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Keywords
cytotoxicity, formaldehyde, vitro, glutaraldehyde, cells, mixtures, human

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In Vitro Cytotoxicity of Formaldehyde and Glutaraldehyde Mixtures in Human Cells

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Abstract

The cytotoxicity of formaldehyde, glutaraldehyde and their binary mixtures was determined using the MTS (tetrazolium salt; Promega) in vitro assay. Cytotoxicity endpoints were investigated in human cells including; pulmonary type II-like epithelial cell lines (A549), hepatoma cell lines (HepG2) and skin fibroblasts. In order to study the cytotoxic effects of airborne formaldehyde, standard atmospheres at concentrations below 10 ppm (12.3 mg/m³) were generated using a dynamic diffusion method. Formaldehyde was bubbled through serum free culture media and cell viability was investigated after treating cells with formaldehyde air samples. HepG2 cells were found to be more sensitive (IC₅₀ = 103.8 ± 23.6 mg/L) to formaldehyde cytotoxicity than both A549 lung derived cells and skin fibroblasts (P < 0.01). For glutaraldehyde, skin fibroblasts were found to be more sensitive (IC₅₀ = 99.9 ± 17.2 mg/L) than A549 cells (p < 0.01). Mixtures of formaldehyde and glutaraldehyde at a specific range of concentrations, approximately 5-20% (w/w) formaldehyde, induced synergistic effects in A549 cells. Exposing of HepG2 cells with formaldehyde air samples (8.75 ppm × 4 h) reduced cell viability to less than 50% (31.6 ± 1.24%). Our findings emphasized the toxic effects of formaldehyde and glutaraldehyde and their potential toxicity interactions should be taken into consideration for occupational risk assessment of health care workers.

Key words: Formaldehyde, Glutaraldehyde, Toxicity interaction, In vitro cytotoxicity.

1. Introduction

Formaldehyde and glutaraldehyde are chemicals that are frequently found together in workplaces such as medical laboratories, hospitals and health services. These two aldehydes have a number of common adverse effects such as respiratory irritation, sensitisation, DNA-protein cross linkage and cytotoxicity. In addition, Formaldehyde has recently classified as a proven human carcinogen on the basis of its ability to cause nasopharyngeal cancer and leukaemia (IARC, 2004). Therefore, the adverse health effects and potential toxicity interactions of these two chemicals is very important to people working in such occupational settings.

Although the toxicity of formaldehyde and, to a lesser extent, glutaraldehyde have been investigated individually there is little toxicity data on the toxicity of binary mixtures of formaldehyde and glutaraldehyde. In this present study the toxic effects of formaldehyde, glutaraldehyde and mixtures of these chemicals was studied using an in vitro cell culture system. Since inhalation is considered as a main route of exposure to vapours of organic chemicals in occupational environments, the cytotoxic effect of airborne formaldehyde were also investigated.

2. Materials and Methods

2.1. Test Compounds

Formaldehyde (CAS No. 50-00-0) 37% solution was purchased from Chem-Supply (Australia) and glutaraldehyde (CAS No. 111-30-8) 50% solution was purchased from Sigma (USA). All chemicals and reagents were of the highest analytical grade available.
2.2. Cell types and culture conditions
Three human cells including: A549, pulmonary type II-like epithelial cell lines (ATCC No. CCL-185), HepG2, hepatoma cell lines (ATCC No. HB-8065) and skin fibroblasts (Westmead Hospital, Sydney, Australia) were used. Cells were cultured in sterile culture flasks with DMEM/F12 (Dulbecco’s modified eagle medium: Ham’s F-12 nutrient mixture; Gibco, USA) culture media supplemented with 5% fetal calf serum (FCS; JS Bioscience, Australia) and 1% antibiotic mixture (Sigma, USA). Cells were kept at 37°C in a humidified 5% CO2 incubator. For experiments, newly confluent cell layers were removed using Trypsin/EDTA (Gibco, USA) and resuspended in culture medium. Cell viability was assessed with trypan blue (Sigma, USA), and cell number was determined.

