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Extraction and Analysis of Per- and Polyfluoroalkyl Substances (PFAS) from Meals Ready-to-Eat (MRE) Films Using GC-MS and LC-MS/MS

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OBJECTIVE: This work was in response to the Defense Logistic Agency's (DLA) Subsistence Network Broad Agency Announcement, BAA-0003-16 addressing 2019 NDAA Section 329 that states packaging materials used for Meals Ready-to-Eat (MRE) that contact food products must be free of per- and polyfluoroalkyl substances (PFAS). This was addressed by determining the presence or absence of PFAS on MREs by extraction followed by gas chromatography mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Any samples positive for PFAS were quantitated using LC triple quadrupole (QqQ) MS at the US Army Engineering and Research Development Center (ERDC) and by high resolution quadrupole time-of-flight (qTOF) MS and GC-MS at Oregon State University (OSU).

BACKGROUND: Per- and polyfluoroalkyl substances, commonly referred to as PFAS, have become of high interest in recent years due to their persistence in the environment and potential to be carcinogenic (Ding and Peijneenburg 2013). PFAS are commonly used in industrial, military, and consumer products, while an effort to phase them out has started in recent years, they are known to be ubiquitous. Specifically, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have potential to be carcinogenic and bioaccumulate in the human body (Gorrochategui et al. 2016). Current advisory limits for drinking water set by the Environmental Protection Agency (USEPA) are 70 ng/L for the total sum of PFAS, however those levels are predicted to lower to single digit ng/L range and potentially lower within the next few years (USEPA 2009). The US Food and Drug Administration (USFDA) has resources regarding the analysis of PFAS in various food products and guidance on potential migration into food (Genualdi and deJagar 2019). Carnero et al. (2021) published a review of the detection of PFAS in various packaging materials and PFAS migration to food, giving insight into what PFAS levels are seen among common food packaging materials. In 2019, a mandate was made that stated materials contacting food in MREs must be PFAS free. The requirement for "PFAS free" is highly limited based on the analytical techniques available. The determination of the presence or absence of PFAS is directly dependent on the limits of detection (LOD) that can be reached. The instrumentation available to quantitate PFAS all have limits, setting a value at which PFAS cannot be detected below. Limits of detection were pushed to reasonable limits to achieve as close to PFAS free as scientifically possible. The detection and quantification of PFAS can be extremely difficult at low concentrations due to their inherent nature to be within analytical instrumentation used for detection. It is imperative to follow strict handling procedures and guidelines to avoid contamination of instrumentation and low-level samples when working with PFAS.

MATERIALS AND METHODS

Chemicals. All PFAS analytes, native and isotopically labeled, were purchased individually at 50 µg/mL or as mixtures at 1 µg/mL from Wellington Laboratories, Inc. (Guelph, Ontario, Canada). A secondary source of PFAS analytes for quality assurance purposes was purchased from Absolute Standards Inc. (Hamden, CT). Ammonium acetate (99.99%) for mobile phase additives, ethylene glycol (≥ 99%) for extraction, and LCMS grade methanol used for extraction and mobile phases were purchased from Sigma Aldrich (St. Louis, MO). Optima (mass spectrometry, MS) grade water for use as high-performance liquid chromatography (HPLC) mobile phase were purchased from Fisher Scientific (Hampton, NH). Methanol (≥ 99%) for GC-MS extraction and analysis was purchased from VWR (Radnor, PA).

Samples. MRE films were provided from three different manufacturers and will be referred to as MRE Films 1 – 3. Films were acquired directly from manufacturers in the form of pouches that have never touched food material. Manufacturers were sent sampling kits to sample films and send them to ERDC in plastic bags and then were distributed to OSU and Notre Dame University (MRE Film 1 only). Plastic bags were tested to ensure they did not have detectable limits of PFAS to verify that any detected PFAS were not due to materials the films contacted during shipping. Pouches were stored double bagged until they were tested.

