

Anesthesiology
80:556-565, 1994
© 1994 American Society of Anesthesiologists, Inc.
J. B. Lippincott Company, Philadelphia

Toxicity of Compound A in Rats

Effect of a 3-Hour Administration

C. T. Gonsowski, B.S.,* M. J. Laster, D.V.M.,† E. I. Eger II, M.D.,‡ L. D. Ferrell, M.D.,§ R. L. Kerschmann, M.D.||

Background: Soda lime converts sevoflurane to $\text{CF}_2 = \text{C}(\text{CF}_3)\text{OCH}_2\text{F}$, an olefin called compound A, whose toxicity raises concerns regarding the safe administration of sevoflurane *via* rebreathing circuits. The present report extends the findings of a previous investigation by others of the toxicity of this olefin, and establishes concentration-response relationships for such toxicity.

Methods: Eighteen groups of ten Wistar rats breathed 0, 25, 50, 100, 200, 300, 350, and 400 ppm of the olefin in oxygen for 3 h. The olefin concentrations were developed in a square-wave manner by injection of saturated vapor followed by a continuous delivery of dilute vapor. The lethal concentration in 50% (LC_{50}) of animals was estimated by logistic regression. Rats 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 using light microscopy.

Results: The LC_{50} equaled 331 ppm (95% confidence limits \pm 13 ppm). No injury resulted to lung or small intestine in either the experimental or the control group (those breathing only oxygen for 3 h). Renal injury (necrosis of the outer strip of the outer medulla, defined in this report as corticomedullary tubular necrosis) occurred at 50 ppm and greater; hepatic in-

jury at 350 ppm and greater; and cerebral injury only at 400 ppm.

Conclusions: The lethal concentration and the threshold for toxicity of the olefin are less than previously reported. The threshold for nephrotoxicity reaches the range of values for the olefin that have been attained in clinical practice. Further studies are required to determine whether these results in rats can be extrapolated to patients. (Key words: Anesthetics, volatile; sevoflurane; toxicity. Compound A.)

SEVERAL reports indicate that alkali such as soda lime and Baralyme (Chemetron, St. Louis, MO) can degrade sevoflurane.¹⁻⁴ Among the degradation products is an olefin [$\text{CF}_2 = \text{C}(\text{CF}_3)\text{OCH}_2\text{F}$, also called compound A⁴] produced by the elimination of hydrogen fluoride from the isopropyl moiety of sevoflurane. In a low-flow system that uses soda lime for the absorption of carbon dioxide, concentrations of the olefin average 8 ppm.⁵ When Baralyme is used, the average concentration equals 20 ppm, and a peak value of 61 ppm has been observed during anesthesia in one of eight patients given sevoflurane.⁵ These concentrations are less than the 400 ppm (female rats) to 420 ppm (male rats) reported by Morio *et al.* to be lethal after a single exposure to the olefin for 3 h.⁴ Although the threshold for injury was not stated in the report of lethality, chronic administration (3 h per day, 3 days per week for 8 weeks) of concentrations of 30, 60, and 120 ppm did not produce histopathologic changes attributable to the olefin.⁴

The study by Morio *et al.* of the toxicity of the olefin in rats did not have as its primary focus the issues of lethality and injury, and a limited supply of this degradation product may have forced the investigators to restrict their applications: (1) The concentration of the olefin was not held constant; rather, a fixed amount was injected into a closed system containing the test animals, resulting in a decrease in concentration over time due to uptake by the exposed rats and degradation by the absorbent for carbon dioxide. (2) Temperature was neither measured nor controlled, nor were mea-

This article is accompanied by an editorial. Please see: Saidman IJ. Unresolved issues relating to peer review, industry support of research, and conflict of interest. ANESTHESIOLOGY 80:491-492, 1994.

* Research Fellow, Department of Anesthesia.

† Staff Research Associate, Department of Anesthesia.

‡ Professor, Department of Anesthesia.

§ Associate Professor, Department of Pathology.

|| Associate Professor and Associate Nephropathologist, Department of Pathology.

Received from the Departments of Anesthesia and Pathology, University of California, San Francisco, California. Accepted for publication December 7, 1993. Supported by grants from Ohmeda and from The Anesthesia Research Foundation. Dr. Eger is a paid consultant to Ohmeda.

Address correspondence to Dr. Eger: Box 0464, University of California, San Francisco, San Francisco, California 94143-0464.

INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

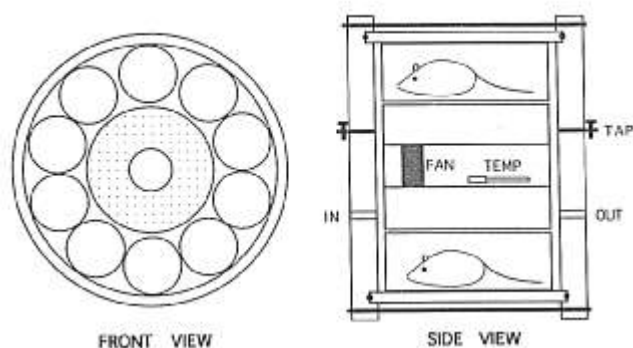


Fig. 1. The chamber used in these studies is shown in two views, a front view (without rats) and the other from the side as a cutaway (with two rats). The external circuit, consisting of the circulating pump, cooled carbon dioxide absorber, liquid (aerosol) trap, and plumbing for replenishment of oxygen, is not shown.

surements made of the concentration of oxygen or carbon dioxide in this closed system. (3) The initial concentration of oxygen in the system was 100%, but oxygen was not replenished during the 3 h of exposure; and (4) Little information was provided on concentration-response aspects of injury. However, these investigators reported pulmonary injury in rats who died within 1 day of exposure, and renal injury in rats dying within 4 days of exposure. Mazze suggested that the findings may indicate a need for further study before the release of sevoflurane into clinical practice in the United States.⁶ The present study focuses on the lethality and toxicity of the olefin product of sevoflurane degradation, describing the concentration-response effects on multiple organ systems.

