Estimation of a Safe Level for Occupational Exposure to Vinyl Chloride Using a Benchmark Dose Method in Central China

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Abstract: Estimation of a Safe Level for Occupational Exposure to Vinyl Chloride Using a Benchmark Dose Method in Central China: Jie Jiao, et al. Department of Occupational Health and Toxicology, School of Public Health, Fudan University, China—Objectives: The aim of this study was to estimate a benchmark dose (BMD) for chromosome damage induced by vinyl chloride monomer (VCM) in VCM-exposed workers in central China and validate the published results in Shanghai. Methods: VCM-exposed workers who had been exposed to VCM for at least one year (n=463) and matched subjects not exposed to VCM or other toxins (n=273) were asked to participate in this study. Micronucleus (MN) frequency based on the cytokinesis-block micronucleus assay (CBMN) was used as a biomarker for chromosome damage induced by VCM exposure. Results: The MN frequency in the VCM-exposed workers was significantly higher than that in the control group, and multivariate Poisson regression suggested that gender, smoking status and VCM exposure were the significant factors influencing the risk of increased MN frequency. When subjects were further stratified according to gender and smoking status, the results showed that female VCM-exposed workers were more susceptible than the males to the risk of increased MN frequency. The MN frequency of smokers was significantly higher than that of nonsmokers in the control group. Our study also suggested that there was a strong dose-response relationship between VCM CED and the increased risk of MN frequency in the total group, males and females. The BMDL₁₀ was found to be 630.6, 670.2 and 273.7 mg-year for all VCM-exposed workers, males and females, respectively. Conclusions: These results invite further scrutiny of the current VCM occupational exposure limits and warrant further study of the risk of VCM genotoxicity and carcinogenicity. (J Occup Health 2012; 54: 263–270)

Key words: Benchmark dose, Chromosome damage, Micronucleus frequency, Vinyl chloride monomer

Vinyl chloride monomer (VCM) is a known cause of angiosarcoma of the liver¹. The International Agency for Research on Cancer (IARC) classified VCM as a confirmed human carcinogen in 1974, 1978 and 1987². VCM is extensively used in the production of vinyl chloride polymer, copolymer resin, packaging materials and wire and cable coatings as well as in industrial and laboratory intermediates³. The wide human exposure to this compound in different industries throughout the world causes great concern for human health. Previous studies⁴−⁵ suggested that the two oxidative metabolites of VCM (chloroethylene oxide (CEO) and chloroacetaldehyde (CAA)) could produce mutagenic effects. In addition to mutations, other genotoxic effects were reported such as gene conversion, sister chromatid exchanges (SCE), micronuclei (MN), mitotic recombination, chromosomal aberrations (CA) and cell transformation³,⁶,⁷. The MN assay has become a well-known and promising indicator to investigate the genotoxicity of a variety of chemicals especially using the cytokinesis-block micronucleus (CBMN) assay⁸,⁹. The CBMN method...
is the most reliable method for measuring MN in peripheral blood lymphocytes (PBL)\(^9\). In this assay, PBL are induced to divide \textit{ex vivo} in culture, and MN are scored in cells that have completed one nuclear division; the latter are recognized as binucleated cells after inhibition of cytokinesis using cytochalasin-B. The CBMN method can assess chromosome damage including chromosome breakage and chromosome loss reliably in occupational and environmental exposures\(^7, 10–16\). Some investigations\(^7, 17\) have demonstrated that the frequency of MN is increased in lymphocytes of individuals occupationally exposed to VCM.

