Evaluating the Safety of Wastewater Reclamation and Reuse Based on the Risk Assessment of Infection caused by Pathogenic Microorganisms

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ABSTRACT

The public health problem of greatest concern in wastewater reclamation and reuse is the possibility of infection and disease caused by pathogenic microorganisms. It is now possible to quantitatively assess the risk from these pathogenic microorganisms based on a numerical model using the risk assessment method similarly utilized for chemical safety assessments. This paper presents a literary review of pathogenic microorganism infection risk assessment methodologies and a summary of examples of their application to drinking water in water supply and wastewater reclamation and reuse.

RISK ASSESSMENT

In the United States, evaluation methods called risk assessments related to human health are used to perform quantitative assessments of the degree to which the concentrations of chemicals and pathogenic microorganisms in drinking water, the atmosphere, etc., affect human health, and ecosystems to regulate these concentrations have been established. The word "risk" in the term "risk assessment" of risk agents in the environment which affect human heath is defined as the probability of an adverse effect on human health.

The basic concepts behind the standardized risk assessment protocol are shown by the procedures presented in Figure 1 from the National Research Council of the United States[1)]. The process begins by defining the source of the risk. In other words, the first step is to clarify the risk agent which is the source of the hazard to be assessed. This is called "hazard identification." The second step, quantitatively describing the effect which this risk agent has on the body of a person who is exposed to it, is called the dose-response assessment. The model used for the dose-response assessment is a numerical model prepared based on epidemiological data or experimental data obtained from either human or animal experiments.

The next step is an assessment of the extent to which a person would be exposed to the risk agent in a certain scenario. This process involves determining how many people would be exposed by what route when a risk agent has been discharged into the environment. When a person has been exposed to a risk agent through ingestion of an environmental medium such as air, drinking water, food, etc., if the concentration of the risk agent in the environmental medium can be directly clarified, the amount ingested with that environmental medium can be clarified as a default value, thus allowing the dose of the risk agent to be calculated.

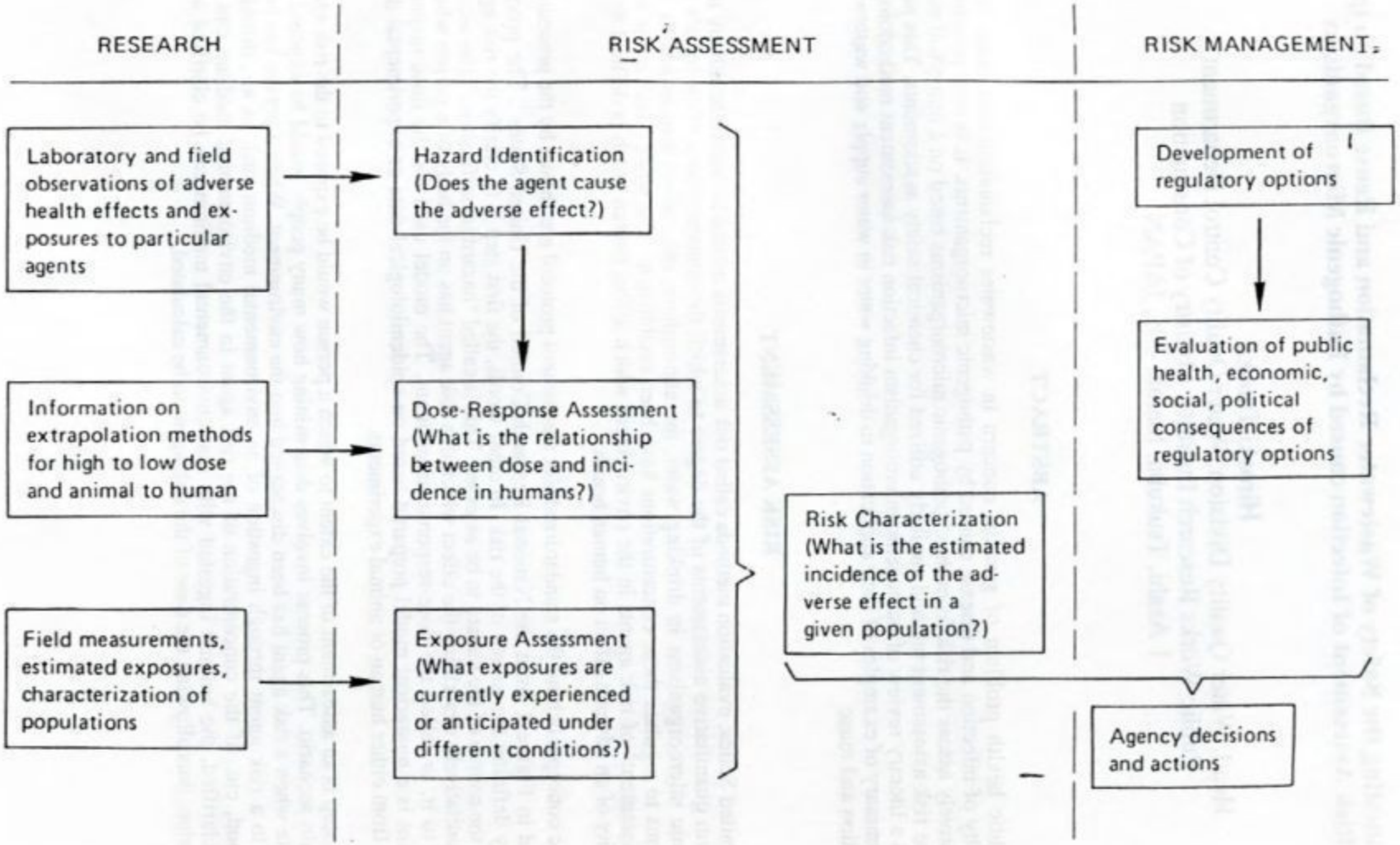


Figure 1 Elements of the risk assessment and risk management processes. From NAS (1983).

If data obtained from monitoring of a risk agent in the environmental medium which the person has ingested is available, this may be used as it is. However, in a case where only the concentration of the risk agent which has been produced in or discharged into the environment from an emission source, or only the concentration of the raw water is known, the concentration in the water or food to which the person is directly exposed is estimated by using a model to predict the transfer and decay processes in the environmental medium and/or the removal process during water or wastewater treatment. The amount to which the person is exposed, in other words the dose, can be calculated based on the amount of water or food ingested and the concentration of the risk agent in this water or food. The final step is the use of a dose-response model based on this dose to measure and characterize the degree of risk. This task is called risk characterization.

The same approach is used to perform a risk assessment for pathogens. The risk of infection is a function of danger (probability of infection from a specified unit dose) and amount of exposure[2,3]. To quantify and characterize the risk, the interaction of the pathogenic microorganisms and the person exposed, in other words, the dose-response relationship and the level of the person's exposure to that pathogenic microorganism must be known. The assessment of risk of infection by a microorganism is usually performed by means of a deterministic evaluation of the microorganism concentration, amount of environmental medium ingested, and the reduction rate in the environment and during treatment. However, because the pathogenic microorganism concentration in the environment, the amounts of water and food ingested, the reduction rate of the pathogenic microorganism in the environment and during various treatment processes, and other factors fluctuate in practice, these all contribute a degree of uncertainty. For this reason, a probability based approach that accounts for the variability of these factors is applied to the exposure assessment, and the risk assessment is based on the results.

A risk assessment of this kind is not only used as a perspective method--finding out what degree of risk is caused by a certain degree of exposure--it is also used as a retrospective method, in other words, when the risk which can be allowed is known, it is used to legally or administratively determine the allowed contamination level[41].

DOSE-RESPONSE MODEL

The central process for risk assessments which transform an amount of exposure to a pathogenic microorganism into risk will now be described in detail. Because the risk to a person from a pathogenic microorganism is categorized into various levels of damage, namely infection, contraction, and death, it is necessary to first clarify which level of risk is to be evaluated. Animal or human data are necessary in order to establish a dose-response model that will quantify the relationship between the amount of a pathogenic microorganism to which a person is exposed and the likelihood that the person will be infected or become ill. Data based on human studies must be used as the basis for assessing the risk from pathogenic microorganisms[2]. The only ways to obtain this information are to either experimentally expose members of a volunteer group to varying amounts of a pathogenic microorganism, or to investigate an accident in which pathogenic microorganisms were mixed with water or food to find out how many people were infected, the type of pathogenic microorganism causing the accident, and the amount of exposure to the pathogenic microorganisms. The only way to determine the morbidity or mortality for a specific pathogenic microorganism is to examine medical records at actual hospitals.

Because dose-response data are often based on small groups of healthy volunteers, the risk is assumed to be higher for children, old people, or other risk groups than the values predicted from the dose-response data based on a healthy volunteer group study. These high-risk groups include many people; as much as 17% of the population of the United States is said to belong to such risk groups[5], and it is not yet clear what degree of safety margin should be allowed in the case of risk assessments for pathogenic microorganisms.

On the other hand, the risk from pathogenic microorganisms is marked by large differences in the seriousness of illness and the mortality between various types of pathogenic microorganisms. The health risk from chemicals is categorized by the United States Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer (IARC) into two groups,

carcinogens accompanied by a high mortality, and non-carcinogens. The criteria for judging a carcinogen is based on whether the agent causes cancer. Unified judgments are made that a non-carcinogen will not harm human health below a certain threshold, but integrated judgments of this kind are not possible regarding pathogenic microorganisms. Discussions of the safety of chemicals deal with the issue of the chronic long-term effects of continuous exposure, while the risk from pathogenic microorganisms is considered as an acute adverse effect, in other words, the risk from each case of exposure[6].

While the dose-response model for chemical risk applied to humans accounts for the weight and body surface area of the person based on data obtained from animal experimentation results, the dose-response model for a pathogenic microorganism is considered to be highly reliable because it is based on data for the direct relationship between humans and pathogenic microorganisms[6]. Another point which distinguishes the assessment of risk from pathogenic microorganisms from the assessment of risk from chemicals is that there are cases where secondary infection is important; in such cases, attention must be paid to the risk of secondary or even tertiary infection of one person by another.

