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CYP2E1 phenotype in Mexican workers occupationally exposed to low levels of toluene

Octavio Jiménez-Garza[a,*], Sergio Márquez-Gamiño[a,1], Arnulfo Albores[b,2], Cipriana Caudillo-Cisneros[a,1], Mariella Carrieri[c,3], Giovanni Battista Bartolucci[c,3], Maurizio Manno[d,4]

[a] Health Sciences Division, Universidad de Guanajuato Campus León, León, Guanajuato, Mexico
[b] Department of Toxicology, CINVESTAV, México, DF, Mexico
[c] Department of Environmental Medicine and Public Health, University of Padua, Italy
[d] Department of Preventive Medical Sciences, University of Naples Federico II, Italy

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ABSTRACT

CYP2E1, an inducible enzyme present in different human tissues, metabolizes several potentially toxic substances including many volatile organic compounds (VOCs). One indirect way to monitor exposure to VOCs may be, therefore, the assessment of CYP2E1 activity *in vivo* using the chlorzoxazone (CHZ) test. *Goal*: To compare CYP2E1 activity in two groups of workers: one with a known occupational exposure to VOCs (exposed group) and the other employed in administrative tasks at two universities (control group) from the city of León, Guanajuato, México. *Material and methods*: (1) Passive diffusion monitors were used to evaluate individual levels of exposure to toluene, benzene and ethylbenzene in 48 persons (24 tannery workers and 24 administrative controls) during a 8 h work shift; (2) after 12 h fasting 500 mg CHZ, a selective probe for assessing CYP2E1 activity, was orally administered and, after 2 h, a venous blood sample was collected for HPLC plasmatic quantitative determination of CHZ and its mean metabolite 6-hydroxychlorzoxazone. *Results*: Toluene mean exposure levels were higher in the exposed group (2.86 ± 2 ppm vs. 0.05 ± 0.005 ppm; $p < 0.001$). Also, in this group CYP2E1 activity was lower ($p < 0.05$) and it decreased as the accumulated months of labor exposure increased (negative correlation, $p < 0.05$). These results are in line with previous findings obtained from shoemakers exposed to various solvents but, interestingly, they are partly in contrast with those of another study in printers. *Conclusion*: In spite of the relatively low levels of toluene exposure found for tannery workers, an effect on CYP2E1 activity was evident. Although the mechanism of this interaction is still unknown, the decrease in CYP2E1 activity *per se* might represent a health risk, considering that these workers may be less protected against other CYP2E1 substrates present in the labor setting or derived from an intentional exposure.

1. Introduction

Volatile organic compounds (VOCs) are a class of chemicals widely used in several industrial applications, like painting, printing, footwear production, and synthetic or natural leather manufacture, among others. People working at any of these workplaces may undergo a significant absorption of VOCs due to their high volatility and lipophilic properties, which make them easily absorbed by the skin and the respiratory tract. Once in the bloodstream, VOCs may reach different target tissues, depending on the compound and the level of exposure, resulting in several detrimental health effects, ranging from acute symptoms to chronic toxicity, including cancer.

Toluene is one of the most widely used VOCs worldwide and its toxicological effects, for the central nervous system in particular, have been well described in humans and animals (Foo et al., 1990; USEPA, 2005). The Threshold Limit Value-Timed Weighted

* Corresponding author at: Blvd. Puente del Milenio 1001, Fracción del Predio San Carlos, 37670 León, Guanajuato, Mexico. Tel.: +52 477 2674900x3626; fax: +52 477 2674900x3660.
E-mail addresses: ojimenezgarza@ugto.mx, ojimenezgarza@yahoo.com (O. Jiménez-Garza), smgamino@fisica.ugto.mx (S. Márquez-Gamiño), aalbores@cinvestav.mx (A. Albores), ccaudilloc@gmail.com (C. Caudillo-Cisneros), mariella.carrieri@unipd.it (M. Carrieri), Giovannibattista.bartolucci@unipd.it (G.B. Bartolucci), maurizio.manno@unina.it (M. Manno).
[1] Blvd. Puente del Milenio 1001, Fracción del Predio San Carlos, 37670 León, Guanajuato, Mexico.
[2] Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, 07360 México, DF, Mexico.
[3] Via Giustiniani 2, 35128 Padova, Italy.
[4] Via S. Pansini, 5, 80131 Napoli, Italy.

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Average (TLV-TWA) recommended by ACGIH for toluene is currently 20 ppm. However, levels of exposure well below this value have been shown recently to induce a significant, although subtle damage in animals (Fujimaki et al., 2009, 2011; Win-Shwe et al., 2010), to increase the production of reactive oxygen species in humans when compared to controls (Moro et al., 2010) and to be associated with sympathetic and peripheral nerve dysfunction and sub-clinical hematological damage during chronic exposure (Shih et al., 2011).

Most of toluene absorbed is metabolized by hepatic CYP2E1, the major cytochrome P450 isozyme responsible for the biotransformation of several low molecular weight VOCs (Guengerich et al., 1991; Nakajima et al., 1991). CYP2E1, a widely studied isoform because of the myriad of compounds it metabolizes, is a polymorphic enzyme. Moreover, its expression and activity depend on various individual factors such as age (Yun et al., 2010; George et al., 1995a), obesity (Varela et al., 2008), hepatic disease (Chtioui et al., 2007; George et al., 1995b) and diabetes (Wang et al., 2003). Furthermore, CYP2E1 is highly inducible by ethanol and other chemicals.

