The Discovery and Analysis of PFAS ('Forever Chemicals') in Human Blood and Biological Materials

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Abstract

This paper reviews the development of analytical techniques used prior to the early 1980s in the identification of organofluorine compounds in human blood. The compounds of interest are industrial products, the so-called Forever Chemicals, which are stable long-chain per- and polyfluoroalkyl substances (PFAS) that are highly resistant to breakdown. Because of their special properties they have been used since the 1950s in a wide range of commercial and domestic products. Academic research in the 1960s and 1970s that originally focused on fluoride in dentistry and human health led to the finding that PFAS were present in the blood of residents of several cities in the United States. This, and concerns over their toxic properties, encouraged industrial research into analytical methods for their detection. Notably, and because long-chain PFAS are nonvolatile, special techniques were developed for analysis by gas chromatography.

Key words: Forever Chemicals; Organic fluorine in biological materials; PFAS; PFOA; PFOS; exposure to PFAS

INTRODUCTION

The concern over widespread contamination of water, and as a result of human blood, with so-called Forever Chemicals is a growing global issue, as reflected in recent years both in the media and scientific journals.[1] It is related to the uses and environmental releases of extremely stable long-chain per- and polyfluoroalkylated compounds containing multiple carbon-fluorine bonds, and is mainly associated with the first, and main, manufacturers, Minnesota Mining and Manufacturing (3M), and DuPont, and in the United States. Other sites of manufacture include several European countries, Japan, and Israel. These compounds are industrial substances, collectively referred to by the acronym PFAS. Perfluoroalkylated substances are fully fluorinated. Polyfluoroalkylated substances are not fully fluorinated. Those compounds discussed here are amphiphilic in nature: At the end of the carbon chain is a functional group, such as a carboxylic acid (-COOH), or carboxylate, or a sulfonic acid (SO_3H), or sulfonate.

PAFS persist in the environment, resisting degradation. They bioaccumulate, impacting on soil and water, and enter the food chain. Public exposure of the outcomes includes the 2019 film "Dark Waters" based on an article published in *The New York Times* during January 2016.[2] This focused on toxic releases from DuPont's manufacturing operations and subsequent litigation. More recently 3M internal documents made available during litigation have been reviewed.[3] In this litigation evidence for the ubiquitous presence of PFAS in human blood and sources of potable water, in addition to matters of toxicology, were prominent.[4] Other accounts in the media go back to the start of the present century. Thus in May 2000, the *Washington Post*/*Los Angeles*

[1] Krafft and Riess, "Per- and polyfluorinated"; Brunn, et al., "PFAS: forever chemicals."

[2] Rich, "The lawyer"; see also Bilott, *Exposure: Poisoned Water*.

[3] Lerner, "3M knew about the dangers"; Williams, "Toxic: 3M knew." See also, Nadi, et al., "The devil they knew."

[4] See, for example, *Reuters*, "3Ms $10.3 billion PFAS settlement."

Times included an article based on a 3M announcement which stated that as a result of contamination of blood in the general population the corporation would discontinue some of its stain-repellant products. According to 3M's senior vice president for research and development: "We have tested it [PFAS, in human blood] pretty widely – not only in this country but in other countries, as well – it's found in very low levels everywhere we test." These fluorinated compounds had been made and used since the 1950s, and 3M health officers had measured their total concentrations in the blood of its workers since the late 1970s, and subsequently of communities at some distance from sites of manufacture. 3M observed: "The surprise wasn't that it was in our workers – that's something we've known for a long time … It was a complete surprise that it was in the blood bank supplies."[5] The general concern has moved way beyond the United States. In the UK for example these compounds, along with microplastics, are investigated as emerging contaminants in potable water supplies. Widespread environmental exposure, occupational and otherwise, with particular reference to blood levels and threats posed to health by PFAS released into water, and present in industrial products, has stimulated major research campaigns. A significant source of water contamination are PFAS surfactants used in the formulation of tough, resilient aqueous film-forming foam (AFFF), manufactured by 3M. They have been widely employed at military bases and airport facilities for extinguishing fuel fires, and especially following aviation accidents.

The main advance in gathering knowledge about the exceptional scale and prevalence of PFAS in the environment came about at the turn of the twenty-first century through the use of electronic instruments with advanced analytical capabilities. These instruments enabled detection of PFAS in low part per trillion (ppt). As a result of ongoing health concerns, in 2009 the US Environmental Protection Agency (EPA) drew up a short term health advisory, and published its first validated method for PFAS compounds in drinking water, with detection limits of less than 2 ppt. How knowledge of these contaminants first came about, as described here, arose from experiments conducted in the 1970s by academic investigators.

PER- AND POLYFLUORALKYL SUBSTANCES (PFAS)

[5] Mayer and Brown, "3M to discontinue."

The most important per- and polyfluoroalkyl substances contain, as manufactured, linear or branched chains of generally eight carbons. In the case of eight-carbon linear chains they are derivatives of perfluorooctane. PFAS are extremely stable, a property that has been exploited in their applications, including for protection of household items and carpets, in addition to the firefighting materials.[6]

Because these compounds are characterized by strong fluorine-carbon bonds, the organic fluorine has been referred to as "fixed" fluorine, and also as "non-exchangeable" fluorine.

The first important commercial alkylated fluorocarbons were the chlorofluorocarbons, or CFCs, and hydrofluorocarbons, including the halocarbon refrigerants trademarked as Freon by Du Pont in 1931. CFCs were followed by organic compounds in which all the hydrogens attached to carbon were substituted by fluorine. They included the tough, nonflammable, and "non stick" polymeric material Teflon (PTFE), or polytetrafluoroethylene, invented by Roy J. Plunkett at Du Pont (later known as DuPont) in 1938.

By 1950, Du Pont was marketing organic fluorine compounds for other uses. For example, in 1950, a report appeared in *Chemical Industries* headed "Fluorineophyte: New Company in New Jersey Turns Out Polytrifluorochloroethylene Oils, Greases and Waxes." This new company purchased the monomer "from Du Pont which reportedly makes it by dechlorination with zinc of Freon… and polymerize it to various degrees for [its] basic products."[7] Also in 1950, *Chemical Industries* made known the availability of Du Pont perfluoroalkyl compounds, with 12 to 41 carbon atoms.[8]

[6] Naturally occurring organic fluorine compounds are extremely rare. Some are present in volcanic and geothermal emissions. See, for biosynthesis of fluoroacetate and 4-fluorothreonin, see O'Hagan and Harper, "Fluorine-containing natural products."

[7] "Fluorineophyte."

[8] *Chemical Industries*, August 1950, p. 266.

To synthesize the eight-carbon chain perfluoro compounds of interest here, Du Pont used, from 1970, the process of telomerization, involving a telogen (organoiodine compound) and a taxogen (tetrafluoroethylene), that participate in a series of addition reactions, to bring about radical polymerization.