The benchmark dose (BMD) was defined by Crump\(^{18}\) as a statistical lower confidence limit corresponding to a moderate increase in risk (1–10\%) above the background risk. Crump suggested that the BMD could be used to replace the no observed adverse effect level (NOAEL) or the lowest observed adverse effect level (LOAEL) for noncarcinogenic effects in the regulatory process for setting acceptable and safe exposure levels of toxic compounds\(^{19, 20}\). The main advantage with the BMD methodology is that it uses all dose-response data from a study\(^{21}\). Gaylor \textit{et al.}\(^{22}\) redefined the BMD as the point estimate of the dose corresponding to a specified low level of risk and suggested that the concept of BMDL (lower confidence limit of benchmark dose) could be used as a replacement for the NOAEL or LOAEL\(^{20}\). The BMDL resulting from this approach is more accurate than a NOAEL or LOAEL. The BMDL is typically calculated using the lower 95\% confidence limit on the dose-response curve to a 1–10\% level of risk above the background. A 10\% benchmark response level (BMR) is conventionally used for dichotomous end points because it is at the low end of the observable range for many common study designs. Various investigations\(^{23–30}\) have performed risk assessments of the non-cancer effects of toxic exposures using the benchmark dose (BMD) method. A previous study\(^{31}\) evaluated the effects of occupational exposure to vinyl chloride monomer (VCM) on the micronucleus and estimated a benchmark dose for chromosome damage induced by VCM in VCM-exposed workers. The objective of the present study was conducted to confirm the finding in the previous study that there was the strong relationship between VCM CED and chromosome damage using the BMD approach; likewise, CBMN was used as an indicator of chromosome damage induced by VCM.

**Materials and Methods**

**Study population**

Workers from a VC polymerization plant in Henan Province, central China, were recruited during routine medical evaluations. After informed consent was obtained, workers underwent an interviewer-administered questionnaire including data on age, lifestyle, professional and medical history and smoking and drinking habits. Eligibility was based on employment records and was defined as occupational exposure to VCM for at least 1 yr. Workers occupationally exposed to VCM who had completed questionnaires and finished the CBMN assay were included in the VCM exposure group (463 in total; 268 males and 195 females, 32.5 ± 8.81 yr). For the control group, we selected 273 (137 males and 136 females, 25.5 ± 7.26 yr) subjects working in the same city as the VCM exposure group and without exposure to VCM and other toxic chemicals as non-occupationally exposed (reference) subjects and constituted the control group. Although the age variable is not comparable between VCM exposure group and the control group, it was adjusted when the authors performed the statistical analysis.

**VCM exposure assessment**

VCM air samples were collected at different work sites of the plant and analyzed by gas chromatography to determine VCM air concentrations for the various work sites and estimate the cumulative exposure dose for each worker. Since the cumulative exposure is related to work location, work duration and many other factors, we can use the same method as described\(^{17, 32}\) previously to assess the cumulative exposure dose of VCM from the VCM air concentrations. In brief, the following equation was used to calculate cumulative exposure dose: cumulative exposure dose (mg) =∑(C × M × T) × A × 70%/10\(^6\), where C (mg/m\(^3\)) was the geometric mean of the VCM exposure concentration for each month in a special workplace, calculated for all different work sites; M was the number of exposure months of each year for a VCM worker; T was the 2 h exposure time in each working day, 20 days in each month, giving 2,400 min of exposure time per month; and A was the alveolar ventilation (male average, 6,500 ml/min; female average, 4,300 ml/min; 30\% dead space).

For the control group, the VCM exposure dose was evaluated according to the published literature\(^{33}\). In brief, general environmental levels of VC from all sources are likely to lead to average exposures of 2–10 µg/day. Smokers may inhale additionally up to 0.5 µg/day. Exposures from food and water are less than 0.1 µg/day, so these exposures were not included when evaluating the total cumulative exposure dose of VCM in the present study.

**CBMN assay**

The CBMN assay was performed according to a standard protocol as described by Fenech\(^{34}\). In
brief, 0.5 ml heparin-anticoagulated whole blood was collected, transferred to 4.5 ml RPMI 1640 medium and incubated at 37°C in 5% CO₂ for 44 h, and cytokinesis was blocked with 6 µg/ml cytochalasin-B (Sigma-Aldrich, St.Louis, MO, USA). Twenty-eight hours after the addition of cytochalasin-B, cells were harvested and fixed with methanol and acetic acid at a ratio of 3:1. Slides were air-dried, stained with Giemsa and scored for CBMN. For each subject, 1,000 binucleated lymphocytes with well-preserved cytoplasm were scored blindly.

**BMD method**

In the present study, VCM cumulative exposure dose was used as the dose parameter. We used the BMDL to estimate the lower confidence limit of the population critical concentration of the cumulative exposure dose of VCM-exposed workers.