A number of models have been proposed that determine the amount of pathogenic microorganisms to which a person is exposed, and the degree of risk of that person becoming infected and of contracting the disease from the amount of the microorganisms to which the person was exposed, in other words, the dose[2,7,8]. Risk assessment models for pathogenic microorganisms are based upon a dose-response relationship for which there is no safe exposure level, that is, a dose level where infection or symptoms do not appear, or a hypothetical threshold value is not set. The model ultimately chosen will be the one which conforms most closely with experimental results, but the exponential model and beta model that are described next were obtained from the results of theoretical considerations of the interaction of pathogenic microorganisms with human beings. Pathogenic microorganisms of different kinds cause various unique symptoms in people. Furthermore, a person's own immunity also affects the pathogenic microorganisms, so that the degrees of susceptibility of various people to a certain pathogenic microorganism are not the same. Thus, even when two or more people are similarly exposed to a pathogenic microorganism, the nature of each person's response varies, so for many groups, a model which expresses the response as infection risk is used.

1) Exponential Model
This type is also called a single hit model. Experiments to find the dose-response are usually performed with the number of pathogenic microorganisms approximated by a Poisson's distribution as a dose. In a case where the percentage of persons who are infected by a particular pathogenic microorganism is constant, that is, the interaction of people with a pathogenic microorganism is fixed, the probability of infection caused by a single exposure to the average number of microorganisms is provided by an exponential model[2,9,10]:

$$P(D) = 1 - EXP(-\gamma D) \qquad (1)$$

Where,
P: Probability of infection by a single exposure
D: Exposure amount, or dose
γ: Parameter

The parameter γ is the infectivity of pathogenic microorganism to persons, and the larger it is, the greater the infectious potential of the microorganism.

2) The Beta Distribution Infection Probability Model
This is a model in which the infectivity varies according to the number of ingested pathogenic microorganisms rather than one which hypothesizes that the capability to infect a person γ of each pathogenic microorganism in a host in the exponential model is constant regardless of the amount of pathogenic microorganisms which have entered the host. Considering that this is a probability

model which expresses this interrelationship as a beta distribution, it is possible to estimate the following infection probability:

$$\bar{P}(D) = 1 - (1 + (D/\beta))^{-\alpha} \qquad (2)$$

Where,
P: Probability of infection with a single exposure
D: Amount of exposure
α, β are parameters.

The larger α the closer to an exponential distribution[8].

Other models proposed include a lognormal distribution model and a logistic model. In a few cases a linear model is hypothesized[12].

3) Lognormal Model
A lognormal distribution model is a model that hypothesizes that there are individual differences in individual immunity to pathogenic microorganisms, and that they are in a lognormal distribution to the amount of pathogenic microorganisms to which each person is exposed:

$$P(D) = \int_0^D \frac{1}{\sqrt{2}\,\pi\,\sigma\,t} \mathrm{Exp}\left(\frac{-1}{2}\frac{(\mathrm{Log}(D) - \mu)^2}{\sigma^2}\right) dt \qquad (3)$$

Where,
P: Probability of infection by a single exposure (risk)
D: Amount of exposure, or dose
α, β, γ, μ, σ: Parameters

4) Logistic Model
The logistic model proposed by Hald[11] is represented as shown below:

$$P(D) = \frac{1}{1 + \mathrm{EXP}\left(-\{M + N\,\mathrm{Log}(D)\}\right)} \qquad (4)$$

Where,
P: Probability of infection by a single exposure (risk)
D: Amount of exposure, or dose
M, N: Parameters

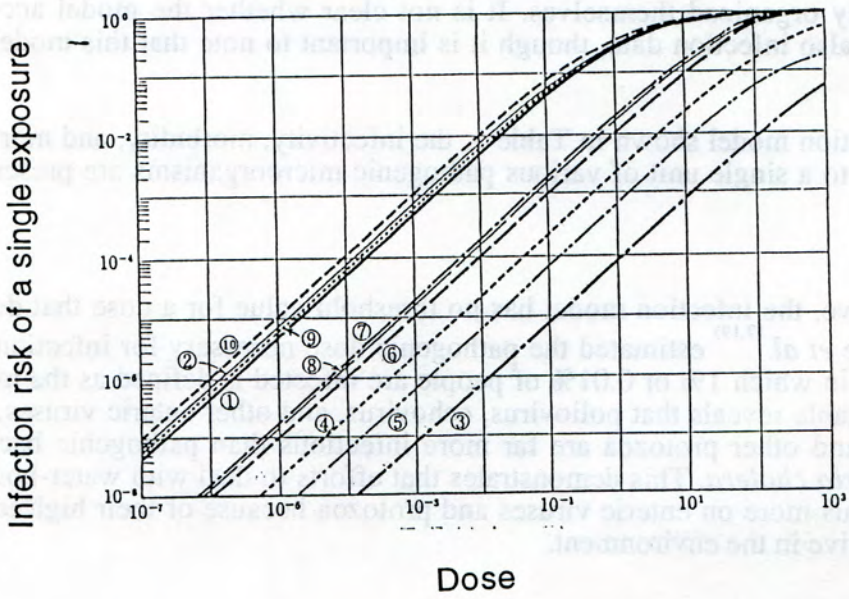
Selection of the most appropriate model and the establishment of the most likely parameters are based on data sets on the dose of pathogenic microorganisms to which people are exposed and the percentage of a group of people who were exposed to this dose and who were actually infected to the total numbers of people who were dosed. These data were obtained either from epidemiological data or from tests of a group of volunteers.

Dose-response models and their estimated parameters for infection from various kinds of pathogenic microorganisms have been reported by Haas[2], Rose *et al.*[7] Regli *et al.*[8], and Haas *et al.*[14], as shown in Table 1. Variations in parameters presented in various literature sources for the same type of pathogenic microorganism are assumed to be a result of the revision of the infection data used for the estimations. Figure 2 shows the dose-response relationships for a number of microorganisms prepared based on this model.

TABLE1 Parameters of Infection Risk Models from Pathogens

Type of Pathogens	References	Type of Model	α	β	γ	Referred number in Fig. 2
Poliovirus 1	2)7)	Beta	15	1000		1
	7)	Beta	0.5	1.14		2
	8)	exponential			0.0091	
	8)	Beta	0.1097	1524		3
	3)	Beta	15	1000		4
	3)	Beta	0.119	200		5
Poliovirus 3	8)	Beta	0.409	0.788		6
	2)	Beta	0.5	1.14		7
Echovirus 12	2)7)	Beta	1.3	75		8
	8)	Beta	0.374	186.69		9
Rotavirus	7)	Beta	0.232	0.247		10
	8)	Beta	0.26	0.42		11
Giardia lamblia	7)8)	exponential			0.0199	
Cryptosporidium parvuum	14)	exponential			0.0047	
Campylobacter	7)	Beta	0.039	55		
Salmonella	7)	Beta	0.33	139.9		
Salmonella typhi	7)	Beta	0.21	5531		
Shigella	7)	Beta	0.16	155		
Shigella dynesteriae 1	2)7)	Beta	0.5	100		
Shigella fleneti 2A ##	2)7)	Beta	0.2	2000		
Vibrio cholera classical	7)	Beta	0.097	13020		
Vibrio cholera El Tor	7)	Beta	2.70E-05	1.33		
Entamoeba coli	2) 7)	Beta	0.17	1.32		
Entamoeba histolytica	7)	Beta	13.3	39.7		

a)



(b)

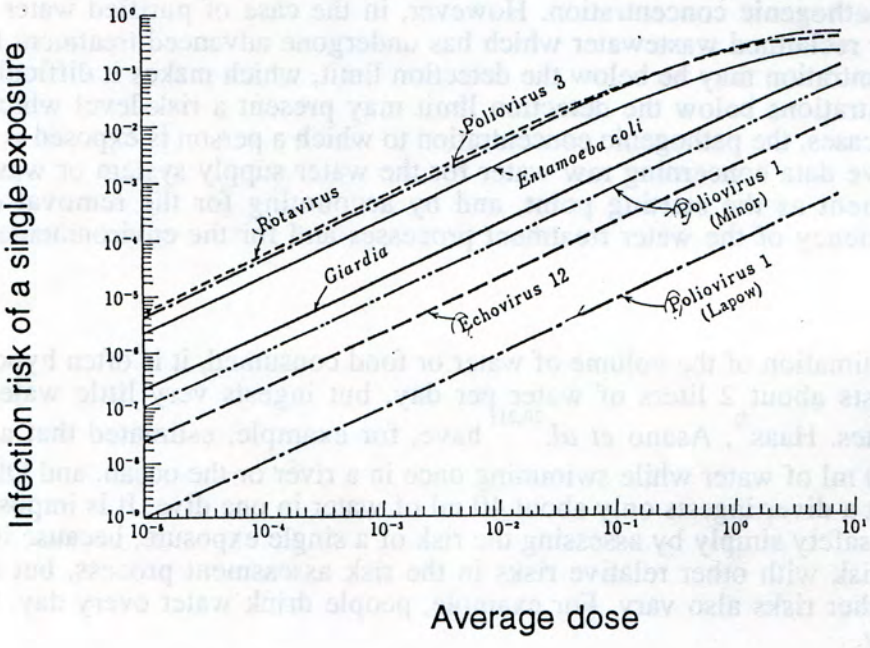


Fig.2 Comparisons of infection risk among various pathogens at low doses
(a) was summarized by Tanaka13) and the numbers in (a) represents viruses tabulated in Table1 as references; (b) was presented by Regli et al.8)

In addition, Cooper *et al.*[15] reported on the parameters for four models as shown in Table 2 based on data which they organized themselves. It is not clear whether the model accounts for the morbidity data or also infection data, though it is important to note that this model handles the risk of morbidity.

Based on the infection model shown in Table 1, the infectivity, morbidity, and mortality when a person is exposed to a single unit of various pathogenic microorganisms are presented in Table 3[5].

As mentioned above, the infection model has no threshold value for a dose that does not cause infection, but Rose *et al.*[17,19] estimated the pathogenic dose necessary for infection as shown in Table 4 for a case in which 1% or 0.01% of people are infected is defined as the minimum dose of infection. This table reveals that poliovirus, echovirus, and other enteric viruses, and *Giardia* cyst, *entamoeba*, and other protozoa are far more infectious than pathogenic bacteria such as *Salmonella* or *Vibrio cholera*. This demonstrates that efforts to deal with water-borne infectious disease should focus more on enteric viruses and protozoa because of their high infectivity and their ability to survive in the environment.

