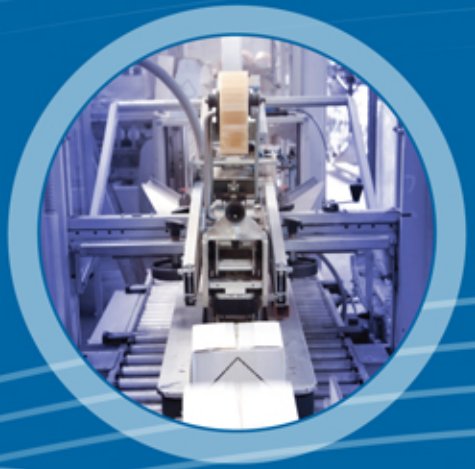
Connecting People, Science and Regulation

Nitrogen Dioxide (NO_2) Sterilization and Decontamination Systems



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PDA Europe
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Objectives

- Review NO_2 processes
  - Sterilization
  - Decontamination
- Give examples of applications
  - Potential benefits
  - Limitations

Nitrogen Dioxide Gas Sterilant

- Nitrogen Dioxide (NO_2) developed for sterilization and decontamination
- Implemented by multiple companies (3 systems on order, 8 cycle validation projects for drug delivery systems)
- Demonstrated efficacy
- NO_2 has broad application to prefilled syringes
  - Decontamination of components
  - Surface sterilization of packaged syringes and delivery systems (with and without vacuum
  - Depyrogenation of components and filling lines

NO_2 Strengths and Limitations

- Surface sterilization process
  - Material permeation is low compared to EO (leads to faster aeration)
  - Low permeability limits device configurations (structures must be open to gas)
- Excellent results with long lumens, complicated geometries, etc.
- Most materials are compatible with the NO_2 process
  - Exceptions: Paper, Delrin, Nylon, Polyurethane

NO_2 Strengths and Limitations

- Biocompatibility – No increase in cytotoxicity
- Common packaging materials are compatible including Tyvek, Mylar, trays and tubs, etc.
- Consumables available including: BI's, CI's, NO_2 detectors, humidity detector, scrubber material, sterilant, etc.

Safety of Sterilant Gases

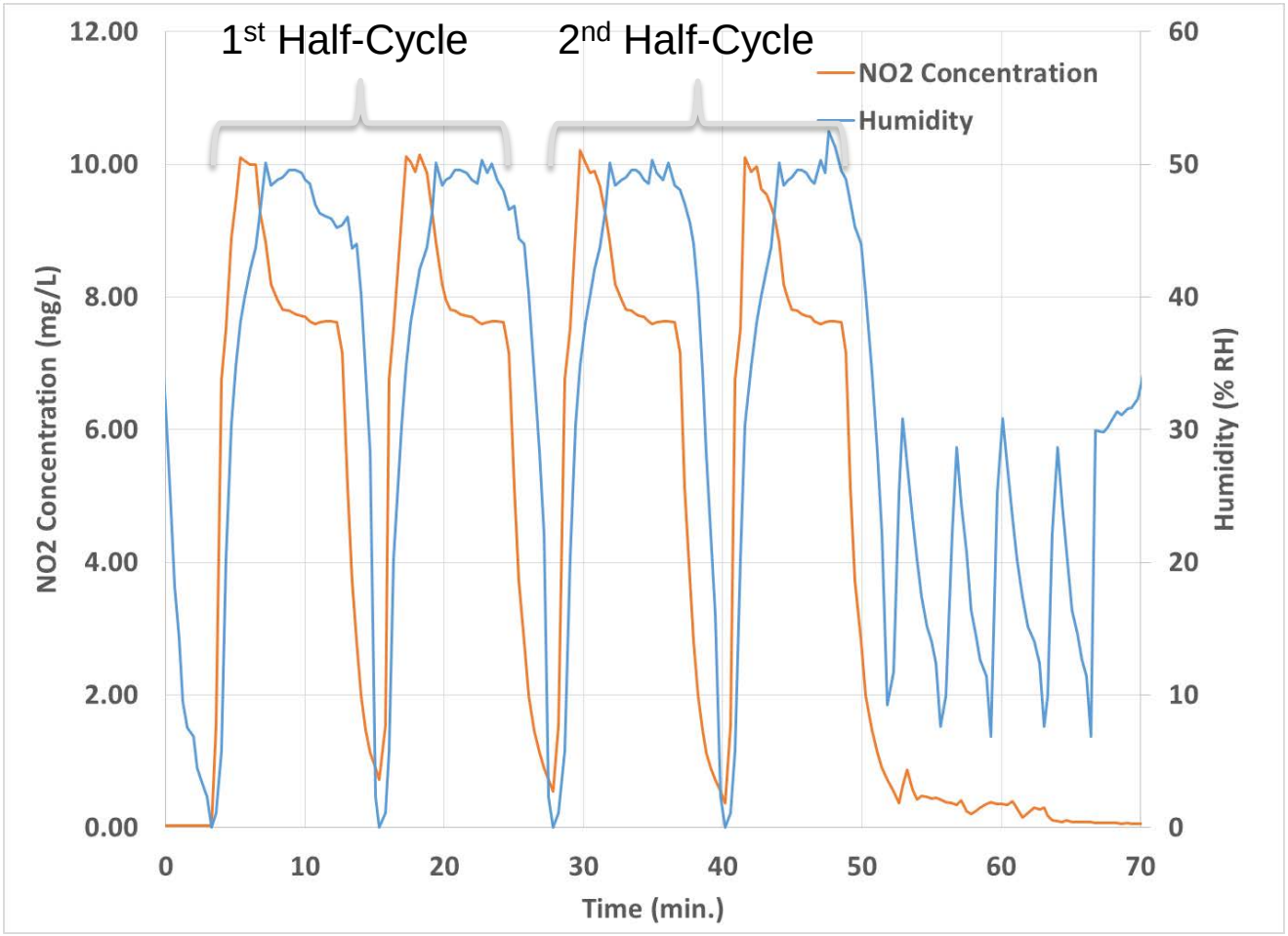
Safety-related Properties	NO2	Hydrogen Peroxide	Ethylene Oxide
Color	Reddish-Brown	Colorless	Colorless
Odor Threshold	0.1 ppm	Odorless	200 – 400 ppm
OSHA PEL (Exposure Limit)	5 ppm	1 ppm	1 ppm
NFPA: Health	3	3	3
NFPA: Flammability	0	0	4
NFPA: Instability	0	1	3

