Reaction of Sevoflurane and Its Degradation Products with Soda Lime

Toxicity of the Byproducts

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Sevoflurane previously has been reported to undergo extensive degradation in the presence of soda lime. To more completely characterize the extent and significance of this reaction, we studied degradation of sevoflurane with and without soda lime, as well as the toxicity and mutagenicity of the degradation products. Two degradation products detected were CF₂ = C(CF₃)OCH₂F (compound A) and CH₃OCF₂CH(CF₃)OCH₂F (compound B). During circulation of 1%, 2%, and 3% sevoflurane in a closed anesthestia circuit for 8 h, peak concentrations of compound A were 13.3 ± 0.27, 30.2 ± 0.10, and 42.1 ± 1.07 ppm at 2 h, respectively. The concentrations of compound B did not exceed 2 ppm. The temperature of the soda lime was 43.3 ± 2.8°C at 1 h and increased gradually to 47.9 ± 1.5°C after 8 h. In closed flasks with soda lime, the magnitude of the decrease in sevoflurane concentrations (3%) and of the increase in compound A concentrations was temperature dependent. The peak concentrations of compound A at 23°C, 37°C, and 54°C were 32.8 ± 6.8 at 2 h, 46.6 ± 1.0 at 0.5 h, and 78.5 ± 2.9 ppm at 0.5 h, respectively. The LC₅₀ (50% lethal concentration) of compound A in Wistar rats was 1,090 ppm in males and 1,050 ppm in females exposed for 1 h. The LC₅₀ was 420 ppm in males and 400 ppm in females exposed for 3 h. The chronic toxicity of compound A in Wistar rats was studied by exposing rats 24 times, for 3 h each, to initial concentrations of 30, 60, or 120 ppm in a ventilated chamber. At all concentrations, there were no apparent effects other than a loss of body weight in females (120 ppm) on the final day (P < 0.01). Compound A did not induce mutation on the reverse (Ames) test at less than 2,500 µg/dish (culture medium 2.7 ml) with activation by 5-9 mixture, and below 1,250 µg/dish (culture medium 2.7 ml) without activation, in four strains of S. typhimurium and in 1 strain of E. coli. Exposure of fibroblasts to 7,500 ppm of compound A for 1 h, compound A did not induce structural change. In a study of acute toxicity of compound B, there was no toxicity in Wistar rats after 3 h of exposure at 2,400 ppm. The reverse (Ames) test for compound B was negative at 625–1,250 µg/dish. We conclude that sevoflurane is extensively degraded in the presence of soda lime and heat and that the LC₅₀ in rats of one degradation product is greater than ten times the peak concentration reached during an 8-h closed circuit anesthetic. (Key words: Anesthesia, volatile; sevoflurane; compound A; compound B. Stability; degradation; soda lime. Toxicity: degradation products.)

In 1987, Hanaki et al.,1 Strum et al.,2 and Eger3 reported the results of an analysis of the reaction between sevoflurane and soda lime. Hanaki et al.1 studied the reaction in the absence of fresh gas (CO₂ supplied) in a closed anesthestia circuit using an anesthesia machine, and Strum et al.2 and Eger3 described the results of the reaction in a static flask system. Hanaki et al.1 reported that there was little reaction, while Strum et al.2 and Eger3 reported that a large amount of sevoflurane was consumed. To resolve the inconsistency between these results, we studied the reaction using a method that is similar, but not identical, to that of Strum et al.2 We also studied the toxicity of the major degradation products of sevoflurane, compound A (CF₂ = C(CF₃)OCH₂F) and compound B (CH₃OCF₂CH(CF₃)OCH₂F).

Materials and Methods

REACTION BETWEEN SEVOFLURANE AND SODA LIME

Standard grade sevoflurane was used in this experiment. Sodasorb® (W.R. Grace, Kexington, MA), Dräger® 800 (Dräger, Luebeck, Germany), Wakolime®, and Wakolime-Ace (Wako Pure Chemical, Osaka, Japan) were used as test CO₂ absorbants. Wakolime-Ace is a new product that contains less potassium (table 1).
TABLE 1. Compositions of Four Brands of Soda Lime

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(OH)₂</td>
<td>78.3</td>
<td>79.2</td>
<td>79.8</td>
<td>80.3</td>
</tr>
<tr>
<td>NaOH</td>
<td>2.0</td>
<td>4.2</td>
<td>1.2</td>
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<td>KOH</td>
<td>5.0</td>
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<td>2.6</td>
</tr>
<tr>
<td>SiO₂</td>
<td>0.1</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Mg(OH)₂</td>
<td>0.7</td>
<td>0.5</td>
<td>—</td>
<td>0.5</td>
</tr>
<tr>
<td>Al(OH)₃</td>
<td>0.1</td>
<td>0.3</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>Water</td>
<td>13.9</td>
<td>15.6</td>
<td>13.6</td>
<td>12.8</td>
</tr>
<tr>
<td>Total</td>
<td>98.1</td>
<td>98.3</td>
<td>97.3</td>
<td>97.9</td>
</tr>
</tbody>
</table>

Values are percentages.

