J. Pharm. Pharmac., 1967, **19**, 231–239 Received December 5, 1966

Impurities in halothane*: their identities, concentrations and determination

J. CHAPMAN, R. HILL, J. MUIR, C. W. SUCKLING AND D. J. VINEY

The following impurities arising during the manufacture of a proprietary halothane have been identified by gas chromatographic, mass spectrometric, nmr and infrared techniques: 2-chloro-, 2,2-dichloro-, 2-bromo-, 2,2-dibromo-, 2,2-dibromo-2-chloro- and 2-bromo-2,2-dichloro-1,1,1-trifluoroethanes, 1,1,2-trichloro-1,2,2-trifluoroethane, 1,2-dichloro-1,1-difluoroethane, 2-bromo-2-chloro-1,1-difluoroethylene, bromodichlorofluoromethane, chloroform, *trans*-2-bromo-1,1,1,4,4,4-hexafluorobut-2-ene and the *trans* and *cis* isomers of 2-chloro- and 2,3-dichloro-1,1,1,4,4,4-hexafluorobut-2-enes. Gas chromatographic methods using chlorinated biphenyl, dinonyl phthalate and polyethylene glycol as liquid phases have been developed for their determination at the ppm level.

THE inhalant anaesthetic halothane B.P. is stabilized 2-bromo-2-chloro-1,1,1-trifluoroethane containing very small amounts of impurities, the nature and biological properties of which have aroused some interest during the past year or so (Cohen, Bellville, Budzikiewicz & Williams, 1963; Corrigan, McHattie & Raventós, 1963; Linde & Butler, 1963; Sexton & Hendrickson, 1963; Albin, Horrocks & Kretchmer, 1964; Butler & Linde, 1964; Cohen & Brewer, 1964; Scherer & Weigand, 1964; Gjaldbaek & Worm, 1965).

This paper deals specifically with the identification and determination of the trace impurities present in Fluothane. It does not purport to cover halothane from other sources that may be manufactured by different routes. The biological properties of the impurities are discussed elsewhere by Raventós & Lemon (1965).

The impurities found to date, many only rarely and in minute amounts, are listed in Table 1 with typical concentrations found by gas-liquid chromatography in the currently manufactured material. Also included in Table 1 are their retention times relative to 1,1,2-trichloro-1,2,2-trifluoroethane, on two stationary phases, namely chlorinated biphenyl and a mixture of dinonyl phthalate and polyethylene glycol.

The presence of most of the impurities listed in Table 1 was not unexpected in the light of the method of manufacture which involves the high-temperature bromination of 2-chloro-1,1,1-trifluoroethane (impurity No. 2). Over-bromination produces a small amount of 2,2-dibromo-2-chloro-1,1,1-trifluoroethane (impurity No. 13). The bromination probably proceeds through a free-radical mechanism, which would also be involved in the formation of the substituted butenes (impurities Nos 1, 3, 4, 7 and 8). These might be formed through a substituted butane as intermediate which, by loss of hydrogen halide, would give the butene,

e.g. $2CF_3{\cdot}CHCl{\cdot} \rightarrow CF_3{\cdot}CHCl{\cdot}CHCl{\cdot}CF_3 \rightarrow CF_3{\cdot}CH : CCl{\cdot}CF_3$.

Alternatively, substituted carbenes might be involved.

Of the other impurities, Nos 5, 9 and 16 occur as trace impurities in the

From Imperial Chemical Industries Limited, Mond Division, Research Department, The Heath, Runcorn.

* The material used in this investigation was Fluothane (I.C.I. Ltd).

