Comparison of the binding efficiency of PCDDs/PCDFs in biomatrices using immunoaffinity columns generated from monoclonal and polyclonal antibodies

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Introduction

Polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs) are environmental contaminants that pose a health concern due to their toxicities (immuno-, hepato-, reproductive, and developmental toxicities, and carcinogenicity). Because PCDDs/PCDFs biomagnify through the food chain, these chemicals need to be surveyed in the presence of a variety of complex sample matrices to ensure food safety. Current dioxin analyses cost over $1000/sample of which half the cost may be attributed to instrumental (HRGC-MS) component and half to sample cleanup to eliminate matrix. Implementation of a broad survey to provide safe food for consumers is limited by the high cost of sample analysis. An efficient, less expensive method for PCDDs/PCDFs analysis is needed.

We have utilized an immunoaffinity column (IAC) for dioxins isolation to explore the potential to minimize solvent usage, provide faster sample preparation time, and reduce the cost for 1,3,7,8- and 2,3,7,8-TCDD analysis in biological samples (1, 2, 3). In this report, columns prepared from either monoclonal or polyclonal antibodies will be compared. In addition, the retention pattern generated by the monoclonal IAC from 15 ^{13}C-labeled PCDD/PCDF congeners will be described.

Material and methods

Antibody and IAC generation: The generation and isolation of anti-dioxins polyclonal IgG from chicken eggs was described previously (1). A monoclonal antibody specific for 2,3,7,8-TCDD was generated by Stanker *et. al.* (4). Monoclonal IgG was isolated from mouse ascites using a protein G column, and the buffer exchanged using an Excellulose™ column (Pierce, Rockford IL, USA). Following the isolation of the monoclonal and polyclonal antibodies, common procedures were used for analysis and preparation of the IACs. The IgG was quantitated by the method of Bradford (5) and purity was confirmed by SDS-PAGE. The non-specific-IgG was obtained from the pre-immunization antibody. The antibodies were immobilized to CNBr-Sepharose according to manufacture's instruction (Pharmacia Biotech, Uppsala, Sweden). The conjugated gel beads were packed in glass columns for sample isolation.

Procedure for IAC purification: The method of using the IAC for the analysis of 1,3,7,8-TCDD spiked bovine milk or serum sample purification was described previously (1, 2, 3). Briefly, a ratio of 10 ng [^{14}C]-1,3,7,8-TCDD per 10 mg specific IgG was used for the retention

studies. Columns were washed with 0.05% non-ionic detergent to remove non-bound interferences and specifically retained compounds were eluted with 0.5% non-ionic detergent from the polyclonal IAC. For the monoclonal IAC the wash and elution solvents were 10% and 50% acetone in water, respectively. The recovery of the ^{14}C-labeled substrate was determined by liquid scintillation counting.

Multiple congener isolation by Monoclonal IAC: A dodecane solution of ^{13}C-labeled and native PCDDs/PCDFs (LCS and PAR) was stripped of solvent using vacuum centrifugation and the sample was reconstituted with acetone. Bovine serum (2 ml) was spiked with the reconstituted solution at ppt levels and IAC isolation was performed. The fractions from the peaks were combined and extracted with dichloromethane and passed through 1g of sodium sulfate. The dichloromethane was evaporated and an internal standard (ISS) and dodecane were added prior to HRGC-MS analysis using EPA method 1613 (The standards: precision and recovery standard (PAR), labeled compound stock solution (LCS), and internal standard spiking solution (ISS) were obtained from Wellington Laboratories, Guelph, Ontario, Canada).

Results and discussion

Table I shows that a monoclonal IAC was effective in isolating 1,3,7,8-TCDD when serum was directly applied to the column with very little lost with the washing process. This clearly demonstrated the column prepared from the monoclonal antibody showed no deleterious matrix effect from serum. The polyclonal IAC was less efficient in isolating 1,3,7,8-TCDD and needed dilution or preferably pre-IAC cleanup to minimize matrix effects. The interference by serum components was demonstrated by the fact that when loading 1,3,7,8-TCDD in buffer, near completion of binding to the column was observed with efficient recovery (92.2%) in the elution process. The specific binding of the analyte was demonstrated by the results observed from the non-specific column which showed no binding even when the sample was applied in buffer.

Table I. Comparison of recovery of serum samples spiked with [^{14}C]-1,3,7,8-TCDD from the wash and the elution fractions from monoclonal, polyclonal and non-specific IAC[a].

