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3M

Laboratory Report
Analysis of FCs in Samples of Children's Sera

Laboratory Report No. FACT-GEN-011
W1724

Testing Laboratory

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Requester

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3M Company
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220-3W-05
St. Paul, MN

REPORT DATE: 05/21/99

1 Introduction

Samples were analyzed to provide a quantitative determination of PFOS and PFHS in a small number of samples of sera collected from children between the ages of 6 and 12. Additionally, samples were screened to provide qualitative data on the presence of several other fluorochemicals including PFDS, PFOSA, PFOSAA, M556, M570, POAA, M463, and M513 (Appendix C contains a list of acronyms).

Less than 100 µL of sera were available for analysis. This small sample volume posed analytical restrictions and method detection limits for this study are significantly higher than reported in earlier studies.

Sera samples were extracted using an ion-pairing extraction procedure. The extracts were quantitatively analyzed for PFOS and PFHS using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HLPC/ESMSMS), and evaluated versus an extracted curve. Qualitative analysis was conducted by comparing peak response in the samples to that obtained from standards, when possible. If standard material was not available, compound identification was based on reasonable HPLC-retention time and predicted mass spectrometer response. Analytical details are available in the study binder maintained by FACT.

Despite the increased detection limits that resulted from the small sample size, several fluorochemicals were detected in these sera samples. PFOS, PFHS, PFDS, POAA, and M463 were detected in each of the samples analyzed; for these analytes, the estimated detection limit is 3 ppb. PFOSAA, M556, and M570 were detected in some of the samples and the detection limit is estimated to be approximately 5 ppb. PFOSA and M513 were not detected in any sample above the estimated detection limit of 8 ppb and 12 ppb, respectively.

The average PFOS concentration was 54 ppb, covering a range of 31-115 ppb. These values are slightly higher than the average values observed previously for adults and some part of that (up to 10%) may be due to improvements to the modified method used to extract these samples.

The average PFHS level determined in these samples was 35 ppb across a range of 5-100 ppb. This average is approximately 10 times higher than the levels previously observed in a small set of adult sera samples. In those samples, the highest value determined in a non-3M worker was 13 ppb. It is unlikely that the minor modifications made to the analytical method could account for this dramatic difference between the two groups of samples.

Although rigorous quality control measures and 3M Environmental Laboratory Standard Operating Procedures were followed, the acquisition of this data was not necessarily collected according to applicable Good Laboratory Practices. This data is not suitable for distribution to any government agency in defense of product suitability or safety. Data presented here is the highest quality data available at this time.

2 Sample Receipt

On 4/27/99, Jean Burris of 3M Occupational Medicine delivered sera samples to the Environmental Lab. Environmental Lab personnel initiated a chain-of-custody upon receipt of the samples.

3 Holding Times

There is no holding time criteria associated with these samples for LC/ESMSMS analysis.

4 Methods - Analytical and Preparatory

Analytical methods

ETS-8-4.0, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

MODIFIED to accommodate small sample size. Reagent volumes were reduced and aqueous extracts were triple rinsed with MtBE. These modifications may result in slightly better analyte recoveries than those achieved using the original method.

ETS-8-5.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical on Serum or Other Fluid Using HPLC-Electrospray/Mass Spectrometry

MODIFIED for PFHS determination by comparing PFHS samples response to PFOS curves, as no standard PFHS was available. This substitution is likely to result in a slight UNDER estimation of PFHS levels.

Sample preparation – aqueous samples, HPLC/ESMSMS: ion-pairing extraction

Analyte is extracted from a sample matrix with ion pairing reagent [tetrabutyl ammonium hydrogen sulfate (TBA)] in a pH-controlled environment. The cationic reagent selectively targets anionic fluorochemicals. Once the anion-TBA pair is formed, the analyte is transferred into a non-polar organic solvent (methyl-*tert*-butyl ether), dried, and reconstituted in methanol for MS analysis.

