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Principle Investigator: Stephen J. Reynolds, Ph.D., CIH
Professor
Dept. Environmental and Radiological Health Sciences
154 EHB
Stephen.Reynolds@Colostate.edu
970-491-3141

Institution: Colorado State University
Ft. Collins, CO 80523-1681

Co-Investigators: Thomas Keefe, Ph.D
Thomas.Keefe@Colostate.edu

John Tessari, Ph.D.
John.Tessari@Colostate.edu

Marv Tillery, Ph.D.
tillerym@sprynet.com

Collaborators at The University of Iowa GPCAH:

Kelley Donham, DVM, PhD
Kelley-Donhma@Uiowa.edu

Patrick O'Shaughnessy, Ph.D.
Patrick-OShaughnessy@Uiowa.edu

Peter Thorne, Ph.D.
Peter-Thorne@Uiowa.edu

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LIST OF TERMS AND ABBREVIATIONS

Inhalable - Aerosol size distribution defined (ACGIH) to have a cut point (50% collection efficiency) at 100 microns.

Respirable -Aerosol size distribution defined (ACGIH) to have a cut point (50% collection efficiency) at 4 microns.

ACGIH – American Conference of Governmental Industrial Hygienists

IOM – Institute of Medicine Inhalable sampler.

Button – Inhalable sampler with porous screen.

CFC – Closed face cassette. 37 mm diameter, plastic.

rFC – Recombinant Factor C endotoxin assay.

LAL – Limulus Ameobocyte Lysate endotoxin assay.

ABSTRACT

Nearly one million U.S. men, women, and children working in agricultural production are at risk for occupational lung disease related to organic dust exposures. The primary goals of this project were 1) to evaluate a novel Recombinant Factor C endotoxin assay using organic dusts from livestock environments, 2) to evaluate new methods for measuring inhalable particulates, endotoxins, and glucans/ergosterols that can be used to help establish occupational exposure guidelines for complex organic dusts in swine, poultry, dairy, equine and sheep environments, 3) to evaluate and develop correction factors for direct-reading aerosol instruments that can be readily used by practitioners for interventions. This project involved close collaboration between the High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) at Colorado State University and the University of Iowa's Great Plains Center for Agricultural Health (GPCAH), and complemented and enhanced the related project conducted at that Center. A unique aspect of this study was the laboratory evaluation of sampler performance when influenced by wind. Overall the IOM had the lowest coefficient of variation (best precision) and was least affected by changes in wind speed. The performance of the Button was negatively impacted in poultry environments where larger (feather) particulates clogged the holes in the screen. In addition to wind speed, MMAD and dust type were important factors affecting performance. Conversion factors for dust (mg/m3) and endotoxin (EU/m3) were estimated based on field studies for ratios of Button/IOM, CFC/IOM, and Cyclone/IOM. This was the first study to develop a method and investigate the use of a new rFC endotoxin assay for agricultural dusts. In general, strong positive correlations exist between the LAL and rFC assays. A GC/EI-MS method for endotoxin analysis was also developed and applied to assessment of 3OHFA distributions. Compared to the parent GC/MS-MS method, it reduces use of toxic chemicals and sample handling, allows sensitive monitoring of the experimental process, and can be used for analysis of very small samples, typical of personal air samples. The direct reading DataRam (PDR) was highly reliable in all field tests and in most of the laboratory tests. Dust type (with different aerosol size distributions) and wind speed are important factors affecting the performance of the PDR. Correction factors were calculated and could be used by researchers and practitioners to calibrate direct reading PDRs, which would be simpler to use in the field and provide results more quickly. This project addressed the need for more research related to organic dusts in agriculture identified by the NIOSH Board of Scientific Counselors, as well as developing practical cost-effective tools for application in engineering and other interventions, also identified as a priority.

HIGHLIGHTS/SIGNIFICANT FINDINGS

This study evaluated the performance of four commonly used aerosol samplers in four agricultural livestock environments. Laboratory studies using a still air chamber and a wind tunnel provided important information regarding the effect of wind velocity on sampler performance. Overall the IOM had the lowest coefficient of variation (best precision) and was least affected by changes in wind speed. The performance of the Button was negatively impacted in poultry environments where larger (feather) particulates clogged the holes in the screen. In addition to wind speed, MMAD and dust type were important factors affecting performance. Variation in aerosol size distribution of endotoxins may have implications for risk of respiratory disease in some environments. Wind velocity had a significant effect on performance for measuring endotoxin as well as dust. Conversion factors for dust (mg/m3) and endotoxin (EU/m3) were estimated based on field studies for ratios of Button/IOM, CFC/IOM, and Cyclone/IOM. These may be used for comparison of results between older and new studies using these samplers, or for conversion between Occupational Exposure Limits based on different samplers.

This was the first study to develop a method and investigate the use of a new rFC endotoxin assay for agricultural dusts. In general, strong positive correlations exist between the LAL and rFC assays. However, responses to assays vary by agricultural environment or dust type. Other than ergosterol contribution, variability between assays could be explained by difference in bacterial composition and other dust components; e.g. the rFC assay may react positively with Actinobacteria.

A GC/EI-MS method for endotoxin analysis has been successfully developed and applied to assessment of 3OHFA distribution in agricultural environments. Compared to the parent GC/MS-MS method, it reduces use of toxic chemicals and sample handling, allows sensitive monitoring of the experimental process, and can be used for analysis of very small samples, typical of personal air samples.

The direct reading DataRam (PDR) was highly reliable in all field tests and in most of the laboratory tests. Dust type (with different aerosol size distributions) and wind speed are important factors affecting the performance of the PDR. Regression models for prediction of PDR performance relative to specific gravimetric samplers based on field studies (real world conditions) explain a large proportion (48% to 96%) of the variation in PDR values. These "correction factors" could be used by researchers and practitioners to calibrate direct reading PDRs, which would be simpler to use in the field and provide results more quickly.

TRANSLATION OF FINDINGS

The new analytical method procedures for rFC endotoxin analysis of agricultural/organic dusts and for GC/MS analysis of chemical markers of endotoxin – 3 OHFAs can be used by researchers and analytical laboratories for evaluation of endotoxin exposures in a wide variety of environments with potential exposures to organic dusts.

The findings regarding performance and comparison of gravimetric and direct reading samplers for measuring dust and endotoxin aerosols can be used by researchers and practitioners to improve exposure assessment through understanding the factors influencing the sampling results from these gravimetric and direct reading devices. The results of this study have already been used by other investigators in California and Saskatchewan to plan and conduct epidemiologic studies of respiratory disease in agricultural workers. Practitioners in particular can use the results to "calibrate" direct reading PDR aerosol monitors to facilitate more accurate and rapid evaluation of exposures in a variety of industries.

The equations predicting relationships between sampler results can be used by organizations or researchers interested in making historical comparisons between data collected using two different samplers, and for establishing Occupational Exposure Limit guidelines.

Findings have been shared in presentations at scientific meetings and in peer reviewed publications. Additional publications and meetings with agricultural producer organizations are scheduled to share findings.

OUTCOMES/RELEVANCE/IMPACT

This study evaluated the performance of four commonly used aerosol samplers in four agricultural livestock environments. Laboratory studies using a still air chamber and a wind tunnel provided important information regarding the effect of wind velocity on sampler performance. Overall the IOM had the lowest coefficient of variation (best precision) and was least affected by changes in wind speed. The performance of the Button was negatively impacted in poultry environments where larger (feather) particulates clogged the holes in the screen. Correlations between the Cyclone and the IOM were relatively good in the laboratory where the range of concentrations tested could be expanded. Correlations in the field were poor due to the narrow range of aerosol concentrations. Therefore, the data obtained in this study resulted in the development of concentration ratios rather than slope values, as a better indicator of relationships between samplers. The CFC/IOM and Cyclone/IOM ratios are important for comparisons between newer and older studies, and for prediction of respirable fractions from Inhalable fractions. In addition to wind speed, MMAD and dust type were important factors affecting ratios. The CFC/IOM relationship appears to be primarily dependent on the type of building (e.g. the aerosol size distribution is stable across geographic regions) and based on the field studies the following ratios may be used for conversion factors for the CFC/IOM: Swine (0.50), Chicken (0.67), Turkey (0.60), Dairy (0.49). Conversion factors for the Cyclone/IOM are again likely best based on the field studies and are: Swine (0.05), Chicken (0.08), Turkey (0.12), Dairy (0.22). Conversion factors for the Button/IOM are: Swine (0.57), Chicken (0.80), Turkey (0.53), Dairy (0.67).

Endotoxin concentrations were higher in dusts from Iowa than in dusts from the same environments in Colorado. Concentrations of endotoxin in dust (EU/mg) and airborne endotoxin (EU/m3) varied significantly with dust type and sampler type. This variation in aerosol size distribution may have implications for risk of respiratory disease in some environments. Wind velocity had a significant effect on EU/m3 and on the rations of EU/m3 measured by the Button, CFC, and Cyclone relative to the IOM. In almost all cases higher wind velocity (1.0 m/s) resulted in lower ratios compared to still air and 0.2 m/s.

Similar to dust concentrations, conversion factors for EU/m3, based on field studies, are suggested to be: Button/IOM: Swine (0.7), Chicken (1.1), Turkey (0.8), Dairy (1.0), Feedlot (0.4), Horse (0.6); CFC/IOM: Swine (0.5), Chicken (0.7), Turkey (0.6), Dairy (0.6), Feedlot (0.7), Horse (0.8); Cyclone/IOM: Swine (0.06), Chicken (0.11), Turkey (0.9), Dairy (0.05), Feedlot (0.06), Horse (0.09). It appears that correction factors for dust and endotoxin are not the same.

In general, strong positive correlations exist between the LAL and rFC assays. However, responses to assays vary by agricultural environment or dust type. LAL may overestimate (or rFC underestimates) endotoxin exposures in chicken and horse dusts and LAL may underestimate (or rFC overestimates) endotoxin concentrations in dairy, swine, and turkey dusts. Our finding showed that ergosterol concentration may not be a major factor of interference in the LAL assay overall, but the magnitude of interference may vary by dust type. Other than ergosterol contribution, this variability could be explained by difference in bacterial composition and other dust components; the rFC assay may react positively with Actinobacteria. Future applications will be expected to increase sample size for ergosterol measurements, to analyze dusts from different agricultural environments, and to investigate presence of potential interference of assays including Actinobacteria and proteins in agricultural dusts.

A GC/EI-MS method for endotoxin analysis has been successfully developed and applied to assessment of 3OHFA distribution in several agricultural environments. Compared to the parent GC/MS-MS method, it reduces use of toxic chemicals and sample handling, allows sensitive monitoring of the experimental process, and can be used for analysis of very small samples, typical of personal air samples.

The direct reading DataRam (PDR) was highly reliable in all field tests and in most of the laboratory tests. Dust type (with different aerosol size distributions) and wind speed are important factors affecting the performance of the PDR. Regression models for prediction of PDR performance relative to specific gravimetric samplers based on field studies (real world conditions) explain a large proportion (48% to 96%) of the variation in PDR values. These "correction factors" will be further analyzed and refined in a peer reviewed publication (Jason Nakatsu's PhD dissertation). The HAM was found to be unreliable in most situations

SCIENTIFIC REPORT

This study evaluated the performance of two inhalable samplers (IOM, Button) and two traditional samplers (37 mm plastic cassette - CFC, cyclone) in agricultural environments, and determined relationships between these sampling devices to allow comparison with historical measurements. Ten sets of samples were collected in swine, chicken, turkey, and dairy facilities in both Colorado and Iowa. Pairs of each sampling device were attached to the front and back of a rotating manniken. Laboratory studies using a still air chamber and a wind tunnel with the same manniken provided important information regarding the effect of wind velocity on sampler performance. Ten sampling sessions were conducted for three different velocities for each dust type. In addition to gravimetric analysis, a novel rFC endotoxin assay was also compared to the more commonly used LAL assay. A GC/MS method for analysis of endotoxin was developed and used to further evaluate the differences between environments and assays. In addition GC/MS for fungal ergosterol was conducted. Results are organized based on the primary goals of this study.

1. *Evaluate and compare the precision and relative predicted dust concentration for the following gravimetric methods for measuring organic dusts in swine, poultry (chicken and turkey), dairy, equine, and sheep environments: Inhalable (IOM) sampler, Inhalable (IOM) sampler with size-selective MultiFoam disc, button aerosol sampler, total dust (37 mm cassette), respirable dust (cyclone) using a laboratory wind tunnel and field studies.*

The IOM with multi-foam disc was dropped after initial studies found high background contamination with endotoxin, and significant quality control problems with gravimetric analysis due to foam deterioration.

Coefficients of variation (CV) of dust concentrations for the four sampling devices are presented in Tables I and II for the laboratory and field studies, respectively. CV values were computed for each pair of samplers as the (positive) range of concentrations measured by each of the two samplers divided by the arithmetic average of the two sampler concentrations. Because these CV values for each sampler pair were log-normally distributed, an overall CV for a sampler type was determined by dividing the geometric mean of all range values by the geometric mean of all arithmetic averages. These CV values therefore represent an indication of intra-sampler variation (precision). In the laboratory, performance of the sampling devices varied depending on the velocity as well as the dust type. In the field, the IOM was most reproducible in any environment, and the Cyclone least reproducible.

Mean dust concentrations for the laboratory and field studies are presented in Tables III and IV, respectively. Under all conditions, the IOM yielded the highest estimate of dust concentrations. In the laboratory the Button consistently showed higher concentrations than the CFC, but in the field the two samplers provided similar concentrations. The Cyclone, designed to collect a smaller fraction of the aerosol from that collected by the other devices, consistently yielded the lowest estimate of dust concentrations as expected. The concentrations produced in the laboratory studies were generally higher than those found in the field, but within the same order of magnitude for turkey dust, and within one order of magnitude for swine and chicken dust. The wider range of concentrations produced in the lab compared with the field allowed a better determination of the correlation between the sampler types. The relationship between sampling devices, evaluated by the Pearson correlation coefficient, was variable, depending on both dust type and velocity in the laboratory (Table V). For the still-air conditions, moderate to strong positive correlations were found for all four devices with all three dust types. Under conditions of increasing wind velocity, far fewer consistent relationships were found. In the field, strong positive correlations were found for the IOM, CFC, and Button in most environments (Table VI). Correlations with the Cyclone were less consistent, with no significant correlations between the Cyclone and any other sampler in swine and turkey environments.

