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Evaluation of the use of an SKC button inhalable aerosol sampler with a Grimm aerosol monitor to determine air concentrations of subtilisin

Study purpose. Currently, there is no OSHA or NIOSH monitoring method for subtilisin. This study evaluated a personal aerosol monitoring method for detection and quantification of subtilisin. The ACGIH ceiling limit and NIOSH short term exposure limit (STEL) for subtilisin is 0.06 μg/m^3 making it the lowest exposure limit established for any exposure by the organizations. This study specifically evaluated the use of an SKC Button inhalable aerosol sampler with a Grimm Aerosol Monitor for monitoring low concentrations of subtilisin particulates in air within a laundry detergent production facility.

Methods. Air samples of detergent with subtilisin were collected using a Button sampler containing a glass fiber filter concurrently with a Grimm model 1.109 Aerosol Spectrometer. Nineteen sampling events of four hours each were performed over a 7-week period, with three Button samplers operating simultaneously. Sampling locations included a detergent containing subtilisin (DCS) production area and a specially constructed enclosure where DCS was introduced in a controlled manner. Sample analysis was conducted with a Konelab Arena 20 analyzer to quantify the amount of subtilisin collected on the filter of each air sample.

Results. A linear regression for the concentration of DCS aerosol measured in the enclosure by the Grimm was compared to the average concentration of DCS aerosol measured on the Button filter in the enclosure, which produced an R^2 value of 0.64 ($p = 0.006$). The Pearson's correlation produced an r value of 0.8 ($p = 0.006$). The linear regression for the average concentrations of DCS aerosol measured on the Button filter media compared to the average subtilisin detected from the aerosol in the enclosure produced an R^2 value of 0.66 ($p = 0.004$). The Pearson's correlation produced an r value of 0.81 ($p = 0.004$).

Discussion. The amount of subtilisin in all air samples in the DCS production area were below the limit of quantitation due to the subtilisin encapsulation at the DCS production area. This caused particles sizes to be greater than the inhalable fraction (100 μm), which is also the limit of the size selectivity of the Button inhalable sampler.

Conclusion. The results of the statistical evaluation from the aerosol monitoring results in the enclosure indicate the Button inhalable sampler compared with the results from the Grimm aerosol monitor may be a valid method for determining airborne subtilisin concentrations for aerosols with diameters less than or equal to the 25 μm particle size. However, due to all results below the limit of quantitation at the DCS production area, it is concluded that there are insufficient results to determine if the Button sampler with the Grimm aerosol monitor can be used in the workplace.

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INTRODUCTION

Subtilisins are large molecular weight proteins (on the order of 27,000 Da) with a density of 1.25 g/ml. More specifically, subtilisins are proteolytic bacterial enzymes that are used primarily in laundry detergent manufacturing industries, contact lens cleaner manufacturers, film processing, and the food processing industries.[1–3] Subtilisins are used in manufacturing of detergents for cleaning clothes because the bacteria's enzymatic properties enhance the stain removal properties.[4] Exposures to particle sizes of aerosolized subtilisin below 100 μm diameter can cause immune-system mediated bronchoconstriction and respiratory symptoms.[2] More specifically, aerosolized subtilisin can cause sore throat, persistent cough, wheezing, nasal congestion, and headache. More serious effects from subtilisin exposure are dermal irritation, asthma, pain in the chest and the potential to incapacitate the victim.[2,5] The California Department of Public Health reported that exposures to subtilisin in the workplace have adversely affected workers in a detergent formulation plant. Workers exposed to subtilisin, when the limits were set to 0.12 μg/m^3 at the detergent plant, were hospitalized.[2] The ACGIH ceiling limit and NIOSH Short Term Exposure Limit (STEL) for subtilisin is 0.06 μg/m^3.[6] Currently there is no 8-h Time Weighted Average (TWA) Occupational Exposure Limit (OEL) or validated personal sampling method for aerosolized subtilisins.[2]

Area sampling methods for subtilisin have been published by Fulwiler[5]; and Fulwiler et al.[7]; and Bruce et al.[8] Fulwiler describes using a high volume sampler and high efficiency glass fiber filter to sample aerosolized subtilisin.[5] Fulwiler et al. demonstrated that a high-efficiency glass fiber filter could collect a high concentration of enzymes when it was used with a high-volume sampling technique.[7] In addition, Bruce et al. described the glass fiber media as the preferred media for collecting enzymes.[8] Fulwiler et al. sampled aerosols using an electrostatic precipitator at 170–283 L/min. However, an adequate sample size could not be colleceted, therefore, a high volume sampling technique was incorporporated with a high efficiency glass fiber medium.[7] Fulwiler et al. does not further discuss the specific type of instrument used as a high volume sampler.[7] Bruce et al. describe using a Galley sampler for sampling enzymes. Their explanation for choosing the Galley sampler as the standard for detecting enzymes in detergent factories was because it was preferred over other area samplers due to its ease of use, and its history for providing accurate data for factories in the United Kingdom.[8] A Galley sampler is a high volume area sampler that operates at 600 L/min and uses a 150-mm glass fiber filter with a pore size of 1.6 μm.[9,10]

