Chlorine residual efficiency in inactivating bacteria from secondary contamination in Isfahan, 2002

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فاعلية الكلور المتبقّي في تعطيل النشاط البكتيري بسبب التلوث الثانوي في أصفهان، 2002

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الخلاصة: لأغراض استقصاء إمكانية تعطيل نشاط المسببات المرضية المحتملة، قام الباحثون بتقييم معدلات البقاء على قيد الحياة لجراثيم الإشريكية القولونية *E. coli*، والمعوية البرازية *Enterococcus fecalis* والغازيَّة المستَرْطِبة *Aeromonas hydrophila* والسلمونيلة التيفية *S. typhi*، وذلك في عينات أخذت من جهاز مياه الشرب في مدينة أصفهان. وتم قياس نسبة الكلور المتبقي، والباهاء pH، ودرجة الحرارة، وإجماليِّ الكربون العضوي. وكان الجرثوم الأكثر حساسية للكلور، هو الغازيَّة المستَرْطبة، إذْ تعطل نشاطها في أقل من 100 دقيقة بمعدل كلور مقداره بين 0.11 مغ/ل و0.90 مغ/ل، بينما أظهرت الأنواع الثلاثة الأخرى من الجراثيم مقاومة أعلى، إذْ تحملت الإشريكية القولونية الكلور بمعدل 0.30 مغ/ل لمدة تزيد على 1000 دقيقة، كما بقيت المعوية البرازية والسلمونيلة التيفية حية في معدل تركيز إجمالي للكلور مقداره 0.50 مغ/ل لمدة 100 دقيقة. واستنتج الباحثون أن مستويات الكلور الإجمالية بمعدل يقل عن 0.71 مغ/ل في أجهزة إمدادات المياه لا يمكنها توفير معدلات السلامة والمأمونية المطلوبة في مياه الشرب.

ABSTRACT To investigate the inactivation of potential pathogens, we evaluated survival rates for ***Escherichia coli***, ***Enterococcus faecalis***, ***Aeromonas hydrophila*** and ***Salmonella typhi*** in samples taken from the Isfahan drinking water system. Chlorine residual, pH, temperature and total organic carbon levels were measured. The organism most sensitive to chlorine was ***A. hydrophila***. It was inactived in < 100 minutes at chlorine levels of 0.11 mg/L to 0.90 mg/L. The other 3 organisms showed higher resistance. ***E. coli*** tolerated 0.30 mg/L chlorine for > 1000 minutes while ***Ent. faecalis*** and ***S. typhi*** survived at total chlorine concentration of 0.50 mg/L for 100 minutes. We concluded that total chlorine levels of less than 0.71 mg/L in water supply systems cannot provide the recommended safety levels.

Efficacité résiduelle du chlore pour inactiver les bactéries dans le cas d'une contamination secondaire à Ispahan, 2002

RÉSUMÉ Afin d'examiner l'inactivation d'agents pathogènes potentiels, nous avons évalué le taux de survie d'***Escherichia coli***, ***Enterococcus faecalis***, ***Aeromonas hydrophila*** et de ***Salmonella typhi*** dans des échantillons prélevés dans le système d'eau potable d'Ispahan. On a mesuré le taux de chlore résiduel, le pH, la température et le taux de carbone organique total. ***A. hydrophila*** était le micro-organisme le plus sensible au chlore. Il était inactivé en moins de 100 minutes à un taux de chlore de 0,11 mg/L à 0,90 mg/L. Les 3 autres micro-organismes présentaient une plus forte résistance. ***E. coli*** tolérait 0,30 mg/L de chlore pendant plus de 1000 minutes tandis que ***Ent. faecalis*** et ***S. typhi*** survivaient à une concentration totale de chlore de 0,50 mg/L pendant 100 minutes. Nous avons conclu qu'une concentration totale de chlore de moins de 0,71 mg/L dans les systèmes d'approvisionnement en eau ne permet pas d'assurer le niveau de sécurité recommandé.

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Introduction

Supplying safe drinking water has a significant effect in protecting and improving public health. Epidemiologic investigations of waterborne gastrointestinal diseases reveal that the water supply distribution systems can play a decisive role in the health of the inhabitants in a region. The annual risk of enteric illness ranges from 1 per 1000 people to 1 per 100 people, and a significant proportion of those illnesses may be caused by organisms in water [*1*]. Worldwide, the risk of illness from waterborne microbial pathogens has been reported as being "probably thousands- to millions-fold greater than that from chemical contaminants in drinking water" [*2*]. A 1993 study indicated that approximately 1000 people (800 children, 200 adults) worldwide were dying every hour from waterborne microbial disease. Most of the increased risk ensues because of localities in which treatment of the domestic water supply is inadequate or non-existent [*3*].

