

## **SECTION 4**

### **RISK EVALUATION CRITERIA**

- 1 - Indicators of viral contamination in aquatic environments
- 2 - Minimum infectious dose - Infection - Disease
- 3 - Risk evaluation

## INTRODUCTION

Knowledge of a certain number of criteria is essential in order to be able to estimate the viral risks linked to the use of waste water or treated sludge in agriculture or aquaculture.

First of all, epidemiological criteria exist which make it possible to determine whether there is a link between reusing waste water and sludge contaminated by viruses in agriculture and aquaculture and the outbreak of epidemics or isolated cases of viral disease in the population (cf. section 1 chapter 3).

Criteria also exist concerning the sanitary quality of aquatic environments. By analysing these environments, the viral contamination level of water, sludge, shellfish, market-garden produce... can be established. This contamination level can be established either directly by revealing the presence of human pathogenic viruses, or indirectly by identifying viral contamination indicators. At the present time, the sanitary quality of aquatic environments is established mainly by identifying and quantifying bacterial indicators of faecal contamination.

Infectiology criteria also exist which cover fundamental notions of minimum infectious dose, infection and disease.

## 1. Indicators of viral contamination in aquatic environments

The virological checking and monitoring of waste water is based mainly on bacteriological and/or parasitological data derived from WHO recommendations (1989) (Table 1-1).

These recommendations do not include a virological criterion, and the only part of the world where the legislation includes this criterion is the State of Arizona (U.S.A.), which decreed the following norms in 1984:

- (1) Standards for areas where irrigation is subjected to restrictive conditions: 125 enteric viruses  $40 \text{ l}^{-1}$ .
- (2) Standards for areas where irrigation is not subjected to restrictive conditions: 1 enteric virus  $40 \text{ l}^{-1}$ .

However, it should be pointed out that the State of Florida (USA) imposes a methodology for the treatment of waste water before its reuse in irrigation. Where the irrigation of market-garden produce and public places is concerned, this treatment consists of a secondary treatment, filtration after coagulation and a high degree of disinfection. The checking of this treatment is based on the identification of faecal coliforms, the level of which must be below  $1 \text{ } 100 \text{ ml}^{-1}$  in 75% of samples, and on the concentration of suspended solids which must be below  $5 \text{ mg } \text{l}^{-1}$ .

Monitoring shellfish and the water in which it is bred is based entirely on bacteriological analyses.

This almost complete lack of virological control is due to the fact that the routine identification and quantification of human enteric viruses in water and shellfish give rise to many problems and have their limitations.

Isolating viruses is a slow, costly process requiring very specialised personnel. Methodologies are not standardised and their yield is low, and therefore they tend to underestimate the quantity of viruses actually present. No appropriate method of viral identification exists for some viruses.

TABLE 1-1

**Recommended micro-biological quality standards for the use of waste water in agriculture (according to WHO, 1989)**

Conditions of use	Exposed group	Intestinal nematodes* (arithmetic mean number of eggs per litre)	Faecal coliforms (geometric mean number per 100 ml)	Type of treatment required in order to achieve microbiological quality
a) Irrigation of products likely to be eaten raw, sports fields and public parks**	Workers Consumers Public	≤ 1	≤ 10 <sup>3</sup>	Successive lagooning in order to achieve required biological depuration, or other equivalent treatment
b) Irrigation of cereals, industrial crops, fruit trees***, fodder, animal feed, reforestation zones	Workers	≤ 1	No norms	Length of minimum retention 8-10 d in a lagooning system or other treatment system with equivalent microbiological efficiency
c) Irrigation using methods whereby neither workers nor public are exposed (ex : underground drip irrigation, etc.)	None	No norm	No norm	Pre-treatment required by irrigation technique and at least primary sedimentation

\* Ascaris, Trichocephales, Ancylostomes.