The linear regressions of the dust concentrations for the CFC, Button, and Cyclone vs. the IOM are presented by dust type and wind velocity in Figures 1 to 3 for the laboratory trials and in Figure 4 for the field trials. Regressions were forced through the origin. An R^2 value is not provided in these figures because the computation of R^2 does not strictly apply to regressions when the intercept is forced through zero. [41] Comparison of the slopes of the regressions provides a clear comparison of sampling devices. The slopes for the various devices vary depending on dust type and velocity. Increased velocity results in smaller slopes for all three devices relative to the IOM for all three dusts included in the laboratory studies, although this effect is less pronounced for chicken and turkey dust at the highest velocity (1.0 m/s). In all cases, the Button vs. IOM slope in the laboratory was greater than the CFC vs. IOM slope, which in turn was greater than the Cyclone vs. IOM slope. In the field, the slope for the Cyclone vs. IOM was consistently smallest. The slope for the Button vs. IOM was similar to the slope for the CFC vs. IOM, except for the dairy environment.

The geometric means of the ratios of the dust concentrations for the CFC, Button, and Cyclone samplers relative to the IOM sampler (denoted as CFC/IOM, Button/IOM, and Cyclone/IOM) provide an additional useful measure of relative performance calculated for each trial (Tables VII and VIII). In the laboratory studies, the ratios vary by dust type and wind velocity, with a trend to the lowest ratios at the highest velocity (1.0 m/s) for all devices and dust types. In general, the ratios for the Button/IOM and CFC/IOM are similar, with the Button/IOM consistently higher. This is also the pattern seen in the field studies, except for the turkey environment for which the CFC/IOM ratio is higher than the Button/IOM ratio.

Tables IX and X present data on the aerosol size distributions for field and laboratory trials respectively. Data are not available for the Colorado swine building because the limited electric power had to be used to operate the mannekin, and for the Iowa dairy barn because dust levels were below detection limits on the stages of the impactor. The MMAD in the Colorado dairy barn was much lower than the MMADs in the other buildings. It is also worth noting the difference in MMADs between the Colorado (29.5) and Iowa (13.4) chicken barns, while the turkey barns were nearly identical (12.2 and 12.5). In general the MMADs created in the wind tunnel at Colorado were larger than the MMADs created in the still air chamber in Iowa. The difference is most dramatic for the chicken and turkey dusts.

ANOVA confirmed that dust type, wind velocity, sampler type, and MMAD all had a statistically significant effect on the $\log_{10}$ratios ($p < 0.05$). The GLM model R^2 for laboratory trials was 0.79, with dust type, velocity, and sampler type, as well as all interactions, having been found to be statistically significant. Pair-wise differences in least square means by sampling device, dust type, and velocity were significant for all three sampler ratios for swine dust, and for the cyclone ratios for chicken and turkey dust. When MMAD was considered, R^2 was 0.93, and final model retained MMAD, sampler type, and their interaction as the only significant effects. MMAD and dust type were weakly correlated ($r = -0.23$, $p < 0.001$), and MMAD and humidity were also correlated ($r = -0.41$, $p < 0.0001$).

The ANOVA model R^2 for field trials was 0.78. Dust type, location (Iowa, Colorado), and sampler type, as well as all interactions except dust type x location, were statistically significant. The $\log_{10}$ratios for Cyclone/IOM measured in Colorado were higher than those measured in Iowa in three environments (swine, chicken, and dairy). When MMAD was considered R^2 was 0.81 and MMAD, sampler type, dust type x sampler type, and location x sampler type remain as significant ($p < 0.05$) effects. In the field trials dust type and MMAD were correlated ($r = -0.30$, $p < 0.0001$), MMAD was also correlated with temperature ($r = 0.41$, $p < 0.0001$) and humidity ($r = 0.15$, $p = 0.003$).

Multiple linear regression was performed to further evaluate the significance of environmental effects on the relationships between specific sampling devices (Tables XI and XII). Even though dust type and MMAD are moderately correlated, both remain in the models for the laboratory trials, but not for the field trials. Dust type is retained in all models. Velocity is also retained in the laboratory models for the CFC and Button.

Overall the IOM had the lowest coefficient of variation (best precision) and was least affected by changes in wind speed. The performance of the Button was negatively impacted in poultry environments where larger (feather) particulates clogged the holes in the screen. Correlations between the Cyclone and the IOM were relatively good in the laboratory where the range of concentrations tested could be expanded. Correlations in the field were poor due to the narrow range of aerosol concentrations. Therefore, the data obtained in this study resulted in the development of concentration ratios rather than slope values, as a better indicator of relationships between samplers. The CFC/IOM and Cyclone/IOM ratios are important for comparisons between newer and older studies, and for prediction of respirable fractions from Inhalable fractions. In addition to wind speed, MMAD and dust type were important factors affecting ratios. The CFC/IOM relationship appears to be primarily dependent on the type of building (e.g. the aerosol size distribution is stable across geographic regions) and based on the field studies the following ratios may be used for conversion factors for the CFC/IOM: Swine (0.50), Chicken (0.67), Turkey (0.60), Dairy (0.49). Conversion factors for the Cyclone/IOM are again likely best based on the field studies and are: Swine (0.05), Chicken (0.08), Turkey (0.12), Dairy (0.22). Conversion factors for the Button/IOM are: Swine (0.57), Chicken (0.80), Turkey (0.53), Dairy (0.67).

Table I. Coefficients of Variation for Dust Concentrations - Laboratory Studies (N = 10 pairs)

Sampler	Swine	Chicken	Turkey
0 m/s			
Cassette	0.237	0.189	0.151
Cyclone	0.343	0.214	0.075
Button	0.131	0.186	0.043
IOM	0.329	0.339	0.185
0.2 m/s			
Cassette	0.148	0.173	0.170
Cyclone	0.235	0.167	0.201
Button	0.128	0.332	0.299
IOM	0.058	0.069	0.446
1.0 m/s			
Cassette	0.273	0.099	0.268
Cyclone	0.394	0.039	0.070
Button	0.170	0.115	0.127
IOM	0.071	0.099	0.265

Table II. Coefficients of Variation for Dust Concentrations - Field Studies (N = 20 pairs)

Sampler	Swine	Chicken	Turkey	Dairy
Cassette	0.071	0.072	0.108	0.294
Cyclone	0.178	0.152	0.138	0.314
Button	0.092	0.048	0.089	0.070
IOM	0.042	0.048	0.086	0.070

Table III. Mean and (Standard Deviation) of Dust Concentrations in mg/m^3 for Lab Sampling with n=10 for each.

	Swine			Chicken			Turkey		
	0 m/s	0.2 m/s	1.0 m/s	0 m/s	0.2 m/s	1.0 m/s	0 m/s	0.2 m/s	1.0 m/s
IOM	14.72 (6.16)	33.37 (6.52)	28.49 (4.52)	13.12 (4.44)	35.12 (12.45)	31.67 (13.50)	6.59 (2.41)	3.46 (1.14)	6.35 (1.45)
Cassette	8.41 (3.25)	10.65 (3.29)	3.99 (1.58)	6.26 (2.45)	17.53 (4.63)	14.19 (6.31)	3.09 (1.04)	1.41 (0.29)	1.77 (0.36)
Button	15.32 (4.93)	13.88 (3.19)	7.43 (1.88)	12.22 (4.04)	22.76 (6.42)	18.85 (8.75)	4.39 (1.84)	2.01 (0.72)	2.47 (0.54)
Cyclone	4.14 (2.06)	1.00 (0.68)	0.46 (0.20)	2.95 (0.92)	1.08 (0.22)	1.09 (0.52)	1.54 (0.39)	0.41 (0.16)	0.33 (0.06)

Table IV. Mean and (Standard Deviation) of Dust Concentrations in mg/m^3 for Field Sampling events.

Sampler	***Swine n=20***	***Chicken n=21***	***Turkey n=20***	***Dairy n=21***
IOM	2.97 (1.04)	2.66 (1.86)	3.52 (1.53)	0.32 (0.33)
Cassette	1.52 (0.64)	1.59 (0.97)	2.05 (0.67)	0.12 (0.08)
Button	1.87 (1.03)	1.97 (1.30)	1.83 (0.63)	0.17 (0.11)
Cyclone	0.20 (0.18)	0.39 (0.45)	0.43 (0.22)	0.08 (0.09)

TABLE V. Pearson Correlation Coefficients for Dust Concentrations - Laboratory (N = 10)

	Still Chamber				0.2 m/s Velocity				1.0 m/s Velocity			
Sampler	IOM	Cassette	Button	Cyclone	IOM	Cassette	Button	Cyclone	IOM	Cassette	Button	Cyclone
Swine												
IOM	1.00	0.67	0.86	0.53	1.00	-0.45	0.3	-0.36	1.00	0.12	0.09	-0.43
		(0.035)	(0.002)	(0.114)		(0.196)	(0.945)	(0.314)		(0.740)	(0.797)	(0.210)
Cassette		1.00	0.76	0.62		1.00	0.44	0.76		1.00	0.43	0.70
			(0.011)	(0.056)			(0.202)	(0.012)			(0.210)	(0.025)
Button			1.00	0.74			1.00	0.13			1.00	0.36
				(0.015)				(0.711)				(0.312)
Cyclone				1.00				1.00				1.00
Chicken												
IOM	1.00	0.81	0.66	0.68	1.00	0.43	0.50	0.82	1.00	-0.07	0.42	-0.02
		(0.008)	(0.053)	(0.046)		(0.211)	(0.140)	(0.004)		(0.851)	(0.256)	(0.635)
Cassette		1.00	0.87	0.43		1.00	0.41	0.24		1.00	0.64	0.75
			(0.001)	(0.213)			(0.238)	(0.502)			(0.048)	(0.013)
Button			1.00	0.53			1.00	0.37			1.00	0.65
				(0.114)				(0.286)				(0.043)
Cyclone				1.00				1.00				1.00
Turkey												
IOM	1.00	0.61	0.77	0.28	1.00	0.63	0.29	0.47	1.00	0.51	0.14	0.57
		(0.061)	(0.009)	(0.432)		(0.049)	(0.424)	(0.171)		(0.131)	(0.703)	(0.087)
Cassette		1.00	0.80	0.72		1.00	0.15	0.22		1.00	0.62	-0.11
			(0.005)	(0.018)			(0.687)	(0.540)			(0.056)	(0.767)
Button			1.00	0.56			1.00	0.03			1.00	-0.52
				(0.091)				(0.940)				(0.121)
Cyclone				1.00				1.00				1.00

TABLE VI. Pearson Correlation Coefficients for Dust Concentrations - Field (N = 20)

Sampler	IOM	Cassette	Button	Cyclone
Swine				
IOM	1.00	0.90 (<0.001)	0.79 (<0.001)	0.10 (0.688)
Cassette		1.00	0.87 (<0.001)	0.22 (0.348)
Button			1.00	0.40 (0.083)
Cyclone				1.00
Chicken				
IOM	1.00	0.92 (<0.001)	0.96 (<0.001)	0.87 (<0.001)
Cassette		1.00	0.93 (<0.001)	0.79 (<0.001)
Button			1.00	0.82 (<0.001)
Cyclone				1.00
Turkey				
IOM	1.00	0.81 (<0.001)	0.81 (<0.001)	0.32 (0.167)
Cassette		1.00	0.70 (0.001)	0.44 (0.054)
Button			1.00	0.40 (0.078)
Cyclone				1.00
Dairy				
IOM	1.00	0.13 (0.567)	0.46 (0.038)	0.14 (0.557)
Cassette		1.00	0.87 (<0.001)	0.90 (<0.001)
Button			1.00	0.90 (<0.001)
Cyclone				1.00

Table VII. Geometric Mean and (Geometric Standard Deviation) for Ratios of Sampler Concentrations in Lab Sampling with n=10 for each.

	Swine			Chicken			Turkey		
	0 m/s	0.2 m/s	1.0 m/s	0 m/s	0.2 m/s	1.0 m/s	0 m/s	0.2 m/s	1.0 m/s
Cassette/IOM	0.59 (1.4)	0.31 (1.6)	0.13 (1.6)	0.44 (1.4)	0.51 (1.4)	0.50 (2.2)	0.48 (1.5)	0.42 (1.3)	0.28 (1.3)
Button/IOM	1.09 (1.3)	0.41 (1.4)	0.26 (1.4)	0.88 (1.4)	0.66 (1.5)	0.65 (1.8)	0.66 (1.3)	0.55 (1.7)	0.39 (1.4)
Cyclone/IOM	0.27 (1.5)	0.03 (2.0)	0.01 (2.3)	0.22 (1.4)	0.03 (1.3)	0.04 (2.2)	0.25 (1.6)	0.12 (1.4)	0.05 (1.2)

Table VIII. Geometric Mean and (Geometric Standard Deviation) for Ratios of Sampler Concentrations in Field sampling events.