NO_2 can be smelled at concentration levels below harmful levels

NO_2 Sterilization Overview

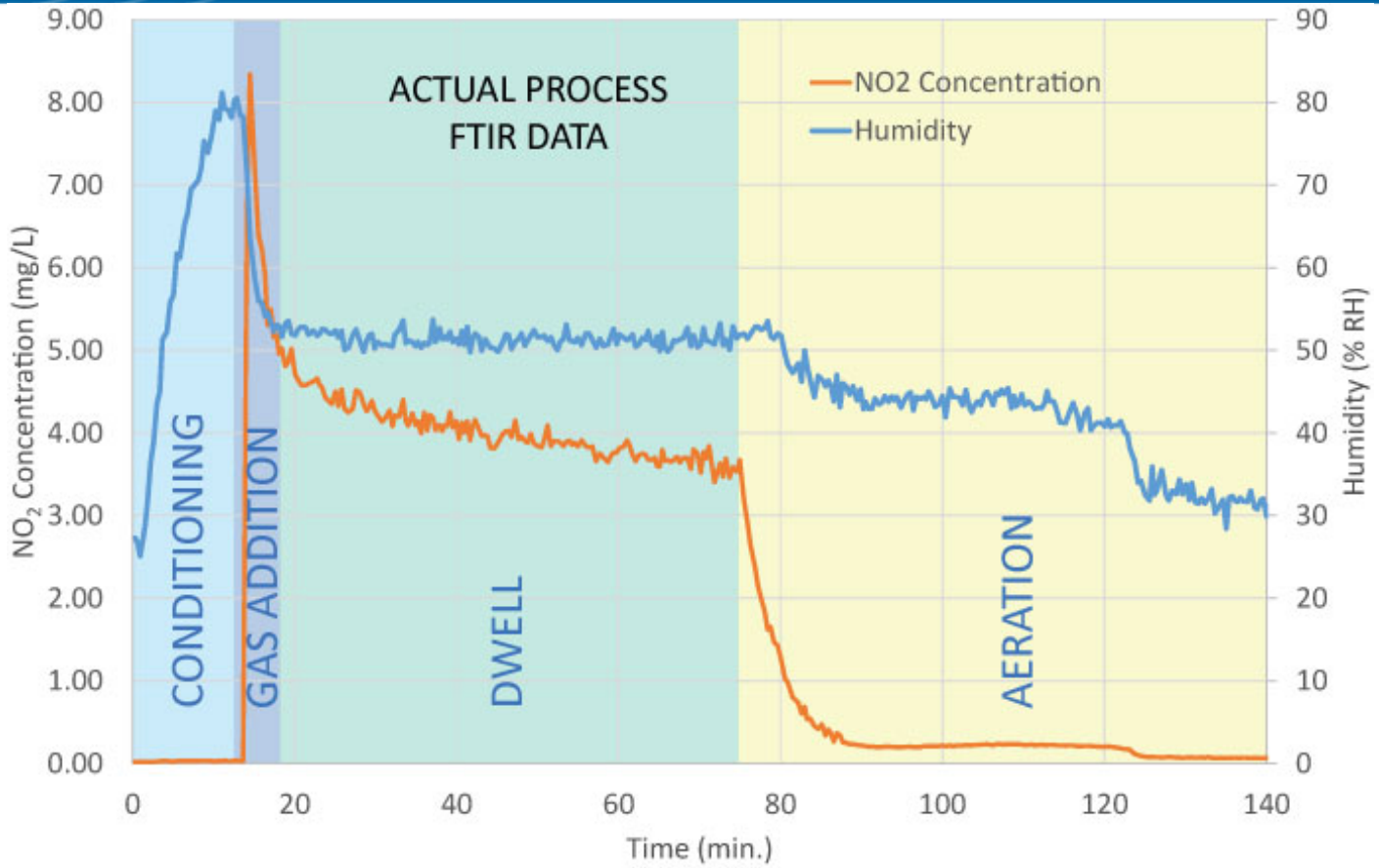
Feature	Parameter Range
Nitrogen dioxide gas sterilant	Boiling point 21°C
Low sterilant concentration	10 mg/L – 15 mg/L (less than 1%)
Room temperature process	Consistent results from 10°C to 65°C
Humidity reduces cycle time	70% - 80% RH provides rapid lethality, 0% RH requires longer exposure time
Fast cycle exposure times	Typically less than 20 minutes
Low sterilant residuals	Residuals often not measureable, no increase in cytotoxicity observed

