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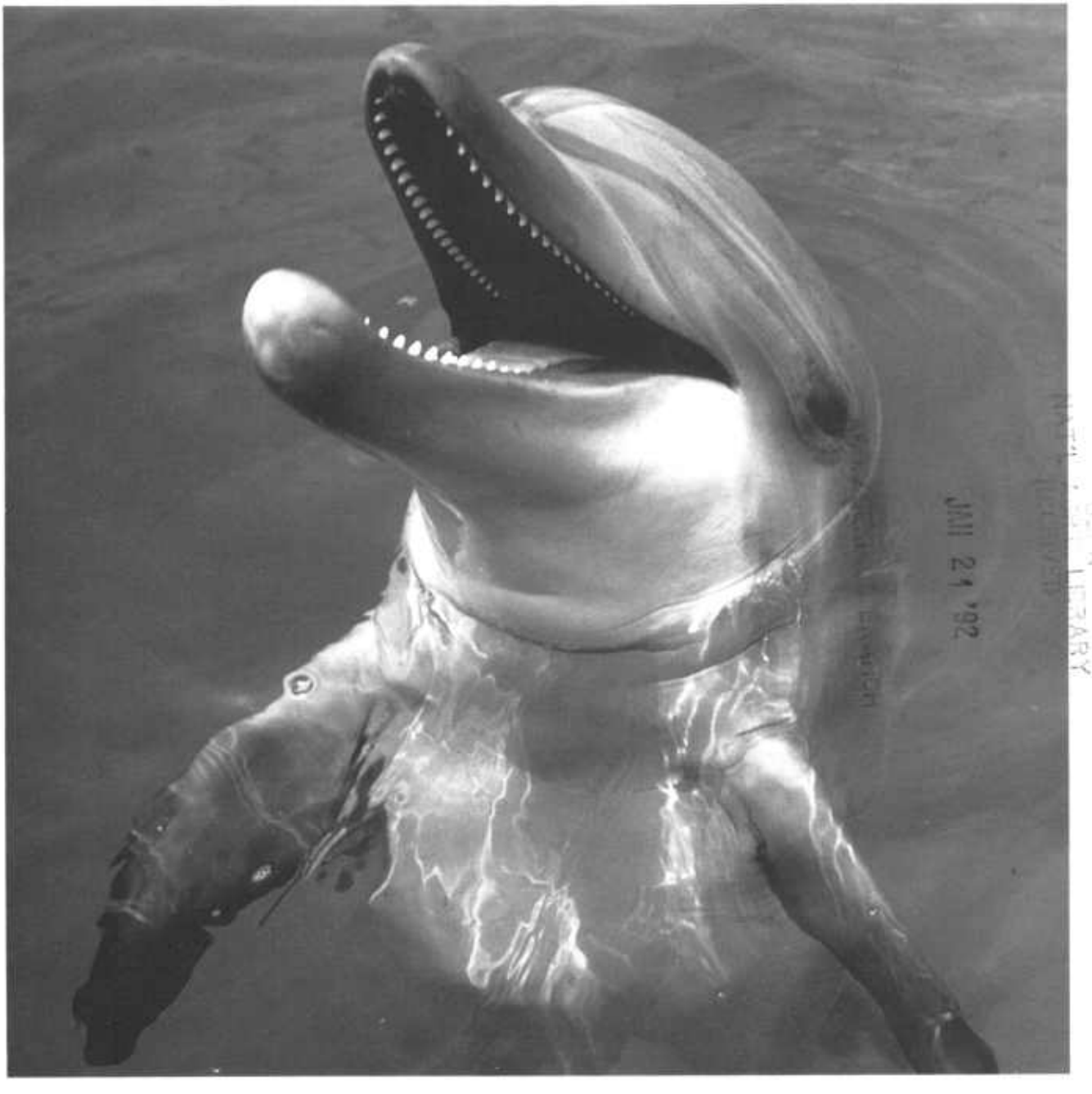
Sterilization of Marine Mammal Pool Waters

Theoretical and Health Considerations



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Sterilization of Marine Mammal Pool Waters

Theoretical and Health Considerations

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If the bowl be square,
The water in it will also be square.

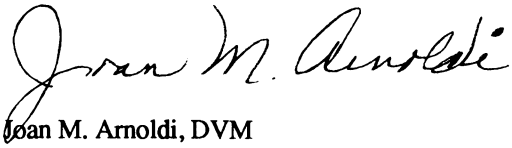
—Chinese proverb

Foreword

Under the provisions of the Animal Welfare Act, the Animal and Plant Health Inspection Service (APHIS) inspects facilities that exhibit captive marine mammals. Since 1979, when we first undertook that responsibility, water quality at such facilities has been the topic of major discussions among APHIS, the National Marine Fisheries Service, the Marine Mammal Commission, the U.S. Department of the Interior's Fish and Wildlife Service, marine mammal researchers, industry personnel, and other concerned parties. These discussions focused on what constitutes good water quality for captive marine mammals and how marine mammal water chemistry works.

I hope that by publishing this manuscript on the sterilization of marine mammal pool waters, APHIS can assist in ensuring that water quality for these animals is better understood. The manuscript will serve as a basic text for APHIS personnel and other interested individuals and also serve as a source of information for future discussions about this subject.

APHIS wishes to thank Dr. Stephen Spotte for writing this manuscript and allowing us to publish it. We also gratefully acknowledge the efforts of the National Agricultural Library in distributing copies through its Animal Welfare Information Center.



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Introduction

Dolphins, seals, and their relatives are creatures of the atmosphere that happen to live in water. Events that helped shape their aquatic existence occurred over millennia and continue still, if we accept natural selection as a stochastic force in biological evolution. Natural selection, however, is not an "optimizing agent" (Gould and Lewontin 1979). Contrary to popular belief, not every feature of an organism's anatomy and physiology is indicative of adaptive evolution, nor is there evidence that all elements of the external environment affect adaptation and survival even indirectly. This is demonstrably so where marine mammals are concerned. Many species have been maintained in apparent good health in captive environments that bear no resemblance to seawater. The hypothesis that ocean waters are somehow superior for the maintenance of captive marine mammals is unsupported by experimental evidence.

The subject of this report is the continuous, nonselective reduction of micro-organisms from marine mammal pool waters by sterilization. It is currently accepted by most husbandry experts that (*1*) superior captive environments are defined partly by low numbers of suspended micro-organisms, and (*2*) the advantages of sterile water outweigh most of the attendant disadvantages associated with sterilization. These assumptions are empirically weak, but not less so than assumptions to the contrary.

Sterilization alters the chemistry of aqueous solutions in ways that dictate subsequent control measures, causing further deviations from the natural state. One example suffices here. As I shall demonstrate, sterilization with chlorine-based oxidants is safer and more efficient at pH values substantially below the natural pH range of seawater. Effective sterilization should therefore determine the most appropriate operating pH, unless it can be shown that a value close to that of seawater has adaptive significance to marine mammals.

Water treatment (including sterilization) encompasses a confusing array of situations that defy both understanding and sensible regulation. The pertinent chemical reactions are difficult to control, poorly described, and protean in scope, dooming those who seek answers to wander dark labyrinths in search of molecular Minotaurs.

Compared with water treatment, water filtration is simple, involving elements of process design that are mostly fixed and amenable to engineering constraints. The only function of a filter is to reduce the number of suspended particles, a matter of no demonstrated relevance to marine mammal health. Devising a reproducible method of measuring the optical properties of water for evaluating filter performance remains the sole problem of theoretical interest. Direct particle counts offer the only hope right now. "Turbidity" is a meaningless measurement, having been based on the false premise that light extinction in turbidimeters is proportional to the concentration of suspended particles (Austin 1974). As observed by Austin et al. (1974), turbidity "is qualitative and relative. . . to the same manner as warmth. One does not measure warmth; one measures temperature."

1.0 Sterilization

The terms sterilization and disinfection are often used interchangeably, but they are not synonyms. *Disinfection* is the selective destruction of infectious organisms; *sterilization* is the nonselective destruction of all life (Sykes 1965). Ultraviolet radiation, chlorine-based oxidants, and ozone are the principal subjects of this report, and they kill micro-organisms nonselectively. Therefore, they are sterilizing agents.

Treating marine mammal pools with sterilizing agents never renders the water sterile because the input of micro-organisms from both the animals and extraneous sources is continuous. Sterility is dynamic, not static, and occurs fleetingly at the point of contact between the agent and the microbe. An adequately designed "sterilization" system merely keeps the numbers of suspended micro-organisms within predetermined limits of controlled disequilibrium, giving an appearance of static conditions. Truly sterile marine mammal pool water is unattainable, and no body of empirical evidence suggests that such an objective is even desirable.

The destruction of micro-organisms by oxidation is actually a secondary process, dependent on dissolution of the oxidant and its subsequent transformation into a suitable chemical state. Ultraviolet (UV) sterilization requires the physical transmission of UV radiation through the water. In both these situations, the pumps, contact chambers, and metering devices are merely technological aids designed to enhance processes that are entirely chemical or physical. In this context, sterilization is controlled not by high technology but by factors such as reaction rates and mass transfer. My principal objective is to describe the boundaries limiting the effectiveness of sterilization. These are drawn by inflexible natural laws, and they alone establish the cadence to which technology marches.

2.0 Water Systems and Water

The water systems of pools constructed to house marine mammals can be open, closed, or semiclosed. *Open* or flowthrough systems require no mechanical filtration devices (fig. 1a), although filters are often used to improve clarity of the influent water. In regions of the world where the water is generally clear, a simple offshore filtration device, such as a large concrete bunker filled with coarse sand, is often an adequate filter. The water supply to an open system enters from a natural source or city tap, flows through the pool, and exits to waste. The exchange is continuous, and control of temperature, color (all waters are pigmented), concentration of suspended particles—and to some extent sterilization—depends on the frequency with which the pool water is replaced.

Semiclosed systems rely on the continuous replacement of the pool water, but at a lower rate than occurs in open systems. Typically, less than than 10 percent of the total volume is replaced every 24 h (fig. 1b). Because the rate of exchange is slow, *granular media filtration* (usually sand or a combination of anthracite, sand, and garnet) is necessary to achieve optimal clarity.

In *closed systems,* all water is recycled through granular media filters and returned to the pool (fig. 1c). Color and temperature control, reduction of particles, and sterilization are based on predetermined protocols and managed as integral components of the overall process design.

Water composition is an important consideration in design and management of marine mammal facilities. Inorganic constituents in particular impinge directly on the *efficacy* of sterilization (fraction or percentage kill of micro-organisms). Marine mammals are maintained in waters of four generic types: freshwaters, brines (sodium chloride dissolved in tap water), artificial seawaters (some or all of the major ions dissolved in tap water), and seawater (table 1). Sometimes seawater has been diluted with freshwater and is brackish.

Table 1—Major elements exclusive of hydrogen and oxygen in seawater and one artificial seawater at the respective salinity and salinity equivalent of approximately 35.0 parts per thousand. Units are mol/L.

Element	Seawater[1]	GP2 Medium[2]
Cl	5.36×10^{-1}	5.45×10^{-1}
Na	4.57×10^{-1}	4.68×10^{-1}
Mg	5.55×10^{-2}	5.32×10^{-2}
S	2.76×10^{-2}	2.82×10^{-2}
Ca	9.98×10^{-3}	1.02×10^{-2}
K	9.72×10^{-3}	2.30×10^{-3}
C (inorganic)	2.33×10^{-3}	2.30×10^{-3}
N (inorganic)	1.07×10^{-3}	1.49×10^{-3}
Br	8.14×10^{-4}	8.40×10^{-4}
B	4.26×10^{-4}	4.08×10^{-4}
Si	1.07×10^{-4}	
Sr	9.13×10^{-5}	9.10×10^{-5}
F	6.32×10^{-5}	

Sources: Bidwell and Spotte (1985, p. 7);[1] Bidwell and Spotte (1985, p. 200–202), Spotte et al. (1984).[2]

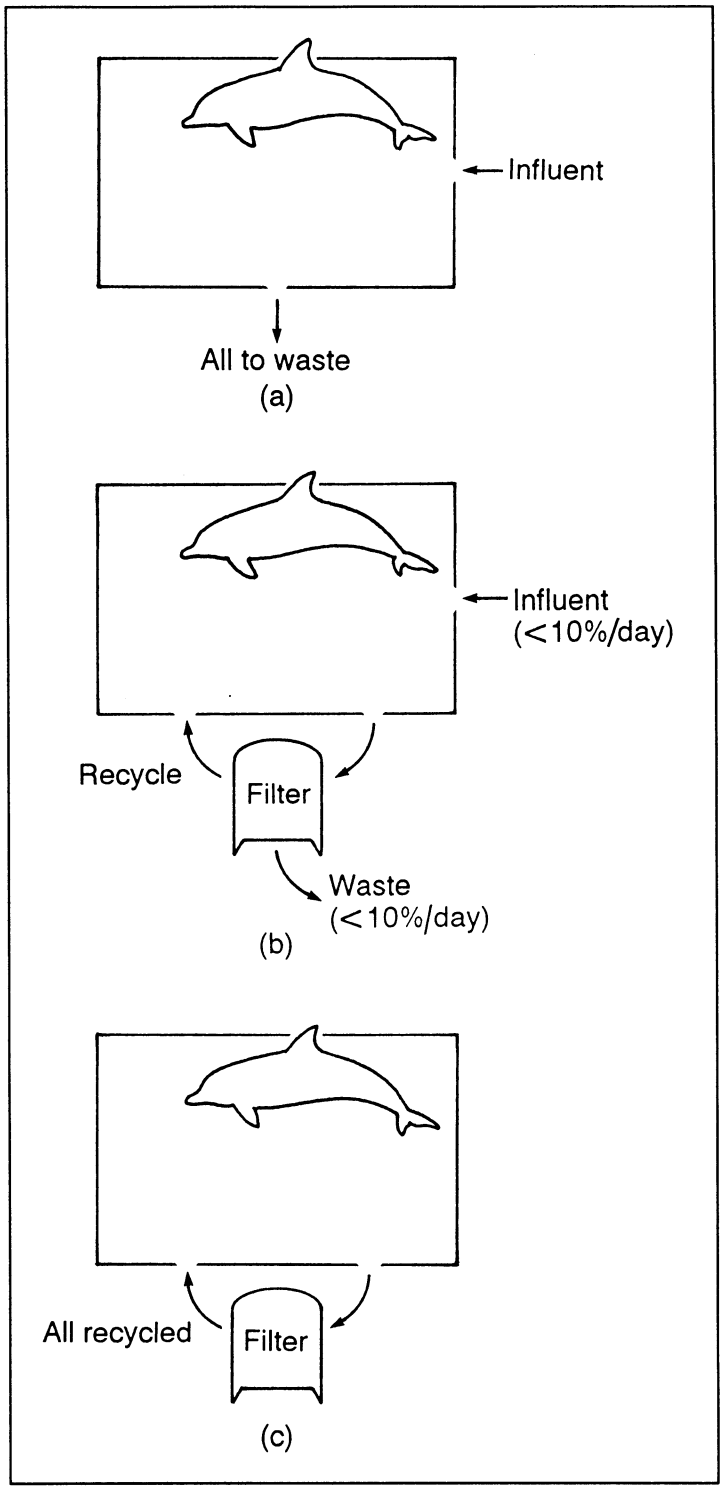

***Figure 1.**—Water system configurations. (a) Open system: a rapid stream of filtered or unfiltered influent flows through the pool to waste. (b) Semiclosed system: a slow stream of filtered or unfiltered influent enters the pool, is recycled through a filter, and a volume equal to that of the influent is discharged to waste. (c) Closed system: all water is filtered and recycled.*
Source: *Stephen Spotte.*

3.0 Micro-organisms

The presence of suspended micro-organisms is not necessarily a signal of impending disease. With effort, numerous microbial species can be cultured from marine mammal pool waters (table 2). These typically are forms shed by the animals themselves and their human attendants

Table 2—Species and numbers of micro-organisms cultured from saline cetacean pool waters at Mystic Marinelife Aquarium (Mystic, CT) and New York Aquarium (Brooklyn, NY).

Micro-organism	Mystic[1]	New York[2]
Acinetobacter sp.	2	0
A. calcoaceticus var. *lwoffi*	1	1
Aeromonas hydrophila	5	1
A. salmonicida	3	0
Alcaligenes sp.	0	1
Aspergillus sp.	1	1
Bacillus sp.	3	3
Bacteroides sp	1	0
Candida sp.	1	0
C. albicans	3	0
C. guilliermondii	0	2
C. rugosa	2	0
C. tropicalis	0	1
CDC V E-1	1	1
Chromobacterium sp.	0	1
Citrobacter diversus	1	0
C. freundii	1	0
Enterobacter amnigenus	0	1
E. cloacae	2	1
Escherichia coli	11	0
Flavobacterium sp.	2	0
F. spiritivorum	2	0
Klebsiella oxytoca	1	0
Micrococcus sp.	5	0
Morganella morganii	2	0
Oxidase +/Gram negative	4	1
Proteus sp.	2	0
P. mirabilis	4	0
Pseudomonas sp.	12	3
Pseudomonas sp. (fluorescent)	1	1
P. aeruginosa	0	1
P. putrefaciens	10	1
Staphylococcus sp.	5	0
S. aureus	1	0
S. cohnii	0	2
S. epidermidis	2	2
S. haemolyticus	1	0
S. hominis	5	2
S. saprophyticus	1	0
S. sciuri	0	1
S. simulans	1	0
S. warneri	4	1
Streptococcus faecalis	3	0
Trichosporon beigelii	3	1
Vibrio sp.	5	2
V. alginolyticus	18	5
V. fluvialis	4	1
V. parahaemolyticus	11	1
V. vulnificus	1	0

Sources: L. Louise Brown and John D. Buck (unpublished data, 1990); 23 total water samples,[1] six total water samples.[2]

(Buck et al. 1987, Buck and Spotte 1986, Dunn et al. 1982, Spotte and Buck 1981) or forms present in raw fishes used as food. Wild and captive marine mammals support microbial populations of comparable species diversity. Micro-organisms cultured from captive belukha whales (*Delphinapterus leucas*) at separate locations are listed in table 3; related information is provided in table 4.

Table 3—Results of blowhole, anal, and skin swabs taken from belukha whales (*Delphinapterus leucas*) at Mystic Marinelife Aquarium and New York Aquarium. All animals had been captured originally in the Churchill River, western Hudson Bay. Also see table 4.