EXPOSURE ASSESSMENT

To perform a single exposure infection risk assessment, it is necessary to assess the pathogenic dose in a single exposure from the pathogenic concentration of water or food directly ingested and the amount of water or food ingested. If any monitoring data are available, they may be used to estimate the pathogenic concentration. However, in the case of purified water in the water supply system or reclaimed wastewater which has undergone advanced treatment for reuse, the pathogenic concentration may be below the detection limit, which makes it difficult to measure. But even concentrations below the detection limit may present a risk level which can not be ignored. In such cases, the pathogenic concentration to which a person is exposed is estimated by taking quantitative data concerning raw water for the water supply system or wastewater after secondary treatment as the starting point, and by accounting for the removal efficiency or inactivation efficiency of the water treatment processes and for the environmental attenuation rate.

Turning to the estimation of the volume of water or food consumed, it is often hypothesized that one person ingests about 2 liters of water per day, but ingests very little water from other transmission routes. Haas[3], Asano *et al.*[20,21] have, for example, estimated that a person only ingests about 100 ml of water while swimming once in a river or the ocean, and Olivieri *et al.*[22] hypothesized that a diver ingests only about 40 ml of water in one dive. It is impossible to fully evaluate relative safety simply by assessing the risk of a single exposure, because it is necessary to compare the risk with other relative risks in the risk assessment process, but the exposure frequencies of other risks also vary. For example, people drink water every day, but swim far more infrequently.

The safety for each reuse application must therefore be considered in terms of the probability of risky events occurring during a certain time period. If the risk caused by a single exposure is defined as P, the following equation can be used to represent this as an annual risk:

$$1 - (1 - P)^n \qquad (5)$$

Here, n is the number of exposure events per year. Similarly, it is possible to also calculate the lifetime risk if n is taken as the frequency of exposure over a person's lifetime.

TABLE2 Parameters of Infection Risk Models from Pathogens

	Logistic Model		Beta Model		Exponetial Model	Lognormal Model	
	M	N	α	β	γ	GM[1]	GSD[2]
Shigella spp.	-7.4577	2.0292	0.16	155	1.03E-03	8.92E+03	31.8
Vibrio cholera classical			0.097	13020	7.45E-06	3.20E+06	14.5
Vibrio cholera El Tor	-24.82	5.39	1.33	2.70E-05	4.99E-05	7.20E-04	5.8
Campylobacter			0.39	55	7.00E-04	30	2.4
Eschenichia coli	-1.2184	0.2406			1.22E-08	4.36E+07	36.6
Salmonella spp.	-1.9927	0.0002	0.33	139.9	2.35E-03		
Salmonella typhi	-7.9934	1.9293	0.21	5531	3.79E-05	3.37E+06	71
Enterovirus						2.50E+02	73
Giardia lamblia			0.18	11.6	1.53E-02	102	17

1. GM: Geometric mean of lognormal distribution in equation (3) μ = Log(GM)
2. GSD: Standard deviation of lognormal distribution in equation (3) σ = Log(GSD)

Table 3 Risk of infection, disease, death due to enteric pathogens[1]

	Infections in 1 million people dosed a single unit of pathogens	Percentage of cases where clinical cares are required after infection(%)	Mortality(%)	Secondary infection(%)
Campylobacter	7,000			
Salmonella typhi	380			
Shigella	1,000			
Vibrio cholera classial			7	
Coxsackieviruses		5-96	0.12-0.94	76
Echovirus 12	17,000		50 0.27-0.29	40
Hepatitis A virus			75 0.6	78
Norwalk virus			0.0001	30
Poliovirus 1	14,900	0.1-1	0.9	90
Poliovirus 3	31,000			
Rotavirus	310,000	28-60	0.01-0.12	
Giardia lamblia	19,800			

1. NRC based by 16), 17), 18)

Table 4 Estimation of minimum dose for infection from enteric pathogens using dose-response models[7)]

	Minimum infection dose	
	1% of infection risk	0.01% of infection risk
Campylobacter	1.4	0.014
Salmonella	4.3	0.042
Salmonella typhi	263	2.6
Shigella	10	0.097
Shigella dysenteriae	20	
Shigella flexneri 2A##	100	1.0
Vibrio cholera classial	1428	13
Vibrio cholera El Tor	667	
Poliovirus 1	0.67	0.0067
Poliovirus 3	0.32	
Echovirus 12	0.59	0.0058
Rotavirus	0.03	
Entamoeba coli	0.04	
Entamoeba histolytica	0.04	
Giardia lamblia	0.5	0.0050

1. NRC based by 16), 17), 18)

RISK ASSESSMENT OF PATHOGENS IN DRINKING WATER

Microorganisms which cause water-borne diseases include viruses, protozoa, and other microorganisms whose tolerance of their environment and behavior during water purification processes differ from those of index organisms: coliform bacteria, fecal coliform bacteria, etc. Because these viruses and protozoa are, as stated in the previous section, endowed with large infectivity, the safety of the public exposed to such microorganisms is a serious problem. Dose-response models have been used for infection risk assessments of pathogenic microorganisms marked by highly variable concentrations in drinking water[2,7,8,19]. This method is used in the development of standards used to determine suitable water purification processes required to reduce the microorganism infection risk.

At the same time as the US EPA controls the effects on the public health of chemicals in drinking water, it also uses the risk assessment method to determine the type of water purification system needed to control bacteria, protozoan cysts, and viruses causing water-borne diseases that harm human health[8,32]. To deal with the increasing incidence of water-borne diseases seen in the United States during the past two decades, the USEPA issued in 1989 the Surface Water Treatment Rule (SWTR)[23]. This rule stipulates the functions which water treatment processes must provide in order to protect water supply systems which draw their water from sources such as surface water or contaminated ground water from *Giardia* cysts and enteric viruses. As a result, all water supply systems using all the surface water, and some groundwater where there is any possibility of contamination, such as shallow ground water aquifers are now required to incorporate sand filters in their treatment. The foundation of this regulation is the risk assessment methodology described above. In other words, as shown in Figure 3, if an allowable annual risk of infection from drinking from the water supply is determined, a dose-response model is used to inversely calculate the microorganism concentration in the drinking that can satisfy this risk of infection. Then, based on pathogenic microorganism measurement data for water supply source water nationwide, the removal efficiency required in the water treatment process can be determined, and the treatment process conditions needed to achieve this efficiency are stipulated.

It is important to note that the allowed infection risk is clearly stipulated. An infection risk at a level of one infection out of 10,000 persons per year, or less than 10^{-4}/year, is allowed. Unlike cancer, water-borne diseases are fatal relatively rarely; Gerba showed that their mortality is roughly 1%[16]. The risk of infection should be reduced to the lowest possible level in order to avoid lost man-hours, treatment costs, and discomfort caused by illness, but it is necessary to consider the extent of other types of risk. An annual risk of infection of the order of 10^{-4}/year is not unreasonable, because according to the US EPA, the actual risk of infection from *Giardia* cysts through exposure from recreational activities unrelated to the water supply, or the actual risk of infection from swimming, are far greater than the annual risk of infection which is the target of the regulations[40].

In the process of setting these standards, Gerba and Haas proposed drinking water quality standards for pathogenic microorganisms using a risk assessment[16]. The beta model used by Haas[2] was used to perform a risk assessment of drinking water ingested at a rate of 2 liters per day to calculate the risk of infection from enteric virus. Table 5 presents the annual infection, disease contraction, and death risk from drinking water containing single virus unit of poliovirus 1 and hepatitis A virus (HAV) in from 10 liters to 1,000 liters. Because no HAV infection model now exists, it was hypothesized that it is about as infectious as poliovirus 1. The HAV morbidity and mortality are higher than those for poliovirus 1, and the annual risk of infection from HAV exceeds one person in 10,000, even when only one virus is found in 1,000 liters of water. So when considering the safety of drinking water, only an extremely low virus concentration in drinking water can be allowed.

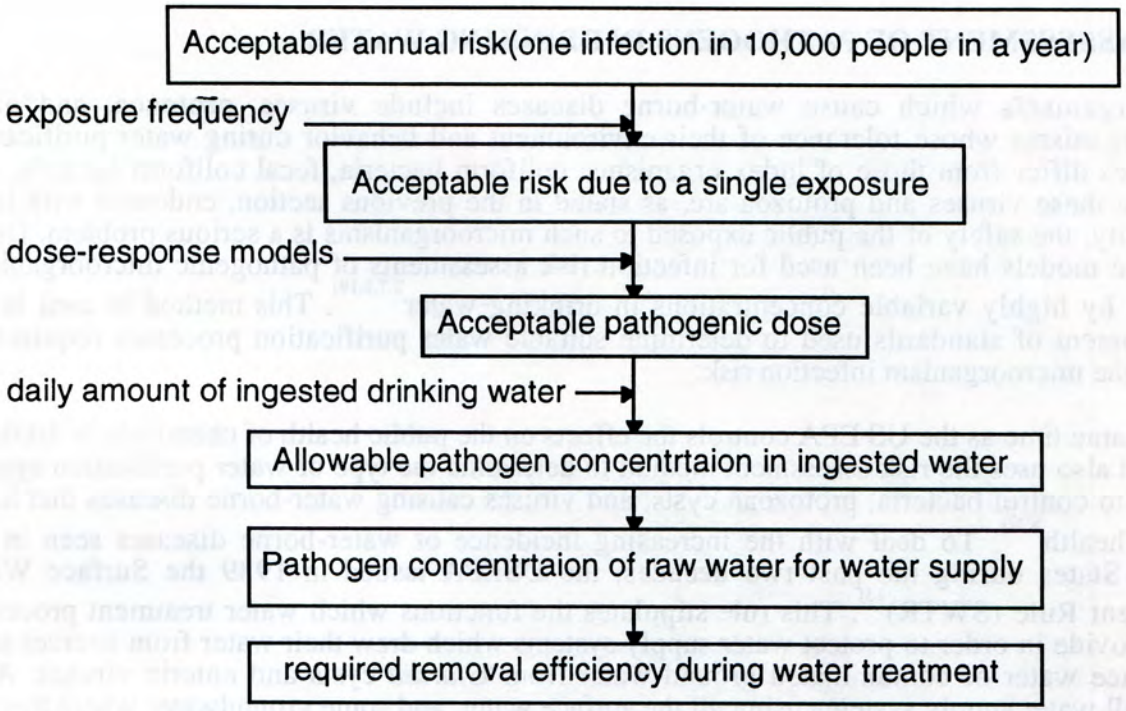


Fig. 3 Procedure to determine a required reduction efficiency of pathogens during water treatment directed in Surface Water Treatment Rule(SWTR)

