

# Genotoxic Effects of Exposure to Formaldehyde in Two Different Occupational Settings

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## 1. Introduction

Formaldehyde (CH<sub>2</sub>O), the most simple and reactive aldehyde, is a colorless, reactive and readily polymerizing gas at room temperature (National Toxicology Program [NTP], 2005). It has a pungent suffocating odor that is recognized by most human subjects at concentrations below 1 ppm (International Agency for Research on Cancer [IARC], 2006).

Aleksandr Butlerov synthesized the chemical in 1859, but it was August Wilhelm von Hofmann who identified it as the product formed from passing methanol and air over a heated platinum spiral in 1867. This method is still the basis for the industrial production of formaldehyde today, in which methanol is oxidized using a metal catalyst. By the early 20<sup>th</sup> century, with the explosion of knowledge in chemistry and physics, coupled with demands for more innovative synthetic products, the scene was set for the birth of a new material-plastics (Zhang et al., 2009).

According to the Report on Carcinogens, formaldehyde ranks 25<sup>th</sup> in the overall U.S. chemical production, with more than 5 million tons produced each year (NTP, 2005). Formaldehyde annual production rises up to 21 million tons worldwide and it has increased in China with 7.5 million tons produced in 2007. Given its economic importance and widespread use, many people are exposed to formaldehyde environmentally and/or occupationally (Nazaroff et al., 2006).

Commercially, formaldehyde is manufactured as an aqueous solution called formalin, usually containing 37% by weight of dissolved formaldehyde.

This chemical is present in all regions of the atmosphere arising from the oxidation of biogenic and anthropogenic hydrocarbons (International Programme on Chemical Safety [IPCS] 1991; Granby et al., 1997). Formaldehyde concentration levels range typically from 2 to 45 ppbV (parts per billion in a given volume) in urban settings that are mainly governed by primary emissions and secondary formation (Chen et al., 2002; Naya & Nakanishi, 2005).

Primary formaldehyde is emitted from motor vehicles and fugitive industrial emissions, while secondary formaldehyde is produced by the photochemical oxidation of volatile organic compounds (VOCs) as the result of intense sunlight, especially during summer months (Odabasi & Seyfioglu, 2005). In addition, it has been postulated that formaldehyde can be produced by reactions involving anthropogenic and naturally occurring alkenes (Chen et al., 2002).

Removal of formaldehyde from the atmosphere can occur by chemical transformations, rain and snow scavenging of vapours and particles, by dry deposition of particles, and by vapour exchange across the air-water interface. Particle/gas phase distribution of formaldehyde is an important factor in determining its atmospheric fate, transport, and transformation (Odabasi & Seyfioglu, 2005).

Considering indoor air presence, homes containing large amounts of pressed wood products such as hard plywood wall paneling, particleboard, fiberboard, and Urea-Formaldehyde Foam Insulation (UFFI) often have elevated levels of formaldehyde emissions exceeding 0.3 ppm (U. S. Environmental Protection Agency [USEPA], 2007). Since 1985, the Department of Housing and Urban Development has only allowed the use of plywood particleboard that conforms to the 0.4 ppm formaldehyde emission limit in the construction of prefabricated and mobile homes (USEPA, 2007). Formaldehyde emission levels generally decrease as products age. In older homes without UFFI, concentrations of formaldehyde emissions are generally far below 0.1 ppm (USEPA, 2007). This value is close to the indoor limit, 0.1 mg/m<sup>3</sup> (0.08 ppm), recommended by the World Health Organization (World Health Organization - Regional Office of Europe [WHO-ROE], 2006), the limit followed by many other countries including the UK (Committee on the Medical Effects of Air Pollutants [COMEAP], 2004), and China (Standardization Administration of China [SAC], 2002).

Moreover, some studies have reported that seasonal variations resulted in higher indoor formaldehyde concentrations during the summer due to increased off gassing promoted by the higher temperatures (Kinney et al., 2002; Ohura et al., 2006; Yao & Wang., 2005). It seems that besides the type of materials used and home age also the season (warmer temperatures) influence formaldehyde concentrations in indoor settings (Viegas & Prista, 2010; Zhang et al., 2009).

Small amounts of formaldehyde are naturally produced in most organisms, including humans, as a metabolic byproduct (IARC, 2006; NTP, 2005), and are physiologically present in all body fluids, cells and tissues. The endogenous concentration in the blood of humans, monkeys and rats is approximately 2-3 mg/L (0.1 mM) (Casanova et al., 1988; Heck et al., 1985). Formaldehyde is also found in foods, either naturally or as a result of contamination (IARC, 2006). Therefore, everyone is continually exposed to small amounts of formaldehyde, environmentally present in the air, our homes and endogenously in our own bodies (Zhang et al., 2009).

Taking into account occupational settings, exposure involves not only workers in direct production of formaldehyde and products containing it, but also in industries utilizing these products, such as those related with construction and household (Zhang et al., 2009). The most extensive use of formaldehyde is in production of resins with urea, phenol and melamine, and also polyacetal resins. These products are used as adhesives in manufacture

of particle-board, plywood, furniture and other wood products (IARC, 2006). Formaldehyde is also used in cosmetics composition and has an important application as a disinfectant and preservative, reason why relevant workplace exposure may also occur in pathology and anatomy laboratories and in mortuaries (Goyer et al., 2004; IARC, 2006; Zhang et al., 2009).

