A PILOT STUDY ON FORMALDEHYDE EXPOSURE RISKS OF BAKERY WORKERS

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Abstract
This study is intended to explore the impacts of formaldehyde (FA) on human health, the use and sources of FA exposure, and the regulations governing acceptable levels of FA in various products across various regions globally. The objective of this pilot study is to evaluate the risk of human FA exposure in traditional bakery units. The study group consisted of 46 subjects working in various areas: the production area (leavening, baking area, storage area), the area where bakery products are sold to customers, and the delivery area to stores. The control group consists of 8 non-exposed, non-smokers subjects. Demographic, medical and anthropometric data were collected based on a questionnaire and the urinary levels of FA were evaluated as a biomarker of exposure. No statistically significant differences regarding the anthropometric and medical parameters of exposed workers and control group were obtained. The highest levels of FA were recorded in smokers working in the fermentation area. The results indicate statistically significant differences of FA levels between the workers in the processing areas and the control group. Likewise, the concentration of FA in the urine of the workers in the processing areas is significantly different compared to the workers in the administrative area. Overall, the measurements have substantiated the occurrence of FA emissions within bakeries, particularly at specific stages of the operational procedures, such as the leavening process.

Resumat
Această cercetare explorează impactul formaldehidei (FA) asupra sănătății umane, utilizarea și sursele de expunere la FA, dar și reglementările care guvernează nivelurile acceptate de FA în diferite produse din diferite regiuni la nivel global. Obiectivul acestui studiu pilot este de a evalua riscul expunerii umane la FA în sectoarele de producție ale brutărilor tradiționale. Grupul de studiu a fost format din 46 de subiecți care au lucrat în diverse domenii: zona de producție (leavening, baking area, storage area), zona în care produsele de pană sunt vândute clienților și zona de livrare către magazine. Grupul de control este format din 8 subiecți neexpuși. Datele demografice, medicale și antropométrice au fost colectate bazându-se pe un cuestionar și concentrația de FA în urina lucrătorilor din diferite sectoare a fost evaluată. Nu s-au obținut diferențe semnificative statistic în ceea ce privește parametrul formaldehidă din urina lucrătorilor expuși și grupul de control. Cele mai ridicate niveluri de FA au fost înregistrate la afumătorii care lucrează în zona de dospire. Rezultatele indică diferențe semnificative statistic ale nivelurilor de FA închisul din zona de producție și grupul de control. De asemenea, concentrația de FA în urina lucrătorilor din zonele de prelucrare și grupul de control. Acest acest studiu pilot este un studiu de baza pentru a înțelege efectele potențiale ale expunerii la FA în brutăriile tradiționale, în special în etapele specifice ale procedurilor operaționale, cum ar fi procesul de dospire.

Keywords: formaldehyde, exposure, smokers, bakery workers, bakeries, health risks.

Introduction
Formaldehyde (FA) is a crucial molecule for the world economy, utilized in building, textiles, paper, plastics and chemical industries [17]. Recent data showed global FA output at 51.8 million metric tons, with China producing 40% of FA worldwide [17]. United States of America, Germany, India, Japan and Brazil are also other large-scale producers [39]. Environmental sources of FA are forest fires and volcanic activity [39]. In multiple uses, FA is used directly in an aqueous solution (formalin) as a disinfectant and preservative. As a natural byproduct of metabolism, FA can be found in many different foods that are naturally...
occurring, such as fruits, vegetables, meats, fish, crustaceans and dry mushrooms [34]. However, it can also be added to some food products as a preservative [18]. FA can appear in several sources other than food materials. Some sources include building materials such as plywood, particleboard and MDF, household products, such as cleaning agents, disinfectants and air fresheners or some essential oils from aromatic plants with antimicrobial activity [7, 21]. FA can also be released when cosmetic items such as nail polish and hair straightening products contain FA-releasing ingredients. Another source of FA is tobacco smoke, as in its composition there is a vast array of around 4700 distinct chemical components and within this category are aldehydes, specifically formaldehyde, propionaldehyde, butyraldehyde, acetaldehyde, acrolein and crotonaldehyde [14, 39]. In comparison to individuals who do not smoke, the act of smoking of a single cigarette result in a 3.5-fold elevation in the concentration of aldehydes in the saliva. Following the consumption of at least ten cigarettes per day, a notable twofold elevation in the concentration of these chemicals was seen in the saliva [14]. In addition, the aerosol produced by e-cigarettes, much like that of traditional cigarettes, also contains reactive aldehydes such as acrolein, acetaldehyde and formaldehyde.

