Toxicity of Compound A in Rats
Effect of Increasing Duration of Administration


Background: An olefin called compound A (CF$_2$=C(CF$_3$)OCH$_2$F) results from the action of soda lime or Baralyme on sevoflurane. We have demonstrated that rats exposed to the olefin for 3 h died at or were injured by olefin concentrations lower than those previously reported to produce these effects. The present report examines the impact of duration of exposure to the olefin on such effects.

Methods: Twenty-three groups of ten Wistar rats breathed 0, 12.5, 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 ppm of the olefin in oxygen for 6 or 12 h. Rats that survived were killed on day 1 or day 4 after breathing the olefin, and specimens of brain, kidney, lung, liver, and small intestine were obtained from all rats for examination by microscopy using hematoxylin and eosin stain and a stain (proliferating cell nuclear antigen) for cell growth (regeneration).

Results: The lethal concentrations in 50% of rats equaled 203 ± 4 ppm (mean ± SE) for a 6-h exposure period and 127 ± 9 ppm for a 12-h exposure period, and both values were lower than the previously determined value of 331 ± 7 ppm for a 3-h exposure period. Compared with results from control rats (those breathing oxygen for 6 h or 12 h), only renal and pulmonary injury were found. Pulmonary injury only occurred at near-lethal concentrations. Renal injury (defined as necrosis of the outer stripe of the outer medullary layer or cortico-medullary junction necrosis) occurred at and above 25–50 ppm for 6-h and 12-h exposures, respectively, a result similar to that previously obtained with a 3-h exposure. Exposure to 25–50 ppm stimulated cell regeneration in a dose-related manner.

Conclusions: In rats, lethal concentrations of the olefin and concentrations producing severe renal injury are inversely related to the duration of exposure to the olefin, exceeding by two- to fourfold peak concentrations that can be obtained in clinical practice. The threshold concentrations for nephrotoxicity (i.e., minimal toxicity) equal concentrations that can be produced in clinical practice. However, even if these threshold effects in rats apply to humans, they probably would not alter renal function. Although dose-related, neither the lethal nor the toxic effects are simply a function of cumulative dose (concentration-time). (Key words: Anesthetics, volatile; sevoflurane; toxicity. Compound A.)

ALKALI such as soda lime and Baralyme (Chemetron, St. Louis, MO) can degrade sevoflurane to an olefin (CF$_2$=C(CF$_3$)OCH$_2$F, also called compound A) by extracting hydrogen fluoride from the isopropyl moiety of sevoflurane.¹ ¹ In a low-flow system that uses soda lime for absorption of carbon dioxide, degradation of sevoflurane produces average concentrations of the olefin equaling 8 ppm.⁵ When Baralyme is used, the average concentration equals 20 ppm, and a peak value of 61 ppm has been observed in one of eight patients tested.⁶ In a closed system used to administer sevoflurane to greyhounds, levels of 90 ± 19 ppm were observed at 2 h of exposure.⁶ Although these levels are less than the 1,050–1,090 ppm found to be lethal in Wistar rats exposed to the olefin for 1 h,⁷ or the 331–420 ppm found to be lethal in rats exposed for 3 h,⁶⁷³ the progressive decrease in the lethal concentration with increasing duration of exposure suggests that longer periods of exposure might result in lethal concentrations that would approach those found in practice. Lethality might be related to cumulative dose (area under the concentration-time curve) rather than concentration.

Similarly, tissue injury might be a function of the area under the curve rather than concentration. Such
INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

a finding would be important because the concentration threshold for organ injury may be an order of magnitude less than the concentration producing death.\(^8\) That is, low concentrations, including those produced in clinical practice, might have measurable noxious effects if given for a sufficient period.

We demonstrated that rats exposed to the olefin for 3 h have renal injury at 50 ppm of the olefin and die at 331 ppm (lethal concentration for 50% \(\text{LC}_{50}\) of rats).\(^7\) In the present study, we assessed whether increasing the duration of exposure from 3 h to 6 or 12 h decreases the olefin concentrations producing death or injury. Using an immunochemical marker for cell proliferation (staining for proliferating cell nuclear antigen or proliferating cell nuclear antigen (PCNA)), we also assessed whether injury was associated with cell regeneration.