Sampler	*Swine n=20*	*Chicken n=21*	*Turkey n=20*	*Dairy n=21*
Cassette/IOM	0.50 (1.2)	0.67 (1.5)	0.60 (1.3)	**0.49 (2.7)**
Button/IOM	0.57 (1.6)	0.80 (1.4)	0.53 (1.3)	**0.69 (2.1)**
Cyclone/IOM	**0.05 (2.0)**	**0.08 (2.3)**	**0.12 (1.7)**	**0.22 (3.8)**

Table IX. Mean MMAD (GSD)- Field [n = 10 for each]

	Swine	*Chicken*	*Turkey*	*Dairy*
Colorado	Missing	29.5 (4.5)	12.2 (2.9)	**7.7 (7.6)**
Iowa	**10.7 (3.5)**	**13.4 (2.3)**	**12.5 (7.5)**	**Not sampled**

Table X. Mean MMAD (GSD)- Lab [n = 10 for each]

Velocity (m/s)	*Swine*	*Chicken*	*Turkey*
0	13.4 (10.0)	7.7 (0.8)	4.0 (1.2)
0.2	19.5 (8.0)	30.4 (10.3)	11.2 (0.2)
1.0	15.6 (16.0)	33.0 (7.5)	14.0 (3.7)

Table XI. Stepwise Multiple Linear Regression Models for the Laboratory Trials

Model	R^2
IOM = 15.50 + 1.43 CFC - 7.15 DustType + 5.75 Velocity - 0.24 MMAD	0.70
IOM = 9.66 + 0.98 Button - 5.82 DustType + 5.27 Velocity	0.72
IOM = 36.54 - 1.46 Cyclone - 10.11 DustType + 0.31 MMAD	0.48

Table XII. Stepwise Multiple Linear Regression Models for the Field Trials

Model	R^2
IOM = 0.55 + 1.57 CFC - 0.15 DustType	0.85
IOM = 1.56 + 1.36 Button - 0.0096 DustType - 0.073 Temperature	0.78
IOM = 2.61 + 3.36 Cyclone - 0.51 DustType	0.44

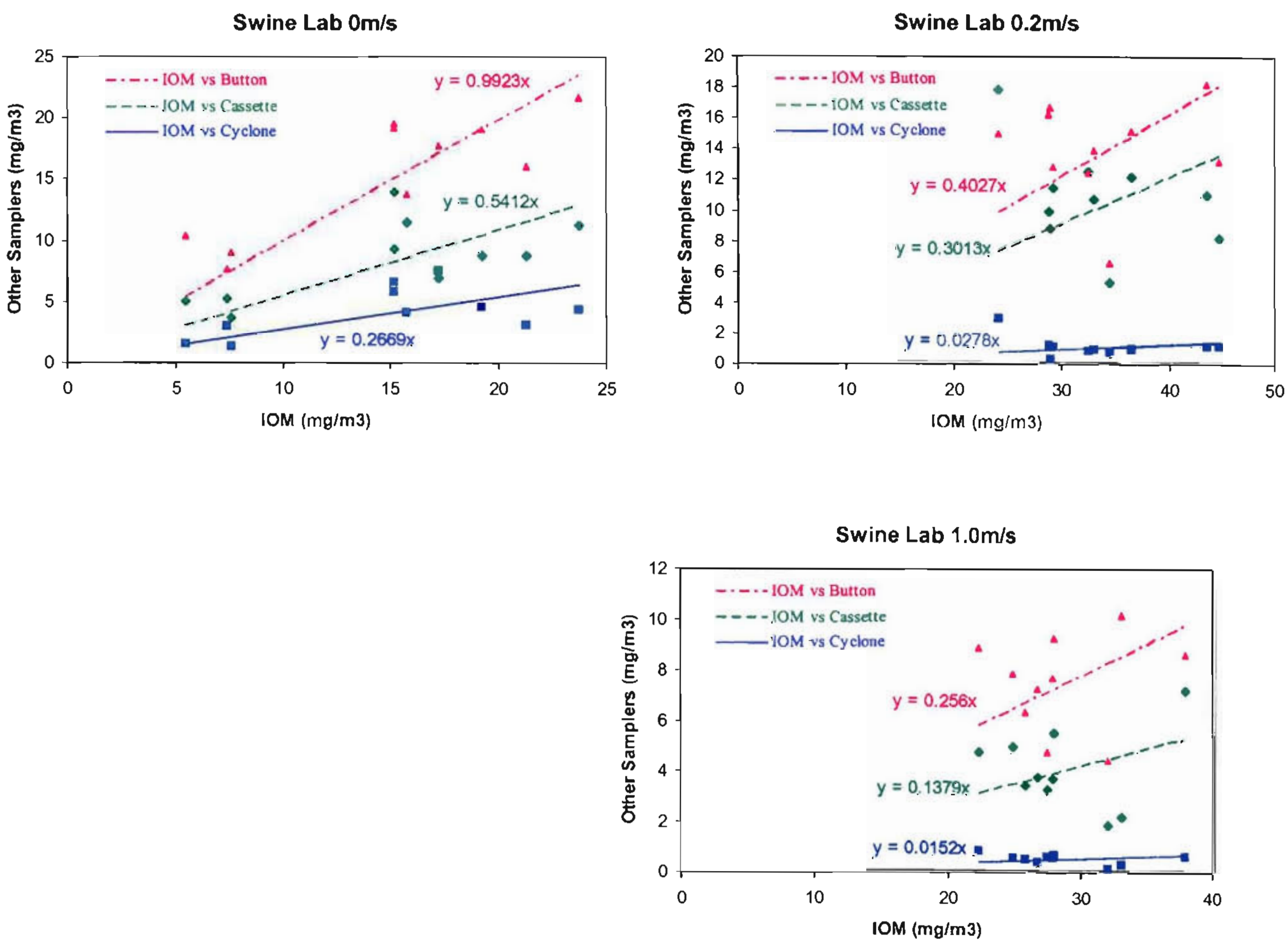


Figure 1. Regression (through 0) of Samplers Relative to IOM for Swine Dust Lab Studies (N = 10)

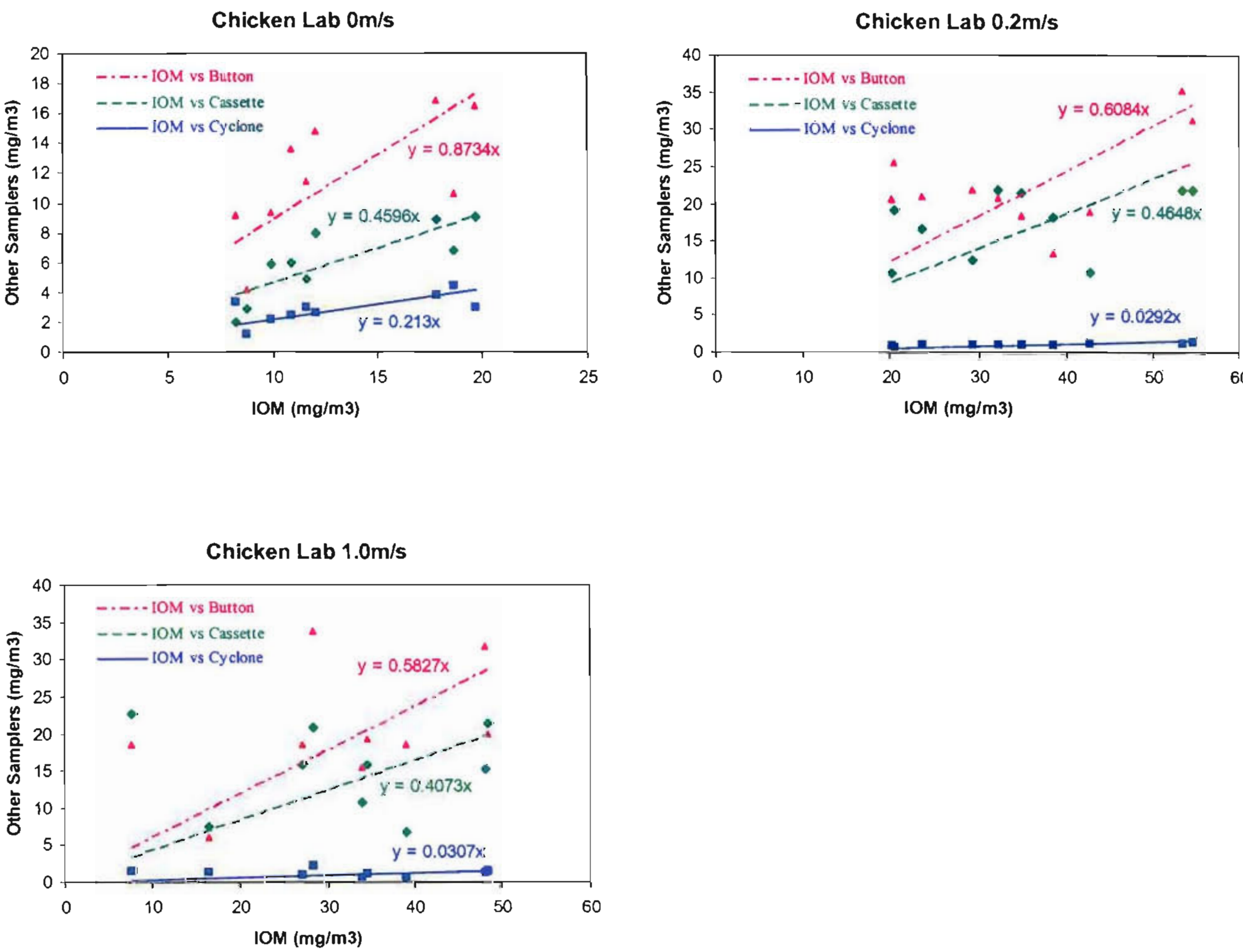


Figure 2. Regression (through 0) of Samplers Relative to IOM for Chicken Dust Lab Studies (N = 10)

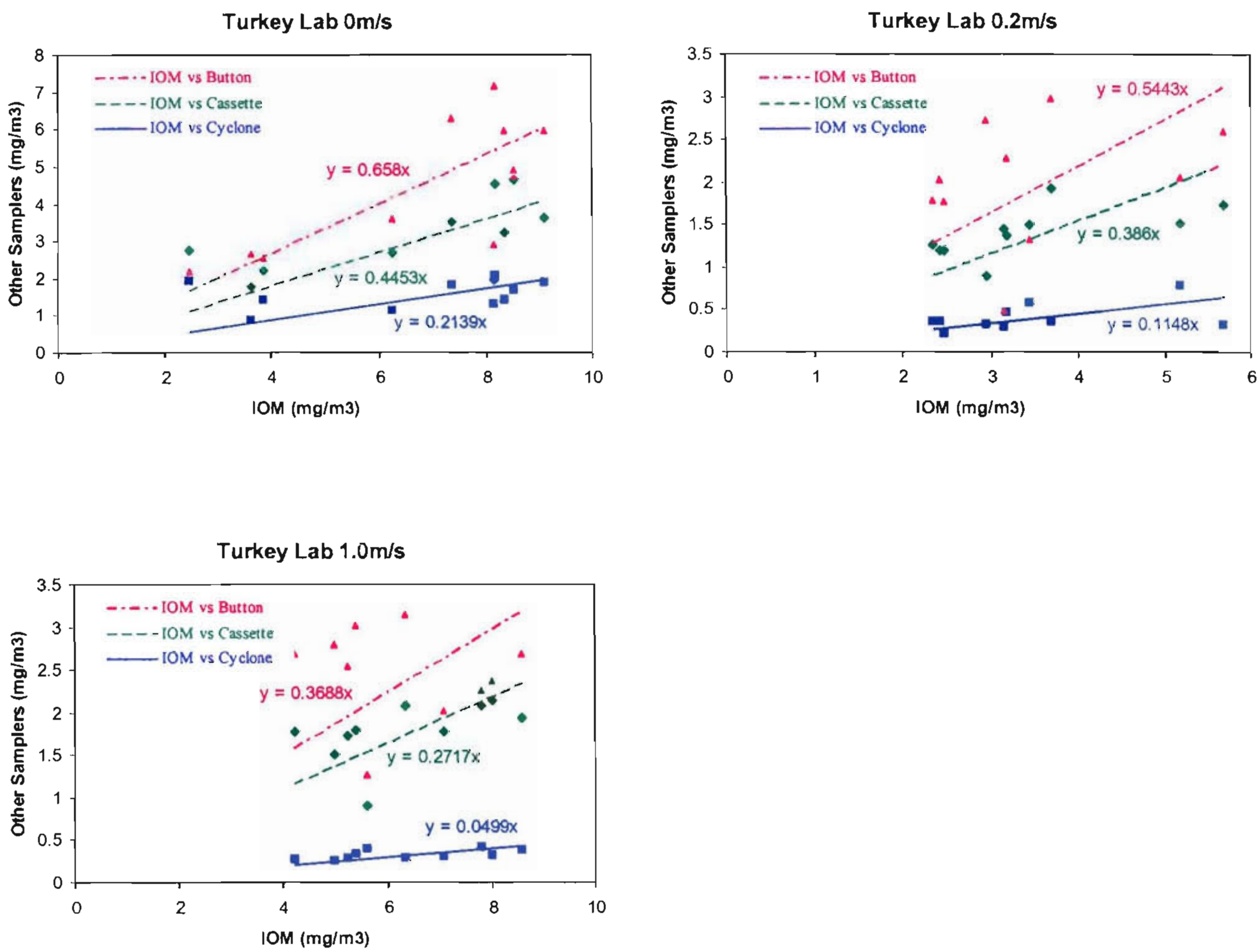


Figure 3. Regression (through 0) of Samplers Relative to IOM for Turkey Dust Lab Studies (N = 10)