TABLE 1. CONCENTRATIONS AND GAS CHROMATOGRAPHIC BEHAVIOUR OF IMPURITIES IN HALOTHANE*

Impurity	Typical concentration (ppm by weight) in the anaesthetic	Retention time relative to $CF_3Cl{\cdot}CFCl_2$ on column of	
		Chlorinated biphenyl at 60°	Polyethylene glycol/ dinonyl phthalate at 60°
1	Not detected (<1)	0·12	0·38
2	1	0·22	1·00
3	Not detected (<1)	0·30	0·73
4	Not detected (<1)	0·40	1·00
5	8	0·62	2·35
6	Not detected (<1)	0·62	2·35
7	Not detected (<1)	0·62	0·73
8	Not detected (<1)	0·78	0·85
9	12	1·00	1·00
10	Not detected (<5)	3·48	Not determined
11	Not detected (<5)	4·85	Not determined
12	Not detected (<5)	6·43	10·9
13	Not detected (<5)	8·73	Not determined
14	1	Masked by halothane	1·50
15	Not detected (<5)	Masked by halothane	3·28
16	Not detected (<5)	Masked by halothane	4·22

* Fluothane (I.C.I.)

1. *trans*-2-Chloro-1,1,1,4,4,4,-hexafluorobut-2-ene.
2. 2-Chloro-1,1,1-trifluoroethane.
3. *cis*-2-Chloro-1,1,1,4,4,4-hexafluorobut-2-ene.
4. *trans*-2-Bromo-1,1,1,4,4,4-hexafluorobut-2-ene.
5. 2,2-Dichloro-1,1,1-trifluoroethane.
6. 2-Bromo-1,1,1-trifluoroethane.
7. *trans*-2,3-Dichloro-1,1,1,4,4,4-trifluorobut-2-ene.
8. *cis*-2,3-Dichloro-1,1,1,4,4,4-trifluorobut-2-ene.
9. 1,1,2-Trichloro-1,2,2-trifluoroethane.
10. Bromodichlorofluoromethane.
11. 2,2-Dibromo-1,1,1-trifluoroethane.
12. Chloroform.
13. 2,2-Dibromo-2-chloro-1,1,1-trifluoroethane.
14. 2-Bromo-2-chloro-1,1-difluoroethylene.
15. 2-Bromo-2,2-dichloro-1,1,1-trifluoroethane.
16. 1,2-Dichloro-1,1-difluoroethane.

2-chloro-1,1,1-trifluoroethane and No. 15 could be formed by bromination of No. 5. Impurities Nos 6, 11 and 15 could all arise in the course of the bromination reaction and No. 14 could be formed by elimination of hydrogen fluoride from halothane. Traces of dichloro- and trichlorofluoromethane have occurred occasionally in 2-chloro-1,1,1-trifluoroethane and one of these could be the precursor of No. 10. The occasional presence of traces of chloroform is more difficult to explain, although it is conceivable that the dichlorofluoromethane already mentioned could disproportionate to form chloroform.

It seemed possible that other compounds might be present which could be overlooked in the chromatographing because of their being masked by the peaks of known impurities or by the peak due to the anaesthetic itself. Seven such possible impurities have been prepared and their gas-chromatographic behaviour determined; this is summarized in Table 2. The columns used are capable of separating all of these compounds from known impurities and from halothane, but none of them has been detected (limit of detection 5 ppm or less) in the anaesthetic. Details of all the compounds in this paper have been published with the exception

TABLE 2. HALOGENATED ETHYLENES AND BUTENES NOT PRESENT IN HALOTHANE*

Compound	Limit of detection (ppm by weight)	Retention time relative to $CF_2Cl{\cdot}CFCl_2$ on column of: Chlorinated biphenyl at 60°	Polyethylene glycol/ dinonyl phthalate at 60°
1	2	0·50	0·57
2	5	masked by halothane	1·68
3	2	0·12	0·80
4	5	1·73	2·13
5	5	2·18	2·52
6	5	5·70	6·66
7	5	6·33	8·30

* Fluothane (I.C.I.)

