WELL

WATER AND ENVIRONMENTAL HEALTH AT LONDON AND LOUGHBOROUGH

WELL Study

Guidelines for wastewater reuse in agriculture and aquaculture: recommended revisions based on new research evidence

Task No: 68 Part 1

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June 2000

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Acknowledgements

The authors would like to thank Dr Rebecca Stott for writing Annex B (Experimental studies on the contamination of wastewater irrigated crops in Brazil and UK) and contributing to the section on the protection of risks from protozoa; the participants of the of the technical meeting on wastewater reuse held in Mexico City of December 4th 1998 for useful comments and contributions which have influenced the recommended revisions to the guidelines and the sections on wastewater treatment and health protection measures; and the Department for International Development (DFID) and Sanitation in Developing Countries (SANDEC) for their encouragement and for financial support for this work .

Executive Summary

Use of wastewater in agriculture is becoming more important due to increasing water scarcity in dry climate regions of the world. Standards for wastewater reuse in many countries have been influenced by the WHO (1989) Health Guidelines and the USEPA/USAID (1992) Guidelines. Since then, epidemiological studies have been carried out by London School of Hygiene and Tropical Medicine, with colleagues in Mexico and Indonesia, and microbiological studies of crops irrigated with treated wastewater have been carried out by Leeds University, with colleagues in Brazil and Portugal, to assess the validity of these guidelines. The WHO (1989) Guidelines for Wastewater Reuse in Agriculture and Aquaculture are reviewed in the light of these and other recent studies.

There are currently three main *approaches for establishing microbiological quality guidelines* and standards for treated wastewater reuse in agriculture which have different objectives as their outcome: (I) the absence of faecal indicator organisms in the wastewater, (II) no measurable excess cases in the exposed population, and (III) a model generated estimated risk below a defined acceptable risk. In this review we use approach II, using empirical epidemiological studies supplemented by microbiological studies on pathogen transmission, in conjunction with approach III, using model-based quantitative microbial risk assessment for selected pathogens in coming to our conclusions. Recommendations have also been made for the use of a disease control approach in the setting of country standards.

The results of studies of consumer risks do not provide any evidence to suggest a need to change the WHO *faecal coliform guideline* of $=10^3$ FC/100ml for *unrestricted irrigation*. Epidemiological studies in a situation where enteric infections are endemic suggest that risks of enteric infections are significant, but low, when the guideline is exceeded by a factor of 10. There was no risk associated with the total consumption of raw vegetables and a two-fold increased risk associated with consumption of specific vegetables (e.g. onion). Risk assessment has indicated that the annual risk of enteric virus and bacterial infection from eating lettuce irrigated with water meeting WHO Guideline level ranges from 10^{-5} to 10^{-9}. However, enteric viruses other than the ones studied may outnumber them by possibly an order of magntiude. Since the US microbial standards for drinking water are based on the criteria that human populations should not be subjected to the risk of infection by enteric disease greater than 10^{-4} (or I case in 10,000 persons/year), then the WHO Wastewater Reuse Guidelines would appear to offer a similar level of protection. In situations where there are insufficient resources to reach 10^3FC/100ml, then a more relaxed guideline of 10^4FC/100ml could be adopted, but should be supplemented by other health protection measures.

The *nematode egg guideline* of =1 nematode egg/litre for *unrestricted irrigation* appears to be adequate to protect consumers of cultivated vegetables spray-irrigated with effluent of consistent quality and at high temperatures, but not necessarily consumers of vegetables surface-irrigated with such effluent at lower temperatures. Studies have shown that lettuces spray-irrigated with water of =1 nematode egg/litre were not contaminated or only lightly contaminated at harvest, and any eggs present were not infective. However, since a few eggs on the harvested plants were viable, crops with a long shelf life represent a potential risk to consumers. Children who ate wild vegetables irrigated with water of =1 nematode egg/litre, however, had significantly increased prevalence of *Ascaris*. It is recommended that a stricter guideline of =0.1 eggs per litre is adopted to prevent transmission of *Ascaris* infection and to allow for the risks to farm workers involved in cultivating the vegetable crops. A guideline of =1 nematode egg/litre may be adequate where crops with a short shelf life are grown (e.g. salad crops) and wild plants are not eaten, or where the aim is disease control (in this case related to intensity not prevalence of infection), instead of prevention of transmission of infection.

In the WHO Guidelines (1989) there was no *faecal coliform guideline* for *restricted irrigation* due to the lack of evidence of a risk of bacterial and viral infections to farm workers and nearby residents. Recent evidence of enteric infections in farming families in direct contact with partially treated wastewater and in populations living nearby sprinkler irrigated fields, when the water quality exceeds 10^6 FC/100ml, suggests that a faecal coliform guideline should now be added. However, where adults and school-aged rural children are in direct contact with the partially treated wastewater originating in an urban area, there may still be at risk of diarrhoeal disease and Human Norwalk-like Virus/Mexico at a level of 10^3-10^4 FC/100ml. A reduced guideline level of =10^3 FC/100ml would be safer where adults are involved in flood/furrow irrigation and children are regularly exposed (through farm work or play). This would also help reduce the risks from epidemic infections which could then be transmitted to effluent-irrigating communities from an outbreak in the source community. Where there are insufficient resources to provide treatment to reach this stricter guideline, a guideline of 10^5 FC/100ml should be supplemented by other health protection measures for children.

The *nematode egg guideline* of ≤1 egg per litre is adequate if no children are exposed, but a revised guideline of ≤0.1 egg per litre is recommended if children are in contact with the wastewater through irrigation or play. Children in contact with effluent from a storage reservoir which met WHO Guidelines had increased prevalence and intensity of *Ascaris* infection, but when the effluent had been stored in two reservoirs and no nematode eggs were detectable, there was very little excess *Ascaris* infection in any age group. A stricter guideline of =0.1 eggs per litre is recommended where children are exposed to irrigation water. Alternatively, a country with limited resources aiming at disease control could adopt a less strict guideline and adopt additional health protection measures; such as, human exposure control and chemotherapeutic intervention.

The evidence reviewed did not support the need for a *separate guideline* to specifically protect against viral infection, but there were insufficient data to evaluate the need for a specific guideline for protozoa.

Regarding *wastewater use in aquaculture*, evidence from epidemiological studies shows that the faecal coliform guideline needs to be below 10^4 FC/100ml. There appears to be sufficient evidence to suggest that the tentative faecal coliform guideline of =10^3 FC/100ml for the fishpond water is the right order of magnitude, and insufficient data to warrant a reduction of this level to 10^2 FC/100ml or a relaxation to 10^4 FC/100ml. This implies that the quality of the feed water can be around 10^4-10^5 FC/100ml, depending on the size of the fishpond and the amount of dilution that occurs. In future, it would be useful to consider adding a bacterial guideline for the quality of the wastewater (SPC/100ml) and for the quality of fish (SPC/g). This will address concerns over the adequacy of faecal coliforms as indicators of health risks from waste-fed aquaculture.

Wastewater treatment technologies suitable for meeting the revised microbiological guidelines for agriculture include the use of waste stabilisation ponds (WSP), wastewater storage and treatment reservoirs (WSTR), or conventional treatment processes. When using WSP, the revised guidelines usually require the use of 1 or more maturation ponds after the anaerobic and facultative ponds. Use of sequential batch-fed storage and treatment reservoirs can be designed to meet the guidelines for unrestricted and restricted irrigation. When conventional treatment processes are used secondary treatment, filtration and disinfection are often needed to meet the revised guidelines. The cost and difficulty in operating and maintaining conventional treatment plants to the level needed to meet the guidelines means that they are not recommended where WSP and WSTR can be used.

A range of *health protection measures* including crop restriction, irrigation technique, human exposure control and chemotherapeutic intervention should all be considered in conjunction with partial wastewater treatment. In some cases, community interventions using health promotion programmes and/or regular chemotherapy programmes could be considered, in particular where no wastewater treatment is provided or where there is a time delay before treatment plants can be built.

In order to meet the faecal coliform guideline for aquaculture, wastewater (or excreta/septage) needs to undergo some form of treatment before it can be used in fishponds. Where WSP are used, effluent from the facultative pond or first maturation pond can be discharged into the fishpond (depending on the effluent quality and size of the fishpond). Where effluent from conventional secondary treatment plants is used, the quality of the effluent may need to be improved by use of a polishing pond prior to the effluent being discharged into a fishpond.

1 Introduction

There has been an increasing interest in reuse of wastewater in agriculture over the last few decades due to increased demand for freshwater. Population growth, increased per capita use of water, the demands of industry and of the agricultural sector all put pressure on water resources. Treatment of wastewater provides an effluent of sufficient quality that it should be put to beneficial use and not wasted (Asano, 1998). The reuse of wastewater has been successful for irrigation of a wide array of crops, and increases in crop yields from 10-30% have been reported (*cited in* Asano, 1998). In addition, the reuse of treated wastewater for irrigation and industrial purposes can be used as strategy to release freshwater for domestic use, and to improve the quality of river waters used for abstraction of drinking water (by reducing disposal of effluent into rivers). Wastewater is used extensively for irrigation in certain countries e.g. 67% of total effluent of Israel, 25% in India and 24% in South Africa is reused for irrigation through direct planning, though unplanned reuse is considerably greater.

During the last decade, there has been growing concern that the world is moving towards a water crisis (Falkenmark, 1989)). There is increasing water scarcity in dry climate regions, for example, in Africa and South Asia, and there are major political implications of water scarcity in some regions e.g. Middle East (Murakami, 1995). Water quantity and quality issues are both of concern. Recycling of wastewater is one of the main options when looking for new sources of water in water scarce regions. The guidelines or standards required to remove health risks from the use of wastewater and the amount and type of wastewater treatment needed to meet the guidelines are both contentious issues. The cost of treating wastewater to high microbiological standards can be so prohibitive that use of untreated wastewater is allowed to occur unregulated.

In the last ten years, new epidemiological, microbiological and risk assessment studies have evaluated the validity of the WHO (1989) Guidelines and other standards, and explored other issues of concern e.g. the need for a viral guideline. This report will review evidence supporting current guidelines for wastewater reuse and the main studies that have contributed to an evaluation of the WHO (1989) Guidelines. Particular emphasis will be given to studies funded by DFID; these include epidemiological studies done by London School of Hygiene and Tropical Medicine in collaboration with the Instituto Nacional de la Nutricion in Mexico City and microbiological studies done by Leeds University with colleagues in Laboratorio Nacional de Engenharia Civil, Lisboa, Portugal, and Estacao Experimental de Tratamentos Biologicos de Esgotos Sanitarios, Universidade Federal da Paraiba, Campina Grande, Paraiba, Brasil. The implications of the studies for the setting of international guidelines for the use of wastewater in agriculture and aquaculture will be considered, along with the wastewater treatment and other health protection measures needed to achieve the guidelines. In a companion document, the implications of the studies for the evaluation of country standards in Mexico will be considered; this case study will be of interest to other Less Developed Countries considering the formulation or review of standards for wastewater use.

2 Agricultural Reuse

2.1 Background

Guidelines for the reuse of effluents, considering methods of wastewater treatment and health safeguards were developed by the World Health Organisation in 1971 (WHO, 1971). These focused on defining appropriate levels of treatment needed for different types of reuse. It was considered that available treatment technologies and use of chlorination could achieve a bacteriological quality of 100 coliform organisms per 100ml, and this would give rise to only a limited health risk if used for the unrestricted irrigation of food crops. When these guidelines were revised (WHO, 1989), more epidemiological and microbiological evidence concerning health risks related to use of untreated and treated wastewater was available, and the guidelines were modified accordingly (Table 1).

The new guidelines have been controversial, particularly relaxation of the guideline for unrestricted irrigation to 1000 faecal coliform[*] per 100ml (geometric mean). Criticisms have included the use of 'partial' epidemiological studies in developing countries, ignoring the acquired immunity of the population involved, and ignoring the health risk assessment methodology used as a foundation for developing drinking water quality standards (Shelef, 1991). Concern has been expressed over the lack of sensitivity of epidemiological methods to detect disease transmission that may not lead to apparent infection in exposed individuals but to secondary transmission from them to cause illness in susceptible individuals (Rose, 1986). Most regulatory agencies in the USA have chosen not to use epidemiological studies as the basis for determining water quality standards (Crook, 1998). The transmission of viral infections through treated wastewater use in industrialised countries has been a particular issue, also related to the relative inefficiency of disinfection processes in removing viruses in comparison with bacteria. Concern has also been expressed over the transmission of emerging parasite infections such as *Cryptosporidium*, *Giardia* and *Cyclospora* which are not easily removed by conventional treatment processes. On the other hand, many countries have welcomed the guidance from WHO, and standards in many countries have been based on WHO (1989) Guidelines e.g. France, Mexico.

2.2. Current WHO Guidelines, and standards for selected countries

WHO (1989) Guidelines for the safe use of wastewater in agriculture took into account all available epidemiological and microbiological data and are summarised in Table 1. The faecal coliform guideline (e.g. =1000 FC/100ml for food crops eaten raw) was intended to protect against risks from bacterial infections, and the newly introduced intestinal nematode egg guideline was intended to protect against helminth infections (and also serve as indicator organisms for all of the large settlable pathogens, including amoebic cysts). The exposed group that each guideline was intended to protect and the wastewater treatment expected to achieve the required microbiological guideline were clearly stated. Waste stabilisation ponds were advocated as being both effective at the removal of pathogens and the most cost-effective treatment technology in many circumstances.

In contrast, US-EPA (1992) has recommended the use of much stricter guidelines for wastewater use in the USA. The elements of the guidelines applicable to reuse in agriculture are summarised in Table 2. For irrigation of crops likely to be eaten uncooked, no detectable faecal coliforms/100ml are allowed (compared to ≤1000 FC/100ml for WHO), and for irrigation of commercially processed crops, fodder crops, etc, the guideline is ≤200 FC/100ml (where only a nematode egg guideline is set by WHO). No nematode egg guideline is

[*] The term `faecal coliforms' is used herein as it is the term most commonly used and understood in the wastewater reuse sector. It may be interpreted as being broadly equivalent to the term `thermotolerant coliforms'. A preferred usage would be `thermotolerant coliforms/*Escherichia coli*; this would allow the eventual use of *E.coli* as the preferred (and exclusively faecal) coliform bacterium (*2*).

& Olivieri, 1998). California has some of the strictest standards, requiring <2.2 total coliforms/100ml for irrigation of food crops (through secondary treatment followed by filtration and disinfection), and <23 TC/100ml for irrigation of pasture and landscape impoundments (through secondary treatment and disinfection) (Crook, 1998). Standards in use in many countries (e.g. Israel, Oman) have been influenced by standards in the USA.

Table 1. The 1989 WHO guidelines for the use of treated wastewater in agriculture[a] (*1*)

Category	Reuse conditions	Exposed group	Intestinal nematode[b] (arithmetic mean no. eggs per litre)[c]	Faecal coliforms (geometric mean no. per 100ml)[c]	Wastewater treatment expected to achieve the required microbiological guideline
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks[d]	Workers, consumers, public	≤ 1	≤ 1000	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	Workers	≤1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal
C	Localized irrigation of crops in category B if exposure to workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by irrigation technology, but not less than primary sedimentation

[a] In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.
[b] *Ascaris* and *Trichuris* species and hookworms.
[c] During the irrigation period.
[d] A more stringent guideline (≤ 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may cone into direct contact.
[e] In the case of fruit trees, irrigation should cease two weeks before fruit is picket, and no fruit should be picked off the ground. Sprinkler irrigation should be used.

specified by US-EPA. Actual standard setting is the responsibility of individual states in the USA, and different States take different approaches (some specify treatment processes, others specify water quality standards) and a range of standards are in use (Table 3, Cooper). Other countries have been influenced by the WHO (1989) Guidelines and some have modified the microbiological criteria to suit local epidemiological and economic circumstances. The revised standards introduced by Mexico in 1996 (Peasey et al, 1999) were designed to be sufficient to protect at risk groups (according to currently available literature) and achievable with the technology and resources available at the time and in the near future in Mexico (Table 4). The standard also imposes limits for wastewater disposal through discharge into rivers and other water sources. The limits imposed are the same as for restricted irrigation i.e. a daily mean of no more than 2000 FC/100ml and a monthly mean of no more than 1000FC/100ml. The standard is aimed to be workable, understandable, compact and clear for the general public and its main objective is to reduce contamination of rivers, lakes, aquifers and other water sources.

Table 2: US-EPA/USAID Guidelines for agricultural reuse of wastewater (adapted from Suggested guidelines for water reuse[1] (US-EPA/USAID, 1992))

Types of Reuse	Treatment	Reclaimed Water Quality	Reclaimed Water Monitoring
Agricultural Reuse – *Food Crops Not Commercially Processed* Surface or spray irrigation of any food crop, including crops eaten raw	• Secondary[2] • Filtration • Disinfection	• = 10 mg/l BOD • No detectable fecal coli/100ml[3] • 1 mg/l Cl_2 residual (min.)	• BOD - weekly • Coliform - daily • Cl_2 residual - continuous
Agricultural Reuse – *Food Crops Not Commercially Processed* Surface irrigation of Orchards and Vineyards	• Secondary[2] • Disinfection	• = 30 mg/l BOD • = 30 mg/l SS • = 200 fecal coli/100ml[4,5] • 1 mg/l Cl_2 residual (min.)	• BOD - weekly • SS - daily • Coliform - daily • Cl_2 residual - continuous
Agricultural Reuse – *Non Food Crops* Pasture for milking animals; fodder, fiber and seed crops	• Secondary[2] • Disinfection	• = 30 mg/l BOD[7] • = 30 mg/l SS • = 200 fecal coli/100ml[4,5] • 1 mg/l Cl_2 residual (min.)	• BOD - weekly • SS - daily • Coliform - daily • Cl_2 residual - continuous
Urban Reuse All types of landscape irrigation (e.g. golf courses, parks, cemeteries).	• Secondary[2] • Filtration • Disinfection	• =10 mg/l BOD • No detectable fecal coli/100ml[3] • 1 mg/l Cl_2 residual (min.)	• BOD - weekly • Coliform – daily • Cl_2 residual - continuous

Footnotes:
1 These guidelines are based on water reclamation and reuse practices in the U.S., and they are especially directed at states that have not developed their own regulations or guidelines. While the guidelines should be useful in many areas outside the U.S., local conditions may limit the applicability of the guidelines in some countries.
2 Secondary treatment processes include activated sludge processes, trickling filters, rotating biological contractors, and many stabilization pond systems. Secondary treatment should produce effluent in which both the BOD and SS do not exceed 30mg/l.
3 The number of fecal coliform organisms should not exceed 14/100 ml in any sample.
4 The number of fecal coliform organisms should not exceed 800/100ml in any sample.
5 Some stablization pond systems may be able to meet this coliform limit without disinfection.