Personal sampling methods for subtilisin have been proposed by Bruce et al. and the American Industrial Hygiene Association.[2,8] Bruce et al. published a method for sampling aerosolized enzymes in a detergent manufacturing plant using the Casella Model A personal sampler and the Anderson stack sampler. They concluded that additional investigation is required since monitoring results could not determine which sampler provided more accurate measurements.[8] Despite these proposed methods, neither NIOSH or OSHA have developed or validated a personal sampling method for subtilisin.[2]

The sampling performance of inhalable samplers are strongly dependent on particle size and ambient air velocity.[11] Kenny et al. evidenced that inhalable samplers displayed a wide variation in sampling efficiency and detected fewer aerosolized particles than was actually present as the air velocity increased.[12] Aizenberg et al. compared the Button sampler to the IOM (Institute of Occupational Medicine), GSP (Gesamt Staub Probenahme) samplers, and the 37-mm closed-face filter cassette.[13] It was evidenced that the Button sampler displayed lower dependence on air velocity than the three other samplers with which it was compared. Aizenberg concluded that the convex geometry of the Button's inlet acts to reduce the effects of wind.[14]

The performance of aerosol samplers can be affected by particle build-up on the filter.[14] The convex geometry of the Button sampler's inlet improves the filter deposition uniformity. Hauck et al. found that measurement results obtained with the Button sampler had lower inter-sample variations of the measured concentration levels and higher uniformity of the particle deposits on the filters than those obtained with the standard cassette. Hauck et al. used a standard closed-face cassette with a 37 mm PVC filter with a 0.8 μm pore size.[15] In addition, Aizenberg et al. evidenced that the much larger collection area of

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the Button sampler prevents it from developing particle surface density build-up on the filter.[16] This is beneficial due to high concentrations of aerosolized DCS that can be present at detergent manufacturing companies. The Button's inlet improves the sampling performance when collecting a large amount of aerosolized particulates. The Button sampler can also be used for monitoring aerosols in locations having low air velocities. Sleeth et al. tested the IOM, the Button Sampler, and the GSP Sampler, also known as the Conical Inhalable Sampler and compared the sampling efficiencies to the inhalable curve at different ultra-low wind speeds of 0.10, 0.24, and 0.42 m/s or approximately 20 fpm, 45 fpm and 80 fpm.[17] The IOM, Button and GSP each provided an increasingly accurate estimate of the inhalable aerosol fraction as air velocity increased. As air velocity decreased each of the three inhalable aerosol samplers consistently provided greater sampling efficiency relative to the inhalable aerosol convention (aerosols <100 μm) and the samplers were significantly different from that criterion at the lowest air velocity of 0.10 m/s.[17] However, their results indicated the air samplers may generally measure higher aerosol concentrations than what would actually be inhaled by humans at ultra-low wind speeds (e.g. 0.10 m/s) and the samplers may provide a more conservative benchmark for use at ultra-low wind speeds.[17] Sleeth et al. concluded that their results suggest the IOM, Button, and GSP provided consistent agreement to

the inhalable curve and may be useful at some ultralow wind speeds.[17] In addition, Kenny et al. explain that inhalable samplers that oversample at low wind speeds are allowed and may be used in practice without correction factors.[12] Simpson et al., Marchand et al. and Duquenne et al. describe sampling methods for organic dusts and endotoxins. They describe using the IOM and Button sampler as the collection method.[18–20] Duquenne further notes that the Button sampler (SKC Inc.) is primarily used in the wood industry, cotton industry, agricultural and animal handling industries and is one of the most frequently used systems for sampling organic dusts and endotoxins.[20]

Because of reported subtilisin health risks and because only high-volume (e.g., about 300 L/min) area filter samplers are currently used to determine workplace enzyme concentrations, a validated subtilisin personal monitoring method would be an invaluable tool for assessing personal worker exposure levels. For these reasons, the purpose of this study was to determine if results from using a Button inhalable aerosol sampler with a Grimm aerosol monitor had acceptably comparable results to calculated aerosol concentrations of DCS in a specially constructed enclosure.

METHODS

Aerosol Monitoring Method

The SKC Button sampler, see Figure 1, was chosen for this study for

evaluation as a personal sampler for subtilisin because the inlet prevents particle build-up on the filter and has lower dependence on air velocity, which enables it to maintain collection efficiency while sampling in dusty, turbulent environments. This Button sampler consists of a convex multi-porous inlet, each with a pore size of 178 μm. The inlet minimizes oversampling of large particles and is designed to collect the inhalable fraction of particulates less than 100 μm. It is designed to operate at a flow rate of 4.0 L/min.[21] Additionally, it was selected because subtilisin currently has the lowest TLV established by ACGIH (0.06 μg/m^3).[22] Therefore, obtaining results above detection would be difficult due to the low TLV. The higher flow rate (4.0 L/min) allows a larger volume and therefore more particulates to be collected relative to what OSHA would use for Particulates Not Otherwise Regulated (PNOR) (2.0 L/min). This increases the likelihood of improving the lower limit of detection and the ability to quantify the enzyme concentration. Based on this information the Button sampler is hypothesized to be a viable method for sampling aerosolized subtilisin.