I = Drägersorb 800; II = Wakoline Ace; III = Sodasorb; IV = Wakoline.

Two degradation products CF₂ = C(CF₃)OCH₂F (compound A) and CH₃OCF₂CH(CF₃)OCH₂F (compound B) were synthesized by Central Glass (Tokyo, Japan). Compound A is an oily, flammable, and with a specific gravity of 1.50 and a boiling point of 43°C at 1 atm. Compound B has an ether-like odor, with a specific gravity of 1.45, and a boiling point of 52.6°C at 56 mmHg or 131°C (unstable partially) at 1 atm. The H₂O/gas coefficients of compounds A and B are 0.18 and 3.19, respectively. Their structure was confirmed by NMR and mass spectrometry, and their purities were determined by gas chromatography. The purities of compounds A and B were 99.0–99.9% and 98.8–99.6%, respectively.

The above-mentioned test CO₂ absorbents were considered “wet type” and the ingredients were analyzed by Central Glass (table I).

Reaction in Anesthesia Circuit

The apparatus and experimental procedures were the same as those used by Hanaki et al. Briefly, a 5-L rubber bag surrounded by an elastic band functioned as a model lung, which was connected to an anesthesia machine (Dräger). The canister was packed with 0.8 kg of soda lime (Wakoline®). Sevoflurane (in 100% O₂) was circulated at 6 L/min (12 × 500 ml) using a ventilator. CO₂ was supplied at 200 ml/min. The initial concentrations of sevoflurane were 1%, 2%, and 3%. Room temperatures were about 25°C and the differences inside the soda lime canister were measured with a thermostor, the tip of which was in the center and 1 cm down from the surface of the soda lime. The degradation products were analyzed by gas chromatography every hour for 8 h. Each experiment was repeated twice.

Conditions for gas chromatography were as follows: glass column, 20% diotylphthalate on chromosorb WAW, 80/100 mesh, 0.4 cm × 0.3 cm × 5 m; column temperature, 110°C; injection port temperature, 130°C; carrier gas, N₂, 40 ml/min; detector, flame ionization detector; injection volume, 1 ml.

Reaction in Glass Flask

The experiments were performed according to the method of Strum et al.² Soda lime, 26 g, and 1.3 ml of water were placed in a 155-ml glass flask, which was sealed with a butyl rubber stopper and then evacuated. Sevo-flurane (24 µl) and isoflurane (21 µl as internal standard) were added to final concentrations of 3% each. The pressure in the flask was then returned to atmospheric pressure with an injection syringe. The room temperature was 23°C. Temperatures of 37°C and 54°C were maintained in a water bath, and the flask was immersed in the water up to the neck during the reaction. The gas was sampled from three flasks after 0.5, 1, 1.5, 2, 3, and 5 h to quantify sevoflurane, isoflurane, and compounds A and B by gas chromatography. For the various brands of CO₂ absorbents, the rates of decrease (%/h) of sevoflurane were calculated at 23°C, 37°C, and 54°C. The concentrations of two degradation products were assayed and compared with their respective calibration curves. As a control for the presence of soda lime, the same volume (35 ml) of Teflon® particles (Chemware®, Norton) was used and residual sevoflurane (%) was determined. Conditions for gas chromatography were the same as described above except for the injection volume of 150 µl.

Soda Lime Affinity of Compounds A and B

The procedures were similar to that described previously in this study. The initial concentrations of compounds A and B were 3% and 0.9% (21 µl and 8 µl), respectively. The brand of soda lime was Sodasorb®. Conditions for gas chromatography were as described in the following section.

TOXICITY OF DEGRADATION PRODUCTS

These studies were approved by the Institutional Animal Committee of Mabushi Pharmaceutical. All studies were carried out under the guidelines of Good Laboratory Practice (Japan) and others.⁵

Acute Toxicity of Compound A

1-H STUDY OF COMPOUND A TOXICITY: Forty-eight male and 48 female Wistar rats (Slc: Wistar), 5 weeks old, weighing 132.0 ± 6.7, and 110.7 ± 4.2 g, respectively, were used. Eight groups of males and females constituted six dose groups, control (O₂) and untreated groups, respectively. Each group of six rats was divided into two and placed in a beaker (5 L). Oxygen was delivered from an oxygen tank to fill the beaker, which was then tightly covered with Saran Wrap® film (polychlorovinylene). Expected concentrations of compound A, in volumes which would yield final concentrations of 700, 930, 1,070, 1,150, 1,220, and 1,400 ppm, were injected into the
beaker with a microsyringe. In this study, CO₂ absorbant was not used. The surviving rats were observed, and their body weights were measured for 14 days, after which they were killed with exsanguination after an overdose of diethyl ether. Urine (glucose, protein, ketone bodies, and pH) and blood (BUN, GOT, GPT, and ALP) were analyzed. The effects of CO₂ generated by the rats in response to toxicity were observed in comparison with untreated controls.

