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Estimation of benchmark dose for micronucleus occurrence in Chinese vinyl chloride-exposed workers☆

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Abstract

In this study, we estimated the possibility of using benchmark dose (BMD) to assess the dose–response relationship between vinyl chloride monomer (VCM) exposure and chromosome damage. A group of 317 workers occupationally exposed to vinyl chloride monomer and 166 normal, unexposed control in Shan-dong Province northern China were examined for chromosomal damage in peripheral blood lymphocytes (PBL) using the cytokinesis-blocked micronucleus (CB-MN) assay of DNA damage. The exposed group (3.47 ± 2.65)‰ showed higher micronucleus frequency than the control (1.60 ± 1.30)‰ ($P < 0.01$). Occupational exposure level based on micronucleus occurrence in all individuals was analyzed with benchmark dose (BMD) methods. The benchmark dose lower limit of a one-sided 95% confidence interval (BMDL) for 10% excess risk was also determined. Results showed a dose–response relationship between cumulative exposure and MN frequency, and a BMDL of 0.54 mg/m^3 and 0.23 mg/m^3 for males and females, respectively. Female workers were more susceptible to MN damage than male workers.

Keywords

Vinyl chloride monomer; Chromosomal damage; Benchmark dose; Micronucleus

Introduction

Vinyl chloride monomer (CH_2 = CHCl, VCM), a major material used in the polymerization process of polyvinyl chloride (PVC), is a human carcinogen according to the classification

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of International Agency for Research on Cancer (1987). Many countries, regions and organizations have established exposure guidelines for vinyl chloride in the workplace (IARC, 2008). The level of VCM in the environment (*i.e.*, air, water, food, etc.) have been summarized in Agency for Toxic Substances & Disease Registry (ATSDR). However, the human exposure of VCM at workplace is still unclear. Previous dose–response studies on VCM exposure in animals have suggested its carcinogenicity (EPA, 2000). In these studies, the end-points were based on tumor incidence or observed liver damage test results. However, the current end points are too late a biomarker that by the time they are observed in workers, the damages have already impaired their working ability significantly. In addition, chromosome damage were induced by VCM at doses much lower than those required to form tumors or cause organ damage. Therefore, the current end-points are inappropriate in assessing human cancer risks.

The frequency of micronucleus (MN) in peripheral blood lymphocytes (PBL) is extensively used as an early biomarker of chromosomal damage and genome stability in human populations. Micronucleus occurrence as an early damage end-point for benchmark dose–response research has been reported in a previous study (Chen et al., 2010). The cytokinesis-blocked micronucleus (CB-MN) assay works by causing cytokinesis inhibition by cytochalasin B (Cyt-B), thereby arresting the cell in the stage soon after completing the first *in vitro* division (the binucleate stage). This assay has facilitated MN analysis exclusively in binucleate cells after test agent treatment. CB-MN assay makes it easy to detect and predict long-term risks associated with human exposure to mutagen and carcinogen in working and non-working environment (Fenech, 2000). Previous epidemiological studies have shown that VCM exposure is associated with increased genotoxicity in human (Luo et al., 2003; Wang et al., 2010; Zhu et al., 2005).

In this study, we used the benchmark dose method to estimate the possible dose–response relationships between MN frequency and VCM exposure. The Benchmark Dose (BMD) was defined by Crump (1984) as a 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 observable adverse effect level (NOAEL) or the lowest observable adverse effect level (LOAEL) for effects that proceeds tumor genesis in setting acceptable daily human exposure to potentially toxic substances. In fact, BMD has already been used as a minimal risk level assessment for damages induced by VCM in previous studies (Thornton et al., 2002). The main advantage with the BMD method is that it uses all collected dose–response data thereby increasing its accuracy and sensitivity. In 1998, Gaylor et al. (1998) introduced the concept of lower confidence limit of benchmark dose (BMDL) and suggested that it could be used instead of NOAEL or LOAEL (Filipsson and Victorin, 2003). The BMDL is typically calculated as the lower 95% confidence limit on a 1–10% risk increase above background, derived from the dose–response curve. 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. In this experiment, we used BMD software and instruction from EPA to estimate reference concentration of VCM exposure that resulted in chromosome damage in occupationally exposed workers.