Table 5 Annual Risk of Infection from Viruses in Drinking Water[16]

	poliovirus			HAV		
Pathogen concentration	Infection	Disease	Death	Infection	Disease	Death
1/10L	6.6x10-1	1.1x10-2	1.1x10-4	6.6x10-1	5.6x10-1	4.9x10-3
1/100L	1.0x10-2	1.1x10-4	9.5x10-7	1.0x10-2	7.8x10-3	4.3x10-5
1/1,000L	1.3x10-3	1.3x10-5	1.1x10-7	1.3x10-3	9.8x10-4	5.8x10-6

Table 6 Allowable pathogen concentration in drinking water satisfying 10-4 of annual infection risk[8]

Pathogens	Allowable concentration number/L	References
Rotavirus	2.22x10-7	
Polio 3	2.65x10-7	
Entamoeba coli	6.25x10-7	
Giardia	6.75x10-6	
Polio 1	1.51x10-5	Minor 24)
Echovirus 12	6.85x10-5	
Polio 1	1.91x10-3	Lepow et al. 25)

Regli *et al.*[8], calculated the concentration of various kinds of pathogenic microorganisms that maintain an annual allowable infection risk of less than 10^{-4}/year when a person drinks 2 liters of water per day, as shown in Table 6. This clearly shows that the dose-response model that best fits *Giardia* and poliovirus 1 is the exponential model, while the beta model is the most appropriate for other pathogenic microorganisms. These results reveal that in order to keep the annual risk of infection at or below 10^{-4}, the pathogen concentration must be extremely low, and that the infection risk varies in the order of 10^{-4} according to the type of microorganism. Thus, the amount of sample water needed to confirm the safety level of *Giardia* is unrealistically low, ranging from 10^{-5} to 10^{-6} liters, which means that a realistic method of finding this safety level is to consider the microorganism concentration of raw water to be purified and the removal efficiency of the purification process. And because there is insufficient information about viruses, it is not appropriate to perform a risk assessment focused on a limited number of varieties of virus; a synthetic virus combining the properties of different viruses must be considered. To combine the properties of the worst viruses, the water quality level must be 2.2×10^{-7}/L when considering the rotavirus with its high infectivity and the hepatitis A virus (HAV), which is difficult to inactivate and remove. Based on the infection model for rotavirus, the relationship of raw water with an annual infection risk of 10^{-4} or less with the virus removal efficiency of the purification process in the 95% reliability area is estimated as shown in Figure 4.

According to Macler *et al.*[6], the most serious consideration must be given to measures to deal with the HAV virus, because of the severity of its symptoms and its strong resistance to disinfection processes, although no practical method of measuring HAV and no dose-response relationship is available. The US EPA set standards for surface water using the concept of a synthetic virus assumed to be the highly infective rotavirus.

Rose *et al.*[19] used the exponential model for *Giardia* to find conditions where the annual infection risk is 10^{-4} from the relationship between the cyst concentration in the water and its removal efficiency. Based on data obtained from measurements of *Giardia* cysts in raw water with considerable contamination from human sources and in raw water containing no such contamination collected from throughout the United States, a dose-response model is used to assess the annual infection risk and daily maximum infection risk, when the level of processing employed by a water supply utility is varied. The results of this study demonstrate that in the case of the raw water containing considerable human contamination, removal of between 4 and 5 Log is necessary, while removal of 3 Log is all that is required by a water purification system handling raw water free from human contamination. Here, the Log removal efficiency, which is also called the logarithmic prevention coefficient, is the absolute value of the log of the percentage of pathogenic microorganisms remaining after processing; for example it is a removal efficiency of 99% for 2 Log, and 90% for 1 Log.

Rose *et al.*[7] conducted a study to find the pathogenic microorganism removal efficiency of the purification process required to satisfy the annual infection risk of 10^{-4} set by the US EPA for the use of surface water as raw water for the water supply system. They calculated the concentration in the raw water to which *Giardia*, poliovirus, and rotavirus must be lowered in order to satisfy the requirement for an annual infection risk of 10^{-4}, for hypothetical purification process removal efficiencies varied from 1 Log to 4 Log. As shown in Figure 5 they estimated the relationship of the pathogen concentration of the raw water with the removal efficiency of the purification process required to reduce the annual infection risk to 10^{-4} under an exposure caused by the ingestion of 2 liters of water for two cases: one where the raw water with the peak concentration is used 40 days per year, and another where water with the average concentration of microorganisms is used 365 days per year. In order to achieve an annual infection risk of 10^{-4}, the process must be controlled so that the concentration of poliovirus is between 0.1 and 0.3/100 liter, that of *Giardia* is 0.23 cysts/100 liters, and there is no more than 10^{-3} cysts of viruses or protozoan cysts per liter. From these results, it was concluded that when raw water with the highest level of contamination is to be used in the water supply system, the removal efficiency of the purification process must be 4 Log.

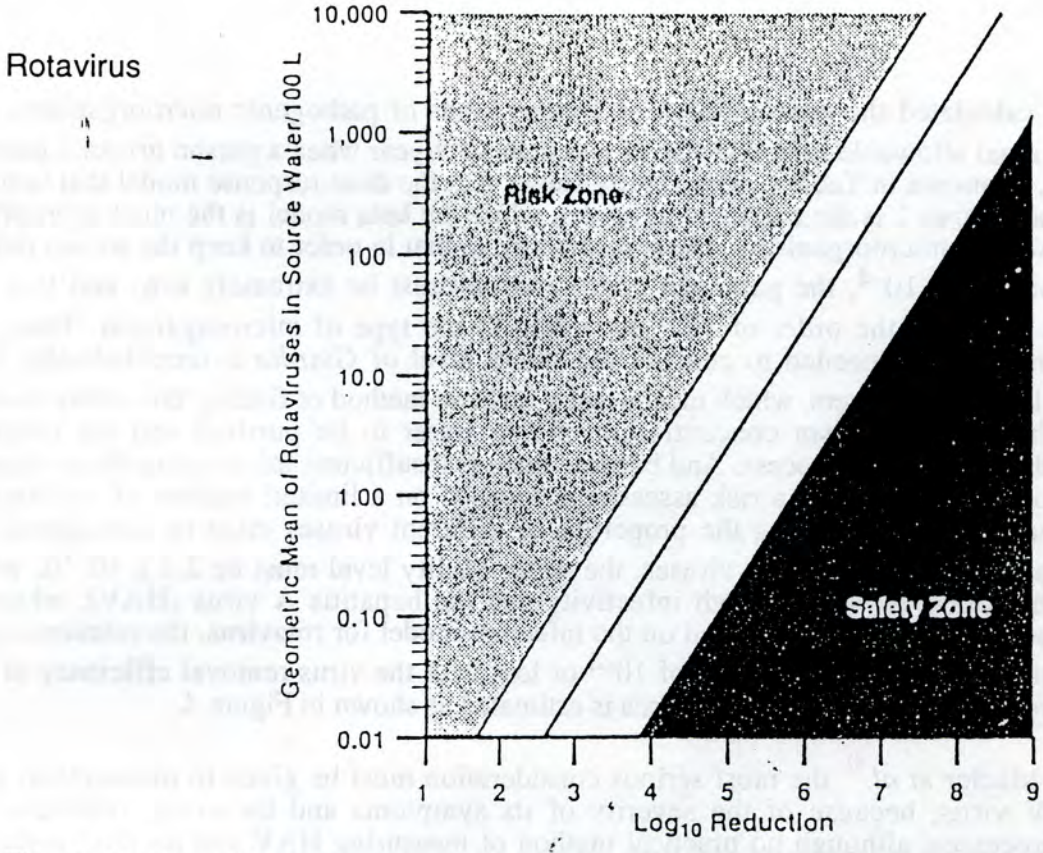


Fig. 4 Relationship between rotavirus concentration in raw water and reduction efficiency during treatment process which satisfies 10-4 of annual acceptable risk level of drinking water(estimated at 95% of reliability level) by Regli et al. [8]

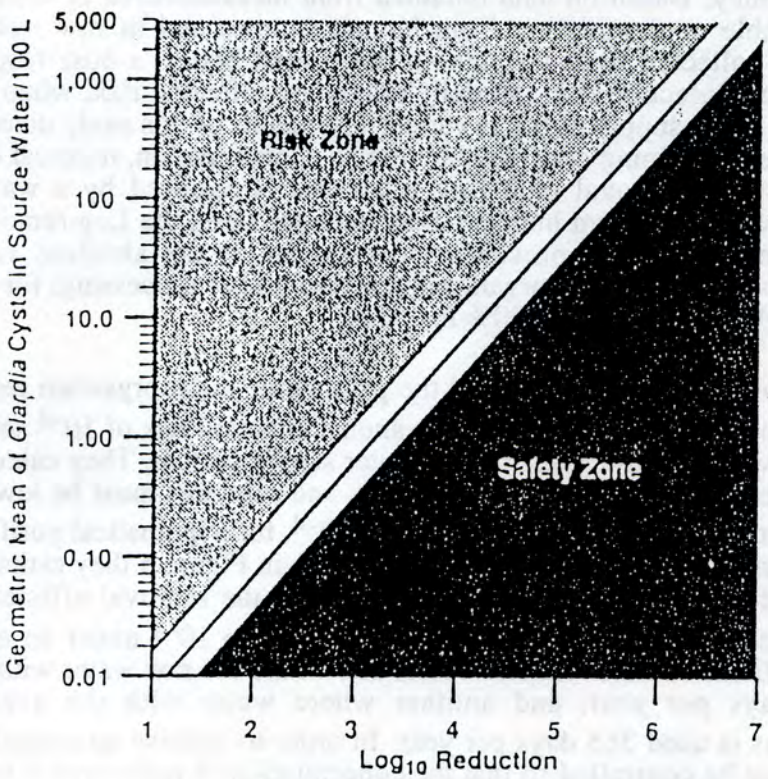


Fig. 5 Relationship between Giardia cyst concentration in raw water and reduction efficiency during treatment process which satisfies 10-4 of annual acceptable risk level of drinking water(estimated at 95% of reliability level) by Rose et al. [19]

Based on the above, the USEPA established the standards shown in Table 7 for the pathogenic microorganism removal efficiency of the purification process needed to achieve water supply control targets calling for an annual risk of one infection or less per 10,000 persons among users of a water supply system using surface water as its raw water[26]. Water supply system operators must provide the filtration and disinfection needed to guarantee the maximum values in this table, and the methods of selecting filtration processes and disinfection processes that satisfy this requirement are also stipulated.