Extraction and analysis. Film samples were tested for both volatile and non-volatile PFAS as well as total fluorine. Analysis of volatile PFAS was completed using GC-MS targeting nine compounds (Table 1). Extraction from film samples followed a previously published extraction method by Rewerts et al. (2018). Methanol-rinsed scissors were used to cut film into 1.5 × 1.5 cm squares. Samples were then placed in a 1.5 mL autosampler vial, mass-labeled standards, and internal standard (7:1 fluorotelomer alcohol) were spiked (150 ng), and methanol added to a final volume of 1500 µL. Vials were then sonicated at 25 °C for 30 min and stored at -15 °C until analysis.

Sample extracts (10 µL) were injected in splitless mode with an inlet temperature of 280 °C. A 4 mm i.d. single-taper Topaz liner with deactivated quartz wool (Restek, Bellefonte, PA) was used. Helium was used as the carrier gas at a constant flow of 1 mL/min. Separations were performed using a deactivated, fused silica capillary column (Agilent Technologies) connected to an Rxi-624Sil MS capillary column (Restek). The initial oven temperature was set to 50 °C and held for 2 min, increased to 188 °C at 5 °C /min, and then increased to 300°C at 15°C/min. The GC-MS method targeted nine analytes. The full list of PFAS targeted in this method, extracted internal standards used for quantitation, and limit of detections can be found in Table 1.

Total fluorine was analyzed for MRE 1 using particle induced gamma ray emission (PIGE) spectroscopy (Muensterman et al. 2022; Ritter et al. 2017). An area of 2 × 2 cm^2 was cut from MRE 1 Film using methanol-rinsed scissors and shipped to Notre Dame University inside a resealable pouch. The sample was mounted to a stainless-steel target frame using clear adhesive tape and was analyzed *ex vacuo*. Total fluorine was calculated based on an external calibration curve with sodium fluoride standards. The accuracy and precision of PIGE ranged from 96% to 106% and ±5.4%, respectively (Peaslee et al. 2020). The limit of quantification was 16 ppm F (Peaslee et al. 2020).

Table 1. List of targets volatile PFAS analyzed using GC-MS. Whole method LOD were based on polyethylene film (sample matrix) spiked with volatile PFAS. Linear dynamic range for GC-MS spanned 1000 – 2000000 ng/L (n = 8). Method LOQ was calculated by multiplying method LOD by 3.3.

Analyte	Isotope Dilution Analog	LOD (ng/g)
4:2 FTOH	d_4-4:2 FTOH	25
6:2 FTOH	d_2-$^{13}C_2$-6:2 FTOH	40
8:2 FTOH	$^{13}C_2$-8:2 FTOH	99
10:2 FTOH	d_2-$^{13}C_2$-10:2 FTOH	31
12:2 FTOH	d_2-$^{13}C_2$-10:2 FTOH	37
N-EtFOSA	d_5-N-EtFOSA-M	44
N-MeFOSA	d_3-N-MeFOSA-M	34
N-MeFOSE	d_7-N-MeFOSE-M	50
N-EtFOSE	d_9-N-EtFOSE-M	58

Non-volatile PFAS were analyzed using two different types of instrumentation, LC-QqQ-MS and high-resolution mass spectrometry using LC-qTOF. High resolution mass spectrometry is capable of non-target analysis and LC-QqQ-MS can achieve lower levels of detection due to increased sensitivity. Both instruments implemented targeted analysis and isotope dilution for quantitation.