Materials and methods

Study subjects

A total of 317 workers, 257 Han Chinese men and 60 women, aged 37.16 ± 8.07 years on average, employed at a PVC polymerization plant in Shandong, China, were included in the study. upon informed consent during routine medical surveillance. The study participants had been occupationally exposed to VCM for at least one year. Their blood samples were drawn, and they were made to complete questionnaires in a one-on-one interview. A total of

136 service workers and managers from the same factory, 98 Han Chinese men and 38 women, with an age distribution similar to the exposure group but lacking direct VCM exposure were enrolled as internal controls. The subjects of the internal control group also provided a blood sample and completed questionnaires. An external control group consisting of residents from the same living area was also recruited. Subjects of this external control group, 14 Han Chinese men and 16 women, with an average age of 37.30 ± 11.43 years, had no known VCM exposure. Both age and gender were included as adjustment variables in all analyses.

Collection and treatment of samples

A detailed questionnaire was completed by each study participant followed by the collection of a 10-ml anticoagulated wholeblood sample. Blood samples were stored at room temperature in an insulated container and were delivered to the laboratory within 12 h of collection. Cytokinesis-blocked micronucleus assays (CB-MN) were performed on the blood samples.

Assessment of vinyl chloride exposure

The VCM plant had been monitoring ambient air VCM concentration at different worksites since the beginning of its establishment. We estimate the cumulative exposure dose (CED) of each worker using the following equation:

$$\mathrm{CED}\,(\mathrm{mg/m^3}-\mathrm{year}) = \sum C\,(\mathrm{mg/m^3}) \times T\,(\mathrm{year})$$

where C is the geometric mean of VCM exposure concentration (in mg/m^3) for each month in a given workplace (calculated for all worksites). By this method, personal cumulative exposure doses (CED) in the VCM exposure group ranged from 0.09 mg/m^3 to 27.02 mg/m^3. We then grouped the VCM exposed subjects into four groups by the quartile cumulative doses (0 mg/m^3~, 0.48 mg/m^3~, 1.13 mg/m^3~, 6.36 mg/m^3~).

CB-MN assay

The CB-MN assay was performed according to standard methods as described by Fenech (1993). Briefly, 0.5 ml heparin-anticoagulated whole blood was added to 4.5 ml of cell culture medium (RMPI1640) and incubated at 37 °C in 5% CO_2. Cytochalasin-B (Cyt-B, Sigma) was added to each cell culture after 44 h at a final concentration of 6 μg/ml to prevent cytokinesis. Twenty-eight hours after the addition of Cyt-B, cells were harvested by cytocentrifugation and fixed with methanol and acetic acid at a ratio of 3:1. Slides were air-dried and stained with Giemsa. For each subject, CB-MN in 1000 binucleated lymphocytes with well-preserved cytoplasm were scored in a blinded fashion by the same reader. MN frequencies are the number of micronucleus observed per 1000 lymphocytes, expressed as a count per thousand (‰).

Statistical methods

Unadjusted MN frequency was reported together with sample characteristics and VCM exposure. The frequency of micronucleus was estimated by computing frequency ratios (FR = e^{β}, e = 2.71828, β: regression coefficient); an increase/decrease in FR suggests proportional change of micronucleus frequency. The statistical analyses were done using the software SAS (SAS Institute).

Benchmark dose estimation

Benchmark Dose Software (BMDS) Version 2.2.1 (U.S. EPA) was used for calculating BMD and BMDL. After fitting the Poisson dose–response model to the MN frequency as outlined in the previous section, a BMD was determined as the dose at which exposure would result in a specified level of increase in adverse response above that of the controls. This specified level of increase in adverse response, typically between 1 and 10%, is called the benchmark response (BMR) level. Alternatively, a lower confidence limit to the BMD estimate, *i.e.* BMDL, is often used to account for uncertainty in the BMD. In the present study, we chose BMR to be 10% above the control adverse response and a 95% confidence level for BMDL.

Results

Subject characteristics and demographics and lifestyle factors

Table 1 presents the gender, age, smoking and drinking status of the study population, along with their associations with MN frequency. There was a small, yet statistically insignificant increase in MN frequency in women compared to men (FR = 1.12, 95% CI: 0.97–1.30). No significant increase in MN was detected in smokers and regular alcohol consumers. However, the older age group (>35 years) exhibited generally a higher MN frequency than the younger age group (≤35) (FR = 1.32, 95% CI: 1.17–1.50; $P < 0.01$).

