the American Academy of Microbiology. Population growth has placed tremendous pressures on water resources. Without appropriate intervention, these pressures will increase. The problems we face include water-borne pathogens, climate change and anthropogenic pressure. There is a need to question the reliability of current indicators of water contamination and to improve risk assessment methodologies. Good surveillance methods are needed, together with the development of databases. Water-borne disease must be made reportable and a mechanism for active surveillance as well as improved risk assessment should be put in place. Surprisingly, the prevalence of water-borne diseases is not well recognised. According to the WHO, however, some 80% of global infectious diseases are attributable to contaminated waters. Thus, use of low-cost, low-technology water treatment systems and education could provide dramatic improvements in human health. However, governments, NGOs, etc., must be made aware of and educated about the social and economic burden of these diseases.

SUMMARY OF WORKSHOP SESSIONS

Current methods used for testing drinking water

Production and distribution of safe water is essential for the protection of public health. The volume of water to be treated is immense and any failure in water treatment plants can produce a health hazard. Pathogens in drinking water are generally faecal in origin, and when common bacteria from faecal matter are present, other pathogens may be as well. Rather than searching for many different pathogens, it is therefore more logical to look for "indicator" micro-organisms that are non-pathogenic in themselves but "indicate" that contamination has taken place. However, bacterial indicator organisms cannot necessarily predict when, or where, treatment failure has allowed the passage of non-bacterial pathogens.

The suitability of selected organisms or groups of organisms as indicators depends on what we wish to measure. Indicator micro-organisms such as coliforms, *E. coli*, bacteriophages, or spore-formers can be used at various stages of treatment or distribution to assess water quality. Classical indicators characterise source water efficiently. However, monitoring of water quality, efficient removal, efficient inactivation, health effects, etc., require other techniques, as the presence of pathogens, especially viruses, and parasites such as *Cryptosporidium* and *Giardia* cannot specifically be tracked by conventional methods.

Many questions require answers. At what stage of the treatment process, from water source to the consumer's tap, should detection and measurement be made? What are the likely pathogens? What volumes of water need to be tested? Furthermore, there are still many gaps in our knowledge of the behaviour of pathogens and how indicators relate to viable pathogens.

A considerable number of pathogens are recognised as increasingly important and as causes of human disease. They include: *E. coli* O157, *Campylobacter*, *Yersinia*, *Aeromonas* and *Helicobacter* species. *Helicobacter*, for example, has high public health relevance since its presence in drinking water is now being reported.

When problems occur, rapid decisions relating to operational aspects and public health and regulatory interventions have to be made. Particularly in emergencies, rapid methods are needed. Unless sampling and measurement times are short in comparison with treatment or transit times, the water will have entered the distribution system or, worse, will have been consumed, before an assessment can be made.

While the rationale for using indicator organisms to detect contamination in source water may be sound, evaluation of treatment efficiency, failures in distribution, post-treatment contamination, etc., may require different indicators. Current measurements often give negative results even when disease outbreaks have occurred, outbreaks which are not necessarily restricted to locations where treatment failures are indicated.

Thus, emerging new molecular technologies need to be evaluated in order to increase our ability to intervene and further protect public health. For example, by using enzymatic methods with a wide range of fluorogenic and chromogenic substrates, enumeration and detection can be done rapidly. Appropriate enzymes (or combinations thereof) can be selected for specific organisms – typically an enzyme is present

in most but not all variants of a strain. Such enzymatic assays may constitute an alternative, specific, sensitive and rapid method for enumerating *E. coli*, coliforms and enterococci.

The fact that outbreaks are often difficult to detect, either because methodologies may be rather insensitive, or because many infections are asymptomatic and some give rise to only minor illness, is a major problem. Sporadic cases of disease are often not reported. Furthermore, disease surveillance may not be universal; for example, surveillance procedures do not exist in all of Europe. In addition, prior exposure to pathogens alters individuals' response to pathogen levels. Prior infections may reduce the severity of illness following subsequent infections, and when endemic levels of water-borne pathogens are high, much of the population may become immune, thus reducing the probability of detecting water-borne infections. On the other hand, if pathogens are removed, populations might become more susceptible to a major epidemic. This requires further study.

For risk assessment, the relationships between dose and response for the epidemiology of infections require more careful consideration. Taking cryptosporidiosis as an example, we need to ask how much risk is posed by a single oocyst. Outbreak data may reveal the persistence of low levels of infection and yet not correlate with reported water levels of oocysts. Furthermore, studies of infection are problematic when the criterion is serological antibody response rather than illness. People may respond serologically at much lower levels of pathogens than those required for illness.

Detection and identification of bacteria

Monitoring of microbial contaminants presently involves filtration and cultivation of indicator bacteria on selective media, followed by colony counting. These traditional procedures are time-consuming and not always sensitive enough to exclude risk of non-specific contamination. Thanks to rapid advances in biotechnological research in the last few years, a wide range of new molecular methods is now available. However, before implementing these methods, some important technical issues need to be addressed. An important issue is how to distinguish between viable and non-viable organisms. The number of viable *E. coli* cells after disinfection, for example, can be overestimated by PCR techniques. Furthermore, rapid techniques may sacrifice sensitivity for speed and are not suitable when the ability to detect very low numbers of microbial cells is essential. Striking the right balance between false positive and false negative results is critical.

