

# Chrysotile and rock wool fibers induce chromosome aberrations and DNA damage in V79 lung fibroblast cells

Yan Cui<sup>1</sup> · Ji Ma<sup>2</sup> · Wei Ye<sup>3</sup> · Zhixia Han<sup>1</sup> · Faqin Dong<sup>4</sup> · Jianjun Deng<sup>2</sup> · Qingbi Zhang<sup>1</sup> 

Received: 16 February 2017 / Accepted: 29 May 2017 / Published online: 7 July 2017  
© Springer-Verlag Berlin Heidelberg 2017

**Abstract** According to global estimates, at least 107,000 people die each year from asbestos-related lung cancer, mesothelioma, and asbestosis resulting from occupational exposure. Chrysotile accounts for approximately 90% of asbestos used worldwide. Artificial substitutes can also be cytotoxic to the same degree as chrysotile. But only a few researchers focused on their genetic effects and mutagenicity information which is useful in evaluating the carcinogenicity of chemicals. In this study, chrysotile from Mangnai, Qinghai, China, and an artificial substitute, rock wool fiber were prepared as suspensions and were tested at concentrations of 50, 100, and 200 µg/ml in V79 lung fibroblasts. Chromosome aberrations were detected by micronucleus assay after exposure for 24 h, and DNA damage were estimated by single cell gel electrophoresis after exposure for 12, 24, or 48 h. According to the results, chrysotile and rock wool fibers caused micronuclei to form in a

dose-dependent manner in V79 cells; olive tail moment values increased in a dose- and time-dependent manner. When V79 cells were exposed to a concentration of 200 µg/ml, the degree of DNA damage induced by chrysotile fibers was greater than rock wool fibers. Our study suggests that both chrysotile and rock wool fibers could induce chromosome aberrations and DNA damage. These materials are worthy of further study.

**Keywords** Chrysotile · Rock wool fibers · Mutagenicity · Single cell gel electrophoresis · Micronucleus test

## Introduction

Asbestos is an abundant, inexpensive, and geographically ubiquitous mineral fiber. Due to its distinctive properties of strength and high temperature tolerance, it is widely used in the textile, building, chemical, machinery, and defense industries. Currently, about 125 million people in the world are exposed to asbestos in the workplace (Ezzati et al. 2004). There are two types of asbestos: hornblende (crocidolite, blue asbestos) and serpentine (chrysotile). Chrysotile accounts for approximately 95% of all asbestos used in the United States (Britton 2002) and 90% of asbestos used worldwide (Geoffrey 2002; Burki 2010). Controversy over the safety of chrysotile use has been substantial (Yarborough 2006; Finkelstein and Meisenkothen 2011; Kagan 2013; Bernstein and Hoskins 2006). As of 2013, more than 50 countries, including all member states of the European Union, had banned the use of all forms of asbestos, including chrysotile (Bernstein et al. 2013). Some non-asbestos producers, especially in western developed countries, advocate use of substitutes, such as rock wool fiber, glass fiber, or ceramic fiber. Despite the bans on chrysotile, however, it is still widely mined and exported in developing countries (Burki 2010), and, in recent years, some

---

Yan Cui and Ji Ma contributed equally to this study

---

Responsible editor: Philippe Garrigues

---

✉ Jianjun Deng  
jianjundeng0801@163.com

✉ Qingbi Zhang  
qingbizhang@126.com

<sup>1</sup> School of Public Health, Southwest Medical University, Luzhou 646000, Sichuan, People's Republic of China

<sup>2</sup> Department of Clinical Laboratory, 404 Hospital of Mianyang, Mianyang 621000, Sichuan, People's Republic of China

<sup>3</sup> B-Ultrasound Room, Yilong County People's Hospital of Nanchong City, Nanchong 637676, Sichuan, People's Republic of China

<sup>4</sup> Key Laboratory of Solid Waste Treatment and the Resource Recycle, Southwest University of Science and Technology, Mianyang 621010, Sichuan, People's Republic of China

countries have maintained or even increased their production or use of chrysotile. China is the largest consumer and second largest manufacturer of chrysotile in the world, and more than one million people in China experience occupational exposure to chrysotile (Wang et al. 2013; Courtice et al. 2012).