Table 3: Examples of microbial quality standards used by various States in USA (from Cooper and Olivieri, 1998)

Exposure Route	Total Coliform per 100ml		Fecal Coliform per 100ml		Enteric Viruses per 40L	
	n[a]	Range of Values	n	Range of Values	n	Range of Values
Spray Irrigation[b]	4	2.0-100	3	2.2-200	1	1[e]
Surface Irrigation[b]	2	100	9	10-1,000	0	-
Parks and Playgrounds[c]	8	2.2-100	3	10-100	1	125
Golf Courses and Open Space[d]	6	2.2-1,000	5	0-100	0	-

[a] Number of states involved out of the 13 selected.
[b] Includes food crop irrigation.
[c] Includes playgrounds
[d] Includes cemeteries.
[e] Arizona is the only state that has a virus standard.

The helminth standard was set at a level achievable through conventional secondary treatment, as financial resources were inadequate to add filtration to the treatment train.

Several countries in the European Union have also been influenced by WHO (1989) Guidelines (Bontoux, 1998). France has used a similar approach in the drawing up of "Sanitary recommendations for the use, after treatment, of municipal waste waters for the irrigation of crops and landscaped areas", published in 1991 (not yet compulsory). The recommendations are similar to those of WHO, defining analogous water categories (A,B and C) and microbiological limits, but complement them with strict rules of application (Bontoux and Coutois, 1997). For example, for category A, the quality requirement must be complemented by use of irrigation techniques which avoid the wetting of fruit and vegetables, and for irrigation of golf courses and open landscaped areas, spray irrigation must be performed outside the opening hours for the public.

Table 4: Mexican Standard governing wastewater reuse in agriculture (NOM-001-ECOL-1996)

Irrigation	FC/100ml (MPN)	Helminth ova/litre
Restricted	1000_m - 2000_d	≤5
Unrestricted	1000_m - 2000_d	≤1

(m=monthly mean, d=daily mean, MPN=most probable number)

Note: Unrestricted irrigation is defined as permitting irrigation of all crops, whilst restricted irrigation excludes salad crops and vegetables that are eaten raw (individual crops are no longer specified in the standard).

Much wastewater reuse in agriculture is indirect and not direct use, that is, the wastewater is disposed of into rivers and the contaminated river water is used for irrigation. However, international guidelines for the microbiological quality of irrigation water used on a particular crop do not exist (Westcot, 1997). The US Environmental Protection Agency (EPA) recommended that the acceptable guideline for irrigation with natural surface water, including river water containing wastewater discharges, be set at 1000 FC/10ml (US-EPA, 1973). This standard has been adopted in some other countries as an irrigation water quality standard, for example, Chile, in 1978 (Westcot, 1997). This standard is also consistent with guidelines for unrestricted irrigation. FAO has now recommended that the WHO (1989) Guidelines are used interim irrigation water standards, until more epidemiological information is available (Westcot, 1997).

2.3 Summary of evidence supporting WHO (1989) Guidelines

Shuval et al (1986) reviewed all the available epidemiological evidence on the health effects of agricultural use of wastewater. Their main conclusions were reported in the technical report of the WHO Guidelines (1989). They are summarised here, with some supporting details.

2.3.1 Effects of use of untreated wastewater

2.3.1.1 Effects on farm workers or wastewater treatment plant workers

Use of untreated wastewater for crop irrigation causes significant excess infection with intestinal nematodes in farm workers, in areas where such infections are endemic. In India, sewage farm workers had a significant excess of *Ascaris* and hookworm infections, compared with farm workers irrigating with clean water (Krishnamoorthi et al, 1973). The intensity of the infections (number of worms per person) and the effects of infection were also higher, e.g. the sewage farm workers suffered more from anaemia, one of the symptoms of severe hookworm infection. There is some evidence that sewer workers may be at increased risk of protozoan infections such as amoebiasis and giardiasis (Dolby et al, 1980, Knobloch et al, 1983) but other studies have not found such an effect (Clark et al,1984). There is no reliable data on the impact on amoebiasis on farm workers in contact with untreated wastewater.

Cholera can be transmitted to farm workers if they irrigate with raw wastewater coming from an urban area where a cholera epidemic is occurring. This was the case in the outbreak of cholera in Jerusalem in 1970, where cholera is not normally endemic and the level of immunity to cholera was low (Fattal et al, 1986a).

There is limited evidence of increased bacterial and viral infections among wastewater irrigation workers or wastewater treatment plant workers exposed to untreated wastewater or wastewater aerosols. Sewage treatment plant workers from 3 cities in the USA did not have excess gastrointestinal illness (compared to controls) but inexperienced workers had more gastrointestinal symptoms than experienced workers or controls (municipal workers); however, these were mild and transitory, and there was no consistent evidence of increased parasitic, bacterial or viral infections from stool examinations or antibody surveys (Clark et al, 1981). In a follow up study, there were no excess seroconversions to Norwalk virus or rotavirus in the inexperienced workers with gastroenteritis, but inexperienced workers had higher rates of antibody to Norwalk virus (Clark et al, 1985).

2.3.1.2 Effects on consumers of vegetable crops

Irrigation of edible crops with untreated wastewater can results in the transmission of intestinal nematode infections and bacterial infections. The transmission of *Ascaris* and *Trichuris* infections through consumption of wastewater irrigated salad crops has been demonstrated in Egypt (Khalil, 1931) and Jerusalem (Fattal et al, 1994), where the infections fell to very low levels when wastewater irrigation was stopped.

Transmission of cholera can occur to consumers of vegetable crops irrigated with untreated wastewater, as during the outbreak of cholera in Jerusalem in 1970. It appears that typhoid can also be transmitted through this route, as seen in Santiago, Chile, where the excess of typhoid fever in Santiago compared with the rest of Chile, and in the summer irrigation months, has been attributed to irrigation with river water containing untreated wastewater (Ferrecio et al, 1984, Shuval et al, 1986). In both cases, transmission has occurred in communities with relatively high sanitation levels where transmission through common routes such as contaminated drinking water and poor personal hygiene has been diminished substantially.

Cattle grazing on pasture irrigated with raw wastewater can become heavily infected with the larval stage of the tapeworm *Taenia saginata* (Cysticercus bovis), as has occurred in Australia. There is no epidemiological evidence of human infection through the consumption of raw or undercooked meat from such cattle, but the risk of infection through this route probably exists.

Many outbreaks of enteric infection have been associated with wastewater contaminated foods, but of the very few which were associated with wastewater irrigation, untreated wastewater was used in all but two cases (Bryan, 1977).

2.3.2 Effects of use of treated wastewater

2.3.2.1 Effects on farm workers or nearby populations

There is very limited risk of infection among workers using partially treated wastewater for irrigation. At Muskegon, USA, workers exposed to partially treated wastewater (from aeration basins and storage lagoons) had no increase in clinical illness or infection with enteroviruses. Only highly exposed workers (nozzle cleaners) had excess antibodies to one enterovirus but no seroconversion and no excess in clinical illness (Linneman et al, 1984).

Sprinkler irrigation with partially treated wastewater can create aerosols containing small numbers of excreted viruses and bacteria but there is no conclusive evidence of disease transmission through this route. Several studies in Kibbutzim in Israel have addressed this question. Here, wastewater is partially treated in oxidation ponds before use for irrigation. The first study (Katzenelson et al, 1976) suggested increases in salmonellosis, shigellosis, typhoid fever and infectious hepatitis in farmers and their families working on or living near fields sprinkler irrigated with effluent from oxidation ponds (retention 5-7 days), but the study was methodologically flawed. The second study (Fattal et al, 1986b) found a twofold excess risk of clinical 'enteric' disease in young children (0-4 years) living within 600-1000m from sprinkler irrigated fields, but this was in the summer irrigation months only, with no excess illness found on an annual basis. The third study (Fattal et al, 1986c and Shuval et al, 1989) found that episodes of enteric disease were similar in Kibbutzim most exposed to treated wastewater aerosols (sprinkler irrigation within 300-600m of residential areas) and those not exposed to wastewater in any form. The wastewater was partially treated in ponds with 5-10 days retention reaching a quality of 10^4-10^5 coliforms/100ml. No excess of enteric disease was seen in wastewater contact workers or their families, as well as in the general population living near the fields. This prospective study is considered to be conclusive, having a superior epidemiological design.

However, it does seem that transmission of enteric viral pathogens to populations living near fields sprinkler irrigated with partially treated wastewater can occur under some circumstances, though this may not result in significant excess clinical infection. In a seroepidemiological study associated with the third Israeli study (Fattal et al, 1986c and Shuval et al, 1989) the results suggested that a non-endemic strain of ECHO 4 virus, which was causing a national epidemic in urban areas, was transmitted to rural communities through aerosols produced by sprinkler irrigated of wastewater, though no excess clinical disease was detected (Fattal et al,1987). The fact that no similar excess of the other viral antibodies studied was found suggests that exposure to wastewater aerosols does not lead to an excess in enteroviral infection under non-epidemic conditions

2.3.2.2 Effects on consumers of vegetable crops

When vegetables are irrigated with treated wastewater rather than raw wastewater, there is some evidence from Germany that transmission of *Ascaris* infection is drastically reduced. In Berlin in 1949, where wastewater was treated using sedimentation and biological oxidation prior to irrigation, rates of *Ascaris* infection were very low, whereas in Darmstadt where untreated wastewater was used to irrigate vegetable and salad crops, the majority of the population was infected (Baumhogger,1949 and Krey,1949). Rates were highest in the suburb where wastewater irrigation was practiced, suggesting farm workers and their families were infected more through direct contact than consumption.

2.4 New evidence of health risks from epidemiological and microbiological studies in Mexico

2.4.1 Study area

Raw wastewater coming from Mexico City to the Mezquital valley, Hidalgo, is used to irrigate a restricted range of crops, mainly cereal and fodder crops through flood irrigation techniques. Some of the wastewater passes through storage reservoirs and the quality of the wastewater is improved before use; this is equivalent to partial treatment. The effluent from the first reservoir (retention time 1-7 months, depending on the time of year) meets the WHO Guideline for restricted irrigation (category B), even though a small amount of raw wastewater enters the effluent prior to irrigation (quality 10^5 FC/100ml and $\leq$1 nematode egg/litre). Effluent from the second reservoir is retained for an additional 2-6 months (>3 months of combined retention), and the quality improved further (quality 10^3 – 10^4 FC/100ml and no detectable nematode eggs). Part of the effluent from the first reservoir enters the river and is abstracted downstream to irrigate a large area of vegetable and salad crops, many of which are eaten raw; the river water is essentially partially treated wastewater (quality 10^4 FC/100ml). These crops are sold in the local markets and eaten by the rural populations in local villages, including those near the second reservoir. In a nearby area, vegetables are irrigated with borehole water.

2.4.2 Results: risks to farm workers related to restricted irrigation and effect of wastewater treatment

2.4.2.1 Exposure to raw wastewater

Farm workers and their children in contact with raw wastewater through irrigation or play have a significantly higher prevalence of *Ascaris* infection than those in a control group, who practice rain-fed agriculture (Fig 1a). The excess infection is greater in children than in adults (Blumenthal et al, 1996, Peasey, 2000). Young children (aged 1-4 yrs) also have a significantly higher rate of diarrhoeal disease (Fig 1b) (Cifuentes et al, 1993).

Figure 1a: Effect of exposure to wastewater on *Ascaris* infection: effect of raw wastewater

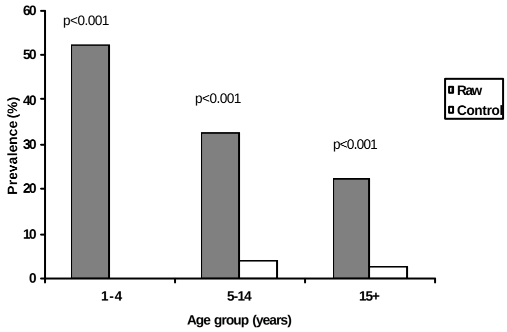
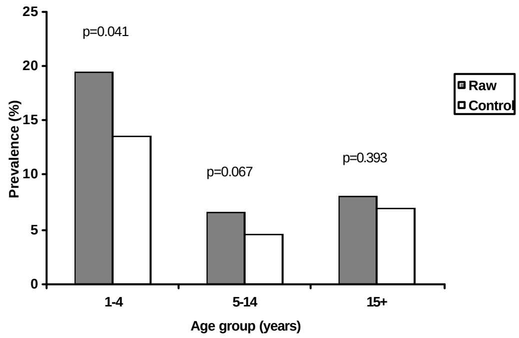


Figure 1b. Effect of exposure to wastewater on diarrhoeal disease : effect of raw wastewater



2.4.2.2 Exposure to partially treated wastewater

Contact with wastewater which has been retained in one reservoir before use (<1 nematode egg/l and 10^5 FC/100ml) results in excess *Ascaris* infection in children, but not in adults, where the prevalence was reduced to a similar level to the control group (Fig 1c) (Blumenthal et al, 1996). Children aged 5-14 years also have significantly higher rates of diarrhoeal disease (Fig 1d) (Cifuentes et al, 1993, Blumenthal et al, 2000a).

Figure 1c. Effect of exposure to wastewater on *Ascaris* infection : effect of retention in one reservoir

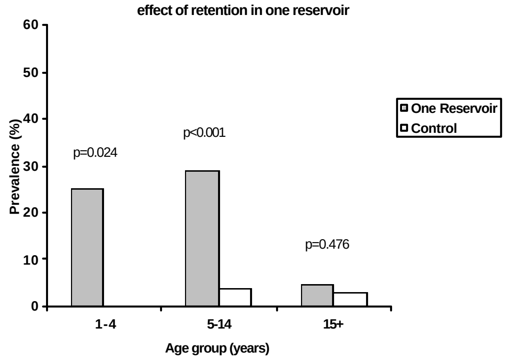
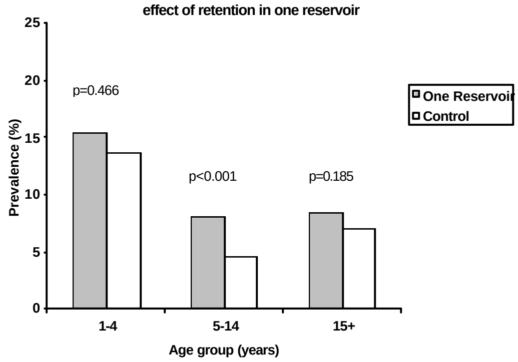


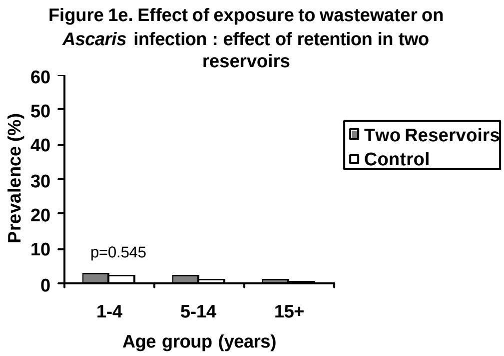
Figure 1d. Effect of exposure to wastewater on diarrhoeal disease : effect of retention in one reservoir

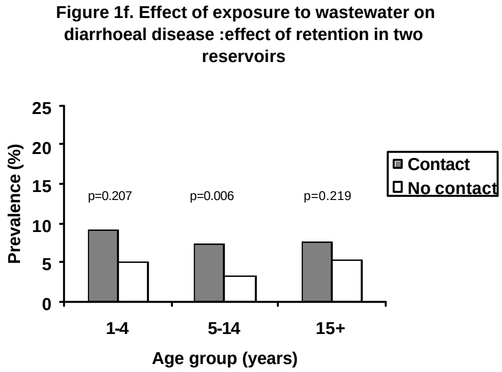


When wastewater has been retained in two reservoirs in series before use (no nematode eggs detected, geometric mean $4x10^3$ FC/100ml, maximum 10^5 FC/100ml) direct contact results in very little excess *Ascaris* infection in any age group (Fig 1e) (Cifuentes et al, 1994, Cifuentes, 1998). However, there is a significant excess of diarrhoeal disease in children aged 5-14 years (Fig 1f), and a four-fold increase in seroresponse to Human Norwalk-like Virus/Mexico in adults with high levels of contact with the effluent from the second reservoir (Annex A, Table 1c) compared with those with no contact with this effluent (Blumenthal et al, 1998, Blumenthal et al, 2000b).

Retention of water in two reservoirs in series, producing water of average quality 10^3 FC/100ml and no detectable nematode eggs, is therefore adequate to protect the children of farmworkers from *Ascaris* infection but not against increased diarrhoeal disease.

The results are presented in more detail in Annex A.





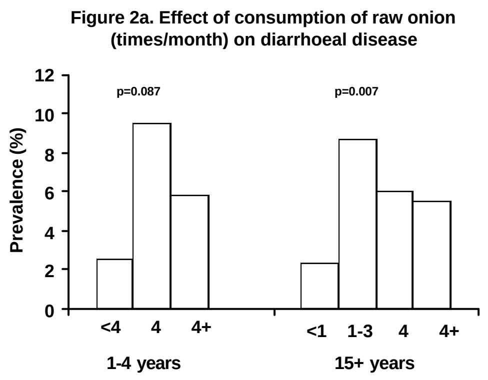
2.4.3 Risks to consumers related to unrestricted irrigation

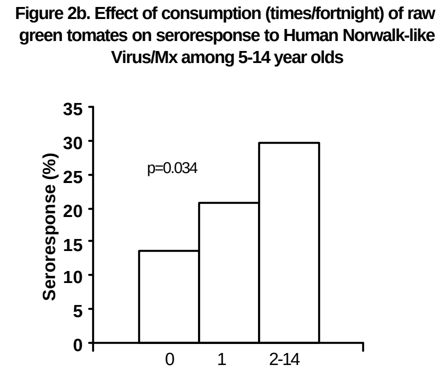
Risks from bacterial and viral infections related to the consumption of specific vegetables (ie. courgette, cauliflower, cabbage, carrots, green tomato, red tomato, onion, chilli, lettuce, radish, cucumber and coriander) and to total consumption of raw vegetables irrigated with partially treated wastewater (average quality 10^4 FC/100ml) were investigated. Consumers (of all ages) had no excess infection with diarrhoeal disease, and no excess infection as measured by serological response to Human Norwalk-like Virus/ Mexico (Hu/NLV/Mx), or *Enterotoxigenic Escherichia coli* (ETEC) related to their total consumption of raw vegetables, that is, the number of raw vegetables eaten each week (Blumenthal et al, 1998, Blumenthal et al, 2000b).