One important issue to be considered when studying the effect on CYP2E1 activity as a result of exposure to industrial chemicals is the fact that almost invariably, workers are exposed to mixtures of chemicals, not to a single agent only. The situation is even more complex if persons are concurrently exposed to several substances which are metabolized by the same enzymatic system (Dennison et al., 2004). In this scenario, individuals occupationally exposed at the same time to high levels of various solvents, such as toluene, benzene, acetone, styrene and others, which are mainly metabolized by this enzyme, may be unable to metabolize them in an efficient manner or, in extreme cases, even causing saturation of CYP2E1 activity in their liver. Another important issue is the fact that these occupationally exposed individuals may also be voluntarily exposed to other chemicals such as ethanol and/or cigarette smoke, or to a certain medications like acetaminophen, all of which metabolized by CYP2E1 as well (González, 2007; Wu and Cederbaum, 2009; Benowitz et al., 2003). Indeed, common mixtures of organic chemicals have long been shown to competitively inhibit oxidative enzymes in a dose-dependent manner (Andersen et al., 1978; Guengerich et al., 1991; Tardif et al., 1995).

When considering toluene exposure in particular, the results of the few studies on CYP2E1 activity and expression actually carried out in a workplace scenario have shown contrasting results. In one study in a population with occupational exposure to several solvents a significant, nearly 50% inhibition of in vivo CYP2E1 enzymatic activity was observed in persons exposed as compared to controls (Lucas et al., 1999), whereas in another work CYP2E1 mRNA content but not CYP2E1 enzymatic activity was found to be increased in exposed persons although with no correlation between toluene exposure level and CYP2E1 activity (Mendoza-Cantú et al., 2006). Moreover, although tannery workers, according to certain non-government international agencies, are reported to be exposed to VOCs in a specific process on leather, the "finishing", to the best of our knowledge there is no report in the literature providing evidence neither of exposure level nor of the kind of chemical mixture actually involved.

The present investigation was intended, therefore, to explore the impact on CYP2E1 activity as a result of the occupational exposure to toluene and other VOCs, i.e. benzene and ethylbenzene in a group of tannery workers as compared to matched controls.

2. Materials and methods

2.1. Study participants and ethical issues

After agreement with the owners of the plant, the workers of two different tanneries from the city of León, Guanajuato, México were invited to participate in the study. In this particular workplaces they perform a specific process of tanning, known as the "finishing" which, according to some international non-profit organizations, is where VOCs are most extensively used (IFC, 2007). Finishing is considered the last part of the tanning process. Here, workers have to apply the dye to the leather and also to give a certain texture depending on whether leather is going to be used for making shoes, belts or other kinds of garment. The two mentioned tanneries differed substantially one from the other considering that one of the two was very traditional (i.e. settled in an old building, with almost null industrial hygiene measures) and the other was settled in a modern place built specifically for industrial use. Concurrently, the administrative personnel of two universities were also invited to participate voluntarily as controls since no VOCs occupational exposure was known for them. Once the invitations were accepted, we asked for and obtained an individual

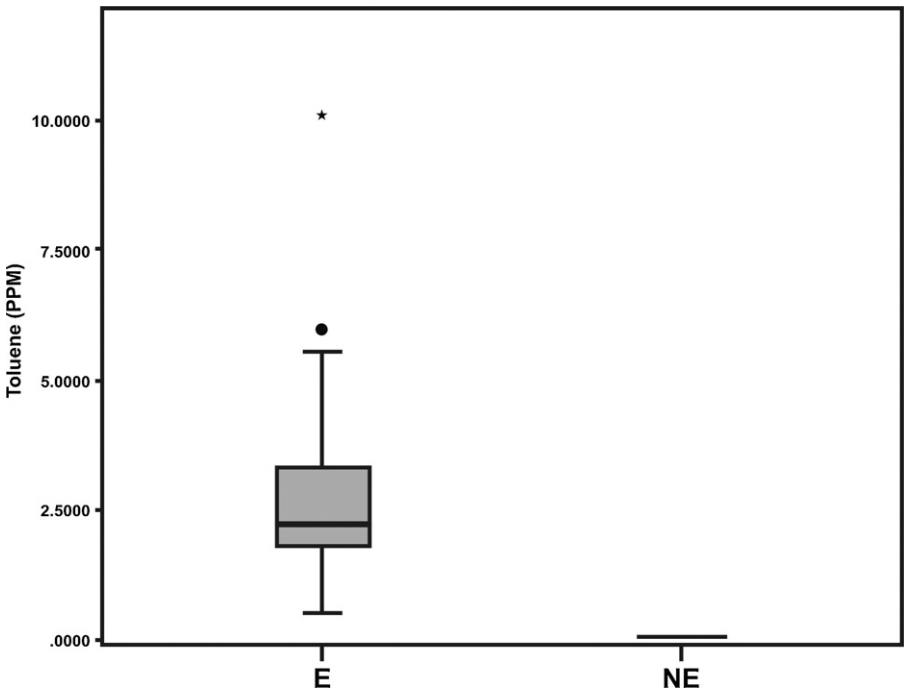

Fig. 1. Individual air toluene exposure levels in the two groups. Levels were higher in E than in NE ($p < 0.001$, Mann Whitney U test). The dot and the asterisk are values marked by the software as "atypical or extreme".