Methods and Materials

Preparation of the Olefin (Compound A) and Test Standards

With approval from our Animal Research Committee, we tested the lethality and potential for injury of a sevoflurane degradation product, the olefin named compound A, in 180 male, specific-pathogen-free Wistar rats using a synthesized olefin (Ohmeda, Liberty Corner, NJ). The olefin was prepared as described previously by incubating sevoflurane with lithium bis(trimethyl-

ylsilyl)amide in tetrahydrofuran at -70°C to -60°C .⁷ The olefin and tetrahydrofuran were separated from the reaction mixture under anhydrous conditions at reduced pressure and at low temperature to minimize secondary reactions. Fractional distillation was used to separate the olefin from tetrahydrofuran. The compound was reported[#] to be >99% pure as determined by gas chromatography, with tetrahydrofuran as the primary contaminant.

Several techniques were used to certify that the compound produced was the olefin. The mass spectrum showed an M^+ of 180 and major m/z fragments of 161, 120, 70, and 69 (base peak). A standard gas chromatogram was indistinguishable from that obtained with the previously produced olefin and gave an area percentage of 99.91% with acetone, CHCl_3 , and tetrahydrofuran impurities. Film FT/IR (Fourier Transform Infrared) showed characteristic bands for $\text{C}=\text{C}$, $\text{C}-\text{F}$ and $-\text{OCH}_2\text{F}$. FT/magnetic resonance imaging at a nominal 1H (proton) strength of 300 MHz showed positive characterization for the olefin from ^1H , ^{13}C , and ^{19}F analyses. The use of two-dimensional fluorine-19 nuclear magnetic resonance to characterize the olefin has been described previously.⁸ Elemental analysis was performed by Schwartzkopf Microanalytical Laboratories (Woodside, NY). The following lists the results as theoretical percentage/experimental percentage: carbon 26.68/26.46; hydrogen 1.12/1.20; and fluorine 63.31/63.03.

We accepted the reported specific gravity of 1.50 and the reported value for the boiling point of 43°C at 1 atm.⁴ We prepared compressed gas cylinders (tanks) containing various concentrations of the olefin in oxygen by injecting precisely measured amounts of the olefin into the cylinders and then adding oxygen to the desired pressure. Care was taken not to inject more liquid olefin than that which would completely vaporize. The concentrations in the tanks were analyzed by gas chromatography, using volumetric primary standards. The gas mixtures in the tanks served both as reference (secondary) standards and as sources of the olefin for the experiments to be described. We tested the stability of the olefin in the tanks by comparing the value found immediately after preparation and mixing, with the value found 5 weeks later, both values being compared against primary standards. No change in concentration was found. Consistent with a purity approaching 100%, using gas chromatography, we found one major peak and four minor peaks. The sum of the areas under the curves for the four minor peaks equaled 0.05% of the area of the major peak.

Halpern D, Sisco R. Unpublished data. 1993.

Exposure Chamber and Ancillary Apparatus

To conserve the limited supply of the olefin available to us, we constructed an exposure chamber large enough to test ten rats concurrently at a minimized internal gas volume (fig. 1). A 30.5-cm ID, 26-cm cylinder of clear acrylic plastic permitted the insertion of ten individual acrylic cylinders (6.4 cm ID, 20 cm) that housed individual rats. Individual cylinders were held in place at the periphery of the large ("parent") chamber with a centrally placed cylindrical nylon plug measuring 15.5 cm (diameter) by 20.5 cm. The plug was pierced lengthwise at its center to create a tunnel with a diameter of 5.25 cm, which contained a fan for circulation of gases and a thermistor probe for measurement of chamber temperature. The parent chamber was sealed at both ends by two clear, 3.3-cm-thick plastic plates that were machined with circular grooves each containing a washer to seal the junction between the chamber and the end plates. The plates were held against the washers and chamber by six external bolted rods. The sealed system could contain 3 atm pressure without leaking. Holes were drilled in the end plates to permit passages for electrical connections to the fan and thermistor and for the input and output of gases. A port (tap) through the end plate permitted sampling of gases exiting from the chamber.

We prepared a circulating gas system external to the chamber (not shown in fig. 1). To minimize the inspired concentration of carbon dioxide, gases leaving the exit pass-through in the end plate were pumped through a 2-l flask containing 150–200 g barium hydroxide in 1,800 ml of water. During each exposure test, the flask containing the barium hydroxide was immersed in ice and water. The absorbent system was cooled to minimize the degradation of the olefin.⁴ Cooling also limited the humidity in the system by causing condensation of water in the cooled solution (condensation did not occur in the parent chamber). The gases then passed through a liquid (aerosol) trap and were returned to the chamber *via* the entrance pass-through. To this external circuit, we also connected a T-port for the delivery of oxygen to replace oxygen consumed by the rats. Oxygen was directed to one arm of a T, the other arm of the T was directed through a water trap to air, and the stem of the T was connected by a one-way valve to the circuit. The absorption of oxygen created a negative pressure that drew gas from the T, thereby maintaining the concentration of oxygen within the chamber. To ensure that laboratory personnel did not breathe the olefin, during

all exposures of rats to the olefin both the parent chamber and the external circuit were placed in a fume hood.

The system was tested for the uniformity of distribution of the olefin within the chamber by assembling all components and adding sampling ports on the inflow side of the chamber and in two unoccupied rat cylinders (see below) placed on opposite sides of the chamber. At 10-min intervals after initiating inflow of the olefin into the chamber, we measured the olefin concentration at these three sites plus the outflow (exiting) site noted in the previous paragraph. The concentrations at the outflow site and in the two rat cylinders were within 7% of the inflow concentration at 10 min after initiating flow and within 5% (usually 2%) thereafter.

