Quantification, Removal, and Recovery of APFO from Contaminated Drinking Water with a Concomitant Endocrinological and Epidemiological Assessment

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ABSTRACT: Ammonium perfluorooctanoate – also called APFO, PFOA, or C8 – is a surfactant associated with the production of Teflon®. APFO does not hydrolyze, photolyze, or biodegrade and is biologically additive with the highest concentrations occurring in individuals whose drinking water has been contaminated. Currently there are five states reporting APFO contamination of air, water, and soil. In addition, the EPA has recently labeled this persistent chemical a potential carcinogen. The purpose of this project was to generate a simple and inexpensive test for the presence and concentration of APFO in drinking water and to develop a method for the removal and recovery of this bioaccumulative chemical.

Testing for the presence and concentration of APFO depends upon the fact that APFO is a surfactant. Agitation of a surfactant in water generates foam. APFO foams heavily in the parts per million range, but contaminated water contains APFO in the parts per billion range. Therefore, sample reduction 1,000-fold was necessary to concentrate APFO and produce foam. For analysis, a sample containing APFO was reduced, agitated to produce foam, photographed, and compared by imaging software analysis to spiked control foam heights. Statistical analysis indicated foam height testing was 92% accurate when compared to confirmed APFO concentrations.

For removal and recovery, the technique chosen depended upon the extremely hydrophobic fluorocarbon tail of the PFO ion. Electrosorption removed APFO and allowed recovery of 3.51% of the APFO at the positive electrode and 2.82% at the negative electrode.

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Both electrodes attracted APFO due to the formation of a diffuse electrical double layer. The use of diffuse electrical double-layer electrosorption for APFO removal and recovery has never before been used, and has currently received a Patent Pending (US60/751,466) from the US Patent and Trademark Office.

APFO is also a peroxisome proliferator. Endocrinological testing on *Culex pipiens* mosquitoes indicated APFO decreased mosquito developmental time. Controls took 13 days to begin to hatch, while APFO contaminated tanks in the ppt ranges took an average of 11.4 days and ppb ranges took only 10.33 days. Pupae size was also markedly different in parts per trillion APFO tanks, with the first day pupae being an average of 147% larger than corresponding controls.

Once evidence of a decrease in time to hatch in mosquitoes was confirmed, data from the Center for Disease Control showing the incidence of mosquito-borne diseases were inspected. Epidemiological analysis of West Nile Virus reports in birds from 2002 to 2004 from APFO-contaminated counties compared to control counties was statistically significant at the P = 0.0004 level. Summed data for all positive reports in humans, mosquitoes, domestic animals, and birds for the same time period was statistically significant at the P= 0.002 level.

KEYWORDS: ammonium perfluorooctanoate, drinking water contamination, electrosorption, endocrinological testing, epidemiological analysis.

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1. INTRODUCTION

Ammonium perfluorooctanoate – also called APFO, PFOA or C8 - is a surfactant associated with the production of Teflon®. APFO has also been found in fire-fighting foams, on coatings applied to fast food wrappers, and as an "inert" ingredient in pesticides, among other applications [1]. APFO does not hydrolyze, photolyze, or biodegrade under environmental conditions [2]. It is biologically additive with highest concentrations occurring in individuals

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whose drinking water has been contaminated [3]. As recently as January 2002, there was only one laboratory in the United States capable of analyzing for APFO, and this lab was under contract to the DuPont company and would not accept samples for analysis without DuPont's approval [4]. This project was designed to generate a simple test for the presence and concentration of APFO in drinking water without expensive equipment or the need to submit samples to a captive laboratory for analysis. This test is based upon the fact that APFO foams heavily in the parts per million (ppm) ranges. Local drinking water is contaminated at parts per billion (ppb) levels [5]. A 1,000-fold concentration brings APFO into foaming range. Since foam height is proportional to concentration, APFO levels in drinking water may be determined by foam height comparisons between the unknown and spiked controls. Recovery of APFO is based upon diffuse electrical double layer electrosorption of the PFO anion (Kelydra Welcker**,** Patent Pending US60/751,466).

