# APPLICATION OF AN IMMUNOAFFINITY COLUMN TO THE ISOLATION OF PCDD/Fs FROM SERUM SAMPLES

Janice K. Huwe*, Weilin L. Shelver*, Donald G. Patterson, Jr.**, and Wayman E. Turner**

*USDA, Biosciences Research Laboratory, P.O. Box 5674 University Station, Fargo, ND, 58105 USA; **Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Center for Disease Control and Prevention, 4770 Buford Highway N. E., Atlanta, GA 30341-3721, USA

## Introduction

In order to better determine health risks which may be related to exposure to persistent organic pollutants such as polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), it is expedient to correlate adverse health effects with body burden levels. Better risk assessment can be expected as the number of correlations increase. A limiting factor in this approach for PCDD/Fs is that current methods of analysis are expensive ($1000 per sample) and time-consuming making widespread sampling costly. An established cleanup method for serum includes liquid-liquid or $C_{18}$ solid-phase extraction followed by multiple chromatography steps using acid, basic, and neutral silica gel, basic alumina, and carbon columns (1,2).

In order to improve throughput, reduce costs, and limit the amount of hazardous solvents used in PCDD/F analyses, we have investigated the use of an immunoaffinity column (IAC) in serum cleanup. We have previously shown that an IAC generated from a monoclonal antibody with specificity towards several of the 2,3,7,8-substituted dioxins effectively isolated 2,3,7,8-TCDD from bovine serum (3). We now report on the specificity of the IAC for other dioxin and furan congeners and present initial results of the IAC cleanup applied to human serum samples.

## Materials and Methods

Immunoaffinity columns were prepared from an anti-dioxin monoclonal antibody (DD3) and used as previously described (3). Two-ml columns were equilibrated with water or prewashed with 10% acetone and 50% acetone before use. Samples tested on the IAC included bovine serum (25 ml) spiked with known amounts of 17 dioxin and furan congeners and 15 $^{13}C_{12}$-labeled congeners and human serum (25 g) containing known amounts of 18 native and $^{13}C_{12}$-labeled dioxin and furan congeners. Serum samples were incubated by rotating together with the column in a Teflon container for 1 hour and then repacking in a glass pipette.

PCDD/Fs which eluted from the IAC were extracted into methylene chloride, passed through dry sodium sulfate (2 g), and concentrated with a keeper solvent (dodecane, 20 ul) containing $^{13}C_{12}$-1,2,7,8-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD as internal standards. Analysis of the dioxins and furans was performed by high resolution GC-high resolution MS.

## Results and Discussion

Table 1 shows the recoveries of dioxins and furans from bovine serum applied to the IAC. Serum I was applied to a column which had not been prewashed and showed recoveries of up to 26% for 2,3,4,7,8-PeCDF. Re-equilibration of the same column (serum II) resulted in a significant improvement in recoveries. The increased recoveries may be due to carryover from the first sample or improved affinity due to "prewashing" from the first sample. When blank bovine

serum (3 ml) was applied to a previously used IAC, incubated, and eluted as before, no dioxins or furans were detected in the eluants by HRGC-HRMS. This indicated that carryover was not a problem from run to run and that the increase in recoveries was probably due to an increased affinity for specific congeners. Prewashing the IAC may rinse out impurities which interfer with the antibody binding or may solvate the IAC beads in a manner which makes the antibody more accessible to binding. Serum III was applied to a new IAC which had been prewashed and shows recoveries over 25% for seven congeners.

Recoveries of five congeners averaged over 35% from the IAC: 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 2,3,7,8-TCDD, and 1,2,3,7,8-PeCDD. Two other congeners (1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD) averaged about 20% recovery. These seven congeners have toxic equivalencies factors 0.1 and represent the major congeners found in human serum.

A quality control sample of human serum was used to determine whether the IAC could be applied to samples with PCDD/F concentrations in the ppq range. Initially samples eluted from the IAC showed interferences in the HRGC-HRMS. A post-IAC cleanup of the samples on a carbon cartridge removed these interfering compounds and provided the results shown in Table 2. Six of the congeners fell within the 95% confidence limits of the analysis: 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 1,2,3,4,7,8-HxCDD. These six congeners accounted for 77% of the toxic equivalency (TEQ) of the sample due to PCDD/Fs.

These initial results demonstrate that immunoaffinity chromatography is a promising method for dioxin cleanup and reduces the time and solvent consumption involved in the traditional method. Further improvements can be made by optimizing the IAC procedure for low level samples, by the use of improved antibodies or mixtures of antibodies with various specificities, and eventually by automation of the process.

