

**Research Article** 

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# Development of a Gaseous Nitrous Acid Generation System for Animal Exposure Experiments

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## Abstract

Although a flow-type generation system for obtaining gaseous nitrous acid (HONO) at parts-per-billion levels has already been reported, higher gaseous HONO concentrations on the order of parts per million (ppm) are necessary for use in animal exposure experiments, which are conducted to examine certain biological effects. We have developed a flow-type generation system to produce gaseous HONO at ppm levels for animal exposure experiments. This system is based on spraying a mixture of aqueous sodium nitrite with an acid in a porous polytetrafluoroethylene tube. Herein, we report the technical details and cautionary notes of the manufacturing of the system. The most technical cautionary note pertains the flow-control method in the tube for the gas and water solution.

**Keywords:** Nitrous acid; Flow-type generator; Animal exposure experiment; Porous PTFE tube

**Abbreviations:** HONO: Nitrous acid; NO<sub>2</sub>: Nitrogen dioxide; NO: Nitric Oxide; PTFE: Polytetrafluoroetylene; ppm: parts per million

# Introduction

Nitrous acid (HONO) exists as a gas in the atmosphere [1,2]. A few inhalation studies [3,4], animal exposure experiments [5,6] and epidemiological investigation [7] have been carried out to examine the biological effects of HONO. All these studies suggest a similar biological impact of HONO. Jarvis et al. suggested that HONO could cause asthma, which is considered to be an effect of nitrogen dioxide  $(NO_2)$  exposure.

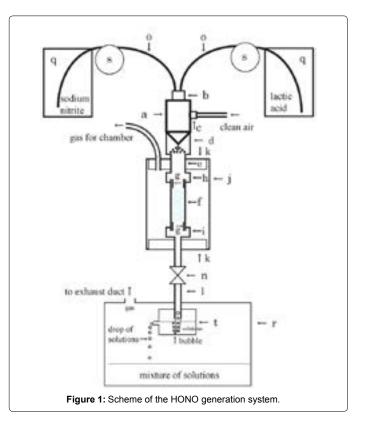
Oka et al. [8] reported continuous HONO generation systems and discussed appropriate HONO generation conditions for use in animal exposure experiments [8]. The study introduced some HONO production methods [9-12], and described the relationship between secondary products and experimental conditions in two types of porous polytetrafluoroethylene (PTFE) tube systems. The systems were called "molecular diffusion method" and "filtration method through the tube wall". We carried out animal exposure experiments by the molecular diffusion method, thereby exposing guinea pigs to 3.6 ppm HONO for four weeks (24 h/day) in the presence of two secondary products: 0.3 ppm NO<sub>2</sub> and 1.6 ppm NO [5]. We also exposed mice to 8.4 ppm HONO for three weeks (24 h/day) in the presence of 2.8 ppm NO<sub>2</sub> and 7.2 ppm NO [6].

Oka et al. [8] demonstrated that the filtration method through the tube wall yields less secondary products than the molecular diffusion method. Therefore, we fabricated a HONO generation system based on the filtration method and operated the generator in our animal exposure experimental system. Our purpose is to demonstrate the fabrication of the new generation system to facilitate HONO animal exposure experiments in many institutes.

## Materials and Methods

# Materials

The following letters describe the corresponding parts in Figures 1 and 2: a. atomizer-nozzle (BN90s-IS[V], SUS316L, 1/8PT, M14;



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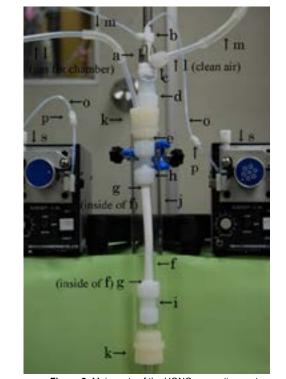


Figure 2: Main parts of the HONO generation system.

