Environmental Research ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Environmental Research

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

Assessment of urinary thiodiglycolic acid exposure in school-aged children in the vicinity of a petrochemical complex in central Taiwan

Po-Chin Huang [a,*], Li-Hsuan Liu [b], Ruei-Hao Shie [c], Chih-Hsin Tsai [a], Wei-Yen Liang [a], Chih-Wen Wang [a,d,e], Cheng-Hsien Tsai [f], Hung-Che Chiang [a,g,*], Chang-Chuan Chan [b,**]

[a] National Environmental Health Research Center, National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan
[b] Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan
[c] Green Energy and Environment Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan
[d] Hepatobiliary Diversion, Department of Internal medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[e] Department of Internal Medicine, Chi-Shan Hospital, Ministry of Health and Welfare, Kaohsiung, Taiwan
[f] Department of Pediatrics, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan
[g] Division of Environmental Health and Occupational Medicine, National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan

ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form
23 September 2015
Accepted 24 November 2015

Keywords:
Children
Thiodiglycolic acid
Petrochemical complex
Urine
Biomonitoring

ABSTRACT

Background: School-aged children living in the vicinity of vinyl chloride (VCM)/polyvinyl chloride (PVC) factories may have an increased risk of exposure to hazardous air pollutants.
Objectives: We aimed to evaluate the urinary thiodiglycolic acid (TDGA) level, as TDGA is a major metabolite of VCM, for students at elementary schools near a petrochemical complex in central Taiwan.
Methods: We recruited 343 students from 5 elementary schools based on distance to the VCM/PVC factory. First-morning urine and blood samples were obtained from our subjects from October 2013 to September 2014. Urine samples were analyzed for urinary creatinine and TDGA using LC/MS–MS. Hepatitis virus infection were assessed using blood samples. We determined their vitamin consumption, resident location, parent's employment, and other demographic or lifestyle characteristics using a questionnaire.
Results: Median urinary TDGA levels for 316 students at 5 elementary schools from the closest (< .9 km) to the farthest (~8.6 km) with respect to the petrochemical complex were 147.6, 95.5, 115.5, 86.8, and 17.3 μg/g creatinine, respectively. After adjusting for age, gender, hepatitis virus infection, vitamin B consumption, passive smoking, and home to source distance, we found that urinary TDGA levels for the closest students was significantly higher than those at other schools. Further, median urinary TDGA levels for students during school time were 4.1-fold higher than those during summer vacation.
Conclusions: After adjusting for confounders, urinary TDGA levels for the school-aged children decreased with increasing distances between the elementary schools and the petrochemical complex.

1. Introduction

Vinyl chloride monomer (VCM) is a colorless gas at room temperature and the major material used to produce ethylene dichloride (EDC) or polyvinyl chloride (PVC) (Sherman, 2009). VCM is less likely to occur naturally or to be present in food and cosmetic products, but it is mostly found in the ambient air in VCM/PVC factories or contaminated ground water (ATSDR, 2006). The No. 6 naphtha cracking complex (namely a petrochemical complex) is owned by the Formosa Petrochemical Corporation (FPC) and is situated in the Mai-Liao Township in central Taiwan (Shie and Chan, 2013; Yuan et al., 2015). For this petrochemical complex, the estimated annual production of VCM and PVC was around 2.76 and 2.93 million tons, respectively, and the estimated annual emission of VCM and 1,2-dichloroethane from the stack and equipment was 24.9 and 11.5 t, respectively.

Since 1987, VCM has been classified as a group 1 human carcinogen by IARC (IARC, 2007). Occupational studies suggested

* Corresponding authors at: National Environmental Health Research Center, National Institute of Environmental Health Sciences, National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan.
** Correspondence to: Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Room 722, No. 17, Xu-Zhou Road, Taipei 100, Taiwan.
E-mail addresses: pchuang@nhri.org.tw (P.-C. Huang), hcchiang@nhri.org.tw (H.-C. Chiang), ccchan@ntu.edu.tw (C.-C. Chan).

http://dx.doi.org/10.1016/j.envres.2015.11.027
0013-9351/

that VCM causes angiosarcoma of the liver (ASL) and hepatocellular carcinoma (HCC) for VCM workers (Cheng et al., 2001; ATSDR, 2006), whereas a few study reports indicated that no association was found between VCM and ASL (Huang et al., 2011). Pulmonary absorption of VCM is rapid, and it is quickly metabolized by the liver of rodents and humans (WHO, 1999; Bolt, 2005). Thiodiglycolic acid (TDGA) is activated by CYP2E1 and has been suggested as a major metabolite of VCM in human urine by ATSDR (ATSDR, 2006). One recent report conducted by the Taiwan IOSH indicated that a *significant correlation* was found between TDGA in the first-morning urine and the personal VCM air level for 32 workers *measured by* liquid chromatography tandem mass *spectrometry* (LC–MS/MS) (Shen and Lee, 2011). Some studies also revealed that a hepatitis virus infection, specific medicine use, nutrition supplement intake, and a current smoker possibly interfered with the TDGA level in humans (Cheng et al., 2001; Navrátil et al., 2007, 2010).