The exposed workers, commonly found in resin production, textiles or other industrial settings, inhale formaldehyde as a gas or absorb the liquid through their skin. Other exposed workers include health-care professionals, medical-lab specialists, morticians and embalmers, all of whom routinely handle bodies or biological specimens preserved with formaldehyde (IARC, 2006; Vincent & Jandel, 2006; Zhang et al., 2009).

Concerning exposure limits in occupational settings, Occupational Safety and Health Administration (OSHA) has established the following standards that have remained the same since 1992: the permissible exposure limit (PEL) is 0.75 ppm (parts per million) in air as an 8-h time-weighted average ( $TWA_{8h}$ ) and the short-term (15 min) exposure limit (STEL) is 2 ppm. American Conference of Governmental Industrial Hygienists (ACGIH) recommended threshold limit value (TLV) is 0.3 ppm as a ceiling value. The National Institute of Occupational Safety and Health (NIOSH) recommends much lower exposure limits of 0.016 ppm ( $TWA_{8h}$ ) and 0.1 ppm (STEL), above which individuals are advised to use respirators if working under such conditions. In Portugal, the Portuguese Norm (NP 1796 - 2007) points also 0.3 ppm as a ceiling value.

The primary metabolite of formaldehyde is formate which is not as reactive as formaldehyde itself and can either enter into the one-carbon metabolic pool for incorporation into other cellular components, be excreted as a salt in the urine, or further metabolized to carbon dioxide (Agency for Toxic Substances and Disease Registry [ATSDR], 1999). The metabolic pathway to formate production is catalyzed by cytosolic glutathione (GSH)-dependent formaldehyde dehydrogenase (FDH). The reaction of formaldehyde with GSH yields (S)-hydroxymethylglutathione which, in the presence of  $NAD^+$  and FDH, forms the thiol ester of formic acid via the action of (S)-formyl glutathione hydrolase (SFGH) (Pyatt et al., 2008).

There is scientific evidence conclusively demonstrating that inhaled formaldehyde does not enter the systemic circulation to modify normally present endogenous levels (ATSDR, 1999; Heck & Casanova, 2004). This is likely due to the high water solubility of formaldehyde and its rapid metabolism. The lack of systemic distribution is evidenced by a variety of studies in rodents, monkeys and humans (Pyatt et al., 2008).

It seems clear that as long as inhaled levels of formaldehyde are below concentrations that can be rapidly metabolized by tissue formaldehyde dehydrogenase and other highly efficient detoxification enzymes, normal endogenous concentrations (0.1 mM) of formaldehyde in the blood do not increase (ATSDR, 1999; Heck & Casanova, 2004).

Human studies have shown that chronic exposure to formaldehyde by inhalation is associated with eye, nose and throat irritation (Arts et al., 2008). Sensory irritation leads to reflex responses such as sneezing, lacrimation, rhinorrhea, coughing, vasodilatation and changes in the rate and depth of respiration. The latter results are a decrease in the total amount of inhaled material resulting in a protective effect to the individual. Trigeminal stimulation is not necessarily an indication of cell or tissue damage. At higher concentrations formaldehyde will lead to cytotoxic reactions; this cytotoxic respiratory tract

irritation is a localized pathophysiological response to a chemical, involving local redness, swelling, or itching (Arts et al., 2006).

Formaldehyde was long considered as a probable human carcinogen (Group 2A chemical) based on experimental animal studies and limited evidence of human carcinogenicity. More recently, several studies report a carcinogenic effect in humans after chronic exposure to formaldehyde, in particular an increased risk for nasopharyngeal cancer (Armstrong et al., 2000; Coggon et al., 2003; Hildesheim et al., 2001; Lubin et al., 2004; Vaughan et al., 2000). Since 2006, IARC classifies formaldehyde as carcinogenic to humans (Group 1), based on sufficient evidence in humans and in experimental animals (IARC, 2006). IARC also concluded that there is a “strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde”.

The “strong” evidence for a causal relationship between formaldehyde exposure and leukaemia comes from recent updates of two of the three major industrial cohort studies of formaldehyde-exposed workers (Hauptmann et al., 2003; Pinkerton et al., 2004). These data have strengthened a potential causal association between leukemia and occupational exposure to formaldehyde, especially for myeloid leukemia (Zhang et al., 2009).

Nevertheless, some authors have argued that it is biologically implausible for formaldehyde to cause leukaemia (Cole & Axten, 2004; Marsh & Youk, 2004). Their primary arguments against the human leukemogenicity of formaldehyde are: (1) it is unlikely to reach the bone marrow and cause toxicity due to its highly reactive nature; (2) there is no evidence that it can damage the stem and progenitor cells, the target cells for leukemogenesis; and (3) there is no credible experimental animal model for formaldehyde-induced leukaemia. This led Pyatt et al., (2008) to recently comment that “the notion that formaldehyde can cause any lymphohematopoietic malignancy is not supported with either epidemiologic data or current understanding of differing etiologies and risk factors for the various hematopoietic and lymphoproliferative malignancies”. Indeed, IARC itself concluded that “based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukaemia in humans” and stated that “this is an area needing more research” (IARC, 2006; Cogliano et al., 2005; Zhang et al., 2009).

