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Fluorocarbon Toxicity: Past, Present, Future

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Synopsis—The history of the development of fluorocarbons and their toxicity is reviewed. It is shown that the toxicity of fluorocarbons is related to the strength of the C–F bond. Animal data on the acute inhalation, topical, and oral toxicity of a variety of fluorocarbons are described. Chronic toxicity data are more sketchy but allow greater insight into the biological activity of these compounds. Finally, some recommendations are made for further toxicological testing of fluorocarbons.

INTRODUCTION

Mankind has attained dominance on this planet essentially because he was—and is—dissatisfied with the prevailing state of affairs. His early fires enabled him to keep warm and, at the same time, control part of a hostile environment. As he grew in knowledge new combinations of elements he wrested from the material world supported increasingly complex human societies; and, where cleverness was combined with knowledge, dominant groups attained hegemony. The history of technology has been to hurdle obstacles which in preceding periods blocked progress. The development of the field of organic chemistry is a striking example of man's intrusion into a naturally stable and at one time inviolable domain. The chemist's success in combining carbon and fluorine underscores man's continuing triumph over nature in view of the rarity of the C–F bond on earth and its strength. Thus, the story of the fluorocarbons is one of purely human technology; and organic fluorine

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compounds, therefore, are in a way not organic in the sense that they are unnatural. On this basis, it is not surprising that a cardinal property of organic fluorine compounds is their extraordinary stability, related to the high bond strength and short interatomic distance between carbon and fluorine.

Fluorine is like hydrogen in that it is not just another substituent for carbon; rather, it forms a whole system of carbon compounds now generally called fluorocarbons. Both hydrogen and fluorine are univalent; and while fluorine is somewhat larger than hydrogen it is small enough to occupy simultaneously all four valence positions of carbon. This is a feat which is accomplished only with difficulty in the case of other more bulky halogens. Nevertheless, the fluorocarbons are significantly different from the hydrocarbons. The greater volume of fluorine, as compared to hydrogen, and the greater bond strength of the C–F union (rupture energy = 114 kcal), contrasted with that of C–H (rupture energy = 93 kcal), provide a protective integument to the carbon-carbon skeleton, resulting in highly stable compounds among the various fluorocarbons. The perfluorinated alkanes highlight this attribute. Chemicals such as CF_4, $F_3C—CF_3$, $F_3C—CF_2—CF_2—CF_3$, and $\underline{CF_2—CF_2—CF_2—CF_2}$ are nonflammable and have virtually no useful synthetic properties. Their durability does, however, make them important commodities of commerce.

Attachment of functional groups or other atoms to a fluorocarbon structure yields classes of compounds, the properties of which may be profoundly influenced by as little fluorine as 1 F atom per molecule. It is notable that the C–F link is more stable than the C–Cl bond (rupture energy = 72 kcal); however, in chlorofluorocarbon, the F atom stabilizes vicinal C–Cl bonds and relieves the steric strain imposed on the molecule by relatively voluminous Cl atoms. The relative greater stability of CCl_3F over that of $CHCl_3$ is illustrative of the settling influence of 1 F atom.

It is natural to relate the foregoing chemical features of the C–F compounds to their biological properties. When this is done it is patent that the high order of stability of fluorocarbons is the dominant biological feature. Chemical stability explains a specific biologic action, or, in contrast, it may be the reason for lack of biological action. Illustrating the first case is the now classical lethal synthesis (1) in which the C–F bond of ω-fluoroacetate is refractory to enzymatic dehalogenation. It consequently enters the tricarboxylic acid cycle intact and passes unscathed

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through to fluorocitric acid. At this point, steric hindrance precludes the action of the enzyme aconitase so that the next step in the chain, conversion to aconitic acid, is blocked. A major part of the toxicity of ω-fluoroacetate, and compounds degraded thereto, is the result of a metabolic blockade caused by a highly stable interloper.

On the other hand, lack of biologic activity is demonstrated by the fluoroanalogues of the chloromustards:

I II

The chlorocompound (I) is a potent vesicant owing to removal of labile Cl which releases a highly active residue. It is the latter which is biologically active, not the fugitive halogen. The fluoroanalogue (II), on the other hand, is inoffensive due to firm C–F bonds and concomitant shielding of otherwise active loci on the molecule.

In the same vein, C–F cohesion is undoubtedly the reason for the low toxicity of the fluoroalkanes. This class is probably the most important group at present in view of its wide use in commerce. Fluoroalkanes are used as refrigerants, propellants, dielectric agents, fire extinguishing compounds, solvents, and freezing agents.