1. 2,2-Dichloro-1,1-difluoroethylene.
2. 2,2-Dibromo-1,1-difluoroethylene.
3. *trans*-1,1,1,4,4,4-Hexafluorobut-2-ene.
4. *trans*-2-Bromo-3-chloro-1,1,1,4,4,4-hexafluorobut-2-ene.
5. *cis*-2-Bromo-3-chloro-1,1,1,4,4,4-hexafluorobut-2-ene.
6. *trans*-2,3-Dibromo-1,1,1,4,4,4-hexafluorobut-2-ene.
7. *cis*-2,3-Dibromo-1,1,1,4,4,4-hexafluorobut-2-ene.

of 2-bromo-3-chloro-1,1,1,4,4,4-hexafluorobut-2-ene. This was prepared by bromination of 2-chloro-1,1,1,4,4,4-hexafluorobut-2-ene in ultraviolet light giving 2,3-dibromo-2-chloro-1,1,1,4,4,4-hexafluorobutane (identified by mass spectrometry) which was dehydrobrominated with potassium hydroxide to the bromochlorohexafluorobutene. Fractional distillation of the crude product gave a mixture of the *cis*- and *trans*- isomers (bp 86°/760 mm), which were then separated by preparative gas-liquid chromatography. Mass-spectrometric analysis of the isomers established their empirical formulae as C_4BrClF_6. Nuclear magnetic resonance investigation showed the coupling constants to be J_{FF} *cis* = 12·8 c/sec and J_{FF} *trans* = 1·6 c/sec, in agreement with theoretically predicted values.

IDENTIFICATION OF IMPURITIES

Mass spectrometry and gas chromatography were the main techniques used in the identification of impurities but microchemical analysis, nuclear magnetic resonance and infrared spectrometry were also used. Gas-liquid chromatographic identifications were made by correlating the retention times with those of authentic specimens of compounds expected to be present either from mass-spectrometric evidence or from the nature of the manufacturing process. Usually these identifications involved the use of liquid phases of widely differing polarity, e.g. chlorinated biphenyl and dinonyl phthalate, polyethylene glycol or a mixture of these two liquids (see Figs 1, 2, 3 and 4).

In most instances a fraction rich in a limited number of impurities, obtained either by laboratory distillation of the crude product or from a suitable point in the process stream, was used as the starting material for identification work.

Impurities of shorter retention time than halothane on a chlorinated biphenyl column. (*a*) 2-Chloro- and 2,2-dichloro-1,1,1-trifluoroethane and 1,1,2-trichloro-1,2,2-trifluoroethane. These compounds (impurities

Nos 2, 5 and 9, Table 1) were concentrated in a low-boiling fraction obtained during distillation of the anaesthetic. They were readily identified, without further separation, from their mass spectra and from their gas-liquid chromatographic behaviour on chlorinated biphenyl and dinonyl phthalate columns (Figs 1 and 2).

(*b*) *trans*- and *cis*-2,3-Dichloro-1,1,1,4,4,4-hexafluorobut-2-ene. These impurities (Nos 7 and 8, Table 1) have higher boiling points than halothane. They were concentrated by fractional distillation before isolation in millilitre quantities by preparative gas-liquid chromatography.

Fractionation was effected on a column, 6 ft long and 5/8 inch internal diameter, packed with 60–72 mesh Celite (7 parts by weight) impregnated with dinonyl phthalate (3 parts by weight). The column was run at 65° with nitrogen as carrier gas at a flow rate of 4 litre/hr. Eluted components, detected by a katharometer, were collected in traps cooled in liquid air. Sample loads of 0·5 to 1 ml were injected into a zone maintained at about 120°, at the column inlet.

Several fractionation runs were made until about 1 ml of each of the impurities had been collected. Both components gave microchemical analyses (C, 20·4; Cl, 36·0; F, 46·5%) and mass spectra consistent with isomers of dichlorohexafluorobutene. Subsequent examination by infrared spectrometry showed that they were identical with the *cis* and *trans* isomers of 2,3-dichloro-1,1,1,4,4,4-hexafluorobut-2-ene previously synthesized and characterized by Dickinson, Hill & Murray (1958).

(*c*) *trans*-2-Chloro-1,1,1,4,4,4-hexafluorobut-2-ene. This impurity (No. 1, Table 1 and present in the first peak of Fig. 1) was isolated by preparative gas-liquid chromatography, under the conditions given above, from a low-boiling distillation fraction from the anaesthetic. Elementary analyses (C, 24·0; H, 0·6; Cl, 18·0; F, 57·1%) of the isolated specimen showed that it had the empirical formula C_4HClF_6. Mass spectrometric and nuclear magnetic resonance spectrometric examinations of the compound established its structure as the required *trans*-isomer.