Micro-organism	Mystic	New York
Acinetobacter calcoaceticus var. *lwoffi*+	+	
Aeromonas hydrophila	+	+
A. salmonicida	+	-
Bacillus sp.	+	-
Candida albicans	+	-
C. ciferrii	+	-
C. guilliermondii	+	-
C. pseudotropicalis	+	+
CDC IV E	+	+
Citrobacter freundii	+	+
Corynebacterium sp.	+	+
Enterobacter aerogenes	+	-
E. agglomerans	+	-
Escherichia coli	+	-
Micrococcus sp.	+	+
Morganella morganii	+	+
Oxidase +/-Gram negative	+	+
Pasteurella multocida	+	-
Pichia wickerhamii	+	-
Plesiomonas shigelloides	+	-
Proteus mirabilis	+	-
P. vulgaris	+	+
Pseudomonas maltophilia	+	+
P. putrefaciens	+	+
Pseudomonas sp.	+	-
Staphylococcus aureus	+	-
Staphylococcus sp.	+	+
S. cohnii	+	
S. epidermidis	+	+
S. haemolyticus	+	-
S. hominis	+	+
S. warneri	+	+
S. xylosus	+	-
Streptococcus faecalis	+	+
Streptococcus sp.	+	-
Vibrio alginolyticus	+	+
V. fluvialis	+	+
V. parahaemolyticus	+	-

Sources: L. Louise Brown and John D. Buck (unpublished data, 1990).

Table 4—Number of samples, isolates, and species of microorganisms (table 3) obtained from belukha whales at Mystic Marinelife Aquarium and New York Aquarium

Whale	Mystic	New York	Times sampled	Isolates	Species
Aurora	x		25	122	31
Cathy		x	2	7	6
Kela	x		32	176	43
Marina		x	5	25	18
Naku	x		38	198	46
Natasha		x	3	5	11
Newfi		x	1	7	5
Winston		x	5	25	18
87001		x	5	28	17
87010		x	5	22	15

Sources: L. Louise Brown and John D. Buck (unpublished data, 1990).

3.1 Coliform Bacteria

The Animal Welfare Act of 1970 was passed more than 2 decades ago to include regulations for marine mammals (Goff 1979). Part of the regulations stipulate that the *most probable number* or MPN (see glossary) of "coliform bacteria" (quotation marks added) in the aquatic portions of marine mammal enclosures (here called pool waters) not exceed 1,000/100 mL. This value can be an average of up to three samples taken at 48-h intervals. A measurement of "coliform bacteria" presumably includes the four genera of bacteria that make up the *total coliforms* (TC): *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter* (Clark and Pagel 1977). Total coliforms have been isolated from soil, vegetation, forest and farm products, and many other environments of nonenteric origin (Geldreich et al. 1964), making TC doubtful indicators of fecal contamination (Cliver and Newman 1987).

The fecal or *thermotolerant coliforms* (TTC), composed mainly of *Escherichia* with a *Klebsiella* component, grow at 44.5 °C and are more specific indicators of fecal pollution. *Escherichia coli* is the only coliform that is unquestionably enteric in homeothermic animals and has no extrafecal sources (Cabelli et al. 1982, Cliver and Newman 1987). The definitive bacterium for demonstrating fecal pollution is therefore *E. coli*, and TTC are preferable to TC for routine monitoring of contaminated waters (Cliver and Newman 1987).

3.2 Interpretation of Data

The MPN is obtained by the *multiple tube fermentation* (MTF) method. Sometimes *membrane filtration* (MF) is substituted for MTF (see glossary) to quantify TC or TTC. If the results are then evaluated in terms of the MPN standard, a problem of interpretation arises. Results obtained by the two methods are not directly comparable because MPN falls outside a normal statistical distribution (El-Shaarawi and Pipes 1982, Maul et al. 1985). An accurate comparison can be made only by transforming MPN to common logarithms or applying a suitable statistical procedure to avoid transformation (e.g., the likelihood ratio test, McNemar's test).

3.3 Coliforms in Aquatic Environments

Coliform bacteria survive poorly in aquatic environments (Geldreich 1970, Joyce and Weiser 1967, McFeters and Stuart 1972, van Donsel et al. 1967). The growth of coliform bacteria is inhibited in seawater (Alivistatos and Papadakis 1975, Cabelli et al. 1982, Carlucci and Pramer 1959, Green et al. 1980, Jones 1937, Olson 1978, Papadakis 1975), particularly aged seawater (Gameson and Saxon 1967); in chlorinated freshwaters (Braswell and Hoadley 1974, Green et al. 1977, Rice et al. 1987); by high concentrations of heterotrophic bacteria and particulate matter (McFeters et al. 1982); by toxic ingredients in selective culture media (Bissonnette et al. 1975, LeChevallier et al. 1983); and by injury (Hurst 1977, McFeters et al. 1982). Injured bacteria are more sensitive to selective media because of damage to the cell envelope (Zaske et al. 1980).

Marine mammals are sometimes kept in waters characterized by high particle counts and often large populations of heterotrophs. These waters are commonly chlorinated, of high ionic strength (up to 0.7 mol/kg, the molality of seawater), and aged (filtered and recycled). Not surprisingly, any combination of culture medium and procedure potentially underestimates the number of coliforms actually present, a situation consistent with water pollution monitoring in general (Dutka and Tobin 1976).

3.4 Coliforms as Indicator Organisms

Pathogenic bacteria have been isolated from drinking waters in the absence of detectable coliforms (Reitler and Seligmann 1957, Seligmann and Reitler 1965), prompting some to question whether coliforms are suitable standard indicator organisms for either drinking or recreational waters (Dufour 1984, Dutka 1973, Haas 1986). Pathogens can be present in marine mammal pool waters when coliforms are low in number or even absent, but I regard this as a separate issue. The intent of the "coliform bacteria" section of the Animal Welfare regulations was to establish standards for monitoring the extent of water contamination, not the presence of waterborne pathogens. These must be isolated by other methods. Moreover, the principal problems of detection are not eliminated by substituting another group of indicator organisms. All culture methods are susceptible to the same interferences: possible loss of injured cells, failure of cells to grow in selective media, and suppression of growth by antagonistic heterotrophs. Accuracy and precision are what matter; the indicator organism used is secondary, so long as its presence has relevance from a husbandry standpoint. The presence of TTC in marine mammal pools is direct evidence of fecal contamination, and the number of these organisms is a suitable unit of measure. The number of TC is less relevant because the sources of contamination can be extrafecal.

4.0 Point-Contact Versus Bulk-Fluid Sterilization

The dynamic processes occurring at the point of contact between sterilizing agents and micro-organisms are a continuing source of confusion. Misinterpretation of the theory has led to widespread misapplication of water process technology, not only in marine mammal husbandry but also in aquaculture (Spotte and Adams 1981).

The objective of water sterilization is to place the sterilizing agent in contact with micro-organisms by the most efficient means possible. Two methods are used, and their respective efficacies depend on physical or chemical properties of the sterilizing agent used, including how it behaves in water. I shall call these methods bulk-fluid and point-contact sterilization (fig. 2). *Bulk-fluid sterilization* relies on the application of agents that interact with other substances, yielding persistent reaction products. When dispersed properly, bulk-fluid sterilizing agents kill micro-organisms in all parts of the water system, including the *bulk fluid* (the main portion where the animals reside). Common examples are compounds containing chlorine (Sect. 6.0).

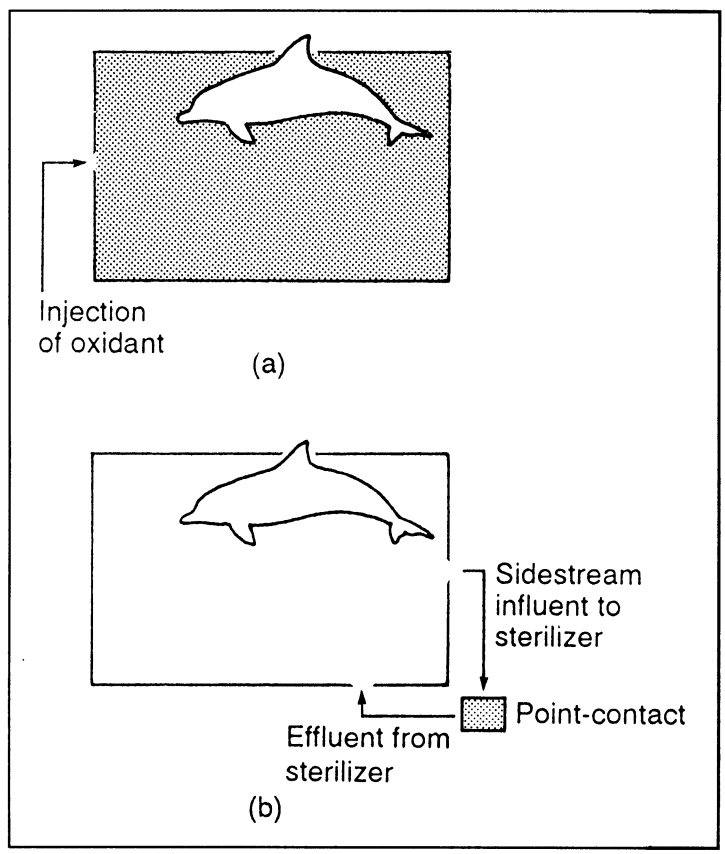

***Figure 2.**—Sterilization by bulk-fluid and point-contact applications. Screened areas are sites of effective sterilization. (a) Bulk-fluid sterilization is effective throughout the water system. (b) Point-contact sterilization is effective at the sterilizer.* Source: *Stephen Spotte.*

Point-contact sterilization relies on contact with a limited portion of the water system (ordinarily not the bulk fluid), and the efficacy of the process diminishes rapidly with increasing distance from the source. Examples of point-contact sterilizing agents are UV radiation (sect. 5.0) and ozone (sect. 7.0), the latter applied to freshwaters; in some saline waters and freshwaters containing bromide (Br^-), ozonation yields persistent reaction products that retain some degree of oxidation potential and demonstrate limited capacity to sterilize the bulk fluid (sect.7.1).

Sterilization by point contact is accomplished at a single location in the water system: at a battery of UV lamps, for example, or the injection point of ozone gas. The bulk fluid is never treated directly; instead, a *side stream* (fig. 2b) is diverted to the point-contact site where sterilization takes place. Treated water is then returned to the bulk fluid.

Animals residing in the bulk fluid feed, defecate, and ultimately become surrounded by their own contaminants. Such is the case unless the rate at which the side stream is sterilized exceeds the rate of bulk-fluid contamination (Spotte and Adams 1981). If the size disparity between the side stream and the bulk fluid is too great, inadequate sterilization results, and this will be true even if the sterilizer is 100 percent efficient (i.e., all micro-organisms in the vicinity of the point-contact site are killed).

Put simply, water sterilized by point-contact methods in closed and semiclosed systems may not protect animals swimming in the bulk fluid from contaminants in their immediate vicinity. The sterilization equipment is remote, located somewhere else in the flow scheme.

Point-contact sterilization was modeled by Spotte and Adams (1981), and the rest of the discussion is based on their report. Three hypothetical water systems composed the model (fig. 3). The curves in figure 4 illustrate the mass of micro-organisms remaining after sterilization.

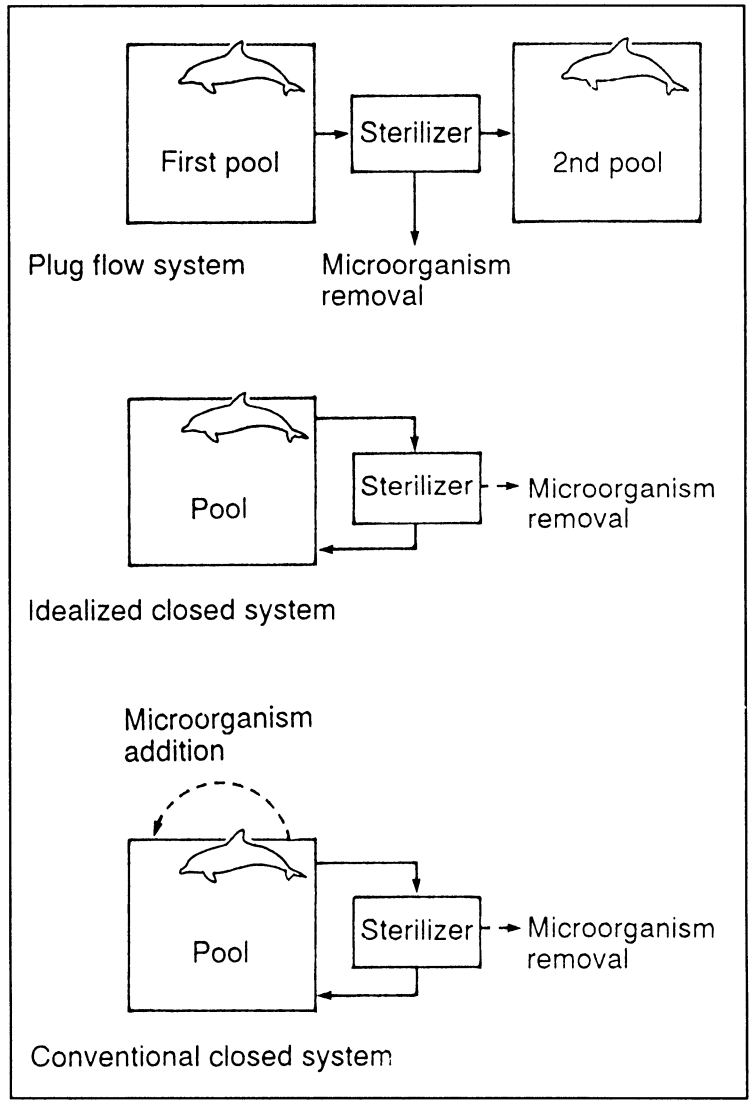

***Figure 3.**—Plug flow system: water flows through the sterilizer in a single pass, and micro-organisms are removed at a constant rate. Idealized closed system: all water is recycled, there is no new addition of pathogens, and micro-organisms are removed at a rate proportional to their concentration. Conventional closed system: all water is recycled, micro-organisms are added at a constant rate, and removed at a rate proportional to their concentration. Efficacy at all three contact sites is assumed to be 100 percent.* Source: *Spotte and Adams (1981).*

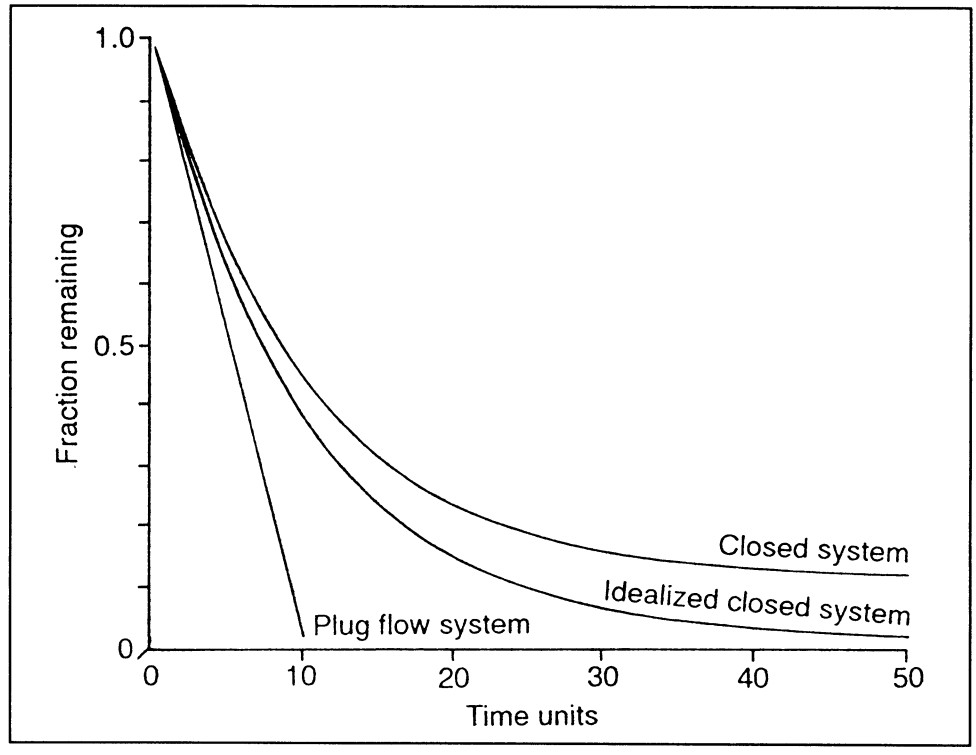

Figure 4.—Mass of micro-organisms remaining in the flow configurations of fig. 3 plotted against time. Complete sterilization is theoretically unattainable in conventional closed systems by point-contact. Source: *Spotte and Adams (1981).*

An effective kill rate of 100 percent has been assumed for all configurations (i.e., no micro-organisms survived contact with the sterilizing agent at the point-contact site). The plug flow scheme shows the total mass of micro-organisms remaining if water flows through the sterilizer in a single pass and is not replaced. This arrangement is similar to an open system with contaminated source water (untreated influent) represented by the first pool. The idealized closed system illustrates a situation in which sterile water is returned from the point-contact site to the bulk fluid with complete mixing but without the influx of additional micro-organisms. The third arrangement represents a conventional closed system (i.e., treated water is sterile, but the influx of micro-organisms from the bulk fluid is constant). Efficacy in the conventional closed system is limited by an equilibrium level at which the kill rate equals the rate of influx. This equilibrium equals the rate of influx divided by the flow rate through the sterilizer.

The efficacy of the sterilizer depends on only two factors: the rate at which micro-organisms are introduced and the effectiveness of the treatment device, as shown below. The same equations can be used to calculate the rate at which chemical contaminants are removed by physical adsorption (sect. 8.0). Symbols are V = total volume of the system (constant), C_0 = initial mass of micro-organisms (constant), t_0 = initial time, F = volume flow rate through the sterilizer (constant), C = mass of micro-organisms, FC/V = kill rate (mass per unit time), and R = influx of micro-organisms (mass per unit time).

In the plug flow scheme, water is pumped through the sterilizer on a single pass and discharged into a second pool. None is recycled. If F = 0.1 V (10 percent of the system volume per hour), 9 h would be required to kill 90 percent of the micro-organisms in the system.

In the idealized closed system, all water is recycled through the sterilizer and returned continuously to the bulk fluid. There is no new influx of micro-organisms. Thus

In the idealized closed system, all water is recycled through the sterilizer and returned continuously to the bulk fluid. There is no new influx of micro-organisms. Thus

$$dC = -C(VF/V)dt$$

$$dC/C = -(F/V)dt$$

$$\ln C = -F/V\ t + k$$

At $t_0 = 0, C = C_0$

$$\ln C_0 = k$$

Therefore, $\ln C/C_0 = -Ft/V$

and $C = C_o e^{-Ft/V}$

If F = 0.1 V per unit time,

$$C = C_0 e^{-0.1t}$$

and

$$C = 0.1\ C_0 \text{ (90 percent kill)}$$

$$e^{-0.1t} = 0.1$$

then t =23 time units.