2. MATERIALS AND METHODS

Initial investigation into the nature and behavior of surfactants disclosed the Gibbs adsorption equation. This equation relates the rate at which surface tension changes with concentration. Utilizing this information as a starting point, a sample of APFO was obtained (Indofine Chemical Company, APFO 98%) and dilutions prepared in both bottled drinking and bottled distilled water (Wal-Mart Brand Drinking Water and Wal-Mart Brand Distilled Water). Samples were tested for foam production using a modified Cylinder Shake Test, originally developed by Stipel in 1941 [6]. Samples prepared and tested ranged from 50 parts per billion to 100 parts per million. Samples tested included: 50 ppb, 100 ppb, 500 ppb, 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, 10 ppm, 12 ppm, 12.5 ppm, 14 ppm, 16 ppm, 18 ppm, 20 ppm, 22 ppm, 25 ppm, 50 ppm, and 100 ppm. Vials were labeled, shaken to develop foam, and photographed a minimum of three times. Ten data points were extracted from each picture using Image J, microscope-imaging software (total 30 data points), and the results statistically analyzed by a heterozygastic t test, graphical regression analysis, and confidence intervals were calculated with an accept/reject level of 0.05.

Water samples from five water districts – four reported as contaminated with APFO > 0.5 ppb and one sample from 25 miles upriver from the source of contamination as a control –

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were obtained and transported in plastic jugs for analysis. Samples were collected on 4 October 2005. Plastic jugs were chosen since APFO is reported to adsorb to glass [7]. Each jug was thoroughly shaken, 500 mL of the water removed and placed in a stainless steel pan, which was placed over a burner on a gas stove, and the liquid reduced by a factor of 1,000 (500 mL reduced to 0.5 mL). The 0.5 mL concentrated sample was carefully removed from the pan and cooled, the minerals allowed to settle, and the liquid fraction transferred into a 5-cm X 1.5-cm flat-bottomed vial. Once samples indicated foaming was present and could reproducibly be measured, two samples 25 ppm and 10 ppm were exposed to electrolysis for periods of time of 3, 6, 9, and 12 hours in an attempt to remove APFO. Electrosorptive testing utilized stainless steel electrodes, 6-Volt Fuji Brand Heavy Duty Batteries, and coated electrode wires with dual alligator clips. Once this process was shown to reduce APFO levels, concentrations were lowered to 500 ppb, 100 ppb, and 50 ppb and the experiments repeated.

When methods for quantification, removal, and recovery were identified, an endocrinological and epidemiological assessment of the effects of APFO on several indigenous invertebrate species was undertaken. Animals such as daphnia, copepods, ostracoda, and *Culex pipiens* mosquitoes were cultured in APFO-contaminated water. Upon discovery that APFO reduced maturation time for *Culex* mosquitoes, potentially leading to an increased risk of mosquito-borne diseases in APFO contaminated areas, data on the incidence of West Nile Virus were downloaded from the Internet. Data were collected from the Center for Disease Control and Prevention website for all areas in the United States reporting APFO contamination of air, water, and land, and examined for correlation of APFO to incidence of West Nile Virus.

3. RESULTS AND DISCUSSION

It is possible to document APFO foams in concentrations of 50 parts per billion and greater. Pure liquids do not foam [8]. The closer a solution comes to a pure solution, the lower the foam that results. In all solutions, however, foam heights are proportional to Concentration, and concentrations of unknowns may be calculated by comparison to spiked controls.

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A fundamental characteristic common to all foams is their very large interfacial area, which is unstable in the thermodynamic sense. Some foams can exist for a long period of time in the absence of external motion, while others collapse very rapidly when the shaking producing the foam ceases. APFO foams are very short-lived, but photographic capture compensates for the short foam life and height analysis using Image J microscope imaging software, allows statistical analysis of the resulting data. Confidence levels with an Alpha of 0.05 for foam analysis of samples with 50 ppb -100 ppm ranged from 0.24 to 0.29, with standard errors of 0.66 to 0.81.

Published reports of APFO levels in the distribution networks of local water districts vary from 0.5 ppb in Belpre, OH, and Tuppers Plains, OH, water districts to 2.2 ppb in the Lubeck, WV, Water District, to over 7 ppb in Little Hocking, OH [5]. Wellhead reports in the Little Hocking area have been recorded as high as 18.60 ppb with test wells at 38 ppb and ground water borings 50 ppb [5].

