

# Ten Day Exposure to Hypochlorous Acid (Wonder Spray) Fog Results in no Detectable Effect on Blood Metabolic Panel and Minimal Lung Pathology

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## Abstract

Hypochlorous acid (HOCl) is a well-established antiseptic with potential for treatment of lung diseases. However, to our knowledge, no studies have been published determining whether the vapor from HOCl leads to lung damage and alters blood metabolism. We therefore exposed groups (n=6) of C57BL/6 mice to a fog of HOCl for 8 h per workday for 2 weeks with no exposure over the weekend. Our results indicate no detectable change in blood metabolic panel and minimal changes in lung pathology. Because the changes in lung pathology were minimal and deemed likely reversible by the pathologist after a high dose exposure, we conclude that HOCl usage should not cause lung damage after use and may lead to new treatments for lung disease.

**Keywords:** Wonder spray; Ten day exposure

## Introduction

HOCl is a potent antiseptic agent that acts faster than sodium hypochlorite (NaOCl; bleach) or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to kill pathogenic bacteria and exhibits lower

cytotoxicity towards mammalian cells [1,2]. HOCl inhibits bacterial replication, cell division, and protein synthesis by oxidizing sulfhydryl enzymes and amino acids, decreasing adenosine triphosphate production; breaking DNA, and depressing DNA synthesis [3-7]. HOCl is in clinical use as a wound cleansing agent with antimicrobial, anti-biofilm, and wound healing activities [1,2,8-10].

While HOCl is used as a cleaning agent [1,2] the possibility of lung damage and secondary effects on blood metabolism by inhaling large quantities of the HOCl has not been tested to our knowledge. We therefore exposed mice to a fog of HOCl for 10 days over 2-weeks (no exposure during weekend) and then assessed its effects on a blood metabolic panel and lung histopathology. We report here that mice subjected to this extended HOCl treatment exhibited no detectable change in the metabolic panel and minimal (likely reversible) lung histopathology.

## Methods

### **Ethics statement**

The Institutional Animal Care and Use Committee of La Jolla Infectious Disease Institute approved all protocols and procedures.

### **Mice**

Female C57BL/6 mice (Jackson Laboratories, 6-8 weeks of age) were housed in micro isolator cages and fed food and water ad libitum. The animals were acclimatized for more than 2 days before beginning the experiment. The mice were housed in mouse micro isolator cages while not exposed to HOCl fog.

### **Exposure of animals to HOCL fog**

The fog was generated by placing a Car Fogger in rat micro isolator cages, which generated a dense, uniform fog in the cage. These misters use ultrasound to generate a cool dry fog. Two misters were filled with either undiluted HOCl (Wonder Spray Inc.; 250 ppm in sterile water) or water to generate the fog in the 2 test cages. The fog was monitored every 2-3 hours to verify correct functioning of the mister. The protocol exposed mice every working day for 8 hours and for 2 weeks (i.e., 10-day exposure) to the fog starting on Tuesday (Table 1). This protocol minimizes the time for healing to overnight because the blood and lungs were harvested on Tuesday after two weeks with exposure on Monday (Table 1). The fog exposure cage was cleaned and dried every use and new food and bedding was added to the cage the next day when the mice were exposed to fog.

## **Blood and lung harvest**

The mice were anesthetized by avertin injection and blood was obtained from the animal via the retro-orbital plexus. The blood was processed to serum and shipped to IDEX for metabolic analysis. The metabolic panel of serum measured: albumin, total protein, creatinine, blood urea nitrogen, Ca, P, K, Na, Cl, and bicarbonate (TCO<sub>2</sub>). After collecting the blood, the lungs were perfused to remove red blood cells and then fixed. Briefly, the left atrium was slit to allow blood to leak out. Approximately 10 ml of cold PBS was perfused through the right ventricle by gravity until the lungs are cleared of blood. Approximately 10 ml of 10% formalin was then perfused to fix the tissue. The lungs were dissected out, placed in a labeled 50 mL tube with 10% formalin, and allowed to fix overnight. On the following day, the lungs were plastic embedded, sectioned, and stained by Hematoxylin and Eosin (H and E). Whole slide images were obtained by using a Panoramic SCAN (3D Histech), displayed, and analyzed electronically via ImageDx by a board-certified veterinarian pathologist with expertise in oxidant damage of lung. Each of the images were judged to be of high quality by the pathologist.