At a flow through the sterilizer equal to 10 percent of the volume of the system per hour, 23 h are required to kill 90 percent of the micro-organisms.

In conventional closed systems, all water is recycled through the sterilizer and returned continuously to the bulk fluid, as in the idealized closed system. However, a continuous influx of pathogens also occurs at rate R, and

$$dC = -C(F/V)dt + Rdt$$

$$dC = [-C(F/V) + R]dt$$

Integration yields

$$C = e^{-Ft/V}(C_0 - RV/F) + RV/F$$

If F = 0.1 V and R = 0.01 for a 90-percent kill, C = 0.1 C_0; then because

$$C = e^{-Ft/V}(C_0 - RV/F) + RV/F$$

in this case

$$C = 0.9\ C_0 e^{-0.1t} + 0.1$$

By substituting different values for t, it can be demonstrated that a 90-percent kill is attainable only after infinite time. Actually, the mass of micro-organisms remaining cannot be less than RV/F, which in this example is 10 percent of C_0.

5.0 Ultraviolet Radiation

Ultraviolet radiation (the process is *irradiation*) has been used to sterilize waters of diverse origin, including large aquarium systems (Herald 1970, Herald et al. 1962, Spotte 1979), fish and shellfish hatcheries (Blogoslawski et al. 1978, Brown and Russo 1979, Bullock and Stuckey 1977, Kimura et al. 1976, Spanier 1978, Vlasenko 1969), wastewaters (Qualls et al. 1985, Whitby et al. 1984, White et al. 1986), drinking waters (Tobin et al. 1983), and hospital whirlpool baths (Gilpin et al. 1985). Radiation emitted from UV lamps designed to sterilize has peak outputs at 254 nm (Spotte 1979). Successful reduction in the number of suspended micro-organisms occurs only if limitations inherent in point-contact sterilization can be surmounted.

The successful treatment of wastewaters and drinking waters by UV irradiation is attributable entirely to flow schematics. The schemes used in both processes are open systems because none of the water is recirculated and recontaminated at any location in the sequence. Untreated influent water flows first to a collection basin, where large particles settle out, then is clarified with flocculants (sect. 8.4). The clarified water is filtered, sterilized, and discharged to receiving streams (wastewater) or city mains for human consumption and industrial use (drinking water).

In public aquarium exhibits, hatcheries, and marine mammal pools, UV irradiation can effectively sterilize untreated influent water near the source—in other words, prior to contact with the bulk fluid. Because UV irradiation is exclusively a point-contact process, its efficacy in closed and semiclosed systems has been challenged on both theoretical (Spotte and Adams 1981) and empirical (Spotte and Buck 1981) grounds. It is doubtful whether any UV sterilizer can process a large enough side-stream flow to sterilize the bulk fluid adequately.

The radiation dosage of a UV sterilizer is equal to the wattage of the unit times the average *contact time* of the water flowing through it (the length of time water is retained at point-contact sites). Because contact time equals the volume of the sterilizer divided by the volume flow rate, the relationship between dosage and flow rate can be established. Efficacy in this situation is a measured comparison of the number of micro-organisms cultured from the influent and effluent streams of the sterilizer. If the flow rate is excessive, contact time inside the sterilizer is reduced, and efficacy is diminished. Even if the efficacy is 100 percent, the comparatively small volume of the side stream is inadequate to sterilize the bulk fluid, and in closed and semiclosed systems the concentrations of suspended micro-organisms in the vicinity of the animals may remain unchanged, whether or not the unit is in operation (Spotte and Buck 1981).

6.0 Chlorine-Based Oxidants

I shall refer to sodium hypochlorite (NaOCl), chlorine gas (Cl_2), mono- and dichloramine (NH_2Cl, $NHCl_2$), chlorine dioxide (ClO_2), and related chlorinated compounds as *chlorine-based oxidants*. All are bulk-fluid sterilizing agents. They yield reaction products that retain some capacity to sterilize over time (sect. 6.3). As a result, bulk-fluid sterilization occurs simultaneously at all locations in the water system, and point-contact is unnecessary. Chloramines are secondary reaction products formed after initial hydrolysis (sect. 6.3).

Chlorine-based oxidants offer several advantages. They (*1*) are inexpensive, (*2*) possess strong antimicrobial properties, (*3*) are soluble for easy addition in controlled amounts, and (*4*) in the gaseous phase can be liquefied under pressure at room temperature for storage and transport. The principal perceived disadvantages are formation of mutagenic and carcinogenic byproducts (Sects. 6.2, 9.0) and products of lower oxidation (i.e., sterilizing) potential (sect. 6.3). Also of importance is the propensity of these oxidants to react with numerous inorganic and organic reducing agents, which heightens *chlorine demand* at the expense of sterilization (i.e., chlorine is consumed in reactions with innocuous elements, leaving less of it available to kill micro-organisms). Chlorine dioxide (ClO_2) is an exception in terms of its advantages and disadvantages (sect. 6.4).

Chlorine gas reacts quickly with water to form hypochlorous acid (HOCl), hydrogen ions, and chloride ions:

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \qquad (1)$$

Reaction of sodium hypochlorite with water yields hypochlorous acid, sodium ions, and hydroxyl ions:

$$NaOCl + H_2O \rightleftharpoons HOCl + Na^+ + OH^- \qquad (2)$$

Hypochlorous acid instantly and reversibly forms hypochlorite and hydrogen ions:

$$HOCl \rightleftharpoons OCl^- + H^+ \qquad (3)$$

Together HOCl and OCl^- compose *free chlorine*. Analytically, their sum is *free available chlorine* (FAC)—in other words, the concentration of HOCl plus OCl^- remaining in an uncombined state, free and available to react. The relative proportions of HOCl and OCl^- depend primarily on the pH of the solution and to a lesser extent on temperature. The concentration of OCl^- increases markedly above pH 7.5, and the amount of HOCl falls proportionately (fig. 5). In practical terms, the efficacy of chlorination diminishes rapidly at pH values >7.5 because HOCl is the principal sterilizing agent in chlorinated water. By one estimate (Brodtmann and Russo 1979), HOCl is 150 to 300 times more effective than OCl^- in destroying viruses, protozoan cysts, and enteric bacteria.

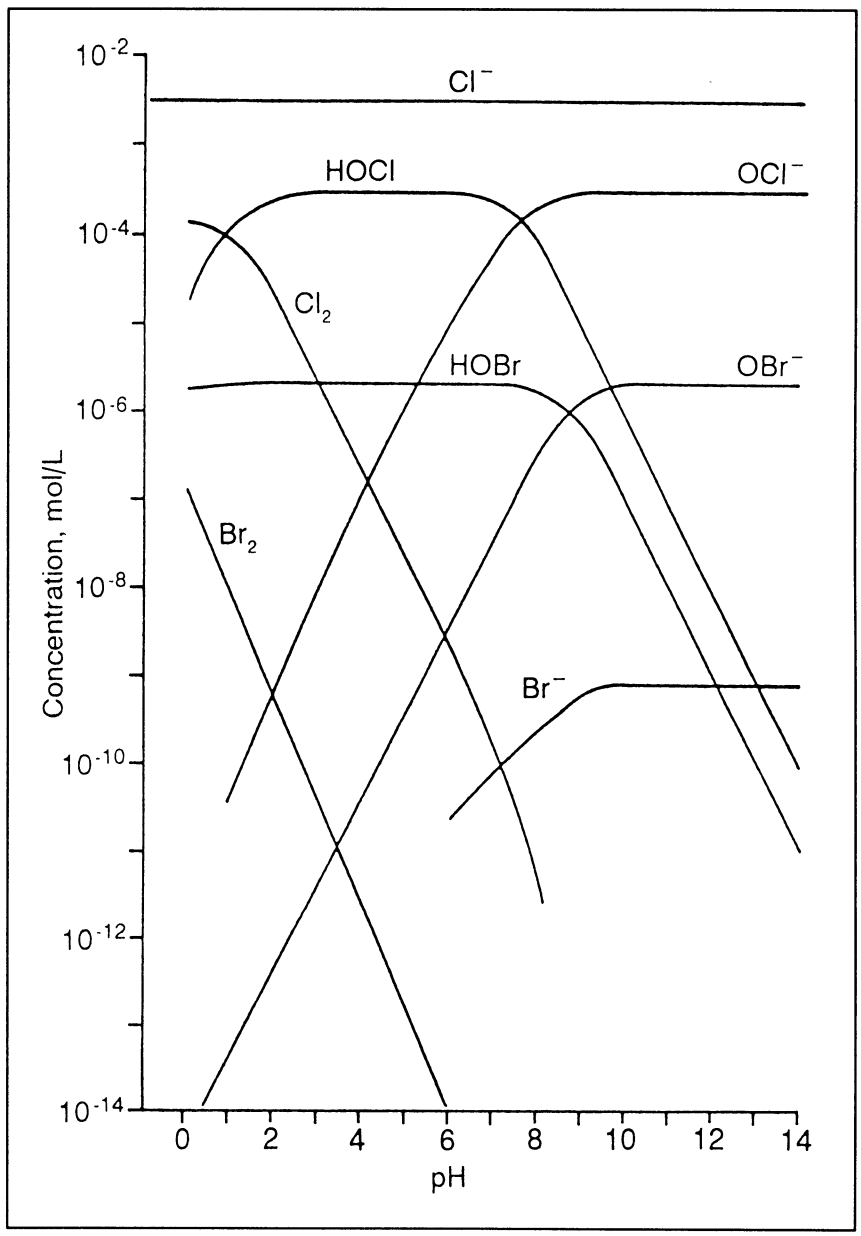

***Figure 5.**—Distribution diagram showing the concentrations of aqueous chlorine and bromine species as a function of pH.* Source: *Rook et al. (1978).*

6.1 Inorganic Reaction Products of Chlorination

Hypochlorous acid reacts with ammonia and amino compounds to form chloramines (sect. 6.3). These persist longer than free chlorine but possess lower oxidizing potential. *Ammonia* in aqueous solutions is the sum of free ammonia (NH_3) and ammonium ion (NH_4^+), ordinarily expressed as the concentration of the nitrogen component (total NH_4-N). Free chlorine reacts with inorganic constituents in water, principally bromide and ammonia (fig. 6), in addition to a plethora of organic compounds other than amines (sect. 6.2). Reactions with organics can be oxidative (i.e., chlorine is not incorporated into the organic matrix) or nonoxidative to yield chlorinated organic compounds.

Any reaction that consumes chlorine exerts a chlorine demand. In water treatment, quantities of chlorine-based oxidants in excess of the chlorine demand must be added continuously to sustain effective, uninterrupted sterilization.

Chlorine-based oxidants react principally with water molecules in freshwaters of low inorganic oxidation potential. The same situation applies with slightly less certainty to brines and unprocessed waters originating from freshwater wells but incompletely describes reactions occurring in estuarine waters, seawater, and artificial seawaters. These last-named solutions contain bromide. Freshwaters cannot be wholly excluded; some contain as much as 2 mg Br^-/L (Sweetman and Simmons 1980). The bromide concentration in seawater is 65 mg/L ($8.14 \cdot 10^{-4}$ mol/L, table 1); in estuarine waters the concentration is proportional to the salinity. Even if bromide is not purposely added to artificial seawaters its presence as a contaminant in other salts is guaranteed.

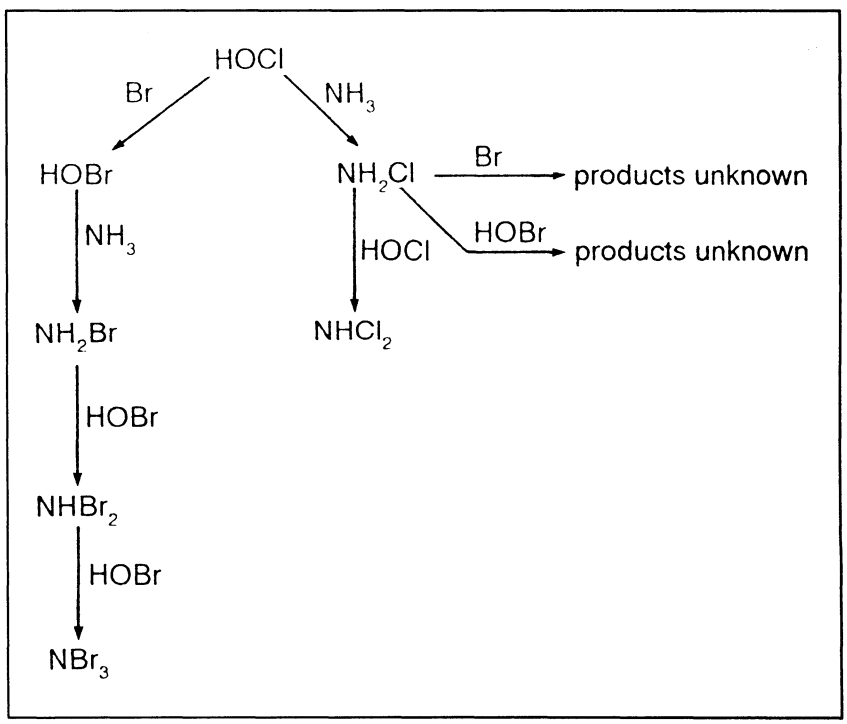

Figure 6.—Major reaction pathways and possible products in chlorinated, ammonia-rich seawater. Source: *Johnson and Inman (1978).*

According to Farkas and Lewin (1947) and Sigleo et al. (1980), chlorination of seawater and estuarine waters results in oxidation of bromide to hypobromous acid (HOBr) and hypobromite ion (OBr^-), together called *active bromine* (fig. 6 and reactions below):

$$HOCl + Br^- \rightleftharpoons HOBr + Cl^- \qquad (5)$$

$$HOBr \rightleftharpoons OBr^- + H^+ \qquad (6)$$

Active bromine disproportionates at higher pH values than does active chlorine (fig. 5). The above reactions are rapid and reach completion within 2.5 min (Wong and Davidson 1977). Time to completion (conversion of HOCl to HOBr) in seawater is <10 s when reactive nitrogen species are absent (Sugam and Helz 1977). Macalady et al. (1977) stated that decomposition of active bromine is analogous to that of chlorine in freshwaters (i.e., to bromide and bromate, BrO_3^-). According to Carpenter and Macalady (1978), one reaction in the series is

$$3HOBr \rightleftharpoons BrO_3^- + 3H^+ + 2Br^- \qquad (7)$$

The formation of bromate is slow because of kinetic restrictions, although sunlight induces conversion of up to 50 percent of the hypobromite to bromate in chlorinated seawater (Macalady et al. 1977).

6.2 Organic Reaction Products of Chlorination

The formation of potentially toxic organohalide reaction products in chlorinated drinking waters was first reported in 1974 by J. J. Rook. Four of these compounds—chloroform ($CHCl_3$), bromoform ($CHBr_3$), bromodichloromethane ($CHBrCl_2$), and dibromochloromethane ($CHBr_2Cl$)—make up the *trihalomethanes* (THM) and are known mutagens or carcinogens (Bellar et al. 1974, Meier and Bull 1985, Oliver and Lawrence 1979, Pereira et al. 1985, Rook 1974). Hundreds of other toxic and potentially toxic chemicals have been isolated from drinking waters in recent years (Leer et al. 1985), including two additional classes of compounds, halogenated acetone and acetonitrile derivitives (Bull and Robinson 1985).

Chloroform is the primary THM formed in chlorinated freshwaters. Some evidence suggests that bromine is more reactive than chlorine, with bromination resulting in greater numbers of bromine-based THM of overall greater mass than chlorination (Ishikawa et al. 1986). In addition, some chlorinated compounds react with bromide to form brominated THM (Ishikawa et al. 1986). Upon chlorination, up to 30 percent of the bromide is converted to bromine that is bound organically and present in THM (Luong et al. 1982). An increase in the bromide concentration increases the percentage of brominated THM because bromine acts as a substitute in haloform reactions during chlorine-bromine interactions (Luong et al. 1982). It has been suggested that the health effects of brominated compounds may engender more serious consequences than those of chloroform (Cooper et al. 1985). This statement, which is entirely inferential, refers to the long-term ingestion of treated drinking waters by humans. Nothing is known about the potential health hazards posed by THM to captive marine mammals (sect. 9.3).

Dissolved humic substances—primarily humic acids of aquatic and terrestrial origin (see glossary)—provide the most important organic substrates that when chlorinated are precursors of THM (Engerholm and Amy 1983, Joyce et al. 1984, Kringstad et al. 1983, Stevens et al. 1976). They represent 30 percent to 50 percent of the dissolved organic carbon in water (Benoit et al. 1979). Humic substances are geopolymers derived from carbohydrates, proteins, fatty acids, and lignin by microbial degradation and enzymatic or autooxidative coupling reactions (Christman and Gjessing 1983, Gjessing 1976, Harvey et al. 1983). Fulvic acids (see glossary) also are known organic THM precursors (Christman et al. 1983, Harvey et al. 1983, Peters et al. 1980, Rook 1977), as are algal materials (Crane et al. 1980, Hoehn et al. 1979, Oliver and Shindler 1980, Rook 1977, Wachter and Andelman 1984).

6.3 Chloramines and Bromamines

Monochloramine (NH_2Cl) is formed during interaction of free chlorine with ammonia and amino compounds by

$$NH_3 + HOCl \rightleftharpoons NH_2Cl + H_2O \qquad (8)$$

Subsequent reactions yield dichloramine ($NHCl_2$)

$$NH_2Cl + HOCl \rightleftharpoons NHCl_2 + H_2O \qquad (9)$$

and nitrogen trichloride (NCl_3)

$$NHCl_2 + HOCl \rightleftharpoons NCl_3 + H_2O \qquad (10)$$

In sum, the chlorine derivatives above make up *combined chlorine*, determined analytically as *combined available chlorine* (CAC). They constitute a reservoir of persistent oxidants that continues to sterilize the bulk fluid after free chlorine has been consumed, although their oxidation potentials are lower, and longer contact times are required to achieve comparable efficacy (Means et al. 1986). In addition, other nitrogenous organic compounds reduce the bactericidal activity of chloramines (Wolfe et al. 1985). As sterilizing agents, chloramines and bromamines (see below) interfere with the efficacy of chlorination. Ammonia, an important reactant, clearly interferes because large amounts of chlorine-based oxidant are necessary for its oxidation before concentrations of free chlorine sufficient for sterilization are generated (Atkinson and Palin 1973).