Methods and Materials

Test Compound and Exposures of Rats to the Olefin

With approval from our Animal Research Committee, we tested the lethality and potential for injury of the olefin (compound A) in 230 male specific-pathogen-free, 5–6-week-old Wistar rats using a synthesized olefin. The means used to define the structure of the compound and the tests for compound purity were described in the companion paper to this one.\(^7\) The olefin was reported to be >99% pure (primary impurity tetrahydrofuran).\(^#\) Our gas chromatography results confirmed a purity approaching 100%. Rats were divided into 23 groups of ten, each group ranging in average weight from 130 to 191 g. Details of the experimental approach were described in our previous report\(^7\) and are summarized as follows.

We used a closed rebreathing system composed of transparent plastic (allowing viewing of the rats) to expose groups of ten animals to oxygen alone (control) or to fixed target concentrations of the olefin (ranging from 12.5 to 250 ppm), achieved by injection of an initial bolus of the olefin followed by delivery of a dilute concentration of the olefin in oxygen. The greatest average deviation from the target concentration (measured by gas chromatography) was always less than 1.0% of the target value, and usually less than 0.25%. Inspired oxygen was 95.2 ± 1.1% (mean ± SD) at the start of exposure and 93.0 ± 2.7% at the end. Oxygen removed by metabolism was replaced. With one exception, inspired carbon dioxide concentrations were maintained at less than 3%. The average maximum inspired carbon dioxide concentration equaled 2.3 ± 0.9% for all experiments. On one occasion (12-h control run) an increase to 5.2% occurred over a 30-min period because of transient obstruction of the system removing carbon dioxide. The average maximum temperature equaled 28.3 ± 0.5°C.

For 4 h after exposure, all rats were observed at hourly intervals (continuously for the higher concentrations) and then, with one exception, every 12 h until they died or were killed (at 1 or 4 days after exposure) by immersion into 100% CO\(_2\). One rat given 225 ppm for 6 h appeared moribund (unresponsive and unable to right himself when turned on his side) and was killed before reaching the target time of 4 days. Rats to be killed on day 1 after exposure were given lower concentrations of the olefin (results used to define tissue injury) than were rats to be killed on day 4 (see next two paragraphs; results used to define LC\(_{50}\)). All rats were weighed before exposure to the olefin and again immediately before autopsy.

We exposed nine groups of rats to the olefin for 6 h. Four groups of rats were exposed to 12.5, 25, 50, or 100 ppm, respectively, of the olefin and were killed on day 1. Five additional groups were exposed to 175, 200 (two groups of ten), 225, or 250 ppm and either died before or were killed on day 4. Three additional groups served as controls (exposed only to oxygen for the 6-h exposure period), and these rats were killed on day 1 after exposure. We did not obtain controls surviving to 4 days because the exposed rats allowed to survive to this time were primarily for the purpose of defining the LC\(_{50}\) and because our previous study for a 3-h exposure\(^7\) revealed little difference between the controls at 1 \textit{versus} 4 days (i.e., there seemed to be no need to expend an additional 60 rats for this and the 12-h study described in the succeeding paragraph).

We exposed eight groups of rats to the olefin for 12 h. Four groups of rats were exposed to 12.5, 25, 50, or 100 ppm, respectively, and were killed on day 1. Four groups of rats were exposed to 75, 100, 125, or 150 ppm, respectively, and either died before or were killed on day 4. Three additional groups served as con-

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Anesthesiology, V 80, No 3, Mar 1994
trols (exposed only to oxygen), and these rats were killed on day 1.

