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LETTER TO EDITOR

(APRIL 17, 2012)

RE: THE BEST SAMPLING TIME IN BUCCAL MICRONUCLEUS CYTOME ASSAY

Dear Sir,

We have received the comments about our article titled "Occupational risk assessment of oxidative stress and genotoxicity in workers exposed to paints during a working week", under the title "The best sampling time in buccal micronucleus cytome assay". We thank our colleague who made an important review concerning the proper assays and sampling conditions that allow us to monitor human genotoxicity.

Perhaps the aim of our work was not made clear enough. In 2009, we published an article [1] showing that pharmacists and nurses handling anti-neoplastic drugs for a long period of time (meaning 34.75 months) presented low DNA breaks and lipid oxidative damages in Monday morning samples when compared to Friday afternoon samples. This data led us to suggest that the weekend rest period could affect the DNA and lipid damage levels, which was important to be taken into account while evaluating continuously exposed workers.

That is why, in our article published by "International Journal of Occupational Medicine and Environmental Health", we aimed to evaluate whether genotoxic damages could differ between Monday and Friday samples. It is important to mention that, as described in our article, the individuals studied had been working with paints for, at least, 0.5 year (to 26 years), which characterizes a long time of continuous exposure for all of them. Thus, the time sampling was at least six months for all the exposed individuals. As we expected, a higher DNA damage index (Comet assay) was found in Friday samples of the exposed group when compared with Monday samples. However, for the micronucleus (MN) assay, such result was not found. We think it does not necessarily represent a false negative result. We suggested that the damages detected by the Comet assay could be repaired and/or the cells stopped the cell cycle to repair the damage, as already related [2]. Although an increase in the MN frequency was reported for painters [3–5], Cárdenas-Bustamante et al. [6] described no difference in this end point for workers exposed to paints. These different results could be related, among others factors, to the composition of the paints. It is hard to know exactly what compounds are present in paints, once manufacturers fail to disclose the complete composition of their products. Besides, it should be taken into account that low concentrations of some solvents present in paints (toluene, benzene and acetone) were unable to induce MN formation [7].

We agree that to get robust results in MN (both in lymphocytes and exfoliated buccal mucosa cells) 2000 cells should be scored in a DNA-specific stain protocol. However, there are various works in the literature that used Giemsa stain for buccal cells [8,9] and that counted 1000 cells for the micronucleus frequency analysis [8–11]. It is important to mention that to prevent false results, the cells were washed many times with a buffer.

As it was mentioned in the title of Table 3, hippuric acid and delta-aminolevulinic acid assays were done in Friday samples. We did not evaluate these parameters in Monday samples. The numbers 0.76 vs. 1.63 refer to delta-aminolevulinic acid values in the control and the exposed group, respectively, both sampled on Friday. Maybe, it was not made clear enough. As for the character (*) in this Table, it is really missing (in 0.52 and 1.63 values). This information was contained in our original version of the

article (as you may see in the legend of the Table), but, maybe, it was deleted during the publication process.

In relation to the Nuclear Division Index (NDI), a biomarker of the proliferative status of the viable cells, we found a difference between the control and the exposed group in Friday samples (Table 5). However, no difference was noted between Monday or Friday samples of the exposed group. Thus, we suggested a possible weak cytostatic effect of the paints. In addition, although the NDI for the control group in Monday samples is below the expected value (1.8), we think it does not compromise our results. However, more studies devoted to this point should be carried out in order to elucidate the data obtained in our work.

In conclusion, we think that our work could help to understand the complex mechanisms involved in the determination of the damages effects in workers continuously exposed to paints.

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