The protection of water quality in the distribution system has, therefore, received constant emphasis and much attention. Although the source water may be safe, once flowing in the distribution mains, it may be detrimentally affected from a variety of sources. Contaminants may enter through sources such as network maintenance and repair operations, illegal connections, shutting and/or opening main valves, abrupt changes in consumption levels, power breaks and pumping stoppage, pressure drops and backflows from homes into the distribution mains. It is, therefore, essential to maintain a recommended level of a stable disinfectant, such as chlorine, in the flow in order to provide safety and to prevent secondary biological contamination by agents which enter the system after the water has been disinfected for drinking purposes [*4*].

It has long been known that a free chlorine residual (chlorine that must be present in the distribution system after the disinfection process to prevent secondary contamination) of 0.75 mg/L in the distribution system at pH ≤ 8.0 and 1 NTU (nephelometric turbidity units) can destroy most gastrointestinal bacteria in less than 30 minutes [*5*].

The standard proposed and practised for microbial control of water in the Islamic Republic of Iran includes the removal/absence of faecal coliform bacteria. The presence of these bacteria is, therefore, used as an indicator of water contamination [*6*].

Secondary contaminants entering the water distribution system are normally controlled through the addition of chlorine and maintaining a certain level of free chlorine residual. The effect of the chlorine residual depends on the chemical and physical conditions as well as on the length of contact time. Although chlorine residual greatly contributes to the inactivation of indicator bacteria, i.e. faecal coliforms, the question awaiting an answer is the level of inactivation of other potential pathogens such as *Enterococcus faecalis*, *Aeromonas hydrophila* and *Salmonella typhi* at the recommended levels of chlorine residual.

The aim of this investigation is to find an answer to this question, focussing on pathogens in the drinking water distribution system in Isfahan, which conveys water from the Isfahan treatment plant to Greater Isfahan [*7*].

Methods

This investigation was carried out during the 10 months from January through October 2002. We took samples from 30 different points along the municipal drinking

water distribution system in Isfahan [*8*]. Two samples were later discarded because of the confusing results obtained from the microbial examination. We took 5 samples from each site in 5-litre Erlenmeyer flasks using the grab sampling method (samples were taken at specified points in time and place and with known volume). Four of the filled containers for each site were tightly capped with aluminium foil and paraffin wax and delivered to the microbiology laboratory in Isfahan University of Medical Sciences for testing. The water in the fifth flask was used at the sampling location to determine total chlorine content using the DPD method [*9,10*].

About 150 mL of this sample was delivered to the laboratory in a small, opaque glass container tightly capped with aluminium foil and sterilized in a dry-heat sterilizer oven for 4 hours to determine total organic carbon (TOC).

The microorganisms selected for investigation were *E. coli*, *Ent. faecalis*, *A. hydrophila*, and *S. typhi*, all strains used in laboratory quality control. They were supplied by the Iranian Pasteur Institute, Tehran. In order to study the survival patterns, several colonies of bacteria recently cultured in specially prepared culture media (brain heart infusion agar, Merck) were mixed with sterile saline and stirred using a vortex mixer in order to prepare a homogeneous stock suspension with 1×10^8 cells/mL (determined by spectrophotometer). Two serial dilutions (1:9) were done, then 1 mL of the suspension was added to 1 of the flasks from each location (i.e. each mL of sample water contained a known number of bacteria).

The survival rates of the bacteria in the prepared samples were determined at 1 minute, 10 minutes, 100 minutes, and 1000 minutes using the serial dilution method from 1 mL of the sample water cultured on brain heart infusion agar incubated at room temperature.

All tests had 1 positive control sample (sterile water + the microorganism under study) and 1 negative control in which a separate culture of the water sample without the addition of the test organism was cultured in an enriched medium (blood agar, Merck) and in differential media (Endo medium and desoxycholate citrate agar, Merck).

The data were analysed using *SPSS* software. Graphs were plotted using *Harvard Graphics*.