**Examination of Tissues by L.D.F. and R.I.K.**

We took specimens of brain, duodenum, kidney, liver, and lung from each rat and immediately placed each specimen in 10% buffered formalin. Specimens were obtained within 5–10 min of killing each rat. Slides were made and stained with hematoxylin and cosin. An initial, unblinded review of tissues revealed renal injury in the olefin-exposed rats and suggested possible pulmonary injury. No evidence of cerebral, hepatic, or intestinal injury was found in rats exposed to the highest concentrations of the olefin, and these tissues were not examined further. The slides containing renal and pulmonary tissue subsequently were randomly ordered and examined for injury by L.D.F., who, for this examination, was blinded to the treatment accorded each rat. In addition to noting whether a tissue was normal or abnormal (injury “yes/no”), we assessed the approximate percentage of the tubule cells in the corticomedullary junction that were damaged, and whether injury extended to the cortex of the kidney (injury “yes/no”). The renal tissues (but not the other tissues) also were examined by R.I.K., who used the same criteria for assessing injury.

**PCNA Proliferation Assay**

**Immunohistochemistry.** The techniques applied paralleled published techniques.11,12 PCNA proliferation analysis was applied to renal tissues from rats killed 1 day after exposure to 0–100 ppm of the olefin for 3, 6, and 12 h. For removal of paraffin, sections were incubated with 3% H2O2, then placed in 10 mm citric acid (pH 6.0) in a plastic Coplan jar in groups of ten and subjected to 10–20 1-min cycles of pulsed microwave heating to boil. After cooling for 20 min, the sections were rinsed in distilled water and then incubated in cascin/phosphate buffered saline (PBS) buffer for 20 min. Sections were incubated at room temperature overnight with primary anti-PCNA antibody (Signet, Cambridge, MA), rinsed in cascin/PBS for 10 min, and incubated for 30 min with horse-anti-mouse IgG, 1:200 (Vector, Burlingame, CA). After an additional rinse in cascin/PBS, sections were incubated for 30 min at room temperature with SA-HRP, 1:10 (Dako, Carpenteria, CA) and rinsed in cascin/PBS. Sections were developed in diaminobenzidine for 5 min, rinsed in distilled water, enhanced with CuSO4 (0.5% in normal saline), and counterstained with Gill’s hematoxylin.

**Nuclear Labeling Index Quantitation.** The PCNA-stained sections were examined blindly after randomization. Sections were viewed on an Olympus BH-2 microscope (Tokyo, Japan) equipped with a tricopic head on which was mounted an 2.5× ocular lens and a Panasonic WV-B1204 black and white video camera (Tokyo, Japan). Under a 10× objective lens, the field size of the captured digitized image encompassed an area of 0.35 mm², roughly spanning the minimum width of the outer stripe of the outer medullary layer, referred to previously as the corticomedullary junction. In this manner, little or no cortex or inner medulla was included in the images. The threshold for each field image was established by increasing the illuminator light intensity at the microscope until the background of renal tissue was no longer visible in the video image. This left only the more intensely stained PCNA-positive nuclei present in the image. By inspection of the PCNA-stained sections before analysis, it was determined that insignificant contamination of the counts would occur because of inclusion of rare PCNA-positive endothelial, inflammatory cell, or other nontubular nuclei. Three images were recorded for each specimen, one field each from the two poles, and one from a lateral location adjacent to the renal papilla. Within these three zones, the area with the highest labeling of nuclei, as judged by scanning under low power, was selected for analysis. Video images were digitized to 256 levels of gray via a RasterOps 364 video card mounted in a Macintosh IIx computer (Mountain View, CA). Images were then converted from gray scale to black and white and counts of stained nuclei in each field image were performed using the “Analyze Particles” feature of the National Institutes of Health graphic analysis program, NIH Image. Counts were recorded as number of PCNA-positive nuclei per three fields.

**Statistical Methods**

We used logistic regression to estimate the LC50 values, and unpaired t test, chi-square analysis, or Fisher’s exact probability test for other comparisons. We accepted P < 0.05 as indicating statistical significance.

**Results**

**Weight Loss and General Observations**

Relative to control rats, rats exposed to the higher concentrations of the olefin (compound A) for 6 h (200 ppm or greater) or 12 h (100 ppm or greater) lost
weight ($P < 0.01$, unpaired $t$ test). These data have limited meaning because they compare weight gains for control rats at 1 day with the weight gains for experimental rats at 4 days after exposure. No rat died during exposure to the olefin, and none appeared to be anesthetized. All moved at intervals during the exposure periods. Exposures to the olefin concentrations of 100 ppm or greater appeared to be irritating. Rats exposed to these concentrations kept their eyes closed, and rats given 150 ppm or greater had tearing. None of the ten rats exposed to 250 ppm (or other rats exposed to lower concentrations) gave evidence of gross neurologic abnormalities.

