

Short term exposure to low amounts of airway irritants in a swine confinement building and inflammatory markers in blood and exhaled air

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Abstract

Introduction and objective. Swine confinement buildings are known to contain large concentrations of airway irritants, and a number of studies have shown acute inflammatory effects in previously unexposed subjects when introduced to the environment in such buildings. However, studies comparing different methods of assessing such reactions are lacking, and it is not known if a measurable response could be found at lower exposure rates. The purpose of this study was to investigate exposure levels in a Norwegian swine confinement building, the airway response to such exposure, and to compare invasive and non-invasive methods of response measurement.

Materials and method. Twelve medical students who were previously unexposed to swine dust stayed in a swine confinement building in Norway for 4 hours, and underwent measurements before and after the start of exposure. The same measurements were also performed beforehand, on the same weekday without exposure, in such a manner that the subjects were their own controls.

Results. The exposure assessment showed considerably lower concentrations of organic dust and endotoxin than earlier studies. However, small, but significant increases in markers of airway inflammation, were found.

Conclusions. Airway response can be measured after lower exposure to airborne irritants in swine confinement buildings than previously known. Further research is needed to assess whether this finding can be utilized for preventive purposes.

Key words

agriculture, swine, organic dust, endotoxin, exposure

INTRODUCTION

The air in swine confinement buildings can contain high concentrations of organic dust, endotoxin and toxic gases such as ammonia and hydrogen sulphide [1, 2, 3]. Biological reactions to such exposures have mostly been investigated by bronchial responsiveness, spirometry, bronchoalveolar lavage (BAL) and blood samples [4, 5, 6, 7]. The reactions shown comprise the recruitment of granulocytes and pulmonary intravascular monocytes/macrophages [8], the release of proinflammatory cytokines from the airway mucosa [9], and the contraction in airway smooth muscles [10]. It has also been shown that levels of leukotrienes in nasal lavage increased significantly in subjects exposed to swine dust [11].

Such indicators of inflammatory reactions have also been related to an increased occurrence of respiratory symptoms and to reduced lung function in people working in swine confinement buildings [12, 13, 14, 15].

There are other markers of inflammation that, to our knowledge, have not previously been tested in relation to

exposures in swine confinement buildings. Among them are exhaled nitric oxide concentration ($F_E\text{NO}$) as a marker of an acute airway inflammatory response [16], and the content of 8-isoprostane in exhaled breath condensate, which is suggested to be indicative of acute oxidative stress as a result of exposure to dust and endotoxin [17].

Our current knowledge on airway inflammatory responses as a result of exposure to swine barn air has mostly been derived from investigations conducted with relatively high exposures. Therefore, it is a challenge to determine the critical dose that can result in an inflammatory response.

Objectives. The purpose of this study was to look at exposure conditions in a modern swine confinement building in Norway and to investigate possible airway responses after short term exposure in such an environment.

MATERIALS AND METHOD

The assessment of the working environment in the swine barn, and the experiments that required presence at the barn were all performed on the premises of a private farm located in central Norway where the farmer, who mostly worked alone, had specialized in swine breeding and production. Our investigations were performed in a room of about 150 m²,

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where 12 sows with approximately 10 piglets each were kept. The breeding room had a mechanical and balanced ventilation system and doors were mostly kept closed. The experiments at the barn were conducted in late October, well into the Norwegian autumn.

Subjects and biological measurements. There were twelve non-smoking and healthy medical students, six female and six male, who were asked to participate in the study. None of them had previously ever stayed in a swine barn environment for any amount of time.

The participants were asked to fill in a form inquiring about their health, and some background factors. They were also asked about any respiratory symptoms consistent with chronic bronchitis, any inflammatory disease affecting joints, lungs or the digestive system, and about known allergies, other respiratory distress, former smoking and current use of medication. Those who answered "Yes" to a question phrased "Have you had a cold during the last 2 to 3 weeks?", were considered to have had a recent cold.

For the biological measurements the participants were divided into two groups (A and B). Measurements were taken on the same weekdays on two occasions during two consecutive weeks for both groups. On the first occasion, the subjects were designated to everyday activities as students (mainly going to lectures and individual studying), while on the same weekday one week later they spent four hours in a swine confinement building. On both days lung function with spirometry and expired NO were measured, and we also took samples for the analysis of exhaled breath condensate (EBC) and blood on three fixed points in time, namely in the morning before starting any particular activity (upon arrival at the university/swine barn), after four hours subsequent to normal activity or when leaving the swine barn, and then again 24 hours after the starting point. In this way, the subjects became their own controls.