However, recently, IARC reaffirmed the classification of formaldehyde in Group I, based on sufficient evidence in humans of nasopharyngeal cancer. Considering the possible association with leukemia the epidemiological evidence has become stronger and IARC has concluded that there is sufficient evidence for leukaemia, particularly myeloid leukaemia (Baan et al., 2009; Hauptmann et al., 2009; IARC, 2006).

Moreover, in 2010 Schwilk and colleagues performed an up-dated meta-analyses focusing in higher exposure groups and myeloid leukemia and included two large recent studies and conclude that formaldehyde exposure is associated with increased risks of leukemia, particularly myeloid leukemia and highlight the importance of focusing on high-exposure groups and myeloid leukemia when evaluating the human carcinogenicity of formaldehyde (Schwilk et al., 2010).

In the case of formaldehyde exposure assessment and considering that health effects seems to be mainly related with the high concentration peaks than with long time exposure at low levels, the strategy to perform exposure assessment in occupational settings must be based on the determination of ceilings concentrations. This option might be the best to evaluate

exposures and to obtain data for risk assessment development (Hauptmann et al., 2009; IARC, 2006).

Manifold *in vitro* studies clearly indicated that formaldehyde can induce genotoxic effects in proliferating cultured mammalian cells (IARC, 2006). Furthermore, some *in vivo* studies detected changes in epithelial cells (oral and nasal) and in peripheral lymphocytes related to formaldehyde exposure (Speit & Schmid, 2006; Suruda et al., 1993).

Frequency of micronucleus (MN) in buccal and/or nasal mucosa cells is being used to investigate local genotoxicity. According to reports concerning experimental genotoxicity studies, MN are the most sensitive genetic endpoints for detection of formaldehyde induced genotoxicity (Merck & Speit, 1998). Thus, MN test with exfoliated cells could be a powerful tool for detection of local genotoxic effects in humans, which is fundamental for hazard identification and risk estimation (Speit & Schmid, 2006).

MN in peripheral blood lymphocytes has been extensively used to evaluate the presence and extend of chromosome damage in human populations exposed to genotoxic agents. As advantages, this MN test provides a reliable measure of chromosomal breakage and loss at lower cost and more easily than chromosomal aberrations. Moreover, the availability of cytokinesis-block technique eliminates potential background caused by effects on cell division kinetics (Bonassi et al., 2001).

Research work has been developed to know occupational exposure to formaldehyde in two different occupational settings (resins production and in pathology and anatomy laboratories) from Portugal and, also, study eventual health effects related with exposure. The objective of this chapter is to describe the work developed and discuss the obtained results.

## **2. Research developed**

### **2.1 Materials and methods**

#### **2.1.1 Subjects**

This study was carried out in Portugal, in 80 workers occupationally exposed to formaldehyde vapours: 30 workers from formaldehyde and formaldehyde-based resins production factory and 50 from 10 pathology and anatomy laboratories. A control group of 85 non-exposed subjects was considered. All subjects were provided with the protocol and with the consent form, which they read and signed.

Health conditions, medical history, medication and lifestyle factors for all studied individuals, as well as information related to working practices (such as years of employment) were obtained through a standard questionnaire.

#### **2.1.2 Exposure assessment**

Two different exposure assessment methods were, simultaneously, applied in the 10 anatomy and pathology laboratories in Portuguese hospitals and in the formaldehyde and formaldehyde-based resins production factory, in order to assess formaldehyde occupational exposure. Environmental monitoring was performed between the period of September 2007 and March of 2008.

In these two occupational settings were identified different exposure groups. In laboratories were defined three, namely pathologists, technicians and technical assistant. Also, in the factory were define three groups – production of resins, impregnation and quality control. These definitions were based essentially on activities similarity.

### 2.1.3 Methods

#### Method 1

In one of the methods 30 environmental samples were obtained by personal air sampling with low flow rate (0.01 to 0.10 L/min) pumps (Zambelli) during a typical working day. The sorbent tubes used were impregnated with 10% (2-hydroxymethyl)piperidine. Sampling time was 6 to 7 hours. Two to three samples were collected in each laboratory by the use of electric flow pumps which were placed in a worker of each exposure group.

Formaldehyde levels were measured by Gas Chromatography (GC). Capillary column: Supelcowax<sup>10</sup> - 30 m x 0.32 mm x 0.5 µm; analyte: oxazolidine derivative of formaldehyde; desorption: 1 mL toluene; 60 min ultrasonic; injection volume: 1 µL splitless; split vent time 30 sec; temperature: injector: 250 °C, detector: 300 °C, column: 70 °C for 1 min; 15 °C/min; hold at 240 °C for 10 min; carrier gas: He – 1.5 mL/min; calibration: formalin solution spiked on sorbent – 4.7 µg/mL, 6.0 µg/mL, 13.0 µg/mL, 25.0 µg/mL, 40.0 µg/mL, 50.0 µg/mL, 100.0 µg/mL e 200.0 µg/mL; calibration curve:  $y=0.008522x - 0.008109$   $r^2 = 0.999968$ , LOD: 1µg/sample . Analysis and time-weighted average ( $TWA_{8h}$ ) estimated according to the National Institute of Occupational Safety and Health method - NIOSH 2541 (National Institute of Occupational Safety and Health [NIOSH], 1994).