Film samples were extracted using a paper and textile extraction method previously published by Robel et al. (2017) that was modified to include a concentration step to lower detection limits and allow for quantitation using isotope dilution. Sample extraction was performed separately for each method of analysis using different pouches from the same film manufacturer. The only change made to the extraction method before the concentration step was the addition of extracted internal standards (EIS) (0.9 ng and 0.7 ng of each ESI for qTOF and QqQ, respectively) before extraction. Before blowing down samples, a small aliquot of ethylene glycol was added to the extract to act as a keeper solvent preventing loss of analytes during the blow down step (Powley et al. 2020). Samples were evaporated to dryness under a gentle stream of nitrogen and reconstituted in methanol (MeOH). An aliquot of the final extract was diluted with MeOH and nonextracted internal standards (NIS), M_2PFOA and M_8PFOS, were added before analysis. During the introduction of a concentration step, multiple dilution factors were tested to balance detection limits and matrix effects. Samples prepared for qTOF analysis were reconstituted in 150 µL of MeOH and a 50 µL aliquot of that extract was diluted to 150 µL using MeOH with 0.3 ng of EIS for analysis. Samples prepared for QqQ analysis were reconstituted in 250 µL of MeOH and a 75 µL aliquot of that extract was diluted to 300 µL using MeOH with 0.2 ng of ESI for analysis. Samples analyzed using QqQ were not able to be concentrated to the same level as the samples analyzed using qTOF due to the increased sensitivity of QqQ. It was observed that when the extracts were concentrated to those prepped for qTOF analysis, PFAS were present in the processing blank > LOQ from the keeper solvent. Matrix effects also affected analysis when using QqQ. Due to the ubiquitous nature of PFAS, additional blanks and controls were used throughout the process ensuring that any analytes identified on MRE films were not from a different source. Processing blanks and blank spikes were used throughout the extraction process. It is important to have processing blanks go through the entire extraction practice to ensure PFAS is not introduced during the extraction process. The QqQ method targeted 31 analytes and the qTOF targeted 50

analytes. The full list of PFAS targeted in these methods, EIS used for quantitation, and LODs can be found in Table 2.

Table 2. List of target nonvolatile PFAS analyzed using LC-QqQ-MS and LC-qTOF and the associated EIS for quantitation. Whole method LOD for LC-QqQ-MS and LC-qTOF were based on polypropylene film (sample matrix) spiked with nonvolatile PFAS. Linear dynamic ranges for LC-QqQ-MS and LC-qTOF spanned 1–200 ng/L and 10–1000 ng/L, respectively (n = 8). Method LOQ was calculated by multiplying method LOD by 3.3.

Analyte	Isotope Dilution Analog[a]	qTOF LOD (ng/g)	QqQ LOD (ng/g)
PFBA	MPFBA[b]	0.04	0.01
PFPeA	M3PFPeA	0.09	0.005
PFHxA	M2PFHxA[b]	0.07	0.002
PFHpA	M4PFHpA	0.06	0.005
PFOA	M4PFOA[b]	0.06	0.002
PFNA	M5PFNA[b]	0.07	0.004
PFDA	MPFDA[b]	0.04	0.004
PFUdA	MPFUdA[b]	0.06	0.003
PFDoA	MPFDoA[b]	0.12	0.002
PFTrDA	MPFDoA	0.05	0.001
PFTeDA	M2PFTeDA	0.02	0.01
PFHxDA	M2PFHxDA	0.07	0.005
PFPrS	M3PFBS	0.05	N/A
PFBS	M3PFBS	0.02	0.005
PFPeS	M3PFBS	0.04	0.003
PFHxS	MPFHxS	0.04	0.002
PFHpS	MPFHxS	0.06	0.001
PFOS	MPFOS[b]	0.03	0.01
PFNS	MPFOS[b]	0.05	0.005
PFDS	MPFOS[b]	0.05	0.006
PFDoS	MPFOS	0.04	N/A
Cl-PFOS	MPFOS	0.04	N/A
PFEtCHxS	MPFHxS	0.03	N/A
FBSA	M8FOSA	0.09	0.005
FHxSA	M8FOSA	0.07	0.006
FOSA	M8FOSA	0.06	0.007
MeFOSA	d-N-MeFOSA-M	0.10	N/A
EtFOSA	d-N-EtFOSA-M	0.09	N/A
FOSAA	d_3-N-MeFOSAA	0.06	N/A
NMeFOSAA	d_3-N-MeFOSAA	0.14	0.005
NEtFOSAA	d5-N-EtFOSAA	0.05	0.005
4:2 FTS	M2-4:2FTS	0.09	0.006
6:2 FTS	M2-6:2FTS	0.12	0.01
8:2 FTS	M2-8:2FTS	0.14	0.006
10:2 FTS	M2-8:2FTS	0.09	N/A
3:3 FTCA	M6:2FTA	0.26	N/A
5:3 FTCA	M8:2FTA	0.20	N/A
7:3 FTCA	M10:2FTA	0.30	N/A
6:2 FTCA	M6:2FTA	0.06	N/A
8:2 FTCA	M8:2FTA	0.24	N/A
10:2 FTCA	M10:2FTA	0.21	N/A