Samples from the four water districts reporting contamination and one control district were concentrated from 500 mL to 0.5 mL by high-heat evaporative sample reduction and the resulting foams compared to spiked controls. Concentrations were then calculated by ratio comparison (Table 1 and Figure 1).

Table 1. Water analysis.

Sample Location	Published Reports for Distribution Networks	Publication Date	Foam Height Analysis
Belpre	>0.5 ppb	2002	2.00 ppb
Lubeck	0.7- 2.2 ppb	1984 - 1989	3.20 ppb
Little Hocking*	3.57 ppb	Jan. 2005	3.30 ppb
Tuppers Plains	> 0.5 ppb	2002	1.90 ppb
Marietta	Control	NA	0 ppb

* http://www.littlehockingwater.org/.

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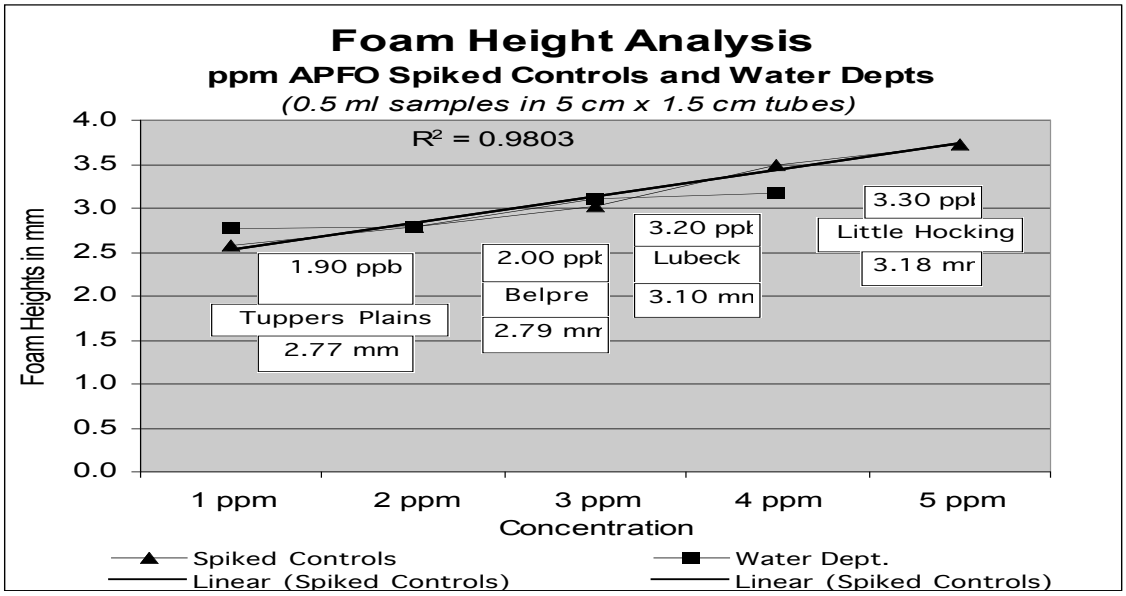

Figure 1. APFO spiked controls and water district samples foam analysis.

Graphical comparison of control and unknown foam heights showed results higher than those published prior to 2005. An internet search of the records, however, provided an explanation of the low levels previously reported. The following statement and accompanying document was found on the Environmental Working Group website [9]:

> "Not only were DuPont's own lawyers uneasy about increased pollution with C-8 and the lack of hard evidence to support claims of C-8 safety, it turns out that the methods that DuPont had supplied to local officials to detect C-8 in tap water were seriously flawed, and that levels of C-8 in the tap water were far higher than water officials knew. In an e-mail to his son (x13316@usma.edu), Reilly recounts how on the evening of May 7, 2001, he received a call at home from engineers working for DuPont who had discovered that the company's method for detecting C-8 in water underestimated actual levels by 75 to 80 percent or more." To quote:
>
>> "We learned recently that our analytical technique has very poor recovery, often 25%, so any results we get should be multiplied by a factor of 4 or even 5. However, that has not been practice, so we have been telling the agencies results that surely are low. Not a pretty situation, especially since we have

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been telling the drinking water folks not to worry, results have been under a level we deem "safe" of 1 ppb. We now fear we will get data from a better technique that will exceed the number we have touted as safe. Ugh."