Scientific evidence found that exposure to asbestos, including chrysotile, can cause cancers of the lung, larynx, and ovary, as well as mesothelioma and asbestosis. According to global estimates, at least 107,000 people die each year from asbestos-related lung cancer, mesothelioma, and asbestosis resulting from occupational exposure (Driscoll et al. 2005a, b). Artificial substitutes can also be cytotoxic, even to the same degree as chrysotile (Gazzano et al. 2007; Paustenbach et al. 2006; Pugnali et al. 2013; Brown and Harrison 2014; Donaldson et al. 2013). Since genotoxicity is generally a prerequisite for the development of malignancy, the relationship between asbestos exposure and chromosomal aberrations and mutations has been explored. Chrysotile fibers induce chromosome aberrations in human lymphocytes, amniotic fluid cells, and peritoneal fluid cells and in bone marrow cells of mice (Stanton et al. 1977; Durnev et al. 1993). In the present study, we compared the genotoxicity of chrysotile from Mangnai, Qinghai, China, and rock wool fiber. Our findings add to the scientific evidence that can be used to evaluate the safety of chrysotile and rock wool fibers.

## Materials and methods

### Chrysotile and rock wool fibers

Chrysotile fiber from Mangnai, Qinghai, China, and rock wool fiber were generously donated by the Southwest University of Science and Technology, Mianyang, Sichuan, P. R. China (He et al. 2014).

### Preparation of suspensions of ultrafine fibers

Fibers were crushed in a ceramic mortar, then cut and ground to 2–3 mm. This material was suspended in ethyl alcohol and ground for 12 h in a horizontal ball mill, and then passed through a 400-mesh screen. Particles smaller than 10  $\mu\text{m}$  were collected and oven-dried to a powder. Fiber powder (0.2 g) was sterilized by incubating at 150  $^{\circ}\text{C}$  for 2 h. RPMI 1640 medium (20 ml) was added, and the mixture was swirled for 30 min to produce a 10 mg/ml ultrafine powder suspension. At the end of this process, no aggregation of the granules was observed, and the average granule diameter was less than 10  $\mu\text{m}$ . Prior to use, suspensions were diluted to 50, 100, or 200  $\mu\text{g}/\text{ml}$  with RPMI 1640 medium supplemented with 2% fetal bovine serum (FBS, Life Technologies/Gibco, Grand Island, NY, USA).

### Cell line and culture conditions

V79 Chinese hamster lung fibroblast cells were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and were maintained in 5%  $\text{CO}_2$  at 37  $^{\circ}\text{C}$  in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin (Life Technologies/Gibco, Gaithersburg, MD, USA). Cells were maintained as monolayers in 25  $\text{cm}^2$  culture flasks at 37  $^{\circ}\text{C}$  in a 5% carbon dioxide humidified atmosphere. At 80–90% confluence, cells were detached with 0.25% trypsin and were passaged at an initial density of  $0.5\text{--}1.0 \times 10^6$  cells/ml.

### Micronuclei (MN) assay

Cells in the logarithmic growth phase were suspended at a concentration of  $10^5$  cells/ml, seeded on culture dishes, and incubated at 37  $^{\circ}\text{C}$  in 5%  $\text{CO}_2$  for 24 h. After the cells adhered, the culture medium was replaced with medium containing suspensions of fiber powder at three different concentrations. Cell culture medium was used as a negative control, and culture medium containing 200  $\mu\text{g}/\text{ml}$  cyclophosphamide was used as a positive control. The culture dishes were incubated for an additional 24 h, were washed with PBS, were fixed with methanol/acetic acid (3:1 [v/v]) for 30 min at 4  $^{\circ}\text{C}$ , and then were covered with coverslips. After staining with Wright-Giemsa stain for 5 min, cell morphology was examined by light microscopy<sup>[26]</sup>. In addition,  $1 \times 10^3$  cells per slide were examined, totaling  $4 \times 10^3$  cells on 3–4 slides per treatment group and the numbers of cells containing MN were counted. The slides were blinded to the scorer. The MN scoring criteria were as follows: diameter no larger than one-third of that of the main nuclei, non-refractile, staining intensity same as or brighter than that of the main nuclei, and located within the cytoplasm but not in contact with the main nuclei. Binucleated cells with overlapping main nuclei were not scored. The results were expressed as percentages of total cells according to the following formula:  $\text{MN} = n_1/n_0 \times 100\%$ , where  $n_1$  was the number of cells containing MN and  $n_0$  was the total number of cells observed.

