UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460
Analytical Chemistry Branch
701Mapes Road
Ft. Meade, Maryland 20755-5350

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: **Verification Analysis for PFAS in Pesticide Products** (ACB Project B23-05b)

FROM: Yaorong Qian, Senior Chemist
David French, Chemist
Analytical Chemistry Branch (ACB)
Biological and Economic Analysis Division (BEAD)
Office of Pesticide Programs (OPP)

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THROUGH: Thuy Nguyen, Branch Chief
Analytical Chemistry Branch (ACB)
Biological and Economic Analysis Division (BEAD)
Office of Pesticide Programs (OPP)

TO: Anne Overstreet, Director
Biological and Economic Analysis Division (BEAD)
Office of Pesticide Programs (OPP)

BACKGROUND

Perfluorooctanesulfonate (PFOS) was reported at concentrations between 4 parts-per-million (ppm) and 19 ppm in six of ten pesticide products tested by Lasee et al. and published in "Targeted analysis and Total Oxidizable Precursor Assay of Several Insecticides for PFAS" (*Journal of Hazardous Materials Letters*, 2022, 3, 100067) [1]. The list of pesticide products tested and the reported PFOS concentration are listed in **Table 1**.

The Analytical Chemistry Branch (ACB) obtained aliquots of the same ten pesticide products listed in **Table 1** from the study author. ACB was also able to purchase four of the six pesticide products from the open market that were reported by Lasee et al. to contain PFOS. Those purchased product brands are marked with an '*' in **Table 1**. All samples were tested by ACB for the presence of perfluoroalkyl substances (PFAS), especially PFOS, using two methods. First, ACB analyzed the pesticide samples utilizing the method described in Lasee's paper (Lasee's method). A second method, one recently developed and validated by ACB (ACB's method), was also utilized to test the same samples (see **Attachment I**).

Table 1. Pesticide products tested by Lasee et al. (2022) and reported PFOS concentration [1]

Pesticide Product	Manufacturer	Active ingredient(s)	PFOS found (mg/Kg, or ppm)
AVID 0.15 EC*	Syngenta	Abamectin	3.92± 0.51
Pedestal*	Chemtura	Novaluron	9.18± 0.34
Ultra-Pure Oil	BASF	Mineral oil	8.64 ±0.67
Marathon 1%*	OHP	Imidacloprid	13.3± 1.4
Oberon*	Bayer	Spiromesifen	19.2± 1.2
Malathion 5EC	Drexel	Malathion	17.8± 0.7
BotaniGard 22WP	LAM International Corp	Beauveria bassiana	ND
Overture 35WP	Valent	Pyridalyl	ND
Conserve	Dow AgroSciences	Spinosad	ND
XXpire	Dow AgroSciences	Spinetoram, Sulfoxaflor	ND

*ACB also purchased these four products from open market and tested for the presence of PFAS, particularly PFOS: AVID 0.15 EC, Pedestal, Marathon 1%, and Oberon.

ND – Not Detected

OBJECTIVES

The primary objectives of this study were:

- To screen for and quantify the potential presence of twenty-nine (29) PFAS compounds (see **Table 2** for the targeted analytes list**)** that might be present in these products
- To verify the presence of PFOS as reported by Lasee et al., in the aforementioned pesticide products

Table 2 - List of twenty-nine (29) PFAS analytes screened in this study utilizing both the Lasee et al. method and ACB's method with the exception of those noted with an *

PFBA	PFOS	PFTeDA	4:2 FTS*
PFBS	PFNA	PFHxDA	6:2 FTS*
PFPeA	PFNS	FOSAA	8:2 FTS*
PFPeS	PFDA	N-MeFOSAA	
PFHxA	PFDS	N-EtFOSAA	
PFHxS	PFUdA	9Cl-PF3ONS	
PFHpA	PFDoA	11C-PF3OUdS	
PFHpS	PFDoS	NaDONA	
PFOA	PFTrDA		

*These three compounds were not analyzed with the dilution method but analyzed in the ACB's pesticide extraction method.