The 3M method of manufacture, which began in around 1950, was based on the process of electrochemical fluorination invented by Joseph H. Simons at Pennsylvania State University in the 1930s. The organic substance to be fluorinated is placed in a cell containing liquid, anhydrous hydrofluoric acid. Fluorine is adsorbed on nickel fluoride at the anode, and hydrogen is evolved at the cathode. Fluorination is believed to take place by a free radical mechanism.[9] In the case of the starting material octanesulfonyl fluoride, one product is perfluorooctane sulfonyl fluoride (POSF), which is converted to perfluorooctane sulfonic acid (PFOS). The electrochemical process favours formation of both straight and (to a lesser degree) branched chains. 3M investigated PFAS and their derivatives for properties that could be applied in commercial products, and synthesized novel compounds in which various functional groups were introduced. Research on certain of these compounds showed that they strongly repelled water and oil. This led to the introduction in 1956 of 3M's Scotchgard and a variety of textile finishes. Certain PFAS were found to act as efficient surfactants, concentrating in the surface of liquids or on the surface of solids. Industrial surfactants serve as anti-foaming agents, as foam builders, as wetting agents, as emulsifiers, as dispersants, and as detergents.[10] From the mid-1960s, 3M PFOS were used extensively in the already mentioned firefighting foams, performance products formulated as complex systems of perfluorochemical surfactants containing emulsifiers. When formulated with polyisocyanates, perfluoroakyl compounds gave urethane (polyurethane) coatings. By the late 1970s, these and other per- and polyfluoro products were, as surface treatments, applied to a variety of other uses, mainly products that involved regular human exposure, including for treatment and protection of carpets (urethane adipates), as well as for paper and packaging products. The finishing products were surface coatings that resisted adhesion: they are anti-adhesive coatings. Low surface free energies as imparted by fluorine were favoured, and the industrial materials were designed to incorporate the required physical

[9] Simons, "Fluorochemicals"; Kissa, *Fluorinated Surfactants*, pp. 31-36.

[10] Kissa, Ref. 9, pp. 1-12.

properties. Certain polymers, as functionalized fluorinated precursors, contain both fluorine and hydroxyl groups (-OH) to confer reactivity and adhesion.

This broad activity continued until the end of the twentieth century, by which time many thousands of per- and polyfluoroalky compounds had been synthesized.

ANALYSIS

The analysis and quantitation of PFAS in biological materials and water is nowadays undertaken with sophisticated instrumental techniques, especially high pressure liquid chromatography (HPLC, today "pressure" is replaced by "performance") hyphenated with tandem mass spectrometry (MS), referred to as HPLC-MS/MS. The modern methods, with sensitivities of parts per trillion, came into widespread use during the last decade of the twentieth century. Before then, the presence and quantitation of PFAS in biological material was achieved by indirect techniques that were originally closely identified with fluoride research in dentistry. This is the purview of this paper which describes the early development of analytical methods for detection and quantitation of fluorine in industrial perfluoro compounds containing various terminal functional groups as discovered in blood and serum. Because of their prevalence, the main focus is on the eight-carbon chain perfluorooctanoic acid (PFOA), and perfluorooctanates, and PFOS and perfluorooctane sulfonates (Figure 1). PFOA and PFOS were manufacture by 3M. Du Pont purchased PFOA from 3M prior to its startup of the telomerization process. Du Pont never made PFOS.

The goal of the analytical research was the ability to isolate, identify, and quantitate the PFAS in blood, serum, and biological materials. The key early technique was based on the release of ionic fluorine (fluoride) by decomposition of organic fluorine compounds. The first methods involved controlled burning, either by ashing in air in a furnace, or combustion using a suitable closed flame apparatus, a "bomb," in order to break the chemical bonds between organic carbon and fluorine (C-F) in alkyl chains (in terminal groups, $-CF_3$; and in chain groups, $-CF_2-$), thereby releasing the fluoride ion (F^-).

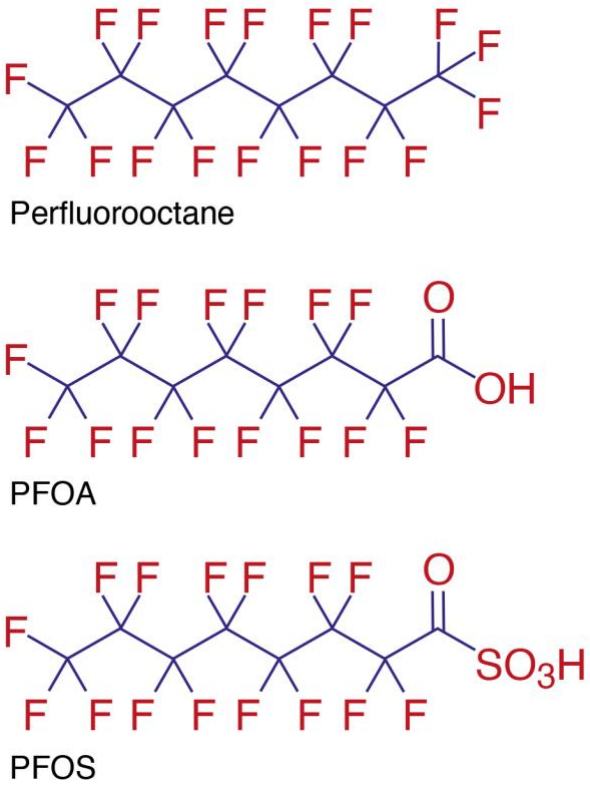

Figure 1. Perfluorooctane, PFOA (perfluorooctanic acid), and PFOS (perfluorooctane sulfonic acid)

Following the decomposition, all the released fixed fluorine, along with previously unbound fluoride, in biological materials and blood, was determined as the fluoride ion. The fluoride ion concentration, without release of the bound fluorine as fluoride, was determined separately. The difference, that is total fluoride less the fluoride measured before decomposition, represented the organic fluorine, as present in PFAS. Other techniques discussed here include the use of fluorine-specific electrodes, derivatization of PFAS to afford products suited to gas chromatography (GC), and decomposition with sodium biphenyl. Significantly, as mentioned here, there was also a diffusion method not involving ashing which though giving erroneous results acted as the stimulus for more fruitful investigations. By the late 1970s the methods of analysis had been refined to the extent that quantitation at low parts per billion (ppb) of total PFAS in blood was achieved. This survey is of special interest to the history of chemical analysis because the 1970s represented a major changeover period from “wet and dry” methods of analysis to instrumental methods of analysis; skills in manipulation on the bench would give way to skills in interpreting

spectral data.[11] Here, as we shall see, both techniques were harnessed to great effect in the trace analysis of organic fluorine in biological materials. Moreover, this is an excellent example of the adaptation of techniques to the special needs brought on by concerns over specific contaminants.[12]

RELEASE OF FLUORINE AS FLORIDE FROM PFAS

The development of methods for the analysis of fluoride followed concerns in the 1930s relating to levels of the ion in potable water and the impact on dental health. Trace measurements of fluoride at the low parts per million (ppm) level were undertaken, and standard methods were developed for measurement of fluoride in water. Analysts combined titrimetry with colorimetry, and other readily available techniques, to quantitate the fluoride. Novel techniques were developed in which the intensity of a colour from a complex produced by reacting the fluoride with a standard indicator colorant was proportional to the concentration of fluoride. In the 1940s, the Scott-Sanchis method, based on the use of Alizarin red S as indicator (an aqueous solution of sodium alizarin sulfonate), was used to determine fluoride in water.[13] By the mid-1950s this method had been improved considerably, particularly for removal of interfering ions. Rapid quantitative analysis was achieved with benchtop instruments, the colorimeters, and also with the spectrophotometers introduced around 1940. These techniques had achieved a high level of sophistication by the late 1950s.[14] Greater specificity was in general obtained with colorimetry. The subsequent interest in the relationship between fluoride and cancer encouraged international research into trace analysis of fluoride during the 1950s and 1960s.

Refinements in combustion included decomposition in the closed Parr oxygen bomb. In 1956, P.

[11] Morris, *The Matter factory*.

[12] Homburg and Vaupel, eds, *Hazardous Chemicals*.

[13] In 1945, the concentration of fluoride ion in potable waters in the United States was adjusted to 1 ppm. This followed the discovery in the early 1930s that fluoride ion at higher concentrations caused dental fluorosis. Dickinson, *The Chemical Analysis*, pp. 109-111; See also *Standard Methods*, pp. 98-107; and *Methods of Analysis*, pp. 311-317.

[14] They included analysis of fluoride based on its reaction with zirconium ions and 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene-disulfonate (SPADNS).