6:2 UFTCA	M6:2FTUA	0.20	N/A
8:2 UFTCA	M8:2FTUA	0.24	N/A
ADONA	M5PFNA[b]	0.07	0.005
9Cl-PF3ONS	MPFOS[b]	0.03	0.003
11l-PF3OUdS	MPFOS[b]	0.05	0.005
HFPO-DA	MHFPO-DA	0.13	0.01
6:2diPAP	M4 8:2 diPAP	0.09	N/A
8:2diPAP	M4 8:2 diPAP	0.13	N/A
diSAmPAP	M4 8:2 diPAP	0.04	N/A

a: Isotope dilution analogs listed are used for quantitation in the QqQ method
b: The qTOF method uses the same base isotope dilution analog with a different label

RESULTS AND DISCUSSION: All three films were analyzed in at least four replicates using three different MS techniques: GC-MS, LC-MS/MS (QqQ), and LC-HRMS (qTOF). There was no target volatile PFAS analytes above LOD present in any of the three films. MRE Film 1 was also sent out for PIGE spectroscopy analysis and fluorine was not present above the limit of quantitation (16 ppm) (Ritter et al. 2017). However, nonvolatile PFAS were present at some level in all three MRE films. It is important to address that in some cases PFBA was present above the limit of quantitation in processing blanks extracted by both ERDC and OSU. Many PFAS can be found throughout the laboratory and contamination is extremely difficult to avoid. There have been other cases of PFBA being present in blanks at various concentrations present in processing blanks when looking for PFAS in food products (Genualdi et al. 2022). Moreover, since fluorine was not detected by PIGE, but nonvolatile PFAS were detected on the MRE films, the introduction of residual PFAS could be through the manufacturing process of the pouches rather than from the application of fluoropolymer films (Muensterman et al. 2022).

The PFAS analytes and concentrations differ between manufacturers and, in some instances, between samples of film from the same manufacturer. Through the extraction of multiple pouches from the same manufacturer, it was determined that the films were heterogeneous, and the composition of films could vary from film-to-film and across a film's surface. The results of non-volatile PFAS analysis for all three MRE films for both types of instrumental analysis are shown in Tables 3–5. Results from qTOF analysis for MRE Films 1 and 2 show dominance of long-chain carboxylic acids (PFNA, PFUdA, and PFDoA). MRE Film 3 has the lowest level of total PFAS, but the highest number of PFAS present for the QqQ analysis with most of them below the limits of quantitation for the qTOF.

Table 3. PFAS analytes detected in MRE Film 1 using LC-qTOF-MS and LC-QqQ-MS (ng/g).

LC-qTOF results for MRE Film 1						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFPeA	<LOD	**<LOQ**	**<LOQ**	<LOD	**<LOQ**	**0.20**
PFHxA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFHpA	**<LOQ**	**1.0**	**2.1**	**0.40**	**1.3**	**0.20**
PFOA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFNA	<LOD	**1.6**	**1.6**	**<LOQ**	**2.2**	**0.20**
PFDA	<LOD	**<LOQ**	<LOD	<LOD	**<LOQ**	**0.20**
PFUdA	<LOD	**4.4**	**0.70**	<LOD	**1.5**	**0.20**
PFDoA	<LOD	**4.6**	**1.8**	**0.48**	**4.5**	**0.20**

PFOS	<LOD	**1.8**	<LOD	**<LOQ**	<LOD	**0.20**
6:2 FTCA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
6:2 diPAP	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
LC-QqQ-MS results for MRE Film 1						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA	**<LOQ**	**0.14**	**0.15**	**0.19**	**0.15**	**0.12**
PFHxA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFOA	<LOD	**0.15**	**<LOQ**	**<LOQ**	**<LOQ**	**0.04**
PFDoA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.04**
PFTrDA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFBS	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.10**
PFHxS	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.05**
PFOS	**<LOQ**	**<LOQ**	**<LOQ**	**0.15**	**<LOQ**	**0.07**
6:2 FTS	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.08**

Table 4. PFAS analytes detected in MRE Film 2 using LC-qTOF-MS and LC-QqQ-MS (ng/g).