#### Method 2

Ceiling values for formaldehyde exposure were obtained using Photo Ionization Detection (PID) direct-reading equipment (with an 11.7 eV lamp) designated by First-Check, from Ion Science. This equipment accurately detects formaldehyde from 1 ppb to 10,000 ppm and performs automatically data log readings from the sensor on a second basis. Measurements were performed in each task and readings were stored in instrument internal memory with a date and time stamp. At the same time it was performed video recording and synchronized with real-time exposure data obtained with PID equipment followed by combination of the exposure profile with the video image of worker activity.

With this method it was possible to establish a relation between worker activities and ceiling values, and to determine principal emission sources.

Eighty three activities were studied in the 10 laboratories and three activities in the factory. All tasks were studied in normal conditions, namely using ventilation dispositive and, as usual, none of the workers was using masks to protect from formaldehyde vapours.

In both methods sampling/measures were performed near workers respiratory system.

Data obtained from NIOSH 2451 method was compared with reference value from OSHA (TLV-TWA=0.75 ppm) because there is no reference in Portugal for this exposure metric. The ceiling values obtained from PID method were compared with reference value from Portuguese Norm 1796-2007 (0.3 ppm).

### 2.1.4 Biological monitoring

To evaluate the effects of the occupational exposure, the study of effect biomarkers was conducted. The biomarkers of effect studied were specifically genotoxicity biomarkers, namely micronuclei in two different biological matrixes - peripheral blood lymphocytes and buccal exfoliated cells.

The protocol used to measure the MN in peripheral blood lymphocytes was the fully validated cytokinesis-blocked micronucleus assay (CBMN), developed by Fenech [20], where it is used cytochalasin B to block the cytokinesis in order to lymphocytes had a binucleated appearance. Heparinized blood samples were obtained by venipuncture from all subjects and freshly collected blood was directly used for the MN test. Lymphocytes were isolated using Ficoll-Paque gradient and placed in RPMI 1640 culture medium with L-glutamine and red phenol added with 10% inactivated fetal calf serum, 50 ug/ml streptomycin + 50U/mL penicillin, and 10 ug/mL phytohaemagglutinin. Duplicate cultures from each subject were incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 44h, and cytochalasin-b 6 ug/mL was added to the cultures in order to prevent cytokinesis. After 28h incubation, cells were spun onto microscope slides using a cytocentrifuge. Smears were air-dried and double stained with May-Grünwald-Giemsa and mounted with Entellan. The frequencies of binucleated cells with MN were determined analyzing 1000 lymphocytes from two slides for each subject.

The optimal protocol of MN test for buccal exfoliated cells was performed after many experiments. In order to reach the optimal protocol, different techniques of collecting the cells and the staining were done.

Concerning to the sample collection, it was considered that the best way of obtaining the sample it was by scrapping the inner checks of the individuals with an endobrush and directly performed a smear in two slides. The samples were immediately fixed with Mercofix®, a methanol based preservative.

The staining protocols selected were based on the affinity of the stains with the nucleus: Hematoxilin-Eosin, Hematoxilin, Giemsa, May-Grunwald Giemsa, Papanicolaou, Feulgen with Light Green and Feulgen.

The reliable results were achieved with Feulgen without counterstain (Nersesyan et al., 2006). This technique consists in a first step of hydrolysis with HCL 5M followed by washing with distillate water, incubation with Schiff Reagent and tap water final washing. The slides were allowed to air dried, mounted with entellan®. Two thousand cells were scored from each individual. Only cells containing intact nuclei, neither clumped nor overlapping were included in the analysis.

The criteria for scoring the nuclear abnormalities in lymphocytes and MN in buccal cells were described by Fenech et al. (1999) and Tolbert et al. (1991), respectively.

## 2.2 Results

### 2.2.1 Characteristics of the studied population

The characterization of the population studied is summarized in Table 1. Controls and exposed workers did not differ significantly in age and in smoking habits. Only for gender distribution a significant difference was found between the two groups ( $p=0.002$ ), due to the larger number of women in the control group.

|                    | Control Group | Exposed Group | P value |
|--------------------|---------------|---------------|---------|
| Number of subjects | 85            | 80            |         |
| Gender             |               |               |         |
| Male               | 31 (36.6%)    | 48 (60.0%)    | 0.002   |
| Female             | 54 (63.5%)    | 32 (40.0%)    |         |
| Age (years)        |               |               |         |
| Range              | 20-55         | 19-56         | 0.180   |
| Mean               | 33.87         | 35.74         |         |
| St. Deviation      | 8.262         | 9.470         |         |
| Smoking status     |               |               |         |
| Non-smokers        | 59 (69.4%)    | 55 (68.8%)    | 0.927   |
| Smokers            | 26 (30.6%)    | 25 (31.3%)    |         |
| Years of exposure  |               |               |         |
| Range              | -----         | 1 – 35        |         |

Table 1. Characterization of the studied population

None of the individuals presented relevant information about health conditions, medical history, medication and lifestyle factors that could influence the results of MN test.