F_ENO. Measurements of exhaled NO were performed using an EcoMedics CLD-88-SP analyzer with a DeNOx 88 accessory to provide NO-free inhaled air (AkuMed AS, Norway). Breathing manoeuvres followed standard recommendations [18]. The subject took two or three preliminary tidal breaths followed by a maximal inspiration from the DeNOx, then exhaled against a calibrated fixed resistance, where flow (50 ml/second) was controlled by observing an analogue readout. For each subject at each measuring point, the F_ENO level was assessed as the average of three measurements of the plateau of the exhaled nitric oxide curve.

Spirometry. Standard spirometry (Spirare sensor model SPS 310 based on tachopneumographic principles) was used, and data were registered by the Spirare 3 software (Diagnostica corp., Norway). Instructions were given to the subject according to The American Thoracic Society/European Respiratory Society recommendations [19]. Spirometry was performed with the subject in a sitting position and wearing a nose-clip. The measurements included forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), peak expiratory flow (PEF), forced expiratory flows at 25, 50, and 75% of the vital capacity (FEF25, FEF50, FEF75), and forced expiratory time (FET). The best curve out of three qualified performances was selected, and the best measurement was defined as the one with the greatest sum of FEV₁ and FVC.

Exhaled breath condensate (EBC). EBC was collected using a breath condenser (EuroScreen; Jaeger, Wuertzburg, Germany) according to existing recommendations [20]. In order to avoid loss of molecules from inflammatory markers to the surface of the sampling tubes, the tubes were coated with 1% bovine serum albumin and 0.01% Tween 20 for 30 minutes. Before the EBC collection, the subjects rinsed their mouths with water. EBC was collected having the subject in a sitting position, wearing a nose-clip, and breathing tidally for 15 minutes through a mouthpiece and a two-way non-rebreathing valve that also served as a saliva trap. The samples were immediately frozen at -80°C and kept so until analysed at the Department of Respiratory Medicine and Allergology at the University Hospital in Lund, Sweden. Owing to low concentrations in the EBC, samples were concentrated (5–10 times depending on the kind of biomarker) by freeze-drying and were resolved in the respective assay buffer. The final concentrations were calculated from the specific freeze-dried volumes. LTB₄ and 8-isoprostane were analysed using EIA kit from Cayman Chemical (Ann Arbor, MI) with a detection limit of 6 and 2.7 pg/ml respectively. IL-1beta was measured using Quantikine HS from R&D Systems (Minneapolis, MN) with a detection limit of 0.05 pg/ml. Activity of α -amylase was checked to exclude saliva contamination. The EnzChek Ultra Amylase Assay Kit (E33651, detection limit 0,2 U/l) from Molecular Probes (Eugene, Oregon) was used according to the manufacturer's protocol.

Blood analyses. IL-6 in serum was measured with the commercial enzyme linked immunoabsorbent assay (ELISA) kit Quantikine HS (R&D systems, UK). After standard incubations the optical density (OD) was measured at 492 nm (Titertek Multiscan Plus MKII, Elfab, Finland). The ODs for each standard and sample were determined by taking the mean of two readings and subtracting the OD of the average zero standard. The IL-6 concentration of each sample was calculated with regression analysis for log transformation on the OD results. All other blood samples were analysed at the Department of Clinical Chemistry at the St. Olavs University Hospital in Trondheim. The fibrinogen concentration in plasma was measured by a Fibri-Prest automate by the clotting method of Glauss [21]. Other blood analyses were done according to the standard procedures of the laboratory.

Exposure assessments. The assessment of exposure in the swine barn was done with both personal sampling equipment and with stationary instruments at a base in the barn.

Individual measurements of exposure. While staying in the swine barn, all twelve participants were equipped with personal sampling pumps (SKC model 224-PCTX8) that sampled air from their breathing zones through a membrane filter with pore size 0.8 μ m at a flow of 2 l/min. The membrane filter was mounted in an IOM filter cassette equipped with a plug of polyurethane foam. This system enables simultaneous sampling of both inhalable (50% cut-point at 10 μ m) and respirable (50% cut-point at 4 μ m) particles and meets international standards such as the ACGIH sampling criteria for inhalable particulate and the ISO/CEN health-related fraction of bioaerosols [22].