Results

Chlorine concentrations of the samples ranged from 0 mg/L to 0.90 mg/L. In 53.58% of the samples tested, total chlorine concentrations were ≤ 0.50 mg/L. (Figure 1). The pH of the samples was 8.0–8.2 and sample temperatures were 10 °C–29 °C.

The TOC of the samples varied from 0.07 mg/L to 3.95 mg/L. The frequency distribution of TOC is shown in Figure 2.

In samples with total chlorine concentrations between 0.51–0.90 mg/L chlorine, *E. coli* was inactivated in 10–100 minutes. This organism remained active for over 1000 minutes in samples with total chlorine concentration of 0–0.30 mg/L (Figure 3).

Survival time for *A. hydrophila* in all samples was less than 100 minutes. Moreover, the organism was completely inactivated within 1 minute in samples with total chlorine concentration between 0.71 mg/L and 0.90 mg/L (Figure 4).

Ent. faecalis showed resistance for over 1000 minutes in samples with total chlorine concentrations ≥ 0.50 mg/L. Greatest sensitivity was observed in samples with total chlorine concentrations of 0.71–0.90 mg/L (Figure 5).

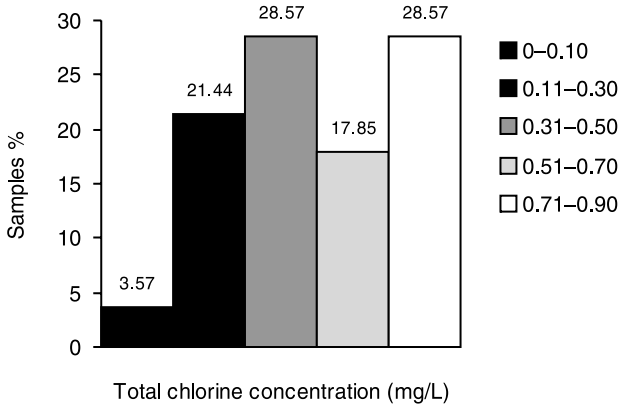

Figure 1 **Distribution of total chlorine concentration in water samplings**

The survival time for *S. typhi* in samples with total chlorine concentrations ≥ 0.50 mg/L was more than 1000 minutes while in samples with chlorine levels of 0.71–0.90 mg/L, survival time was reduced to less than 100 minutes (Figure 6).

In order to characterize and enumerate the original bacteria in the samples, water samples were used for direct bacterial culture. Ten of the samples contained between 1 and 24 colonies of aerobic bacteria, including Gram-positive cocci, Gram-posi-

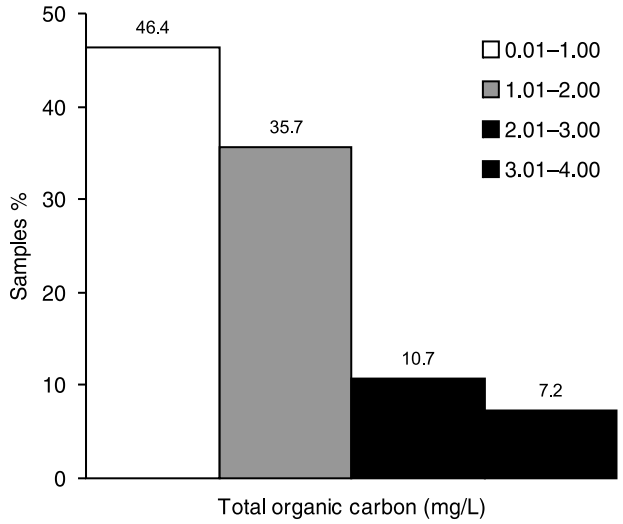

Figure 2 **Distribution of total organic carbon concentration in water samplings**

