Exposure to Mutagenic Aldehydes and Particulate Matter During Panfrying of Beefsteak with Margarine, Rapeseed Oil, Olive Oil or Soybean Oil

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Objectives: The aim of the study was to see if a cook could be exposed to mutagenic aldehydes in fumes from frying of beefsteak using margarine, rapeseed oil, soybean oil or virgin olive oil as frying fat. In addition, levels of particle exposure were measured to make the results comparable to other studies. Methods: The levels of higher aldehydes and total particles were measured in the breathing zone of the cook during the panfrying of beefsteak with the four different frying fats. In addition, the number of particles in the size intervals 0.3–0.5, 0.5–0.7 and 0.7–1.0 μm in the kitchen was registered. Results: Measured levels of mutagenic aldehydes were between non-detectable and 25.33 μg m⁻³ air. The exposure level of total aerosol was between 1.0 and 11.6 mg m⁻³. Conclusions: Higher aldehydes were detected in all samples from this study, and mutagenic aldehydes were detected in most of the samples. Frying with margarine gave statistically significantly higher levels of mutagenic aldehydes and particles in all three size fractions than frying with the three different kinds of oil.

Keywords: aldehydes; cooking fumes; cooking oil; margarine; trans,trans-2,4-decadienal

INTRODUCTION

Studies have shown that cooking with gas or electric stoves produces a range of harmful and potentially mutagenic compounds (Vainiotalo and Matveinen, 1993; Metayer et al., 2002; Svendsen et al., 2002) as well as high levels of fine and ultrafine particles (Abt et al., 2000; Dennekamp et al., 2001; Wallace et al., 2004; Afshari et al., 2005).

Cooking fumes, especially from frying, contain several specific agents which may give adverse health effects in the lung. Studies have shown that cooking fumes contain aldehydes such as formaldehyde, acetaldehyde and acrolein (Vainiotalo and Matveinen, 1993; Svendsen et al., 2002). Also, during frying at high temperatures, fat will enter the atmosphere as an aerosol. Both aldehydes and fat aerosols are irritating to the airways when inhaled (Svendsen et al., 2003).

In addition, cooking fumes contain substances with mutagenic activity and they may be a risk factor in lung cancer (Chen et al., 1992; Metayer et al., 2002). Emissions from high-temperature frying have recently been classified as ‘probably carcinogenic to humans (Group 2A)’ by the International Agency for Research on Cancer (IARC, 2006). An increased risk of respiratory tract cancer in cooks and bakers has been reported (Coggon et al., 1986; Lund and Borgan, 1987). Among the compounds which have been identified as mutagenic in cooking fumes are polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) (Chiang et al., 1999a,b). These are mainly found in fumes obtained at temperatures >250°C (Lijinsky, 1991; Wu et al., 2001). The thermal stressing of cooking oils rich in polynsaturated fatty acids also generates various higher aldehyde species, i.e. aldehydes with a higher number of carbon atoms, such as trans-2-alkenals, trans,trans-alka-2,4-dienals and n-alkanals, arising from the fragmentation of conjugated hydroperoxy-diene precursors (Gertz, 2000). Alkanals are saturated aldehydes (i.e. without double bonds), and alkenals are unsaturated aldehydes with one or more double bonds.
Mutagenic compounds in fumes from peanut oil heated to temperatures \(\sim 100^\circ\text{C}\) have been studied (Wu et al., 2001). The following compounds were identified as the ones with the strongest mutagenicity in the Ames test (in descending order): trans, trans-2,4-decadienal, trans,trans-2,4-nonadienal, trans-2-decenal and trans-2-undecenal. Trans,trans-2,4-decadienal has also been detected in cooking fumes resulting from heating other oils, such as rapeseed oil and soybean oil, and is considered to be the major mutagenic and cytotoxic compound in oil fumes (Zhu et al., 2001).

Trans,trans-2,4-decadienal has been reported to inhibit human erythroleukemia cell growth, to affect cell viability, to reduce the cellular glutathione level and to be involved in the beginning of DNA fragmentation \textit{in vitro} (Nappez et al., 1996). Other studies indicate an association of this aldehyde with genotoxic effects due to the reaction with nucleic acid bases (Loureiro et al., 2000).