On August 2013, the Syu-Cuo Branch of Ciao-Tou elementary school was relocated from its old campus to new campus, which were 2.3 km and .9 km away from the VCM and PVC factories of a petrochemical complex in central Taiwan. Based on a 2-year (May 2012–June 2014) air-monitoring database established by the local EPA of Yun-Lin County, data revealed that the annual concentration of VCM at the old campus of Syu-Cuo Branch was 2.19 ppb with an hourly maximum of 165.8 ppb. As individuals located near or downwind from the production facilities may be exposed to atmospheric levels of VCM higher than those of ambient background levels (ATSDR, 2006), it raised the question whether exposure to increased VCM levels affected students attending surrounding elementary schools. The objective of this study was to evaluate whether urinary TDGA levels in elementary students decreased based on the distance of their elementary school to a nearby petrochemical complex using TDGA as a surrogate biomarker.

2. Methods

2.1. Ethic statement

The protocol of this study was approved by the Research Ethics Committee of the National Health Research Institutes (No. EC1020607). All participants provided informed consent, and the parents of the children signed an additional agreement before study enrollment.

2.2. Subjects' recruitment

All 5 selected elementary schools were within 10 km of the closed No 6. petrochemical complex in central Taiwan, including the new campus of Syu-Cuo Branch (School A, ~.9 km), Feng-An (School B, ~2.7 km), Ciao-Tou (School C, ~5.5 km), Mai-Liao (School D, ~6.9 km), and Lun-Feng (School E, ~8.6 km) elementary schools. The inclusion criteria for our subjects were as follows: students attending grade 1–6 of elementary schools who lived locally for >1 y, and students aged >6 y. Because of the number of students attending the elementary schools varied from approximately 100 (A and B) to >400 or nearly 1000 (C, D, and E), we invited 6 volunteer boys and 6 volunteer girls from each grade (1–6) from schools A and B. Then, we randomly matched students by gender for our subjects at schools C through E using the school identification number for each student.

2.3. Sample collection

Our sampling occurred from October 2013 to September 2014, and the sampling date for each school was as follows: A (October 16, 2013), B (November 16, 2013), C (March 5, 2014), D (April 9, 2014), and E (September 24, 2014). We collected a urine sample for participants attending school A during summer vacation (August 18, 2014). A first-morning urine sample (<60 mL) was collected using a plastic urine bag (PP) on Wednesday morning for each participant, immediately transferred into amber glass bottle and stored at -80 °C. A 20-mL blood sample was drawn from our participants by clinical nurse at the same time, *centrifuged and* stored at -80 °C.

2.4. Assessment of urinary TDGA levels

We analyzed the TDGA levels in the urine sample of the participating students using LC/MS–MS. Formic acid ($>98\%$), the standard for TDGA ($>98\%$), and 4-nitrobenzoic acid (4-NBA, $>98\%$) were purchased from Sigma Aldrich Lab, Inc, St. Louis, MO, USA. Briefly, we added 50 μL of the urine sample and 150 μL of 4-NBA (internal standard) to 1.3 mL of acetonitrile (ACN, $\geq 99.9\%$, Merck, Darmstadt, Germany) in a 2-mL vial. The mixed sample was centrifuged at 9000 rpm (4 °C) for 10 min; then, we used a PVDF filter for sample filtration and stored it at -20 °C for instrument analysis. We used an Atlantis Silica HILIC (100 Å; 2.1 mm × 150 mm; 3 μm, Waters, MA, USA) analytical column. We used a negative ion mode and multiple reaction monitoring with the following conditions to obtain the best performance: nebulizer gas; nitrogen; ion spray voltage, -4.5 kV; temperature of turbo gas, 350 °C; collision gas pressure, 5 psi; and curtain gas pressure, 10 psi. The first and second optimized ion TDGA pairs were 149/105 and 149/61, respectively, and the 4-NBA optimization was 166/122. The TDGA concentration in the blank sample was defined as less than 2 times the minimal detectable level (MDL). The recovery and relative difference percentage of the spiked (1, 5, and 20 ng/mL) and repeated (precision) sample was defined as within $\pm 15\%$ and $\pm 30\%$, respectively, for each batch. The executed performance for accuracy and precision was 85–104%.

We used the following formula to calculate the TDGA concentration in the urine sample:

$$C_x = \frac{A_x c_{is}}{A_{cs} \bar{RRF}}$$

C_x = TDGA Concentration in the urine sample.
A_x = Response area of the sample ($TDGA_{149/105}$).
A_{cs} = Response area of the internal standard ($4\text{-}NBA_{166/122}$).
C_{is} = Spiked concentration for the internal standard.
$\bar{RRF}$ = Mean RRF for the calibration curves.

2.5. Health examination

All subjects were invited to participate in serial health examinations including a biochemical examination of a blood sample and an abdominal ultrasound for fatty liver. Some clinical health indices including hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), anti-core antigen antibody of hepatitis B (Anti-HBc), and anti-core antigen antibody of hepatitis C (Anti-HCV) in the serum sample, as well as urinary creatinine in the urine samples, were measured using a Taiwan Accreditation Foundation certified laboratory (No.: 1447 & 1673), which had been recognized by the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement.

2.6. Questionnaire

With the assistance of the participating children's parents or primary caregivers, we used a self-reported questionnaire to obtain the following: personal information, environmental exposure scenario, physical activity, disease history, and children's diet. The

Please cite this article as: Huang, P.-C., et al., Assessment of urinary thiodiglycolic acid exposure in school-aged children in the vicinity of a petrochemical complex in central Taiwan. Environ. Res. (2015), http://dx.doi.org/10.1016/j.envres.2015.11.027