### Single cell gel electrophoresis

Single cell gel electrophoresis (SCGE), also known as the alkaline comet assay, was performed as described (Behm et al. 2009). Briefly,  $0.5\text{--}1.0 \times 10^6$  V79 cells were seeded onto 12-well culture plates and were incubated with a fiber suspension at 50, 100, or 200  $\mu\text{g}/\text{ml}$  in culture medium for 12, 24, or 48 h. Medium containing no fiber was used as a negative control, and medium containing 0.1 mM  $\text{K}_2\text{Cr}_2\text{O}_7$  was used as a positive control. For electrophoresis, microscope slides with frosted ends were covered with normal-melting-point agarose (1% in distilled water) before the experiment. For

the second layer, 50  $\mu\text{l}$  of cell suspension mixed with 100  $\mu\text{l}$  of low-melting-point agarose (0.5% in PBS, Sigma) was pipetted onto the slides, was spread by use of a cover slip, and was maintained on an ice-cold flat tray for 5 min for solidification. After removal of the cover slips, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris (pH 10), and 1% sodium sarcosinate), with 1% Triton-X 100 and 10% DMSO added immediately before the reaction, for at least 2 h at 4 °C. The slides were removed from the lysing solution and were placed in a horizontal gel electrophoresis tank. The tank was filled with fresh electrophoresis solution (1 mM Na<sub>2</sub>EDTA and 300 mM NaOH, pH 13) to a level approximately 0.5 cm above the slides. The slides were allowed to remain in the cold (4 °C) buffer for 20 min to denature cellular DNA. Electrophoresis was performed at 4 °C for 30 min at 300 mA and 25 V (~0.74 V/cm). After electrophoresis, the slides were washed with Tris buffer (0.4 M Tris, pH 7.5) 3 times for 5 min each. To stain the DNA, ethidium bromide (65  $\mu\text{l}$  of 20  $\mu\text{g}/\text{ml}$ ) was added to each slide. Slides were covered with a cover slip, were placed in a humidified, airtight container to prevent drying of the gel, and were analyzed within 3–4 h. Cells were visualized with a fluorescent microscope equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. For analysis, 100 cells were selected randomly using two slides. The slides were blinded to the scorer. CASP comet analysis software was used for quantification of DNA damage. Olive tail moments (OTM = [distance between the center of gravity of DNA in the tail and the center of gravity of DNA in the head]/[percent of DNA in the tail]) were measured and calculated automatically.