Among available amplification techniques, the polymerase chain reaction (PCR) is a molecular technique which can be used to identify specific bacterial strains within a mixed population. A method has been developed for strict and opportunistic pathogenic bacteria, such as *Salmonella*, *E. coli* and *Aeromonas*, in raw and treated water which, compared to the traditional culture techniques, has greater specificity and sensitivity. Furthermore, the simple and rapid protocol of the proposed PCR technique provides results in a fraction of the time required by conventional culture methods. PCR amplification of genes and computer-aided analysis now allow for finding short, unique sequences that help identify groups of bacteria or single species. These methods make possible the simultaneous detection and identification of several organisms, including indicator and pathogen species.

However, while detection by PCR is faster, more specific and more sensitive, the reaction can be inhibited by contaminants in the sample. Furthermore, as mentioned, detection by PCR does not provide information on viability. To overcome this limitation, an indirect approach has been developed for assessing the viability of PCR-detected bacteria by analysing each sample in combination with a culture. In addition, reliability of the PCR has been tested with suspensions of *E. coli* exposed to ozone or UV that kill the micro-organisms. This treatment produced residues and debris that make interpretation difficult. Results suggest that dead cells with partially intact DNA may be detected.

As mentioned, enumeration of coliforms, traditionally used as indicator organisms, is far from ideal. Therefore, other micro-organisms, such as *Bacteroides*, which are present in faeces in high numbers, have been proposed. These outnumber coliforms perhaps 100-fold. Their use would allow identification of source of contamination and provide better data on the extent of contamination. Using group-specific probes, as few as 5-10 cells can be detected in human and animal faeces and in environmental samples.

A PCR method for detecting *Listeria* has also been designed. Samples have been tested to compare this technique with immunological assays. The PCR method gave no false negatives but immunological methods did. The PCR method is now being tested to screen food samples for contamination.

In addition, rapid immunological tests have been developed that recognise *Enterobacteriaceae* and *Legionella*. A molecular biological test based on 23S-rDNA-targeted probes is still under development and has been tested for enterococci and *E. coli*. A microbial analysis system, which combines fluorescent labelling and laser scanning, has been developed and has the capacity to provide ultra-sensitive results down to one cell within 90 minutes of sampling.

Detection and identification of viruses

Enteroviruses give rise to a wide variety of diseases and may survive disinfection of drinking water. It is possible to find enteroviruses in samples with no apparent faecal contamination. In a study in Switzerland, 24 out of 98 river water samples were found to contain cytopathogenic viruses. These were identified as human or bovine enteroviruses, reoviruses and rotaviruses. The G8 type of rotavirus was recovered from two locations in the same river. This was the first report of the presence of this virus type in Switzerland. These viruses have also been detected in some groundwater samples. Additional molecular epidemiological studies will be needed to identify the original host. However, traditional detection techniques require laborious and time-consuming propagation in specific cell cultures.

To detect viruses faster, more sensitive molecular technologies are now available. PCR, for example, can be used to detect very low viral titers within a few hours. This technique would be less expensive because it significantly reduces operating time. As some areas of the viral genome are highly conserved, a detection technique can be applied for groups of viruses. Although this technique cannot discriminate between infectious and non-infectious viruses, it offers a rapid way to screen multiple samples. However, selective detection of infectious viruses is necessary for surveillance purposes, and solutions to the problem of low specificity are being sought. It appears that shorter incubation steps might be a solution.

Detection and identification of protozoa and algae

Cryptosporidium is a protozoan parasite which causes diarrhoea in man and animals. The oocysts can contaminate surface waters and are extremely resistant to chlorine. Fluorescence microscopy and flow cytometry methods used to detect oocysts in water samples are time-consuming. In addition, counting techniques can give rise to huge variations; a sample count of ten oocysts may, in reality, represent any number from one to 100. It is important to improve concentration methods. For this reason, better separation methods, such as immunomagnetic separation, are being developed. Furthermore, it is essential, from the public health viewpoint, to determine whether oocysts are live or not; a method for distinguishing live oocysts, such as *in vitro* excystation, is required. Although PCR technology would be quicker, results show inconsistencies when it is used for quantitative analysis. Other techniques, such as immunoanalysis, are more sensitive, faster, and easier to use, and can be applied to larger volumes of water.

The protozoon *Giardia*, also a parasite of public health significance, occurs primarily in two species: *Giardia muris,* which infects rodents, and *G. intestinalis*, which infects humans. Traditional techniques

cannot be used to distinguish between these species. The use of random amplification of polymorphic DNAs may be a solution; a mixture of primers of both species would enable detection of *Giardia muris* as well. This would indicate possible *Giardia* contamination, even in the absence of disease outbreak.

Acanthamoebae occur worldwide, primarily in water. These small free-living amoebae can cause conjunctivitis in contact lens users and can act as a vehicle for *Legionella* in aquatic environments. Approximately 80% of the human isolates studied so far belong to seven of the 22 known genotypes, an indication that virulence may be associated with specific groups. Knowledge of the genetic identity of pathogenic strains may thus give new insights.

