THE IMPURITIES IN FLUOTHANE*: THEIR BIOLOGICAL PROPERTIES

BY

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SUMMARY

Fluothane (halothane BP), one of the purest anaesthetics, contains traces of some impurities amounting to a total of 0-05 per cent w/w, none of them exceeding 0-01 per cent w/w. Once these impurities had been identified they were synthesized and tested for anaesthetic activity and toxicity. The results of these experiments showed that most of these impurities have low toxicity but Fluothane contains traces of four unsaturated hydrocarbons that are relatively toxic. When Fluothane is administered in anaesthetic concentrations, its impurities are diluted to the same extent and therefore cannot be administered in toxic concentrations.

Although Fluothane (halothane BP) is one of the purest of all anaesthetics (see BP and USP for purity of other anaesthetics) it contains traces of some by-products formed during its manufacture. The first ICI standard of purity, established in 1956, allowed only 0-1 per cent w/w of total volatile impurities with a maximum limit of 0-05 per cent w/w for any particular one. This standard was maintained until recently when further improvements in its manufacture have reduced the total amount of volatile impurities to 0-05 per cent and to 0-01 per cent w/w for any individual by-product so making Fluothane one of the purest available pharmaceutical substances of any kind. The improvements and development of new analytical techniques, particularly vapour-phase chromatography and mass-spectrography, have now allowed the identification of the trace impurities; they were then synthesized and tested for anaesthetic activity and toxicity. The isolation and identification of these trace impurities will be described by Chapman and associates.

Arising from recent reports of hepatotoxicity following administration of Fluothane, these substances have been re-examined, since some of them are structurally related to certain unsaturated hydrocarbons shown by Clayton (1962) to possess high toxicity. In particular, CF₃-CCl=CCl-CF₃ has been extensively studied since Cohen and associates (1963) have suggested that the concentration of this butene may increase tenfold under conditions of clinical use, partially by enrichment because of a lower volatility relative to halothane and partially by a chemical reaction of halothane with the metal surface of the Copper Kettle vaporizer in presence of oxygen. These claims have been thoroughly investigated by our chemical colleagues, by Butler and Linde (1964) and by Albin, Horrocks and Kretchmer (1964) who consider that they are without foundation. A preliminary report of our experiments with this substance has been already published in this journal (Corrigan, McHattie and Raventós, 1963).

This butene is present in halothane as a mixture of cis and trans forms in a ratio of approximately 1:6. Most of the experiments reported here have been done with material containing the two isomers in this proportion, but the mixture has been resolved by preparative scale gas liquid chromatography, and some experiments have been carried out with samples of both pure cis and trans forms.

Materials. Samples of the materials used in these experiments were prepared in the Research Departments of the Mond and Pharmaceuticals Divisions of Imperial Chemical Industries Limited. All these samples were at least 99-5 per cent w/w pure.

METHODS

In the experiments with mice and rats, batches of ten animals each were placed in a chamber which was flushed continuously with mixtures of known

*Fluothane, an ICI trade mark, is the name given to the halothane BP manufactured by ICI.
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### Table I

**Acute toxicity of by-products of Fluothane.**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Bp. (°C)</th>
<th>Concentration in liquid Fluothane (% w/w)</th>
<th>AC50 (% v/v)</th>
<th>LC50 (% v/v)</th>
<th>LC50 (%)</th>
<th>AC50 (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF₃-CCl=CCl-CF₃ (cis-trans mixture)</td>
<td>66</td>
<td>&lt;0.0015</td>
<td>4.7</td>
<td>c. 12.0</td>
<td>c. 3.0</td>
<td></td>
<td>Delayed deaths with concentrations above 0.005% v/v. See text for further details.</td>
</tr>
<tr>
<td>CF₃-CH=CCl-CF₃ (trans)</td>
<td>35</td>
<td>&lt;0.003</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>Convulsions. No anaesthesia. No delayed deaths with 1.5% v/v.</td>
</tr>
<tr>
<td>CF₃-CH=CBr-CF₃ (trans)</td>
<td>52</td>
<td>&lt;0.025</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td></td>
<td>Convulsions with concentrations above 0.06% v/v.</td>
</tr>
<tr>
<td>CF₃=CClBr</td>
<td>42.0</td>
<td>&lt;0.005</td>
<td>—</td>
<td>0.025</td>
<td>—</td>
<td></td>
<td>Renal lesions. See text for details.</td>
</tr>
<tr>
<td>CF₃Cl-CFCl₂</td>
<td>48</td>
<td>&lt;0.0005</td>
<td>5.7</td>
<td>&gt;10.0</td>
<td>c. 2.0</td>
<td></td>
<td>Convulsions. Delayed deaths with concentrations above 6.0%.</td>
</tr>
<tr>
<td>CF₃-CHCl₃</td>
<td>27</td>
<td>&lt;0.0015</td>
<td>2.4</td>
<td>7.4</td>
<td>3.1</td>
<td></td>
<td>No side effects.</td>
</tr>
<tr>
<td>CF₃-CH₂Cl</td>
<td>6.1</td>
<td>&lt;0.001</td>
<td>4.3</td>
<td>15.0</td>
<td>3.5</td>
<td></td>
<td>Convulsions.</td>
</tr>
<tr>
<td>CF₃-CHBr₃</td>
<td>72</td>
<td>&lt;0.001</td>
<td>0.53</td>
<td>1.2</td>
<td>2.2</td>
<td></td>
<td>No side effects.</td>
</tr>
<tr>
<td>CF₃-CClBr₂</td>
<td>93</td>
<td>m.p.43°</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>No deaths with 100 mg/kg i.v.</td>
</tr>
<tr>
<td>CFCI₃Br</td>
<td>52.5</td>
<td>&lt;0.001</td>
<td>&lt;2.0</td>
<td>&gt;2.0</td>
<td>&gt;1.0</td>
<td></td>
<td>Convulsions.</td>
</tr>
<tr>
<td>CF₃-CCl₄Br</td>
<td>69.5</td>
<td>m.p.28°</td>
<td>&lt;0.002</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Administered intravenously. No anaesthesia. LD₅₀ = 175 mg/kg.</td>
</tr>
<tr>
<td>CF₃-CH₂Br</td>
<td>26.5</td>
<td>&lt;0.0005</td>
<td>2.51</td>
<td>9.76</td>
<td>3.9</td>
<td></td>
<td>Convulsions.</td>
</tr>
<tr>
<td>CF₃Cl-CH₂Cl</td>
<td>46.8</td>
<td>&lt;0.0005</td>
<td>1.29</td>
<td>4.9</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCl₄</td>
<td>61.2</td>
<td>&lt;0.001</td>
<td>1.2</td>
<td>2.1</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₄HClF₄</td>
<td>—</td>
<td>&lt;0.003</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>No tested because uncertainty of chemical structure.</td>
</tr>
</tbody>
</table>