Olefin Analysis

For analysis of the olefin, we used a Gow-Mac 580 flame ionization detector gas chromatograph (Bridge-water, NJ) equipped with a 2.2-mm ID, 4.6-m column containing 10% SF-96 on 68/80 mesh Chromosorb WHP maintained at 44°C, while the gas sampling loop (the injection port) was maintained at ambient temperature. The carrier flow of nitrogen was 8.5 ml/min. The detector (maintained at 120°C) received 20 ml/min hydrogen and 200 ml/min of air. Because of a slight alinearity, we prepared standards for each attenuation used.

Test Animals and Exposure Conditions

We tested 12 groups of ten rats at the olefin concentrations ranging from 25 to 400 ppm. Six additional groups of ten rats exposed only to oxygen served as control rats (see below). All rats were exposed to a given concentration of the olefin (0 ppm in the case of control rats) for a period of 3 h, after which they were removed from the chamber. The rats were 5–6 weeks old and weighed 123.5–159.4 g (range of average weights for the groups of ten with the exception of one group of ten control rats with an average weight of 203 g). Each rat was weighed before insertion into an individual cylinder. The bottom of each cylinder was lined with blotting paper to absorb urine. After placement of ten rats within the chamber, the chamber was sealed and the system flushed with oxygen for 10–15 min. Oxygen concentrations were monitored using a Beckman model E (Pauling-type) analyzer (Fullerton, CA) calibrated with 100% O₂ and 100% N₂. The system was not closed until the oxygen concentration reached 97–98%.

INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

After preoxygenation, we sealed the chamber and injected a bolus of saturated olefin vapor to achieve the target test concentration in the 1st min of exposure. The test concentration was sustained by a continuing flow of dilute vapor from one of the tanks containing the olefin/oxygen mixture. Sampling of gas from the exit port of the chamber began at 3 min after bolus injection and continued at 5–10-min intervals during the first 30–90 min of study, then at 10–20-min intervals until the end of the period of exposure (180 min). All samples were analyzed for the olefin, and some for the concentrations of oxygen and carbon dioxide (Beckman LB2 infrared analyzer). We concurrently measured (thermistor probe) and recorded the temperature within the chamber. The clear plastic used in the construction of the exposure chamber and the individual chambers holding the rats permitted us to view each rat throughout the 3-h exposure period.

The concentration of the olefin was brought to the target concentration by increasing or decreasing the inflow of dilute vapor. To ensure that the average concentration equaled the target concentration, we developed a computer program that allowed calculation of a running average by applying the rhomboid rule to give the average concentration for each time interval. The concentration for time zero (the time of injection of the bolus of the olefin) was estimated by backward extrapolation from the first two measurements. The greatest averaged deviation from the target test concentration was always less than 2% (usually less than 1%) of the targeted value. For the first 4 h after exposure, all rats were observed at 1-h intervals (continuously for the higher concentrations) and thereafter at 12-h intervals. Rats that appeared moribund (unresponsive and unable to right themselves when turned on their sides) were killed by immersion in 100% CO₂. Rats surviving to 1 or 4 days (see below) were killed in the same manner.

Three of the six control groups were killed at day 1 and three at day 4 after exposure to oxygen alone. Six groups of rats were exposed to 50, 100, 200, 300, 350, and 400 ppm of the olefin, respectively. Those that did not die before day 4 after exposure were killed, and results for all six groups analyzed to define the lethal concentration for 50% of animals (LC₅₀). The final six groups of rats were exposed to 25, 50, 100, 200, 300, and 400 ppm, respectively, and either died or were killed on day 1 after exposure. The results from this final group were used to define tissue injury and to determine if the degree or site of injury differed at 1 *versus* 4 days after exposure.

Tissue Analyses

All rats were weighed immediately before autopsy. Within 5–10 min after killing each rat, we took specimens of brain, duodenum, kidney, liver, and lung and immediately placed each specimen in 10% buffered formalin. Standard paraffin processing and sectioning at 5 μ was performed, and sections were stained with hematoxylin and eosin. An initial, unblinded review of tissues revealed that results for duodenum and lung did not differ between exposed (with particular attention to results for the highest levels of exposure) and control groups, and these tissues were not examined further. Injury was found in brain, kidney, and liver in rats exposed to the highest concentrations of the olefin. The slides for these tissues subsequently were randomly ordered and examined for injury by two of the authors (L.D.F. and R.L.K.), who, for this examination, were blinded to the treatment accorded each rat (R.L.K. examined renal tissue only). Examinations were conducted separately to avoid any bias of one pathologist affecting the interpretation of the other. In addition to noting whether a tissue was normal or abnormal (injury "yes/no"), we visually assessed the approximate percentage of damaged (necrotic) renal tubular cells in the outer stripe of the outer medulla⁹ (for this report, we name this the corticomedullary junction), and determined whether injury extended to the outer cortex of the kidney (injury "yes/no"). That is, we visually estimated the fraction of tubular cells injured (necrotic) but did not count the necrotic *versus* normal cells.

Data Analysis

We used logistic regression to estimate the LC₅₀ values, and unpaired *t* test, chi-square analysis, or Fisher's exact probability test to assess differences between groups. We accepted *P* < 0.05 as indicating statistical significance.

