Elevated urinary levels of kidney injury molecule-1 among Chinese factory workers exposed to trichloroethylene

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Introduction

Trichloroethylene (TCE) is a volatile chlorinated organic compound commonly used in industrial settings as a degreaser for metal parts and general-purpose solvent for lipophilic compounds. In 1995, it was estimated that more than 400,000 workers were exposed to TCE on an annual basis in the United States (1). Further, as a consequence of its presence in workplaces for many years and relative stability, TCE has become a widespread environmental water contaminant and one of the most frequently observed chemicals at Superfund sites.

TCE has recently been classified by the US EPA as carcinogenic in humans by all routes of exposure, whereas the National Toxicology Program classified TCE as reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity from studies in humans, and sufficient evidence of carcinogenicity from studies in experimental animals and information suggesting TCE acts through mechanisms that indicate it would probably cause cancer in humans (2,3). Evidence for the carcinogenicity of TCE in humans is strongest for liver and kidney cancers and non-Hodgkin's lymphoma (4).

A recent meta-analysis of 23 studies on kidney carcinogenicity of TCE in humans showed a probable relationship between TCE exposure and kidney cancer (5). TCE is bioactivated to reactive intermediates through the renal glutathione S transferase (GST) and cysteine conjugate beta-lyase enzymes to form cysteine S conjugates, which are believed to be responsible for its nephrotoxic and nephrocarcinogenic effects (6,7). Therefore, the recent findings of an increased risk among TCE-exposed subjects especially in those subjects carrying polymorphisms in GST and cysteine conjugate beta-lyase genes has provided strong biological plausibility of an association between TCE and renal cancer in humans (7).

Long-term animal studies have shown low incidences of renal adenoma and adenocarcinoma in a number of rat strains (8). Rats given TCE orally or by inhalation also developed non-neoplastic lesions in the kidneys at relatively low dose levels. It has been suggested that kidney tumours in rats arise as a result of persistent cytotoxicity and regeneration. However, although nephrotoxicity occurs also in mice they do not seem to develop renal neoplasms. As such, kidney toxicity seems to be a possible but insufficient contributing factor for rodent renal carcinogenesis. It is worthy to note that mutagenicity has been proposed to contribute, independently of cytotoxicity and regeneration, to renal tumorigenesis in the rat (7).

Data from human studies on TCE and nephrotoxicity are limited but have suggested that there might be a toxic effect of TCE on the kidneys at relatively high exposure levels (>35 ppm) (9–12). Information on nephrotoxicity at lower TCE doses is generally lacking.

Recently, several new sensitive markers of kidney toxicity have been developed including glutathione S transferase alpha and pi (Alpha-GST and Pi-GST) and kidney injury molecule-1 (KIM-1), which enables the identification of low level kidney toxicity. To address questions about TCE’s potential to cause kidney cancer, we carried out a cross-sectional study to evaluate the impact of occupational exposure to TCE on kidney injury using a panel of nephrotoxicity markers (i.e. Alpha- and Pi-GST, KIM-1, vascular endothelial growth factor (VEGF), N-acetyl-beta-(D)-glucosaminidase (NAG) activity).

Methods

Study design

To select factories for study, we conducted an initial screening of more than 40 potential study factories over a 1 year period using Dräger tubes and 3M organic vapour monitoring (OVM) badges to measure TCE and other chemicals including benzene, styrene, ethylene oxide, formaldehyde, methylene chloride, chloroform, perchloroethylene and epichlorohydrin. Factories were included if they used TCE in manufacturing processes, had no detectable benzene, styrene, ethylene oxide, formaldehyde or epichlorohydrin levels, and low to negligible levels of methylene chloride, chloroform or perchloroethylene. Ultimately, six study factories with metal (n = 4), optical lenses (n = 1) and circuit boards (n = 1) cleaning operations were identified that fulfilled the above selection criteria (13).

In June and July, 2006, we carried out a cross-sectional study of 80 workers currently exposed to TCE in the six study factories with TCE cleaning operations and 45 unexposed controls. Control subjects were enrolled from a clothing manufacturing factory and a food production factory that did not...
use TCE and were in the same geographic region as the factories that used TCE. Controls were frequency matched by age (±5 years) to exposed workers. Exclusion criteria for both TCE-exposed and unexposed workers included history of cancer, chemotherapy and radiotherapy, as well as previous occupations with notable exposure to benzene, butadiene, styrene and/or ionizing radiation. The study was approved by Institutional Review Boards at the US National Cancer Institute and the Guangdong National Poison Control Center, China. Participation was voluntary and all subjects gave written informed consent.