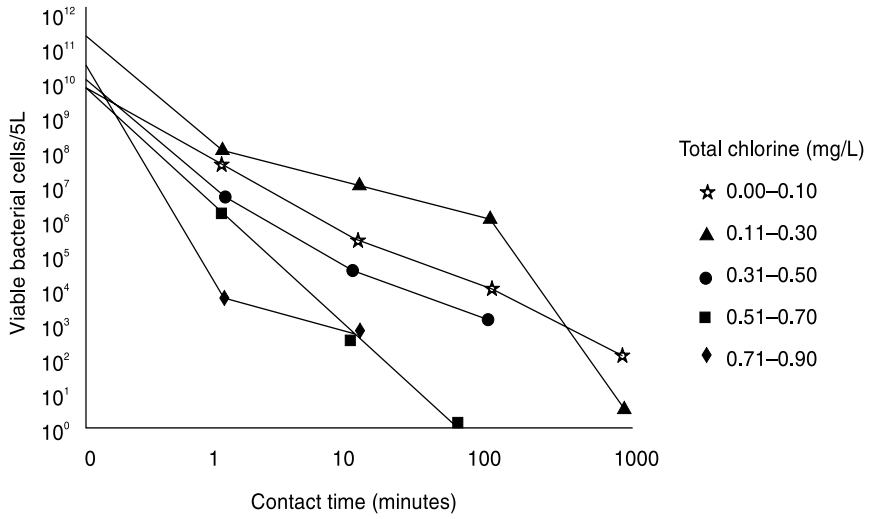

Figure 3 **Mean survival rates for *Escherichia coli* in chlorine at five different concentrations (both scales are logarithmic)**

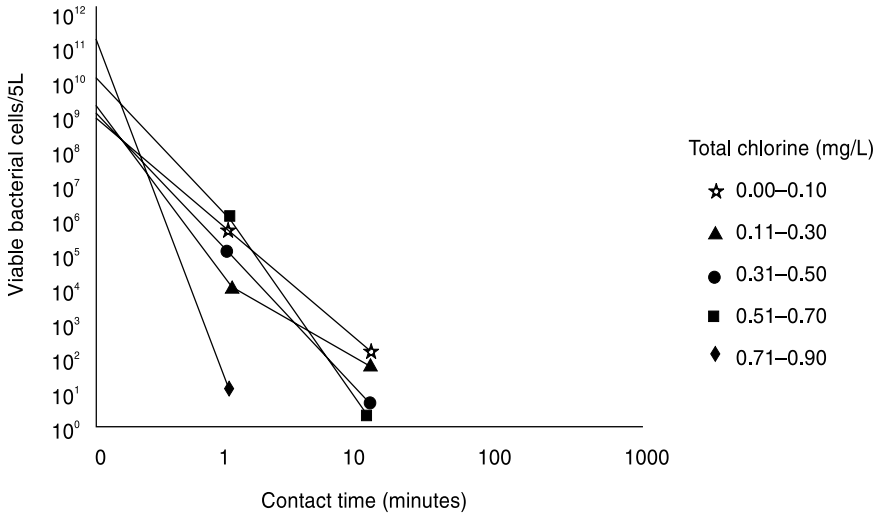

Figure 4 **Mean survival rates for *Aeromonas hydrophila* in chlorine at five different concentrations (both scales are logarithmic)**

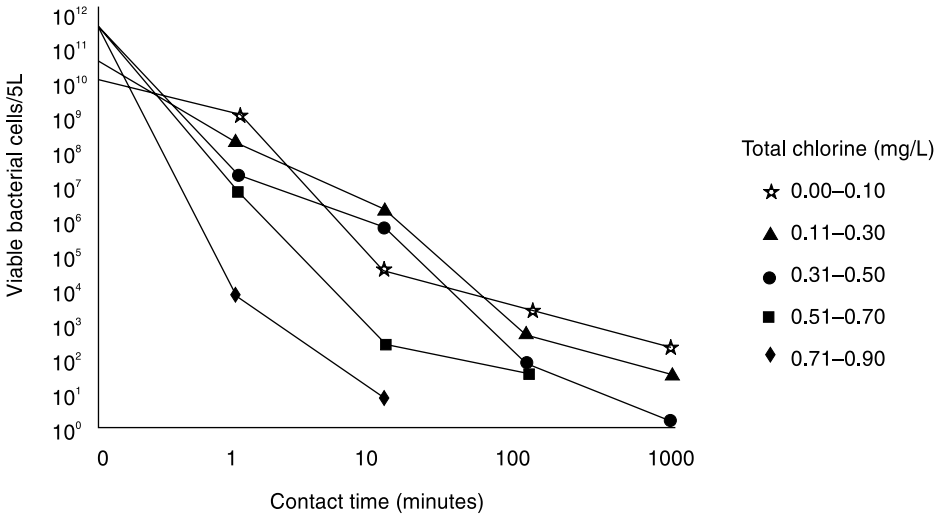

Figure 5 **Mean survival rates for *Enterococcus faecalis* in chlorine at five different concentrations (both scales are logarithmic)**