(*d*) *cis*-2-Chloro-1,1,1,4,4,4-hexafluorobut-2-ene. This impurity (No. 3, Table 1) is included in the third peak of Fig. 1 and is also included in the *trans*-dichlorohexafluorobutene peak obtained on a polyethylene glycol or polyethylene glycol/dinonyl phthalate column (see Figs 3 and 4). This impurity was first observed in the chromatogram, from a polyethylene glycol column, of a fraction free from the *trans*-dichlorohexafluorobutene. It was shown by mass spectrometry to have the structure $CF_3{\cdot}CH : CCl{\cdot}CF_3$, and as it appears as a separate peak from the compound already identified as *trans*-2-chloro-1,1,1,4,4,4-hexafluorobut-2-ene it follows that it must be the *cis*-isomer.

(*e*) *trans*-2-Bromo-1,1,1,4,4,4-hexafluorobut-2-ene. Although this compound (impurity No. 4, Table 1) has a slightly higher boiling point than halothane, it is concentrated in the lower-boiling fractions from the latter. It was isolated from such a fraction, in which it was the only detectable impurity, by preparative gas chromatography as described above. Mass spectrometry and microchemical analysis (C, 19·9; H, 0·4; Br, 34·2; F, 45·9%) of the isolated impurity established its molecular

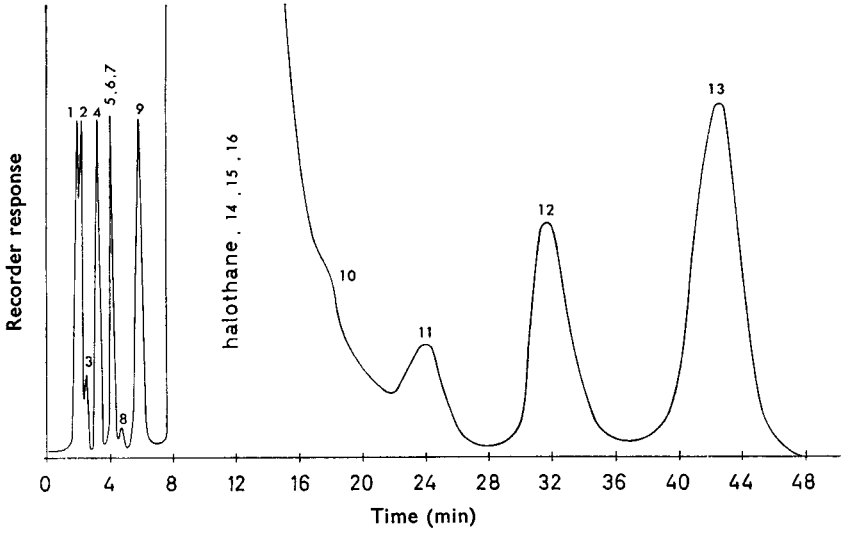

FIG. 1. Halothane impurities: chromatogram from a chlorinated biphenyl column at 60°. Impurities are numbered as in Table 1.

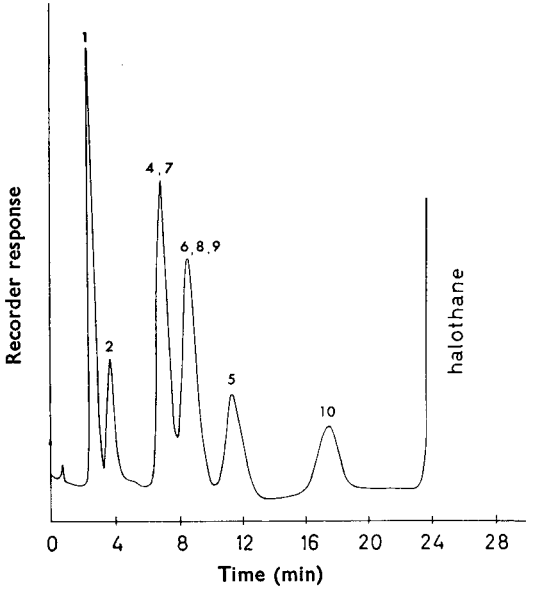

FIG. 2. Halothane impurities: chromatogram from a dinonyl phthalate column at 55°. Impurities are numbered as in Table 1.