LC-qTOF results for MRE Film 2						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFPeA	<LOD	**0.27**	**0.41**	**0.24**	**<LOQ**	**0.20**
PFHxA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFHpA	**<LOQ**	**4.8**	**5.0**	**3.8**	**1.5**	**0.20**
PFOA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFNA	<LOD	**1.4**	**1.7**	**1.3**	**0.37**	**0.20**
PFDA	<LOD	<LOD	<LOD	<LOD	<LOD	**0.20**
PFUdA	<LOD	**0.29**	**0.45**	**0.30**	<LOD	**0.20**
PFDoA	<LOD	**0.51**	**0.62**	**0.55**	<LOD	**0.20**
6:2 FTCA	<LOD	<LOD	**0.34**	<LOD	<LOD	**0.20**
LC-QqQ-MS results for MRE Film 2						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA	**<LOQ**	**0.13**	**0.14**	**0.11**	**0.15**	**0.12**
PFHxA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFOA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.04**
PFDoA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.04**
PFTrDA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFBS	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.10**
PFHxS	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.05**
PFOS	**<LOQ**	**2.3**	**1.1**	**1.1**	**0.85**	**0.07**
6:2 FTS	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.08**
HFPO-DA	<LOD	**0.44**	**<LOQ**	**<LOQ**	**<LOQ**	**0.13**

Table 5. PFAS analytes detected in MRE Film 3 using LC-qTOF-MS and LC-QqQ-MS (ng/g).

LC-qTOF results for MRE Film 3						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA	**<LOQ**	**0.53**	**0.37**	**0.49**	**<LOQ**	**0.30**
PFPeA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**0.35**	**0.30**
PFHpA	<LOD	**0.47**	**<LOQ**	**0.40**	**0.33**	**0.30**
PFOA	<LOD	**<LOQ**	<LOD	<LOD	**<LOQ**	**0.30**
PFNA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	<LOD	**0.30**
PFOS	<LOD	**<LOQ**	<LOD	<LOD	**<LOQ**	**0.30**
6:2 diPAP	**<LOQ**	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**0.30**
LC-QqQ-MS results for MRE Film 3						
Analyte	**Processing Blank**	**n:1**	**n:2**	**n:3**	**n:4**	**LOQ**
PFBA[a]	<LOD	<LOD	**0.35**	**0.14**	<LOD	**0.12**
PFPeA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFHxA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**
PFOA	<LOD	**<LOQ**	**<LOQ**	**0.063**	**<LOQ**	**0.04**
PFNA	<LOD	<LOD	**<LOQ**	<LOD	<LOD	**0.04**
PFDA	<LOD	<LOD	<LOD	**<LOQ**	<LOD	**0.05**
PFDoA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.04**
PFHxS	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.05**
PFHpS	<LOD	**<LOQ**	<LOD	**<LOQ**	<LOD	**0.06**
PFOS	<LOD	**0.39**	**0.15**	**0.62**	**0.17**	**0.07**
PFNS	<LOD	<LOD	<LOD	**<LOQ**	<LOD	**0.06**
NEtFOSAA	<LOD	**<LOQ**	**<LOQ**	**<LOQ**	**<LOQ**	**0.06**

a: Analyte concentrations were reported after background subtraction.