Typical Vacuum Cycle



The number of pulses is a cycle variable and depends on the specific load

Typical Without Vacuum



- The gas fill and aeration depend on load conditions.
- This cycle is for surface sterilization of syringes. Isolator decontamination requires less dwell time

Mechanism of Sterilization

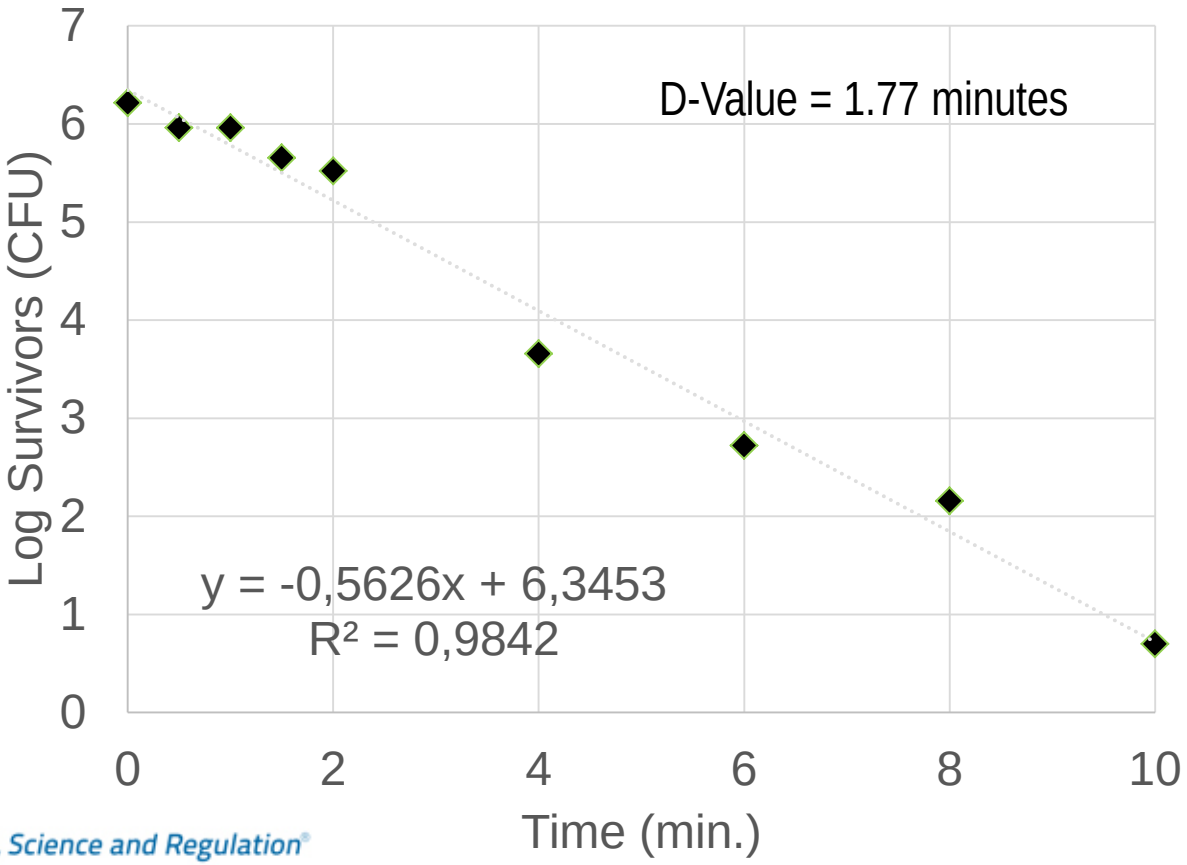
- The NO_2 sterilant has a much lower oxidation potential than other sterilants (not principle mechanism of inactivation)
- The NO_2 process degrades DNA. The degradation is not reversible
- NO_2 *exposure is not carcinogenic or mutagenic for humans*

Oxidation Potential of Selected Gases	
Oxidant	Oxidation potential, (V)
Flourine	3.0
Hydroxyl radical	2.8
Ozone	2.1
Hydrogen peroxide	1.8
Noxilizer's Sterilant, NO_2	-0.8

Biological Process	**NO_2 Results**
DNA Integrity	Fragmented
ATP Production	Inhibited
Germination Process	Disrupted
Reproduction	None

Microbial Inactivation: D-Value

- Linear inactivation of microbes with NO_2
  - Typical methods of D-value, SAL, etc. apply
  - *Geobacillus stearothermophilus* is the indicator organism used



Material Compatibility

Compatible Materials			
Stainless Steel	Polyethylene	Polyetherimide	Thermoplastic Elastomers
Aluminum	Polypropylene	Polycarbonate	
Gold (Plating)	PET / PETG	Cyclic Olefins	Polylactides Bioresorbables
Glass / Ceramic	Polystyrene	PVC*	
Fluoropolymers	Polysulfones	Silicone*	This list is not exhaustive.
Viton (Gaskets)	PEEK / PAEK	Hypalon	

* Depends on grade.

