Effectiveness of Alternative Antimicrobial Agents for Disinfection

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National Collaborating Centre for Environmental Health
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Outline

- Regulation of disinfectant products in Canada
- Brief introductory review of several alternative agents
- ‘Alternative agents’ and ‘Traditional disinfectants’
What are they?

‘Alternative’ (in this presentation)

- Tea tree oil
- Thyme oil
- Electrolyzed water
- Ozonated water
- Silver
- Vinegar, lemon juice, baking soda
- Microfibre (cleaning)

‘Traditional’ *

- Bleach
- Quaternary ammonium compounds (QUATs)
- Iodophors
- Alcohol

*For more on ‘traditional’ disinfectants please see: NCCEH evidence review on *Disinfectants and Sanitizers for Use on Food Contact Surfaces* (http://www.ncceh.ca/en/practice_policy/ncceh_reviews/food_contact_sanitizers)
Regulation of Disinfectants in Canada

• „Disinfectants’ are “drugs” defined by the Food and Drugs Act
  – any substance or mixture of substances manufactured, sold or presented for use in:
    […] disinfection in premises where food is manufactured, prepared or kept

• IF the product has „disinfectant claims’, must have a Drug Identification Number (DIN)
  – Not all sanitizers have „disinfectant claims’, hence no DIN; they may still have substantial antimicrobial properties

Regulation of Disinfectants in Canada

Health Canada

- Therapeutic Products Directorate
  - Non-food contact, environmental surfaces, medical devices
- Bureau of Chemical Safety, Food Directorate
  - Residue levels, food contact sanitizers
- Non-Food Chemical Program
  - Chemicals used in federally registered food establishments
  - “...used in a food preparation environment and its intended use is not to become part of the food product”

Canadian Food Inspection Agency
Drug Identification Number (DIN)

What is a DIN?

A Drug Identification Number (DIN) is a computer-generated eight digit number assigned by Health Canada to a drug product prior to being marketed in Canada. It uniquely identifies...

Product Name: BENEFECT BOTANICAL DISINFECTANT

DIN: 02242474

Electronic Product Monograph is not available

Company: SENSIBLE LIFE PRODUCTS
347 Innovation Drive
Flamborough
Ontario
Canada L9H 7H9

Class: Disinfectant

Dosage Form(s): Spray

Route(s) of Administration: Disinfectant (Domestic)
Disinfectant (Food Premises)
Disinfectant (Hospital/Hc Facilities)
Disinfectant (Institutional/Industrial)

Number of Active Ingredient(s): 1

Schedule(s): OTC

American Hospital Formulary Service (AHFS): 38:00.00 DISINFECTANTS (FOR AGE
Anatomical Therapeutical Chemical (ATC): V07AV TECHNICAL DISINFECTANTS

Active Ingredient Group Number: 0101107005

Active Ingredient(s)
THYMOL (THYME OIL)

Health Canada - Drug Product Database

Reference Listing of Accepted Construction Materials, Packaging Materials and Non-Food Chemical Products

Use of "marketing claims" as advertising related to the Canadian Food Inspection Agency (CFIA) or other government agencies:

Please note that any references related to CFIA logo, Agriculture and Agri-Food Canada or Health Canada certifying acceptance, approval, evaluation, registration, homologation or a category in the CFIA’s reference listing, e.g. sub-category N1, are not acceptable and should not be used as marketing tools on a private label and or any advertising tools. This is also applicable to any other communication mediums with the clientele, e.g.: website, catalogue, brochure etc., in regards to the mandatory pre-market evaluation of non-food chemicals, construction materials and packaging materials by the present program prior to their use in registered food establishments in Canada.

Therefore our policies do not permit the use of the CFIA’s name or any other government agencies as mentioned above by a commercial entity. This practice may be considered as an endorsement from our Agency or other government agencies and consequently should be deleted from any marketing tools used by a Canadian or a foreign manufacturer.

Using the Reference Listing

Last Update: 2011/12/05

Search by Category and Sub-category

To view the list of companies and their products, please select a category and a sub-category.

Categories

Disinfectants

Sub-Categories

--- Select All ---

No entry in any field will return the entire list.

