Reports

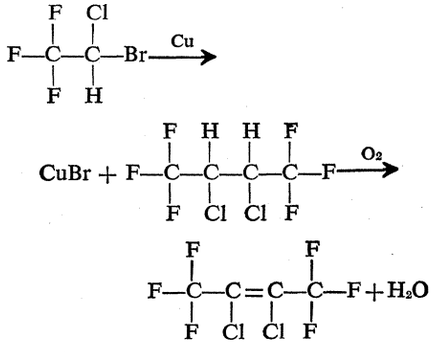
Impurity in Halothane Anesthetic

Abstract. *A halogenated butene has been isolated from commercially available halothane (Fluothane). Its concentration increases under the conditions in which halothane is commonly used in clinical work.*

Because of recent reports (*1*) of hepatic necrosis following the administration of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), and on request of the Subcommittee on the National Halothane Study of the Committee on Anesthesia of the National Academy of Sciences–National Research Council, an examination of this drug was undertaken for the presence of other halogenated compounds. Gas chromatographic analysis of halothane (hydrogen flame detector; di-isodecyl phthalate column; 70°C; helium flow 50 ml/min) showed the presence of a compound with a retention time of 35 seconds; halothane has a retention time of 75 seconds. The compound was present in each bottle of halothane that was examined, whether freshly opened or not. The average concentration in freshly opened bottles was approximately 0.01 percent. It was also shown, in a number of samples, that the concentration of this compound may increase under conditions in which halothane is used clinically. On one occasion it had increased to 0.1 percent during 5 days' use when the compound was stored continuously in a "copper kettle" vaporizer. Therefore a series of laboratory studies were undertaken to determine the conditions which would cause the concentration of this compound to increase.

Halothane, when refluxed in the presence of copper filings in an oxygen atmosphere, yielded an increased concentration. Heat further accelerated the reaction. In the absence of either copper or oxygen the concentration of the compound did not increase. Fractional distillation also yielded an increase of the compound, with radical enrichment observed, in the residual volume.

Identification of the compound was achieved by mass spectrometry following preparative gas chromatography with collection of the effluent under liquid nitrogen. The substance thus identified is 2,3-dichloro-1,1,1,4,4,4-hexafluorobutene-2, existing as the *cis* or *trans* isomer. Its boiling point is 67.8°C with a molecular weight of 232 (*2*). A feasible chemical mechanism of formation of the compound from halothane might be:



The pharmacologic properties of this compound have been only partially studied. Lu *et al.* noted that the compound produced convulsions at the point of anesthesia in two of four rats studied. All the rats had postanesthetic analgesia and died within 18 hours (*3*). This compound is closely related structurally to fluorocarbons of high toxicity. Clayton has shown that the presence of a double bond in the polyfluoroalkenes is associated with an increase in chemical activity and toxicity over that shown by the alkanes. In addition, the alkenes show an increasing toxicity with an increase in the number of chlorine atoms (*4*). However, since relationships between molecular configuration and toxicity are still obscure, investigations are currently under way to delineate the toxicity of this compound. A preliminary investigation in our laboratory has shown that it is acutely toxic to the dog when inhaled in anesthetic concentration. Delayed onset of anesthesia was followed by convulsions and death within 1 hour and 40 minutes. Another investigation has shown toxic symptoms in Wistar strain rats, after exposure for 4 hours to a 0.01-percent concentration. Severe degenerative changes were observed in the lung, liver, and kidney (*5*).

Of pertinent interest is a recent article (*6*) entitled "Halothane Hepatitis, an American Disease?" The wide use of copper vaporizers in this country as opposed to nickel-plated or glass vaporizers in many other countries may be significant.

Although the relative toxicity of 2,3-dichloro-1,1,1,4,4,4-hexafluorobutene-2 still remains to be established, a note of caution would include the suggestion that halothane be removed from the vaporizer at the end of each day's use to prevent a continuous concentration by evaporation and to further reduce to a minimum the time of contact within the copper container.