Materials tested for multiple cycles with no observed degradation

Materials Compatibility

Noxilizer is not compatible with a few medical device materials.

Incompatible Material	Design Alternatives
Polyurethane	Thermoplastic Elastomers (TPE)
Nylon	Polyester, Polyolefin
Delrin (polyacetal)	PEEK, PSU, PEI
Cellulose-based (some paper)	Polyester or styrene label stock
Copper-containing alloys*	Stainless steel

*Requires lower humidity cycles.

- NOTE: Each device needs to be evaluated on an individual basis, as geometry and load configuration can affect sterilization.

Applications

- Sterilization of vials prior to filling
- Syringe tub decontamination system
- Surface sterilization of prefilled syringes
- Needle lumen sterilization (drug delivery system)
- Sterilizer unit for medical devices

Sterilization of Syringes and Vials

- Prior to filling, syringes in tubs and vials in trays can be rapidly sterilized or packaging decontaminated.
- Important considerations are:
  - Minimal sterilant residuals on surfaces that contact the product
  - Processing speed to maintain production rate
- Test for sterilant residuals on surface of vials and syringes

PassPort Residuals Test

Cycle Parameters

Parameter	Set Point
NO_2 Concentration	10 mg/L
Relative Humidity	60 % RH
Dwell Time (per pulse)	10 min.
Number of Pulses	4

- Double the cycle used to sterilize vials: worst-case scenario

Results

- Ion chromatography on water extract *
  - Below LOD: **$[NO_3^-] < 2.0$ µg/mL**
- pH measurement on 0.01 M NaCl extract
  - Converted to NO_2 on surface: **0.49 nmol NO_2 / cm^2**
- Residuals are below NO_3^- amount permitted in WFI

PassPort Residuals Test

Cycle Parameters

Parameter	Set Point
NO_2 Concentration	10 mg/L
Relative Humidity	60 % RH
Dwell Time (per pulse)	10 min.
Number of Pulses	4

- Cyclic olefin copolymer (COC) vials, 3-mL
- West butyl rubber stoppers

Results

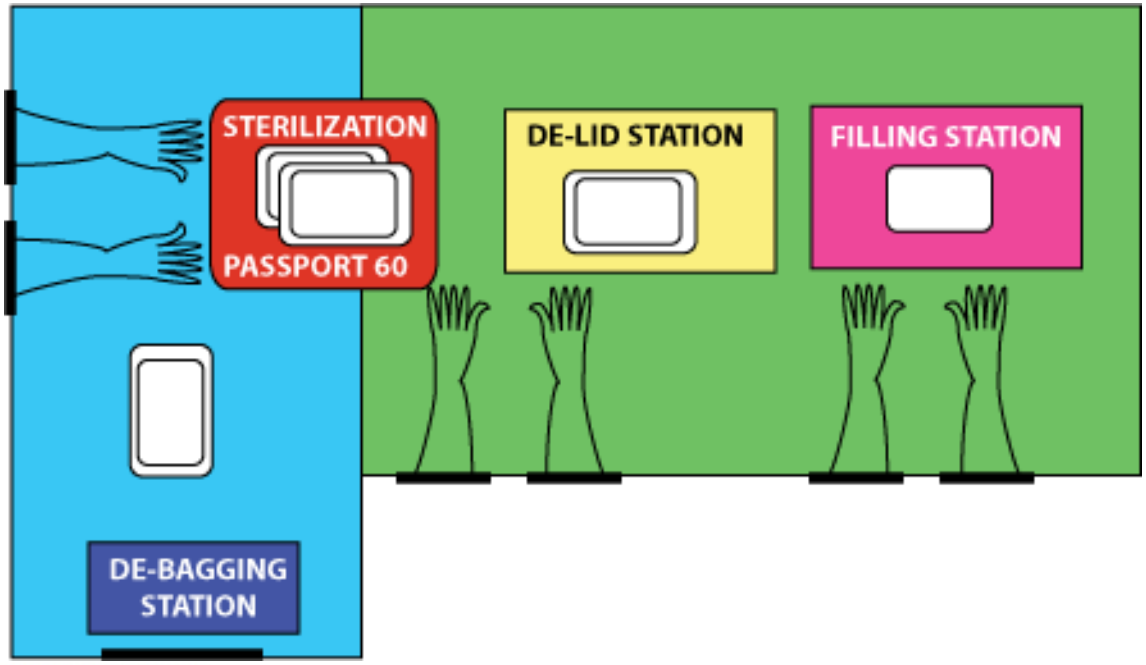
Test	COC Vial Result	Butyl Rubber Stopper Result
MEM Elution Cytotox.	0	0
Direct Contact Cytotox.	0	0

- Non-cytotoxic response
  - COC vials
  - Butyl rubber stoppers

PassPort 60 System

- Nominal 60 liter chamber
- Accommodates 4 tubs per cycle.