STUDY RESULTS

As mentioned above, all samples in this study were analyzed by two different methods, Lasee's method and ACB's method, for presence of PFAS, especially PFOS. The main difference between the two methods is in the sample preparation step. The sample preparation step in Lasee's method is a simple dilution in a solvent/water solution to dilute the matrix using a single instrument for analysis. ACB's method involves a more intense extraction and clean up procedure to isolate PFAS compounds from the sample matrix before instrumental analysis, thus reducing matrix interference which results in better/more accurate detection limits. Instrumental analysis for both methods is based on the EPA SW 846 method 8327.pdf (epa.gov)[2] for detection of PFAS, which calls for using isotopically labeled (mass labeled) surrogates (standards added during sample preparation step) and isotopically labeled internal standards (standards added prior to instrument analysis). A mass labeled compound contains one or more carbon (^{12}C) atom(s) which is replaced by ^{13}C isotope atom(s). Since their molecular masses are slightly different, the mass spectrometer can differentiate the mass labeled from the non-labeled PFAS during sample analysis. Use of mass labeled PFAS is to monitor the performance of the method and to accurately quantify the recovery of non-labeled PFAS. Instrument response of an identified non-labeled PFAS compound is compared to the response of its isotopically labeled analog for quantification. Finally, ACB utilized two instruments to identify and quantify targeted analytes using liquid chromatography / tandem mass spectrometry (LC/MS/MS) and liquid chromatography/high resolution accurate mass spectrometry (LC/HRAMS) techniques.

LASEE'S METHOD:

A. Methodology

The analytical procedures described in the published paper (Lasee et al., 2022) followed a simple solvent/water dilution technique for sample preparation, and the SW846 method 8327 for instrumental analysis of the prepared samples.

The ACB tested the samples using the same procedures, except for the final product solution, which was made at 100 µg/ml in methanol. The 100 µg/ml solution is 10x more concentrated than that of Lasee et al. (2022) and would ensure that the PFAS compounds, if present as reported, would be detected.

Three different sets of samples were prepared, and mass labeled PFAS (surrogates), including mass labeled PFOS, were fortified in each sample to measure the recovery of PFAS. In addition, ACB spiked both mass labeled and non-labeled PFAS (including PFOS) in two sets of samples as additional measurements for the detection and recovery of PFAS by the method.

While Lasee's paper only discussed the use of an LC/HRAMS instrument for their samples, as noted above, ACB used two different analytical instruments, an LC/HRAMS and an LC/MSMS for confirmation of results, and mass labeled internal standards for quantification.

B. Results and Comments

None of the 29 PFAS compounds (**Table 2**), including PFOS, was detected in any of the samples above the instrument's background levels, either in those obtained from Lasee or in those purchased on the open market by ACB, by either LC/MSMS or LC/HRAMS. The method's background level of each PFAS is 10 parts per trillion (ppt) or less (not taking the dilution factor into consideration).

As part of our quality control, two sets of QC samples were fortified/spiked with PFAS at known concentrations (1 and 9 ppm equivalent in the products), either with mass labeled PFAS standards (a total of 12, including two differently labeled PFOS) or non-labeled PFAS (a total of 26, including PFOS). Recoveries of PFAS in samples were greater than 60% for the 9 ppm spiking level, and greater than 40% for the 1 ppm level, using both analytical instruments (LC/MSMS and LC/HRAMS). Presence of the matrix in the diluted samples did not affect the detection of the spiked PFAS. The techniques used by ACB would have detected the PFAS if any of the pesticide products contained reported PFAS.

The method detection limits ranged from 0.2-1 ppm (0.5 ppm for PFOS, based on sample weight) for different PFAS in these pesticide products, taking into consideration of the dilution factor.