3-H STUDY OF COMPOUND A TOXICITY: Thirty male and 30 female Wistar rats (Slc: Wistar), 5 weeks old, weighing 117.2 ± 4.2 and 103.7 ± 3.6 g, respectively, were used. Five groups of male and female rats consisted of one control (O₂) and four dose groups. One group was placed in a 25-L plastic chamber (fig. 1) connected to a CO₂ absorbant, that is, a 5-L solution of 50 mM Ba(OH)₂, and was filled with oxygen. Compound A, to expected concentrations of 390, 510, 670, and 880 ppm, was introduced into the chamber with a microsyringe. The concentrations of compound A were measured by gas chromatography at intervals of 30 min. Rats were exposed to the circulating gas for 3 h. Dead animals were dissected immediately after death. The surviving rats were observed, and their body weight was measured and urine analyzed. At autopsy, the liver and kidney were excised and fixed in formalin. The observed concentrations (as an integrated concentration) for 3 h were 110, 250, 350, and 490 ppm in males and were 160, 290, 340, and 460 ppm in females, respectively.

Acute Toxicity of Compound B

3-H STUDY OF COMPOUND B TOXICITY: The chamber and the methods were similar to that described above. Twenty-four male and 24 female Wistar rats (Slc: Wistar), 5 weeks old, weighing 116.3 ± 7.0 and 98.2 ± 2.4 g, respectively, were used. Four groups (n = 6) consisted of three dose groups and a control (O₂) group. The observed concentrations of compound B were 800, 1,500, and 2,500 ppm in males and 400, 1,200, and 2,500 ppm in females.

Chronic Toxicity of Compound A

Sixty male and 60 female 6-week-old Wistar rats weighing 146.0 ± 6.3 and 114.1 ± 4.5 g, respectively, were placed in a 120-L chamber. The CO₂ absorbant was a 10-L solution of 50 mM Ba(OH)₂. Four groups of 15 males and females constituted the 50, 60, and 120 ppm compound A group and a control (O₂) group. The rats were exposed for 3 h/day, 3 days/week for 8 weeks. The exposure times were selected according to the Food and Drug Administration guidelines for general anesthetics and other procedures were followed according to Japanese guidelines. The initial concentrations were set and measured every 0.5 h for 3 h. Body weight as well as food and water consumption were measured. The eyes (cornea, iris, and fundus) were examined. Urine (volume, occult blood, glucose, protein, ketone bodies, and pH) and blood (GOT, GPT, ALP, total protein albumin, A/G ratio, glucose, total cholesterol, total bilirubin, BUN, creatinine, uric acid, inorganic phosphorus, CPK, Ca, Na, K, and Cl) were analyzed. At the end of 24 times exposure, 10 rats were killed immediately and 5 rats were killed 14 days later for recovery observation. The method of killing rats was similar as described above. The heart, lung, liver, spleen, kidney, brain, pituitary gland, thyroid gland, submandibular gland, thymus, adrenals, testis, prostate, seminal vesicle, ovaries, and uterus were weighed. For histopathologic examination, all excised organs and femoral bone marrow were fixed in neutral buffered formalin.

Reverse Test (Ames test) of Compounds A and B

The procedures were carried out according to the guidelines of the Ministry of Labour (Japan). In these guidelines, E. coli and a pre-incubation procedure were added to the original Ames method.

The test strains were S. typhimurium TA98, TA100, TA1535, TA1537 and E. coli WP2uvrA. Dimethyl sulfide (DMSO, DOTAITO) was used as the negative control and as the solvent of the compounds A and B. The positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, N-ethyl-N'-nitro-N-nitroso-guanidine, 9-aminocaridine HCl, 4-nitroquinoline-N-oxide, Benzo(a)pyrene, and 2-aminoanthracene.

The doses were 5,000, 2,500, 1,250, 625, 313, and 156 μg in each dish (2.7 ml). The pre-incubation procedures were as follows:
1. Compounds A and B were dissolved in 0.1 ml of DMSO and added to a sterile culture tube.
2. S-9 Mix (0.5 ml) or the same volume of 0.1 M phosphate buffer (pH 7.4, 0.5 ml) was added.
3. Bacteria suspension 0.1 ml (37°C, 16–18 h) was added and mixed well.
4. The mixture (screw tap) was pre-incubated for 20 min at 37°C in a shaking incubator (60 cycles/min).

Preheated (45°C) soft agar, 2 ml, was then added to the mixture and poured onto a glucose-agar plate. The plate was incubated for at least 48 h at 37°C.

The number of revertant colonies formed by mutation was counted. If the number of revertant colonies was at least twice that of the negative control, and if there was a dose-dependent increase, the drug was considered mutagenic.