### 2.2.2 Exposure assessment

Formaldehyde exposure values were determined using the above described methods: NIOSH 2541 for average concentrations ( $TWA_{8h}$ ) and PID method to obtain ceiling concentrations (Tables 2 and 3).

| Laboratories | Exposure Groups     | FA $TWA_{8h}$<br>n=29<br>(ppm) | FA Ceiling *<br>n=83<br>(ppm) | Range<br>Ceiling values<br>(ppm) | Mean<br>Ceiling values<br>(ppm) |
|--------------|---------------------|--------------------------------|-------------------------------|----------------------------------|---------------------------------|
| A            | Technical Assistant | 0.27                           | 2.51                          | 1.05 - 2.51                      | 1.78                            |
|              | Pathologist         | <LOD                           | 3.19                          | 0.34 - 3.19                      | 1.04                            |
|              | Technician          | 0.16                           | NM                            | -----                            |                                 |
| B            | Technical Assistant | 0.15                           | 0.62                          | 0.62                             | -----                           |
|              | Pathologist         | 0.24                           | 2.71                          | 1.49 - 3.36                      | 2.23                            |
|              | Technician          | 0.16                           | 3.36                          | 1.91 - 3.36                      | 2.31                            |
| C            | Technical Assistant | 0.12                           | 0.53                          | 0.53                             | -----                           |
|              | Pathologist         | 0.47                           | 2.93                          | 1.53 - 2.93                      | 2.18                            |
|              | Technician          | 0.51                           | 2.28                          | 2.22 - 2.28                      | 2.25                            |
| D            | Technical Assistant | < LOD                          | NM                            | -----                            |                                 |
|              | Pathologist         | 0.07                           | 2.31                          | 2.09 - 2.31                      | 2.21                            |
|              | Technician          | 0.11                           | 0.85                          | 0.85                             | -----                           |
| E            | Technical Assistant | < LOD                          | NM                            | -----                            | -----                           |
|              | Pathologist         | 0.06                           | 1.10                          | 0.95 - 1.10                      | 1.03                            |
|              | Technician          | 0.07                           | 0.85                          | 0.85                             | -----                           |
| F            | Technical Assistant | 0.09                           | NM                            | -----                            | -----                           |
|              | Pathologist         | 0.23                           | 0.34                          | 0.22 - 0.34                      | 0.28                            |
|              | Technician          | 0.12                           | 0.28                          | 0.28                             | -----                           |

| Laboratories | Exposure Groups     | FA TWA <sub>8h</sub><br>n=29<br>(ppm) | FA Ceiling *<br>n=83<br>(ppm) | Range<br>Ceiling values<br>(ppm) | Mean<br>Ceiling values<br>(ppm) |
|--------------|---------------------|---------------------------------------|-------------------------------|----------------------------------|---------------------------------|
| G            | Technical Assistant | 0.16                                  | 0.71                          | 0.64 - 1.71                      | 0.67                            |
|              | Pathologist         | 0.05                                  | 2.81                          | 0.18 - 2.81                      | 0.56                            |
|              | Technician          | 0.04                                  | 1.26                          | 1.26                             | ----                            |
| H            | Technical Assistant | 0.25                                  | 0.68                          | 0.68                             | ----                            |
|              | Pathologist         | 0.11                                  | 2.08                          | 1.21 - 2.08                      | 1.65                            |
|              | Technician          | 0.25                                  | 0.68                          | 0.68                             | ----                            |
| I            | Technical Assistant | 0.05                                  | 0.95                          | 0.95                             | ----                            |
|              | Pathologist         | < LOD                                 | 0.47                          | 0.21 - 0.47                      | 0.34                            |
|              | Technician          | 0.06                                  | NM                            | ----                             | ----                            |
| J            | Technical Assistant | NM                                    | NM                            | ----                             | ----                            |
|              | Pathologist         | 0.13                                  | 5.02                          | 1.15 - 5.02                      | 3.24                            |
|              | Technician          | 0.08                                  | 4.32                          | 4.32                             | ----                            |

\* Higher values for each exposure group

< LOD - Below the Detection limit

FA-formaldehyde

NM - Not measured

Table 2. Formaldehyde exposure results in laboratories

| Exposure groups      | FA<br>TWA <sub>8h</sub><br>n=3<br>(ppm) | FA Ceiling *<br>n=3<br>(ppm)               | Range<br>Ceiling<br>values<br>(ppm) | Mean<br>Ceiling<br>values<br>(ppm) |
|----------------------|---|--|-------------------------------------|------------------------------------|
| Production of resins | NM                                      | Collecting a sample of the reactor<br>1.02 | 0.01 - 1.02                         | 0.15                               |
| Impregnation         | < LOD                                   | Operation of impregnation machine<br>1.04  | 0.00 - 1.04                         | 0.21                               |
| Quality control      | < LOD                                   | analyze a resin sample<br>0.52             | 0.01 - 0.52                         | 0.08                               |

\* Higher values for each exposure group

< LOD - Below the Detection limit

NM - Not measured

FA -formaldehyde

Table 3. Formaldehyde exposure results in the factory

All of the results for time-weighted average concentrations (TWA<sub>8h</sub>) not exceeded OSHA reference value (0.75 ppm), with the majority of values falling below the method detection limited.