The reported PFOS levels by Lasee et al. ranged from 3.9 ppm to 19.2 ppm in the tested products (**Table 1**). These levels are well above the estimated method detection limit of 0.5 ppm for PFOS, and the spiking levels of our QC samples. If present, PFOS would have been detected in these products.

ACB'S METHOD:

A. Method

All the pesticide products listed in **Table 1** were processed and analyzed with a pesticide extraction method recently developed and validated recently at ACB for PFAS. This method is specific to these products, which are formulated in non-volatile oil and contain non-ionic surfactants. Aliquots of purchased pesticide products were also spiked at about 0.5 ppb by ACB with PFAS to monitor the performance of the method. All sample extracts were analyzed using the SW846 method 8327 and the same LC/MS/MS and LC/HRAMS as with Lasee's dilution method. This pesticide extraction method has a detection limit of approximately 0.2 ppb, which is more than 1000x lower than that of the dilution method. Both mass labeled surrogates and internal standards were used.

B. Results and Comments

None of the 29 PFAS compounds, including PFOS, was detected in any of the samples above the method detection limits, either in those obtained from Lasee or in those purchased by ACB, by either LC/MSMS or LC/HRAMS.

As part of our quality control, samples were fortified/spiked with known concentration of PFAS (2 ppb), either with mass labeled PFAS standards (a total of 12, including two differently labeled PFOS) or with non-labeled PFAS (including PFOS), then processed and

analyzed using the ACB's method. All spiked compounds were successfully recovered (greater than 50% of the fortification level) from the extracts of the pesticide products in the analyses with both analytical instruments (LC/MSMS and LC/HRAMS). These techniques used by ACB would have detected the PFAS if any of the pesticide products contained reported PFAS at or above 0.2 parts per billion (ppb) levels.

Detailed information on ACB's method is in **Attachment I**

CONCLUSION

BEAD's Analytical Chemistry Branch could not confirm the presence of PFOS as reported in Lasee's publication (3.9 ppm to 19.2 ppm), nor detect any PFAS above the method detection limits (0.2 ppb) in those pesticide products. Some background levels of PFAS were seen at less than 10 ppt (based on instrument response only, and not taking into consideration any dilution factor or sample preparation factor).

Although the SW846 Test Method 8327 is applicable for analyzing PFAS in samples that have been previously prepared using solvent dilution or extraction, due to the complex nature of pesticide products, preparation by solvent dilution is not an appropriate method. A more robust preparation method is necessary. Furthermore, since low amounts of PFAS are readily observed in the environment, incorrectly interpreted background data could be multiplied by a large dilution factor (if dilution was used as sample preparation), resulting in reporting of an overexaggerated concentration of a background PFAS or a false-positive identification. These large dilution factors utilized by Lasee et al. could have contributed to the high results obtained in that study.

REFERENCES

1. *Steven Lasee, Kaylin McDermett, Naveen Kumar, Jennifer Guelfo, Paxton Payton, Zhao Yang, Todd A. Anderson,* Targeted analysis and Total Oxidizable Precursor assay of several insecticides for PFAS - ScienceDirect. *Journal of Hazardous Materials Letters*, 2022, 3, 100067

2. EPA Method 8327. Per- and polyfluoroalkyl substances (PFAS) by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS). https://www.epa.gov/system/files/documents/2021-07/8327.pdf

ATTACHMENT

ATTACHMENT I – ACB Method for Pesticide Formulation Containing Non-ionic Surfactants and Non-volatile Oils

Scope of Method and Application

This method is for the analysis of poly- and per-fluorinated alkyl substances (PFAS) in pesticide formulations containing non-ionic surfactants and oil. It is based on a QuEChERS (Quick, Easy,

Cheap, Effective, Rugged and Safe) extraction approach, followed by Solid Phase Extraction (SPE) cleanup to remove excess oily substances, and analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). This method is not applicable if formulations contain ionic surfactants (such as sodium lauryl sulfate, quaternary ammonium compounds, etc.) or only organic solvents/liquids (petroleum distillates, mineral oil, etc.). A different method Analysis of PFAS in Oily Matrix (epa.gov) can be used for pesticide products formulated in organic solvents/oils.