\*\* Stricter norms (<200 fecal coliforms per 100 ml) will be applied to the irrigation of lawns, e.g. hotel lawns, with which the public can be in direct contact.

\*\*\* Irrigation will have to stop two weeks before fruit is gathered and windfalls must not be picked up. Sprinkling will not be authorized.

Bearing these problems in mind, monitoring should be based on the detection of indicator organisms which reveal the presence of human enteric viruses.

These organisms should, on the one hand, reveal the presence of human enteric viruses and, on the other, be indicators of the functioning and efficiency of treatment or disinfection systems.

The ideal viral contamination indicator should satisfy the following conditions:

- the indicator must always be present when enteric viruses are present,
- the incidence and persistence of the indicator and the enteric viruses must be similar,
- the indicator and the enteric viruses must be in a constant ratio and the count of the indicator must give a good estimate of the number of pathogenic viruses present,
- the indicator must be present at higher levels than those of the enteric viruses,
- the indicator must be able to withstand environmental pressures and disinfectants to the same degree as the enteric viruses,
- the indicator must be non-pathogenic and easily quantifiable,
- the indicator must be easy to detect in all types of samples.

### **1.1 Bacterial indicators**

The only bacterial indicators of faecal contamination used at the present time are faecal coliforms and faecal streptococci. These bacteria are bad viral contamination indicators because wide variations in survival patterns exist between viruses and bacteria both in aquatic environments and after treatment and disinfection. In fact enteric viruses persist for longer periods than faecal coliforms and streptococci. The latter are more resistant than coliforms to disinfection treatments and persist for a longer time, particularly in a marine environment. BOSCH *et al.*, (1988) have noted a correlation between the levels of enteroviruses and of faecal streptococci in sediment in the case of recent pollution. However, faecal streptococci are not

good viral indicators since their sensitivity to disinfecting agents is higher than that of enteric viruses.

As a general rule, bacterial indicators are more sensitive to disinfection than enteric viruses and other pathogens, such as mycobacteria, sporulated bacteria and protozoan cysts (SOBSEY, 1989).

A large quantity of data, both analytical (BERG and METCALF, 1978; GERBA *et al.*, 1979; MARZOUK *et al.*, 1980; GOYAL *et al.*, 1978; VAUGHN *et al.*, 1980; SCHWARTZBROD *et al.*, 1985) and epidemiological (PORTNOY *et al.*, 1975; CRAUN, 1978) exist which confirm that water, and seafood which had satisfied bacteriological norms, did in fact contain human enteric viruses.

## **1.2 - Viral indicators**

The best indicator of viral contamination is without any doubt a virus. It is therefore possible to envisage using as indicator either a human enteric virus or a specific bacteriophage of an enteric bacterium.

The vaccinal type 1 poliomyelitis virus has been proposed as an indicator. However, the quantities found in aquatic environments have turned out to be too small and too variable for this virus to be a good indicator.

Any incidence of a specific cytopathic effect of enteroviruses after inoculation of the water sample to be analysed on cell cultures could be used as an indicator of viral presence. However, relying on this type of indicator would involve using the relatively complicated and costly cell culture methodology.

When precise data become available concerning the exact significance and quality of the results obtained by molecular biology techniques (P.C.R.), it may be possible to consider using the presence of viral nucleic acids in water, sludge or shellfish as proof of viral contamination. However, this can only be envisaged as a possible solution for the future.

Many authors suggest that, for the time being, specific bacteriophages of enteric bacteria be used as indicators. They have the advantage of being viral and of being easily and rapidly identifiable.

Three groups of phages have been proposed: somatic coliphages, the (F-RNA) specific bacteriophages and the bacteriophages which infect *Bacteroides fragilis*. A certain number of arguments point to using coliphages. KOTT (1981) considers that:

- phages are found abundantly in residual water and in contaminated water,
- populations of coliphages are much larger than those of enteroviruses,
- coliphages are incapable of reproduction outside the host bacterium,
- coliphages can be isolated and counted using simple methods,
- the length of time between taking the sample and obtaining the final result is shorter for coliphages than for enteroviruses,
- certain coliphages are as resistant to inactivation and disinfection as enteroviruses.

