Effects of Carbon Dioxide With and Without Bioeffluents on humans

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ABSTRACT
Carbon dioxide (CO2) has traditionally been assumed innocuous at the typical levels indoors, and merely an indicator of metabolic emissions from humans (bioeffluents). Recent studies suggest that exposure to pure CO2 at concentrations of 2,500 to 4,000 ppm, the levels that occur periodically indoors, can have negative effects on mental performance in form of reduced ability for making decisions, typing and proofreading. Present study aimed to examine further these effects. Twenty-five human subjects were exposed to elevated CO2 with and without bioeffluents in a chamber. The exposure levels were as follows: background exposure with CO2 at 500 ppm, exposure to pure CO2 (without bioeffluents) at 1,000 and 3,000 ppm, exposure to metabolically generated CO2 (with bioeffluents) at 1,000 and 3,000 ppm. Each exposure lasted 4.25 hours and their order was balanced. Subjects rated perceived air quality and acute health symptoms. They performed several cognitive tasks. Their physiological responses were monitored. No effects were seen during exposures to elevated levels of pure CO2 (without bioeffluents). Exposures to elevated levels of bioeffluents (with CO2) reduced significantly perceived air quality assessed upon entering the chamber, increased significantly intensity of neurobehavioral symptoms, and reduced mental performance of addition in terms of lowering the speed and increasing error rates the latter two effects particularly so when metabolic CO2 was at 3,000 ppm. Physiological measurements and the performance of Tsai-Partington test suggested that exposures to bioeffluents increased arousal/stress and this result together with the stronger intensity of neurologic symptoms and complaints could be the underlying mechanisms of reduced performance. Present results confirm the expected negative effects of exposure to elevated levels of bioeffluents thereby providing basis for ventilation requirements. The effects of exposures to pure CO2 up to 3,000 ppm seem to be imperceptible and undetectable by the used cognitive tests, but the role of CO2 in the mixture with bioeffluents shall not be underestimated and further examined.

KEYWORDS
Carbon dioxide; Human bioeffluents; Perceived air quality; Acute health symptoms; Cognitive performance; Physiological responses

1 INTRODUCTION
Since the 19th Century, the carbon dioxide (CO2) concentrations indoors have been used as an indicator of air quality in buildings (Pettenkofer, 1858). CO2 is a product of metabolic processes in human body and exhaled in abundance allowing simple measurements and tracking, but only in the presence of humans. In numerous studies, CO2 has therefore been measured and associated significantly with subjectively assessed acute health symptoms such as headaches, fatigue, eye, nose and respiratory tract symptoms. In these studies, CO2 is merely considered as an indicator of exposures to other pollutants and insufficient ventilation with outdoor air and not as a causative agent causing the observed effects (Seppänen et al., 1999). The concentration of CO2 at which these effects were observed in the buildings were <5,000 ppm and usually <3,000 ppm.
Recent studies suggest that exposure to pure CO\textsubscript{2} (without bioeffluents) at the levels relevant for indoor non-industrial environments can cause significant negative effects on mental performance. One study by Kajtár and Herczeg (2012) showed that performance of proofreading test was negatively affected when taken by ten subjects during exposures to CO\textsubscript{2} without bioeffluents at 3,000 ppm for 2 to 3 hours. In parallel to the effects on performance, Kajtár and Herczeg (2012) observed that diastolic blood pressure increased and mid-frequency components of heart rate variability decreased. The effects were attributed to increased mental effort during these exposures. Another study by Satish et al. (2012) showed systematic reduction in the decision-making performance with increasing pure CO\textsubscript{2} (without bioeffluents) from 600 to 1,000 and 2,500 ppm during a 2.5-hour exposure; this effect reached statistical significance at 2,500 ppm. No physiological measurements were however made in this study to examine the potential underlying mechanism of the observed effects. That these low CO\textsubscript{2} levels could be of importance have been additionally showed by Maddalena et al. (2014). They exposed subjects for 4 hours this time to 1,800 ppm metabolically generated CO\textsubscript{2} (with bioeffluents) and found that the decision-making performance was also significantly reduced compared with 900 ppm. The magnitude of effects was however much lower than in the study of Satish et al. (2012). Maddalena et al. (2014) did not measure physiological responses either. They asked subjects to rate their acute health symptoms and perceptions of environment; no differences were however observed in these ratings between different exposures examined.

The results of Kajtár and Herczeg (2012) and Satish et al. (2012) suggest that CO\textsubscript{2} at the levels measured normally indoors may have negative effects on human responses. They suggest thus that CO\textsubscript{2} should no longer be considered as only a surrogate for indoor air quality but also as a pollutant. Consequently, present study was performed to examine further the validity of this postulate.