RISK ASSESSMENT OF PATHOGENS IN DISCHARGE FROM WASTEWATER TREATMENT PLANTS

The safety of treated wastewater based on risk assessments were first discussed by Hutzler *et al.*[12]. Based on pathological statistical data, they performed infection risk assessments of a case where hepatitis A virus (HAV) shed by patients into wastewater was discharged to the environment. It is difficult to quantify HAV, and the discussion was based on the amounts of infective feces instead of the virus concentration. Feces containing HAV were deliberately provided, and the relationship between the fecal dose and the appearance of HAV patients created clinically in this way is summarized in Figure 6. Because it was impossible to detect the HAV virus, the dose-response relationship is expressed by treating the amount of infective feces as the dose. A typical dose of 0.1 g of feces causes disease occurrence requiring medical action at a probability of 50% from the results of an estimation of the upper confidence limit (UCL) of 95% area at that time. Up to this UCL, it was assumed that the dose-response relationship is linear in the model. HAV virus is shed in the feces of an HAV patient for about 1 month, and about 80 persons in a population of 100,000 shed HAV. Each patient sheds between 100 and 200 grams of feces each day, so if 450 liters of water are consumed per person, then the wastewater will contain 0.03 mg/liter of HAV infected feces. 0.02 mg/liter of feces infected with HAV is discharged from sewage treatment plants, and is inactivated with 99.9% reduction in the receiving waters. The dilution factor in the waters is 10 times, so if a person swimming in the area ingests 10 ml of water, that person's infection risk is as follows:

$$\text{risk} = \text{UCL} \times \frac{d}{de} = 0.5 \times \frac{2 \times 10^{-8}\text{g}}{0.1\text{g}} = 10^{-7} \qquad (6)$$

Haas[2] studied a dose-response model for enterovirus etc., and calculated the risk of infection to a swimmer downstream from where enterovirus is discharged into the river by a sewage treatment plant[3]. Based on the reported geometric mean of virus in the water, namely 5,650/liter, and assuming that HAV is reduced at 67.5% in the grit tank and primary sedimentation tank, at 86% by the subsequent activated sludge process, and finally at 90% in the chlorination tank, then the concentration of viruses remaining in the treated wastewater is 25.7/liter. The dilution factor at the point where treated wastewater is discharged is 100 times, and it is hypothesized that the decay constant of the viruses during the two days it takes for the water to flow downstream is 0.69/day. Assuming that a person swimming at this downstream location ingests 100 ml of water and that 1% of all those persons infected during downstream recreational use of the river will become ill as a result of the infection, the risk of illness from a single exposure is estimated to be 6.5×10^{-5} in a case where disinfection is performed and 6.3×10^{-4} when it is not.

Table 7 Designated reduction in water treatment processes which is regulated in Surface Water treatment Rule(SWTR) [Q6]

Daily average concentration of pathogen in raw water	Required reduction during water treatment	
	Giardia	viruses
<=1	3 Log	4 Log
>1-10	4 Log	5 Log
>10-100	5Log	6Log

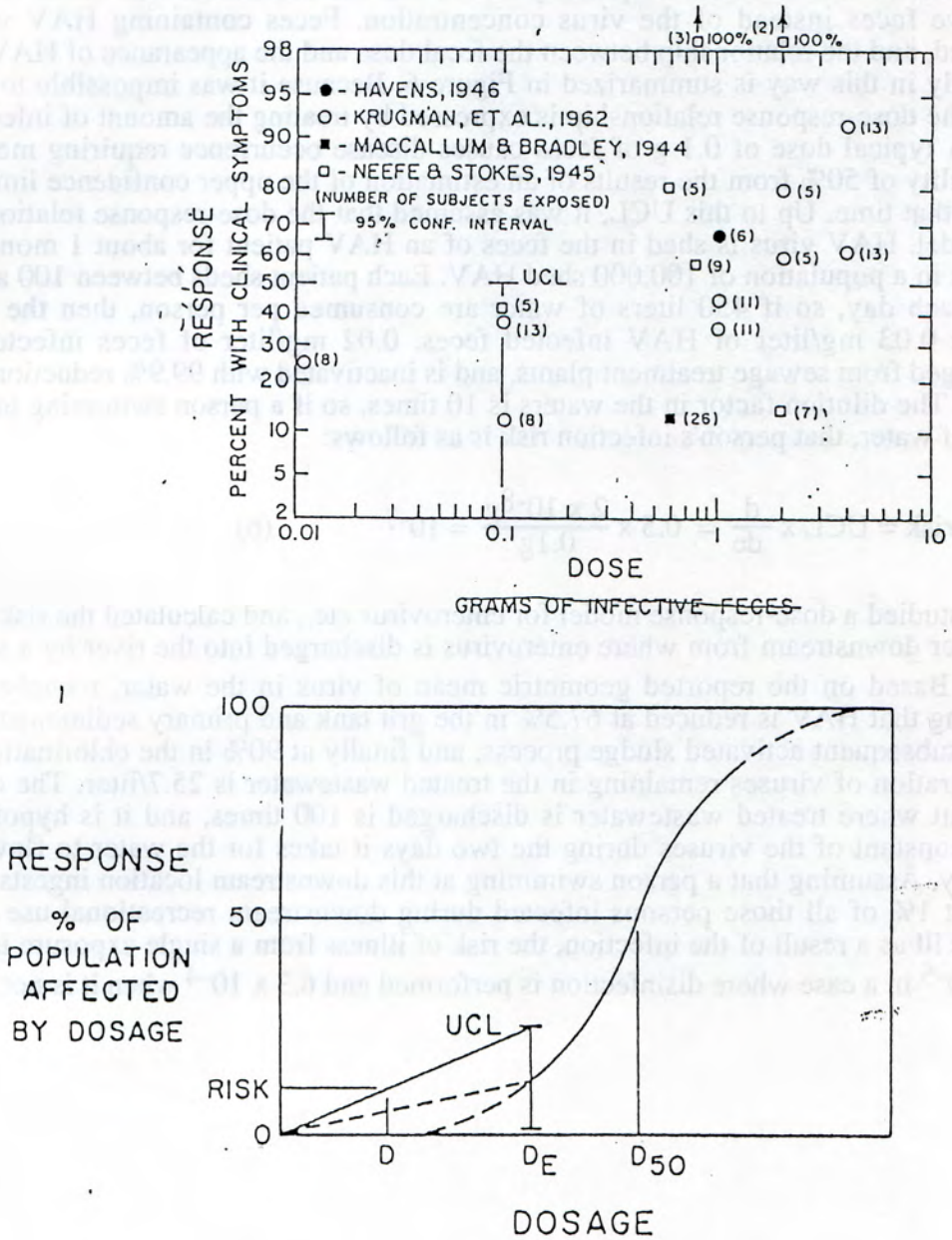


Fig. 6 Estimation of HAV infection model [12]
Upper figure represents dose interms of weight of infective feces, and lower graph shows that the dose-response relation is linear as high as a 95% upper confidence limit

Olivieri *et al.*[22] used the Monte Carlo method developed by Cooper *et al.*[15] to calculate the infection risk to a diver from virus in wastewater discharged into the ocean from a sewage treatment plant where sedimentation is practiced but no biological treatment is performed. This Monte Carlo method uses a model which accounts for variations in the concentration of pathogenic microorganisms, in the amount of water ingested, and in the processing of the pathogenic microorganisms or the attenuation properties of the environment. Olivieri *et al.*[22] assumed that a diver ingests 40 ml of water (10% of standard deviation) and set the initial dilution factor at the diffuser used to discharge the water into the ocean at 100 times and the dilution rate at the edge of the kelp bed at an initial diffusion of 3,000 times, and estimated the risk at those two points. Kehr and Butterfield[28] performed a study of the frequency of the contraction of typhoid fever based on data concerning the relationship of the number of coliform bacteria groups with *Salmonella typhoid* in wastewater or in contaminated water(Fig. 7). They discovered a relationship between the percentage of *Salmonella typhi* to total coliform bacteria groups and the incidence of typhoid fever, and showed that the number of pathogenic microorganisms per 1 million coliform groups is related to the number of persons per 100,000 who contract typhoid fever, and that this can be found experimentally with the following formula[28]:

$$y = a\, r^{n} \qquad (7)$$

Where,
a, n: Constants, 3 and 0.46 respectively
y: Number of pathogenic microorganisms per 1 million coliform groups
r: Contraction rate per 100,000 persons

Olivieri *et al.*[22] employed the Kehr-Butterfield formula to experimentally find the relationship of the number of persons becoming ill per unit of population with the number of coliform groups which are pathogenic microorganisms using monitoring data concerning the coliform groups and *Salmonella spp.* in the water discharged from the wastewater treatment plant presented in Table 8 and enterovirus at Santee in San Diego.

Then using the rate of contraction for *S. typhi*, *Salmonella spp.*, and *Shigella spp.* per 100,000 persons for the entire United States, which are 0.18, 360, and 160 respectively, Olivieri et al. estimated the concentration of pathogenic microorganisms at the diffuser and at the edge of the kelp bed as shown in Table 9. This was used to evaluate the risk of contracting an illness from diving once.

But enterovirus conforms to the concentration detected in the wastewater, and it is assumed that it is reduced to 1/10 in the sedimentation tank in the sewage treatment plant. This result indicated that viruses present the greatest risk of infection, and that the morbidities at the diffuser and at the edge of the kelp bed are 3 persons in 100 and 1 person in 1,000 respectively.