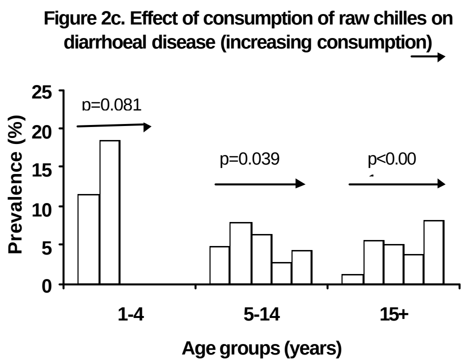
However, there was an excess of diarrhoeal disease in those in the exposed area who ate increased amounts of onion compared with those who ate very little (Fig 2a). The effect was seen particularly in adults and children under 5 years of age. There were also higher levels of serological response to Hu/NLV/Mx in school-aged children who ate green tomato (Fig 2b) and in adults who ate salsa (containing green tomato). The increase in diarrhoeal disease associated with eating increased amounts of raw chillies (Fig 2c) was not related to use of partially-treated wastewater as the chillies eaten by the study population were grown in raw wastewater. Only the risks from eating onion and green tomato can be associated with using partially treated wastewater in irrigation. In the final analysis, consumption of onion, or green tomato, once a week or more was associated with at least a two-fold increase in diarrhoea or Hu/NLV/MX respectively. Enteroviruses were found on onions at harvest, giving support to this epidemiological evidence. The effects described were seen after allowance was made for other risk factors for diarrhoeal disease**.** No excess serological response to enterotoxigenic *E. coli* was related to raw vegetable consumption.

Consumption of vegetable crops irrigated with water of quality 10^4 FC/100ml therefore causes a significant risk of enteric infection in consumers.

The results are presented in more detail in Annex A.







2.5 New evidence of health risks from studies in other sites

In this section, new studies that shed light on the appropriateness of the WHO (1989) Guidelines are reviewed (evidence from studies that were not fully published at the time of the WHO Scientific Group meeting in 1987 is included).

2.5.1 Effects on farm workers or wastewater treatment plant workers

Evidence of the beneficial effect of wastewater treatment, and particularly of the positive effect of wastewater storage in reservoirs, was found in the Lubbock Infection Surveillance Study, a study of farm workers and residents living near the Lubbock land treatment system in Texas, USA. Here, a rural community was exposed to sprinkler application of partially treated wastewater from a much larger urban community (Camann et al, 1986). For the first year, mainly primary effluent and trickling filter effluent was used to irrigate cereals and industrial crops (quality 10^6 FC/100ml and virus 100-1000pfu/l)), and in the second year, the effluent was stored in reservoirs before use (quality 10^3-10^4 FC/100ml and virus <10pfu/l) (Camann et al, 1988).

There was no clear association between self-reported clinical illness episodes and exposure to wastewater (Camann et al, 1986). However, in the data on seroconversion to viral infections, a high degree of aerosol exposure was related to a slightly higher rate of viral infections (risk ratio of 1.5-1.8). A dose-response relationship was observed over the four irrigation seasons; the episodes of viral infection associated with wastewater exposure mainly occurred in the first year, before the reservoirs had come into use. More supporting evidence was found for the role of the wastewater aerosol route of exposure than for direct contact with wastewater. Of the many infection episodes observed, few were conclusively associated with wastewater exposure and none resulted in serious illness. However, the authors could not determine whether wastewater exposure or identified alternative explanations were the actual risk factors for the enteric viral infections. Analysis of clinical viral infection data (from faecal specimens) also showed that aerosol exposure (high) was associated with new viral infections in the summer of the first year of irrigation, but the effect was of borderline significance ($p=0.06$)(Camann and Moore, 1987). However, when allowance was made for alternative risk factors, eating at local restaurants was identified as an alternative explanation for the viral infection episodes. In a specific study of rotavirus infection, wastewater spray irrigation had no detectable effect on the incidence of infection (Ward et al, 1989). Altogether, the results do suggest that aerosol exposure to wastewater of quality 10^3-10^4 FC/100ml does not result in excess infection with enteric viruses. There is some evidence that exposure to wastewater of quality 10^6 FC/100ml results in excess viral infection (but not disease) but this is not conclusive.

A new study of wastewater treatment plant workers (Khuder et al, 1998) suggests that they have a significantly higher prevalence of gastroenteritis and gastrointestinal symptoms than controls (college maintenance and oil refinery workers). There was no association between extent of exposure and prevalence of symptoms. However, these results are not reliable since workers were asked about symptoms over the previous 12 months (retrospectively). The previous studies (Clark et al, 1981 and 1985, see Annex A) are more credible, involving ongoing collection of illness information and human samples (prospectively).

2.5.2 Effects on consumers of vegetable crops

No further epidemiological studies have been located which assess the risk of enteric infections to consumers of vegetable crops irrigated with treated wastewater.

2.5.2.1 Evidence from microbiological studies of crops irrigated with treated wastewater

Studies on bacterial contamination of vegetable crops

Work in Portugal during 1985 - 1989 (Vaz da Costa Vargas et al., 1996) explored the effect of the irrigation of salad crops with treated wastewater of various qualities. When poor quality trickling filter effluent (10^6 FC per 100 ml) was used to spray-irrigate lettuces, the initial level of indicator bacteria on the lettuces (10^6 FC/100g) reflected the bacteriological quality of the irrigation water and exceeded the ICMSF (1974) recommendations for foodstuffs eaten raw ($<10^5$ FC per 100 g fresh weight, preferably $< 10^3$ FC per 100 g). Once irrigation ceased, FC levels were similar to the level seen in lettuces irrigated with fresh water after 7 to 12 days. Final levels were below the recommendations of ICMSF (1974) and the quality was better than that of lettuces on sale in the local markets (10^6 FC per 100 g) irrigated with surface waters.

In studies of drip and furrow irrigation of lettuces and radishes with waste stabilization pond effluent which had a FC count slightly higher than the WHO recommendation of 1000 per 100 ml (1700 - 5000 FC per 100 ml geometric mean count) crop contamination levels varied considerably. Under dry weather conditions they were, at worst, of the orders of 10^3 and 10^4 *Escherichia coli* per 100g for radishes and lettuces respectively, and salmonellae were always absent. The quality was better than that of locally sold lettuces (which had a geometric mean FC count, based on 172 samples, of 1×10^6 per 100g) and fell within the recommendations of ICMSF (1974). However, when rainfall occurred, *E. coli* numbers increased and salmonellae were isolated from lettuce surfaces (Bastos and Mara, 1995).

Experiments in UK assessed the effect of irrigation with final effluent from a conventional treatment plant (10^5-10^6 FC/100ml). When furrow irrigation was used, the quality of lettuces in covered plots improved to acceptable levels (10^3 FC/100g) within 3 days of cessation of irrigation and were *E.coli* free after 9 days. However, results indicated that crops in uncovered plots were recontaminated with bacteria from contaminated soils after significant rainfall and regrowth of *E.coli* on crop surfaces was observed. Radishes were prone to low level long term contamination with *E.coli* (up to 20 days).

These studies show that irrigating salad crops with effluent from conventional treatment plants can result in unacceptable levels of bacterial contamination of crops (unless a period of cessation of irrigation occurs before harvest) whereas use of better quality effluents from waste stablilisation ponds results in acceptable levels of bacterial contamination.

Studies in Israel have investigated the use of effluent from wastewater storage reservoirs in unrestricted irrigation of vegetable and salad crops (Armon et al, 1994). When vegetables were irrigated with poor quality effluent (up to 10^7 FC/100ml of eluant solution) high levels of faecal indicator bacteria were detected (up to 10^5 FC/100ml). However, when vegetables were irrigated with better quality effluent (0-200 FC/100ml) from a storage reservoir with a lower organic loading, faecal coliform levels on crops were generally very low, less than 10^3 FC/100ml and often lower (the data presented do not allow for greater specificity about the levels) with a maximum of 10^4 FC/100ml. The authors concluded that it is necessary to treat wastewater effluents to an extent that no residual contaminants are detected on the irrigated crops, but could alternatively be interpreted as showing that use of treated wastewater meeting WHO (1989) Guideline levels results in acceptable levels (ICMSF, 1974) of bacterial contamination on crops.

Studies on contamination of vegetable crops with nematode eggs
Experimental studies in NE Brazil and Leeds UK, investigated the consumer risk from nematode infection (*Ascaris lumbricoides* and *Ascaridia galli* respectively) from wastewater-irrigated lettuces (Ayres et al., 1992; Stott et al., 1994). In Brazil, when raw wastewater (>100 nematode eggs/l) was used to spray-irrigate lettuce, harvested crops were contaminated with mean values of up to 60 eggs / plant after 5 weeks irrigation. Irrigation with effluent from the anaerobic pond of a series of waste stabilisation ponds (>10 eggs/l) reduced levels of nematode contamination on lettuce to around 0.6 eggs/plant at harvest and produced a better quality of lettuce than that sold in the local market. When facultative pond effluent (<0.5 eggs/l) was used for irrigation, no eggs were detected on crops. Lettuces irrigated with maturation pond effluent (0 eggs/l) were also not contaminated despite growing uncovered plants in heavily contaminated soil containing >1200 *Ascaris* eggs/100g indicating that neither irrigation nor rainfall resulted in recontamination of crops.

In the UK trials, spray-irrigation of lettuce with poor quality wastewater (50 nematode eggs/l) resulted in contamination of around 2.2 eggs/plant at harvest. Improving the wastewater quality to 10 eggs/l resulted in reduced levels of nematode contamination on lettuce to a maximum of 1.5 eggs/plant. When wastewater at the WHO quality of = 1 eggs/l was used for irrigation, very slight contamination was found on a few plants at around 0.3 eggs/plant. However, no transmission of *A. galli* infection was found from wastewater irrigated crops using animal studies although the infective dose is very low at less than 5 embryonated eggs.

The results collectively show that irrigation with wastewater of WHO (1989) Guideline quality resulted in no contamination of lettuce at harvest (0.5 eggs/l) or very slight contamination on a few plants (6%) with eggs that were either degenerate or not infective. However, a few nematode eggs on harvested plants were viable, but not yet embryonated (20% *A. lumbricoides* on >100 eggs/l irrigated crops; <0.1 *A. galli* eggs/plant irrigated with 1-10 eggs/l) and so crops with a long shelf life can represent a potential risk to consumers as these eggs might have time to become infective.

The results are presented in more detail in Annex B.

2.5.2.2 Evidence from risk assessment studies
Asano and Sakaji (1990) used the risk assessment methodology described by Haas

(1983) to estimate the risks of consumption of market-garden produce irrigated with water containing 1 enteric virus in 40 litres (the Arizona standard). An individual's annual risk of infection was between 10^{-4} (ie. one case per 10,000 persons) and 10^{-8} (though when 100ml of such water is accidentally ingested the risk of infection is between 10^{-3} and 10^{-7}). Asano et al (1992) estimated the risk of infection with 3 enteric viruses (poliovirus1 and 3, echovirus 12) related to use of *chlorinated tertiary effluents* and four scenarios of exposure to wastewater; (i) irrigation of market-garden produce, (ii) irrigation of golf courses, (iii) recreational uses of water and (iv) groundwater recharge. They used estimates of the amount of water ingested via the various scenarios, for example, 1 ml/day for 2 days per week all year by golfers handling and cleaning golf balls, 10ml per day for consumers of food crops. Allowance was made for viral reduction in the environment, for example, through stopping irrigation of crops 2 weeks before harvest. The annual risk of infection related to consuming irrigated market-garden produce was between 10^{-6} and 10^{-11} when the effluent contained one viral unit in 100, and between 10^{-4} and 10^{-9} when water with a maximum concentration of 111 viral units/100litres was used. The risk from the irrigation of golf courses is higher, between 10^{-2} and 10^{-5}. Even when *unchlorinated secondary effluents* were investigated (data taken from plants in California), risk assessment showed that for food crop irrigation and groundwater recharge, the annual risk of viral infection was less than 10^{-4} more than 95% of the time (Tanaka et al,1998). For golf courses, the risks are at acceptable levels when chlorinated secondary effluent (3.9 log removal) is used (10^{-4} - 10^{-6}) but not when it is not chlorinated (10^{-1}-10^{-2}). The estimated risks are higher when treated wastewater is used in recreational impoundments used for swimming.

More recently, Shuval et al (1997) used the drinking water model for infection risk developed by Haas et al. (1993) and combined this with laboratory data on the degree of viral contamination of vegetables irrigated with wastewaters of various qualities. The annual risk of becoming infected with hepatitis A from eating cucumbers which had been irrigated with untreated wastewater was 10^{-3} but when the cucumbers were irrigated with treated wastewaters containing =1000 FC per 100 ml the risk was 10^{-6} - 10^{-7}; for rotavirus infection the risk was 10^{-5} - 10^{-6}. Data from waste stabilisation ponds in northeast Brazil (Oragui et al, 1987) suggests that rotavirus numbers are likely to be less than 30 per 100 litres when the faecal coliform content is below 10^{4} per 100ml. The results of these studies are therefore consistent with those obtained by Asano et al (1992).

2.6 Discussion and implications of results for international guidelines and policy concerning wastewater use in agriculture

2.6.1 Implications of the results for international guidelines for safe use of wastewater in agriculture

2.6.1.1 Approaches to setting microbiological guidelines

There are currently several alternative approaches to establishing microbiological guidelines for wastewater reuse, which have different outcomes as their objective:

I The absence of faecal indicator organisms in the wastewater,
II No measurable excess cases in the exposed population, and
III A model-generated risk which is below a defined acceptable risk

Their assumptions appear to be as follows:

I *The absence of faecal indicator organisms in the wastewater*

In this approach, there should be no detectable indicators of faecal pollution in the wastewater. This approach is based on the premise that it is impractical to monitor reclaimed water for all the pathogenic microorganisms of concern, and that use of surrogate parameters, such as faecal indicator organisms, is acceptable. Total and faecal coliforms* are the most commonly used indicator organisms and these are often used in conjunction with specified wastewater treatment requirements. Where this occurs, the assumption is made that the need for expensive and time-consuming monitoring of treated water for pathogenic microorganisms is eliminated. In practice, this approach has led to guidelines which require zero faecal coliforms per 100ml for the irrigation of crops to be eaten raw, in association with a requirement for secondary treatment, filtration and disinfection. USEPA/USAID (1992) have taken this approach and consequently has recommended very strict guidelines for wastewater use in the USA.

II *No measurable excess cases in the exposed population – the epidemiological perspective*

The objective here is that there should be no *actual* risk of infection - that is, no measurable excess risk of infection attributable to wastewater reuse, based on scientific evidence, especially from epidemiological studies. This approach was adopted in setting the 1989 WHO guidelines (Table 1), where epidemiological evidence was used (where available) and supported by information from microbiological studies. Allowance can be made for local epidemiological, socio-cultural and environmental factors and the guidelines modified accordingly.

III. *A model-generated risk which is below a defined acceptable risk*

In this approach an acceptable risk of infection is first defined, as in the case of microbial contamination of drinking water supplies, for example, for which the USEPA has set annual risk of 10^{-4} per person (Haas et al, 1993). Once the acceptable annual risk has been established by the regulator, a quantitative microbial risk assessment (QMRA) model is then used to generate an estimated annual risk of infection based on exposure assessment (including data on the concentrations of microorganisms in wastewater, the quantity of treated wastewater remaining on crop surfaces following irrigation pathogen-indicator ratios and pathogen die-off between food crop harvest and consumption) and “dose-response” data (i.e. data from human infection trials on pathogen dose and resulting infection, if any). A microbiological quality guideline would then be set so that the QMRA model produces an estimate of annual risk which is below the regulator’s acceptable annual risk. This risk assessment approach is especially powerful when the acceptable risk is below the level that can be measured in most epidemiological studies (unless extremely large populations are studied).

These three approaches are considered further elsewhere (Blumenthal et al, 2000c)

In our assessment of the implications of the evidence on the health risks from wastewater use on international guidelines we combine approach II with approach III. We use evidence from studies since 1989 (including evidence from studies that were not fully published at the time of the WHO Scientific Group meeting in 1987) to evaluate the 1989 WHO guidelines, and propose alternative guidelines where the evidence supports a change (Table 2). We use empirical epidemiological evidence where it is available, as these studies measure the result of real exposures that occur over time, and do not depend on the use of estimates of mean daily microbial doses and dose-response analyses based on experiments with healthy volunteers where the data are extrapolated to provide low dose estimates. Epidemiological studies are particularly useful in highly endemic areas for enteric diseases where risks of infection are high enough to be easily measurable with current techniques. Where the epidemiological evidence is incomplete we have used evidence from microbiological studies. Quantified microbial risk assessment studies are particularly useful in areas with a low endemicity of enteric diseases, where risks of infection are low, and where regular monitoring of pathogens in wastewater occurs and produces good data sets for use in exposure assessment. The evidence is strongest where both approaches lead to the same conclusions. If different results are obtained, further analysis of the studies should help to where identify weaknesses and parts of the methodology that need improvement. We believe that this is a rational approach, which it is likely to be cost-effective in most settings. It does not achieve a 'no risk' scenario, and a low level of risk may remain (below that which is detectable through most epidemiological studies). Using the 'no risk' approach (approach I), however, and setting a standard of 0 FC/100ml results in very high additional costs per case of infectious disease averted compared with a standard of 1000 FC/100ml (section 2.5, Shuval et al, 1997). Individual countries may wish to spend money on reducing risks to these very low levels, but it is not necessary for international guidelines to encourage other countries to do so.

A *fourth* approach may be considered in future in the setting of country standards. This is necessary because the implications of wastewater reuse for many infections are not best addressed through using infection as the assessment criteria. Using the 'burden of disease' associated with wastewater reuse would be a better assessment criterion. For example, for diarrhoeal disease this would take into account the incidence of diarrhoeal disease, the number of hospitalisations and the mortality associated with wastewater reuse. However, at present there are no data on which to base such an assessment. Instead, a 'disease control' approach could be adopted (see section 2.6.1.3).

2.6.1.2 Proposed revised guidelines based on using the epidemiological perspective

In light of the epidemiological and microbiological studies reviewed above, it is possible to evaluate the WHO (1989) Guidelines, and propose alternative guidelines where the evidence supports a change (see Table 5).