The aerosol sampling set-up consisted of an SKC AirChek XR5000 air sampling pump (SKC Inc., Eighty Four, PA), with 18″ of 1/4″ inside diameter Tygon tubing (Ryan Herco Flow Solutions, Sandy, UT), attached to the Button aerosol samplers (SKC Inc., Eighty Four, PA) equipped with one die-cut 25-mm (1.6 μm pore size) glass fiber filter, (VWR International, Radnor, PA). A Grimm 1.109 direct read instrument (Grimm Aerosol Technik GmbH & Co., Ainring, Germany) was operated concurrently, adjacent to three Button samplers. The glass fiber filter used in the Button sampler was cut from the glass fiber filter used in the Galley sampling system at the laundry detergent manufacturer in order to enable analysis of subtilisin.

Sampling was conducted for 19 different events for four hours each over a seven-week time period at a laundry detergent manufacturer. DCS aerosol sampling was conducted in a small, specially constructed enclosure with

Figure 1. SKC Button Sampler.

Figure 2. The Inside of Enclosure.

the capability to methodically inject DCS into the enclosure. It was located in a room adjacent to an active DCS production area. The following procedures were performed before each sampling event to assure acceptable accuracy of flow volumes associated with each sample. Four SKC XR5000 air sampling pumps were prepared, but only three pumps were used to collect air samples. The fourth SKC XR5000 air pump served as a backup in case one of the pumps malfunctioned. Each filter was first desiccated for at least 12 hours prior to the initial weighing and placed in a clean container the day before sampling. On the day of sampling, the pumps were calibrated and the sampling air flow rate was set to 4.0 L/min for all pumps using a Bios-DryCal DC Lite primary gas flow calibrator (Bios International Corporation, Butler, NJ).

One pre-weighed and desiccated glass fiber filter was designated as a blank (or control) sample for quality assurance and quality control (QA/QC) purposes. A blank filter was used for each sampling event and each was kept in a clean container before and during each sampling event.

Sampling was then conducted at either the DCS production areas or the enclosure. Seven sampling events were conducted in the DCS production areas and 12 sampling events were conducted in the enclosure. The three Button samplers were placed near to each other at a distance of five inches

(12.7 cm) apart, facing the same direction and on a metal rack five feet (1.5 m) off the ground as shown in Figure 2. During sampling the SKC air pumps were checked every 12 minutes to ensure they were operating because resolvable malfunctions within this time period are equal or less than 5 percent of the total sampling time of four hours (12 minutes is 5% of four hours).[23] Ambient air monitoring for DCS aerosol was also conducted using a Grimm 1.109 direct read instrument (Grimm Aerosol Technik GmbH & Co., Ainring, Germany) for all sampling events.

After completing a sampling event, the air pumps were not turned off until the air flow of each pump was post-calibrated. This was done to calculate the percent difference between the pre and post air flow values (calibration). The combined pre and post calibration flow rates in L/min were then averaged and multiplied by the time in minutes to calculate sample volume. The filters were removed from the Button samplers and placed in a desiccator overnight (for at least 12 hours). Air sampling pumps, Tygon tubing, Button samplers and other associated sampling equipment were cleaned using detergent and water, dried with paper towels and placed in a cabinet until the next sampling event. After desiccation, the glass fiber filters were post-weighed using a microbalance, placed in sterilized containers, and stored in a refrigerator at 2.8 °C.

Method for Enzyme Analysis of Samples

The filters were submitted to the laundry detergent manufacturer's product quality laboratory for enzyme analysis. All filters were analyzed for subtilisin using a Thermo Scientific Konelab 20 analyzer (Thermo Fisher Scientific, Vantaa, Finland). The Konelab 20 arena analytical method chosen is similar to the Bruce, Dunn et al. method and was selected because their method provides high sensitivity (results above detection), which is of particular value in dust analysis.[8] With this method, enzymes are first extracted into a buffered solution and the solution's spectrophotometry activity is measured. The analytical method for the enzyme's activity involves a reaction with casein, in which the enzyme hydrolyzes the casein whose products contain free amino groups. The amino groups are allowed to react with 2,4,6-trinitrobenzenesulfonic acid (TBSA), which forms a colored product. Higher concentrations of enzymes in the solution produce the colored compound at a faster rate. By measuring the change in absorbance at 420 nm over time and comparing this rate to that of known standard enzyme solutions, the mass of subtilisin on the filters is determined. The mass of subtilisin obtained from the product quality laboratory was divided by 0.96 m^3 (4.0 LPM × 240 minutes) in order to calculate the concentration (μg/m^3).

