Effect of Inhalable Endotoxin Exposures on Cytokine Levels in Workers in Northern Colorado Dairies

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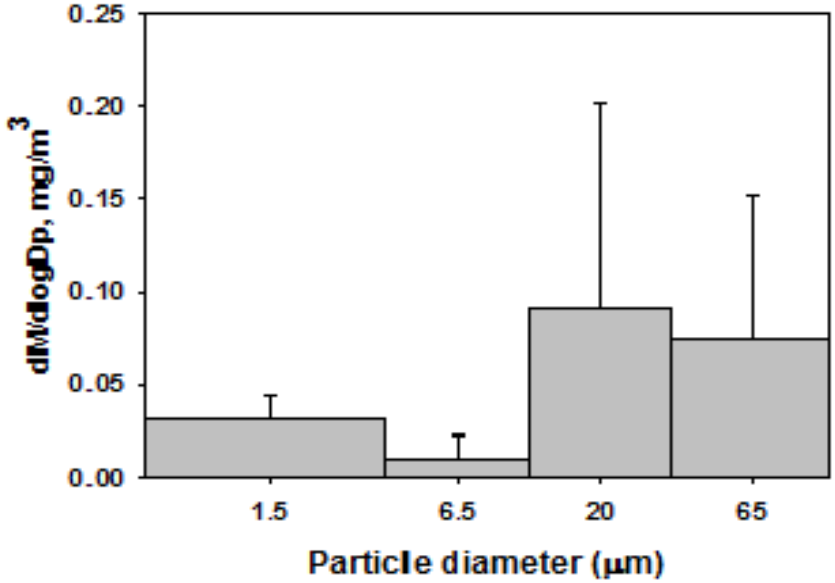
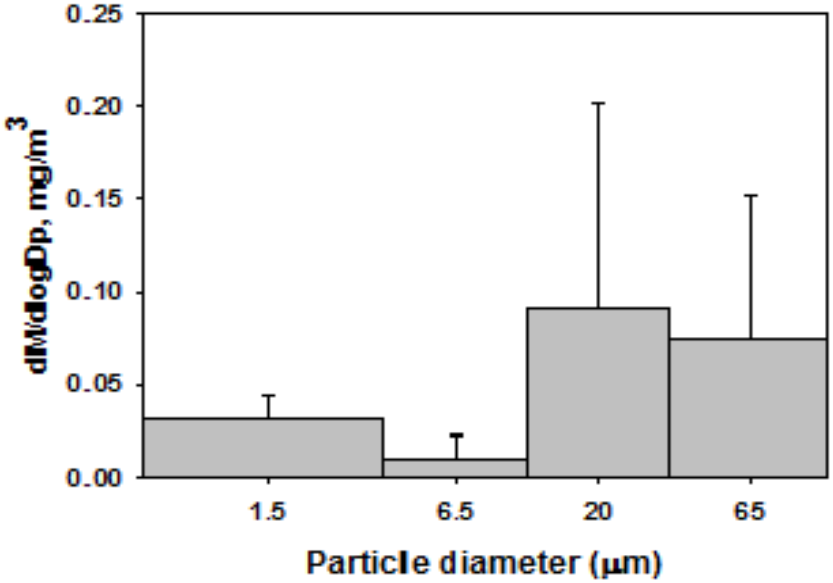
Background

- Cross-shift and longitudinal lung function decline
- Asthma
- Chronic bronchitis
- Wheeze
- Cough



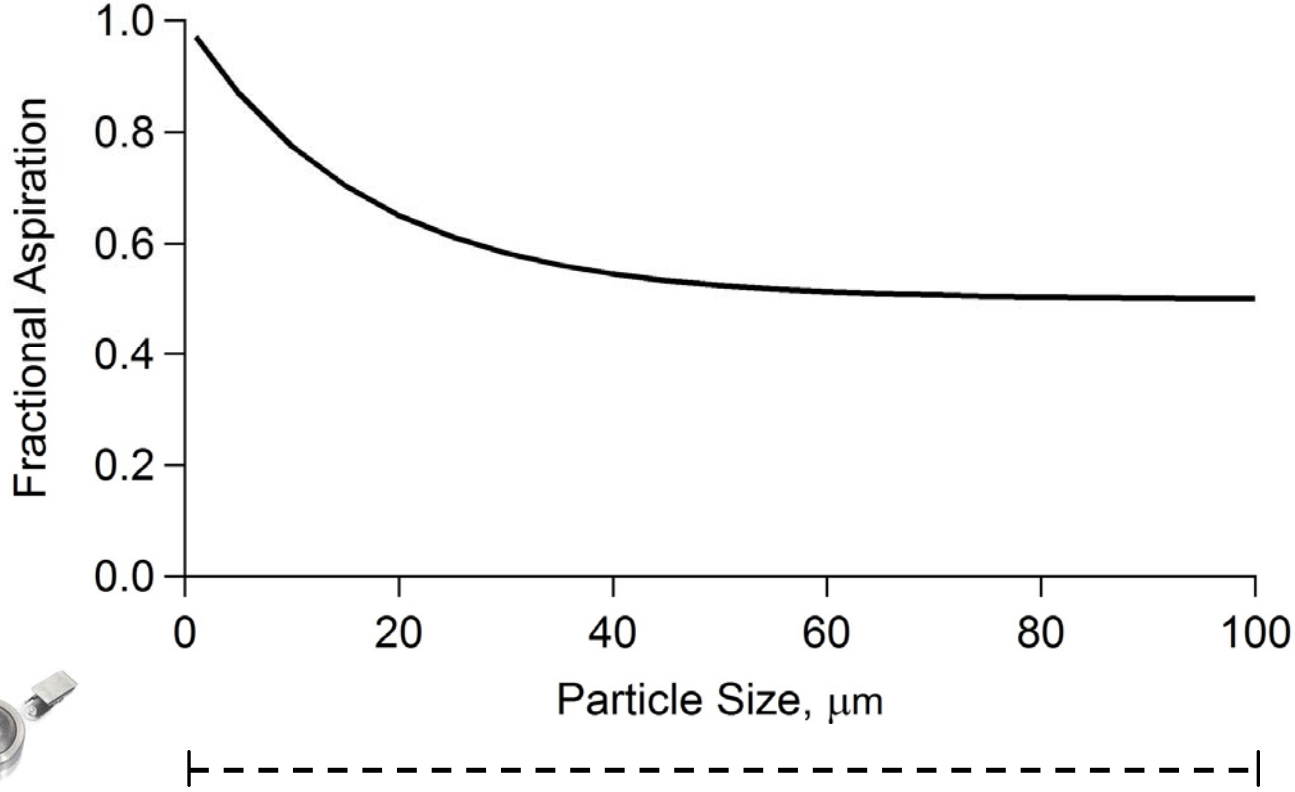
Aerosol mass size distribution in a Colorado dairy measured by the ISEL cloud impactor. Data represents 8hr time-weighted averages taken across several days.

Background



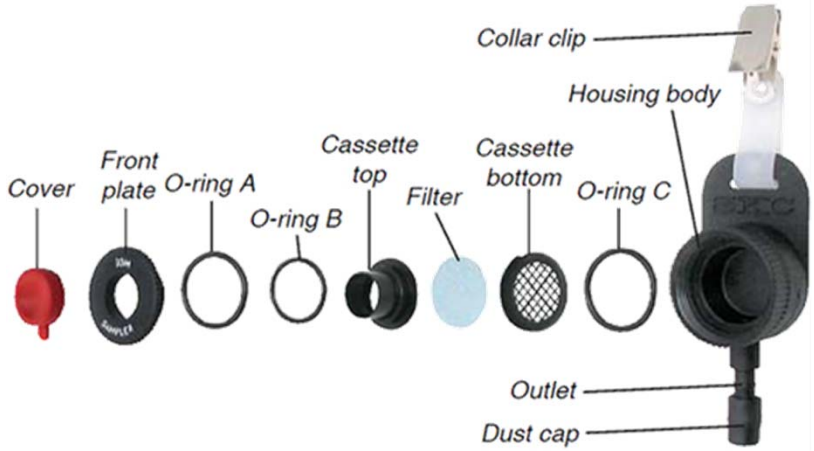
Aerosol mass size distribution in a Colorado dairy measured by the ISEL cloud impactor. Data represents 8hr time-weighted averages taken across several days.

Inhalable Particulate Matter Criterion



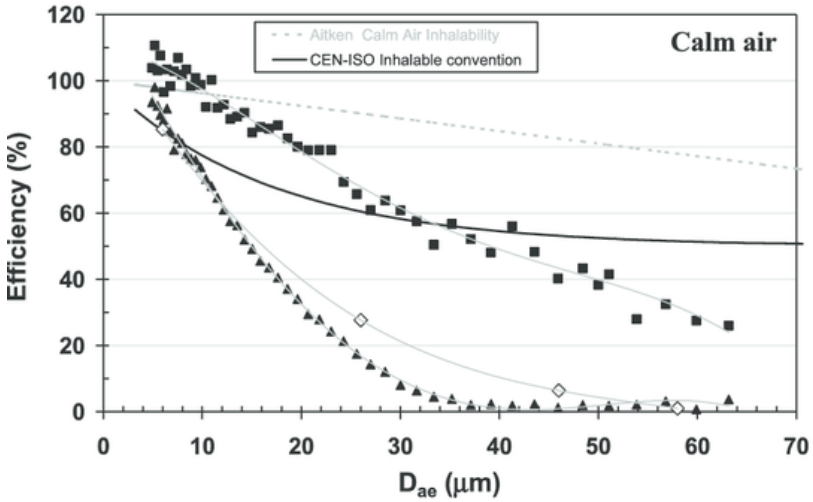
Limitations of existing inhalable samplers

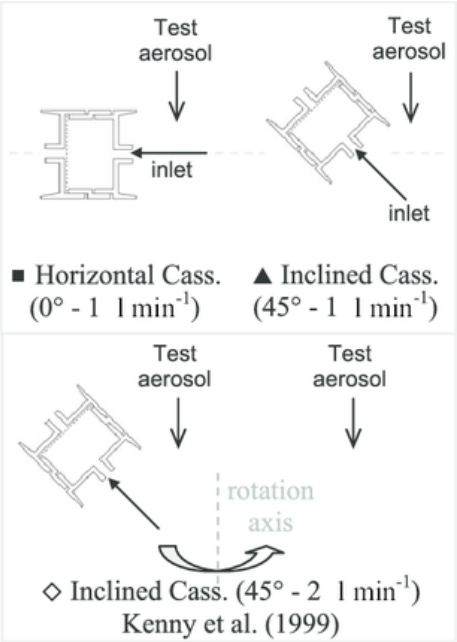
- Inhalable samplers are designed to have sampling efficiencies matching the Inhalable Particulate Matter Criterion
- Current state-of-the-art: IOM Inhalable Dust Sampler
  - $85 conductive plastic
  - $269 stainless steel
- Button Sampler
  - $275