The amounts of dust in each sample were obtained by pre and post-weighing the membrane filters according to standard laboratory practice.



Stationary measurements of exposure. During the participants' stay in the barn, a base for stationary sampling and measurements was placed approximately 1.5 m above the floor level in an area where normal work procedures were performed at regular intervals. It was assumed that the pollution conditions at the stationary base were similar to the conditions in other parts of the barn since ceiling-mounted fans provided a continuous mixing of the air.

The base was equipped with the following instruments and sampling devices:

- 1 pump (SKC model 224-PCTX8, SKC Inc., USA) for the sampling of organic dust (total dust) on a fibreglass filter for analysis of endotoxins.
- 1 pump (SKC model 224-PCTX8) with a SKC aluminium cyclone for the collection of organic dust (respirable fraction) with a fibreglass filter for the analysis of endotoxins.
- 1 direct displaying device for the measurement of hydrogen sulphide (H_2S): Dräger Indicator Tubes for hydrogen sulfide, code 8101991 (Dräger Safety AG, Germany). Measurements were done every 30 minutes.
- 1 direct displaying, logging instrument for the measurement of ammonia (NH_3): ToxiRAE (PGM30), photoionization gas detector (RAE Systems Inc., USA) calibrated with 100 ppm isobutylene in air and electronically adjusted to give direct readings of ammonia concentrations. Logging period was set to 1 minute.
- 1 direct displaying instrument for the measurement of carbon dioxide (CO_2) levels: Model Telaire (General Electric Company, USA)
- 1 direct displaying instrument for the measurement of temperature and relative humidity: Vaisala Indicator HMI 31 (Vaisala OY, Finland)
- 1 direct displaying, logging instrument for the measurement of respirable dust concentrations: MIE personalDataRAM (Thermo Andersen Inc., USA) with a particle size-selective inlet cyclone. The air pump connected to this instrument was set to sample air at a rate of 2.5 l/min, and according to the calibration curve for the cyclone, this gave readings of respirable particles (50% cut-point at 4 μm). Logging time was set to 1 minute.

The measurement period was approximately 5 hours on each of the two days. During that time, the direct displaying instruments were read twice an hour. In addition, the measured ammonia level on the direct displaying instrument was controlled with colouring indicator tubes (Dräger Indicator Tubes for ammonia, code 6733231, Dräger Safety AG, Germany).

Samples to determine the concentration of endotoxins in the air were collected on two fibreglass filters each day. One of the samples collected total dust, the other respirable dust. These samples were sealed and shipped off with blind samples to Pegasus Lab AB, Uppsala, Sweden where endotoxin levels were determined. A standard LAL-test (Limulus amoebocyte lysate) (Lonza Sales Ltd., Basel, Switzerland) for the determination of endotoxins was used.

Statistics. Statistical analyses were performed using PASW Statistics version 17.0 for Windows (SPSS Inc., Chicago, USA). Since the subjects were their own controls, differences between the measured values with and without exposure were analysed in a paired manner by the use of Wilcoxon's signed rank test.

Ethical considerations. The study was approved by the Ethical Committee for Medical Research in Central Norway. All the subjects in the study were given written information about the project, and they all signed a written consent to participate. Their participation in the study was entirely voluntary, and everyone was informed that they could withdraw from the experiments and have all data on them deleted at any time without any need to explain why. The participants all received a symbolic allowance for their participation.

There were no known conflicts of interest for any of the authors.

RESULTS

The individual characteristics of the participants in regard to gender, age, height, weight and some health conditions, as well as their individual levels of exposure to inhalable dust, are provided in table 1. There were equal numbers of participants from each gender, and their mean age was 23.8 years. For one subject, the individual measures of dust exposure was not analysed due to pump failure. The arithmetic mean concentration of inhalable particles in the eleven samples analysed was 2.92 (SD 1.05, range 1.44–4.59) mg/m^3 . A respirable dust fraction was detectable in only three of the eleven samples, and only in very small amounts (0.08–0.73 mg/m^3).