MN frequency and VCM exposure dose

The exposed group had a higher MN frequency (3.47 ± 2.65)‰ than the external controls (1.60 ± 1.30)‰ ($P < 0.01$) based on simple Poisson regression. Simple Poisson regression also showed an increasing MN frequency in each of the four exposure levels (0 mg/m^3~, 0.48 mg/m^3~, 1.13 mg/m^3~, 6.36 mg/m^3~) compared with the external controls (FR = 2.07, 95% CI: 1.61–2.83; $P < 0.01$); (FR = 1.80, 95% CI: 1.36–2.41; $P < 0.05$); (FR = 2.43, 95% CI: 1.86–3.23; $P < 0.01$); (FR = 2.88, 95% CI: 2.21–3.82; $P < 0.01$), respectively (Table 2).

Prevalence of MN frequency at different exposure levels across groups

To further confirm the dose–response relationship between cumulative VCM exposure and MN frequency, as well as to facilitate benchmark dose computation, we adopted a threshold, which is the 95-percentile of the controls' MN frequency. MN frequency above this threshold suggests chromosomal damage. This corresponds to classifying subjects with MN frequency equal or greater than 5‰ as being chromosomally damaged. The number of chromosomally damaged cases (MN frequency ≥ 5‰) in four different exposure levels was then calculated. The results demonstrated that MN frequency increases with increasing cumulative VCM exposure (Table 3).

BMD model selection

In our study, the goodness of fit was determined through application of six dichotomous dose–response models (Gamma, Logistic, Probit, Quantal-Linear and Weibull) by using total study population. P values ≥0.05, which indicates the fitness of the model, could be found in all models, providing an initial indicator of goodness of fit. However, the BMDL from the log-probit model was too low to be accepted considering the real exposure level in workplace. Comparing the Akaike Information Coefficient (AIC) values, where smaller values indicate better fits, the data suggested the use of the Log-logistic model (Table 4).

Benchmark doses based on dose–response of micronucleus damage prevalence

Benchmark Dose Software (version 1.4.1; U.S. EPA, 2000) was used to perform dose–response evaluation on the incidence of MN using the Log-logistic model (Table 5). The

study population was divided into four dose exposure levels by using the quartile VCM-cumulative exposure dose. Among each of the exposure levels, study subjects were further classified by their MN frequency. Individuals with equal or greater than 5‰ of MN frequency were labeled as chromosome damaged. Below this frequency, individuals were considered as normal. Based on the AIC values, the Log-logistic model was used, where the formula is given by $P[\text{response}] = c + (1 - c)/[1 + \text{EXP}(-a - b * \log(\text{dose}))]$.

From the fitted dose–response model, we estimated the BMD and its 95% lower confidence bound BMDL. The BMDL of VCM-cumulative exposure dose was 0.54 mg/m^3 and 0.23 mg/m^{3}for males and females, respectively.

Discussion

The detection of MN in binucleated cells by means of *t ex vivo*/*in vitro* cytokinesis-block micronucleus (CBMN) assay (Fenech et al., 2005) has been successfully employed as a reliable biomarker of exposure to chemical agents (Danitsja et al., 2008; Bonassi et al., 2007). Previous studies have also reported differences in MN frequency among different VCM exposed workers (Fucić et al., 1994; Miao et al., 2009; Qiu et al., 2011). Based on these previous studies, we explored the micronucleus frequency in VCM workers. Our study found that workers who were cumulatively exposed to VCM faced a significantly higher frequency of micronucleus, when compared to controls. Previous epidemiologic studies have also investigated the effects of various lifestyle and biological factors on MN frequency in human lymphocytes. The dominating role of age and gender among variables affecting MN frequency in peripheral blood lymphocytes (PBL) is well documented (Fenech and Bonassi, 2011; Kazimírová et al., 2009). Our study found that the most consistent demographic variable influencing the MN frequency was age. There was significant increase in MN frequency among older workers compared with younger workers. However, no significant difference between female and male workers was found. A possible reason may be the limited number of female workers present in this study. There was no significant effect of smoking or alcohol consumption on MN frequency. The most plausible interpretation for this lack of association is that the magnitude of association with VCM exposure was so strong that relationships with smoking or alcohol drinking were masked. Alternatively, blood concentrations of cigarette or alcohol-related genotoxins might be too low to cause chromosomal damage in lymphocytes (Bonassi et al., 2003).