In this study, the analysis of regression and curve estimation were performed using the Benchmark Dose Software (Version 2.1.2) from the US Environmental Protection Agency (EPA). A BMR of 10% was used in calculating the BMD. This value is consistent with 10% of the BMR suggested by the US EPA (2003). The normal cut-off point was defined as being based on the 95% limit value in the control group. The BMD analysis for the total group was used as an example to explain the models selected. We fit the data to a Probit model, Logistic model and Log-logistic model, respectively (Table 5). p values ≥0.05 can be found in all models above mentioned. We chose the log-logistic model to analyze the data because the AIC value of this model was the smallest among the three models. The BMDL was specified as the lower 95% confidence limit of the dose corresponding to the BMR in the study, and the smooth option of the model was applied (figure not shown).

The equation of the log-logistic model is

\[ P \{\text{chromosome damage}\} = \frac{c+(1-c)}{1+\exp(-a-b \times \log(CED))}. \]

**Statistical analysis**

The multivariate Poisson regression model was performed to examine the relationship between the cumulative exposure dose and MN frequency. Potential confounding variables included in the final models were age (continuous variables), smoking status, drinking status, gender and VCM exposure status (continuous variables). The risk of chromosome damage was estimated by calculating frequency ratios (FR) (herein and in the following text, FR is the IRR (incidence rate ratio)) and 95% confidence intervals (CI), with FR= e^β (e≈2.71828). For categorical variables, the FR denotes the proportional increase of the micronucleus frequency in a comparison group relative to the reference group; for example, an FR of 1.48 for females versus males means a 48% increase in the micronucleus frequency in females. For continuous variables, the FR represents the proportional increase of micronucleus frequency due to the increase of one unit of the variable evaluated; for example, an FR for age of 1.11 means 11% increase in the micronucleus frequency per year of age. All p values presented are 2-sided, and all analyses were performed using the SAS Version 9.2 Software (SAS Institute).

**Results**

**VCM exposure assessment**

We estimated that the exposure dose in the VCM exposure group ranged from 34.49 mg to 113.06 mg in air, with the median dose being 58.4 mg. The personal cumulative exposure dose in the VCM exposure group ranged from 886 mg to 127,915 mg, with the median dose being 18,910 mg.

Based on the above data, we assessed the VCM cumulative exposure dose (CED) in mg-year. In the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>FR (95% CI)</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−0.162</td>
<td>−</td>
<td>4.93</td>
<td>0.027</td>
</tr>
<tr>
<td>Age</td>
<td>−0.059</td>
<td>0.94 (0.82–1.08)</td>
<td>0.72</td>
<td>0.396</td>
</tr>
<tr>
<td>Smoke*</td>
<td>0.173</td>
<td>1.19 (1.01–1.40)</td>
<td>4.11</td>
<td>0.043</td>
</tr>
<tr>
<td>Drink</td>
<td>0.121</td>
<td>1.13 (0.97–1.32)</td>
<td>2.37</td>
<td>0.123</td>
</tr>
<tr>
<td>Gender**</td>
<td>−0.256</td>
<td>0.77 (0.68–0.88)</td>
<td>14.97</td>
<td>0.001</td>
</tr>
<tr>
<td>VCM exposure**</td>
<td>0.952</td>
<td>2.60 (2.21–3.03)</td>
<td>139.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MN: micronucleus; VCM: vinyl chloride; FR: frequency ratio of mean MN frequency between a comparison and a reference group, the results derived from Multivariate Poisson regression models when these impact factors were adjusted by each other.

*, **: p<0.05, p<0.01 with regard to the corresponding group, respectively.
subsequent analysis, as shown in Table 4, four ordinal CED groups were used: 0<CED≤167, 167<CED≤667, 667<CED≤1,667 and 1,667<CED. The group CED levels were represented by the group median CED level.

MN frequency and its association with risk factors

The mean and range of CBMN frequencies in the VCM-exposed group and control group were 1.99 and 0–22 and 0.79 and 0–4, respectively. Herein and in the following text, the scale of CBMN frequencies concretely refer to per mille. MN frequency in the VCM-exposed workers was significantly higher than in the control group [FR (95% CI): 2.52 (2.17–2.93)]. As shown in Table 1, multivariate Poisson regression suggested that gender, smoking status and VCM exposure were the significant factors influencing the risk of increased MN frequency. All the data exclusive of age and VCM exposure status (age and VCM exposure status are continuous data) were analyzed as binary data in the Poisson regression analysis.