2 METHODS

The experiment was carried out in a 3.6 \times 2.5 \times 2.5 m stainless steel chamber (30 m\textsuperscript{3} volume with recirculation ducts) (Figure 1), which was described in detail by Albrechtsen (1988). The construction minimizes the emissions and sorption of pollutants and ensures that the chamber volume is tightly sealed. The chamber has its own system for supplying and conditioning outdoor air. There were six workstations in the chamber for the subjects and the experimenter, each consisting of a table, a chair, a laptop PC and a desk lamp.

Twenty-five subjects were recruited through advertisements placed on the university campus. They were all students. Ten males and fifteen females were included with a mean age of 23±2 (mean ± SD) years, mean height of 173±11 cm and mean weight of 74.9±21.8 kg. During experiments, the subjects wore the same type of self-selected garment (with mean thermal insulation estimated to 0.75 clo) in order to remain thermally neutral during each exposure.

In three of the five exposures examined in the present experiments, the outdoor air supply rate was high enough to remove bioeffluents, creating a reference condition with CO\textsubscript{2} at 500 ppm (referred to as B500), while chemically pure CO\textsubscript{2} was added to the supply air to create exposure conditions of 1,000 ppm or 3,000 ppm (referred to as P1000 and P3000). In two other conditions, the outdoor air supply rate was restricted (reduced) to allow the metabolically produced CO\textsubscript{2} to reach 1,000 ppm or 3,000 ppm (referred to as M1000 and M3000), thereby ensuring that other bioeffluents reached concentrations corresponding to those in the occupied rooms with CO\textsubscript{2} at these levels. Temperature and noise level were kept constant during the exposures, however, due to the lack of a dehumidifier, the relative humidity (RH) increased by a few percentage at M3000.
Figure 1 Schematic figure of the chamber, where the experiments were carried out (left) and a view of the inside of the chamber (right): ① supply fan, ② two stage filter G3/F7, ③ heating coil, ④ cooling coil, ⑤ dampers, ⑥ filter box for charcoal filters (empty), ⑦ filter box (empty), ⑧ recirculating fan, ⑨ electric heating coil, ⑩ exhaust fan, ⑪ HOBO logger (temperature & relative humidity sensor) with CO₂ sensor, ⑫ desk lamp, ⑬ laptop, ⑭ temperature and humidity sensor of the chamber control system, ⑮ multi-gas analyser, ⑯ flowmeters, ⑰ pressure regulator, ⑱ CO₂ gas cylinders (30L), ⑲ sampling point

Figure 2 Experimental procedure

Twenty-five subjects were exposed in the climate chamber for 255 minutes in groups of five persons at a time in a Latin-square design; each group participated in the experiment in one week from Monday to Friday. Mental performance was examined by the test battery (TB) including multiple tasks somewhat resembling office work (proofreading test, addition, subtraction and text typing), as well as by neurobehavioral tests (redirection, digit span memory, grammatical reasoning, Stroop and Stroop with feedback), Tsai-Partington test and d2 attention test. Subjective ratings of comfort and acute health symptoms were rated by the subjects during exposures, as well as physiological responses (PM) were monitored and saliva samples were
collected to analyse levels of stress biomarkers: $\alpha$-amylase and cortisol. Figure 2 shows in details the experimental procedure during each exposure.

The effects of exposures on different outcomes were analysed using a mixed ANOVA model; the significance level was set to 0.1 for random effects and to 0.05 for fixed effects. Experimental conditions (C), time at which different assessments were made during the day (T), condition x time interaction (C x T), order of exposure of conditions (O) and gender (G) were included as fixed factors. Subjects (S), groups (Gr), subject x condition interaction (S x C) and subject x time interaction (S x T) were included as random factors in the model. In addition to mixed ANOVA model, Page test for trend was used for these outcomes that changed monotonically with CO$_2$ levels with significance level set to 0.05 (1-tail).

3 RESULTS AND DISCUSSION

Figure 3 (left) shows that the acceptability of the air quality upon entering the chamber polluted by human bioeffluents prior to and after exposure was assessed to be lower than in the other three exposure conditions; ratings at M3000 were statistically significantly different from the other assessments except these at M1000. There were no significant differences in the acceptability of the air quality between background exposure (B500) and exposures to artificially raised CO$_2$ concentrations (P1000 and P3000). Assessments of odour intensity confirmed that air quality was worse only during exposures with elevated levels of bioeffluents (M1000 and M3000). Using assessments of acceptability of air quality the % dissatisfied with air quality was estimated and compared with the relationship developed by Fanger (1988); the results from the present study matched well the previously established relationship except for the low levels of CO$_2$ (Figure 3, right).