Limitations of total dust samplers

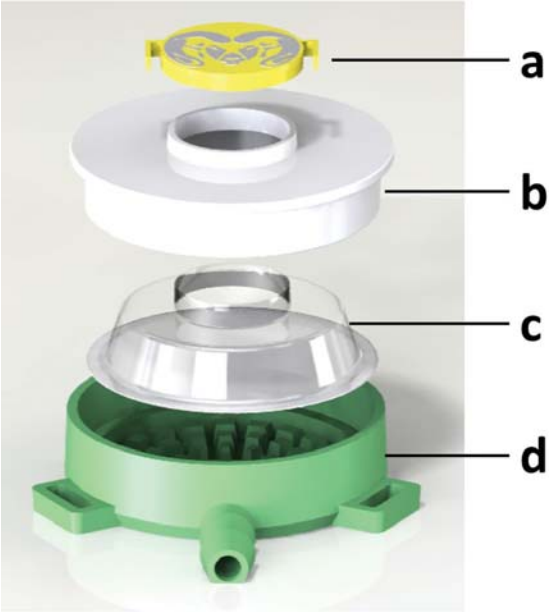
- $10.25/10 pk





Görner, Peter, et al. "Laboratory study of selected personal inhalable aerosol samplers." *Annals of occupational hygiene* 54.2 (2010): 165-187.

What is the PHISH sampler?



Exploded view of the new sampling system (a) inlet cover, (b) inlet, (c) capsule and filter, and (d) housing.

- **P**ersonal **H**igh-flow **I**nhalable **S**ampling **H**ead (PHISH)
- Inhalable sampler that operates at 2 to 10 L min^{-1}
- Sampling efficiency is designed to match the Inhalable Particulate Matter Criterion
- Designed to overcome the limitations of existing inhalable and total dust samplers

Use of 3D printing for manufacturing

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Opinions expresse

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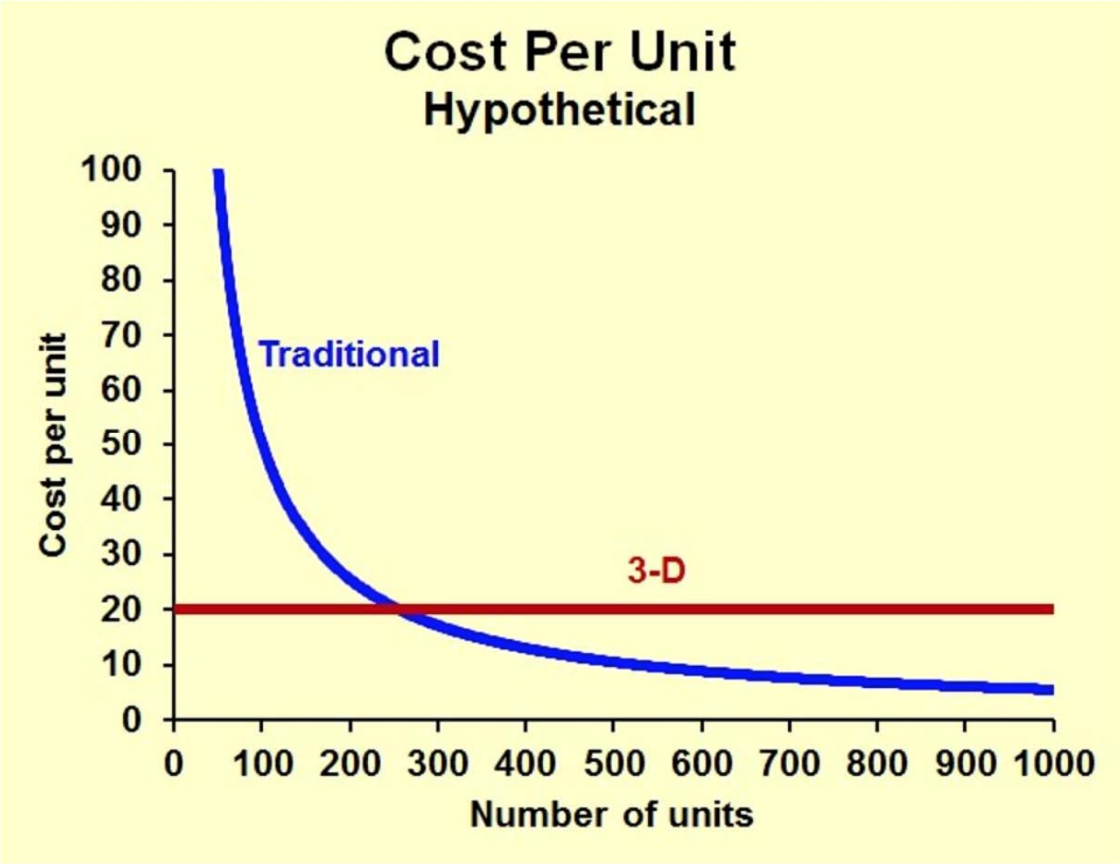
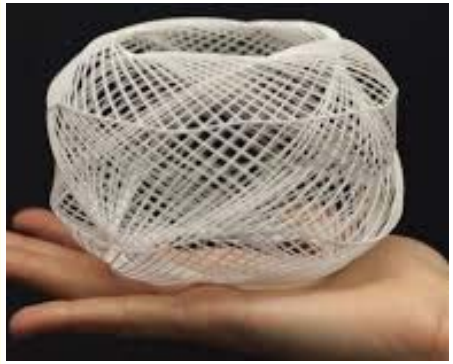
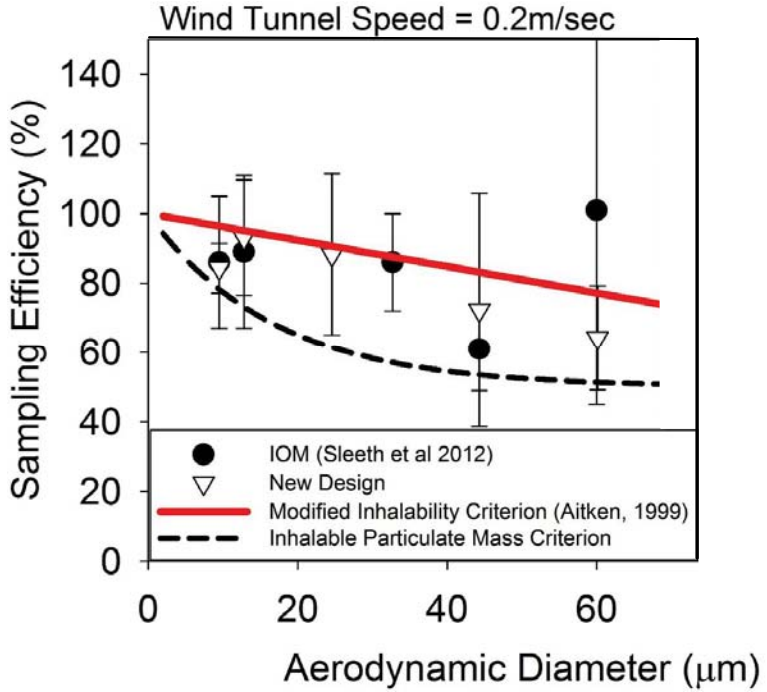




Wind tunnel testing of the PHISH sampling efficiency

- Sampling efficiency was evaluated using wind tunnel and computer modeling experiments
- Tested a range of flowrates from 2 to 10 l min^{-1}



Sampling efficiency of capsules compared to previous IOM studies and the modified inhalability criterion. Error bars represent pooled variance of all samplers and replicate tests at each particle size evaluated