Cyanobacteria may also be a problem in water supplies. Cyanobacterial blooms occur under various environmental conditions, such as influxes of nutrients. Cyanobacteria produce a variety of toxins, some of which can be lethal. A two-year monitoring of three locations in rivers in the Paris area has demonstrated levels of toxin-producing cyanobacteria that may present human health risks. In order to evaluate risk, new methods, based on a two-step approach, are being developed: first, observation of microalgal samples in parallel with the detection of the most abundant toxins; and, second, the isolation of algal strains for the development of molecular probes.

The key issues for detection and identification

Drinking water is derived from many sources: water collected on the surface, groundwater resulting from percolation of surface water, and recycled wastewater. Subsequent to catchment, water is usually treated to remove toxic chemicals and pathogens (disease-causing organisms) and distributed to consumers; however, many small water supplies still distribute untreated groundwater. While it is often assumed that groundwater is pathogen-free, this is often incorrect, as groundwater can become contaminated by infiltration of surface water or sewage water. Therefore, it may need to be treated like water from other sources.

Pathogenic organisms in water may be conveniently divided into three groups – bacteria, viruses and protozoan parasites. In most cases, pathogen contamination of water is derived from human and animal faeces, and the pathogens enter humans and animals when water is drunk or used to wash food. While water-borne pathogens may cause gastroenteritic diseases such as diarrhoea, they can also be the source of longer-term illnesses and death.

It may be valuable, especially if cost-effective methods can be developed, to detect and monitor the levels of pathogens at each stage of the water collection, treatment and distribution process, from the source to the consumer's tap. In some cases (but not all), the maximum allowable levels of pathogens are stipulated by national regulations. It is also necessary to be alert to the presence of pathogens in their hosts in order to investigate, and prevent, the spread of disease. Some easily detected pathogens are used as surrogates, or indicators, for the presence of others that are less easily monitored.

Different scales and types of treatment require different monitoring schemes. Thus, the needs of small communities differ from those of a megacity, and heavily industrialised locations, where water may be recycled many times, differ from rural locations. In all cases, however, drinking water and wastewater need integrated management. The choice of methodologies, both of treatment and measurement, and the development of novel techniques, must always be set in the context of an end-point: "safe" drinking water. The meaning of "safe" and the consequent quality levels, are considered later.

Molecular methods for detection

We probably know less than 1% of the bacterial species that occur in the natural environment, either because the many others have never been isolated and cultured or exist in a "viable but non-culturable" state. Thus, detection by culture on agar media gives a distorted view of bacterial diversity. This particularly applies to water.

Molecular methods targeting nucleic acids offer tools for revealing this diversity. When applying these techniques to bacteria in water, however, obstacles are encountered. For instance, stressed bacteria are less

reactive and often occur as tiny cells. Also, some organic matter may bind probes, and it is important to know whether detected organisms are alive. Some technological advances – gene amplification, viability tests, cell concentration and image analysis – may help address these problems, but it should be remembered that if cysts or oocysts, live or dead, are found in tap water, treatment systems have already broken down.

After 100 years of taxonomic microbiology, the definition of species is still a matter of debate. Strains of a species are not genomically identical; there may be a divergence of 2-3% in the DNA of similar strains. Furthermore, an organism is assigned to a taxon only if the latter is already known. An organism which has not been previously isolated cannot be identified. It must first be recognised as novel and then classified within the framework of existing taxonomy. Classification is facilitated if the organism contains genes and expresses unique gene products. These unique properties may be targeted by non-culture-dependent molecular approaches (probes, antisera, PCR products, etc.).

For example, the discovery of a large number of bacterial toxins and other virulence factors has led to a better understanding of bacterial mechanisms of pathogenicity and to powerful molecular methods (gene probes and PCR) for detecting and identifying toxigenic bacteria. However, for many pathogenic bacteria, the factors or genes responsible for virulence are unknown. New pathogens have emerged and many have yet to be identified. However, even in the case of unknown organisms, it may be possible to determine pathogenicity by probing for specific toxin genes. In the future, this analysis will be facilitated by the use of multigene sensors on a biochip, a technique that may be used in risk assessment to screen for the presence of toxin genes.

Enzyme immunoassays (EIA) are widely used to detect and quantify pathogens. Many different EIA methods are available for both quantitative and qualitative analysis. Fully automated tests exist which can be done in the laboratory, and simple diagnostic kits can be used by non-specialists in less well-equipped environments. Such tests have demonstrated that it is possible to detect and quantify viruses and *Cryptosporidium* oocysts. Cross-reaction can be avoided by making antibodies against specific parts of the cell wall.

Fluorescent *in-situ* hybridisation (FISH) is a potentially important alternative technique. It is presently limited to detection of fast-growing cells, as stressed/starved bacteria are less reactive. There are, however, limitations: bacterial debris in water may interfere with fluorescence, and inanimate material may bind to probes. FISH, even if less sensitive, is faster and cheaper than PCR (but cannot be used for the detection of virulence and other genes). Both methods fail to distinguish clearly between “viable-non-culturable” and dead organisms.

These techniques require expensive equipment and therefore may not be suitable for use in the field or in developing countries. However, progress is being made towards rapid on-site tests. Such tests are already available for the detection of *Cryptosporidium* in faecal samples.

Epidemiology and statistical implications of low numbers of organisms

An important issue to consider when interpreting the results obtained from samples of drinking water supplies is the large variation in counts which may affect compliance with standards. The large degree of variation in micro-organism counts raises the question of what a zero reading may mean. First, a zero reading does not exclude the existence of regions with higher counts within the water supply system, and, second, in some instances it may reflect “near misses”.