The spatial configuration of unsaturated hydrocarbons may give rise to two isomeric forms, cis and trans, depending on the position of the substituents relative to the plane determined by the double bond as it is illustrated below.

\[
\begin{align*}
R-C=\underset{Cl}{C}=\underset{Cl}{R} \\
\text{cis} \\
\end{align*}
\quad
\begin{align*}
R-C=\underset{Cl}{C}=\underset{Cl}{R} \\
\text{trans}
\end{align*}
\]

**CORRECTIONS**

“Atypical” should replace “typical” or “a typical” in the following places:
- page 723, column 2, line 18;
- page 724, column 1, line 31, and column 2, line 18;
- legends to figures 14 and 15.

“Alveoli” should replace “alveolar” in legend to figure 14.
concentrations of the compounds under test in oxygen prepared by the method of Raventós (1956). The animals inhaled the compounds for 1 hour or for longer periods of time. Two of the impurities found in Fluothane, $\text{CF}_3 - \text{CBr}_2\text{Cl}$ and $\text{CF}_3 - \text{CBrCl}_2$, are solids with low melting and boiling points. These compounds were administered intravenously to mice as solutions in a fat emulsion (Infonutrol, Astra).

Rhesus monkeys (Macaca Mulata) were anaesthetized with an intravenous injection of pentobarbitone (30 mg/kg) and placed in a chamber of 30 l. capacity through which the vapour mixtures were passed at a rate of 10 l./min. Further doses of pentobarbitone were administered if the animals showed any signs of recovery.

The same chamber was used in experiments carried out in rabbits but in this case it was not necessary to administer pentobarbitone.

Dogs (beagles) were anaesthetized with pentobarbitone, intubated using an endotracheal tube with an inflatable cuff and the vapour mixtures were administered by an open circuit method through a set of unidirectional respiratory valves. As with the monkeys, further doses of pentobarbitone were administered if necessary.

All animals were kept under observation for 15 days when it was considered that they would survive from the treatment. Some animals were killed with a large dose of thiopentone when it was evident that they were in pain or had no chance of survival. Postmortem examinations were generally carried out immediately after death and specimens of the tissues were fixed with Zenker-acetic and with formol-saline solutions. In other cases the examinations were done as soon as possible after death in order to reduce the postmortem changes to a minimum. The histological sections of these tissues were stained with iron haematoxylin and eosin, and for fat with Fettrot.

**Biochemical methods.**

Liver function tests have been made in some animals by Dr. D. S. Platt of ICI biochemistry department. Serum transaminase levels (s.g.0.t.) were determined by the method of Reitman and Frankel (1957); alkaline phosphate was measured by the method of Bessey, Lowry and Brock (1958) and bilirubin by the method of Lathe and Ruthven (1958).

**Statistics.**

The results of some of our experiments were analyzed statistically by Dr. O. L. Davies.

**RESULTS**

The impurities known to be present in Fluothane were tested in mice for anaesthetic potency and acute toxicity and the results of these experiments are summarized in table I. The values of median anaesthetic (AC50) and median lethal (LC50) concentrations have been calculated from the incidences of anaesthesia and deaths recorded after the inhalation of vapour mixtures for 30 minutes.

It is evident that only the four unsaturated compounds from this table have a relatively high toxicity and therefore a large number of experiments were carried out with these substances, especially with $\text{CF}_3 - \text{CCl} = \text{C} = \text{CCl} - \text{CF}_3$.

$\text{CF}_3 - \text{CCl} = \text{C} = \text{CCl} - \text{CF}_3$ (2:3 dichloro-1:1:4:4:4 hexafluorobutene-2).

In the preliminary experiments reported in table I it was found that this compound was a mild anaesthetic with an AC50 of about 4-7 per cent v/v and a LC50 of about 12-0 per cent v/v (table I), but the mice that survived after exposure to its vapour for 1 hour died a few hours after recovery. These early results were confirmed in recent experiments where a wide range of concentrations of the butene was examined.

In this later series of experiments it was found that mice exposed for 1 hour to concentrations of the butene above 0-08 per cent v/v died during the first 24 hours following the experiments. Those exposed to lower concentrations for the same time survived for longer periods of time but most deaths were recorded between the third and fifth day after exposure. As a rule no deaths were observed after this time.

In some experiments the effects of the inhalation of the butene for different periods of time up to 6 hours were investigated. The results of these tests show that the toxicity of this compound is roughly proportional to the duration of the experiments. In Table II, which summarizes these results, the LC50 of the butene decreases from 0-0055 per cent v/v after 1 hour to 0-0020 per cent v/v after 6 hours inhalation.