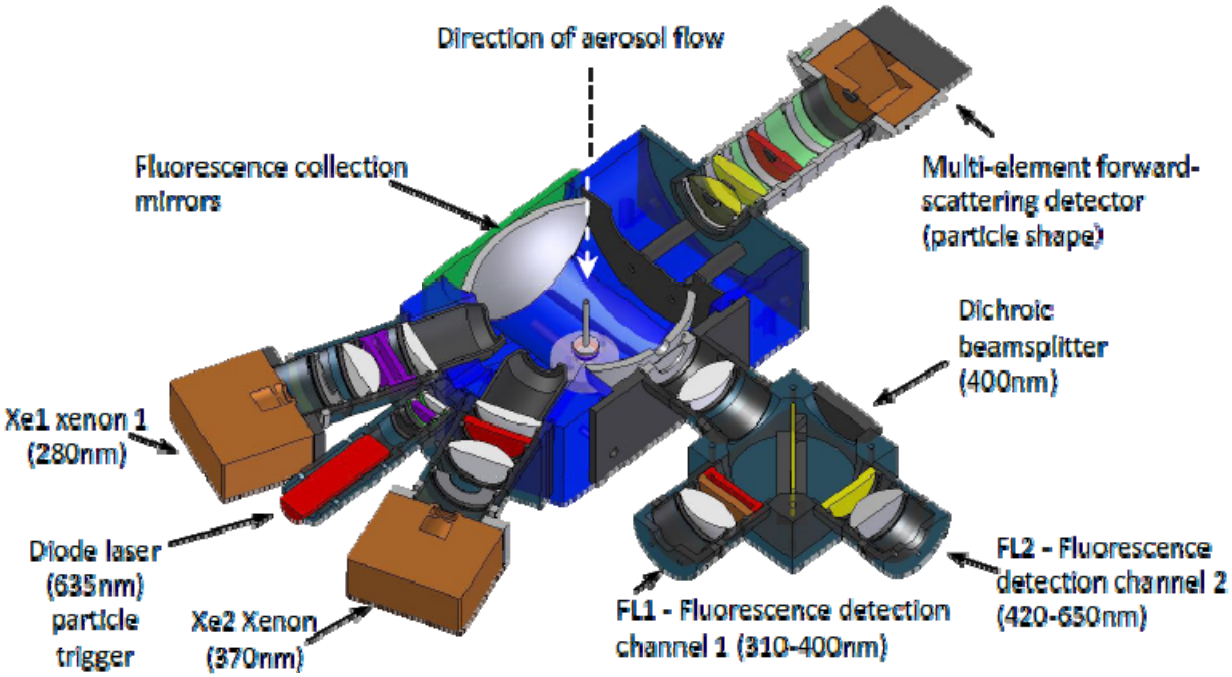
PHISH Sampler

1. Anthony, T. R., Landázuri, A. C., Van Dyke, M., & Volckens, J. (2010). Design and computational fluid dynamics investigation of a personal, high flow inhalable sampler. *Annals of Occupational Hygiene*, meq029.
2. L'Orange, C., Anderson, K., Sleeth, D., Anthony, T. R., & Volckens, J. (2015). A simple and disposable sampler for inhalable aerosol. *Annals of Occupational Hygiene*, mev065.
3. Anthony, T. R., Sleeth, D., & Volckens, J. (2016). Sampling efficiency of modified 37-mm sampling cassettes using computational fluid dynamics. *Journal of occupational and environmental hygiene*, *13*(2), 148-158.
4. Anthony, T. R., Cai, C., Mehaffy, J., Sleeth, D., & Volckens, J. (2016). Performance of Prototype High-Flow Inhalable Dust Sampler in a Livestock Production Facility. *Journal of Occupational and Environmental Hygiene*, (just-accepted).
5. Stewart, J., Sleeth, D. K., Handy, R. G., Pahler, L. F., Anthony, T. R., & Volckens, J. (2016). Assessment of increased sampling pump flow rates in a disposable, inhalable aerosol sampler. *Journal of Occupational and Environmental Hygiene*, (just-accepted), 00-00.

Wideband Integrated Bioaerosol Sensor (WIBS-4)

- Real-time fluorescent instrument
- Dual-wavelength excitation (280 and 370 nm) and dual-band emission measurements (310-400 and 420-650 nm)
- Improved discrimination among fluorescent particle types compared to single wavelength excitation methods
- Single-particle measurements of fluorescence, optical size and shape factor
- Deployed in a wide range of challenging environments including aircraft[1] and tower installations in forested regions[2]

Wideband Integrated Bioaerosol Sensor (WIBS-4)



Dairy Parlor Field Testing

Objectives:

1. Measures of upper respiratory inflammation will be more strongly associated with exposures measured with the PHISH sampler compared to those made using the 37-mm CFC.
2. Evaluate the temporal trends in particle number concentration, size, and fluorescent signal of bioaerosols on a dairy

Methods

Exposure measures

- PHIS
- 37-m
- Butt
- WIB ed PHISH sam

Health effects data

- Pre/post shift questionnaires
- Pre/post shift pulmonary function testing
- Post shift nasal lavage
  - TNF-α, IL-1β, IL-6, IL-8, IL-10, and IFN-γ
  - Cytokines measured using Luminex, a bead-based multiplexing technology, and MagPix plate reader

Methods: Nasal Lavage and Cytokines

- Collect from worker following his/her shift and on day off using sterile saline.
- Cells separated for cellular differentiation using light microscopy.
- Cytokines measured using Luminex, a bead-based multiplexing technology, and MagPix plate reader.
- Measured concentrations of six markers of upper respiratory inflammation: TNF-α, IL-1β, IL-6, IL-8, IL-10, and IFN-γ.

Methods

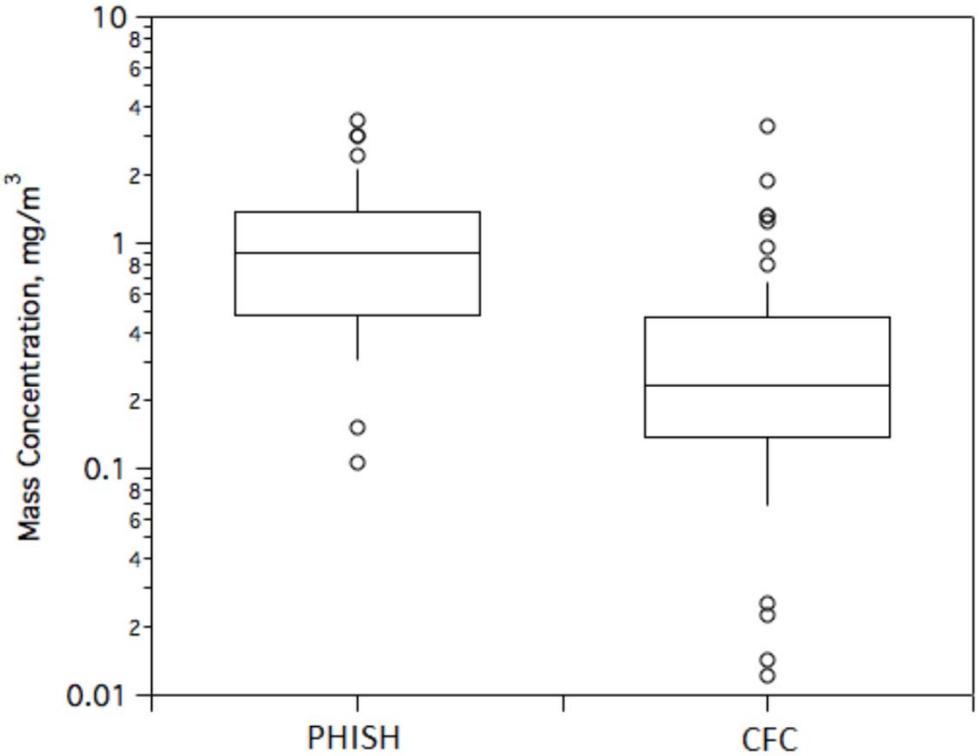
- Four dairies in Northern Colorado
  - Dairies 1 and 4 were ~2000 cows
  - Dairies 2 and 3 were >5000 cows
  - All were organic dairies
  - Elevated parallel parlor arrangements
- 38 workers participated
- 27 workers with 3 full sampling days and day-off
- 121 matched CFC and PHISH samples
- 1 continuous week of area monitoring

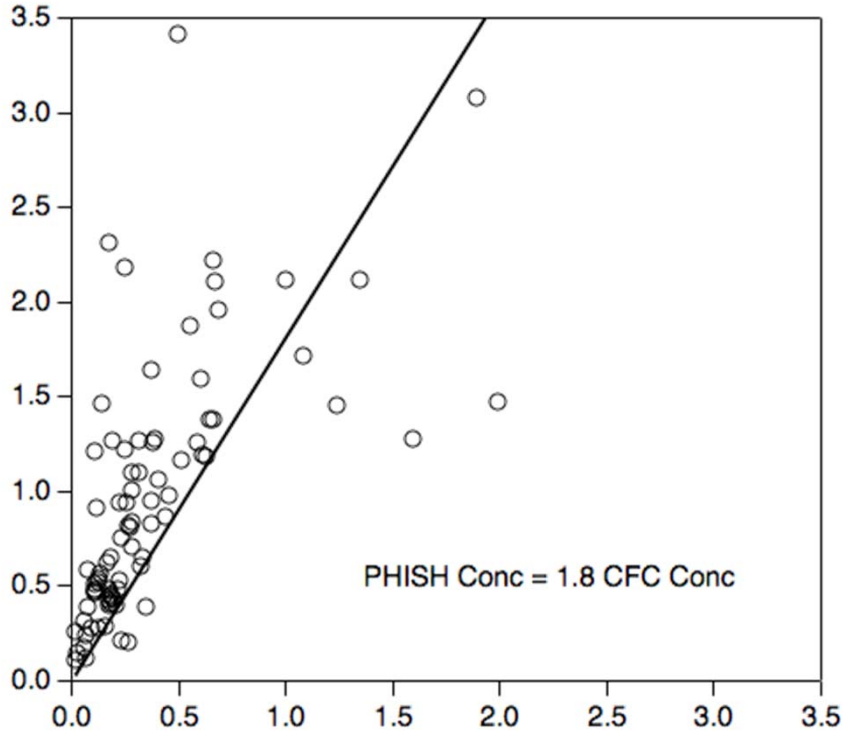
Participant Demographics

		N	% Participant
Gender	Female	7	19
	Male	29	80
Ethnicity	Hispanic	33	92
	Not Hispanic	3	8
Smoking Status	Never	16	44
	Smoker	20	55
Live On a Farm	Yes	23	64
	No	13	36