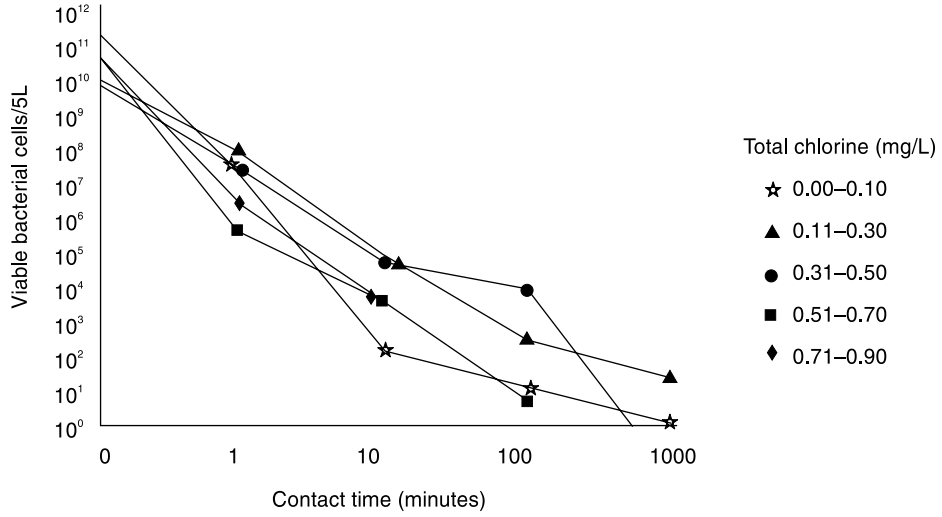

Figure 6 **Mean survival rates for *Salmonella typhi* in chlorine at five different concentrations (both scales are logarithmic)**

tive bacilli, or both. None of the bacteria used in our investigation were found in the raw water samples.

The findings were analysed using the chi-squared test and assuming $P < 0.05$, the possible significant relationships between the dependant variable (microorganism survival) and the independent variable (total chlorine) was determined. The minimum concentration of total chlorine which ensured the safety of the drinking water in our samples was 0.71 mg/L.

Discussion

The recommended total chlorine concentration after an exposure time of half an hour under non-emergency conditions and at appropriate pH levels is 0.5–0.8 mg/L at the end-of-line points [*9*]. In our study, 53.58% of the samples had chlorine concentrations below this level (under emergency conditions such as epidemics of gastrointestinal infection and natural disasters the recommended level is 1.0 mg/L, mainly because of the distance water has to travel from its point of origin, the treatment plant, to its point of consumption).

Haas found that the chlorine residual in distribution systems should be maintained at no less than 0.5 mg/L since at this chlorine concentration secondary contaminants would be eliminated and heterotrophic growth prevented [*11*].

In our study, the chlorine concentration in half the samplings in the service area was not only below that required for emergency conditions but that also below the level recommended for normal conditions.

The pH levels of the samples ranged between 8.0 and 8.2, which is within the recommended range of 7.0–8.5 [*9*].

In our samples, TOC concentrations were > 2.0 mg/L in 17.9% of cases (Figure 2). Organic carbon can create a nutrient environment favourable to bacterial survival and also contribute to the formation of chlorine by-products. Since the municipal drinking water distribution system is large and covers a vast service area, the effects of TOC will be amplified. It is, therefore, essential to reduce TOC levels to the maximum recommended level of 2.0 mg/L (the recommendation in D/DBPS Act) [*12*].

The most chlorine-sensitive microorganism studied was *A. hydrophila*, which did not remain stable for more than 100 minutes in any of the samples. Thus, a vast range of chlorine concentrations are effective on this bacteria and the presence of chlorine disinfectant in the water distribution system at concentration 0.11–0.90 mg/L will suffice for the removal of secondary contamination by this microorganism in less than 100 minutes. In a previous study, Massa et al. found that *A. hydrophila* TW11 was never completely inactive at various concentrations (0.11–0.50 mg/L) and strain TW27 was generally more sensitive than strain TW11, showing a 99% higher mortality rate after 3 minutes contact at 0.5 mg/L chlorine [*13*]. Ozbas and Aytac found that concentrations up to 2.5 mg/L free chlorine had no effect on *A. hydrophila* A306, even after 30 minutes exposure [*14*]. Cattabani registered the survival rate of 4 strains of *A. hydrophila* after an exposure of 10 minutes at a concentration of 0.3 mg/L free chlorine and inhibition after 5 minutes at a concentration of 2.5 mg/L [*15*].

