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Removal of malodorous substances at an animal facility in a subtropical region equipped with COSA/TRON® system

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ABSTRACT

The animal facility affiliated with the School of Medicine, University of the Ryukyus, located in a subtropical area in Japan, was previously equipped with the COSA/TRON® air recovery system in order to sustain a well balanced laboratory environment. In 1988, we measured the concentrations of ammonia, hydrogen sulfide, methyl mercaptan and dimethyl sulfide in the air of animal rooms at this animal facility. In this previous study, all of these odors were maintained at very low levels, a situation to which the COSA/TRON system was considered to have contributed. However, we anticipated that the air conditioning system would by now have become decrepit and deteriorated in its function for the elimination of malodorous substances and microorganisms. In the present study, we measured the same malodorous substances as those examined previously. The concentration of ammonia was determined by the indophenol method, and those of sulfur compounds by gas chromatography. The concentrations of ammonia, methyl mercaptan and dimethyl sulfide were increased as compared with those measured in 1988. The results obtained indicated that an increase in these malodorous substances (which could not be effectively eliminated by cleaning) had occurred, and that the COSA/TRON system continued to be useful for the elimination of sulfur compounds. However, efficiency of the system for eliminating ammonia at low concentrations may have gone down. *Ryukyu Med. J., 17(3)135~141, 1997*

Key words: malodorous substances, COSA/TRON system, laboratory animals

INTRODUCTION

Malodorous substances in animal rooms exert various kinds of physiological and pathophysiological effects on laboratory animals. It has been reported that high levels of ammonia induce inflammatory changes in the trachea and lung of guinea-pigs and rats[1,2], while comparatively low levels of ammonia tend to slow down the ciliary movement in the trachea of laboratory rats and make such animals more susceptible to respiratory airway infections[3-6]. In addition, ammonia inhibits the phagocytic activities of macrophages[7]. As a result, malodorous substances disrupt the health of laboratory animals. Therefore, it is very important for animal facilities to regulate and control malodorous substances[8-10].

This animal facility, located in a subtropical area of Japan, was previously equipped with a contaminant excitation field, COSA/TRON® (control of secondary air electronically) air recovery system (COSA/TRON system), in which air pollutants are neutralized electronically by adsorption onto charged corpuscles generated by the system. Grown corpuscles adsorbing air pollutants are trapped by the filtration system, and the air is recirculated (Manufactory's booklet, Sinko Kogyo Co., Osaka, Japan). In 1988, on measuring levels of ammonia and sulfur compounds (hydrogen sulfide, methyl mercaptan and dimethyl sulfide) which are laid down by Akushuu Bousi Hou, the Japanese law for the prevention of malodorous substances, all of these compounds were shown to be maintained at very low levels as compared with both the standard values ordained in that law and those reported at other animal facilities[11]. On investigating the cleanliness of the mouse room for conventional use in the animal facility by means of the trapping of airborne microorganisms, it was found that the count of trapped microorganisms had not increased after 7 days under non-cleaning and non-disinfecting conditions. These results were similar to those observed under cleaning and disinfecting conditions[12]. It was considered

Table 1 Husbandry and feeding of animals

Animal room	Room sizes	Housing equipment*	Feed	Water
First mouse room	5.9×2.3×2.5 m	Box-type plastic cage (21×31×13 cm)	Commercial diet (CE-2, CLEA Japan, Inc., Tokyo, Japan)	For all animals, water was supplied *ad libitum* with an automatic watering system
Rat room	5.9×5.4×2.5 m	FRP rat cage (26×38.2×20 cm)	Commercial diet (CE-2, CLEA Japan, Inc., Tokyo, Japan)	
Monkey room	4.7×2.6×2.5 m	Box with drainboard made of stainless steel (59×60×66 cm)	Sweet potato and Commercial diet (PS, Oriental Yeast Co., Tokyo, Japan)	
Rabbit room	5.9×5.4×2.5 m	FRP rabbit cage (35×52.7×35 cm)	Commercial diet (RC-4, Oriental Yeast Co., Tokyo, Japan)	
Guinea-pig room	5.9×2.3×2.5 m	Wire mesh type cage (35×42×20 cm)	Commercial diet (RC-4, Oriental Yeast Co., Tokyo, Japan)	
Rat isolation room	4.5×2.8×2.5 m	FRP rat cage (26×38.2×20 cm)	Commercial diet (CE-2, CLEA Japan, Inc., Tokyo, Japan)	

*: All cages were purchased from CLEA Japan, Inc., Tokyo, Japan.

that the COSA/TRON system had contributed to the high levels of cleanliness at the facility.

