By-products of Disinfection and Ultraviolet and Eyes in Marine Mammal Life Support Systems

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We stand on the shoulders of giants

We stand on the shoulders of giants

some of us don’t get too far

Reasons for Using Disinfectants

- Prevent disease
- Improve clarity and appearance
- Meet regulatory requirements

Sterilants that are Commonly Used

- Chlorine
- Ozone
- Bromine
- Ultraviolet

- Generally act by oxidation / reduction reactions that break and reform chemical bonds

Other techniques of Sterilization

- Heat
  - Pasteurization
- Ultrafiltration
- Distillation

Best discussion for treatment of marine mammal pool water

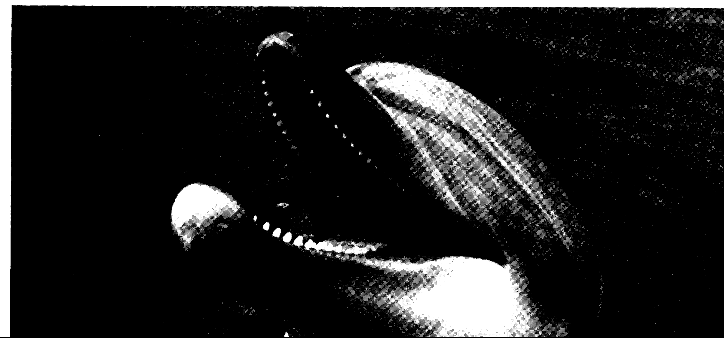
United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Technical
Bulletin No. 1797

Sterilization of Marine Mammal Pool Waters

Theoretical and Health Considerations



Down sides of Sterilants

- May eliminate biological processes in Life Support Systems
- May select for resistant pathogens
- Mycobacteria, fungi
- May cause injury directly
- Related to improper use
- Significant costs in using and monitoring
- Can produce unexpected by-products
- Can mimic biological processes

Natural waters may not be clear

Boulder Creek, Colorado
Creekside view window

small fish at Vancouver Waterfront

Natural waters are not sterile

What fish eating mammal could live in sterile water?

Some water appears to be sterile

Would you want to live in a reflecting pool?

Justification for sterilants

- From APHIS Technical bulletin No. 1797: Sterilization of Marine Mammal Pool Waters, Theoretical and Health Considerations. Stephen Spotte, Ph.D. October 1991

"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."

Unfortunately the hazards from sterilants may be greater than has been thought

Elimination or avoidance of pathogens is important, but adding other dangers may not be helpful

The goal is healthy animals, the question is how to achieve that

Since October of 1991 a lot of new information about biological mechanisms has been learned

Dangers from Sterilants related to their mechanisms

- Main mechanism is to produce very reactive chemical compounds (free radicals) which react with the surface of the organisms we desire to eliminate. Generally oxidizers.
- These reactions cause injury to the organism and kill it.
- The reactive chemical compounds have effects on other compounds in the system as well.
- Free radicals = Loose cannons

Can These Compounds Cause Harm?

- In my limited case load of Harbor Seals and California sea lions living in chlorinated systems several disease manifestations seem over-represented
- Ocular disease including keratitis, uveitis, cataracts, and lens luxation
- High levels of hepatic iron and high ferritin
- Transitional cell carcinoma in situ and metastatic
- Interstitial and tubular nephritis

Oxidant sterilants and pure water.

- Potential injury related to concentration of oxidant compound and sensitivity of animals in the water
- if the Sterilant is low enough no injury should result.

There is more than water in a life support system

- Normal organic matter "NOM"
- Wastes, uneaten food, material from the environment
- Nitrogenous wastes
- Urea, ammonia, proteins, amino acids, nucleic acids, hormones
- Inorganic compounds
- Bromine, carbonates, etc.