SUBMIT
Regulation of Disinfectants in Canada
“Intended Use”

General criteria in evaluation:
• Efficacy
• Safety
• Quality

Application-specific
• Hospital, institutional, food premise, residual activity, target organisms
• Need to read and understand labels and manufacturer instructions
HOUSEHOLD ALLERGENS: Pre-clean (heavily) soiled surfaces. Discard wipe. Use a fresh wipe to remove dust mite debris, pet dander, pollen particles, dust, dirt and hair that may cause sneezing. Unplug small appliances before cleaning. NOT recommended for polished or bare wood surfaces. NOT intended for personal hygiene. DO NOT use on utensils, dishes or glasses.

To Sanitize (Kill 99.9%): Salmonella enterica, Escherichia coli (E. coli), Staphylococcus aureus (Staph), Streptococcus pyogenes (Strep), Methicillin Resistant Staphylococcus aureus (MRSA), Campylobacter jejuni, Listeria monocytogenes, and Enterobacter aerogenes, allow surface to remain wet for 30 seconds.

*To Disinfect (Kill 99.99%+): Salmonella enterica, Staphylococcus aureus, Pseudomonas aeruginosa, Respiratory Syncytial Virus, Swine Influenza A (H1N1) virus allow surface to remain wet for 10 minutes. Allow surface to air dry.

For surfaces that come in contact with food: Use only on hard non-porous surfaces and rinse thoroughly with water.

To Disinfect Toys: Use only on hard non-porous surfaces. Use enough fresh wipes to thoroughly wet surfaces. Wipe surface and allow to remain wet for 10 minutes. Allow surface to air dry. Toss dirty wipe away. Rinse thoroughly with water after use.
Criteria Test Organisms for Disinfectants

- **Spores:**
  - *Bacillus subtilis* and *Clostridium sporogenes*

- **Mycobacteria:**
  - *Mycobacterium bovis*

- **Virus:**
  - Poliovirus, Type 1 (Sabin)

- **Fungi:**
  - *Trichophyton mentagrophytes*

- **Bacteria:**
  - *Salmonella choleraesuis* (*S. enterica*), *Pseudomonas aeruginosa*, and *Staphylococcus aureus*
### Performance Criteria for Microbicidal Efficacy

<table>
<thead>
<tr>
<th>Type of organism (claim)</th>
<th>Level of Disinfection</th>
<th>Reduction Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial spores (sporicide)</td>
<td>Chemosterilization (CS), high level disinfection (HLD)</td>
<td>≥ 6 log (99.9999%)</td>
</tr>
</tbody>
</table>
| Mycobacteria (mycobactericide) | CS, HLD Intermediate level disinfection | ≥ 6 log (99.9999%)  
| | | ≥ 4 log (99.99%) |
| Virus (virucide) | Disinfection (general) | Viral "infectivity titer must be reduced by at least 3 log$_{10}$ beyond the level of cytotoxicity" |
| Fungi (fungicide) | Disinfection (general) | ≥ 4 log (99.99%) |
| Bacteria (bactericide) | Disinfection (general) | ≥ 5 log (99.999%) |

1. Tea Tree oil

Description

• Essential oil extracted from leaves of a plant (*Melaleuca alternifolia*)
• Present in cosmetic products
  – Deodorants, shampoos, hair products, skin products,
• Often used as topical anti-inflammatory:
  – skin infections, acne, ringworm, scabies, and athlete’s foot
• Int. standards for chemical composition (ISO 4730)
Impair cell membrane integrity

- Leakage of cell contents
- Increase susceptibility to sodium chloride
- Inhibit cellular respiration
Tea Tree Oil – antimicrobial efficacy

Log reduction, 5 min contact time

Data from: Messager et. al., 2005
## Tea Tree Oil – antimicrobial efficacy

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (% v/v)</th>
<th>MBC/MFC (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>0.3</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.08 – 2</td>
<td>0.25 – 4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1– 8</td>
<td>2– &gt;8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.5–1.25</td>
<td>1–2</td>
</tr>
<tr>
<td>MRSA</td>
<td>0.04–0.35</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>0.31–0.7</td>
<td>2–4</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>0.016–0.4</td>
<td>2–8</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>0.11–0.44</td>
<td>0.25–0.5</td>
</tr>
</tbody>
</table>

MIC = Minimum inhibitory concentration (inhibit growth/replication)
MBC/MFC = Minimum bactericidal/fungicidal concentration (kills microbe)
Data from: Carson et al., 2006
Tea Tree Oil – toxicity

- Ingestion of undiluted TTO
  - Confusion, inability to walk, disorientation, ataxia, unconsciousness, coma

- Dermal exposure (cosmetic products)
  - Allergic skin reactions, irritation
Tea Tree Oil – endocrine disruptor?