Table 1. Individual characteristics of the twelve participants in the study

| Subject No. | Inhalable dust exposure (mg/m^3) | Gender* | Age (years) | Height (cm) | Weight (kg) | Recent cold | Known allergy |
|-------------|--------------------------------------|---------|-------------|-------------|-------------|-------------|---------------|
| 1 | -# | F | 24.6 | 171 | 59 | Yes | No |
| 2 | 4.09 | M | 23.4 | 179 | 80 | No | No |
| 3 | 4.05 | F | 20.4 | 172 | 58 | Yes | No |
| 4 | 2.38 | F | 22.3 | 162 | 50 | Yes | Yes |
| 5 | 4.59 | M | 28.6 | 189 | 75 | Yes | No |
| 6 | 3.47 | M | 25.5 | 182 | 74 | No | Yes |
| 7 | 2.41 | M | 22.8 | 192 | 91 | Yes | Yes |
| 8 | 2.51 | M | 24.8 | 189 | 80 | Yes | No |
| 9 | 1.44 | M | 22.3 | 183 | 80 | No | No |
| 10 | 1.88 | F | 24.5 | 182 | 81 | No | No |
| 11 | 3.39 | F | 22.1 | 173 | 64 | Yes | No |
| 12 | 1.89 | F | 24.5 | 167 | 70 | No | Yes |

* F=female, M=male. # n.a. due to pump failure.

The four participants who reported to have allergies were allergic to pollen (grass or birch). None of the subjects were, however, affected in any way by these allergies during our experiments conducted in the autumn. Apart from the common cold, none of the participants answered "yes" to the questions regarding chronic inflammatory disease, symptoms of chronic bronchitis, or respiratory distress. In regard to current medications, one of the participants used a non-steroid anti-inflammatory drug (piroxicam) at the time of the experiments owing to a strain injury.

Stationary measures of exposure to some specific factors taken during the course of the day spent in the barn for both groups are presented in table 2. Concentrations of both dust

Table 2. Stationary measures of exposure to environmental factors during stays in the barn for group A and B

| Exposure factors | Exposure day for group A | Exposure day for group B |
|---|--------------------------|--------------------------|
| Mean concentration of respirable dust fraction by use of direct reading instrument (mg/m ³) | 0.102 | 0.088 |
| Peak concentration of respirable dust fraction by use of direct reading instrument (mg/m ³) | 0.258 | 0.238 |
| Hydrogen sulphide (ppm) | 0.2 | 0.2 |
| Endotoxin concentration in respirable fraction (ng/m ³) | 20 | 9 |
| Endotoxin concentration in total dust sample (ng/m ³) | 16 | 12 |
| Mean ammonia concentration (ppm) | 2.7 | 3.1 |

and endotoxin were slightly higher for group A (participants 1–6) than for group B (participants 7–12). The individual measurements of inhalable dust were also higher for group A, having a mean value of 3.7 mg/m³ versus 2.3 mg/m³ for group B, while ammonia concentration was a little higher for group B. The temperature inside the building was stable at between 15 and 16 degrees Celsius on both days, and the relative humidity was also stable at 60 per cent. Carbon dioxide concentrations varied between 700–750 ppm during the stay inside the building for both groups. All in all, we judged that the exposure levels on the two days of exposure were within acceptable variation in order to allow us to analyse the participants' results combined.

In table 3 median values with the interquartile range from the individual outcome measurements of EBC, inflammatory markers in blood, exhaled NO, and spirometry on days with and without exposure of the study group are presented. For some of the parameters there were considerable differences in the measurements at zero hours, reflecting a day to day variation. Therefore, we analysed changes between days with and without exposure rather than absolute values (tab. 4). The levels of α -amylase were low in all samples (data not shown), confirming that the levels of leukotrienes and 8-isoprostane were not due to saliva contamination [23]

Changes in the concentration of inflammatory markers between the measurements at the different time points

(4h–0h and 24h–0h) on days with and without exposure are presented in table 4. Many of the changes developed a statistically significant difference on the day with exposure compared to the day without exposure.