Atomax Co., Shizuoka, Japan b. T joint (30-3MRT-2C, 3mm, 1/8PT; Sanplatec Co., Osaka, Japan) c. joint (HN-1010, 6 mm, 1/8PT; Sanplatec Co., Osaka, Japan) d. joint (PTFE union, 3/8 -1/2PT) e. PTFE tube (7 mm×9 mm) f. porous PTFE tube (TB-1008, approximately 15 cm; Sumitomo Electric Fine Polymer, INC., Kumatori, Japan) g. PTFE tube (6 mm×8 mm, length approximately 30 mm) h. joint (PTFE union, 3/8PT) i. joint (PTFE union, 1/4 - 3/8PT) j. acrylic pipe (30 mm×35 mm, length approximately 250mm) k. silicon cork (32 mm×27 mm, height 35 mm) l. PTFE tube (4 mm×6 mm) m. silicon tube (5 mm×9 mm) n. PTFE needle valve (NVS1, for 6 mm tube; Sanplatec Co., Osaka, Japan) o. PTFE tube (2.4 mm×3.0 mm) p. joint (30-3U-C; Sanplatec Co., Osaka, Japan) q. bottle (5 L) r. bottle (10 L) s. tubing pumps for PTFE tube (MRP-1X; Nikko Engineering Concept, Inc., Kanagawa, Japan) t. instrument for fluid resistance (air valve generator ingredient).

## Structure of the HONO generation system

A scheme of the HONO generation system is shown in Figure 1. Aqueous sodium nitrite and lactic acid solutions were injected into the atomizer-nozzle (a) by means of tubing pumps (s). Clean air for the animal exposure chambers was sprayed with the mixture inside a porous PTFE tube (f). The gas filtered by the porous PTFE tube was then sent to the animal exposure chambers, and the mixture inside the tube was discharged with a small part of the gas.

The discharge velocity of the mixture was controlled by a needle valve (n) and an air valve (t). The needle valve was effective for controlling the flow velocity of the aqueous solution, whereas the air valve was used to regulate the flow velocity of the gas. The discharged gas, together with the aqueous solution, was exhausted outdoors after ventilation of the soda lime column. The HONO concentration in the animal exposure chamber was regulated by the concentration of the aqueous solution.

#### Example of assembly

A photo of the HONO generation system is shown in Figure 2. For convenience, the PTFE tubes were connected with joints (p) and silicon tubes (m). The atomizer-nozzle (a) and the joint (d) were connected using a nozzle mounting screw, and the joints (h, i) and the porous PTFE tube (f) were connected by placing the PTFE tube (g) into the porous PTFE tube (f). The circumference of the porous PTFE tube (f) was sealed using an acrylic pipe (j) and silicon corks (k).

#### Example of the running conditions

Aqueous sodium nitrite solution: Three sodium nitrite (>98.5% pure; Wako Pure Chemical Industries, Ltd., Osaka, Japan) solutions (2, 6, and 18 mMol) were prepared using milliQ water for dose-dependent animal exposure experiments.

Aqueous lactic acid solution: A concentrated lactic acid (>85% pure; Wako Pure Chemical Industries, Ltd., Osaka, Japan) solution (18–25 mMol) was prepared using milliQ water for dose-dependent animal exposure experiments.

Tubing pumps for the PTFE tube: The flow velocity was maintained at approximately 0.45 ml/min.

Blast conditions for the exposure chamber: The air for the HONO generation system was supplied by the filtration of room air with a container of around 5 kg granular activated charcoal and 15 sheets of American air filters for vinyl isolators (Clea Japan, Inc., Tokyo, Japan). We also used air compressors (0.4LE-8S; Hitachi Industrial Equipment Systems Co., Ltd., Tokyo, Japan), dehumidifiers (RAX3F; Orion Machinery Co., Ltd., Nagano, Japan), and high pressure regulator valves (model No. 44–2263-241; Kojima Instruments Inc., Kyoto, Japan) with a maximum supply pressure of around 0.08 MPa. The air flow to the atomizer-nozzle was 16 L/min, regulated by mass flow controllers (model 8350MC-0-1-1; Kojima Instruments Inc., Kyoto, Japan). The animal exposure chambers were supplied with air from the HONO generation system.

Temperature of the porous PTFE tube: The acrylic pipe of the HONO generation system was heated to 30°C using a water bath.