Yet while C–F firmness insures stability and favors low toxicity, it does not imply complete lack of biological activity. Trifluoroiodomethane, tagged with I^{131}, may be safely used to trace the circulatory paths of the brain, thereby demonstrating that, while the compound undergoes no detectable biological degradation, it is biologically active in penetrating the blood-brain barrier. Similar considerations apply to fluorinated anesthetics in which metabolic degradation is not a *sine qua non* for the narcotic activity. The story is much the same for other fluoroalkanes—low in toxicity but active in the organism to varying degrees and for different reasons.

Commercial History of Fluorocarbons

Because of their commercial importance in cosmetics the fluoroalkanes are of interest to cosmetic chemists and naturally the general public. Historically, three developments reflect the increasing importance of knowledge of the toxicity of the fluorocarbons and particularly the fluoroalkanes. The first was the advent in the 1920's of mechanical refrigeration. Early refrigerants were unsatisfactory. Ethylene was flammable; SO_2 and NH_3 were corrosive and toxic. It was about the

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same time that Le Beau and Damiens (2) prepared CF_4, the simplest perfluorocarbon. Shortly thereafter, CCl_2F_2 was prepared specifically for refrigerant use. This compound provided not only the requisite temperature-pressure relationships but also nonflammability and low degree inhalation toxicity. So convinced of the low toxicity of CCl_2F_2 was Thomas Midgley that at the 1930 American Chemical Society Meeting, to demonstrate nonflammability, he inhaled CCl_2F_2 and on exhalation extinguished a burning candle.

The second historical development is indicated by the publication in 1928 by F. Lehmann (3) of experiments on the pharmacological action and influence of the trifluoromethyl group. This was the first in the development of a number of fluorinated compounds which possessed pharmacological activity.

Thirdly, and probably eclipsing the first two, was the synthesis of a number of derivatives of ω-fluoroacetates emerging from chemical warfare research during World War II. Wartime security obscured the development of other fluorocarbons; but during this period, the first fluoropolymer, a tetrafluoroethylene resin, was developed by Du Pont. This resin, because of its resistance to the most aggressive chemical agents, was largely committed to the atomic energy programs of that era. Post-war commercial development of fluorocarbons saw a decline of the toxic fluoroacetate compounds (except as pesticides) and a rapid rise of polyfluorinated organic compounds because of their high stability and low toxicity. The major fluorocarbons in this extensive market penetration have been the fluoroalkanes.

Prominent among the uses cited above is the aerosol propellant market. Nonfood aerosols have shown a phenomenal growth, doubling since 1960 to about 1.45 billion units in 1965. Food aerosols are not as large in volume (60–70 million units yearly), but applications in this area are increasing. For example, chloropento-fluoroethane and octafluorocyclobutane, the fluoroalkanes, Freon® 115 and Freon® C-318, are approved by FDA for food propellants.

Acute Inhalation Toxicity

Because of rapid developments on the marketplace and the increasing likelihood of consumer exposure, the need for toxicological information has grown. Midgley's dramatic demonstration fortunately did not set a precedent for the conduct of toxicity experiments, although it did focus attention on the inhalation hazard as primary in the early studies on the fluoroalkanes used in refrigeration units and also as fire extinguishing

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compounds. Accordingly, to evaluate the practical hazard, the first toxicity designs were predicated on the brief exposure to a relatively high concentration that a householder, a refrigeration repairman, or a fire fighter might encounter. The Underwriters' Laboratories designed an inhalation study in which groups of 12 guinea pigs were exposed to a graded series of concentrations of the test compound. At each sampling time (five minutes, 30 minutes, one hour, and two hours) three guinea pigs, if surviving the exposure, were removed from the chamber for observation or pathologic examination of vital organs within a two to ten day period after exposure. In addition to the Underwriters' work on the then new fluorocarbon refrigerants, they also included refrigerants in use at the time, e.g., ammonia and sulfur dioxide. The comparison provided by this inclusion was invaluable in comparing novel refrigerants with materials already in use. Studies on several refrigerants in the decade from 1931 to 1941 led to a classification system which has been used to grade the safety of new compounds. In this system, class 1 compounds are the most toxic, causing death or serious injury when inhaled continuously by guinea pigs at 0.5 to 1.0% (by vol.) for five minutes, e.g., sulfur dioxide. Class 2 is comprised of materials judged hazardous (on the same basis) when concentrations of 0.5 to 1.0% (by vol.) are inhaled for thirty minutes, e.g., ammonia and methyl bromide. Classes 3 and 4 are distinguished by an exposure which proves toxic at 2.0 to 2.5% for one hour and two hours, respectively, e.g., carbon tetrachloride and chloroform (class 3), dichloroethylene and methyl chloride (class 4). Class 6 includes materials which during a two-hour exposure at 20% produce no injury, e.g., dichlorodifluoromethane, dichlorotetrafluoroethane, and monobromotrifluoromethane. Classes 4-5, 5a, and 5b are not precisely defined by the above parameters; they encompass compounds more toxic than those in class 6 but less toxic than class 4 materials, e.g., trichlorotrifluoroethane and methylene chloride (class 4-5); trichlorofluoromethane, chlorodifluoromethane, and CO_2 (class 5a); ethane, propane, and butane (class 5b).