B. Sweetser of Du Pont's Chemical Department employed a version of the combustion method to determine quantitatively the fluorine in a number of fluorine-containing organic compounds, including Teflon and the sodium salt of trifluoroacetic acid. Sweetser used R. Wickbold's oxyhydrogen combustion method to decompose the organic fluorine to carbon dioxide and hydrogen fluoride (HF). The fluoride was absorbed in sodium hydroxide solution, and titrated with thorium nitrate, using Alizarin red S as indicator.[15] Sweetser preferred the Wickbold method because, he explained, with the alternative Parr bomb method, "in the case of highly volatile compounds and compounds with high fluorine content there has been difficulty in the decomposition step." The method was found suitable for "compounds notorious for their stubbornness in resisting decomposition."[16] Chemists at 3M later achieved greater success in the use of the Parr bomb to determine organic fluorine in serum.[17] In general, however, the Wickbold method was preferred. These, as we shall see, were important observations.

The introduction of fluoridation for dental purposes stimulated research into new methods for determination of the presence of fluoride in different media. Ashing of biological material for the microdetermination of fluoride, at the low parts ppm using titrimetric techniques had been adopted by analytical chemists from the late 1930s. They included Wallace David Armstrong (1905-1984) at the Department of Physiological Chemistry, University of Minnesota.[18] In the 1950s, Armstrong, then with the Department of Biochemistry, The Medical School, was a senior figure in fluoride analysis. With Leon Singer, the latter at Minnesota's School of Dentistry, he undertook investigations using a diffusion technique, namely electron transfer catalysis. The work was published in 1954. It did not involve ashing or combustion.[19]

Armstrong assisted the Indian researchers Pothapragada Venkateswarlu and D. Narayana Rao, at the Department of Biochemistry, the Government Medical College, Trivandrum (in the state then

[15] Matuszak and Brown, "Thorium nitrate." See also McClure, "Microdetermination."

[16] Sweetser, "Decomposition," p. 1768; and Wickbold, "Die quantitative,"

[17] Belisle and Hagen, "Method for the determination of the total."

[18] For Armstrong, see Singer and Posner, "Wallace D. Armstrong (1905-1984)."

[19] Singer and Armstrong, "Fluoride determination by electron transfer catalysis."

known as Travancore-Cochin). They published on the estimation of fluorine (actually fluoride) in biological materials, also in 1954.[20] Venkateswarlu (1926-2015), who plays a role here, studied for his undergraduate degree in biochemistry at Andhrra University during 1946-1948. It was probably in the late 1950s that he joined Armstrong at Minnesota in order to study for his doctorate in physiological chemistry and microbiology, which was awarded in 1962. Over the following years Venkateswarlu studied fluoride analysis in biological materials and later contributed to methods used in trace determinations of fluorine in organic perfluoro compounds.

In 1959, Singer and Armstrong had used ashing to determine the presence of fluoride in blood serum. The presence of organic fluorine in blood was almost certainly not then appreciated, nor even a consideration, in this work. It was enough to release fluoride quantitatively from biological material. The sample was ashed with magnesium oxide, as fixative, and total fluoride was determined by colorimetric analysis, using the fading in color of a zirconium-Eriochrome Cyanine R lake. They observed that "absorbance of the solutions can be measured with commonly available spectrophotometers."[21] Around this time specific-ion electrode coulometric titration for halogens had come into general use, and thus was applicable to fluoride. The end point was determined by a silver-saturated calomel combination.

THE DONALD R. TAVES VERSUS ARMSTRONG AND SINGER CONTROVERSY

The introduction of several new commercial products containing the organofluorine compounds stimulated the further application of analytical techniques, particularly in industry, for determining fluorine when bound to carbon. However it had little or no impact on the trace determination of organic fluorine in biological materials. That would change with Singer and Armstrong's studies after their results were questioned by Donald R. Taves, at the University of Rochester, New York. Taves, subsequently with his doctoral student Warren S. Guy (b. 1942), and in collaboration with NMR expert Wallace Siegfried Brey (1922-2023), at the University of

[20] Venkateswarlu and Rao, "Estimation of fluorine." Venkateswarlu also published under V. Pothapragada. From 1956, Travancore-Cochin was known as Kerala.

[21] Singer and Armstrong, "Determination of fluoride in blood serum," p. 106. See also Singer and Armstrong, "Regulation of human plasma."

Florida (Gainsville), would in the mid-1970s provide unequivocal evidence for the ubiquitous presence of PAFS in human blood.

Donald Taves (b. circa 1928) requires a brief introduction. He received his BS degree in 1949 and his MD in 1953, both from the University of Washington. In 1954 he was appointed a public health resident with Washington's Clark-Skamania Health Department. He was awarded the MPH (Masters in Public Health) in 1957 from the University of California. In 1960, when he was still a public health officer, he enrolled on a PhD programme to study bone mineralization, under William F. Neuman, joint head of the Department of Radiation Biology and Biophysics at the University of Rochester. However, his main interest was in the impact of fluoridation, a topic in which he would later become a leading expert.

Taves' recent reminiscences inform us on his first interactions with Armstrong and Singer and his introduction to trace fluorine analysis in biological material.[22] According to Taves, since the late 1950s he had been interested in the impact of sodium fluoride introduced to the body by fluoridation on renal disease, and in methods for measuring serum fluoride at the levels required to perform the proper renal clearance studies. In 1959, he applied in writing to Harold Hodge, head of the new Department of Pharmacology at Rochester, for a doctoral post in order to continue this work, but Hodge did not respond, perhaps, because as a proponent of fluoridation, he opposed Taves' questioning of the 1950s fluoridation policy of the California Department of Public Health. That was the reason for his joining Neuman. Taves first met with Armstrong and Singer around 1960 at a scientific meeting held in San Francisco. "I made a trip to San Francisco to meet with Armstrong and learn about the new method that he and Singer were developing for measuring serum fluoride." This method was based on diffusion of the fluoride. Taves:

> I was hoping that I could use their method to study the effects of renal disease on serum fluoride. Wallace, better known as Wally, was one of the few MDs who belonged to the American Dental Association (ADA), and the ADA's journal published many of the early biochemical studies. I knew he was going to talk about their new method of analysis and outline the preliminary results of using it on serums from fluoridated and non-fluoridated cities at a meeting in San Francisco. I was particularly interested in their finding that there was no difference in serum fluoride due to fluoridation and at the same time finding a big difference in terms of dental caries. I found that worrisome because I hypothesized that serum levels should correlate

[22] Taves, "Fluoride: From nutrient."

> with the amount that was ingested. We exchanged our viewpoints for about an hour in the hallway outside of the meeting without convincing the other…"[23]

Following completion of his PhD dissertation in 1963 on "Factors Controlling Calcification," Taves received a post as assistant professor with Neuman's department. He was now "free to pursue the fluoride analysis question," and visited Singer's lab "where they [Armstrong and Singer] were still working on their new [diffusion] method," but were facing difficulties. Taves remembers: "As we were talking, his lab technician interrupted to say that the values she was obtaining were still too low relative to their earlier methods. His response was to increase the length of time of diffusion." Taves, in retrospect, observes: "Rather than extending the time, they should have checked to see if all the fluoride had diffused. If they had done that, they would have known that they were dealing with a contaminant rather than fluoride and, with more diffusion time, they would obtain higher values due to the contamination." There were certainly problems of both identity and quantitation.