**Chromosome Aberration Test of Compound A with Mammalian Cells in Culture**

The procedures were followed according to the guidelines of the Ministry of Health and Welfare of Japan. A fibroblast cell line derived from newborn Chinese hamster lungs was used. The negative controls were air and non-exposed culture medium.

Compound A, in gaseous form, was exposed to the fibroblasts. Since we had no good gaseous positive control, N-methyl-N-nitrosoguanidine (MNNG) was used as the positive control in the culture medium described below. The gaseous concentrations of compound A were 15, 1,500, and 7,500 ppm, in volumes of 0.76, 76, and 380 µl, respectively. The culture medium was Eagle's MEM (minimum essential medium, NISSUI) supplemented with heat-inactivated newborn calf serum (HINCS, 10% Boehringer Mannheim) and N-2-hydroxypropyl piperazine-N'-2-ethane sulfonic acid (HEPES, DONTAT 20 mM) without Kanamycin (pH 7.2). Trypsin-EDTA (0.125% and 0.01% in PBS 1×) solution was used to disperse cells in the Petri dish (35 m/m). About 1.2 × 10⁴ cells in 2 ml medium were seeded in Petri dishes (35 m/m) and cultured in a CO₂ incubator (CO₂ 5%, 37°C, 98% R.H.) for 3 days. Fibroblast cells grew on the Petri dish bottom. Lids were removed and the culture medium was aspirated. Inverted Petri dishes were placed on the mesh in the exposure chamber (airtight, 10 l, Acrylic/resin). Compound A was injected through butyl packing at room temperature. The cells were exposed directly to each concentration of compound A for 60 min. Thereafter, 2 ml of culture medium was added to the fibroblasts, which were incubated under CO₂ for 24 or 48 h.

The negative control was prepared by exposing the plates as described above to air without compound A for 60 min. The positive control was prepared by adding 2 ml of culture medium containing MNNG (1.4 µg/ml).

After incubation for 22 and 46 h, 0.04 µl of 0.1% Colcemid (GIBCO) was added to the 2 ml of medium to give a final concentration of 0.2 µg/ml. Cell division was thus fixed at metaphase. The fibroblasts were dispersed with 1 ml of Trypsin-EDTA, transferred to tubes and centrifuged (800 rpm) for 3 min. The supernatant was discarded. Four milliliters of 75 mM KCl hypotonic solution was added to the cells and the suspension was incubated for 15 min at 37°C. The cells were fixed for 10 min by adding three drops of ice-cold methanol: glacial acetic acid, 3:1. A further 4 ml of the fixative was added and the 8-ml mixture was allowed to stand for 15 min. After centrifugation (800 rpm) for 3 min, the same procedure (4 ml addition of the fixative) was repeated two times. After the last centrifugation, 0.5 ml of fixative was added and dispersed. One or two drops of the dispersed mixture was placed on a glass slide, fixed with a flame, then stained with 1.3% Giemsa solution.

One hundred cells at metaphase, with well-spread chromosomes, were examined microscopically (×400). The frequencies of polyploidy and structural aberration were recorded. Aberrations were classified as follows: gaps, breaks, exchanges, inversion, fragmentation, and ring formation. If one cell had more than one type of aberration, each abnormality was counted separately. The frequencies of polyploidy and structural aberration in the treated groups were scored according to the method of Ishidate, that is, if the frequency was <5% then the score was −; 5–10%, ±; 10–20%, +; 20–50%, ++; and >50%, +++.

**Micronucleus Test of Compound A (Preliminary Test)**

Fifteen male BDF mice (Slc; BDF1), 8 weeks old, weighing 26.1 ± 0.9 g, were divided into groups of three and placed in 5-L beakers. Each mouse was exposed to initial concentrations of 150 ppm of compound A for 3 h (II.1.4). After 18, 24, 30, 48, or 72 h, the mice were killed with an overdose of diethyl ether and bone marrow smears were prepared.

The incidence of micronuclei was counted in 1,000 polychromatic erythrocytes from each animal. The frequency of polychromatic erythrocytes in the total number of erythrocytes was calculated.
DATA ANALYSIS AND STATISTICS

All data were expressed as mean ± SD. Analysis of variance (ANOVA) and t tests with the Bonferroni correction were used to test for degradation rate difference between soda limes or temperatures. In acute toxicity studies of compound A for 1 h, LC₅₀ was calculated according to the Probit method and Student’s t test were used to test for BUN, GOT, GPT, and ALP. In chronic toxicity of compound A, Dunnett or Scheffe of multiple comparison were used to test for difference between exposed groups.

Results

REACTION BETWEEN SEVOFLURANE AND SODA LIME

Reaction in Anesthesia Circuit

The initial sevoflurane concentrations of 1%, 2%, and 3% slowly decreased with time, equalling 0.88 ± 0.08, 1.74 ± 0.08, and 2.72 ± 0.15% at 4 h, and 0.81 ± 0.07, 1.57 ± 0.04, and 2.61 ± 0.17% at 8 h.