### Statistical analyses

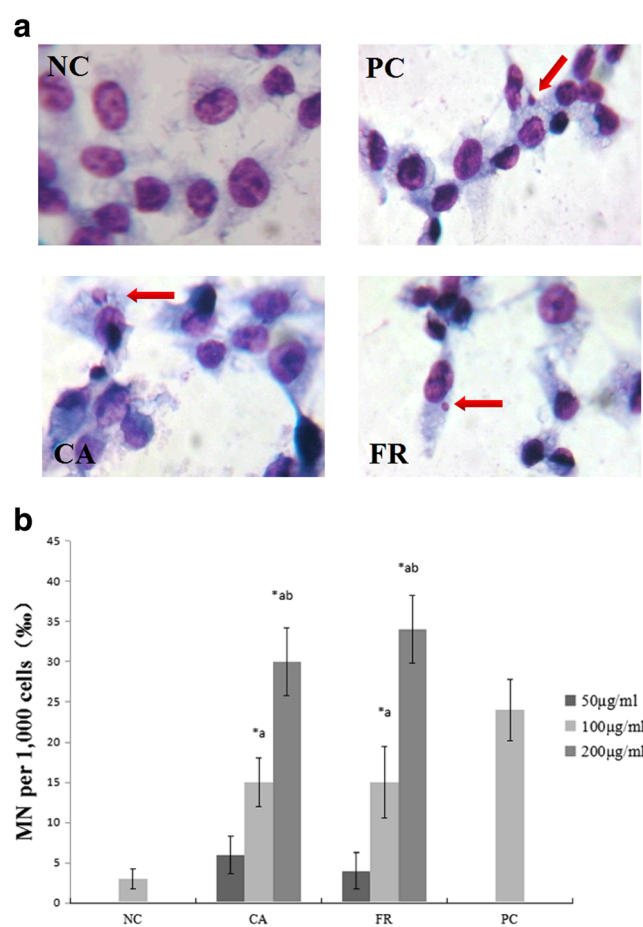
SPSS 20.0 was used for all data analyses. Results were expressed as means  $\pm$  standard deviations. One-way analysis of variance (ANOVA) was used for comparisons of SCGE results and MN frequencies from experimental and control samples. Post hoc analysis of group differences was performed by the Fisher's least significant difference (LSD) test. The limit for statistical significance was fixed as  $p < 0.05$ .

## Results

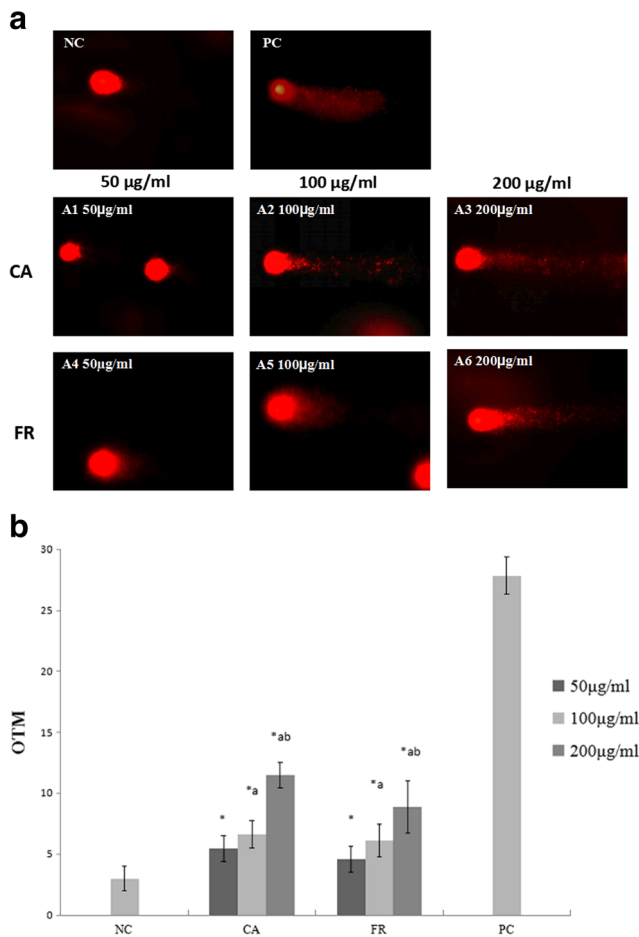
### Chrysotile and rock wool fibers induce chromosome aberrations in V79 cells

MN, cytoplasmic chromatin masses with the appearance of small nuclei, arise from chromosome fragments or an intact chromosome lagging behind in the anaphase stage of cell division (Dopp et al. 1997). Their presence in cells is a

reflection of structural and/or numerical chromosomal aberrations during mitosis. To determine if chrysotile and rock wool fibers cause an increase in MN of V79 cells, the frequencies of MN were determined after 24 h of exposure to 50, 100, or 200  $\mu\text{g}/\text{ml}$  fiber suspensions and were compared with those for untreated cells. Images of cells with and without MN are shown in Fig. 1a. Chrysotile and rock wool fibers caused increases in MN. For these fibers, there was no statistical difference in MN rates between untreated cells and cells treated with 50  $\mu\text{g}/\text{ml}$  fiber suspension. There was, however, a significant increase in MN in cells treated with 100 or 200  $\mu\text{g}/\text{ml}$  fiber suspension (Fig. 1b). Thus, for V79 cells, chrysotile, and rock wool fibers induce increases in MN in a dose-dependent manner.