FA can be also produced in food while cooking, as many foods undergo the Maillard reaction. Cooking temperature, time and food moisture affect the Maillard reaction and FA levels in food increase with cooking temperature and time [31]. Therefore, FA can occur when bread is baked [23]. Significant effects on FA release are exerted on the production of flavouring substances in bakery depending on the type of flour, cereals used, processing phases and preparation method. During the investigation of a case of nasopharyngeal cancer in a subject who had always worked in a bakery in Tuscany (Italy), a previously unknown exposure was discovered: the release of FA during the fermentation and baking of bread [3]. In one research, a dependable and precise testing methodology was introduced for the purpose of monitoring FA levels in dairy products. The primary rationale behind employing this testing methodology stems from the significant toxicological impacts of FA on human health when ingested through food consumption [12]. Another method for detection of FA in foods is HPTLC (high performance thin layer chromatography). This method is used to efficiently and economically determine the levels of FA in milk samples [11]. The HPTLC method possesses significant advantages in terms of cost-effectiveness and flexibility. It necessitates very small quantities of solvents, resulting in minimum environmental damage [33, 35]. FA can cause skin irritation, sensitization, allergic responses and respiratory issues. FA is banned in some countries and limited in others to reduce risk [23]. According to the harmonized classification and labelling approved by the European Union, FA is toxic if swallowed, is toxic in skin contact, causes severe skin burns and eye damage, is toxic if inhaled, may cause cancer, is suspected of causing genetic defects, and may cause allergic skin reactions [13].

FA’s frequent use raises health concerns as this molecule has an important sensitizing effect based on its ability to interact with proteins. Asthma is one of the problems associated with the sensitizing potential [17, 23]. Long-term FA exposure, particularly at work, has been linked to degenerative diseases, upper and lower airway irritation, cough, wheezing, body sores, chest problems, abdominal issues and appetite loss [17]. Long-term occupational FA exposure can result in pharyngeal congestion, chronic pharyngitis, chronic rhinitis, loss of olfactory function, lacrimation and corneal problems, heartburn, tremor, lethargy and other symptoms [10]. A variety of illnesses could be brought on by FA’s ability to alter micro-RNA and gene expression patterns [12].

Neurasthenia symptoms from FA exposure include headaches, dizziness, insomnia and memory loss. According to research, exposure to FA increased headache and dizziness by 30% to 60% [29]. Exposure to FA was shown to increase lung damage, minor airway anomalies and pulmonary ventilation resistance [18]. Some studies indicate that FA exposure can cause a gradual decline of WBC (white blood cells), platelets and Hb counts [15, 21]. The DNA and chromosomes of human peripheral blood cells can be harmed by FA resulting in genotoxicity, particularly once biotransformation capability is reached [4, 38]. According to Health Canada and WHO (World Health Organization), FA is only mildly genotoxic, with most effects manifesting in vivo in cells from tissues or organs following initial contact [1]. FA has been identified as a human carcinogen, and its correlation with nasopharyngeal cancer and leukaemia has been documented in numerous epidemiological investigations [28, 30, 32]. In 2004, International Agency for Research on Cancer (IARC) has classified formaldehyde as group 1 carcinogen with sufficient evidence for nasopharyngeal carcinoma [9, 17, 20, 27]. A funeral industry case-control research connected FA to myeloid leukaemia mortality [39]. Recent evidence indicate that genetic factors could be involved in the toxicity of FA. Nowadays it is recognized that aldehyde dehydrogenase 2 (ALDH2) metabolizes reactive aldehydes reducing their toxicity. The presence of the genetic variant ALDH2*2 significantly reduces the ability to detoxify reactive aldehydes in humans [31]. Therefore, an increased health risk for diseases such as cancer or cardiovascular disease could exist with frequent exposure to aldehydes, in case of inefficient aldehyde metabolism.
The analysis of a limited number of pollutants is conducted in relation to a specific phenotype, employing a hypothesis-driven approach to comprehend the connections between exposure and disease [24]. The negative health impact of FA exposure is becoming an increasing health issue, necessitating further research and mitigation as the FA industry expands globally to support economic growth and it affects millions of people [29]. The present study aims to evaluate the FA exposure in traditional occupational sectors such as bakeries and to compare the risk of FA exposure depending on the specific stages of the operational procedures. The influence of smoking habit of the exposed subjects was also evaluated.

Materials and Methods

Subjects
The study group consists of 46 subjects working in bakeries, in various areas: production area (leavening area, baking area, storage area) and administrative areas such as the area where bakery products are sold to customers, the delivery area to stores. Bakeries were from three Romanian areas, and samples were collected over the period April - May 2023. The control group consisted of 8 non-smoking subjects. All individuals who agreed to participate in the study were fully informed about the study and each subject signed an informed consent form prior to the study. The study was conducted in accordance with the Declaration of Helsinki.