The temperature in the soda lime canister was 43.3 ± 2.8°C after 0.5–1 h and increased to 47.9 ± 1.5°C after 8 h.

The two degradation products were compounds A and B.

The concentrations of compounds A and B in the circuit are shown in figure 3. Compound A peaked at 2 h and then gradually decreased. The rates of decrease (%/h) of the concentrations of compound A with 1%, 2%, and 3% sevoflurane were 5.96 ± 0.17 (2–8 h), 3.86 ± 0.06 (2–7 h), and 6.48 ± 1.10 (2–8 h), respectively. Compound B did not exceed 2 ppm.

Reaction in Glass Flask

The residual sevoflurane (%) with soda lime in the flask is shown in figure 4. In the control study with Teflon®, sevoflurane concentrations were not changed (coefficient of variation < 2%). The rates of decrease (%/h) of sevoflurane significantly increased as temperature increased (P < 0.05, table 2). The residual (%) of sevoflurane at 37°C with time was 89.1 ± 0.2% at 0.5 h, 72.6 ± 0.8% at 3 h, and 61.1 ± 1.3% at 5 h, respectively (fig. 4). The two degradation products of sevoflurane were compounds A and B and their concentrations (ppm) are shown in figure 5. The concentrations of compound A after 0.5 h at 23°C, 37°C, and 54°C significantly increased to 25.9 ± 3.2, 46.6 ± 1.3, and 78.5 ± 2.8 ppm as temperature increased (P < 0.01). The concentrations of compound A at 37°C with time were 36.9 ± 1.1 at 3 h and 32.2 ± 2.2 ppm at 5 h, respectively. Compound B was detected only after 2 h at 54°C, and its concentration increased to 27.3 ± 3.8 ppm at 5 h. The rates of decrease (%/h) of sevoflurane with various brands of CO₂ absorbers are shown in table 2 and are compared with those from Strum et al.2 and Eger.3 Table 2 also shows the rate of sevoflurane decrease with Drägersorb® 800 and Wakolime®-Ace. Although the rate of decrease is less with Wakolime®-Ace, the concentrations of compound A with Wakolime®-Ace were of the same magnitude as those with Sodasorb® (fig. 6).

Soda Lime Affinity of Compounds A and B

Compound A levels rapidly decreased in the glass flask. Residual compound A (%) decreased to 26.2 ± 2.05% after 3 min, 1.67 ± 0.42% after 15 min, and 0.027 ± 0.01% after 30 min at room temperature (23°C). The rate of decrease (%/min) was 25.61 ± 1.1. In contrast, compound B levels decreased slowly in the system. The residual (%) of compound B was 55.8 ± 1.1% after 28 h.

TOXICITY OF THE TWO DEGRADATION PRODUCTS

Acute Toxicity of Compound A

1-H study of compound A toxicity: The following symptoms were observed: decreased locomotor activity, prone position, loss of righting reflex, decreased respiratory rate, cyanosis, lacrimation, and piloerection, which were similar to symptoms observed in animals of the inhalational acute toxicity studies of isoflurane and sevoflurane.4 Animals did not die during exposure, but died from 30 min to 9 days thereafter. At least 930
ppm, no animals died until 2 weeks later. At autopsy, lung congestion and hyperemia were observed in the animals that died on the day of exposure. The kidneys of animals that died on the next day after exposure were discolored and had rough surfaces. BUN was increased in the females (3/6) that survived at 1,150 ppm. The BUN of the control was 20.9 ± 1.6 and of the 1,150 ppm group was 27.4 ± 3.4 (P < 0.01). Occult blood, sugar, protein, and ketone bodies were found in the urine of dead animals. Histopathologic examination revealed degeneration and necrosis of renal tubules in animals that died after the next exposure day and slight necrosis was found in those that survived. There were no other histopathologic changes that could be attributed to compound A.

**TABLE 2. Rates of Decrease (%/h) of Sevoflurane with Various Brands of Soda Lime**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Wakolime Ace</th>
<th>Drägerorb 800</th>
<th>Sodasorb</th>
</tr>
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<tbody>
<tr>
<td>23°C</td>
<td>0.79 ± 0.011¥</td>
<td>2.51 ± 0.04¥</td>
<td>1.60 ± 0.541¥</td>
</tr>
<tr>
<td>37°C</td>
<td>2.22 ± 0.04¥</td>
<td>6.01 ± 0.28†</td>
<td>8.41 ± 0.66‡</td>
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<tr>
<td>54°C</td>
<td>12.07 ± 0.31</td>
<td>13.59 ± 1.68</td>
<td>39.43 ± 1.89</td>
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* P < .05.
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‡ Difference from 37°C (‡P < .05, ¥P < .01).

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LC₅₀ was calculated as 1,090 ppm in males and 1,050 ppm in females. From other results (micronucleus test), we estimated that the decrease of compound A was less than 10% in the beaker for 1 h.