Production and Introduction of “Wastes”

- Body excretion and secretion
- Environmental sources
  - Wind and water carried
  - Birds and other organisms
- Wasted food
- Growth of organisms in water
  - Algae, bacteria

Public water Report for my home water system

- Discusses by-products of disinfection
  - "Some people who drink water, containing TTHM (total tri-halomethanes) in excess of the MCL (maximum control level) over many years experience problems with their liver, kidneys, or central nervous system, and may have an increased risk of getting cancer."
- MCL for TTHMs is 80 ppb
- It is easy to exceed that level with chlorination in water containing more than 3 mg/l of total organic carbon

NOM in Life Support Systems

- Marine mammal systems tend to be heavily loaded.
- In a system I work with housing 4 sea lions and fed about 20 kg of fish daily I estimated that the calorie content of the fish would equal the caloric needs for 20 humans.
- That is like 20 people in a room and never leaving it to urinate or defecate!
- It is easy to exceed 3 mg/l TOC

Oxidants + other compounds = by-products

- A tremendous range of possible compounds
- Major mechanism is attachment of chlorine or bromine atoms
- Bromine more likely to produce persistent products
  - Bromine is in natural waters
- The simplest and easiest to measure are tri-halomethanes
  - Chloroform, bromoform, bromodichloromethane, and chlorodibromomethane
  - Halomethanes and haloacetic acid levels are regulated for public water supplies

By-products of disinfection

- A host of possible compounds
- Some such as chloroform and bromoform are volatile
  - Can sit just above pool surface
  - May want to vent protein skimmers outside!
- Some are non-polar and small so they can penetrate mucosal or even skin surfaces directly
- Some such as bromate are not removed by any filtration technique in use

Summary of disinfection by-products.

Table 3
Disinfectants and disinfection by-products (adapted from ICPS, 2000)

Disinfectant	Significant organohalogen DBP	Significant inorganic DPBs	Significant non-halogenated DPBs
Chlorine	THM, HAA, HAN, CH, CP, CPh, *N*-chloramines, halofuranones, bromohydrins	Chlorate (mostly from hypochlorite use)	Aldehydes, cyanoalkanoic acids, alkanoic acids, benzene, carboxylic acids
Chlorine dioxide		Chlorite, chlorate	Unstudied
Chloramine	HAN, cyanogens chloride, organic chloramines, CH, chloramino acids, haloketones	Nitrate, nitrite, chlorate, hydrazine	Aldehydes, ketones, nitrosamines
Ozone	Bromoform, MBA, DBA, dibromoacetone, cyanogens bromide	Chlorate, iodate, bromate, hydrogen peroxide, HOBr, epoxides, ozonates	Aldehydes, ketoacids, ketones, carboxylic acids

THM: trihalomethanes, HAA: haloacetic acids, HAN: haloacetonitriles, HK: haloketones, MBA-CP: chlorophenols, CH: chloral hydrade.

ICPS 2000. Disinfectants and Disinfectant By-Products. World Health Organization, Geneva.

- Public water system managers use flocculants, dissolved gas flotation and other methods to remove suspended organic material before chlorination to reduce formation of disinfection by-products
- My local water system had the highest TTHM readings when the Total Organic Carbon exceeded 3 mg/l during the summer