Note: Due to the wide occurrence of PFAS in the environment, it is highly recommended to verify that all supplies and equipment are free of PFAS above the limit of detection. Certain PFAS compounds have been found in SPE cartridges, SPE manifold, and filters during the method development.

This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of LC-MS/MS instrumentation, and the interpretation of the associated data. EPA has validated this method through the Analytical Chemistry Branch (ACB) of the Biological and Economic Analysis Division, Office of Pesticide Programs.

Sample Preparation

Solvents:

- Milli-Q water
- Ethyl acetate
- Hexane
- Methanol

Materials:

- QuEChERS salt mix (6 g $MgSO_4$/1.5 g NaCl)
- Ammonium acetate
- Solid Phase Extraction cartridge –Florisil 1 g/6 mL column
- Polypropylene test tubes 15 and 50 mL

Solutions:

- Mobile phase A: Aqueous 20 mM ammonium acetate
- Methanol/water (99/1, v/v)
- Hexanes/ethyl acetate (9/1, v/v)

Standards:

- Extraction Standard: Mixture of isotopically labeled PFAS standards, different from injection standards
- Injection Standard: Mixture of isotopically labeled PFAS standards, different from Extraction standards
- Native PFAS standard: Mixture of all the target PFAS compounds.

Equipment:

- Geno/Grinder or equivalent
- Centrifuge
- N-Evap or equivalent
- Sonicator
- Liquid chromatography/tandem mass spectrometry (LC-MS/MS)

Extraction Procedure:

1. Weigh approximately 4 grams of pesticide products into 50 mL polypropylene centrifuge tubes.
2. For the procedural blank, transfer approximately 4 grams of Milli-Q water into a 50 mL tube.
3. For blank spikes and matrix spikes, weigh approximately 4 grams of Milli-Q water and pesticide product, respectively, into 50 mL tubes.
4. Add appropriate amount of "Extraction Standard" into each sample.
5. Add appropriate amount of spiking solution containing PFAS to spike samples.
6. Mix by vortexing or shaking and then let samples equilibrate after addition of PFAS standards for 15 minutes.
7. Add 5 mL of Milli-Q water and 25 mL of ethyl acetate to each sample.
8. Shake each sample on Geno/Grinder for 20 minutes at 1000 rpm.
9. Add QuEChERS salt mix (6 g MgSO4/1.5 g NaCl) to each sample, shaking by hand to break all salt clumps.
10. Shake all samples on Geno/Grinder for 20 minutes at 1000 rpm, followed by centrifugation for 10 minutes at 4000 rpm.
11. Transfer 20 mL of organic supernatant to a new 50 mL centrifuge tube and concentrate to dryness under N_2 flow at 50°C-60°C. *Note*: Some oil may remain after concentration depending on product formulation.
12. Add 20 mL of hexane/ethyl acetate (9/1, v/v) to the dried extracts and sonicate for 30 minutes, followed by a round of brief hand-shaking and then centrifugation at 4000 rpm for 10 minutes.
13. For solid precipitates: Decant entire supernatant into a new 50 mL tube.
14. For biphasic layers: Carefully transfer 20 mL of organic supernatant to a new 50 mL tube.
15. Concentrate samples as much as possible as in Step 11. Then combine with 5 mL of hexane/ethyl acetate (9/1, v/v) and proceed to SPE cleanup.
16. Attach Florisil SPEs to manifold and condition with 10 mL of methanol, followed by 10 mL of hexane/ethyl acetate (9/1, v/v).
17. Load sample onto SPE, and wash with 10 mL of hexane/ethyl acetate (9/1, v/v). Do not let the column run dry.
18. Place collection tubes under the manifold and elute samples with 10 mL of methanol.
19. For all samples: Add appropriate amounts of "Injection Standard" mixture to all solutions.
20. Concentrate all samples to dryness. Reconstitute with 1 mL of methanol/water (99/1, v/v). *Note:* If precipitate is visible in tube, centrifuge the tubes.
21. Transfer the solutions to LC vials for instrument analysis with LC-MS/MS.

