Toxicology Letters 212 (2012) 222–227

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Acute effects of exposure to vapors of hydrogen peroxide in humans

Lena Ernstgård*, Bengt Sjögren, Gunnar Johanson

Work Environment Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-171 77 Stockholm, Sweden

HIGHLIGHTS

- We studied the acute effects of inhaled hydrogen peroxide in humans.
- Volunteers were exposed to 0 ppm, 0.5 ppm or 2.2 ppm hydrogen peroxide for 2 h.
- Our study suggests that hydrogen peroxide is slightly irritating at 2.2 ppm.

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 23 May 2012
Accepted 29 May 2012
Available online 5 June 2012

Keywords:
Irritation
Inflammation
Human exposure

ABSTRACT

Hydrogen peroxide is a reactive chemical mainly used for bleaching, as a disinfectant, and as a general oxidizing agent. The aim of this study was to investigate subtle acute effects of inhaled hydrogen peroxide vapors. Eleven healthy volunteers were exposed to 0 (clean air), 0.5 and 2.2 ppm for 2 h at rest. Symptoms related to irritation and central nervous system effects were rated with Visual Analog Scales. The ratings varied considerably but were generally low and with no significant differences between exposure conditions, although the ratings of smell ($p = 0.09$, Friedman's test), nasal irritation ($p = 0.06$) and throat irritation ($p = 0.06$) showed borderline tendencies to increase at 2.2 but not at 0 and 0.5 ppm. Nasal airway resistance increased after exposure to 2.2 ppm hydrogen peroxide ($p = 0.04$, paired t-test) but not after 0.5 ppm. No exposure-related effects on pulmonary function, nasal swelling, breathing frequency and blinking frequency were detected. Furthermore, no clear effects were seen on markers of inflammation and coagulation (interleukin-6, C-reactive protein, serum amyloid A, fibrinogen, factor VIII, von Willebrand factor and Clara cell protein in plasma). In conclusion, our study suggests that hydrogen peroxide is slightly irritating at 2.2 ppm, but not at 0.5 ppm.

1. Introduction

Hydrogen peroxide is a colorless, reactive unstable chemical with pungent odor. It is an oxidizing agent and will react or decompose to oxygen and water in the presence of most metals, alkaline solutions or catalase. The decomposition is highly exothermic. Hydrogen peroxide is mainly used in chemical industrial processes and in paper industry for bleaching. In addition, it is used for bleaching textiles and hair, as a disinfectant and for water treatment. The use has increased sharply in Europe during the 1990s as hydrogen peroxide replaced chlorine in various bleaching processes. In Sweden, it has increased from 86 000 ton 1999 to 159 000 ton in 2008 (SPIN, 2000). Due to instability and risk of explosion, hydrogen peroxide is generally kept in aqueous solution. In most consumer products such as a hair bleach, chlorine free bleaches, and contact lens disinfectants, hydrogen peroxide is more diluted (1–6%) than the solutions used in industry (commonly 30–70%).

In modern major industrial settings closed automated production systems are often used and this implies a lower potential risk of occupational exposure to workers during normal working conditions. However, spills and leaks are occupational hazards associated with manual handling of hydrogen peroxide in old and/or small industrial settings. In minor industrial uses, the safety management systems are often not implemented in the process, or for storing or transporting the hydrogen peroxide inside the factory (EU-RAR, 2003).

Hydrogen peroxide is known to be irritating to the mucous membranes and the airways (Watt et al., 2004) and this is considered to be the critical effect when setting an occupational exposure limit. The Swedish occupational exposure limit is presently 1 ppm (1.4 mg/m^3) for an 8-h work-shift, with 2 ppm (3 mg/m^3) given as a 15-min ceiling value (SWEA, 2011). An 8-h threshold limit value (TLV) of 1 ppm is also given by the American Conference of Governmental Industrial Hygienists (ACGIH, 2012), whereas the German Research Foundation has set a maximum 8-h concentration (MAK) of 0.5 ppm (DFG, 2011). However, the documentation

* Corresponding author. Tel.: +46 8 524 82226; fax: +46 8 336981.
E-mail addresses: Lena.Ernstgard@ki.se (L. Ernstgård), Bengt.Sjogren@ki.se (B. Sjögren), Gunnar.Johanson@ki.se (G. Johanson).