Results

Exposure Characteristics

Oxygen concentrations at the end of exposure (95.2 \pm 1.5%; mean \pm SD) decreased slightly from those at the start of exposure (96.7 \pm 1.4%). The constancy of the oxygen concentrations indicated that there were no significant inward leaks into the exposure chamber. The maximum temperature in the chamber never exceeded 29.5°C (mean maximal temperature 27.8 \pm

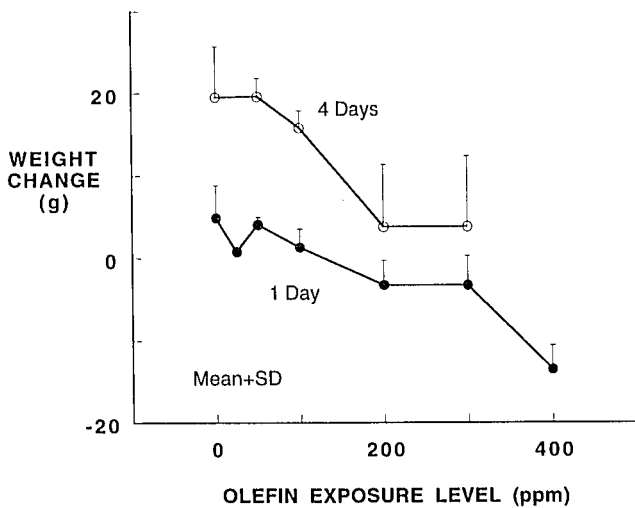


Fig. 2. Increasing concentrations of the olefin produced less weight gain or even weight loss as illustrated by the weight change from after versus before exposure to the olefin for rats killed at 1 or 4 days. The weight change was significantly less than the weight gain in control rats for all rats exposed to 100 ppm or to higher concentrations of the olefin ($P < 0.01$). Values following exposure to 350 or 400 ppm for rats killed at 4 days are not shown because few, if any, of these rats survived.

0.9°C). Similarly, the maximum concentration of carbon dioxide never exceeded 3.9% (mean maximal concentration $2.0 \pm 0.6\%$). Rats exposed to higher concentrations of the olefin lost weight (killed at 1 day) or gained less weight (killed at 4 days) than those exposed to the lower concentrations (fig. 2). All groups given 100 ppm or more of the olefin (compound A) had a significantly smaller weight gain than their control peers ($P < 0.01$ by unpaired t test).

Gross Observations and LC₅₀ Results

No rat died during exposure to the olefin, and no rat appeared to be anesthetized, even at the highest exposure concentration (400 ppm). All rats moved in their cylinders from time to time. Exposures to olefin concentrations of 100 ppm or greater appeared to be irritating: rats exposed to these concentrations closed their eyes, and rats given 200 ppm or greater teared. Some rats given 350 or 400 ppm had what appeared to be convulsive activity (rigid extension of the hind limbs, sometimes proceeding to clonic movements of the forelimbs) during the latter portion of the exposure period. Of the 20 rats exposed to 400 ppm, 13 displayed this behavior (6 during exposure, 6 afterward, and 1 both during and afterward), and additional rats

appeared to be tremulous. Of the ten rats exposed to 350 ppm, five displayed this behavior after exposure, and an additional rat made circling movements that appeared abnormal. No rat exposed to 300 ppm had convulsive activity, but four rats were tremulous and ataxic immediately after exposure. None of the 20 rats exposed to 200 ppm gave evidence of gross neurologic changes.

Deaths occurred only in rats exposed to 300 ppm (one death) or greater. The single death after 300 ppm occurred on day 3. Eight rats given 350 ppm died, and all rats given 400 ppm died before 4 days had passed. At 350 ppm, only 1 of 8 died on the 1st day compared with 10 of 20 rats given 400 ppm. Of the remaining seven given 350 ppm, two died on day 2, three died on day 3, and two died on day 4. Logistic regression revealed an LC_{50} of 331 ± 7 ppm (mean \pm SE) with 95% confidence limits of 318–344 ppm (fig. 3). These data include one rat given 400 ppm of the olefin and one given 350 ppm that were deemed moribund and were killed. Had they survived, the LC_{50} value would be slightly increased.

Results of Microscopic Examination by L.D.F.

Blinded microscopic examination of the brain revealed injury in 3 of 20 rats exposed to 400 ppm (two of these rats were noted to have convulsive-like activity); 1 of 10 rats exposed to 350 ppm (and this rat also convulsed); but in no rat at lower exposure concen-

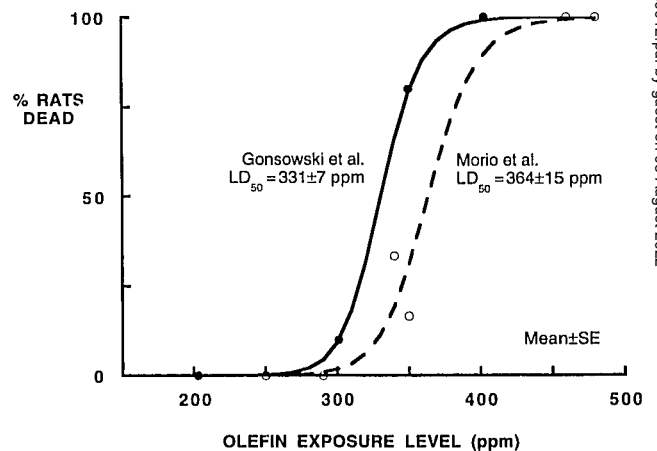


Fig. 3. The curve defined by logistic regression provided an LC_{50} of 331 ± 7 ppm (mean \pm SE) for the rats we studied. Included in this graph are the data and regression curve for the combined female and male results published by Morio et al.⁴ The LC_{50} from this analysis (364 ± 15 ppm) was only slightly (10%) greater ($P < 0.05$) than the LC_{50} we found.

INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

trations. Comparing the results for 400 ppm with those for the 60 control rats (no injury) demonstrated a significant difference ($P = 0.028$, two-tailed Fisher's exact test). Injury consisted of small pyknotic neuronal nuclei with eosinophilic cytoplasm.

Hepatic injury was found in rats exposed to 300 ppm (1 of 20 rats, this rat having been killed on day 1), 350 ppm (3 of 10 rats, one each dying on days 1, 2, and 3), and 400 ppm (2 of 20 rats, both rats dying on day 2 after exposure). For the rats given 350 ppm and for the combined data for rats given 350 and 400 ppm, the results differed significantly from those obtained in control rats ($P < 0.05$; 0 of 30 control rats had injury). Hepatic injury was found in 2 of 30 rats (7%) killed or dying on the 1st day after exposure and 4 of 20 (20%) in rats killed or dying at later times (difference not significant). Injury consisted of periportal fatty infiltration, hepatic cellular swelling, and occasional pyknotic hepatocyte nuclei.