Table 8 Pathogen concentration in wastewater treatment plant [22]

	pathogen concentration in primary effluent(number/100mL)			Untreated wastewater (PFU/L)
	Coliform bacteria group	Fecal Coliform group	*Salmonella* spp.	enterovirus
Average	44.5×10^6	6.5×10^6	0.97	19.883
Geometric mean	7.64	6.81		4.29
Standard deviation	1.99	1.7	0.56	7.9
Geometric standard deviation	0.299	0.23		

Table 9 Estimating diver's risk of disease due to ocean outfall of sewage in San Diego [22]

	At the location of diffuser		At the Edge of Kelp Beds	
	Pathogen concentration n/L	Risk of disease in one dive	Pathogen concentration n/L	Risk of disease in one dive
Salmonella spp.	0.003	0.3x10-4	0.0001	0.9x10-6
S. typhi	0.13	0.9x10-6	0.004	0.7x10-7
Shigella spp.	0.1	0.1x10-4	0.003	0.9x10-6
Pathogenic *E.Coli*	330	0.3x10-6	11	0.1x10-6
Enterovirus	200	0.3x10-2	7	0.1x10-2
Total risk level		0.3x10-2		0.1x10-2

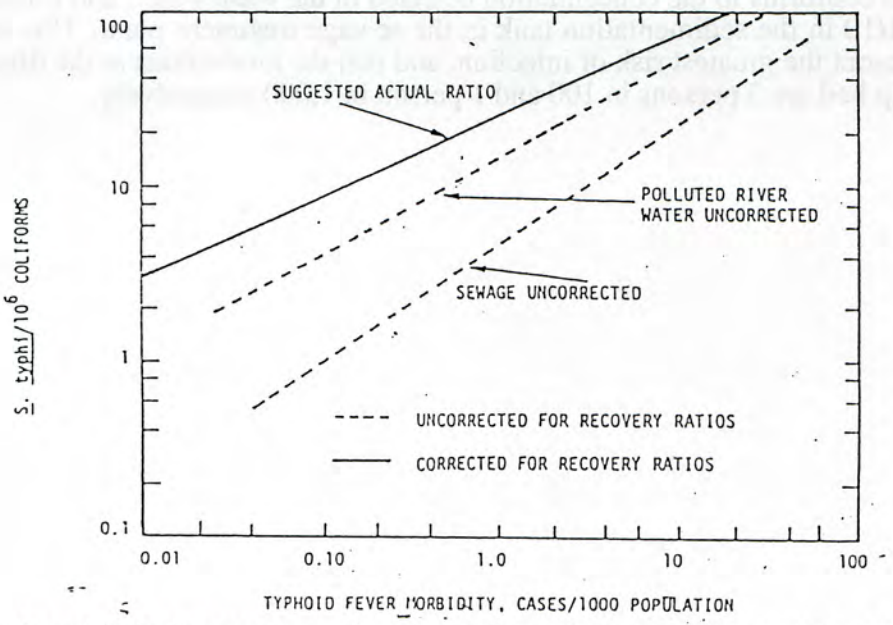


Fig.7 Relationship between the ratio of number of *Salmonella typhi* to Coliform bacteria group counts and occurance of typhi fever in population [28]

RISK ASSESSMENT OF PATHOGENS FOR WASTEWATER RECLAMATION AND REUSE

Rose and Gerba[17] evaluated the safety of microorganism standards in Arizona for viruses and *Giardia* when wastewater is reused, and the infection risk from enteric virus when reclaimed wastewater is ingested based on data concerning viruses and *Giardia* in reclaimed wastewater. Arizona is the only state in the United States where standards have been set for unrestricted use which exposes an unspecific public to viruses or *Giardia* or other parasites; standards such as one enteric virus in 40 liters and one cyst or one oocyst of *Giardia* or *Cryptosporidium* in 40 liters. In the case of reuse where there is no possibility of the public being exposed on the other hand, a standard of 125 enteric viruses in 40 liters has been set. Based on data indicating that the virus concentration of 100 liters of secondary effluent from wastewater treatment plants in Arizona and Florida was a maximum of 245 and 210 PFU, and an average of 13 and 130 PFU, and that the virus concentration after filtration of the secondary effluent was a maximum of 45, and 1.0 PFU and an average of 1.25 and 0.13 PFU, they used the infection model set by Haas[2] to calculate the infection risk from viruses or *Giardia* in a case where a person has accidentally directly ingested 100 ml of reclaimed wastewater. From the results summarized in Table 10, the infection risk from accidentally ingesting the secondary effluent would be about 2×10^{-3}, but the subsequent filtration could reduce this risk to 2×10^{-4} and 2×10^{-6} for *Giardia*. Thus, it was concluded that if the filtration and disinfection can be counted on to have a disinfection effect, it would be possible to reuse this water for unrestricted use as irrigation water.

Based on data concerning pathogenic microorganisms in water discharged from a wastewater reclamation and reuse plant in St. Petersburg in Florida, Rose *et al.*[30] assessed the infection risk of accidentally ingesting 100 ml of this reclaimed water as shown in Table 11. In this case, rotavirus and echovirus are used as examples of having extremely high infectivity and moderate infectivity, respectively. Because there was no *Cryptosporidium* infection model available at the time of that study, the *Giardia* infection model was applied to *Cryptosporidium* without modification.

The next study introduced here is that by Asano *et al.*[21]: a risk assessment of virus infection in many applications of wastewater reclamation and reuse based on the most reliable available measurement data for viruses at five sewage treatment plants in California. Scenarios for assessing the safety of wastewater reclamation and reuse are established to assess the infection risk in the reuse applications listed in Table 12; ones highly likely to result in contact with the reclaimed water by the public. The reuse scenarios correspond to the following types of wastewater reclamation and reuse which are becoming increasingly common in California.

[1] Spray Irrigation of Golf Courses
Golf courses are watered at night, and it is assumed that each golfer plays a course an average of twice a week all year. It is hypothesized that about 1 ml of reclaimed water remaining on the grass adheres to golfers' hands when they pick up their ball, exposing their mouths to this water. But it is also assumed that if a golfer comes into contact with this reclaimed water containing viruses about 1 day after spraying, the virus will have attenuated in the first order reduction at a decay rate of 0.69/day.

[2] Sprayed on Fields Growing Food Crops
This scenario considers the risk to consumers when food products sprayed with reclaimed water are eaten uncooked. In this case, it is assumed that the total amount of reclaimed water remaining on the food products a person eats in a single day is equivalent to about 10 ml, food is shipped to the market about 2 weeks after spray irrigation is stopped, then ingested by consumers. It is also hypothesized that during this period, the viruses gradually die in the first order reduction at a decay rate of 0.69/day under the effects of drying and the sun's rays.

Table 10 Single exposure risk when a person ingests 100 mL of secondary effluent from wastewater treatment plant by mistake[P7]

Virus /protozoa concentration in 100L	Risk from viruses/protozoa in Arizona(AZ), Florida(FL)	Risk of infection
10^4		2 x 10-1
10^3		2 x 10-2
10^2	AZ* FL*	2 x 10-3
10^1	AZ*/ FL**	2 x 10-4
1	AZ* FL**	2 x 10-5***
0.1	FL**	2 x 10-6***

* peak and average concentration in secondary effluent
**levels at peak and average of filtrate following secondary effluent
***Acceptable annual risk by a single exposure

Table 11 Risk of infection when 100 mL of reclaimed water is ingested by mistake[ÇRÇOA]

	Pathogen concentration in reclaimed water	Dose in 100mL	Rotavirus model	Echovirus model	*Giardia* model
Viruses	0.01 PFU	1.0x10-5	6.2x10-6	2.0x10-3	
	0.13 PFU	1.3x10-4	6.0x10-5	2.7x10-7	
Giardia	0.49 cysts	4.9x10-4			9.8x10-6
	0.89 cysts	8.9x10-4			1.88x10-5
	1.67 cysts	1.77x10-3			3.3x10-5
	3.3 cysts	3.3x10-3			6.6x10-5
Cryptosporidium	0.75 oocysts	7.5x10-4			1.5x10-5
	5.35 oocysts	5.35x10-3			1.1x10-4

[3] Reuse of Water for Recreation (Swimming)
The flow volumes of Southern California's rivers are extremely low in the summer; reclaimed water discharge d to the receiving water is used for swimming with almost no dilution. The infection risk is calculated for a person who swims 40 days a year and is assumed to ingest about 100 ml each time.

[4] Artificial Groundwater Recharge to Supply Drinking Water
Reclaimed water is sprayed on the surface of the ground and thus percolates into the groundwater. In this case, it is assumed that it passes through about 3 m of an unsaturated stratum, and enters the groundwater. aquifer. Next, it is diluted by the addition of an equal amount of rain water. Then six months later, it is used as drinking water. It is assumed that if the thickness of the unsaturated stratum is considered to equal (L) m, the viruses remaining after the unsaturated stratum will equal $10^{-0.7}$L, and the viruses will attenuate at a decay rate of 0.69/day in the saturated groundwater aquifer.

Based on the above scenarios, Asano *et al.* assessed the infection risk from three kinds of enteroviruses with dose-response models found by Haas[2].

This tertiary treatment plant reduces viruses at a removal efficiency of 5 Log as shown in Figures 8 and 9 [31]. The annual infection risk from echovirus 12, poliovirus 1, and poliovirus 3 in a case where water processed at this tertiary treatment facility is reused was assessed along with the annual infection risk in the case of the reuse of tertiary effluent with an actual measured maximum virus concentration of 111 vu per 100 liters and a detection limit of 1 vu per 100 liters. Tables 13 shows the annual infection risk for echovirus 12, the virus with the highest infectivity of the three[21].

Tanaka *et al.*[32] performed a statistical analysis of enteric virus data for treated wastewater at four sewage treatment plants in California, clearly demonstrating that at treatment plants where the virus concentration in the secondary effluent was identical, the virus concentration varied from day to day in accordance with the lognormal distribution, and that the enteric virus concentration in the secondary effluent was widely distributed among treatment plants (Figure 10). They then studied how safe this reclaimed wastewater which had completed only secondary treatment process or the following tertiary treatment process was for reuse for various purposes, and the safety in comparison with the safety from pathogenic microorganism infection established for water systems in the United States, in other words, the standard allowances stipulating one infection or less per 10,000 people per year.

The hypothetical reuse scenarios presented above are based on Asano *et al.*[21] They used the Monte Carlo method to calculate the annual infection risk in a case of rotavirus, which presents the greatest infection risk in a low dose. As shown in Figure 11, in the reuse scenarios, when the tertiary treatment promising removal efficiency of 5.2 Log is applied to unrestricted use in California, it will be lower than the allowable annual infection risk of 10^{-4} hypothesized by the USEPA for the water supply, which means it provides sufficient safety. However, it clearly exceeds the allowable risk in the case of recreational use, where contact with the human body is assumed to occur. When an estimation is made of the virus removal efficiency during the tertiary treatment required to satisfy the USEPA allowable annual infection risk for the water supply, namely a risk level of 10^{-4} at a reliability of 90% or 95%, it is estimated that the tertiary treatment removes viruses at the level shown in Figure 12 at probability of time.

Risk assessments by Asano *et al.*[21] had considerable repercussions when the wastewater reclamation and reuse standards in California were revised. The Peer Review Committee established by the Department of Public Health of the State of California believed that the infection risk was highest for reuse applications involving human contact with reclaimed water, and that although the allowable infection risk for wastewater reclamation and reuse is preferable at the level of one person in 10,000 per year, allowable risk standards would not be set, and that there was not enough data to set pathogenic microorganism standards at that time.