Similar experiments were carried out in rats. In these tests it was found that the LC50 of the
butene was lower than that found in mice and higher toxicity was recorded in experiments where the rats were exposed to the butene for periods from 1 to 4 hours. As in mice, the rats exposed to concentrations around the LC\textsubscript{50} died during the third to fifth day after the experiments (table II).

A few rabbits were exposed to vapour mixtures of the butene in different concentrations for periods of 1 to 4 hours. Although the number of experiments (fifteen rabbits) is small, it is evident that this species is more sensitive than mice and rats and its LC\textsubscript{50} after exposure for 1 hour is probably between 0.003 and 0.004 per cent v/v (table III).

<table>
<thead>
<tr>
<th>Concentration % v/v</th>
<th>Mice Duration of exposure (hr)</th>
<th>Rats Duration of exposure (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.008</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.0055</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>0.0041</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.0033</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.0021</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>0.0016</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\text{log (1000 \times \text{LC}_{50})} = 0.741 - 0.586 - 0.420 - 0.310 = 0.672 - 0.444 - 0.206 \\
\pm \text{ standard error} = 0.018 - 0.017 - 0.035 - 0.020 = 0.025 - 0.026 - 0.023 \\
\text{LC}_{50} \% \text{ v/v} = 0.0055 - 0.0039 - 0.0026 - 0.0020 = 0.0047 - 0.0028 - 0.0016
\]

Twenty dogs, mostly in groups of three, were exposed for different lengths of time to various concentrations of the butene. The time of death of each animal was recorded. With those surviving for 14 days after the experiment it was considered that they would not die from the toxic effects of the butene. Similar experiments were carried out with twenty monkeys (Rhesus). In table IV the results of both series of experiments are compared. From this table it is evident that dogs are more resistant to the butene than monkeys and that the LC\textsubscript{50} found in dogs is much higher than that found in monkeys.

The statistical analysis of these results show that if the total dose of butene is expressed as the concentration multiplied by the time of exposure, then the comparative log LD\textsubscript{50} may be obtained by adding the log of the exposure time to the log LC\textsubscript{50} given in table IV.

When this is done one can see that the values for LD\textsubscript{50} do not differ significantly for the different times of exposure. In other words the toxicity is probably dependent on the total amount of compound taken up by the animals within the range of values of these experiments.

The relationship between per cent survivors and the dose may be made linear by using the logarithm of the dose and the transformation \( y = \ln[s/100-s] \), \( s \) being the percentage of survivors. Apart from a factor \( \frac{1}{s} \), this transformation is the well-known logit transformation (Fisher and Yates), which is very similar to the Probit transformation but more convenient to handle.

The resulting equations are

\[
\text{Monkeys} = (y + 1.164) = -9.29 \log (x - 10.0308) \\
\text{Dogs} = (y + 1.311) = -36.75 \log (x - 10.0745).
\]

\[ 
\]
TABLE IV
Toxicity of CF₃–CCl=CCl–CF₃ in monkeys (Rhesus) and dogs. Individual survival times in days after different exposure times.

<table>
<thead>
<tr>
<th>Concentration % v/v</th>
<th>Duration of exposure (hours)</th>
<th>Monkeys</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1                2      3</td>
<td>1      2  4  6</td>
<td></td>
</tr>
<tr>
<td>0.013</td>
<td>5                 —      —</td>
<td>1.1    —  —  —</td>
<td></td>
</tr>
<tr>
<td>0.0315</td>
<td>7–10  10–1  14–6</td>
<td>9– &gt;14</td>
<td>1.1–1  1.1  —  —</td>
</tr>
<tr>
<td>0.0166</td>
<td>10– &gt;14–  6– &gt;14–</td>
<td>&gt;14    &gt;14  &gt;14  &gt;14</td>
<td></td>
</tr>
<tr>
<td>0.008</td>
<td>&gt;14– &gt;14  &gt;14– &gt;14–</td>
<td>&gt;14    &gt;14  &lt;14  &gt;14</td>
<td></td>
</tr>
<tr>
<td>log (1000 x LC₅₀)</td>
<td>1.271            1.143  0.956</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± standard errors</td>
<td>±0.084           ±0.101  ±0.093</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC₅₀ as % v/v</td>
<td>0.0186           0.0139  0.0090</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

where x is the dose, i.e. the product of the concentration and the time of exposure in the limits mentioned above.

The ratio of the LD₅₀ calculated for dogs and monkeys is 3 with 95 per cent confidence limits of 1.7 to 5.0. The slope is much higher for dogs than for monkeys indicating that the first are less variable in their response to the butene.

These results have also been analyzed with respect to time of death. In this analysis a maximum survival time was assigned to those animals which survived for 14 days after the experiments. Here again the dose could be expressed as concentration x time of exposure. The survival time was sensibly linear with the logarithm of the dose and the fitted equations were as follows:

Monkeys = ST – 8.79 = –11.25 log (x/0.0308)

Dogs = ST – 7.19 = –21.35 log (x/0.0745)

where ST = survival time

and x = concentration as per cent v/v multiplied by time of exposure in hours.

As in the previous analysis the slope for dogs is much higher than for monkeys and the ratio of the comparative doses for dogs to monkeys to give the same response, i.e. survival time, is 2:1 which is consistent with the ratio of the LD₅₀'s.

Toxicity of the cis and trans forms of CF₃–CCl=CCl–CF₃.

The toxicity of pure samples of cis and trans forms of this butene was measured in mice by our standard method, but because the material was limited the mice were exposed to the compounds for 1 hour only. These tests showed that the cis form was about three times less toxic than the trans but the difference in toxicity of the pure trans form and the cis-trans mixture, used in the rest of the experiments, was not statistically significant (see table V).

TABLE V
Toxicity of cis and trans forms of CF₃–CCl=CCl–CF₃ in mice after exposure for 1 hour.