Subjects were further stratified according to gender and smoking status. The results for FR derived from Poisson regression analysis (Table 2) showed that among VCM-exposed workers, the MN frequency of females was significantly higher than that of males [FR (95% CI): 1.17 (1.03–1.34)], but there was no similar association in the control group. The MN frequency of smokers was significantly higher than that of nonsmokers in the control group [FR (95% CI): 1.38 (1.02–1.85)].

MN frequency and its association with VCM exposure

In this study, as we mentioned above, we found that subjects occupationally exposed to VCM had a higher risk of having an increased MN frequency than the control group. VCM-exposed workers were further divided according to different CEDs (Table 3). The results derived from Poisson regression suggested that there was a strong dose-response relationship between VCM CED and the risk of increased MN frequency.

The values of BMD and BMDL of VCM cumulative exposure dose as chromosome damage indicators

We defined the normal cut-off point based on the 95% limit value in the control group such that if a

Table 2. MN frequency of VCM exposure and control group by gender and smoking status

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Exposure</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>MN (%)</td>
<td>FR (95%)</td>
<td>N</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>137</td>
<td>0.70 (0.5)</td>
<td>1</td>
<td>268</td>
</tr>
<tr>
<td>Female</td>
<td>136</td>
<td>0.88 (0.5)</td>
<td>0.80 (0.61–1.05)</td>
<td>195</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>212</td>
<td>0.73 (0.5)</td>
<td>1</td>
<td>280</td>
</tr>
<tr>
<td>Smoker</td>
<td>61</td>
<td>1.00 (0.5)</td>
<td>1.38 (1.02–1.85)*</td>
<td>183</td>
</tr>
</tbody>
</table>

MN: micronucleus; VCM: vinyl chloride; FR: frequency ratio of mean MN frequency between a comparison and a reference group, the results derived from Poisson regression models fitted separately to the control or exposure group with a single risk factor under consideration; *: The numbers in parenthesis in MN column mean minimum and maximum MN frequency, respectively.

Table 3. Differences in MN* frequency by CED* of VCM*

<table>
<thead>
<tr>
<th>CED (mg-year)</th>
<th>N</th>
<th>MN (%)</th>
<th>χ²</th>
<th>p</th>
<th>FR* (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–328</td>
<td>328</td>
<td></td>
<td></td>
<td>1</td>
<td>1.12 (0.9)*</td>
</tr>
<tr>
<td>167–</td>
<td>111</td>
<td>1.40 (0.10)</td>
<td>2.02</td>
<td>0.1549</td>
<td>1.25 (0.92–1.69)</td>
</tr>
<tr>
<td>667–</td>
<td>76</td>
<td>1.86 (0.10)</td>
<td>9.87</td>
<td>0.0001</td>
<td>1.66 (1.21–2.27)</td>
</tr>
<tr>
<td>1,667–</td>
<td>222</td>
<td>2.13 (0.22)</td>
<td>32.46</td>
<td>&lt;0.0001</td>
<td>1.90 (1.53–2.38)</td>
</tr>
</tbody>
</table>

*MN: micronucleus; *CED: cumulative exposure dose of vinyl chloride monomer; *VCM: vinyl chloride; *FR: frequency ratio of mean MN frequency between a comparison and a reference group, the results derived from Poisson regression models fitted separately to the control or exposure group with a single risk factor under consideration; *: The values in parenthesis in MN mean minimum and maximum MN frequency, respectively.
value was higher than this, we would consider the level of chromosome damage as abnormal (positive). In this study, chromosome damage was defined as a MN frequency $\geq 3\%$. VCM: vinyl chloride monomer; CED: the median cumulative exposure dose to vinyl chloride in each CED group was used as the dose level in chi-square trend test. For example, the median CED were 3.8, 346.6, 1,134.2, 5,132.2 mg-year for both genders combined.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Model</th>
<th>BMD</th>
<th>BMDL</th>
<th>AIC</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total*</td>
<td>Probit</td>
<td>1,125.8</td>
<td>882.9</td>
<td>754.95</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>Logistic</td>
<td>1,150.8</td>
<td>914.9</td>
<td>755.10</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>Log-logistic</td>
<td>913.3</td>
<td>630.6</td>
<td>753.74</td>
<td>0.11</td>
</tr>
</tbody>
</table>