Although dose–response evaluation for VCM exposure induced toxicity has already been conducted in human and animals (Til et al., 1991; Simonato et al., 1991), previous studies used incidence of hepatic angiosarcoma or observed liver damage as the evaluation end point (Wong et al., 1991). To us, this evaluation endpoint may still not be sufficient for establishing occupational exposure levels (OEL) of VCM to safeguard against non-cancer risk such as genotoxicity.

In our study, we first demonstrated a dose–response relationship between cumulative VCM exposure and MN frequency by using chi-square tests (Table 3). In order to assess the possibility of using BMD method in determining the dose–response relationship between VCM exposure and chromosomal damage, we adopted a threshold, which is the 95th-percentile of the control group's MN frequency. MN frequency above this threshold suggests chromosomal damage. This corresponds to classifying subjects with MN frequency equal or greater than 5‰ as being chromosomally damaged, which is in accordance with the value suggested by the Human MicroNucleus (HUMN) project. We then applied this MN frequency threshold in the four VCM exposure groups (0 mg/m^3~, 0.48 mg/m^3~, 1.13 mg/m^3~, 6.36 mg/m^3~) to calculate their frequency of chromosomal damage The proportion of chromosomal damaged subjects within each exposure groups were then determined (Table

3) and compared against their exposure levels. A significant dose–response relationship was observed. As VCM exposure increased, the proportion of chromosomally damaged subjects also increased.

The goodness of fit for different BMD calculations was determined through application of six dichotomous dose–response models. By comparing the Akaike Information Coefficient (AIC) values, where smaller values indicate better fits, the data suggested the use of the Log-logistic model to estimate BMD/BMDL values. The BMD/BMDL computation was based on an aggregated risk model, and this kind of BMD/BMDL value reflected the population's average risk. Subsequently, a smaller BMD value for females also suggests that females are more susceptible to VCM induced chromosome damage than males (0.54 mg/m^3 vs 0.23 mg/m^3). This is in accordance with previous studies (Wang et al., 2011). The possible reason is that the X chromosome has a greater tendency to be lost as an MN compared with other chromosomes. The fact that females have two copies of the X chromosome while males have only one copy, as a result, may lead to greater incidence of MN, and hence lower tolerance to VCM induced chromosome damage.

Despite of technological advances in reducing VCM exposure in Chinese factories, China still has higher occupational limit threshold limit value-time-weighted average (TLV-TWA = 10 mg/m^3), compared with USA OSHA Permissible Exposure Limit (PEL) for VCM in General Industry (<1 ppm, almost 2.79 mg/m^3) (OSHA). Our BMD results suggest that chromosome damage occurs at exposure levels under current national standard. Based on data from all study subjects, we obtained a BMD_{10} value of 1.68 mg/m3-year and a BMDL value of 1.08 mg/m^3-year. By dividing the BMDL value (1.08 mg/m^3-year) by forty employment years (Table 5), we obtained a reference TLV-TWA of 0.03 mg/m^3, which is much smaller than the current PEL level suggested by OSHA and means the current PEL and TLV-TWA level are still too high to protect workers from chromosomal damage.

While a large number of occupational studies reported an association between VCM and liver angiosacoma, few proposed an exposure level adequate for occupational exposure purposes. Simonato et al. (1991) reported on the results of a large cohort study of 12,706 VCM workers. A significant increase in liver cancer mortality was observed. Furthermore, Wang et al. (2011) derived concentration of average time-weighted-average (TWA) as 7.20 ppm (20.09 mg/m^3) from Simonato's study. They reviewed these angiosarcoma cases and observed the lowest cumulative exposure of 288 ppm-year (803.52 mg/m^3-year, 1 ppm = 2.79 mg/m^3) in a 45-year-old worker with an exposure period of 10 years who died 16 years after his first exposure. This CED of 288 ppm-years is equivalent to a time-weighted-average (TWA) of 7.20 ppm/year for 40 years (288 ppm-year/40 years = 7.20 ppm, or 20.09 mg/m^3).