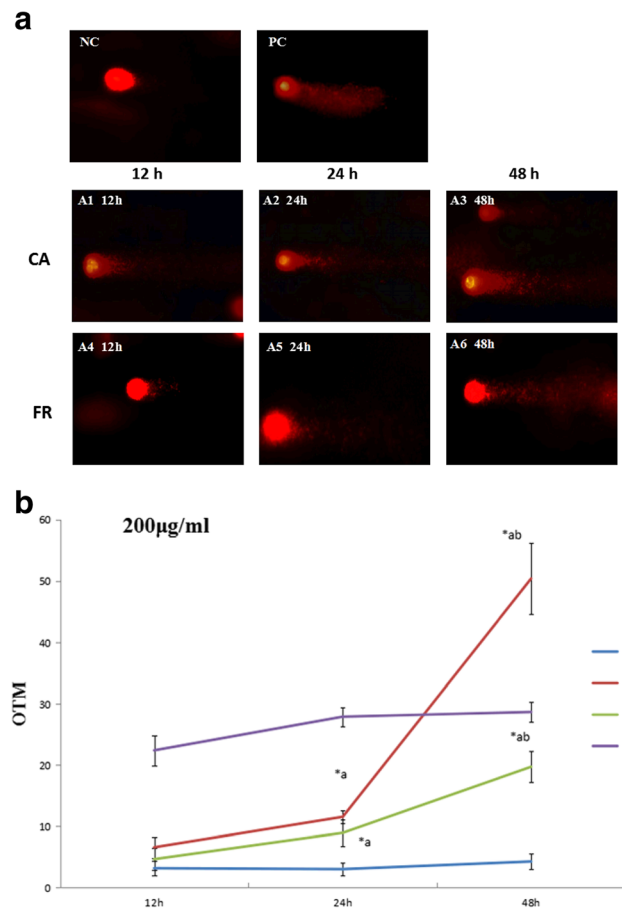
**Fig. 1** In V79 cells, chrysotile and rock wool fibers induce chromosome aberrations in dose-dependent manner. *NC* negative control group, cell culture medium with no fiber added, *PC* positive control group, medium containing 200  $\mu\text{g}/\text{ml}$  cyclophosphamide. *MN* micronuclei, *CA* exposed to chrysotile fibers, *FR* exposed to rock wool fibers. V79 cells were exposed to 50, 100, or 200  $\mu\text{g}/\text{ml}$  suspensions of chrysotile or rock wool fibers for 24 h. **a** MN in cells exposed to chrysotile or rock wool fibers (100  $\mu\text{g}/\text{ml}$ , 40 $\times$ ). **b** The frequency of MN in cells exposed to chrysotile or rock wool fibers for 24 h. \* $P < 0.05$ , significantly different from the negative control. <sup>a</sup> $P < 0.05$  significantly different from 50  $\mu\text{g}/\text{ml}$  dose group and <sup>b</sup> $P < 0.05$  significantly different from 100  $\mu\text{g}/\text{ml}$  dose group



**Fig. 2** In V79 cells, chrysotile and rock wool fibers induce DNA damage in a dose-dependent manner. *NC* Cell culture medium with no fiber added was used as a negative control, *PC* medium containing 0.1 mM K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was used as a positive control. *CA* exposed to chrysotile fibers, *FR* exposed to rock wool fibers. V79 cells were exposed to 50, 100, or 200 µg/ml suspensions of chrysotile or rock wool fibers for 24 h. **a** Typical DNA comet images, 400×. **b** DNA fragmentation was quantified by determining OTMs using CASP comet analysis software. \**P* < 0.05, significantly different from the negative control. <sup>a</sup>*P* < 0.05 significantly different from 50 µg/ml dose group and <sup>b</sup>*P* < 0.05 significantly different from 100 µg/ml dose group