The primary purpose of this system was to gauge the acute inhalation hazard, and it was evident that the fluoroalkanes possess low toxicity on the basis of the above grouping. Subsequent work has confirmed this judgment, and experience in the production and marketing of fluoroalkanes attests to a low order of toxicity for man. Toxicological data, Underwriters' Class, and Threshold Limit Values (TLV) of the American Conference of Governmental Industrial Hygienists for a number of fluoroalkanes are summarized in Table I.

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As far as threshold limit values (TLV) are concerned, the first industrial hygienic standards for airborne contaminants proposed for some fluoroalkanes by Cook (4) in 1945 ranged from 5000 ppm for dichlorofluoromethane to 100,000 ppm for dichlorodifluoromethane. The values were subsequently lowered to 1000 ppm not because of toxicity but on the premise that good engineering practice demands that no environmental contaminant should be allowed to exceed 1000 ppm (on an eight-hour average), an engineering standard rather than an indicator of tox-

Table I

Inhalation Toxicity of Fluoromethanes

		Exposure				
Group	Structure	Conc. (%)	Hours	Fatal (+ or −)	U.L.[a] Class	TLV[b]
A	$CHCl_3$	2.0	2	+	3	50
	$CHCl_2F$	10.0	1	+	4–5	1000
	$CHClF_2$	20.0	2	−	5a	(1000)
	CHF_3	20.0	2	−	6[c]	(1000)
B	CCl_4	2.0	2	+	3	10
	CCl_3F	10.0	2	−	5a	1000
	CCl_2F_2	20.0	2	−	6	1000
	$CClF_3$	20.0	2	−	6[c]	(1000)
	CF_4	20.0	2	−	6[c]	(1000)
C	CH_3Cl	2.0	2	+	4	100
	CH_2Cl_2	5.0	2	+	4–5	500
	$CHCl_2F$	10	1	+	4–5	1000
	CCl_2F_2	20	2	−	6	1000

[a] U. L. signifies Underwriters' classification. The higher the value the lower the toxicity.

[b] TLV = Threshold Limit Value published by the American Conference of Governmental Industrial Hygienists, 1966 Values. Figures in parentheses indicate provisional values.

[c] Based on data from Haskell Laboratory.

icity or health hazard. Only carbon dioxide has a TLV greater than 1000 ppm, *viz*, 5000 ppm. Thus, while the TLV of 1000 ppm is commensurate with the low toxicity of the fluoromethanes, it is primarily a good housekeeping standard. Industrial experience has established its validity and utility.

Much of the early toxicity work on the fluoroalkanes was purely taxonomic, i.e., the assignment of individual compounds to categories. Toxicology admits of no foolproof deductive method by which the toxicity of individual compounds can be determined from chemical structures or properties. This applies especially to the fluorocarbons. In spite of the strength of the C–F bond and its intramolecular stabilizing

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influence, chemical stability is not a reliable or predictive determinant of toxicity. As data became available, however, a general principle of toxicity could be ascertained, and the data assembled in Table I illustrate this principle: a lower degree of toxicity corresponds with an increasing number of fluorine atoms in the molecule. All three groups, A, B, and C of Table I, reveal this, with possibly the most striking example evident in comparing CCl_4 with CF_4 of group B. The influence of fluorine and its interactions with other atoms in the molecule are also apparent in this compilation. As shown by the contrasting toxicities of $CHCl_3$ and $CHCl_2F$ (group A), the inclusion of even a single F atom effects a marked lowering of toxicity, explained possibly by the increased stability of neighboring Cl atoms (fostered by the C–F linkage) and subsequent intractability to enzymatic dehalogenation. Addition of further F atoms to this molecule produces continued reduction of toxicity. Group B provides similar evidence of this stabilizing force of F as compared to Cl in the methane series. Group C adumbrates yet another feature relating to the principle under discussion, namely, the interplay of hydrogen, fluorine, and chlorine. The substitution of Cl for H appears, in some instances, to lower toxicity, as may be seen by comparing CH_3Cl with CH_2Cl_2; and the replacement of H in CH_2Cl_2 and $CHCl_2F$ with F to give $CHCl_2F$ and CCl_2F_2, respectively, also eventuates in lowered toxicity. Thus, while it is sometimes true that increasing the Cl complement tends to decrease toxicity, fluorine is far more effective than chlorine, as illustrated by the lower toxicity of CH_2F_2 compared with that of CH_2Cl_2. Henne (5) in 1937 reported no toxic effects in guinea pigs inhaling 20% CH_2F_2 for hours, whereas 2% CH_2Cl_2 was fatal in two hours. The intermediate compound, CH_2ClF, assumes a mid-position so that the order of toxicity would be:

$$CH_2Cl_2 > CH_2ClF > CH_2F_2$$

This stabilizing attribute of F may also be noted in connection with bromine. The data of Table II indicate the following toxicity trend: $CBr_2F_2 > CBrClF_2 > CBrF_3$. From this sequence it may be concluded that the brominated compounds are more toxic than the chlorinated ones and the highly fluorinated are least toxic. It should not be inferred that the fluorination blocks the escape of a toxophoric halogen (Br or Cl). It is not the fugitive halogen atom which is offensive but the ensuing active residue. The dehalogenation of CH_3Br to form the alkylating CH_3-active group is illustrative. The CH_3-fragment reacts *in vivo* with sulfur-containing compounds, thereby producing biologically active com-

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Table II

Acute Inhalation Toxicity of Fluoromethanes Containing Bromine

Compound	Exposure		Lethal Concentration[a] (vol. %)
	Animal	Time (min)	
CBr_2F_2	Rat	15	5.5
$CBrClF_2$	Rat	15	32
$CBrF_3$	Rat	15	83

[a] Vapor mixed with air.

Table III

Acute Inhalation Toxicity of Several Fluoroethanes

Structure	Approximate Lethal Concentration		
	Vol. (%)	Exposure (hours)	Animal
$CCl_2F—CCl_2F$	1.5	4	Rat
$CClF_2—CCl_3$	1.5	4	Rat
$CCl_2F—CClF_2$	10	4	Rat
$CClF_2—CClF_2$	>20	8	Guinea pig
$CHCl_2—CF_3$	3.5[a]	4	Rat
$CClF_2—CHF_2$	>20	2	Guinea pig
$CClF_2—CF_3$	>80[b]	4	Rat
$CHF_2—CF_3$	>10	4	Rat
$CF_3—CF_3$	>80[b]	4	Rat

[a] LC_{50}.

[b] 80% fluorocarbon and 20% O_2.

Table IV

Comparison of Bromine and Chlorine in the Acute Inhalation Toxicity of Fluoroethanes

Compound	Lethal Concentration[a] (vol. %)
1. $CH_2Cl—CF_3$	25.0
2. $CH_2Cl—CHF_2$	7.5
3. $CH_2Br—CF_3$	11.7
4. $CH_2Br—CHF_2$	4.6

[a] Mice exposed for ten minutes.

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pounds. In this regard, CH_3Cl is not as active as CH_3Br; and, as would be anticipated, CH_3F is least active of the three. Thus toxicologically the order is: $CH_3Br > CH_3Cl > CH_3F$.

A correspondence of low toxicity with increasing fluorination is disclosed by inhalation experiments with fluoroalkanes of longer chain length. Table III summarizes relevant data on fluoroethanes, some with chlorine substituents; Table IV depicts the comparative roles of Cl and Br in fluoroethane (compare compound 1 with 3 and 2 with 4). In the latter table, the relation of H and F is evident from a comparison of compound 1 with 2 and of 3 with 4. Table V summarizes the acute inhalation toxicity for several fluoropropanes. Again, the principle of declining toxicity with increasing fluorination is observed. The toxicity of octafluorocyclobutane with its full complement of fluorine would seem to be the epitome of toxicological inertness demonstrated in Table VI.

Acute Oral and Topical Toxicity

In the decade when the fluoroalkanes were employed primarily as refrigerants and fire extinguishing agents, study of their acute inhalation toxicity seemed adequate to evaluate the safety for their intended use. However, expanding applications of these materials in pharmaceuticals, cosmetics, foods, and the aerospace industry necessitated a reappraisal of the basis for toxicological judgment. This, of course, reflects an evolutionary trend typical for any chemical or class of materials whenever penetration of the marketplace becomes a potential public health issue. In this way, fluorocarbons participated with other chemicals in society's requirement for a wider base on which to judge safety and health effects. Thus, uses of the fluorocarbons meant new routes of contact, and experimental procedures were adapted accordingly.