Unrestricted irrigation - Category A

The results of studies of consumer risks do not provide any evidence to suggest a need to change the WHO *faecal coliform guideline* of $=10^3$ FC/100ml for irrigation of vegetable and salad crops eaten uncooked (*Category A1*). Epidemiological studies in an area in Mexico where enteric infections are endemic suggest that risks of enteric infections are significant, but low, when the guideline is exceeded by a factor of 10 (Blumenthal et al, 1998, Blumenthal et al, 2000b). There was no risk associated with the total consumption of raw vegetables but consumption of onions, eaten by the majority of the study population, was associated with at least a two-fold increase in diarrhoeal disease Microbiological studies also suggest that a guideline of $=10^3$ FC/100ml is appropriate in hot climates, where crops irrigated with water just exceeding the guideline value fell within the quality recommendations of ICMSF (1974) (Vaz da Costas Vargas et al, 1996). Recontamination of crops in uncovered plots after significant rainfall, however, suggests that a stricter guideline may be necessary in countries where significant rainfall occurs during the growing season. However, risk assessment studies in Israel (Shuval et al, 1997) have indicated that the annual risk of enteric virus and bacterial infection from eating lettuce irrigated with water meeting the WHO Guideline level ranges from 10^{-5} (rotavirus) and 10^{-6} (hepatitis A virus) to 10^{-9} (cholera). Data from risk assessment in the USA (Asano et al, 1992) support these conclusions, finding the annual risk of infection from enteric viruses was between 10^{-4} and 10^{-9} when water with a maximum viral concentration of 111 units per 100 litres was used to irrigate market garden produce. Data from waste stabilisation ponds in northeast Brazil (Vaz da Costas Vargas et al, 1996) suggest that rotavirus numbers are likely to be less than 30 per 100 litres when the faecal coliform content is below 10^4 per 100ml. However, other enteric viruses such as adenovirus may significantly outnumber rotaviruses and enteroviruses, possibly by an order of magnitude (*30*). It can therefore be extrapolated from these data that use of water meeting the WHO guideline level of 1000 FC per 100 ml is likely to produce an annual risk of viral infection of less than 10^{-4}. Since the US microbial standards for drinking water are based on the criteria that human populations should not be subjected to the risk of infection by enteric disease greater than 10^{-4}, then the WHO (1989) wastewater reuse guidelines would appear to offer a similar level of protection. Furthermore, additional treatment to a FC level more stringent than 1000 per 100 ml is not cost effective, for example, Shuval et al*.* (1997) showed that the cost per case of hepatitis A avoided by irrigation with zero FC per 100 ml (as recommended by USEPA and USAID, 1992), rather than with 1000 FC per 100 ml, was of the order of US$ 3-30 millions.

The *nematode egg guideline* of =1 nematode egg/litre appears to be adequate to protect consumers of cultivated vegetables spray-irrigated with effluent of consistent quality and at high temperatures, but not necessarily consumers of vegetables surface-irrigated with such effluent at lower temperatures. Studies have shown that lettuces spray-irrigated with water of =1 nematode egg/litre (mean maximum temperatures exceeding 28^{o}C) were not contaminated (when quality <0.5 eggs/litre) or only lightly contaminated at harvest, and any eggs present were not infective (Annex B; Ayres et al, 1992; Stott et al, 1994). However, since a few eggs on the harvested plants were viable, crops with a long shelf life represent a potential risk to consumers. Epidemiological studies of wastewater-related risk factors for *Ascaris* infection in central Mexico showed that there was an increase of *Ascaris* infection among men consuming crops surface-irrigated with raw wastewater infection compared to those who did not each such crops, but there was no increased risk when crops were irrigated with sedimented wastewater (from a reservoir) with ≤1 nematode egg per litre. However, children under 15 years who ate crops from local fields had a two-fold increase in *Ascaris* infection compared with those who did not eat such crops, when either raw wastewater or sedimented wastewater was used in irrigation (Peasey, 2000). The increased risk in these circumstances may have been influenced by the irrigation method (surface, rather than spray), and the lower mean temperature (due to high altitude and semi-desert conditions). It would be sensible, therefore, to adopt a stricter guideline of ≤ 0.1 eggs per litre to prevent transmission of *Ascaris* infection in circumstances where conditions favour the survival of helminth eggs (lower temperatures, surface irrigation), and also to allow for the risks to farmworkers involved in cultivating the vegetable crops (see below). In situations where crops with a short shelf life are grown in hot and dry conditions, and where workers are adequately protected from infection through direct contact with wastewater or soil, the original guideline of ≤1 nematode egg per litre would appear to be adequate. However, use of the revised guideline may be considered prudent even in these circumstances, adding a greater margin of safety.

Restricted irrigation - Category B
In the WHO (1989) guidelines there was no *faecal coliform guideline* for restricted irrigation due to the lack of evidence of a risk of bacterial and viral infections to farm workers and nearby residents. Recent evidence of enteric infections in farming families in direct contact with partially treated wastewater (Mexico) and in populations living nearby sprinkler irrigated fields (USA) when the water quality exceeds 10^6 FC/100ml suggests that a faecal coliform guideline should now be added. Data from Israel (Shuval et al, 1989) and Lubbock, USA (Camann et al, 1986) on situations where spray/sprinkler irrigation is used suggest that a level of $=10^5$ FC/100ml would protect both farm workers and nearby population groups from infection via direct contact or wastewater aerosols (*Category B1*).

However, data from Mexico on a situation where flood irrigation is used showed that there was a significant excess of diarrhoeal disease in children aged 5-14 years, and a four-fold increase in seroresponse to Human Norwalk-like Virus/Mexico in adults with high levels of contact with the effluent from two sequential storage reservoirs (containing partially treated wastewater with 10^3-10^4 FC per 100ml) compared with those with no contact with this effluent (Blumenthal et al, 1998, Blumenthal et al, 2000b). There was also an excess of diarrhoeal disease in adults (OR=1.5) but this did not reach significant levels (p=0.12) probably due to sample size factors. A reduced guideline level of $\leq 10^3$ FC per 100ml would be safer where adult farmworkers are engaged in flood or furrow irrigation (*Category B2* in Table 2) and where children are regularly exposed (*Category B3* in Table 2). This would also help to reduce the risks from epidemic infections which could be transmitted to effluent-irrigating communities from an outbreak in the source community (Fattal et al, 1987). Where there are insufficient resources to provide treatment to reach this stricter guideline, a guideline of 10^5 FC per 100ml should be supplemented by other health protection measures (for example, health education concerning avoidance of direct contact with wastewater, and the importance of handwashing with soap after wastewater contact).

The *nematode egg guideline* of =1 nematode egg/litre does not appear to sufficiently protect farm workers and their families, especially children (under 15 years of age). This is particularly the case where wastewater treatment systems produce an effluent of variable quality, where the partially treated wastewater may be contaminated with small quantities of wastewater, and where children of farm workers come into direct contact with the effluent. In such a situation in Mexico, children in contact with effluent from a storage reservoir which met the WHO Guideline (even though it was contaminated with small quantities of raw wastewater) had increased prevalence and intensity of *Ascaris* infection. When the effluent had been stored in two reservoirs and no nematode eggs were detectable, there was very little excess *Ascaris* infection in any age group (Cifuentes, 1998, Blumenthal et al, 2000a). Similar situations would arise where raw wastewater is allowed to bypass conventional treatment plants, especially during periods of peak flow, allowing untreated wastewater containing nematode eggs (where nematode infections are endemic) into the effluent that is reused for agriculture. Since this is often the case in reality, a stricter guideline of =0.1 eggs per litre is required for restricted irrigation where children are exposed to irrigation water (*Category B3*). This would also be useful in circumstances where stable treatment systems, such as waste stabilisation ponds are in use, and workers may come into contact with the soil, since eggs in soil can accumulate to high numbers (Annex B).

Table 5. Recommended revised microbiological guidelines for treated wastewater use in agriculture[a]

Category	Reuse Conditions	Exposed group	Irrigation technique	Intestinal nematodes[b] (arithmetic mean no of eggs per litre[c])	Faecal coliforms (geometric mean no per 100ml[d])	Wastewater treatment expected to achieve required microbiological quality
A	*Unrestricted irrigation* A1 Vegetable and salad crops eaten uncooked, sports fields, public parks[e]	Workers, consumers, public	Any	$\leq 0.1^{f}$	$\leq 10^3$	Well designed series of waste stabilization ponds (WSP), sequential batch-fed wastewater storage and treatment reservoirs (WSTR) or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration and disinfection)
B	*Restricted irrigation* Cereal crops, industrial crops, fodder crops, pasture and trees[g]	B1 Workers (but no children <15 years), nearby communities	(a) Spray/sprinkler	≤ 1	$\leq 10^5$	Retention in WSP series inc. one maturation pond or in sequential WSTR or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration)
		B2 As B1	(b) Flood/furrow	≤ 1	$\leq 10^3$	As for Category A
		B3 Workers including children < 15 years, nearby communities	Any	≤ 0.1	$\leq 10^3$	As for Category A
C	Localised irrigation of crops in category B if exposure of workers and the public does not occur	None	Trickle, drip or bubbler	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation.

In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.

b Ascaris and Trichuris species and hookworms; the guideline is also intended to protect against risks from parasitic protozoa

c During the irrigation season (if the wastewater is treated in WSP or WSTR which have been designed to achieve these egg numbers, then routine effluent quality monitoring is not required).

d During the irrigation season (faecal coliform counts should preferably be done weekly, but at least monthly).

e A more stringent guideline ($\leq$ 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

f This guideline can be increased to $\leq$1 egg per litre if (i) conditions are hot and dry and surface irrigation is not used, or (ii) if wastewater treatment is supplemented with anthelmintic chemotherapy campaigns in areas of wastewater re-use.

g In the case of fruit trees, irrigation should cease two weeks before fruit is picked and no fruit should be picked off the ground. Spray/sprinkler irrigation should not be used.

2.6.1.3 Implications of a disease control approach in the setting of country standards

Where economic constraints limit the level of wastewater treatment that can be provided, a country may choose disease control as the objective, where a certain risk of infection is accepted and the objective is to stop disease levels being reached, or where only the most vulnerable groups e.g. young children, are protected. The implications of the studies are less clear, due to the paucity of disease data, and are discussed below.

Unrestricted irrigation

If the objective is to prevent clinical enteric disease (and not enteric infection), the studies in Mexico suggest that it may be possible to set a *faecal coliform guideline* for unrestricted irrigation of 10^4 FC/100ml in areas where enteric infections are endemic, immunity to viral infections exists and crops are eaten locally. At this level, the serological studies in Mexico suggest there was transmission of viral infection but do not necessarily reflect a significant increase of disease. Risks of diarrhoeal disease were related to the consumption of onion and green tomato but not of other crops. If the guideline were set at this lower level, crop restrictions could be added e.g. to prevent growing of onion. However, it may be prudent to keep the guideline at 10^3 FC/100ml in order to (i) prevent the spread of infections causing national epidemics being transmitted to rural communities through sprinkler irrigation of partially-treated wastewater (Fattal et al, 1987), and (ii) grow crops which may be exported to countries where enteric infections are not highly endemic.

A *nematode egg guideline* for unrestricted irrigation of =1 nematode egg/litre may be adequate where crops with a short shelf life are grown (eg. salad crops) and wild plants are not eaten. The very few viable eggs that are likely to be present would have less chance of developing to infectivity in these circumstances. It is possible that a relaxed guideline of 10 eggs/litre may be considered to be adequate if the goal is to prevent high intensities of helminth infections (worm load) rather than infection itself.

Restricted irrigation

In highly endemic areas, if the objective were to prevent enteric disease in the vulnerable children (under 5's) and not necessarily in older children, a *faecal coliform guideline* of $=10^5$ FC/100ml would be adequate. Contact with wastewater of 10^5 FC/100ml led to increased diarrhoeal disease in older children and not young children (Cifuentes, 1995, Table A1 section b, Annex A). School-aged children involved in farming activities would need to be protected using other measures, and children discouraged from playing in the fields. Where there is a difficulty in doing this, a relaxation of the guideline would not be recommended.

Where the goal is to prevent high intensities of helminth infections, it is conceivable that a less strict nematode egg guideline and additional health protection measures could be used. In Mexico, the current standard for restricted irrigation is 5 eggs/litre, designed to be achievable by conventional treatment plants. There is currently no epidemiological evidence, however, on which to base such a relaxed guideline. In fact, data from Mexico suggest that intensities of infection in school-aged children are as high when they are exposed to wastewater of =1 egg/litre as to raw wastewater (Table A2, Annex A) suggesting that a stricter standard is necessary if treatment is the only health protection measure used. It is possible that a relaxed guideline could be used if it is supplemented by other measures, such as, twice yearly chemotherapy for school-aged children (who have the highest intensity infections)(see section 2.6). This would only be suitable in countries where anti-parasite campaigns exist and can be successfully extended to cover areas where wastewater is used in agriculture. Disease control would be dependent on chemotherapy regularly reducing intensities of infection, which can easily return to pre-treatment levels after 6 months.

2.6.1.4 Risks from enteric viruses and parasitic protozoa – are specific guidelines

necessary?

Protection against risks from enteric viruses through a viral guideline
The faecal coliform guideline in most guidelines and standards for wastewater reuse is intended to address risks of enteric infections due to both bacterial and viral pathogens yet it may not be adequate to protect against viral infections because (i) conventional treatment processes involving disinfection are much less efficient in removing viruses than indicator bacteria – and, as improved (molecular) techniques for viral detection have become available, this becomes even more apparent (Blackmer et al. 2000), and (ii) median infectious doses for enteric viruses are very low (below 50 infectious particles) in comparison with those for most enteric bacteria (Haas et al. 1993, and Scwartzbrod, 1995). A further point is that wastewater virology is a rapidly expanding research area, with the range of routinely considered faecal viruses being extended to include, for example, adenoviruses and astroviruses (Chaperon et al. 2000), and these may survive longer in treated wastewaters than enteroviruses.

There are few data available on the risks of viral infection from either direct contact or crop consumption. Nevertheless, the following currently available findings have implications for the evaluation of current guidelines with respect to viral risks:

(1) Use of risk assessment approaches have indicated that (a) when the concentration of viruses (poliovirus 3, echovirus 12 and poliovirus 1) in chlorinated tertiary effluent was a maximum of 111 pfu per 100 ml, the estimated annual risk of enteroviral infection from spray irrigation of food crops was 10^{-4} – 10^{-7} (Asano et al. 1992); (b) use of chlorinated secondary effluents (3.9 log virus removal) to irrigate food crops resulted in an estimated annual risk of enteroviral infection to consumers of 10^{-7} - 10^{-9} and even the use of unchlorinated secondary effluents resulted in an estimated annual risk of enteroviral infection of 10^{-3} – 10^{-5} (Tanaka et al. 1998); and (c) use of effluent of 1000 FC per 100 ml to irrigate salad crops resulted in an order-of-magnitude estimate for the annual risk of viral infection of less than 10^{-4} (Shuval et al. 1997). However, these studies are recognised to have deficiencies (see Section 1, part III) compared to more advanced QMRA techniques.

(2) Epidemiological studies have indicated that (a) when there was spray irrigation with effluent containing fewer than 10^{5} FC per 100 ml, there was no significant risk of enteroviral infection to the surrounding population (Shuval et al. 1989, and Camann et al. 1986); and (b) when there was surface irrigation with effluent of 10^{3}-10^{4} FC per 100ml, there was a significant risk of infection with Norwalk-like virus (Hu/NLV/MX) to farmworkers with high levels of contact with the wastewater (Blumenthal et al. 2000b); however (c) when there was surface irrigation with effluent of 10^{4} FC per 100ml there was little risk of infection with Hu/NLV/MX associated with consumption of vegetable crops eaten raw (Blumenthal et al, 2000c).

Taken together, these results suggest that (i) use of tertiary treatment plus disinfection may not be needed to protect against viral risks from consumption of vegetable crops eaten raw, and that (ii) the faecal coliform guideline of $\leq$1000 FC per 100ml is adequate and no extra viral guideline is currently justified.

Adequacy of protection against risks from parasitic protozoa by the nematode egg guideline
There is increasing concern about the role of wastewater in the environmental transmission of protozoan pathogens such as *Giardia*, *Cryptosporidium* and *Cyclospora*. The 1989 WHO guidelines assumed that if helminth egg levels were reduced to the level of the helminth egg guideline, then other "easily settlable" pathogens such as protozoan (oo)cysts would also be reduced to levels that did not cause excess infection in exposed populations. However, recent studies have shown that the removal of helminth eggs does not correlate with that of protozoan (oo)cysts (Stott et al. 1997, Grimason et al. 1993 and

Alouini, 1998). There is evidence that protozoan (oo)cysts are not effectively removed by conventional wastewater treatment processes with reported efficiencies varying from 26-100% (Bukhari et al. 1997, Sykora et al. 1990, and Robertson). In addition, the infectious dose can be low; human feeding studies have shown that the median infectious dose for *Giardia* is between 10 and 100 cysts, and for *Cryptosporidium* between 30 and 1000 oocysts (Cooper, and Olivieri, 1998).

Most of the evidence of water-related outbreaks of enteric protozoan diseases indicate they are associated with ingestion of contaminated drinking water and immersion in recreational waters (Craun, 1990, Fricker and Crabb, 1998, and Ortega et al. 1998) and consumption of contaminated foods (Smith, 1993, and Rose and Slifko, 1999). There are few data on the importance of wastewater reuse in agriculture, particularly the use of treated wastewater, in the transmission of parasitic protozoan infection, and these other routes of transmission and poor domestic hygiene are probably more important, especially in developing countries. Even though oocysts of both *Cryptosporidium parvum* and *Cyclospora cayetanensis* have been detected on market vegetables in an endemic area (Ortega et al. 1997), there is no epidemiological evidence to implicate direct use of wastewater used for irrigation as a risk factor for either pathogen.

Epidemiological studies done in Mexico have shown that there is a small risk of amoebic infection (OR=1.3) in those in contact with untreated wastewater but not in those in contact with settled wastewater retained in two reservoirs before use, which meets the WHO nematode egg guideline (Cifuentes, 1995). Initial analysis indicated that there was no risk of *Giardia intestinalis* in agricultural workers and their families related to contact with raw wastewater, but a small risk related to contact with wastewater retained in two reservoirs (Cifuentes et al. 1991/2). However, when these data were analysed further, allowing for the effect of other transmission routes, the risk related to contact with the reservoir effluent did not remain significant (Cifuentes et al, 2000). A study in India has also shown that there was no significant risk of *Giardia* infection in agricultural workers using untreated or treated wastewater, compared to controls (Sehgal and Mahanjan, 1991).

These studies indicate that there is at present no evidence to suggest that use of treated wastewater meeting the WHO nematode egg guideline for irrigation results in an increased risk of parasitic protozoan infection or that which exceeds acceptable levels, and therefore no evidence to support the establishment of a separate guideline for protozoa. However, it may be that risks from protozoan parasites are of greater public health importance in industrialised countries than the risks from helminthic infections.

2.6.2 Implications for wastewater treatment and other health protection measures

There are a number of health protection measures that can be adopted, including wastewater treatment, crop restrictions, irrigation techniques, human exposure control and chemotherapeutic interventions. In practice these are usually used in combination, and not singly. The most commonly used combination is partial wastewater treatment plus crop restrictions, and this is reflected in the wastewater guidelines (Table 5). Partial wastewater treatment can, however, be combined with one or more of the other measures.

2.6.2.1 Wastewater treatment

A full discussion of wastewater treatment methods appropriate to meet the proposed revised guidelines for wastewater reuse (Table 5) is given in Annex C. The main points are summarised here.