Table 12 Summary of Exposure Scenarios Used in Risk Assessment on Wastewater reclamation And reuse[21)]

Application purposes	Risk group receptor	Exposure frequency	Amount of water ingested in a single exposure	Reduction in the environment
Scenario I Golf course irrigation	Golfer	Twice per week	1 mL	Irrigation one day before playing
Scenario II Crop Irrigation	Consumer	Every day	10 mL	Stop Irrigation two weeks before harvest and shipment. Viral reduction due to sunlight
Scenario III Recreational Impoundment	Swimmer	40 days per year - summer season only	100 mL	No virus reduction
Scenario IV Groundwater recharge	Groundwater consumer	Every day	1000 mL	3 m vadose zone and 6 month retention in aquifer. Virus inactivation coefficient = 0.69/d

1. Adapted from Asano *et al.* (1992)

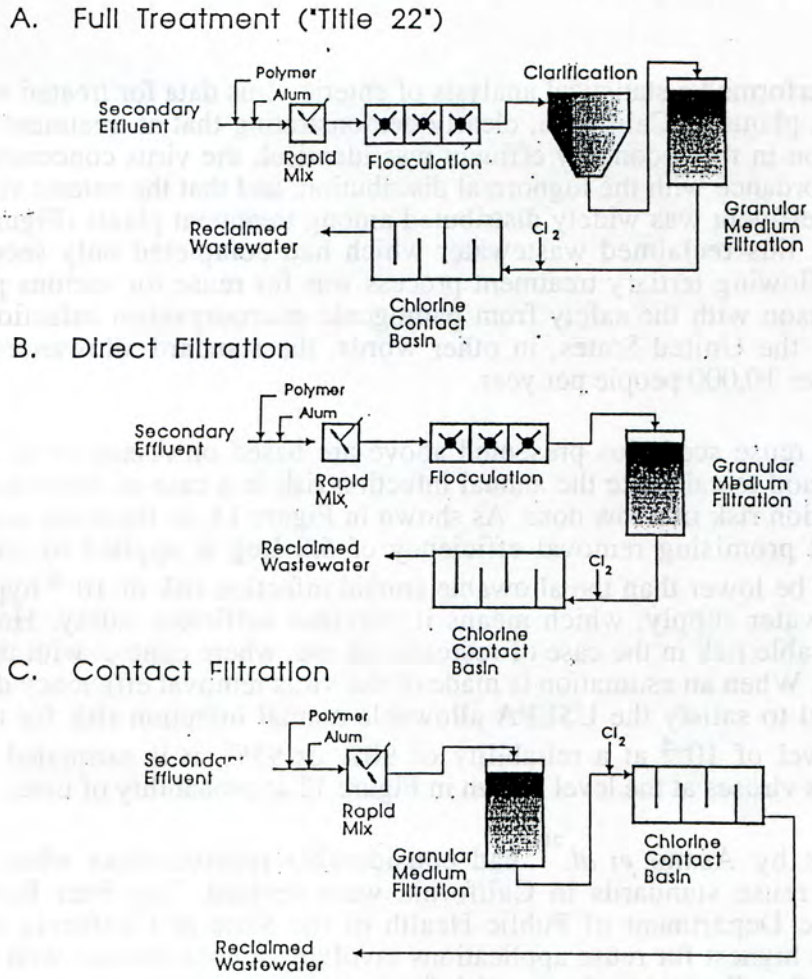


Fig.8 Outline of tertiary treatment for unrestricted wastewater reclamation and reuse applications required by California State

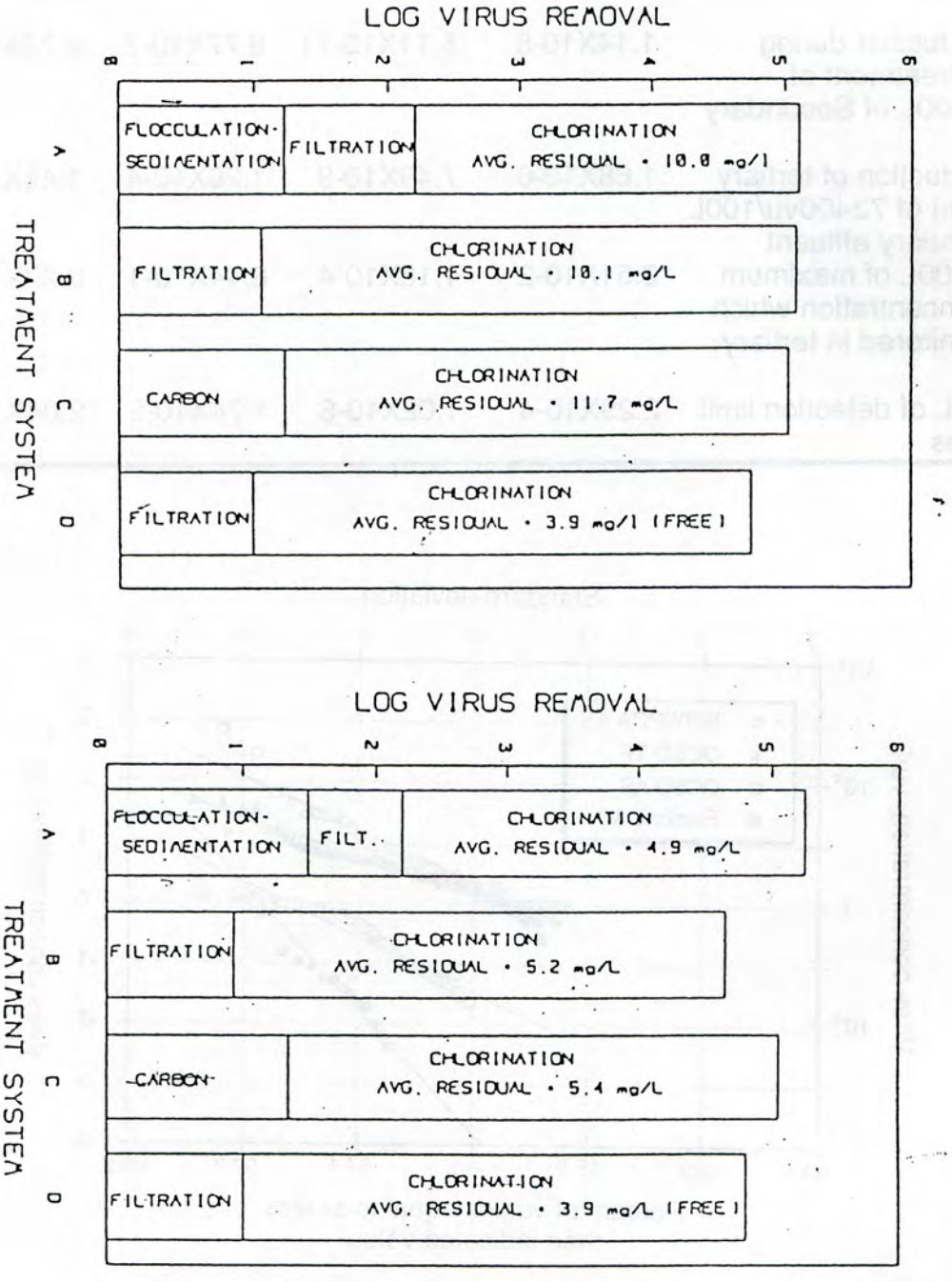


Fig. 9 Virus reduction efficiency during tertiary treatment applicable to unrestricted application of wastewater reclamation and reuse in the Sate of California [31)]
Upper and lower figures indicate chlorination processes at 10 and 5 mg/L of residual chlorine, respectively where systems A to D represent as follows: A: Full treatment; B:Direct filtration; C: Direct filtration applying activated carbon; D: Contact filtration

Table 13 Comparisons annual infection risks depending on different reuse applications[çoçpA]

Evaluated reclaimed water	Golf course irrigation	Food crop irrigation	Recreational impoundment	Groundwater recharge
5 log reduction during tertiary treatment of 500vu/100L of Secondary effluent	1.14X10-8	5.11X10-11	8.77X10-7	9.73X10-15
5 log reduction of tertiary treatment of 73400vu/100L of Secondary effluent	1.68X10-6	7.49X10-9	1.29X10-4	1.46X10-12
111vu/100L of maximum virus concentration which was monitored in tertiary effluent	2.51X10-2	1.13X10-4	8.44X10-1	2.27X10-8
1vu/100L of detection limit of viruses	2.29X10-4	1.02X10-6	1.74X10-2	2.04X10-4

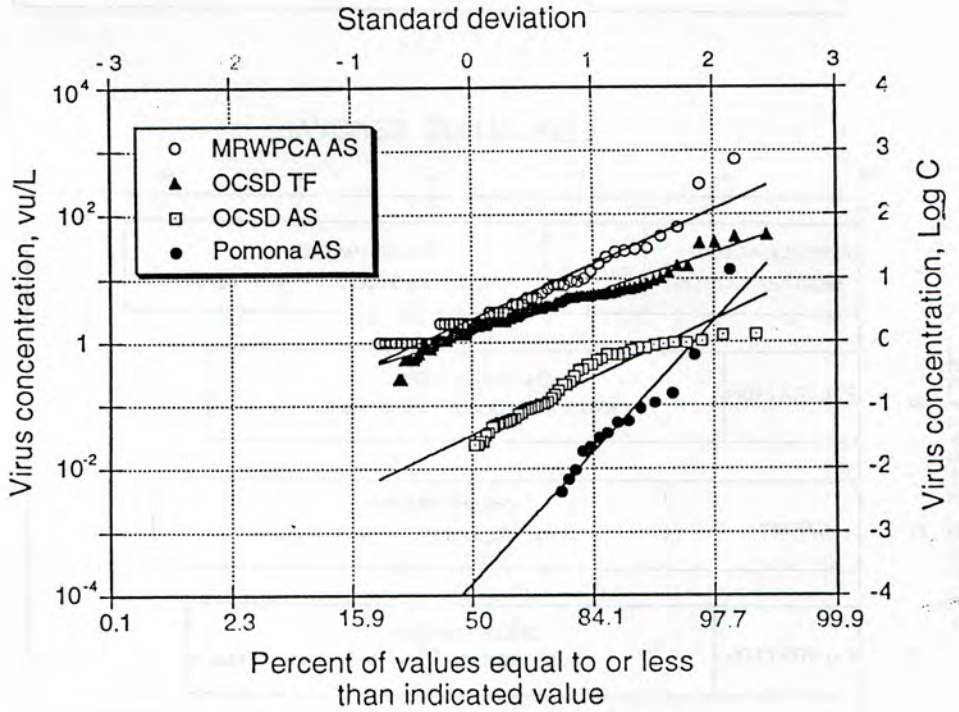


Fig. 10 Distribution of virus concentrations of unchlorinated secondary effluents from wastewater treatment plants in California State[32]