**Lethality Studies**

For the 6-h studies, death occurred only in rats exposed to 175 ppm (two deaths) or greater. Logistic regression gave an LC$_{50}$ of 203 ± 4 ppm (mean ± SE; fig. 1). For the 12-h studies, death occurred only in rats exposed to 100 ppm (one death) or greater. Logistic regression gave an LC$_{50}$ of 127 ± 9 ppm. The results for the 6-h LC$_{50}$ differed significantly from those for the 12-h LC$_{50}$ ($P < 0.001$; unpaired $t$ test).

**Tissue Injury Observed by L.D.F.**

Eleven of the 50 rats exposed to 175–250 ppm of the olefin for 6 h and killed on day 4 had pulmonary congestion and/or edema compared with 2 of 50 control rats ($P < 0.01$, chi-square analysis). Only 3 of 40 rats exposed to 12.5–100 ppm for 6 h and killed on day 1 had pulmonary injury (not different from control). In the 12-h group, the incidence of pulmonary injury in rats exposed to 12.5–100 ppm and killed on day 1 (6 of 40 rats) or to 75–150 ppm and killed on day 4 (7 of 40 rats) did not differ significantly from that found in control rats (1 of 30).

The kidney demonstrated damage (cell swelling and/or necrosis) directly related to the concentration of the olefin (fig. 2). As in our previous study, we accepted necrosis of at least 1% of the tubule cells in the renal corticomedullary junction as indicative of injury. The present results appeared to suggest a leftward shift of the threshold for nephrotoxicity relative to that at 2 h. However, examination of the specific data indicated that the shift was small. For the 6-h exposure period, injury occurred at the 1% level in 5 of 30 control rats (compared to the previous study results of 0 of 30 for 3 h, $P = 0.052$, Fisher's exact test), 2 of 10 rats exposed to 12.5 ppm, 0 of 10 rats exposed to 25 ppm, 5 of 10 exposed to 50 ppm, and 10 of 10 exposed to 100 ppm. Relative to control results, the result at 50 ppm gave $P < 0.1$ by chi-square analysis, and at 100 ppm, $P < 0.001$.

In the 6-h group, no corticomedullary junction tubule injury greater than 1% occurred at 50 ppm or less, whereas at 100 ppm injury ranged from 5% to 85%. The mean values for the percentage of corticomedullary junction tubule cells injured in rats killed at day 1 are

**Fig. 2.** The percentage of injured tubule cells in the corticomedullary junction increased as a function of concentration for each of the exposure periods. The data for rats killed at day 1 after exposure are indicated by continuous lines and closed symbols. Data for rats killed at day 4 (or dying before this time) are indicated by dashed lines and open symbols. Results from the evaluation by L.D.F. are given by the heavier lines and circles; those from R.L.K. are given by lighter lines and triangles.
provided in figure 2 (continuous lines). Renal injury was more severe in rats killed at day 4 (or dying before day 4), as might be anticipated from the higher exposure concentrations (fig. 2, dashed lines). Higher concentrations also produced injury in the renal cortex, again in a concentration-related manner.

In the 12-h study group, the incidence of cortico-
medullary junction injury of 1% or greater did not differ for the 12-h control group (4 of 30; 1 with 10% injury, 3 with 1% injury) compared with rats exposed to 12.5 ppm (0 of 10) or to 25 ppm (4 of 10; fig. 2). None of these results differed significantly from the results obtained for control values for the 6-h or 3-h study groups. However, at 50 ppm, eight of ten rats exposed for 12 h had injury (compared to the 12-h control, \( P < 0.001 \), chi-square analysis), which ranged from 0–20% of necrotic tubule cells. The percentage of injured renal tubule cells, especially those at the corticomedullary junction, increased with increasing concentrations of the olefin (fig. 2: continuous lines indicate results for rats killed at day 1, and dashed lines, day 4). Higher concentrations also produced injury in the renal cortex, again in a concentration-related manner.

