Halothane Impurities and the Copper Kettle

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Halothane samples taken from freshly opened bottles and after long term clinical use in a Copper Kettle vaporizer were subjected to gas chromatography and mass spectrometry analysis. Of the three halogenated impurities found, two were identified as cis or trans 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene and 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. While the third impurity could not be positively identified because of its very low concentration, mass spectrometer peaks corresponding to CF_3, C_3, F_3, and Cl were obtained. 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene has been reported having toxic properties. No information is available concerning the toxicity of 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. It was found that there was no significant enrichment of either butene in the halothane samples taken from the Copper Kettle vaporizers after long-term use.

CURRENT controversy concerning the significance of the impurities found in halothane was initiated by the report of Cohen and his co-workers.[1] They reported the presence of a compound identified as *cis* or *trans* 1,1,1,-4,4,4-hexafluoro-2,3-dichloro-2-butene in a concentration of about 0.01 per cent in the freshly opened bottle of halothane. The concentration of the butene was found to have increased to 0.1 per cent during the five days use when halothane was kept continuously in a Copper Kettle vaporizer. This compound was found to be extremely toxic in rats causing death within 18 hours after exposure to 2.0 ml. of the vapor.[2] Sexton and Henderson of Imperial Chemical Industries and Ayerst Laboratories offered in rebuttal their findings that of 100 specimens of halothane drawn from all types of vaporizers only one specimen was found to have the butene derivative in a concentration as high as 0.058 per cent while the mean concentration of all specimens was 0.028 per cent, well below the quality control specification of 0.05 per cent.[3]

The purpose of this report is to further analyze the impurity levels in halothane before and after exposure in the Copper Kettle vaporizer.

Method

Halothane Samples. The residuals of two Copper Kettle vaporizers were drained, stored in amber colored bottles for analysis and labeled I^c and II^c. It had been previously determined that no agent other than halothane had ever been used in these Kettles and that the Kettles had not been completely drained for at least four months. By charting consumption, it was estimated that the residual represented at least 1.5 liters of halothane per Kettle. A sealed bottle of halothane was opened, an aliquot taken for analysis, labeled III^f, and the remainder poured into a Copper Kettle vaporizer that had been previously emptied and cleaned. The Kettle was then sealed and after a week's clinical use was drained, the residual halothane labeled III^{fc}. Another sealed bottle was opened and the sample taken for analysis, labeled IV^f. Sample V^f was taken from a freshly opened bottle of halothane that was stored in a stoppered glass test tube and kept in the dark for one week before analysis.

Analytical Technique. Halothane samples were analyzed for trace impurities using an Aerograph Hy-Fi Model 600 gas chromatograph* equipped with a flame ionization detector. A stainless steel column (5 feet by 1/8 inch) packed with 5 per cent SE-30 silicone on Chromosorb W was used at a temper-

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* Wilkins Instrument & Research Inc., Walnut Creek, California.

ature of 38° C. The carrier gas was nitrogen at 8 p.s.i. For estimation of impurity concentrations, it was assumed that the mass response of each component was the same. The sensitivity was reduced 100 times for evaluation of the mass response of halothane. Chromatograms were also obtained using a Barber-Colman Model 10 gas chromatograph† equipped with a flame ionization detector. Glass columns, 6 feet by 3 mm., packed with 3 per cent SE-30 silicone on 120/140 mesh Gas Chrom P or packed with 5 per cent Apiezon L on 120/130 mesh Anakrom ABS were used at 68° C. The results were similar to those obtained with the Aerograph Hy-Fi Model 600.

Selected samples of halothane were chromatographed on a Burrell Kromotog‡ equipped with thermal conductivity detector and a Bendix mass spectrometer. A 2.5 mm. by 6 mm. glass column packed with 6 per cent polyethylene glycol 300 on 30/60 mesh Celite was programmed from 45° to 140° C. A similar glass column packed with 6 per cent Apiezon L on 30/60 mesh firebrick was programmed from 45° to 290° C.

† Barber-Colman Co., Rockford, Illinois.
‡ Burrell Corp., Pittsburgh.