Of the other mutagenic aldehydes identified (Wu et al., 2001), trans-2-decenal is also shown to cause significant oxidative damage in human A-549 cells (Wu and Yen, 2003). In addition, it has been shown that extracts of oil fumes from soybean oil, sunflower oil and lard cause cytotoxicity and oxidative DNA damage in human A-549 cells. These fume extracts contained not only trans,trans-2,4-decadienal but also trans-2-decenal, trans,trans-2,4-nonadienal and trans-2-undecenal (Dung et al., 2006).

Exposure to fine (aerodynamic diameter < 2.5 \(\mu\text{m}\)) and ultrafine (aerodynamic diameter < 0.1 \(\mu\text{m}\)) airborne particles has been identified as an important factor affecting human health (Oberdörster et al., 1994; Seaton et al., 1995). Ultrafine particles are supposed to cause oxidative stress in pulmonary cells (Li et al., 2003; Beck-Speier et al., 2005) and to enhance allergic reactions and pulmonary inflammations (Brown et al., 2001; Oberdörster, 2001; Alessandrini et al., 2006). The fine particle fraction may cause the same negative effects, though not to the same extent (Oberdörster, 2001; Li et al., 2003).

The aim of the study was to see if higher mutagenic aldehydes could be detected in fumes from frying of beefsteak, collected in the breathing zone of the cook. The frying was performed according to an experimental, standardized procedure, mimicking actual cooking conditions in a private household in Norway. The levels of higher aldehydes (trans,trans-2,4-decadienal, 2,4-decadienal, trans,trans-2,4-nonadienal, trans-2-decenal, cis-2-decenal, trans-2-undecenal, 2-undecenal, as well as various alkanals and alkenals) were measured during pan-frying of beefsteak with four different frying fats: margarine, soybean oil, virgin olive oil and rapeseed oil. The level of total particulate matter in the breathing zone of the cook was also measured. In addition, we measured the number of particles in the size intervals 0.3–0.5, 0.5–0.7 and 0.7–1.0 \(\mu\text{m}\) in the kitchen.

To our knowledge, data on personal exposure to higher mutagenic aldehydes in cooking fumes produced under real-life conditions have not previously been reported.

**METHODS**

The measurements were performed during frying of beefsteak following a standardized procedure representing real-life conditions, that is, conditions similar to a common Norwegian home with regard to the location, ventilation conditions and the frying procedure.

**Materials**

Beefsteak from the shoulder of bovine ox, margarine, virgin olive oil, soybean oil and rapeseed oil were purchased from a local grocery store in Trondheim, Norway. The margarine used contained soybean oil, rapeseed oil, coconut oil, palm oil and vitamins A and D and was without hydrogenated fats.

**The location**

The experiment was performed in a room of 19 m\(^2\), equipped with an electric stove. The stove was placed centrally on one of the walls in the room. A modern kitchen hood, exhausting outside the building, was hung 50 cm above the stove. During frying, the hood was run on medium capacity, extracting 335 m\(^3\) air h\(^{-1}\). The basic ventilation in the kitchen room was 119 m\(^3\) h\(^{-1}\) of air supply and 112 m\(^3\) h\(^{-1}\) outlet (without kitchen hood ventilation).

**The frying procedure**

The beefsteak was fried following a standard procedure which was designed for this study. The pan was heated on the hotplate (210 mm diameter) on the top of the electric stove, using the maximum effect setting (2100 W) until the surface temperature in the middle of the pan reached 100°C. At 100°C, 20 g of margarine or 20 ml of rapeseed oil or olive oil or soybean oil was added to the pan. After heating the margarine/oil for \(\sim 30–40\) s, two pieces of beefsteak were added, each of them \(\sim 150\) g with a thickness of 1.5–2.0 cm. At that moment, the temperature in the pan was 190–200°C. The beefsteak was left for 2 min and was then turned. At 3 min, the heat was reduced to medium effect, and at 4 min, the beefsteak was turned for the second time. After this, the steak was turned once a minute until the frying ended 10 min after it started. Eight minutes after adding the steak to the pan, 10 g of margarine or 10 ml of oil was added. The temperature on the side of the beefsteak facing the pan was 280–300°C during frying. As the beefsteak was removed from the pan, the pan was also removed from the heat and the
electricity was turned off. The temperature in the pan was measured with a TES 1322A (TES Electrical Electronic Corp., Taipei, Taiwan) remote non-contact thermometer, using a laser pointer to measure surface temperatures.