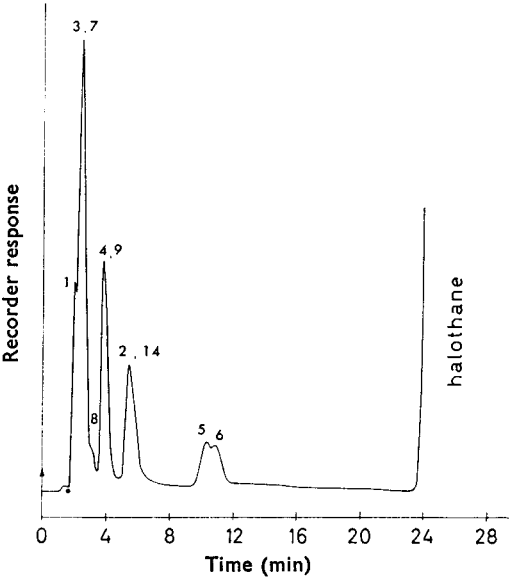

FIG. 3. Halothane impurities: chromatogram from a polyethylene glycol 400 column at 50°. Impurities are numbered as in Table 1.

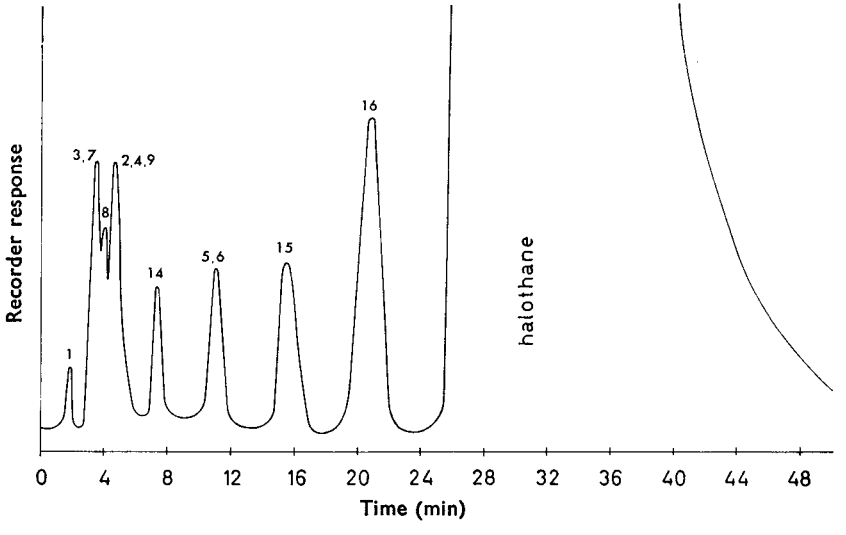

FIG. 4. Halothane impurities: chromatogram from a polyethylene glycol/dinonyl phthalate column at 60°. Impurities are numbered as in Table 1.

formula as C_4HBrF_6. It was shown to be the required *trans*- isomer by nmr spectrometry.

(*f*) 2-Bromo-1,1,1,-trifluoroethane. This impurity (No. 6, Table 1) is eluted together with *trans*-dichlorohexafluorobutene and 2,2-dichloro-1,1,1-trifluoroethane from a chlorinated biphenyl column (Fig. 1). Its presence was first noticed when a sample of the anaesthetic was chromatographed on a polyethylene glycol column which separated it completely from the former, and partially from the latter (see Fig. 3). It was tentatively identified by mass-spectrometric examination of a small fraction obtained from an analytical-scale polyethylene glycol column. Confirmation of its identity was achieved by comparing its mass spectrum and chromatographic behaviour with that of an authentic specimen of 2-bromo-1,1,1-trifluoroethane.