In 1965, Armstrong and Singer published details of a modified diffusion method which "eliminated the requirement for ashing biological materials with magnesium oxide as fixative."[24] However, Taves, now an expert in fluoride analysis, was unable to accept their results. The reported fluoride level was far too high. Taves drew attention in *Nature* to the fact that, as compared with his method, which involved ashing, the reported Singer and Armstrong determinations of "fluoride from serum indicate that the generally accepted value for normal humans is too high by as much as a factor of ten."[25]

Singer and Armstrong responded to Taves after undertaking ashing by the method that they had used in 1959. They claimed that from the two methods of analysis, diffusion and ashing, they obtained similar results, using human plasma (sera that has had clotting agents removed), reporting, in June 1967, in *Nature*:

> Taves has indicated that our method for estimation of serum fluoride content based on diffusion of hydrogen fluoride gives results which are about ten times too large, but our values of human plasma

[23] Taves, Ref. 22.

[24] Singer and Armstrong, "Determination of fluoride: Procedure."

[25] Taves, "Normal human serum."

> fluoride content, which he quotes, were not obtained by the diffusion method but by an entirely different procedure which requires ashing of the sample with magnesium oxide followed by separation of the fluoride by distillation from perchloric acid. The agreement of the results for fluoride analyses of urine, bovine plasma, dentine, liver and muscle obtained by the two procedures [of Singer and Armstrong], which are quite different in principle, furnishes mutual support of the reliability and accuracy of both methods. Nevertheless, we have carried out further experiments, some of which are like those reported by Taves, and our evidence is that both of our methods give results for plasma fluoride content which are not markedly in error.[26]

Taves was quick to reply, in September 1967, again in the columns of *Nature*: "In 1966 I published results showing that the normal concentration of fluoride in human serum is about one-tenth the generally accepted value—that of Singer and Armstrong."[27] That was from their diffusion method. Taves, however, could not fault their ashing method. It was to his credit that he could now explain the unexpectedly high results obtained by Singer and Armstrong using ashing.

Taves established that Singer and Armstrong had, by resorting to complete ashing, measured both the free fluoride ion normally present and the far greater amount of fluoride ion that had been released from a hitherto unexpected organic fluorine-containing compound. Taves' own results, following his modification of the ashing procedure, to ensure complete ashing, were now in good agreement with those of Singer and Armstrong. Here it should be pointed out that because of the extremely strong fluorine-carbon bonds, earlier ashing experiments were not always carried out to the point where all the carbon-fluorine bonds were broken. This had been noted by Sweetser at Du Pont in 1956. Armstrong had, unwittingly, brought about release of most or all of the fluorine as fluoride.

[26] Singer and Armstrong, "Normal human serum fluoride."

[27] Taves, "Use of urine to serum fluoride." "Part of the evidence supporting my results was the 'at least five-fold discrepancy' that could be shown in their [Singer and Armstrong] data which is most easily explained by an error in the measurement of the serum fluoride. The discrepancy involves the ratio of urine to plasma fluoride concentrations when determined by fluorine-18 as compared with stable fluoride analyses. Because the concentration of fluoride in urine is well established in contrast to that in serum where analysis is much more difficult, it is reasonable to question the latter when there is a discrepancy. Singer and Armstrong, however, in their recent rebuttal, think that I came to erroneous conclusions because I directed attention to only two of their analyses, which they now consider aberrant."

It is also important to emphasize here that this led to the clearest early experimental evidence for the presence of industrial per- and polyfluoro compounds in blood and biological materials.

In March 1968, Taves, now an associate professor at Hodge's Department of Pharmacology, associated with Rochester's School of Medicine and Dentistry, explained:

> It has been assumed that there is only one form of fluoride in serum, the inorganic F ion. It would therefore seem that either the value for serum fluoride which I found (1 μM) … or that found by Singer and Armstrong (7.5 μM) … must be in error. While the diffusion method of Singer and Armstrong has been shown to produce erroneous values, the same cannot be said for their ashing and distillation procedure. The evidence that the serum fluoride is about 1 μM in a fluoridated community does not rule out the possibility that more fluoride could be made available from serum by ashing... [28]

Taves investigated the available methods for analysis of fluoride at low levels of detection, including use of the fluorescence of a morin-thorium complex, and a fluoride ion-specific electrode. The latter was used to establish, following ashing, the presence of perfluoroalkyl compounds in blood plasma. Taves found "excellent agreement between the findings with the fluoride electrode and those employing the morin-thorium reagent." Moreover,

> These results are consistent with the hypothesis that there are two forms of fluoride in serum, exchangeable [fluoride] and non-exchangeable [fluorine]…. In 1950, Smith, Gardner, and Hodge found normal values ... for serum fluoride in a fluoridated community, implying that they were measuring only exchangeable fluoride [the fluoride ion]. They distilled fluoride from blood… and then ashed the distillate. *If in fact there is a non-exchangeable fluoride in serum, it did not break down or diffuse under these conditions, implying a large stable molecule. These findings are consistent with the presence of a fluorocarbon molecule* (emphasis added).[29]

Having validated the method of Armstrong and Singer when they used ashing, Taves continued to refine his experimental techniques to establish, also with ashing as the first step, the presence of perfluoroalkyl compounds in blood plasma. In one case, hexamethyldisoloxane, which accelerated the release of fluoride, was used in the next step, diffusion separation with electrophoresis, which was highly sensitive to the presence of the fluoride ion.[30] According to Taves, he had eliminated interference from contaminants that had caused the Armstrong and Singer diffusion method to give high readings. In his November 1968 paper in *Nature*, Taves

[28] Taves, "Evidence,' 1968a.

[29] Taves, Ref. 28.

[30] Taves, "Separation of fluoride," 1968b.

reported "Evidence has been given that there are two types of fluoride in human serum. The observation that 80-90 per cent of the fluoride is not made available as exchangeable fluoride until after ashing suggests that it might be bound in some fashion to the serum proteins. I have therefore determined the distribution of exchangeable and non-exchangeable fluoride relative to the serum proteins, after electrophoresis…. Free-flow curtain electrophoresis was used because the concentration of fluoride in serum is very low, making it necessary to use large volumes of serum. The serum was obtained from a normal human who obtained his water from a fluoridized supply."[31] The "normal human" was Taves himself. He now interpreted his result: It provided unequivocal evidence for the presence of a perfluorocarbon compound, likely of industrial origin, in serum. Shortly after, Taves assigned the further investigation of fluorine in serum to Warren S. Guy.

By 1975, Guy and Taves had isolated the fluorine in human blood obtained from blood banks in five districts, three in New York State and two in Texas. Taves and Guy released fluoride by ashing, and the total fluoride was determined with the fluoride electrode. For analysis of the unashed serum they used preparative silicic acid chromatography. This revealed a dominant peak. Following four separations, the same fractions that gave this peak were combined and re-chromatographed as a cleanup step. The NMR expert Wallace Brey was asked to undertake fluorine NMR (^{19}F-NMR) analysis of the material that gave the sharp peak. This indicated the presence of one or more organic perfluoro compounds. Brey's ^{19}F-NMR suggested a chain of atomic groupings of the type $–CF_2–$; a $–CF_2–$ grouping located next to a terminal $–CF_3$ grouping; and the terminal grouping itself $–CF_3$. Apart from the $–CF_2–X$ grouping where X was an unknown functional group (perhaps = COOY, a carboxylic acid, where Y= H, or another atom or atomic grouping), this was consistent with a perfluoro carboxylic acid or derivative: $CF_3–(CF_2)_n–CF_2–CO–Y$. There was also the suggestion of the presence of branched isomers. A structure such as $CF_3–(CF_2)_n–CF_2–CO–Y$ ruled out other perfluoro compounds of commerce consisting of smaller molecules. Thus whereas Taves had identified a perfluorocarbon compound(s) in his 1968 paper in *Nature*, Guy, Taves, and Brey had in 1975 more specific evidence of a long-chain

[31] Taves, "Electrophoretic mobility," 1968c.

perfluorocarbon compound, or compounds, of six or seven carbons, which, they speculated, was a perfluoro carboxylic acid or some other closely related compound.[32]

Certain of the chemical shifts in the NMR spectrum were in close agreement with the spectra obtained from linear perfluorooctanic acid used as standard. However, there was one notable difference. The chemical shift for the $–CH_2–$ grouping next to the –COOH in perfluorooctanic acid, run as the standard, was -120.2 ppm, whereas in the sample it was -114.3 ppm. Could the 3M corporation assist with complete identification?