The standard frying procedure was repeated two or three times during every day of frying. After every repetition, the kitchen was ventilated until the level of particles in the air was within 7–14 particles cm⁻³ in the size fraction 0.3–0.5 µm, measured with the Met One particle counter.

**Personal exposure measurements**

**Aldehydes.** The levels of higher aldehydes were measured by use of stainless steel tubes with 220 mg Tenax TA. The sampling flow rate was 100 ml min⁻¹. The pump used was a SKC Pocket Pump model 210-1002. The sampling was performed as personal measurements, with the tubes placed on the left shoulder of the person frying the beefsteak (the cook). The sampling started when the beefsteak was added to the pan and ended when the pan was removed from the heat, a total of 10 min. After sampling, the tubes were closed with endcaps and stored in room temperature until analysis.

The automatic thermal desorber (ATD) tubes were analyzed by thermic desorption in an ATD 400 (Perkin Elmer, Waltham, MA, USA) and gas chromatography–mass spectrometry in a Focus GC–DSQ (Thermo-Electron Corporation, Waltham, MA, USA) following standard procedures for qualitative/semiquantitative MS full-scan analyses (MDHS 72, 1993). The identified aldehydes were quantified as equivalents based on the response of hexanal. The analyses were performed by a certified commercial laboratory.

Results were presented as the quantity of the aldehydes trans, trans-2,4-decadienal, 2,4-decadial, trans-2-decenal, cis-2-decenal, 2-undecenal, alkanals and alkenals measured in the breathing zone of the cook during frying of beefsteak. The group ‘alkanals’ consisted of eight different alkanals (butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal and undecanal). The group ‘alkenals’ consisted of 2-heptenal, 2-nonenal and trans, trans-2,4-heptadienal.

**Total particles.** The sampling of total particles was performed using preweighed, double Gelman AE glassfiber filters (37 mm). The two filters were placed in a closed-face clear styrene acrylonitrile cassette connected to a pump (Casella Vortex standard 2 personal air sampling pump, Casella CEL, Bedford, UK) with an airflow of 2 l min⁻¹. The filter cassette was placed on the left shoulder of the cook. The sampling was run continuously through 1 day of frying, each day including either two or three repetitions of the standard frying method. On days with two repetitions, the sampling ran 65 min (on average) and on days with three repetitions, the sampling ran 110 min (on average). Between the repetitions of the frying procedure, the kitchen was ventilated (as described previously). The intensity of frying was equal during every 10-min frying period. Thus, when calculating the mass concentration in mg m⁻³ air, the results are comparable, even though the sampling time was not the same during the whole study. Before and after sampling, the filters were conditioned in an excitor for 24 h. The filters were analyzed gravimetrically using a Mettler balance (0.01 mg resolution). An inner calibration was performed on the Mettler balance before every weighing. Blank filters were included in the analysis in order to control for deviations caused by temperature or humidity.

**Stationary measurements in the kitchen**

The number of particles was determined by the use of a Met One model 237B (Pacific Scientific Instruments, Grants Pass, OR, USA) particle counter in the kitchen. This particle counter registers the number of particles in 0.1 ft³ (2832 cm³) of air in the particle sizes 0.3–0.5, 0.5–0.7 and 0.7–1.0 µm. The instrument also registers particles in the fractions 1.0–2.0, 2.0–5.0 and >5.0 µm. These were not included in the present study because they contained a small percent (2.6–3.4%) of the total number of particles registered. All Met One measurements were started manually and lasted for 1 min. Measurements were performed before frying and 1, 4, 7, 10, 12, 15 and 18 min after the start of frying. The inlet of the particle counter was located 1 m above the floor and 1.3 m to the side of the stove.

