The Human and Environmental Toxicity of Microbicidal Chemicals

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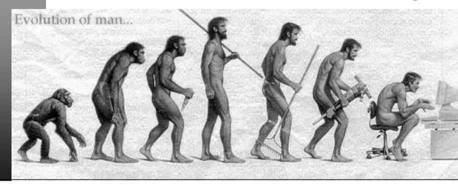
A mighty creature is the germ,
Though smaller than the pachyderm.
His customary dwelling place
Is deep within the human race.
His childish pride he often pleases
By giving people strange diseases.
Do you, my poppet, feel infirm?
You probably contain a germ.
Ogden Nash



Objectives of today's discussion

- focus on toxicity & downside of microbicides
  - for humans
  - for the environment
- interactions of disinfectant chemicals with bacterial pathogens and the host
  - Simultaneous or sequential exposures
- how microbicides affect microbial ecology and why that matters

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Introduction

CHEMICALS
- many recognized as environmental/health threats
- generally good analytical tools available
- effects can be acute, chronic or cumulative
- risk usually increases with exposure

MICROBES
- pathogens – potrentially dramatic health effects
- much more complex & difficult to work with
- effects - acute or chronic; can replicate
- risk generally declines with exposure

CHEMICAL-MICROBE INTERACTIONS??
- innumerable such interactions – mostly unknown
- potential for direct or indirect effects on humans
- knowledge constrained by current regulations

Focus on deliberate interactions

- >20,000 registered products containing 620 different pesticides in use in the U.S. alone
- chemicals used specifically for microbial control
  - ~8000 registered antimicrobial products; >50% of total pesticides
  - does not include chemicals for water treatment
  - >300 registered actives; ~14 in >90% of products
  - chemicals as preservatives in foods, medicines etc. and in treated articles (sublethal?)
- widespread use of antibiotics
  - medicine
  - animal husbandry and aquaculture
  - fruit trees etc.

Characteristics of microbicides

- many different types of natural chemicals have specific antimicrobial potential
- relatively few simple classes exploited commercially as microbicides
- designed to kill essentially everything – toxic by nature
- broadly reactive – many interactions
- compare with drugs, antibiotics – often single site

Modern society and chemicals

- live in world of chemicals – relatively few of which have been assessed for toxicity
- formerly high occupational exposures
- now very broad range and mostly lower
- lifestyles dictate certain exposures
- hospitals now rely on microbicides
- risks from direct exposure, byproducts, combined chemical-microbial risks, and from changes in microbial populations

Microbicides & the environment

- large quantities used in healthcare & industry
- importance of spent microbicide disposal for environment not yet widely recognized
- all discarded to environment – primarily water through sewage & land through sludge
- already concern over antimicrobials like antibiotics and trichlosan in drinking water
- some microbicides used deliberately in water and sewage treatment

Reducing exposure by safe handling

- personnel exposure – mainly skin & inhalation
- Patients through residuals, inhalation and or accidental spills
- cautious handling and storage always
- many reports of poisonings – children
- majority exposures from regular use
  - hypersensitivity
  - contact dermatitis
- California study – 4 types of microbicides responsible for most occupational illnesses
  - Hypochlorite, quats, chlorine gas, glutaraldehyde
- glutaraldehyde replacements
  - OPA, oxidizers like hydrogen peroxide

Disinfectant byproducts (DBP)

- microbicides produce many DBP - high reactivity
- DBP can be more toxic than original microbicide
- only studied well for hypochlorite – now under study for other chlorine chemistries
- regulated for water treatment
- significant issue not widely considered outside of drinking water
- higher concentrations used in food production & discharged from processing
- paper production, sewage and many industries
- need work on DBP for other microbicides

Chlorine-based products

- broadly used in water treatment, food sanitation and many industries
- hypochlorite & chloramines give chlorinated DBPs – many toxic, some mutagenic
- DBPs can be measured in breath of swimmers
- chlorine dioxide gives only oxidised DBP but needs on-site generation – not used in healthcare
- effective microbicides but readily neutralized and need careful use to ensure efficacy

Glutaraldehyde & OPA

- glutaraldehyde well recognized as sensitizer, respiratory irritant and cause of occupational asthma; less data on opa
- used at relatively high concentrations for instrument reprocessing and some exposure might be inevitable
- in europe also used for environmental surface disinfection – possibly more respiratory exposure

Quaternary ammonium compounds

- the most commonly used actives in microbicidal products
- act at membrane level – pole holes in membranes and make them leaky
- relatively low human toxicity but still known to result in contact dermatitis and occupational asthma
- relatively refractory to environmental breakdown but can be used as carbon sources by variety of bacteria

Non-chlorine oxidisers

- hydrogen peroxide, peracetic acid, ozone
- chemicals often thought to be more environmentally benign than some microbicides because 'they do not leave a toxic residue'
- nevertheless oxidized DBPs will be present and not much is known about them
- extremely hazardous at high concentrations and if respired due to highly reactive nature

Quantitative structure activity relationships (QSARs)

- toxicity of many chemicals remains untested
- Nature of the chemical often used to predict its toxicity from its structure and knowledge of the toxicity of related chemicals
- Used in human health and ecological risk assessment