With the lapse of 9 years from the previous study in 1988, we expected that the air conditioning system would by now have become decrepit, with a consequent deterioration in function for the elimination of malodorous substances and microorganisms. In the present study, we measured the same malodorous substances as those examined previously. The results will be used to secure an effective administration of this animal laboratory.

MATERIALS AND METHODS

Animals and husbandry

We followed the "Standards Relating to the Care and Management of Experimental Animals" (Notification No. 6, March 27, 1980, from the Prime Minister's Office, Tokyo, Japan) for the care and use of the animals, and the guide for animal experiments of the University of the Ryukyus. This animal experiment was approved by the Committee on animal experiments of the University of the Ryukyus. Details of the husbandry and feeding of the animals are summarized in Table 1.

Air conditioning of animal rooms

In the animal facility, air conditioning of each animal room is maintained to give a room temperature of 24±1℃, at 55±10% humidity, and the ventilation rate of room air was 20 changes per hour. As mentioned, this facility is equipped with a COSA/TRON system, and 80% of total air exhausted from animal rooms is provided for recirculation to each animal room. In order to remove fine dust and particles in the air of the animal rooms, 'roughing' filters (prefilters) and medium efficiency filters are installed in the air conditioning system. The medium efficiency filters are changed at 4-5 year intervals.

Cleaning of animal rooms

In the animal facility, cleaning of each animal room is carried out every day, except for Sundays, from 08:30 to 12:00 in the morning. The procedures include sweeping away wood chips of bedding and feces ejected from the cages, scrubbing the floor, brushing away adhesives, and disinfecting the racks and floor with hypochlorite solution. To house the mice, commercially processed wood chips disinfected by heating (Kyudo Co., Kumamoto, Japan) are used as bedding. The cages and bedding are changed twice a week. In the rat room, rabbit room, guinea-pig room and rat isolation room, debris is swept away automatically with a current water at an interval of one hour.

Sampling of the room air

For the measurement of ammonia and sulfur compounds, the sampling point was placed at 125 cm above the floor in the center of the following animal rooms: first mouse room, rat room, monkey room, rabbit room, guinea-pig room, and rat isolation room. The sampling was carried out before and after cleaning of the animal rooms on the same day. In the case of the rat isolation room, besides the sampling at the above point, additional sampling was carried out from the following two other points: within the exhalation orifice on