Impurities of longer retention time than halothane on a chlorinated biphenyl column. (a) Bromodichlorofluoromethane. A specimen of this compound (impurity No. 10, Table 1) was isolated by preparative gas-liquid chromatography from an enriched distillation fraction. The fractionation conditions were those described on p. 234, the impurity being eluted before the main component (see Fig. 2). The mass spectrum of the isolated material was consistent with the formula $CBrCl_2F$. Subsequent examination, by mass-spectrometric and gas-liquid chromatographic techniques, of an authentic specimen of bromodichlorofluoromethane confirmed this.

(b) 2,2-Dibromo-1,1,1-trifluoroethane and chloroform. The identities of these impurities (Nos 11 and 12, Table 1) were indicated by mass-spectrometric examination of the appropriate fractions obtained from an analytical-scale chlorinated biphenyl column and confirmed by correspondence of their retention times with authentic specimens.

(c) 2,2-Dibromo-2-chloro-1,1,1-trifluoroethane. This impurity (No. 13, Table 1) was isolated from crude halothane by fractional distillation and identified from its mass spectrum and elementary analysis.

Impurities masked by halothane on a chlorinated biphenyl column. There are three impurities in this category, namely 2-bromo-2,2-dichloro-1,1,1-trifluoroethane, 2-bromo-2-chloro-1,1-difluoroethylene and 1,2-dichloro-1,1-difluoroethane. All three were separated from each other and from other components on a column containing a mixture of polyethylene glycol 400 and dinonyl phthalate as the liquid phase (see Fig. 4). It was from such a column that small fractions were isolated for mass-spectrometric examination. Tentative identifications by this technique were confirmed when authentic specimens of the materials were examined by gas chromatography.

GAS-LIQUID CHROMATOGRAPHIC DETERMINATION OF IMPURITIES

By far the greatest problem in the determination of impurities in the anaesthetic was the selection of liquid phases that would not only separate them from the main component but would also give sufficient resolution of the impurities to permit quantitative estimation of each one. Of the

many possible liquid phases examined, no single one separated all the known impurities.

Before the discovery of the presence of 2-bromo-1,1,1-trifluoroethane, two columns were developed, one containing chlorinated biphenyl and the other a mixture of polyethylene glycol and dinonyl phthalate, which between them provided adequate information for the determination of the fifteen impurities then known to be present (see Figs 1 and 4). Because the bromotrifluoroethane behaves in the same way as 2,2-dichloro-1,1,1-trifluoroethane on both columns, the combined concentrations of these two impurities are obtained. This information is usually sufficient, but when individual concentrations are required an additional chromatogram must be run using polyethylene glycol as the liquid phase (see Fig. 3).

METHOD

Apparatus. Gas chromatographs fitted with flame ionization detectors, e.g. Pye Series 104, Model 4.

Reagents. 2-Bromo-2-chloro-1,1,1-trifluoroethane free from detectable impurities. Specimens of the compounds listed in Table 1; the purity of these materials need not be higher than 95%. Chromosorb P, 60–80 mesh and Celite, 72–85 mesh (Messrs. "JJ's" of King's Lynn, Norfolk). Aroclor 1254 (chlorinated biphenyl) (Monsanto). Dinonyl phthalate and polyethylene glycol 400 (May & Baker).

Gas chromatography columns and conditions. Stationary phase mixtures of Aroclor 1254 and Chromosorb P, dinonyl phthalate and Celite, and polyethylene glycol and Celite were prepared, each mixture containing 30% by weight of liquid phase. The stationary phases were then packed in copper or stainless steel tubes, 3/16 inch internal diameter to give columns of: A, 6 ft Aroclor/Chromosorb; B, 6 ft polyethylene glycol/Celite; C, 6 ft polyethylene glycol/Celite joined to 3 ft dinonyl phthalate/Celite.

The Aroclor column was operated at 60° with a carrier gas (nitrogen) flow rate of 30 ml/min; the polyethylene glycol column at 50° with a gas flow rate of 50 ml/min and the mixed polyethylene glycol/dinonyl phthalate columns at 60° with a gas flow rate of 40 ml/min, all being measured at atmospheric pressure.

All columns were conditioned at the operating temperature for 16 hr or until a stable recorder base line was obtained.

