Environmental Research 150 (2016) 645–650

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Determination of total urinary 2,5-hexanedione in the Chinese general population

Pan Xing-Fu [a], Qian Ya-Ling [b,1], Zhao Wei [a], Tang Hong-Fang [b], Ruan Zheng [b], Wu Bang-Hua [c], Huang Han-Lin [c], Zheng Yu-Xin [a], Yan Hui-Fang [a,*]

[a] Key Laboratory of Chemical Safety and Health, National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, China
[b] Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang Province, China
[c] Guangdong Prevention and Treatment Center for Occupational Diseases, Guangzhou, Guangdong Province, China

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form
15 May 2016
Accepted 17 May 2016
Available online 26 May 2016

Keywords:
Urine
2,5-Hexanedione
Resident

ABSTRACT

Objective: Determination of the urinary levels of 2.5-hexanedione (2,5-HD) was performed in subjects belonging to the Chinese general population to define the reference value for this metabolite.
Methods: Urine samples were collected from 8235 individuals (4216 men and 4019 women) from the healthy general population who had not been occupationally exposed to n-hexane or methyl-n-butyl ketone. The determination was performed by a gas chromatography mass spectrometry method using an ion-trap mass spectrometer.
Results: The result showed that the urinary 2,5-HD median level was 0.159 mg/L for the total samples. Males had statistically significant higher excretion of 2,5-HD in urine than females (median 0.171 mg/L compared to 0.147 mg/L, $Z=-8.21$, $P<0.001$). There was a statistically significant difference in urinary 2,5-HD levels among age groups. The excretion of 2,5-HD in urine was related to increasing age ($r=-0.160$, $P<0.05$). There was statistically significant difference in urinary 2,5-HD levels among people from difference provinces. The results showed that there was also a statistically significant effect in urinary 2,5-HD levels between current smokers and non-smokers.
Conclusion: Finding a measurable amount of 2,5-HD in urine does not mean that the level of 2,5-HD causes an adverse health effect. Biomonitoring studies on levels of urinary 2,5-HD can provide physicians and public health officials with reference values so that they can determine whether people have been exposed to higher levels of 2,5-HD than are found in the Chinese general population. These data can also provide a foundation for scientists to make a plan for further study.

1. Introduction

2,5-HD is the main neurotoxic metabolite of methyl-n-butyl ketone (MBK) and n-hexane, and known to cause polyneuropathy (Marrs, 1999; Spencer et al., 2014). MBK and n-hexane are used as solvents in the manufacture of tires, adhesives, shoes, varnishes and textiles. 2,5-HD is commonly used as a biomarker to assess exposure to n-hexane (Perbellini et al., 1981; Cardona et al., 1993; Kawai et al., 1992; Mutti et al., 1993), which is excreted in urine. Urinary 2,5-HD can be measured in individuals exposed to n-hexane or MBK and is regularly used for the biomonitoring of exposed workers. These workplaces exposures have been of great concern. The important route of exposure is mainly by inhalation.

Urinary 2,5-HD has not only been detected in workers with occupational exposure to n-hexane or MBK, but is also found at low concentrations in the general population (Fedtke and Bolt, 1986a; Perbellini et al., 1986; Kawai et al., 1990, 1991, 1993; Italian, 1993; Maestri et al., 1994; Bavazzano et al., 1998). The aim of our study was to measure the urinary 2,5-HD concentration of the Chinese general population and to describe the characteristics of urinary 2,5-HD demographic distribution–thus providing basic data for the establishment of criteria to distinguish between daily contact and occupational exposure.

2. Experimental

2.1. Subject selection

By using a cluster sampling method, we selected study participants in the following order. Initially, we divided China into three

* Corresponding author.
E-mail address: yan_huifang@hotmail.com (Y. Hui-Fang).
[1] First co-author: Pan Xing-Fu and Qian Ya-Ling contributed equally to this work.