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DDT and Dieldrin in Rivers: A Report of the National Water Quality Network

Abstract. *As a part of the water-quality surveillance activities of the National Water Quality Network at 101 sampling stations, insecticides were identified in 38 samples from ten rivers during the period May through December 1962. Both DDT and dieldrin were identified by infrared and gas chromatographic analysis of carbon adsorption extracts.*

Since the introduction of DDT as an insecticide during World War II, the use of organic pesticides has increased enormously. It has been estimated that more than 9000 commercial pesticide preparations are available in the United States (*1*). Such com-

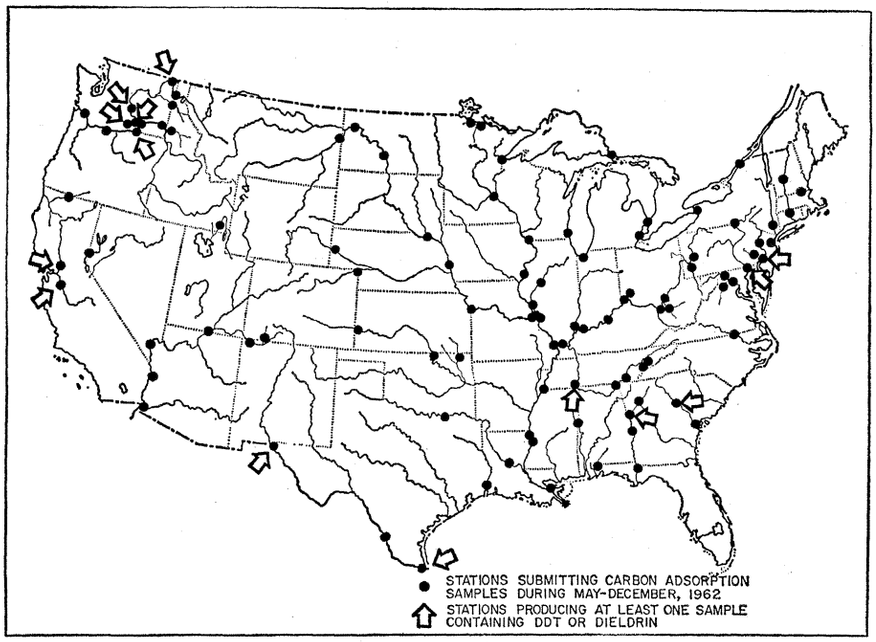

Fig. 1. Identification of DDT and dieldrin by the National Water Quality Network, May–December 1962.

pounds, applied to foliage, soil, and water courses may be expected to move with rainfall and runoff into rivers and their tributaries (*2*), provided the compounds are sufficiently resistant to degradation by biochemical and physical action. Many of the chlorinated insecticides, including DDT and dieldrin, resist such action (*3*).

As a part of the sampling and analytical program of the Public Health Service National Water Quality Network (*4*), the carbon adsorption method (*5*) is used for qualitative screening of river water samples taken monthly for the determination of a wide variety of organic pollutants. As a dividend of this program which is designed to assess water quality in terms of numerous pertinent parameters, qualitative screening for pesticides has also been possible. This report, which covers the period from May through December 1962, includes pesticide identifications made on samples received from 101 stations. The geographical locations of these stations are shown in Fig. 1.

Samples were obtained by passing up to 19,000 liters (5000 gal) of water through a 7.6 × 45.7 cm (3 × 18 in.) glass cylinder containing activated carbon (*5*) at a flow rate of 1.9 liters (0.5 gal) per minute. The chloroform extract (CCE) of the dried carbon was separated, according to solubility, into neutral and other fractions. An aromatic subfraction was separated from the neutral fraction by column chromatography with benzene as the mobile solvent (*6*). The infrared spectrum of each chloroform extract and aromatic fraction was examined for absorption bands characteristic of DDT and dieldrin as well as of other pesticides. Each aromatic fraction was also subjected to gas chromatographic analysis (*7*). The results are summarized in Table 1.

The aromatic fraction of each sample producing infrared absorption bands characteristic of DDT also gave at least two of the three chromatographic peaks associated with technical grade DDT. Similarly, those aromatic fractions which generated infrared spectra characteristic of dieldrin also produced a chromatographic peak identical to that of commercial dieldrin. The carbon adsorption–infrared method is qualitative; however, by this method these compounds have been detected at a concentration of 10 μg per liter (*8*). Our experience has often indicated that sensitivities of less than 1 μg per liter can be obtained. In pilot studies with carbon (*9*) it has been reported that 98 percent of the DDT in an emulsion (5 mg/liter) is adsorbed and 80 percent is recovered by extraction. Since the aromatic subfraction, from which the infrared identifications were made, represents a concentration of 1 to 2 μg per liter in water, it is reasonable to assume that the concentration of pesticide was not appreciably greater than this value. Such concentrations are well below those known to be toxic to fish (*9*) or presumed to be hazardous to man (*10*).