<table>
<thead>
<tr>
<th></th>
<th>log (1000 x LC₅₀)</th>
<th>LC₅₀ % v/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>± standard error</td>
<td></td>
</tr>
<tr>
<td>Trans</td>
<td>0.786 ± 0.026</td>
<td>0.0061</td>
</tr>
<tr>
<td>Cis</td>
<td>1.253 ± 0.028</td>
<td>0.0179</td>
</tr>
<tr>
<td>Trans-cis mixture</td>
<td>0.740 ± 0.023</td>
<td>0.0055</td>
</tr>
</tbody>
</table>


Mixtures of 1.5 per cent v/v Fluothane and increasing concentrations of the butene were administered to mice and rats for periods from 1 to 4 hours and the results of these tests were compared to those obtained in similar tests where only the butene had been used. These tests show that the presence of Fluothane in anaesthetic concentration decreases significantly the toxicity of the butene, by 1.6 in mice and 1.8 for rats, but the differences between these ratios is not quite statistically significant (see table VI). The reason for this reduction in toxicity of the butene is not clear. However, it is possible that the respiratory
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Fig. 1
Macroscopical aspect of the lungs, heart and liver of a dog killed 5 days after the administration of 0.0166 per cent v/v of CF₃-CCl=CCl-CF₃ for 6 hours.

Fig. 2
Lungs and heart of the same dog.
**Fig. 3**
Lung of a dog treated with 0.0166 per cent v/v of CF₂Cl-CCl₃=CCl-CF₃ for 2 hours. This section shows patchy thickening of the alveolar walls. (×150)

**Fig. 4**
Liver of same dog; normal appearance. (×150)
depression produced by 1.5 per cent Fluothane decreases the uptake of the butene and in this way the animals can tolerate higher or a given concentration of the butene for longer periods of time.

Pathology.

All animals treated with relatively high concentrations of the butene showed intense dyspnoea and cyanosis. The onset of these symptoms was rapid in the animals which inhaled high concentrations of this compound, but they appeared only after 2 or 3 days in the animals which inhaled the butene in concentrations around the LC50.

Two interesting observations were recorded, one in dogs and the other in female monkeys. Dogs treated with concentrations of butene around 0.06 per cent v/v had intense haemorrhages of the large intestine. Female monkeys which inhaled this compound in sublethal concentrations showed uterine bleeding 2 or 3 days after the experiment lasting for another 3 or 5 days.

In the postmortem examination of all the animals changes of different intensity were observed in the lungs. As a rule the lungs were congested and oedematous, but in some cases, such as in the dog illustrated in figures 1 and 2, which inhaled the butene in a concentration of 0.0166 for 6 hours, intense hepatization and haemorrhage of the lungs, congestion of the liver and an enlarged heart were found. The livers appeared to be congested but any macroscopical evidence of fatty degeneration, so frequently seen in animals which have been anaesthetized with chloroform, was never found.

Histological examination of the specimens obtained from mice and rats treated with the butene showed that the major pathological changes were localized in the lungs. These showed an irritative reaction characterized by intense congestion and oedema lasting for 24 hours or more, during which time polymorphonuclear leucocytes increased in numbers in the alveolar walls and then filled small groups of alveoli to give scattered pneumonic patches. The latter gradually resolved until the lungs appeared normal in the animals killed 10 to 14 days after exposure. Only in one animal, a rat which inhaled 0.002 per cent v/v butene for 4 hours and survived for 3 days, was there any suggestion of the typical regeneration of bronchial epithelium commonly seen in large animals.

In spite of the wide range of concentrations of butene and lengths of exposure used in these experiments, no single case of fatty degeneration of the liver was found. Careful scrutiny of the sections revealed nothing more than occasional degenerating liver cells or a mild increase in the number of cells showing lipid-positive granules.

A few rats which inhaled the butene in concentrations of 0.055 per cent v/v and 0.033 per cent v/v for 1 hour showed renal changes consisting of small thromboses in some glomerular capillaries,
vacuolation and degenerative changes in the first part of the proximal convoluted tubules, and an increase in the number of colloid casts. The total damage was not severe.

Mice which died 24 hours after exposure showed acute degeneration of the inner or X zone of the adrenal cortex. This was less marked in animals which survived for longer periods of time.

As with rodents, in dogs and monkeys the pathological changes due to the inhalation of the butene are found mainly in the lungs and their intensity is roughly proportional to the duration of the inhalation. Figures 3 to 8, taken from three dogs which inhaled 0.0166 per cent v/v butene for 2, 4 and 6 hours respectively, illustrate this point. The first two dogs survived for 14 days after the administration of the butene and showed residual lung damage consisting of a thickening of the alveolar walls (figs. 3 and 5). The livers of these two animals were within normal limits (figs. 4 and 6).

The third dog (figs. 1 and 2) was sacrificed because of an intense lung haemorrhage on the fifth day after treatment. In this illustration (fig. 7) the haemorrhage has masked other lung changes which were similar to those seen in the first two animals. Again, the liver showed no suggestion of fatty or toxic lesions although congestion is present (fig. 8).

The lungs of dogs treated with higher concentrations of the butene showed initial degeneration of bronchial epithelium, followed by a typical hyperplasia. Damage to the alveolar walls produced, among other changes, large emphysematous bullae. Sometimes the alveoli were lined by a fibrinous membrane. Four other dogs besides the one mentioned above, died from internal haemorrhages between 1 and 5 days after the administration of the butene. Figures 9 to 13 show the pathological changes produced in a dog which was sacrificed, when moribund, because of an intense haemorrhage of the large intestine, 30 hours after the inhalation of 0.065 per cent v/v butene for 2 hours. Figure 9 shows this lesion. Its adrenal glands showed acute degeneration of the zona fasciculata and recticularis (fig. 10) whilst the glomerulosa appeared almost normal. The usual changes were found in the lungs (fig. 11) but no toxic or degenerative lesions could be seen in the liver (fig. 12) in spite of congestion. The coronary arteries showed acute fibrinoid necrosis of the media (fig. 13) and some small areas of degeneration of the myocardium were present.