**3-H Study of Compound A Toxicity**: The following symptoms were observed: ear and tail flush, nictitation, decreased locomotion, recumbent position, prone position, decreased respiratory rate, cyanosis, tremor, ptosis, and piloerection, which were similar to symptoms observed in animals of the inhalational acute toxicity studies of isoflurane⁴ and sevoflurane.⁸ In males, six of six animals

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died at 490 ppm during 2.5-3 h of exposure and one animal died at 350 ppm after 0.5 h from the end of exposure. In females, four of six animals died during 2.5-3 h of exposure and two animals died after 1 h from the end of exposure at 460 ppm. Two of six animals died at 340 ppm 4 days after exposure. At less than 250 ppm in male and 290 ppm in female, all animals remained alive for 2 weeks. Sugar and ketone bodies were found in the urine of dead animals. The kidneys of a few dead and surviving (350 ppm, five of six males; 340 ppm, four of six females) were discolored. Histopathologic examination revealed lung congestion, hyperemia, and hemorrhage in the animals that died on the day of exposure, and degeneration and necrosis of renal tubules were found in those that died 4 days after exposure. No other histopathologic changes could be attributed to compound A. The LC₅₀ ranged from 350 to 490 ppm (mean value 420 ppm) in males and from 340 to 460 ppm (mean value 400 ppm) in females.

**Acute Toxicity of Compound B**

3-H Study of Compound B Toxicity: None of the animals died. The following symptoms were observed during exposure: staggering gait, decreased locomotor activity, slightly decreased respiratory rate, and prone position. No changes that could be attributed to compound B were found in the urine or at autopsy.

**Chronic Toxicity of Compound A**

The initial concentrations of compound A (120 ppm) in the chamber gradually decreased to about 90 ppm after 1.5 h.

The general behavior, food and water consumption, ophthalmologic examination, urinalysis, hematologic evaluation, organ weight, and autopsy were normal after exposure to 30, 60, or 120 ppm. The body weights of the females exposed to 120 ppm were significantly less on the last day of exposure and on the following day (P < 0.01) but had returned to normal by the final day of recovery. No histopathologic changes could be attributed to compound A in any of the animals exposed to 120 ppm group.

**Reverse (Ames) Test of Compounds A and B**

1. Compound A did not induce reverse mutation either with or without S-9 Mix. Inhibition of growth occurred at 1,250–5,000 µg/plate. The values of Compound A are shown in table 3.

2. Compound B did not induce reverse mutation either with or without S-9 Mix. Inhibition of growth occurred at 1,250–5,000 µg/plate.

3. All positive controls had an obvious increase in the number of reverse mutations (table 3).

**Chromosome Aberration Test of Compound A with Mammalian Cells in Culture**

Compound A did not induce chromosome aberration. The incidence of polyploidy was not affected either by compound A or by the positive control, MNNG. There were structural aberrations in 3% of those incubated for 24 h and 2% of those incubated for 48 h and in 44% of the positive controls (MNNG) incubated for 24 h and 29% of those incubated for 48 h (table 4).

**Micronucleus Test of Compound A**

Compound A did not increase the number of micronuclei in bone marrow cells (0.1 ± 0.1%). The decrease of compound A concentrations (150 ppm) for 3 h was estimated at 18.4% from the control mice.

**Discussion**

**Quantitative and Qualitative Analyses of Degradation Products**

Other than methanol, Hanaki et al. reported that there are five compounds formed as degradation products of the reaction between sevoflurane and soda lime. Using qualitative analysis of degradation products, Kujo et al. and Miyano et al. also detected these five compounds. However, under the present experimental conditions, compound A was commonly found and compound B was sometimes present, but compounds C, D, and E were not detected (fig. 7). This difference may be related to the duration of the reaction times, which were 5 and 8 h in our study and >7 and 17 h in their studies, for reactions...
TABLE 3. Reverse Mutation Test of Compound A