DDT and dieldrin were not found in the drainage from many agricultural areas including the Mississippi and Missouri rivers. It is possible that these compounds were present in concentrations below those which can be detected by the screening method used. Moreover, according to Berck (*2*) chlorinated hydrocarbons are adsorbed on the suspended solids of river water. Thus, the silt common to some rivers may effectively remove pesticides from water.

The presence of DDT or dieldrin at 14 of 101 locations most probably represents runoff from localized upstream applications of these pesticides to land areas.

Table 1. Identification of DDT and dieldrin in river water in 1962. The figures in parentheses after the number of samples denote the beginning date of the first sample and the ending date of the final sample. Approximately one week was required to collect each sample.

NWQN station No.	River	Location	No. of samples	Substance identified
75	Susquehanna	Conowingo, Md.	4 (8/1–11/14)	DDT
12	Delaware	Phila., Pa.	4 (5/17–11/20)	DDT
48	Savannah	N. Augusta, S.C.	6 (5/4–12/16)	Dieldrin
120	Chattahoochee	Lanett, Ala.	2 (9/7–11/19)	DDT
99	Tennessee	Pickwick Ldg., Tenn.	6 (7/17–12/27)	DDT
46	Rio Grande	El Paso, Texas	1 (July and Sept.)*	DDT
71	Rio Grande	Brownsville, Texas	1 (11/19–12/4)	DDT
122	San Joaquin	Vernalis, Calif.	2 (8/29–10/24)	DDT
116	Sacramento	Greens Ldg., Calif.	3 (5/23–10/9)	DDT
89	Yakima	Richland, Wash.	3 (6/19–11/20)	DDT
112	Columbia	Northport, Wash.	1 (10/20–10/27)	DDT
10	Columbia	Wenatchee, Wash.	1 (Oct.–Dec.)*	DDT
9	Columbia	Pasco, Wash.	3 (10/5–12/13)	DDT
81	Columbia	McNary Dam, Ore.	1 (11/5–11/19)	DDT

* Composite.

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4. The National Water Quality Network, established in 1957, is operated by the Public Health Service as a cooperative project with federal, state, and local agencies as well as industry. It now consists of 128 geographically dispersed, surface water sampling stations located on major rivers and the Great Lakes. Some 175 individual agencies participate in its operation and are herewith acknowledged as contributors to this report. Analytical work is accomplished in the field and in Network laboratories located in Cincinnati, Ohio, and includes organic, radiological, biological, microbiological, and physical analyses.
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7. Each aromatic fraction was dissolved in 0.2 ml CCl_4. Volumes ranging from 0.2 to 0.5 μl were injected into a Perkin-Elmer model 800, flame ionization, dual column, temperature controlled, gas chromatograph equipped with a 91 cm, 0.32-cm O.D. stainless steel column packed with 5-percent Dow-11 on 60- to 80-mesh Chromosorb W. Helium, the carrier gas, was fed at 60 ml/minute. Corroborating identifications of DDT were made by increasing the temperature between 125° to 200°C at 5°C per minute. Dieldrin was determined isothermally at 170°C.
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24 June 1963

Acetylcholine-like Activity in Sciatic Nerve

Abstract. *Extracts of sciatic nerve exhibit acetylcholine-like activity that is only partly attributable to acetylcholine. The extracts show relatively greater activity on the rectus abdominis muscle of the frog than on the ileum of the guinea pig. To prevent the action of the extract on the frog rectus abdominis muscle, a greater concentration of* d*-tubocurarine is required than is necessary to prevent the action of known acetylcholine.*

The different sensitivities of the ileum of the guinea pig and the rectus abdominis muscle of the frog to acetylcholine and its congeners have been exploited to show the presence of choline esters other than acetylcholine in tissue extracts (*1*). In measuring acetylcholine-like activity in acetone extracts of sciatic nerve prepared (*2*) from rabbits treated with physostigmine, we observed that a higher value was obtained in measurements on the frog muscle than that obtained on the guinea pig ileum (*3*). When assayed on the frog muscle, the sciatic nerve contained activity equivalent to 2.55 ± 0.43 μg of acetylcholine per gram of fresh weight; on the guinea pig ileum, 1.57 ± 0.41 μg acetylcholine per gram of fresh weight.