The first experiments at Haskell Laboratory in the 1950's were conducted with dichlorodifluoromethane, CCl_2F_2, and symmetrical dichlorotetrafluoroethane, $CClF_2—CClF_2$. For both of these the acute oral toxicity, as judged by single doses of the fluorocarbon in oil, was low. The approximate Lethal Dose (ALD) was CCl_2F_2 greater than 1000 mg/kg, $CClF_2—CClF_2$ greater than 2250 mg/kg. In other words, maximum feasible single doses were not sufficient to kill rats. Repeated doses of CCl_2F_2 at 430 mg/kg and $CClF_2—CClF_2$ at 1300 mg/kg administered daily for ten days produced no signs of toxicity or pathological change in male rats. Organ weights were within control limits, and histology of major organs, including the liver, was unaffected by these dosages of CCl_2F_2 or $CClF_2—CClF_2$. Quevauviller (6) confirmed these observa-

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Table V

Toxicity of Several Fluoropropanes for Mice Exposed for Ten Minutes

Structure	Anesthetic Concentration (%)	Approx. Lethal Concentration (%)
$HCF_2—CF_2—CClF_2$	10	20
$HCF_2—CF_2—CBrF_2$	4	10
$HCF_2—CF_2—CHClF$	2.5	3
$HCF_2—CF_2—CHCl_2$	0.5	2
$CClF_2—CF_2—CH_2F$	10	15
$CClF_2—CF_2—CHF_2$	10	20

Table VI

Toxicity Studies on Octafluorocyclobutane (OFCB)

Exposure				
OFCB Concentration (vol. %)	Duration (hours)	No. of Exposures	Animals	Toxic Effects
80[a]	0.25	1	Mice	None
80[a]	4	1	Rats	None
1.0	6	19	Rats	None
10	6	4	Rats, mice, guinea pigs, dogs	None
10	6	90	Rats, mice, rabbits, dogs	None

[a] 80% OFCB + 20% oxygen.

Table VII

Acute Oral Toxicity of Various Fluoroalkanes

Compound	ALD (mg/kg)[a]
CCl_2F_2	> 1,000[b]
$CHCl_2CClF_2$	7,500
CCl_2FCCl_2F	>25,000
$CClF_2CCl_3$	>25,000
$CClF_2CCl_2F$	45,000 (LD_{50} = 43,000 mg/kg)
$CClF_2CClF_2$	>2,250 mg/kg[b]

[a] Rats, ten to fourteen day survival period.

[b] Maximum feasible dose of fluorocarbon dissolved in peanut oil.

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tions on $CClF_2$—$CClF_2$ by showing that a daily dose of 2000 mg/kg was not toxic for rats receiving the dosage for twenty-three to thirty-three days.

In work by Haskell Laboratory, chloropentafluoroethane, $CClF_2$—CF_3, dissolved in cottonseed oil, was administered orally to rats five times a week for two weeks. Five milliliters of test solution was given at each treatment, and the individual daily doses were in the range of 140–172 mg/kg. A group of control rats received the oil alone. During the treatment period, there were no differences between the test and control rats with respect to appearance and rate of weight gain. This was also true of a recovery period of two weeks. Test and control rats were killed four hours or fourteen days after the tenth dose. Gross and microscopic examination of major tissues revealed no change attributable to $CClF_2$—CF_3. Therefore, under the conditions of this experiment, there was no evidence of cumulative toxic effects exerted by $CClF_2$—CF_3. This work demonstrated a low order of toxicity *via* the oral route of administration both for acute and short-term repeated dosing. Hepatoxic chlorinated materials, CCl_4 and $CHCl_3$, would produce liver cell injury at comparable dosage. Table VII summarizes the acute oral toxicity of several other fluoroalkanes.

Points of contact other than oral are a subject of consideration, and toxicity data on the symmetric and asymmetric isomers of tetrachlorodifluoroethane and of 1,1,2-trichlorotrifluoroethane illustrate what may be required to establish a safe use of these fluoroalkanes as solvents. Data on isomers of tetrachlorodifluoroethane are shown in Table VIII. The two isomers were shown by this work to have a low order of acute oral, dermal, and inhalation toxicity. The Approximate Lethal Dose for rats by the oral route is greater than 25,000 mg/kg for both isomers.