Targets for toxicity

- unless swallowed, exposure usually insufficient to see acute organ effects
- effects more subtle and show in most sensitive systems at cellular level
- immune system/defence mechanism effects
- genetic effects – mutations
- potential for carcinogenesis
- potential for birth defects
- targets similar in humans and other species

Bacteria and toxins

- ironically, bacteria often used to assess toxicity, mutagenicity of chemicals for humans, but almost no attention paid to the effects on bacteria
- e.g., if a bacterial test shows that a product is mutagenic, then it might be mutagenic for humans, but it is certainly mutagenic for bacteria
- bacterial-toxin interactions not generally seen as important for human health
- probably least explored interactions but may be very important
  - high surface area to volume ratio
  - intimate contact & rapid reaction

How bacteria deal with toxins

- knowledge mostly from antibiotic resistance studies; limited number of basic strategies
- EXCLUSION (works for all…..BUT)
  - increased barrier or reduced permeability
  - increased external sequestration
- REMOVAL (works for all)
  - increased efflux
  - close association with other high-efflux organisms
- DETOXIFICATION (limited by metabolic reactions)
  - breakdown or sequestration inside cell
  - close association with other detoxifying or resistant microbe(s)
- ALTERED TARGET(S): (single target e.g. antibiotics)
  - removal, modification, amplification

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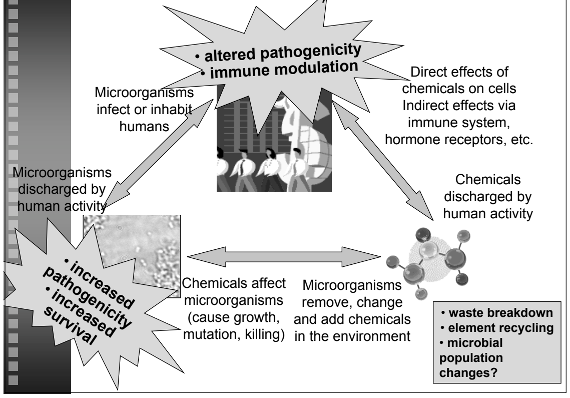
Interactions between humans, microbes and chemicals



The chemical-microbe interface

- most concern for toxins mutagenic to bacteria
- very plastic genome – readily adapts
- genotoxic chemicals – mutations; higher rates under starvation stress
- rapidly evolving mutator strains – increased conjugation/gene exchange
- mutator strains implicated in many infections and in pathogen evolution
- 'precondition' microbes under chemical stress to greater survival
- increased capacity for microbial survival
- potentially common mechanisms for resistance to variety of toxins and to antibiotics
- increased pathogenicity of microbe for host?

Examples of microbial adaptations

- low levels of microbicides can promote sporulation in *Clostridium difficile,* a major cause of diarrhea
- cross resistance between biocides and antibiotics: -
  - Pine oil – *Staphylococcus aureus* – Price et al. (2002)
  - Biocides, consumer products and bacterial efflux pumps
    - *Stenotrophomonas maltophilia* – Sanchez et al. (2005)
    - *Escherichia coli* – Rickard et al. (2004)
  - Triclosan – targets highly conserved enzyme (enoyl reductase) important in fatty acid biosynthesis – more like a drug
    - *E. coli* – Braoudaki and Hilton (2004)
    - *Salmonella enterica* – Randall et al. (2004)
  - multiple antibiotic resistant isolates developed in situ in biofilm by *E. coli* in response to low levels of chlorine in drinking water
- *E. coli* response to Cd – widespread changes in gene expression, shift to anaerobic metabolism, upregulation stress response & energy metabolism
- cross resistance between Cd and peroxide
  - *Xanthomonas campestris* – Bandjerdkij et al., 2005

Combined effects on the host

- living cells - homeostatic but interact with environment
- toxins (chemicals) & pathogens (microorganisms)
- host response affected by genetics, age (hormones, immunity), nutrition
- chemicals - modify membranes, genes, enzymes etc. – might predispose to infections
- chemicals might cause reactivation of latent virus infections
- inflammation/endotoxins- increase toxicity of chemicals
- infections can inhibit enzymes that breakdown toxics
- multiple effects can occur simultaneously
- can result in immune system modulation or autoimmunity
- joint chem-micro exposures - role in chronic and degenerative diseases?