The pathological lesions found in monkeys treated with the butene in concentrations from 0.13 per cent v/v to 0.008 per cent v/v were similar in nature to those found in dogs. All monkeys, except those treated with the lowest mentioned concentration, had marked atypical hyperplasia of the bronchial epithelium which extended into the alveoli. Frequently there was metaplasia of this epithelium to a stratified squamous type containing prickle cells. The other alveoli were far from normal; their walls were thickened and the normal architecture was grossly distorted; they were often lined by large cells, sometimes obviously derived from invading bronchial epithelium, but in other cases perhaps typical wall cells or macrophages. The alveolar lumen might be lined with a deposit of fibrin, filled with exudate or contain cast-off cells, macrophages and polymorphonuclear leucocytes (figs. 14, 15 and 16). Secondary changes such as atelectasis, emphysema, haemorrhage and fibrosis were present in some animals.

Some monkeys showed mild centrilobular fatty vacuolation of the liver (figs. 17 and 18), presumably secondary to a degree of right ventricular failure. Mild damage to the kidneys was found in some monkeys treated with high concentrations of the butene, and one of them had acute fibrinoid degeneration of the coronary arteries similar to that seen in dogs. Uterine bleeding was observed during the second to fifth day after the inhalation of this substance, but this had disappeared when the animals were sacrificed and no cause for this haemorrhage could be seen in the histological sections.

Biochemistry.

Samples of blood of the dogs and monkeys treated with the butene were obtained before and every second day after the experiments. A final blood sample was taken either immediately before death or during the fourteenth day after the treatment when they were sacrificed. The samples were centrifuged at once and analyzed for bilirubin, transaminase (s.g.o.t.) and alkaline phosphatase. All the results of these tests were within normal limits, which confirms the absence of hepatic lesions found in the histological sections of the livers of these animals.
FIG. 5
Lung of a dog treated with 0.0166 per cent v/v of CF₃—CCl—CCl—CF₃ for 4 hours. This section shows more marked thickening of the alveolar walls than fig. 3. (×150)

FIG. 6
Liver of same dog; normal appearance. (×150)
FIG. 7
Lung of the dog of figs. 1 and 2, treated with 0.0166 per cent v/v of \( \text{CF}_3 - \text{CCl} = \text{CCl} - \text{CF}_3 \) for 6 hours. The thickening of the alveolar walls is masked by profuse haemorrhage into the lung. (×150)

FIG. 8
Liver of the dog of figs. 1 and 2 showing congestion, also dilatation of the spaces of Disse, but no fatty changes. (×150)
Large intestine of a dog treated with 0.065 per cent v/v of CF<sub>3</sub>---CCl<sub>2</sub>---CCl---CF<sub>4</sub> and killed 30 hours later. This section shows the transition from congested but fairly normal mucosa to an area of mucosal necrosis with haemorrhage. (×150)

Adrenal of dog of fig. 9 showing degenerative changes in the zona fasciculata (ZF) where few cells remain intact. (×620)
FIG. 11
Lung of dog of fig. 9. This section shows some alveolar thickening with infiltration by polymorphonuclear leucocytes which is difficult to discern in the photograph. (× 150)

FIG. 12
Dog of fig. 9. The liver of this animal is normal except for congestion in spite of the severe changes in other organs. (× 150)

In contrast to hexafluorodichlorobutene the corresponding monochloro derivative does not anaesthetize mice even in the highest concentration used in these experiments, i.e. 1.6 per cent v/v, but it produces convulsions in the animals which inhale it in concentrations above 0.5 per cent v/v. No deaths were recorded during the 14 days following the experiments. Some of the mice used in these tests were sacrificed and their organs were examined histologically. Their lungs showed some congestion and their livers had small foci of necrosis with polymorphs; some cells stained positively for lipids. Typical fatty degeneration, however, was not found.


\[ CF_3-CH=CBr-CF_3 \] is not an anaesthetic and produces convulsions in mice treated with concentrations as low as 0.06 per cent v/v. Its acute lethal concentration is about 0.5 per cent v/v. The histological examination of the tissues of mice sacrificed some days after the experiments showed more or less the same pathological changes as those found in mice treated with the monochloro homologue, i.e. congestion and oedema of the lungs, a small increase in the number of liver cells staining positively for lipids but no true fatty degeneration.

\[ CF_2=CClBr \] (2:bromo 2:chloro 1:1 difluoro ethylene).

This substance which is present in Fluothane in trace quantities is also formed when Fluothane vapours are recirculated over some brands of soda lime under conditions similar to those of closed-circuit anaesthesia. In in-vitro experiments, carried out by the pharmaceutical department of ICI it was found that about 0.02 per cent w/w of the Fluothane was transformed into \( CF_2=CClBr \) in 4 hours. The results of these experiments will be published elsewhere by Fernie and Hudson (in preparation).

Therefore, during anaesthesia with 1.0 per cent v/v Fluothane in closed circuit one could expect that the ethylene would reach a concentration of about 0.0002 per cent v/v or 2 p.p.m. This expectation was confirmed in the animal experiments described below.

The toxicity of this compound is higher than that of the last mentioned, and as with \( CF_3-CCl-CCl-CF_3 \) there is a relationship between inhaled concentration and time of death of the animals. Mice exposed to concentrations between 0.5 per cent v/v and 0.1 per cent v/v died in 48 hours but those exposed to lower concentrations died between 3 and 10 days after the test. The LC50 of this compound calculated from these experiments is around 0.025 per cent v/v.