Examination of subcellular fractions (*4*) obtained by differential centrifugation of the sciatic nerve revealed another discrepancy. When assayed on the frog muscle, the acetylcholine-like activity was almost equally divided between the soluble fraction of the nerve, which contained 47.3 ± 4.6 percent of the activity, and the total particulate material. When assayed on the ileum, 79.3 ± 0.60 percent of the activity was in the soluble fraction.

These observations suggested that a choline ester other than acetylcholine may be present in the sciatic nerve extracts, since the frog muscle is sensitive to low concentrations of many choline esters, whereas the guinea pig ileum is especially sensitive to acetylcholine and relatively insensitive to other choline esters (*1*).

An alternative explanation—that the extracts contained material that either sensitized the frog muscle to acetylcholine or prevented the response of the ileum to acetylcholine—was ruled out by showing that on both muscles the effects of the extract (boiled or unboiled) and of known acetylcholine are additive.

The following evidence is consistent with the idea that the guinea pig ileum responded to acetylcholine (or a closely related compound) in the nerve extract. The slopes of the dose-response curve for the extract and for acetylcholine were identical.

The effects of equally active doses of the extract and of acetylcholine were prevented by atropine at the same dose, 6 μg/liter; neither pyrilamine maleate, 5 μg/liter, nor lysergic acid diethylamide, 50 μg/liter, which are, respectively, antagonists of histamine and 5-hydroxytryptamine, affected the activity of the extract or of acetylcholine. The atropine-treated ileum recovered its sensitivity concurrently with both the extract and acetylcholine. The action of the extract on the ileum, like that of aceytlcholine, was destroyed both by boiling for 10 minutes at *p*H 10 and by incubation with acetylcholinesterase prepared from bovine erythrocytes. Finally, when the extract was chromatographed on paper (*5*), all material with activity was eluted in the area, R_F 0.00 to 0.20, that included known acetylcholine (R_F 0.12).

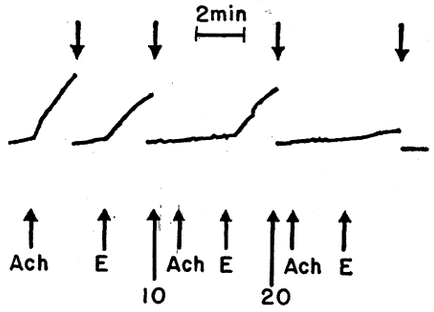

Fig. 1. The effect of *d*-tubocurarine on the action of 200 ng of acetylcholine (Ach) and an extract (E) equivalent to 90 mg of sciatic nerve on the frog rectus abdominis muscle. Numbers refer to μg of *d*-tubocurarine chloride pentahydrate, which was permitted to act for 10 minutes. The upper arrows indicate a change of bathing solution.

That the rectus abdominis muscle of the frog responds to substances other than acetylcholine in the sciatic nerve extract is evident from the following observations. The action of the extract was not prevented by a concentration of *d*-tubocurarine that prevented the action of acetylcholine (Fig. 1). Doubling the concentration of *d*-tubocurarine, however, did prevent the action of the extract (Fig. 1). The differential action of these two concentrations of *d*-tubocurarine was noted in six of seven experiments. Furthermore, only 66.5 ± 15.6 percent of the material acting on the frog muscle was recovered in the eluate of material with R_F 0.00 to 0.20; the lower dose of *d*-tubocurarine completely prevented the action of this eluate.

The extract of sciatic nerve was inactive on both preparations if the rabbits were not treated with physostigmine. It is likely that the extract contains, besides acetylcholine, another choline ester to which the frog rectus abdominis muscle is especially sensitive. Other than acetylcholine, the only known, naturally occurring choline ester with potent activity on this preparation is propionylcholine, but since this compound has an R_F value of 0.15 in the solvent system used in this work, it would be included in the examined eluate and hence cannot be the active material (*6*).

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