When this information is taken into account and the previously reported results multiplied by a factor of 4, the APFO concentrations found by foam analysis fit the data ranges that resulted. Comparing the most recent concentrations published in January 2005 for Little Hocking, samples showed concentrations obtained by foam height analysis to be 92% accurate.

Once quantification of APFO from water samples proved feasible, removal and recovery procedures were initiated. For the removal and recovery of APFO, there are three factors that lead to the concentration of APFO at the electrodes. The first is the obvious attraction of the electrode to its oppositely charged ion (the PFO anion is attracted to the positive electrode, while NH_4 cation is attracted to the negative electrode). An electrical double layer forms at each electrode attracting the counter ions. Thus NH_4 cation forms the secondary layer at the positive electrode and the PFO anion forms the secondary layer at the negative electrode. Second, APFO is a surfactant and is drawn to any interface, in this case the boundary between the electrode and the solution. Third, APFO has an extremely hydrophobic fluorocarbon tail, so that if the first two factors create a local concentration high enough, micellular formation could further concentrate the APFO at the surface of the electrode. Setting aside these theoretical considerations, the fact is that APFO is indeed concentrated at the electrodes leading to a method of APFO removal and recovery of extremely dilute aqueous solutions and measuring the reduction in foam height illustrates the removal of APFO (Figures 2 and 3).

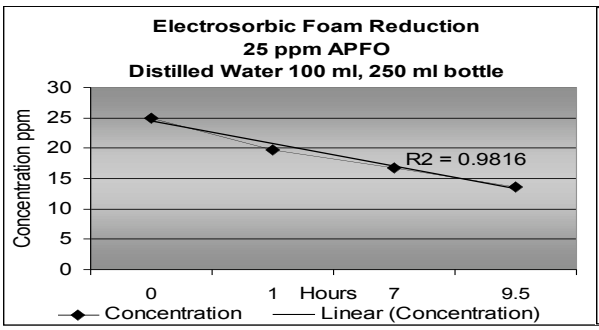

Figure 2. APFO removal 25 ppm.

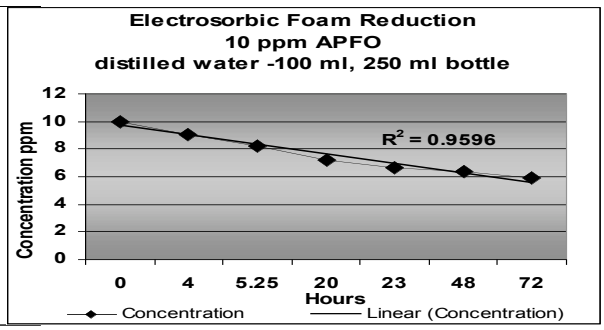

Figure 3. APFO removal 10 ppm.

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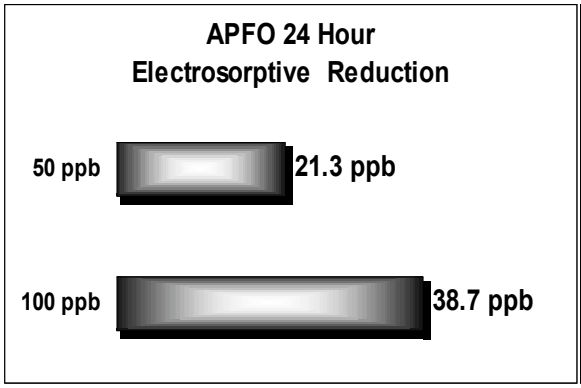
Running a regression analysis on the concentration reduction occurring in 25 ppm and 10 ppm samples showed that the resulting data points were within 96-98% of the line best connecting the these points. Since drinking water concentrations of APFO are in the parts per billion ranges, the electrosorption technique was tested on APFO concentrations in that range as well. Over a 24-hour test, samples of 50 ppb and 100 ppb decreased to 21.3 and 38.7 ppb, respectively (Figure 4). Examination of all the data and calculating the percent electrosorptive reduction in 12 hours shows that higher concentrations decreased at a more rapid rate (Figure 5).