Two case histories of outbreaks were reported, one in Japan and the other in Mexico. In Japan, inadequacies in turbidity monitoring and coagulation/flocculation resulted in failure to protect against a

Cryptosporidium outbreak. Even low exposure to oocysts appeared to cause disease. From dose response curves, 30 oocysts could infect one in five persons.

In Mexico, outbreaks were associated with re-use of water for irrigation. In Mexico, treated water which complies with bacterial quality standards based on faecal indicators is being used for many purposes, among them as a way of conserving fresh water supplies for drinking. Preliminary data were presented on the health impact of such water in a land reclamation project in a suburb of Mexico City. The area receives the effluent from a secondary treatment plant which is to be upgraded and used for crop irrigation and aquifer recharge. The effluent complies with current quality regulations, but local sewage discharges from illegal settlements are reducing the efficacy of treatment. *Giardia* cysts have been detected in the effluent and from large wells.

The situation of water supply in Israel was also reported. A study covering ten cities was undertaken to assess the prevalence and concentration of *Giardia* cysts and *Cryptosporidium* oocysts in raw wastewater and to assess the efficiency of removal by secondary wastewater treatment. Drinking water sources are probably affected by farm animals, as there are generally no barriers to access. All tested raw wastewater samples were positive for both parasites while secondary treatment removed 99% of *Giardia* and 90% of *Cryptosporidium* oocysts. The study concluded that there are high concentrations of both parasites in wastewater and that they may find their way into sources of drinking water.

The key issues for treatment

To define treatment regimes, it is necessary to know the concentration of biologically derived contamination. Pathogens are not continuously present, however, even in contaminated water sources. Moreover, owing to the large number of possible pathogens, it has not been feasible to develop special detection methods for every organism. Current techniques principally monitor indicator organisms. For example, faecal coliforms (a group of easily detectable bacteria commonly present in high numbers in the gut) satisfactorily represent a wide range of other bacterial pathogens at the pre-treatment stage. Their presence in source water indicates faecal contamination and the possible presence of pathogens. However, current indicator organisms are less suitable for monitoring efficacy of treatment because of the wide range of susceptibility of different organisms to treatment techniques.

Measurements based on carefully selected indicators are perfectly valid in appropriate circumstances and have the advantage of being both inexpensive and easy to carry out. This does not mean that there is no room for improvement. While faecal coliforms may be suitable for pre-treatment estimates, a number of other indicators among the viruses or protozoa may be more suitable for post-treatment monitoring. There is much debate over which organisms can best fulfil this role. The identification of specific indicators for each group might be a useful follow-on activity from the Interlaken workshop. In addition, the appearance of new, mutant pathogens and the presence of the physiological form of bacteria often called “viable but non-culturable” mean that new indicators are needed in the “toolkit”.

The desired end product is “safe” drinking water. This raises the question of what is meant by “safe” and what represents a tolerable level of pathogens. Some regulatory authorities have specified a “zero” tolerance, in other words, no pathogens. The possibility of measuring “zero” raises many questions. No public water supplies at present or in the foreseeable future are seen as having a zero risk from occasional or opportunistic pathogens. It is not practical to supply or plan to deliver sterile water to consumers’ taps. Regulations should be science-based, and both qualitative and quantitative risk assessment is needed to ensure water quality.

Policy issues raised by the introduction of new methods: What does it take to bring a method into use?

Despite substantial advances in recent years, access to safe drinking water remains a major public health challenge. Recent years have seen renewed recognition of the importance of the microbiological quality of drinking water. Changes in legislative approaches include a revision of the microbiological component of the WHO *Guidelines for Drinking Water Quality*. New molecular methods for safe drinking water may assist the WHO in this process.

Furthermore, The American Society for Microbiology is about to release a report, *Microbial Pollutants in our Nation's Water*. From this report, it appears that control of water pollution in the United States over the past two decades has focused on chemical risks, thereby overshadowing the more significant risks associated with microbial pollutants. Water-borne micro-organisms pose increasing threats to public health. Outbreaks of infectious disease indicate that there are watersheds and communities at high risk from contaminated water.

Water contaminated with pathogenic micro-organisms leads not only to human suffering but also to significant economic losses. Children, the elderly, and certain other groups are at higher risk from microbial pollutants in water than the rest of the population. In the United States, more than 70% of diarrhoea-related deaths occur among the rapidly growing age group of 55 years and older.

Microbial pollution of water in the United States represents a growing environmental and public health crisis that is being insufficiently addressed at national level. Responsibility for the nation's water supply is fragmented among numerous federal, state, and local government bodies, all of which apply different methods and standards. The lack of an integrated regulatory approach threatens the nation's water supply.

Indicator systems such as coliform bacteria which are currently used to assess microbial risks are inappropriate for many of the micro-organisms that threaten US water supplies. Effective risk assessment requires an adequate database of information on exposure and outcomes, but this database does not exist.

Action should be taken through co-ordinated research and policy-making efforts by all agencies and institutions involved in water quality issues. Proposed actions include:

- Begin an independent scientific assessment to address the microbial safety of the nation's water.
- Determine the appropriate and necessary human and financial resources needed for research, development, and implementation of water protection programmes focused on water-borne micro-organisms.
- Identify the education and training programmes needed to improve surveillance of water in the United States.
- Determine which programmes and methods must be developed or expanded for sufficient monitoring of the microbial quality of water.
- Define the databases for occurrence and health effects needed to establish appropriate water safety policies, as they relate to microbial pathogens.