However, this view is perhaps too optimistic since, in certain cases, coliphages are present in residual water and effluent in which no enteroviruses can be isolated, whereas, in other cases, enteroviruses are isolated in water samples in which no coliphages can be found. Finally, and even more important, certain coliphages can multiply outside the human digestive tract at the expense of *E. coli* or resident enterobacteria.

It would appear that somatic coliphages are not suitable because the resistance factor of members of this heterogeneous group is very variable and because some of them can multiply in water.

However, the specific F-RNA bacteriophages (MS2, f2...) seem to have more potential. They use sexual pili as receivers, their physical structure resembles that of enteroviruses and they seem to multiply only in the digestive tract of homeothermic animals, although some results obtained from waste water can only be correctly interpreted if these phages do in fact reproduce in this environment (HAVELAAR, 1987). Specific F-RNA phages are found as frequently in the digestive tract of animals as in that of man (DHILLON *et al.*, 1976).

The use of specific *Bacteroides fragilis* phages as indicators has been studied recently by JOFRE *et al.*, (1986); TARTERA and JOFRE (1987); TARTERA *et al.*, (1989). They have

definite advantages over the specific F-RNA bacteriophages because, if a suitable indicator strain is used, the isolated phages are very specific to the human digestive tract and they do not reproduce outside the human body. Nevertheless they are found in smaller quantities than the specific F-RNA bacteriophages in human stools, but in larger quantities than enteroviruses. Furthermore, the techniques used at the present time to detect them are more complicated and take longer than those used for specific F-RNA bacteriophages.

### 1.2.1 Behaviour of bacteriophages in aquatic environments

The survival of bacteriophages in aquatic environments varies according to the environment and the type of phage.

Therefore, according to JOFRE (1991), the T99 in the following environments is:

- Surface water, 31 days for the T7 somatic coliphage and 10 days for the F specific MS2 coliphage (NIEMI, 1976; SCHEUERMANN *et al.*, 1987).
- Ground water, 12 days for the F specific MS2 coliphage and 3.2 days for the F specific f2 coliphage (BITTON *et al.*, 1983; YATES *et al.*, 1985).
- Sea water, 5 days for the f2 phage, 6 days for the *Bacteroides fragilis* phage and 20 days for the MS2 phage (AYRES, 1977; JOFRE *et al.*, 1986).

The specific F-RNA bacteriophages and the *Bacteroides fragilis* bacteriophages could be considered as good viral indicators. When NASSER *et al.*, (1992) were evaluating the potential of F specific coliphages as indicators of the persistence of the hepatitis A virus (HAV) and of the poliovirus in waste water, they showed that the temperature-related inactivation of F specific coliphages was identical or lower than that of the poliovirus and the HAV.

In the same way, SPRINGTHORPE and SATTAR (1992) have shown that the survival in river water of the F specific MS2 coliphage was similar to that of human pathogenic viruses.

Finally, JOFRE *et al.*, (1986) have reported that the presence of *Bacteroides fragilis* bacteriophages is correlated with that of enteroviruses and rotaviruses.

Therefore, the specific F-RNA bacteriophages and the *Bacteroides fragilis* phages can be considered as good indicators of the presence of enteric viruses in aquatic environments;

however, further research must be carried out on the ecological aspects of these phages and their ability to multiply in warm water.

### **1.2.2 Behaviour of bacteriophages during waste water treatment**

The proportion of phages eliminated during waste water treatments varies according to the type of treatment.