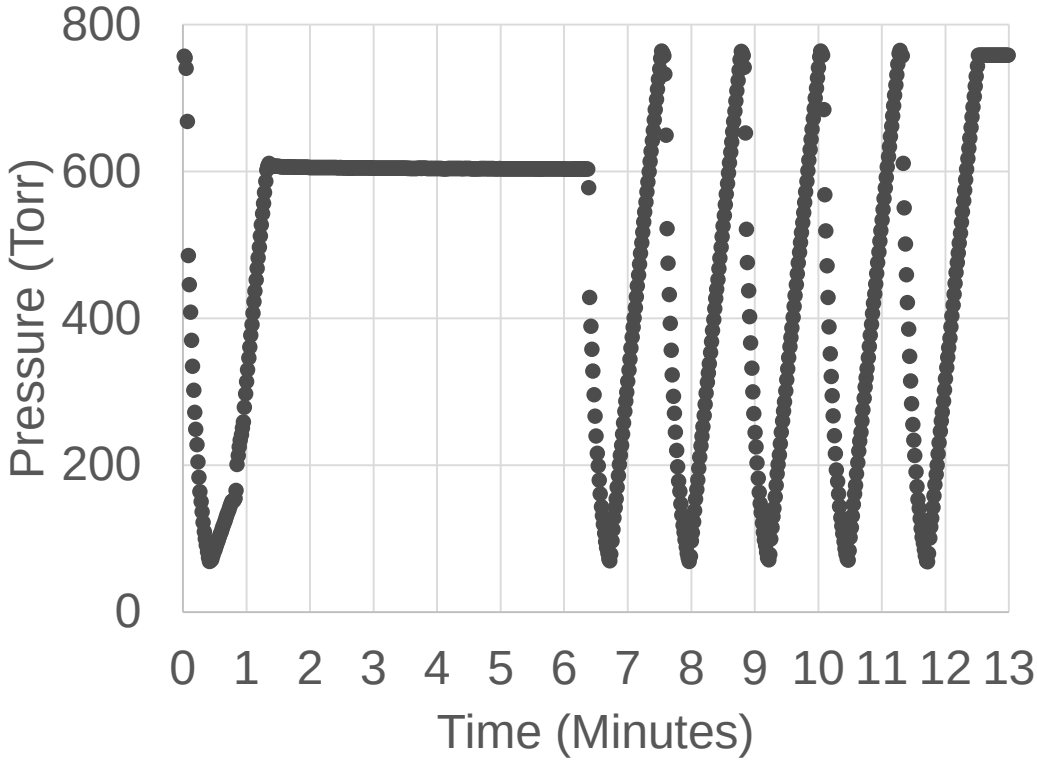
  15 minute cycle = 16 tubs / hr = 1600 syringes per hour



Noxilizer PassPort System

- This system is designed to be used when introducing work items into an isolator
- Versions can have various sizes, from 60 L volume (large enough for two syringe tubs) to 300 L (for up to 16 syringe tubs)
- Versions can be designed with or without vacuum capability
- Requirements:
  - Less than 15 minute process
  - Six log reduction
  - Aerate to < 5 ppm
  - No residuals