Sample collection
The urine samples were collected in sterile polyethylene containers (50 mL) and frozen at -20°C until analysis.

Demographic, medical and anthropometric data
Simultaneously with the collection of urine samples, demographic, medical and anthropometric data were recorded from participants using a pre-designed questionnaire, including the following items: sex, age, BMI, weight, height, the presence of allergies, general medical history, prescription usage, smoking and dietary habits. Systolic blood pressure (BP), diastolic BP, hearth rate, blood oxygen saturation were measured for each subject.

Detection of FA concentrations in urine samples
The analysis was performed with Formaldehyde assay kit (catalogue number MAK131, Sigma-Aldrich), where formaldehyde is derivatized with acetoacetanilide in the presence of ammonia resulting in a fluorescent (λex = 370, λem = 470 nm) product, proportional to the FA present in samples.

Statistical analysis
We used the Statistical Package for Social Sciences software (SPSS, version 15, 115 Chicago, IL, USA). Results are presented as mean ± standard deviation (SD). Comparison between groups was performed using Student’s unpaired t-test. The level of significance was set at 0.05.

Results and Discussion
Two distinctive groups of subjects were established according to the working place: bakery workers (n = 24) and administrative or managerial workers (n = 22). Non-smoker and smoker categories were revealed both the group within the group of bakery and administrative workers, while the control group consists of non-smoker subjects. The studied sample population was homogenous as regards the age or BMI (Table I). Following the analysis of the cardiovascular parameters, it was observed that the highest values of hearth rate were recorded among smokers and workers in the administrative area. At the same time, the BP values tend to be at the limit of the high levels for the administrative workers. Regarding the blood oxygen saturation level, the lowest values were observed among smokers and administrative workers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 8)</th>
<th>Smokers (n = 33)</th>
<th>Non-smokers (n = 13)</th>
<th>Bakery workers (n = 24)</th>
<th>Administrative workers (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>43.63 ± 7.52</td>
<td>46.6 ± 9.48</td>
<td>45.04 ± 9.48</td>
<td>47.12 ± 5.69</td>
<td>46.95 ± 9.39</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.5 ± 15.24</td>
<td>71.86 ± 8.72</td>
<td>67.61 ± 14.08</td>
<td>69.12 ± 11.89</td>
<td>71.09 ± 9.46</td>
</tr>
<tr>
<td>Height, cm</td>
<td>161.09 ± 6.86</td>
<td>166 ± 6.28</td>
<td>163.66 ± 7.54</td>
<td>166.12 ± 6.58</td>
<td>165.45 ± 7.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28 ± 10.32</td>
<td>26 ± 2.76</td>
<td>26 ± 7.47</td>
<td>25 ± 3.81</td>
<td>26 ± 3.12</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>133.4 ± 11.8</td>
<td>130.4 ± 21.14</td>
<td>129.22 ± 13.11</td>
<td>121.37 ± 16.7</td>
<td>137.27 ± 17.63</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73.3 ± 6.1</td>
<td>79.86 ± 5.91</td>
<td>76.11 ± 5.91</td>
<td>77.91 ± 10.19</td>
<td>80.63 ± 10.92</td>
</tr>
<tr>
<td>Heart rate</td>
<td>72.54 ± 4.65</td>
<td>79.86 ± 12.52</td>
<td>69.11 ± 5.07</td>
<td>70.62 ± 10.12</td>
<td>77.59 ± 13</td>
</tr>
<tr>
<td>Blood Oxygen saturation</td>
<td>96.9 ± 6.45</td>
<td>95.7 ± 1.7</td>
<td>97.11 ± 1.02</td>
<td>97 ± 1.02</td>
<td>95.4 ± 1.99</td>
</tr>
<tr>
<td>Allergy</td>
<td>4/8</td>
<td>6/33</td>
<td>4/13</td>
<td>3/24</td>
<td>6/22</td>
</tr>
</tbody>
</table>

Table I

Demographic, anthropometric, behavioral and clinical characteristics, recorded in study subjects selected from bakery and administrative workers vs. control group.

The concentration of FA ranged from 16.01 μg/L to 46.13 μg/L, indicating a significant interindivual variations.