The percentage varies from 0% to 83% for primary sedimentation (SHERMAN *et al.*, 1975; IGNAZZITO *et al.*, 1980), from 11% to 98% for activated sludge (NAPARSTED *et al.*, 1976; BITTON *et al.*, 1983), from 20% to 90% for biological filters (SHERMAN *et al.*, 1975; KOTT *et al.*, 1978) and from 0% to 94% during lagooning (BELL, 1976; BAYLET *et al.*, 1980). In fact, according to AYRES (1977) and NIEUWSTAD *et al.*, (1988) the percentage of phage elimination is lower than that of indicator bacteria elimination and very close to that of animal viruses.

According to HAVELAAR (1987) the specific F-RNA bacteriophages would be good indicators of the efficiency of depuration treatments in ridding residual water of enteric viruses.

Finally, according to JOFRE *et al.*, (1989) the *Bacteroides fragilis* phages react in the same way as enteric viruses to waste water treatments.

### **1.2.3 Behaviour of bacteriophages during disinfection treatments**

The possibility of using bacteriophages as indicators of waste water disinfection has been considered, but results are not easily comparable since many different types of phage, water and treatment are involved. However, it has been found that the resistance of phages to the normal processes of disinfection (chlorine, ozone, ultra-violet rays) is greater than that of the indicator bacteria (SHAH and CAMISH, 1972; PETRASEK *et al.*, 1980; SNEAD *et al.*, 1980; TAYLOR 1982; HAVELAAR *et al.*, 1987; HAVELAAR, 1987; TARTERA *et al.*, 1988). Therefore, with a treatment using 3 to 5 mg of chlorine per litre of treated waste water, the specific F-RNA bacteriophages are reduced by a factor of 0.05 to 0.5 log, the somatic coliphages by a factor of 0.4 to 1.3 log and the *E. coli* by a factor of 2 to 4 log (HAVELAAR, 1987).

HALL and SOBSEY (1992), working with a phosphate buffer not requiring an oxidant, at 5° C, have shown that with 2 mg l<sup>-1</sup> of ozone, the inactivation of the hepatitis A virus (HAV)

and of the F specific MS2 coliphage was higher by a factor of 4 log and 3.8 log respectively after 5-10 seconds.

KESWICK *et al.*, (1985) have stated that, with doses of 3.75 or 6.25 mg l<sup>-1</sup> of free chlorine for 30 minutes, the Norwalk virus and the f2 bacteriophage are not completely inactivated, unlike the poliovirus and the Wa rotavirus. Only the f2 bacteriophage has a level of sensitivity similar to that of the Norwalk virus.

TARTERA *et al.*, (1988) have shown that, after treating tap water with 2.3 mg of chlorine per litre for 10 minutes, the *Bacteroides fragilis* phages are reduced by 90%, the poliomyelitis viruses by 99% and the f2 coliphages by 99.9%.

Where exposure to ultra-violet rays is concerned, the f2 coliphage (specific F-RNA bacteriophage) is more resistant than the poliomyelitis viruses (1.7 to 3.5 times) and the *E. coli* (2.3 to 7 times) (HAVELAAR *et al.*, 1987). In the same way, BOSCH *et al.*, (1989) have stated that the f2 phage is more resistant to UV than the poliovirus 1, the SA 11 rotavirus and the *Bacteroides fragilis* phage.

Finally, according to BURGE *et al.*, (1981), the f2 coliphages can be considered as excellent indicators of the destruction of pathogenic micro-organisms during composting.

When all these different results have been taken into consideration, it would appear that the specific F-RNA bacteriophages and the *Bacteroides fragilis* phages can be considered as good disinfection indicators, their resistance to disinfection agents being greater or equal to that of human pathogenic enteric viruses. However, it must be remembered that bacteriophages, like viruses, are partially protected from the inactivating effect of disinfectants when they are linked to solid particles (STAGG *et al.*, 1978).

## 2 Minimum infectious dose, infection and disease

When an individual is infected by an enteric virus, the latter multiplies in the body, mainly in the intestine, and large quantities of viral particles are excreted in the stools. This viral presence in stools is a sign of infection.