Showing the course of the development of LTB₄ in EBC and IL-6 in plasma on the two days with and without exposure in the swine barn, figure 1 visualizes the data from table 3 and 4. The different developments on the two days are highly

Table 4. Median (interquartile range) of the differences between measurements from exhaled breath condensate (EBC), blood, NO in exhaled air (F_ENO), and spirometry at 4 and 24 hours and baseline values, on days with and without exposure

| | Median (IQR) | Day without exposure (baseline value) | | Day with exposure (baseline value) | |
|-------------|--------------------------|---------------------------------------|---------------|------------------------------------|-------------------------|
| | | 4h-0h | 24h-0h | 4h-0h | 24h-0h |
| EBC | LTB ₄ (pg/ml) | -2.36 (3.76) | -2.12 (8.62) | 1.10(2.69) p=0.05* | 3.10(4.75) p=0.06 |
| | 8-isoprostane (pg/ml) | -6.18 (6.55) | -4.42 (8.63) | 0.35(3.91) p=0.005 | 0.87(5.33) (p=0.041) |
| | IL1 | -1.0 (3.95) | -1.0 (5.25) | -0.1 (1.45) | 0.9 (2.25) |
| Blood | Fibrinogen (g/l) | 0.0(0.30) | 0.0(0.38) | 0.0(0.20) | 0.30(0.25) p=0.058 |
| | IL-6 (pg/ml) | 0.0004 (0.18) | -0.013 (0.10) | 0.47 (0.85) p=0.004 | 0.39 (0.50) p=0.002 |
| | D-dimer (mg/l) | 0 (0.1) | 0 (0.1) | 0.1 (0.2) | 0.1 (0.2) p=0.059 |
| | CRP (mg/l) | | 0 (0.51) | | 0.74 (2.31) |
| Exhaled air | F _E NO (ppb) | -1.40 (1.90) | -1.75 (3.75) | -2.45 (1.73) (p=0.045) | 0.45 (5.95) |
| | FVC (l) | -0.03 (0.14) | -0.01 (0.19) | -0.07 (0.13) | -0.07 (0.15) p=0.034 |
| Spirometry | FEV ₁ (l) | -0.06 (0.14) | -0.05 (0.23) | -0.04 (0.16) | -0.07 (0.09) |
| | FEV ₁ /FVC | -0.009 (0.02) | -0.008 (0.02) | 0.0007 (0.02) p=0.041 | -0.003 (0.02) |
| | PEF (l/min) | -4.5 (36.0) | 0.5 (54.75) | -8.0 (39.5) | -12.0 (48.5) |

* Comparison between development on day with exposure and day without exposure by paired analyses with the Wilcoxon's signed rank test.

Table 3. Median (interquartile range) values from measurements from exhaled breath condensate (EBC), blood, NO in exhaled air (F_ENO), and spirometry in study group at the start (0h), after 4 hours (4h), and 24 hours (24h) on days with and without exposure

| Medium | Variables | Day without exposure | | | Day with exposure | | |
|-------------|------------------------------|----------------------|--------------------|---------------------|--------------------|--------------------|---------------------|
| | | 0h Median (IQR) | 4h Median (IQR) | 24h Median (IQR) | 0h Median (IQR) | 4h Median (IQR) | 24h Median (IQR) |
| EBC | LTB ₄ (pg/ml) | 8.57 (3.49) | 5.85 (3.63) | 5.56 (5.59) | 3.13 (4.27) | 4.37 (3.62) | 5.45(5.58) |
| | 8-isoprostane (pg/ml) | 14.32 (4.69) | 7.08(4.91) | 7.19(5.11) | 3.39(4.56) | 3.83(2.65) | 4.99(2.22) |
| | IL-1 | 2.2(4.6) | 1.5(2.4) | 1.9(1.1) | 1.2(1.5) | 0.9(0.6) | 1.2(1.3) |
| Blood | Fibrinogen (g/l) | 2.6 (1.0) | 2.45 (1.2) | 2.4 (1.3) | 2.7 (1.1) | 2.7 (1.2) | 2.95 (1.0) |
| | IL-6 (pg/ml) | 0.12(0.20) | 0.13(0.21) | 0.12(0.20) | 0.11(0.08) | 0.71(0.86) | 0.47(0.63) |
| | D-dimer (mg/l) | 0.4 (0.1) | 0.4 (0.2) | 0.4 (0.1) | 0.4 (0.3) | 0.5 (0.3) | 0.5 (0.2) |
| | High sensitivity CRP (mg/l)* | 0.48(1.39) | | 0.57(0.68) | 0.71(2.17) | | 2.49(2.80) |
| Exhaled air | F _E NO (ppb) | 12.05(9.95) | 10.50(9.95) | 9.45(8.65) | 12.35(7.25) | 10.45(5.95) | 15.30(9.20) |
| | FVC (l) | 5.0(1.69) | 5.08(1.78) | 5.14(1.65) | 5.07(1.73) | 5.03(1.69) | 5.00(1.45) |
| Spirometry | FEV ₁ (l) | 4.03(0.98) | 4.03(1.10) | 4.01(1.14) | 4.03 (0.94) | 4.05 (1.02) | 3.98 (0.89) |
| | FEV ₁ /FVC | 0.84 (0.1) | 0.83 (0.07) | 0.82 (0.12) | 0.86 (0.08) | 0.85 (0.10) | 0.84 (0.08) |
| | PEF (l/min) | 579 (183) | 579 (183) | 562 (145) | 557(189) | 560 (219) | 576 (169) |