Results

Chromatography and mass spectrometry of samples I^c and III^f showed the presence of three minor impurities equally concentrated in both samples (fig. 1). A notched peak (A) immediately following air was identified by mass spectrometry as 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene. The presence of both *cis* and *trans* forms is indicated by the notching. The second impurity (fig. 1B) was identified as 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. Because of the very small concentration, the third impurity (C) could not be positively identified. However, mass spectrometer peaks corresponding to CF_3, C_3, F_3, and Cl were obtained. The total impurities were estimated at 0.03 and 0.02 per cent, respectively (table 1) in these two samples indicating that litttle or no enrichment of butene impurities had occurred in the vaporizer.

Chromatograms obtained with the Barber-Colman and Aerograph Hy-Fi apparatus also had the same 3 impurity peaks (fig. 2). Peak C was low or absent in all samples except II^c in which it was the major component of the impurities. It was slightly higher in sample III^{fc} after one week in the Copper

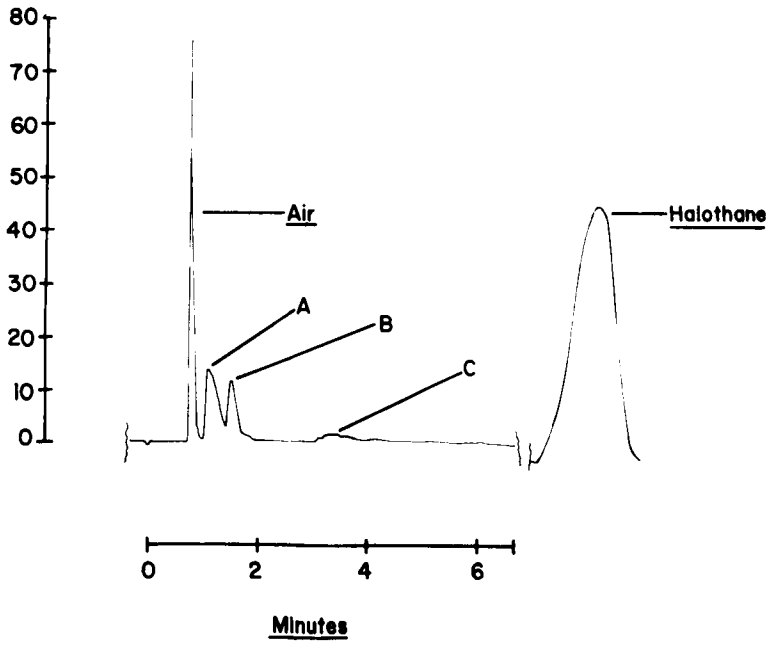

FIG. 1. Chromatogram of halothane sample from Copper Kettle vaporizer (I^c). (A) 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene. (B) 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. (C) unidentified halogenated compound. Thermal conductivity detector. Identification of peaks by Bendix mass spectrometer at 70 electron volts.

TABLE 1. Total Impurity Levels in Halothane

Samples	Total Impurities, Percentage
Residue I[c]	0.03
Residue II[c]	0.17
Fresh III[f]	0.02
Residue III[fc]	0.03
Fresh IV[f]	0.05
Fresh V[f]	0.06

Kettle. It was highest in sample V[f] of the fresh samples. Peak B was low in all samples. Peak A was the major impurity component in all samples except II[c]. No evidence was obtained for a significant increase in the level of peak A during normal operating conditions which included refilling of the Copper Kettle vaporizer without drainage.

Discussion

While we have been able to positively identify two halogenated impurities and point to the existence of a third in halothane, the clinical toxicological significance of 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene has not been clearly delineated. We have also not been able to find any information relating to the toxicity of the second impurity 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. We are presently trying to identify the third impurity and also investigating the analysis of these impurities in blood and tissue using the electron capture detector.[4,5]

The gas chromatography conditions reported by Cohen and his associates[1] are similar to those illustrated in figure 2. It is apparent that the impurity peaks are extremely close and B and C are not well resolved. Under these conditions, it would be difficult to exclude the presence of contaminants if an increased concentration of impurities were found. The possibility of another contaminant was revealed in analyzing Sample II[c], the long standing residue from a Copper Kettle vaporizer. Chromatography showed a large peak corresponding to the position of impurity C,