Compared with free chlorine, chloramines are capable of reducing concentrations of THM substantially (Argaman et al. 1984, Brodtmann and Russo 1979, Reed 1983)—according to one report, by as much as 80 percent (Mitcham et al. 1983). In still another report (Stevens et al. 1978), it was shown that ammonia treatment of raw water entering a drinking-water treatment plant produced THM levels of <20 µg/L, whereas exposure to free chlorine alone resulted in concentrations > 160 µg/L. White (1972) stated that monochloramine, the dominant chloramine species in water, is produced in seconds at pH 7 to 9 and most efficiently if the chlorine/nitrogen mass ratio is <5. Johnson (1978) wrote that monochloramine is the principal chlorine-based sterilizing agent in waters containing 0.1 to 1.0 mg/L NH_4-N. As the chlorine:nitrogen ratio increases or the pH decreases, monochloramine disproportionates in water to form ammonia and dichloramine. Dichloramine formation is maximum at chlorine:nitrogen mass ratios of 5 to 7.6. The formation of $NHCl_2$ can also occur by the reaction of monochloramine with HOCl, so long as the chlorine:nitrogen mass ratio is >5.

Some drinking water plants rely on *chloramination*, which involves the flash-mixing of a chlorine-based oxidant (e.g., NaOCl) with ammonia to yield reaction products of extended oxidation potential. Sterilization and prevention of the formation of THM are sensitive to whether ammonia is added to the flowing stream first or concurrently (Means et al. 1986). The only advantage of chloramination is the potential reduction of THM, but this is not offset by its disadvantages, notably lower oxidation potential than free chlorine and the need for complicated mixing and monitoring systems to assure that ammonia and chlorine are consumed in correct proportions. In addition, ammonia is highly soluble in water and potentially lethal to some fishes and aquatic invertebrates at the µg/L level (Spotte 1979). At sublethal concentrations in fishes, ammonia induces neuroendocrine responses indicative of stress (Spotte and Anderson 1989). The use of ammonia in the vicinity of fish exhibits is not recommended.

When seawater is chlorinated, processes resulting in NH_2Cl formation compete with bromide oxidation (i.e., reactions 5 and 11 compete):

$$HOCl + NH_4^+ + H^+ \rightleftharpoons H_2Cl + H_2O \qquad (11)$$

The rates of reactions 5 and 11 are strongly pH dependent and nearly equal in seawater at pH 8.1 when the concentration of total NH_4-N is approximately 60 µg/L (Haag 1980). Squires (1977) noted that bromine compounds react with ammonia and suggested that some bromine could be liberated from the seawater of marine mammal pools even at pH 8.0.

Importantly, bromide oxidation (reaction 5) predominates in seawater, with subsequent production of bromamines (Jolley and Carpenter 1983). Mono- and dibromamine are formed by

$$NH_2Cl + HOBr \rightleftharpoons NHBrCl + H_2O \qquad (12)$$

and

$$NHBrCl + HOBr \rightleftharpoons NBr_2Cl + H_2O \qquad (13)$$

Ultimately, however, monochloramine becomes the dominant species at total NH_4-N concentrations up to at least 1 mg/L (Johnson and Inman 1978).

Salinity influences the differential formation of hypobromous acid and monochloramine (fig. 7) because salinity and the concentration of bromine are proportional. As shown, both species occur in artificial seawater of pH 8.1 that contains 0.3 to 0.4 mg/L total NH_4-N. At higher ammonia concentrations (e.g., 0.8 mg/L total NH_4-N), monochloramine prevails.

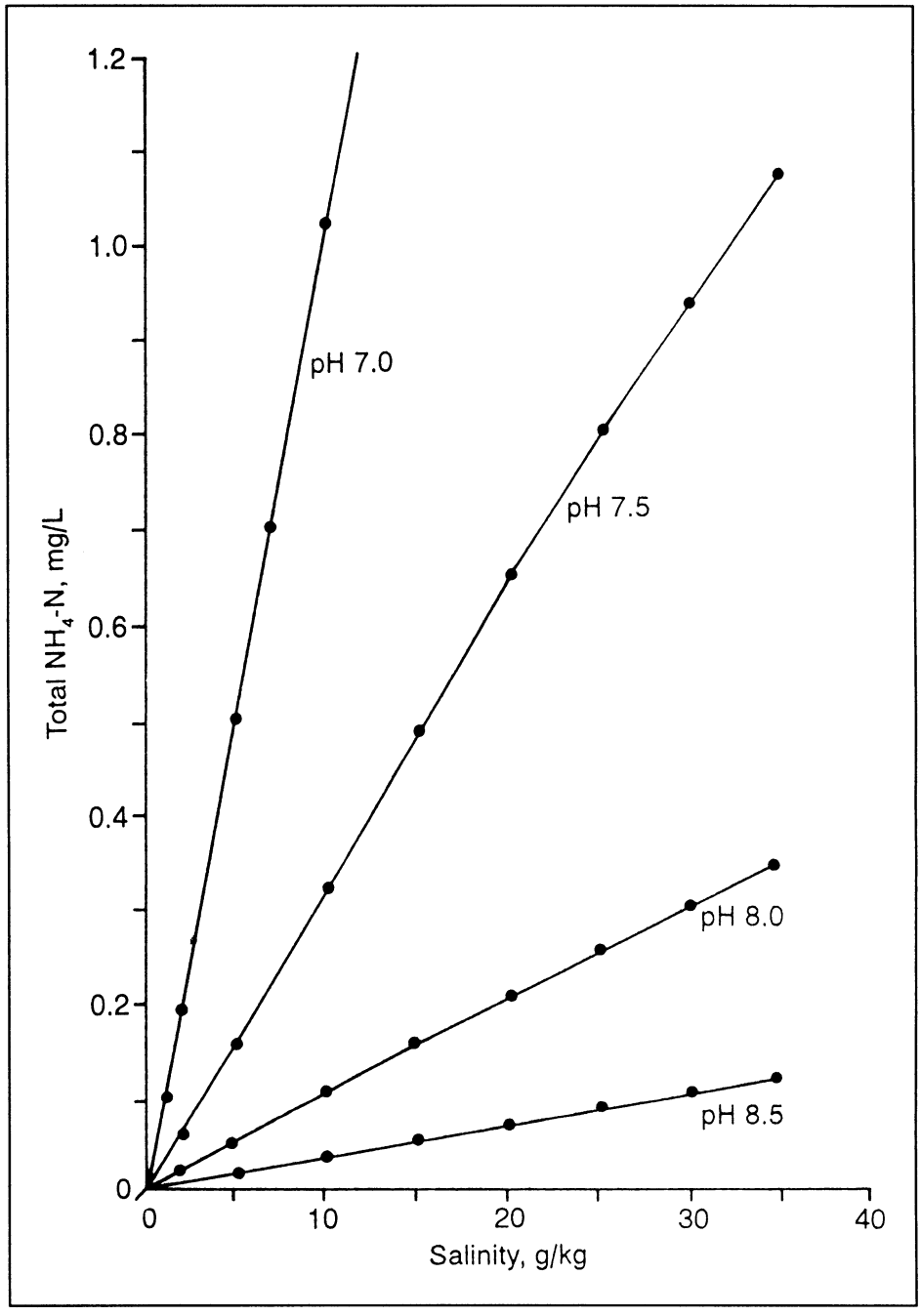

***Figure 7.**—Plot of total ammonia nitrogen (total NH_4-N) versus "salinity" in artificial seawater to give an equal probability of forming hypobromous acid (HOBr) or monochloramine (NH_2Cl) at a stipulated pH. Combinations falling above a pH line produce conditions favorable for NH_2Cl formation; combinations below a line enhance formation of HOBr.* Source: *Johnson and Inman (1978).*

6.4 Chlorine Dioxide

As described by Atkinson and Palin (1973), chlorine dioxide (ClO_2) is produced onsite by mixing a strong chlorine solution (500 to 1,000 mg/L Cl_2) with sodium chlorite at pH 2.5 by the reaction

$$2NaClO_2 + Cl_2 \rightleftharpoons 2NaCl + 2ClO_2 \qquad (14)$$

Numerous other reactions occur, depending on pH (Granstrom and Lee 1958).

Chlorine dioxide offers several advantages over other chlorine-based oxidants. It *(1)* apparently does not yield THM directly (Lange and Kawczynski 1978), *(2)* is a strong sterilizing agent over a broader pH range (Cronier et al. 1978), *(3)* does not react with ammonia to form chloramines (Ingols and Ridenour 1948), *(4)* often achieves comparable sterilization at lower dosage levels and contact times (Cooper et al. 1985), *(5)* is superior at controlling odors (Cooper et al. 1985), and *(6)* readily removes iron and manganese (Cooper et al. 1985). This last factor is especially advantageous where the source water originates from wells. In addition to these other advantages, chlorine dioxide does not react with bromide; thus hypobromous acid and hypobromite are not formed. As a result, bromoform and other THM of bromine origin are not produced when waters containing humic compounds are placed in contact with ClO_2 (Masschelein 1979). Finally, chloroform formation is inhibited by chlorine dioxide in the presence of HOCl, the extent of inhibition depending on the ClO_2:HOCl ratio (Abdel-Rahman 1985). However, chlorine is often a trace contaminant of the ClO_2 generation process (Cooper et al. 1985).

Chlorine dioxide has several disadvantages. It is sensitive to temperature, pressure, and light, and can be explosive even at low temperatures (Cooper et al. 1985, Masschelein 1979). Such instability means that ClO_2, like ozone (sect. 7.0), must be generated onsite. Although ClO_2 is not known to form THM directly, their formation is possible (Colclough et al. 1983). Finally, ClO_2 is more expensive than other chlorine-based oxidants.

7.0 Ozone

Ozone, an allotrope of the element oxygen, has three oxygen atoms. Consequently, its chemical properties differ from those of molecular oxygen. The unstable nature of the O_3 molecule results in its rapid decomposition; therefore, ozone cannot be stored and must be generated where it is to be used. Ozone is commonly employed as a sterilizing agent in large aquarium systems (Honn 1979, Honn and Chavin 1976, Ramos and Ring 1980, Sander and Rosenthal 1975, Schlesner and Rheinheimer 1974, Spotte 1979, Stopka 1975), aquaculture installations (Blogoslawski 1977, Menasveta 1980, Moffett and Shleser 1975, Oakes et al. 1979, Rosenlund 1975, Rosenthal 1981, Rosenthal and Otte 1979, Tipping 1987), wastewater effluents (Rakness et al. 1984, Stover and Jarnis 1981), drinking waters (Carmichael et al. 1982, Lawrence and Cappelli 1975 unpubl., Rice et al. 1981), and to kill larvae of fouling organisms (e.g., barnacles, mussels) in power plant cooling waters (Carpenter et al. 1979, Sengupta et al. 1975). As with UV irradiation, misapplication of the process technology can affect the results adversely.

7.1 Inorganic Reaction Products of Ozonation

During ozonation, the immediate reaction products formed at the point-contact site are short-lived free radicals, hydroperoxide species, and unstable ozonide intermediates (Hoigné and Bader 1976, Peleg 1976, Prengle 1983). The rate at which these substances appear (i.e., the rate at which ozone decomposes) is affected by pH and temperature. Prolonging the decomposition of ozone enhances sterilization: the process becomes more effective at low pH because more undissociated ozone is retained (Farooq et al. 1977a), and the rate at which microorganisms are killed is dampened with decreasing temperature (Farooq et al. 1977b).

The chemistry of ozone in water is influenced substantially by large amounts of bromide (Haag and Hoigné 1983). Ozonation of seawater oxidizes Br^- to active bromine and ultimately to bromate (Crecelius 1979, Kalmaz et al. 1985, Kosak-Channing and Helz 1979, Williams et al. 1978). Bromate is not oxidized further (Haag and Hoigné 1983). The conversion is quantitative both in freshwaters and in seawater (Crecelius 1979, Haag and Hoigné 1983), so long as reactive organic compounds are absent (Cooper et al. 1986).

Ozone oxidizes bromide to form hypobromous acid; hypobromite reacts with ozone to form either bromate or a species that results in formation of bromide (Haag and Hoigné 1983). The reactions are

$$O_3 + Br^- \rightleftharpoons O_2 + OBr^- \qquad (15)$$

$$O_3 + OBr^- \rightleftharpoons 2O_2 + Br^- \qquad (16)$$

$$2O_3 + OBr^- \rightleftharpoons 2O_2 + BrO_3^- \qquad (17)$$

The process is slow at low pH, and active bromine reaches greater maximum concentration, which is roughly proportional to contact time (Haag and Hoigné 1983). In freshwaters, active bromine forms after several minutes; oxidation to bromate also is slow, and the time is extended at low pH. During typical conditions used to process drinking waters (1 mg O_3/L, pH 7 to 9), the maximum concentration of active bromine is attained within 20 min (Haag and Hoigné 1983). In seawater the reaction is rapid because of the high Br^- concentration, and the half life of O_3 is 5.3 s at 20 °C (Haag and Hoigné 1983). Ozonation in the presence of bromide is less efficient because Br^- is regenerated from OBr^-, an intermediate oxidation product, resulting in catalytic destruction of O_3 and increasing the ozone demand.

"Reactive" or "residual" halogens and other products with equivalent redox potentials can sometimes be measured in water after ozonation. The oxidant species produced by ozonation and chlorination are indistinguishable by amperometric techniques (Richardson and Burton 1981). Traces produced by ultraviolet spectroscopy are different when freshwater is ozonated or chlorinated but similar when the analysis is performed using seawater (Squires 1977). A method of expressing "reactive" ozone is as OCl^- equivalents. In one series of experiments in which seawater and artificial seawater were bubbled with O_3 for 1 h (maximum concentration of 13.5 mg O_3/kg after 5 min), OCl^- concentrations were the same (Williams et al. 1978). The artificial seawater (salinity equivalent of approximately 35 parts per thousand, Sect. 10.1) contained nine inorganic ions comprising >99.8 percent of the inorganic ion composition of seawater. A simple brine solution produced no detectable OCl^-, whereas a solution of NaBr yielded a lower OCl^- concentration than either the seawater or artificial seawater. The extent to which reaction products are formed by ozonation is clearly greater in seawater and at least one artificial seawater than in brines.

7.2 Organic Reaction Products of Ozonation

Hypobromous acid reacts with organic solutes to produce brominated compounds and other substances of potential toxicity. Examples are bromophenols, bromoform, formaldehyde, and acetaldehyde (Sweetman and Simmons 1980, Yamada and Somiya 1980). Bromoform ($CHBr_3$) arises in the presence of bromide and humic acids (Cooper et al. 1986, Haag and Hoigné 1983). Little bromoform is produced at low O_3 concentrations because of competitive reactions that generate hypobromous acid (Haag and Hoigné 1983). The greatest amounts of bromoform appear at moderate O_3 concentrations and low pH values, conditions at which HOBr is not oxidized to bromate (Haag and Hoigné 1983). Overall, bromoform formation depends on pH and the O_3:Br^- ratio. Small differences in either variable are critical and account for the disparate results reported in the literature (Haag and Hoigné 1983). For example, concentrations of bromoform generated by ozonating low-saline waters at low O_3:Br^- ratios are similar to concentrations produced by chlorination (Cooper et al. 1985). Conversely, no $CHBr_3$ was reported to arise in mixtures of synthetic humic acid and bromide at high O_3:Br^- ratios and high pH (Haag and Hoigné 1983).

Ozonation of water in the presence of humic substances produces mutagens and carcinogens (Gruener 1978, Kool and Hrubec 1986), as does posttreatment of chlorinated water with ozone (van Hoof 1983). Many of these substances are indistinguishable from those produced by chlorination (Bull 1980, Kool et al. 1985). Treating seawater with ozone instead of chlorine-based oxidants in the presence of bromide yields compounds that are probably more toxic than those formed by chlorination (Williams et al. 1978).

8.0 Control of Organic Carbon

The addition of organic matter to marine mammal water systems is continuous, and this can be confirmed by measuring *total organic carbon* (TOC), the sum of *dissolved organic carbon* (DOC) and *particulate organic carbon* (POC). The distinction between DOC and POC is arbitrary. Ordinarily, DOC is defined as the fraction of the TOC that passes through a filter of stipulated pore size (e.g., 0.45 μm, Reuter and Perdue 1977). Material trapped in such a filter is POC. Removing a portion of the TOC reduces oxidant demand, which enhances the efficacy of sterilization. Trihalomethanes, which are part of the DOC, are reduced simultaneously. Three approaches have been used in drinking-water treatment to remove THM or reduce their concentrations (Wolfe et al. 1984): (*1*) pretreating the raw influent to reduce the levels of THM precursors, (*2*) applying a sterilizing agent that does not generate THM, and (*3*) removing THM after they form. The first option is of little use in closed and semiclosed systems because TOC is added continuously (sect. 8.1). The second option has been discussed (e.g., chlorine dioxide). This section deals with the third, except for application of chloramines (sect. 6.3).

8.1 Organic Carbon in Marine Mammal Water Systems

Much of the DOC in waters of all types exists as compounds that are refractory to removal by conventional process technology. Among the more durable components are humic and fulvic acids. In nature, many of these substances are so stable that they exist unchanged for thousands of years. The age of most soil humus ranges from 50 to 3,000 years: the alkaline-extractable fraction (i.e., fulvic acid) is 50 to 250 years old, whereas the portion resistant to extraction is considerably older, at least 2,000 years (Gjessing 1976). Dissolved organic carbon in subsurface waters of the oceans has an apparent ^{14}C age of several thousand years; because this exceeds the mixing time of the oceans, the bulk of organic matter dissolved in seawater appears to have been recycled several times without degradation (Bada and Lee 1977).

Chlorination of estuarine (i.e., brackish) waters increases the proportion of dissolved organic compounds of low molecular weight, suggesting scission of large molecules (Sanders 1982). Chlorination of estuarine water results in approximately 1/3 mole of CO_2 formed per mole of Cl_2, evidence that oxidation of carbon exerts a large chlorine demand (Sigleo et al. 1980). On an equal molar basis, an oxidative decarboxylation reaction can be written

$$Cl_2 + R\text{-}CH(NH_2)\text{-}COOH + H_2O \rightleftharpoons R\text{-}CHO + NH_3 + 2H^+ + 2Cl^- + CO_2 \qquad (18)$$

The reaction is illustrated using Cl_2; reactive chlorine species are more likely to be HOCl, HOBr, and so forth (Sigleo et al. 1980).