KNOWLEDGE TRANSFER TO 3M

With their results to hand, Guy, Taves, and Brey made preparations to give a joint presentation on organic fluorocompounds in human tissues on 25 August 1975 at the Chicago meeting of the American Chemical Society. Anxious to establish, prior to the meeting, the exact nature of the compound isolated from human blood, Guy made two phone calls to 3M for assistance in identification. On 14 August 1975, Guy made his second phone call, to G. H. Crawford of 3Ms Photographic Products Division. A few days later, Crawford summarized his version of the phone conversation. “I got John Pendergrass [of 3M’s Medical Department] on the line and Guy brought in a Dr Tays [sic.].” Brey’s interpretation from his NMR data indicated that the compound

> is fluorocarbon carboxylic acid with a C_6 or C_7 fluoroalkyl group. Dr Brey suspects a branched end on the chain, e.g. perfluoro t-butyl … The discussion involved Dr Guy’s speculative questions as to where such a ‘universal’ presence of such compounds in human blood could come from…. Somewhere he got the information that 3M’s fluorocarbon carboxylic acids are used as surfactants and wanted to know if they were present in ‘Scotchgard’ or other items in general use by the public… We plead ignorance but advised him that ‘Scotchgard’ was a polymeric material not a F.C. [fluorocarbon] … They have done experiments involving water boiled in Teflon cookware with negative results. We suggested obtaining plasma specimens from uncivilized areas, e.g. New Guinea where they don’t use too much ‘Teflon’ cookware or ‘Scotchgard’.

Crawford and Pendergrass

> adopted a position of scientific curiosity and desire to assist in any way possible and suggested that our own analytical people might be able to clarify Dr Brey’s NMR findings (I know Wallace Brey from way back. He is highly respected, conservative and not given to frivolous speculations)…My recommendation … is to get Richard [Newmark, at 3M Central Research Analytical] in touch with Brey, obtain spectra for

[32] Spectral data was reproduced in Guy, Taves, and Brey, “Organic fluorocompounds.”

> his own interpretation perhaps samples to run on our equipment, etc., in other words, keep scientists talking to scientists in the spirit of cooperative scientific enquiry. On the positive side—if it is confirmed to our satisfaction that everybody is going around with fluorocarbon surfactants in their bloodstreams with no apparent ill-effects, are there some medical possibilities that would bear looking into?[33]

A copy of the paper given by Taves, Guy, and Brey at the August ACS meeting was sent to 3M, no doubt in the hope that the corporation would assist with identification. The document included the NMR information, which was acted upon at 3M.

According to an internal 3M chronology, during 17 to 21 September 1975, Newmark at Central Research Analytical compared the ^{19}F-NMR spectra of PFOA, PFOS, and related compounds, with that obtained by Brey. After further NMR studies, Newmark advised his colleagues on November 6: "$C_8F_{17}SO_3H$ spectra matches that presented by Guy, et al." The 114.3 ppm peak that had confounded the academic workers was close to a peak of 114.2 ppm from PFOS. Thus, Newmark, on the basis of instrumental evidence, was of the opinion that Guy and Taves likely had isolated PFOS as the main component of the fraction. As it turned out, he was correct. However, 3M did not inform Guy and Taves of Newmark's analysis. More important, in the long term, was the fact that 3M was now alerted to the fact that populations outside the factory wall were exposed to PFAS. The outcome was that 3M immediately undertook internal investigations into techniques for measuring their presence in human blood. In 16 December 1975, 3M representatives visited Rochester to consult with Guy and Taves: "3M proposes, and Guy and Taves agree that 3M will attempt to isolate and identify organic fluorine in human blood." On 17 February 1976, 3Ms "Central Research Analytical develops an accurate analytical method for determining parts per billion quantities of organic fluorine compounds in human blood. Method tested on blood from American Red Cross and value agrees with those in literature."[34] Tests on 3M personnel conducted during April to October 1976 gave concentrations of "organic fluorine compound" from 50 to 1,000 times normal values.

[33] Environmental Working Group. G. R. Crawford to J. A. Pendergrass, "Record of a telephone conversation – August 14, 1975," 3M Interoffice Correspondence, 20 August 1975, pp. 270-272.
[34] Ref. 33, pp. 276-278.

The detailed account of the findings of Guy, Taves, and Brey appeared during 1976 in their chapter on "Organic Fluorocompounds in Human Plasma: Prevalence and Characteristics." This was similar to the document provided to 3M following the August 1975 ACS meeting. The paper described how, "In order to further characterize the organic fluorine fraction, it was purified from 20 liters of pooled human plasma and characterized by fluorine nmr." Figure 5 was a reproduction of Brey's ^{19}F-NMR spectra.The blood was obtained from blood banks of 106 individuals from five cities where there had been no change in fluoride in potable water for at least five years. Fluoride was obtained by ashing and determined with the fluoride electrode. The values for inorganic fluoride (F^-) and organic fluoride (R-F) "show that the average fluoride concentration in plasma is directly related to the fluoride concentration in the water supply, and that the average organic fluorine concentration in plasma is not." Moreover, "A series of compounds having a structure consistent with that found here for the predominant form of organic fluorine in human plasma is widely used commercially for their potent surfactant properties."[35]

On 25 February 1977, at the Denver meeting of the American Association for the Advancement of Science, Guy, then at the Childrens' Hospital, Cincinnati, Ohio, lectured at a Fluoride Symposium on "Perfluorooctanoic Acid in Human Plasma." The July issue of *Fluoride*, journal of the International Society of Fluoride Research, included "a special report on the symposium: Guy announced that with Taves he "had isolated in 1976 by spectroscopic analysis, perfluorooctanoic acid, a major component in pooled plasma which accounts for at least 1/3 of the organic fluoride content."[36] Moreover, "Guy and Taves again report finding $C_7F_{15}CO_2H$ in pooled plasma and attribute its presence to industrial products."[37] Among them was 3Ms Scotchgard.

However, the academic workers were not made aware of Newmark's findings. In September and October 1976, there were a number of exchanges between 3M and Taves over the analysis of PFOA, while in late October Singer requested samples of PFOA with the intention of improving

[35] Guy, Taves, and Brey, Ref. 32, pp. 118, 125, 131.

[36] Waldbott and Yiamouyiannis, "Special Report."

[37] Waldbott and Yiamouyiannis, Ref. 36.

on the analysis method of Taves. By this time chemists at 3M were making advances in PFAS analysis. Some years later, Taves stated: "They [3M] would come check with me periodically – they wouldn't tell me what they were doing, but they wanted to know what I knew."[38] It probably suited 3M, as almost certainly the sole manufacturer of PFOS (FC-95), for the academic workers to believe that PFOA was the major component of the material isolated by Guy and Taves during their chromatographic separation. Notwithstanding the reluctance on the part of 3M to reveal its findings in 1976, two years later its chemists started to publish their methods for PFAS. But here we must backtrack a few years for a brief summary of Venkateswarlu's research in the 1970s

Reverse Extraction In 1971, Venkateswarlu and co-worker P. Sita, around that time affiliated, respectively, with the Department of Biochemistry at Sri Venkateswara Medical College, and the Postgraduate Institute of Medical Education and Research Chandigah, in India, published on "A New Approach to the Microdetermination of Fluoride." This was based on an adsorption-diffusion technique. Fluoride was adsorbed on calcium phosphate, and it was found "possible to concentrate traces of fluoride from a large amount of sample low in fluoride, a feature which permits more reliable determination of fluoride."[39] By late 1971, no doubt as a result of their achievements in fluoride analysis, Venkateswarlu and Sita had received posts in the United States, at, respectively, the Department of Biochemistry, The Medical School, University of Minnesota, and the University of Minnesota Hospital. With Singer and Armstrong, Venkateswarlu adapted the calcium phosphate method to "the isolation and concentration of ionic and ionizable fluoride from interfering substances in biological fluids and [it] eliminates the requirements of ashing and diffusion." They speculated that in plasma the ionic fluoride is "in equilibrium with a loosely bound fluoride [ionizable fluoride]… furnishing additional ionic fluoride."[40]

Notwithstanding this suggestion, in 1974, Venkateswarlu published what would become his first significant contribution to trace PFAS analysis, a reverse extraction technique for increasing the

[38] Bryson, *The Fluoride Deception*, pp. 234 and 352-353 (notes 11 and 12).