On the opposite, for ceiling concentrations all the higher results obtained for each exposure group in each occupational setting exceeded the reference value (0.3 ppm). In laboratories, values lied between 0.18 ppm and 5.02 ppm, with a mean of 2.52 ppm. In the factory the concentration values registered each second lied between 0.0 and 1.02 ppm.

The three activities studied in the factory have result above the reference value for ceiling concentrations (0.3 ppm).

In production of resins the higher concentration value was obtained during the collection of a sampling in resins reactor performed by a production operator. In this case and during operation of impregnation machine there were not local exhaust ventilation dispositive. Only in the "quality control" exposure group there was a small hotte that is not normally used to perform quality analysis of resins.

In the case of laboratories, all of them had, at least, one task with a higher result than the reference value (0.3 ppm) (Figure 1).

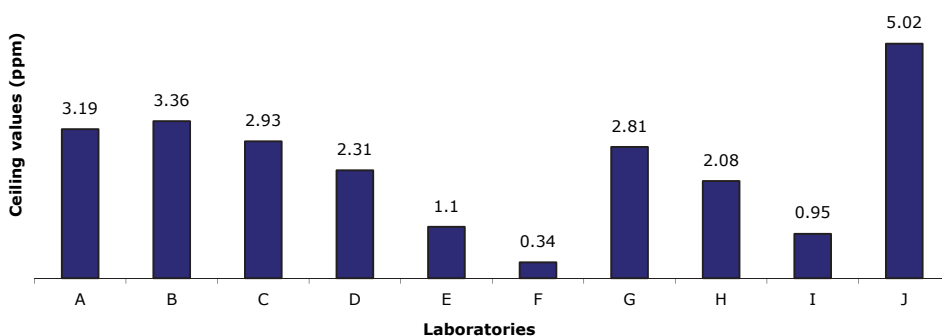


Fig. 1. Higher ceiling value obtained in each laboratory

Considering all of the 83 tasks studied in the laboratories (Table 4), 93% of the results were higher than the reference value for ceiling concentrations (0.3 ppm).

Highest exposure level was observed during "macroscopic examination" of formaldehyde-preserved specimens. This task is developed in a macroscopic bench with local exhaust ventilation. In all the laboratories studied was verified that ventilation was functioning normally.

The task "data registration" showed also a high formaldehyde concentration value, being important to note that this task occurs during macroscopic examination (Table 4).

Concerning the 69 macroscopic examinations, the most frequent task develop in this laboratories, it was possible to verify that near 93% of formaldehyde concentration values were higher than 0.3 ppm.

In this occupational setting, highest score for ceiling values was identified in the results of the exposure group "Pathologists" and the highest mean was obtained for the "Technicians" group (Table 5).

It is important to consider that none of the workers of the two occupational settings were using appropriate respiratory protection during the tasks studied.

| Tasks                                   | Number | Ceiling Values (ppm) | Exposed Workers                     |
|---|--------|----------------------|-------------------------------------|
| Macroscopic examination                 | 69     | 5.02                 | Pathologist                         |
| Disposal of specimen and used solutions | 5      | 0.95                 | Technicians and Technical Assistant |
| Jar filling                             | 2      | 2.51                 | Technical Assistant                 |
| Data registration                       | 3      | 4.32                 | Technicians                         |
| Specimen wash                           | 2      | 2.28                 | Technicians                         |
| Biopsy                                  | 2      | 1.91                 | Technicians                         |

Table 4. Formaldehyde exposure during laboratories tasks

| Exposure Groups     | Tasks studied* | Range (ppm) | Mean (ppm) | St. Deviation (ppm) |
|---------------------|----------------|-------------|------------|---------------------|
| Technical Assistant | 9              | 0.28 - 2.51 | 0.86       | 0.58                |
| Pathologist         | 65             | 0.21 - 5.02 | 1.42       | 1.07                |
| Technician          | 14             | 0.68 - 4.32 | 2.04       | 0.95                |

\* some activities involved the simultaneously exposure of two groups

Table 5. Ceiling results for each exposure group

### 2.2.3 Biological monitoring

Table 6 showed that the frequency of MN in occupationally exposed workers was significantly higher in comparison with the control group, both in peripheral blood lymphocytes ( $p < 0.001$ ) and in epithelial buccal cells ( $p < 0.001$ ).

|  | Controls        | Exposed         |                                    |                 |
|--|-----------------|-----------------|------------------------------------|-----------------|
|  |                 | Factory         | Pathology and anatomy laboratories | Total           |
| MN PBL <sup>1</sup><br>Mean $\pm$ Std. Dev | 1.17 $\pm$ 1.95 | 1.76 $\pm$ 2.07 | 3.70 $\pm$ 3.86                    | 2.97 $\pm$ 3.42 |
| MN EBC <sup>2</sup><br>Mean $\pm$ Std. Dev | 0.13 $\pm$ 0.48 | 1.27 $\pm$ 1.55 | 0.64 $\pm$ 1.74                    | 0.88 $\pm$ 1.69 |