When wastewater is treated with the intention of using the effluent for agricultural irrigation and not disposal in receiving waters, the important quality criteria are those relevant to human health rather than environmental criteria and those related to the health of fish in receiving waters. Therefore, faecal coliform removal and nematode egg removal are more important than BOD removal. In many situations, the most cost-effective wastewater treatment option is waste stabilization ponds (WSP), as suggested in WHO (1989). The advantages of WSP are low cost, simplicity of construction, operation and maintenance, and high efficiency especially with respect to the removal of nematode eggs and faecal bacteria. Properly designed (Mara, 1997; Mara and Pearson, 1998), WSP can easily meet the helminthological and bacteriological quality requirements for both restricted and unrestricted irrigation (Table 5 and Table 6). There are many existing WSP that do not achieve these qualities (see, for example, Maynard et al*.,* 1999), but they may not have been so designed or are overloaded or poorly maintained.

Land availability or the cost of land can limit the use of WSP's, especially when dealing with effluent from large cities (population > 1 million), or in countries where lower temperatures mean that longer retention times, and therefore larger land areas, are required to meet the FC guideline for unrestricted irrigation. For example, for a flow of 1000 m^3 per day of a wastewater with a BOD_5 of 350mg/l and a faecal coliform count of $5x10^7$ FC/100ml, the total pond area required to produce an effluent containing = 1000 FC/100ml would be 8,000 m^2 at 25^oC, 13,700 at 20^oC, and 25,400 at 15^oC.

Table 6: Mean annual performance of five waste stabilization ponds in series in northeast Brazil

Source (at 24-27^oC)	Retention (days)	BOD_5 (mg/l)	Suspended solids (SS) (mg/l)	Faecal Coliforms (per 100 ml)	Human intestinal nematode eggs (per litre)
Raw wastewater	-	240	305	4.6×10^7	804
Effluent from:					
Anaerobic pond	6.8[a]	63	56	2.9×10^6	29
Facultative pond	5.5	45	74	3.2×10^5	1
First maturation pond	5.5	25	61	2.4×10^4	0
Second maturation pond	5.5	19	43	450	0
Third maturation pond	5.8	17	45	30	0

Source: Mara and Silva (1986)

[a] Later work showed the same performance for BOD and SS removals at retention times of ~ 1 day (Silva, 1982)

Wastewater storage and treatment reservoirs (WSTR) are particularly useful in arid and semi-arid regions where agricultural production is limited by the quantity of water, including treated wastewater, for irrigation, since WSTR permit the whole year's wastewater to be used for irrigation, rather than just that produced during the irrigation season. Recent research in Brazil has shown that sequential batch-fed WSTR's (in pilot scale) can remove faecal coliforms to less than 1000 FC/100ml by three weeks into the rest phase (Mara et al, 1996) whereas single WSTR's in Israel produce an effluent suitable for restricted irrigation. Sequential storage reservoirs in the Mezquital Valley in Mexico produce an effluent with a mean quality of 10^3 FC/100ml, the quality varying depending on the retention time which varies according to irrigation demand (Cifuentes, 1995).

In situations where conventional treatment is being considered, it is essential to assess the cost of operation, maintenance and personnel training, all of which are considerably higher than for non-conventional treatment systems. Conventional wastewater treatment systems (such as activated sludge, trickling filters) can only achieve a 2 $\log_{10}$ unit reduction of faecal coliforms, so they do not meet the microbiological requirements for agricultural reuse unless supplemented by tertiary treatment processes. They can be used in circumstances where WSP are not suitable; extended aeration plants, such as oxidation ditches, are generally the best option in this case as their costs are lowest (Arthur, 1983). Conventional secondary STP's are better at removing helminth eggs due to the retention time in primary and secondary sedimentation. Data from Mexico suggest that these are reduced to around 3 eggs/litre by advanced primary treatment. Filtration can be used to reduce the egg levels further but this can add as significant extra cost to the plant. However, maturation ponds (sometimes called "polishing" ponds in this context) can be used to upgrade conventional effluents prior to either restricted or unrestricted irrigation. Reservoirs can also be used for this purpose e.g. in the USA, reservoirs have produced a 2-3 log reduction in faecal coliform levels in trickling filter effluent, from 10^6 to 10^3-10^4 FC/100ml (Moore et al, 1988).

Sludge from conventional treatment plants or WSP must be treated or disposed of carefully as pathogens are concentrated there. Helminth eggs can survive and remain viable for nearly 12 months. Sludge can be injected into the subsoil or placed in furrows and covered with a layer of earth before the planting season and no tuberculous crops planted along such trenches. Alternatively there are a variety of treatment methods to make sludge safe including storage for 6-12 months at ambient temperature in hot climates, anaerobic digestion and forced aeration co-composting of sludge (Hespanhol, 1997).

The wastewater treatment system chosen needs to be able to deal with large differences in seasonal flows of wastewater, including peaks during the rainy season; WSP can do this. Bypassing conventional treatment plants with untreated or semi-treated wastewater which is then used in agriculture is a particular source of health risks. Issues of the training of treatment plant personnel and the running costs of each system, as well as treatment efficiency, should guide the choice of treatment facility.

It is important when defining wastewater treatment policies to remember that treatment is not the only measure available to protect health; crop selection and restriction, different irrigation techniques and human exposure control are equally important health protection measures. These non-treatment options should be considered as part of an integrated approach to health protection where wastewater irrigation policies are being proposed or modified.

2.6.2.2 Crop restriction

Crop restriction is often practiced in conjunction with wastewater treatment so that lower quality effluents can be used to irrigate non-vegetable crops (see Table 5). Although this appears straightforward, in practice it is often difficult to enforce. It can only be done effectively where a public body controls the use of wastewater and laws providing for crop restricted are strictly enforced, where there is adequate demand for the crops allowed under crop restrictions and where there is little market pressure in favour of excluded crops. (i.e. salad and other crops eaten uncooked). Crop restriction requires much less costly wastewater treatment and may be favoured for this reason alone (but wastewater treatment engineers need to discuss this clearly with the appropriate regulatory agency and local farmers). Wastewater irrigation with crop restrictions is practiced in Mexico, Chile and Peru. In Chile, wastewater from the city of Santiago was used to irrigate salad crops and vegetables until 1992. However, as part of a national campaign to prevent and control cholera, crop restriction was enforced, together with a general hygiene education program. The result was a reduction in cholera cases by over 90% attributable to the consumption of salad crops or vegetables (Monreal, 1992).

Crop restriction is not effective to control health risks from indirect reuse, where wastewater-contaminated surface waters are used directly by the farmers and do not come under the control of public bodies. Much unrestricted irrigation actually uses wastewater-contaminated surface waters rather than wastewater itself (either untreated or treated) and constitutes a particular challenge to the regulatory and public health authorities.

2.6.2.3 Irrigation technique

The irrigation technique can be chosen to reduce the amount of human exposure to the wastewater. In general, health risks are greatest when spray/sprinkler irrigation is used, as this distributes contamination over the surface of crops and exposes nearby population groups to aerosols containing bacteria and viruses (the opposite occurs with nematode eggs, which tend to be washed off during spray irrigation (Annex B)). This technique should be avoided where possible, and if used, stricter effluent standards apply (see Table 5). Flood and furrow irrigation exposes field workers to the greatest risk, especially if earth moving is done by hand and without protection. Localised irrigation (inc. drip, trickle and bubbler irrigation) can give the greatest degree of health protection by reducing the exposure of workers to the wastewater. A period of cessation of irrigation before harvest (1-2 weeks) can allow die-off of bacteria and viruses such that the quality of irrigated crops improves to levels seen in crops irrigated with fresh water, as shown by Vaz da Costas Vargas et al (1996). However, it is not practical in unregulated circumstances since farmers will probably not cease irrigation of leafy salad crops 5 days or more before harvest. Replacing partially-treated wastewater with fresh water for a week or so before harvest is not a reliable way of improving crop quality since re-contamination of the crops from the soil has been found to occur (Vaz da Costas Vargas et al, 1996). Use of cessation of irrigation before harvest is more viable with fodder crops which do not need to be harvested at their freshest, and could enable the use of lower quality effluents.

2.6.2.4 Human exposure control

The groups potentially most at risk from wastewater reuse in agriculture are the farm workers, their families, crop handlers, consumers of crops, and those living near wastewater-irrigated areas. The approach required to minimize exposure depends on the target group. Farm workers and their families have higher potential risks of parasitic infections. Protection can be achieved by low-contaminating irrigation techniques (as above), together with wearing protective clothing (e.g. footwear for farmers and gloves for crop handlers) and improving levels of hygiene both occupationally and in the home can help to control human exposure. Provision of adequate water supplies for consumption (to avoid consumption of wastewater) and for hygiene purposes (e.g. for handwashing) is important. Consumers can be protected by cooking vegetables, and by high standards of personal and food hygiene.

Studies are needed to see whether hygiene promotion could possibly be included in the work of agricultural extension services or by the health authorities where wastewater reuse occurs e.g. to promote handwashing with soap after irrigation. It is possible that health promotion programmes could be linked to existing health related services. For example, in Mexico, hygiene promotion could be linked to desparasitation campaigns the National Vaccination Council carries out for among 2-14 year olds in previously designated high-risk areas, and to their health education programmes for women (Peasey et al., 1999). The national diarrhoea control programme in Mexico has already increased sales of ORS ten fold in 11 years (Gutierrez et al., 1996); promotion of ORS was linked to the childhood immunization programme.

The effectiveness of current promotional techniques in environmental health, however, is not very encouraging, as few have had an impact on behaviour change or health status (Cave and Curtis, 1999). Better intervention design is needed and only a few specific behaviours should be targeted. Behaviour change can be slow and require intensive or prolonged intervention. In addition, the promotion of specific protective hygiene behaviours is generally now thought more effective when tackled separately from disease and risk.

Studies have demonstrated that such behaviours are more easily and efficiently modified for social and cultural reasons, rather than through a fear of possible illness (Curtis and Kanki, 1998).

2.6.2.5 Chemotherapeutic intervention

Chemotherapy, especially for helminth infections, can be considered in countries where the Ministry of Health is involved in periodic anthelminthic campaigns in areas of high infection levels, as occurs in Mexico. Areas where inadequately treated wastewater is reused (directly or indirectly) could be targeted, along with known areas of high prevalence of helminth infections. Treatment of children every 4 to 6 months is needed to prevent infection reaching pre-treatment intensities of infection. Adults and children from farming families could be particularly targeted.

The use of regular chemotherapy programmes and human exposure control, including hygiene promotion, should be considered as interim measures in cases where no wastewater treatment is provided or where there is a time delay before treatment plants can be built.

3 Aquacultural Reuse

3.1 Background and WHO Guidelines

Fish farming is becoming an increasingly important as a source of income for farmers as it is a high value crop and consumer demand for fish is increasing. Interest in wastewater-fed fish farming is based on its cost-effectiveness and the interest in resource recovery from the investment in wastewater treatment e.g. through the use of effluent from waste stabilisation ponds in fish ponds.

Tentative effluent guidelines for aquaculture were proposed by WHO (1989) following a review of the literature on the survival of pathogens in and on fish by Strauss (1985). A tentative *bacterial guideline* was set at $=10^3$ faecal coliforms per 100ml (geometric mean) for fishpond water, which can be achieved by treating the wastewater feed water to 10^3-10^4 FC/100ml. This was to protect against the risk of bacterial infections and was aimed at ensuring that the invasion of fish muscle was prevented. A *helminth quality guideline* was set at the absence of viable trematode eggs, aimed at preventing the transmission of trematode infections such as schistosomiasis, fasciolopsiasis and clonorchiasis.

New data are available to allow assessment of the bacterial guideline. The validity of the trematode egg guideline will not be reviewed here.

3.2 Summary of evidence supporting WHO (1989) tentative guidelines

The main evidence used to support the WHO (1989) Bacterial Guideline was evidence on the quality of fish grown in wastewater fed fishponds of different qualities. Strauss (1985) concluded that:

(1) Invasion of fish muscle by bacteria is very likely to occur when the fish are grown in ponds containing $>10^4$/100ml and $>10^5$/100ml faecal coliforms and salmonellae respectively. The potential for muscle invasion increases with the duration of exposure of the fish to the contaminated water.

(2) There is some evidence to suggest that there is little accumulation of enteric organisms and pathogens on, or penetration into, edible fish tissue when the faecal coliform concentration in the fishpond water is $< 10^3$/100ml.

(3) Even at lower contamination levels, high pathogen concentrations may be present in the digestive tract and the intraperitoneal fluid of the fish.

There were no epidemiological data on the health effects to populations consuming fish raised in wastewater fed fishponds.

3.3 New evidence of health risks from studies in Indonesia

The use of human excreta in aquaculture is a traditional practice in most of highland areas of West Java, Indonesia and occurs through latrines overhanging the 'home garden' fishpond. These ponds are generally small in size (on average about 200 m^2) and are usually situated alongside the houses, although some are larger and run on a commercial basis. A cross-sectional study of the risk of diarrhoeal disease associated with the use of excreta in such fishponds was carried out in West Java (Blumenthal et al, 1991/92.; Abisudjak, in preparation).

The population were exposed through consuming fish originating in an excreta-fed pond, but also in many other ways. Several types of exposure were identified; domestic exposure, (from the use of water which originated from excreta-fed fishponds for bathing and washing of kitchen utensils or food), recreational exposure (from contact with pond water while playing or swimming) and defecation exposure (from use of fishpond latrines for defecation). Three study groups were set up. The exposed group included those with domestic and defecation exposure; the semi-exposed group included those with defecation exposure but no domestic exposure and the non-exposed (control) group included those without domestic or defecation exposure. The effect of defecation exposure on the rate of diarrhoeal disease was determined by comparing the exposed and semi-exposed groups, and the effect of domestic exposure was determined by comparing the exposed and control groups. Recreational and occupational exposure occurred in both exposed and semi-exposed groups and consumer exposure in all groups. Multivariate analysis was used to estimate the risks associated with each exposure.

The quality of fishpond water used by selected households in the study was markedly worse than well water, with an overall geometric mean faecal coliform count of 3.9×10^4 FC/100 ml. Fishponds were classified by size and by source of excreta but it was not possible to define the quantity of excreta input to each pond. Although there was a great range in water quality, there was no evidence that smaller fishponds were more contaminated than large ones, or that directly excreta-fed ponds were more contaminated than indirectly excreta-fed ponds.

The one-week prevalence of diarrhoea in children under 5 years was 12.1%, 7.6% and 7.9% in the exposed, semi-exposed and non-exposed groups respectively, and was significantly different between exposure groups. The prevalence in those over 5 years was 1.4%, 1.2% and 1.4% and showed no difference between exposure groups.

For children under 5 years, a multiple logistic regression analysis was carried out to examine the effect of the exposures after allowing for several potential confounding factors (crowding, age, keeping of food and treatment of kept food). There was no risk of diarrhoea related to defecation exposure (odds ratio=0.81). There was a two-fold increase in diarrhoea related to recreational exposure (OR=1.91), a 1.6 fold increase related to domestic exposure and a 1.4 fold increase associated with consumer exposure (although the latter was of borderline significance). When the risk related to consumption of fish was explored separately in the three study areas, there was a two-fold increase in diarrhoea related to consumption in the control area (OR=2.35 95% C.I. 1.01-5.29) a 1.5 fold increase in the semi-exposed area (which was not statistically significant), and no increase in the exposed area.

The results show that recreational and domestic contact with water from excreta-fed fishponds with a mean quality of 4×10^4 faecal coliforms causes an excess risk in exposed children under 5 years of age, but not in persons over 5 years of age. Consumption of fish from such ponds is a risk to persons living in areas with no ponds and with less exposure to contamination.

3.4 Discussion and implications of studies for international guidelines

The epidemiological study in West Java indicates that exceeding the WHO tentative guideline level by 40 fold is problematic for vulnerable population groups like young children in this situation, but does not invalidate the tentative guideline, which could be around the right level.

The ponds where the fish were raised were neither commercial fish farms nor maturation ponds in a waste stabilisation pond series. They were fertilised with excreta from overhang latrines, either on the pond itself or on a pond further up the hillside. In such circumstances the risk could be higher than in wastewater fed fishponds where the influent is treated effluent (e.g. from WSP) and the fish are not in contact with raw wastewater or excreta. It therefore could represent a ‘worst case’ scenario. In fish farms or combined WSP/aquaculture systems the risks from consumption of the fish and contact with the pond water are relevant (equivalent to recreational contact above).

Studies of the microbiological quality of fish raised in wastewater-fed aquaculture systems have been used to recommend criteria for acceptable bacterial levels in fishpond water and fish muscle. Buras et al (1987) raised fish (Tilapia and silver carp) in experimental ponds over a whole growing season and concluded that the ‘threshold concentration’ (i.e. the concentration that caused the appearance of bacteria in muscles) was $1x10^4$ bacteria/100ml based on SPCs (standard plate counts). The role of faecal coliforms as adequate indicators of fish contamination was questioned, as they were not always detected in the muscles of fish whereas other bacteria were recovered; the use of bacteria (SPC) as an indicator was proposed. However, it is useful to review the level of faecal coliforms for comparative purposes; at this threshold concentration, the level of faecal coliforms in the water was around $3x10^2$ FC/100ml. Moscoso and Florez (1991), however, found that when Tilapia were grown in a combined WSP/aquaculture system in Peru, bacteria penetrated the fish muscle when the water exceeded 10^5 FC/100ml, and concluded that maximum level of faecal coliforms in the pond water should be $1x10^4$ FC/100ml. This would be achievable by a maximum concentration of $1x10^5$ FC/100ml in the effluent of the WSP used to feed the aquaculture pond. These two studies therefore come to different conclusions regarding the threshold concentration.

Current guidelines or standards for the microbiological quality of fish (reviewed in Strauss, 1995, and Leon and Moscoso, 1996) show that the standard plate count is used in conjunction with an *E.coli* or coliform level in most cases. The rejectable levels set for the quality of fish were10^6 SPC/g and *E.coli* 500 per gram (ICMSF, 1995), 10^7 SPC/g (FAO/IAEA/WHO, 1989) $5x10^4$ SPC/g and $0.7x10^3$ coliforms/g (USA, in Leon and Moscoso, 1996), and 10^5 SPC/g and 10^1 *E.coli*/g (Sweden, in Strauss, 1995). These levels are less strict than those proposed by Buras (1987) for fish raised in excreta-fed systems, who recommended that the total aerobic bacterial concentration in fish muscle should not exceed 50 bacteria/g. This is probably because ICMSF regulations are for fish contaminated mainly by handling and were not set up to include fish raised in excreta fed systems (Edwards, 1992). Many regulatory agencies do not specify microbiological standards for freshly caught fish, but specify standards for processed products, therefore ensuring adequate personal and institutional hygiene during transport, processing and marketing, and treatment for conservation of raw, unprocessed products prior to sale (Strauss, 1995).