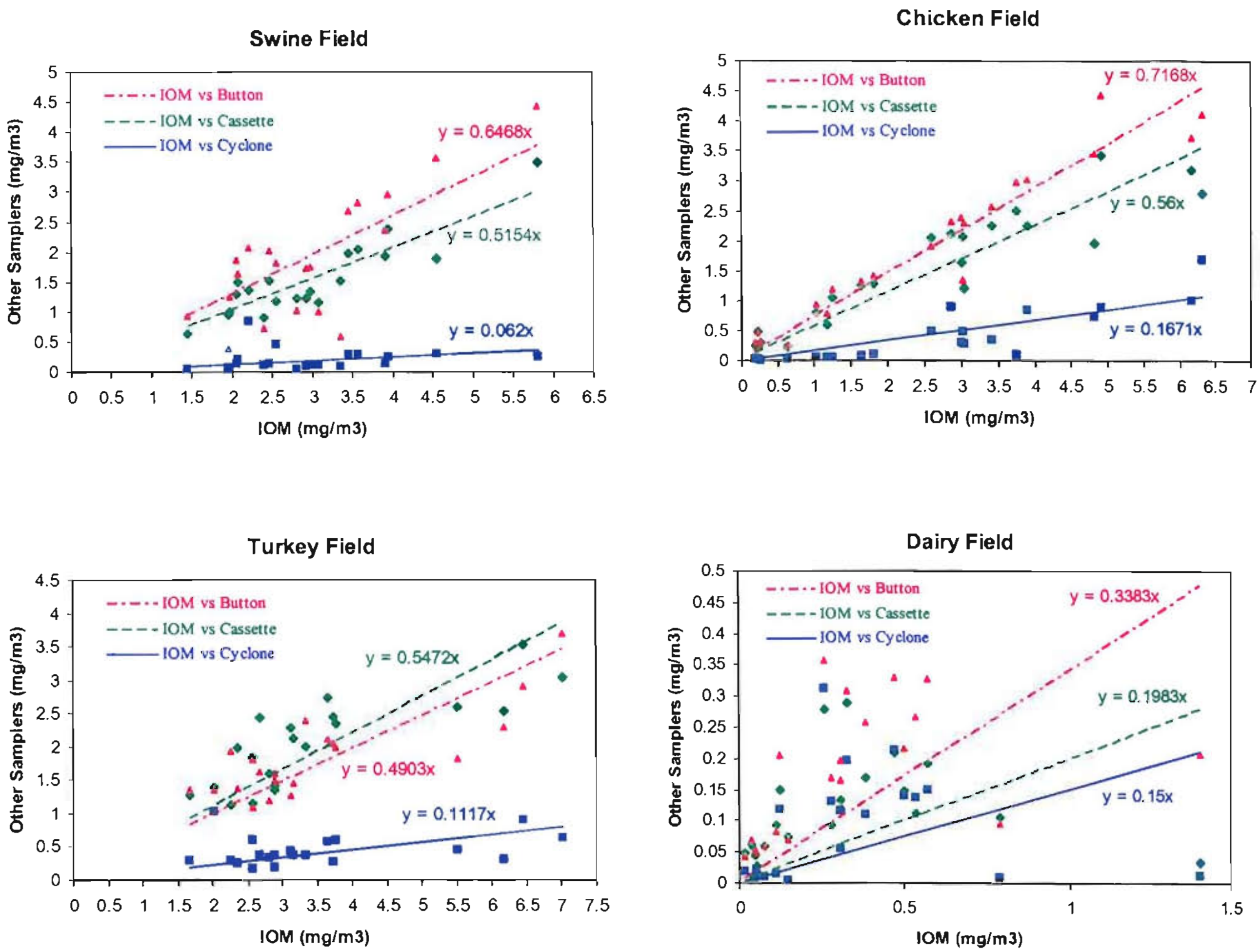
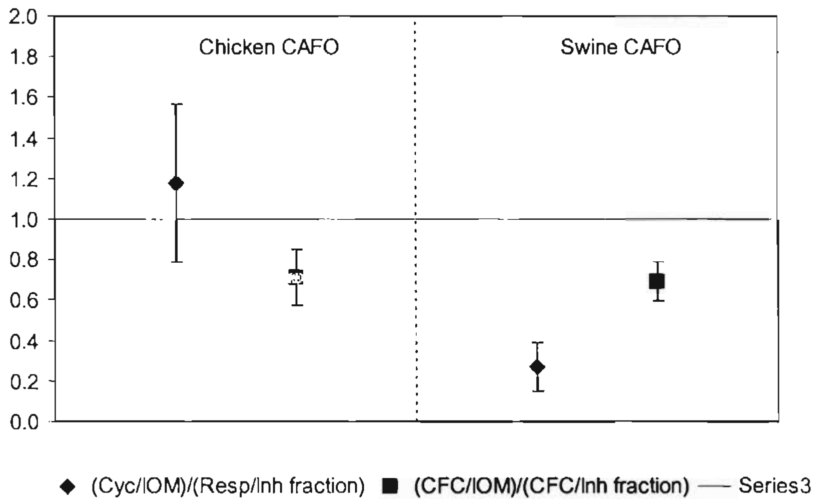


Figure 4. Regression (through 0) of Samplers Relative to IOM for Field Studies (N = 20)

Figure 5. Actual/Expected Ratios - CAFOs

Means +/- 95% CI



The CFC collected about 80% of expected in both CAFOs. The Cyclone undercollected from expected in Swine CAFO

O'Shaughnessy PT, Lo J, Golla V, Nakatsu J, Tillery MI, Reynolds SJ: [2007] Comparison of Aerosol Samplers Relative to the Inhalable and Respirable Collection Criteria. JOEH 4:237-245.

Reynolds SJ, Nakatsu J, Tillery MI, Keefe T, Mehaffy J, Thorne PS, Donham KJ, Nonneman M, Golla V, O'Shaughnessy PT: [2007 submitted] Field and Wind Tunnel Comparison of Four Aerosol Samplers using Agricultural Dusts. JOEH.

2. *Evaluate and compare the precision and relative predicted dust concentration of these devices and collection media for measurement of endoxotins and glucans/ergosterols in these same environments using a new Recombinant Factor C fluorometric endotoxin assay, the chromogenic LAL assay and chemical methods.*

This is the first study to report on the new rFC endotoxin assay development and application to agricultural dusts. Object 3 (below) describes the method, and comparison to traditional LAL assay. Since the rFC and LAL correlated very well, results were combined for this analysis. ANOVA confirmed that there was no difference between the two assays for these results.

Geometric mean concentrations of endotoxin content of dust (EU/mg), airborne endotoxin content (EU/m3), and the ratio of EU/m3 measured by the three sampling devices (CFC, Button, Cyclone) relative to the IOM are presented in Tables XIII for Field Studies and XIV for Laboratory studies. Again, no laboratory trials were conducted for Dairy dust since it was not possible to collect enough dust from the field. In addition, analyses on Horse and Cattle Feedlot dusts were only completed for samples collected in Colorado.

For field studies ANOVA confirmed that the concentrations of endotoxin in dust (EU/mg) varied significantly with dust type, sampler type, collection location (CO or IA), and their interactions (R2 = 0.75, all p <0.0003). Endotoxin content was higher in samples from Iowa. In Horse and Feedlot dust EU/mg was highest in CFC and Button samples. In Dairy the cyclone had the lowest concentrations, but the other samplers gave similar results. For Chicken, Swine and Turkey dust endotoxin content was distributed about equally. For laboratory studies ANOVA also confirmed a significant difference by dust type and sampler type, with slightly different distributions by sampler type (R2 = 0.45). Velocity was not a significant factor for EU/mg.

EU/m3 varied significantly by dust type, sampler type, location (IA/CO), and their interactions (ANOVA R2 = 0.92) for field studies. Again airborne concentrations of endotoxin were higher in Iowa than in Colorado. The IOM yielded significantly higher concentrations for Swine dust, and the Cyclone yielded significantly lower concentrations for Chicken, Turkey, and Dairy dusts. For Feedlots and Horse dusts the order of concentrations by sampler was: IOM>CFC>Button>Cyclone. For laboratory studies velocity had a significant effect on EU/m3 in addition to dust and sampler type (R2 = 0.82). In all cases, as the velocity increased to 1.0 m/s, EU/3 went down. The change in velocity also had an impact on the relationships between samplers with the IOM and Button yielding similar concentrations in most dust types.

Ratios of EU/m3 for CFC, Button, and Cyclone divided by EU/m3 for IOM were also found to vary significantly by dust type and sampler (field ANOVA R2 = 0.84), and in addition velocity (lab ANOVA R2 = 0.87). Figures 6 and 7 present regressions of sampler ratios for both field and lab studies. These are similar to the results for gravimetric analyses (see above), however in most cases the Button/IOM produced higher ratios than the CFC/IOM for EU/m3 for both field and lab studies. Similar to dust concentrations, conversion factors for EU/m3, based on field studies, are suggested to be: Button/IOM: Swine (0.7), Chicken (1.1), Turkey (0.8), Dairy (1.0), Feedlot (0.4), Horse (0.6); CFC/IOM: Swine (0.5), Chicken (0.7), Turkey (0.6), Dairy (0.6), Feedlot (0.7), Horse (0.8); Cyclone/IOM: Swine (0.06), Chicken (0.11), Turkey (0.9), Dairy (0.05), Feedlot (0.06), Horse (0.09). It appears that correction factors for dust and endotoxin are not the same. Again wind velocity had an important effect on these ratios – in almost all cases higher wind velocity (1.0 m/s) resulted in lower ratios compared to still air and 0.2 m/s.

Manuscript in preparation.

TableXIII. Endotoxin Results – Field Studies

Swine

		Colorado			Iowa		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	1371.265	2.167274	20	4470.872	1.964238
	EU/m^3	20	3522.59	2.391399	20	13521.63	2.210542
	IOM/IOM	10	1	0	10	1	0
Cassette	EU/mg	20	2173.434	1.543206	19	3420.689	1.672629
	EU/m^3	20	2465.804	1.793051	19	6070.643	1.705614
	Cassette/IOM	10	0.631262	1.34665	10	0.381784	1.545497
Button	EU/mg	20	1963.627	1.709354	20	4225.11	2.314361
	EU/m^3	20	2012.83	2.218718	20	10194.91	2.491015
	Button/IOM	10	0.551386	1.565628	10	0.797901	1.450717
Cyclone	EU/mg	20	2401.904	1.986663	20	3263.886	2.723846
	EU/m^3	20	197.7313	2.195793	20	766.225	2.44417
	Cyclone/IOM	10	0.055176	1.603099	10	0.052703	1.965371

Chicken

		Colorado			Iowa		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	310.1396	2.246351	22	1214.048	1.448365
	EU/m^3	20	239.3473	1.925685	22	4857.764	1.558529
	IOM/IOM	10	1	0	11	1	0
Cassette	EU/mg	20	311.543	1.560688	22	1204.128	1.594939
	EU/m^3	20	202.5886	2.016543	22	2543.119	1.986894
	Cassette/IOM	10	0.85295	1.203411	11	0.565994	1.478909
Button	EU/mg	20	354.057	1.999593	22	1621.225	1.64327
	EU/m^3	20	261.0229	2.643572	22	4226.307	2.401487
	Button/IOM	10	1.221999	1.89666	11	0.943963	1.669038
Cyclone	EU/mg	20	287.9566	1.681424	22	1185.48	2.18354
	EU/m^3	20	8.910503	2.399661	22	806.0842	1.871251
	Cyclone/IOM	10	0.036852	1.520767	11	0.168098	1.609338

Turkey

		Colorado			Iowa		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	2046.332	1.663521	19	7235.111	2.010661
	EU/m^3	20	6447.134	1.785259	19	23770.4	1.921839
	IOM/IOM	10	1	0	10	1	0

Cassette	EU/mg	20	2604.468	1.612879	20	5909.808	1.657539
	EU/m^3	20	4597.723	1.686801	20	12554.53	1.731014
	Cassette/IOM	10	0.713817	1.435701	10	0.498981	1.367407
Button	EU/mg	20	3301.926	1.282657	17	8290.339	2.133284
	EU/m^3	20	5621.917	1.481088	17	14996.7	2.268215
	Button/IOM	10	0.853154	1.246906	10	0.689869	1.694222
Cyclone	EU/mg	20	1990.122	1.493063	20	3558.437	2.212299
	EU/m^3	20	651.4605	1.814248	20	1610.833	2.214604
	Cyclone/IOM	10	0.099039	1.507134	10	0.067277	1.375493

Dairy

		Colorado			Iowa		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	17	528.9227	5.056401	22	335.1481	1.76973
	EU/m^3	19	49.36895	2.277166	22	109.234	1.781131
	IOM/IOM	10	1	0	11	1	0
Cassette	EU/mg	19	567.1622	1.909941	22	412.0668	1.880357
	EU/m^3	19	29.29639	2.279088	22	65.02295	1.545714
	Cassette/IOM	10	0.619495	1.784244	11	0.592632	1.428869
Button	EU/mg	20	403.7942	1.714144	22	563.9396	1.67135
	EU/m^3	19	26.65265	1.820839	22	142.1774	1.475111
	Button/IOM	10	0.57326	1.795004	11	1.296828	1.508202
Cyclone	EU/mg	13	271.4835	2.305633	18	14.03363	2.166924
	EU/m^3	14	3.096159	2.012547	18	1.828878	1.905221
	Cyclone/IOM	10	0.071234	2.203738	11	0.015213	2.389072

Feedlot

		Colorado		
		n	GM	GSD
IOM	EU/mg	10	599.0023	2.169573
	EU/m^3	10	178.2959	1.977691
	IOM/IOM	10	1	0
Cassette	EU/mg	10	1010.271	1.430567
	EU/m^3	10	128.7889	1.89284
	Cassette/IOM	10	0.722333	1.461863
Button	EU/mg	10	1035.471	1.34991
	EU/m^3	10	72.92538	1.684764
	Button/IOM	10	0.409013	1.615429
Cyclone	EU/mg	10	537.3149	2.077463
	EU/m^3	10	10.44598	2.582113

	Cyclone/IOM	10	0.058588	2.350283

Horse

		Colorado		
		n	GM	GSD
IOM	EU/mg	10	442.7057	1.394412
	EU/m^3	10	252.1645	1.267778
	IOM/IOM	10	1	0
Cassette	EU/mg	10	597.6165	1.253225
	EU/m^3	10	194.1922	1.323859
	Cassette/IOM	10	0.770101	1.431013
Button	EU/mg	10	437.9359	1.460462
	EU/m^3	10	162.8485	1.202105
	Button/IOM	10	0.645802	1.19855
Cyclone	EU/mg	10	343.1783	1.427982
	EU/m^3	10	22.10962	1.468864
	Cyclone/IOM	10	0.087679	1.363883

Table XIV. Endotoxin Results – Laboratory Studies

Swine

		0 m/s			0.2 m/s			1.0 m/s		
		n	GM	GSD	n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	2504.501	2.171344	20	2005.503	1.868846	20	1862.569	4.531379
	EU/m^3	20	28050.25	3.362383	20	65252.19	1.992615	20	37430.93	2.355313
	IOM/IOM	10	1	0	10	1	0	10	1	0
Cassette	EU/mg	19	3603.569	2.247776	20	5289.455	2.822437	20	4068.857	2.920152
	EU/m^3	19	27730.69	2.532003	20	40273.21	2.236227	20	14792.27	3.00689
	Cassette/IOM	10	0.828394	1.926456	10	0.643569	1.49357	10	0.414826	1.751334
Button	EU/mg	20	3645.413	2.596573	20	4752.22	1.828606	20	4278.285	2.018962
	EU/m^3	20	52320.26	2.633035	20	63522.58	1.815141	20	30529.6	2.251002
	Button/IOM	10	1.801639	1.98853	10	0.916945	1.793189	10	0.794688	1.57652
Cyclone	EU/mg	20	3606.938	2.880322	18	3432.545	3.346765	20	1766.765	4.343532
	EU/m^3	20	11992.14	4.607391	19	3366.015	3.148213	20	633.0264	3.102242
	Cyclone/IOM	10	0.424383	2.686423	10	0.06811	1.938556	10	0.020893	1.858464