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose (µg/plat)</th>
<th>S-9 Mix</th>
<th>TA98</th>
<th>TA100</th>
<th>TA1535</th>
<th>TA1557</th>
<th>E. coli WP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>—</td>
<td>34.2 ± 7.3</td>
<td>185.7 ± 19.8</td>
<td>8.0 ± 1.5</td>
<td>6.0 ± 2.0</td>
<td>22.5 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Compound A</td>
<td>156.25</td>
<td>33.5 ± 6.2</td>
<td>221.3 ± 39.7</td>
<td>9.0 ± 1.3</td>
<td>4.2 ± 2.3</td>
<td>23.2 ± 3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>312.5</td>
<td>34.7 ± 5.5</td>
<td>183.0 ± 25.7</td>
<td>8.3 ± 3.1</td>
<td>5.8 ± 3.1</td>
<td>25.7 ± 5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>625</td>
<td>35.3 ± 4.4</td>
<td>210.8 ± 48.1</td>
<td>6.8 ± 1.3</td>
<td>7.0 ± 2.4</td>
<td>24.3 ± 6.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,250</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>16.8 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>2,500</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ΔF-2</td>
<td>0.1</td>
<td>443.7 ± 25.6</td>
<td>610.2 ± 39.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>—</td>
<td>44.7 ± 9.3</td>
<td>38.22 ± 43.6</td>
<td></td>
<td></td>
<td>403.7 ± 29.1</td>
</tr>
<tr>
<td>ENNG</td>
<td>2</td>
<td>—</td>
<td>5</td>
<td>5</td>
<td>44.7 ± 9.3</td>
<td>38.22 ± 43.6</td>
<td>403.7 ± 29.1</td>
</tr>
<tr>
<td>ACR</td>
<td>80</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>5</td>
<td>44.7 ± 9.3</td>
<td>38.22 ± 43.6</td>
</tr>
<tr>
<td>DMSO</td>
<td>5</td>
<td>—</td>
<td>5</td>
<td>5</td>
<td>44.7 ± 9.3</td>
<td>38.22 ± 43.6</td>
<td>403.7 ± 29.1</td>
</tr>
<tr>
<td>Compound A</td>
<td>156.25</td>
<td>55.5 ± 4.3</td>
<td>243.7 ± 22.7</td>
<td>11.3 ± 3.9</td>
<td>16.5 ± 3.5</td>
<td>24.8 ± 4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>312.5</td>
<td>51.0 ± 5.9</td>
<td>229.2 ± 11.5</td>
<td>8.8 ± 2.9</td>
<td>15.0 ± 4.9</td>
<td>22.5 ± 4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>625</td>
<td>52.5 ± 4.9</td>
<td>220.0 ± 7.3</td>
<td>11.5 ± 3.1</td>
<td>15.2 ± 3.0</td>
<td>25.7 ± 4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,250</td>
<td>53.2 ± 6.0</td>
<td>225.2 ± 28.7</td>
<td>10.5 ± 1.9</td>
<td>15.2 ± 2.5</td>
<td>24.7 ± 5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,500</td>
<td>51.5 ± 6.3</td>
<td>202.3 ± 48.3</td>
<td>8.2 ± 2.1</td>
<td>15.3 ± 2.7</td>
<td>20.5 ± 4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>45.7 ± 6.7</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>B(a)P</td>
<td>5.0</td>
<td>760.8 ± 41.8</td>
<td>1012.0 ± 43.1</td>
<td>79.5 ± 4.3</td>
<td>70.5 ± 5.8</td>
<td>862.8 ± 44.6</td>
<td></td>
</tr>
<tr>
<td>2AA</td>
<td>2.0</td>
<td>—</td>
<td>5</td>
<td>5</td>
<td>44.7 ± 9.3</td>
<td>38.22 ± 43.6</td>
<td>403.7 ± 29.1</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

* Growth inhibition.

in static (glass flask) and dynamic (anesthesia circuit) systems, respectively.

In an anesthesia circuit under conditions of this study, compound A was detected at 30–40 ppm, and compound B did not exceed 2 ppm when the concentrations of sevoflurane were 2–3%.

Wakoline®-Ace was less reactive than was Sodasorb® with sevoflurane at 37° C and 54° C, although the concentrations of compound A produced with similar as those with Sodasorb® (fig. 6). Kudo et al.5 and Katayama6 reported that Wakoline®-Ace produced more compound A in comparison to Wakoline®. Using the same brand of soda lime at 37–54° C, the rate of decrease (%/h) of Sevoflurane 2–3%.

Strum et al.2 (31.04) is greater than that of Eger3 (12.9/ 40° C) and this study (8.4).

ANESTHESIA CIRCUIT (DYNAMIC) VERSUS GLASS FLASK (STATIC)

We found that a similar reaction and magnitude of decrease occurred in both the static (glass flask) and dynamic (anesthesia circuit) systems by analyzing the correlation between the decline in the amount of compound A generated and the decrease in sevoflurane concentrations in both systems. In the glass flask, there were strong correlations between the residual percentages of sevo-

TABLE 4. Number of Chromosomal Aberrations in 100 Cells at Metaphase

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Dose</th>
<th>Time (h)</th>
<th>Quantitative Aberration Polyplody (%)</th>
<th>Structural Aberration (%)</th>
<th>No. of Structurally Aberrant Cells</th>
<th>Rate of Aberrant Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exposure</td>
<td>—</td>
<td>24</td>
<td>G: 1 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Air exposure</td>
<td>—</td>
<td>24</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound A</td>
<td>15 ppm</td>
<td>24</td>
<td>G: 1 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Compound A</td>
<td>1,500 ppm</td>
<td>24</td>
<td>G: 1 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MNNNG</td>
<td>1.40 µg/ml</td>
<td>24</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No exposure</td>
<td>—</td>
<td>48</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Air exposure</td>
<td>—</td>
<td>48</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound A</td>
<td>15 ppm</td>
<td>48</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound A</td>
<td>1,500 ppm</td>
<td>48</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MNNNG</td>
<td>1.40 µg/ml</td>
<td>48</td>
<td>G: 0 B: 0 E: 0 R: 0 F: 0 O: 0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

G = gaps; B = breaks; E = exchanges; R = inversion; F = fragmentation; O = ring formation.