For the use of wastewater in aquaculture, it seems appropriate for guidelines to specify the water quality that is acceptable for aquaculture, taking into account both the likely microbiological quality of the fish grown in such water and the likely health effects to consumers of the fish and workers in contact with the fishpond water. It is important to note that concentration of bacteria in the digestive tract is always higher than that in the fish muscle, and there is therefore potential for cross-contamination of fish muscle during gutting and preparation of the fish. Evidence from the epidemiological studies can take this latter risk into account. The study in Indonesia (above) shows that the water quality needs to be below 10^4 FC/100ml before the risks are reduced to acceptable levels. On balance, there appears to be sufficient evidence to suggest that the tentative faecal coliform guideline of $=10^3$ FC/100ml (WHO, 1989) for the fishpond water is the right order of magnitude, and insufficient data to warrant a reduction of this level to 10^2 FC/100ml or a relaxation to 10^4 FC/100ml. This implies that the quality of the feed water can be around 10^4-10^5 FC/100ml, depending on the size of the fishpond and the amount of dilution that occurs. However, the water quality should stay constant over the growing season as where large fluctuations in the quality of the influent water occur, this reduces the quality of the fish (Buras et al, 1987). The water quality should therefore be monitored weekly if there are likely to be fluctuations in its quality. In future, it would be useful to consider adding a bacterial guideline for the quality of the wastewater (SPC/100ml) and for the quality of fish (SPC/g).

Wastewater treatment is not enough. Attention should also be paid to protecting aquaculture workers and populations living nearby the ponds from contact with the pond water, and to ensuring that high standards of hygiene are maintained during fish handling and gutting. The use of health promotion programmes, by the Fisheries Department or by the health services, to address such behaviours needs further research (as for wastewater reuse in agriculture, section 2.6.2.4).

The implications of guidelines at this level are that wastewater (or excreta/septage) needs to undergo some form of treatment before it can be used in fishponds. Guidance on the design of WSP for wastewater-fed aquaculture is given in Annex C. Anaerobic and facultative ponds are designed on the basis of surface nitrogen loading and the facultative pond effluent discharged into the fishpond. Checks are made to see that the fishpond does not contain more than 1000 FC/100ml (Mara et al, 1993, Mara, 1997). If the quality is >1000 FC/100ml, the retention time in the fishpond should be increased or a maturation pond could be added to the WSP. All the trematode eggs settle out in the anaerobic and facultative ponds. Where effluent from conventional secondary treatment plants is used, the quality of the effluent may need to be improved by use of a polishing pond prior to the effluent being discharged into a fishpond.

4 Conclusions

The review of recent studies on the health effects of wastewater reuse in agriculture has led to an evaluation of the WHO (1989) Guidelines and to recommendations for revised microbiological guidelines for wastewater use in agriculture and aquaculture. The conclusions are as follows:-

1. For *unrestricted irrigation*, there is evidence to support the validity of *the faecal coliform guideline* of =1000 FC/100ml and no evidence to suggest that it needs to be revised. It is supported by data from epidemiological, microbiological and risk assessment studies. However, there is epidemiological evidence that the *nematode egg guideline* of =1 egg/litre is not adequate in conditions which favour the survival of nematode eggs (lower mean temperatures, surface irrigation) and needs to be revised to =0.1 egg/litre where those conditions apply.

2. For *restricted irrigation*, there is evidence to support the need for *a faecal coliform guideline* to protect farm workers, their children, and nearby populations from enteric viral and bacterial infections. The appropriate guideline will depend on which irrigation method is used and who is exposed. For example, if adult farmworkers are exposed through spray/sprinkler irrigation, a guideline of $\leq 10^5$ FC per 100ml is necessary. A reduced guideline of $\leq 10^3$ FC per 100ml is warranted where adult farmworkers are engaged in flood or furrow irrigation, and where children under 15 years are regularly exposed (through farm work or play). Where there are insufficient resources to meet this stricter guideline, a guideline of $\leq 10^5$ FC per 100ml should be supplemented by other health protection measures. The *nematode egg guideline* of ≤ 1 egg per litre is adequate if no children are exposed, but a revised guideline of ≤ 0.1 egg per litre is recommended if children are in contact with the wastewater through irrigation or play.

3. The risks to exposed populations are dependent on the irrigation method used. Health risks from irrigated crops are greatest when spray/sprinkler irrigation is used and risk to field workers are greatest when flood or furrow irrigation are used. The proposed guidelines take these risks into account.

4. The evidence reviewed did not support the need for a separate guideline to specifically protect against enteroviral infections, but there were insufficient data to evaluate the need for a specific guideline for parasitic protozoa.

5. There are three different approaches for establishing microbiological quality guidelines and standards for treated wastewater reuse in agriculture which have different objectives as their outcome: (I) the absence of faecal indicator organisms in the wastewater, (II) no measurable excess cases in the exposed population, and (III) a model generated estimated risk below a defined acceptable risk. The above conclusions were based on use of approach II, using empirical epidemiological studies supplemented by microbiological studies on pathogen transmission, in conjunction with approach III, using model-based quantitative microbial risk assessment for selected pathogens.

6. The use of a disease control approach can be considered for the setting of country standards, especially where economic constraints limit the level of wastewater treatment that can be provided. Here, the aim would be to protect populations against excess disease rather than excess infection. This could result in the relaxation of microbiological guidelines and the use of other health protection measures to supplement wastewater treatment.

7. The revised microbiological guidelines can be met through the use of waste stabilisation ponds, wastewater storage and treatment reservoirs, or through conventional treatment processes. When using WSP, the revised guidelines usually

require the use of 1 or more maturation ponds after the anaerobic and facultative ponds. Use of sequential batch-fed storage and treatment reservoirs can be designed to meet the guidelines for unrestricted and restricted irrigation. When conventional treatment processes are used secondary treatment, filtration and disinfection are often needed to meet the revised guidelines. The cost and difficulty in operating and maintaining conventional treatment plants to the level needed to meet the guidelines means that they are not recommended where WSP and WSTR can be used.

8. Crop restriction, irrigation technique, human exposure control and chemotherapeutic intervention should all be considered as health protection measures to be used in conjunction with partial wastewater treatment. In some cases, community interventions using health promotion programmes and/or regular chemotherapy programmes could be considered , in particular where no wastewater treatment is provided or where there is a time delay before treatment plants can be built.

9. Regarding wastewater use in aquaculture, evidence from epidemiological studies shows that the faecal coliform guideline needs to be below 10^4 FC/100ml. There appears to be sufficient evidence to suggest that the tentative faecal coliform guideline of $=10^3$ FC/100ml (WHO, 1989) for the fishpond water is the right order of magnitude, and insufficient data to warrant a reduction of this level to 10^2 FC/100ml or a relaxation to 10^4 FC/100ml. This implies that the quality of the feed water can be around 10^4-10^5 FC/100ml, depending on the size of the fishpond and the amount of dilution that occurs. In future, it would be useful to consider adding a bacterial guideline for the quality of the wastewater (SPC/100ml) and for the quality of fish (SPC/g). This will address concerns over the adequacy of faecal coliforms as indicators of health risks from waste-fed aquaculture.

10. In order to meet the faecal coliform guideline, wastewater (or excreta/septage) needs to undergo some form of treatment before it can be used in fishponds. Where WSP are used, effluent from the facultative pond or first maturation pond can be discharged into the fishpond (depending on the effluent quality and size of the fishpond). Where effluent from conventional secondary treatment plants is used, the quality of the effluent may need to be improved by use of a polishing pond prior to the effluent being discharged into a fishpond.

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ANNEX A

Epidemiological studies of wastewater reuse in Mexico

A series of epidemiological studies were conducted in Mexico to assess, firstly, the occupational and recreational risks associated with exposure to wastewater of different qualities, and secondly, the risks of consuming vegetable crops irrigated with partially treated wastewater. In the first set of studies, infections (from helminths, protozoa and diarrhoeal disease) in persons from farming families in direct contact (through irrigation or play) with effluent from storage reservoirs or raw wastewater, were compared with infections in a control group of farming families engaged in rain-fed agriculture. In the studies on consumer risks, infections with diarrhoeal disease, Human Norwalk-like Virus/Mx and *Enterotoxigenic E. coli (LT)* in persons from a rural population eating raw vegetables irrigated with partially treated wastewater were compared with infections in persons (in the same area) not eating these vegetables. Comparison was also made with infections in persons in a nearby area where vegetables were irrigated with borehole water. In all studies, the effects of wastewater exposure were assessed after adjustment for many other potential confounding factors (including socio-economic factors, water supply, sanitation and hygiene practices).

1.1 Study area

Raw wastewater coming from Mexico City to the Mezquital valley, Hidalgo, is used to irrigate a restricted range of crops, mainly cereal and fodder crops through flood irrigation techniques. Some of the wastewater passes through storage reservoirs and the quality of the wastewater is improved before use; this is equivalent to partial treatment. The effluent from the first reservoir (retention time 1-7 months, depending on the time of year) met the WHO guideline for restricted irrigation (category B), even though a small amount of raw wastewater enters the effluent prior to irrigation. Some effluent from the first reservoir passes into the second reservoir and is retained for an additional 2-6 months (>3 months of combined retention), and the quality improved further. Local farming populations are exposed to the wastewater and effluent through activities associated with irrigation, domestic use (for cleaning, not for drinking) and play. Part of the effluent from the first reservoir enters the river and is abstracted downstream to irrigate a large area of vegetable and salad crops, many of which are eaten raw; the river water is essentially partially treated wastewater. These crops are sold in the local markets and eaten by the rural populations in local villages, including those near the second reservoir. In a nearby area, vegetables were irrigated with borehole water.

1.2 Wastewater quality

Untreated wastewater contained a high concentration of faecal coliforms (10^6-10^8 FC/100ml) and nematode eggs (90-135 eggs per litre). Retention in a single reservoir reduced the number of helminth ova substantially, to a mean of = 1 eggs/litre (so meeting the WHO Guideline for restricted irrigation) whereas faecal coliform levels were reduced to 10^5 FC/100ml (average over the irrigation period) or 10^4 FC/100ml, with annual variations depending on factors such as rainfall. The concentration of helminth ova remained below 1 ova/litre (monthly monitoring) even after a small amount of raw wastewater entered the effluent downstream of the reservoir. Retention in the second reservoir reduced the faecal coliform concentration further (mean 4×10^3 FC/100ml) and no helminth ova were detected. Faecal coliform levels varied over the year depending on the retention time in each reservoir which varied according to demand for irrigation water.

The geometric mean quality of the river water at the point where it is abstracted for use in irrigation was 4×10^4 FC/100ml, with little variation occurring over the year. Enterovirus and hepatitis A virus were present for most of the year (95% and 69% monthly samples respectively), whereas rotavirus was detected during the peak months for rotavirus cases. Limited data on virus levels on crops at harvest showed that enterovirus was detected on all crops tested (onion, radish, lettuce, cauliflower and coriander) whereas hepatitis A virus was detected on lettuce, radish and onion (on which rotavirus was also detected).

1.3 Risks to workers related to restricted irrigation and effect of wastewater treatment

1.3.1 Exposure to raw wastewater

Exposure to raw wastewater over one year (following chemotherapy) was associated with a significantly increased prevalence (percentage) and intensity of *Ascaris* infection (mean egg load) in all age groups (Table A1 section a). Exposure was related to a 20 fold increase in infection in children (compared to the control group) and a 10 fold increase in adults (Blumenthal et al, 1996, Peasey, 2000). Increased morbidity, as shown by increased wheezing and difficulty in breathing, was detected among those with higher intensity infections. The specific behaviour which was most risky for adults was irrigating chillies (6 fold increase) which was done by furrow irrigation and involved earth moving, done by hand or by spade. For children, the most risky behaviour was eating local plants (irrigated with wastewater). Exposure to raw wastewater was shown to account for over 80% of *Ascaris* infection in the commmunity.

Table A1: Effect of direct contact with wastewater of different qualities on enteric infections

Infection	Age Group (years)	Odds Ratio	95% C.I.	p value
(a) Untreated Wastewater				
Ascaris[1]	2-14	19.41	6.93-54.39	<0.0001
	15+	10.01	4.00-25.02	<0.0001
Diarrhoea[2]	0-4	1.75	1.10-2.78	0.01
	5+	1.34	1.00-1.78	0.04
(b) Partially Treated (one reservoir)				
Ascaris[1]	2-14	13.89	4.94-39.08	0.030
	15+	2.71	0.96-7.65	<0.0001
Diarrhoea[2]	0-4	1.13	0.70-1.83	0.466
	5+	1.50	1.15-1.96	0.003
(c) Partially Treated (two reservoirs)				
Ascaris[3]	0-4	1.29	0.49-3.39	0.544
	5+	1.94	1.01-3.71	0.01
Diarrhoea[4]	0-4	2.00	0.75-5.32	0.38
	5-14	2.18	1.13-4.24	0.02
	15+	1.51	0.91-2.48	0.28
Human Norwalk-like	1-4	0.60	0.44-1.54	0.54
Virus/Mexico[4]	5-14	0.72	0.50-1.11	0.14
	15+			
	Level of +	1.23	0.55-2.77	
	Contact ++	4.21	1.62-10.96	0.0096

[1]Source: Peasey, 2000., Peasey et al, 2000a
[2]Source: Cifuentes, 1995., Blumenthal et al, 2000a
[3]Source: Cifuentes et al, 1994., Cifuentes, 1998
[4]Source: Blumenthal et al, 1998., Blumenthal et al, 2000b

Over 1.5 times as many young children (aged 1-4 yrs) exposed to raw wastewater had an episode of diarrhoeal disease (in the last two weeks) than in the control group (Table A1 section a) (Cifuentes, 1995, Blumenthal et al, 2000a). The increase was less in those aged 5-14 years (1.3 times). A small increase in infection with *Entamoeba histolytica* was seen in children aged 5-14 years, but this was probably not exclusively disease-causing amoebic infection (Cifuentes et al 1994). Rates of *Trichuris* and hookworm were very low and unrelated to wastewater contact.

1.3.2 Exposure to partially treated wastewater

Exposure over one year to wastewater which was retained in one reservoir resulted in a 14 fold increase in *Ascaris* infection in children (especially those aged 5-14 years) and a much smaller increase (3 fold) in infection in adults (Table A1 section b) (Peasey 2000 and Peasey et al, 2000). For adults, planting chillies was associated with increased infection. The intensity of *Ascaris* infection in adults was reduced to the level in the control group, but in children was similar to levels in the raw wastewater group (Table A2) (Blumenthal et al, 1996). Older children (aged 5-14 years) also had significantly higher rates of diarrhoeal disease (Table A1 section b) (Cifuentes, 1995, Blumenthal et al, 2000a)

Table A2: Intensity of infection (mean egg load in eggs per gram of faeces $\pm$ standard error of the mean).

Age Group (years)	*Ascaris* intensity in those exposed to:		
	Raw Wastewater	**Wastewater retained in one reservoir**	**Control group**
2-4	2,726 $\pm$ 1127 [a]	35 $\pm$ 20	0 $\pm$ 0
5-14	1,954 $\pm$ 513 [c]	2,110 $\pm$ 664 [b]	3 $\pm$ 2
$\geq$15	638 $\pm$ 223	88 $\pm$ 47	197 $\pm$ 131

[a] = <0.05, [b] = <0.01 and [c] = <0.001 *Source: Blumenthal et al 1996*

When wastewater was retained in two reservoirs in series, direct contact with the effluent resulted in very little excess *Ascaris* infection in any age group (Cifuentes et al,1994). In those over 5 years, the prevalence was twice as high as in the control group, but the excess infection was less than 1% (Table A1 section c). Initially, it was found that there was no excess of diarrhoeal disease related to exposure with this water (Cifuentes et al, 1994, Cifuentes, 1998) compared to the level in the control group, where rain-fed agriculture was practised. However, in a later study, when children with contact with the effluent from the second reservoir were compared with children from the same population but with no contact with the effluent, a two-fold or greater increase in diarrhoeal disease in children aged 5-14 years, and a four-fold increase in seroresponse to Human Norwalk-like Virus/MX in adults with high levels of contact was found (Table A1 section c) (Blumenthal et al, 1998, Blumenthal et al, 2000b).

Retention of water in two reservoirs in series, producing water of average quality 4×10^3 FC/100ml and no detectable nematode eggs, is therefore adequate to protect the children of farm workers from *Ascaris* infection but not against increased diarrhoeal disease.

1.4 Risks to consumers related to unrestricted irrigation

In the above studies, there was some evidence that eating local plants (wild greens such as spinaches) was associated with an increased risk of *Ascaris* infection in children (2-14 years) in families exposed to raw wastewater and to effluent from one reservoir.

Risks from bacterial and viral infections related to the consumption of specific cultivated vegetables (ie. courgette, cauliflower, cabbage, carrots, green tomato, red tomato, onion, chilli, lettuce radish, cucumber and coriander) and to total consumption of raw vegetables irrigated with partially treated wastewater (quality 10^4 FC/100ml) were investigated in a separate study. The results indicated that consumers of all ages had no excess infection with symptomatic diarrhoeal disease (Table A3), and no excess serological response (defined as 50% increase in antibody titre over one year) to Human Norwalk-like Virus/MX or Enterotoxigenic *E. coli* related to their total consumption of raw vegetables, that is, the frequency of eating raw vegetables. (Blumenthal et al, 1998, Blumenthal et al, 2000b).

However, there was an two-fold or greater excess of diarrhoeal disease in those who ate increased amounts of onion compared with those who ate very little (Table A4). The effect was particularly seen in adults and children under 5 years of age. Similar results were found for the consumption of chillies.

Table A3: Effect of total consumption of raw vegetables on prevalence of diarrhoea (%)

Age Group (years)	No of days on which vegetables eaten during the last week				
	0	1-2	3-5	6+	p value
1-4	20.7	13.8	14.5	16.8	0.407
5-14	9.0	5.6	4.9	4.2	0.279
15+	5.5	3.6	3.8	4.7	0.047

Source: Blumenthal et al, 1998., Blumenthal et al, 2000

Data on the consumption of foods prepared from raw vegetables also supported these results, since foods containing chilli or onion were associated with increased infection. Frequently eating 'salsa' (chilli sauce) was associated with increased diarrhoea in adults and older children, an increased seroresponse to Human Norwalk-like Virus/Mexico and a significant rise in antibody titre to ETEC in children (1-14 years). Consumption of 'picadillo' (choppped onions, chilli and red tomato) by adults was associated with increased diarrhoea whereas frequent consumption of 'guacamole' was associated with a significant rise in antibody titre to ETEC in children (1-14 years). There were also higher levels of serological response to Human Norwalk-like Virus/Mexico in school-aged children who ate green tomato, but this effect was not seen in other age groups (Table A5). No excess serological response to enterotoxigenic *E. coli* was related to individual raw vegetable consumption; the increased seroresponse related to eating foods prepared from raw vegetables could be due to contamination introduced via the chillies, but it could also have been introduced during preparation and bacteria multiplied to reach an infective dose during storage.