Previous studies also offered strong evidence in support of a dose–response relationship between VCM exposure and the serum biomarker for plasma oncoproteins expression (p21 or p53). Luo's (Luo et al., 2003) study results showed that 12 out of 58 (16.9%) exposure workers were tested positive for one oncoprotein (p21 or p53) expression. The odds ratio for positivity in this subgroup in comparison with the unexposed controls was 4.35. One p21-positive worker had an estimated cumulative exposure of only 0.56 ppm-years (mg/m^3). Another two (p21 and p53) positive worker had an estimated cumulative exposure of 1.07 ppm-years (mg/m^3). Du et al. (1995) found that serum levels of gamma-glutamyl transferase (GGT), but not other indicators of liver function, correlated with exposure in a group of 224 VC workers with time-weighted average (TWA) exposure ranging from 0.36 to 74 ppm (0.92–189 mg/m^3). Note that our estimated value is an smaller than these value even smaller than 0.56 mg/m^3 based on the cancer risk estimated by Li et al. (1998) This difference may imply that genotoxicity is a more sensitive endpoint than the cancers and our estimated

TLV-TWA value may serve as a point of departure when additional safety factors are applied to adjust for uncertainties.

Based on past toxicological study results of VCM, EPA reported the a NOAEL value of 4.4 mg/m^3 for significant liver cell degeneration and LOAEL value of 43.9 mg/m^3 in mice with chronic consumption of VCM (EPA, 1998). The chronic dietary toxicity study of Til et al. (1991) in rats is the principal study for both dietary and inhalation reference value (RfC). The LOAEL based upon cell degeneration is at the highest dose of 1.3 mg/kg-day and the NOAEL at dose of 0.13 mg/kg-day based on the Clewell's study (Clewell et al., 1995).

However, the NOAEL is usually derived from animal data, and is defined as the highest experimental dose level for which the response is not significantly different compared to the response in the control group. Then, an uncertainty factor must be used for extrapolating the animal study results in human application. Generally, a factor is also used to account for differences in sensitivity to chemical agent in the human population. Additional uncertainty factors may also be used depending on characteristics of the study. Much previous researches estimated the application of BMD in different toxicant such as manganese, cadmium (Santamaria and Sulsky, 2010; Lei et al., 2007; Piersma et al., 2008; Suwazono et al., 2011). Previous studies also have suggested the possibility of using Benchmark analysis to assess the toxicity of VCM (Bi et al., 1985). The main advantages of the use of the BMD over NOAEL and LOAEL in our study was the more complete use of dose–response data by BMD methods not limited to being one of the experimental dose levels. Also, uncertainties about the value of a BMD can be quantified using statistical methodology. The uncertainty of a BMD may be expressed as a confidence interval, in which the lower end of a one-sided 95% confidence interval is termed BMDL. Also, the use of BMD method can provide an estimation of the potential chromosome damage associated with VCM exposure.

Despite of advantage of our present study, the application of MN frequency as a non-disease-specific marker remains limited because confounding factors such as age, smoking habits, alcohol consumption, and other chemical exposure could also cause changes in MN frequency. Moreover, our ability to accurately control for these confounding factors was limited due to the nature of study data collection. Besides, this study did not directly measure individual VCM exposure. Rather, we calculated the exposure level based on job category, work duration, air concentration of VCM at worksites, and exposure information taken from the published literature. Therefore, variation might be introduced in assigning subjects in the four exposure groups. These all contributed to the overarching uncertainty surrounding the dose–response model and the final BMD estimates.

Although there are uncertainties about our BMD and BMDL estimates, especially concerning exposure assessment and unknown mode of action involving MN, our study is sufficient to warrant further studies on VCM induced genotoxicity. The evidence also invites scrutiny of the current TLV-TWA to enhance occupational safety and regulatory standards.

Conclusion

Our study showed an increase in chromosome damage with increasing VCM cumulative exposure (CED). We also demonstrated that females are more susceptible than males to chromosomal damage caused by VCM. Further study into the role of MN frequency as a sensitive biomarkers, better VCM individual exposure assessment are desirable in order to improve the quantification of occupational exposure limits for VCM with respect to MN frequency.

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Table 1

Micronuclei (MN) frequency of VCM-exposed workers and unexposed controls by demographic characteristics.