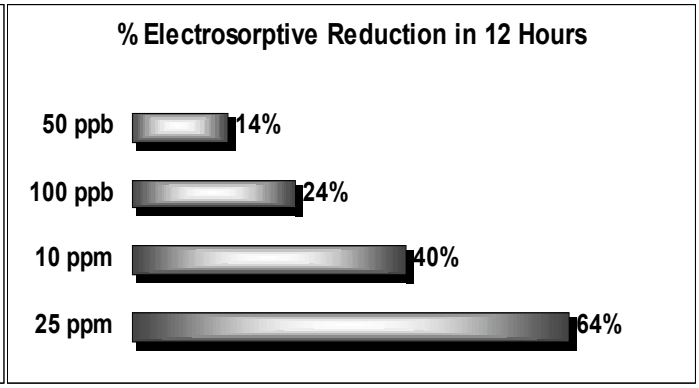


Figure 4. APFO ppb 24-hour test. **Figure 5. Percent reduction in 12 hours.**

Once quantification of APFO in drinking water and a method of removal were identified, recovery of the APFO was undertaken. APFO is attracted to and may be removed from solution by electrosorption to the electrode through the diffuse electrical double layer (Table 2).

Table 2. Statistical analysis of electrode recovery.

Statistical Analysis - 30 data points	Positive Electrode	Negative Electrode
t test comparing control foam height to positive and negative foam heights	$P = 2.35 \times 10^{-16}$	$P = 4.72 \times 10^{-24}$
t test comparing foams generated by rinsing positive and negative electrodes	$P = 5.37 \times 10^{-9}$	

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The t test shows that foams generated by rinsing positive and negative electrodes were statistically significantly different when compared to the foam generated by a control and that the foams generated from rinsing the positive and the negative electrodes were statistically different from each other. The positive electrode attracted a statistically greater amount of APFO, which was reflected by a greater foam height.

The electrical double-layer thickness decreases as the concentration of ions increases. Since surfactants decrease surface tension, however, the EDL decrease due to increased concentration of ions seems offset by the lowered surface tension induced by the APFO. Foam heights generated by the pipette removal of 5-mL liquid over the each electrode, dramatically illustrates the greater attraction of the APFO to the positive electrode (Figure 6).

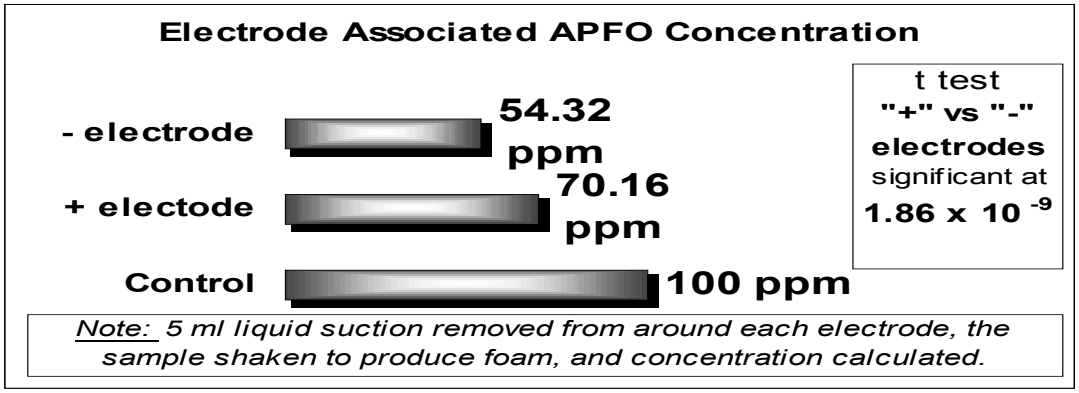

Figure 6. Electrode associated concentration.

Recovery rates were 3.51% due to electrosorption at the positive electrode, and 2.82% due to the electrical double-layer attraction to the negative electrode.