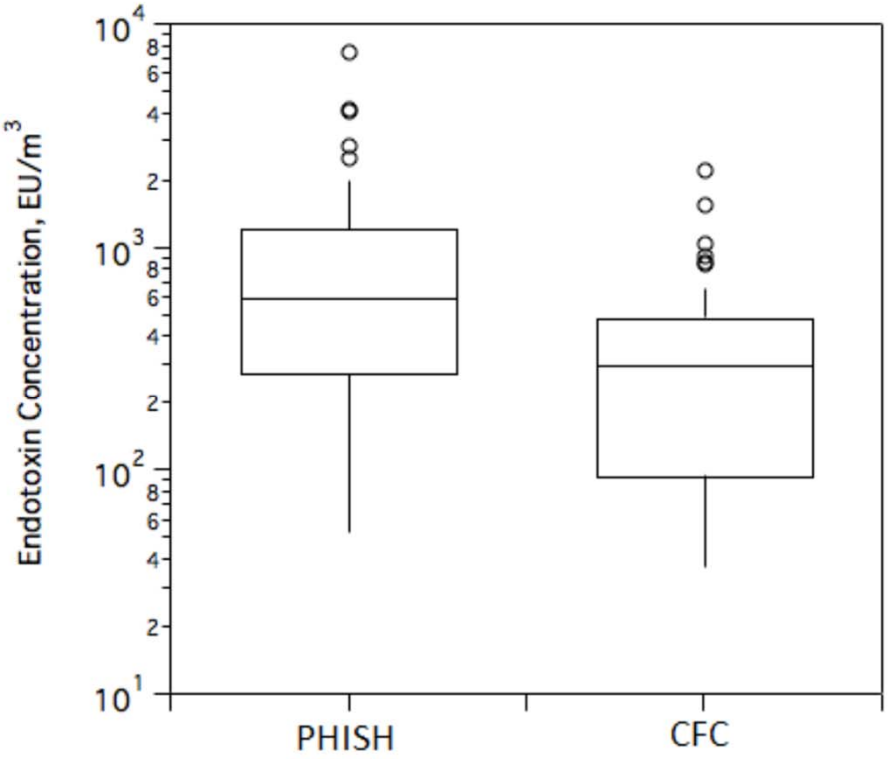
Results

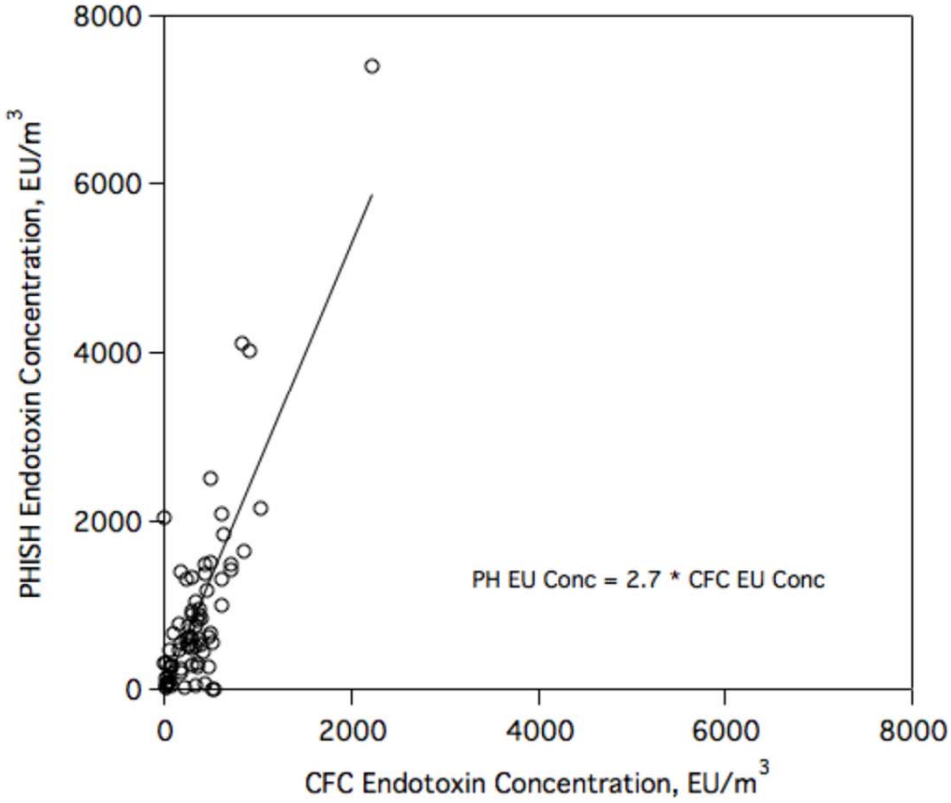
Mass Concentration
PHISH sampler compared to 37-mm CFC





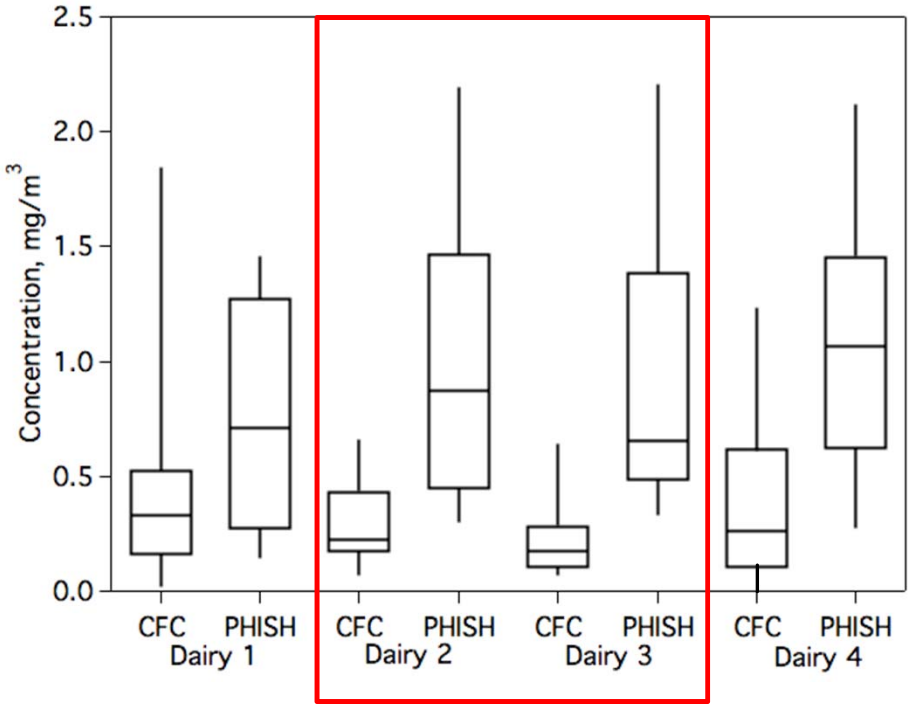
Endotoxin Concentration
PHISH Sampler Compared to 37-mm CFC