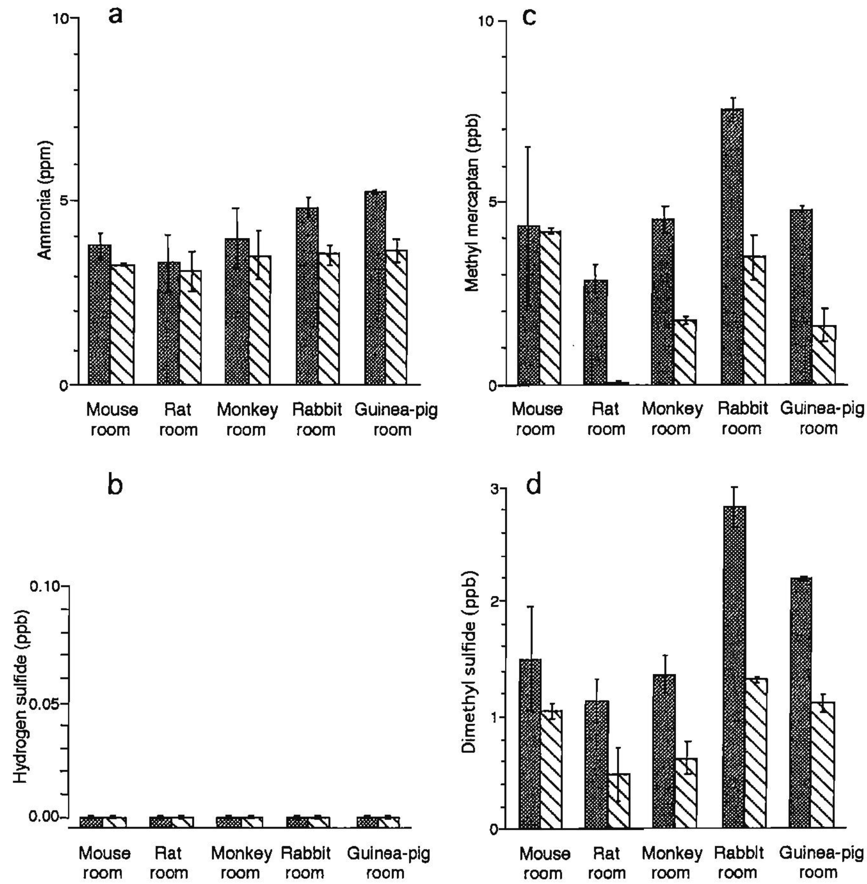

Fig. 1 Concentrations of ammonia (a) and sulfur compounds (b-d) before and after cleaning of the animal rooms. Solid bars: before cleaning, hatched bars: after cleaning. Data show each value of 2 measurements and mean values.

the ceiling, and just under the exhaust orifice on the floor. The sampling was carried out under operating conditions of the COSA/TRON system. Subsequently, in the rat isolation room, this system was shut down for 7 days, and after day 8, sampling was again carried out. All sampling in this study was carried out under the air conditioned by medium efficiency filters which had worked for 4-5 years.

Determination of the concentration of ammonia

The concentration of ammonia was determined by an indophenol colorimetric method according to Akushuu Bousi Hou, the Japanese law for the prevention of malodorous substances[13-15].

Determination of the concentrations of sulfur compounds

The concentrations of hydrogen sulfide, methyl mercaptan and dimethyl sulfide were measured by gas chromatographic analysis in accordance with the law described above[13, 14, 16].

Statistical analysis

Statistical analysis was performed by the paired t test.

RESULTS

Concentrations of ammonia and sulfur compounds before and after cleaning of the animal rooms

The concentrations of ammonia and sulfur compounds before and after cleaning of the animal rooms are shown in Fig. 1. Before the cleaning, the least concentration of ammonia was 3.29 ppm in the rat room, while the highest value was 5.21 ppm in the guinea-pig room. After the cleaning, the concentration of ammonia was reduced in all animal rooms (Fig. 1a).

Hydrogen sulfide was not detected at any of the animal rooms both before and after the cleaning (Fig. 1b).

Before the cleaning, methyl mercaptan was detected in all animal rooms, and the highest value was 7.53 ppb in the rabbit room. After the cleaning, the concentration of methyl mercaptan was reduced in all animal rooms; the values then ranged between 0.07 ppb and 4.14 ppb (Fig. 1c).

Before the cleaning, dimethyl sulfide was also detected in all animal rooms, and the values ranged between 1.13 ppb and 2.82 ppb. After the cleaning, the values were decreased to between 0.48 ppb and 1.31 ppb (Fig. 1d).