The results obtained by L.D.F. and R.L.K. are summarized in an incidence table (table 1). Increasing concentrations of the olefin and increasing durations of exposure to a given concentration of the olefin each increased the severity of injury. L.D.F. and R.L.K. agreed on the category assignment for 83% of slides, differing by one category for 16% and by two categories for 1%. Approximately half of the disagreements (18 of 38) occurred at injury scores of 0–1%.

PCNA Determinations
Counts were obtained for all sections; in general, counts were lowest in the control groups and increased with increasing concentrations of the olefin (fig. 3). Concentrations of 50 ppm of the olefin produced counts significantly greater than those found in control rats for all durations of exposure (\( P < 0.05 \) 12-h exposure to \(<0.001 \) 3-h exposure, unpaired \( t \) test), and a concentration of 25 ppm given for 12 h also produced a significantly greater count (\( P < 0.005 \)).

Discussion

Our results indicate that an increase in the duration of exposure to the olefin (compound \( A \)) decreases the concentration of the olefin that results in death (fig. 1) and decreases the concentration that produces severe renal injury (fig. 2 and table 1). The validity of the \( LC_{50} \) values is suggested by the parallel steepness of the curves on which they are based (fig. 1), the smallness of the standard error for each measurement, and the agreement with the results from the study by Morio et al. for a 3-h exposure.\(^4\) The validity of the data for the threshold for renal injury is suggested by the agreement between the independent evaluations of the pathologists (L.D.F. and R.L.K.; fig. 2 and table 1), and by the agreement of their evaluations with the estimate of cell regeneration using PCNA staining to define cells in a growth cycle (fig. 3). All these results indicate a threshold for renal injury of 25–50 ppm, the lower value for the longest exposure to the olefin.

Both we and Morio et al.\(^5\) find that the product of the \( LC_{50} \) and the duration of exposure (i.e., the dose of the olefin) is not constant, instead increasing with increasing duration of exposure. For example, the product of exposure duration and the \( LC_{50} \) increases from 993 ± 21 ppmXh (mean ± SE) for 3 h, to 1,224 ± 24 ppmXh (23% greater than the 3-h value) for 6 h, to 1,524 ± 108 ppmXh (53% greater than the 3-h

Anesthesiology, V 80, No 3, Mar 1994
INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

Table 1. Categorization of Data by % Corticomedullary Junction Injury for Specific Exposure Durations and Concentrations

<table>
<thead>
<tr>
<th>No. of Rats for Each Exposure Duration and Concentration</th>
<th>30</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olefin (ppm)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>100</td>
<td></td>
<td>85</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2–5</td>
<td>0</td>
<td></td>
<td>15</td>
<td>25</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.1–25</td>
<td>0</td>
<td></td>
<td>0</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>25.1–50</td>
<td>0</td>
<td></td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>&gt;50</td>
<td>0</td>
<td></td>
<td>0</td>
<td>25</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

% Injury | Percentage of rats showing a particular % injury

3-h exposure
0–0.1      | 100  | 100 | 85 | 60 | 0  | 0  | 0  |
0.2–5       | 0    | 15  | 25 | 10 | 0  | 0  | 0  |
5.1–25      | 0    | 0   | 0  | 15 | 10 | 5  | 0  |
25.1–50     | 0    | 0   | 0  | 0  | 55 | 0  | 0  |
>50         | 0    | 0   | 0  | 0  | 0  | 25 | 95 |
6-h exposure
0–0.1       | 93   | 95  | 100| 65 | 0  |    |    |
0.2–5       | 7    | 5   | 0  | 35 | 25 |    |    |
5.1–25      | 0    | 0   | 0  | 0  | 35 |    |    |
25.1–50     | 0    | 0   | 0  | 0  | 0  | 20 |    |
>50         | 0    | 0   | 0  | 0  | 0  | 20 |    |
12-h exposure
0–0.1       | 95   | 100 | 65 | 40 | 0  |    |    |
0.2–5       | 5    | 35  | 30 | 15 |    |    |    |
5.1–25      | 0    | 0   | 0  | 30 | 20 |    |    |
25.1–50     | 0    | 0   | 0  | 0  | 20 |    |    |
>50         | 0    | 0   | 0  | 0  | 45 |    |    |