BMD: benchmark dose; BMDL was specified as the lower 95% confidence limit on the cumulative exposure dose of vinyl chloride corresponding to the benchmark dose reaction in this study; *Total: the subjects of genders combined.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>N</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>BMD-0.1</th>
<th>BMDL-0.1</th>
<th>AIC</th>
<th>$p$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>737</td>
<td>–9.01</td>
<td>1</td>
<td>0.11</td>
<td>913.3</td>
<td>630.6</td>
<td>753.74</td>
<td>0.11</td>
</tr>
<tr>
<td>Male</td>
<td>406</td>
<td>–13.63</td>
<td>1</td>
<td>0.22</td>
<td>1,420.6</td>
<td>670.2</td>
<td>465.72</td>
<td>0.63</td>
</tr>
<tr>
<td>Female</td>
<td>331</td>
<td>–8.26</td>
<td>1</td>
<td>0.11</td>
<td>431.4</td>
<td>273.7</td>
<td>301.92</td>
<td>0.57</td>
</tr>
</tbody>
</table>

VCM: vinyl chloride monomer; Model: $p=c+(1-c)/[1+\exp(-a-b*\log(CED))]$, here CED is the median cumulative exposure dose to vinyl chloride in each CED group was used as the dose level in chi-square trend test; a, b and c are coefficients derived from the data; Excess risk at BMD is 0.10, *$p$ values were obtained from chi-square test; use the Pearson goodness of fit test; if $p>0.05$, means the equation is succeed fit.

The log-logistic model as implemented in BMDS permits only aggregated data. Thus we used group prevalence data of chromosome damage (Table 6), ignored individual risk factors and utilized the median VCM CED of each group as the “dose” for each individual in that group. Concretely, 3.8, 346.6, 1,134.2 and 5,132.2 mg-year were the medians of the four CED groups in total (gender combined); 3.9, 356.5, 1,273.4 and 6,254.8 mg-year were the medians for males; and 3.7, 322.4, 1,052.5 and 3,848.7 mg-year were the medians for females.

The estimated parameters and corresponding values of BMDL$_{0.1}$ are presented in Table 6. $p$ values provide a goodness-of-fit measure for the models. We found
that BMDL\textsubscript{10} values were 630.6 mg-year, 670.2 mg-year and 273.7 mg-year for total VCM-exposed workers, male VCM-exposed workers and female VCM-exposed workers, respectively.

**Discussion**

MN is expressed in cells when either acentric chromosome fragments or whole chromosomes fail to be segregated to the daughter nuclei during mitosis\textsuperscript{35}. It also seems that the CBMN assay can be used to measure not only whole chromosome loss or chromosome breaks but also excision-repair events\textsuperscript{8, 22}. Previous studies\textsuperscript{7, 17, 35} suggested that MN frequency could be one suitable index to evaluate the clastogenic and aneuploid effects in relation to occupational and environmental exposure to VCM. Consistent with previous studies\textsuperscript{7, 15–17}, our study suggested that VCM-exposed workers did have a risk of increased MN frequency that was significantly higher than that of the unexposed control group and that there was a significant dose-response relationship between MN frequency and VCM exposure.

This study also suggested that smoking status is one of the factors influencing the risk of MN formation in that MN frequency in smokers was significantly higher than in nonsmokers within the control group, which is consistent with previous investigations\textsuperscript{36, 37}; however, there was no significant association between smoking status and MN frequency in the VCM exposure group. A reasonable explanation might be that VCM exposure masked the association between smoking and MN frequency, while in the control group, without the interaction with VCM exposure, the association became apparent.

This study showed that MN frequency in female VCM-exposed workers was significantly higher than in male VCM-exposed workers, which is consistent with other evidence\textsuperscript{18} showing that MN frequency tended to be greater in females relative to males. The increase in MN frequency in females can be accounted for by the greater tendency of the X chromosome to be lost as an MN relative to other chromosomes and to the fact that females have two copies of the chromosome compared with only one in males\textsuperscript{39, 40}. In this study, the fact that the BMD\textsubscript{10} value for females was lower than that for males may also be attributable to female VCM-exposed workers being more susceptible than male VCM-exposed workers.