Once tests for quantification, removal, and recovery were identified, an endocrinological and epidemiological assessment of the effects of APFO was undertaken. A mixed microbiological culture starting with 6 each of daphnia, copepods, and ostracoda were cultured for six weeks in concentrations of APFO from 0-500 ppb and 0-500 ppt. After six weeks, microscopic ten fields analysis illustrated an overall greater effect on survival and reproduction in ppt concentrations of APFO on the microinvertebrates tested than in ppb concentrations. Chi-square analysis between ppt and ppb was statistically significant P=0.0001.

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Culex pipiens mosquitoes were also cultured and hatched in APFO concentrations from 0-500 ppt and 0-500 ppb. Mosquitoes were fed daily with finely ground, Wardley Brand Fish food containing 46% protein. Mosquitoes were examined before hatching for pupal size and for development time, and after hatching for hatch rates, gender ratio, adult size, and wingbeat frequency. *(Note: In mosquitoes, wingbeat frequency is species specific and gender determined and endocrine disruptive chemicals have been observed causing variations.)*

Mosquito developmental time was decreased in the parts per trillion and parts per billion concentration levels. Controls took 13 days to begin to hatch, while APFO-contaminated tanks in the ppt ranges took an average of 11.4 days while the ppb ranges took only 10.33 days. Pupae size was also markedly different in parts per trillion APFO tanks with the first day pupae being an average of 147% larger than corresponding controls. Mosquito gender ratios, hatch rates, adult size, and wingbeat frequencies were not affected by APFO in the concentrations tested.

The decrease in time to hatch initiated interest in the incidence of mosquito-borne diseases in APFO-contaminated areas. In the United States, there is information on two DuPont and one 3M facility that have reported APFO contamination of air, water,and soil. The DuPont facilities reporting APFO emissions are in Washington, West Virginia, and Decatur, Alabama, while the 3M facility is in Cottage Grove, Minnesota (Appendix A). Contamination into neighboring states has occurred at both the West Virginia and Minnesota sites [10].

Data were collected from the Center for Disease control web site for the years 2002, 2003, and 2004. Of the positive tests of West Nile reported to the CDC from APFO-contaminated areas for the years 2002, 2003, and 2004, the greatest number of those were for birds (Table 3).

Table 3. Positive reports of West Nile in birds.

2002		2003		2004	
Control Counties	Contaminated Counties	Control Counties	Contaminated Counties	Control Counties	Contaminated Counties
67	197	31	203	5	103
t test		t test		t test	
P = 0.012		P = 0.048		P = 0.015	
t test comparing • all years: P = 0.0004					

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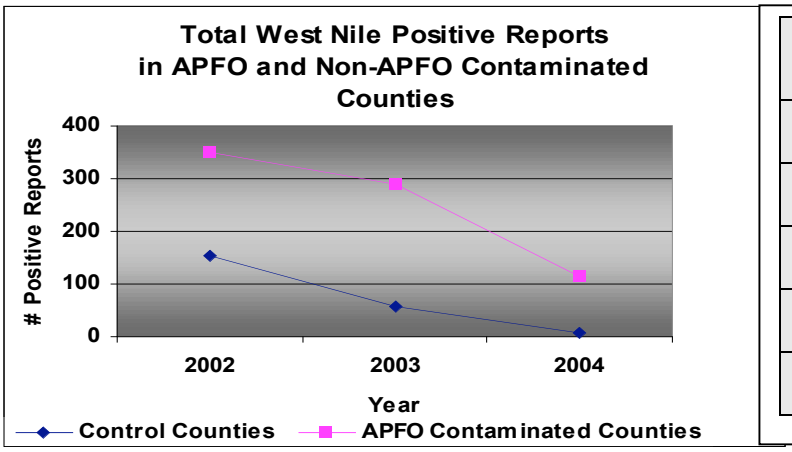
A graph of the total number of reported positive cases of West Nile in birds in control counties of all states compared by year shows a steady decline (R^2=0.9914) while APFO-contaminated areas show a much less intense decline R^2=0.7023. Total positive human, mosquito, and veterinary reports of West Nile for 2002, 2003, and 2004 in the both APFO and Control counties were low.Taken individually, none was statistically significant. However, combining all positive reports of West Nile in the control counties and then comparing them to all positive reports in the APFO contaminated counties throughout the United States generated data that were statistically significant at the P = 0.002 level using the t test (Figure 7).



