How Research is Determining Policy: 

Can UV Protect the Public from Adenovirus in Drinking Water?

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Disinfection 2011: 
April 10-12, Cincinnati, OH 
Addressing the Full Spectrum of Global Disinfection Challenges
Co-Investigators

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- Phyllis Posy, Atlantium, Bet Shemesh, Israel
- Jim Malley, University of New Hampshire
- Anne Eischeid, PhD, Duke University
- Jeanette Thurston, USDA, Washington DC
- Kathy Spindler/Shanna Ashley, Univ of Michigan
- Doug Rogers, University of Nebraska
- Joel Meyer, Duke University
- Ray Schaefer, Phoenix Science and Technology
Overview

• Why UV? Why Now?
• The Adenovirus case
• UV Disinfection of Adenoviruses
• DNA Damage Assessment
• Field Scale 4-log Validation Studies
• Other Studies of Relevance
• Implications for Regulatory Action
Why UV? Why Now?

**Disinfection**
- Common in wastewater treatment in US
- Common in water treatment in Europe
- Growing use for municipal drinking water in US
  - *Cryptosporidium parvum* inactivation

**Chemical Contaminant Removal**
- In Europe, triazine herbicides
- In US and Canada, N-nitrosodimethylamine (NDMA)
  - **Water Reuse**
UV Mechanism of Action - Overview

- Physical Process
- Light Energy Absorbed by DNA
- Dimer Formation
- Inhibits Replication
- Organism that Cannot Replicate, Cannot Infect
- Still Metabolically Active
Low Pressure (LP UV)
Mercury vapor
Monochromatic (253.7 nm)

Medium Pressure (MP UV)
Mercury vapor - Polychromatic
Adenoviruses and UV Disinfection

- Cause respiratory and enteric illness
- 52 human serotypes in six subgroups
  - Group C: Ad2 and Ad5
  - Group F: Ad40 and Ad41
- Double Stranded DNA virus (dsDNA)
- Impact on regulations!
  - EPA Candidate Contaminant List (CCL3)
  - LT2ESWTR
  - Groundwater Rule

UV disinfection requirements for ALL viruses are currently governed by adenovirus
UV Treatment of Viruses

Low Pressure UV 254 nm

LT2 and GWR Regulations for UV inactivation of viruses is based on adenovirus

Gerba et al., 2002
USEPA Adenovirus UV Data

![Graph showing UV dose vs. log inactivation for various studies.](image)
Background: UV Irradiation

- Large data set for UV disinfection
- Includes monochromatic (LP) 254 nm
- No polychromatic (MP) 200-400nm data (yet)
- Many serotypes studied
- Some studies combine disinfectants
  - UV + Chlorine species (free and combine chlorine)

- No Prior UV-Virus Full-Scale Studies
UV Effectiveness for Pathogens

![Graph showing UV dose effectiveness for various pathogens.](image)

- UV Dose for 4-log
- E. coli, Legionella, Salmonella, Shigella, V. cholerae, Hepatitis, Poliovirus, Rotavirus, Cryptosporidium, Giardia, Bacillus Spores, Adenovirus
Why is Adeno so UV “Resistant”?

- Studies only performed with LP UV
- Specific DNA damage from UV 254 nm
  - Thymine dimer
- **Hypotheses**
  - Significant dimer-DNA damage occurs
  - Damage is repaired in host cell
  - Infectivity is restored/DNA replicated
  - Adeno is a dsDNA virus (same as host)
  - Higher UV doses required to inflict enough DNA damage to minimize repair
Motivation for Our Research

- Curiosity over why adenovirus showed such different dose-response behavior compared to other viruses
- Interest in studying polychromatic UV light for disinfection - may have advantages over LP UV?
- Belief that UV (LP or MP) should be able to provide a similar level of virus inactivation as chlorine
- Ground Water Rule suggests UV cannot be validated for 4-log virus inactivation in a UV reactor
  - We set out to prove it could and to augment previous lab work
LP UV and MP UV: Lamp Output

Low Pressure (LP UV)
- Mercury vapor
- Monochromatic (253.7 nm)
- Inactivates pathogens by damaging their DNA/RNA

Medium Pressure (MP UV)
- Mercury vapor - Polychromatic
- Inactivates pathogens by damaging DNA and possibly additional macromolecules (proteins) via polychromatic
UV Disinfection Technologies
AD Type 40

UV inactivation much higher than USEPA data set; for MP and P-UV

Disinfection at <240 nm: Germicidal Action Spectra for Adenovirus

Impact of Cell Culture??