Sample Analysis and Procedure

Calibration:

- Prepare a calibration curve of at least 5 levels in the range of 0.02 – 20 ng/mL of "Native" compounds.
- Each calibration point should also have "Extraction Standards" and "Injection Standards" at, for example, 0.50 ng/mL.

Data Analysis Note:

- Quantitation calculations are based on the response ratio of "Native PFAS" signal to "Extraction Standard" signal.

- Matrix effects can be assessed by comparing responses of "Injection Standards" in samples and calibration sets.

LC-MS/MS Specifications/Parameters

Equipment: Agilent 6470 LC-MS/MS or Equivalent
Mobile Phase A: Aqueous 20 mM Ammonium Acetate
Mobile Phase B: Methanol
Flow Rate: 0.400 mL/min
Solvent Gradient: 70% Mobile Phase A to 5% Mobile Phase A in 13 min.
Total Run Time: 26 minutes + 5 minutes Post Time Equilibration
MS Operation Mode: Electrospray Negative Ionization (ESI-) mode

List of Analyzed PFAS Compounds

Acronym	Chemical Name	Limits of Quantitation (ppb)	Comments
PFBA	Perfluoro-n-butanoic acid	0.40	High background
PFPeA	Perfluoro-n-pentanoic acid	0.40	High background
PFHxA	Perfluoro-n-hexanoic acid	0.40	
PFHpA	Perfluoro-n-heptanoic acid	0.40	
PFOA	Perfluoro-n-octanoic acid	0.40	
PFNA	Perfluoro-n-nonanoic acid	0.40	
PFDA	Perfluoro-n-decanoic acid	0.40	
PFUdA	Perfluoro-n-undecanoic acid	0.40	
PFDoA	Perfluoro-n-dodecanoic acid	0.40	
PFTrDA	Perfluoro-n-tridecanoic acid	0.40	
PFTeDA	Perfluoro-n-tetradecanoic acid	0.40	
PFHxDA	Perfluoro-n-hexadecanoic acid	0.40	
PFODA	Perfluoro-n-octadecanoic acid	2.00	Low recovery
PFPeS	Perfluoro-1-pentanesulfonate, Potassium Salt	0.40	
PFHxS	Perfluoro-1-hexanesulfonate, Sodium Salt	0.40	
PFHpS	Perfluoro-1-heptanesulfonate, Sodium Salt	0.40	
PFOS	Perfluoro-1-octanesulfonate, Sodium Salt	0.40	
PFNS	Perfluoro-1-nonanesulfonate, Sodium Salt	0.40	
PFDS	Perfluoro-1-decanesulfonate, Sodium Salt	0.40	
PFDoS	Perfluoro-1-dodecanesulfonate, Sodium Salt	0.40	
FOSAA	Perfluoro-1-octanesulfonamidioacetic acid	2.00	
N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	0.40	
N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	2.00	
11Cl-PF3OUDS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate, Potassium Salt	0.40	
9-CL-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate, Potassium Salt	0.40	

Acronym	Chemical Name	Limits of Quantitation (ppb)	Comments
4:2 FTS	1H, 1H, 2H, 2H-perfluorohexanesulfonate, Sodium Salt	2.0	
6:2 FTS	1H, 1H, 2H, 2H-perfluorooctanesulfonate, Sodium Salt	2.0	High background
8:2 FTS	1H, 1H, 2H, 2H-perfluorodecanesulfonate, Sodium Salt	0.40	
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate, Sodium Salt	0.40	

Note: PFBA, PFPeA, and 6:2 FTS have high background levels in this procedure. PFODA have low recovery by this extraction procedure.