Micronuclei in Peripheral Blood Lymphocytes in Formaldehyde Occupationally Exposed Workers

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INTRODUCTION

FORMALDEHYDE

Formaldehyde (CH_2O) the most simple and reactive of all aldehydes, is a colorless, reactive and readily polymerizing gas at normal temperature *(Zang et al., 2009)*. It has a pungent, suffocating odour that is recognized by most human subjects at concentrations below 1 ppm *(Speit et al., 2007)*.
According to the Report on Carcinogens, formaldehyde (FA) ranks 25th in the overall U.S. chemical production with more than 11 billion pounds (5 million tons) produced each year (NTP, 2005). Is an important industrial compound that is used in the manufacture of synthetic resins and chemical compounds such as lubricants and adhesives *(WHO, 1989)*. It has also applications as a disinfectant, preservative and is used in cosmetics. Estimates of the number of persons who are occupationally exposed to FA indicate that, at least at low levels, may occur in a wide variety of industries *(Kauppinen et al., 2000)*. The occupational settings with most extensive use of formaldehyde is in the production of resins and in anatomy and pathology laboratories *(IARC, 2006)*.
Several studies reported a carcinogenic effect in humans after inhalation of FA, in particular an increased risk for nasopharyngeal cancer. Nowadays, the International Agency for Research on Cancer (IARC) classifies FA as carcinogenic to humans (group 1), on the basis of sufficient evidence in humans and sufficient evidence in experimental animals *(IARC, 2006)*. Manifold in vitro studies clearly indicated that FA is genotoxic. FA induced various genotoxic effects in proliferatin cultured mammalian cells. A variety of evidence suggests that the primary DNA alterations after FA exposure are DNA-protein crosslinks (DPX). Incomplete repair of DPX can lead to the formation of mutations *(Speit et al., 2007)*.

MICRONUCLEUS ASSAY

MN are highly relevant because they represent true mutagenic events. According to the experience with experimental genotoxicity studies, MN belong to the most sensitive genetic endpoints for the detection of FA induced genotoxicity *(Merk & Speit, 1998)*. The MNT with exfoliated cells could be a powerful tool for the detection of local genotoxic effects in humans, and the detection of these effects is of fundamental importance for hazard identification and risk estimation (Speit & Schmid, 2006). The use of MN in peripheral blood lymphocytes (PBL) has been extensively used to evaluate the presence and the extend of chromosome damage in human populations exposed to genotoxic agents in various occupational settings, in the environment, on as a consequence of lifestyles.
Zang *et al.* (2009) hypothesize that FA may act on bone marrow directly or, alternatively, may cause leukaemia by damaging the haematopoietic stem or early progenitor cells that are located in the circulating blood or nasal passages, which would then travel to bone marrow and become leukemic stem cells.
However, with actual knowledge we can conclude that, regard to risk estimation, local toxic effects at site of first contact seem to be most relevant *(Herausgegeben, et al., 2006; Speit & Schmid, 2006; IARC, 2006)*.
As advantages the PBL MN test provides a reliable measure of chromosomal breakage and loss at lower cost and with less work than chromosomal aberrations and the availability of the cytokinesis-block technique has removed the potential confounding caused by effects on cell division kinetics (Bonassi *et al.*, 2001).

AIM OF THE STUDY

To evaluate genotoxic effects related to formaldehyde occupational exposure using MN test in peripheral blood lymphocytes.
To evaluate the differences of information by the application of two environmental monitoring methodologies.

MATERIAL AND METHODS

This study was carried out in Portugal, in 89 workers occupationally exposed to FA : 34 workers in a FA production and FA-based resins factory and 55 in 10 pathology anatomy laboratories. A control group of 95 non-exposed subjects was considered. All subjects were provided the protocol and consent form, which the subjects read and signed. Exposure assessment was done by applying simultaneously two techniques of air monitoring. In one of the methodologies environmental samples were obtained by personal air sampling with low flow pumps during a typical working day. Sampling time was 6 to 8 hours. Formaldehyde levels were measured by Gaseous Chromatography (GC) analysis and time-weighted average (TWA8) estimated according to the NIOSH method (NIOSH 2541) *(NIOSH, 1994)*. The second methodology was aimed to measure ceiling values of FA using Photo Ionisation Detection (PID) equipment (with 11,7 eV lamps) with simultaneously video recording.
Evaluation of genotoxic effects was performed by application of MN test in peripheral blood lymphocytes. Heparinized venous blood was collected between 10 and 12 a.m., from each subject, and was processed by application of cytokinesis block micronucleus assay. All samples were stained with May-Grunwald Giemsa and coded and analysed under blind conditions. The criterion of scoring the cells with MN was the same as the described in "The Human MicroNucleus Project" *(*Fenech *et al., 2003)*.

RESULTS

Table 1. General characteristics of exposed and control subjects

Characteristics	Exposed	Controls
Gender		
Males	60%	36,6%
Females	40%	63,5%
Age	35,74	33,87
Range	19-56	20-55
Smoking habits		
Smokers	41%	31%
Non-smokers	59%	69%

Table 2. FA levels of the exposed groups

	Factory Workers (n = 34)	Laboratory workers (n= 46)
FA exposure level (TWA) (ppm)		
Range	0,20 – 6,3	0,05 – 0,51
Mean	3,25	0,28
FA ceiling concentration (ppm)		
Range	0,003 – 1,04	0,02 – 5,02
Mean	0,52	2,52
Exposure duration		
Range	1 – 27	1 – 33
Mean	6,26	13,55

Table 3. Micronucleated cells in peripherial blood lymphocytes

Groups	Factory Workers (‰)	Laboratory Workers (‰)
Exposed	2,1	3,98
Exposed Mean	3,28	
Controls Mean	1,25	

CONCLUSIONS

FA exposure assessment shows that the two groups of workers (factory and laboratory) were exposed to high peak concentrations. This is consonant with several studies that point pathology and anatomy laboratories and resins production as the occupational settings were the workers are frequently exposed to high peak concentrations of FA *(Shaham et al., 1997; Burgaz et al., 2002; Goyer et al., 2004; Albuquerque et al., 2005; Orsière et al., 2006)*. This is very important question considering that health effects resulting from FA exposure are more related with peaks of high concentrations than with long time exposure at low levels *(IARC, 2006)*. Similar to Shaham et al. (2002) that reported for 14 pathology laboratories a low level for TWA (0,4 ppm) and a high ceiling level (2,24 ppm) our results in the laboratories also shows the same kind of difference between the two metrics, 0,28 and 2,52 ppm, respectively. These data draws attention to the importance of metric selection. In the case of FA, ceilings concentrations might be a better strategy to develop exposure assessment.

Our findings in blood lymphocytes can be an indication that cytogenetic effects can be seen in tissues distant from the area of initial contact (nasopharyngeal) and even reach the bone marrow and cause toxicity, giving relevance to the thesis of Zang and colleagues *(2009)*. In conclusion, the population studied is exposed to high peak concentrations of FA and a long-term exposure and these two aspects cumulatively can be the cause for the effects observed (increase in MN PBL). The association of these cytogenetic effects with FA exposure gives important information to risk assessment process and may also be used to assess health risks for exposed groups. These results suggest that must be applied preventive and protective measures aim to reduce occupational exposure to this chemical agent in these two occupational settings in Portugal.

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