The numbers presented in this study are the mean number of particles produced during frying with the different kinds of frying fat, i.e. the baseline values measured before frying started are subtracted.

**Statistical analyses**

All analyses were performed using SPSS version 14.0. The comparisons of means were calculated by univariate analyses in a general linear model.

**RESULTS**

**Personal exposure measurements**

**Aldehydes.** Table 1 describes the quantity of the aldehydes trans, trans-2,4-decadienal, 2,4-decadial, trans-2-decenal, cis-2-decenal, 2-undecenal, alkanals and alkenals measured in the breathing zone of the cook during frying of margarine, rapeseed oil, soybean oil and virgin olive oil. Trans, trans-2,4-nonenal and trans-2,4-undecenal were not registered above the detection limit in any of the samples collected.

In a series of measurements where some values were non-detectable, these were substituted by \( L/2^{1/2} \), where \( L \) is the detection limit (Hornung and Reed, 1990). For
example, when measuring trans,trans-2,4-decadienal, levels above the detection limit were registered in all samples when frying with margarine. When frying with rapeseed oil, levels above the detection limit were measured in one of five samples. Frying with soybean oil gave levels above the detection limit in two of six samples. When frying with olive oil no single results were above the detection limit.

The aldehydes included in the group alkanals were present in various quantities (Table 1). The aldehydes included in the group alkenals were present in small quantities (Table 1).

Frying with margarine gave the highest quantities of most aldehydes (trans,trans-2,4-decadienal, 2,4-decadienal, trans-2-decenal, 2-undecenal, alkanals and alkenals) (Table 1). The statistical comparison of the levels of aldehydes measured show that the quantities of trans,trans-2,4-decadienal, 2,4-decenal, trans-2-decenal, 2-undecenal, alkanals and alkenals were significantly higher during frying with margarine than during frying with all kinds of oil (rapeseed oil, soybean oil and virgin olive oil). When comparing the three different types of oil, the differences in aldehyde quantities were not statistically significant.

Total particles. The mean levels of total particles measured in the breathing zone of the cook during the different experiments are given in Table 2.

Stationary measurements in the kitchen

The mean numbers of particles in the size fractions 0.3–0.5, 0.5–0.7 and 0.7–1.0 μm (particles cm$^{-3}$ air) measured in the kitchen during a standard frying procedure with the different frying fats are given in Table 3.

Compared to the number of measurements performed during frying with margarine referred in Table 1, six additional registrations of particles are included in Table 3. These were measurements performed under the same conditions as presented here, but before the measurements of higher aldehydes were initiated.

Table 1. Higher aldehydes (μg m$^{-3}$) measured in the breathing zone of the cook during panfrying of beefsteak using four different kinds of frying fat

<table>
<thead>
<tr>
<th>Frying fat</th>
<th>Number of repetitions</th>
<th>t,2-Decadienal [mean (SD)]</th>
<th>t,2-Decenal [mean (SD)]</th>
<th>2-Decenal [mean (SD)]</th>
<th>2-Undecenal [mean (SD)]</th>
<th>Alkanals [mean (SD)]</th>
<th>Alkenals [mean (SD)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine</td>
<td>3</td>
<td>10.33 (2.52)</td>
<td>25.33 (4.51)</td>
<td>25.33 (9.70)</td>
<td>20.67 (7.64)</td>
<td>55.70 (11.00)</td>
<td>426.00 (70.00)</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>5</td>
<td>0.63 (1.32)</td>
<td>n.d.</td>
<td>3.60 (6.40)</td>
<td>0.82 (1.08)</td>
<td>107.00 (75.00)</td>
<td>1.80 (4.00)</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>5</td>
<td>0.52 (0.82)</td>
<td>n.d.</td>
<td>2.20 (5.29)</td>
<td>2.02 (3.62)</td>
<td>128.00 (53.00)</td>
<td>4.00 (2.70)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3.33 (2.34)</td>
<td>3.33 (2.34)</td>
<td>121.00 (85.00)</td>
<td>0.90 (1.30)</td>
</tr>
</tbody>
</table>

The results are given as arithmetic mean [standard deviation (SD)] for every 10-min frying period. The number of repetitions of the standard frying procedure using each kind of frying fat is listed.

n.d. = no single results above detection limit, detection limit = 0.05 μg m$^{-3}$.