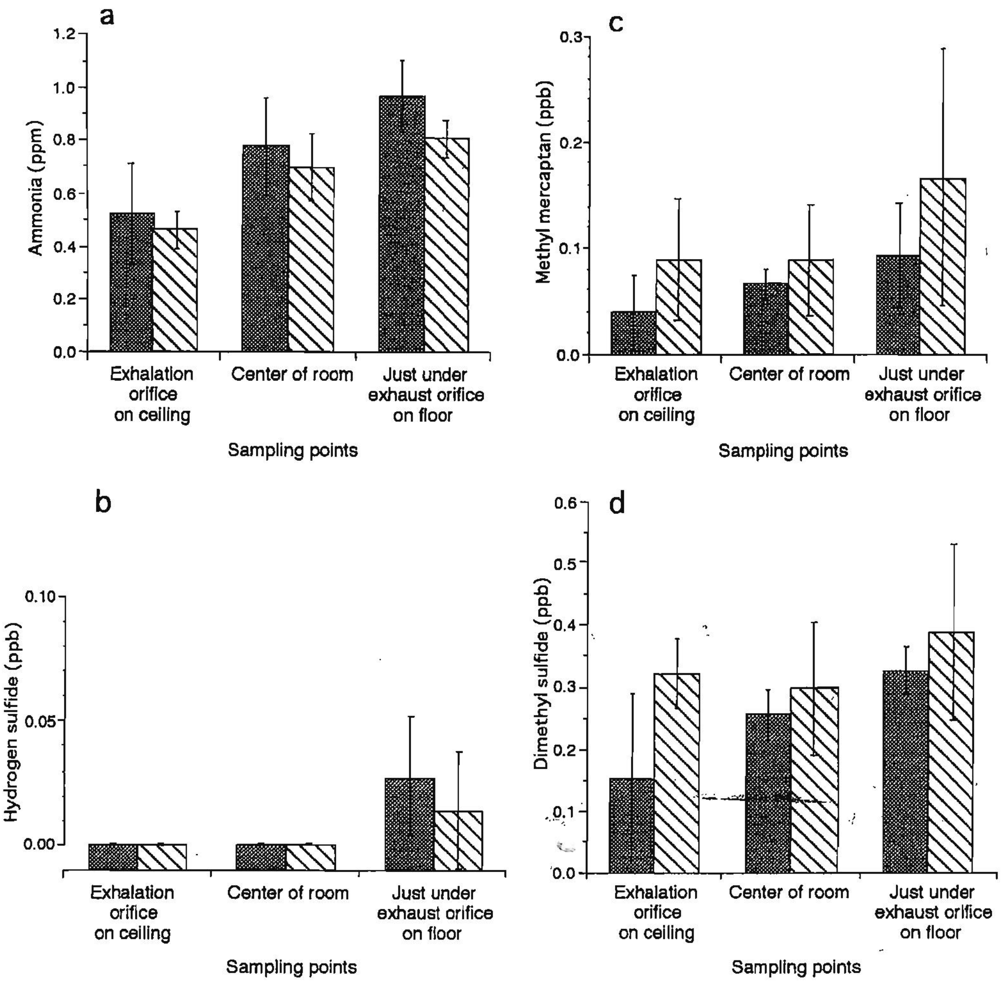

Fig. 2 Concentrations of ammonia (a) and sulfur compounds (b-d) under operating and nonoperating COSA/TRON system conditions. Solid bars: operating COSA/TRON system conditions, hatched bars: nonoperating COSA/TRON system conditions. Data show means ± SD of 3 measurements.

Concentrations of ammonia and sulfur compounds under operating and nonoperating COSA/TRON system conditions

The concentrations of ammonia and sulfur compounds under operating and nonoperating COSA/TRON system conditions in the rat isolation room are shown in Fig. 2. Under operating conditions, the concentrations of ammonia were 0.520 ppm within the exhalation orifice on the ceiling, 0.776 ppm in the center of the room, and 0.961 ppm just under the exhaust orifice on the floor. Under nonoperating conditions, the concentrations of ammonia were decreased at all sampling points (Fig. 2a).

Under operating and nonoperating conditions, hydrogen sulfide was not detected except at the point just under the exhaust orifice on the floor, where a trace of hydrogen sulfide was detected, with values of 0.027 ppb under operating conditions and 0.014 ppb under nonoperating conditions (Fig. 2b).

The concentrations of methyl mercaptan were 0.039 ppb within the exhalation orifice on the ceiling, 0.065 ppb in the center of the room and 0.091 ppb just under the exhaust orifice under operating conditions. Under nonoperating conditions, the concentrations were increased at all sampling points, and that within the exhalation orifice on the ceiling was 0.089 ppb (Fig. 2c).

The concentrations of dimethyl sulfide were 0.153 ppb within the exhalation orifice, 0.255 ppb in the center of the room and 0.325 ppb just under the exhaust orifice during operating conditions. Under nonoperating conditions, the concentrations were increased at all sampling points, and that within the exhalation orifice was increased to 0.321 ppb (Fig. 2d).

A statistical evaluation, however, showed that the differences between the values measured under operating conditions and those under nonoperating conditions were not significant at any sampling point ($P<0.05$).