**Chrysotile and rock wool fibers induce DNA damage in V79 cells**

The SCGE assay is also known as the comet assay because, upon electrophoresis, damaged DNA trails behind intact DNA in the nucleus, resembling a comet. SCGE is a widely used procedure for detection of DNA fragmentation due to single- or double-strand breaks (Gagné et al. 1996). To compare DNA damage, SCGE was performed for V79 cells exposed to chrysotile or rock wool fibers. Cells were incubated for 24 h with 50, 100, or 200 µg/ml of each fiber suspension before quantifying DNA damage. As reflected by the extended migration of fragmented DNA in comet olive tails, the treated cells showed an increase in DNA damage that dose-dependently correlated with the concentrations of chrysotile and rock wool fibers (Fig. 2a). After 24 h of exposure, a significant increase in



**Fig. 3** In V79 cells, chrysotile and rock wool fibers induce DNA damage in a time-dependent manner. *NC* Cell culture medium with no fiber added was used as a negative control, *PC* medium containing 0.1 mM K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was used as a positive control. *CA* exposed to chrysotile fibers, *FR* exposed to rock wool fibers. V79 cells were exposed to 200 µg/ml suspension of chrysotile or rock wool fibers for 12, 24, or 48 h. **a** Typical DNA comet images, 400×. **b** DNA fragmentation was quantified by determining OTMs using CASP comet analysis software \**P* < 0.05, significantly different from the negative control. <sup>a</sup>*P* < 0.05 significantly different from exposed to for 12 h and <sup>b</sup>*P* < 0.05 significantly different from exposed to for 24 h

OTM was observed for all concentrations of chrysotile and rock wool fibers. In addition, there was DNA migration for the V79 cells treated with 200 µg/ml chrysotile or rock wool fibers for 12, 24, or 48 h (Fig. 3a), and the magnitude of OTMs increased in a time-dependent manner. Chrysotile fibers had a greater effect than rock wool fibers (Fig. 3b). These results indicate that chrysotile and rock wool fibers induce DNA damage of V79 cells in a dose- and time-dependent manner and that the degree of DNA damage induced by chrysotile fibers is greater than that induced by rock wool fibers.

**Discussion**

Chrysotile accounts for more than 90% of the asbestos used worldwide (Geoffrey 2002; Burki 2010). Studies concerning

the mutagenicity of chrysotile, mainly epidemiological investigations, focus mainly on the incidence of lung cancer and mesothelioma of populations exposed to chrysotile (Driscoll et al. 2005a, b; Goodman et al. 2014). As determined by investigations of the pathology of mesotheliomas, chrysotile fibers, along with various other types of asbestos fibers, are present (Yano et al. 2009; Straif et al. 2009; Huang et al. 2007; Ishihara 2001). Thus, evaluation of the mutagenicity of chrysotile fibers alone may not reflect the cause of mesotheliomas. Further evidence is needed to establish the cause of these cancers.

Mutation refers to “permanent changes in the structure and/or amount of the genetic material of an organism that leads to heritable changes in its function, and includes gene mutations as well as structural and numerical chromosome alterations” (Eastmond et al. 2009). Cancer is the result of accumulation of mutations that result in autonomy, unlimited growth, and metastasis of target cells. Therefore, an evaluation of mutagenicity is used as a test for carcinogenicity. Although the SCGE and MN are conventional methods to assess carcinogenicity, the genetic endpoints of these methods are different. DNA damage were detected by SCGE, and MN assay were used to evaluate the chromosome damage induced by DNA breaking agent and Aneuploidy mutagen. In the present study, the tests were used to evaluate the genetic effects of chrysotile from Mangnai, Qinghai, China and rock wool fiber.