The total sum of alkanals/alkenals other than the ones listed separately in the table.

Table 2. The levels of total particles (mg m$^{-3}$) measured in the breathing zone of the cook during panfrying with four different kinds of frying fat

<table>
<thead>
<tr>
<th>Frying fat</th>
<th>Number of samples</th>
<th>Total particles [mean (SD)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine</td>
<td>2</td>
<td>11.6 (0.7)</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>3</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3</td>
<td>1.4 (0.7)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>3</td>
<td>1.0 (1.1)</td>
</tr>
</tbody>
</table>

The levels are given as arithmetic mean [standard deviation (SD)] level of total particles measured during 1 day of frying (one sample day$^{-1}$), each day including two or three repetitions of the standard frying procedure. The number of samples for each kind of frying fat is listed.
Table 3. The number of particles in the size fractions 0.3–0.5, 0.5–0.7 and 0.7–1.0 μm, measured in the kitchen during panfrying of beefsteak using four different kinds of frying fat

<table>
<thead>
<tr>
<th>Frying fat</th>
<th>Number of repetitions</th>
<th>Number of particles in the different size fractions [mean (SD)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine</td>
<td>9</td>
<td>4.6 × 10² (2.0 × 10²)²</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>6</td>
<td>1.2 × 10² (2.5 × 10⁰)</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6</td>
<td>1.8 × 10² (4.7 × 10⁰)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>6</td>
<td>1.8 × 10² (6.4 × 10⁰)</td>
</tr>
</tbody>
</table>

The results are given as arithmetic mean (SD) number of particles cm⁻³ air produced during a 10-min frying period. The number of repetitions of the standard frying procedure using each kind of frying fat is listed.

The level of submicrometer particles increased rapidly in the kitchen when frying started, regardless of the use of the kitchen hood. The main size fraction of the particles was <0.5 μm (Table 3).

The statistical comparison of the mean number of particles showed a significantly higher number of particles in all size fractions during frying with margarine than during frying with all three kinds of oil. When comparing the three different types of oil, the differences in numbers of particles were not statistically significant (Table 3).

**DISCUSSION**

**Aldehydes**

During frying of beefsteak under real-life conditions with four different kinds of frying fat, we were able to detect higher aldehydes in all samples, and mutagenic aldehydes were detected in most of the samples. The highest levels of mutagenic aldehydes were measured during frying with margarine, with the mean levels of trans,trans-2,4-decadienal and 2,4-decadienal as the highest (25.33 μg m⁻³, Table 1).

The aldehyde supposed to be the most carcinogenic (trans,trans-2,4-decadienal) was mainly detected in fumes from frying with margarine (Table 1). The levels may, however, have been slightly higher than the ones reported in Table 1 since the laboratory reported difficulties in distinguishing between the different isomers of 2,4-decadienal. Thus, the category 2,4-decadienal also may include some trans,trans-2,4-decadienal.

Others (Dung et al., 2006) have also studied levels of mutagenic aldehydes in cooking fumes collected in the breathing zone of the cook, but the collecting and reporting methods are different from the ones used in the present study. This makes it difficult to make direct comparisons of the exposure levels.

Several studies have focused on evaluating the mutagenicity and finding the mutagenic components in cooking fumes (Qu et al., 1992; Chiang et al., 1999b; Wu et al., 2001). PAHs and HCAs have been identified as the main mutagenic compounds (Vainiotalo and Matveinen, 1993; Li et al., 1994; Chiang et al., 1999b). A study on the mechanisms behind the association between exposure to cooking oil fumes (COFs) and lung cancer indicates that COF induces anti-apoptotic effects, contributing to the cell survival and proliferation of A-549 lung cancer cells. The results also indicate that trans,trans-2,4-decadienal from COF may make a more important contribution than PAHs (benzo[a]pyrene) to the cell survival and the proliferation of A-549 lung cancer cells (Hung et al., 2007).