Enclosure

Sampling was conducted in the enclosure in order to monitor in a controlled environment where a specific concentration of DCS was maintained as an aerosol. DCS aerosol concentration levels in the enclosure were maintained by adding the DCS at different intervals throughout the sampling event. During each sampling event a different DCS concentration was introduced into the enclosure. Additions of the DCS mixture were made into the enclosure through a window located at the bottom (see Figure 3) and by using 18″ length of 3/8″ inside diameter Tygon tube that was pre-loaded with equal portions of the DCS. A can of compressed air (Falcon DPXL3 Dust-Off, 10oz.) was used to inject the DCS, thus producing aerosolized DCS in the
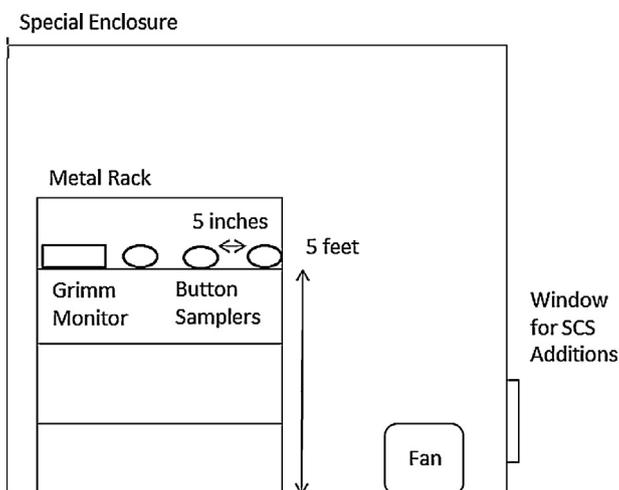
Figure 3. Specially Designed Enclosure, 1.87 m × 2.15 m × 1.85 m, Total volume 7.4 m³.
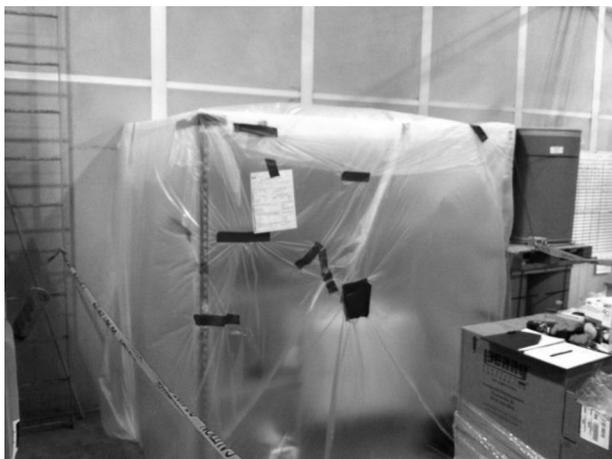

Figure 4. Outside of Specially Designed Enclosure, 1.87 m × 2.15 m × 1.85 m, Total volume 7.4 m³.

enclosure. After the introduction of the DCS a plastic shield was then placed over the window to seal it. For each event, an equal portion of the total DCS was incrementally introduced into the enclosure. A similar DCS concentration was introduced for all three samples collected per each sampling event. The Grimm 1.109 instrument monitoring was used to determine the range of particle sizes present for each monitoring event.

The enclosure was constructed with a metal frame and flexible plastic sheets for the ceiling and walls which were sealed with duct tape (see Figures 2 and 3). The small enclosure measured 1.87 m wide × 2.15 m tall × 1.85 m deep, and the enclosure air volume was 7.4 m³ (262.4 ft³). It was located in the detergent manufacturer's production building. Air movement in the enclosure was generated by a Honeywell Turbo Force Air Circulator Fan (Honeywell Inc., HT-900, Morristown, NJ), which was placed on the floor, rotated to an angle of 60° from the horizontal, and the fan speed was set to the medium air velocity selection, which was found to generate 353 cfm at the fan face and 199 cfm at one foot distance from the fan face (see Figures 3 and 4).

For sampling in the enclosure, the detergent and subtilisin were separately ground with a coffee grinder and mortar and pestle, sieved through a 25 μm sieve and then mixed together. After mixing, the blend was stored in a refrigerator at 2.8 °C in order to prevent enzyme decomposition. The subtilisin and detergent were manually mixed to attain a blend of 2 percent subtilisin and 98 percent detergent by weight. This was done in order to be precise in monitoring 2 percent subtilisin to detergent in the enclosure, which is the typical mixture in the DCS production area. The 25 μm size for the DCS particles was decided on to assure sufficient aerosolization of particles in the enclosure for monitoring purposes and were of a size able to be measured by both the button sampler and the Grimm 1.109.

DCS Production Area
Sampling locations in the DCS production area were selected to represent typical air concentrations of DCS in the production area. The DCS production areas were located at the laundry detergent manufacturer where observed ambient levels of aerosolized DCS resulted from (1) the DCS mixture being poured into the consumer packaging containers at a worker line and (2) DCS that was poured into bags in a housed bulk bag filling area. Six sampling events occurred at the consumer packaging line and one event occurred in the housed bulk bag filling area. All sampling events for these locations will be referred to as the DCS production area for convenience.

The DCS composition at the laundry detergent manufacturer is produced to meet a 2 percent subtilisin concentration. The subtilisin particulates at the DCS production area are encapsulated and typical size ranges of the encapsulated subtilisins are from 50 μm to greater than 100 μm. The blending process, to meet a 2 percent subtilisin to detergent concentration, involves encapsulated subtilisin that is metered from a holding bin and conveyed to an agglomerator. Agglomerators are rotary, heated cylinders (121 °C) and are sloped at the discharge end in order to tumble and mix all of the DCS ingredients. The DCS then flows from the agglomerator to a rotating condenser that further mixes and cools the DCS.

Data Analysis for the Enclosure

The statistical analyses used to analyze the sample results were a linear regression and Pearson's correlation. These were accomplished by using STATA version 12.0 (College Station, TX). For the first linear regression, the independent variable was the amount of DCS measured in the enclosure by the Grimm and the dependent variable was the average amount of DCS measured on the Button filter. For the second linear regression the independent variable was the average amount of DCS measured on the Button filter and the dependent variable was the average amount of subtilisin detected.