Public water system example

	Contaminant	Any MCL of TT violation?	Sample Date (or highest detected)	Max Level Detected	Unit	MCLG	MCL	Range	Likely Source of Contamination
	2009 Table of Detected Contaminates: Inorganics								
entry point	FLUORIDE	No	10/3/08	1.35	ppm	N/A	2.2	0 - 1.35	Erosion of natural deposit; water additive that promotes strong teeth; Discharge from fertilizer and aluminum factories
	NITRATE-N	No	8/6/09	0.22	ppm	10	10	N/A	Runoff from fertilizer use; Leaching from septic tanks, sewage, Erosion of natural deposits.
	**SODIUM	No	5/23/07	11	ppm	N/A	**		Naturally-occuring; Road salt; Water softeners; Animal waste
	SULFATE	No	5/23/07	23		N/A	250		Naturally-occuring mineral
	ZINC	No	4/20/04	0.032		N/A	N/A		
	Contaminant	**Any MCL of TT violation?**	**Sample Date (or highest detected)**	**Max Level Detected**	**Unit**	**MCLG**	**AL**	**Range**	**Likely Source of Contamination**
distribution	COPPER[1]	No: 90%=.074ppm	9/9/08	0.28	ppm	1.3	1.3	ND - 0.28	Corrosion of household plumbing; erosion of natural deposits; leaching from wood preservatives.
	LEAD[1]	No: 90% = 6.0ppb	7/3/08	7.6	ppb	0	15	ND - 7.6	Corrosion of household plumbing; erosion of natural deposits.
	2009 Table of Detected Contaminates: Microbiological								
	Heterotrophic Bacteria	No	12/15/09	150	count / ml	N/A	N/A	0 - 150	Naturally occurring
Entry Point	Turbidity[1]	No	6/9/09	1.98	NTU		TT >5.0 NTU	.06 - 1.98	soil runoff
		No	1/1/09	0.65			TT: 95% samples not ≤0.5NTU	0.03 - 0.33	
	Heterotrophic Bacteria	No	4/20/09	5	count / ml		N/A	0 - 5	Naturally occurring
	2009 Table of Detected Contaminates: Disinfection By-products formed by chlorination of tap water, and their precursors								
	Total Chlorine	No	4/14/09	2	ppm	N/A	N/A	1.04 - 2	Water additive used to control microbes
	Free Chlorine	No	4/14/09	1.56		(MRDLG) 4.0	(MRDL) 4.0	0.74 - 1.56	
		No	10/27/09	1.27				0.1 - 1.27	
distrib	TTHM[1]	No: RAA= 37.78 ppb	8/6/09	66.1	ppb	N/A	TT=80ppb	17.3 - 66.1	By-product of drinking water chlorination needed to kill harmful microorganisms. TTHM's are formed when source water contains large amounts of organic matter.
	HAA	No RAA= 16.0 ppb	8/6/09	22.1			TT=60ppb	8.8 - 22.1	By-product of drinking water disinfection needed to kill harmful microorganisms
Entry Point	TOC	No	6/23/09	3.4	ppm		TT = TOC & SUVA >2.0	1.67 - 3.4	Precursors to by-products of drinking water chlorination
	SUVA	No	1/12/09	1.23	L/mg-m			0.04 - 1.23	
	Other Properties of Tap Water 2009								
	Total Solids	No	10/30/09	175	ppm	N/A	N/A	153 - 175	Naturally occurring minerals
	Total Dissolved Solids	No	3/20/09	172				160 - 172	
	Alkalinity (as CaCO3)	No	3/13/09	96.53				78.7 - 96.5	
	Calcium Hardness (as CaCO3)	No	2/20/09	126				89 - 126	
	pH	No	8/19/09	8.2	SU			7.4 - 8.2	Naturally occurring
dist	pH	No	12/4/09	8.2	SU			7.5 - 8.2	Naturally occurring

From my home public water supply annual report

Contaminant	Any MCL of TT violation?	Sample Date (or highest detected)	Max Level Detected	Unit	MCLG	MCL	Range
Total Chlorine	No	4/14/09	2	ppm	N/A	N/A	1.04 - 2
Free Chlorine	No	4/14/09	1.56	ppm	(MRDLG) 4.0	(MRDL) 4.0	0.74 - 1.56
	No	10/27/09	1.27				0.1 - 1.27
TTHM[1]	No: RAA= 37.78 ppb	8/6/09	66.1	ppb	N/A	TT=80ppb	17.3 - 66.1
HAA	No RAA= 16.0 ppb	8/6/09	22.1			TT=60ppb	8.8 - 22.1
TOC	No	6/23/09	3.4	ppm		TT = TOC & SUVA >2.0	1.67 - 3.4
SUVA	No	1/12/09	1.23	L/mg-m			0.04 - 1.23

Chlorine total max 2ppm
free chlorine max 1.56ppm
Total trihalomethanes max 66.1ppb
Haloacetic acids max 22.1 ppb
TOC max 3.4 ppm (mg/l)

Note: These values are not measured daily, these values are the highest they found when measuring at regular intervals

TOC in Life Support Systems

- Marine mammal systems tend to be heavily loaded.
- In a system I work with housing 4 sea lions and fed about 20 kg of fish daily I estimated that the calorie content of the fish would equal the caloric needs for 20 humans. (2000 kcal/kg herring x 20kg and assuming 2000kcal/person/day)
- That is like 20 people in a room and never leaving it to urinate or defecate!
- It is easy to exceed 3 mg/l TOC

Acceptable levels for drinking water

- Based on toxicity in animal experiments but designed to minimize risk to to humans drinking or bathing in the water
  - Usually assumes drinking 2 liters daily and bathing once daily
  - The worry is cancer not organ injury
- Risk assessment for animals must be different because of more constant exposure and breathing air over the water all the time!!!