**Particles**

In this study, we measured particles down to the size of 0.3 μm during frying in the kitchen. The main fraction of particles detected was in the range 0.3–0.5 μm. This is consistent with previous studies (Abt et al., 2000), demonstrating that cooking and frying mainly emits particles in the size fraction 0.02–0.5 μm. Unfortunately, instruments to measure ultrafine particles were not available during the experimental period of this study.

The frying of beefsteak with margarine produced the highest numbers of particles in all three size fractions registered (Table 3). The differences in numbers of particles between margarine and the three kinds of oil were statistically significant. The margarine used in this study contained 17% water (±1%). In the three oils that were used, water content is normally <0.07% (according to the distributors: Mills, Unil and Hills Foods). This may indicate that the larger particle numbers measured during frying with margarine were caused by the production of water vapor. However, the measurements of total particles in the breathing zone of the cook also showed higher levels during frying with margarine than with the different kinds of oil (Table 2). The filters used in these measurements were dried before the gravimetric analysis. This may imply that the higher numbers of particles measured during frying with margarine were not related to water content alone.

The highest mean level of particles in the size fraction 0.3–0.5 μm measured in the kitchen was 1.3 × 10⁵ particles cm⁻³ (Table 3), measured with
the kitchen hood running on a medium level (335 m$^3$ air h$^{-1}$). In a previous study (Sjaastad et al., 2008), we measured a maximum level of $1.1 \times 10^5$ particles cm$^{-3}$ in the size fraction 0.3–0.5 μm during frying according to the same procedures as in the present study. However, in the latter study, the kitchen hood was run on maximum capacity, extracting almost 600 m$^3$ h$^{-1}$.

The level of total particles in the breathing zone of the cook was measured to be able to compare the personal exposure during frying in private homes with cooking fume exposure of professional cooks, measured in previous studies.

The mean level of total particles (based on all values in Table 2) measured in the breathing zone of the cook was 3.8 mg m$^{-3}$. In a study conducted on professional cooks in restaurants, personal exposure levels were in the range 0.08–2.95 mg m$^{-3}$ (Svendsen et al., 2002). These measurements were made during 1.5–2.5 h of a work shift, with varying amounts of time spent on frying meat and the use of various kinds of frying fats. The levels measured during frying with cooking oils in the present study are comparable to those measured in the restaurants.

The highest level of total particles measured in the present study was 11.6 mg m$^{-3}$. Compared to the restaurant levels, this seems very high. However, this level was measured during frying with margarine. The main type of frying fat used in the restaurants was different types of vegetable oil (Svendsen et al., 2002).

Overall, it seems that the exposure to cooking fumes during one single frying episode in a domestic kitchen may be similar or even higher than during such an episode in restaurant kitchens.

It has been shown that exposure to cooking fumes under domestic conditions has a significant effect on urinary mutagenicity in healthy non-smoking subjects (Pavanello et al., 2007). Studies on both Asian (Zhong et al., 1999; Metayer et al., 2002) and Western (Sivertsen et al., 2002; Svendsen et al., 2003) style cooking have indicated adverse health effects (lung cancer included) from exposure to cooking fumes. Furthermore, Norwegian statistics have indicated increased mortality from respiratory diseases and diseases of the circulatory system in employees in hotel and restaurants (Borgan and Kristoffersen, 1986) as well as low life expectancies in cooks compared to employees in other occupations (Borgan, 2004).

CONCLUSIONS

To our knowledge, the levels of mutagenic aldehydes in the breathing zone of the cook have previously not been measured under real-life conditions. The measurements in the present study are expected to represent levels found in the average Norwegian home during the frying of beef with different kinds of frying fat. They are also supposed to be representative for the exposure professional cooks are subject to during frying of beefsteak.

The reduction of the level of pollution caused by frying may be an important health factor, both in the working environment of professional cooks and in private homes. According to the present results, frying with rapeseed oil, soybean oil or virgin olive oil instead of margarine is one way of reducing personal exposure to some of the components in cooking fumes that may cause adverse health effects.

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