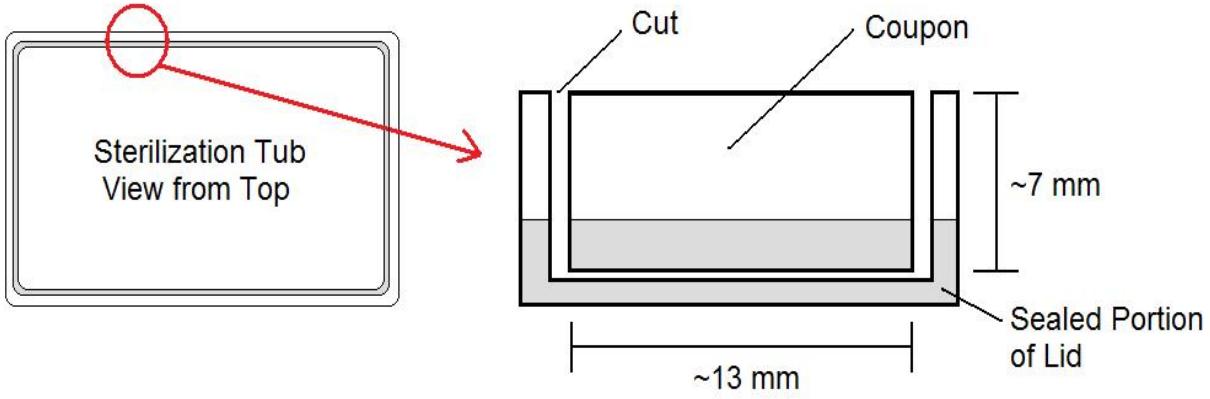
PassPort System Cycle Time

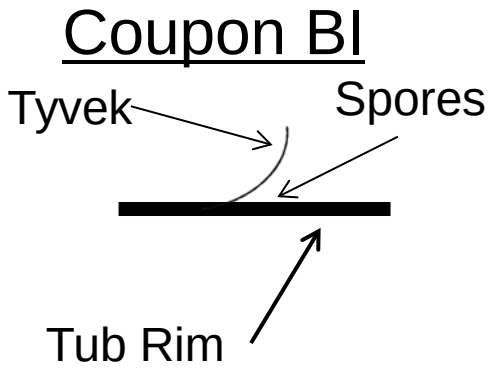


- This cycle is recorded from a 50 L chamber used for testing
- A scrubber is used in the exhaust path (between the chamber and the pump) to remove NO_2

PassPort Lethality Test

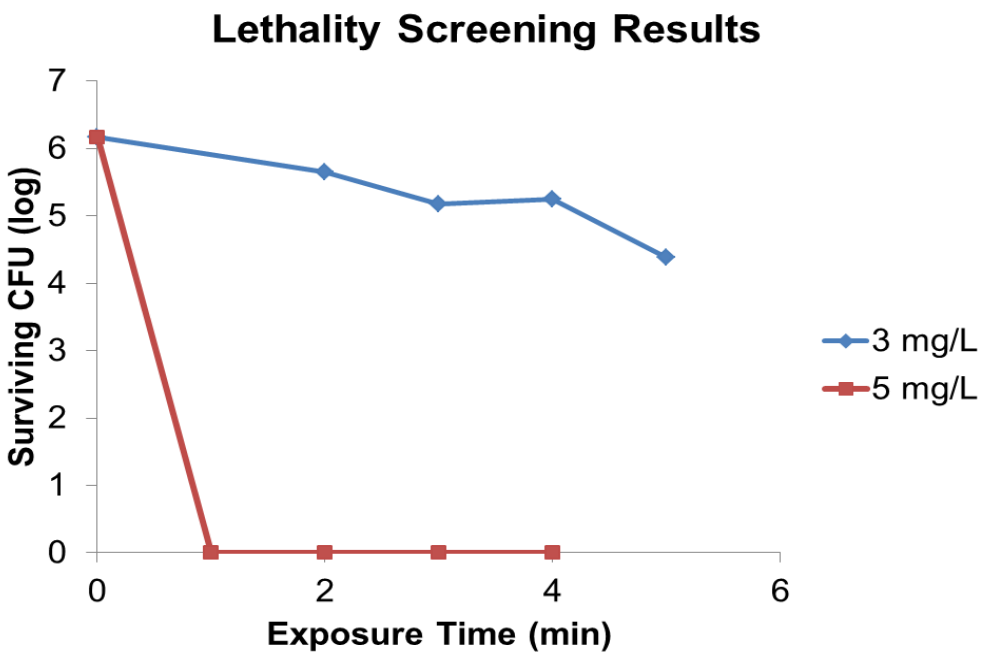
- Two types of BI's used: 'normal' BI's (in pouch) and coupon BI's
- Coupon BI's are cut from the tub rim
- To form the biological indicator (BI), the spore suspension, with > 10^6 spores, was placed under the Tyvek flap that remained on the tub





PassPort Lethality Test

Enumeration Results



Fraction Negative Results (5 mg/L)

Exposure Time	BI Results (# Pos./# Tested)	Tub BI Results (# Pos./# Tested)
2 Min	0/5	5/5
3 Min	0/5	1/5
4 Min	0/5	3/5
5 Min	0/50	0/5
6 Min	0/40	0/5

- Single exposure pulse used for this lethality ranging study
- A single exposure at 5 minutes with 5 mg/L NO_2 concentration will decontaminate tubs

Pre-filled Syringes

- After filling, syringes are packaged and sterilized* for ophthalmic applications, used during surgery, etc.

*Only the surface of the container are sterilized. The compound in the container is not sterilized.

- Important considerations are:
  - Temperature of the sterilization process
  - Contamination of the API due to sterilant ingress (permeability of the closure system)
- Test ingress with WFI filled syringes

Pre-filled Syringes

Cycle Parameters

Parameter	Set Point
NO_2 Concentration	15 mg/L
Relative Humidity	60 % RH
Dwell Time	75 min.

- Approximately double the cycle used to sterilize syringes
  - Worst-case scenario

Results

- Ion chromatography on water extract *
  - Below LOD: **[NO_3^-] < 2.0 μg/mL**
- pH measurement on 0.01 M NaCl extract
  - Control = 6.89, Exposed = 6.65
  - Converted to ppm NO_3^- : **0.002 ppm NO_3^-**
- Residuals are low in concen.:
  - WFI limit: 0.2 ppm NO_3^-

* Test was performed by an external laboratory.