0378-4274/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2012.05.025

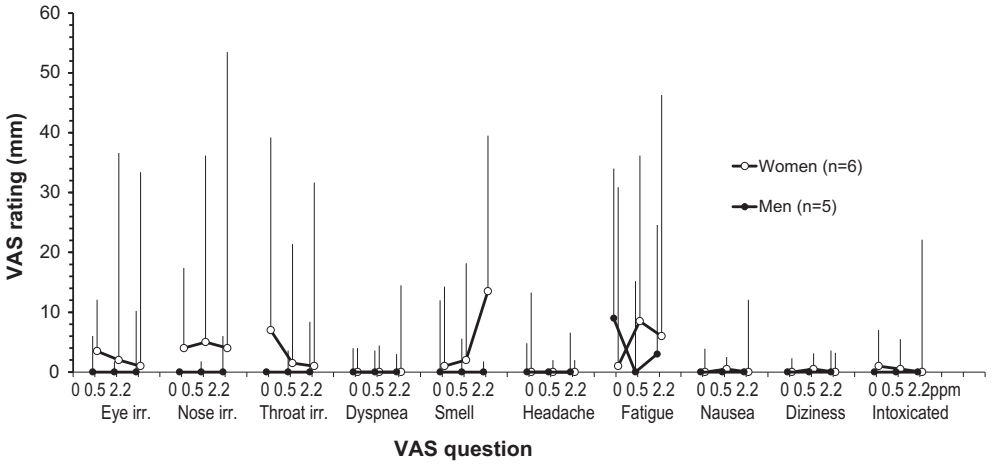

Fig. 1. Median ratings of the ten symptoms in the Visual Analog Scale (VAS) form during 2 h exposures to 0 ppm (control), 0.5 and 2.2 ppm hydrogen peroxide. Female (o, $n = 6$) and male (●, $n = 5$) data are given separately. Vertical bars indicate the 90th percentiles.

is poor regarding effect levels and we found few relevant studies in the scientific literature (Kondrashov, 1977, cited by EU-RAR, 2003; Riihimäki et al., 2002; Mastrangelo et al., 2005, 2009). Thus, neither a No-Observed Adverse Effect Level (NOAEL), nor a Lowest Observed Adverse Effect Level (LOAEL) could be identified.

The aim of this study was to investigate acute irritative and inflammatory effects in humans of short term inhalation exposure to hydrogen peroxide.

Measurements of acute effects included symptom ratings related to irritation and effects of the central nervous system. Irritation in the eyes, nose, and airways was performed by means of instruments which we have previously used in several studies of acute effects of volatile chemicals (see e.g. Ernstgård et al., 2006a,b,c, 2009). In addition, some markers of inflammation and coagulation; Interleukin-6, C-reactive protein, serum amyloid A, fibrinogen, factor VIII, von Willebrand-factor and Clara cell protein, were measured in blood. These markers were chosen as they react rather rapidly to inflammatory stimuli.

2. Methods

2.1. Subjects

Eleven volunteers, six women and five men, with a mean age of 26 years (range 20–38 years) participated in the study. The volunteers were students recruited by advertisement at Karolinska Institutet. Inclusion criteria were; between 20 and 50 years old, healthy, non-smoker and without chronic diseases. A medical examination, including clinical blood chemistry tests, was performed prior to exposure. To avoid unintended fetal exposure, females performed a pregnancy test (Clinitest hCG, Simens, USA) immediately before each exposure. None of the volunteers were allowed to use contact lenses in the exposure chamber. The subjects were informed about the design of the study, the possible hazards, and their right to immediately and unconditionally interrupt the exposure. Each participant signed a written consent after the presentation of oral and written information. The study was performed according to the Helsinki declaration and was approved by the Regional Ethical Review Board in Stockholm.

2.2. Experimental design

Two subjects at the same time were exposed at 3 separate occasions to vapors of hydrogen peroxide at 0.5, 2.2 ppm and to clean air as control exposure. The subjects were exposed for 2 h during resting conditions while seated in an exposure chamber (20 m^3) with controlled climate (average temperature 24 °C, 30% relative humidity, 18–20 air changes/h). The exposure sessions were separated by at least one week and the concentrations were administered according to a balanced design. The subjects were instructed not to discuss their symptoms or assumed exposure conditions with anyone until after the final exposure session.