LP UV + adenovirus + cells="resistance"

MP UV + adenovirus + cells = susceptible

LP UV + adenovirus = ???
MP UV + adenovirus = ???
DNA damage is the same for both LP and MP: interesting…

See: Eischeid, Meyer and Linden, Appl Environ Micro, Jan, 2009
Structure of Adenovirus

- **Major coat proteins on outer surface of viral capsid**
  - Hexon, penton, fiber

- **Minor coat proteins on inner surface of viral capsid**
  - IIIa, VI

- **Core proteins in viral core**
  - major core and minor core

http://www.tulane.edu/~dmsander/WWW/335/Adenoviruses.html
Infectious Cycle of Adenovirus

- Gradual shedding of viral coat and core proteins as virus travels to nucleus
- Transcription of viral DNA in host nucleus
- Viral PROTEINS important for successful infection of host cell!

www.tulane.edu/~dmsander/WWW/335/Adenoviruses.html
Other Studies

• Recently Published in *Appl. Environ. Micro.*
  – Adeno 5, 40, 41
  – Three different cell lines
  – LP and MP UV

• FINDINGS
  – MP much more effective than LP for inactivation
  – 40 and 41 more susceptible to MP than 5
  – Repair deficient cell lines result in very low doses for UV inactivation

So, What do we know now…..

- LP UV is (apparently) not very effective – requires high dose
- MP UV is much more effective – in line with other viruses for UV doses
- Same amount of DNA damage occurs from LP and MP (LP is effective??)
- When assayed in cell culture, MP is more effective than LP
Given what we know,

• What is it about cell culture assay that alters the DNA damage found in LP but not MP?

• Do the viruses repair the DNA damage in cell culture and restore infectivity?

• Why do MP exposed viruses not show infectivity in cell culture? – The role of the adenovirus proteins

• Would we expect the same response \textit{in vivo} compared to the \textit{in vitro} cell culture?

• Is it possible to demonstrate 4-log adenovirus inactivation at field-scale?
Field Test: Experimental Design

- Bench Experiments to verify efficacy of MP UV vs LP
- Establish a UV Reactor test plan to cover a range of UVT and flows
- Interact with outside experts (US EPA and State Regulators) to get feedback on the plan
- Carry out CB and field-scale reactor tests using an Atlantium Technologies MP UV System, evaluated data

This study, published in Journal AWWA in 2009, won the 2010 AWWA Publications Award for Best Paper

Adenovirus Assay

- **Adenovirus Type 2**
  - Respiratory, Easier to propagate than 40/41
    - Similar inactivation, used in data set for EPA rule
- **Propagation**
  - Used A549 cells, freeze thaw, extracted with chloroform
- **Enumeration**
  - Quantified by the TCID$_{50}$ method
  - Log inactivation calculated from TCID$_{50}$ data
Collimated Beam Results

- 5 sets of LP and MP CB tests
- LP: 4-log at ~170 mJ/cm²
- MP: 4-log at ~60-80 mJ/cm²
Field Testing Site: UV Center

Post-UV Reactor
Chlorine Injection

Clean Water Staging Tank
0.75 Mgal

Effluent Disposal Tank
1.3 Mgal

Adenovirus Spike
1.3 Mgal

HydroQual NY State UV Validation and Research Center

Control Trailer
With LSA and MS2 Injection Loop

Potable Water

Sodium Sulfite Injection

Pump Bank and Manifold

Flow Meter

Injection Premix Loop

Control Panel

Test Unit

Shelter

Ge...
Consistent agreement between MS2 biodosimetry and DMS testing in Atlantium Reactor.
RED for MP Adenovirus Inactivation

- Calculate RED from LP UV dose-response curve
- Obtain a LP RED for the MP reactor
- EPA Goal of 186 mJ/cm^2 + VF for 4-log virus credit
- Achieved clear data for 4.1 log inactivation, indicating ~186 LP RED after VF