[39] Venkateswarlu and Sita, "A new approach," 1971a, p. 760.

[40] Venkateswarlu, Singer, and Armstrong, "Determination of ionic (plus ionizable)," 1971b, p. 356.

availability of released fluoride. The total fluoride, following extraction as a fluorosilane, according to the 1968 method of J. A. Fresen, F. H. Cox, and M. J. Witter, of the University of Groningen, was determined, with the preexisting fluoride, with the hanging drop fluoride electrode.[41] The method enabled detection in serum, with "a two- to threefold gain in the concentration of fluoride compared with that in the original sample."[42]

It was probably in 1977 that Venkateswarlu, as a result of his skills in fluoride analysis, joined the 3M Commercial Chemicals Division, where he continued with research into improvements in trace analysis. In that year he published a comprehensive review of methods for analysis of fluorine in biological materials.[43] His chapter was completed before Guy, Taves, and Brey announced their findings in August 1975. In 1979, Guy published a chapter on "Inorganic and Organic Fluorine in Human Blood," in which he surveyed methods for analysis of fluorine, and especially misleading information from diffusion-colorimetry. Guy agreed with Venkateswarlu, who had opined: "The values so obtained reflect ionic fluoride plus interfering substances, the latter masquerading as nonionic fluorine … in unashed body fluids… this practice should be discontinued." Of the organic fluorine compounds present in human plasma, Guy, still ignorant of 3Ms findings, concluded that "The major type is probably a derivative of perfluoro-octanic acid, presumably a synthetic environmental contaminant."[44]

GAS CHROMATOGRAPHY AND DERIVATIZATION

The main analytical work on PFAS at 3M was carried out from around October 1975 by chemists Jon Belisle and Donald F. Hagen at Central Research. In this, they had a special interest

[41] Fresen, Cox, and Witter treated trimethyl chlorosilane with water, to release the corresponding silanol (R_3SiOH, where R = methyl), which reacted selectively with fluoride ion to form the stable, volatile trimethylfluorosilane (CH_3SiF), suited to GC analysis. Fresen, Cox, and Witter, "The determination of fluoride."

[42] Venkateswarlu, "Reverse extraction technique," on p. 880.

[43] Venkateswarlu, "Determination of fluorine in biological materials," 1977, on pp. 93-201.

[44] Guy, "Inorganic and organic fluorine in human blood," on pp. 136-137 and 141. Guy quoted from Venkateswarlu, "Fallacies in the determination." Later reviews by Venkateswarlu include "Determination of fluorine," 1994.

in the application of gas chromatography to the determination of PFAS, and of PFAO and its derivatives.

From the time of its introduction in the early 1950s, gas chromatography (GC) became the method of choice for separating volatile organic compounds. Early instruments were built inhouse in industrial and academic laboratories. From the mid-1950s, they became available from mainly American manufacturers of electronic instruments.[45] In the 1960s, two highly sensitive detectors were introduced into GC, the flame ionization detector (FID) and electron capture detector (ECD); both were ideal for analysis of halogens. Narrow capillary gas chromatography columns improved resolution and were preferred in organic trace analysis of pesticides, water, and contaminants in the atmosphere. Identification was achieved with mass spectrometry (MS).[46]

Interestingly, in 1971, using GC, there was further evidence of the stability and environmental presence of chlorofluorocarbon compounds. James Lovelock, at the University of Reading, UK, used his invention, the ECD, to measure "Atmospheric Fluorine Compounds as Indicators of Air Movements." Lovelock worked with volatile organofluorines the great stability of which was such that he suggested the use of these "industrial stable compounds as indicators of air movements and wind indicators."[47]

GC is suited to the analysis of volatile, as well as stable, organic compounds. PFAS compounds are not volatile enough and are thus unsuited to GC. Derivatization in which organic fluorine is first converted into fluoride was found to provide a route to volatile products that could be readily detected by GC. In 1967, a method for quantitative measurement of a volatile fluorosilane, was published by R. Bock and H. J. Semmler at the University of Mainz.[48] It

[45] Ettre, "American instrument companies."

[46] In MS unknown molecules are subjected to fragmentation. The recorded fragmentation patterns, by use of special "atlases," with tables showing peaks corresponding to structural features of groups of atoms (fragments), enables the determination of complete structures.

[47] Lovelock, "Atmospheric fluorine."

[48] Bock and Semmler, "Abtrennung und Bestimmung."

involved conversion of organic fluorine to inorganic fluoride, followed by quantitative measurement of the volatile, stable fluorosilane. It was similar to the method of Fresen, Cox and Witter, which Venkateswarlu had used in 1974.[49] In 1978, Belisle and Hagan at 3M were of the opinion that the Bock and Semmler procedure "presented a new approach for the separation and determination of fluoride."[50]

Using the Bock and Semmler procedure, the two 3M chemists undertook research to determine the total organic fluorine content in blood, and serum/plasma. They employed oxygen bomb combustion, and reaction of the released fluoride with the extractant triethylsilanol to afford the triethylfluorosilane, which was then analyzed by GC. As applied to perfluorooctanoic acid (PFOA), the total fluoride was determined with a flame ionization detector. Using an internal standard, they determined less than 1 ppm, and as low as 0.010 ppm. "The application of this method to a sample (both with and without combustion), allows one to determine the concentration of both organic (bound fluorine) and inorganic (ionic fluoride) in the sample." [51]

According to Belise and Hagen: "It should be possible to increase the lower limits of detectability for fluoride by concentration techniques such as reverse extraction," the method published in 1974 by their colleague Venkateswarlu, who, they noted, had demonstrated that "perfluorooctanoic acid would be a good compound to use in evaluating the new method for the decomposition and recovery of fluoride." With the oxygen bomb procedure and the use of whole blood, 95 + or – 5% at the microgram level of added perfluorooctanoic acid was recovered. Similar results were obtained with *p*-fluorobenzoic acid.

Belisle and Hagen concluded: "The determination of total fluoride in biological samples requires the more vigorous oxygen bomb decomposition technique for quantitative results. Gas chromatographic measurement of the fluoride ion level via the resultant fluorosilane reaction is accurate, dependable, specific, and applicable over a wide dynamic range. This combination

[49] Venkateswarlu, Ref. 41.

[50] Belisle and Hagen, Ref. 17, p. 545.

[51] Belisle and Hagen, Ref. 17.

appears to provide the most ideal method yet developed for the determination of organic and inorganic fluoride contents."[52]

In 1980, 3M medical director F. A. Ubel and colleagues published a preliminary report on the exposure to PFAS of its plant workers using analysis of a triethylsilane derivative. It was limited to detection of total organic fluorine from the blood stream at 0.5 ppm. Higher than normal levels were found in the ambient air. "No ill health effects attributable to exposure" to ammonium perfluorooctanate, 3M's FC-143, "were found among these workers…. Through certain modifications in the process steps and improvements in engineering controls, a substantial reduction in the airborne fluorochemical levels within the plant was achieved."[53] This lack of ill health effects would be the corporation's standpoint, at least in the public domain, for the next two decades, and, as detection limits were lowered, would include individuals without occupational exposure.