Data on the source of vegetables show that the chillies eaten by the study population were grown in raw wastewater, so the risk of diarrhoea associated with eating chillies (Table A4 section b) was related to *raw* wastewater irrigation. Therefore, it is only the risks from eating onion and possibly green tomato that can be associated with using *partially-treated* wastewater for irrigation. However, since 83% of adults and 56% of children under 5 years of age ate onion more than once a month, the majority of the study population had a two-fold or greater risk of diarrhoea. Enteroviruses were found on onions at harvest, giving support to this epidemiological evidence.

In contrast, we have evidence that eating some other raw vegetables was associated with a decrease in diarrhoea. The evidence is strongest for eating carrots, which was associated with a 60% or greater reduction in diarrhoea in all age groups (Table A4 section c). Protective effects of 50% or greater were also related to eating red tomato, salad and the total amount of raw vegetables eaten by the older children. Consuming a high number of foods containing raw vegetables was also associated with a 75% reduction in seroresponse to Human Norwalk-like Virus/Mexico.

In summary, these results indicate that there is a year round potential for transmission of enteric infections through consumption of vegetable crops irrigated with water of quality 10^4 FC/100ml, and consumption of some vegetables is associated with a significant risk of enteric infection in consumers in the rural population studied. However, the risks associated with consumption of some vegetables, particularly onion, may be balanced by the protective effects associated with consumption of other vegetables.

In the communities studies, factors other than consumption of contaminated vegetables are equally or more important as risk factors or protective factors against these infections. In particular, there is evidence supporting the importance of hygiene behaviour; hand washing is protective against diarrhoea (symptomatic) and Human Norwalk-like Virus/Mx, especially in adults and when soap is used (Table A6). There is also evidence of risk associated with drinking water from public supplies. Chlorination of drinking water supplies in the area is often inadequate such that the water is often effectively untreated. Prevention of contamination in the home is also important.

Table A4: Effect of consumption of specific raw vegetables on risk of diarrhoea

Age Group (years)	Consumption (times per week/month)	Odds Ratio	C.I.	p value
(a) Onion				
1-4	<4/month	1.00		
	4/month	3.80	1.24-11.68	
	>4/month	2.19	0.54-8.89	0.047
15+	<1/month	1.00		
	1-3/month	3.99	1.62-9.82	
	4/month	2.54	1.05-6.39	
	>4/month	2.24	0.88-5.71	0.007
(b) Chilli				
1-4	=4/month	1.00		
	>4/month	1.72	0.95-3.12	0.081
5-14	<1/month	1.00		
	1-3/month	1.84	0.72-4.70	
	1/week	1.40	0.53-3.69	
	2-4/week	0.63	0.22-1.75	
	>4/week	0.92	0.32-2.62	0.039
15+	<1/month	0.19	0.06-0.63	
	1-3/month	1.00		
	1/week	0.91	0.52-1.60	
	2-4/week	0.68	0.39-1.21	
	>4/week	1.55	0.91-2.64	<0.001
(c) Carrot				
1-4	0	1.00		
	1-7/week	0.36	0.11-1.19	0.055
5-14	<1/month	1.00		
	≥1/month	0.33	0.14-0.76	0.006
15+	0	1.00		
	1-7/week	0.41	0.16-1.03	0.032

Source: Blumenthal et al, 1998, Blumenthal et al, 2000

Table A5: Effect of consumption of green tomato on seroresponse to Human Norwalk-like Virus/Mexico in children of 5-14 years

Times/2 Weeks	Odds Ratio	C.I.	p value
0	1.00		
1	1.44	0.76-2.75	
2-14	2.52	1.03-6.13	0.034

Source: Blumenthal et al, 1998, Blumenthal et al, 2000

Table A6: Effect of handwashing on diarrhoea and seroresponse to Human Norwalk-like Virus/Mexico in adults

Infection	Method	Odds Ratio	p value
Diarrhoea	Water	1.00	
	Water + Detergent	0.90	
	Water + Soap	0.58	0.04
Calicivirus-Mx	Water	1.00	
	Water + Detergent	1.06	
	Water + Soap	0.46	0.06

Source: Blumenthal et al, 1998

Annex B

Experimental studies on microbiological contamination of wastewater–irrigated crops in Brazil and UK

Experimental studies in northeast Brazil and Leeds, UK were conducted to investigate the risks to consumers from nematode infection (*Ascaris lumbricoides* and *Ascaridia galli* respectively) from crops irrigated with wastewater of varying qualities. In both studies, spray irrigation of lettuce was chosen to reflect the worst case situation for consumers in which the most contaminating irrigation method is used for a raw edible crop. Irrigation trials related wastewater quality to levels of crop contamination during irrigation and at harvest, survival of nematode eggs on irrigated plants (in terms of egg development and viability) and transmission of nematode infection from ingestion of irrigated crops using animal models to assess the potential risk to consumers.

1. Experimental design

In Brazil, raw and treated waste waters from an anaerobic, facultative and maturation pond of a pilot-scale series of waste stabilisation ponds were used to spray-irrigate lettuce crops. A manual irrigation system was employed to imitate that used locally for commercial production. Lettuces were irrigated from transplanting (as 4-week old seedlings) twice a day for the first week and then once a day thereafter for five weeks until harvest. Lettuces were sampled at weekly intervals and enumerated for eggs using a specifically developed washing method. The number, species and viability of nematode eggs on plants was determined during irrigation to evaluate the potential risks from ingestion of wastewater irrigated crops (Ayres, 1991; Ayres et al., 1992).

In complementary studies in the UK lettuces were spray-irrigated with wastewater containing eggs of the chicken roundworm *Ascaridia galli* (as a nematode model for *Ascaris lumbricoides*). Crops were irrigated using a hand-held irrigation system with treated effluent artificially seeded with eggs of *A.galli* to reflect waste waters of poor medium and WHO qualities. Crops were irrigated at least three times a week from transplanting for five weeks until harvest. The transmission of nematode infection from ingestion of irrigated crops was assessed using chicken bioassays: two harvested plants were fed to a pair of immunosuppressed chickens once a week for five weeks. Chickens were examined for *A.galli* infection six weeks from the first feeding date and worm burdens used as the criterion of infection (Stott et al., 1994; Stott, 1995).

2. Wastewater quality

In Brazil, a variety of helminth ova were found in waste waters including eggs of *A.lumbricoides*, *Trichuris trichiura*, hookworm, *Hymenolepis nana* and *Hymenolepis diminuta*. All species of nematode eggs were found in raw and anaerobic pond waste waters, but only *Ascaris* eggs were detected in facultative pond effluent. Eggs of *A.lumbricoides* predominated (>95%) in all waste waters. Raw wastewater contained a high number of nematode eggs (166-202 eggs per litre). Treatment in the anaerobic pond greatly reduced the number of nematode ova to around 14-18 eggs/l. The concentration of nematode ova in facultative pond effluent was on average <0.5 eggs/l (thus satisfying WHO nematode quality criteria). Retention in a maturation pond consistently removed all nematode ova during the irrigation programme.

In UK studies, final effluent was collected from a local conventional treatment plant. The effluent was seeded with an appropriate sample from a homogenous suspension of *A.galli* eggs to produce mean wastewater qualities of 50, 10 and 1 egg/l.

3. Nematode egg contamination on wastewater-irrigated crops

Wastewater quality had a significant effect on crop contamination with greater levels of contamination found on plants irrigated with higher numbers of eggs. In Brazil, only eggs of *A. lumbricoides* were found on the wastewater-irrigated crops. When raw wastewater (>100 eggs/l) was used for irrigation the level of contamination increased with time. However, the increase in the total number of eggs on the plant was in proportion to the increase in weight and plant surface area, and egg density in terms of eggs per gram fresh weight stayed the same indicating that no accumulation per se was found on plants. At harvest, raw-wastewater irrigated crops were contaminated with on average <60 eggs/plant (Table B1). When anaerobic pond effluent (>10 eggs/l) was used for irrigation, contamination levels on the plants were greatly reduced to around 0.6 eggs/plant. No contamination was found on lettuces spray-irrigated with facultative pond effluent (<0.5 eggs/l) nor on lettuce irrigated with maturation pond effluent (0 eggs/l).

Table B1: Mean number of *A.lumbricoides* eggs per lettuce after irrigation for five weeks with raw and treated WSP waste waters containing 0-202 eggs per litre (NE Brazil)

	WSP effluent			
	Raw wastewater >100 eggs/l	**Anaerobic pond >10 eggs/l**	**Facultative pond <0.5 eggs/l**	**3rd Maturation pond 0 eggs/l**
Mean no. of eggs per lettuce (Trial 1):	59.74	0.56	0	0
Mean no. of eggs per lettuce (Trial 2):	29.26	0.58	0	0

The quality of irrigated crops was found to be significantly improved by rainfall or clean water irrigation prior to harvesting. Contamination on raw wastewater irrigated crops was reduced by 98% following heavy rainfall and all nematode eggs were removed from plants irrigated with anaerobic pond effluent. When clean water was used to spray irrigate crops contaminated by raw or partially treated wastewater irrigation, the majority of eggs were removed after 3 days and all eggs were removed from raw wastewater irrigated crops after 7 days. However, lower levels of contamination were removed more readily from crops irrigated with anaerobic pond effluent; all eggs were removed within a single application of clean water irrigation. These results suggest that, at least in the case of *A.lumbricoides* eggs, crop recontamination does not occur as a result of rain or splash from overhead irrigation systems unlike that suggested for bacterial recontamination of crops. No eggs were found on lettuce crops irrigated with facultative or maturation pond effluent despite being grown in contaminated soil containing up to an average of 1200 *Ascaris* eggs per 100 g.

In the UK studies, levels of crop contamination were also related to wastewater quality. Levels of contamination increased during irrigation on crops irrigated with wastewater containing >10 eggs/l. However, there was no evidence for egg accumulation on the plants. Low levels of contamination were found on plants harvested after 5 weeks irrigation. When poor-quality wastewater (50 eggs/l) was used to irrigate lettuce crops, 23 percent of the plants were contaminated with around 2.2 eggs/plant at harvest. Irrigation with better quality effluent (10 eggs/l) improved the quality of crops as the levels of nematode contamination were reduced to around 1.5 eggs/plant and the incidence of contamination was also reduced to 15%. When the plants were irrigated with wastewater at the WHO Guideline of ≤1 egg/l, only very slight contamination was found on a few plants (6%). Levels of contamination were around 0.3 eggs/plant at harvest suggesting that a few eggs may remain on plant surfaces despite successive irrigation or the effects of environmental weathering.

The number of eggs on the plants was found to be highly aggregated in the UK studies: the majority of plants were uncontaminated and only a few plants were contaminated with eggs. Irrigation with 50 eggs per litre significantly increased the level of nematode contamination compared to plants irrigated with the WHO Guideline of ≤ 1 egg/l. There was also weak evidence to suggest that irrigation with 10 eggs per litre resulted in a mean increase of 0.5 egg per plant, compared with plants irrigated with water containing the WHO Guideline level.

Collectively the results show that levels of nematode contamination on crops at harvest do not reflect the nematode quality of the irrigation wastewater (Ayres et al., 1992). Irrigation with raw or poor-quality wastewater containing high numbers of eggs did not result in heavily contaminated crops at harvest. Cultivating plants under apparently highly contaminating conditions may not lead to great levels of contamination on plants. A similar observation has been reported in Morocco where raw wastewater containing 90-2200 *A.lumbricoides* per litre was used to irrigate tomatoes and resulted in a contamination level at harvest of only 2 eggs per kg (Rhallabi et al., 1990). The lack of accumulation on plants during irrigation suggests that the irrigation water itself might have a “wash on/off” effect by removing and replacing eggs at the next application. Irrigation with partially treated wastewater (>10 eggs/l) improved the quality of irrigated crops compared to plants irrigated with poor quality waste waters (>50 eggs/l) although plants were still contaminated at harvest albeit with low levels of contamination. Irrigation with wastewater of the WHO Guideline quality resulted in no contamination of lettuce at harvest or very slight contamination on a few plants.

4. Development of nematode eggs on wastewater-irrigated crops

Eggs recovered from contaminated plants were examined for stages of development in order to interpret the risk of infection from eggs found on plants. Studies in Brazil and UK found that the eggs did not develop to the infective stage on wastewater-irrigated crops. The majority of eggs remaining on plants at harvest were either unembryonated or developing, but the farthest stage of development reached on plants was the gastrula intermediate stage. No embryonated eggs were recovered from wastewater-irrigated crops.

The absence of embryonated eggs on the plants may have been due to the eggs being continually washed off and replaced from subsequent irrigation, or eggs degenerating before crop harvesting. Mean maximum temperatures ranged from 28-33°C for each harvesting occasion in Brazil and temperatures in the glasshouse in the UK study were usually in excess of 33°C, suggesting that environmental factors, particularly desiccation, may facilitate rapid egg degeneration and removal from the plants.

5. Viability of nematode eggs on wastewater irrigated crops

The viability of nematode eggs remaining on wastewater-irrigated crops decreased significantly with weeks of irrigation in both the Brazil and UK irrigation trials indicating a rapid degeneration of eggs on the plants. However, a few nematode eggs on harvested plants were still viable. In particular, plants irrigated with WHO quality wastewater (1 egg/l) were contaminated with very low numbers of viable eggs (0-0.15 egg per plant). Since viable eggs can remain on crops for up to 35 days, there is a risk that crops harvested within the egg survival period may be contaminated with viable eggs. Wastewater-irrigated vegetables may thus represent a potential risk to consumers.

6. Transmission of nematode infection from wastewater-irrigated crops

In the UK studies, ingestion of lettuces spray-irrigated with wastewater containing embryonated eggs of *A.galli* resulted in worm infections in immunocompromised chickens. The threshold level of infection was found to be low, with an infective dose of fewer than 10 embryonated eggs per pair of birds being required to establish an infection. However, whilst studies showed that there was an actual risk of infection from edible crops contaminated with embryonated eggs, no actual risk of infection was found from crops spray-irrigated with unembryonated eggs. No transmission of *A.galli* infection was found in chickens fed contaminated crops spray-irrigated with wastewater, although the estimated egg dose received from spray-irrigated plants of 1.2-20 eggs per plant exceeded the minimum infective dose of <5 embryonated eggs for *A.galli*. The results indicate that the potential risk of nematode infection to consumers from contaminated plants appears to be minimal at or shortly after harvest.

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ANNEX C

Wastewater Treatment

This Annex briefly describes wastewater treatment in:

- waste stabilisation ponds,
- wastewater storage and treatment reservoirs, and
- polishing ponds for upgrading conventional effluents,

for the production of effluents with a microbiological quality suitable for either restricted or unrestricted irrigation. For wastewater-fed aquaculture a system for minimal wastewater treatment and maximal fish production is also described.

1. Waste Stabilisation Ponds

Waste stabilisation ponds (WSP) are shallow man-made basins into which wastewater continuously flows and from which, after a retention time of many days (rather than several hours in conventional treatment processes), a well treated effluent is discharged. WSP systems comprise a series of anaerobic, facultative and maturation ponds, or two or more such series in parallel. In essence, anaerobic and facultative ponds are designed for BOD removal and maturation ponds for pathogen removal, although some BOD removal occurs in maturation ponds and some pathogen removal occurs in anaerobic and facultative ponds. The functions and modes of operation of these three different types of pond are described in Sections 1.2 – 1.4.

1.1 Advantages of WSP

The advantages of WSP systems, which can be summarized as *simplicity, low cost* and *high efficiency*, are as follows:

Simplicity. WSP are simple to construct: earth moving is the principal activity; other civil works are minimal – preliminary treatment, inlets and outlets, pond embankment protection and, if necessary, pond lining. WSP are also simple to operate and maintain: routine tasks comprise cutting the embankment grass, removing scum and any floating vegetation from the pond surface, keeping the inlets and outlets clear, and repairing any damage to the embankments. Only unskilled, but carefully supervised, labour is needed for pond operation and maintenance.

Low cost. Because of their simplicity, WSP are much cheaper than other wastewater treatment processes. There is no need for expensive electromechanical equipment (with its attendant problems, in developing countries, of foreign exchange and spare parts), nor for a high annual consumption of electrical energy.

The cost advantages of WSP were analysed in detail by Arthur (1983) in a World Bank Technical Paper. Arthur compared four treatment processes – trickling filters, aerated lagoons, oxidation ditches and WSP, all designed to produce the same quality of final effluent, and he found that WSP systems were the cheapest treatment process at land costs of US$ 50,000-150,000 (1983 $) per hectare, depending on the discount rate used (5-15 percent). These figures are much higher than most land costs, and so land costs are unlikely to be a factor operating against the selection of WSP for wastewater treatment (but, of course, land availability may be).

High efficiency. BOD removals > 90 percent are readily obtained in a series of well designed ponds. The removal of suspended solids is less, due to the presence of algae in the final effluent (but, since algae are very different to the suspended solids in conventional secondary effluents, this is not cause for alarm: indeed the European directive on urban wastewater treatment (Council of the European Communities, 1991) permits WSP effluents to contain up to 150 mg suspended solids/l, and it also allows sample filtration prior to BOD analysis to remove the algae). Total nitrogen removal is 70-90 percent, and total phosphorus removal 30-50 percent.

WSP are particularly efficient in removing excreted pathogens, whereas in contrast all other treatment processes are very inefficient at this and require a tertiary treatment process, such as chlorination (with all its inherent operational and environmental problems see; Feachem et al., 1983) or ultra-violet treatment (which may not always be effective — see *Report*, 1998), to achieve the destruction of faecal bacteria. Activated sludge plants may, if operating very well, achieve a 99 percent removal of faecal coliform bacteria: this might, at first inspection, appear very impressive, but in fact it only represents a reduction from 10^8/100 ml to 10^6/100 ml (that is, almost nothing). A series of WSP, on the other hand, can easily be designed to reduce faecal coliform numbers from 10^8/100 ml to below the guideline value for unrestricted irrigation of 1000 per 100 ml, which is a removal of 99.999 percent (or 5 $\log_{10}$ units). WSP can also easily achieve the current and proposed guideline values for restricted irrigation of no more than 1 and 0.1 intestinal nematode egg per litre (Table 5, Main Document). A general comparison between WSP and conventional treatment processes for the removal of excreted pathogens is shown in Table C1; detailed information is given in Feachem et al. (1983).

Table C1: Removals of excreted pathogens achieved by waste stabilization ponds and conventional treatment processes

Excreted Pathogen	Removal in WSP	Removal in conventional treatment
Bacteria	up to 6 log units *	1 - 2 log units
Viruses	up to 4 log units	1 - 2 log units
Protozoan cysts	100%	90-99%
Helminth eggs	100%	90-99%

Source : Feachem et al. (1983)

* 1 log unit = 90 percent removal; 2 = 99 percent; 3 = 99.9 percent, and so on.