Chicken

		0 m/s			0.2 m/s			1.0 m/s		
		n	GM	GSD	n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	998.9453	1.587847	20	1001.669	1.590172	20	754.378	4.042087
	EU/m^3	20	14451.45	1.506569	20	33060.67	1.614361	20	25865.72	1.670526
	IOM/IOM	10	1	0	10	1	0	10	1	0
Cassette	EU/mg	20	1317.181	1.949031	19	1087.228	1.397992	20	814.2502	1.348322
	EU/m^3	20	7021.506	2.355459	19	18561.23	1.681112	20	9726.395	1.920521
	Cassette/IOM	10	0.515355	1.740384	10	0.498602	1.786773	10	0.370052	1.410701
Button	EU/mg	20	1415.946	1.376162	16	966.6659	2.681073	20	973.5913	1.440087
	EU/m^3	20	16129.1	1.664136	16	21929.01	2.833789	20	15206.66	1.97062
	Button/IOM	10	1.128389	1.636723	9	0.497237	3.347105	10	0.59883	1.526238
Cyclone	EU/mg	20	1271.462	1.425276	20	1010.236	1.31793	19	742.4551	1.465152
	EU/m^3	20	3516.243	1.739306	20	1052.443	1.383181	19	699.5936	1.932195
	Cyclone/IOM	10	0.252331	1.45641	10	0.030783	1.248618	10	0.026056	1.640866

Turkey

		0 m/s			0.2 m/s			1.0 m/s		
		n	GM	GSD	n	GM	GSD	n	GM	GSD
IOM	EU/mg	20	676.3665	2.294267	19	1581.114	3.141655	19	1969.261	2.209076
	EU/m^3	20	3071.996	2.216856	19	3827.856	1.605786	19	12054.09	2.00281
	IOM/IOM	10	1	0	10	1	0	10	1	0
Cassette	EU/mg	20	690.4393	1.918148	17	685.0854	1.793009	20	790.5936	1.465993
	EU/m^3	20	1579.021	2.054185	18	1040.488	1.718781	20	1345.36	1.754895
	Cassette/IOM	10	0.526801	2.007383	10	0.279285	1.340461	10	0.110412	2.030908
Button	EU/mg	20	787.113	1.975851	20	1158.683	1.809216	20	1423.124	1.336984
	EU/m^3	20	5759.333	1.7195	20	2056.638	2.19687	20	3396.038	1.50878
	Button/IOM	10	1.92314	1.823737	10	0.577329	1.592179	10	0.265261	1.955371

Cyclone	EU/mg	20	675.4422	2.198724	19	452.722	3.161399	20	510.6648	1.858573
	EU/m^3	20	920.8901	1.954285	19	176.3147	2.713434	20	161.0291	1.692905
	Cyclone/IOM	10	0.300134	1.656996	10	0.047079	2.420472	10	0.013557	1.892891

Feedlot

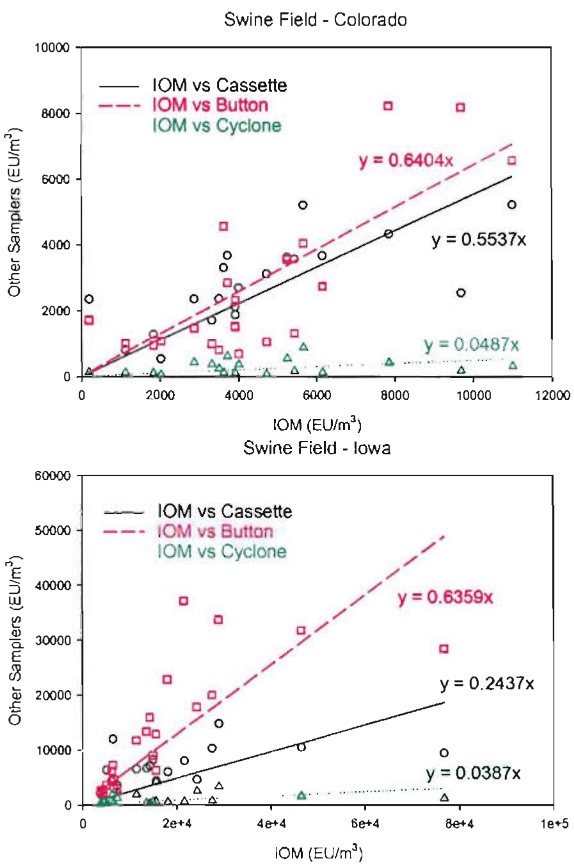
		0.2 m/s			1.0 m/s		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	10	158.1986	2.026567	10	316.6803	4.009817
	EU/m^3	10	1504.644	2.46431	10	1060.953	1.394769
	IOM/IOM	10	1	0	10	1	0
Cassette	EU/mg	10	311.5618	1.442404	10	159.5695	1.546993
	EU/m^3	10	462.8957	2.951411	10	254.8932	1.854884
	Cassette/IOM	10	0.307645	1.356929	10	0.240249	1.958866
Button	EU/mg	9	281.1341	1.525349	10	240.8197	1.39196
	EU/m^3	9	1050.326	2.068321	10	678.7645	1.354623
	Button/IOM	9	0.735357	1.390831	10	0.639768	1.484048
Cyclone	EU/mg	9	359.3617	3.5028	10	144.6872	1.569796
	EU/m^3	10	67.80754	1.662875	10	66.52805	1.640701
	Cyclone/IOM	10	0.045065	2.117922	10	0.062706	1.729178

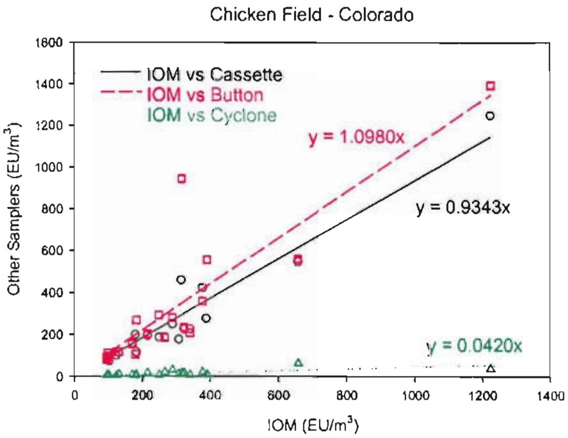
Horse

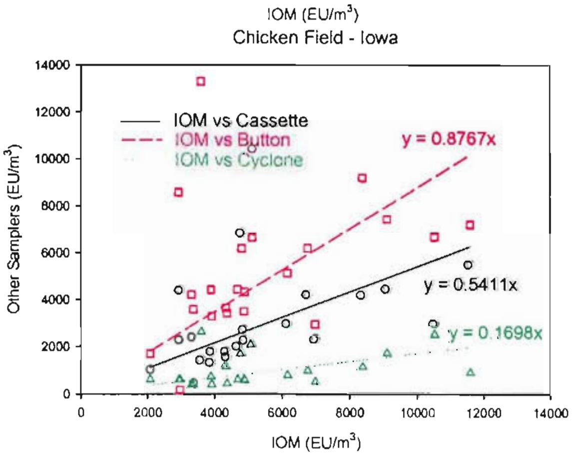
		0.2 m/s			1.0 m/s		
		n	GM	GSD	n	GM	GSD
IOM	EU/mg	10	226.3682	1.197335	9	412.6005	2.75056
	EU/m^3	10	4991.92	1.275045	10	3373.591	4.341287
	IOM/IOM	10	1	0	10	1	0
Cassette	EU/mg	10	402.8334	1.130855	9	340.8629	1.551121
	EU/m^3	10	2447.833	1.379084	9	3777.011	1.257481
	Cassette/IOM	10	0.490359	1.478124	9	1.118827	5.022417

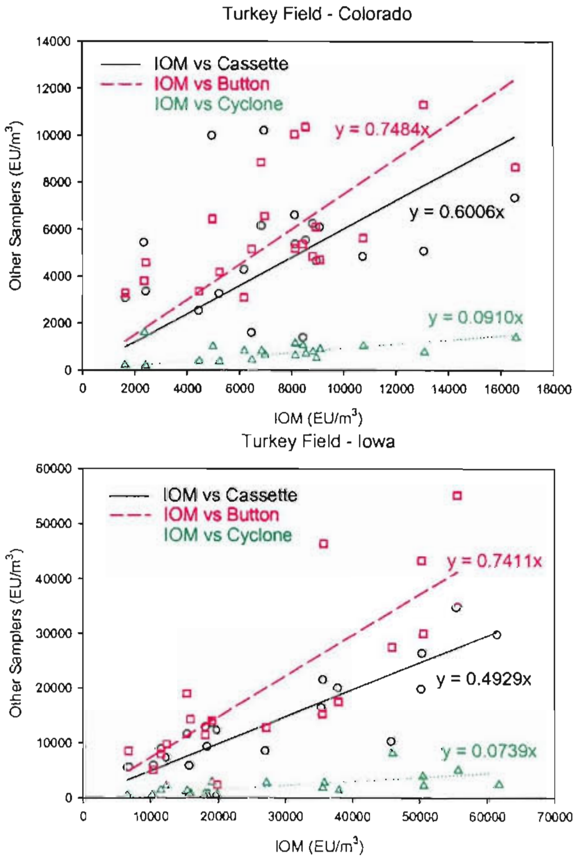
Button	EU/mg	10	388.3647	1.156565	10	386.4218	1.692812
	EU/m3	10	3260.938	1.369248	10	3187.424	1.202124
	Button/IOM	10	0.653243	1.458278	10	0.944816	4.514505
Cyclone	EU/mg	10	337.8434	1.239909	10	244.5571	2.894658
	EU/m3	10	533.6099	1.565158	10	458.1591	1.410157
	Cyclone/IOM	10	0.106895	1.627541	10	0.135808	4.277274

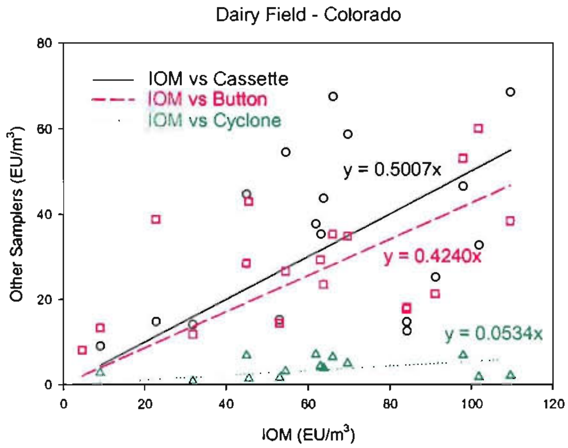
Figure 6. Regression (through 0) of Samplers Relative to IOM (EU/m3)- Field

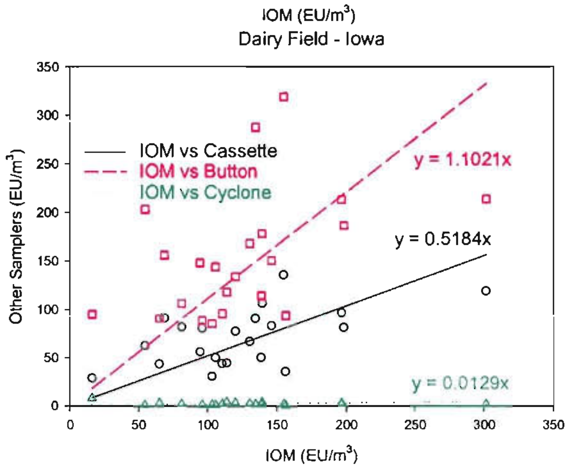


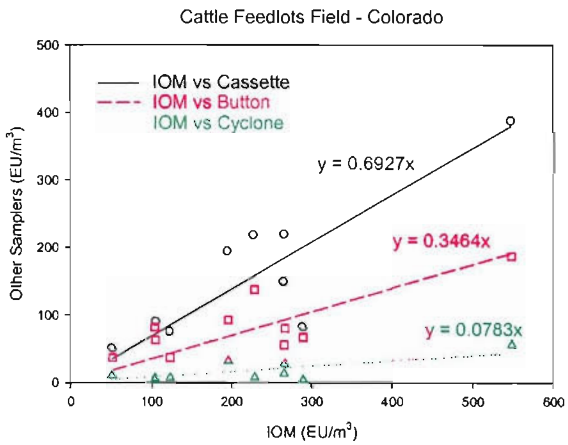

Chicken Field - Colorado
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 1.0980x
y = 0.9343x
y = 0.0420x
Other Samplers (EU/m³)
IOM (EU/m³)


Chicken Field - Iowa
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.8767x
y = 0.5411x
y = 0.1698x
Other Samplers (EU/m³)
IOM (EU/m³)


Turkey Field - Colorado
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.7484x
y = 0.6006x
y = 0.0910x
Other Samplers (EU/m3)
IOM (EU/m3)
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
Turkey Field - Iowa
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.7411x
y = 0.4929x
y = 0.0739x
Other Samplers (EU/m3)
IOM (EU/m3)
0
10000
20000
30000
40000
50000
60000
70000


Dairy Field - Colorado
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.5007x
y = 0.4240x
y = 0.0534x
Other Samplers (EU/m³)
IOM (EU/m³)
0
20
40
60
80
0
20
40
60
80
100
120


Dairy Field - Iowa
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 1.1021x
y = 0.5184x
y = 0.0129x
Other Samplers (EU/m³)
IOM (EU/m³)
0
50
100
150
200
250
300
350
0
50
100
150
200
250
300
350


Cattle Feedlots Field - Colorado
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.6927x
y = 0.3464x
y = 0.0783x
Other Samplers (EU/m3)
IOM (EU/m3)
0
100
200
300
400
500
600