Exposure measurement and sample collection
For TCE-exposed workers (n=80), full-shift personal air exposure measures, 2–3 per subject resulting in 235 measurements, were taken in a 2-week time period in the factories using 3M OVM badges before biological sample collection. For control workers (n=45), a single OVM badge was collected before biological sample collection. All OVM badges were analysed for TCE by GC-FID (LOD 0.12 ppm) and a subset (48 from TCE-exposed workers) was analysed for a panel of organic hydrocarbons including benzene (LOD 0.08 ppm), methylene chloride (LOD 0.14 ppm), perchloroethylene (LOD 0.1 ppm) and epichlorohydrin (LOD 0.1 ppm) by GC-MS. As part of the quality control procedures, duplicate badges were analysed for TCE in Guangdong, China and the United States and showed similar results with a Pearson correlation of 0.99.

Subjects were interviewed using a questionnaire that requested information about demographic and lifestyle characteristics and occupational history. They were also asked to provide a peripheral blood, buccal cell mouth rinse and urine samples, and undergo a brief physical exam that included measurement of blood pressure, height, weight and temperature.

Urinary analyses
Post-shift urine samples were stored at 4°C until being processed within 10h of collection. Samples were centrifuged and 1.4 ml of urine supernatant was then mixed with 0.3 ml freezing buffer (NEPHKIT® Urine Stabilizing Buffer; Argutus Medical) to stabilize proteins for storage and freezing. Samples were subsequently stored at −80°C.

Post-shift spot urine samples were analysed for creatinine, Alpha-GST, Pi-GST, VEGF, KIM-1 and NAG concentrations. Creatinine was determined by an enzyme substrate–based colorimetric assay. Assay CVs were 5% for Pi-GST, 10% for NAG, 15% for Alpha-GST and KIM-1, and 20% for VEGF.

Statistical analysis
Levels of TCE exposure and urinary markers were natural log transformed to normalize their distributions. Student t-test was used to test for difference in the natural logarithm (ln) of each endpoint between unexposed control and exposed workers. In addition, exposure response analyses were performed by linear regression. In these analyses, current TCE air levels in part per million (ppm) were based on the arithmetic mean of an average of two to three measurements per subject. Cumulative exposure in ppm years was calculated by multiplying the individual arithmetic mean TCE levels with duration of employment at the current job. Linear regression models included in addition to the natural log-transformed exposure variables the frequency-matching factor age (as a continuous variable) and were corrected for ln(creatinine) (as a continuous variable). In addition, potential confounders that have been shown previously to influence one or more of the studied markers in this report were included in a model for a given marker if the regression coefficient was altered by ±15%, and included sex, current smoking (yes/no), current alcohol consumption (yes/no) and body mass index (BMI). As current smoking and sex were largely collinear (i.e. only men smoked) we only included sex in the final models. All analyses were carried out using SAS version 9.2 software (SAS Institute, Cary, NC, USA).

Results
Demographic characteristics including age, sex, BMI, current smoking and alcohol status were comparable between the unexposed control and exposed subjects (Table 1). Mean TCE exposure among the exposed was 22 ppm (SD 35.9) while TCE exposure was negligible in the control factories. On average, the exposed subjects worked for 2 years in the TCE facilities while unexposed subjects worked for 2.3 years in the control factories.

Table I. Demographic characteristics and TCE exposure level

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Unexposed (n=45)</th>
<th>Exposed (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>24.9±6.0</td>
<td>25.2±6.6</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>21.5±2.8</td>
<td>21.5±2.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n (%)</td>
<td>29 (64.4)</td>
<td>57 (71.3)</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>16 (35.6)</td>
<td>23 (28.8)</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes n (%)</td>
<td>15 (33.3)</td>
<td>34 (42.5)</td>
</tr>
<tr>
<td>No n (%)</td>
<td>30 (66.7)</td>
<td>46 (57.5)</td>
</tr>
<tr>
<td>Current alcohol use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes n (%)</td>
<td>19 (42.2)</td>
<td>26 (32.5)</td>
</tr>
<tr>
<td>No n (%)</td>
<td>26 (57.8)</td>
<td>54 (67.5)</td>
</tr>
<tr>
<td>TCE exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current (ppm) mean ± SD</td>
<td>&lt;0.03</td>
<td>22.2±35.9</td>
</tr>
<tr>
<td>Cumulative (ppm, years)mean ± SD</td>
<td>&lt;0.1</td>
<td>35.8±66.2</td>
</tr>
</tbody>
</table>

Exposure to TCE appears to have influenced one or more of the studied markers in this report; however, the relatively short tenure of most exposed workers (average 2 years) cumulative TCE exposure was not sufficient to result in statistically significant changes in the urinary markers.