Air pollution

- SO_2, NO_2, automobile fumes, ozone, many unknown chemicals
- important particles in respirable range esp. <2.5 µm
- small particulates, laden with chemicals and microbes, can pass directly into cells
- cellular & immune system effects
- might predispose to- or exacerbate infections
- asthma & atopy – new cases or exacerbate symptoms?
- chronic obstructive pulmonary disease (COPD)
- hypersensitivity pneumonitis
- increases parasitism of soil invertebrates by protozoa
  - uncontaminated sites 0-20%, up to 80% at contaminated
- indoor air pollution (fungi, bacteria, endotoxins, chemicals)
  - link between dampness, virus infection and allergen exposure
  - woodsmoke, tobacco smoke, virus infections and cancer
  - latent virus infection and cigarette smoke

Water and food pollution

- air Scrubbing leads to similar spectrum of chemicals in water & foods
- effects of chemicals exacerbated by pathogen 'packages'
- simultaneous exposure to microbes and:
  - pesticides and fertilizers - food crops
  - antibiotics, hormones and drugs - food animals
  - genotoxic contaminants in potable water include metals, low levels of pesticides, PCB's etc., disinfectant residuals, disinfection byproducts

Metals and metalloids

- acute or chronic exposure may predispose to infections; augment or suppress immune response or make autoimmune. Examples:
- Mercury (induced autoimmunity and neurotoxicity) exacerbates virus infections and increases malaria in Hg-exposed
- Copper
  - reduced resistance - catfish to *Aeromonas hyrophila* infection; rainbow trout to infectious hematopoietic necrosis virus and bacteria; Salmonids to *Yersinia ruckeri* (redmouth) – also viruses; Zebrafish – copper and zinc protective at low levels against *Listeria monocytogenes*, infection increase at higher levels
  - Human infections – elevated serum Cu in human brucellosis, and in infertile men with *Ureaplasma urealyticum* (cause or effect?); *Actinomyces israelii* 2-12 % in Cu IUD users

Metals and metalloids cont.

- Cadmium (kidney, systemic toxin)
  - heavy metal gradient (smelter) inc. infections at higher metal (esp. cadmium)
  - Cd inc. stress response - scavenger enzymes protect bacteria from host
  - Single airborne Cd^{++} challenge increased mortality by Pasteurella multocida in mice but decreased it with Influenza A compared to Al^{+++} control challenge
  - Cadmium – increased *Listeria* infections in mice
- Zinc, Copper, Iron, selenium – metabolism may be altered during infections
- Selenium needed in diet for proper immune function; Se deficiency increases viral pathologies in mice
- Arsenic – greater mortality on challenge to streptococcal aerosol; reduced pulmonary bactericidal activity to *Klebsiella pneumoniae*

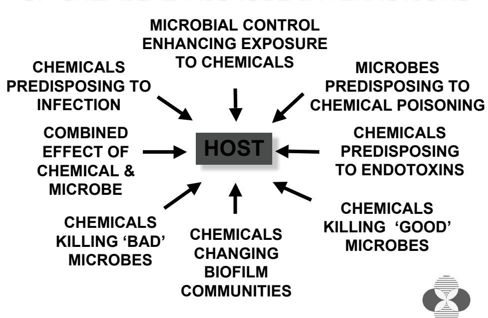
Other combined effects of chemicals and pathogens on host

- reactivation of infections
- Numerous reports of increased drug toxicity when administered during infections
- At least 7 virus – chemical combinations reported as co-carcinogens
- Nothing yet known about combined effects for microbicides, or their byproducts, and pathogens

Microbial population changes

- microbicides & antibiotics kill more than targets
- kill all other susceptible bacteria that are carrying out useful and protective functions
- once the ecosystem is cleared of susceptible bacteria, resistant bacteria can multiply and dominate the environment due to lack of competition
- sometimes resistant bacteria are pathogens (e.g. mycobacteria)
- in general, microbial communities respond to presence of antimicrobial by shifts from those organisms that are sensitive to those that are tolerant or resistant
- sublethal exposure to microbicides – can link to antibiotic resistance?

DIRECT & INDIRECT HEALTH EFFECTS OF CHEMICAL-MICROBE INTERACTIONS



Whether for humans, pathogens or the environment ....
THE DOSE MAKES THE POISON
.... but many effects subtle and mediated through immune systems/defense mechanisms; overt toxicity is relatively rare

The microbial advantage

- the ability of pathogens to rapidly evolve resistance to toxic chemicals in their environment gives them an unassailable advantage
- even microbicides themselves are not impossible to colonize

their unique outer membrane that constitutes an effective barrier to the passage of germicides, and/or efflux systems. While the concentrated solutions of the disinfectants have not been demonstrated to be contaminated at the point of manufacture, Newman et al. found that an undiluted phenolic may be contaminated by a *Pseudomonas* sp. during use [315]. In most of the reports that describe illness associated with contaminated disinfectants, the product was used to disinfect patient-care equipment such as cystoscopes, cardiac catheters, and thermometers. The germicides used as disinfectants that were reported contaminated include chlorhexidine, quaternary ammonium compounds, phenolic, and pine.

- "What does not kill me, makes me stronger".

Friedrich Nietzsche 1888

Concluding remarks

- simultaneous/sequential exposures to pathogens and chemicals increasing, especially in healthcare settings
- toxicology of many chemicals, & virulence factors of most microbes, only partially understood
- major gaps in knowledge of combined health impact of real-life exposures to chemicals & microbes
- microbial control can create problems; microbicides useful but potentially dangerous – double edged sword
- need prudent use for efficacy and safety; strategies for microbicide use to avoid sublethal exposures
- Are truly safe & effective biocides possible?
- multidisciplinary work & sustained funding needed

Thank you for your attention

"Soap and water and common sense are the best disinfectants"

William Osler (1849-1919)
Canadian physician

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