Table 2 Malodorous substances in animal rooms before cleaning

a. Concentration of ammonia

	Ammonia (ppm) First mouse room	Rat room	Rabbit room	Monkey room	Guinea-pig room
At our animal facility:					
this study (1995)	3.78	3.29	4.74	3.90	5.21
previous study (1988)	1.24	1.11	2.02	2.52	1.60
Report by Yamauchi	19.0	1.8	26.7	23.7	–

b. Concentration of hydrogen sulfide

	Hydrogen sulfide (ppb) First mouse room	Rat room	Rabbit room	Monkey room	Guinea-pig room
At our animal facility:					
this study (1995)	n.d.	n.d.	n.d.	n.d.	n.d.
previous study (1988)	n.d.	–	–	n.d.	–
Report by Yamauchi	0.1	0.5	0.4	3.4	–

c. Concentration of methyl mercaptan

	Methyl mercaptan (ppb) First mouse room	Rat room	Rabbit room	Monkey room	Guinea-pig room
At our animal facility:					
this study (1995)	4.30	2.87	7.53	4.49	4.72
previous study (1988)	n.d.	–	–	tr.	–
Report by Yamauchi	0.1	0.1	0.1	0.8	–

d. Concentration of dimethyl sulfide

	Dimethyl sulfide (ppb) First mouse room	Rat room	Rabbit room	Monkey room	Guinea-pig room
At our animal facility:					
this study (1995)	1.48	1.13	2.82	1.34	2.19
previous study (1988)	0.162	–	–	0.255	–
Report by Yamauchi	0.2	0.2	0.6	0.3	–

n.d.: Not detected, tr.: trace, –: not done.

Table 3 Number of housing animals

	Mice	Rats	Rabbits	Guinea-pigs	Monkeys
This study	917	274	86	93	9
Previous study	583	234	43	71	10

DISCUSSION

Table 2 summarized the concentrations of ammonia, hydrogen sulfide, methyl mercaptan and dimethyl sulfide measured in the present study, listed together with those reported in our previous study[11] and those reported by Yamauchi[17]. Clearly, the concentrations of ammonia, methyl mercaptan and dimethyl sulfide in the present study were increased as compared with those measured in 1988. Although a trace of hydrogen sulfide was noted just under the exhaust orifice in the rat isolation room, no hydrogen sulfide was detected at any other sampling point, just as in the previous study. However, the concentrations of methyl mercaptan were increased to between 2.87 ppb and 7.53 ppb, which were appreciably higher values than those in the 1988 study and those reported by Yamauchi. The concentrations of dimethyl sulfide were also increased in the present study. Compared with the number of animals housed during the 1988 study, the number of mice and rabbits was almost double (Table 3). It was thought that the increased number of animals housed might raise the concentration of malodorous substances in the mouse and rabbit rooms. However, the fact that the concentration of malodorous substances had increased in other animal rooms besides the mouse and rabbit rooms indicated that some factors may be involved in the increases in malodorous substances.

After cleaning of the animal rooms, the concentrations of ammonia, hydrogen sulfide, methyl mercaptan and dimethyl sulfide were decreased in comparison with those measured before the cleaning, confirming that these malodorous substances are eliminated by cleaning of the animal rooms. However, although the concentration of ammonia had been reduced by 50% after the cleaning in the previous study, it was reduced by only 13.5%-25.5% after the cleaning in the present study. These findings suggest that an increase in ammonia which could not be effectively eliminated by the cleaning had occurred.

Among the measurements made in the rat isolation room, the concentration of ammonia just under the exhaust orifice showed the highest value; a neutral value was observed in the center of the room, and the least value within the exhalation orifice. Similar results were obtained for the measurements of hydrogen sulfide, methyl mercaptan, and dimethyl sulfide. These findings indicate that the air in the rat isolation room tended to flow from the exhalation orifice on the ceiling to the exhaust orifice on the floor, and also that malodorous substances were exhausted with the flow of ventilated air. It was evident therefore that ventilation of air in the rat isolation room was being carried out effectively.