		Unexposed workers		Controls		Exposed workers		FR (95% CI)
		N	Mean MN ± SD (‰)	*N*	Mean MN ± SD (‰)	*N*	Mean MN ± SD (‰)	
Gender	Male	98	2.46 ± 1.80 *	14	1.57 ± 1.40	257	3.39 ± 2.67 **	Reference
	Female	38	2.63 ± 2.34 *	16	1.63 ± 1.26	60	3.82 ± 2.52 **	1.12(0.97–1.30)
Age	≤35	17	1.47 ± 1.37	15	1.40 ± 1.35	128	2.91 ± 2.44 **	Reference
	>35	119	2.66 ± 1.99	15	1.80 ± 1.26	189	3.85 ± 2.72 **	1.32(1.17–1.50)
Smoke	No	74	2.49 ± 2.02 *	28	1.64 ± 1.31	158	3.44 ± 2.56 **	Reference
	Yes	62	2.53 ± 1.90	2	1. 00 ± 1.41	159	3.50 ± 2.74 *	1.02(0.90–1.15)
Drink	No	61	2.31 ± 2.11	26	1.77 ± 1.27	202	3.46 ± 2.77 **	Reference
	Yes	75	2.67 ± 1.83 *	4	0.50 ± 1.00	115	3.48 ± 2.43 *	1.01 (0.89–1.14)

MN, micronuclei; FR, frequency ratio, compare with reference in exposed group MN; VCM, vinyl chloride monomer; ‰, per thousand lymphocytes; SD, standard deviation.

* Compare with control group $P < 0.05$.

** Compare with control group $P < 0.01$.

Table 2

Differences in micronucleus (MN) frequency by VCM exposure.

CED (mg/m^3)	Number	MN Freq ± SE (‰)	FR (95%CI)
Control	30	1.60 ± 1.30	1
Exposure	317	3.47 ± 2.65 **	2.31(1.81–3.01)
0~	76	3.16 ± 2.50 **	2.07(1.61–2.83)
0.48~	71	2.68 ± 2.31 **	1.80(1.36–2.41)
1.13~	87	3.62 ± 2.65 **	2.43(1.86–3.23)
6.36~	83	4.29 ± 2.84 **	2.88(2.21–3.82)

MN, micronuclei; FR, frequency ratio, compare with pooled unexposed and control group MN; VCM, vinyl chloride monomer; ‰, per thousand lymphocytes; SD, standard deviation; CED, cumulative exposure dose, VCM exposed subjects were divided into four groups with quarte cumulative doses.

** Compare with control group $P < 0.01$.

Table 3

Trend test at different exposure level in all groups.

CED (mg/m^3)	Total			Male			Female		
	Damage	Normal	%	Damage	Normal	%	Damage	Normal	%
0~	23	219	9.5	17	149	10.2	6	70	7.9
0.48~	11	60	15.5	10	54	15.6	1	6	14.3
1.13~	19	68	21.8	10	46	17.9	9	22	29.0
6.36~	29	54	34.9	29	54	34.9	0	0	–
Linear trend									
X^2		29.12			20.91			7.99	
P		<0.001			<0.001			0.005	

Micronucleus damage was defined as micronucleus frequency ≥5‰. %: damage case frequency in each group; CED, cumulative exposure dose, VCM exposed subjects were divided into four groups with quarte cumulative doses.

Table 4

Different models were used to estimate the BMD and BMDL for VCM cumulative exposure dose for total population in this study.

Model	BMD	BMDL	AIC	*P*-value
Gamma	1.96	1.36	429.81	0.18
Logistic	3.04	2.43	432.07	0.05
Log-logistic	1.68	1.08	429.13	0.26
Probit	3.28	2.53	439.84	0.05
Quantal-linear	2.37	1.60	438.20	0.12
Weibull	2.37	1.60	438.20	0.12

AIC, Akaike Information Coefficient.

Table 5

BMD based on a Log-logistic dose–response model for micronucleus damage.

Group	*N*	b_0	b_1	BMD_{10} (mg/m^3)	$BMDL_{10}$ (mg/m^3)
Total	483	−2.66	1.00	1.68	1.09
Male	369	−2.13	1.00	1.23	0.54
Female	114	−1.45	1.82	0.67	0.23

Model: P[MN damage] = c + (1 − c)/[1 + EXP (−a − b * log (dose))]. Excess risk at BMD is 10% above reference risk in the remaining normal population. Goodness-of-fit test (P-value) indicated the model fitted the data well. BMD: benchmark dose; BMDL: lower confidence bound on the BMD. Total: all individuals in this study.