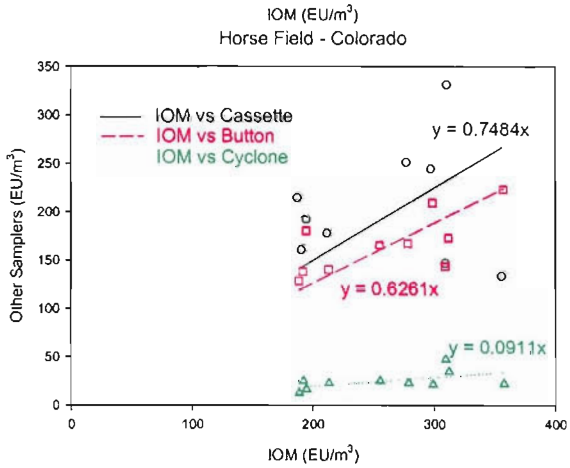
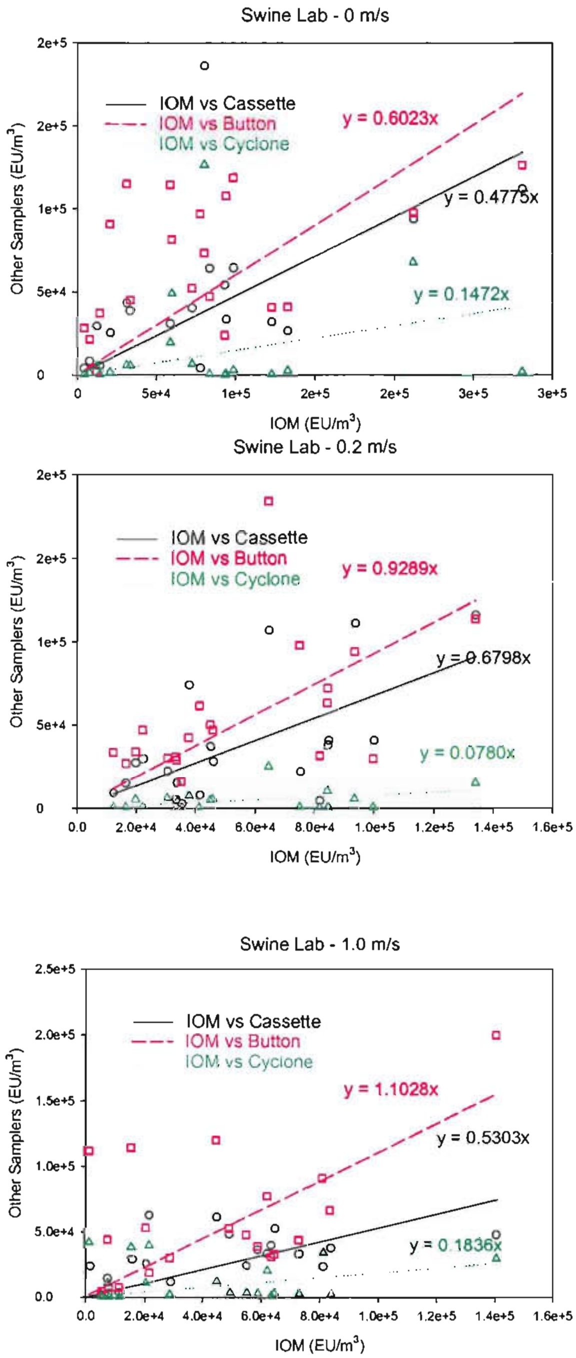
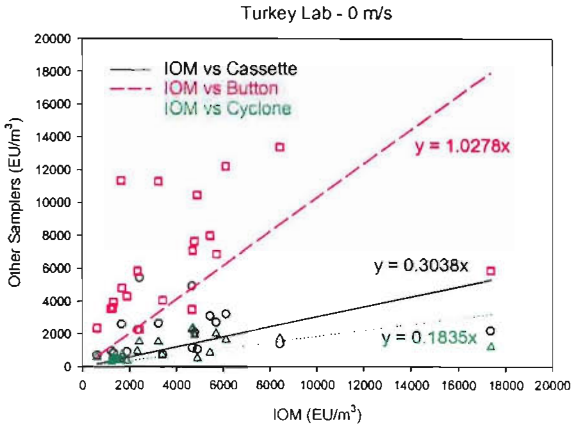
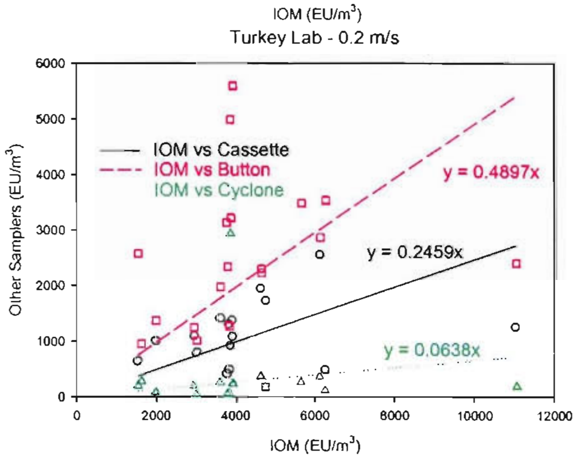
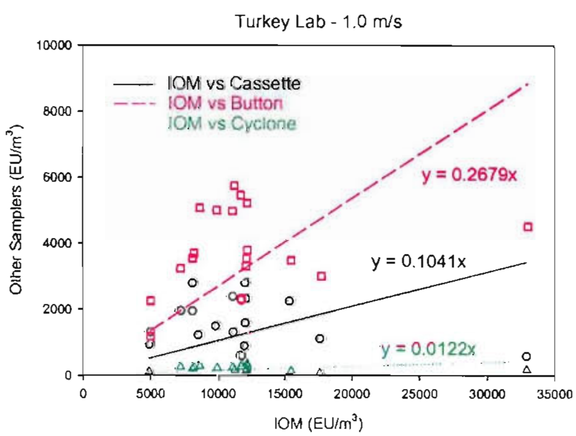

Horse Field - Colorado
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.7484x
y = 0.6261x
y = 0.0911x
Other Samplers (EU/m3)
IOM (EU/m3)
0
50
100
150
200
250
300
350
400

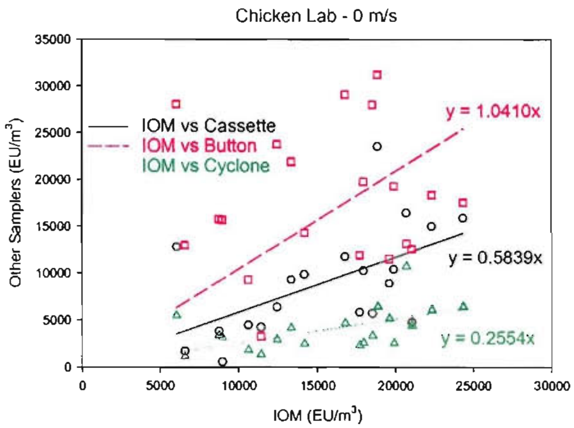
Figure 7. Regression (through 0) of Samplers Relative to IOM (EU/m3)- Laboratory

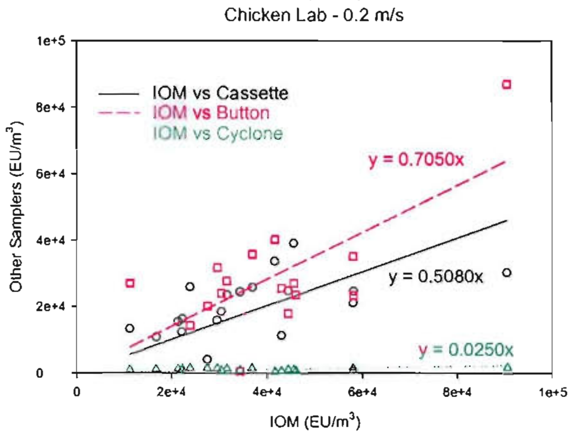


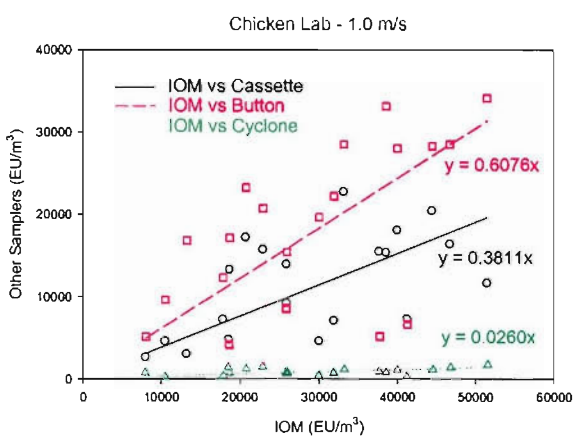

Turkey Lab - 0 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 1.0278x
y = 0.3038x
y = 0.1835x
Other Samplers (EU/m³)
IOM (EU/m³)


Turkey Lab - 0.2 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.4897x
y = 0.2459x
y = 0.0638x
Other Samplers (EU/m³)
IOM (EU/m³)

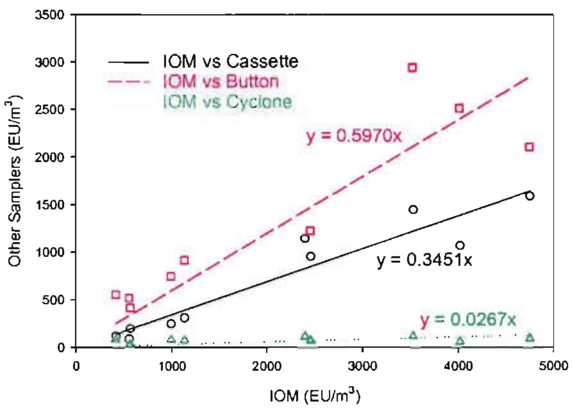

Turkey Lab - 1.0 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.2679x
y = 0.1041x
y = 0.0122x
Other Samplers (EU/m³)
IOM (EU/m³)


Chicken Lab - 0 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 1.0410x
y = 0.5839x
y = 0.2554x
Other Samplers (EU/m³)
IOM (EU/m³)

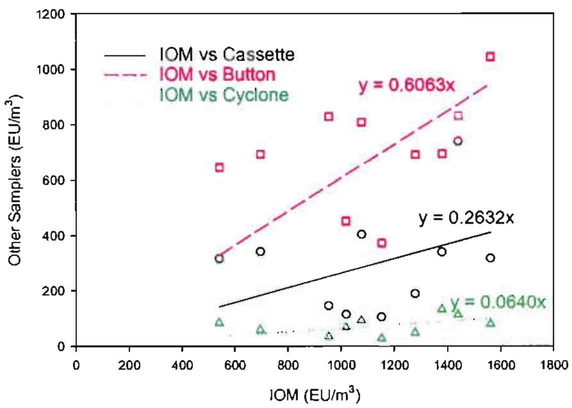

Chicken Lab - 0.2 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.7050x
y = 0.5080x
y = 0.0250x
Other Samplers (EU/m³)
IOM (EU/m³)

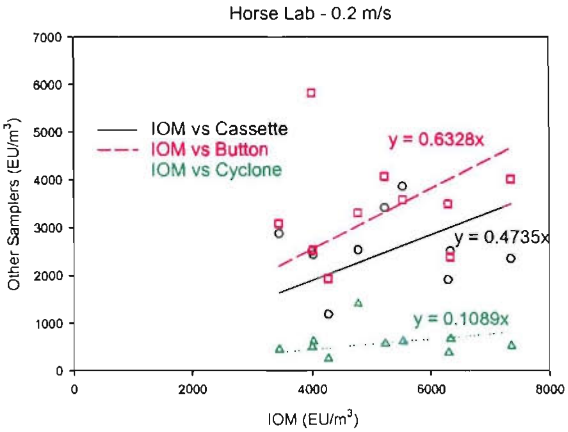

Chicken Lab - 1.0 m/s
IOM vs Cassette
IOM vs Button
IOM vs Cyclone
y = 0.6076x
y = 0.3811x
y = 0.0260x
Other Samplers (EU/m³)
IOM (EU/m³)

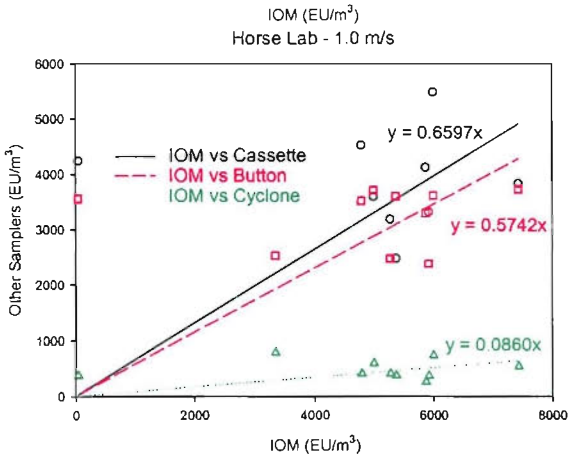
Cattle Feedlot Lab - 0.2 m/s



Cattle Feedlot Lab - 1.0 m/s







3. *Determine the performance of the new Recombinant Factor C fluorometric endotoxin assay, and compare the relationships between chemical analyses and biological assays for endotoxins and glucans in these dust samples, and identify specific chemical fractions that best correlate with assay measures. Characterize the distributions of endotoxins and ergosterols by specific chemical components and size fraction in these various organic dust environments.*

Of a total of 720 samples collected in this study, 713 samples were analyzed by rFC assay and 689 samples were analyzed by LAL assay. A subset of 405 samples were analyzed by GC/MS for 3-OHFAs (chemical marker of endotoxins), and 194 samples were analyzed for ergosterol (chemical marker of fungi). Unanalyzed samples were eliminated due to the breakage of tubes during shipping or for samples below detection limits for GC/EI-MS analysis.

Figure 8 shows GM and GSD of dust and endotoxin levels and the ratio of rFC/LAL responses. A significant difference between agricultural dust types was discovered ($p < 0.0001$ for all variables). Among the five livestock environments, swine had the highest endotoxin levels per mg dust in both rFC and LAL assays, followed by chicken, turkey, and horse. The lowest endotoxin level per mg dust was found in dairy. A similar trend was observed for endotoxin concentrations in the air with slight difference in rank orders between LAL and rFC results. For LAL, endotoxin concentration in chicken dust was the second highest followed by turkey dust; this order is opposite in rFC. The rFC/LAL ratio results indicate that LAL provided approximately 1.3 to 1.5 times higher responses to endotoxin than rFC in chicken and horse dusts; rFC was 1.1 to 1.4 times higher than LAL in turkey, swine, and dairy dusts. The rank order of the rFC/LAL ratio was dairy >swine > turkey >chicken >horse.

ANOVA (GLM) showed that there was a trend of difference between Colorado and Iowa samples ($p = 0.0999$). Overall, Iowa samples contained higher geometric mean endotoxin concentrations with both LAL and rFC assays, but the rFC/LAL ratio was slightly higher for Colorado samples (1.05) than for Iowa samples (0.93). ANOVA showed no significant difference between laboratory and field samples ($p = 0.1899$), thus, laboratory and field samples were combined for each agricultural dust.

The correlations between dust and endotoxin concentrations are shown in Figure 9 and Table XV. Both LAL and rFC measurements were significantly positively correlated to dust concentrations in all five environments ($p < 0.0001$). Horse dust had the highest correlation between dust concentration and LAL ($r = 0.98$), whereas chicken dust was the highest for rFC ($r = 0.93$). Dairy dust showed the lowest correlations between dust and endotoxin exposures in LAL and rFC ($r = 0.54$ and 0.55, respectively). Correlations between LAL and rFC were highly positive and significant. However, the magnitude of correlations between LAL and rFC varied by environment. The rank order of LAL and rFC correlations was chicken (0.96) >horse (0.92) >Dairy (0.91) >Turkey (0.84) >Swine (0.81).