To explore the biologic exposure limit of chromosome damage induced by occupational VCM exposure, the BMD and BMDL of the VCM cumulative exposure dose were calculated as the 10% response level of chromosome damage using the log-logistic model of BMDS. The present study is the first use of the BMDL\textsubscript{10} method to establish VCM occupational exposure safety values in central China. As we mentioned above, the results of BMD\textsubscript{10} also suggest that female VCM-exposed workers are more prone to VCM-induced chromosome damage than males. According to the current VCM permissible exposure limit of 1 ppm (2.79 mg/m\textsuperscript{3}) in most developed countries and assuming a typical work duration of 40 yr, a worker’s VCM cumulative exposure dose would be 3,225 mg-year, which is higher than the BMDL\textsubscript{10} for all VCM-exposed workers and for male VCM-exposed workers and much higher than the BMDL\textsubscript{10} for female VCM-exposed workers in this study. This result is consistent with other biomarker studies of VCM risk assessment. For example, a study of VCM-specific mutant protein biomarkers in male VCM workers in France and Taiwan\textsuperscript{41} suggested that the current exposure limit was too high to protect against a statistically significant increase in these biomarkers and that an acceptably safe level would be about one quarter of the current level or the equivalent of an approximately 800 mg-year cumulative exposure dose for males, which compares favorably to the 670 mg-year cumulative exposure dose for males arrived at in this present study. So, although there were uncertainties about our BMD and BMDL estimates, the evidence we have presented is sufficient to warrant further studies on VCM risk of genotoxicity and further scrutiny of the current VCM occupational exposure limits.

This study has several strengths. First, it is an occupational epidemiological study with a relative large sample size. Second, for the first time, the study performed an analysis of the dose-response relationship between VCM cumulative exposure dose and chromosome damage risk using the BMD method in central China and to confirm the previous results in a Shanghai study, and based on this, we derived the VCM-exposed workers’ BMDL\textsubscript{10} including for males and females, for chromosome damage on the basis of MN frequency. The population and location of the two studies are different and independent, and the results of both are similar, although the FRs in the present study are not the same as those from the previous study. Likewise, the covariates in this model are not exactly the same as those in the previous study (for example, for Table 1 in the present study, multivariate Poisson regression models were used when these covariates were adjusted by each other), but the two studies can be used as evidence supporting that VCM-exposed workers did have a risk of increased MN frequency that was significantly higher than the unexposed control group and that there was a strong dose-response relationship between VCM cumulative exposure dose (CED) and the increased risk of a higher MN frequency in the VCM exposure group. The BMD method can be used to evaluate the
relationship between the genotoxicity indicator and the exposure dose of chemical carcinogens. We did derive the values of BMD and BMDL of the VCM cumulative exposure dose as a chromosome damage indicator in the present study, although they were not the same as those in the previous study; however, we did see that the BMD value for females was lower than that for males in both the previous and present study, which suggested that female VCM-exposed workers were more susceptible than male VCM-exposed workers. The estimated values in the two studies can be used to guide policy for how to establish the VCM occupational exposure limits.

In the meantime, our study has several limitations. First, the present study is a case-control study, VCM-exposed workers constituting the cases and controls being school workers who had no exposure, so the study results could be distorted by some bias in that the school workers must be different from the plant workers in many aspects. Second, our study did not directly measure personal VC exposure; instead, exposure assessment was based on job description, work duration, air concentration of VCM at work sites through environmental monitoring and exposure information taken from the published literature. Furthermore, multi-group classification of cumulative exposure introduced additional variation to the exposure assessment. These all contributed to the overarching uncertainty surrounding the dose-response model and the final estimates. Third, unlike hepatic angiosarcoma, MN remains a nonspecific disease marker, and other confounding factors may influence it, such as age, gender, diet, lifestyle factors, disease and genetic factors34. For example, our study did not have quantitative data about smoking habits, and in the absence of such data, the simple comparison of smokers versus nonsmokers might be misleading.

In conclusion, VCM exposure was shown to have likely induced chromosome damage in VCM-exposed workers at exposure levels much lower than the current VCM occupational exposure limits, which invites further investigations on a safety level to protect against genotoxicity than the current standard affords. As a biomarker for genotoxicity, chromosome damage data can be used for dose-response assessment and for estimating benchmark doses for the purposes of deriving OELs. Future work is highly desirable to investigate noncarcinogenic health effects induced by VCM and to produce better dose-response data, hence better estimation of BMDs and better quantification of OELs.

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