In the United States, the Environment Protection Agency (EPA) determines maximum contaminant level goals for both chemicals and micro-organisms. Enforceable levels are set as close to these goals as practicable after consideration of costs, availability of treatment options and of suitable analytical methods. The Clean Water Act mandates the EPA to develop risk-based criteria and analytical techniques for enforcing water quality standards. The current focus is on updating and standardising techniques for quantification and speciation of microbial contaminants in water.

In the United Kingdom, the Drinking Water Inspectorate makes recommendations regarding methodology which are not strictly mandatory. Laboratories may use alternatives provided they show equivalent or better performance. The key issues are unequivocal, scientifically valid definitions of the organisms detected and acceptable criteria for determining equivalence of methods. New methods must meet current

requirements, and validation data must permit comparisons with presently approved techniques. In Germany, the adoption of new methods requires intensive co-operation with local health authorities and validation against standard procedures.

Thus, adoption of new methods cannot be immediate and raises several key questions. What is the advantage of looking for new methods rather than improving water plant performance and operation? Should one not ask how else the goals of safe drinking water can be achieved and what specific information is needed rather than search for improved techniques? For example, is it the presence or the concentration of *Legionella* that is important? Ultimately, it is necessary to find the approach that provides the greatest safeguard for preventing water-borne microbial infections.

Indicator methods are simple and easy to use, cheap, and cover a wide range of pathogens. However, current indicators leave little room for improvement. These methods have served relatively well but are less accurate and informative than what is needed. The main limitations are lack of specificity and slow response. Furthermore, current indicators are poor for addressing parasites and viruses. However, the introduction of new technological advances is always a slow process, and there is the problem of validating new procedures and managing the additional information, as molecular methods may identify a broader and more specific group of organisms than conventional tests.

Furthermore, most current measurements of microbial water contaminants are in fact retrospective. More effective real-time measurements are needed so as to improve the capacity to prevent the spread of water-borne pathogens. By the time contamination is confirmed, water has already gone into circulation. *Cryptosporidium* outbreaks, for example, cannot be prevented by routine monitoring. Real-time measurements would be essential for feedback control of treatment processes.

Nevertheless, it may not be appropriate to set quality standards for either drinking water or the environment on the basis of the most sensitive techniques available. The adoption of the precautionary principle has led the EU to set stringent standards for the maximum permitted levels of pesticides, in effect a surrogate zero level. In England and Wales the adoption of this standard has led to capital investment of over £1 billion. It is highly debatable whether this level of expenditure can be justified on toxicological grounds. Before standards relating to more sensitive techniques are introduced, the impact on water customers should be assessed, balancing the benefits against the potential costs.

Regarding the economic aspects, there are as yet few reliable figures on the relative costs of conventional and new technologies. However, some general remarks are possible. First, the costs of new technologies are almost always highest at first and come down over time. Second, cost considerations must also include not only the initial costs of using the new technology but also the long-term economic costs of not using it ("opportunity costs"). For serious diseases such as cholera or hepatitis, the costs of a large-scale outbreak to the health care system and the economy will be much higher than the costs of a new, more expensive technology that could have prevented it. In addition, there is growing evidence that water-borne infections may lead to long-term heart and liver disease, among others. This means that the long-term costs of not using the best available technologies may be very high indeed. Third and finally, the "therapeutic gap" between detection and prevention of diseases varies and this has major economic implications. If, for medical and technical reasons, the way from detection to prevention is long and arduous, the economic costs to detection may be high. However, prevention might be faster and easier for water-borne diseases than for many others.

R&D priorities

R&D priorities should address specific targets of epidemiological relevance, sensitivity, specificity and cost. “Technical needs” may be summarised as follows:

- Risk assessment requirements:
  - New indicators for source waters
  - Quality control for plant operation
  - Molecular fingerprinting for disease outbreaks
  - Risk analysis to define sample volume
  - Recovery of target micro-organisms
  - Removal of contaminants (interfering factors)
- Analytical aspects:
  - Improved sensitivity and specificity
  - Distinguish viable from non viable
  - Distinguish pathogenic (infective) from non pathogenic
- Detection:
  - Methods capable of automation (colorimetric preferred)
  - Standardisation of methods
  - New methods will have to show they are better either in terms of results or cost (costs are likely to fall, however, if the market is there)
  - Sample collection/processing has cost implications
  - Techniques that could be handled by chemists could lower costs.
  - Link the measurement to disease outbreaks
  - New methods must be applicable to drinking water quality

The case for new molecular methods

Surveillance of water quality is a public health requirement and requires robust, reliable detection techniques. However, because indicator organisms are not suitable for this purpose, specific pathogens need to be used. Molecular methods are not yet sufficiently developed to handle routine surveillance tasks, and priority should be given to the development of new tools for these tasks and for investigation of outbreaks to link the pathogens in water to those in humans. Most samples analysed give negative results even if low numbers of organisms are actually present. This again raises the question of the definition of "zero".