	Control	APFO
Birds	103	531
Humans	10	32
Mosquitoes	10	64
Vet	97	124
Total	220	751

Figure 7. Total West Nile reports.

4. CONCLUSIONS

In areas where APFO has been detected in drinking water, individuals may determine the approximate concentration of APFO at their own home by simply concentrating a 500 mL sample 1,000-fold and shaking the 0.5 mL concentrated sample in a small test tube with a 1 cm interior diameter. This simplified test method is useful for initial surveying of potentially contaminated areas since number of millimeters of foam is approximately equal to the parts per billion of APFO in the original sample. More exact concentrations may be calculated by photographic capture followed by image analysis comparison to spiked controls. In addition,

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APFO may be removed from water by electrosorption and the reduction in concentration illustrated by before and after comparisons of foam heights.

Positive-electrode electrosorptive APFO recovery rates for 100 ppm static tanks are approximately three and one-half percent per day. While this rate may appear low, the current technology adsorbs APFO onto activated carbon, which then requires removal and disposal. Electrosorptive removal and recovery are potentially cheaper and more environmentally friendly than activated carbon. In addition, the fact that APFO decreased mosquito development time led to an investigation, which confirmed a statistically significant increase in West Nile Virus reports in APFO-contaminated areas.

In conclusion, although drinking water is currently contaminated and there is an increased risk of WNV in APFO-contaminated areas, any problem technology can cause, technology can cure. APFO is necessary for the production of Teflon® and Teflon® products are crucial in everything from Nomex® Heat Resistant clothing, to Kevlar® vests, to Gore-Tex® jackets. Removing APFO from water is important, but it is simply an engineering problem that requires a solution so future generations can to continue to obtain "better things for better living through chemistry."

5. ABBREVIATIONS AND ACRONYMS

APFO = ammonium perfluorooctanoate -also called C8 or PFOA by news organizations
CDC = Center for Disease Control and Prevention
EWG = Environmental Working Group
EDL = Electrical Double Layer
ppm = parts per million = 1×10^{-6}
ppb = parts per billion = 1×10^{-9}
ppt = parts per trillion = 1×10^{-12}
WNV = West Nile Virus

6 ACKNOWLEDGMENTS

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Credits

This research was conducted during the months of August through December 2005. I would like to thank Dr. Michael Norton and Dr. James Joy of Marshall University for reading and commenting on my completed paper. In addition, I would like to thank my teacher, Mrs. Susan Smith for glassware and chemicals and my parents for the years of patience and love which has given me the confidence to try and the courage to fail. For without the bitterness of failure I could never have appreciated the sweetness of success.

Author

Named for the American snapping turtle (*Chelydra serpentina*) that lives in the cool and green waters of the mighty Ohio River, Kelydra Welcker was born with a drive to protect the waters of this ancient river. From participating as a toddler in the Ohio River Cleanup campaigns to working to show the impact of industrial outfalls on the health of tiny clams and snails, Kelydra has spent her entire life as a part of this commercially contaminated ecosystem. Therefore, when a locally produced chemical became worldwide news and the small river town of Parkersburg, West Virginia, became the focus of lawsuits, EPA fines, and an enormous endocrinological study, Kelydra decided that simply studying the effects of this chemical wasn't enough – it was time to fix the problem.

Taking news reports stating that the contaminating chemical was a DuPont Teflon® surfactant called C8 and using old-fashioned wet-chemical techniques, Kelydra developed an easy test for the presence and concentration of this chemical in local drinking water and then moved on to removing and recovering this contaminant. After months of research, Kelydra developed a technique that was simple, safe, and successful – so successful in fact, that on 3 January 2006 she received a patent (pending) from the US Patent Office for her technique.

In addition to her love for water, Kelydra also participates in her school's community service programs, Quiz Bowl, and Science Bowl, is an active member of the National Honor Society, and was a 2002 finalist in the Discovery Channel Young Scientist Challenge.

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What does the future hold? Kelydra intends to pursue her interest in fluorosurfactants and would like to be a chemical engineer involved in preventing and repairing environmental contamination. Oh – and, like the snapping turtle she was named for – you can bet she will never be far from water!