Chlorinated waters of closed-system marine mammal pools contain organic compounds that resist ozonation (Adams and Spotte 1980). Logically, much of this material must also be refractory to chlorine-based oxidants if the water has been chlorinated continuously. I suspect that easily oxidized organics in marine mammal pool waters are transformed routinely by either ozonation or chlorination. Over time, refractory components of the DOC increase, eventually predominating.

The control of DOC in marine mammal water systems is desirable for two reasons. First, humic and fulvic acids are chromophoric and impart an unsightly yellowish color to the water. Second, these compounds interact with ozone and chlorinated reaction products to yield substances of mutagenic and carcinogenic potential, although the likelihood of any adverse effects on the health or lifespans of captive marine mammals is remote (sect. 9.0).

Organic compounds enter marine mammal pools from a number of sources, including influent water, urine and feces, and uneaten food. Large, granular media filters are seldom-considered sources of humic matter. By trapping and concentrating POC, large filters exert enormous oxidant demand. In granular media filters of all types, this effect is manifested primarily at the entry point of the unfiltered influent stream. Most POC in rapid sand filters is trapped in the top 2.5 cm (Tchobanoglous 1970) and at deeper levels in units containing dual media (anthracite and sand) and multimedia (anthracite, sand, and garnet). Any oxidant remaining in the influent stream is removed quickly, permitting large, viable populations of micro-organisms to exist despite continuous bulk-fluid sterilization. The lysing of micro-organisms attached to filtrant grains, combined with the shedding of their polysaccharide matrices caused by mechanical shear forces as water streams through the filtrant under pressure, results in the continuous generation and recycling of organic matter (Spotte 1979, in press). There is ample evidence that much humic matter in natural waters originates from lysing of bacterial cells (Schnitzer and Khan 1972). Logically, the same effects generate humic compounds in processed waters (Spotte in press).

The concentrations of organic carbon present in marine mammal pool waters have been studied only superficially. We know very little about the rate at which organics accumulate and even less about their composition. Spotte and Adams (1979) measured TOC daily for several weeks in two closed-system marine mammal pools, called systems 1 and 2 (fig. 8). Both contained

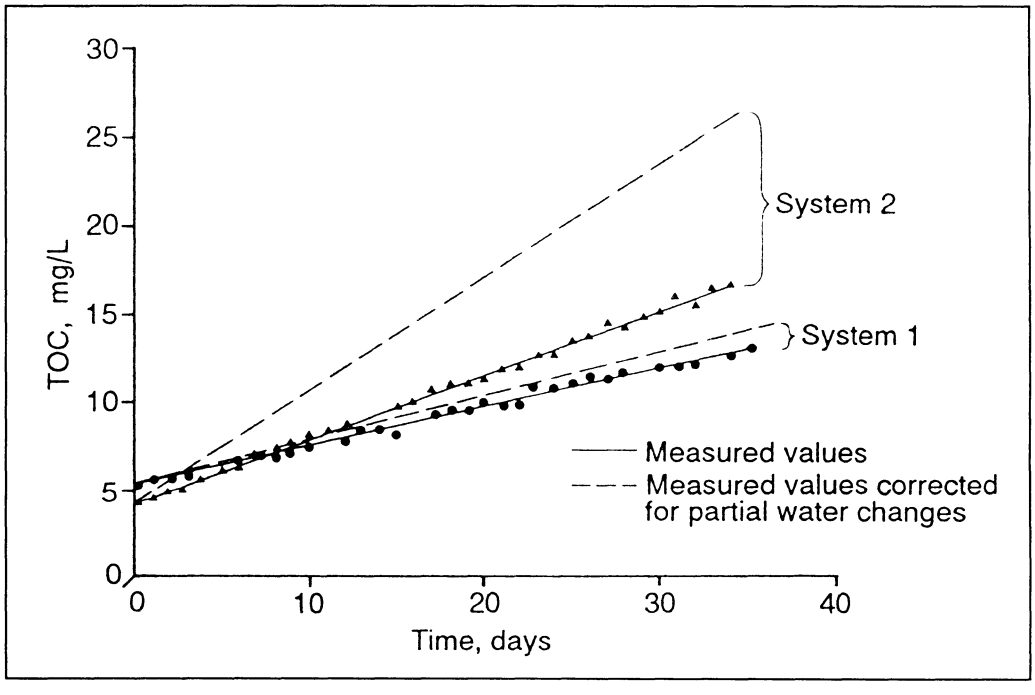

***Figure 8.**—Accumulation of total organic carbon (TOC) with time in two closed-system marine mammal pools (see text descriptions).*
Source: *Spotte and Adams (1979).*

chlorinated brine, but the methods of filtration were different. System 1 was processed by rapid sand filters; system 2, by diatomaceous earth added to the filters continuously in a slurry and supplemented with powdered activated carbon. Some types of activated carbon are excellent for removal of organic matter from water (sect. 8.2). The diatomaceous earth with its accumulated organic load was discarded periodically when the filters were backwashed. In the filters of system 1, accumulated organic matter was removed by periodic backwashing, although the

filtrant was retained. Total organic carbon increased linearly for 20 d in both systems: in system 1 at the rate of 0.23 mg/(d•L) to approximately 16 mg/L, equivalent to an estimated 6.07 percent of the carbon fed to the animals; in system 2 at 0.37 mg/(d•L) to approximately 12.5 mg/L, representing an estimated 5.13 percent of the organic carbon added as food. In the authors' opinion, the waters and filters of closed-system marine mammal pools are sinks for TOC originating from raw fish fed to the animals. As a point of reference, 95 percent of municipal drinking waters in the United States contain <5 mg TOC/L (Condie and Bercz 1985).

Refractory organic compounds inevitably accumulate in closed systems. However, some are partially decomposed during oxidation, which renders them more susceptible both to bacteriological mineralization in the filters and tertiary treatment processes such as physical adsorption (sect. 8.2). If an objective of water management is to keep the concentration of THM low, humic matter must be removed from solution continuously. Unfortunately, humic compounds are among the most refractory components of the DOC. Their removal is difficult and expensive. As stated previously, humic compounds are important precursors of THM. The fact that chlorination and ozonation yield reaction products culminating in THM formation is indirect evidence that at reasonable contact times and dosage levels humic matter is largely refractory to both oxidation processes.

8.2 Removal by Activated Carbon

Activated carbon (see glossary) is among the most effective materials for the physical adsorption of organic carbon (including THM) from water (Anderson et al. 1981, Loper et al. 1985), precursors of THM (Jodellah 1985, Lee et al. 1981, McCreary and Snoeyink 1980, Semmens et al. 1986), and chloramines (Komorita and Snoeyink 1985, Weber et al. 1983). Granular forms have particle sizes >0.1 mm; smaller particles are termed powdered activated carbon. During physical adsorption the substance removed is the *adsorbate* (e.g., DOC), and it is adsorbed onto surfaces of the *adsorbent* (activated carbon in this case). Physical adsorption is affected by several factors, notably (*1*) mass transfer of the adsorbate into the adsorbent; (*2*) contact time; (*3*) concentration and composition of the adsorbate; (*4*) particle size, pore surface area, and selectivity of the adsorbent; and (*5*) nature of the biological film on the surfaces of the adsorbent (Spotte 1979). Adsorption of molecular organics takes place by diffusion into the extensive micropore system of activated carbon granules. The rate of diffusion is slow (Schuliger 1978). In closed and semiclosed systems, the process is restricted by the same factors limiting the effectiveness of point-contact sterilization, unless contact chambers are large enough to accommodate a sizable side-stream flow.

In one series of experiments (Spotte and Adams 1984), the adsorptive capacities of different types of activated carbons evaluated under identical conditions (artificial seawater) differed by orders of magnitude (fig. 9). This finding suggests that characteristics of the adsorbent (the fourth limiting factor in the previous paragraph) are subject to substantial variability. Unfortunately, the high cost of the most effective activated carbons, such as hardwoods, prohibits their use. From a design standpoint only contact time and concentration and composition of the adsorbate are controllable, and neither can be brought on line without extensive pilot testing of the actual water to be treated. This means that physical adsorption processes are impossible to engineer economically until untested water systems containing marine mammals are operational.

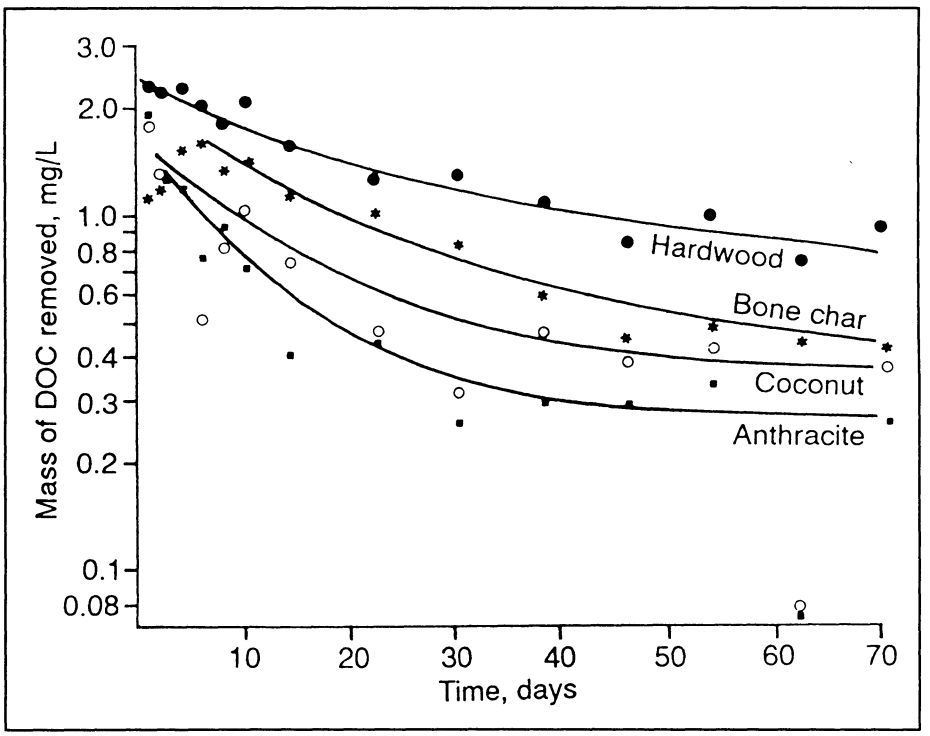

Figure 9.—Efficacy of four granular activated carbons in removal of dissolved organic carbon (DOC) over 70 d. Source: *Spotte and Adams (1984).*

Activated carbon has a finite life, and replacement cost is a serious disadvantage. After a time—often no more than a few weeks—the adsorptive capacity of the material becomes exhausted. Chemical regeneration of spent activated carbon is sometimes effective (Martin and Ng 1984), but treatment by high heat (870–980 °C) for about 30 min in a multiple hearth furnace or rotary kiln is the most common method (Adams et al. 1986, Lyman 1978, Sontheimer 1974). Steam is introduced at a rate of 1 kg/kg of activated carbon. Organic matter in the pores is vaporized and driven off. Regeneration is cost effective only in large industrial applications; otherwise, the material must be discarded and replaced. Exhausted material must be treated as hazardous waste for disposal purposes. Limited evidence indicates that pretreatment of the filtered effluent with ozone prior to contact with activated carbon may prolong the life of the material (Stopka 1978).

Adsorption of dissolved organics with activated carbon is a viable method of reducing TOC in marine mammal pool waters. In samples of chlorinated brine from a closed-system marine mammal pool, contact for 10 min resulted in reduction of the initial TOC concentration (12.91 mg/L) by approximately 37 percent (fig. 10). The amount removed was approximately equivalent to 20 days' accumulation at the previously determined rate of increase (Spotte and Adams 1979). However, only an additional 11 percent was removed in 60 min, indicating that most nonrefractory compounds had been removed during initial contact.

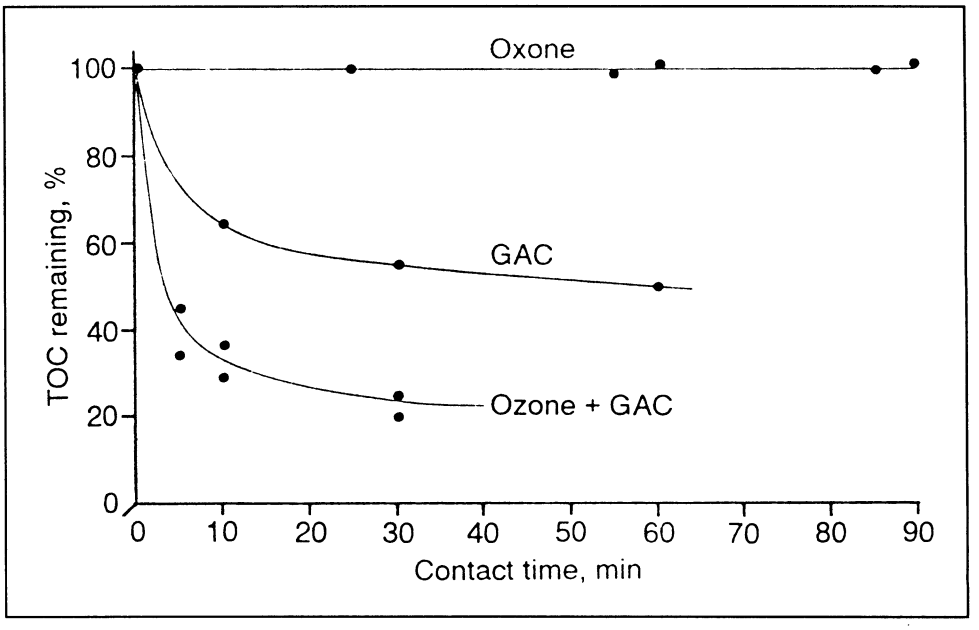

Figure 10.—Effect on total organic carbon (TOC) of oxidation by ozonation, adsorption by granular activated carbon (GAC), and preozonation followed by GAC contact. Source: *Adams and Spotte (1980).*

8.3 Alteration by Ozonation

Ozonation of drinking waters, wastewaters, and seawater for the purpose of removing organic matter has yielded mixed results. This is attributable to the composition of the water being treated, the ozone concentration, contact time, design of the contact system, and other variables. Direct removal of an organic compound by ozonation is predicated on its complete oxidation. The nonrefractory component, however, is not susceptible to direct oxidation by O_3 at reasonable contact times and dosages (Gruener 1978, Meijers 1977, Nebel et al. 1973, Williams et al. 1978). The Williams team reported that treatment of filtered seawater with 8.6 mg/L O_3 contacted at 200 mL/min reduced the TOC by 25 percent in 10 min. Complete oxidation of even the nonrefractory organics was slow. Moreover, preferential reactions with bromide reduced the actual contact time to 1 min at the same flow rate. Treated wastewaters behave similarly. Elsewhere it has been reported that ozonation decreased the TOC in wastewater by only 25 percent after 15 min, and to <50 percent after 1 h (Gruener 1978).

In the only quantitative study published to date in which water from a marine mammal pool was examined (a chlorinated brine), ozonation had no measurable effect on the TOC concentration during sequential contact periods of 25, 30, and 30 min (fig. 10). Evidently, the entire organic component (13.66 mg/L TOC) was refractory to complete oxidation by ozone. Pretreatment of drinking waters and wastewaters by ozonation enhances the physical adsorption of TOC by activated carbon (Glaze and Wallace 1984, Guirguis et al. 1978, Rice 1980, Somiya et al. 1986), and the same apparently is true for marine mammal pool waters. Ozonation of chlorinated brine taken from a marine mammal pool, followed by contact with granular activated carbon, removed 60 percent of the TOC in 5 min, 70 percent in 10 min, and 78 percent in 30 min (fig. 10). The initial TOC concentration was 12.54 mg/L.

Perhaps the most valuable function of ozonation is to render some of the refractory organics nonrefractory and more amenable to mineralization by micro-organisms attached to filtrant or activated carbon grains (Stephenson et al. 1980). Little is known about the actual mechanisms involved, but the process is believed to render certain compounds more biodegradable by lowering their molecular weights (Suzuki et al. 1978) and perhaps by introducing oxygenated groups into the structures of humic compounds and other refractory molecules, providing sites where microbiological degradation can commence (Nebel et al. 1976).

Ozonation decolorizes yellowish, humic waters in seconds (Anonymous 1975, Farooq et al. 1977a, Gruener 1978, Meijers 1977). In marine mammal pool waters, this process results in a pleasing cosmetic effect. As discussed previously, decolorization is not equated with the removal of a substantial portion of the TOC at reasonable dosage levels and contact times.

8.4 Flocculation

Flocculants remove TOC, reducing the oxidant demand of treated waters and potentially lowering the amount of oxidant necessary to achieve adequate sterilization (Robinson 1979). Filtration and related processes become more efficient as the size of the particulate matter increases (Gregory 1989). Consequently, flocculation improves filtration efficiency by aggregating and enlarging a fraction of the POC and converting some of the DOC to particulate matter. Much of this material, which is colloidal in size (1 nm to 1μm, see Glossary), would otherwise pass unchanged through the filters and be recycled to the bulk fluid.

During flocculation, organic matter altered by colloid destabilization is precipitated and trapped in the filters as flocs and sediments. *Flocs* are usually classed as permanent aggregates (Gregory 1989), although a standard terminology does not exist; for example, coagulation, flocculation, and sedimentation are sometimes considered to be synonyms (e.g., Randtke 1988). For simplicity, I shall refer to the principal process (colloid destabilization) as coagulation, the final result as flocculation (i.e., precipitation), and the subtances formed as flocs. Gregory preferred aggregation to describe the overall process.

Alum and cationic polyelectrolytes (see below) are the materials most often applied to water for removal of humic and fulvic acids. The relationship between the concentration of anionic organics and flocculant demand is stoichiometric, and organic matter is removed most efficiently at predetermined flocculant concentrations. Upon initial contact, colloids and organic matter compete for reactive sites on the flocculant, with sodium salts of organic acids being first to react through carboxylate and phenolate groups (Narkis and Rebhun 1977). Only after these and other reactions have been completed does precipitation commence (Narkis and Rebhun 1977). By most accounts the process reaches peak effectiveness at acidic pH values.

Alum or aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$ (see glossary), is the most widely used flocculant in water treatment (Anonymous 1983). Alum was reportedly used for clarifying water in ancient China and 16th-century Egypt. In more modern times, alum was first used in England in 1757, in France by the 1820's, and in the United States in 1885. Under certain conditions, adding alum reduces the concentrations of humic material in processed drinking waters by as much as 50 percent (Hoehn et al. 1984).