Minimizing By-Product levels

- Clean water
  - reduce NOM (normal organic matter)
  - reduce Organic Carbon levels in water to be treated
  - Before chlorination in public water supplies TOC levels are reduced by filtration, coagulation, Dissolved gas flotation or other means
  - Should be less than 2 or 3 mg/l TOC before chlorination or ozonation to avoid exceeding potable water regulatory requirements
  - Measuring TOC in saline waters requires expensive equipment. Chloride intereferes with simple tests.
- Reduce bromine

Inhalation may be the main route of entry

- Measuring blood levels of chloroform in humans after drinking 1 liter of water, a 10 minute bath or a 10 minute shower gave the highest readings after the bath and almost as high after the shower.
- Similar results were found with bromodichlormethane and dichlorobromomethathane

Household exposures to drinking water disinfection by-products: whole blood trihalomethane levels
LORRAINE C. BACKER,[a] DAVID L. ASHLEY,[b] MICHAEL A. BONIN,[b] FREDERICK L. CARDINALI,[b] STEPHANIE M. KIESZAK[a] AND JOE V. WOOTEN[b] Journal of Exposure Analysis and Environmental Epidemiology (2000) 10, 321±326

Example compound: chloroform

- Volatile so it can be in the air over the water and can be inhaled
- Can easily penetrate mucosal surfaces and even skin
- Toxicity requires processing into toxic metabolites.
- Organs affected are related to presence of Cytochrome p450 enzyme systems in sufficient levels to produce toxic metabolites.

Chloroform toxicity

- Target organ usually liver or kidney because of the levels of p450 enzymes
- There are p450 enzymes in the eye at levels that I suspect may be sufficient to lead to toxicity
- In mice different individuals can have different relative levels of p450 enzymes in liver or kidney so one individual will have liver damage and another kidney damage from the same amount of chloroform

Chloroform toxicity mechanism

- Chloroform : $HCCl^3$
- Broken down by Cytochrome p450 enzyme system
- Initial step produces Carbonyl Chloride
  - also known as phosgene
  - very reactive compound once used as a chemical weapon
- Also produces HCl
- HCl and Carbonyl Chloride can cause injury
- Normally these intermdiate products are bond to anti-oxidants such as glutathione to prevent injury

Cytochrome p450 system

- Mechanism to breakdown compounds
- Considered to be a detoxification system but also has functions in the normal cell
  - It breaks down hormones and other compounds normally in the body
  - Once a hormone molecule reaches its target tissue and does its job what happens to it?
  - Without p450 enzymes the hormone levels would continue to rise as they were produced
- These systems are present in all organisms
- Cytochrome p450 enzymes are present in the eye

Glutathione

- Major cell anti-oxidant
- glutathione is maintained in the reduced state so it can quickly react with oxidizing free radicals and control potential damage from oxidizing compounds.
- Once oxidized it is reduced by NADPH to repeat the process which requires energy
- Part of the mechanisms that maintain the reducing environment in the cell
- Very important in the eye
  - Reduced glutathione levels in the lens can cause changes quickly[1]

1 Epstein and Kinoshita, Invest. Opthalmology vol 9 no 8 1970

Intracellular Redox Level

- Normally a reducing environment in the cell
- Oxidation reactions damage cell components
- Controlled oxidation in mitochondria produces the cells energy
- The capacity of the anti-oxidant mechanisms in the cell meets normal requirements
- Anything that increases the load on these systems can lead to oxidative damage.

Oxidative stressors are additive

- Anything that affects the reducing environment in the cell can increase the oxidative damage to membranes, etc.
- Normal processes in the cell produce free radicals which the protective mechanisms such as the glutathione system handle
- In the skin and eye UV radiation reacts with chemical bonds leading to production of free radicals which lead to increased oxidative stress.

U-V light acts by breaking bonds and leading to oxidation so it is an oxidative stressor

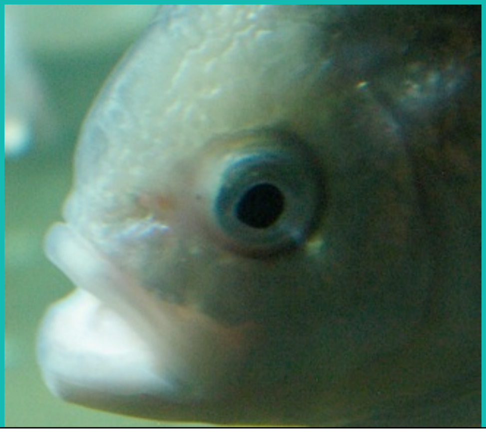
- Short wave length light very energetic
- Blue light also more energetic than red light
- Short wavelengths easily scattered
  - why the sky is blue
  - Less directional, even out of direct sun can be significant
- Penetrates clear water
  - <10% loss in 2 meters.