This infection can, from a clinical point of view, remain totally invisible and cause no pathological symptom, or it can result in disease and the onset of clinical symptoms.

The onset of clinical symptoms depends on numerous factors, such as the state of immunity of the individual, his age, state of health, the type and strength of the virus. Whether the disease manifests itself or not by clinical symptoms, the infected individual will excrete large quantities of virus in his stools and is therefore a contaminator.

It must be emphasised that a water-borne viral infection transmitted to one or several individuals can be transmitted to other individuals, either by contact between individuals, or by contamination of various products through which people can become infected. This was clearly demonstrated during a water-borne epidemic caused by the Norwalk virus, during which the level of secondary attack was calculated at 30% (GERBA *et al.*, 1985).

Many enteric viruses can cause infections which are not apparent. When infections are caused by enteroviruses, clinical symptoms rarely appear. The frequency of symptomatic infections is estimated at 1% for polioviruses, for example. When infections are caused by the hepatitis A virus, the age of the infected person is the most important factor. Therefore, if the infection occurs during childhood, the percentage of individuals with clinical symptoms is 5%, whereas it is 75% if the first infection occurs during adulthood.

On the other hand, where rotaviruses are concerned, clinical symptoms are much more frequent in childhood than in adulthood.

However, for an individual to become infected, he must ingest a sufficient quantity of viral particles, i.e. the minimum infectious dose (MID).

This minimum infectious dose (MID) varies according to a large number of parameters such as the virus type, the state of dispersion of the viral suspension, the nature and the pH of the ingested food...

The existence of these various parameters no doubt explains why such wide disparities occur between different MID's reported in the literature. According to WARD and AKIN (1984), the ingestion of one or two viral particles (PFU) could cause an infection in a sensitive person. On the other hand, other authors have reported higher MID's. MINOR *et al.*, (1981) found that, after administering oral doses of 50 TCID<sub>50</sub> of the type 1 poliovirus to 2-month old children, only 50% of the children were infected. SCHIFF *et al.*, (1984) administered to 100 voluntary adults different doses of Echo 12 virus added to drinking water, and worked out the percentage of infection by isolating the viruses in the stools. The statistical interpretation of these results made it possible to estimate the minimum infectious dose at 17 PFU. GRAHAM

*et al.*, (1987), working with dwarf piglets, have shown that the MID for the porcine rotavirus was 1 PFU.

It should be pointed out that these estimated MID's concern only enteroviruses and do not take into account the fundamental enteric viruses in water-based virology, i.e. the gastro-enteritis and hepatitis viruses. However, it would appear that there is a consensus when estimating that the MID for enteric viruses is low and is below 50 infectious particles (GERBA and HAAS, 1988).

Another factor which must be taken into consideration is the risk of mortality after infection with enteric viruses. The risk of mortality for the hepatitis A virus has been estimated at 0.6% by the Centre for Disease Control (1985). Mortality levels for various enteroviruses have been listed in table 2.1.

**TABLE 2.1**  
**Levels of mortality caused by enteroviruses**  
**(according to ASSAAD and BORECKA, 1985)**

Virus	Mortality rate %
Poliovirus 1	0,9
A2 Coxsackievirus	0,5
A9 Coxsackievirus	0,26
Coxsackievirus B virus	0,59 to 0,94
Echovirus 6	0,29

### 3. Risk evaluation

Evaluation of the risks linked to the presence of viruses in aquatic environments is of fundamental importance not only when establishing norms but also when identifying those treatment processes which will make it possible for these norms to be respected.