<table>
<thead>
<tr>
<th>Log Reduction</th>
<th>LP RED mJ/cm^2</th>
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<tbody>
<tr>
<td>1.6</td>
<td>73</td>
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<tr>
<td>2.3</td>
<td>139</td>
</tr>
<tr>
<td>2.9</td>
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<td>&gt;3.3</td>
<td>&gt;156</td>
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<tr>
<td>&gt;4.0</td>
<td>&gt;180</td>
</tr>
<tr>
<td>4.1</td>
<td>233</td>
</tr>
</tbody>
</table>

Note: These results are specific to this reactor but method is translatable
Comparison of AD and MS2 REDs

- MS2 RED data corresponds to Adenovirus RED data
- MS2 can serve as a surrogate when comparative data are collected

What you get when it’s 3rd party validated with EPA protocols

In response to your letter of January 13, 2011, we are pleased to hereby accept the above referenced Ultraviolet Disinfection Unit for meeting the requirements of 4-log virus inactivation for the Village of Mohawk public water system in Herkimer County. The system will continue to require chlorination at a minimum to maintain

Dear Ms. Posy,

In response to your letter of January 13, 2011, we are pleased to hereby accept the above referenced Ultraviolet Disinfection Unit for meeting the requirements of 4-log virus inactivation for the Village of Mohawk public water system in Herkimer County. The system will continue to require chlorination at a minimum to maintain a distribution system residual and also maintain the ability to chlorinate at current levels in the event that the UV system must be taken off line.

This acceptance is based on the significant full scale validation testing that your company has completed through HydroQual including validation using live adenovirus, MS-2, T-1, Q-Beta and Dyed Microspheres and Hydroqual’s third party certification of the unit’s capability.

The unit must continue to be operated within the parameters for which it was validated and we support the

8.50 x 11.00 in

Challenges for Adeno Testing

- **Titer able to achieve in Reactor (1-5 x 10^5)**
  - Hard to obtain high titer adenovirus stocks
- **Working with a pathogenic virus**
  - Required special handling and precautions
- **Achieving high log reduction in reactor**
  - Governed by influent titer and assay
- **Meeting a dose with validation factors**
  - >1 validation factor for UV reactor conditions
  - Means higher dose than 186 needs to be met
Implications of Adenovirus
Type 2 or 5 vs. 40 or 41

• High titer required for field studies is not possible using Ad40 or Ad41
  – Need to use lower # adeno, respiratory Ad2 or Ad5

• Does the dose-response for Ad2/5 indicate equivalent inactivation of Ad 40/41?
  – WaterRF study 3105 found no enhancement for Ad 40 under MP UV. Since retracted due to experimental problems

• Recent AEM Study proves that Ad 40/41 is more sensitive to MP than Ad5
Implications of Adenovirus Type 2 or 5 vs. 40 or 41

Take Home Messages

• Adenovirus studies contain inherent variability

• Overwhelming evidence shows that all adenoviruses are more sensitive to MP than LP

• There is no proven difference in the UV resistance of enteric vs. respiratory viruses
  – 40/41 shown to be more sensitive to MP than 5

• Practicality makes the use of lower number adenoviruses essential, but not less useful
Implications for EPA Guidance

• MP UV definitely is more effective and should be addressed separately for adenovirus and virus credit

• Can demonstrate dose of 186 mJ/cm² via LP benchmark and >4 log reduction of viruses
  – Adds to the body of published data that MP UV needs a lower UV dose for adenovirus inactivation

• How will States and EPA view these data?
  – We can validate UV dose and get 4 log inactivation economically – NOW accepted in New York State!

• Acceptance of UV for virus control in ground water
  – Should be an available tool for small systems and others to use
Next Steps

- Successfully completed another field-scale adeno challenge to validate another Atlantium UV reactor
- WateReuse Research Foundation
  - Continue Mouse adenovirus studies
- National Science Foundation (NSF)
  - Study DNA repair post cell culture for LP and MP
  - Continue protein damage studies
Current-Investigators

- Sarah Bounty, Graduate Student, CU-Boulder
- Roberto Rodriguez, Post-doctoral Scholar, CU
- Mark Hernandez, Professor, CU-Boulder
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Off Spec Calculation Worksheet

- Water is off spec on the R200 in 4 log virus mode if:
  - The proper credited dose is not achieved
  - The flow rate exceeds 900 gpm
  - The UVT goes below 92%
  - The UV intensity sensor is not in calibration and no correction factor has been implemented
  - The UVT analyzer fails the calibration check four times in a row