The correlations between LAL and rFC in each sampling device were evaluated (Figure 10). The high and significant correlations were found in all devices ($r > 0.90$). In general, cassette had the highest correlation between LAL and rFC assays ($r = 0.93$) and cyclone was the lowest ($r = 0.91$). ANOVA (GLM) showed there was a significance difference in sampling devices for the rFC/LAL ratio ($p < 0.0001$). Multiple comparison test showed there was a difference between cyclone and other devices, but no difference among other three sampling devices.

Stepwise forward multiple linear regressions were performed to evaluate the relationship between assay results and GC/EI-MS accounting for effects of individual 3-OHFAs, chemical markers of endotoxins, at the same time. The results of the evaluation of maximum R^2 for the combination of 3-OHFAs with each LAL and rFC assay at $\alpha = 0.05$ are shown in Table XVI. Both LAL and rFC assay results had the same combination of 3-OHFAs (C9:0, C12:0, C13:0, and C14:0), but R^2 was lower for LAL (0.2046) than for rFC (0.4456). In addition, total 3-OHFAs (sum of all 3-OHFAs) was significantly correlated with LAL and rFC in all environments, but correlation coefficients were consistently higher for rFC (Table XVII).

Correlations between chemical marker of fungi (ergosterol) and rFC, LAL, and the rFC/LAL ratio were calculated to evaluate the magnitude of potential interference from fungi (Figure 11). Ergosterol was moderately significantly correlated with both LAL and rFC. There was no correlation between ergosterol and the rFC/LAL ratio. Ergosterol results are shown in Table XVIII.

In general, strong positive correlations exist between the LAL and rFC assays. However, responses to assays vary by agricultural environment or dust type. LAL may overestimate (or rFC underestimates) endotoxin exposures in chicken and horse dusts and LAL may underestimate (or rFC overestimates) endotoxin concentrations in dairy, swine, and turkey dusts. Our finding showed that ergosterol concentration may not be a major factor of interference in the LAL assay overall, but the magnitude of interference may vary by dust type. Other than ergosterol contribution, this variability could be explained by difference in bacterial composition and other dust components; the rFC assay may react positively with Actinobacteria.

Future applications will be expected to increase sample size for ergosterol measurements, to analyze dusts from different agricultural environments, and to investigate presence of potential interference of assays including Actinobacteria and proteins in agricultural dusts.

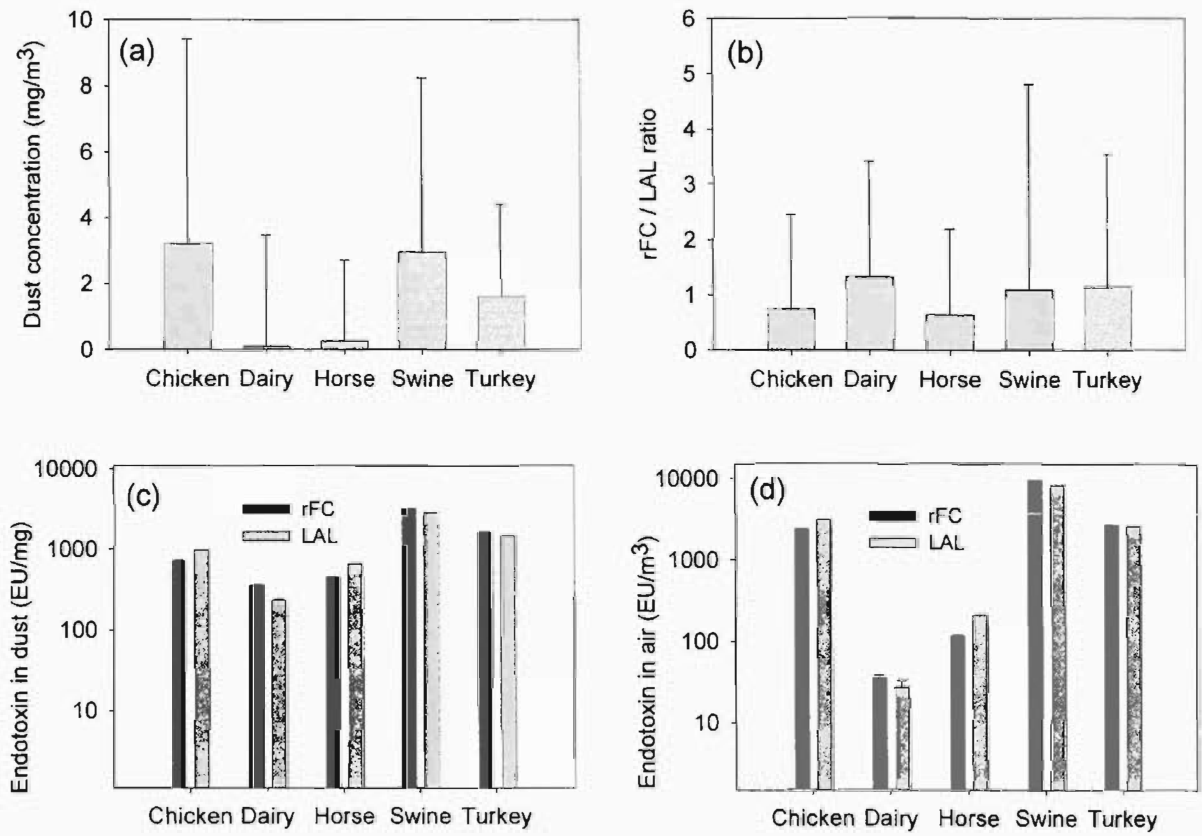


FIGURE 8. Geometric means and geometric standard deviations of (a) dust concentration, (b) rFC/LAL ratio, (c) endotoxin level per mg dust, and (d) endotoxin concentration per m^3 air in each dust type

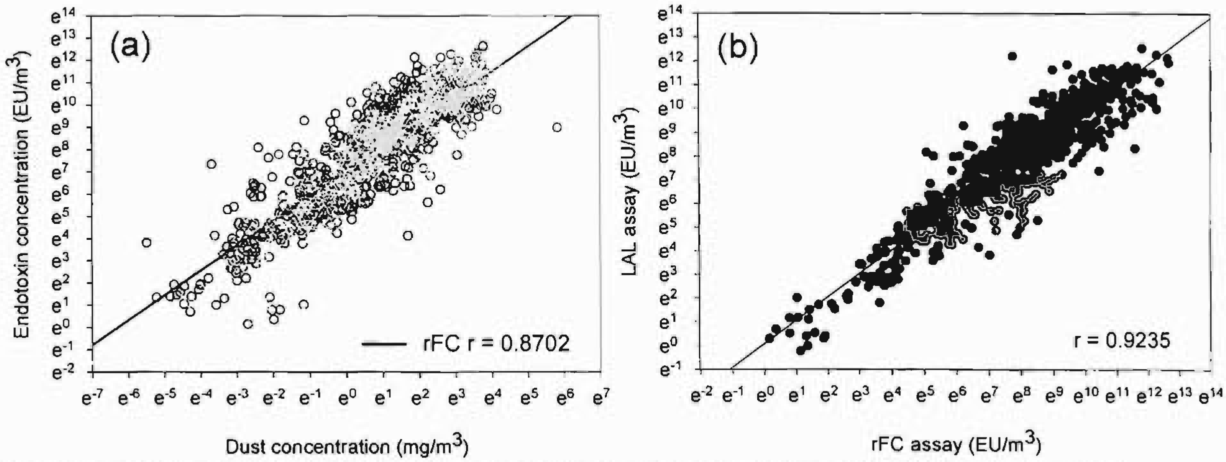


FIGURE 9. Correlation (a) between dust and endotoxin concentrations and (b) between LAL and rFC assays

TABLE XV. Correlation between dust (mg/m^3) and endotoxin concentrations (EU/m^3) with p-values (bold = $p < 0.05$)

	Dust concentration vs. Endotoxin Concentration		
	rFC	LAL	rFC vs. LAL
Chicken	0.9333	0.9426	0.9558
(n = 204/202)[A]	**<0.0001**	**<0.0001**	**<0.0001**
Dairy	0.5470	0.5378	0.8359
(n = 78/76)[A]	**<0.0001**	**<0.0001**	**<0.0001**
Horse	0.9309	0.9777	0.9153
(n = 40/15)[A]	**<0.0001**	**<0.0001**	**<0.0001**
Swine	0.8415	0.8869	0.8073
(n = 198/197)[A]	**<0.0001**	**<0.0001**	**<0.0001**
Turkey	0.6733	0.7649	0.8417
(n = 193/199)[A]	**<0.0001**	**<0.0001**	**<0.0001**

[A] First number shows rFC sample size and second number shows LAL sample size

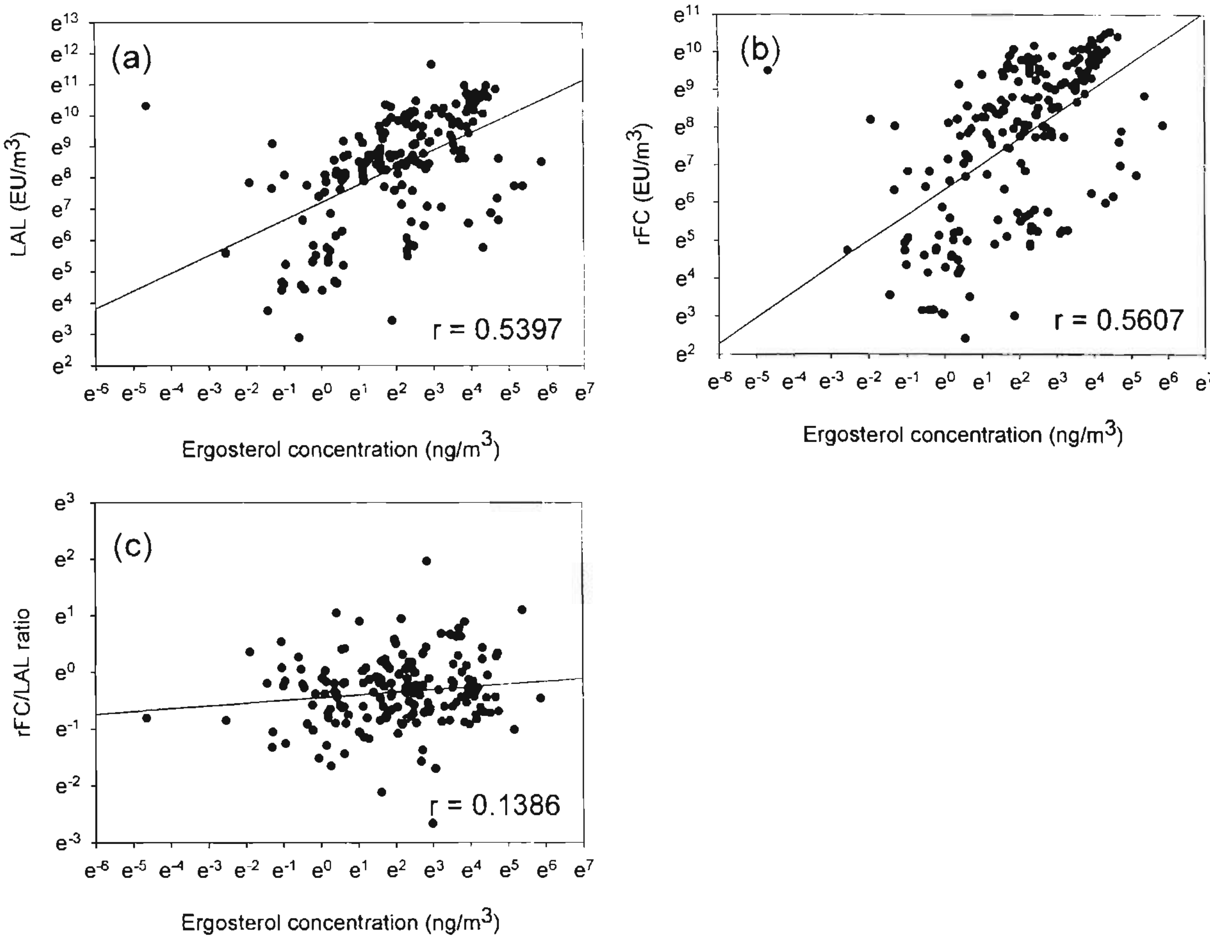


FIGURE 10. Correlations between LAL and rFC assays for each sampling device: (a) cassette (n = 182), (b) cyclone (n = 181), (c) IOM (n = 181), and (d) button (n = 177).