http://dx.doi.org/10.1016/j.envres.2016.05.030
0013-9351/

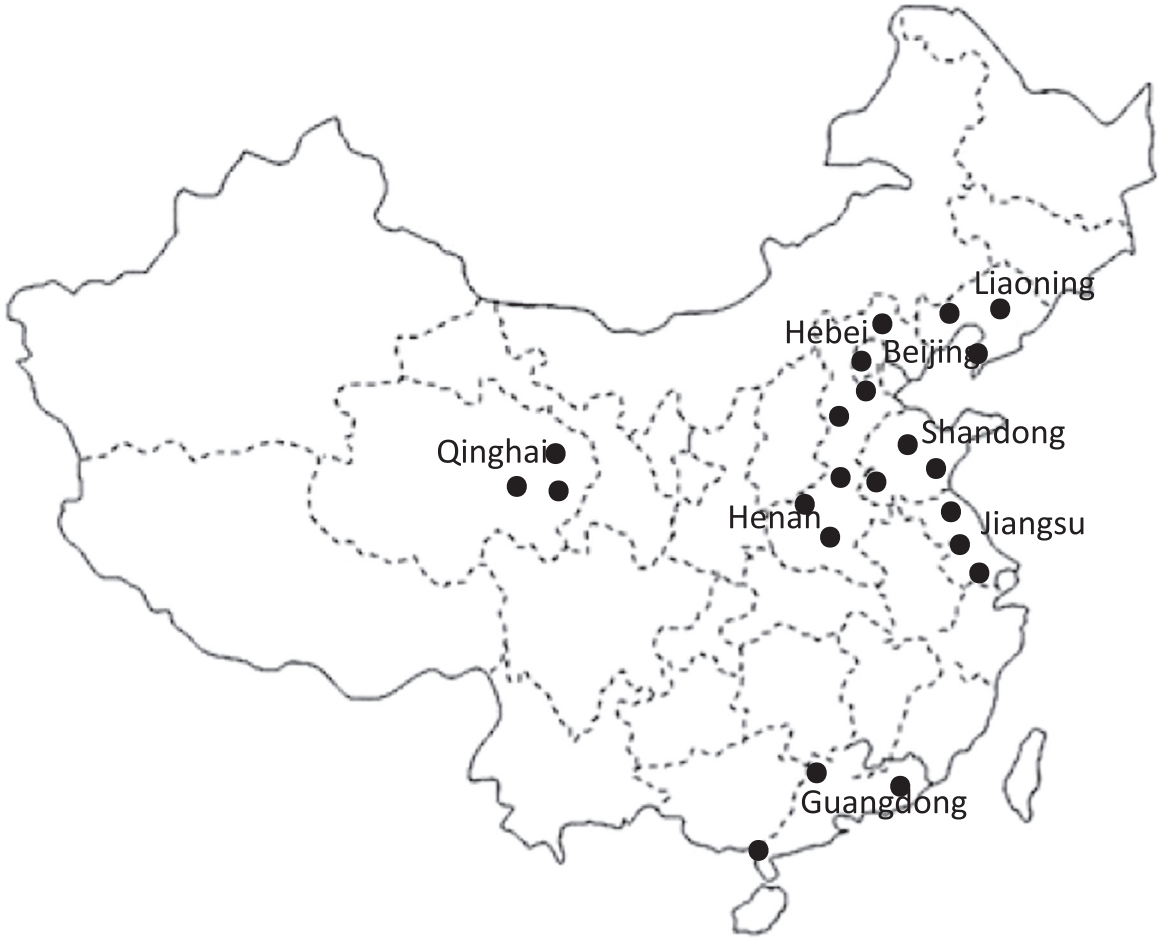

Fig. 1. Location of the study population. The black spots stand for the sampling point. Mainly distributed in the eastern coastal area, including the provinces of Liaoning, Beijing, Hebei, Shandong, Jiangsu, and Guangdong. In the central region was the Henan province and in the western was the Qinghai province.

geographical regions: eastern coastal, central, and northwest. We then randomly selected difference provinces (Fig. 1) and randomly selected neighborhood communities and schools within three cities from each province [i.e., six provinces (Liaoning, Hebei, Beijing, Shandong, Jiangsu, Guangdong) from the eastern coastal region, one province (Henan) from the eastern region and one province (Qinghai) from the northwest region]. Finally, we randomly selected subjects from each community and school for this study. Urine specimens were collected from participants during their annual medical check-up from 2009 to 2010 and stored cold (2–4 °C) or frozen until shipment via cold chain transport to the CDC's National Institute of Occupational Health and Poison Control. About 20 mL of urine samples were collected from each participant, varying in specific density from 1.005 to 1.029 g/L. The urine samples were stored at −80 °C with PTFE (polytetrafluoroethylene) tubes until analysis.

The following case should to exclude: (1) living in these areas as mentioned above fewer than five years; (2) living in an industrial area; (3) history of liver and kidney diseases, diabetes, hyperthyroidism, or cancer; (4) having taken calcium, iron, Zn, or other trace elements in dietary supplements within the past three months; and (5) age out the range of 12–60 years old.

This study was performed according to the Helsinki Declaration of 1975 through a review by the Ethical Committee of the National Institute of Occupational Health and Poison Control of the Chinese CDC. Informed written consent was obtained from all participants; for those <18 years of age, consent was obtained from their parents or guardians.

2.2. Laboratory methods

Samples collected for 2,5-HD measurements were shipped via cold chain transportation to the CDC's National Institute of Occupational Health and Poison Control.

2.2.1. Equipment

The gas chromatography mass spectrometer (GC/MS 4000, Varian, Walnut Creek, CA USA) was equipped with positive chemical ionization and an ion-trap mass spectrometer and autosampler; Low temperature centrifuge; Electric thermostatic water bath oscillation; Vortex oscillator; Teflon 15 mL centrifuge tube; 5 mL medical syringe; Volumetric flask; Pipette.

2.2.2. Materials

2,5-HD standards were purchased from Sigma (USA, $\geq 99.5\%$). We obtained ethyl acetate from Tedia Company Inc. (USA, Chromatography grade). Hydrochloric acid and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other compounds used for analysis can be regularly found in a laboratory. All other solvents used were of analytical grade.

The stock solutions of 2,5-HD were prepared by weighing out 10 mg of 2,5-HD and dissolving in 50 ml of distilled water. Stock solutions were stored at −20 °C. Five calibration standard solutions, covering a range of 0.02–1.00 mg/L, were prepared by diluting with distilled water in 100 ml volumetric flasks. The calibration standard solution was made freshly before analytical run.

The samples shifted from −80 °C temperature to room