On skin application, rabbits exhibited local irritation but did not succumb nor exhibit any systemic response to either isomer, although a maximum feasible dose was applied. These were greater than 7500 mg/kg of CCl_2F—CCl_2F and greater than 11,000 mg/kg of $CClF_2$—CCl_3. Application of the symmetric isomer, CCl_2F—CCl_2F, to the skin of guinea pigs produced mild irritation but no skin sensitization. It was mildly irritating to the rabbit eye without damaging the cornea or iris. Washing the eye with water reduced the duration of irritation. These experiments demonstrated that the biological action of the two isomers was similar at the same dose levels. Orally, the two compounds acted on the gastrointestinal tract and produced a mild liver reaction at high doses without histological injury. Repeated oral doses of both isomers,

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Table VIII

Comparison of the Toxicity of the Symmetric and Asymmetric Isomers of Tetrachlorodifluoroethane

Symmetric Isomer ($CCl_2F—CCl_2F$)	Asymmetric Isomer ($CClF_2—CCl_3$)
Oral Toxicity	
Approximate lethal dose (rat)	
>25,000 mg/kg	>25,000 mg/kg
Diarrhea, weight loss at 11,000 mg/kg and above	Diarrhea, weight loss at 11,000 mg/kg and above
Liver and major organs normal histologically	Slight increase in liver weight at 25,000 mg/kg
	Liver and kidney normal histologically
Ten-day repeated (rat)	
5000 mg/kg daily	5,000 mg/kg daily
Mortality: 1/5; diarrhea; initial body weight loss; tremor inactivity; slight increase in liver weight, slight reversible histological change in liver	Mortality: 0/6; diarrhea initial body weight loss; tremor inactivity; slight increase in liver weight; liver normal histologically
Dermal Toxicity	
Approximate lethal dose (rabbit)	
>7500 mg/kg	>11,000 mg/kg
Skin erythema; no signs of systemic toxicity; no histological changes in major organs	Severe skin irritation in ethanol; weight loss; histological change in skin musculature, none in major organs
Inhalation Toxicity	
Approximate lethal concentration (rat)	
15,000 ppm (vol.)	15,000 ppm (vol.)
Respiratory and CNS effects; slight liver change histologically	Respiratory and CNS effects, slight to moderate liver changes histologically

5000 mg/kg daily for 10 days, also act similarly: These resulted in a slight reversible liver response, as judged histologically.

Trichlorotrifluoroethane, $CCl_2F—CClF_2$, as indicated by rat studies, has a low order of oral toxicity with an LD_{50} of 43,000 mg/kg. Odou (7) has administered a massive oral dose to a female dog lightly anesthetized with sodium pentobarbital. The total dose given was 92,000 mg/kg, and death occurred in one and one-half hours. Some dogs have survived oral doses of this magnitude, and Odou (7) reported that a human under anesthesia accidentally received about 1 l of cold $CCl_2F—CClF_2$ in the stomach. This produced vomiting and transient cyanosis. The individual survived and reported only severe rectal irritation and diarrhea for three days thereafter.

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Trichlorotrifluoroethane is also low in toxicity, as judged by skin contact studies with rabbits. Application of $CClF_2$—CCl_2F to the skin of rabbits gave an ALD greater than 11,000 mg/kg. The only effect was local irritation of the skin at the site of application, and histology disclosed alterations in the dermis and adjacent connective tissues. There were no systemic changes attributable to treatment. Applications of CCl_2F—$CClF_2$ at the very high dose of 5000 mg/kg each day for five days resulted in fluctuations in weight and damage to the skin which were evident grossly and histologically. Slight liver changes were observed microscopically, but no other systemic changes were disclosed. When instilled undiluted into the eyes of three rabbits and washed out with water twenty seconds later, $CClF_2$—CCl_2F produced mild, transient conjunctivitis in one rabbit only, and the others showed no reaction. Instillation into rabbit eyes without water washing produced minimal corneal dullness in only one rabbit, which subsided in forty-eight hours.

Similar results from eye studies on dichlorodifluoromethane were reported by Downing and Madinabeitia (8) citing work conducted at Haskell Laboratory. In this study a 50% solution of CCl_2F_2 in refined mineral oil was sprayed into rabbits' eyes from a distance of about 15 cm. Controls consisted of rabbits receiving mineral oil alone. The animals receiving the fluorocarbon with mineral oil in the eye showed the same eye reaction as the controls did. In both groups, slight conjunctival irritation developed, but this disappeared in twenty-four hours.

