Evaluation of Perfluorooctanesulfonate (PFOS) in Rat Brain

Christopher Lau 1, Julie Thibodeaux 1, David Ehresman 2, Sue Tanaka 2, John Froehlich 3, and John Berenholtz 2

1 USEPA, Research Triangle Park, NC; 2 3M Company, St. Paul, MN; 3 Pace Analytical, St. Paul, MN

Abstract

This study evaluated whether there is a differential distribution of PFOS within the brain, and compared male rats with non-pregnant rats at age when formation of the blood-brain barrier is not yet complete (postnatal day 7). Male and female Sprague-Dawley rats (60-70 day-old, 4x) were treated with 3 mg PFOS per kg per day by gavage or given a single oral dose of 70 mg PFOS/kg on postnatal day 0. A blood sample was obtained from tail-blood prior to sacrifice, and the brain was removed and homogenized in 0.1 N HCl to extract PFOS from all brain regions. A control group of rats was similarly treated with distilled water. Brain tissues were homogenized in 70% methanol and PFOS concentration in the supernatant was measured by LC/MS-MS analysis. The objectives of the present study were therefore to: 1) develop a reliable method for quantitative analysis of rat brain tissue; 2) determine the relative difference between males of PFOS in perfused and non-perfused rat brain tissue; 3) determine the concentration of PFOS in cerebrospinal fluid (CSF) of rats relative to serum PFOS concentration; and 4) determine the regional brain mean PFOS concentrations expressed as percentage of mean serum concentration by analyzing brain tissues. This study examined whether there is a differential distribution of PFOS within the blood-brain barrier is not yet complete (postnatal day 7). Male and female Sprague-Dawley rats (60-70 day-old, 4x) were treated with 3 mg PFOS per kg per day by gavage or given a single oral dose of 70 mg PFOS/kg on postnatal day 0. A blood sample was obtained from tail-blood prior to sacrifice, and the brain was removed and homogenized in 0.1 N HCl to extract PFOS from all brain regions. A control group of rats was similarly treated with distilled water. Brain tissues were homogenized in 70% methanol and PFOS concentration in the supernatant was measured by LC/MS-MS analysis. The objectives of the present study were therefore to: 1) develop a reliable method for quantitative analysis of rat brain tissue; 2) determine the relative difference between males of PFOS in perfused and non-perfused rat brain tissue; 3) determine the concentration of PFOS in cerebrospinal fluid (CSF) of rats relative to serum PFOS concentration; and 4) determine the regional brain mean PFOS concentrations expressed as percentage of mean serum concentration by analyzing brain tissues.

Methods

Dosing and Sample Collection

Objective 1, 2, and 3

Adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were not previously exposed and were either a single oral dose of 70 mg PFOS/kg on postnatal day 0 or 3 mg PFOS per kg per day for 28 days by gavage. Adult male and female Sprague-Dawley rats (60-70 day-old, 4x) were treated with 3 mg PFOS per kg per day for 28 days by gavage or given a single oral dose of 70 mg PFOS/kg on postnatal day 0. A blood sample was obtained from tail-blood prior to sacrifice, and the brain was removed and homogenized in 0.1 N HCl to extract PFOS from all brain regions. A control group of rats was similarly treated with distilled water. Brain tissues were homogenized in 70% methanol and PFOS concentration in the supernatant was measured by LC/MS-MS analysis.

Anatomical Procedures

Sample Preparation

Brain Tissue

Brain tissues from PFOS and vehicle treated rats were stored at -80°C until ready to begin analysis. Samples were analyzed by liquid chromatography coupled with tandem mass spectrometry on a triple quadrupole mass spectrometer equipped with an electrospray ionization source. The ionization source was operated in negative ion mode. A regular gradient was used for the analysis of PFOS with a dwell time of 200 ms. For each sample, a 10 µL injection was introduced to the mass spectrometer through a high performance liquid chromatography system. All quantitative calculations for PFOS were based on the ion ratios between PFOS and the stable-isotope-labeled internal standard (dual substituted PFOS standard). Our mobile phase was 51% acetonitrile and 49% 2 mM ammonium acetate. All source parameters were optimized under these conditions according to manufacturer's guidelines.

Sealed vials were removed from each tube and placed in a steel vial vial. Each sample was stored at -80°C until ready to begin analysis. All brain samples were homogenized in 70% methanol and PFOS concentration in the supernatant was measured by LC/MS-MS analysis. The objectives of the present study were therefore to: 1) develop a reliable method for quantitative analysis of rat brain tissue; 2) determine the relative difference between males of PFOS in perfused and non-perfused rat brain tissue; 3) determine the concentration of PFOS in cerebrospinal fluid (CSF) of rats relative to serum PFOS concentration; and 4) determine the regional brain mean PFOS concentrations expressed as percentage of mean serum concentration by analyzing brain tissues.

Results

Objective 1 - Brain Tissue/Method Development

The brain extracts were analyzed by the API 4000 mass spectrometer from Applied Biosystems/MDS Sciex Instrument Corporation. The ion spray (IS) was operated at 5500 V (positive mode) and 2000 V (negative mode) at a source temperature of 450°C. For the negative mode 51% acetonitrile and 49% water was used. For the positive mode 50% methanol and 50% water was used.

The extracted ion chromatograms from the mass spectrometer were analyzed by the Analyst software version 1.3. The retention time for PFOS was approximately 2.5 minutes.

Results

Objective 2 and 3 - Comparison of PFOS Concentrations in Perfused versus Non-Perfused Brains and CSF Relative to Serum PFOS Concentration

Although brain tissue PFOS concentration in neonatal rats was approximately 100 times less than corresponded serum or CSF concentrations, PDOS, the present study demonstrates that PFOS is able to be measured in perfused brain tissue. This method employs a base digestion followed by a solid phase extraction at an acidic pH for optimal recovery. This technique involves the use of a base digestion followed by a solid phase extraction at an acidic pH for optimal recovery. This method employs a base digestion followed by a solid phase extraction at an acidic pH for optimal recovery. This method employs a base digestion followed by a solid phase extraction at an acidic pH for optimal recovery.

Discussion and Conclusions

Objective 2 - Brain Tissue PFOS Concentrations after Perfusion Relative to Serum PFOS Concentrations

The objectives of the present study were therefore to: 1) develop a reliable method for quantitative analysis of rat brain tissue; 2) determine the relative difference between males of PFOS in perfused and non-perfused rat brain tissue; 3) determine the concentration of PFOS in cerebrospinal fluid (CSF) of rats relative to serum PFOS concentration; and 4) determine the regional brain mean PFOS concentrations expressed as percentage of mean serum concentration by analyzing brain tissues.

Conclusions

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Table 1. Comparison of PFOS Concentrations in Perfused versus Non-Perfused Brains and CSF Relative to Serum PFOS Concentration

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PFOS [µg/mL]</th>
<th>% of Serum [PFOS]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>26.2 ppm</td>
<td>100%</td>
</tr>
<tr>
<td>Brain</td>
<td>6.9 ppm</td>
<td>26.2%</td>
</tr>
</tbody>
</table>

Citations