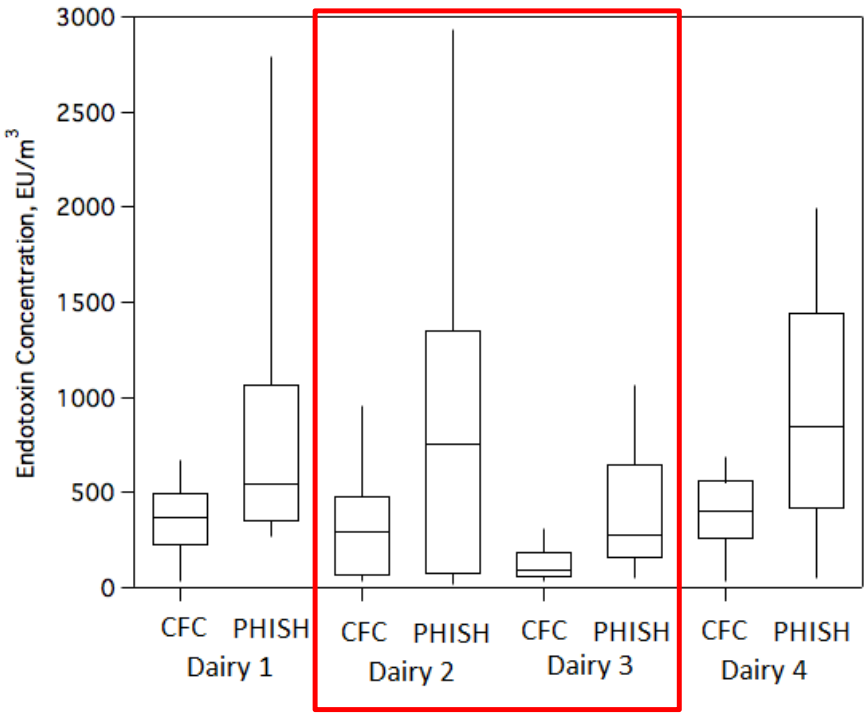


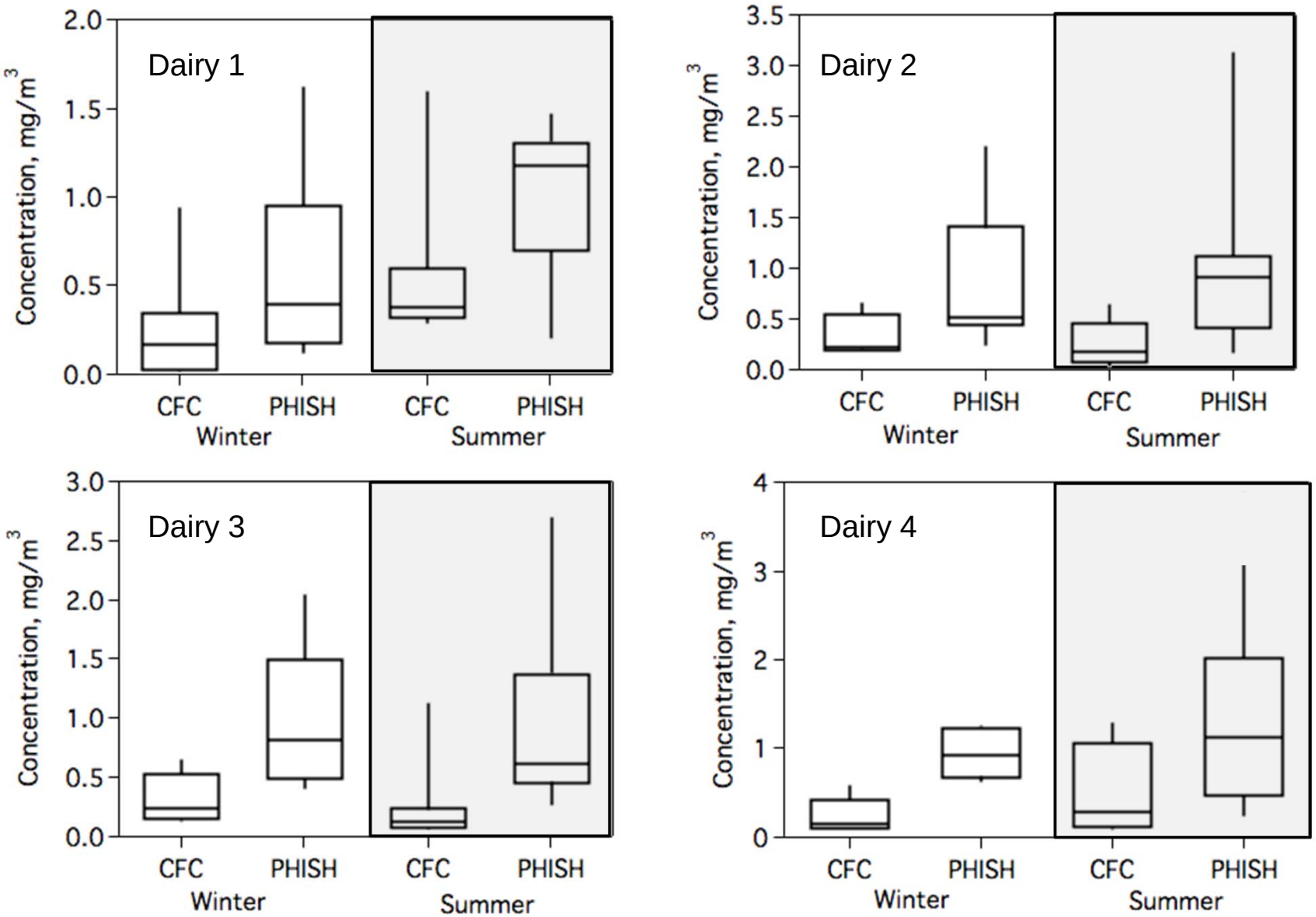
Average Concentration by Dairy

Mass Concentration, mg/m^3

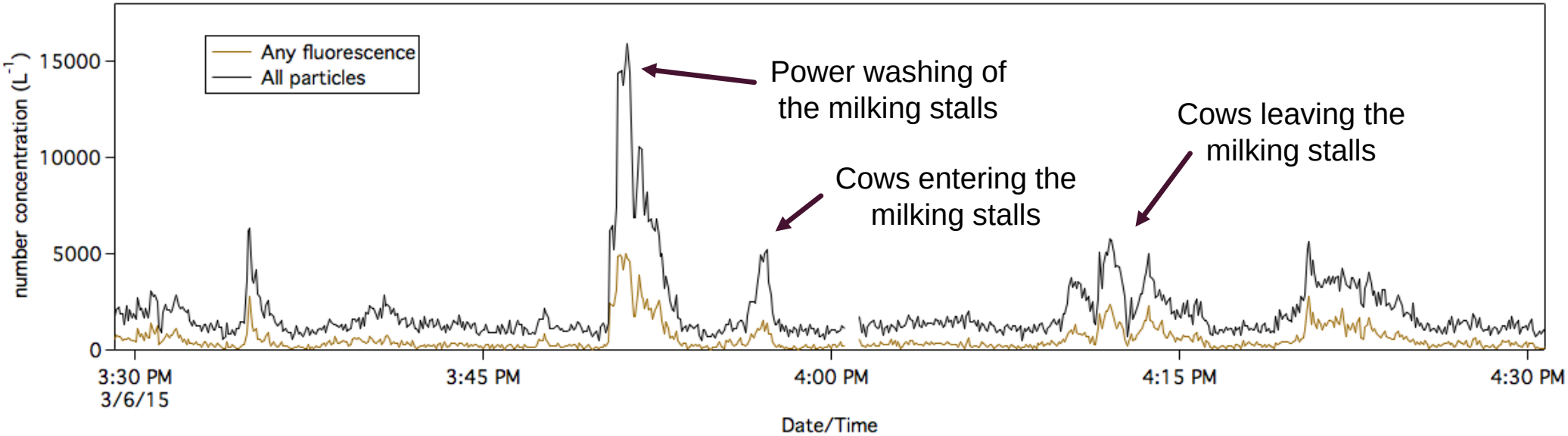


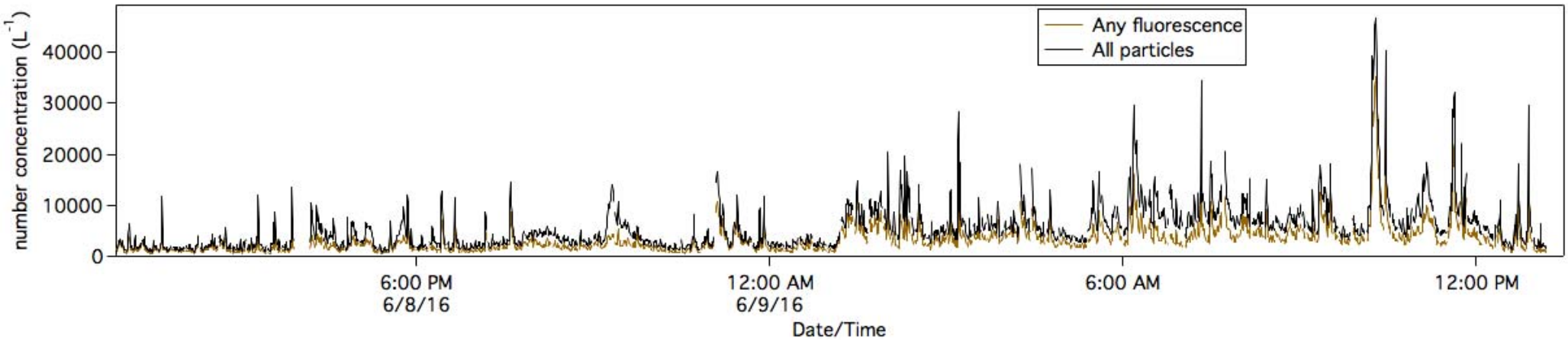
Endotoxin Concentration, EU/m^3

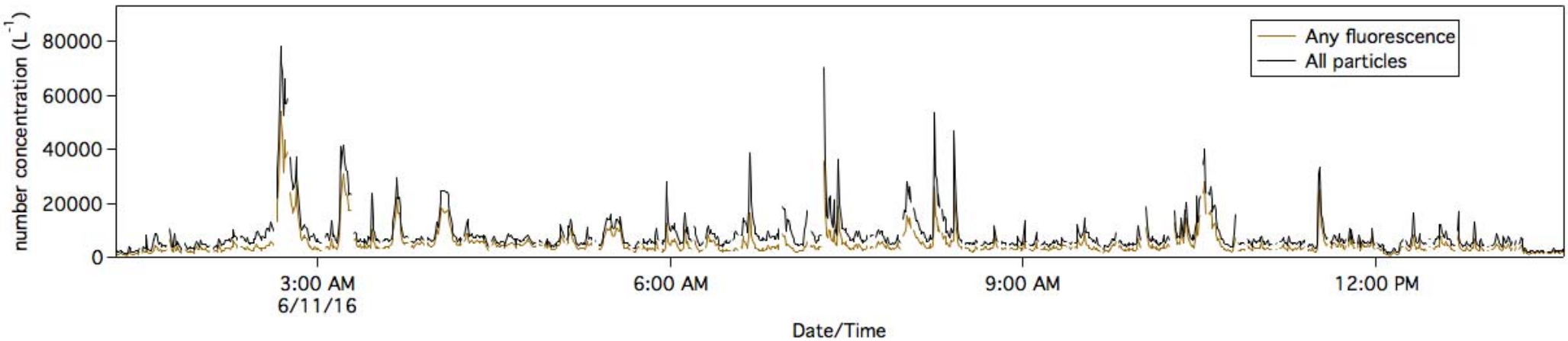


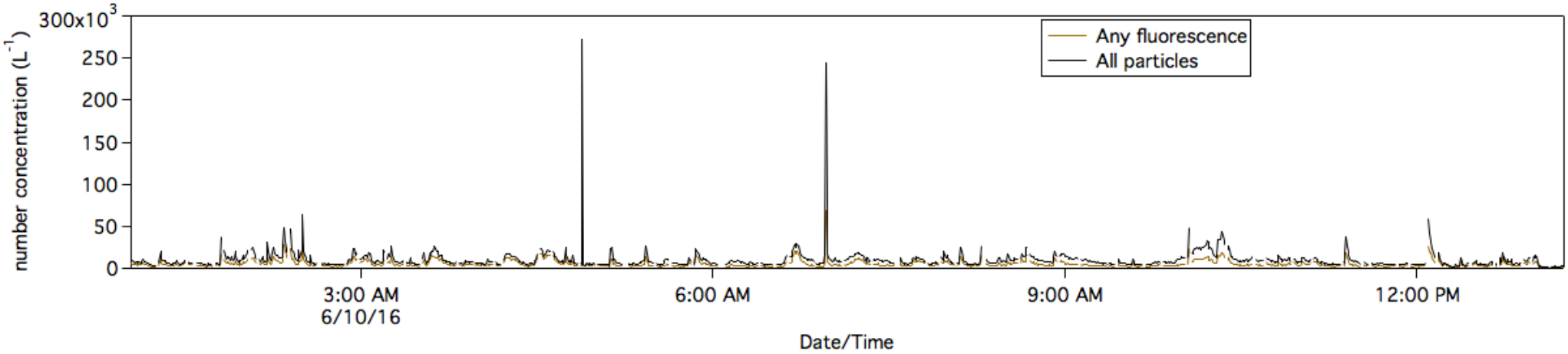

Dairy 1
Dairy 2
Dairy 3
Dairy 4
Concentration, mg/m3
CFC
PHISH
CFC
PHISH
Winter
Summer