TABLE XVI. Stepwise forward multiple linear regression analyses for 3-OHFAs and each endotoxin assay

		N	3-OHFA combination[A]	R^2	P-value
rFC	Overall	405	13,9,14,12	0.4456	<0.0001
	Chicken	148	13,8,9,14,12	0.4611	<0.0001
	Dairy	35	17,14,9	0.6754	<0.0001
	Horse	37	None	-	-
	Swine	35	13,12,8	0.4621	0.0002
	Turkey	150	13,15,14,18	0.4614	<0.0001
LAL	Overall	376	13,12,9,14	0.2046	<0.0001
	Chicken	145	15,8,13	0.2488	<0.0001
	Dairy	33	15	0.2783	0.0016
	Horse	14	None	-	-
	Swine	35	None	-	-
	Turkey	149	15,13,12,17	0.2151	<0.0001

[A] In order of entrance to the model

TABLE XVII. Pearson correlations of total 3-OHFAs (pmol/m^3) with each endotoxin assay (EU/m^3) with p-values (bold = $p < 0.05$)

	Total 3-OHFAs and rFC	Total 3-OHFAs and LAL
Overall	0.86297	0.7851
(n = 405/376)[A]	**<0.0001**	**<0.0001**
Chicken	0.93418	0.88944
(n = 148/145)[A]	**<0.0001**	**<0.0001**
Dairy	0.39461	0.33706
(n = 35/33)[A]	**0.0209**	0.0592
Horse	0.78841	0.74225
(n = 37/14)[A]	**<0.0001**	**0.0024**
Swine	0.74661	0.7164
(n = 35/35)[A]	**<0.0001**	**<0.0001**
Turkey	0.39788	0.20048
(n = 150/149)[A]	**<0.0001**	**0.0142**

[A] First number shows n for rFC and second number shows n for LAL

Table XVIII. GC/MS - Ergosterol Results

	IOM			Cassette		
	n	GM	GSD	n	GM	GSD
Chicken	18	1.005257	9.510212	7	3.273852	1.918531
Feedlot	7	11.34685	1.890642	4	39.15098	1.116434
Horse	24	19.41193	1.904121	10	17.78457	2.090424
Swine	-	-	-	-	-	-
Turkey	5	3.083731	1.643357	8	13.82753	4.102517

		IOM			Cassette		
		n	GM	GSD	n	GM	GSD
Chicken	Field	-	-	-	-	-	-
	Lab	18	1.005257	9.510212	7	3.273852	1.918531
Feedlot	Field	1	5.557826	-	-	-	-
	Lab	6	12.78018	1.834137	4	39.15098	1.116434
Horse	Field	7	20.81657	2.10728	5	26.13208	2.046449
	Lab	17	18.86147	1.859602	5	12.10355	1.792035
Swine	Field	-	-	-	-	-	-
	Lab	-	-	-			
Turkey	Field	3	2.707465	1.789409	3	3.105353	1.114534
	Lab	2	3.748408	1.533548	5	33.87853	2.451989

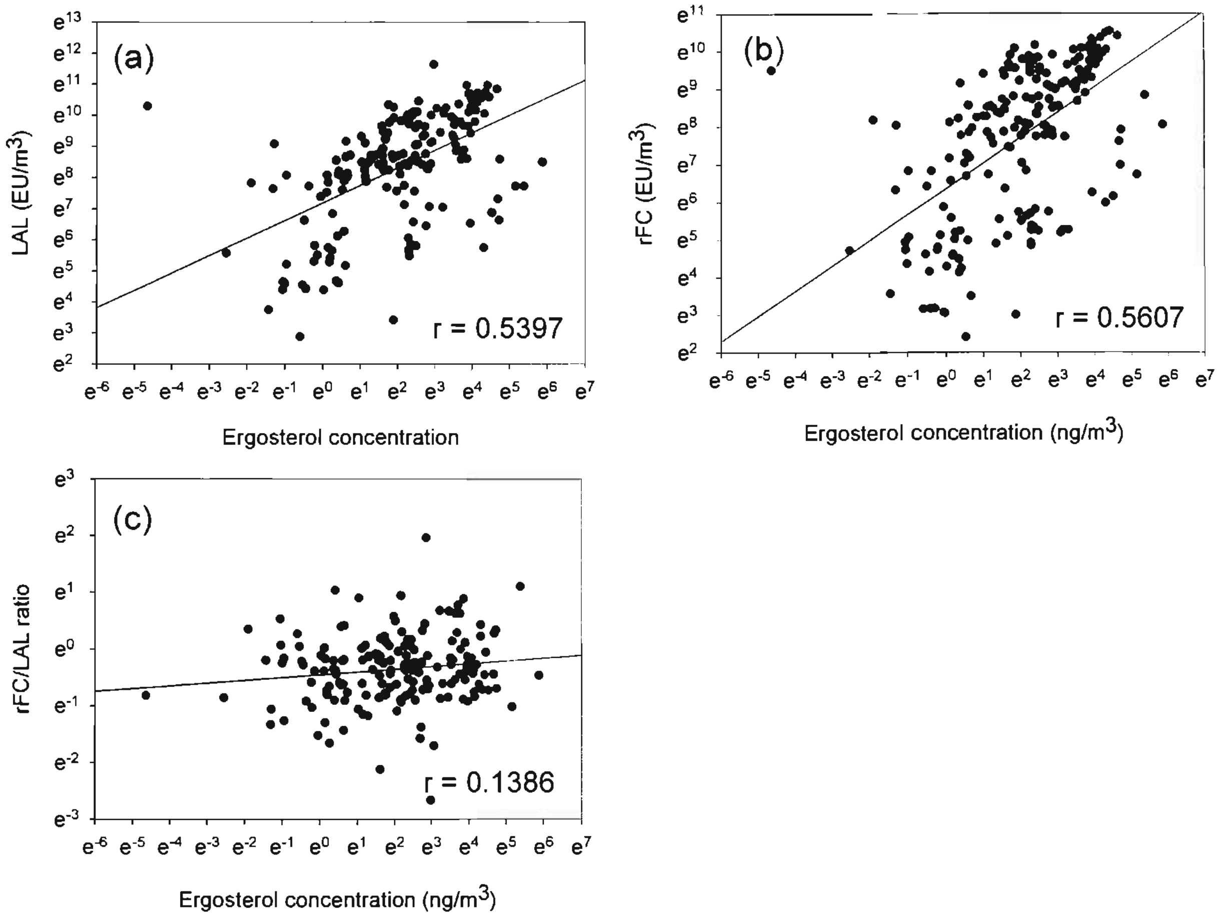


FIGURE 11. Correlations between ergosterol and (a) LAL, (b) rFC, and (c) rFC/LAL

Saito R, Reynolds SJ, Thorne PS, Mehaffy J, Cranmer BK, Keefe T, Metwali N, O'Shaughnessy PT: [2007 submitted] Comparison of recombinant Factor C (rFC) and Limulus amebocyte lysate (LAL) assays for endotoxin exposure in five livestock dusts. JOEH

GC/MS Method Development

Two calibration curves were created for each experimental set: one for lower concentrations (2, 6, and 20 ng) and one for higher concentrations (20, 100, and 500 ng). The calibration curves yielded R^2 of 0.9966 with standard deviation of 0.0071. Signal-to-noise ratios of the chromatograms showed that the method provided an LOD of 1 to 3 pmol (equivalent to 0.5 ng spike) and an LOQ of 2 to 6 pmol (equivalent to 1 ng spike), depending on carbon chain length of 3-OHFAs measured.

The reproducibility of C11:0 3-OHFAs surrogate recovery was less than 12 % CV (n = 24). Since this method contained two derivatization steps including one overnight reaction, CV of less than 12 % was considered satisfactory. Correlation between 3OHFAs taken through the entire sample preparation process and 3OHFA methyl esters converted directly to trimethylsilyl analogs, was poor (data not shown). Thus spike recovery was not evaluated in a traditional manner. Instead, C11:0 surrogate coupled with calibration linearity were used to ensure strong quality control of data reporting. As stated above, fatty acid standards were submitted to the entire sample workup and covered the analytical working range (2 to 500 ng spikes of individual 3-OHFA). This method effectively assessed relative spike recovery at 5 levels for each compound and yielded excellent calibration linearity.

A GC/EI-MS method for endotoxin analysis has been successfully developed and applied to assessment of 3OHFA distribution in several agricultural environments. Compared to the parent GC/MS-MS method, it reduces use of toxic chemicals and sample handling, allows sensitive monitoring of the experimental process, and can be used for analysis of very small samples, typical of personal air samples.

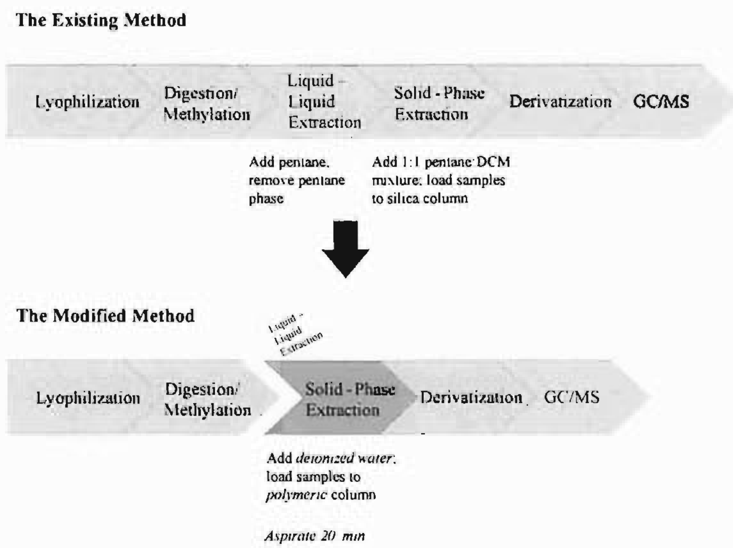


FIGURE 12. Changes in experimental procedures for GC/EI-MS analysis

Saito R, Cranmer BK, Tessari JD, Larsson L, Reynolds SJ: [2007submitted]Recombinant Factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxins in four agricultural dusts. JOEH

4. *Evaluate the precision and relative predicted dust concentration for direct reading aerosol instruments including the DataRam (PDR), HAM, and their relationship to the gravimetric methods in these organic dust environments. Calibrate these devices compared to traditional gravimetric methods and derive correction factors if needed for adjustment of data produced by these devices. Characterize aerosol size distributions using Grimm direct-reading particle counter. Evaluate the usability and utility of these direct reading aerosols devices for practical applications in these agricultural environments.*

The HAM was found to be unreliable in most situations. The two HAMs seldom corresponded with each other in any environment, and seldom correlated with the gravimetric methods.

The DataRam (PDR) was highly reliable, with the two instruments providing strongly correlated readings in all field tests and in most of the laboratory tests. Table XIX. The PDR correlated well with the Cyclone for all dusts in the field. It correlated well with the IOM in 3 dust types in the field – Chicken, Swine, Turkey, but not with the others. The CFC and Button correlated well with the PDR for all except Horse and Dairy Dusts. Lab studies, where wind velocity varied, demonstrated fewer correlations between gravimetric devices and the PDR, in some cases demonstrating negative correlations. It does appear that the dust type (with different aerosol size distributions) and wind speed are important factors affecting the performance of the PDR. Table XX presents regression equations for prediction of PDR performance relative to specific gravimetric samplers based on field studies (real world conditions). In most cases these models explain a large proportion (48% to 96%) of the variation in PDR values. Manuscript in preparation.

Table XIX. PDR Correlations, Chicken Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	0.96	0.96	0.97
PDR - IOM	ns	ns	0.96
PDR - CFC	0.70	ns	0.98
PDR - Button	ns	ns	0.98
PDR - Cyclone	ns	ns	0.85

PDR Correlations, Swine Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	0.89	ns	0.83
PDR - IOM	ns	ns	0.71
PDR - CFC	0.70	ns	0.77
PDR - Button	ns	ns	0.73
PDR - Cyclone	ns	ns	ns

PDR Correlations, Turkey Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	1.00	0.99	0.98
PDR - IOM	-0.75	ns	0.85
PDR - CFC	ns	ns	0.88
PDR - Button	0.73	ns	0.78
PDR - Cyclone	- 0.74	ns	0.92

PDR Correlations, Horse Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	0.90	ns	0.97
PDR - IOM	ns	ns	ns
PDR - CFC	ns	ns	ns
PDR - Button	ns	ns	ns
PDR - Cyclone	ns	ns	0.94

PDR Correlations, Cattle Feedlot Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	ns	0.97	0.63
PDR - IOM	ns	0.70	ns
PDR - CFC	ns	0.93	0.74
PDR - Button	ns	0.81	0.79
PDR - Cyclone	ns	0.71	0.69

PDR Correlations, Dairy Dust

Pearson Correlations	Lab 1.0 m/s	Lab 0.2 m/s	Field
PDR - PDR	Insufficient dust	Insufficient dust	0.98
PDR - IOM			ns
PDR - CFC			ns
PDR - Button			ns
PDR - Cyclone			0.78

Table XX. Estimated prediction (regression) equations for PDR using Field Studies

Dust Type	Intercept	Sampler	Regression R^2
Chicken PDR =	0.020	0.048 IOM	0.92
	0.011	0.076 CFC	0.96
	0.014	0.064 Button	0.94
	0.016	1.41 Cyclone	0.76
Swine PDR =	0.052	0.030 IOM	0.49
	0.051	0.068 CFC	0.60
	0.087	0.038 Button	0.53
Turkey PDR =	0.193	0.103 IOM	0.72
	0.095	0.248 CFC	0.75
	0.203	0.194 Button	0.62
	0.106	1.275 Cyclone	0.84
Horse PDR =	- 0.071	1.438 Cyclone	0.89
Feedlot PDR =	- 0.008	0.276 CFC	0.54
	- 0.003	0.328 Button	0.62
	0.003	1.176 Cyclone	0.48
Dairy PDR =	- 0.0067	1.937 Cyclone	0.58

PUBLICATIONS

Journal Articles

O'Shaughnessy PT, Lo J, Golla V, Nakatsu J, Tillery MI, Reynolds SJ: [2007] Comparison of Aerosol Samplers Relative to the Inhalable and Respirable Collection Criteria. JOEH 4:237-245.

Saito R, Cranmer BK, Tessari JD, Larsson L, Reynolds SJ: [2007submitted]Recombinant Factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxins in four agricultural dusts. JOEH

Reynolds SJ, Nakatsu J, Tillery MI, Keefe T, Mehaffy J, Thorne PS, Donham KJ, Nonneman M, Golla V, O'Shaughnessy PT: [2007 submitted] Field and Wind Tunnel Comparison of Four Aerosol Samplers using Agricultural Dusts. JOEH.

Saito R, Reynolds SJ, Thorne PS, Mehaffy J, Cranmer BK, Keefe T, Metwali N, O'Shaughnessy PT: [2007 submitted] Comparison of recombinant Factor C (rFC) and Limulus amebocyte lysate (LAL) assays for endotoxin exposure in five livestock dusts. JOEH

Additional manuscripts on Direct Reading instruments studies and on Corrections for OELs are in progress, but not yet submitted.

Proceedings/Presentations

Reynolds SJ: [2007] Colorado Agriculture Big and Small. Health and Safety Risk Management. Greely, CO Feb. 22.

Reynolds SJ: [2007] Rural Health Grand Rounds, University of Colorado School of Medicine. Agricultural Health and Safety. Denver, CO. February 13.

Reynolds SJ: [2006] ENDOTOXIN and ORGANIC DUST LUNG DISEASE, UC Davis Ag Center Seminar, Davis, CA May.

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Dissertations/Theses

Jason Nakatsu – PhD Dissertation Spring 2008

Rena Saito - PhD Dissertation Spring 2008

INCLUSION OF GENDER/MINORITY – NOT APPLICABLE

INCLUSION OF CHILDREN – NOT APPLICABLE

MATERIALS AVAILABLE FOR OTHER INVESTIGATORS

Two Analytical Method Protocols have been developed and are available for other researchers or analysts. One is for the rFC Endotoxin Assay applied for agricultural dusts. The other is for GC/MS analysis of agricultural dusts for chemical markers of endotoxin – 3 OHFAs. Contact Dr. Reynolds at Stephen.Reynolds@Colostate.edu.