A linear regression for the enclosure results was used to determine how well the Button sampler can estimate the variability of subtilisin air concentrations in the workplace by providing an R^2 value. R^2 is used to determine if one variable (independent) can predict the variability of another variable (dependent). According to the *Principles of Biostatistics* an R^2 value of 1.0 indicates the variability of the dependent variable is predicted 100 percent, a strong positive is $(0.77 \leq R^2 < 1.0)$, a moderate positive is $(0.55 \leq R^2 < 0.77)$, and a weak positive is $(0 \leq R^2 < 0.55)$. The slope of the line from the linear regression results were considered statistically different than zero if the p value was less than 0.05.[24]

A Pearson's correlation (r value) was used to determine the correlation between the amount of DCS mixture measured in the enclosure and the amount of subtilisin collected and analyzed. The r value is used to determine the strength of association or correlation between two variables. According to the *Principles of Biostatistics* a perfect positive correlation has an r value of 1.0, a strong positive correlation is $(0.6 \leq r < 1)$, a moderate positive is $(0.3 \leq r < 0.6)$, and no correlation is $(0 \leq r < 0.3)$. A Pearson's correlation was considered to be statistically significant if the p value was less than 0.05.[24]

Ten of the 12 sampling events were used for statistical analyses. A total of 60 data points (30 data points from the amount of DCS measured on the filter and 30 data points from the amount of

subtilisin detected) were collected in order to provide a large number for statistical evaluation and for statistical power.

Data Analysis for the DCS Production Area

The equation of the line for the second linear regression in the enclosure was intended to be utilized to predict the amount of subtilisin at the DCS production area. Seven (7) sampling events ($n = 21$ samples) were collected in the DCS production areas.

RESULTS

The amount of DCS introduced ($\mu g/m^3$), the total particulates measured by the Grimm ($\mu g/m^3$) and the average concentration and standard deviation (SD) measured in micrograms per cubic meter ($\mu g/m^3$) by the Button samplers (number of samples or $n = 3$ for each event) in the enclosure for all sampling events are shown in Table 1. The average amount of DCS collected on the filter and average amount of subtilisin detected in the enclosure for all sampling events are shown in Table 2. The average amount of DCS collected on the filter and average amount of subtilisin detected for

the DCS production area are shown in Table 3.

The limit of quantitation (LOQ) for subtilisin was 0.0142 micrograms. Blank samples that have a positive subtilisin value measured by the product quality laboratory or value above the LOQ are displayed in the Tables, as shown in Tables 2 and 3. All other blank samples (one for each event) not listed, were below the LOQ. Sample results below the LOQ were adjusted by calculating 50 percent of the LOQ/m^3 (0.0074 $\mu g/m^3$) for statistical analysis.

Sample events 3, 7, 8, 10–16, 18 and 19 were performed in the enclosure as shown in Tables 1 and 2. Sample events 1, 2, 4–6, 9 and 17 were performed in the DCS production area as shown in Table 3.

Two sampling events were omitted from statistical comparisons. The amount of DCS aerosol collected was not obtained for sample event 12 percent and 100 percent subtilisin was introduced instead of DCS for sample event 15 in the enclosure, so both sampling events were omitted (see Tables 1 and 2). There was one sampling event in which a low concentration of subtilisin was detected above the LOQ of 0.0142 μg on the blank sample filter (0.024 μg for enclosure sample

Table 1. Concentration of DCS Measured by the Grimm and Average Concentration Measured by the Button Filter in the Enclosure.

Sample Number	DCS Introduced ($\mu g/m^3$)	Grimm Total Particulates ($\mu g/m^3$) (X-axis)	Average DCS and (SD) measured on filter by Button samplers* ($\mu g/m^3$) (Y-axis)
3	32,247	345.01	585 (4.0)
7	27,640	333.77	920 (105)
8	9,872	121.25	220 (27)
10	32,905	477.99	586 (3)
11	17,111	237.97	351 (26)
12	11,846[c]	171.0[c]	[a,c]
13	38,828	365.18	469 (9)
14	16,108	119.25	162 (22)
15	14,865[b,c]	188.22[b,c]	241 (77)
16	30,946	186.39	360 (38)
18	10,811	97.78	165 (94)
19	7,514	119.32	186 (5)

* Adjusted for blanks.
[a] QA laboratory analyzed samples before they were post-weighed.
[b] Subtilisin only.
[c] Not included in analyses.

Table 2. Average Concentration of DCS Measured by the Button Filter and Average Subtilisin Concentrations Detected on the Button Filter for Enclosure Samples.