* only 2 measurements



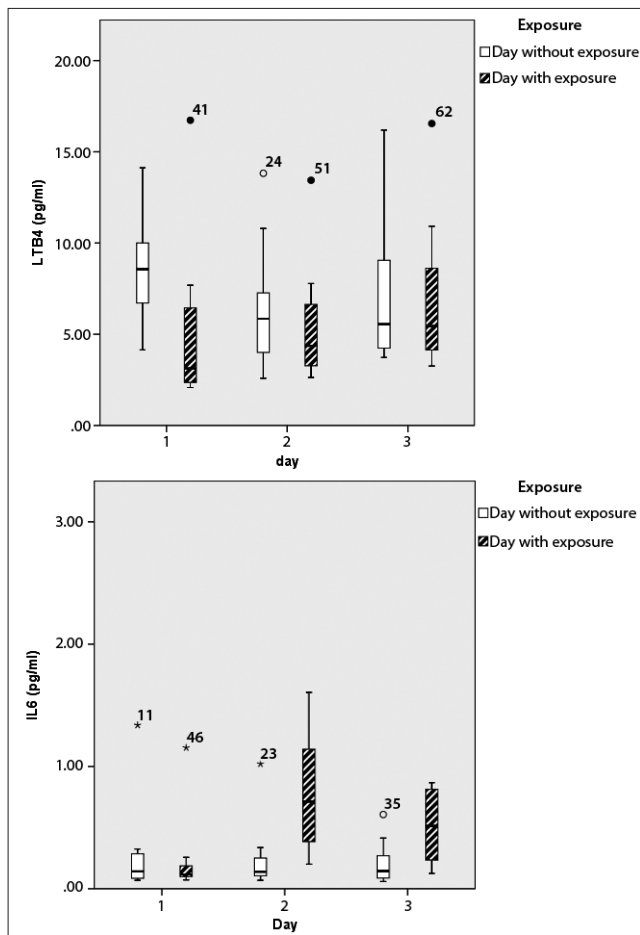


Figure 1. Course of development of LTB4 in EBC and IL-6 in plasma on the 3 measurements time points: 1) in the morning before entering the barn, 2) 4 hours after entering the barn, 3) 24 hours after entering the barn) for all participants on days with and without exposure to swine barn air

statistically significant for IL-6 and of borderline significance for LTB4.

DISCUSSION

In this study, voluntary healthy subjects who were previously unexposed to the environment in swine confinement buildings, showed inflammatory responses following a four-hour stay inside a swine barn where it was shown that there were relatively low levels of airborne dust and other hazardous substances.

Exposure levels in our study, in particular to endotoxin and organic dust, were considerably lower than in earlier studies from swine barns, where the mean concentrations of total dust have been reported to be 13.5–17.0 mg/m³ and the endotoxin concentrations were 28–600 µg/m³ [5, 6]. The good ventilation system and the thorough cleaning procedures in the building that we found at the farm which was studied are considered to be important factors in reducing the concentration of airway irritants in swine confinement buildings.

While the levels of inhalable dust in personal samples varied from 1.9 to 4.6 mg/m³, the small amounts of respirable dust detected could be an indication that the dust particles in such buildings are in general relatively large. Earlier works [24] concluded that the predominant particle size fraction

of organic dust in swine confinement buildings is in the size range 9.6–26 µm. The results of the present study seems to be in line with that, both according to the individual measures and to the low values of respirable dust measured by the direct displaying, logging instrument. The relatively large spread in the amount of dust between the individual measures might be explained by the fact that some of the subjects during the experiment were engaged in relatively dusty tasks, while others did not.