Table II presents the descriptive data on the level of urinary FA across various categories. The highest
values (means ± SD) among the analysed groups were recorded among smokers and bakery workers. The difference of urinary FA level was significantly greater (p < 0.001) in the smokers (33.2 ± 8.24 μg /L), than in the control group (19.38 ± 2.94 μg /L). Also, statistically significant differences were also recorded in the case of non-smokers bakery workers (27.57 ± 10.05 μg /L), compared to the control group (19.38 ± 2.94 μg /L). The urinary concentration of FA was similar in smokers and non-smokers bakery workers, and both were significantly different form control group. These results indicate an exposure to FA, both among smokers and among non-smoking bakery workers. The results suggest that there are no no significant differences between smokers and non-smokers bakery workers, regarding the levels of FA in urine while both differ significant from the control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 8)</th>
<th>Smokers (n = 33)</th>
<th>Non-smokers (n = 13)</th>
<th>Bakery workers (n = 24)</th>
<th>Administrative workers (n = 22)</th>
<th>All subjects (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary formaldehyde level, μg /L</td>
<td>19.38 ± 2.94</td>
<td>33.2 ± 8.24*</td>
<td>27.57 ± 10.05*</td>
<td>37.78 ± 7.49*</td>
<td>27.84 ± 6.49*</td>
<td>30.39 ± 9.49</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD (standard deviations); n = number of subjects; * p < 0.001 vs. Control Group; § p < 0.05 (smokers vs. non-smokers); § p < 0.001 (bakery workers vs. administrative workers)

The occupational exposure to FA occurs in a variety of different sectors, such as healthcare sector, the wood processing industry and other industry sectors. A recent study confirmed the release of aldehydes (FA and acetaldehyde) in bakeries and pastry industries, especially in some phases of the work process, such as leavening [26]. The current pilot study is the first that evaluate the FA exposure in the traditional production sectors of bakeries in Romania. For some of the analysed parameters such as age, weight, height, body mass index, BP, the analysed groups did not register statistically significant differences. In other studies, on smoking, it was revealed that smoking has an impact on the heart rate of young individuals who smoke, leading to an elevation in resting heart rate [25] or the effect of smoking involves an elevation in both systolic and diastolic BP, as well as an increase in heart rate and peripheral vascular resistance [11, 26]. More than half of the subjects included in the study do not suffer from allergies, a few cases were registered to be allergic to some medicines such as acetylsalicylic acid, iodine and antibiotics from the cephalosporin class. The subject included in the study suffer from chronic diseases such as hypertension, the presence of kidney stones, hypercholesterolemia, depressive syndrome. Most of the subjects included in the study live in a rural area, and their dietary habits consist of consuming food products obtained in their own household. The peculiarity of the work shifts is that, for the people who work in the production area, it was imposed to work on 3 shifts, including during the night. The presence of carbon monoxide in smoke diminishes the blood's ability to transport oxygen, hence contributing to increased strain on the heart. It is worth noting that the aforementioned risks are heightened in the presence of diabetes, hypertension, high cholesterol and glucose intolerance [25]. In case of bakery workers, the level of FA exposure can be influenced by many variables: ventilation, the size of the production rooms, the floor space, the flour type and yeast used, the temperature setting throughout the leavening process [10, 22]. The published data on the FA levels in urine as a marker for exposure to formaldehyde are limited. Some attempts were made to use the formic acid in urine as a biomarker for exposure to low levels of FA, but the conclusion was that biological monitoring of FA exposure (via formic acid shifts) at these low levels was not a feasible technique [16]. In contrast, a significant increase of formic acid level in FA-exposed workers compared to controls (p < 0.05) was shown in a study that evaluate the occupational exposure to FA of anatomy-pathology laboratory workers [20]. In other study, urinary formic acid concentrations were found to correlate strongly with occupational exposure to FA [26]. However, the use of formic acid as a biomarker of FA exposure is still considered questionable, given the poor understanding of the normal variation of formiate concentration in the urine [19]. Therefore, it is suggested that the urine FA levels could be also used as a biomarker of exposure to FA in various occupational settings. The results of our study indicate that the urine FA concentrations of bakery workers is significantly increased compared to control. No effect of smoking habits was found, in agreement with other recent studies [20]. Although the urine FA levels are limited use to evaluate the exposure to FA, recent studies indicate that urine FA levels are involved in various pathology. Therefore, Alzheimer’s disease progression was correlated with urine FA levels [19], a correlation was found between urine FA and cognitive abilities in the clinical spectrum of Alzheimer’s disease [36, 38], and urine
FA level was shown to be inversely correlated to mental state examination scores in senile dementia [37].

Conclusions
The results of this pilot study provided new information on the occupational exposure of bakery workers to FA and revealed no effect of smoking habits in this setting. The levels of urinary FA were increased in bakery workers compared to administrative workers and they were significantly different from the control group suggesting a significant occupational exposure to FA in traditional bakery production sectors. This exposure may have some pathogenetic significance as FA is a highly toxic compound and it is recognized as a human carcinogen. Therefore, safety issues and appropriate measures should be indicated to protect workers. The need to inform workers, the measures to reduce pollution at workplace during bread production, mainly in the leavening phase are important to reduce exposure and to protect the worker’s health.

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Conflict of interest
The authors declare no conflict of interest.

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