In 1980, also, derivatization of PFOA to give a volatile product was achieved by esterification, in which, for example, the acid group, -COOH was changed to the methyl ester, $–COCH_3$. The methyl esters were prepared by *N*,*N*-dimethylformamide dimethyl acetal derivatization. The method was first described in 1977 by Donald E. Elliott, at National Foam System, Inc. (Lionville, Pennsylvania). The methyl esters, were, for comparative purposes, analyzed by GC with a FID and a thermal conductivity detector (TCD). The samples analyzed covered the C-5 to C-12 range.[54]

A similar method of derivatization was used by Hagen and Belisle for detection of PFOA and other free acids of 3M compounds at the low parts per billion. They determined PFOA in blood and other biological samples by conversion to the volatile methyl ester (via the diazomethane). The ester was analyzed at 3M using GC, this time with the electron capture detector (ECD).[55]

[52] Belisle and Hagen, Ref. 17.

[53] Ubel, Sorenson, and Roach, "Health status."

[54] Elliott, "Anomalous response."

[55] Belisle and Hagen, "A method for the determination."

In 1981, Hagen, Belisle, Venkateswarlu, and James D. Johnson, a drug metabolism expert at Riker Laboratories, reported that that they had developed a quantitative microanalytical method based on this methylation route using a sensitive microwave plasma detector, specifically for perfluorooctoanate in blood. At that time, "only a few public donor samples were analyzed."[56] Blood samples were taken from 3M workers and other employees. In the mid-1980s, hyphenated GC-MS was used to quantitatively determine perfluorooctanic acid, as its benzyl ester, in plasma and urine.[57]

However, it should be emphasized, esterification of was not suited to perfluorinated sulfonates, which included PFOS.

SODIUM BIPHENYL DECOMPOSITION

Another technique for measuring organic fluorine involved sodium biphenyl reductive decomposition, as originally described by L. M. Liggett, of Wyandotte Chemicals Corporation in 1954.[58] Only in the 1970s was the sodium biphenyl reagent found suitable for trace determination of fluorine in blood. T. P. Stein and colleagues at Pennsylvania School of Medicine, and Herbert W. Wallace, at Exxon Research and Engineering Company, Linden, New Jersey, undertook determination of fluorocarbon compounds in blood as part of a programme for using fluorocarbon emulsions as artificial oxygen carriers. One product they used was FC-47, perfluorotributylamine. Their publication described the use of a method which they claimed had wide applicability. This involved sodium biphenyl decomposition, and fluoride specific ion electrode coulometric titration.[59]

Venkateswarlu, while continuing with development of his reverse extraction technique for the determination of fluoride in biological materials, introduced reductive cleavage of the C-F bonds using the sodium biphenyl reagent and determined the resulting fluoride ions with the hanging

[56] Hagen, et al., "Characterization of fluorinated metabolites," p. 336.

[57] Ylinen, et al. "quantitative gas chromatographic."

[58] Liggett, "Determination of organic halogen."

[59] Stein, et al, "Determination of fluorocarbon," pp. 480, 483.

drop fluoride electrode. The fluoride specific (selective) ion electrode was suggested as an alternative to GC analysis; Venkateswarlu had earlier made the valid point that the method involving reverse extraction was simpler and cheaper than GC or MS.[60]

According to Venkateswarlu in 1982, the use of the sodium biphenyl reagent for determination of fluorine in organic compounds in blood serum had to await "the present availability of sodium biphenyl reagent having an adequately low fluoride blank."[61] Sodium biphenyl featured in another paper, coauthored by Venkateswarlu, "Automated Molecular Absorption Spectrometry for Determination of Fluorine in Biological Samples." It described a method for "rapid screening of blood serum samples from plant workers for organic fluorine." The 3M authors of this paper reductively cleaved the C-F bonds at room temperature with the sodium biphenyl reagent, extracted the fluoride with diphenylsilanediol, and quantitatively determined total fluorine by aluminum monofluoride molecular absorption spectrometry. However, the method "has not, so far, been used by us to determine organic fluorine in normal human or animal blood sera, in which the organic fluorine levels would be relatively lower than those in the samples from plant workers exposed to organic fluorochemicals."[62]

3M's somewhat sarcastic remark to Guy suggesting that he should obtain blood samples from the inhabitants of New Guinea would soon backfire. By 1980, 3M needed a better understanding of the pervasiveness of PFAS, and with the help of the People's Republic of China obtained serum from inhabitants of a rural district in China where 3Ms products were unlikely to be found. In 1981, Belisle published on fluorine determination in serum obtained from these donors. His conclusion: "It is clear that nearly everyone (greater than 98 percent) has both forms of fluorine in his blood and that the reported values are somewhat dependent on the method of analysis." However, in his published opinion: "As yet, we have no conclusive evidence to indicate that the prevalence of trace amounts of organic fluorine in human blood is primarily the result of industrial fluorochemicals."[63]

[60] Venkateswarlu, Ref. 42, p. 878.

[61] Venkateswarlu, "Sodium biphenyl," p. 1132.

[62] Venkateswarlu, et al., "Automated molecular absorption," p. 2236.

[63] Belisle, "Organic fluorine in human serum."

Table 1. Timeline for techniques of PFAS analysis until 1983 (see the end of the manuscript)

TOTAL ORGANIC FLUORINE, STANDARDS, AND LIMITS OF DETECTION

As has been described in the foregoing, the early investigations into organic fluorochemicals in blood and biological materials presented many challenges and required some innovative thinking. In 1968, Donald Taves, determined using an ashing method what he believed to be an organofluorine typical of products made in industry. In 1975, Taves and Guy isolated the major fraction of PFAS present in pooled samples of blood and tentatively identified it, with the help of the NMR expert Wallace Brey, with PFAS compounds, perhaps PFOA or similar compound manufactured by 3M. At that point, chemists at 3M took up the story and established, on the basis of NMR comparisons with the data of Brey, the likely presence of PFOS in the pooled blood of city dwellers. 3M developed techniques using pure samples for determination of the limits of detection of pure PFOA. Methods of standardization were validated. By around, 1980 derivatization followed by gas chromatography enabled quantitation of PFOA and other perfluorocarboxylic acids. For comparative purposes, studies were made with different GC detectors. The detection limit of PFOA in a 10 ml sample of blood plasma was 0.015 ppm. However, PFOS was not amenable to methyl ester derivatization. By 1981, following concerns raised by environmental releases of PFAS from finishing processes, Du Pont had developed derivatization - gas chromatographic techniques for measuring trace amounts of PFOA in blood and, significantly, water. Most activities at this time were concerned with standardization.

POSTSCRIPT: COMBINING NEW TECHNOLOGIES IN INSTRUMENTAL ANALYTICAL CHEMISTRY

There was little further progress in techniques until the 1990s, when for analysis of PFAS this decade saw a tremendous leap forward with the application of high pressure liquid chromatography (HPLC) for separation, combined with tandem mass spectrometry (MS/MS) for detection.[64] Quantitative measurement of PFOA, POSF and other PFAS was achieved without

[64] MacDonald, "Waters Corporation."

the need for decomposition and derivatization became possible. The hyphenation of HPLC with MS required a means for overcoming the large pressure difference between the HPLC apparatus and the high vacuum MS spectrometer. This involved the introduction of new devices, and ultimately electrospray ionization, the value of which was appreciated at 3M around 1990.[65] Though problems remained with selectivity and sensitivity the combined techniques provided evidence of widespread environmental exposure.[66] Specific PFAS compounds in small volumes of blood were identified in the early 1990s. Improvements were made in the accuracy of measurements. By 1997, the detection limit for PFOS was down to 50 ppb.