Syringe and Vial Summary

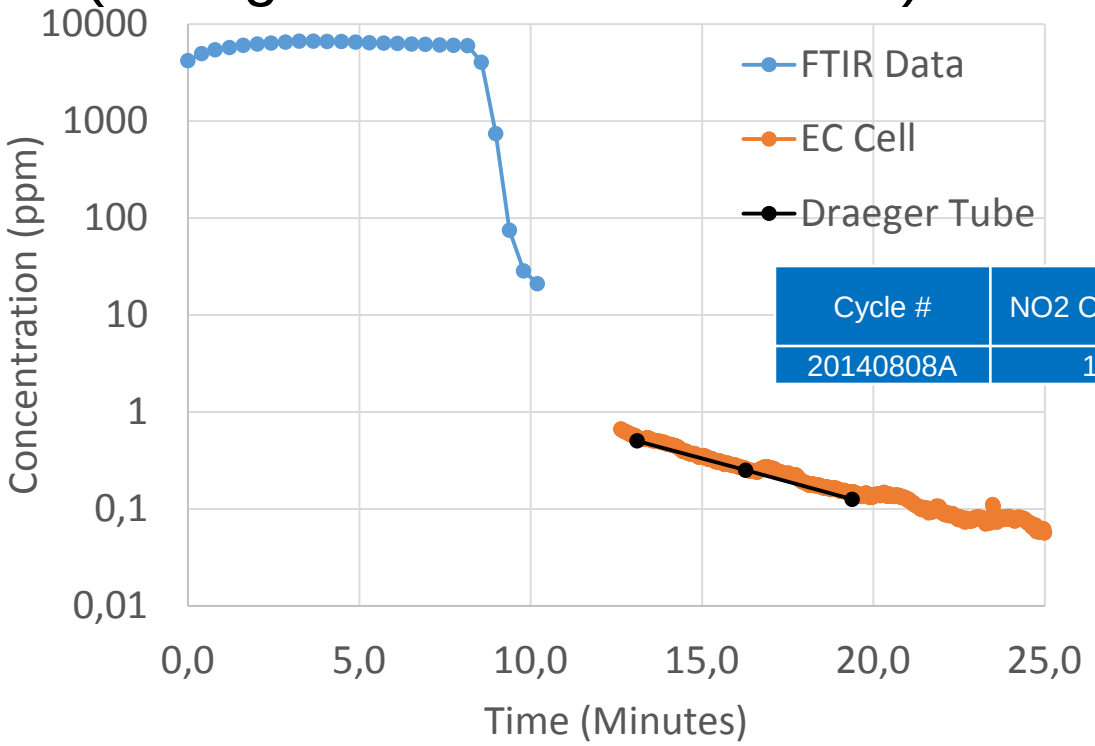
- NO_2 is a new method of sterilization/decontamination
- This method provides a viable method for sterilizing syringes, vials, etc.
- Results show:
  - Sterility assurance level of 10^{-6} possible
  - Material compatibility
  - Low level of sterilant residuals (WFI)
- Rapid cycle time for efficient manufacturing
  - In-line sterilization or batch processing

Isolator Decontamination and Aeration

Data recorded in a heavily loaded isolator made be Walker Barrier Systems

NO_2 Aeration of 800 L Isolator

- Aeration begins at 8.5 minutes
- Concentration in the isolator is below 1 ppm in 5 minutes of aeration and below **100 ppb in 13 minutes** (Draeger tubes and EC Cells)

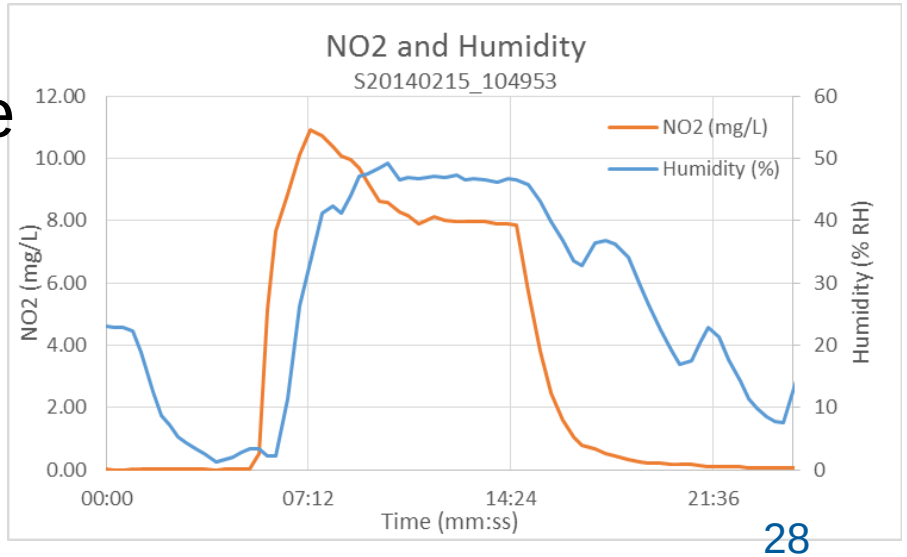


Cycle #	NO2 Concentration	Dwell (Minutes)	Positive BI's SSTL
20140808A	14 mg/L	7.5	0/12