Sample Number	Average and (SD) of DCS Measured on the Filter by Button Samplers[*] (μg/m^3) (X-axis)	Average and (SD) Subtilisin Detected (μg)	Average and (SD) Subtilisin Detected[*] (μg/m^3) (Y-axis)
3	585 (4.0)	0.108 (0.008)	0.113 (0.008)
7	920 (105)	0.094 (0.027)	0.098 (0.028)
8	220 (27)	0.016 (0.009)	0.017[d] (0.01)
10	586 (3)	0.101[d] (0.021)	0.105 (0.022)
11	351 (26)	0.069 (0.001)	0.072 (0.001)
12	[a,c]	0.034 (0.013)	0.035[c] (0.013)
13	469 (9)	0.106 (0.002)	0.11 (0.002)
14	162 (22)	<0.014 (0)	0.0074[d] (0)
15	241[b,c] (77)	0.57 (0.031)	0.594[b,c] (0.032)
15 blank		0.002	
16	360 (38)	0.035 (0.007)	0.037 (0.008)
18	165 (94)	0.036[d] (0.05)	0.038[d] (0.052)
18 blank		0.024	
19	186 (5)	<0.014 (0)	0.0074[d] (0)

[*] Adjusted for blanks.
[a] QA laboratory analyzed samples before they were post-weighed.
[b] Subtilisin only.
[c] Not included in analyses.
[d] Samples were not blank adjusted due to an error of enzyme found on blanks.

Table 3. Average Concentration of DCS Measured by the Button Filter and Average Subtilisin Detected at the DCS Production Area.

Sample Number	Average and (SD) of DCS Measured on the Filter by Button Samplers[a] (μg/m^3)	Subtilisin Detected on Blanks (μg)	Average Subtilisin Detected[a] (μg/m^3)
1	346 (150)	(<LOQ)[b]	(<LOQ)
2	3,086 (181)	(<LOQ)[b]	(<LOQ)
4	1,873 (239)	(<LOQ)[b]	(<LOQ)
5	5,888 (187)	(<LOQ)[b]	(<LOQ)
6	330 (65)	(<LOQ)[b]	(<LOQ)[c]
6 blank		0.012	
9	1,240 (168)	(<LOQ)[b]	(<LOQ)
17	1,812 (1,306)	(<LOQ)[b]	(<LOQ)

[a] Adjusted for blanks.
[b] LOQ = 0.0142 μg.
[c] Samples were not blank adjusted due to an error of enzyme found on blanks.

event 18). It may be possible that results found on the blank sample was due to carryover of sample residue between analyses, which has been noted for analyses of samples for agents having high molecular weights, which is a characteristic of the subtilisin. All sample filters that collected DCS were blank adjusted by adding the mass (mg) lost or subtracting the mass gained (mg) from the blank to each sample filter (see Tables 1–3). Sample filters from sample events six, 15 and 18 were not blank adjusted due to subtilisin found on blanks. The average mass lost (15 blank samples) for a blank sample due to sample handling was 4.0 μg with a standard deviation of 0.6. The average mass gained (two blank samples) for a blank sample due to sample handling was 2.0 μg with a standard deviation of 2.0. Five samples at the DCS production area were found to have a positive concentration of subtilisin by the laboratory; however, the results were below the LOQ and were adjusted to be 50 percent of the LOQ (0.0074 μg/m^3) for statistical analysis. Samples not detected by the laboratory were adjusted to 0.0074 μg/m^3 (50% of the LOQ/m^3 (LOQ = 0.0142 μg, m^3 = 0.96)) and is used for statistical purposes only.

Button samples having analytical results below the LOQ occurred at the enclosure and the DCS production area; see Tables 2 and 3. Nine Button samples (30%) collected in the enclosure were below the LOQ. In addition, higher subtilisin concentrations were detected for Button samples collected in the enclosure when compared to the DCS production area, as seen in Tables 2 and 3. All Button samples (100%) collected at the DCS production area were below the LOQ as shown in Table 3.

Enclosure Statistical Analyses Results

The concentrations of DCS measured by the Grimm and the average concentrations measured by the Button samplers in the enclosure, listed in Table 1, were statistically evaluated using a linear regression and Pearson's correlation as shown in Figure 5. This shows an equation for the line, an R^2 value, and an r value. The X-axis is the concentration of DCS measured by the Grimm in the enclosure and the Y-axis is the average concentration of DCS measured on the sample filter by the Button Sampler.

The equation of the line listed in Figure 5 is $y = 1.468x + 47.43$, and has an R^2 value of 0.64 ($p = 0.006$). Therefore, the slope of the line is statistically different than zero and due to an R^2 value greater than 0.55, the variability of the average concentration of DCS mixture measured on the Button filter is moderately predicted by the concentration of DCS measured by the Grimm in the enclosure. The Pearson's correlation was also performed for the data listed in Table 1. The r value was 0.8 ($p = 0.006$), the power is 0.99, and the confidence interval is from 0.55 to 0.92 which indicates a strong positive correlation and is statistically significant.