- Clinical report:
  - 3 cases of prepubertal gynecomastia (abnormal growth of breast tissue)
    - Age 4, 7, and 10 yrs
    - Otherwise healthy boys
  - All had exposure to cosmetic products w/ lavender oil and/or TTO.
    - Discontinued use resolved condition
Tea Tree Oil – endocrine disruptor?

**Human cell culture study** *(Henley et al., 2007)*

- Both lavender and TTO induced “*estrogenic and antiandrogenic activities*”

**Bioavailability of TTO components known to penetrate skin** *(Nielson, 2008)*

“The estrogenic potency of TTO was confirmed, but none of the bioavailable TTO constituents demonstrated estrogenicity.”

“[…] cautious *in vitro* to *in vivo* extrapolations from the mixtures of constituents with potentially varying bioavailabilities.”
Highlights - TTO

Substantial antimicrobial properties
- Inhibits growth of variety of bacteria, fungi

Oral and dermal toxicity may limit use
- Accidental ingestion by children
- Skin sensitizer

Low solubility in water
- Issues with formulation and use on surfaces?
2. Thyme Oil

Description

- Essential oil from herb, *Thymus spp.*
  - Thymol, carvacrol
  - GRAS status (US)

- Uses
  - Food additive
  - Mouthwash (thymol)
  - Other (insufficient evidence to support effectiveness):
    - Sore throat, cough, bronchitis
  - Disinfectant (thymol, carvacrol)
Thyme Oil – antimicrobial efficacy

- Primary active ingredient in several registered disinfectants
  - At least one has been accepted for use in federally registered food establishments (issued *no objection letter*)
Thyme Oil

Mechanism of action

- Impair cell membrane integrity
  - Leaky cell
  - Decrease in ATP (molecule that drives chemical reactions in cell)
    - Reduction in proton motive force (generates ATP)
Thyme Oil – antimicrobial efficacy

- **Yes**, after 5 min exposure to 0.31% thyme oil
- **No**, after 24h exposure to >10% thyme oil
- **Yes**, after 15 min exposure to 2.5% thyme oil

Can it achieve 5 log (99.999%) reduction?

Data from: Mayaud et al. 2008
Thyme Oil – antimicrobial efficacy

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (% v/v)</th>
<th>MBC (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.045–0.35</td>
<td>0.31</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>0.0156–0.62</td>
<td>0.62</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>0.045–&gt;2</td>
<td>–</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.02–0.29</td>
<td>–</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>9.35</td>
<td>25</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>9.35</td>
<td>22.5</td>
</tr>
<tr>
<td><em>Stachybotrys chartarum</em></td>
<td>6.2</td>
<td>12.6</td>
</tr>
</tbody>
</table>

MIC = Minimum inhibitory concentration (inhibit growth/replication)
MBC/MFC = Minimum bactericidal/fungicidal concentration (kills microbe)
Data from: Burt, 2007; Hammer et al., 1999; Mayaud et al., 2008; Segvic Klaric et al., 2007
Highlights – Thyme oil

- Primary active ingredient in disinfectant: Use in at least one HC registered disinfectant
- Minimum Risk Pesticide (US EPA): Low oral and dermal toxicity, GRAS status, Exempt from certain pesticide registration requirements
- Listed as a sensitizer and asthmagen by the Association of Occupational and Environmental Clinics (AOEC): Concerns with occupational exposure?
3. Electrolyzed Water

![Diagram of electrolyzed water generation]

**FIGURE 1.** Schematic of electrolyzed water generation. The basic chemical reactions at the anode can be summarized as follows: $2H_2O \rightarrow 4H^+ + O_2↑ + 4e^-$, $2NaCl \rightarrow Cl_2↑ + 2e^- + 2Na^+$, and $Cl_2 + H_2O \rightarrow HCl + HOCl$. At the cathode, the main chemical reactions are $2H_2O + 2e^- \rightarrow 2OH^- + H_2↑$ and $2NaCl + 2OH^- \rightarrow 2NaOH + Cl^-$. 