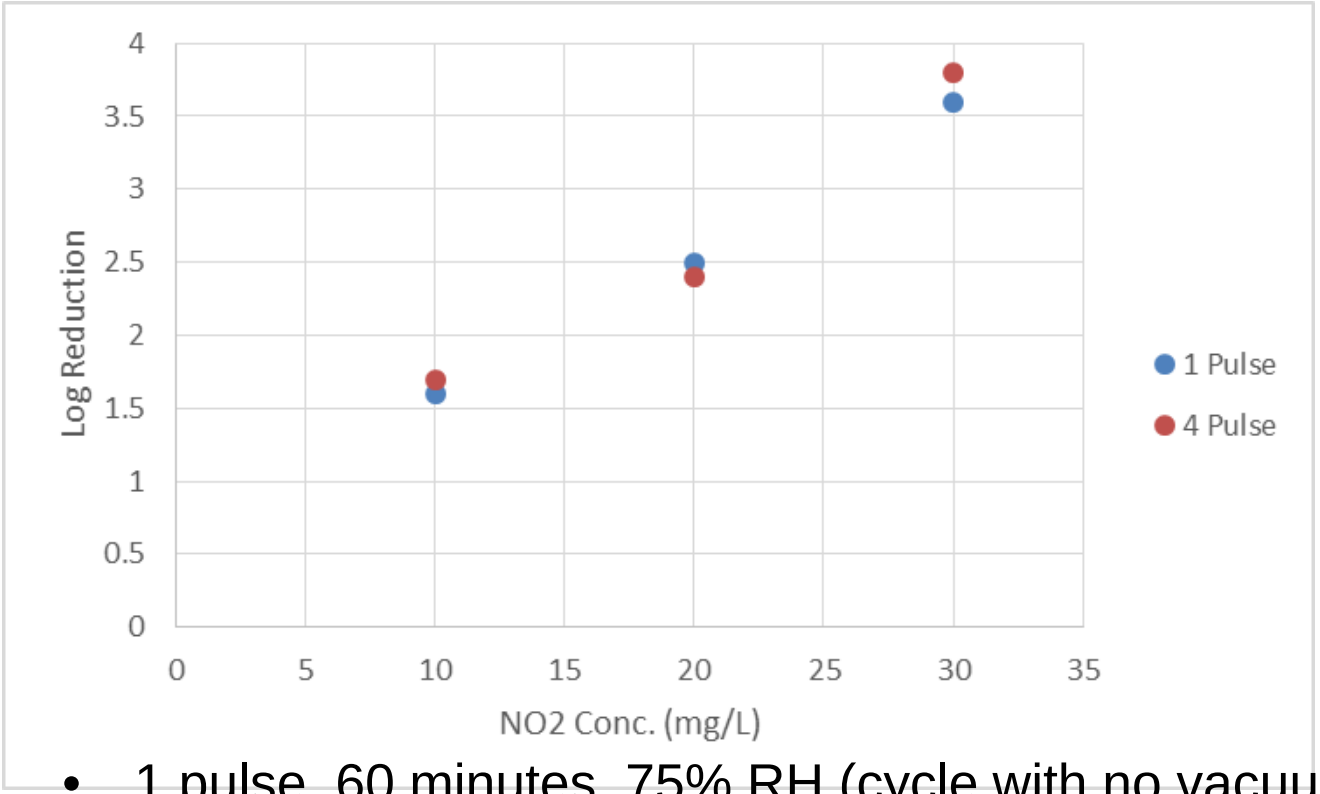
Syringe Needle Sterilization

- Needle lumen is most challenging location
- Demonstrate inoculation and recovery in syringe needles
- Average inoculation = 1.09 x 10^6 per syringe
- Needles had between 10^4 and 10^5 spores in the lumen
- 12 inoculated syringes in each cycle, in tub, three cycles
- Cycle parameters were: **6 min. dwell**,10 mg/L, 75% RH

- All 36 syringes and syringe needles are sterilized
- SAL requires a second half-cycle)



Endotoxin Dose Response



- 1 pulse, 60 minutes, 75% RH (cycle with no vacuum)
- 4 pulses, 10 minutes, 75% RH (fast cycle)

- Cycle validated on an aseptic filling line

Vacuum Sterilization Units

- RTS 360: Simple, effective and economical in-house sterilization
  - Operates at Room Temperature
  - Maintains Material Properties
  - Shorter Cycle Time
  - No Pre-Conditioning
  - No Lengthy Aeration Required
  - No Residuals
  - Free Standing/Non-Hazardous Bi-Product
  - Safer than Ethylene Oxide
  - Fully Scalable Multi-pallet system available

Patented Noxilizer Sterilization System

	Nox. RTS-360	Typical EO System
Chamber Capacity	360 Liters	2200 Liters
Standard Cycle Time	80 Minutes	12-18 Hours *
Manufacturing to Release Time	On-Site, Immediate Use	Off-Site, 7-25 Day Turnaround

NO_2 Processes

- NO_2 process offers a new solutions

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