The average concentrations of DCS measured on the sample filters and average concentrations of subtilisin
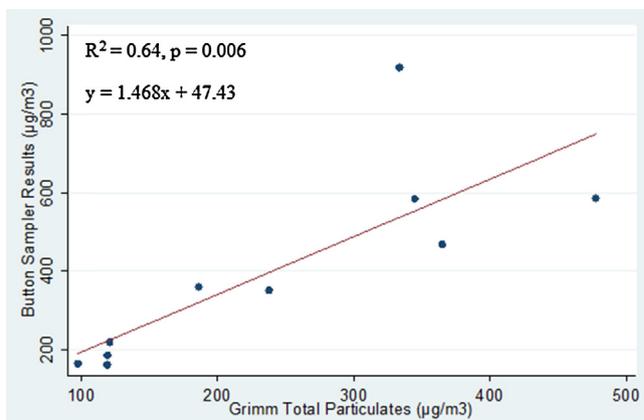
Figure 5. Concentration of DCS Measured in the Enclosure by the Grimm and Average Concentration Measured on the Button Filter ($n = 3$) for Each Sampling Event.
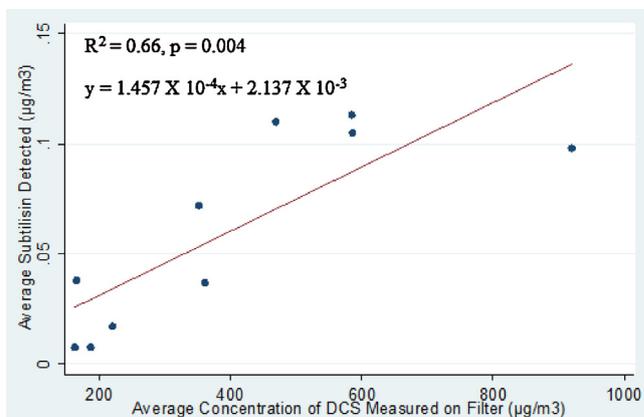

Figure 6. Average Concentration of DCS Measured on Button Filter ($n = 3$) for Each Sampling Event and Average Subtilisin Detected from Enclosure Samples.
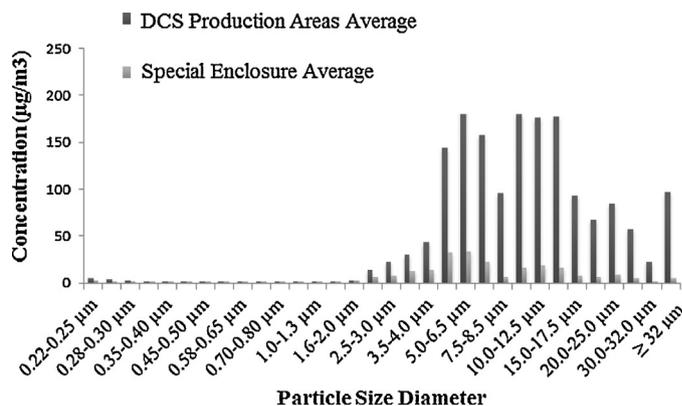

Figure 7. DCS Particle Size Distribution, Enclosure and DCS Production Area (Average).

detected for the enclosure, listed in Table 2, were statistically evaluated using a linear regression and Pearson's correlation as shown in Figure 6. It shows an equation for the line, an R^2 value, and an r value. The X-axis is the average concentration of DCS measured on the sample filter and the Y-axis is the average concentration of subtilisin detected.

The equation of the line listed in Figure 6 is $y = 1.457 \times 10^{-4}x + 2.137 \times 10^{-3}$ and has an R^2 value of 0.66 ($p = 0.004$). Therefore, the slope of the line is statistically different than zero and due to an R^2 value greater than 0.55, the variability of the average concentration of subtilisin detected in the enclosure is moderately predicted by the average concentration of DCS measured on the filter in the enclosure. The Pearson's correlation was also performed for the data listed in Table 2. The r value is 0.81 ($p = 0.004$), the power is 1.0, and the confidence interval is from 0.57 to 0.92 which indicates a strong positive correlation and is statistically significant. Therefore, the Button sampler may be useful in determining the air concentration of subtilisin for particle sizes less than or equal to 25 μm.

DCS Production Area Results

Due to all sample results at the DCS production area below the LOQ, the linear regression equations from the enclosure could not be used to predict the amount of subtilisin at the DCS production area. The DCS particle size distribution by different particle size ranges detected in the ambient air by the Grimm 1.109 direct read instrument for all sampling events in the enclosure and DCS production area are shown in Figure 7.

The enclosure and DCS production area had a similar distribution of particle sizes, however, for the largest particle size range of 32 μm and greater, a higher concentration of DCS was found in the DCS production area when compared to the enclosure, see Figure 6. The DCS production area displayed a concentration of 100 μg/m^3 for particle sizes greater than 32 μm and the enclosure displayed a concentration of 5 μg/m^3 for particle sizes greater than 32 μm. It should be noted that Figure 6 does not provide a direct comparison for the DCS particle sizes of 50 μm and greater at the DCS production area, but it does provide an indication that a greater concentration of larger particles were present at the DCS production area. The higher concentration of larger DCS particles at the DCS production area may have caused less subtilisin to be collected

by the Button samplers when compared to the enclosure and caused results to be below the LOQ.

DISCUSSION

The comparison of the average concentration of DCS measured by the Grimm and average DCS measured by the Button sampler in the enclosure, Figure 5, identified a moderate R^2 value (moderate = 0.55–0.77) for linear regression and a strong positive correlation (+0.6–1) for the Pearson's correlation, which was statistically significant ($R^2 = 0.64$, $r = 0.8$ and $p = 0.006$). The comparison of the average concentration of DCS measured and average subtilisin detected in the enclosure, Figure 6, identified a moderate R^2 value (moderate = 0.55–0.77) for linear regression and a strong positive correlation (+0.6–1) for the Pearson's correlation, which was statistically significant ($R^2 = 0.66$, $r = 0.81$ and $p = 0.004$). Statistical analysis results from the enclosure demonstrate the Button inhalable sampler with the Grimm may be useful in determining the aerosolized subtilisin concentrations for particle sizes less than or equal to 25 μm. However, the DCS subtilisin concentrations for samples collected in the DCS production area were not predicted by the linear regression equation from the enclosure due to all subtilisin results for this area being below the LOQ.