HPLC/ESMSMS: for detailed qualitative work

In HPLC, an aliquot of extract is injected and passed through a reverse phase liquid chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. For example, in a standard solution PFOS may elute at 8.0 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched on the HPLC system to within 1%.

Following HPLC separation, ESMSMS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray, an ionization technique used primarily for detection of molecular ions, is generally operated at relatively mild temperatures. Molecules are ionized, and a primary ion, characteristic of the analyte, is selected. This ion is bombarded with high-energy gas; subsequent collisions create smaller secondary ionic fragments unique to the primary ion, which are detected.

For example, for PFOS ($C_8F_{17}SO_3^-$) analysis, ion 499 is selected as the characteristic primary ion. This ion is fragmented into other ions such as 80 amu (corresponding to SO_3^-), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected and can be used to differentiate PFOS from other compounds that might have the same characteristic 499 amu primary ion, but different chemical compositions and secondary ion fragmentation patterns.

5 Analysis

5.1 Calibration

For quantitative determinations, a mid-level, extracted matrix calibration check standard is analyzed every 5 samples to monitor instrumental drift. Calibration check standards are compliant if instrumental response is within +/- 10% of the expected value.

Quantitation of the target analytes is based on linear regression analysis (weighted 1/x) of two extracted matrix curves bracketing each group of samples. Quantitation of each analyte is based on the response of one or more specific daughter ions using the multiple response-monitoring mode of the instrument.

5.2 Blanks

Two extraction blanks, utilizing water as surrogate matrix, are extracted with each batch of samples. Additionally, two samples of blank matrix or surrogate matrix, used for the extracted calibration curves, are extracted along with each batch of samples and curves.

Blanks are compliant if no target analyte is detected above the limit of detection for a specific analyte. In this study, all blanks were compliant.

5.3 Surrogates

Tetra-hydro perfluorooctane sulfonate is used as a surrogate in this analysis. Surrogate response is monitored to confirm gross instrumental failure.

5.4 Matrix Spikes

Duplicate matrix spike analyses utilizing all target analytes for which quantitative data is presented were prepared and analyzed for one control animal. For sera analysis, recoveries were within the acceptable range of 85-115%.

5.5 Laboratory Control Samples

Laboratory Control Samples are not a component of this study.

5.6 Sample Related Comments

Due to limited sample volume, it was not possible to conduct matrix spike studies on any of these samples. Although the current understanding of the analytical methods indicate that rabbit serum is a suitable surrogate matrix, matrix spike studies for this matrix are recommended. Standard materials for PFDS and M463 were not available for compound identification at the time of analysis. Compound identification is based on reasonable retention time and mass spectral data consistent with compound structure.

6 Data Summary

Despite the increased detection limits that resulted from the small sample size, several fluorochemicals were detected in these sera samples. PFOS, PFHS, PFDS, POAA, and M463 were detected in each of the samples analyzed; for these analytes, the estimated detection limit is 3 ppb. PFOSAA, M556, and M570 were detected in some of the samples and the detection limit is estimated to be approximately 5 ppb. PFOSA and M513 were not detected in any sample above the estimated detection limit of 8 ppb and 12 ppb, respectively.

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This report reflects the most accurate data available at this time.

7 Data / Sample Retention

Samples and extracts will be retained for future evaluation, if necessary.

REPORT DATE: 05/21/99

8 Attachments

8.1 **Attachment A:** Quantitative Results of PFOS and PFHS Determination
8.2 **Attachment B:** Qualitative Summary of FCs identified in Samples of Children's Sera
8.3 **Attachment C:** Table of FACT Acronyms
8.4 **Attachment D:** Chain of Custody
8.5 **Attachment E:** Analytical Data for PFOS and PFHS determination

attachments
sent separately

9 Signatures

Lisa Clemen, Analytical Chemist — Date: 5/21/99

Kris Hansen, Ph.D., Study Director — Date: 5/21/99

REPORT DATE: 05/21/99