Several studies have been carried out since 1988 concerning the evaluation of viral risks linked to the consumption of drinking water. GERBA and HASS (1988) have worked out the risk of infection, disease and death for persons consuming water which contains low concentrations of poliovirus 1 and the hepatitis A virus (HAV). They were therefore able to estimate that, when 2 litres were consumed per day of water containing 1 virus per 1000 litres of water, the annual risk of infection was  $10^{-2}$  for the poliovirus 1 and HAV, the risk of disease was  $1.1 \cdot 10^{-4}$  for HAV and  $7.8 \cdot 10^{-3}$  for the poliovirus and the risk of mortality rose to  $9.5 \cdot 10^{-7}$  for the poliovirus and  $4.3 \cdot 10^{-5}$  for HAV. They therefore found that risks varied very considerably depending on the type of enteric virus concerned.

ROSE and GERBA (1991b), basing their work on the fact that the Environmental Protection Agency (EPA) consider as acceptable the risk of one infection for 10 000 per year for

infectious agents transmitted by drinking water, have calculated that the number of polioviruses and rotaviruses should not exceed, respectively, 0.1 and 0.3 PFU 100 l<sup>-1</sup> of drinking water per day.

In another context, PAYMENT *et al.*, (1991) measured the annual incidence of gastro-enteritis linked to the consumption of tap water obtained from surface water satisfying the norms for drinking water. They reported an incidence of 0.76 among persons drinking tap water, compared to 0.50 among persons drinking filtered water.

Where the evaluation of viral risks linked to the reuse of waste water is concerned, ROSE and GERBA (1991a) studied the viral contamination of water which had been treated for reuse in Arizona and Florida. They observed average viral concentrations after secondary treatment (treatment by activated sludge and disinfection) of respectively 13 and 130 PFU 100 l<sup>-1</sup>.

They also noted that if the water were filtered before being disinfected, the reduction in the number of viruses was greater and resulted in water containing 1 PFU 100 l<sup>-1</sup> on average.

The risk of infection after accidental ingestion of 100 ml of water is between 2 10<sup>-3</sup> and 2 10<sup>-4</sup> when it is a question of secondary, chlorinated effluent, and goes down to between 2 10<sup>-4</sup> and 2 10<sup>-5</sup> when it is secondary effluent which has been filtered and disinfected.

ASANO and SAKAJI (1990) in California have analysed the risks to health of using the methodology described by HAAS (1983). They have reported that, according to their estimates, with water containing 1 enteric virus for 40 litres (Arizona standards), the probability of infection is between 10<sup>-3</sup> and 10<sup>-7</sup> when 100 ml of water are consumed. If the same water is used to irrigate market-garden produce, the risk of infection is between 10<sup>-4</sup> and 10<sup>-8</sup>.

It should be pointed out that the risk of infection from market-garden produce is considerably reduced by the fact that enteric viruses are naturally inactivated in the environment (reduction by a factor of 1 log in 4 days). This observation provides a very strong argument in favour of instigating a latency period between irrigation and harvesting.

When ASANO *et al.*, (1992), were evaluating norms for the reuse of waste water in California, they studied four scenarios of exposure to waste water and identified the risks of infection. The scenarios are: irrigation of market-garden produce, irrigation of golf courses, recreational uses of water and the replenishment of ground water. The risks have been identified for 3 enteric viruses (poliovirus 1 and 3, echovirus 12) and for water which has undergone

various treatments and which therefore contains varying quantities of virus. As way of example, the risk of contracting an infection after exposure to water containing one infectious viral unit for 100 litres is indicated in table 3.1.

It can be seen that the annual risk of infection, after exposure to effluent containing one viral unit for 100 litres, during recreational activities such as swimming or golf, is between  $10^{-2}$  and  $10^{-7}$  (depending on the type of virus), whereas the risk is between  $10^{-6}$  and  $10^{-11}$  from irrigated market-garden produce or replenished ground water. It is obvious that the risk of infection is greater, the higher the viral concentration level of the water. For example, with water having a maximum concentration of 111 viral units for 100 litres, the annual risk is between  $10^{-1}$  and  $10^{-5}$  for recreational water and golf courses, and between  $10^{-4}$  and  $10^{-2}$  for market-garden produce and ground water replenishment.