As this species belongs to the family Vibronaceae, its survival characteristics may be generalized to the genera in the family; however, this generalization must be taken cautiously until corroborated by further studies.

E. coli showed a higher resistance to the disinfectant than *A. hydrophila*. In samples with chlorine concentrations < 0.31

mg/L, *E. coli* survived for over 1000 minutes. There was a significant correlation between *E. coli* survival rates and chlorine concentration ($P < 0.05$). Furthermore, *E. coli* lost its resistance in less than 1000 minutes at total chlorine concentrations 0.31–0.90 mg/L. In a previous study on 3 selected *A. hydrophila* strains, Knochel found that *A. hydrophila* E9 was the most resistant isolate in monochloramine solution, followed by *E. coli* and the 2 other strains [*16*].

It has also been reported that *E. coli* was chlorine sensitive but that microorganisms such as *Clostridium perfringens* and viruses did not become inactive in most total chlorine concentrations [*17,18*]. Our results for *E. coli* are in agreement with these. Since in microbiological analyses of water, *E. coli* is in effect an indicator bacterium, it is essential to bear in mind the fact that indicator bacteria are rapidly inactivated in water distribution systems and this may act as a limiting factor to drawing sound, realistic conclusions regarding the quality of water in epidemic outbreaks.

Ent. faecalis also showed considerable resistance to low total chlorine concentrations, remaining active for more than 1000 minutes at chlorine concentrations < 0.51 mg/L but survival dropped at 0.51–0.90 mg/L ($P < 0.05$). It follows that the survival rate for this bacterium over periods up to 1000 minutes depends on chlorine concentration. As this species is also considered an indicator of faecal contamination of water owing to its frequent occurrence in faecal waste, *Ent. faecalis* should be studied with regard to inactivation in terms of contact time, inactivation rate and chlorine concentration. Torkian reported that the presence of free chlorine disinfectant in the municipal water distribution system would help to remove secondary contaminants [*19*]. Our results are in agreement with this.

Total chlorine concentration ≤ 0.50 mg/L led to reductions in the *S. typhi* population in drinking water but not to its total removal. It becomes inactive in < 1000 minutes at chlorine concentrations 0.51–0.70 mg/L and in < 100 minutes at chlorine concentrations 0.71–0.90 mg/L. In the chi-squared test performed to determine the correlation between *S. typhi* survival rates and total chlorine concentration, it was found that P was < 0.05, and consequently, the survival rate of this microorganism depends on total chlorine concentration over 1000 minute periods.

An investigation to evaluate the inactivating power of chlorine residuals in water distribution systems indicated that *E. coli* remained active in drinking water samples over periods up to 1000 minutes. All other organisms tested in this study remained resistant to the disinfectant [*5*]. These findings conform with our own findings on microorganisms in the Isfahan water distribution system.

Recommendations

- The presence of chlorine disinfectant in water distribution systems is essential but the exposure time and chlorine concentration are critical.
- It is essential to bear in mind that, since *E. coli* is the basic faecal indicator in microbial investigations of water quality, in waterborne outbreaks of epidemics chlorine residuals in water may inactivate indicator bacteria but not eliminate other important bacteria in water.
- With regard to the spread of waterborne epidemics in the Islamic Republic of

Iran and reduced safety in water distribution systems, chlorine concentration must be constantly maintained at levels higher than 0.70 mg/L.

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Joint WHO-CEHA and ISESCO regional training course for trainers for sanitary inspection of public water supply systems

The World Health Organization, Regional Centre for Environmental Health Activities (WHO/CEHA) organized a joint WHO-CEHA and Islamic Educational, Scientific and Cultural Organization (ISESCO) regional training course for trainers for sanitary inspection of public water supply systems in Amman, Jordan from 16 to 18 May 2005. The objectives of the training course were to:

- provide training on the methods of sanitary inspections to avoid sources of pollution;
- strengthen water quality monitoring;
- minimize water pollution;
- assess long-term planning for water resources;
- minimize waterborne diseases;
- highlight the use of sanitary inspection as an integral part of water quality surveillance.

Participants from Egypt, Islamic Republic of Iran, Iraq, Jordan, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Pakistan, Palestine, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia and Yemen participated in the course.