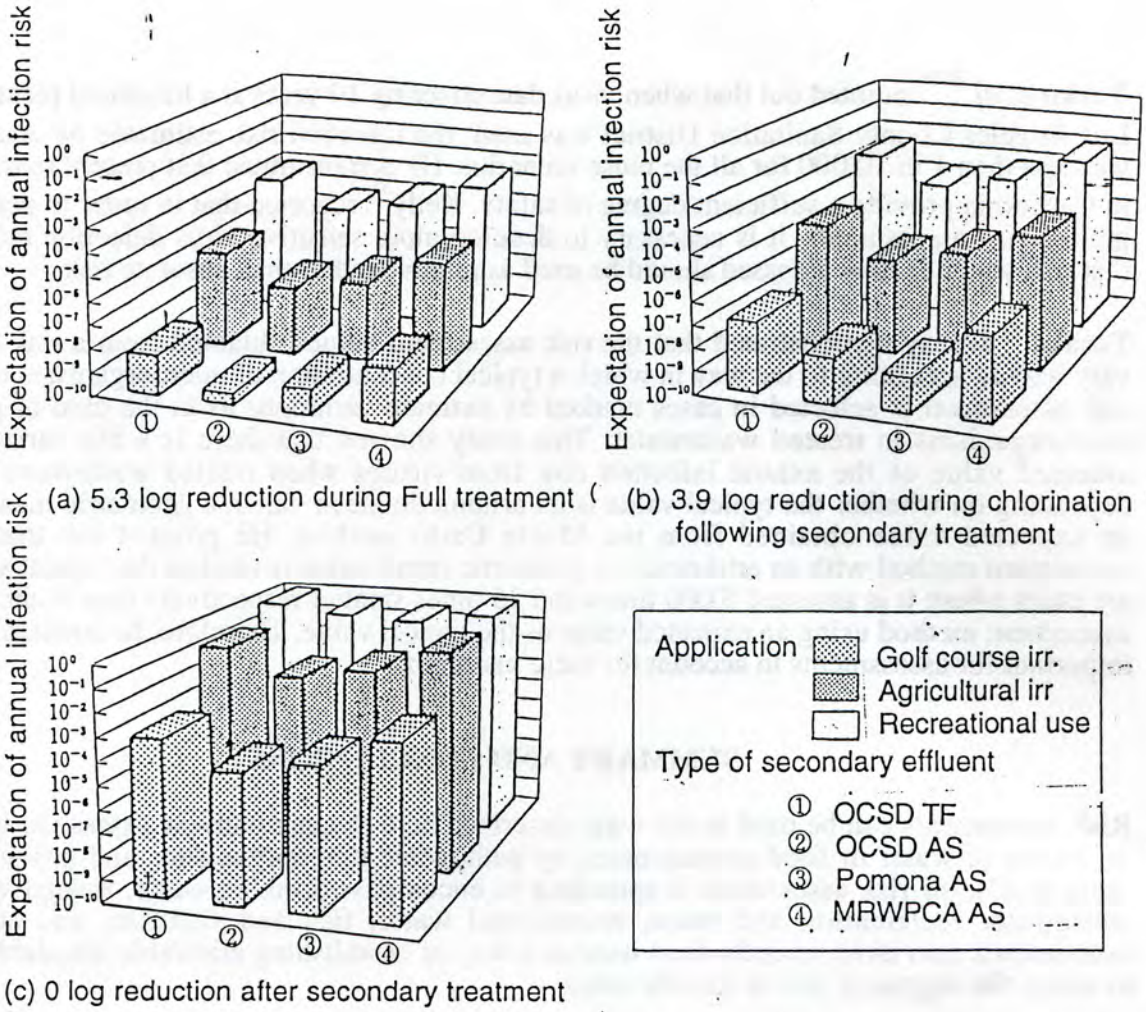


Fig. 11 Estimation of annual risk of infection from various reuse applications based on virus data in wastewater treatment plants in California
Assumptions on treatment levels are (a)tertiary treatment satisfying reuse criteria called Title 22; (b) chlorination following secondary treatment; (c) only secondary treatment

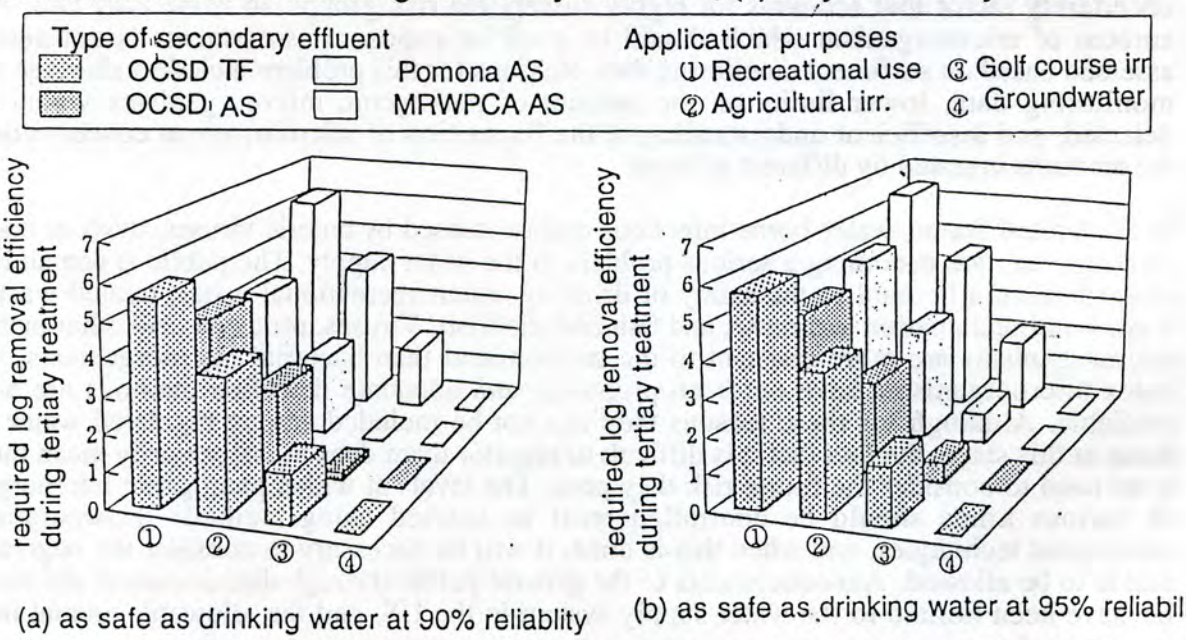


Fig. 12 Required virus inactivation efficiency during tertiary treatment for wastewater reclamation and reuse which satisfies the safety equivalent to the acceptable annual risk supposed by USEPA[3]

Yanko *et al.*[34,38] pointed out that when virus data covering 10 years at a treatment plant run by the Los Angeles County Sanitation District was used, the infection risk estimated by Asano *et al.*[21] was less than 1 in 10,000 for all the reuse scenarios. He demonstrated that present reuse standards in California provide a sufficient degree of safety. Kelly[33] reported that in order to perform more precise risk assessments, it is necessary to develop more sensitive virus detection methods, and that risk which is now assessed should be used as relative rather than absolute risk.

Tanaka[35] clearly demonstrated that the risk assessment values obtained from a risk assessment vary widely according to the way in which a typical concentration of microorganisms used for the risk assessment is selected in cases marked by extreme variations as in the case of pathogenic microorganisms in treated wastewater. This study showed that there is a big variation in the assessed value of the annual infection risk from viruses when treated wastewater is reused, depending on whether the typical value is an arithmetic mean value, a geometric mean value, or an expected value obtained from the Monte Carlo method. He pointed out that when an assessment method with an arithmetic or geometric mean value is used as the typical value, there are cases where it is assessed 5,000 times and 35 times smaller respectively than in the case of an assessment method using an expected value as the typical value. Therefore, he concluded that it is important for assessments to account for these variations.

SUMMARY AND CONCLUSIONS

Risk assessments can be used in the ways described here to quantitatively assess the latent effect on health of water or food contaminated by pathogenic microorganisms, and provide a useful method. Use of risk assessment is spreading to encompass drinking water, treated wastewater, wastewater reclamation and reuse, recreational water, fish and shellfish, and so on. Risk assessments also have recently been used as a way of establishing allowable standards, not only to assess the degree of risk in specific cases.

But procedures constituting a methodology for microorganism risk assessments are not as firmly established as they are for risk assessment methods for chemicals. Researchers and organizations performing risk assessments disagree about many issues, such as whether it is correct to use a most probable value model to represent the dose-response relationship or how to predict the uncertainty factor that accounts for highly susceptible risk groups, to name only two. And the amount of microorganism which should be used for exposure assessments is not necessarily assessed based on sufficient monitoring data. Reasons for this problem include a shortage of basic monitoring data, lower limits on the amount of pathogenic microorganisms which can be detected, and insufficient understanding of the fluctuation of microorganism concentrations and the amounts ingested by different persons.

In the United States, water-borne infectious disease caused by enteric viruses, cysts or oocysts of protozoa, etc. are becoming a serious problem in the water supply. The public is demanding that closer attention be paid to the safety of drinking water, recreational water, treated wastewater, wastewater reclamation and reuse, and fish and shellfish. Viruses, protozoa, and other pathogenic microorganisms are more resistant to the environment than bacterial microorganisms, suitable index microorganisms have not been proposed, and adequate detection methods are not even available. Although for these reasons they can not be included among regulated water quality items at this stage, the fact that it is difficult to monitor them does not necessarily mean that there is no need to consider the future risk they pose. The levels at which pathogenic microorganisms of various kinds should be controlled must be studied using methods incorporating risk assessment techniques, and when this is done, it will be necessary to consider the degree of risk that is to be allowed. Announcements to the general public through discussions of the issue thus far have been limited to the water supply system in the US, and the allowable annual infection risk of 10^{-4} introduced in this report, but it is also argued that such standards are needed for all water utilization categories[33]. In recreational use, swimming and other activities are being conducted with extremely high risk of infection and morbidity[39]. Because cultural and historical conditions vary from country to country, it is also necessary to fully consider determining just what the degree of relative risk should be compared with.

Research on and the application of microorganism infection risk assessment methods have just started, and great progress certainly lies ahead.

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