2.3. Cytotoxicity testing protocol
The MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay was used to measure the cytotoxicity of test chemicals (Promega, 2001). Cells were added to serial dilutions of the test chemicals in 96-well microtiter plates in four replicates. Backgrounds for each test concentration were also prepared. For each experiment, two internal controls were set up including an IC0 (0% inhibitory concentration) and an IC100 (100% inhibitory concentration), (Malich et al., 1997). MTS/PMS (Phenazine methosulfate) reagents were added to cell cultures in a ratio of 1:5. After 4 hrs incubation, absorbance was measured at 492 nm, using a microtiter plate reader (Multiskan MS, Labsystems, Finland).

2.4. Cytotoxicity testing of mixtures
In order to study the cytotoxicity of formaldehyde and glutaraldehyde mixtures, human A549 lung cell lines were incubated for 4 hrs with varying concentrations of the two chemicals. Cell viability was investigated using the MTS assay.

2.5. Cytotoxicity testing of airborne formaldehyde
Test atmospheres of formaldehyde (< 10 ppm) were produced, using a dynamic diffusion method (Saltzman, 1997). The generated concentration was monitored by air sampling, using two midget impingers (SKC, USA) in series that contained an absorption solution (1% sodium bisulfite; 10 mL). In parallel, air sampling for the in vitro study was carried out using two other impingers in series. The first one contained serum-free culture media (10 mL) and was used for cytotoxicity assessments. The second impinger contained an absorption solution used for measuring any breakthrough of formaldehyde. After 1 or 4 hrs of air sampling at constant air flow (0.3 L/min), cells were incubated for 4 hrs with serial dilutions of formaldehyde air samples in culture media. Cell viability was assessed using the MTS assay. Three other air samples were subjected to chemical analysis using the 3500 NIOSH method (NIOSH, 1994).

3. Results
The NOAEC (no observed adverse effect concentration), IC10 (10% inhibitory concentration), IC50 (50% inhibitory concentration) and TLC (total lethal concentration) values of formaldehyde and glutaraldehyde in three cell types with the MTS assay are presented in Table 1. Each value represents the mean (M) and standard deviation (SD) of at least three experimental replicates

A dose response analysis for various concentrations of formaldehyde and glutaraldehyde was performed (Fig. 1). Concentrations were calculated as total concentration of formaldehyde and glutaraldehyde. Both individual test chemicals showed similar IC50 values in A549 cells (IC50 ~ 200 mg/L). Formaldehyde 43.2% (w %) induced a less than additive effect. While 87.3% (w %) induced antagonistic effects (right shift of the dose-response curve), 7.8% (w %) formaldehyde induced synergistic effects (left shift of the dose response curve) in A549 cells with the MTS assay.

Cytotoxic effects of airborne formaldehyde in HepG2 and A549 cells with the MTS assay resulting from identical airborne concentration and sampling times are compared in Fig. 2. The cell viability of HepG2
cells were significantly reduced after exposure to formaldehyde air samples \( (p<0.0001) \). Dose-dependent effects of airborne formaldehyde were observed in HepG2 cell lines with the MTS assay (Fig. 3).

4. Discussion

The cytotoxicity of formaldehyde, glutaraldehyde and mixtures of these two chemicals was studied in human cell types including HepG2 liver derived, A549 lung derived and skin fibroblasts with the MTS assay. Cytotoxicity endpoints of formaldehyde and glutaraldehyde were determined in all cell types, indicating both test chemicals were toxic to human cells (Table 1). HepG2 cells were found to be more sensitive \( (IC_{50} = 103.8 \pm 23.6 \text{ mg/L}) \) to formaldehyde than both A549 cells and skin fibroblasts when analysed by multiple comparisons \( (p < 0.01) \). The hepatotoxicity of formaldehyde may relate to the presence of cellular enzymes in liver cells such as NAD-dependent formaldehyde dehydrogenase (Newell, 1983; WHO, 2002). However, for glutaraldehyde skin fibroblasts were found to be more sensitive target cells \( (IC_{50} = 99.9 \pm 17.2) \) than the A549 lung derived cells \( (p < 0.01) \).