The heterogeneity of the film material contributes to differences in results between the two instruments considering different pouches of the same film were used for each analysis. This was further tested using extracts from MRE Film 1 by comparing the percent relative standard deviation (% RSD) of six individual extracts to the % RSD of a pool of those extracts injected in triplicate. The results for the qTOF and QqQ analysis can be found in Table 6. There was a clear difference between the two analyses, supporting the hypothesis that the MRE pouches are not homogeneous. The heterogeneity of PFAS on the films gives cause to believe that the PFAS present on the films could be from a release agent throughout the process of making the film or assembling the pouches. With PFAS potentially being introduced though the assembly of the pouches, it is possible that the difference in results between the two instruments is a result of the release agent in the process not in contact with the entire length of the film. Due to the heterogeneity of the films, it was determined to better compare results between the two instrumental techniques, films should be extracted by one individual and then be split for analysis. The technique of splitting extracts eliminates the heterogeneity of the film contributing to the differences between the two instruments.

Table 6. % RSD comparison for both instrumental techniques between replicate samples versus replicate injections of the same sample.

LC-qTOF		
Analyte	**% RSD (n = 6)**	**% RSD Pooled sample**
PFHpA	100	1.1
PFNA	43	2.2
PFDA	1.1	26
PFDoA	51	N/A
LC-QqQ-MS		
Analyte	**% RSD (n = 6)**	**% RSD Pooled sample**
PFHpA	79	5.0
PFNA	39	10
PFDoA	36	1.7

The limits of detection reached with this extraction method, specifically using the QqQ, makes it possible to detect single digit ng/L quantities of PFAS in the extract. While PFAS are present above limits of quantitation in all films tested, the levels are low, thus requiring a need to determine if there is a quantity of PFAS present in packaging material that would be considered negligible.

Calculations can be made from the results to estimate the highest possible concentration of PFAS that could be present in the food packaged in these films. Using the 0.3 g sample of film used for extraction, the hypothetical total amount of PFAS contained in a full pouch was estimated assuming the sample represents the concentration in the overall pouch. That estimation was then used to estimate the potential amount of PFAS introduced to the food through contact assuming that 100% of the PFAS leached into the food product using the average weight of an MRE for the weight of the food. The total PFAS detected in n:1 from MRE Film 1 by qTOF was used for these calculations because it had the highest concentration of total PFAS present. The film itself has a total concentration of 13.4 µg/kg of PFAS and an estimated 0.129 µg/kg highest possible concentration of PFAS in the food. These values are at the low end of packaging materials when compared to levels found in food covered in a recent review concerning PFAS in food packing material and their potential migration into food (USFDA 2020). It is unlikely that 100% of PFAS present in the film would leach into the food products considering that the highest rate of migration of PFAS to food is seen in paper materials (Carnero et al. 2021; USFDA 2020). Additionally, the migration of shorter chain PFAS is more likely than longer chain PFAS and short chain PFAS were not as prevalent in the MRE films tested (Carnero et al. 2021). It is important to address the feasibility of analytical confidence that a product is "PFAS free;" instead, reporting that PFAS are not present above a specific limit is practical. This research pushed the limits of detection for the extraction of PFAS from film material so LODs were as low as was reasonable without causing additional analytical issues such as matrix effects and background levels of PFAS in the material used for extraction.

SUMMARY: Volatile PFAS were not present above limits of detection in any of the three films tested. However, non-volatile PFAS were detected in all three films analyzed in this study, with most detections below limits of quantitation. MRE Films 1 and 2 had many of the same PFAS detected between QqQ and qTOF analyses, while MRE Film 3 did not contain the same analytes. Films 1 and 2 both predominantly contained long-chain carboxylic acids (PFNA, PFUdA, and

PFDoA), which were present in Film 3 below the limits of quantitation. Film 3 contained shorter chain carboxylic acids (PFBA, PFHxA, and PFOA). With the levels of PFAS observed in the films, it is believed that they are a result of releasing agents present in the manufacturing process of the films and pouches. The presence of PFAS because of releasing agents supports the heterogeneity observed in the film materials since the releasing agent would not be consistent throughout the film. This research has shown that PFAS are present on multiple films used to assemble MREs and the next step would be to determine the source of these PFAS. This would require testing the starting materials for the films and then following the films through the assembly line and testing them throughout the process. To expand this work, qTOF data can be reanalyzed to determine if PFAS not targeted in the two analytical methods are present, thus allowing for a more comprehensive picture on the presence of PFAS in the films.

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