Discussion
We found that exposure to relatively low levels of TCE (i.e. average 22 ppm) was associated with a dose-dependent increase in nephrotoxicity markers KIM-1 and possibly Pi-GST. The association was seen for both current TCE exposure (ppm) and cumulative TCE exposure (ppm years). However, given the relatively short tenure of most exposed workers (average 2 years) cumulative TCE exposure.
was mostly driven by exposure intensity. An analysis with duration among the exposed workers indeed did not show an effect with any of the markers suggesting that the observed effect was driven predominately by current TCE exposure levels. Altogether, these data suggest that kidney toxicity is found at occupational levels of TCE exposure below the current Occupational Safety and Health Administration permissible exposure limit of 100 ppm and below the current National Institute of Occupational Safety and Health recommended exposure limit of 25 ppm.

TCE exposure has been shown to be related to nephrotoxicity in animal studies. These studies suggest that kidney damage due to TCE can occur due to persistent cytotoxicity and regeneration (6). As in the rats, kidney toxicity is believed to be a contributing factor to the development of renal cancer in humans following exposure to TCE (6). TCE-induced nephrotoxicity in humans, however, has not been demonstrated conclusively partly due to methodological limitations on exposure assessment and the use of insufficient sensitive markers (10). In the present study, we collected extensive exposure information and used novel sensitive nephrotoxicity markers. Our findings of kidney toxicity at relatively low TCE levels are of importance as it indicates that toxic metabolites are formed at these concentration levels adding to the plausibility of the epidemiological findings linking TCE and kidney cancer.

We did not observe an association between TCE exposure and NAG or VEGF. NAG is mainly found in the proximal tubular brush border cells and is shed into the urine in case of kidney damage (15,16). VEGF is a protein involved in wound healing (16). Both biomarkers are indicative of severe kidney damage resulting in functional loss. The absence of an association between TCE exposure and VEGF and NAG indicates that TCE exposure at the levels and duration encountered in this study does not lead to severe loss of function of the kidneys.

Among the more sensitive nephrotoxicity markers (Pi- and Alpha-GST and KIM-1), we found a positive association with KIM-1 and possibly Pi-GST. KIM-1 is a kidney-specific membrane protein. In healthy cells, KIM-1 is expressed at a very low level, mostly in the proximal tubules. KIM-1 is strongly upregulated in injured kidney cells throughout the kidneys (17). When kidney cells are damaged, the KIM-1 ectodomain is cleaved and shed into the urine (18). Recently, urinary KIM-1 has been shown to outperform traditional biomarkers of kidney injury in preclinical biomarker qualification studies (19). In addition, in a variety of acute and chronic rodent kidney injury models resulting from drugs and environmental toxicants (i.e. metals), KIM-1 has been shown to be a very sensitive and early diagnostic indicator of kidney injury (19,20). KIM-1 has not been previously measured in studies on nephrotoxicity among TCE-exposed subjects. PiGST is a glutathione-S-transferase expressed in the distal tubules and collecting duct cells. PiGST is shed into the urine in case of distal tubular damage (21). Pi-GST has been measured in a retrospective study among subjects with symptoms and controls by Bruning et al. (22). In contrast to our study, a positive association with Alpha-GST was found but not with Pi-GST. However, given the retrospective design of this study, it is difficult to compare the results of our cross-sectional study with the study of Bruning et al. Green et al. (10) measured Alpha-GST but not Pi-GST in a study among current-exposed subjects and did not observe a difference in urinary Alpha-GST levels between exposed and unexposed subjects. As such, the results on Alpha- and Pi-GSTs among TCE-exposed subjects are largely inconsistent and therefore, our borderline statistical finding of Pi-GST being related to TCE exposure should be interpreted with caution.

We corrected our exposure-response models by including ln(creatinine) as a continuous variable in the model. This allows for a correction of urinary flow that is not necessarily directly proportional to the urinary creatinine concentration and which can vary by marker. We repeated our analyses by expressing the biological parameter concentration corrected for ln(creatinine), sex, current alcohol use and BMI between the unexposed and the two exposure categories.
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Disclosure

JVB is the co-inventor on KIM-1 patents assigned to Partners Healthcare, which has licensed them to a number of companies including Genzyme, Johnson and Johnson, BiogenIdec, BioassayWorks and R and D systems. He is a consultant to Genzyme.

References


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