<sup>1</sup> Peripheral Blood Lymphocytes

<sup>2</sup> Epithelial Buccal Cells

Table 6. Frequency of MN in the studied population

When analyzing each occupational setting separately, we found significant differences in MN frequencies in peripheral blood lymphocytes ( $p < 0.001$ ) and in epithelial buccal cells ( $p < 0.005$ ) between the laboratories and control groups. Concerning the factory group,

significant differences in MN frequencies were only detected in epithelial buccal cells ( $p < 0.001$ ).

Finally, it was compared MN frequencies between the two exposed groups and found that MN frequency in peripheral blood lymphocytes was significantly higher in the laboratories group ( $p < 0.005$ ), but respecting to epithelial buccal cells there was no significant difference between them ( $p = 0.108$ ).

In what concern to the three exposure groups studied in the pathology anatomy laboratories, the pathologists group has higher MN mean in lymphocytes and the technician had higher MN mean in buccal cells (Table 7).

|   | Pathology and anatomy laboratories |                  |                     |
|---|------------------------------------|------------------|---------------------|
|   | Pathologist                        | Technician       | Technical Assistant |
| MN PBL<br>Mean $\pm$ Std. Error of Mean | 5.00 $\pm$ 1.24                    | 3.76 $\pm$ 0.647 | 4.13 $\pm$ 1.55     |
| MN EBC<br>Mean $\pm$ Std. Error of Mean | 0.58 $\pm$ 0.434                   | 1.18 $\pm$ 0.406 | 0.88 $\pm$ 0.611    |

Table 7. Frequency of MN in the exposure groups of laboratories

Factory results reveal quality control group with higher MN mean in lymphocytes and also in buccal cells (Table 8).

|   | Factory           |                 |                 |
|---|-------------------|-----------------|-----------------|
|   | Resins Production | Impregnation    | Quality control |
| MN PBL<br>Mean $\pm$ Std. Error of Mean | 1.85 $\pm$ 2.48   | 1.16 $\pm$ 1.04 | 4.5 $\pm$ 0.7   |
| MN EBC<br>Mean $\pm$ Std. Error of Mean | 0.66 $\pm$ 0.94   | 1.75 $\pm$ 1.79 | 3.5 $\pm$ 0.5   |

Table 8. Frequency of MN in the exposure groups of factory

### 2.3 Discussion

As indicated by several studies (IARC, 2006; Orsière et al., 2006; Shaham et al., 2003) exposure assessment in present investigation demonstrates that both occupational settings studied involve exposure to high peak formaldehyde concentrations.

The importance of this consideration lies in the fact that health effects (cancer) linked to formaldehyde exposure are more related with peaks of high concentrations than with long time exposure at low levels (IARC, 2006; Pyatt et al., 2008). Moreover, the choice of exposure metric should be based on the most biologically relevant exposure measure in order to diminish misclassification of exposure, thus leading to attenuated exposure-response relationships (Preller et al., 2004). Furthermore, high exposures of short duration (peaks) are of special concern, because they can produce an elevated dose rate at target tissues and organs, potentially altering metabolism, overloading protective and repair mechanisms and

amplifying tissue responses (Preller et al., 2004; Smith, 2001). In addition, Pyatt et al. (2008) pointed out, as a limitation in most epidemiological studies, the lack of data about exposure to peak concentrations. Therefore, in those studies, health effects resulting from occupational exposure to formaldehyde are associated to exposure exclusively based on time-weighted average concentrations (Pyatt et al., 2008). Until 2004 only two studies concerning the association between exposure to formaldehyde and nasopharyngeal cancer that presented data on exposure to ceiling concentrations obtained higher relative risk values compared with the other studies (Hauptmann et al., 2004; Pinkerton et al., 2004; Zhang et al., 2009).

Recently Hauptmann and colleagues have found that mortality rate from leukemia increased significantly not just with number of years of activity, in this case embalming, but also with increasing peak formaldehyde exposure (Dreyfuss, 2010; Hauptmann et al., 2009).

Results in laboratories indicate “macroscopic examination” as the task involving the highest exposure. This is probably because precision and very good visibility is needed and, therefore, pathologists must lean over the specimen with consequent increase of proximity to formaldehyde emission sources. Studies developed by Goyer et al., (2004) and Orsière et al., (2006) support that proximity to impregnated specimens promotes higher exposure to formaldehyde. “Pathologist” is normally the exposure group that performs this task. However, the “Technician” group obtained, simultaneously, higher  $TWA_{8h}$  and higher mean of ceiling values. This can be due to the fact that this is the group involved in more tasks related with formaldehyde exposure, during the working day.

In the case of the factory, the task “collecting a sample of the reactor” involved a manual process. Probably the proximity and reactor open promote exposure.

It is important to refer that these type of information (exposure determinants, emission sources and exposed workers) was only possible to obtain because video recording could be performed simultaneously with concentration measurements.