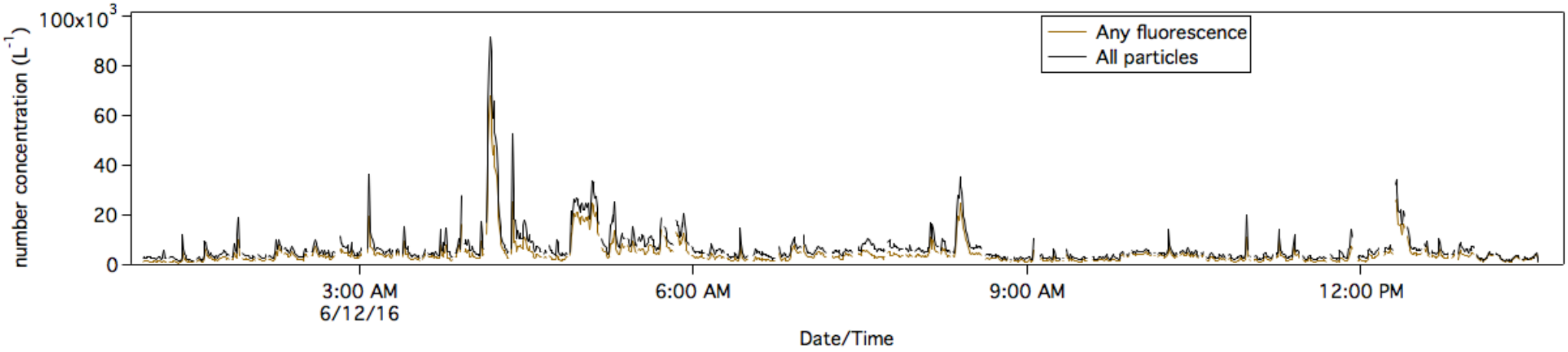
Time-series data



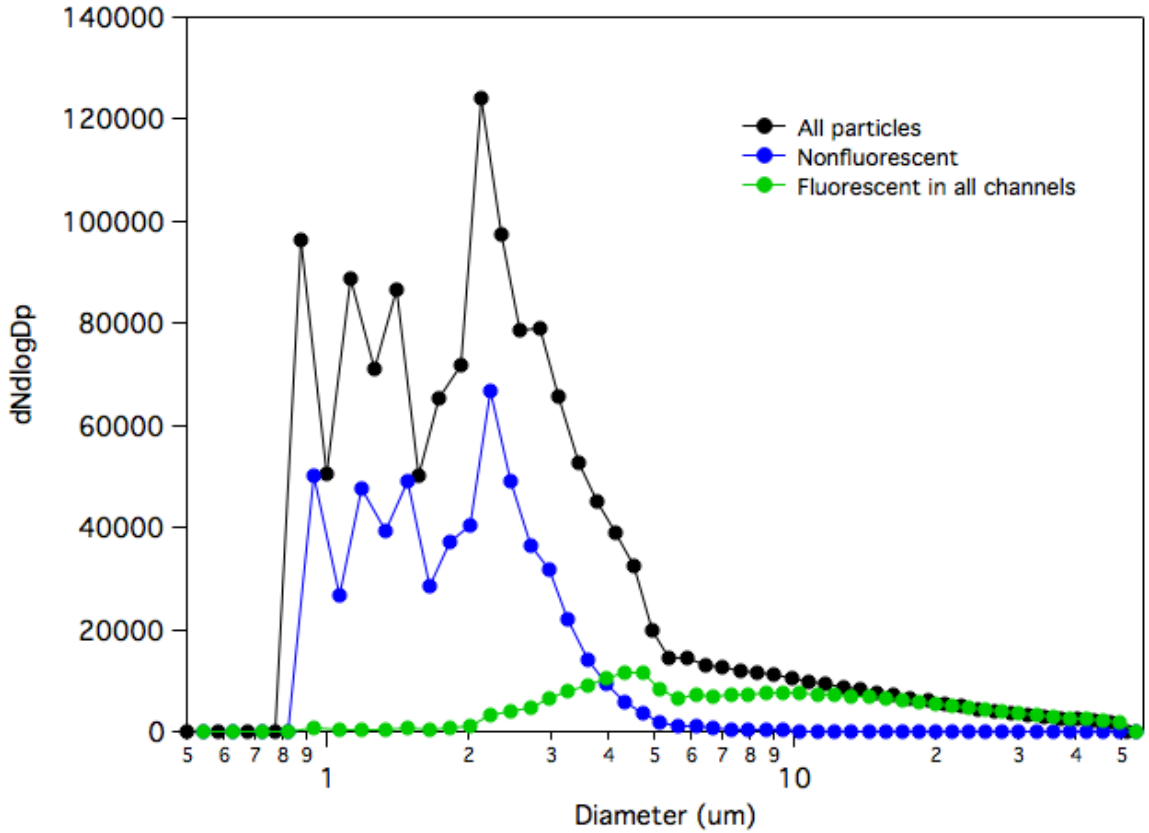

Any fluorescence
All particles
number concentration (L^{-1})
40000
30000
20000
10000
0
6:00 PM
6/8/16
12:00 AM
6/9/16
6:00 AM
12:00 PM
Date/Time


Any fluorescence
All particles
number concentration (L^{-1})
80000
60000
40000
20000
0
3:00 AM
6/11/16
6:00 AM
9:00 AM
12:00 PM
Date/Time

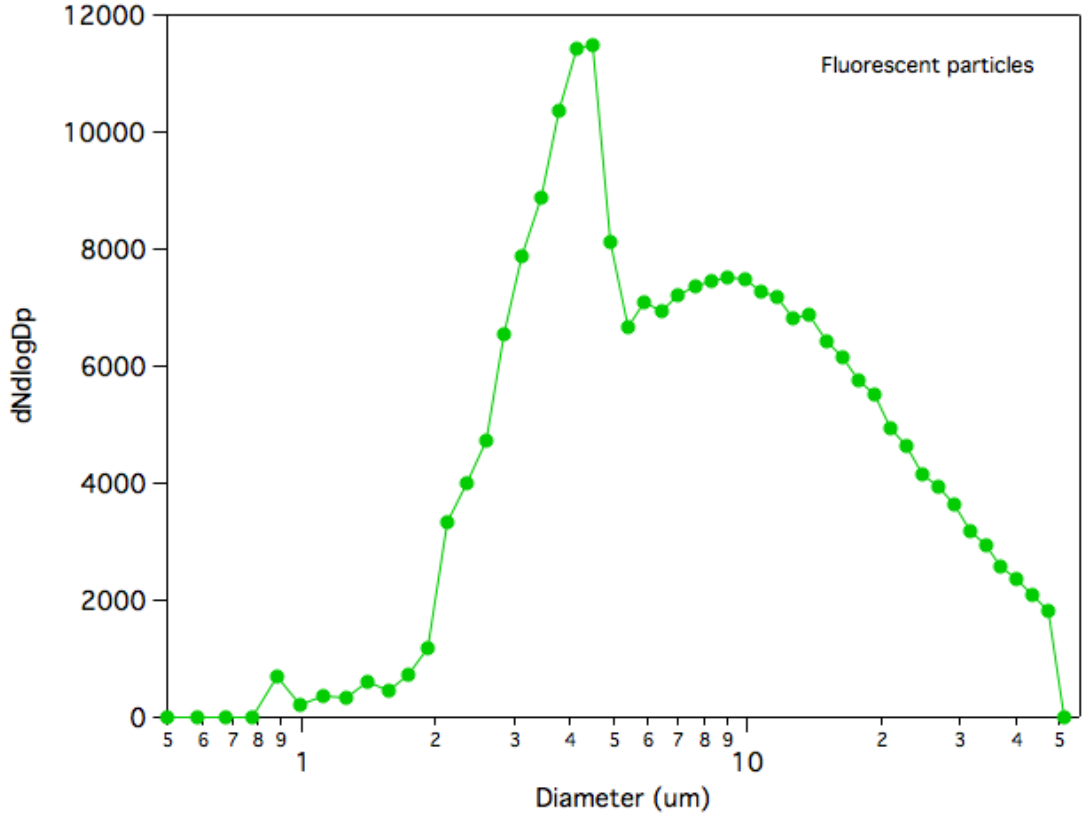

300x10^3
250
200
150
100
50
0
number concentration (L^-1)
Any fluorescence
All particles
3:00 AM
6/10/16
6:00 AM
9:00 AM
12:00 PM
Date/Time


100x10^3
80
60
40
20
0
number concentration (L^-1)
Any fluorescence
All particles
3:00 AM
6/12/16
6:00 AM
9:00 AM
12:00 PM
Date/Time

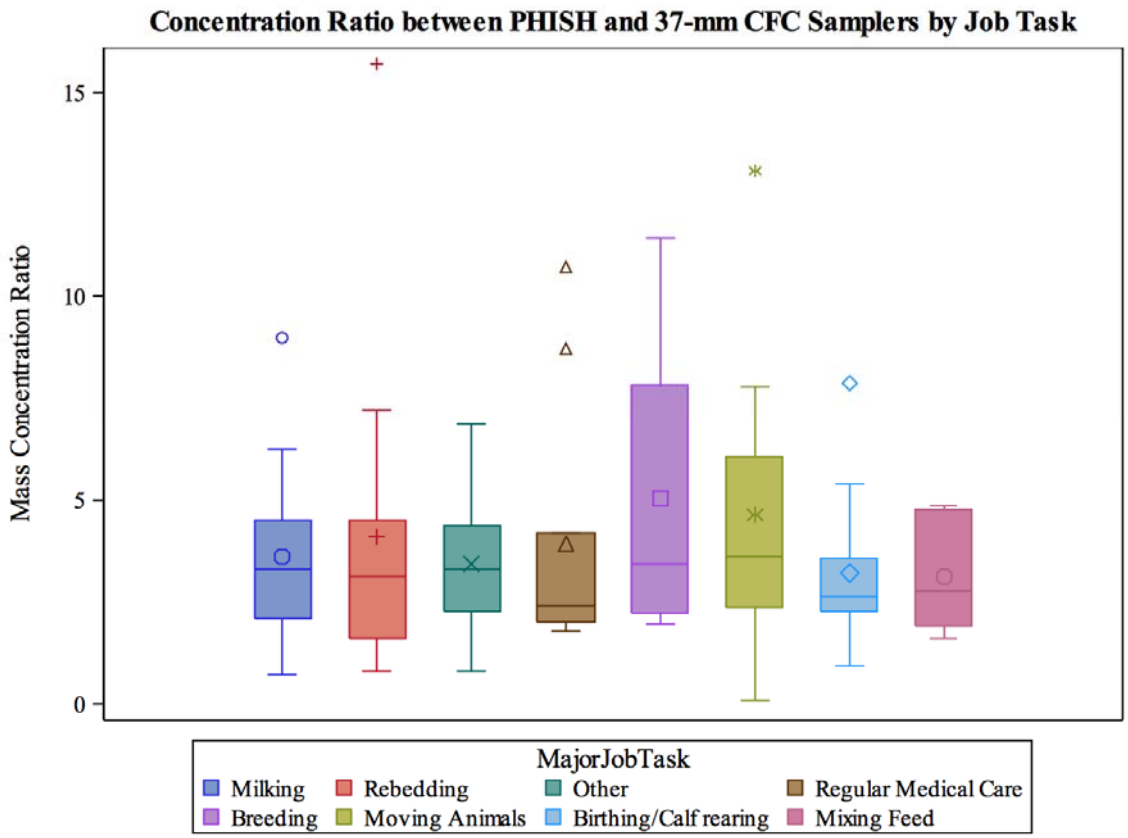
Size distribution of fluorescent and nonfluorescent particles