Figure 4 shows that difficulty in thinking clearly, headache, fatigue and sleepiness were significantly higher at M3000, while there was a very small difference in the intensity of these symptoms between other exposure conditions. Only difficulty in thinking clearly was marginally worse in M1000 than in the other exposure conditions but the difference did not reach formal statistical significance.
Among the many tests examining mental performance, there were only few, for which the performance differed significantly between conditions (Table 1). Speed at which subjects added units was lower (P=0.023) for different conditions compared with B500, the difference reaching statistical significance at M3000. Analysis of variance showed that % of errors made by the subjects varied also between different conditions (P=0.049). It was however not possible to determine, at which conditions the difference was statistically significant through the post-hoc tests. Raw data showed the highest % of errors at B500 and M3000. Analysis of variance showed also that the difference in performance of proof reading performed in various exposure conditions approached statistical significance (P=0.062): Speed, at which subjects proof-read the text was varying across different conditions but was the lowest at M3000 while highest at P3000; there were no effects on errors or false positives. Page test showed that speed increased systematically between B500, P1000 and P3000 (P<0.05); systematic effect was also seen in reduction of speed between B500, M1000 and M3000 but this trend did not reach formal statistical significance.

The results of Tsai-Partington test showed that the number of correct links made by the subjects was lower for different conditions compared with B500, the difference reaching statistical significance at M1000 (P<0.001) (Figure 5). The performance of Tsai-Partington test depends on the level of stress and is improved (more links are made) at lower stress and large attention
field (Eysenck and Willett, 1962). Thus this result suggests that the arousal of subjects was higher at elevated CO₂ levels, particularly so when other bioeffluents were present. This is to some extent confirmed by the results of redirection test, subjects responding significantly quicker at M3000 compared with P1000 (P=0.015) as would be expected at higher arousal (Duffy, 1957).

Table 1 Performance of mental tasks that differed between conditions at P<0.1 (LS Mean± SE)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Condition</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>B500</td>
<td>P1000</td>
</tr>
<tr>
<td>Units completed per minute in addition</td>
<td>3.6±0.2</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>% Errors made in addition</td>
<td>9.4±1.3</td>
<td>7.1±1.3</td>
</tr>
<tr>
<td>Number of correct links in Tsai Partington test</td>
<td>14.6±0.6</td>
<td>14.2±0.6</td>
</tr>
<tr>
<td>Lines proof-read per minute in proof-reading test</td>
<td>10.5±0.5</td>
<td>11.0±0.5</td>
</tr>
<tr>
<td>Response time in redirection test (s)</td>
<td>165.4±6.3</td>
<td>177.0±6.3</td>
</tr>
</tbody>
</table>

Figure 5 Average performance of Tsai-Partington at different exposure conditions; ** shows the differences that reached statistical significance; bars show standard error

Heart rate was higher at the beginning of each exposure when compared with the subsequent sessions. In particular, heart rate was significantly higher at M3000 and P3000 compared with B500 at 128 min and at M3000 vs. B500 at 147 min (Figure 6).

Figure 6 Change of heart rate along the course of exposure; the figure shows average heart rate for four typing sessions; ** indicates where the differences between some conditions reached statistical significance (see text for details); bars show standard error
Diastolic blood pressure increased after the exposure compared with the pre-exposure level in all conditions; the increase after exposure to CO₂ with bioeffluents at 3,000 ppm (M3000) was statistically significant. Moreover, the magnitude of increase in diastolic blood pressure at M3000 was significantly higher than that in the other four exposures (Figure 7). Higher diastolic blood pressure can be caused by vasoconstriction as a reaction of the sympathetic nervous system to higher stress/arousal. This result is consistent with the findings of Kajtár and Herczeg (2012), who observed that diastolic blood pressure increased after exposure to CO₂ without bioeffluents at 5,000 ppm compared with 600 ppm. In addition, increased heart rate could also be a result of activation of sympathetic nerve and manifestation of higher physiological stress.

The results of analysis of biomarkers in saliva seem to confirm higher arousal/stress. Although salivary α-amylase increased significantly, while cortisol levels decreased significantly after 4.25 h exposure, independently of conditions, which is likely due to diurnal changes in these two biomarkers, the exposure to CO₂ with bioeffluents at 1,000 ppm and 3,000 ppm (M1000 and M3000) increased alpha-amylase more than would be expected due to diurnal rhythm as in other exposures (Figure 8).

**CONCLUSIONS**
Exposure to CO\textsubscript{2} without bioeffluents at or below 3,000 ppm did not produce any adverse subjective ratings concerning air quality and acute health symptoms, neither decreased the performance of mental tasks during 4.25-hour exposures.

Exposure to metabolically generated CO\textsubscript{2} with bioeffluents at 3,000 ppm reduced perceived air quality, increased the intensity of neurobehavioral acute health symptoms, and significantly affected performance of some mental tasks.

Increased arousal level and neurologic symptoms during exposures to bioeffluents with CO\textsubscript{2} at 3,000 ppm may be the reason for the observed effects on mental performance.

5 ACKNOWLEDGEMENTS

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