* No exposure (medium change).

† Air exposure (no compound A).

‡ Quantitative aberration + structural aberration.
flurane and of compound A at 37°C and 54°C (r = 0.98 and 0.97, respectively).

The ratios of compound A to sevoflurane concentrations at given temperatures in the flask were independent of the reaction time (0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 h) as follows: 23°C, (1.13 ± 0.02) × 10⁻²; 37°C, (1.73 ± 0) × 10⁻²; 54°C, (3.33 ± 0.1) × 10⁻³.

The rates at which the amounts of sevoflurane and compound A decreased in the flask at 37°C were almost same, i.e., 8.41 ± 0.66 and 8.24 ± 1.28%/h, respectively (Figs. 4 and 5). Even in the anesthesia circuit the rates for compound A at 1%, 2%, and 3% sevoflurane were 5.96 ± 0.17, 3.86 ± 0.06, and 6.48 ± 1.10%/h, respectively. To calculate the ratios of sevoflurane to compound A concentrations, we observed that sevoflurane concentrations after 2 h were 0.91%, 1.78%, and 2.78% and that compound A concentrations were 13.3, 30.2, and 42.1 ppm. The ratios were then 1.46 × 10⁻², 1.70 × 10⁻³, and 1.51 × 10⁻³, suggesting that the system was similar to that in the flask at 37°C (1.73 × 10⁻²). In the anesthesia system, the reaction proceeded at <37°C.

These ratios imply that the rate of production of compound A is proportional to the concentration of sevoflurane and that the rates of adsorption or destruction of compound A are proportional to its concentration. This also follows from the other information provided by this study (Fig. 3). The actual concentration of compound A must result as a balance between its production and destruction. Furthermore, these data indicate that compound A is unlikely to accumulate with the passage of time, even in a closed circuit.

**THE TOXICITY OF DEGRADATION PRODUCT CONCENTRATIONS IN A CLOSED ANESTHESIA SYSTEM**

Since in the closed circuit system used in the present experiment only a small amount of compound B was produced (less than 2 ppm) and since its toxicity was also low, compound B can be excluded from this discussion of the safety of sevoflurane through soda lime.

In the circuit experiment with 3% sevoflurane, the compound A concentration increased to 30 ppm after 1 h and peaked at 40 ppm after 2 h (Fig. 3).

Soma et al.⁷ reported no abnormalities in a study of chronic toxicity of sevoflurane in closed-circuit anesthesia of Cynomolgus monkeys.

Clinical trials of safety under closed or low flow anesthesia (in vivo) have been implemented by Bitoh et al.⁸ and Brown et al.⁹ with Sodasorb®, in Japan and the United States, respectively. Bitoh et al.⁸ induced anesthesia in a closed circuit with low flow nitrogen and oxygen in 10 adult patients and measured the degradation products in inspired air every hour. They found peak concentrations of 10–25 ppm of compound A and 1.5 ppm of compound B and the value of blood biochemistry on days 7 and 14 were normal. Brown et al.⁹ also described that the concentration of compound A detected in the low flow anesthesia (<750 ml/m) was 15 ppm.

In the rat studies, compound A affected the kidney but not the liver at the lethal concentrations of 1,000 ppm for 1 h and 400 ppm for 3 h. However, compound A did not affect the kidney at 700 ppm for 1 h and 250 ppm for 3 h, conditions under which all animals survived. Closed circuit or low-flow circuit anesthesia containing 10–25 ppm of compound A were considered safe for humans by Bitoh et al.⁸ and Brown et al.⁹.

Degradation products of sevoflurane with soda lime were determined as CF₂ = C(CF₃)OCH₂F (compound A) and CH₃OCF₂CH(CF₃)OCH₂F (compound B) by NMR.

The LC₅₀ of compound A in Wistar rats was 1,090 ppm in males and 1,050 ppm in females exposed for 1 h. The LC₅₀ was 420 ppm in males and 400 ppm in females exposed for 3 h. The chronic toxicity of compound A (30, 60, or 120 ppm, 24 times for 3 h, each) was negligible other than a loss of body weights in females.

The LC₅₀ of compound A for 3 h using 3% sevoflurane in a closed circuit for 8 h is greater than ten times the peak concentration reached.

Compound A did not induce mutation on the reverse (Ames) test at less than 2,500 µg/dish.

**References**

5. Kudo M, Kudo T, Matsuki A: Reaction products of sevoflurane
Footnotes


d Food and Drug Administration: Guidelines for the clinical evaluation of general anesthetics. HHS (FDA) 82-3052, 1982.


b Ishidate M: Chromosomal aberration test in vitro; Data from studies on the induction of chromosome aberration in cultured Chinese hamster cells. Tokyo, Realize Inc., 1986, pp 1-X.