1.2 Anaerobic Ponds

Anaerobic ponds are 2-5 m deep and receive such a high organic loading (usually > 100 g BOD/m^3 d, equivalent to > 3000 kg/ha d for a depth of 3 m) that they contain no dissolved oxygen and no algae. They function much like open septic tanks, and their primary function is BOD removal. They work extremely well in warm climates: a properly designed and not significantly underloaded anaerobic pond will achieve around 60 percent BOD removal at 20°C and as much as 75 percent at 25°C. Retention times are short: for waste waters with a BOD of up to 300 mg/l, 1 day is sufficient at temperatures > 20°C. Indeed, as noted by Marais (1970), "pre-treatment in anaerobic ponds is so advantageous that the first consideration in the design of a series of ponds should always include the possibility of anaerobic treatment".

1.3 Facultative Ponds

Facultative ponds are designed for BOD removal on the basis of a relatively low surface loading (100-400 kg BOD/ha d) to permit the development of a healthy algal population as the oxygen for BOD removal by the pond bacteria is mostly generated by algal photosynthesis. Due to the algae facultative ponds are coloured dark green, although they may occasionally appear red or pink (especially when slightly overloaded) due to the presence of anaerobic purple sulphide-oxidising photosynthetic bacteria. The concentration of algae in a healthy facultative pond depends on loading and temperature, but it is usually in the range 500-2000 µg chlorophyll *a* per litre. The algae are responsible for introducing conditions that kill faecal bacteria; Curtis et al*.* (1992) found that pH values >9 and the combination of a high dissolved oxygen concentration and a high visible light intensity were rapidly fatal to faecal coliforms.

Helminth eggs, which can number up to 2000 per litre of wastewater depending on the endemicity of intestinal nematode infections, are removed by sedimentation and thus most egg removal occurs in the anaerobic and facultative ponds. It is sensible to check whether the facultative pond effluent complies with the recommendations for restricted irrigation (Table 5, Main Document); if it does not, then one (or more) maturation ponds will be necessary to reduce egg numbers to $\not>$ 1 or 0.1 per litre and, if required, faecal coliform numbers to $\not> 10^5$ per 100 ml.

BOD removal in facultative ponds is usually in the range 70-80 percent based on unfiltered samples (that is, including the BOD exerted by the algae), and above 90 percent based on filtered samples.

1.4 Maturation Ponds

A series of maturation ponds receives the effluent from the facultative pond, and the size and number of maturation ponds is governed mainly by the required bacteriological quality of the final effluent (Table 5). The removal of excreted pathogens is extremely efficient in a properly designed series of ponds (Table C2). Maturation ponds achieve only a small removal of BOD, usually around 10-25 percent in each pond. The method of Marais (1974) is generally used to design a pond series for faecal coliform removal — see Section 1.6.

Table C2: Geometric mean bacterial and viral numbers per 100 ml in raw wastewater and the effluents of five waste stabilization ponds in series in northeast Brazil at 26°C

Organism	RW*	A	F	M1	M2	M3
Faecal coliforms	2×10^7	4×10^6	8×10^5	2×10^5	3×10^4	7×10^3
Campylobacters	70	20	0.2	0	0	0
Salmonellae	20	8	0.1	0.002	0.01	0
Enteroviruses	100	60	10	4	0.5	0.09
Rotaviruses	8	2	0.7	0.3	0.1	0.03

Source: Oragui et al*.* (1987).

* RW, raw wastewater; A was an anaerobic pond with a mean hydraulic retention time of 1 day; F and M1-M3 were a facultative pond and maturation ponds, respectively, each with a retention time of 5 days.

1.5 Pond design for helminth egg removal

Helminth eggs are removed in WSP by sedimentation very efficiently. Ayres et al*.* (1992) give the following design equation for egg removal in a single pond:

$$R = 100\,[1 - 0.4 \exp(-\,0.49\theta + 0.0085\theta^2)] \qquad (1)$$

where R = percentage egg removal
θ = mean hydraulic retention time (defined as pond volume/flow), days

This equation is applied to each pond in the series in turn. In practice anaerobic and facultative ponds have to be first designed on the basis of the maximum design BOD loading allowed to be applied to them (which depends on temperature); this, together with the BOD of the wastewater entering them, establishes their retention time, and hence the percentage egg removal they achieve. If the facultative pond effluent contains more than the required egg numbers (Table 5, Main Document), then one or more maturation ponds are added to the series.

A design example is given in Box A.

Helminth eggs in pond sludges. Anaerobic and facultative ponds need to be desludged every 2-3 and 10 years, respectively. As *Ascaris* eggs can remain viable for > 5 years, the sludges removed from WSP must be disposed of carefully by, for example, on-site burial, in a sanitary landfill or deep ploughing into agricultural land.

1.6 Pond design for faecal coliform removal

Faecal coliform bacteria are removed in a series of WSP according to the equations given by Marais (1974):

$$N_e = N_i/(1 + k_T\,\theta_a)\,(1 + k_T\,\theta_f)\,(1 + k_T\,\theta_m)^n \tag{2}$$

$$k_T = 2.6\,(1.19)^{T-20} \tag{3}$$

where N_e = required number of faecal coliforms per 100 ml of final effluent;
N_I = number of faecal coliforms per 100 ml of raw wastewater;
k_T = first order rate constant for faecal coliform removal, day^{-1} (see equation 3 above);
θ = retention time, days; subscripts a, f and m refer to the anaerobic, facultative and maturation ponds, respectively;
n = number of maturation ponds (assumed at the design stage to be equally sized).
T = temperature, ^{o}C.

A design example is given in Box B.

2. Wastewater Storage and Treatment Reservoirs

Wastewater storage and treatment reservoirs (WSTR), also called effluent storage reservoirs, are especially useful in arid and semi-arid areas. They were developed in Israel to store the effluent from a WSP system during the period (8 months in Israel) when it is not required for irrigation (Juanico and Shelef, 1991). It is thus a method of conserving wastewater so that, during the irrigation season, the whole year's wastewater can be used for irrigation. Thus 2-3 times the land area can be irrigated and 2-3 times the quantity of crops produced. WSTR would be used in preference to WSP when the economic value of water is high enough to justify their use.

Current Israeli practice is to treat the wastewater in an anaerobic pond and discharge its effluent into a single 5-15 m deep WSTR with an 8-month retention time. This is perfectly satisfactory, as the WSTR effluent is only used to drip-irrigate cotton and so this usage complies with the guideline in Table 5 (main document) for restricted irrigation category B2, since any helminth eggs settle out in the anaerobic pond and the WSTR. If the restricted irrigation category is B1, rather than B2 as above, then there is the additional requirement that the faecal coliform number should not exceed 10^5 per 100 ml. The above single WSTR cannot achieve this during the irrigation season since the anaerobic pond effluent is discharged into a continuously decreasing WSTR volume, such that towards the end of the irrigation season — i.e. closest to crop harvest — the irrigation water is of increasingly poorer bacteriological quality and will eventually contain > 10^5

faecal coliforms per 100 ml. The solution in this case is to have two batch-fed reservoirs in parallel, each half the volume of the above single reservoir. The contents of one reservoir are used for irrigation until it is half empty, when the contents of the other are used and the anaerobic pond effluent discharged into the first reservoir until the second reservoir is half empty, when the cycle is repeated.

Box A: Egg removal in WSP – design example

Assume that the number of eggs in the raw wastewater is 100, its BOD (L_i) is 300 mg/l and the design temperature is 20^{o}C.

Solution – outline only; further details are given in Mara et al. (1991), Mara (1997) and Mara and Pearson (1998).

Anaerobic pond. For 20^{o}C the design volumetric BOD loading (λ_v) is 300 g/m^3 day, and the retention time is given by

$$\theta_a = L_i / \lambda_v$$

$$= 300 / 300, = 1 \text{ day}$$

From equation 1 the percentage egg removal for 1 day retention time is 75 percent, so the number of eggs in the anaerobic pond effluent is 25 per litre.

Facultative pond. BOD removal in the anaerobic pond is taken as 60 percent at 20^{o}C, so its effluent BOD is 120 mg/l. The design surface BOD loading on the facultative pond is 250 kg/ha day at 20^{o}C. Taking its depth (D) as 1.8 m, then its retention time is given by:

$$\theta_f = 10\, L_i\, D / \lambda_s$$

$$= 10 \times 120 \times 1.8 / 250, = 8.6 \text{ days}$$

Thus, from equation 1 R = 98.9 percent and so the facultative pond effluent contains 0.3 egg per litre. This is < 1 egg per litre and so suitable for reuse categories A2, B1 and B2 (Table 5, Main Document), but not categories A1 and B3, for which the guideline is 0.1 egg per litre. To achieve this, a maturation pond is needed; the minimum retention time in maturation ponds is 3 days, for which R = 89.8 percent and thus the number of eggs in its effluent is 0.03 per litre, which is satisfactory for categories A1 and B3.

If the number of eggs in the raw wastewater were 10 per litre, then the facultative pond effluent in the above example would contain 0.03 per litre, which is suitable for all categories. If the egg count was 1,000 per litre, then the facultative pond effluent would contain 3 per litre. Thus one 3-day maturation pond would be required for categories A2, B1 and B2; and two would be required for categories A1 and B3.

For temperatures <20^{o}C the design BOD loadings are lower; this results in longer retention times and thus higher egg removals. At higher temperatures egg removals are lower as a result of the lower retention times (but minimum retention times of 1 and 5 days are used for anaerobic and facultative ponds, respectively). Similarly, for stronger waste waters (i.e. L_i > 300 mg/l), retention times are longer and thus egg removals higher; and *vice versa* for weaker waste waters (for example, for a BOD of 500 mg/l), θ_a and θ_f would be 1.7 and 14.4 days, respectively, for which R = 81.7 and 99.8. Thus for 100 eggs per litre of raw wastewater, the facultative pond effluent would contain 0.04 egg per litre. If the egg count were 1000 per litre, then a 3-day maturation pond would be necessary for ≤ 0.1 egg per litre, but not ≤ 1 egg per litre).

Box B: Faecal coliform removal in WSP – design example

Assume that the number of faecal coliforms in the raw wastewater is 5×10^7 per 100 ml, with all other parameters as in the example in Box A. Thus θ_a = 1 day and θ_f = 8.6 days. For 20°C the value of k_T is given by equation 3 as 2.6 day^{-1}.

Solution – outline only; further details are given in Mara et al. (1991), Mara (1997) and Mara and Pearson (1998).

Design for 10^5 faecal coliforms per 100 ml – i.e. for reuse category B1

The number of faecal coliforms per 100 ml of facultative pond effluent is given by the following version of equation 2:

$$\begin{aligned} N_e &= N_i / (1 + k_T\, \theta_a)\,(1 + k_T\, \theta_f) \\ &= 5 \times 10^7 / [1 + (2.6 \times 1)]\,[1 + (2.6 \times 8.6)] \\ &= 5.9 \times 10^5 \end{aligned}$$

This is too high for any of the reuse categories (Table 5), and so maturation ponds are required. A single 3-day pond would reduce the count to:

$$\begin{aligned} N_e &= 5.9 \times 10^5 / [1 + (2.6 \times 3)] \\ &= 6.7 \times 10^4 \text{ per 100 ml, which is satisfactory.} \end{aligned}$$

If the number of faecal coliforms had been 1×10^7 per 100 ml, then the facultative pond effluent would just be suitable for reuse category B1. If the temperature had been 26°C or above, then the facultative pond effluent would also be suitable for this category. Stronger waste waters result in longer retention times in the anaerobic and facultative ponds, and therefore faecal coliform removals are slightly higher. For example, if L_i = 500 mg/l, then θ_a = 1.7 day and θ_f = 14.4 days, so the effluent from the facultative pond would contain 2.5×10^5 per 100 ml, and a 3-day maturation pond would be required, as above.

Design for 1000 faecal coliforms per 100 ml – i.e. for reuse categories A1 and B3

The following version of equation 2 can be used to design the series of *n* maturation ponds:

$$N_e = N_i / (1 + k_T\, \theta_m)^n$$

where N_i is now the number of faecal coliforms per 100 ml of facultative pond effluent and N_e = 1000 per 100 ml of final effluent.

This equation is rearranged as follows:

$$\theta_m = [(N_i / N_e)^{1/n} - 1] / k_T$$

Here $N_i = 5.9 \times 10^5$, N_e = 1000 and k_T = 2.6; thus:

$$\theta_m = [(5.9 \times 10^5 / 1000)^{1/n} - 1]/2.6$$

Box B, continued

This equation is solved for n = 1, 2, 3 etc. until θ_m is < 3 days (the minimum retention time in maturation ponds):

For n = 1, θ_m = 227 days
= 2 = 9
= 3 = 2.8

In this example the chosen series of maturation ponds would comprise three ponds each with a retention time of 3 days.

Effect of temperature

As shown by equation 3, the value of k_T is extremely sensitive to temperature, changing by 19 percent for each change in temperature of 1 degC. If the above example were for 25°C, then the anaerobic pond retention time would remain at 1 day (the design minimum), the facultative pond retention time would decrease to 4.6 days (as the BOD removal in the anaerobic pond would increase to 70 percent and the permissible loading on the facultative pond would increase to 350 kg BOD/ha day) and the value of k_T would be 6.2 day^{-1}. The number of faecal coliforms in the facultative pond effluent would be given by:

$$N_e = 5 \times 10^7 / [1 + (6.2 \times 1)]\,[1 + (6.2 \times 4.6)]$$

$$= 2.4 \times 10^5 \text{ per 100 ml}$$

Only two 3-day maturation ponds would now be required to achieve < 1000 faecal coliforms per 100 ml:

$$N_e = 2.4 \times 10^5 / [1 + (6.2 \times 3)]^2$$

$$= 625 \text{ per 100 ml}$$

So the overall retention time at 25°C would be 11.6 days, rather than 18.6 days at 20°C.

Restricted or unrestricted irrigation?

The 1-day anaerobic pond and 8.6-day facultative pond achieve ≤ 1 egg per litre (assuming 100 eggs per litre of raw wastewater), as shown in Box A, i.e. a total retention time of 9.6 days for reuse categories A2, B1 and B2. A 3-day maturation pond is required for ≤ 0.1 egg per litre for categories A1 and B3, i.e. a total retention time of 12.6 days.

For unrestricted irrigation the above example shows that three 3-day maturation ponds are needed to follow the anaerobic and facultative ponds, i.e. a total retention time of 18.6 days, which is 48-94 percent more than required for unrestricted irrigation. Thus it is very important to decide — in conjunction with the local farmers — whether to select restricted irrigation or unrestricted irrigation as this has such a huge influence on pond land area requirements and hence costs.

If the WSTR effluent is to be used for unrestricted irrigation, or for unrestricted irrigation category B3, then it should contain ≤ 1000 FC per 100 ml, which the above single WSTR cannot achieve, at least not during the irrigation season (Liran et al*.*, 1994). Instead several batch-fed WSTR in parallel are required (Mara and Pearson, 1992). These receive anaerobic pond effluent and are each operated on a sequential cycle of fill, rest and use, with faecal coliform die-off to < 1000 per 100 ml occurring during the fill and rest periods. Recent research in northeast Brazil (Mara et al*.*, 1996) has shown that sequential batch-fed WSTR are very efficient at removing faecal coliforms: at temperatures of 25°C die-off to < 1000 per 100 ml throughout the whole reservoir depth of 6 m occurred 3 weeks into the rest phase. WSTR were found to behave much like deep facultative ponds with an algal biomass of around 500 µg chlorophyll *a* per litre (as with WSP, such algal concentrations in WSTR effluents are beneficial for crop irrigation as the algae act as slow-release fertilisers in the soil). The much greater depth of WSTR (5-15 m, compared with 1-2 m for WSP) reduces evaporative losses; in northeast Brazil such losses amounted to under 14% of the inflow to a 6 m deep WSTR during a 4-month rest phase in the hottest part of the year (25-27°C), with a corresponding increase in electrical conductivity to 160 mS/m. Waste waters of such conductivity have been successfully used to irrigate local cash crops, including lettuce.

WSTR are a very flexible system of wastewater treatment and storage. Juanico (1995) details several arrangements, including two WSTR in series, with effluent from the first being used for restricted irrigation and that from the second, for unrestricted irrigation. An alternative "hybrid" WSP-WSTR system is to treat the wastewater in anaerobic and facultative ponds, the effluent from the latter being discharged into a WSTR during the non-irrigation season, but used for restricted irrigation during the irrigation season when the WSTR contents are used for unrestricted irrigation (Mara et al., 1996).

Design procedures for WSTR are given in Mara (1997) and Mara and Pearson (1998).

3. Upgrading Conventional Effluents

The effluents from conventional secondary wastewater treatment systems (activated sludge and its variants such as aerated lagoons and oxidation ditches, and trickling filters) do not meet the microbiological quality requirements for agricultural use (Table 5, Main Document), unless supplemented by a tertiary treatment process. These include chlorination, UV disinfection (both problematic — see Feachem et al*.,* 1983; and *Report*, 1998), sand filtration and maturation ponds (often calling polishing ponds in this context). Sand filtration can remove helminth eggs to < 0.1 per litre, and can also reduce faecal coliform numbers to < 10^5 per 100 ml (see Strauss et al*.,* 1995; and Chen et al., 1998), but it requires very careful operation and maintenance. Maturation ponds will often be the most appropriate way to reduce both helminth eggs and faecal coliforms to the required levels (see Ayres et al*.,* 1992; Mara, 1997; Mara and Pearson, 1998).

4. Wastewater-fed Aquaculture

Anaerobic and facultative ponds are used as described in Sections 1.2 and 1.3, and the facultative pond effluent is discharged into fishponds. These are designed on the basis of surface nitrogen loading, and then checks are made to see that they do not contain more than 1000 FC per 100 ml, which is the WHO Guideline for aquacultural reuse, nor more than 0.5 mg free ammonia per litre as higher values are toxic to the fish. All the trematode eggs settle out in the anaerobic and facultative ponds.

The optimum nitrogen loading on the fishpond is 4 kg total N per ha per day (Mara et al., 1993). Too much nitrogen causes too high an algal biomass, with the resultant risk of deoxygenation at night and consequent fish kills; and too little nitrogen results in too low an algal biomass and consequently low fish yields. Design procedures for wastewater-fed fishponds are given in Mara (1997) and Mara and Pearson (1998).

Fish yields in excess of 10 tonnes/ha year can be obtained in small (up to 1 ha), carefully managed wastewater-fed fishponds. If these are stocked with fingerlings at the rate of 3 per m^2, then three months later the fingerlings will have grown to 150-250 g. Partially draining the pond will ensure that almost all the fish can be harvested. This cycle can be done three times per year. Allowing for a 25% fish loss due to mortality, poaching and consumption by fish-eating birds, the annual yield is:

$(3 \times 200$ g fish per $m^2)$ $(10^{-6}$ tonnes/g) $(10^4$ m^2/ha)

$\times$ (3 harvests per year) (0.75, to allow for the 25% loss)

= 13.5 tonnes of fish per hectare per year.

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