## **Microscopic evaluation of h and e mouse lung sections**

Thirteen (H and E) stained digital images of mouse lungs were submitted (6 from each of groups and one untreated lung). Lung samples were well-fixed (lungs were inflated and had no collapsed areas) and well-prepared as H and E slides.

A board-certified veterinary pathologist with experience in laboratory animals and toxicologic pathology evaluated the H and E slide digital images for any findings and scored the findings on a scale from 0 to 5 (0=within normal limits or WNL, 1=minimal findings or the least change discernible, 2=mild findings, 3=moderate, 4=marked, and 5=severe or to the greatest extent possible (e.g. all tissues in all fields affected)). Findings were denoted as focal (only in a focal area) or diffuse as appropriate.

Lungs were evaluated overall for size and shape (macroscopic outline) as well as microscopically for any findings on pleural surfaces, in major airways (bronchi), bronchioles, and in blood vessels. Lung parenchyma, including alveoli (walls, spaces) and interstitium were examined for any evidence of inflammation or damage including but not limited to interstitial and alveolar (airway) leukocytes, edema, and hyaline membranes (protein exudates), and breaks in septa. Lungs exposed to agents by inhalation (rather than particles or systemic) would be anticipated to have findings in the airways and alveoli. Lungs exposed to systemic pulmonary toxins might be expected to have more vascular-centric findings rather than airway/alveolar findings.

## Statistical analysis

Analysis of variance with the Prism program (GraphPad) with Tukey's post-hoc test was performed to statistically compare all metabolic panel measurements with a P value cut-off of 0.05. Comparison of the pathological parameters was performed by the Mann-Whitney nonparametric test with a P value cut-off of 0.05. The mean and standard deviation of the results are reported in text.

## Results

HOCl-fog does not elicit any detectable changes in metabolic panel. All the components of the metabolic panel were similar in HOCl- and control water-fog treated groups. The values were also similar to the untreated mouse that was included as a reference (data not shown).

The untreated control slide for Animal #5 was within normal limits for all parameters evaluated. The water-fog treated animals were mostly in the range of normal; however, some prominent alveolar macrophages were observed.

Lungs from HOCl-fog treated animals exhibited only minimal focal changes, specifically in prominent alveolar macrophages, scattered minimal protein exudates/hyaline membranes in alveolar spaces/ respiratory bronchioles, disruption of alveolar walls. These findings were very subtle and focal: i.e., not to the level of minimal to mild or diffuse through the entire lung fields and are likely reversible. In one animal, scattered focal hemorrhages were observed. The minimally increased foci of minimally prominent macrophages (macrophages in alveolar spaces or airways) were very small and focal; these were only more prominent than what was seen in other lung sections in this set of tissues (compared with sections from water fog-treated animals) and would not have been listed as a finding without the comparator tissues. The minimal focal disruption of alveolar walls was also in comparison to the unaffected mouse lungs. These areas were very focal (often only one or two alveoli affected).

## Discussion

The anti-septic properties of HOCl are well established [1,2,11] but whether the vapor elicits lung and blood toxicity after use remains to be determined. We therefore exposed groups of mice to a dense fog of HOCl or water for 8 hours per day for 10 days over a 2-week period. Our results indicate no detectable changes in the metabolic panel and only minimal subtle changes in lung pathology that the pathologist judged were reversible.

## Conclusion

We therefore conclude that Wonder Spray is safe to use as a disinfectant and possible for medical use. Because HOCL is well tolerated when inhaled, studies are underway to explore the use of inhaled HOCL for the treatment of pneumonia and COVID-19 infection.

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