The results show that the chrysotile and rock wool fibers cause chromosome aberrations, as indicated by an increase in MN frequency, in a dose-dependent manner. Further, in V79 cells, both chrysotile and rock wool cause DNA damage in a time- and dose-dependent manner. The SCGE results indicate that chrysotile exposure leads to single- and/or double-strand breaks in DNA. This is in agreement with a previous study showing that asbestos causes a variety of DNA damage, including DNA cross-linking, DNA single- or double-strand breaks, and damage to bases (Yano et al. 2009). Our results are also in agreement with results of a study showing that exposure of V79 cells to chrysotile leads to an increase in reactive oxygen species and MN and to decreases in cell viability; it also causes genetic toxicity in a time- and a dose-dependent manner (Dopp et al. 2005).

Assessment of the severity of DNA damage, as measured by the comet assay, showed that the effects of chrysotile were greater than those for rock wool. Further research is required to determine the structural features and surface properties and to clarify how they are related to genotoxicity. Glass fibers have cytotoxic and genotoxic effects, even at low concentrations (Rapisarda et al. 2015). Further studies, including epidemiological investigations and experiments with animals, should be accomplished in regard to the safety of chrysotile and rock wool fibers.

## Conclusion

For V79 cells, chrysotile and rock wool fibers cause chromosome aberrations in a dose-dependent manner and DNA damage in a dose- and time-dependent manner. Measurements of DNA damage show that chrysotile has a greater effect than rock wool. Further investigation of the safety of chrysotile and other fibers is warranted.

**Acknowledgements** The authors wish to thank Donald L. Hill (University of Alabama at Birmingham, USA), an experienced, English-speaking scientific editor, for editing.

## Compliance with ethical standards

**Funding** This research was funded by the Key Program of National Nature Science Project of China (No. 41130746), the National Natural Fund Project of China (No. 41472046), and the Department of Sichuan Province Natural Science Foundation of China (14JC0126).

**Conflict of interest** All authors declare that there are no conflicts of interest in this study.

## References

- Behm C, Degen GH, Follmann W (2009) The fusarium toxin enniatin B exerts no genotoxic activity, but pronounced cytotoxicity in vitro. *Mol Nutr Food res* 53:423–430
- Bernstein DM, Hoskins JA (2006) The health effects of chrysotile: current perspective based upon recent data. *Regul Toxicol Pharmacol* 45:252–264
- Bernstein D, Dunnigan J, Hesterberg T, Brown R, Velasco JA, Barrera R, Hoskin J, Gibbs A (2013) Health risk of chrysotile revisited. *Crit rev Toxicol* 43:154–183
- Britton M (2002) The epidemiology of mesothelioma. *Semin Oncol* 29: 18–25
- Brown TP, Harrison PT (2014) Crystalline silica in heated man-made vitreous fibres: a review. *Regul Toxicol Pharmacol* 68:152–159
- Burki T (2010) Health experts concerned over India’s asbestos industry. *Lancet* 375:626–627
- Courtice MN, Lin S, Wang X (2012) An updated review on asbestos and related diseases in China. *Int J Occup Environ Health* 18:247–253
- Donaldson K, Poland CA, Murphy FA (2013) Pulmonary toxicity of carbon nanotubes and asbestos—similarities and differences. *Adv Drug Deliver res* 65:2078–2086
- Dopp E, Schuler M, Schiffmann D (1997) Induction of micronuclei, hyperdiploid and chromosomal breakage affecting the centric/pericentric regions of chromosomes 1 and 9 in human amniotic fluid cells after treatment with asbestos and ceramic fibers. *Mutat res* 377: 77–87
- Dopp E, Yadav S, Ansari FA, Bhattacharya K, Von Recklinghausen U, Rauen U, Rahman Q (2005) ROS-mediated genotoxicity of asbestos-cement in mammalian lung cells in vitro. *Particle and Fibre Toxicology* 2:9
- Driscoll T, Nelson DI, Steenland K, Leigh J, Concha-Barrientos M, Fingerhut M, Prüss-Üstün A (2005a) The global burden of non-malignant respiratory disease due to occupational airborne exposures. *Am J Ind med* 48:432–445