The postmortem examination of these animals showed intense renal tubular degeneration already apparent in mice dying 24 hours after the inhalation of 0.5 and 0.1 per cent. Many of the convoluted tubules were completely atrophic; others showed varying degrees of degenerative change, while the collecting tubules were choked with basophilic casts (figs. 19 and 20).

Mice which inhaled concentrations between 0.1 per cent v/v and 0.006 per cent survived for longer periods of time and had similar but less severe renal lesions. Some animals treated with 0.12 per cent v/v and sacrificed on the fourteenth day after the tests, showed marked recent fibrosis spreading out from the cortico-medullary junction; whilst some renal tubules were atrophic, others were lined by flat basophilic undifferentiated epithelium, and were presumably regenerating.

In order to see if during closed-circuit anaesthesia the breakdown of Fluothane could produce toxic concentrations of \( CF_2=CClBr \), we anaesthetized two monkeys and two dogs in closed circuit using a brand of soda lime which we knew to degrade Fluothane. The concentration of Fluothane, and in two cases the concentration of \( CF_2=CClBr \), in the rebreathing bag were monitored by gas chromatography.

One monkey was anaesthetized by this technique three times for 2, 4 and 6 hours respectively on alternate days, with a concentration of Fluothane in the reservoir bag between 0.8 per cent v/v and 1.3 per cent v/v. The second monkey was anaesthetized for 6 hours on each of two successive days with similar concentrations of Fluothane. Both animals were killed on the third day after the last anaesthesia. No abnormalities were found either during the postmortem examinations or in the microscopical sections of their organs.
In the experiments* with dogs, besides monitoring the concentration of Fluothane, samples of the gas mixtures in the reservoir bag were analyzed for their CF$_2$=CClBr content at regular intervals. The dogs were anaesthetized for 4 hours with concentrations between 0-8 per cent v/v and 1-0 per cent v/v of Fluothane in the reservoir bag and the amounts of the ethylene in the circuit, which were hardly detectable (0-00005 per cent v/v) at the beginning of the experiment, rose to 0-001 per cent v/v at the end of the anaesthesia. As with the monkeys, these animals were sacrificed on the third day after the experiments and no macroscopical or microscopical abnormalities were found in their organs.

These two sets of results show that CF$_2$=CClBr does not build up and accumulate to toxic concentrations during closed-circuit anaesthesia with Fluothane.

Other impurities.
Fluothane also contains some halogenated ethane and methane derivatives in trace amounts. The toxicity of these substances is very much lower than that of the unsaturated impurities described above.

CF$_3$Cl-CFCI$_2$ (1:2:2-trichloro-1:1:2-trifluoroethane). It has an AC$_{50}$ of 5-7 per cent v/v and a LC$_{50}$ higher than 10-0 per cent v/v. The anaesthesia produced by this compound is associated with convulsions and some of the exposed to concentrations above 6-0 per cent v/v died a few days after treatment. This compound has been also examined by Burns and colleagues (1961) and by Clayton (1962).

CF$_3$-CHClBr (2:2 dibromo 1:1:1-trifluoroethane). This compound has an AC$_{50}$ only slightly lower than its LC$_{50}$, i.e. both around 2-0 per cent v/v. Convulsions were recorded in mice during the inhalation of this compound.

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CF$_3$-CHBr$_2$ (2:2 dibromo 1:1-1-trifluoroethane). This substance has also been studied by Robbins (1946). It has a very high anaesthetic potency (AC$_{50}$ = 0-53 per cent v/v) but its acute toxicity is proportionally high (LC$_{50}$ = 1-2 per cent v/v). No side effects or delayed deaths were observed in the mice treated with this compound.

CF$_3$-CClBr$_2$ (2:2 dibromo 2 chloro 1:1:1-trifluoroethane). This compound is a solid with a melting point of 43°C and a boiling point of 93°C. Because of its physical characteristics this compound was administered intravenously dissolved in a fat emulsion (Infonutrol, Astra). No anaesthesia or deaths were observed in mice when this compound was injected in doses up to 100 mg/kg.

CFCl$_2$Br (bromo dichlorofluoromethane). It has an AC$_{50}$ only slightly lower than its LC$_{50}$, i.e. both around 2-0 per cent v/v. Convulsions were recorded in mice during the inhalation of this compound.

CF$_3$-CCl$_2$Br (2 bromo 2 dichloro 1:1:1-trifluoroethane). This solid, melting point 28°C, boiling point 69.5°C, was also administered intravenously to mice dissolved in the fat emulsion mentioned above. No anaesthesia was produced with any of the dose levels used in these experiments but it has a LD$_{50}$ of 175 mg/kg. It did not produce delayed deaths.

CF$_3$-CH$_2$Br (2 chloro 1:1:1-trifluoroethane). The anaesthetic action of this compound has been studied by Robbins (1946) and by Poznak and Artusio (1960). The results on experiments with mice showed that it has a AC$_{50}$ of 2-51 per cent v/v and a LC$_{50}$ of 9-76 per cent v/v. In rabbits it produces mild convulsions and cardiac irregularities, i.e. arrhythmias and ventricular extrasystoles.

CF$_2$CI-CH$_2$Cl (1:2 dichloro 1:1-difluoroethane). This is another compound studied by Robbins in 1946. Our results with mice are similar to those of Robbins, i.e. AC$_{50}$ = 1-3 per cent v/v and LC$_{50}$ = 4-3 per cent v/v. However, he reported that it produced delayed toxic effects and deaths, a result that we have been unable to confirm. It produces convulsions, cardiac arrhythmias and lung lesions, when administered to rabbits in concentrations around 3-0 per cent v/v.