The kidney revealed the greatest damage (figs. 4–7). Damage was most extensive in the outer strip of the outer medullary layer (corticomedullary junction). Damage extended to the outer cortex and medulla only when most of the corticomedullary junction was necrotic. We accepted involvement of at least 1% of the corticomedullary junction of the kidney as indicative of injury (fig. 5). This level was accepted because it exceeded the findings for all control rats: Abnormalities were detected in less than 0.1% of these cells in 9 of 30 control rats killed on day 1 and 1 of 30 killed on day 4 ($P < 0.02$ by chi-square analysis), the remaining rats showing no abnormalities. At 25 ppm, five of ten rats had evidence of corticomedullary junction tubular abnormalities in less than 0.1% of cells, and five had no evidence of injury (not significantly different from control). At 50 ppm, 3 of 10 rats killed on day 1 had evidence of injury in 1% of corticomedullary junctional cells, a finding that differed significantly from the absence of injury in the 30 rats in the 1-day control group ($P = 0.024$, two-tailed Fisher's exact test). At concentrations of 100 ppm or greater, all rats killed at 1 day (or dying before 1 day) had renal injury comprised of swelling and/or necrosis, particularly of tubular cells in the corticomedullary junction. The percentage of injured corticomedullary tubular cells increased with increasing concentrations of the olefin (figs. 6 and 7). Higher concentrations also produced injury in the cortex, again in a concentration-related manner. In all cases, it appeared that the percentage of renal cells injured diminished by day 4 after exposure.

Results of Microscopic Examination by R.L.K.

In general the results obtained by R.L.K. confirmed those provided by L.D.F. As indicated in methods, both pathologists examined the randomly allocated slides blindly. R.L.K. defined injury as necrosis or necrosis with regeneration. His examination uncovered no incidence of necrosis in the kidneys of control rats or in rats given 25 ppm of the olefin. At 50 ppm, 3 of 10 rats killed on day 1 had evidence of necrosis in corticomedullary junctional cells, a finding that differed significantly from the absence of injury in the 30 rats in the 1-day control group ($P = 0.024$, two-tailed Fisher's exact test). At 50 ppm, two of ten rats killed on day 4 showed necrosis with regeneration (not significantly different from control; $P = 0.12$, Fisher's exact test). At concentrations of 100 ppm, eight of ten rats killed on day 1 showed necrosis, and eight of ten rats killed on day 4 showed necrosis with regeneration ($P < 0.001$ compared to control rats). At concentrations of 200 ppm or greater, all rats killed at 1 day (or dying before 1 day) had corticomedullary junction necrosis (day 1) or necrosis with regeneration (day 4). The percentage of injured corticomedullary tubular cells increased with increasing concentrations of the olefin (figs. 6 and 7). R.L.K.'s estimates nearly equaled those of L.D.F. for rats killed on day 1 (fig. 6). At the 200-ppm exposure level for rats killed on day 4 (fig. 7), R.L.K.'s estimates of injury appeared higher than those of L.D.F., but the difference was not significant. As did L.D.F., R.L.K. found that higher concentrations of the olefin produced injury in the renal cortex, again in a concentration-related manner.

Discussion

Our results indicate that administration of the olefin (compound A) to rats for 3 h may injure the brain, liver, and kidney but not the lung or duodenum. Injury to brain and liver occurred at higher applied concentrations (350–400 ppm), whereas injury to corticomedullary renal tubular cells occurred at lower (50 ppm) as well as higher concentrations.

Qualitative and quantitative differences and similarities exist between our results and those of Morio *et al.*⁴ Using logistic regression, we find an LC_{50} of 331 ppm, a value less than the value of 420 ppm that they found for male rats using probit analysis. We have reanalyzed their data for female and male rats. Regression analysis revealed no difference attributable to gender. Accordingly, we combined their data, obtaining an LC_{50}