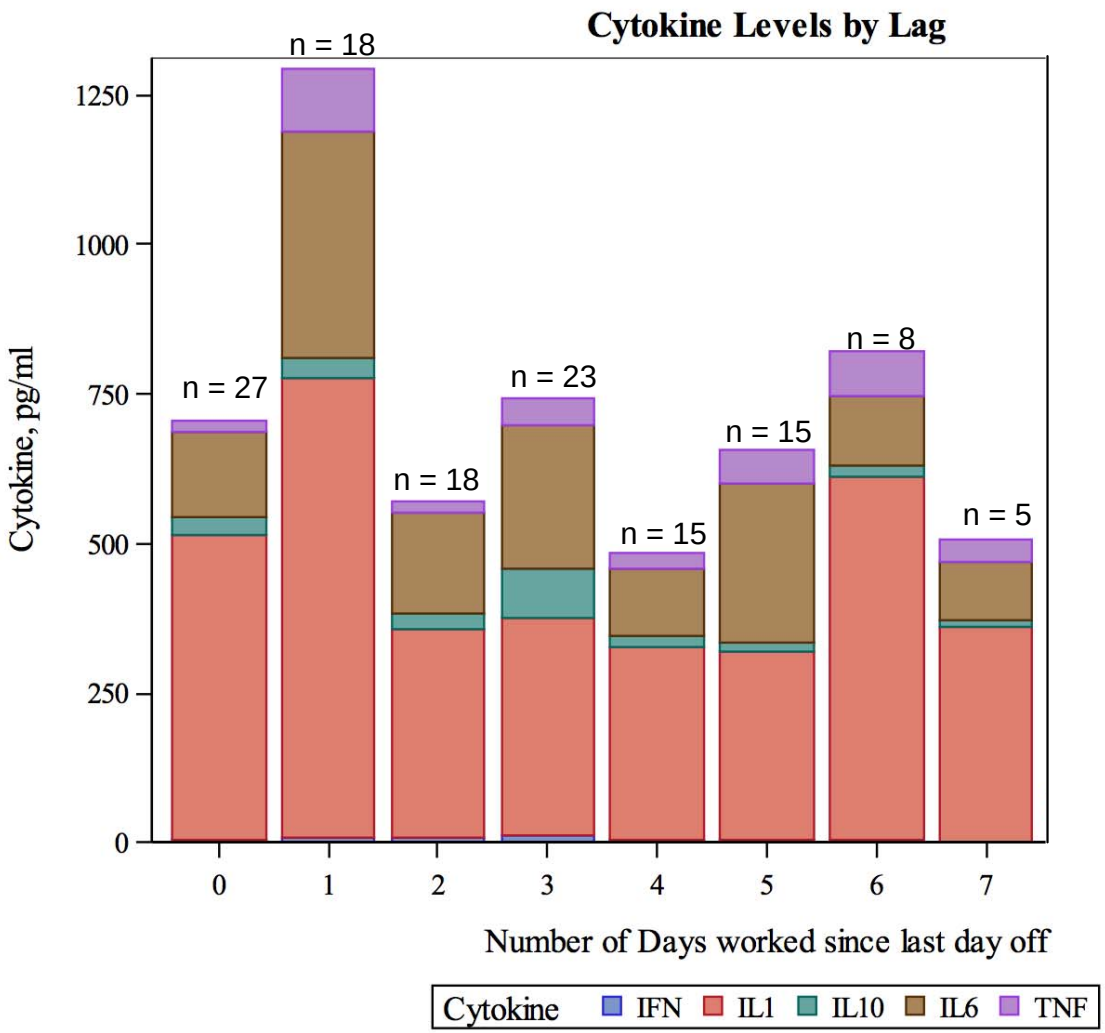
Size distribution of fluorescent particles

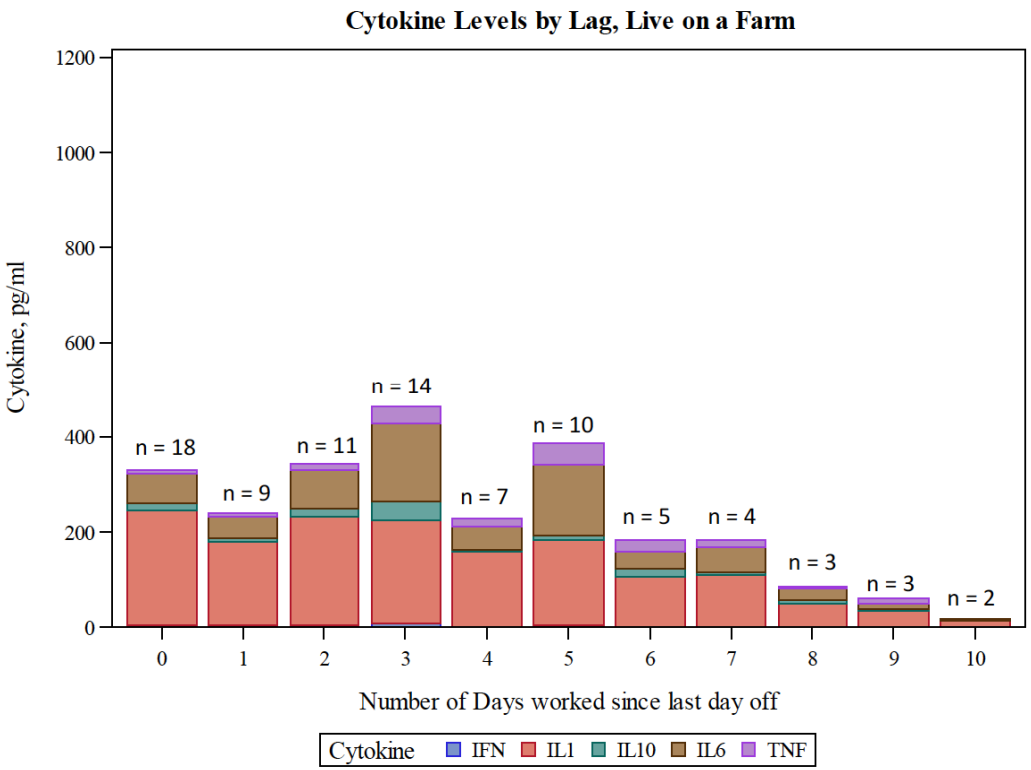


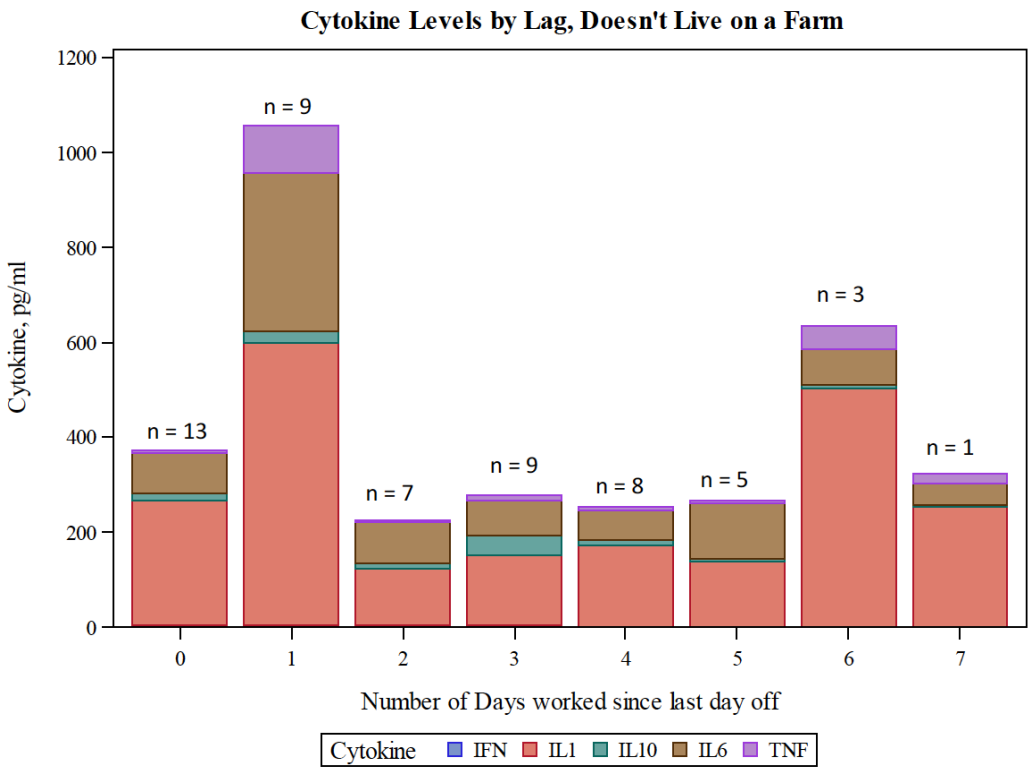
Job Task and Mass Concentration Ratios between PHISH and 37-mm CFC samplers



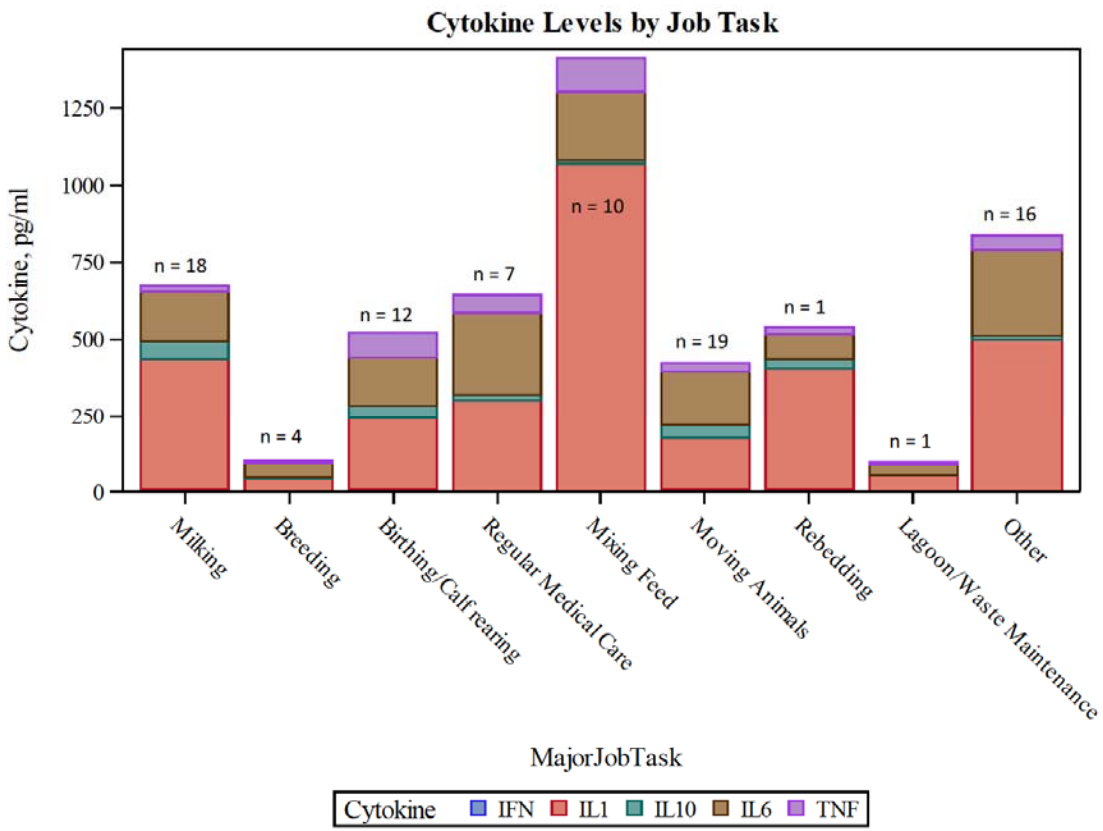
Pearson Correlation Coefficients, N = 201 Prob > |r| under H0: Rho=0						
	TNF	IL1	IL6	IL8	IL10	IFN
TNF	1.00000	0.77255 <.0001	0.53365 <.0001	0.69388 <.0001	0.01861 0.7932	0.29455 <.0001
IL1	0.77255 <.0001	1.00000	0.31536 <.0001	0.73114 <.0001	-0.03419 0.6299	-0.05243 0.4598
IL6	0.53365 <.0001	0.31536 <.0001	1.00000	0.39452 <.0001	-0.01667 0.8143	0.40006 <.0001
IL8	0.69388 <.0001	0.73114 <.0001	0.39452 <.0001	1.00000	0.07113 0.3156	-0.03554 0.6165
IL10	0.01861 0.7932	-0.03419 0.6299	-0.01667 0.8143	0.07113 0.3156	1.00000	0.08354 0.2384
IFN	0.29455 <.0001	-0.05243 0.4598	0.40006 <.0001	-0.03554 0.6165	0.08354 0.2384	1.00000