The amount of subtilisin detected at the DCS production area was below the LOQ because, unlike the subtilisin ground for use in the enclosure, the subtilisin in the DCS production process mixture was encapsulated and is known as an enzyme complex or agglomerate as indicated by the following statement: The enzyme complex totally encapsulates the subtilisin and retains the enzyme's activity as an engineering control and increases the size of the enzyme as a dust suppression method. "The enzyme complex is composed primarily of phosphates and is pre-coated with a liquid non-ionic surfactant. The non-ionic surfactant prevents the enzyme complex granule from fragmenting to form dust and acts as glue causing the enzyme to adhere

to a larger base granule."[5] Based on the information from the manufacturer's technical representative, the ranges of sizes of the encapsulated subtilisin particulates at the DCS production area are typically from 50 μm to greater than 100 μm. The inhalable Button sampler minimizes sampling of particles greater than 100 μm and particles larger than 100 μm are not likely to be inhaled.[21] In addition, due to four hours sampling times, an adequate concentration of DCS may not have been collected in order to detect subtilisin above the LOQ. Due to the four hours sample time, the enzyme complex or agglomerate decreasing the amount of aerosolized subtilisin, and the size selectivity of the inhalable sampler, all of the results from the DCS production area were below the LOQ and therefore it is not surprising that results were not statistically significant.

The statistical results from Table 2 at the enclosure show a positive linear regression R^2 value and Pearson's correlation because the DCS mixture introduced was limited to a particle size no greater than 25 μm, which was easily aerosolized, see Figure 7. The smaller particles of DCS allowed more subtilisin to be collected by the inhalable SKC Button sampler in the enclosure when compared to the DCS production area, which caused the results to be above the LOQ.

A strength for this study was a large sample size of 30 samples (21 samples or 70% above the LOQ) collected in the enclosure, which provided a power of 1.0 for the Pearson's correlation ($r = 0.81$). Another strength was that the method of analysis was sensitive enough (results were above the LOQ) to detect the aerosols on the filter for subtilisin particles less than or equal to 25 μm. Limitations of this study were the four hours sampling time and the high percentage of results below the LOQ (30% of samples in the enclosure and 100% in the DCS production area). Even though 2 percent subtilisin with detergent was introduced into the enclosure, 2 percent subtilisin was not detected when compared to the amount of DCS measured, see Table 2. The 2 percent subtilisin in the DCS was not detected at the enclosure due to the

possibility that the detergent and subtilisin was not perfectly blended. The slope of the linear regression line in Figure 6 displays a value of 1.457×10^{-4} which indicates less than 2 percent subtilisin was detected when compared to the DCS measured. In addition, the subtilisin enzymatic activity may have been reduced at the enclosure due to grinding the encapsulated subtilisin to ≤25 μm and potentially destroying the encapsulation which retains the subtilisin's activity. The particle settling velocity of the ≤25 μm subtilisin in the enclosure was 9.256 cm/s (using Stoke's law) and an air velocity of 1,822 fpm was needed to maintain particulate aerosolization in the enclosure. Another limitation was the low but detectable concentration of subtilisin detected on one blank filter by the product quality laboratory which was above the LOQ of 0.0142 μg (0.024 μg). The Button samplers were placed five inches apart and the face velocity at this distance is approximately reduced to less than 7.5 percent and could potentially have a small interference with the adjacent Button samplers.[25] Furthermore, two limitations of the Button sampler are the inlets can become clogged and particles can bounce off the face of the inlet.[26] However, an analysis was not conducted to investigate if this occurred during sampling of DCS.

Additional research is suggested in order to corroborate laboratory analysis results because one blank sample was reported from the laboratory to have subtilisin above the LOQ. In addition, further research is suggested in order to, verify the DCS was well blended with 2 percent subtilisin to detergent, increase the distance between Button samplers, and increase the four hours sampling time to six or eight hours to investigate if the Button sampler will collect sufficient subtilisin for the results to be above the LOQ.

CONCLUSION

Results at the DCS production area were insufficient to determine if the SKC Button sampler with the Grimm monitor can be useful in the workplace. This was because all subtilisin

concentrations at the DCS production area were below the LOQ. Subtilisin at the DCS production area was encapsulated and a large portion of the DCS was likely to be larger than the 100 μm inhalable fraction. Less aerosolized subtilisin and of a smaller particle diameter was collected when results were compared to monitoring in the enclosure. The four hours sampling time may have also impeded the amount of subtilisin collected at the DCS production area. These are likely the reasons that all results from the DCS production area were below the LOQ and a correlation could not be accurately determined. DCS introduced into the enclosure was ground to less than or equal to 25 μm which caused results to be above the LOQ. A strong positive Pearson's correlation was determined and a moderate R^2 was determined for linear regression for all results collected in the enclosure. The samples collected in the enclosure evidenced that the Button sampler may be useful in determining the aerosolized subtilisin concentrations for particle sizes less than or equal to 25 μm. While the Button sampler provided a correlation of exposure in the controlled environment, it is inconclusive that sampling with the SKC Button sampler will provide useful information in the workplace environment.

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