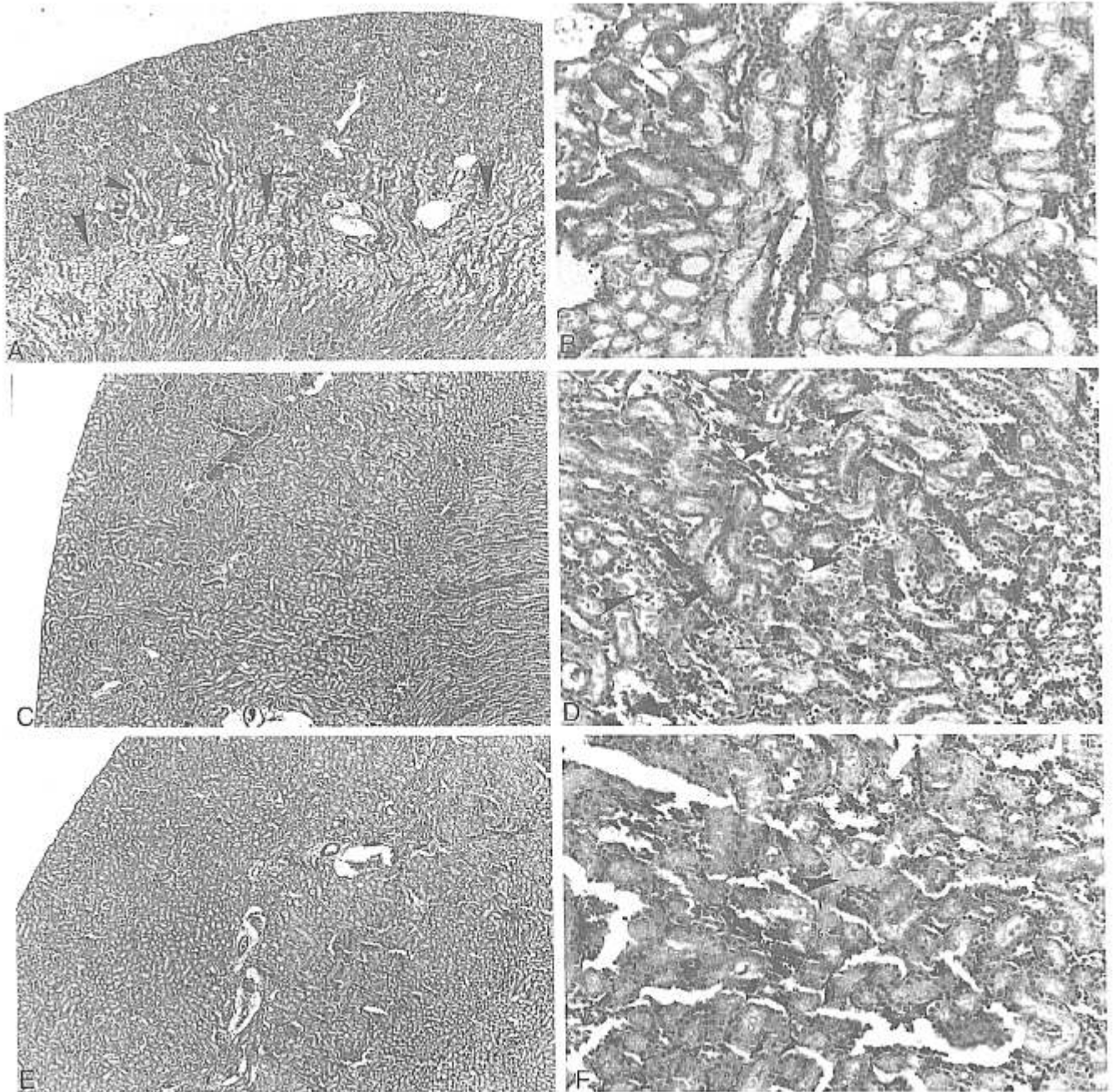


Fig. 4. Photomicrographs of renal tissue at a low (10 \times) original magnification (*left*) and high (50 \times) original magnification (*right*), all stained with hematoxylin and eosin, illustrate the concentration-related corticomedullary necrosis seen in this study. The pair of photomicrographs at the top indicate the destruction evident at 300 ppm. At low magnification, the kidney shows diffuse, extensive necrosis of tubules at the corticomedullary junction (the paler staining zone below the cortex; larger arrows) with tubular necrosis extending focally into the cortex (smaller arrows). Higher magnification shows damaged tubules lined by pale cellular debris, with no nuclei present in the necrotic tubules, but intact nuclei in the intervening viable tubules. At 100 ppm (middle pair), low magnification reveals no obvious abnormalities, but higher magnification shows several scattered tubules lined by necrotic cells (arrows) and containing necrotic debris. At 50 ppm (lowest pair), again the lower magnification exhibits nothing notable. Higher magnification discloses a rare tubule lined with necrotic cellular debris (arrow).

INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

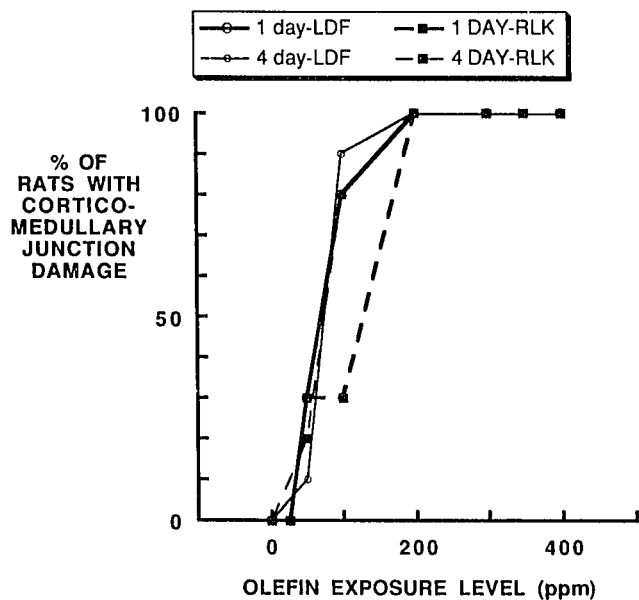


Fig. 5. The incidence of renal injury (the percentage of rats that showed necrosis in corticomedullary junctional cells) was related to the olefin concentration. For rats killed 1 day after exposure, a significant percentage of animals showed renal injury at 50 ppm, and 80–100% of rats given 100 ppm or more showed significant injury. Similar results were obtained for rats surviving to 4 days. The results obtained by L.D.F. did not differ significantly from those obtained by R.L.K.

of 364 ± 15 ppm (fig. 3). This value is only slightly (10%) greater than ours. Given the differences in experimental approaches, the minimal difference in LC_{50} values is reassuring and supports the validity of both measurements.