Legal requirements and/or internal and external quality standards dictate treatment performance levels. If the plant is functioning correctly, there should, in principle, be no problems of pathogens spreading. There is, however, a need for real-time on-line monitoring of plant operations. Currently, this can only be done by using physico-chemical characteristics, such as turbidity, which effectively act as indicators for treatment failure. Current biological methods are far too slow for on-line monitoring – by the time the pathogen is detected, it has been released into the distribution system. The development of suitably rapid biological feedback methods would make it possible to monitor the treatment process effectively.

No single detection method will fulfil all requirements. Evaluating source water is quite different from controlling the finished product. In addition, the testing of groundwater intended for immediate use must be more complete than monitoring of source water entering a full treatment plant.

It is necessary to track pathogens to source by sampling water, faeces, etc. Tests must be specific and sensitive, and it is in this area that molecular methods are currently being used. Molecular typing and species identification are most relevant in outbreak situations. To assess management options, information on dose/response relationships (bearing in mind that they are affected by a population's immune status), demonstration of cause and effect, and incidence is needed. Novel data collection methods are required, as is easy data exchange.

There is a wide range of novel methodologies, principally based on the detection and manipulation of polynucleotides using the polymerase chain reaction. In their current, laboratory-based form, these techniques are suitable for investigative work, but are not yet inexpensive or robust enough for monitoring in the field. Molecular methods can successfully detect some "viable but non-culturable" organisms and can also be used to track pathogenic determinants – genes that give rise to pathogenic properties (the biosynthesis of toxins, for example).

If current techniques are inadequate, it is because they are not pathogen-specific, insufficiently sensitive, and too slow or too expensive. The molecular methodologies are improving rapidly, especially through reduction in sample size and automation. However, they face a dynamic situation in which novel pathogens regularly appear. Detection and monitoring must keep pace. Good new concentration methods which would reduce pressures to find techniques for detecting very low numbers of pathogens would also be valuable.

Regulatory authorities are naturally conservative when faced with novel methodologies. Flexible systems for introducing and accrediting new techniques are needed.

MAJOR PUBLIC POLICY ISSUES

Information and education

The general public wishes to be fully informed but does not necessarily want all the details. People should have confidence in and trust their decision makers. They should have enough information to understand the implications of water contamination and to make community-wide decisions about alternative treatments and standards. This requires informing and educating the widest range of people, from young children to professionals and administrators.

Direct education of the public is a role for individual governments. However, the OECD might provide documents to help with this task: an educational tool such as a briefing booklet for policy makers, for example, setting out what can and cannot be done.

Regulation and standards

Different countries have different problems – some have plenty of water and some a scarcity, so that water must be re-used. Differences in the availability of water and economic resources have led to regional and national differences in the levels of waterborne pathogens deemed acceptable. Furthermore, the most exquisitely sensitive detection techniques currently require sophisticated laboratory support, and developing countries do not generally have the necessary infrastructure.

The concept of "tolerable risk" varies from one country to another and this is normal. Even in the individual states of the United States, there is no uniformity concerning acceptable risks. It is necessary to differentiate between risk assessment, which reflects the science, and standards, which depend on national realities, social values, economic costs and environmental factors. Since financial and water resources are finite, risk assessment can be universal, but countries will need flexibility in setting standards.

There are legal limits on levels of specific micro-organisms in drinking water (for example, the EU directives) but only nationally agreed measurement protocols. The WHO has developed relevant international quality/safety standards for drinking water, and these should be well publicised.

The need for international co-operation

Although different parts of the world will have different standards and levels of acceptability, common surveillance tools for water-borne disease are essential so that investigation and reporting will result in comparable information. Currently, it is possible to draw quite different conclusions from different sets of similar data. The OECD could co-ordinate the standardisation of reporting.

The OECD may be a suitable organisation for following up on some of the policy implications indicated below. As many organisations are working on aspects of provision of drinking water, it would be both more efficient and less costly to join forces. The OECD could bring together Europe, North America,

Mexico, Japan, Australia and New Zealand to work on economic as well as health aspects, which are intimately linked. An appropriate collaboration would be with the WHO.

The OECD is already involved in the wider aspects of water management (its recent publication, *Water Consumption and Sustainable Water Resources Management*, contains data based on country reviews), but has concentrated primarily on chemicals. There is now a need to put more emphasis on biological aspects.

Wider information exchange is crucial so that research can be better co-ordinated. Several groups often work on the same problem, and this can be a waste of valuable resources. Mechanisms like the Interlaken workshop are needed for research groups to communicate with each other.

It was felt that the OECD could provide the framework for an integrated assessment of microbiological pollution in water by encouraging the co-ordination of studies, conducted in different countries, on assessment of risk, national guidelines, regulations and standards. The OECD might also determine the appropriate human and financial resources needed for research, development and implementation of water protection programmes.

Policy implications

The assessment of microbial quality of drinking water is currently based largely on culture techniques which do not detect specific waterborne pathogens but rely on the monitoring of indicator bacteria that reveal the potential presence of microbial pathogens of faecal origin. While these indicators are clearly suitable for protecting against various bacterial pathogens, such as Salmonella and Shigella, it has become evident that they are not reliable for viruses and protozoa, especially after treatment for disinfection. To investigate epidemics under outbreak conditions, indicator organisms cannot be used. In this case, the new molecular technologies are the best choice, but they require additional development.