## Determination of nitrogen oxides

HONO Determination: HONO was collected by the Harvard EPA Annular Denuder System [13,14], with sodium carbonate and glycerol coating two annular denuders (URG-2000-30×150-3CSS; URG Corporation, NC). The amount of HONO was measured by ion chromatography (700 series; Metrohm Japan LTD., Tokyo, Japan).

 $NO_2$  and NO Determination: The concentrations of contaminated  $NO_2$  and NO were measured by a NOx analyzer (ECL-77A; J-science, Inc., Kyoto, Japan) after passing the sample through the sodium carbonate annular denuders within the same sampling period.

# Results

Table 1 shows the concentrations of HONO and secondary products in the exposure chambers. The HONO and secondary products samplings were not continuous. One sampling was carried out for 30 min with an air flow of 1 L/min, and the total sampling periods were less than 2% throughout the experimental period. Therefore we estimated the nitrogen oxides concentrations as one digit under the decimal point (ppmv).

	HONO (ppmv)	NO (ppmv)	NO <sub>2</sub> (ppmv)
clean air chamber	0.0	0.0	0.0
chamber of NaNO <sub>2</sub> 2 mM	0.1	0.0	0.0
chamber of NaNO <sub>2</sub> 6 mM	0.4	0.1	0.0
chamber of NaNO <sub>2</sub> 18 mM	1.7	0.2	0.0

HONO: Ion chromatography measured HONO gathered by sodium carbonate denuder. Sodium carbonate denuder gathers 100 % of HONO, 0 % of NO and 2 - 3 % of NO, [15].

NO and NO<sub>2</sub>: NOx analyzer measured NO and NO<sub>2</sub> by way of sodium carbonate denuder.

 Table 1: Concentrations of nitrogen oxides in the chambers for the animal exposure experiments.

# Discussion

The concentrations in the chamber of using 18 mM NaNO<sub>2</sub> were: HONO=1.7 ppmv, NO=0.2 ppmv, and NO<sub>2</sub>=0.0 ppmv, obtained using the HONO generation system based on the filtration method through the tube wall. The concentrations obtained in our previous experiment, which was carried out using the HONO generation system based on the molecular diffusion method, were reported as HONO=3.6 ppmv, NO=1.6 ppmv, and NO<sub>2</sub>=0.3 ppmv [5]. We consider that the filtration method through the tube wall yields less secondary products than the molecular diffusion method.

The most devised point was the development of a method for stabilizing the drainage flow inside the porous PTFE tube. While a gas is necessary to prevent solution leakage, excessive gas emission could make the gas flow in the chamber unstable. Oka et al. [8] showed that the emission of a large amount of gas with the drainage may reduce the concentration of secondary products NO<sub>2</sub> and NO. Because our air flow system could not emit such a large amount of gas with the drainage, a stable drainage flow system was devised for low gas emission. The needle valve controlled only the solution flow, and therefore, an air valve generator ingredient was installed to control the gas flow. The air valve generator ingredient was not resistant to the solution flow.

At low temperature, the HONO generation conditions were unstable, and therefore, the acrylic pipe of the generation system was heated to 30°C. The boiling point of HONO is unknown. In our experiments, HONO generation was suppressed under low-temperature conditions, whereas highly-dense HONO was detected at elevated temperatures. The effects of the indoor temperature on HONO generation could not be recognized, except for low temperatures. The length of the porous PTFE tube was not important for HONO generation.

The concentration of the aqueous lactate solution did not cause a difference in the HONO generation condition from same concentration of sodium nitrite water solution to 10 times of it. The lactate concentration was increased than the sodium nitrite concentration to disregard solution sending speed difference of two tubing pumps. The capacity of our exposure chambers was 0.2 m<sup>3</sup>, and the air was passed through the chambers at 16 L/min. A suitable atomizer-nozzle was chosen for our system.

The costs depend on the animal exposure experimental facility, and improvements are not possible. Therefore, we did not investigate the best conditions previously reported by Oka et al. [8]. However, we could adapt the filtration method for our animal exposure system, and our results suggest that the filtration method through the tube wall yields less secondary products than the molecular diffusion method.

#### Acknowledgement

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