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**References**

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| | $^{12}$C-Spikes | $^{12}$C-Analytes from serum (ppt) | | | | % Recovery from serum | | | |
|---|---|---|---|---|---|---|---|---|---|
| Congener | (ppt) | I | II | III | Ave. | I | II | III | Ave. |
| 2378-TCDF | 0.8 | 0.99 | 0.95 | 0.94 | 0.96 | 18.6 | 85.7 | 52.2 | 52.2 |
| 12378-PeCDF | 4 | 6.08 | 5.57 | 5.80 | 5.82 | 2.7 | 9.5 | 11.9 | 8.0 |
| 23478-PeCDF | 4 | 4.69 | 4.74 | 4.93 | 4.79 | 26.1 | 133.4 | 74.6 | 78.0 |
| 123478-HxCDF | 4 | 5.12 | 4.58 | 4.60 | 4.77 | 1.0 | 3.5 | 5.9 | 3.4 |
| 123678-HxCDF | 4 | 6.27 | 5.38 | 4.80 | 5.48 | 0.3 | 1.0 | 2.2 | 1.2 |
| 234678-HxCDF | 4 | 5.08 | 5.19 | 4.99 | 5.08 | 10.4 | 60.6 | 39.1 | 36.7 |
| 123789-HxCDF | 4 | 12.87 | 11.63 | 5.61 | 10.03 | 0.1 | 0.3 | 0.6 | 0.3 |
| 1234678-HpCDF | 4 | 9.18 | 6.85 | 4.70 | 6.91 | 0.2 | 0.7 | 2.0 | 1.0 |
| 1234789-HpCDF | 4 | 14.19 | 9.75 | 4.79 | 9.58 | 0.1 | 0.5 | 1.0 | 0.5 |
| OCDF | 8 | 10.47 | 29.74 | nf | 20.10 | | | | |
| | | | | | | | | | |
| 2378-TCDD | 0.8 | 0.84 | 0.80 | 0.82 | 0.82 | 16.2 | 83.8 | 69.0 | 56.3 |
| 12378-PeCDD | 4 | 4.44 | 4.45 | 4.14 | 4.34 | 15.0 | 78.8 | 60.2 | 51.3 |
| 123478-HxCDD | 4 | 4.36 | 4.24 | 4.13 | 4.24 | 5.1 | 25.7 | 27.4 | 19.4 |
| 123678-HxCDD | 4 | 5.44 | 4.68 | 4.41 | 4.84 | 4.5 | 27.4 | 29.7 | 20.5 |
| 123789-HxCDD | 4 | 3.47 | 2.57 | 2.59 | 2.88 | | | | |
| 1234678-HpCDD | 4 | 11.37 | 5.84 | 5.10 | 7.44 | 3.4 | 14.4 | 15.1 | 10.9 |
| OCDD | 8 | 186279 | 561.64 | nf | 93420 | 0.4 | 0.2 | nf | 0.3 |

Table 1. Recoveries and quantitation of spiked PCDD/F congeners from an IAC cleanup of bovine serum; nf = not found.

| QC sample | QC I | QC II | QC I | QC II | Within 95% |
|---|---|---|---|---|---|

| Congener | mean ppq, n=43 | ppq | ppq | % rec12overy | % recovery | confidence limit |
|---|---|---|---|---|---|---|
| 2378-TCDF | 234 | 209 | 204 | 15 | 19 | yes |
| 12378-PeCDF | 237 | 138 | 136 | 4 | 6 | |
| 23478-PeCDF | 226 | 216 | 224 | 19 | 24 | yes |
| 123478-HxCDF | 236 | nf | 77.2 | 4.4 | 6 | |
| 123678-HxCDF | 247 | 86 | 84 | 3.6 | 5 | |
| 234678-HxCDF | 252 | 246 | 240 | 11 | 14 | yes |
| 123789-HxCDF | 235 | nf | nf | 2.6 | 5 | |
| 1234678-HpCDF | 704 | 1300 | 982 | 2.1 | 4 | |
| 1234789-HpCDF | 256 | 142 | 166 | 2.1 | 4 | |
| OCDF | 223 | 8090 | 5500 | 0.8 | 1 | |
| | | | | | | |
| 2378-TCDD | 226 | 194 | 212 | 22 | 28 | yes |
| 12378-PeCDD | 244 | 228 | 254 | 19 | 24 | yes |
| 123478-HxCDD | 209 | 205 | 199 | 9 | 11 | yes |
| 123678-HxCDD | 556 | 1770 | 1460 | 7.4 | 11 | |
| 123789-HxCDD | 247 | 419 | 353 | 4.4 | 6.3 | |
| 234678-HxCDD | 1340 | 80800 | 72600 | 3.1 | 6 | |
| 1234678-HpCDD | 178 | 59300 | 51400 | 3.1 | 6 | |
| OCDD | 10200 | 592000 | 526000 | 0.6 | 1.3 | |

Table 2. Recoveries and quantitation of spiked PCDD/F congeners from human serum using a prewashed
IAC followed by a carbon column for cleanup; nf = not found.