When alum is added to water of neutral pH, aluminum hydroxides form rapidly. Initially, these are colloidal precipitates, but they subsequently grow into large flocs. Flocculation with alum is most effective within the pH range 7.5 to 6.5, or near the region of minimum solubility of the hydroxide (Gregory 1989)

Natural and synthetic polymers are effective flocculants but more expensive than alum. The synthetic kinds used in water treatment are available as neutral or ionizable molecules. The latter are *polyelectrolytes* (see glossary). Long-chain neutral polymers of high molecular weight (>10^6) adsorb POC (mainly nonpolar particles) at many points along the chain. A single polymer molecule subsequently is attached to several particles, effectively *bridging* them by adsorption (Gregory 1989). Polymer bridging is important from an applications standpoint because it strengthens and enlarges the floc (Gregory 1989). Strengthening helps flocs resist destabilization caused by shear forces in the filters (Ghosh et al. 1985). Ideally, a large portion of the floc surface is free of adsorbed polymer and extends into the water (fig. 11a). Adsorption of excess polymer inhibits bridging by reducing the surfaces available for contact (fig. 11b). Consequently, polymers of all types are best entrained at an optimal dose, which ordinarily is related to the concentration of POC (Gregory 1989).

Cationic polyelectrolytes (positively charged polymers) reduce the concentrations of humic and fulvic acids. The removal mechanism is probably a variant of the electrostatic patch effect, although bridging may also be important under certain conditions (Gregory 1989). The configuration of adsorption by the polyelectrolyte onto a negatively charged particle is such that "electrostatic patches" of excess charge occur on unoccupied surfaces (fig. 12).

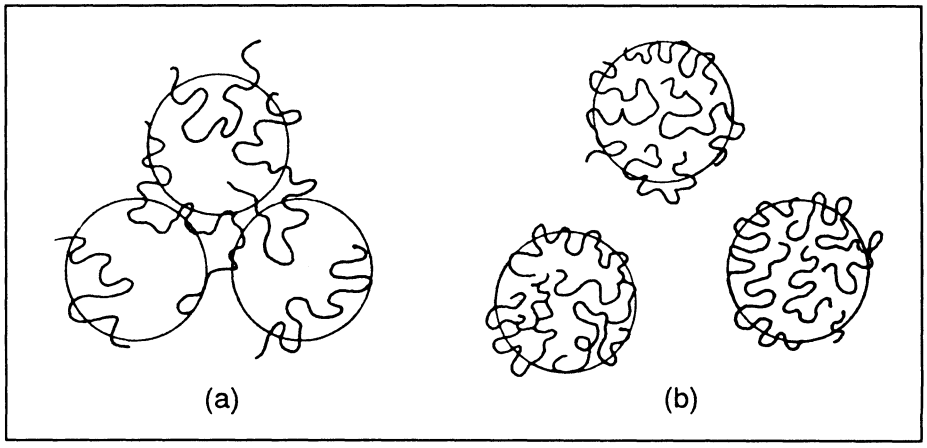

***Figure 11.**—Schematic illustration of (a) bridging flocculation and (b) restabilization by excess adsorbed polymer.* Source: *Gregory (1989).*

Unfortunately, no reliable method of selecting the appropriate polymer exists (Ghosh et al. 1985). Moreover, particulate organic compounds in marine mammal pool waters have not been described. The following comments are, therefore, speculative. The waters contained in most closed systems used to house marine mammals are dilute suspensions (i.e., low POC concentration) of high color potential. According to Amy and Chadik (1983), the best waters for coagulation with polyelectrolytes have low POC levels, moderate to high color, and low pH (6.5 to 4.5). If negatively charged particles predominate in marine mammal pool waters, as I shall assume, cationic polyelectrolytes are the polymers of choice. These materials should be selected on the basis of charge density, not molecular weight. The molecular weight of a polyelectrolyte has little influence on performance (Gregory 1989). The quantity required for optimal flocculation is roughly that needed to reduce the surface charges of the particles (zeta potential) to zero. As such, the most effective polyelectrolytes have the highest charge densities (Gregory 1989). Polyelectrolytes of low molecular weight (approximately 30,000), however, are sensitive to overdosing, resulting in restabilization (i.e., the reverse of colloid destabilization) of the organic acid and failure to remove it from solution (see literature cited in Amy and Chadik 1983). In addition, low molecular weight polyelectrolytes are themselves precursors of THM if an unprecipitated residual remains after treatment (Kaiser and Lawrence 1977).

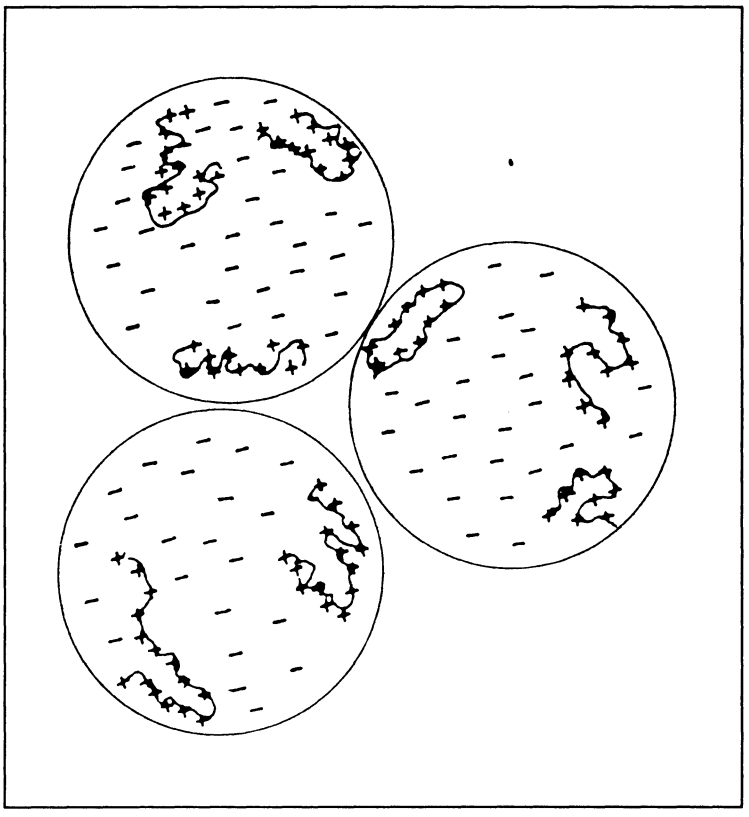

Figure 12.—Electrostatic patch model for interaction of negatively charged particles with adsorbed cationic polyelectrolyte. Source: *Gregory (1989).*

8.5 Bacteria as Colloids

Bacteria possess colloidal characteristics, including certain light-scattering and surface-charge properties, that place them in the "coarse dispersion" category of colloid classification (Rubin et al. 1969). The surfaces of bacteria are negatively charged, making them susceptible to coagulation by inorganic cations and cationic polyelectrolytes. In this respect, bacteria conform with the Schulze–Hardy rule, which states that the coagulating power of a counter ion increases with its charge. Magnesium and other divalent cations in seawater enhance the coagulation of suspended bacteria and hasten their removal.

8.6 Removal by Packed Column Aeration

Trihalomethanes are volatile and amenable to removal by aeration (Roberts and Levy 1985, Roberts et al. 1984, Umphres et al. 1983), so long as the process design promotes efficient air-water contact to enhance the rate of mass transfer between the liquid and gas phases (Roberts and Levy 1985). For THM removal to be efficient, in other words, the air:water ratio must be very high. This eliminates from consideration simple aeration and also airstripping, which relies on injection of fine air bubbles into a water-filled column (Spotte 1979). In both processes, rising air bubbles become saturated rapidly with THM. Because the air:water ratio is low, adsorbed compounds are not driven into the atmosphere (Roberts et al. 1984).

Packed column aeration is an effective means of reducing THM in wastewaters and drinking waters. Until now, no one has suggested using the process to remove these compounds from marine mammal pool waters. Operating principles of the packed column aerator are simple; from an operational standpoint, the units are trouble free and energy efficient. A typical design consists of a tower made of fiberglass-reinforced plastic (fig. 13) filled with plastic packing (fig. 14). A centrifugal fan blows air upward into the tower as water trickles down. Treated water is collected in a plenum at the base of the column and recycled to the bulk fluid.

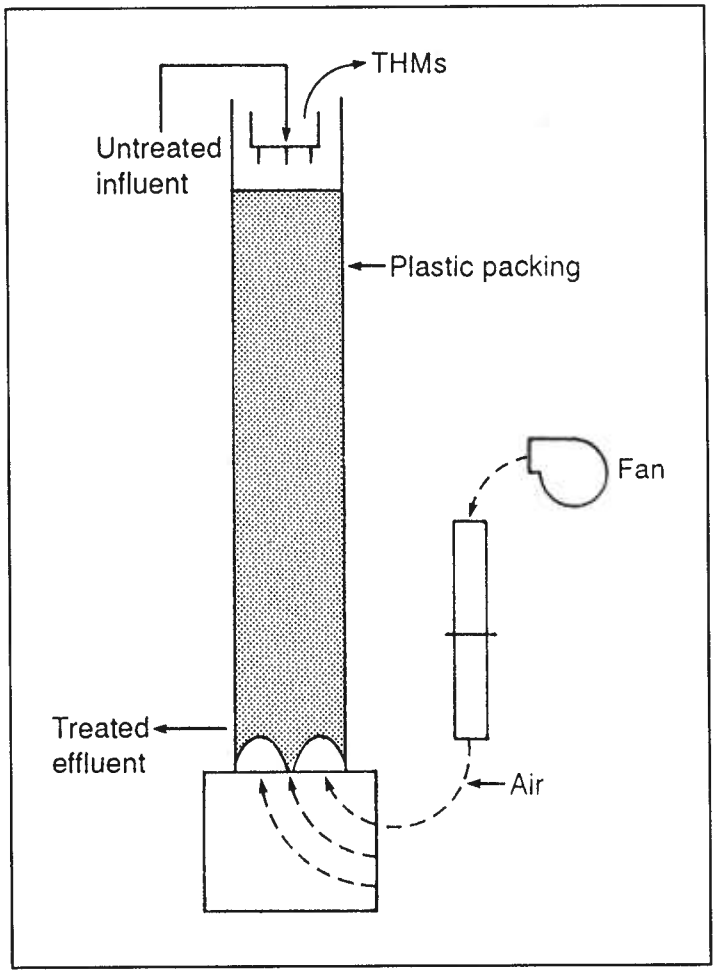

Figure 13.—Diagrammatic illustration of a packed column aeration device for removal of trihalomethanes (THM) and other highly volatile organic compounds from water. Source: *Modified from Umphres et al. (1983).*

Figure 14.—Representative plastic packing, in this case in the shape of cylinders. Source: Norton Chemical Process Products, Akron OH.

Packed column aeration also removes nitrogen trichloride and other compounds of high volatility. Nitrogen trichloride typically forms during conditions of low pH (<4.4) but appears in chlorinated waters at higher values (to pH 8) if the initial chlorine:nitrogen ratio is large (reaction 10). Nitrogen trichloride is always associated with excess amounts of free chlorine and never with large amounts of monochloramine (Atkinson and Palin 1973). Nitrogen trichloride imparts an unpleasant odor to treated water reminiscent of poorly managed indoor swimming pools. Removing it is desirable.

8.7 Removal by Other Processes

At least 50 processes have been documented for use in the removal of organics from wastewater effluents, the most effective of which is reverse osmosis (DeWalle et al. 1982). This process removes precursors of THM (Taylor et al. 1987), but nothing has been published on its potential for the treatment of marine mammal water systems. Ordinarily, reverse osmosis is associated with high pumping costs. Another highly rated process in wastewater treatment is the physical adsorption of organics onto polymeric resins. In the only study in which marine mammal water (chlorinated brine) was evaluated, the two polymeric resins tested were ineffective: both reduced the concentration of TOC by 9 percent, but only after 96 and 77 h (Adams and Spotte 1982).

9.0 Trihalomethanes and Risk Assessment

The use of reactive chemicals to sterilize water for consumption by humans or to maintain captive marine mammals involves possible long-term risks, although any assessment of the degree of risk is meaningful only if considered within a rigid contextual framework. Confounding variables inherent in epidemiological data are among the factors that impinge on this framework. The credibility of bioassays is weakened by extrapolation of results across species lines (e.g., laboratory rodents to humans) and by the uncertain correlation between mutagenesis and carcinogenesis. Risk assessment carries with it the inherent danger of simplistic rulemaking. Procedures used to assess risk in the public health sector are designed to address the single question of whether the hazard has potentially serious consequences for everyone (Bull and McCabe 1985). However, known benefits should also be considered. In the case of treated drinking waters and wastewaters, these benefits involve a marked reduction in waterborne diseases and are well documented (Craun and Gunn 1979, Haas 1986). Any health hazards, in contrast, can only be inferred (Bull and McCabe 1985). A comparable risk assessment where captive marine mammals are concerned is not possible at this time because (*1*) the presumed advantage of sterile water (i.e., reduced incidence of waterborne infectious diseases) has not been evaluated experimentally, and (*2*) the relative incidence of mutagenesis and carcinogenesis in wild versus captive marine mammals is unknown.

At issue is whether THM impair the health or shorten the lifespans of captive marine mammals by placing them at risk. Extensive epidemiological data on human risk show no clear trend with increasing exposure (i.e., dose-response gradient) to THM in drinking waters (Crump and Guess 1982). Although comparable data for marine mammals have not been gathered, the eventual emergence of a clear trend is no less doubtful. Before presumed dangers of THM to captive marine mammals culminate in unwarranted concern, it might be prudent to consider the context in which toxicological data are obtained and then used in risk assessment.

9.1 Mutagenicity and Carcinogenicity

Regulations governing the *maximum tolerated dose* of suspected hazardous chemicals (i.e., the highest dose administered without causing obvious toxicity) rely heavily on rodent bioassays and presume a strong positive correlation between mutagenicity and carcinogenicity (see glossary). The conceptual basis of this relationship is badly flawed, viewed either as a mechanistic link at the cellular level or by extrapolation from bioassay data to humans and other species.

Not surprisingly, the use of rodent bioassays to decide maximum tolerated dose has come under strong attack. According to Ames and Gold (1990), "more than half of all chemicals tested (both natural and synthetic) are carcinogens in rodents, and a high percentage of these carcinogens are not mutagens." Abelson (1990) described the situation more bluntly: "The principal method of determining potential carcinogenicity of substances is based on studies of daily administration of huge doses of chemicals to inbred rodents for a lifetime. Then by questionable models, which include large safety factors, the results are extrapolated to effects of minuscule doses in humans." These comments set the stage for a brief discussion of what the presence of mutagens and carcinogens—known or potential—might mean in marine mammal husbandry.

The principal limitations of long-term (chronic) animal bioassays are inherent insensitivity and variability (Wilkinson 1987). These are compounded by uncertainty associated with extrapolating data across species lines and different sets of conditions (e.g., high dose and short duration compared with low dose and long duration). Test animals are routinely exposed to high doses of suspected toxicants in an attempt to increase the sensitivity of the tests. However, in a group of 52 chemicals determined to be positive in the National Toxicity Program in which

animals were exposed under long-term conditions, two-thirds would not have fallen into this classification had the maximum tolerated dose been 50 percent of the concentration used (Wilkinson 1987). Wilkinson asked: "Does this increased 'power of detection' really reflect true carcinogenic potential, or is it a false positive resulting from cytotoxicity or dose-dependent differences in metabolism and pharmacokinetics? Does it have any relevance to assessing low-dose effects in any species?"

Data used to classify potential mutagens and carcinogens in the environment have been obtained from (*1*) *in vitro* short-term tests, (2) long-term animal bioassays mainly with rats and mice, and (*3*) epidemiological studies. *Mutagenicity testing* measures the capacity of a chemical to alter DNA. *Short-term tests* are mutagenicity assays targeted to bacteria or mammalian cell cultures. They measure *potential* carcinogenicity on the premise that an important event in both mutagenesis and carcinogenesis is alteration of DNA (Meier and Daniel 1990). A mutagen (in this context a potential carcinogen) identified by short-term testing can then be concentrated and fed to rodents as a test of carcinogenicity. However, long-term rodent bioassays can extend to 2 years, making them expensive. Consequently, preliminary screening by short-term testing has gained favor. Short-term tests are poor quantitative predictors of risk, and the results have limited use in risk assessment (Meier and Daniel 1990). It bears mentioning that, when extrapolated to humans or marine mammals, short-term test results are even less applicable than results of rodent bioassays.

More than 200 short-term tests are currently available (Meier and Daniel 1990). One used widely for identifying mutagens in water is the Ames *Salmonella* assay (or simply the Ames test). Information in this paragraph pertains to findings derived from its application as summarized by Noot et al. (1989). The amount of mutagenic material produced by water sterilization methods follows, in relative order, ozone < chlorine dioxide < chloramines < chlorine. In general, chlorination produces high levels of mutagenic activity; the use of chloramines or chlorine dioxide results in less activity. Data from ozonation experiments are inconsistent and have yielded mutagenicity levels ranging from below detection to the equal of chlorination. In several experiments, the concentrations of ozone required to eliminate mutagenicity ranged from 10 to >33 mg/L. Such levels not only exceed operational limits but are potentially hazardous to humans and marine mammals.

At least four factors, two involving the Ames test, suggest caution when comparing the results of ozonation and chlorination: (*1*) the Ames test correlates less well with carcinogenicity than thought previously (Glaze et al. 1989), (*2*) mutagenicity data from complex solutions (e.g., all natural waters) are extremely difficult to interpret (Glaze et al. 1989), (*3*) preconcentration methods may recover ozonation byproducts less well than byproducts of chlorination (Glaze et al. 1989), and (*4*) *Salmonella* strains vary widely in sensitivity (Noot et al. 1989).

9.2 The Risks Drinking Waters Pose to Humans

Oxidant reaction products in water have been tested for mutagenic and carcinogenic potential in organisms representing a number of phyla. Attendant to the idea of relevance in bioassay is the implication that the substance evaluated follows the same biochemical pathways in test organisms as in the organism of concern (e.g., humans, marine mammals); otherwise, the toxicological endpoints are not comparable (Wolff 1977). Most carcinogens require metabolic activation, and correct interpretation of bioassay results is influenced strongly by the test organism selected to supply metabolizing enzymes (Stevens et al. 1985). For example, the carcinogen dimethylnitrosamine is mutagenic in *Salmonella* in the presence of hamster liver S-9, but not when the S-9 is derived from mice or rats (Prival and Mitchell 1981). In addition, any apparent association between exposure and disease is clouded by observational bias if the test organisms were similar but experimental designs differed (Craun 1985).