Our results qualitatively differ from those of Morio *et al.* in three ways. First, Morio *et al.* found lung congestion, hyperemia, and hemorrhage in animals that died on the day of exposure. We did not find pulmonary injury or, at least, found no difference between control and experimental animals. This may be a consequence of the different approaches to exposure. Pulmonary injury may require administration (albeit transient) of the higher concentration used by Morio *et al.* Second, we found cerebral injury, whereas Morio *et al.* did not. The cerebral injury in our study was associated with evidence of cerebral irritability, manifested by what appeared to be seizures. Similarly, Morio *et al.* noted tremor in some of their rats. Whether cerebral injury in our study occurred by direct injury to the brain or an indirect insult resulting from hypoxia secondary to seizures is unclear. Third, we demonstrated hepatic injury, whereas Morio *et al.* found none. Again whether

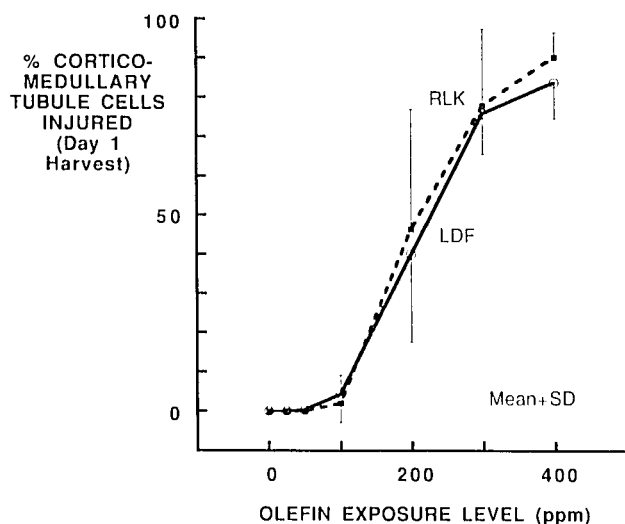


Fig. 6. In kidneys harvested on day 1 after exposure to the olefin, the percentage of tubular cells in the corticomedullary junction showing injury increased with increasing concentration of the olefin. Such cells appeared to be the primary target of the olefin. The results obtained by L.D.F. were remarkably similar to those of R.L.K. Values are mean + SD.

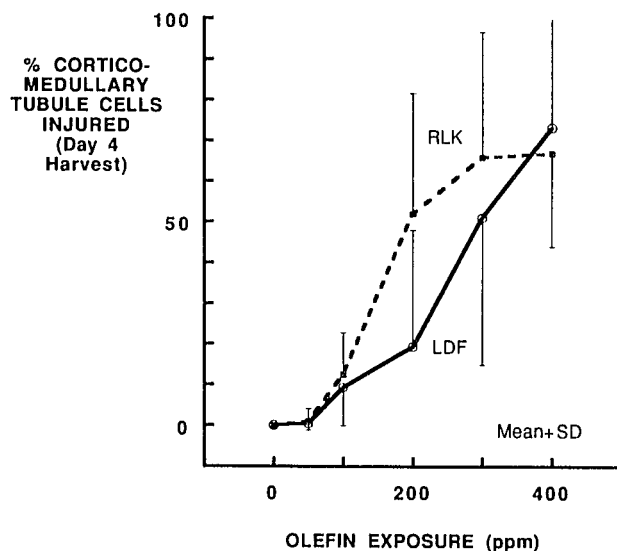


Fig. 7. In kidneys harvested on day 4 after exposure to the olefin, the percentage of tubular cells in the corticomedullary junction showing injury increased with increasing concentration of the olefin. Such cells appeared to be the primary target of the olefin. Although the results obtained by L.D.F. suggested less injury at 200 ppm than that found by R.L.K., the difference was not significant. Values are mean + SD.

hepatic injury resulted from direct injury or indirect seizure-related hypoxia is unclear; however, one rat sustained hepatic injury at 300 ppm in the absence of convulsive activity, suggesting a direct component of injury. Both cerebral and hepatic manifestations of a toxic effect were found only at the higher test concentrations.

Our results for the kidney differ quantitatively from those of Morio *et al.* Morio *et al.* did not indicate the concentration producing renal injury in their acute 3-h exposure group (equivalent to our exposure groups), probably because of their more truncated approach. All rats that survived their exposure were killed on day 14, by which time tubular injury in such rats likely would have been repaired. That is, examination of tissues in rats dying soon after exposure only applied to rats given higher concentrations, concentrations in excess of 250–290 ppm. They note that “. . . 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.” Our results show necrosis of corticomedullary tubules in all rats given 200 ppm for 3 h.

However, in a separate study in the same report, Morio *et al.* administered nominal concentrations of 30, 60, and 120 ppm of the olefin 3 h per day, 3 days per week for 8 weeks to groups of ten rats killed on day 1 after the last exposure. These were initial concentrations, and the average concentrations appear to have been approximately 25% lower. Morio *et al.* found no histologic changes attributable to the olefin in any of the animals exposed to nominal concentrations of 120 ppm (the average concentrations probably were 90 ppm). In contrast, we found evidence of renal injury after a single exposure to 50 ppm of the olefin, with a concentration-related increase in injury at higher concentrations. Furthermore, Morio *et al.* also reported degeneration and necrosis of renal tubules in rats that died 4 days after acute exposure. Although we, too, found injury 4 days after exposure, injury tended to be more severe in animals killed on day 1 after exposure (results from L.D.F.) or consistently showed regeneration (R.L.K.). It appears that repair had decreased evidence of renal injury by day 4.

The differences between our findings for the kidney and those obtained by Morio *et al.* may have resulted from subtle differences in experimental design. First, as noted earlier, the olefin test concentration profile differed for their study and ours. Second, we sustained the partial pressure of oxygen in the exposure chamber, whereas Morio *et al.* did not replenish the oxygen as

it was consumed. Perhaps the higher partial pressure in our study enhanced the potential for injury. Consistent with this possibility, L.D.F. found rare (<0.1% of the kidney) renal injury in 9 of 30 control rats killed 1 day after exposure to 3 h of oxygen, but in only 1 of 30 rats killed 4 days after exposure, a finding consistent with repair of injury. Also consistent with repair, L.D.F. found that the fraction (%) of renal cells injured 4 days after exposure to the olefin tended to be less than the percentage after 1 day, and R.L.K. found regeneration in rats killed on day 4 but not in rats killed on day 1. Third, in their acute studies, Morio *et al.* examined groups of 6 rats, whereas we examined groups of 10 and increased that to 30 control rats for the 1- and the 4-day survival studies. In addition, Morio *et al.* examined tissues from day 14 in rats surviving the acute exposure. Our greater numbers may have permitted statistical differences to become apparent. However, this difference would apply with less force to the chronic studies by Morio *et al.*, in which groups of ten were studied and rats were killed on day 1. Fourth, by blinding L.D.F. and R.L.K. to the treatments accorded each rat, we attempted to remove bias from our histologic examinations. Morio *et al.* conducted unblinded examinations.