The hydrogen peroxide vapor was generated by pumping liquid hydrogen peroxide (30%, hydrogen peroxide ACS reagent, including 0.5 mg/l stannate-containing compound and 1 mg/l phosphorus-containing compound to stabilize the solution, Sigma–Aldrich) by means of an HPLC pump to a preheated tube connected to the inlet air of the exposure chamber. The hydrogen peroxide vapor was mixed with clean air and dispersed into the entire exposure chamber through the ceiling. Five fans situated within the chamber further ensured an even distribution. The air concentrations in the chamber were continuously monitored and logged in parallel on two electrochemical detectors (Sensor XS EC H_2O_2, PAC III, Draeger, Germany) which displays were blinded from the volunteers. The correctness of the factory-calibrated EC detectors was checked by adding known amounts of hydrogen peroxide to Tedlar™ bags filled with 9.6 l of clean air to final concentrations of 0.5, 1.0, and 2.0 ppm. The response of the two detectors was similar and proportional to the spiked concentration. However, it was slightly lower than 100%, the average ratios being 88% (0.95% CI, 79–101%, $n = 15$) and 80% (72–89%), respectively. The final concentrations of hydrogen peroxide in chamber air were corrected for the detector response. The hydrogen peroxide concentration were, on average, 0.48 (sd 0.05), and 2.2 (sd 0.19) ppm, respectively. The air temperature averaged 23.4 °C at all three exposure conditions, with little variation (sd 0.05 °C). The humidity was somewhat lower (27% Rh) than the pre-set value of 30% Rh and the variability was 5% Rh (sd), but there was no statistical difference between the sessions.

The methods for measurements of symptoms and effects in the airways, nose and eyes were the same as previously used (Ernstgård et al., 2006a,b,c, 2009) but are summarized below for convenience.

2.3. Symptom ratings

Symptom ratings were performed using a 0–100 mm Visual Analog Scale (VAS) graded from "not at all" to "almost unbearable" (Fig. 1). The 10 symptoms to be rated in the questionnaire were (1) "discomfort in the eyes: burning, irritated, or runny eyes"; (2) "discomfort in the nose: burning, irritated, or runny nose"; (3) "discomfort in the throat or airways"; (4) "breathing difficulty"; and (5) "solvent smell", (6) "headache"; (7) "fatigue"; (8) "nausea"; (9) "dizziness"; and, (10) "feeling of intoxication". The questionnaire was elaborated for vapor exposure and has been used in several similar inhalation studies performed in our laboratory (Ernstgård et al., 2006a,b,c, 2009; Iregren et al., 1993; Sundblad et al., 2004). Symptom ratings were performed immediately before, during exposure (at 3, 60, and 118 min), and after exposure (at 145, 330, and 1440 min from onset of exposure).

2.4. Airway measurements

Pulmonary function parameters were measured prior to, immediately after, and at 3.5 h post exposure. The measurements included vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF) and forced expiratory flow at 25%, 50% and 75% of FVC (FEF_{25}, FEF_{50}, FEF_{75}). The measurements were performed with the subjects standing in an upright position and according to the recommendations of the American Thoracic Society (ATS, 1987). The highest values of three slow and three forced exhalations were used. The parameters FEV_1/FVC and FEV_1/VC were calculated from the spirogram. The pulmonary function tests were performed using a spirometer (Vitalograf 21210; Buckingham, United Kingdom) along with designated computer software (Spirotrac 3, v 2.0). The spirometer was calibrated every morning with a known volume (2 l) according to the computer software.

A peak expiratory flow meter (Mini-Wright, Clement Clarke International Ltd, London, UK) was used to assess nasal and mouth PEF rates. During nasal exhalation, the flow meter was connected to a face mask and the subject exhaled maximally into the flow meter with his mouth closed (Nihlén et al., 1998). PEF measurements were performed prior to, immediately after, and at 3.5 h post exposure. The highest of three measurements was kept at each occasion. The blocking index, a measure