*Done with the collaboration of Mr. D. S. Corrigan of the ICI Pharmaceuticals Department.
Fig. 13
Dog of fig. 9. Coronary artery. This section shows acute fibrinoid degeneration of the media (F). (×620)

Fig. 14
Lung of Rhesus monkey treated with 0.0166 per cent v/v CF₃CCl=CCl=CF₃ for 1 hour and killed 10 days later. This section shows: (1) a typical hyperplasia of the bronchial epithelium extending into the alveolar (H); (2) thickening of the alveolar walls; and (3) presence of oedematous fluid and large numbers of cells, macrophages or cast-off alveolar wall cells, in the alveoli (×150)
Lung of Rhesus monkey treated with 0.0166 per cent v/v of CF$_3$-CCl=CCl-CF$_3$ for 2 hours and killed 14 days after. This section shows similar changes to those of fig. 14, but the typical hyperplasia of the bronchial epithelium is more extensive and severe, and metaplasia to a stratified squamous type is evident. (x 150)

Enlargement of part of fig. 15 showing the hyperplastic epithelium with more detail. (x 540)
Fig. 17
Liver of Rhesus monkey of fig. 14. The section shows vacuolation of cells in centrilobular areas. When stained for fat, the centrilobular cells showed a mild increase in small lipid-positive granules in their cytoplasm but vacuoles did not take up the stain. (×150)

Fig. 18
Section of the liver of the Rhesus monkey of fig. 15, showing normal histology. (×150)
FIG. 19
Kidney of a mouse treated with 0·025 per cent v/v of CF$_2$-CClBr and killed 14 days later. The section shows acute degeneration of the convoluted tubules, many of which are atrophic. Casts are present in the collecting tubules.

FIG. 20
High power view of the kidney of the mouse of fig. 19 showing complete disintegration of many convoluted tubules.
CHCl₃ (trichloromethane, chloroform). Chloroform has been identified in Fluothane in concentrations of about 0.001 per cent w/w. As its toxic effects are so well known we feel that it is not necessary to describe them.

C₄HClIF₆. The chemical structure of this impurity has not yet been elucidated, but our chemical colleagues have identified it as an isomer of C₄HClIF₆ by the mass spectrum of its chromatographic peak. For this reason it has not been tested in animals.

**DISCUSSION**

In any complicated chemical synthesis, such as the method of manufacture of Fluothane, besides the main product other substances are also produced. These by-products or contaminants must be removed by physical or chemical methods if one wants to have a product of the purity required in anaesthetic agents, but in some cases the last traces of these impurities are difficult to eliminate and may be detected in the final product if the analytical methods are sufficiently sensitive. If a compound can be made by two different methods, the amounts and constitution of the impurities present could be different, therefore if halothane is made by a method different from that used by ICI Ltd., it may contain other contaminants (Scherer and Weigand, 1964; Gjaldbaak and Worm, 1965).

Once the contaminants of Fluothane manufactured by ICI Ltd. had been identified, it was decided to prepare samples of them with a purity of at least 99.5 per cent w/w and investigate their effects and toxicity. Some of these substances were known and had been studied by other authors (Robbins, 1946; Lu, Ling and Krantz, 1953; Burns et al., 1961, 1962; Clayton, 1962). In general our results confirm those already published, but because of the differences between the methods used by us and other authors, only our results have been reproduced in table I. From this table it is evident that only the unsaturated fluorohydrocarbons present in Fluothane are relatively highly toxic, but they are present in such small amounts that when diluted with oxygen or air during anaesthesia they are administered in concentration many times smaller than their toxic concentrations.

Lu, Ling and Krantz (1953) studied the action of CF₃—CH=CCl—CF₃ on two rats and found that it had delayed toxic effects. It is difficult to compare this result with those reported here, mainly because the authors did not mention the method used in their experiment, but if one assumes that they followed the technique described in one of their previous articles (Krantz et al., 1940) it is possible that in their experiment they used CF₃—Cl=Cl—CF₃ in a concentration around 10 per cent v/v. Besides this, they did not mention if they used a mixture of the cis and trans form or a pure sample of one of the isomers.

In the experiments reported here it was found that this butene is highly toxic and that there are marked differences in the sensitivity of different species to this substance. Rabbits are perhaps the most sensitive animals, rats and mice are slightly less sensitive, and monkeys and dogs are between five and ten times more resistant than rodents.

It was interesting to note that in spite of these differences in sensitivity the pathological lesions produced by this butene were always found in the lungs and were of the same type irrespective of the species used in the experiments. No important lesions were found in any of the other organs and no liver dysfunction was observed by means of liver function tests.

When this butene is synthesized or extracted from Fluothane one obtains a mixture of its cis and trans forms in the proportion of 1:6. This material was used in most of our experiments but Drs. D. J. Gilman and G. V. McHattie resolved this mixture by the method of Dickinson, Hill and Murray (1958) and provided us with small pure samples of the two isomers which allowed us to measure their toxicity in some short experiments in mice. The toxicity of the trans form, with an LC₅₀ of 0.0061 per cent v/v after exposure of 1 hour, is not statistically different from the toxicity of the cis-trans mixture, but the cis form, with an LC₅₀ of 0.0179 per cent v/v, is about three times less toxic than the other two materials. The pathological lesions produced by the three materials were the same.

The monochloro butene, CF₃—CH=CCl—CF₃, was studied by Robbins (1946) and although he does not reproduce his results in the tables in his paper, it is mentioned in the text as the only fluorinated hydrocarbon that produces convulsions and has no anaesthetic activity. Because of the lack of information of the concentrations used in his experiments it is impossible to compare his results with those reported here.
Another contaminant of Fluothane with convulsive properties is CF$_3$—CH=CBr—CF$_3$. No anaesthesia was produced in the mice which inhaled this compound in concentrations between 0-02 per cent v/v and 0-65 per cent v/v, but convulsions were observed in the animals treated with concentrations higher than 0-06 per cent v/v. This compound has been identified by Albin, Horrocks and Kretchmer (1964) in samples of Fluothane obtained from Copper Kettles and from freshly opened bottles of Fluothane.