Source: Hricova et al., 2008
Electrolyzed Water

• Bottom line…

Salt + Water + Electricity → HOCl
Electrolyzed Water

• HOCl (hypochlorous acid)
  – Strong oxidizing agent with antimicrobial activity
  – Active agent in household bleach, pool disinfectant (e.g., sodium hypochlorite)
  – Typically 10-90 ppm

• Why not just use bleach?
  – EW has other properties…
Electrolyzed Water – typical properties

**Two solutions**

- **Acidic EW:** pH = 2 - 3
- **Basic EW:** pH = 10 - 13

**Oxidation reduction potential** (measure of oxidizing activity)*

- **1100 mV (AEW),**
- **-800 mV (BEW)**

**Conditions for survival of pathogens**

- **pH 4.5 - 9**
- **ORP: +500 to -250 mV**

*For comparison, Hydrogen peroxide ORP = 1.77 V; Ozone gas ORP = 2.07 V
Electrolyzed Water – antimicrobial efficacy

Data from: Huang et al., 2008; Hricova et al., 2008; Park et al., 2002; Venkitanarayanan et al., 1999; Vorobjeva et al., 2004; Deza et al., 2005
Electrolyzed Water

• Other applications
  – Sanitizer (AEW, HOCl)
  – Degreaser (BEW, NaOH)
  – Decontamination of cattle hides
    • 47% reduction in hides positive for *E. coli* O157:H7
      (Bosilevac et al., 2005)
  – Decontamination of shell eggs
    • >6 log reduction in *S. enteritidis* in 1 min
      (Cao et al., 2009)
Highlights – Electrolyzed Water

Promising alternative agent
- Many potential applications
- Reviews available

Low oral toxicity, but may be corrosive
- Lower concentrations of HOCl
- No special handling of hazardous chemicals
- Acidic EW vs. Neutral EW

Rapid loss of antimicrobial activity
- Hours to days
4. Ozonated Water

- Ozone gas dissolved in water
  - unstable (30 min half life)
- US FDA approved
  - Antimicrobial agent
  - Treat, store, process foods
- NSF registered devices
  - Food processing areas
- CFIA
  - acceptable use in food establishment
Ozonated Water – antimicrobial efficacy

Data from: Bialoszewski et al., 2010

3 ppm ozone, 30s

Log reduction ranges (CFU/ml or g)

C. albicans
E. hirae
P. aeruginosa
S. aureus
E. coli
Ozonated Water

Applications

- Large scale operations
- Hard surfaces
- Hand wash
- Decontamination of cattle hides
- Lowering chemical oxygen demand of process waters
- Washing and extending shelf life of produce
### Highlights – Ozonated Water

| Promising alternative agent | - Many potential applications  
|                            | - Devices recognized by NSF, U.S. FDA, CFIA |
| No toxic residues          | - No special handling of hazardous chemicals |
| High start up and operating cost | - Corona discharge, high energy UV |
| Rapid loss of antimicrobial activity | - Minutes |
5. Silver

Description

- Silver ion (Ag+)
  - Interfere w/ cellular respiration
  - Interacts with DNA
  - Disruption of proteins
- Disinfectant
  - Silver dihydrogen citrate
  - Residual antimicrobial activity

Li et al., 2011
Silver

Antimicrobial coatings

Hard surfaces

Meat/produce packaging

Residual antimicrobial

Medical devices (e.g., catheters)

Wound dressings

Applications
Silver – antimicrobial efficacy

• Silver impregnated meat/melon liners
  – 1 log reduction (meat)
  – 3 log reduction (melon)

• Silver-zeolite coatings
  – 5 log reduction in 24 hours (S. aureus, E. coli, P. aeruginosa, and L. monocytogenes)
  – B. cereus were reduced by 3 logs after 24h, but spores viable at 48 h
Silver – Concerns?