Each number indicates the average of the percentage of rats assigned by L.D.F. and R.L.K. to a given category of severity of injury at a specific exposure concentration applied for a specific period of time. We accepted 0–0.1% as essentially no injury, 0.2–5% as minimal injury, 5.1–25% as mild injury, 25.1–50% as moderate injury, and >50% as severe injury. For these 230 rats, L.D.F. and R.L.K. agreed on the category assignment for 192/230 (83%) of the slides, differing by one category for 26/23 (16%) or two categories for 2/23 (1%) for the remaining slides. All data are for rats killed one day after exposure. Blanks indicate that no data were available for the indicated exposure concentration.

value) for 12 h. Each succeeding value significantly exceeds the previous value (P < 0.01, unpaired t test). An opposite result might have been predicted from the knowledge that the alveolar concentration of the olefin tends to increase with time and that the LC50 values are based on a constant inspired concentration. Thus, at a constant inspired partial pressure of the olefin, the partial pressure in a rat's body should increase with time. If toxicity is related to the cumulative effect of this partial pressure, our calculated areas under the curve should have decreased, not increased.

The increase in area implies either that the rats became tolerant to the noxious effects of the olefin or that reparative processes had sufficient time over the longer exposures at lower concentrations to counteract part of that noxious effect. Tolerance also is suggested by a comparison of our previous results and those of Morio et al. Morio et al. did not find injury from exposures to 30, 60, and 120 ppm of the olefin for 3 h per day, 3 days per week for 8 weeks, whereas we found injury after a single 3-h exposure to as little as 50 ppm.

Our findings are not consistent with those from other studies that relate the threshold for toxicity to the LC50 for other olefins halogenated solely with fluoroine. For such studies, the ratio of concentrations (or doses) that are lethal to the threshold concentrations (or doses) are roughly a factor of 10. Although we found a comparable result for 3-h exposure of rats to the olefin (a ratio of 331/50, or 6.6), the results for 6-h (203/100, or 2.0, for L.D.F.'s evaluation and 203/50, or 4.1, for the evaluation by R.L.K.) and 12-h (127/50 or 2.5 for L.D.F. and 127/25 or 5.0 for R.L.K.) exposures suggest a convergence of lethal and threshold values. With increasing duration of exposure, the LC50 values tend to decrease proportionately more than the threshold values. The results provided in figures 2 and 3 suggest that the nephrotoxicity threshold of approximately 50 ppm changes relatively little with increasing duration of exposure. This is not true of the concentration producing severe injury (e.g., damage to 50% of corticomedullary junction cells); the concentration producing such damage decreases approximately in proportion to the decrease.
in concentration causing death (compare figs. 1 and 2). Note that the data for day 4 in figure 2 may underestimate the injury relative to day 1 because some repair likely occurred by day 4. That is, the dashed lines in figure 2 may be shifted slightly to the right.

We speculate that the limited change in the threshold for renal injury is not a result of repair of injured tissue. The time for repair is too short; moreover, repair would change absolute rather than proportional differences. Perhaps the mechanism by which the olefin produces injury is opposed by a normal protective chemical (e.g., glutathione) whose rate of replenishment determines the concentration producing injury; injury occurs when the supply of the olefin (or an olefin degradation product) exceeds the supply of the protective chemical. Such a possibility also is consistent with the large range of injury seen at a given concentration in the present study. Rats will differ in their rates of production of the protective chemical.