This resource gives opportunity to directly relate performance with exposure (Mcglathlin, 2005; Ryan et al., 2003; Rosén et al., 2005). Additionally, real-time measurements are useful also for evaluating engineering controls and their efficacy (Yokel & MacPhail, 2011).

In addition, and in agreement with other studies (Kromouht, 2002; Meijster et al., 2008; Susi & Schneider, 1995), it is possible to conclude that  $TWA_{8h}$  measurements give poor information and is of less utility in the identification of tasks that should be targeted for control.

Long exposures to formaldehyde, as those to which some workers are subjected for occupational reasons, are suspected to be associated with genotoxic effects that can be evaluated by biomarkers (Conaway et al., 1996; IARC, 2006; Viegas & Prista, 2007). In this study, the results suggest that workers in pathological anatomy laboratories are exposed to formaldehyde levels that exceed recommended exposure criteria and a statistically significant association was found between formaldehyde exposure and biomarkers of genotoxicity, namely MN in lymphocytes and buccal cells.

Chromosome damage and effects upon lymphocytes arise because formaldehyde escapes from sites of direct contact, such as mouth, originating nuclear alterations in lymphocytes of those exposed (He & Jin, 1998; Orsière et al., 2006; Ye et al., 2005; Zhang et al., 2009).

Our results corroborate previous reports (Ye et al., 2005) that lymphocytes can be compromised by long term exposures. Moreover, the changes in peripheral lymphocytes can be a sign that the cytogenetic effects triggered by formaldehyde can reach tissues faraway from the site of initial contact (Suruda et al., 1993). Long term exposures to high concentrations of formaldehyde indeed appear to have a potential for generalized DNA damage. In experimental studies with animals, local genotoxic effects following formaldehyde exposure have been previously demonstrated to give rise to DNA-protein cross links, structural chromosomal aberrations, and aberrant cells (IARC, 2006). In our research work the MN frequency in peripheral blood lymphocytes was significantly higher in the laboratories group in comparison with the factory, probably because the years of exposure are higher in the first group.

In humans, formaldehyde exposure is associated with an increase in the frequency of MN in buccal epithelium cells (Burgaz et al., 2002; Speit et al., 2007) as corroborated by the results presented here.

Suruda et al. (1993) claims that although changes in oral and nasal epithelial cells and peripheral blood cells do not indicate a direct mechanism leading to carcinogenesis, they do indicate that DNA alterations took place. It thus appears reasonable to conclude that formaldehyde is a risk factor for those that are occupationally exposed in these two occupational settings (IARC, 2006).

In human biomonitoring studies it is important to assess the influence of major confounding factors such as gender, age and smoking habits in the endpoints studied. However, in our results no significant differences were obtained in MN frequencies between women and men (both in peripheral blood lymphocytes and epithelial buccal cells). However, in other studies an increase in MN frequencies in women was found. Current knowledge on the effect of gender on genetic damage determines a 1.5-fold greater MN frequency in females than in males (Fenech et al., 2003; Wojda et al., 2007), which can be explained by preferential aneugenic events involving the X-chromosome. Surrallés et al. (1996) reported an excessive overrepresentation of this chromosome in micronucleic lymphocytes cultured from women.

Tobacco smoke contains a high number of mutagenic and carcinogenic substances and is causally linked to an elevated incidence of several forms of cancers (IARC, 1985). Hence, smoking is an important variable to consider in biomonitoring studies and, particularly in this study since formaldehyde is present in tobacco smoke (IARC, 2006). The effect of tobacco smoking on MN frequency in human cells has been object of study. In most reports the results were unexpected, as in many instance smokers had lower frequencies of MN than non-smokers (Bonassi et. 2003; Orsière et al., 2006). In the present study no significant differences were found in MN (peripheral blood lymphocytes and epithelial buccal cells) between smokers and non-smokers. These findings are similar to results obtained in the study of Bonassi et al., (2003). These authors recommend that quantitative data about smoking habit should be collected because the sub-group of heavy smokers ( $\geq 30$  cigarettes per day) can influence the results. For notice, the questionnaire results of this study revealed no heavy smokers in these two workers groups.

### 3. Conclusion

Some preventive measures can be applied to reduce exposure to formaldehyde in these two occupational settings. In the case of anatomy and pathology laboratories exposure reduction can be achieved by the use of adequate local exhausts ventilation, relocation of the specimen containers to areas with isolated ventilation and using hooded enclosures over such containers.

For the factory, preventive measures must consider automating some processes like sampling in reactors and, additionally, promote the use of the existing located ventilation dispositive.

Exposure assessment methods applied in the research developed permitted to conclude that TWA<sub>8h</sub> measurements give poor information concerning to preventive measures priority and CBMN assay applied to assess genotoxic effects is a screening technique that can be used for clinical prevention and management of workers under occupational carcinogenic risks, namely exposure to a genotoxic agent such as formaldehyde.

The most recent studies suggest that future research is warranted to more effectively assess the risk of leukemia arising from formaldehyde exposure and to better explain some inconsistencies in mode of action and, also, to understand the role of short-term peak exposures.

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