Inhalative Exposure to Vanadium Pentoxide Causes DNA Damage in Workers: Results of a Multiple End Point Study

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BACKGROUND: Inhalative exposure to vanadium pentoxide (V_2O_5) causes lung cancer in rodents.

OBJECTIVE: The aim of the study was to investigate the impact of V_2O_5 on DNA stability in workers from a V_2O_5 factory.

METHODS: We determined DNA strand breaks in leukocytes of 52 workers and controls using the alkaline comet assay. We also investigated different parameters of chromosomal instability in lymphocytes of 23 workers and 24 controls using the cytokinesis-block micronucleus (MN) cytome method.

RESULTS: Seven of eight biomarkers were increased in blood cells of the workers, and vanadium plasma concentrations in plasma were 7-fold higher than in the controls (0.31 μg/L). We observed no difference in DNA migration under standard conditions, but we found increased tail lengths due to formation of oxidized purines (7%) and pyrimidines (30%) with lesion-specific enzymes (formamidopyrimidine glycosylase and endonuclease III) in the workers. Bleomycin-induced DNA migration was higher in the exposed group (25%), whereas the repair of bleomycin-induced lesions was reduced. Workers had a 2.5-fold higher MN frequency, and nucleoplasmic bridges (NPBs) and nuclear buds (Nbuds) were increased 7-fold and 3-fold, respectively. Also, apoptosis and necrosis rates were higher, but only the latter parameter reached statistical significance.

CONCLUSIONS: V_2O_5 causes oxidation of DNA bases, affects DNA repair, and induces formation of MNs, NPBs, and Nbuds in blood cells, suggesting that the workers are at increased risk for cancer and other diseases that are related to DNA instability.

KEY WORDS: comet assay, cytokinesis-block micronucleus assay, DNA damage, occupational exposure, vanadium pentoxide. *Environ Health Perspect* 116:1689–1693 (2008). doi:10.1289/ehp.11438 available via *http://dx.doi.org/* [Online 31 July 2008]

Vanadium pentoxide (V_2O_5) is used for the production of metal alloys, for the manufacturing of lithium batteries and high-pressure lamps, and for the synthesis of chemicals [International Agency for Research on Cancer (IARC) 2006]. Its annual production worldwide is in the range of 60,000 tons (IARC 2006; Woolery 1997). Occupational exposure to the oxide occurs at production sites, during processing and refining of vanadium ores and sludges, during manufacturing of vanadium-containing products, in the course of combustion of vanadium-rich fuels, and by handling of catalysts in the chemical industry (Plunkett 1987). Environmental exposure to the metal and its oxides occurs via inhalation in the vicinity of metallurgical plants or through consumption of contaminated foods (Barceloux 1999; IARC 2006). Although foods contain low concentrations, nutrition is the major source of exposure for the general population (Barceloux 1999).

The National Toxicology Program (NTP 2002) found an increase of lung adenomas and carcinomas in mice after inhalative exposure to V_2O_5; this was paralleled by an increased incidence of hyperplasia in lung tissue. In male rats, the number of tumors was elevated (nonsignificantly), whereas in females no increase was found (IARC 2006; NTP 2002; Ress et al. 2003). These findings led to a reevaluation of the metal oxide (IARC 2006) and to its classification as a group 2B ("possible human") carcinogen. One of the problems encountered in the risk assessment of V_2O_5 is the lack of human data. According to IARC (2006), inhalative exposure to V_2O_5 in vanadium plants exists worldwide, and several hundred workers may be affected; furthermore, workers of other industries may also be exposed. The occupational exposure limit in Austria for V_2O_5 in air is 0.05 mg/m^3 (Bundesminister für Wirtschaft und Arbeit 2003). The Senate Commission of the German Research Foundation [Deutsche Forschungsgemeinschaft (DFG)] decided to suspend the maximum allowed concentration of V_2O_5 in workplace air because of its suspected carcinogenicity (DFG 2006). In the United States, the National Institute for Occupational Safety and Health and the American Conference of Governmental Industrial Hygienists established an occupational exposure limit of 0.05 mg/m^3 air (Occupational Safety and Health Administration 2006). Measurements of air concentrations in vanadium factories yielded values in the range of 0.02–5 mg/m^3 (IARC 2006).

Results of *in vitro* and animal studies indicate that the oxide causes formation of reactive oxygen species (Ingram et al. 2003, 2007; Wang et al. 2003; Zhang et al. 2001) and aneugenic effects (Migliore et al. 1993; Ramirez et al. 1997; Zhong et al. 1994) and interferes with DNA synthesis and repair (IARC 2006). Because DNA damage and aneugenic processes are known to play a role in the onset of human cancer (Duesberg et al. 2005; Pitot 1986), evidence of genetic damage in exposed humans would support the assumption of increased cancer risks. At present, only one study on the influence of occupational exposure to V_2O_5 on DNA stability has been published; in that study, Ivancsits et al. (2002) investigated DNA migration in leukocytes using the standard single-cell gel electrophoresis (SCGE) assay. The authors observed no indication of damage and found no elevation in the frequencies of sister chromatid exchanges (SCEs) or the concentration of 8-hydroxy-2′-deoxyguanosine in leukocytes. Lener et al. (1998) found no SCEs or chromosomal aberrations in blood cells of children living in the vicinity of a vanadium production site.

Our goal in the present study was to comprehensively evaluate the impact of inhalative V_2O_5 exposure on genetic stability. We monitored DNA migration in leukocytes of workers and matched controls with the standard SCGE assay, and we monitored oxidized bases using lesion-specific enzymes (Collins et al. 1993). Furthermore, we measured the sensitivity toward bleomycin (BLEO) and the repair of lesions induced by this cytostatic agent (Rajaee-Behbahani et al. 2001; Schmezer et al. 2001; Wei et al. 2005). BLEO sensitivity was initially monitored in chromosomal aberration assays (Hsu et al. 1989; Szekely et al. 2003) and later in SCGE experiments (Schmezer et al. 2001).

Additionally, we conducted cytokinesis-block micronucleus cytome (CBMN Cyt) assays with lymphocytes. This test is widely used for the detection of DNA damage in

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