An extension of this thought, suggested by Gandolfi** is that the problem is not a lack of glutathione, but a nephrotoxic conjugate of glutathione. This possibility has been explored by Gandolfi and others for other fluorinated alkene**. The product of hepatic conjugation of alkene with glutathione is metabolized to the corresponding cysteine S-conjugate. Acetylation of the cysteine conjugate in the kidney and subsequent excretion of the acetylated compound would prevent a toxic effect, but if the acetylation process is exhausted, the cysteine conjugate can be degraded to cytotoxic metabolites (particularly an unstable thiol) by γ-glutamyl transpeptidase. Monks and Lui note that the kidney possesses the highest level of γ-glutamyl transpeptidase in the body. The threshold for renal necrosis is not the threshold for an effect on renal function. The loss of 1% or a far greater fraction of renal tubules does not necessarily indicate a decrease in function. Essentially normal function can be obtained with only one kidney. Thus, even if the injury seen in the present applies to humans (and that remains to be determined), it may be difficult to define such injury by tests of function.

The pulmonary injury found in rats exposed to the olefin for 6 h and killed on day 4 (i.e., the highest concentration of the olefin for this exposure duration) confirms a similar earlier finding by Morio et al. for exposures of shorter duration. It is not clear why this result was not obtained in our 12-h exposure group or in our previous 3-h exposure group. Indeed, because the injury appeared to be larger in our 6-h groups (exposed to roughly twice the olefin concentration given to the 12-h groups, i.e., 250 ppm vs. 150 ppm as the highest concentrations), we reexamined the slides for the 3-h group (similarly, exposed to roughly twice the concentration given to the 6-h groups). Using a blinded examination of randomly sorted slides for rats given 200, 300, and 400 ppm for 3 h and killed on day 1; for 200, 300, 350, and 400 ppm for rats killed on day 4; and for the slides for the respective control groups, we again found no significant difference nor even a trend to significance. For example, in this repeated examination, congestion was seen in 8 of 60 control slides and 11 of 69 experimental slides. Possibly our failure to find a difference in pulmonary injury between experimental and control rats for the 3-h study resulted from a fortuitously high incidence in the control groups.

The results of the present study may be interpreted in the context of several issues. In the future, cost constraints may require more extensive use of lower gas flows. Using a low fresh gas flow (0.8 l/min), Frink et al. demonstrated that anesthesia with sevoflurane results in olefin concentrations that vary as a function of the carbon dioxide absorbent, i.e., soda lime or Baralyme. The olefin concentration also varied among patients who breathed from circuits in which the same absorbent was used. The highest olefin concentration found by Frink et al. in 16 patients was 61 ppm, with most peak concentrations less than 20 ppm. These observations by Frink et al. combined with the LC50 of

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Fig. 3. The proliferating cell nuclear antigen (PCNA)-stained cell counts from three fields (upper and lower poles plus a lateral section at the mid-kidney) showed increasing numbers of counts with increasing concentrations of the olefin. The exception was the counts at the 12-h exposure to 100 ppm of the olefin, when the extensive destruction of tubule cells may have limited the availability of cells for regeneration.
400 ppm reported by Morio et al. \(^4\) prompted the editorial by Mazze in the same issue of this journal.\(^{17}\) Mazze noted the 8:1 ratio between the olefin’s lethality and concentrations attained during anesthesia, suggesting that this margin of safety was a cause for concern. The present results indicating an IC\(_{50}\) less than that reported by Morio et al. (400 ppm) suggest a still smaller safety ratio than that which prompted Mazze’s comments. These data in conjunction with the data on the olefin concentrations during anesthesia increase concern regarding the safety of sevoflurane when administered using low flows of background gas.

Further investigations may be needed to address the issue of safety. Our results in rats may not apply to humans. Thus, we need data from studies in primates (or at least other animal models) on the threshold for toxicity of the olefin. To define the range of the olefin concentrations attainable during low-flow anesthesia, we need data from greater numbers of patients than those studied by Frink et al. Such studies might examine the effect of different flow rates and patient sizes, as well as the duration of anesthesia and the choice of carbon dioxide absorbent. Perhaps the problem could be avoided if we found ways to minimize the production of the olefin from sevoflurane. Finally, if some protective mechanism limits toxicity, we need to define those conditions that might impair or improve such a protective mechanism in patients. This may involve some metabolic defense. Perhaps, as suggested by Gandolfi,\(^{2}\) the presence of sevoflurane will ameliorate or abolish the injury seen.

**Gandolfi AJ: Personal communication. 1993.**

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Anesthesiology, V 80, No 3, Mar 1994