If animal bioassay data are used to make a case for cancer in humans, the assessed risk is not alarming. One summary (Crump and Guess 1982) provided the following information based on a number of epidemiological studies conducted in high-risk locations in the United States. Initially, low-dose carcinogenic potencies from animal bioassays were estimated. Next, animal risk estimates were converted to "human values" according to surface-area differences between the bioassay animals and humans. Calculated carcinogenic potencies were then used to estimate an upper limit of human risk for each chemical at its maximum drinking-water concentration within the most highly contaminated areas of the country. When the data were collated, the resulting estimate of human lifetime excess risk was <0.1 percent. The national lifetime risks for rectal, colon, and bladder cancer—cancers most commonly associated with carcinogens in drinking waters—are each at least 1 percent. Consequently, a lifetime risk of <0.1 percent corresponds to less than a 10-percent increase in human cancer risk at any of the locations studied. The authors concluded that these values had probably been overestimated. For example, low-dose animal potencies were estimated from upper confidence limits based on a model that assumes a linear increase in risk associated with low-dose levels. Many bioassays are conducted at megadose levels for brief periods, frequently many orders of magnitude above concentrations likely to be encountered in realistic situations over a period of years, even if cumulative toxicity is taken into account. Actual concentration and temporal effects are overwhelmed, or at least masked. In one study (Scully et al. 1985), 4 mL of sodium hypochlorite at concentrations of 200 or 1,000 mg/L were administered by gavage to rats of 250-400 g, then recovered within 30 min. The purpose was to determine if *N*-chloramines are formed and subsequently enter the blood after ingestion of HOCl. Both processes occurred. Taken at face value, these conclusions would be cause for concern. However, bioassays by design are reductionistic. The study just described was intended to test a possible physiological mechanism. Nothing else was suggested by the authors, and nothing more can be assumed. Sodium hypochlorite is a powerful oxidant. In drinking waters and marine mammal pool waters, concentrations of free chlorine seldom exceed 0.5 mg/L. Hypochlorous acid is one of two reaction products of hydrolysis, and its concentration is even less.

Among other limitations, short-term tests that use megadose concentrations sidestep the possibility that many cancers are induced indirectly as a result of numerous variable factors interacting subtly in inhibitory, additive, synergistic, and antagonistic combinations. In humans, cancers associated with consumption of chlorinated waters over many years often do not appear until age 60 or more (Cragle et al. 1985)—in other words, until nearly a lifetime of daily ingestion of water containing tiny amounts of carcinogens.

If a particular attribute of a toxicant is associated with a consistently observed effect (e.g., the appearance of a disease), and the same effect is seen after exposure, data relating exposure to effect may convey a false appearance of association because of confounding bias. Experimental error is not the problem; rather, confounding bias is a basic element of all epidemiological studies (Craun 1985). In an extensive review of epidemiological findings, rectal, bladder, and colon cancer risks associated with chlorinated drinking waters were 1.1 to 2.0 times higher than the risk from unchlorinated water (Crump and Guess 1982). These discrepancies are sufficiently large to attract notice, but small enough to be inseparable from confounding risks related to other environmental factors (e.g., smoking, diet, air pollution, occupation, pollution by agricultural and industrial runoff in different drinking waters within the test zones).

Failure to control confounding variables in public health risk assessment raises some astonishing possibilities. Epidemiological studies involving the risk of chlorinated reaction products in drinking waters provide an interesting example. Crump and Guess (1982) noted that when the data are collated, the degree of risk actually exceeds the portion attributable to THM, even assuming that *all* THM have the same carcinogenic potency as chloroform.

The notion of what constitutes an adequate margin of safety with respect to toxicant exposure requires careful evaluation. If the data are appropriate and reliable, a safety margin of 10 could be applied in the case of humans to the maximum no-effect dose to obtain a maximum "safe" level (Pendygraft et al. 1979). When applied to chloroform ingestion, the maximum no-effect concentration for liver damage in humans is 0.3 to 0.9 mg/(d•kg). A safety margin of 10 applied at the low end of the curve gives a value of 0.03 mg/(d•kg), or 2.1 mg of chloroform for a 70-kg person. When the compound in question is a carcinogen, a safety margin of 5,000 is applicable to the minimum-effect dose. If data for kidney carcinoma are considered as an example, and 90 mg/(d•kg) is estimated as the minimum-effective dose, the maximum daily dose becomes 0.02 mg/(d•kg), or 1.4 mg/d for a 70-kg person. This value is assumed to represent a risk to human health that is either nonexistent or negligible (Pendygraft et al. 1979).

9.3 Assessment of Risk to Marine Mammals

If the values cited above are applied to a closed-system marine mammal pool for which data are available, and certain assumptions are made, a qualitative statement can be made about possible maximum-effect doses of THM. The next paragraph represents an exercise in logic and nothing else: THM concentrations have not been measured in marine mammal pool waters, and marine mammals are not humans.

The water system (system 1 described previously; Adams and Spotte 1980, Spotte and Adams 1979) was 1.55×10^6 L and contained eight marine mammals (four cetaceans, four pinnipeds) with respective total masses of 2,747 and 343 kg. The TOC concentration increased by 0.23 mg/(d•L) to about 12 g/L after 30 d (1.8×10^2 g total). Assume for sake of discussion that 2 percent of the TOC consisted of chloroform—an inordinately high value—and that the animals drank pool water containing 10 mg/L TOC. The upper concentration of TOC in most U.S. drinking waters is <5 mg/L, as stated previously (Condie and Bercz 1985); chlorinated Missouri River water contains chloroform levels of <0.1 to 311 µg/L (Stevens et al. 1976). In this example, I selected a middle value of 100 µg/L $CHCl_3$ in 5 mg/L TOC (2 percent of the TOC). In comparison, the relative proportion in the water system was considered to be 2 percent of 10 mg/L TOC, or 0.2 mg/L $CHCl_3$. Finally, assume a maximum-effect dose of 0.02 mg/(d•kg), which is believed to constitute no risk to humans (sect. 9.2). By extrapolation, the value is 6.86 mg/d for each animal in the system. To obtain this dose and still remain in the no-risk category, an animal would have to drink 33 L of pool water daily.

A bottlenose dolphin (*Tursiops truncatus*) of 150 kg is estimated to ingest about 1.5 L/d if maintained in freshwater and about 0.5 L/d in seawater (Ridgway 1972). Seawater ingestion by two captive common dolphins (*Delphinus delphis*) of 55 and 59 kg was estimated at 12 and 13 mL/(d•kg) (Hui 1981). If values for these species can be considered typical, it is unlikely that 33 L/d is ever ingested, but even so an affected animal would still fall within the safe dose category even in waters containing chloroform far in excess of expected concentrations.

The threat to human health represented by undesirable chemicals in drinking water is minimal (sect. 9.2). Logically, risks to marine mammals immersed in waters containing these compounds also are minimal, if they exist at all.

10.0 Salinity and pH

With the exception of temperature, salinity and pH are perhaps the most frequently measured operational factors in marine mammal husbandry. I shall discuss them from the standpoint of their theoretical properties and importance—if any—as isolated measurements. "Ideal" salinity and pH values do not exist for two reasons. First, any effort to identify them would be confounded by too vast an array of variables. Second, because the effects are both interactive and multifactorial, what constitutes an ideal value in one situation is nonideal in another. This is especially true of pH. Captive marine mammals can survive in apparent good health throughout a wide range of salinity and pH values.

10.1 Salinity

By classic definition, *salinity* is the total mass in grams of all dissolved substances in a kilogram of seawater after carbonate has been converted to oxide, bromine and iodine are replaced with chlorine, and the organic matter has been oxidized at 480 °C. The four constituents just named are present in seawater in minor concentrations (i.e., <1 mg/kg). Consequently, salinity varies only slightly (<0.05 percent) from the total mass of dissolved solids. This relationship does not necessarily hold true for most artificial seawaters because carbonate, bromine, iodine, and organic matter may not match seawater concentrations (Bidwell and Spotte 1985). Paired samples of seawater and artificial seawater, in other words, can have the same measured total solute concentration and the same ionic strength but differ in aspects of chemical composition essential to the definition of salinity. As such, the term is appropriate only when referring to seawater but never in reference to artificial seawaters and brines (Bidwell and Spotte 1985).

The "salinity" of a sample of seawater can be measured indirectly with a salinometer or refractometer, or by conductivity. These instruments can also be used to determine the salinity equivalent of an artificial seawater or brine. Hydrometer readings with a temperature correction (i.e., specific gravity) are appropriate for all saline waters. The temperature correction step is rigorous (Spotte in press), poorly understood by husbandry personnel, and seldom performed correctly. Specific gravity can be converted to salinity or salinity equivalents by procedures in Spotte (in press).

10.2 pH

There can be no such factor as "average pH." The notion of a series of pH values centered about the mean is inherently incorrect. Measuring the pH of a series of water samples and then dividing by the number of measurements yields a numerical value, but that value is not the mean. By definition, pH is the logarithm of the reciprocal of H^+ normality. The "p" stands for *puissance* (power), and "H" is the symbol for hydrogen. As noted by Kinney (1973), summing a series of pH values multiplies the reciprocals of the normalities of the hydrogen ion concentrations. Dividing by the number of samples gives the *n*th root of the product. The mean can be determined only by changing the original pH determinations to hydrogen normalities and dividing by the number of measurements. The logarithm of the reciprocal is then "mean pH." An acceptable pH for marine mammal pool waters (or any other waters) should obviously be stated as a range of values.

Isolated pH measurements have no direct utility. Knowing the pH of a marine mammal pool is useful only for controlling important chemical reactions that are pH-mediated. To state, for example, that the pH in two water systems declined by the same amount is misleading in terms of the possible effects (e.g., chlorination reactions), unless the starting values were identical. Consider water systems with starting pH values of 8.5 and 7.5. Over time, the pH in both declines to final values of 8.0 and 7.0. The change from 8.5 to 8.0 resulted in an increase in the hydrogen ion concentration of 6.8×10^{-9} mol/L; the change from 7.5 to 7.0 produced an addition 10 times this amount even though the unit change was the same (0.5 pH unit).

The practical effect of pH on chlorination is substantial; the effect of temperature is less (fig. 15). As shown, the concentration of free available chlorine (FAC) required to sustain 0.5 mg/L HOCl rises steeply with increasing pH. As a sterilizing agent, HOCl is far more effective than OCl^-, but its proportion of the FAC—and consequently its concentration—diminishes as the pH rises. A greater mass of chlorine-based oxidant must be added to waters of high pH to achieve adequate sterilization. The figure shows that at 20 °C and pH 8.5, approximately 5.8 mg/L FAC are required to maintain 0.5 mg/L HOCl. In contrast, only 1 mg/L FAC is necessary at this temperature and pH 7.5.

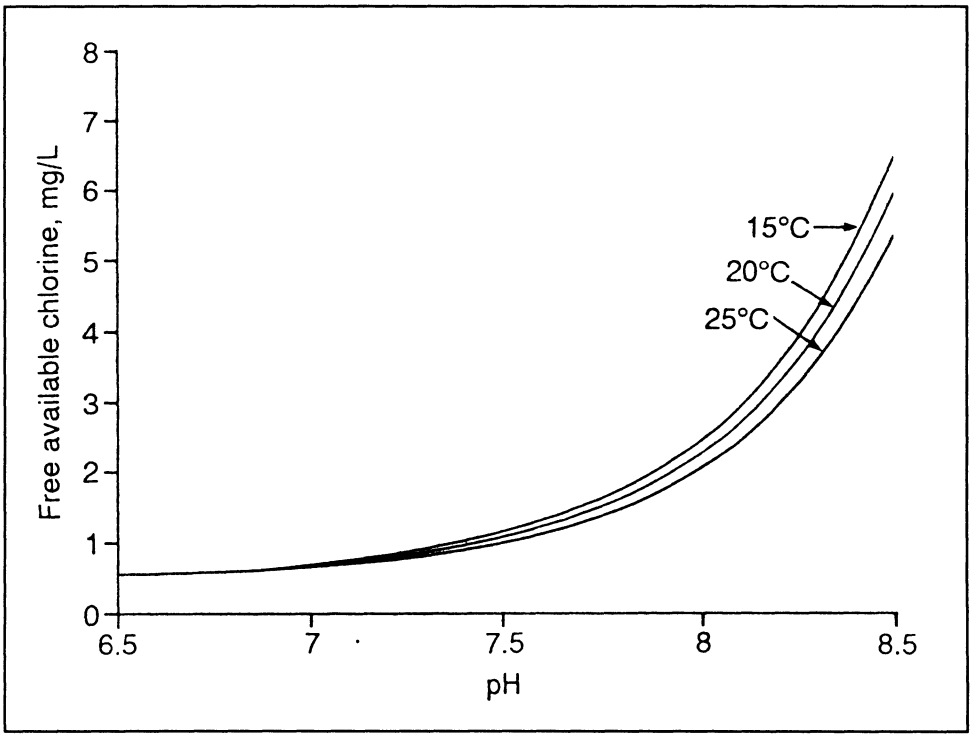

Figure 15.—Free available chlorine (FAC) concentration required to yield 0.5 mg/L HOCl at different temperature and pH values, excluding demand from ammonia and reducing compounds. Source: *Gary Adams, Sea Research Foundation, calculated from the dissociation constant of HOCl in freshwater (Rich 1973), which varies linearly from 2.0 x 10^{-8} at 0 °C to 3.7 x 10^{-8} at 25 °C.*

Greater concentrations of THM are produced at higher pH values (Stevens et al. 1989). This is caused partly by the greater mass of chlorine-based oxidant required to achieve sterilization (i.e., the oxidation potential of the water is increased). If THM are of concern, pH values should be kept near the low end of the working range.

The pH of offshore seawater is approximately 8.2, a fact of no demonstrated ecological or physiological relevance to marine mammals. Many species inhabit estuaries and other environments in which the pH is considerably less. Marine mammals have been managed safely over a wide pH range, at least 6.5 to 8.5, indicating that any direct effects of pH per se are relatively unimportant. The intuitive truth of this statement is evident from the pH scale, which is logarithmic. Water at pH 6.5 contains 10 times more hydrogen ions than it does at pH 7.5 and 100 times more than at pH 8.5. The pH range recommended to achieve efficient sterilization and flocculation is 7.0 to 6.0.

10.3 pH Control

Chlorination raises the pH of water slightly; flocculation with alum lowers it. The control of pH is therefore a dynamic process. The pH can be lowered with muriatic acid (hydrochloric acid) and raised by adding carbonate salts (sodium bicarbonate, $NaHCO_3$, or sodium carbonate, Na_2CO_3). In seawater and brackish waters, alkalinity is the sum of the analytical concentrations of the anions of carbonic and weaker acids. The only other weak acid of importance is boric acid. Alkalinity can be expressed

$$A = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4)] + [OH^- - H^+] \qquad (19)$$

where brackets represent molar concentration. Not uncommonly, the pH of closed and semiclosed marine mammal pool waters declines. This is often evidence that alkalinity has been depleted. Adjusting only the pH treats the effect of the problem but not its cause. Bicarbonate and carbonate salts raise both pH and alkalinity.

In recirculated waters, the long-term trend is toward acidity as alkalinity is depleted, especially if acidic flocculants (e.g., alum) are added continuously. In such situations there is danger of a sudden and precipitous fall in pH. Bicarbonate and carbonate salts restore alkalinity. The reaction between aluminum and bicarbonate can be expressed in simple form as

$$Al^{3+} + 3HCO_3^- \rightleftharpoons Al(OH)_3(s) + 3CO_3^{2-} \qquad (20)$$

where *s* denotes a solid (i.e., hydroxide precipitate). By this reaction, 10 mg of alum, $Al_2(SO_4)_3 \cdot 18H_2O$, consume 7.6 mg of $NaHCO_3$ (Letterman et al. 1979).

11.0 Conclusions

1 The sole function of granular media filtration is to reduce the concentration of suspended particles. This has cosmetic importance but is without known relevance to marine mammal health. A reliable method of measuring the concentration of particulate matter in water has not been devised. "Turbidity" is a meaningless measurement, having been based on the false premise that light extinction in turbidimeters is proportional to the particle concentration.

2 Truly sterile marine mammal pool water is unattainable, and no body of empirical evidence suggests that such an objective is even desirable.

3 The presence of thermotolerant coliform bacteria (TTC) in marine mammal pools is direct evidence of fecal contamination, and the number of these organisms is a suitable unit of measure. The number of total coliforms (TC) is less relevant because the sources of contamination can be extrafecal.

4 The utility of ultraviolet (UV) irradiation is probably limited to sterilizing contaminated influent waters of open and semiclosed systems.

5 The relative proportions of HOCl and OCl^-, the components of free chlorine, are highly pH dependent. The efficacy of chlorination diminishes rapidly at pH values >7.5 because disproportionation of free chlorine favors OCl^-, the weaker sterilizing agent.

6 Chloramines, although persistent oxidants, interfere with the efficacy of chlorination by increasing the quantity of chlorine-based oxidant required to achieve sterilization. On the positive side, chloramines are effective in reducing THM.

7 Sterilization of a water supply, whether it be drinking water, wastewater, or a marine mammal pool, involves compromises. No single process or combination of processes yields completely satisfactory results.

8 Chlorine-based oxidants and ozone are the principal sterilizing agents applied to marine mammal waters. None can be endorsed or dismissed unequivocally. Their efficacy depends on many variables, including process design elements (e.g., flow rate, contact time), composition of the water being treated (e.g., bromide and TOC concentration), and thermodynamic factors.

9 The filters and waters of closed-system marine mammal pools are sinks for organic compounds, and TOC increases linearly. The accumulated portion is refractory (resistant to biological and chemical degradation).

10 A reliable method of selecting the appropriate polymer for flocculation has not been devised. Alum and cationic polyelectrolytes would appear to be effective flocculants for marine mammal pool waters. Cationic polyelectrolytes should be selected on the basis of charge density, not molecular weight.

11 Packed column aeration, as a process technique for volatile DOC removal (including THM), has not been applied to marine mammal pool waters but should be effective. Packed column aerators are simple to design and operate, easy to maintain, and energy efficient.

12 The risk to marine mammals from ingestion of THM is minimal to nonexistent.

13 The operating pH of marine mammal pools should be stated as a range of values; "mean pH" does not exist in a conventional sense (i.e., a series of pH values cannot properly be averaged).