1. *The quality/safety criteria for each stage of the water collection, treatment and distribution process need to be defined in order to set monitoring standards and establish adequate detection methods.*
2. *Guidelines for tolerable levels of risk should be agreed upon, bearing in mind that different countries will have their own regulatory standards.*
3. *The information required, both at each processing stage and for surveillance and epidemiology, should be identified, and the degree of specificity/sensitivity laid down. This should include the choice of specific indicators for selected groups of organisms.*
4. *A full assessment of the need for individual measurements, based on risk analysis, should be made. This should build on the output of the Interlaken workshop.*
5. *The scope and robustness of both current (indicator-based) techniques and molecular methods need to be improved, bearing in mind that at least some will have to be used in the field and in developing countries. New molecular techniques are urgently needed, particularly for investigation of outbreaks.*
6. *More research and development proposals that target identified issues and application gaps should be carried out. These proposals would give the research community in individual countries something to which to respond.*
7. *Methodologies need to be standardised and validated, preferably on a international basis. A mechanism for sharing validated methods is required.*
8. *A flexible system for introducing new methodologies, with accreditation in agreement between regulators and industry, should be set up.*
9. *The development, for example by the WHO, of international water quality/safety standards, should be publicised.*
10. *International collaboration, starting with collaboration between the OECD and the WHO, should be encouraged. International co-operation might lead to the following:*
    - *Investigation of disease outbreaks and lessons learned from the evidence from existing studies.*
    - *Improved qualitative and quantitative microbiological risk assessment and the transition from one to the other.*
    - *Ongoing work in small communities, relative to water supply safety, sustainable integrated water management and safety.*
    - *Criteria and procedures for the adoption of new methods.*
11. *It is proposed that the Interlaken Workshop be followed by a focused working group to propose and implement future activity. Priorities for future activities should be set, bearing in mind the limited resources of agencies active in this area.*
12. *Briefing materials should be provided to governments to assist their efforts in educating and informing the public.*

ANNEX 1

OECD WORKSHOP INTERLAKEN '98 ON MOLECULAR TECHNOLOGIES FOR SAFE DRINKING WATER

PROGRAMME

Date	5-8 July 1998
Place	Congress-Center Interlaken, Switzerland
Chair	Prof. Dr. Alexander J.B. Zehnder, Director, Swiss Federal Institute for Environmental Science and Technology (EAWAG), Switzerland
Deputy Chair	Dr. David Harper, Chief Scientist, Department of Health, United Kingdom
Plenary Session I Chair	Dr. Rita Schoeny, Associate Director, Health and Ecological Criteria Division, Office of Water, US Environmental Protection Agency (EPA), Washington, DC, United States
Plenary Session II Chair	Dr. Marie-Marguerite Bourbigot, Research Director/International Technical Director, Compagnie Générale des Eaux (CGE), France
Plenary Session III Chair	Dr. Thomas Egli, Department of Microbiology, Swiss Federal Institute for Environmental Science and Technology (EAWAG), Switzerland
Rapporteurs	Dr. Peter Gosling (United Kingdom) Dr. Mike Griffiths (United Kingdom)
Participants	Approx. 80
Organisers/Sponsors	OECD and Switzerland

Day 1, Sunday, 5 July 1998

Prof. Dr. med. Thomas Zeltner, Director, Federal Office for Public Health, Switzerland
Welcome address by Switzerland

Prof. Dr. Alexander J.B. Zehnder, Director, EAWAG, Switzerland
Scope of the Workshop

Mr. Risaburo Nezu, Director, Directorate for Science, Technology and Industry, OECD
Welcome address by the OECD

Dr. David Harper, Chair, OECD Working Party on Biotechnology
Welcome address by the OECD Working Party on Biotechnology

Dr. Joan Rose, University of South Florida, United States (presented by Prof. Payment)
Waterborne disease education and control methods

Day 2, Monday, 6 July 1998

Plenary Session I: Current Methods Used for Testing of Drinking Water
Chair: Dr. R. Schoeny (United States)

Dr. N. Lightfoot (EC/United Kingdom)
The concepts of indicators and colony counts

Prof. M. Manafi (EC/Austria)
New approaches for the fast detection of indicators, in particular enzyme detection methods (EDM)

Dr. F. Frost (United States)
Endemic versus epidemic waterborne disease

Prof. P. Payment (Canada)
Waterborne viruses and parasites: resistance to treatment and disinfection

Special Session 1: Detection and Identification of Bacteria
Chair: Prof. P. Grimont (EC/France)

Dr. H. Meier (EC/Germany)
Detection and identification of indicator bacteria using rRNA targeted probes

Ms. M.R. de Roubin (France)
Detection of viable pathogenic bacteria from water samples by PCR

Dr. J. Menaia (EC/Portugal)
Bacteroides spp. as alternative indicator organisms: monitoring through PCR 16S-rRNA amplification

Dr. M. Snozzi and Mr. B. Zimmermann (Switzerland)
PCR detection of E. coli *after disinfection with UV or ozone*

Dr. E. Frahm (Germany)
Application of new methods in regular water hygiene control

Dr. M. Savill (New Zealand)
From design through to routine implementation of a PCR system for Listeria monocytogenes

Dr. P. Cornet (France)
The ChemScan® RDI, a real-time and ultra-sensitive microbial analysis system