UV can sterilize water

- One can place sewage contaminated water in a clear or transparent blue container, add sugar and nutrients for a rehydration solution, leave it in the sun for a day and then put it away for storage.
- Used to prepare 'WHO Juice' to prepare rehydration solutions for diarrheic diseases in children
- Does not work with brown or green bottles
- Orientation of bottle does not matter, can be upright or lying down.
- see: http://almashriq.hiof.no/lebanon/600/610/614/solar-water/unesco/

U-V and blue light affects the eye.

- Oyster fishermen have high incidence of cataracts.
- Uveitis from sun on snow or sand
- May cause pterygium formation



See how the overhead light is focused at the limbus and below the eye in this fish at the Vancouver Aquarium

U-V and blue light affects the eye.

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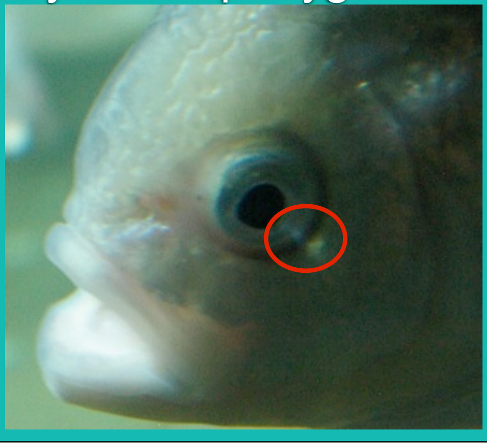
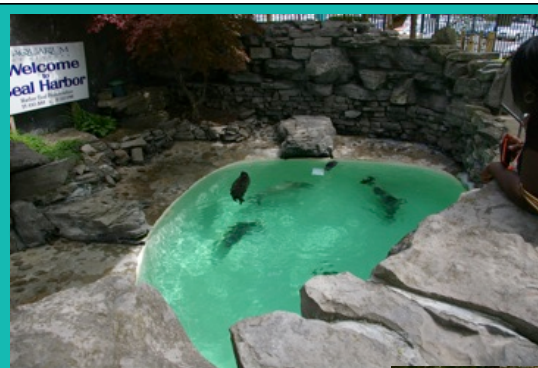


See how the overhead light is focused at the limbus and below the eye in this fish at the Vancouver Aquarium

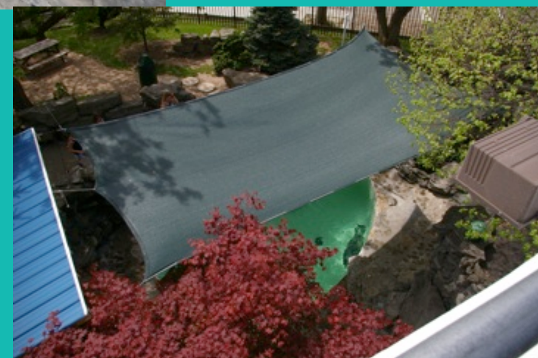
Ways to reduce UV injury

- Sun shade
  - over head shades
  - trees or plantings
  - rock or wall shading
- Don’t paint pools blue!
  - tan or brown colors may be best
- Don’t require animals to look at the sun when being trained or fed
-



Pool painted a sand color. Blue may be more highly reflective for UV light

Sun shade over pool. Reduces intensity of sunlight



Examples of things that can be done to reduce uv injury

Conclusions

- By-products of disinfection are produced when oxidant sterilants are used in water containing organic matter
- Many of these by-products cause injury mainly after reactions with systems in the cell
- These compounds can add to oxidative stress already experienced in the body
- the benefits must be weighed against the unrecognized potential for injury

Recommendations

- Reduce TOC (total organic carbon)
  - filtration
    - get the poop out before it hits a pump
  - foam fractionation
  - flocculation
- Reduce or eliminate chlorine, never use Bromine,
- Consider TOC level and Bromine level before using ozone
  - Reduce other oxidative stress such as UV
    - Shade, color, proper handling

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