Calibration of columns. Using pure halothane as the main component, artificial mixtures of the following types were prepared: type 1 containing impurities Nos 1, 2, 4, 7–13 of Table 1, type 2 containing impurities Nos 5, 7, 14, 15 and 16 and type 3 containing impurities Nos 5 and 6. Two or three mixtures of each type were made up so as to cover the impurity concentration ranges encountered in practice (0–25 ppm).

Mixtures of types 1 and 2 were chromatographed on the Aroclor column, and those of types 2 and 3 on both the polyethylene glycol/dinonyl phthalate and polyethylene glycol columns. Five μl aliquots were used in each instance. The amplifier attenuation was set at $\times 20$ and the output signal was fed to a 1 mV potentiometric recorder. Calibration graphs

were constructed by plotting component peak heights against appropriate concentrations.

Sample analysis. Samples (5 μl) were chromatographed on each of the three columns. The concentrations of impurities Nos 1, 2, 3, 4, 8–13 of Table 1 were obtained directly from appropriate peak height measurements on the Aroclor chromatogram. (Impurity No. 3, i.e., *cis*-2-chloro-1,1,1,4,4,4-hexafluorobut-2-ene was measured as impurity No. 4 as no standard was available.) The combined concentrations of impurities Nos 5–7 were also measured. Similarly, from the polyethylene glycol-dinonyl phthalate chromatogram, the concentrations of impurities Nos 14–16, together with the combined concentration of Nos 5 and 6 measured as the former, were obtained. Individual concentrations of impurities Nos 5 and 6 were obtained from the polyethylene glycol chromatogram.

The height of the peak containing impurity No. 7 in the Aroclor chromatogram was corrected for the presence of impurities Nos 5 and 6 (measured as impurity No. 5), and, from the corrected value, the concentration of impurity No. 7 was obtained.

Discussion

In view of the exhaustive search for impurities that has been made in this proprietary brand of halothane, it seems unlikely that there is present at concentrations above about 10 ppm, any impurity other than those reported; at this level and below they would not be expected to have any significance in anaesthetic practice (see Raventós & Lemon, 1965).

Gas-liquid chromatographic methods for the determination of all the known impurities are tedious and when frequent analyses are required, as in process control, a large amount of apparatus is fully occupied. It seems probable that the use of coated capillary columns in place of packed columns would reduce the time required for an analysis and might also lead to a reduction in the number of columns required because of the higher resolving power of coated capillaries.

Acknowledgements. The authors wish to acknowledge the invaluable help of their colleagues, Drs. D. T. Cropp, J. C. Goodchild, J. I. Hollies and D. G. Stevenson and Messrs. R. L. McGinty, B. Postlethwaite and C. Powney, either in the synthesis or in the measurement of physical properties of the compounds studied.

References

Albin, M. S., Horrocks, L. A. & Kretchmer, H. E. (1964). *Anesthesiology*, **25**, 672–5.
Butler, R. A. & Linde, H. W. (1964). *Ibid.*, **25**, 397–8.
Cohen, E. N., Bellville, J. W., Budzikiewicz, H. & Williams, D. H. (1963), *Science, N.Y.*, **141**, 899.
Cohen, E. N. & Brewer, H. W. (1964). *J. Gas Chromat.*, **2**, 261–262.
Corrigan, D. S., McHattie, G. V. & Raventós, J. (1963). *Br. J. Anaesth.*, **35**, 824–825.
Dickinson, F., Hill, R. & Muray, J. (1958). *J. chem. Soc.*, 1,441–1,443.
Gjaldbaek, J. C. & Worm, K. (1965). *Dansk Tidsskr. Farm.*, **39**, 141–151.
Linde, H. W. & Butler, R. A. (1963). *Anesthesiology*, **24**, 887.
Raventós, J. & Lemon, P. G. (1965). *Br. J. Anaesth.*, **37**, 716–737.
Scherer, von O. & Weigand, W. (1964). *Anaesthesist*, **13**, 313–314.
Sexton, W. A. & Hendrickson, W. G. (1963). *Science, N.Y.*, **142**, 621–622.