- Driscoll T, Nelson DI, Steenland K, Leigh J, Concha-arrientos M, Fingerhut M, Prüss-Üstün A (2005b) The global burden of disease due to occupational carcinogens. *Am J Ind med* 48:419–431
- Durnev AD, Daugel-Dauge NO, Korkina LG, Seredenin SB (1993) Peculiarities of the clastogenic properties of chrysotile-asbestos fibers and zeolite particles. *Mutation Research/Genetic Toxicology* 319:301–308
- Eastmond DA, Hartwig A, Anderson D (2009) Mutagenicity testing for chemical risk assessment: update of the WHO/IPCS harmonized scheme. *Mutagenesis* 24:341–349
- Ezzati M, Lopez AD, Rodgers A, Murray CJL (2004) Comparative quantification of health risks. Global and regional burden of disease attributable to selected major risk factors. Geneva: World Health Organization 2004:1651–1801
- Finkelstein MM, Meisenkothen C (2011) Malignant mesothelioma among employees of a Connecticut factory that manufactured friction materials using chrysotile asbestos. *Ann Occup Hyg* 54:692–696
- Gagné F, Blaise C, Bermingham N (1996) Lethal and sublethal effects of marine sediment extracts on rainbow trout hepatocytes. *Toxicol Lett* 87:85–92
- Gazzano E, Turci F, Foresti E, Putzu MG, Aldieri E, Silvagno F, Fubini B (2007) Iron-loaded synthetic chrysotile: a new model solid for studying the role of iron in asbestos toxicity. *Chem res Toxicol* 20:380–387
- Geoffrey T (2002) Asbestos and its lethal legacy. *Nat rev Cancer* 2:311–315
- Goodman JE, Peterson MK, Bailey LA, Kerper LE, Dodge DG (2014) Electricians' chrysotile asbestos exposure from electrical products and risks of mesothelioma and lung cancer. *Regul Toxicol Pharmacol* 68:8–15
- He J, Dong F, Deng J, Jing H (2014) Contrastive study on toxicities of chrysotiles with different origins on V79 cells. *Journal of Environmental and Occupational Medicine* 31:476–478 **(in Chinese)**
- Huang FD, Dong FQ, Wu FC, Ma J, Deng JJ (2007) Study on cytotoxicity of chrysotile and substitute fibres. *J Toxicol* 21:498–500 **(In Chinese)**
- Ishihara Y (2001) In vitro studies on biological effects of fibrous minerals. *Ind Health* 39:94–105
- Kagan E (2013) Asbestos-induced mesothelioma: is fiber biopersistence really a critical factor? *Am J Pathol* 183:1378–1381
- Paustenbach DJ, Madl AK, Donovan E, Clark K, Fehling K, Lee TC (2006) Chrysotile asbestos exposure associated with removal of automobile exhaust systems (ca. 1945–1975) by mechanics: results of a simulation study. *Journal of Exposure Science and Environmental Epidemiology* 16:156–171
- Pugnaloni A, Giantomassi F, Lucarini G, Capella S, Bloise A, Di Primio R, Belluso E (2013) Cytotoxicity induced by exposure to natural and synthetic tremolite asbestos: an in vitro pilot study. *Acta Histochem* 115:100–112
- Rapisarda V, Loreto C, Ledda C (2015) Cytotoxicity, oxidative stress and genotoxicity induced by glass fibers on human alveolar epithelial cell line A549. *Toxicol in Vitro* 29:551–557
- Stanton MF, Layard M, Tegeris A, Miller E, May M, Kent E (1977) Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. *J Natl Cancer Inst* 58:587–603
- Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, Coglianò V (2009) A review of human carcinogens-part C: metals, arsenic, dusts, and fibres. *The Lancet Oncology* 10:453–454
- Wang X, Courtice MN, Lin S (2013) Mortality in chrysotile asbestos workers in China. *Curr Opin Pulm med* 19:169–173
- Yano E, Wang ZM, Wang XR, Wang MZ, Takata A, Kohyama N, Suzuki Y (2009) Mesothelioma in a worker who spun chrysotile asbestos at home during childhood. *Am J Ind med* 52:282–287
- Yarborough CM (2006) Chrysotile as a cause of mesothelioma: an assessment based on epidemiology. *Crit rev Toxicol* 36:165–187