**TABLE 3.1**  
**Risk of contracting an infection after exposure to water containing**  
**one infectious viral unit for 100 litres**

	<b>Irrigation of golf course</b>	<b>Irrigation of market garden produce by sprinkling</b>	<b>Recreational water for un- restricted use</b>	<b>Replenishment of ground water</b>
<b>Risk during life time</b>				
Echovirus 12	2,71 10 <sup>-4</sup>	2,82 10 <sup>-6</sup>	2,74 10 <sup>-2</sup>	3,72 10 <sup>-8</sup>
Poliovirus 1	9,31 10 <sup>-6</sup>	9,70 10 <sup>-8</sup>	9,52 10 <sup>-4</sup>	3,40 10 <sup>-9</sup>
Poliovirus 3	6,84 10 <sup>-3</sup>	7,15 10 <sup>-5</sup>	5,04 10 <sup>-1</sup>	1,43 10 <sup>-8</sup>
<b>Annual risk</b>				
Echovirus 12	9,04 10 <sup>-6</sup>	4,04 10 <sup>-8</sup>	6,93 10 <sup>-4</sup>	5,31 10 <sup>-10</sup>
Poliovirus 1	3,10 10 <sup>-7</sup>	1,39 10 <sup>-9</sup>	2,38 10 <sup>-5</sup>	4,86 10 <sup>-11</sup>
Poliovirus 3	2,29 10 <sup>-4</sup>	1,02 10 <sup>-6</sup>	1,74 10 <sup>-2</sup>	2,04 10 <sup>-10</sup>
<b>Daily risk</b>				
Echovirus 12	8,69 10 <sup>-8</sup>	1,11 10 <sup>-10</sup>	1,73 10 <sup>-5</sup>	1,46 10 <sup>-12</sup>
Poliovirus 1	2,98 10 <sup>-9</sup>	3,80 10 <sup>-12</sup>	5,95 10 <sup>-7</sup>	1,33 10 <sup>-13</sup>
Poliovirus 3	2,20 10 <sup>-6</sup>	2,80 10 <sup>-9</sup>	4,38 10 <sup>-4</sup>	5,60 10 <sup>-13</sup>

Few studies have been carried out to evaluate the risks to public health from using waste water, but those which exist are extremely important. They should be strongly encouraged and developed because they form an invaluable foundation on which to base norms or recommendations. The evaluation of risks according to the different ways in which individuals can be exposed to waste water should also make it possible to lay down clear guidelines as to how this type of water can be used.

## Conclusion

In order to be able to evaluate fully the risks linked to the use of waste water and treated sludge in agriculture and aquaculture, it is essential to take into account both epidemiological criteria and criteria concerning the sanitary quality of the environment, as well as the concepts of minimum infectious dose, infection and disease.

At the present time, sanitary quality checks on waste water, shellfish breeding waters, market-garden produce and seafood are based entirely on bacterial contamination indicators (except in the state of Arizona).

These bacterial indicators (faecal coliforms and streptococci) are proving to be bad indicators, not only of viral presence, but also of the efficiency of disinfection where enteric viruses are concerned. Numerous studies are being carried out at the present time to try and find an effective indicator of viral contamination in aquatic environments, which could be one or several bacteriophages. It would appear that the specific F-RNA bacteriophages and the *Bacteroides fragilis* phages could fulfil this role but further studies are necessary, particularly concerning the ecological aspects of these potential indicators, before any favourable conclusions can be put forward.

The minimum infectious dose for enteric viruses is now considered to be low and is certainly below 50 infectious particles.

Evaluation of the risks associated with viral presence in water for irrigation, market-garden produce or seafood is of fundamental importance.

Since 1988, various studies have been carried out to evaluate the viral risks linked to the consumption of drinking water and to the reuse of waste water, and they have provided some thought-provoking figures concerning the viral concentration of water and the uses to which it is put. It has thus been shown that the risk is greater when a golf course has been watered than when market-garden produce has been irrigated.