Swine confinement buildings have earlier been shown to have larger concentrations of both hydrogen sulphide and ammonia than was the case in our study [1, 2, 3]. Hydrogen sulphide is mainly formed in the manure storage which in Norway usually is located below the floor of the confinement room. The low and constant values measured in our study can be considered to be due to sufficient ventilation of the manure storage with a negative pressure compared to the barn room. During both days of experiments, the temperature inside the building was set to 15°C, and the ventilation was run automatically with this as a basis. As the outside temperature varied slightly between the test days, this might explain the small observed difference in ammonia concentration between the two days. A corresponding increase in carbon dioxide concentration was, however, not observed even though this should also be expected to be ventilation dependent. Another study [25] reported considerable seasonal differences with concentrations of carbon dioxide of 600 ppm in summer and 2,500 ppm in winter, indicative of reduced/insufficient ventilation during winter time. All in all we find that our measurements of exposure factors showed acceptable variation between the two test days, that they were probably representative, and that the exposure levels were generally lower than in previous similar studies [4, 5, 6, 7].

Even with the relatively low exposure levels, the experiment resulted in some differences between the measurements of outcome variables performed on days with and without exposure. In this regard we find it remarkable that both inflammatory markers in EBC and blood showed statistically significant different developments in the hypothesized direction on days with and without exposures. We do not, however, know with sufficient certainty yet what pathophysiological significance such changes have, since we do not know the exact meaning of the observed reactions. In regard to lung function variables there were also some statistically significant differences, but as the changes are small, and well within the normal test variability, their clinical significance can be regarded as dubious.

The investigation of exhaled nitric oxide in the participants showed a statistically significant decrease at 4 hours after the start of exposure as compared to the control day. This finding is contrary to an earlier study in which F_ENO levels increased after exposure to swine dust [16]. In the study mentioned, exposure levels were, however, considerably higher than in our case, suggesting that a higher level of exposure may be needed to cause a significant increase in F_ENO levels.

In accordance with an earlier study [7], the results showed an increase in both IL-6 and fibrinogen after exposure when compared to the day without exposure. It was, however, only the increase in IL-6 concentrations that was statistically significantly higher. In our measurements, it is remarkable how IL-6 in blood shows an increase during the first four hours on the exposed days and that the concentration of fibrinogen follows with an increase from 4 to 24 hours. A

similar pattern has also been shown in a previous study of tunnel construction workers [26] and might reflect that the rise in IL-6 is due to a direct reaction in the airway mucosa, while the increase in fibrinogen is a reaction in the liver, secondary to the increase in acute inflammatory mediators, in particular IL-6. Furthermore, our measurements showed an almost statistically significant increase in d-dimer concentration at 24 hours after exposure when compared to the control day. This result could be interpreted as an activation of a coagulation process over the 24 hours after the start of exposure, triggered by an inflammatory response to the environment in the building, which would be in line with the findings in another study investigating immunological responses after exposure to ultrafine particles [27].

An earlier study has shown an increase in levels of leukotriene B4 (LTB4) in nasal lavage as a result of exposure to swine dust [11]. In our study, we found an increase in LTB4 levels in exhaled breath condensate when comparing to the control levels. The difference was statistically significant at 4 hours after the start of exposure, and almost significant at 24 hours, indicating an inflammatory response. Such a finding has, to our knowledge, not been published before. The increase in 8-isoprostane levels at 24 hours was also significantly different from the levels at the control measurements, which might confirm the hypothesis that swine dust exposure triggers an acute oxidative stress response [17]. A study conducted among COPD patients showed a considerable day-to-day variability in EBC levels of LTB4 and 8-isoprostane [28], which might explain some of these differences as individual variation.

In this study we made use of both EBC and exhaled nitric oxide which are two relatively simple and, most of all, non-invasive methods for measuring inflammatory reactions in the airways. With the development of new devices for such measurements, we think this could be developed into acceptable means for the early detection of inflammatory reactions in exposed workers, and thus for preventive purposes.

CONCLUSIONS

Our findings indicate that there are changes in inflammatory markers in both EBC and blood after short term exposures in swine confinement buildings, even when the exposure levels are relatively low. There is a need to elucidate further what is the biological meaning of such changes. It should, however, also be considered whether such inflammatory reactions, measured by relatively simple and non-invasive means, can be used to monitor early airway reactions for preventive purposes.

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