In the study on the determination of particulate matters in the same rat isolation room[18], the count values of the particulate matters (with aerodynamic diameters of one micrometer or less) were below 10,000 counts/ft^3 under both operating and nonoperating COSA /TRON system condition. Since the ionic radius of the ammonia molecule is smaller than that of sulfur compounds, and the ammonia concentration in the rat isolation room was lower than 1 ppm, it was speculated that the charged corpuscles generated by the COSA/TRON system would fail to grow substantially in diameter. Therefore, they would not be trapped by the filtration system, and might continue to float within the room in the form of ammonium aerosol. Consequently, the total amount of ammonia might have increased under operating COSA/TRON system conditions. The medium efficiency filters installed in the air conditioning system may have become decrepit because of 4-5 years work, which might also affect the increases in malodorous substances in this study.

REFERENCES

1) Weatherby J.H.: Chronic toxicity of ammonia fumes by inhalation. Proc. Soc. Exp. Biol. Med. 81: 300-301, 1952.
2) Gamble M.R. and Glough G.: Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. Lab. Anim. 10: 93-104, 1976.
3) Broderson J.R., Lindsey J.R. and Crawford J.E.: The role of environmental ammonia in respiratory mycoplasmosis of rats. Am. J. Pathol. 85: 115-127, 1976.
4) Richard D., Bouley G. and Boudene C.: Effects of ammonia gas continuously inhaled by rats and mice. Bull. Eur. Phisiopathol. Resp. 14: 573-582, 1978.
5) Shaerdel A.D., White W.J., Lang C.M., Dvorchik B.H. and Bohner K.: Localized and systemic effects of environmental ammonia in rats. Lab. Anim. Sci. 33: 40-45, 1983.
6) Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences: Laboratory Animal Husbandry. In: Guide for the Care and Use of Laboratory Animals, pp10-31, National Institutes of Health Bethesda, Maryland, 1985.
7) Gamble M.R.: The importance of environmental control in animal houses. Journal of the Institute of Animal Technicians 26: 9-15, 1975.
8) Osima Y. and Kasahara A.: On a simplified test for ammonia concentration in the laboratory animal room. Exp. Anim. 8: 12-15, 1959 (in Japanese).
9) Sato N.L., Fujisawa N., Maeda Y. and Fukui M.: A method to eliminate odor from recirculating air in the animal house. Exp. Anim. 41: 39-45, 1992.
10) Kurosawa T., Yoshida K., Okamoto M. and Tajima M.: Invention of an air forced ventilated micro-isolation cage and rack system. - Environment within the cages: ventilation, air flow - . Exp. Anim. 42: 547-554, 1993 (in Japanese).
11) Miyazaki I., Kosugi T., Koga T., Tohme Y. and Kinjo S.: Investigation of malodorous substances in the Institute for Animal Experiments, Faculty of Medicine, University of the Ryukyus. J. Exp. An. Tech. 23: 48-52, 1988 (in Japanese).
12) Koga T., Tanabe M., Kinjo S., Tohme Y., Miyazaki I. and Kosugi T.: Effects of the contaminant excitation field on the counts of airborne microorganisms and adherent microorganisms in mouse room. J. Exp. An. Tech. 23: 57-64, 1988 (in Japanese).
13) Kankyouchou Kankyouhourei Kenkyuukai: Akushuu Bousi Hou. In: Kankyou-roppou, pp234-266, Chuuou Houki Shuppan, Tokyo, 1992 (in Japanese).
14) Ishiguro T.: A review of current analytical methods and survey for odorants. J. Japan Soc. Air Pollut. 23: 171-182, 1988 (in Japanese).
15) Harwood J.E. and Huyser D.J.: Some aspects of the phenol-hypochlorite reaction as applied to ammonia analysis. Water Res. 4: 501-505, 1970.
16) Stevens R.K., Mulik J.D., O'Keefe A.E. and Krost K.J.: Gas chromatography of reactive sulfur gases in air at the part-per-billion level. Anal. Chem. 43:

827-831, 1971.
17) Yamauchi C.: Environment and control of laboratory animals, pp78-87, Shuppan-kagaku Sogo-kenkyujo, Tokyo, 1985 (in Japanese).
18) Ueda T., Koga T., Tohme Y., Kinjo S., Kinjoh K. and Kosugi T.: Number of airborne dust particles in a conventional rat room equipped with running water flushing units. J. Exp. An. Tech. 30: 37-42, 1995 (in Japanese).