These evaluations of viral risks have led to the formulation of practical conclusions such as the necessity for a latency period between the irrigation and harvesting of market-garden produce. The duration of this latency period can be calculated according to the viral concentration of the water, the sprinkling method, weather conditions (temperature, sunshine, rainfall) and the level of acceptable risk.

Few studies of this kind exist at the present time but they must be developed because, on the basis of their conclusions, it will be possible to lay down norms and recommendations concerning viruses in waste water, based on objective criteria.

As present knowledge stands, it would appear to be too early to edict norms and recommendations concerning standards for viruses in waste water.

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## **SECTION 5**

### **RECOMMENDATIONS AND SUGGESTED AVENUES OF RESEARCH**

- 1 - Recommendations
- 2 - Suggested avenues of research

## **1. Recommendations**

1.1. Information is sorely lacking concerning the virology of water in developing countries. It is therefore recommended that a very serious attempt be made to obtain precise data for these countries concerning the following:

- the level of viral contamination for waste water, surface water and sea water;
- the virological quality of water and the conditions for its use in irrigation;
- the level of viral contamination in areas involved in aquaculture and the breeding of seafood.

1.2. It would appear that it is too soon to put forward proposals for the establishment of standards or recommendations concerning viruses in waste water and sludge. This can only be done when precise data becomes available on the evaluation of risks linked to the use of waste water and sludge and on the level of acceptable risk.

However, in the meantime, the recommendation could be made that only waste water which has already undergone treatment to eliminate 80 to 90% of settleable solids, which is the normal substrate for enteric viruses, be used for irrigation.

1.3. The setting up of quality controls within laboratories concerned with the virology of aquatic environments.

## **2. Suggested avenues of research**

2.1. The continuation and development of studies with the intention of proposing an indicator or pool of indicators of viral contamination in aquatic environments.

In particular, it is important to determine whether the *bacteroides fragilis* phages and the specific F-RNA bacteriophages are good indicators of the presence of enteric viruses in aquatic environments, whatever the climate of the country (there is the problem of tropical waters where specific F-RNA bacteriophages can not only be present when there is no faecal contamination at all, but can also multiply) and good indicators of disinfection on a virological level.

2.2. Development of research into the use of molecular biology techniques, and in particular PCR, for the analysis of aquatic environments.

- \* Evaluation of the different techniques proposed for PCR (with preliminary concentration, without preliminary concentration, with immunocapture...) using a "round-robin" in order to select and then standardize one or several methodologies.
- \* Assessment of the exact significance of a positive reaction in PCR (where the isolation of infectious viruses is concerned) from the point of view of public health, risk, and epidemiology.
- \* If the results of the above researches are satisfactory, it would be possible to envisage organising a large-scale study in parallel with PCR, carried out by registered laboratories, working with a large number of samples collected especially in developing countries.

2.3. Development of studies concerning the evaluation of risks linked to the reuse of waste water, and, in particular, the risks linked to the consumption of irrigated market-garden produce.

2.4. Carrying out epidemiological surveys of the transmission of enteric viruses via waste water, irrigated market-garden produce and seafood, especially in developing countries.

2.5. Research into the mechanisms of inactivation of enteric viruses in the environment.

2.6. Research not only into the mechanisms of seafood decontamination, but also into methodologies for depurating these products which can be standardised and controlled from the point of view of virological efficiency.

2.7. Carrying out, **in the field**, an experimental programme concerning waste water (before and after different depuration treatments), soil and irrigated market-garden produce. For all these elements, this programme would include the evaluation of contamination levels and survival levels for pathogenic viruses (enteroviruses, rotaviruses, Norwalk virus), bacteriophages (specific F-RNA and *Bacteroides fragilis* phages) and bacteria (faecal coliforms and streptococci, salmonellae).