The last unsaturated hydrocarbon studied in this series, CF$_2$=CClBr, is present in Fluothane in trace amounts, in other words one can identify it but it is difficult to measure its concentration with any degree of accuracy. In some experiments where 10 per cent v/v Fluothane was recirculated over soda lime under conditions similar to those found in closed-circuit anaesthesia it was found that, after 4½ hours recirculation, about 0-02 per cent of the Fluothane present in the circuit was degraded into CF$_2$=CClBr. If the same degradation takes place during closed-circuit anaesthesia in practice and assuming that between 1 and 2 per cent v/v Fluothane is present in the circuit, at the end of a 4-hours anaesthesia the concentration of CF$_2$=CClBr may rise to between 0-0002 and 0-0004 per cent v/v or to about between 1/125 and 1/60 of its lethal concentration for mice. It was confirmed in experiments with monkeys which were anaesthetized several times for periods up to 6 hours with Fluothane administered in closed circuit, and using a brand of soda lime that decomposed the anaesthetic, that toxic concentrations are not reached under conditions of practical use.

These contaminants are present in liquid Fluothane in amounts which can be measured only by very sensitive methods such as gas chromatography. When one administers anaesthetic concentrations of Fluothane in air or oxygen, the contaminants are diluted approximately in the same proportion and, therefore, it is impossible to give them in toxic concentrations during Fluothane anaesthesia.

CONCLUSIONS

The by-products formed during the manufacture of Fluothane have been identified and tested for anaesthetic activity and toxicity.

These impurities are present in Fluothane in very small amounts. The ICI standard of purity allows only 0-05 per cent w/w of total volatile impurities and no single one can exceed 0-01 per cent w/w, so that during anaesthesias with 1 to 2 per cent v/v Fluothane the patients may inhale between 0-0005 to 0-001 per cent v/v of total impurities.

These impurities can be divided into two groups, i.e. saturated and unsaturated fluoro-hydrocarbons. The first have low toxicity and these with anaesthetic activity have a narrow margin of safety. The four unsaturated fluorocarbons present in Fluothane have relatively high toxicity.

CF$_3$—CCl=C=CICl$_3$ (cis-trans mixture) produces intense lung lesions. Its toxic concentration after 1 hour administration ranged from 0-004 per cent v/v for rabbits to 0-0725 per cent v/v for dogs. Its trans form is about three times more toxic than the cis form. No hepatic lesions are produced with this substance.

CF$_3$—CCl=CH—CF$_3$ (trans) and CF$_3$—CBr=CH—CF$_3$ (trans) are convulsant.

CF$_2$=CClBr produces intense renal tubular degeneration in mice exposed to concentrations between 0-006 and 0-1 per cent v/v for 1 hour. This compound is formed when Fluothane is recirculated over some brands of soda lime. However, it does not build up and accumulate to toxic concentrations during closed-circuit anaesthesia.

APPENDIX

During recent months, since the article was submitted for publication, a new method of purification of Fluothane has been developed by ICI Ltd. By this process the total content of impurities present in Fluothane has been reduced from 0.05 per cent to <0.0075 per cent w/w or to <75 p.p.m. Any single unsaturated impurities are present only in amounts of <5 p.p.m. and each of the saturated impurities is present in quantities of <20 p.p.m.

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THE IMPURITIES IN FLUOTHANE: THEIR BIOLOGICAL PROPERTIES

REFERENCES


LES IMPURETES DANS LE FLUOTHANE: LEURS PROPRIETES BIOLOGIQUES

SOMMAIRE

Le Fluothane (halothane BP), l'un des anesthésiques les plus purs, contient des traces de quelques impuretés qui peuvent atteindre un total 0,05 pour cents w/w, sans qu'aucune d'elles ne dépasse 0,01 pour cents w/w. Une fois ces impuretés identifiées elles ont été synthétisées et examinées du point de vue de leur activité comme anesthésique et de leur toxicité. Les résultats de ces examens ont montré que la plupart de ces impuretés sont peu toxiques mais que le Fluothane contient des traces de quatre hydrates de carbones non saturés qui sont relativement toxiques. Lorsque le Fluothane est administré à des concentrations anesthésiques ses impuretés sont diluées de la même façon et ne peuvent donc être administrées à ces concentrations toxiques.

DIE VERUNREINIGUNGEN IM FLUOTHANE: IHRE BIOLOGISCHEN EIGENSCHAFTEN

ZUSAMMENFASSUNG

Fluothane (Halothane BP), eines der Anästhetika mit hochstem Reinheitsgrad, enthält geringe Verunreinigungsspuren bis zu einer Gesamtmenge von 0,05 Gewichtsprozenten, keine der Verunreinigungsunterstanzen überschreitet 0,01 Gewichtsprozenten. Sowohl diese Verunreinigungen identifiziert worden sind, wurden sie synthetisiert und auf ihre anästhetischen Eigenschaften und ihre Toxizität untersucht. Die Ergebnisse dieser Untersuchungen zeigen, daß die meisten dieser Verunreinigungen eine geringe Toxizität aufweisen, aber Fluothane enthält Spuren von vier ungesättigten Kohlenwasserstoffen, die relativ toxisch sind. Wenn Fluothane in den gebräuchlichen Anästhetikumkonzentrationen verabfolgt wird, werden die Verunreinigungen im gleichen Ausmaß verdünnt, so daß toxische Konzentrationen nicht verabreicht werden können.