Special Session 2: Detection and Identification of Viruses
Chair: Prof. Dr. K. Botzenhart (Germany)

Dr. D. Tougianidou and Prof. Dr. K. Botzenhart (Germany)
Molecular techniques for the detection of enteroviruses in water

Dr. A. Metzler (Switzerland)
Molecular characterisation of enteric viruses detected in surface water

Special Session 3: Detection and Identification of Protozoa and Algae
Chair: Dr. A. Metzler (Switzerland)

Dr. P. Krüger, Dr. A. Wiedenmann, and Prof. Dr. K. Botzenhart (Germany)
Detection of Cryptosporidium oocysts in water: Comparison of the conventional microscopic immunofluorescence method with PCR and TaqMan PCR

Dr. T. Endo (Japan)
Clustering of Acanthamoeba isolates by means of mtDNA digestion patterns

Dr. G. Ionas (New Zealand)
Species differentiation of Giardia by PCR

Prof. S. Puiseux-Dao (France)
Toxic cyanobacteria in resource waters: monitoring of their occurrence and of the toxin detection

Day 3, Tuesday, 7 July 1998

Plenary Session II: Molecular Methods for Detection
Chair: Dr. M-M. Bourbigot (France)

Prof. E. Stackebrandt (EC/Germany)
Molecular detection and identification of micro-organisms

Dr. L. Torrance (EC/United Kingdom)
Immunological detection and quantification methods

Prof. J. Frey (Switzerland)
Target genes for the identification and detection of potentially hazardous bacteria

Prof. P. Grimont (EC/France)
Adaptation of methods to natural waters

Special Session 4: Epidemiology and Statistical Implications of Low Numbers of Organisms
Chair: Dr. M. Snozzi (Switzerland)

Dr. P. Gale (United Kingdom)
Mathematical models for the distribution of organisms in water distribution systems

Dr. H. Yoshikura (Japan)
Detection of microbes in water treatment stations in Japan

Dr. E. Cifuentes (Mexico)
The case of protozoa: Measures for water safety and reuse, Mexico

Dr. A. Nasser (Israel)
Prevalence of Cryptosporidium and Giardia in waste- and surface water in Israel

Plenary Session III: Policy Issues Raised by the Introduction of New Methods and Follow-up
Chair: Dr. T. Egli (Switzerland)

Prof. C. Fricker (EC/United Kingdom)
"Old" versus "new" methods

Prof. R. Atlas (United States)
Microbial pollutants in water: Environmental and public health issues. A call to action by the American Society for Microbiology

Panel Discussion: What Does It Take to Bring a Method to Use?
Introductory statements by
Dr. R. Schoeny (United States)
Mr. M. Waite (United Kingdom)

Day 4, Wednesday, 8 July 1998

Plenary Session III: Policy Issues Raised by the Introduction of New Methods and Follow-up (continued)

Prof. R. Atlas (United States)
R&D priorities and technical issues for molecular technologies for safe drinking water

Dr. J. Bartram (WHO, Geneva)
Policy and administrative issues

Dr. R.J. Tye (United Kingdom)
Sensitive detection techniques – financial implications for water customers

Panel Discussions

Dr. S. Wald (OECD/STI)
Economic and Cost Aspects

Dr. S. Wald, Ms. M-C. Huet, Dr. E. Ronchi, Dr. M. Yakowitz (OECD)
Possible OECD follow-up (test guidelines, other)

Final Conclusions and Recommendations

ANNEX 2

LIST OF CHAIRS, SPEAKERS, STEERING GROUP MEMBERS AND OTHER PARTICIPANTS

Chairs

Prof. Alexander J.B. ZEHNDER, Chair	**Switzerland**
Dr. David HARPER, Deputy Chair	**United Kingdom**
Dr. Marie-Marguerite BOURBIGOT, Plenary Session Chair	**France**
Dr. Thomas EGLI, Plenary Session Chair	**Switzerland**
Dr. Rita SCHOENY, Plenary Session Chair	**United States**

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Prof. Pierre PAYMENT	**Canada**
Mr. William ROBERTSON	
Ms. Petra KOJECKA	**Czech Republic**
Mr. Petr PUMANN	
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Dr. Nadine DUMOUTIER	
Prof. Patrick GRIMONT	
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Dr. Monique POMMEPUY	
Prof. Simone PUISEUX-DAO	
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Dr. Jens JACOB	
Dr. Petra KRÜGER	
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Prof. Dr. Bruno GOTTSTEIN
Mr. Daniel HÄFLIGER
Dr. Ursula JENAL-WANNER
Mr. Andres KÄCH
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Dr. Alfred METZLER
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Dr. Kurt VORBURGER
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Dr. Paul GALE	**United Kingdom (contd.)**
Dr. Judith HILTON	
Dr. Rowena J. TYE	
Mr. Mike WAITE	
Prof. Ron ATLAS	**United States**
Dr. Floyd FROST	
Dr. I.V. ECONOMIDIS	**EC**
Dr. Colin FRICKER	**EC (United Kingdom)**
Prof. Patrick GRIMONT	**EC (France)**
Dr. Nigel LIGHTFOOT	**EC (United Kingdom)**
Prof. Dr. Mammad MANAFI	**EC (Austria)**
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Dr. Lesley TORRANCE	**EC (United Kingdom)**
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