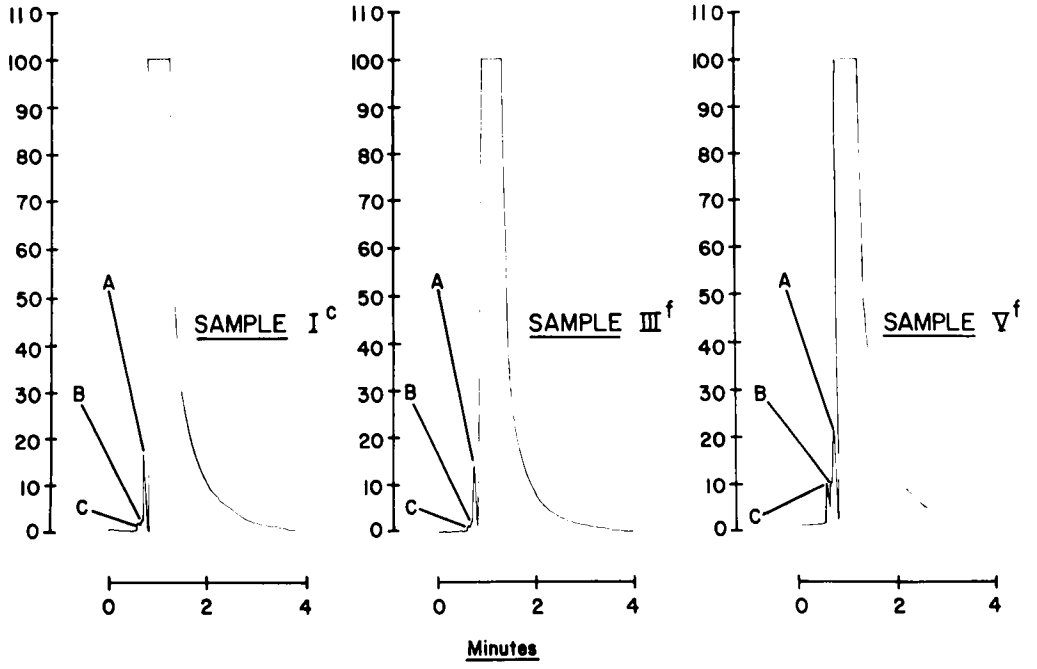

FIG. 2. Records obtained by gas chromatographic analysis of halothane. Sample I[c]—Residue from Copper Kettle vaporizer. Sample III[f]—Freshly opened bottle of halothane. Sample V[f]—From freshly opened bottle of halothane stored in a glass for one week before analysis. (A) 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene. (B) 1,1,1,4,4,4-hexafluoro-2-bromo-2-butene. (C) Unidentified halogenated compound.

the unidentified halogenated compound, the total impurities measuring 0.17 per cent. We were impressed by this finding, thinking that we had found an enrichment of this compound. Another aliquot of Sample II[c] was then subjected to gas chromatograph mass spectrometer analysis and the peak was positively identified as cyclopropane. Evidently, cyclopropane had diffused back into the Copper Kettle vaporizer. This emphasizes the necessity of identification of impurity peaks.

The quality control specifications for halothane[3] require it to be 99.9 per cent pure, and no other impurity may be present in amounts greater than 0.05 per cent. It would be desirable for the manufacturers to make their total impurity analysis available so that further toxicological studies can be made.

Summary

Gas chromatography and mass spectral analysis of halothane from both freshly opened bottles and samples taken from a Copper Kettle vaporizer after long term clinical use revealed the presence of three halogenated impurities: 1,1,1,4,4,4-hexafluoro-2,3-dichloro-2-butene; 1,1,1,4,4,4,-hexofluoro-2-bromo-2-butene; and an unidentified third impurity. There was no significant enrichment of either butene in halothane samples taken from the Copper Kettle vaporizer after long-term use.

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VISUALLY EVOKED RESPONSE Effects of premedication, methoxyflurane, halothane or cyclopropane anesthesia and succinylcholine on visually evoked responses (VER) were recorded from various scalp electrodes including the central, parietal and occipital areas in 12 surgical patients. Preanesthetic medication with scopolamine tended to reduce wave three of Ciganek and promote adaptation or "habituation," though l-hyoscyamine or atropine had no effect. Light surgical anesthesia (Stage III, upper plane 1) prolonged, enhanced and sometimes merged waves one, two, and three. Slightly deeper anesthesia reduced these responses and enhanced wave four. Succinylcholine, injected intravenously, and controlled ventilation did not markedly alter the VER. (*Domino, E. F., Induced Muscle Paralysis, Ann. N. Y. Acad Sci. 112: 226 (May) 1964.*)