Cytokine Levels by Lag
n = 27
n = 18
n = 18
n = 23
n = 15
n = 15
n = 8
n = 5
Cytokine, pg/ml
0
250
500
750
1000
1250
0
1
2
3
4
5
6
7
Number of Days worked since last day off
Cytokine
IFN
IL1
IL10
IL6
TNF

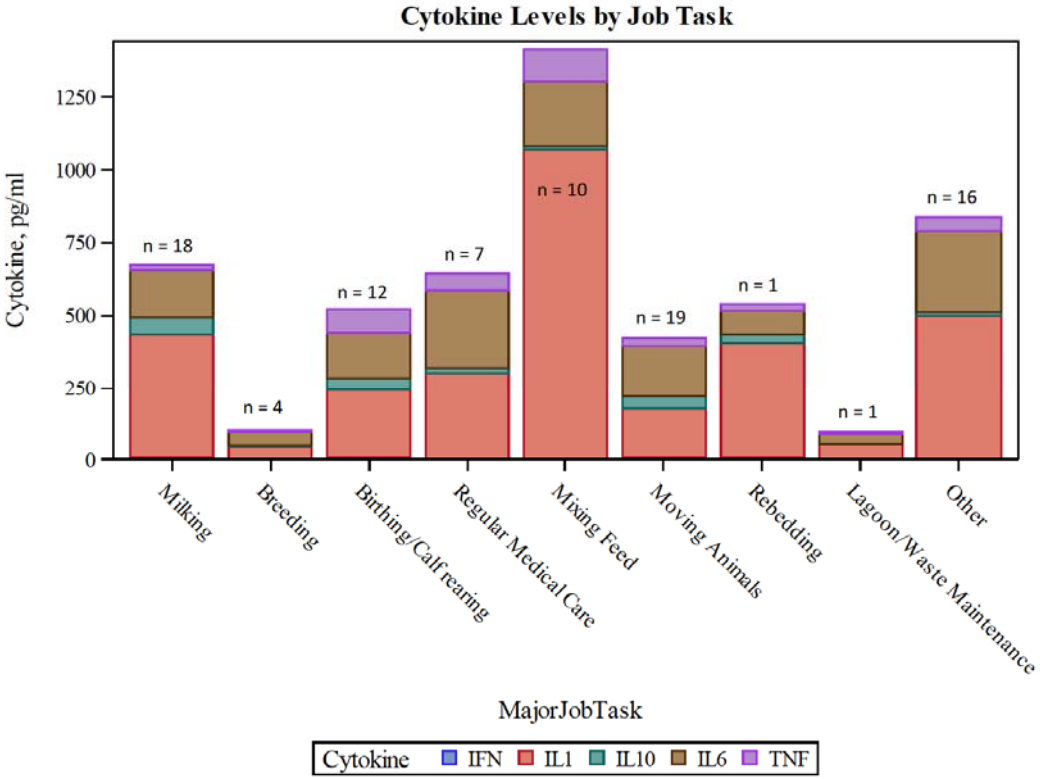

Cytokine Levels by Lag, Live on a Farm
Cytokine, pg/ml
1200
1000
800
600
400
200
0
n = 18
n = 9
n = 11
n = 14
n = 7
n = 10
n = 5
n = 4
n = 3
n = 3
n = 2
0
1
2
3
4
5
6
7
8
9
10
Number of Days worked since last day off
Cytokine
IFN
IL1
IL10
IL6
TNF

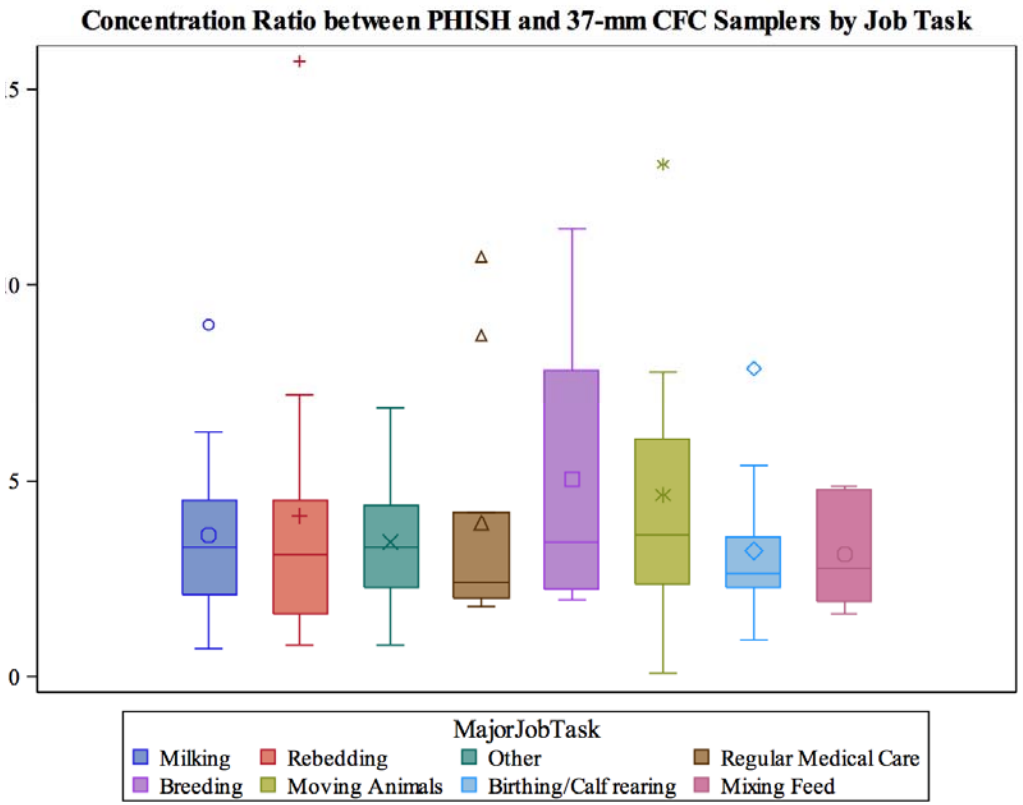

Cytokine Levels by Lag, Doesn't Live on a Farm
Cytokine, pg/ml
1200
1000
800
600
400
200
0
n = 13
n = 9
n = 7
n = 9
n = 8
n = 5
n = 3
n = 1
0
1
2
3
4
5
6
7
Number of Days worked since last day off
Cytokine
IFN
IL1
IL10
IL6
TNF

Cytokine Profile by Job Task



Cytokine Profile by Job Task





Pulmonary Function Testing

- FEV_1 and FVC were significantly lower (P = 0.003) post-shift compared to pre-shift
- Significant Predictors of FEV_1:
  - CFC mass concentration (P = 0.02)
  - Age (P = 0.0137)
  - Job task (P = 0.0004)
  - Dairy (P = 0.0192)
  - Interaction of age and gender (P = 0.0421)

Upper respiratory symptoms

- Proportion of reported symptoms ***increased*** between pre and post shift for:
  - Eye irritation
  - Nose irritation
  - Headache
- ***Decreased*** for
  - Blurred vision
  - Mucous
  - Shortness of breath
  - Wheezing
  - Throat irritation
  - Cough.

Changes were not statistically significant

Significant predictors of TNF-α levels

TNF-α levels were associated with:

- PHISH mass concentration ($P<0.0001$)
- Dairy ($P<0.0001$)
- Job task ($P<0.0001$)
- Gender ($P <0.0001$)
- Number of days worked since last day off ($P = 0.0211$)
- Interaction between dairy and job task ($P<0.0001$)

Significant predictors of IL-8 levels

IL-8 levels were associated with:

- PHISH endotoxin concentration (P=0.05)
- Dairy (P = 0.055)
- Job task (P = 0.026)
- Interaction between job task and dairy (P = 0.009)

Conclusions

- PHISH mass concentrations were 1.8 times higher compared to 37-mm CFC
- PHISH endotoxin concentrations were
- Concentration ratios varied by job task
- TNF-α, IL1-β, IL6, IL8, IL10, and IFN-γ were strongly correlated with one another
  - IL10 inhibits TNF-α and IFN-γ
  - IL6 and IL8 activate each other in cascade reactions
- Living on site resulted in lower cytokine levels
- Cytokine profile varied by job task
- Temporal variation in particle number concentration

Acknowledgements

Design, Evaluation, and Validation of a Next Generation Inhalable Aerosol Sampler CDC/NIOSH R01 OH10295
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Dr. Steve Reynolds
Dr. Josh Schaeffer

University of Denver:
Dr. Lee Newman
Brian Tooker
Stephen Brindley

Field study team:
Jessy Tryon
Mary Bradford
Amanda VanDyke

WIBS research/field team:
Dr. Gavin McMeeking
Dr. Nicholas Good

Questions?