BY-PRODUCTS OF DISINFECTION OF WATER AND POTENTIAL MECHANISMS OF OCULAR AND OTHER ORGAN INJURY AND HEMOSIDEROSIS IN MARINE MAMMALS

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Disclaimer and further information

This presentation is based on my observations, lab results and literature review. My purpose is to give you things to think about and information to use when advocating for life support system improvements

Swimming in living pools , not living in swimming pools

A more complete version and some links to more information is available at WWW.CentralParkAH.com

Can't Marine Mammals be kept in water their food fish could live in?

It is possible!

Manatees and fish at Columbus Zoo

Overview

- Safe levels of sterilants and by-products of disinfection may be lower for marine animals than for humans
- Sterilants may mimic biological processes and trigger unexpected inflammatory processes
- Chloramines may have significant biological effects
- Heme Oxygenase a stress protein protective against oxidant damage may produce elevated levels of free and bound iron in tissues

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

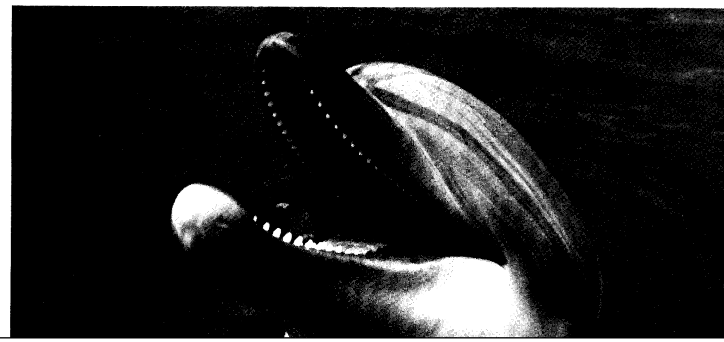
United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Technical
Bulletin No. 1797

Sterilization of Marine Mammal Pool Waters

Theoretical and Health
Considerations



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

Some water appears to be sterile

Would you want to live in a reflecting pool?

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

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
- Old age or something more?

Oxidant sterilants and pure water.

- Potential injury related to concentration of oxidant compound and sensitivity of animals in the water
- if the level of 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.

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

Public water Report for my city 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

- 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

Mechanisms of injury

- Direct injury
  - Spikes above planned levels of oxidants
  - Improper administration, mixing, or control of use
- By-products of disinfection
- Mimicking normal biological compounds and triggering unexpected results

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, and dissolved gas flotation
  - Should be less than 2 or 3 mg/l TOC before chlorination or ozonation to meet regulatory requirements
  - Measuring TOC in saline waters requires expensive equipment. Chloride interferes with simple tests.
- Biological filtration to reduce nitrogenous compounds
- Reduce bromine

Intracellular Redox Level

- Normally a reducing environment in the cell
- Oxidation reactions can damage cell components
- 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.

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

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
- 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.

Oxidants + other compounds = by-products

- 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 in drinking water

Example compound: chloroform

- Volatile so it can be in the air over the water and can be inhaled
- Can easily penetrate mucosal and respiratory 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 may be sufficient to lead to toxicity
- In mice different strains or sexes 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 cause the injury

By-products of disinfection

- A host of possible compounds
- Some such as chloroform and bromoform are volatile
  - Can sit just above pool surface because they are heavier than air
  - May want to vent protein skimmers outside!
- Some are non-polar and small so they can penetrate mucosal or respiratory 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.

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 aquatic animals must be different because of more constant exposure and breathing air over the water all the time!!!

Recent Information

- Many cells in the body produce Hypochlorous acid
  - Neutrophils produce hypochlorous acid to kill bacteria and oxidize dead tissue
- Myeloperoxidase is the enzyme producing the Hypochlorous acid and is implicated in a multitude of degenerative and inflammatory diseases
- Heme Oxygenase-1 has been identified as a stress protein which has a protective effect against myeloperoxidase
  - breaks down the heme molecule used by myeloperoxidase to produce hypochlorous acid
  - produces biliverdin, free iron, and carbon monoxide
- Chloramines produced by the hypochlorous acid in tissues have major physiologic effects

Myeloperoxidase

- Uses a heme molecule to produce Hypochlorous acid
- Implicated in kidney disease, heart disease, and other degenerative diseases
- Adding hypochlorous acid to the water may mimic some of the degenerative effects

Hypochlorous acid

- The active compound in chlorinated disinfection processes
- HOCL <=> H+ + OCl-
- At physiologic pH of 7.3 to 7.4 HOCl, the strongest oxidizer predominates
- In tissue HOCl combines to form chloramines such as histidine chloramines which diffuse through tissues and expand the area of inflammation
- Chloramines are weaker oxidizers so they take longer to react and can spread more widely and expand the affected area

Heme Oxygenase

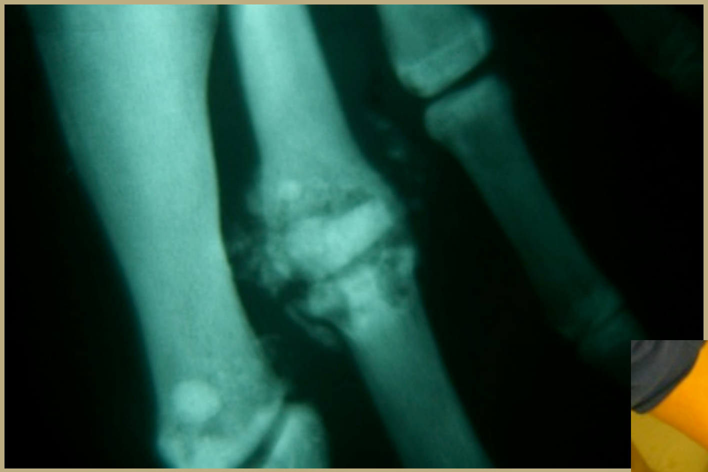
- Breaks down heme to Biliverdin, Iron and Carbon Monoxide
- Protects against the effects of Myeloperoxidase
- One of the “stress proteins”
- Induced by a multitude of compounds or environmental conditions.
  - more than 50 compounds or conditions have been found to induce it in more than 30 different tissue culture models across multiple species

Biliverdin

- Biliverdin oxidized to bilirubin
  - reversible reaction
  - Provides an anti-oxidant effect similar to glutathione

Iron

- Free iron is reactive and must be returned to use or stored in ferritin molecules.
- Iron in ferritin not reused can lead to hemosiderin formation
- High iron in tissues may represent an after effect of the protective activity of Heme Oxygenase



In humans high iron is associated with phalangeal joint enlargement and arthritis

Carbon Monoxide

- Carbon monoxide has been found to be an important physiologic compound with special effect on vascular systems
- Has been found to be of value in reducing Ischemia/Reperfusion (I/R) injury
- Diving mammals undergo I/R stress routinely

Conclusions

- Due to constant exposure safe levels of by-products of disinfection are likely lower for marine mammals than for humans
- Oxidant sterilants may produce unexpected physiologic effects
- High levels of stored iron may represent a sign of chronic exposure to oxidant stress which stimulates the action of the stress protein heme oxygenase
- The numerous potential by-products may represent a risk factor for transitional cell neoplasia

What to do?

- Reduce NOM (normal organic matter)
  - BIOLOGICAL FILTRATION!
  - What would you do if the system contained fish?
- Avoid constant exposure to oxidant sterilants
- Consider UV exposure as an additive stress and reduce exposure
  - shade
  - consider using colors other than blue
  - Avoid making animals look into the sun when training and feeding
- Avoid bromine

Thank you!