14 The notion that the natural pH of seawater (about 8.2) has survival value for marine mammals has no empirical basis.

15 Sterilization and flocculation processes are most effective at the pH range 7.0 to 6.0.

12.0 Summary

1 *Disinfection* is the destruction of infectious organisms; *sterilization* is the nonselective destruction of all life. Ultraviolet radiation, chlorine-based oxidants, and ozone kill micro-organisms nonselectively. Therefore, they are sterilizing agents.

2 Marine mammal water systems can be *open* (flowthrough), *closed* (filtered and recycled), or *semiclosed* (filtered and recycled with a small volume of replacement influent water, ordinarily <10 percent of the total volume per 24 h).

3 Marine mammals are maintained in waters of four generic types: freshwaters, brines (sodium chloride dissolved in tap water), artificial seawaters (some or all of the major ions dissolved in tap water), and seawater. Brackish water is dilute seawater.

4 The objective of water sterilization is to place the sterilizing agent in contact with micro-organisms by the most efficient means possible. Two methods are used: point-contact and bulk-fluid sterilization (see 11 and 12).

5 The presence of suspended micro-organisms is not necessarily a signal of impending disease. With effort, numerous microbial species can be cultured from marine mammal pool waters. These typically are forms shed by the animals themselves and their human attendants, or present in raw fish used as food. Wild and captive marine mammals support diverse microfloral populations.

6 The Animal Welfare Act stipulates that the most probable number (MPN) of "coliform bacteria" in marine mammal pool waters not exceed 1,000/100 mL. A measurement of "coliform bacteria" presumably includes the four genera of bacteria that are the total coliforms (TC): *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter*. Total coliforms have been isolated from soil, vegetation, forest and farm products, and many other environments of nonenteric origin, making them doubtful indicators of fecal contamination.

7 The fecal or thermotolerant coliforms (TTC), comprised mainly of *Escherichia* with a *Klebsiella* component, are more specific indicators of fecal pollution. *Escherichia coli* is the only coliform that is unquestionably enteric in homeothermic animals and has no extrafecal sources; consequently, it is the definitive bacterium for demonstrating fecal pollution of water, and TTC are preferable to TC for routine monitoring of contaminated waters.

8 Data needed to compute MPN are obtained by multiple tube fermentation. Sometimes membrane filtration (MF) is substituted for multiple tube fermentation. However, the two methods are not directly comparable because MPN falls outside a normal statistical distribution. An accurate comparison can be made only by transforming MPN to common logarithms, or applying a suitable statistical procedure to avoid transformation.

9 Coliforms survive poorly in aquatic environments. Their growth is inhibited in seawater (particularly aged seawater), in chlorinated freshwaters, by high concentrations of heterotrophic bacteria and particulate matter, by toxic ingredients in selective culture media, and by injury. Marine mammal pools are sometimes characterized by high particle counts and large populations of heterotrophs, are chlorinated, are of high ionic strength, and are aged (filtered and recycled). Not surprisingly, any combination of culture medium and procedure potentially underestimates the number of coliforms actually present, a situation consistent with water pollution monitoring in general.

10 Pathogenic bacteria have been isolated from drinking waters in the absence of detectable coliforms. It should be recognized that pathogens could be present in marine mammal pools when coliforms are low in number or absent, but this is a separate issue. The intent of the "coliform bacteria" section of the Animal Welfare regulations was to establish standards for monitoring the extent of water contamination, not the presence of waterborne pathogens. Moreover, the principal problems of detection are not eliminated by substituting another group of indicator organisms.

11 *Point-contact sterilization* kills micro-organisms at a central source. Water diverted in a side stream is placed in brief but continuous contact with the sterilizing agent, then recycled to the *bulk fluid* (the main part of the system where animals reside). Point-contact methods have limited utility unless the numbers of micro-organisms in the bulk fluid are simultaneously lowered to acceptable limits. This is true even if 100 percent of the micro-organisms at the contact site are killed. Ultraviolet (UV) radiation (all waters) and ozone (freshwaters and brines only) are point-contact sterilizing agents.

12 *Bulk-fluid sterilization* kills micro-organisms throughout the entire water system and relies less on flow rate, side-stream configuration, and other engineering constraints. Chlorine-based oxidants are bulk-fluid sterilizing agents, as is ozone applied to waters containing bromide (seawater, brackish waters, and some freshwaters). In the presence of bromide, ozone forms persistent oxidants that serve as weak bulk-fluid sterilizing agents.

13 Chlorine-based oxidants react with water to form hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), together called *free chlorine* and determined analytically as *free available chlorine* (FAC). Hypochlorous acid is by far the superior sterilizing agent.

14 Chlorine-based oxidants react with numerous inorganic and organic reducing agents, resulting in increased chlorine demand and reduced efficacy (i.e., percentage kill of micro-organisms).

15 In seawater, brackish waters, and some freshwaters, bromide reacts with chlorine and increases chlorine demand.

16 Chlorine-based oxidants react with organic compounds to produce *trihalomethanes* (THM)—chloroform, bromoform, bromodichlorimethane, and dibromochloromethane—which are mutagens and carcinogens. Chloroform is the principal trichloromethane formed in freshwaters.

17 The most important precursors of THM are humic substances, mainly humic and fulvic acids. These constitute 30–50 percent of the dissolved organic carbon in natural waters.

18 Hypochlorous acid reacts with free ammonia (NH_3) to form monochloramine (NH_2Cl). Subsequent reactions yield dichloramine ($NHCl_2$) and nitrogen trichloride (NCl_3). In sum, these are *combined chlorine,* determined analytically as *combined available chlorine* (CAC).

19 Combined chlorine is a persistent oxidant and continues to sterilize the bulk fluid after free chlorine has been consumed. However, the oxidation potential is less than that of free chlorine.

20 Bromine oxidation predominates in seawater, with subsequent production of bromamines. Like chloramines, bromamines are persistent oxidants of limited oxidation potential.

21 Chlorine dioxide (ClO_2) offers several advantages over conventional chlorination: it does not yield THM directly, is a strong sterilizing agent over a broader pH range, does not react with ammonia to form chloramines, and is often more effective at lower dosages and shorter contact times.

22 Chlorine dioxide has several disadvantages, including instability. As such, it must be generated on site.

23 Ozone (O_3) must also be generated where it is to be used. Ozone reacts strongly and preferentially with bromide in seawater and brackish waters to form hypobromous acid (HOBr) and hypobromite ion (OBr^-).

24 Hypobromous acid reacts with organic substances to produce mutagenic and carcinogenic compounds (e.g., bromoform, formaldehyde). These are perhaps more toxic than reaction products of chlorination.

25 Trihalomethanes are reduced by techniques that lower the concentration of total organic carbon or TOC (the sum of dissolved organic carbon, DOC, and particulate organic carbon, POC). In the process oxidant demand is lowered and the efficacy of sterilization increased.

26 Complete removal of humic substances prior to chlorination or ozonation is not possible, although activated carbons effectively adsorb THM, precursors of THM, and chloramines. High cost prohibits the use of activated carbons in the treatment of marine mammal pool waters.

27 Ozonation decolorizes water rapidly. However, at typical dosage levels and contact times organic matrices are not oxidized completely, and TOC is not reduced. Pretreatment with ozone results in increased adsorption of DOC by activated carbon. Ozonation also enhances mineralization of DOC by bacteria in the filters.

28 Flocculants remove TOC, lowering oxidant demand and potentially reducing the amount of oxidant necessary to achieve adequate sterilization.

29 Alum and cationic polyelectrolytes (positively charged polymers), two common flocculants, should be effective in the removal of humic and fulvic acids from marine mammal pool waters.

30 Bacteria possess colloidal characteristics, making them susceptible to flocculation.

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Glossary

Activated carbon Adsorbents manufactured from a number of carbon-based materials (e.g., coal, animal bone, wood). Manufacturing starts with char formation to drive off hydrocarbons. This is followed by activation, during which the material is reheated to about 900 °C in the presence of an oxidizing gas.

Active bromine Sum of hypobromous acid (HOBr) and hypobromite ion (OBr^-).

Adsorb To take up (as molecules) and hold on the surface of a solid or liquid.

Adsorbate Substance adsorbed during a physical adsorption process. During DOC-activated carbon contact, DOC is the adsorbate.

Adsorbent Substance adsorbing the adsorbate. During DOC-activated carbon contact, activated carbon is the adsorbent.

Alkalinity Net negative charge of all ions that interact with hydrogen ion. Important in long-term stability of pH. Maintained by addition of sodium bicarbonate or sodium carbonate.

Alum The octadecahydrate form of aluminum sulfate, called cake alum or patent alum, used as a flocculant for clarifying water.

Ames test Short-term test of mutagenicity based on *Salmonella.*

Ammonia In aqueous solutions, the sum of free ammonia (NH_3) and ammonium ion (NH_4^+), ordinarily expressed as the nitrogen component (total NH_4-N).

Brine Industrial grade sodium chloride dissolved in tap water.

Bromamines Mono- and dibromamine (NHBrCl and NBr_2Cl).

Bulk fluid Main portion of a water system (e.g., the portion where the animals reside).

Bulk-fluid sterilization Process method in which all sections of the bulk fluid are sterilized simultaneously. Examples are chlorination of all waters and the ozonation of waters containing bromide.

Carcinogen An agent that promotes development of cancer.

Chloramines Constituents of the combined chlorine.

Chloramination Flash-mixing of a chlorine-based oxidant (e.g., NaOCl) with ammonia to yield reaction products of extended oxidation potential.

Chlorine-based oxidants Sodium hypochlorite (NaOCl), chlorine gas (Cl_2), mono- and dichloramine (NH_2Cl, $NHCl_2$), chlorine dioxide (ClO_2), and related chlorinated compounds used in water sterilization.

Chlorine demand Reaction of chlorinated compounds with inorganic and organic reducing agents. The generic term is oxidant demand. Oxidant is consumed at the expense of sterilization.

Chlorine dioxide (ClO_2) Oxygenated chlorine gas produced on site by reacting a strong chlorine solution (500 to 1,000 mg/L Cl_2) with sodium chlorite at pH 2.5.

Closed system System in which all water is recycled through granular media filters and returned to the pool. Also see Open and Semiclosed systems.

Colloid A phase dispersed so extensively that surface forces are important in defining its properties. Colloids range from 1 nm to 1 μm.

Colloid destabilization Principal process initiating flocculation.

Combined available chlorine Analytical determination of combined chlorine (see below).

Combined chlorine Sum of monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and nitrogen trichloride (completely substituted ammonia, NCl_3).

Contact time Length of time water is retained in a closed space (i.e., a contact chamber) for purposes of adsorption or point-contact sterilization.

Disinfection Selective distruction of infectious organisms. Also see Sterilization.

Dissolved organic carbon (DOC) Fraction of the total organic carbon (TOC) that passes through a filter of stipulated pore diameter (e.g., 0.45 μm).

Electrostatic patch effect An alternative mechanism to polymer bridging in which a polyelectrolyte adsorbs an oppositely charged particle forming "patches" of excess charge. Also see Polymer bridging.

Efficacy As used here, the percentage reduction of micro-organisms or measured reduction in the concentration of a class of chemical compounds (e.g., TOC).

Escherichia coli Principal fecal or thermotolerant coliform; the only coliform unquestionably enteric in homeothermic animals and without extrafecal sources.

Fecal coliforms See *Escherichia coli* and Thermotolerant coliforms

Flocculation Alteration of organic matter in water by precipitation following colloid destabilization. Flocculated particles are larger in size and more readily trapped in granular media filters.

Flocs Permanent aggregates resulting from flocculation.

Free available chlorine (FAC) Concentration of free chlorine (see below) determined analytically.

Free chlorine Sum of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). The relative proportions of HOCl and OCl^- depend primarily on the pH of the solution. The concentration of OCl^- increases markedly above pH 7.5, and the amount of HOCl falls proportionately.

Fulvic acid Fraction of the humic material in water that is water-soluble and stays in solution after humic acid has precipitated. Also see Humic acid.

Granular media filtration Filtration by sand (rapid sand filtration); a combination of anthracite and sand (dual media filtration); or anthracite, sand, and garnet (multimedia filtration).

Humic acid Fraction of the humic material in water that is soluble in dilute base and insoluble in alcohol and acid. Also see Fulvic acid.

Maximum tolerated dose (MTD) The highest dose that can be administered to a laboratory animal without causing excessive weight loss or other life threatening signs of toxicity.

Membrane filter (MF) method Method of bacterial culture in which a measured volume of water is drawn by vacuum through a membrane filter of known retentive capability. The filter is placed on agar culture medium or on a cellulose pad saturated with liquid culture medium. Bacterial colonies are counted directly and expressed as the number of colony-forming units/ 100 mL of water. The MF method provides results in less time than the MPN method and yields direct counts of coliform bacteria. It is a standard method and accepted by Federal regulatory agencies responsible for water pollution monitoring. Also see Most probable number (MPN).

Molal scale Concentration scale (mol/kg of water), which is temperature- and pressure-independent.

Molar scale Concentration scale (mol/L of final solution), which is temperature- and pressure-dependent.

Most probable number (MPN) Estimate of viable numbers of bacteria (e.g., coliforms) after incubation in liquid medium using the multiple tube fermentation technique. The sample ideally is diluted to a level at which some (but not all) portions contain a bacterial cell. A series of tubes is inoculated with identical portions at the dilution, some with cells and some without. After incubation, those with cells show turbidity and gas production. By counting the number of tubes indicating gas production at each dilution a viable count can be estimated using statistical tables. The MPN method requires more glassware, culture media, and time than the membrane filter method. It is a standard method and accepted by Federal regulatory agencies responsible for water pollution monitoring. Also see Membrane filter (MF) method.

Mutagen An agent that induces mutation.

Multiple Tube fermentation Method of bacterial culture based on most probable number (MPN).

Muriatic acid A solution of hydrogen chloride gas (HCl) in water. Also called hydrochloric acid. Added to marine mammal pool waters to lower the pH.

Open system System in which water enters from a natural source or city tap, flows through a pool, and exits to waste. Also see Closed and Semiclosed systems.

Ozone (O_3) Allotrope of oxygen; solubility in freshwater at 20 °C is 0.57 g/L. The odor of ozone gas is pungent and fresh at concentrations <5 mg/L, unpleasant and acrid at higher levels. Toxic in gaseous phase. Concentrations of 0.02 to 0.05 mg/L are easily detected, but olfactory fatigue develops quickly. In humans, coughing is the first symptom; severe effects include depression, cyanosis, nausea, and pulmonary edema. Rooms with ozone generators should be well ventilated.

Packed column aeration Process method for removing volatile organics (e.g., trihalomethanes, nitrogen trichloride) from water. A packed column aerator consists of a fiberglass-reinforced plastic tower filled with plastic packing. A centrifugal fan blows air upward into the tower as water trickles down. Treated water is collected in a plenum at the base of the column and recycled to the bulk fluid. A very high air:water ratio promotes mass transfer from solution to the atmosphere.

Particulate organic carbon (POC) Fraction of the total organic carbon (TOC) retained on a filter of stipulated pore size (e.g., 0.45 μm).

pH Logarithm of the reciprocal of H^+ normality. The "p" stands for puissance (power), and "H" is the symbol for hydrogen.

Point-contact sterilization Process method in which water in a side stream is sterilized at a central source before being recycled to the bulk fluid. Examples are UV irradiation and ozonation of waters containing low concentrations of bromide. Also see Side stream.

Polyelectrolytes Natural or synthetic polymers with positive or negative charges. Most polymers used as flocculants in water clarification are synthetic, composed of polyacrylamide and its derivatives. Aqueous polymerization of acrylamide yields products of almost infinite molecular weight (up to 20 million). Polyacrylamide is nominally nonionic, but hydrolysis can yield anionic products, and copolymerization of acrylamide with an appropriate cationic monomer results in cationic polyelectrolytes. A variety of products varying in molecular weight and charge density are available commercially. Highly charged cationic polyelectrolytes are appropriate for treating marine mammal pool waters. The molecular weight of the product selected is not relevant.

Polymer bridging Adsorption of particles at many points along the chain of long-chain polymers (i.e., the particles are "bridged" by adsorbed polymer). Occurs during flocculation. Also see Electrostatic patch effect.

Refractory compounds Compounds that in aqueous solution resist biological and chemical degradation.

Salinity Total mass in grams of all dissolved substances in a kilogram of seawater after carbonate has been converted to oxide, bromine and iodine replaced with chlorine, and the organic matter oxidized at 480 °C. Properly used only in reference to seawater and brackish waters, never artificial seawaters or brine.

Semiclosed system System in which water is replaced continuously but at a lower rate than in open systems, often less than than 10 percent of the total volume per 24-h period. Because of the slow exchange, filtration is necessary to achieve optimal clarity. Also see Closed and Open systems.

Short-term test Mutagenicity test targeted at bacteria or mammalian cell cultures. Measures *potential* carcinogenicity on the premise that an important event in both mutagenesis and carcinogenesis is alteration of DNA.

Side stream Diverted flow of water from a large to a small diameter pipe for purposes of point-contact sterilization (e.g., UV irradiation, ozonation) or physical adsorption (e.g., activated carbon contact).

Specific gravity Ratio of the densities of two solutions (e.g., seawater to pure water at 4 °C, the latter presumed to be 1.000 g/cm^3). Obtained from ahydrometer reading after correction for temperature.

Sterilization Destruction of all life. Also see Disinfection.

Thermotolerant coliforms (TTC) Also called fecal coliforms. Comprised mainly of *Escherichia* with a *Klebsiella* component; grow at 44.5 °C; are more specific indicators of fecal pollution than total coliforms (TC).

Total coliforms (TC) Group that includes four genera of bacteria: *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter*. Total coliforms have been isolated from many sources of nonenteric origin and are doubtful indicators of fecal contamination.

Total organic carbon (TOC) Sum of dissolved organic carbon (DOC) and particulate organic carbon (POC).

Trihalomethanes (THM) Known mutagens and carcinogens present at trace concentrations in chlorinated and ozonated waters. Precursors are humic and fulvic acids. The four chemical species are chloroform ($CHCl_3$), bromoform ($CHBr_3$), bromodichloromethane ($CHBrCl_2$), and dibromochloromethane ($CHBr_2Cl$).

Ultraviolet (UV) irradiation Application of UV radiation to sterilize water or air. Microorganisms are killed directly by inactivation of DNA, or indirectly by chemical changes in the water that result in production of toxicants.

Ultraviolet (UV) radiation Rays in the portion of the electromagnetic spectrum falling between visible light and the X-rays. The most active wavelengths biologically are between 190 and 300 nm; the most lethal (i.e., "germicidal") are at 260 nm. UV lamps are manufactured to have peak outputs at 254 nm.

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