Our system for removing carbon dioxide was modeled after that described by Morio *et al.* Our system differed in that we circulated the gases in the exposure chamber, separated our rats from each other, cooled the absorbent (a solution of barium hydroxide), and measured the concentrations of carbon dioxide that developed. The inspired concentrations of carbon dioxide that we found would not be expected to increase arterial concentrations more than a few millimeters of mercury because of the ventilatory response to carbon dioxide. If the concentrations of inspired carbon dioxide were higher and/or if metabolism were higher in the study by Morio *et al.*, the greater associated ventilation would have produced higher alveolar concentrations of the olefin. Higher levels of metabolism (and hence carbon dioxide) might have occurred because Morio *et al.* did not cool their absorbent. This might explain the slightly higher LC₅₀ that they found. However, it would not explain the absence of renal injury in their rats.

Our findings are consistent with those of other studies that relate the threshold for toxicity to the LC₅₀ value for olefins halogenated solely with fluorine.^{10–12} For such studies, the ratio of concentrations or doses that are lethal to the threshold concentrations or doses are approximately a factor of 10, a value similar to our ratio of 331/50.

INJURY FROM A SEVOFLURANE BREAKDOWN PRODUCT

Studies conducted preliminary to beginning these investigations suggested certain challenges that must be addressed in future studies. First, in the absence of a continuing delivery of the olefin to the test system, the concentration of the olefin decreases rapidly and dramatically from the concentration early in exposure. We found that the olefin concentration could decrease more than threefold in 0.5 h. Second, in the absence of active cooling of the barium hydroxide, temperature in the parent chamber could increase to greater than 30°C. Hyperthermia may have caused the injury seen in one of the early studies of the toxicity of sevoflurane.¹³ Third, we found that oxygen consumption in control rats exceeded $30 \text{ ml} \times \text{kg}^{-1} \times \text{min}^{-1}$, indicating that without replacement of the oxygen consumed, the partial pressure of oxygen must decrease during testing.

Our results indicate that the concentrations of the olefin produced in clinical practice (up to 61 ppm⁵) can reach the threshold for renal injury (50 ppm in our study) in young rats. Such injury may not be readily apparent, because it would not likely produce any functional changes. Considerably higher concentrations would be required to produce hepatic and cerebral injury or death. Whether these results in young rats can be applied to humans remains to be determined.

The authors thank Winifred von Ehrenburg and Drs. Dennis M. Fisher and Richard B. Weiskopf for their critiques of the manuscript. In addition, they thank Dr. Fisher for his assistance in some of the analyses.

References

1. Eger EI II: Stability of 1-653 in soda lime. *Anesth Analg* 66: 983-985, 1987
2. Strum D, Johnson B, Eger EI II: Stability of sevoflurane in soda lime. *ANESTHESIOLOGY* 67:779-781, 1987
3. Wallin R, Regan B, Napoli M, Stern I: Sevoflurane: A new inhalational anesthetic agent. *Anesth Analg* 54:758-765, 1975
4. Morio M, Fujii K, Satoh N, Imai M, Kawakami U, Mizuno T, Kawai Y, Ogasawara Y, Tamura T, Negishi A, Kumagai Y, Kawai T: Reaction of sevoflurane and its degradation products with soda lime: Toxicity of the byproducts. *ANESTHESIOLOGY* 77:1155-1164, 1992
5. Frink EJ Jr, Malan T, Morgan S, Brown E, Malcomson M, Brown BR Jr: Quantification of the degradation products of sevoflurane in two carbon dioxide absorbents during low-flow anesthesia in surgical patients. *ANESTHESIOLOGY* 77:1064-1069, 1992
6. Mazze R: The safety of sevoflurane in humans (editorial). *ANESTHESIOLOGY* 77:1062-1063, 1992
7. Huang C, Venturella V, Cholli A, Venutolo F, Silbermann A, Vernice G: Detailed investigation of fluoromethyl 1,1,1,3,3,3-hexafluoro-2-propyl ether (sevoflurane) and its degradation products: I. Synthesis of fluorinated, soda lime induced degradation products. *J Fluorine Chem* 45:239-253, 1988
8. Cholli A, Huang C, Venturella V, Pennino D, Vernice G: Detailed investigation of fluoromethyl 1,1,1,3,3,3-hexafluoro-2-propyl ether (sevoflurane) and its degradation products: II. Two-dimensional fluorine-19 NMR characterization of fluoromethyl 1,1,3,3,3-pentafluoro-2-propenyl ether. *Appl Spectroscopy* 43:24-27, 1989
9. Montgomery CA Jr, Seely J: *Kidney, Pathology of the Fischer Rat*. Edited by Boorman GA, Eustis SL, Elwell MR, Montgomery CA Jr, MacKenzie WF. New York, Academic, 1990, p 129
10. Potter C, Gandolfi A, Nagle R, Clayton J: Effects of inhaled chlorotrifluoroethylene and hexafluoropropene on the rat kidney. *Toxicol Appl Pharmacol* 59:431-440, 1981
11. Gad S, Rusch G, Darr R, Cramp A, Hoffman G, Peckham J: Inhalation toxicity of hexafluoroisobutylene. *Toxicol Appl Pharmacol* 86:327-340, 1986
12. Kennedy G: Toxicology of fluorine-containing monomers. *Crit Rev Toxicol* 21:149-170, 1990
13. Hitt B, Mazze R, Cook T, Beppu W, Kosek J: Thermoregulatory defect in rats during anesthesia. *Anesth Analg* 56:9-14, 1977