Possible toxicity interactions of formaldehyde and glutaraldehyde were investigated in human A549 lung derived cells. Based on our results formaldehyde and glutaraldehyde were showed to have approximately similar cytotoxicities \( (IC_{50} \approx 200 \text{ mg/L}) \) in A549 cells. Results of this study showed that mixtures of formaldehyde and glutaraldehyde may cause both synergistic and antagonistic effects in human A549 cells with the MTS assay depending on the different concentration range of each component. For example, while formaldehyde 87.3% \( (w \%) \) induced antagonistic effects (right shift of dose-response curve), 7.8% \( (w \%) \) formaldehyde induced antagonistic effects (left shift of the dose-response curve) in lung derived cells (Fig. 1). The occurrence and the nature of any toxicity interaction depend upon various factors such as biological endpoint, concentration of each component within a mixture and specific experimental conditions (Schlesinger, 1995). Even in one study measuring a specific endpoint, the type of toxicity interactions occurring may vary depending on the exposure dose (Calabrese et al., 1988) or exposure time (Schlesinger, 1995). Our results suggest that the concentration of chemical components in a mixture may influence the type of combined toxic effects.

The cytotoxicity of airborne formaldehyde was investigated after treating human cells with formaldehyde air samples collected in serum free culture media. While the viability of HepG2 cells was significantly reduced after exposing with formaldehyde air samples \( (p<0.01 \text{ or less}) \), no statistically significant differences were measured between cell viability of treated A549 cells at the concentrations tested, compared with controls (Fig. 2). In particular, the concentrations of airborne formaldehyde tested were toxic to HepG2 hepatoma cell lines. Dose-dependant cytotoxic effects of airborne formaldehyde were observed in HepG2 cell lines (Fig. 3).

The average collection efficiency of airborne formaldehyde in serum free culture media was calculated \( (96.8\%) \). This result suggested that the impingement method has a potential to be used for toxicity testing of soluble airborne contaminants such as formaldehyde (Bakand et al., 2005a). Further, direct exposure of human cells at the air/liquid interface using porous membranes has been developed in our laboratory for toxicity testing of both soluble and insoluble airborne contaminants (Bakand et al., 2005b; Bakand et al., 2006a; Bakand et al., 2006 b).

Our findings emphasized that the toxic effects of formaldehyde, glutaraldehyde and their potential toxic interactions need to be taken into consideration for occupational risk assessment of health care workers. Further, these results suggest that in vitro cytotoxicity test methods using human cells has a potential to be used as a promising method for cytotoxicity testing of individual chemicals, various chemical mixtures and airborne contaminants that found in workplace environments. All these considerations are essential in toxicity testing and risk assessment of workplace chemicals.
References:


Table 1. Cytotoxicity endpoints of test chemicals in three cell types with the MTS assay

<table>
<thead>
<tr>
<th>Test Chemicals</th>
<th>Cell types</th>
<th>NOAEC</th>
<th>IC_{10}</th>
<th>IC_{50}</th>
<th>TLC</th>
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<tbody>
<tr>
<td></td>
<td>A549</td>
<td>1.49±0.18</td>
<td>30.15±1.45</td>
<td>198.36±9.54</td>
<td>1506.50±77.25</td>
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<tr>
<td></td>
<td>HepG2</td>
<td>1.36±0.31</td>
<td>15.78±3.58</td>
<td>103.79±23.55</td>
<td>708.40±181.07</td>
</tr>
<tr>
<td></td>
<td>Fibroblast</td>
<td>4.99±0.86</td>
<td>29.90±5.58</td>
<td>196.68±36.73</td>
<td>1155.21±278.47</td>
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<tr>
<td>Glutaraldehyde</td>
<td>A549</td>
<td>2.30±0.13</td>
<td>30.66±3.50</td>
<td>201.73±23.01</td>
<td>1412.08±201.49</td>
</tr>
<tr>
<td></td>
<td>HepG2</td>
<td>3.51±1.40</td>
<td>22.37±5.76</td>
<td>147.19±37.88</td>
<td>875.90±208.91</td>
</tr>
<tr>
<td></td>
<td>Fibroblast</td>
<td>2.14±0.32</td>
<td>15.18±2.61</td>
<td>99.89±17.15</td>
<td>609.67±127.83</td>
</tr>
</tbody>
</table>
Fig. 1. Comparison of dose response curves of formaldehyde and glutaraldehyde mixtures in varying Concentrations (FA, Formaldehyde).

Fig. 2. Comparison of cell viability of HepG2 and A549 cell lines exposed to formaldehyde air samples (** p < 0.0001).
Fig. 3. Dose-dependent effects of airborne formaldehyde in HepG2 cells (* p < 0.01; ** p < 0.0001).