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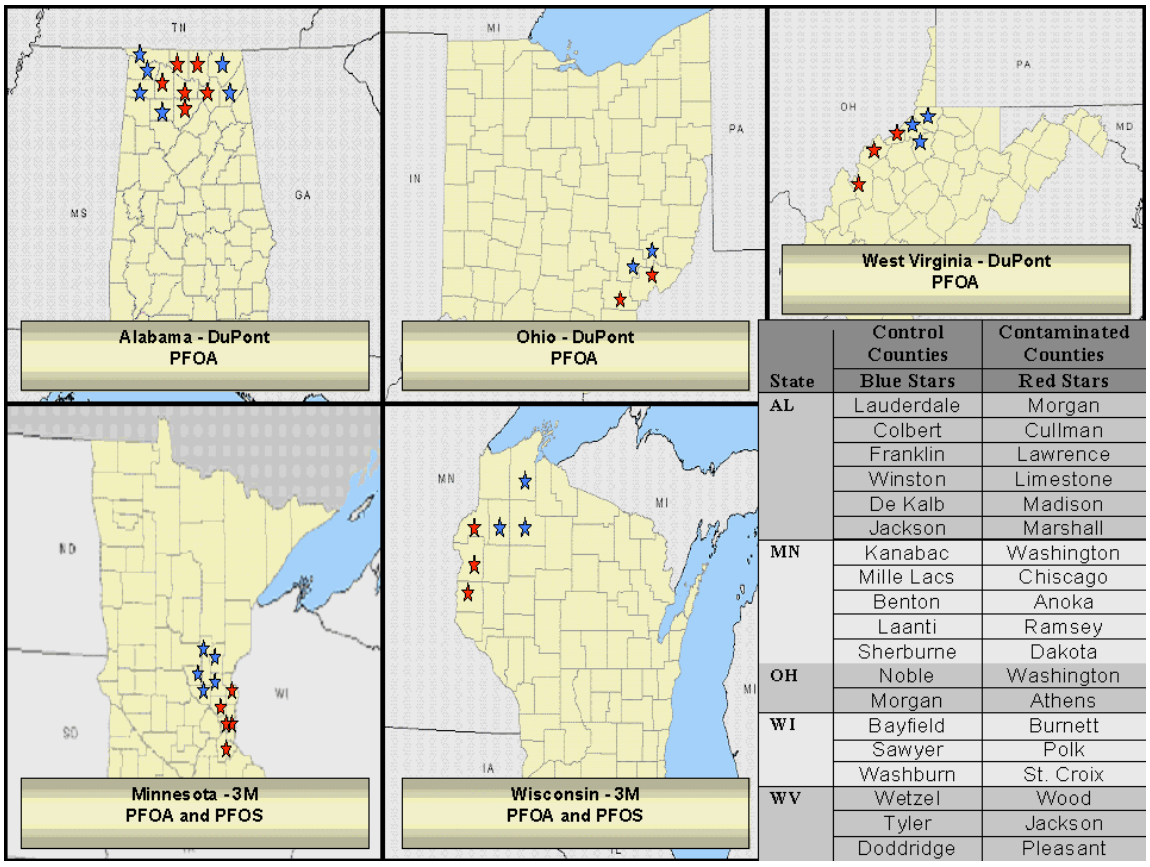
9. APPENDIX—MAP OF APFO CONTAMINATION



State	Control Counties Blue Stars	Contaminated Counties Red Stars
AL	Lauderdale	Morgan
	Colbert	Cullman
	Franklin	Lawrence
	Winston	Limestone
	De Kalb	Madison
	Jackson	Marshall
MN	Kanabac	Washington
	Mille Lacs	Chiscago
	Benton	Anoka
	Laanti	Ramsey
	Sherburne	Dakota
OH	Noble	Washington
	Morgan	Athens
WI	Bayfield	Burnett
	Sawyer	Polk
	Washburn	St. Croix
WV	Wetzel	Wood
	Tyler	Jackson
	Doddridge	Pleasant

Note: Red stars are confirmed APFO contamination of air, water, and soil. Blue stars indicate control counties clusters of similar size.

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