Quevauviller *et al.* (9) and Quevauviller (6) have investigated the effects of various fluoroalkanes on the skin, tongue, soft palate, and auditory canal of rats; the eye of the rabbit; and the speed of healing of wounds and burns in the rat. Five materials were evaluated by these tests: CCl_3F; CCl_2F_2; CCl_3F and CCl_2F_2 mixed; CCl_3F and $CHClF_2$ mixed; and $CClF_2$—$CClF_2$. The animals were treated once or twice daily, five days a week for five or six weeks. In the case of wounds and burns the treatment was continued until healing was complete. The compounds or mixtures were released from pressurized containers for five or ten seconds from a distance of 10–20 cm onto the experimental site.

The skin of the rats became irritated, evincing an edema and slight inflammatory reaction. The reactions were most marked with the $CCl_3F/CHClF_2$ mixture and $CClF_2$—$CClF_2$. Older rats were more severely affected than younger rats.

The application of the five test substances to the tongue, soft palate, and auditory canal of rats produced no significant abnormalities. In one

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rat treated with $CClF_2$—$CClF_2$ the auditory canal showed desquamation of the epithelium and inflammation.

The rabbit eye reacted to the five materials with hyperemia and lacrimation. There was an inflammatory reaction of the eyelid noted in rabbits exposed to CCl_3F and $CClF_2$—$CClF_2$. There were no other histologic alterations.

The healing of wounds and burns made experimentally on the skin of rats was retarded, compared to controls. There appeared to be little if any difference in this retardation among these five compounds or mixtures evaluated.

Chronic Toxicity

Experiments to evaluate the chronic toxicity have not been extensive, nor do they approach the refinement achieved in the investigation of food additives. Clayton (10) has reviewed the situation with respect to chronic effects of fluoroalkanes, and it appears that experimental designs for evaluating this aspect of toxicity are now capable of arriving at an evaluation of safety for particular uses.

Prior to 1960, chronic toxicity studies were concerned primarily with effects of repeated inhalation. This was natural because the majority of fluoroalkanes in use were gases at ambient temperature, and the applications involved an inhalation hazard (10). In 1960, however, the food additive problem was broached by DuPont's introduction of octafluorocyclobutane (OFCB) as a food propellant (11). In this instance, because of the compound's extremely low solubility in body fluids and the technical impracticability of "feeding a gas," it was agreed by the Food and Drug Administration that repeated inhalation exposures employing a variety of mammals six hours a day for 90 exposures at 100,000 ppm would suffice to demonstrate the safety of OFCB as a food propellant. Single and short term repeated exposures had demonstrated the "inertness" of OFCB (Table VI). The 90 exposures disclosed no effects, clinically or pathologically, on the animals inhaling OFCB.

A similar problem was faced when chloropentafluoroethane (CPFE) was proposed as a food propellant. As with OFCB, a 90 exposure 100,000 ppm test revealed the compound's biologic inactivity. However, CPFE was also administered in cottonseed oil solution to male rats daily for ten days (see below). No evidence of toxicity was discerned from this experiment, which added to the assurance of safety gained by the inhalation phase of the investigation.

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For the evaluation of the inhalation hazard peculiar to life in a nuclear submarine which may operate continuously submerged, Siegel (12) conducted continuous exposures of rats, guinea pigs, rabbits, monkeys, and dogs for a ninety-two-day period to 810 ppm of dichlorodifluoromethane. Some liver changes were observed, but there was no clinical intoxication, and hematological values were within normal limits.

Biological Activity

What emerges from these chronic studies is evidence of a low degree of biologic activity of the fluoroalkanes investigated. Furthermore, it is apparent that each of the studies was specially designed for a particular need. There are, of course, decided advantages to this approach, which answers specific questions. However, there remains the question of the mechanism of action of the fluoroalkanes. Generally these compounds act on the central nervous system with a mild effect, if any, on the liver. Each of these actions is dependent largely on the number of fluorine atoms in the molecule—a higher complement being associated with lower activity. This is usually related to the strength of the C–F bond. Assurance of low toxicity is therefore to be inferred from these observations, and this is of great practical importance to the manufacturer and consumer. On the other hand, with the increasing penetration of fluoroalkanes in the marketplace, there is a growing need to study the body's handling of this class of fluorochemicals to elucidate modes of biological action.