- Antimicrobial resistance
- Chronic ingestion of silver (gram amts)
  - Argyria:
    - Irrev. blue discolouration
    - Condition is not currently associated with pathological effects
  - Unproven health claims
  - Lifetime NOAEL 10 g
    - Levels in water is thousands, if not millions lower
Highlights – Silver

- Silver dihydrogen citrate
- Many potential applications
- Silver coatings/nanotechnology
- May lose antimicrobial activity once all Ag ions released
6. Vinegar, lemon juice, baking soda

Organic acids

Vinegar
(acetic acid)

Lemon juice
(citric acid)

Mechanism

pH 2-3

Release protons in cell; causes cell death

Conditions for survival of pathogens

pH 4.5 - 9
Baking Soda

- **Mechanism**
  - Unclear...
  - pH 8.34…unlikely inhibits growth of pathogens
Vinegar, lemon juice, baking soda – antimicrobial efficacy

Organic acids

- Some efficacy against Shigella, Salmonella, E. coli, P. aeruginosa, Y. enterocolitica
- Less against S. aureus, L. monocytogenes

Baking Soda

- Notable virucidal activity against feline calicivirus (norovirus surrogate)
- Ineffective against E. coli, P. aeruginosa, S. aureus, Salmonella

Please see NCCEH evidence review for tabulated data
Log reductions
Vinegar, Baking Soda

Data from: Rutala et al., 2000
Highlights – Vinegar, lemon juice, baking soda

Applications are limited
- Poor antimicrobial efficacy, difficult to compare
- **Household** vs. commercial

Strong odour/taste
- May damage organoleptic properties of foods

Low toxicity
- Food additives
7. Microfibre

Description

• Fibres with extremely small weight to length ratio
• <1 g per 9000 m; 1/16th thickness of human hair
• Unique structure, electrostatic, capillary effect
  – Used without detergents
  – 40X more surface area than cotton
Microfibre – cleaning efficacy

Figure 1. Mean number of bacteria (log$_{10}$ reduction, cfu) removed from all surface types by each microfibre cloth.

Smith et al., 2011
“...no significant difference in the decontamination efficacy...”

*S. aureus* or *E. coli* log CFU remaining on test surface after decontamination with different cloth types

Data from: Diab-Elschahawi et al., 2010
Microfibre – economical?

• University of California Davis Medical Center (UCDMC) in Sacramento, CA
  – study compared between using conventional cotton mops vs. microfibre mop system (U.S. Environmental Protection Agency, 2002)

<table>
<thead>
<tr>
<th>Total Costs</th>
<th>Microfiber mops use 95% less water and chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$468 to $470 per 100 rooms per day</td>
<td>$497 per 100 rooms per day</td>
</tr>
</tbody>
</table>

Source: U.S. Environmental Protection Agency, 2002
Microfibre – economical?
Microfibre

Program Results
UCDMC first used the microfiber mops in a pilot test beginning in summer 1999, and within one year it completely replaced conventional loop mops with the microfiber alternative in all patient care areas. The program resulted in three measurable economic benefits:

- 60% lifetime cost savings for mops
- 95% reduction in chemical costs associated with mopping tasks
- 20% labor savings per day
Highlights – Microfibre

Unique properties
- Effective for cleaning
- Lighter material
- Reduce use of water, chemicals

No active antimicrobial properties
- Issues with cross contamination if used with water only

Damaged by heat, chlorine
- Special laundry procedure
- Compatibility with cleaning agents/disinfectants?
Overall impressions…

• Electrolyzed water and Ozonated water
  – Potential for many applications

• Silver
  – Unique residual antimicrobial activity

• Thyme oil
  – Can be an alternative, but costly?

• Tea tree oil
  – Oral and dermal toxicity concerns

• Vinegar, Lemon juice, Baking soda
  – Perhaps use in household setting, but not commercial

• Microfibre
  – Potential for cost-effective cleaning alternative, economical?
Thank You

Questions?
Comments?

www.ncceh.ca | www.ccnse.ca

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Additional Resources

- **Disinfectants:**
  - NCCEH - Disinfectants and Sanitizers for Use on Food Contact Surfaces ([http://www.ncceh.ca/en/practice_policy/ncceh_reviews/food_contact_sanitizers](http://www.ncceh.ca/en/practice_policy/ncceh_reviews/food_contact_sanitizers))

- **Registration of Disinfectants in Canada:**
References

**Tea tree oil**

- Nielsen JB. What you see may not always be what you get--bioavailability and extrapolation from in vitro tests. Toxicol In Vitro. 2008 Jun;22(4):1038-42.


References

- **Thyme oil**
References

• Electrolyzed water
References

• Ozonated water
References

• Silver
References

- **Vinegar, lemon juice, baking soda**
  - Palou L, Smilanick JL, Crisosto CH. Evaluation of food additives as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruit. J Food Prot. 2009;72(1037-1046).
References

• **Microfibre (cleaning)**