By 2009, when there was sufficient evidence of public health threats created by trace PFAS, the US Environmental Protection Agency (EPA) drew up a short term health advisory for exposure of 200 ng/L for POSF, and 400 ng/L for PFOA, and published its first validated method (537) for fourteen PFAS compounds in drinking water, with detection limits for POSF of 1.4 ppt, and for PFOA of 1.7 ppt.[67] Increasing knowledge of PFAS toxicity, increasingly stringent regulatory activities, and increasingly lowering thresholds of concern, especially in the United States, led to a change from C-8 PFAS to less toxic products based on C-6 and other compounds in reformulation of fluorinated surfactants. This in particular applied to the fluorinated surfactants used in aqueous film-forming foams AFFF that were found in groundwater near military bases and airports from the late 1990s.[68] Nevertheless, there were concerns that the new products were not without risks to the environment

All analytical methods introduced since the early 2000s are refinements of HPLC-MS/MS hyphenation. Inter-laboratory studies, new standards, and updated validated EPA methods

[65] Fenn, "Electrospray ionization."

[66] The literature on PFAS analysis since around 2000 is extensive. See for example Villagrasa, de Alda, and Barcelo, "Environmental analysis," and Yahnke and U. Berger, "Trace analysis." For concerns from within the scientific community see for example, Blum, et al., "The Madrid Statement."

[67] US EPA "Determination of selected perfluorinated."

[68] Moody and Field, "Perfluorinated surfactants," and, more recently, Dubocq, et al., "Characterization of the chemical contents."

evolved in attempts to tackle the environmental legacy of Forever Chemicals. By the second decade of the twenty-first century individual contaminants present in trace amounts could be identified and quantified.[69] Further EPA promulgations and advisories led to a landmark ruling on 8 January 2024, aimed at preventing companies from starting or resuming the manufacture or processing of 329 PFAS "that have not been made or used for many years without a complete EPA review and risk determination."[70]

SUMMARY AND CONCLUSION

This account suggests itself as a useful example of the roles of error and failure in the development of scientific knowledge, and of puzzle solving providing a heuristic cue. The main stimulus in the investigation of PFAS compounds in human blood and biological materials arose from the publication of what appeared to be erroneous results arising from fluoride analysis by a diffusion technique.

The diffusion technique was developed around 1960 by Armstrong and Singer who were interested in measuring fluoride in biological materials. However, their published results were questioned by Taves, who believed that the fluoride content was far less. The interactions between Armstrong and Singer, on the one side, and Taves, on the other, took place in the columns of *Nature*. Armstrong and Singer resorted to ashing, which appeared to support their findings. Unlike most previous workers, however, they, wisely as it turned out, extended the time of combustion. They did not realise it at the time but they had brought about quantitative release of organic fluorine in biological material by its decomposition, that is, the conversion of organic fluorine into the ionic form, fluoride (F^-). They measured quantitatively the total fluoride. It was Taves who, through repeating the ashing method, in 1968 explained the apparent anomalous results when he established that two forms of fluorine, fixed (organic) and exchangeable (ionic), existed in blood, and could be clearly distinguished. The organic fluorine was calculated by

[69] See for example, on particle-induced gamma-ray emission (PIGE), Ritter, et al., "PIGE as a screening tool."

[70] EPA press office, "Biden-Harris administration." For the situation in Europe, see Brunn, et al., Ref. 1.

subtracting fluoride, as analyzed quantitatively in the compound *without* ashing, from total fluoride. Taves considered that the source of the organic compound(s) he had isolated were large molecules, with a high fluorine content, not unlike the industrial perfluorocarbon compounds. It was a promising explanation.

In 1975, Taves and his associate Guy amassed sufficient evidence, following chromatographic separation of a major fraction, to confirm that there were perfluorocarbon compounds in the blood of communities located beyond manufacturing sites. They decided tentatively on the basis of ^{19}F-NMR data supplied by NMR expert Brey that what they had isolated was a perfluoro acid, or a closely related compound.

Guy made contact with 3M requesting assistance in identification. This was not forthcoming. However, based on internal NMR analysis, 3M decided that the substance isolated by Taves and Guy was PFOS. This was a matter of considerable concern because 3M was the primary manufacturer of PFOS. It was this definitive evidence for the presence of PFAS compounds in human blood that led 3M to undertake intensive research into trace PFAS analysis by a number of techniques. These included conversion of perfluoroalkanoic acids into volatile derivatives that were suited to analysis by GC. This work benefited from earlier studies in organic fluorine analysis, and newly available instrumental techniques, in particular fluorine-specific electrodes, NMR, and GC detectors suited to trace analysis of halogens.

The novel applications of methods described here enabled analysis for trace organic fluorine in blood and water. Derivatization without decomposition was useful for PFOA, but not suited to PFOS. This was the situation until the 1990s, when quantitation of PFAS and of its individual components was achieved following hyphenation of HPLC with MS. It brought about a complete transformation in methods of analysis, if not a paradigm shift. During the first decade of the twenty-first century HPLC-MS/MS, aided by successive improvements, would become the method of choice in rapid, trace PFAS analysis, including compound identification, particularly in water, which is a key concern today. In the United States and parts of Europe the pervasiveness of PFAS contamination has increasingly attracted public attention, and action, but, it seems, not to the same levels elsewhere. However, testing and research into methods for the

removal of these persistent organic pollutants from water and waste is an ongoing international effort.

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Table 1. Timeline for techniques of trace PFAS analysis until 1983

TOF = Total organic fluorine LOD = Limit of Detection

Year	Reference	Matrix	Technique	Result

1968	Taves, *Nature* 217 (1968, March 16):1050-1051; and Taves, *Nature* 220 (1968, November 9):582-583.	Blood	Ashing, electrophoresis	Evidence of PFAS (covalent fluorine) in serum
1974	Venkateswarlu, *Anal. Chem.* 46(7) (June 1974):878-882		Reverse extraction, fluoride specific electrode	Threefold increase in trace fluoride recovery
1976	Guy, Taves, and Brey, in Filler, ed., *Biochemistry Involving Carbon-Fluorine Bonds* (1976), 117-134.	Blood banks	Isolation of a PFAS from human blood, and ^{19}F-NMR	Tentative evidence for PFOA or similar compound(s) in blood
1977	Elliott, *J, Chromatogr. Sc.* 15 (10)(1977):475-477	Standards	*N*,*N*-dimethylformamide-dimethyl acetyl derivatization, GC	0.5 mg in 0.5 mL. No quantification
1978	Belisle and Hagen, *Anal. Biochem.* 87(2) (July 1978):545-555	Standards	Combustion, silane derivatization, GC	For PFAS. LOD: Less than 1 ppm, and as low as 0.010 ppm
1980	Belisle and Hagen, *Anal. Biochem.* 101(2) (1980):369-376	PFAS in plasma, urine, liver	Diazomethane derivatization, GC	For PFOA. LOD: 0.02 ppm in 10 ml sample; 0.015 ppm for plasma; 0.0015 ppm for urine; 0.06 ppm for liver tissue.
1981	Belisle, *Science* 212 (4502) (1981):1509-1510.	Blood of Chinese donors		For TOF. Blank of 0.002 ppm in a 10 ml serum sample
1981	Hagen, Belisle, Johnson, and Venkateswarlu, *Anal. Biochem.* 118 (1981):336-343.		Methylation, GC, with microwave plasma detector	For perfluorooctanate
1982	Venkateswarlu, *Anal. Chem.* 54(14) (1982):1132-1137; and Venkateswarlu, et al., *Anal. Chem.* 55(14) (1983):2232-2236.	Blood of 3M plant workers	Decomposition with sodium biphenyl, automated molecular absorption spectrometry	For TOF