Some insight into the biologic activity of the fluorocarbons may be gained by considering a few of the salient features of the biology and chemistry of the halogenated hydrocarbons and comparing them with those of the fluorocarbons. Dehalogenation in aliphatic systems is well established for many compounds, and it is the basis of the mechanism of action of a number of halogenated compounds (13). Both enzymatic and nonenzymatic processes have been observed in this regard. It is noteworthy that the biologic activity is not related to the halogen moiety but rather to the halogen-free part of the molecule. As previously cited, the dehalogenation of CH_3Br to form the methylating residue $CH_3\cdot$ is typical of this concept. It is the methyl radical which can react with sulfur-containing compounds *in vivo*, thus producing a biologically active compound. CH_3Cl is not as active as CH_3Br, and CH_3F is even less active than CH_3Cl. The labile halogens of $COCl_2$, which gives rise to an active $-\overset{\oplus}{\underset{|}{C}}=\overset{\ominus}{O}$ group, are another example. The

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corresponding fluorocompounds CH_3F and COF_2 exhibit much lower activity than the chlorocompounds, largely due to a firm C–F bond.

Further addition of F to the molecule tends to stabilize carbon-halogen bonds. It is known that di- and tri-halogenated compounds differ markedly from the monosubstituted analogues owing to shortened interatomic distance and increase in steric effects from the added bulk in the former compounds. These kinds of molecular changes result from increasing fluorination and are probably the basis for the low toxicity of the highly fluorinated anesthetics, i.e., CF_3—CHBrCl (halothane), CH_3—O—CF_2—$CHCl_2$, CF_3—CHBrF (teflurane), HCF_2—CF_2—CH_2Cl, and HCF_2—CF_2—CH_2Br (14). Although dehalogenation of these may occur it would appear to be of minor significance. The anesthetic activity of these materials may be best explained by the formation of "physical adducts" according to Chenoweth and McCarty (13) and is comparable to the action of nitrogen and xenon. These inert materials may become part of *in vivo* "crystal" formations in accord with Pauling's hypothesis (14). Another aspect of these postulated *in vivo* actions is the requirement of binding forces and a dipole moment. Completely halogenated molecules or partially halogenated, symmetrical compounds, because of the lack of the requisite dipole function, would not readily form "crystals" and therefore in most cases would be poor anesthetics. CCl_4 is a notable exception, although CF_4 certainly is not. Hexafluoroethane and monochloropentafluoroethane are poor anesthetics, possibly because of their symmetrical morphology. Hydrogen situated at the end of a three-carbon chain enhances chemical stability and anesthetic potency, whereas a centrally placed hydrogen exerts the opposite effect (15).

Although highly fluorinated compounds may be poor anesthetics, they are active in sufficiently high concentration. The mechanism of action in these instances may be akin to the physical adducts alluded to above; however, this remains speculative until relevant studies are conducted. The mild hepatotoxic action of some of the chlorofluorocarbons falls into the same category. The idea that lipophilic properties of halogenated compounds influence biologic activity, as in the preceding hypotheses, must be regarded as a chance correlation. Any relation between this physical property and biological effects is uncertain.

Future Research

It is evident at the present stage of knowledge of the toxicology of the fluorocarbons that experiments to elucidate biologic mechanisms

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are needed. The following format of investigation is suggested which is designed to bring together relevant toxicological facts and mechanism of action.

I. Preliminary Toxicity Tests
   (a) Designed to determine single dose toxicity for several routes of administration employing different mammalian species
   (b) Observations of clinical response and tissue change should be included
   (c) Objective of this phase is to characterize the toxicity and to discern target organs or systems

II. Repeated Dose Studies
   (a) Designed to ascertain cumulative effects
   (b) Duration, routes, and species should be sufficient to provide adequate information for intended use
   (c) Clinical observations and pathology should be included

III. Mechanism Studies
   (a) Designed to determine major routes of excretion and excretory products for mammals
   (b) Determination of *in vivo* handling (intermediary metabolism)
   (c) Determination of the mode of action
   (d) Conduct of appropriate human metabolism studies

IV. Chronic Toxicity
   (a) Designed to ascertain lifetime effects, utilizing several species of animals
   (b) Design based on use pattern in terms of duration and kinds of contact
   (c) Observations included in lifetime studies should be based on knowledge derived from mechanism experiments. Objective is to determine any alteration in mechanism of action during the various phases of the life cycle

It is not the intention of this schema to impose undue strictures on the investigator nor to provide a regimen to be followed religiously by the amateur. Parts III and IV should be weighted differently depending upon usage of a product. Part III would seem to take priority, however, if intelligent extrapolations are to be made with reference to public health. While mechanism studies may be minimized in the face of a clear necessity to conduct prolonged and involved chronic studies,

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they should not be completely by-passed. The present status of the toxicology of the fluorocarbons definitely places mechanism studies in first rank in future investigations.

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