Application Matrix	*Monoclonal IAC*		*Polyclonal IAC*		*Non-specific IAC*	
	Wash	Elute	Wash	Elute	Wash	Elute
Serum	11.5	91.6	59	24.1	69.1	6.4
Serum 1:20			12.6	72.2		
Serum after extraction and Carbograph Cleanup			5.8	91.7		
Buffer			2.1	92.2	94.6	3.2

[a]Data represent the mean of 2-4 replications, the "wash" represents the % of applied radioactive sample recovered from the sample loading and column washing steps and "elute" represents the % of radioactivity recovered when the column was washed with the elution solvent.

Table II demonstrates the increased difficulty of isolating 1,3,7,8-TCDD from milk. Dilution to minimize matrix effects does not significantly improve binding efficiency for either monoclonal or polyclonal IACs of this dioxin in milk. Only after pre-column cleanup using a Carbograph column does the affinity column perform well. The high lipid content of milk competes with 1,3,7,8-TCDD for the antibody-binding site, explaining the sample matrix problems.

Table II. Comparison of recovery of milk samples spiked with [^{14}C]-1,3,7,8-TCDD for wash and elute fractions from monoclonal, polyclonal and non-specific IAC[a].

Application Matrix	*Monoclonal IAC*		*Polyclonal IAC*		*Non-specific IAC*	
	Wash	Elute	Wash	Elute	Wash	Elute
Milk 1:10	52.6	24.3			88.1	7.7
Milk 1:20	42.8	28.4	60.6	32	88.4	15.3
Milk 1:50	31.2	29.7	36.6	57	73.6	23.9
Milk after extraction and Carbograph Cleanup			5.7	89.3	86.7	6.9

[a]Data represent the mean of three replications.

Because the monoclonal IAC showed excellent results with the serum, this column was used to explore the binding pattern of multiple congeners of PCDDs and PCDFs. One sample was run at the normal concentrations used for precision and recovery (PAR) checks in the EPA method 1613. Another sample was run at ten times the concentration to check the concentration dependency of the recovery. Concentration dependency could result from two factors. First if the high concentration showed higher recovery one would anticipate the procedure was below the concentration limits where good analytical performance could be expected. If the recovery of the high concentrations were lower than the low concentrations the binding capacity of the IAC columns would have been exceeded. Finally, the sensitivity of ELISA analyses are shown, which should correlate with the recovery from the IAC study because the same antibody used for the IAC experiments was used for the ELISA assay. The results are shown in Table III.

Table III. Comparison of recovery patterns of LCS samples from monoclonal IAC and sensitivity of monoclonal antibody DD3 in an ELISA.

Congeners	*% recovery-1X*	*% recovery-10X*	*Sensitivity(ng)*[a]
2,3,7,8-TCDF	15.7	13.2	0.7
1,2,3,7,8-PeCDF	5.2	4.5	
2,3,4,7,8-PeCDF	34.9	35.0	0.3
1,2,3,4,7,8-HxCDF	2.3	1.7	
1,2,3,6,7,8-HxCDF	0.7	0.7	
2,3,4,6,7,8-HxCDF	35.2	24.3	
1,2,3,7,8,9-HxCDF	0.4	0.5	
1,2,3,4,6,7,8-HpCDF	0.8	0.7	
1,2,3,4,7,8,9-HpCDF	0.5	0.5	
2,3,7,8-TCDD	53.0	49.1	2.5
1,2,3,7,8-PeCDD	52.2	53.6	0.8
1,2,3,4,7,8-HxCDD	19.9	13.9	20
1,2,3,6,7,8-HxCDD	34.6	27.9	>200
1,2,3,4,6,7,8-HpCDD	13.8	11.1	
OCDD	0.3	0.4	>200

[a] Data obtained from reference (4), experiment was performed by competition ELISA, sensitivity was base on IC_{50}.

Table III clearly demonstrates that there was little or no concentration effect on the recovery of any of the compounds with the exception of 2,3,4,6,7,8-HxCDF and 1,2,3,6,7,8-HxCDD, which showed a slightly higher recovery at the lower concentration. Within the dibenzofuran series significant binding (> 10%) occurred for three congeners, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 2,3,4,6,7,8-HxCDF. The ELISA results confirm this for two of the isomers for which data is available. In contrast, compounds of this series with a 1-chloro substituent apparently bind very poorly to the antibody and show very low recovery in the IAC experiments. This is particularly true in the higher substituted compounds, hexa- and hepta- series (0.4-2.3%).

In the dibenzodioxin series the presence of a 1-chloro substitution does not have a similar effect. Congeners 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD have three fold affinity differences in the ELISA assay but show nearly equal recoveries in the IAC experiments. The addition of more chloro groups (> 5) generally decreases binding and decreases recovery. With six chlorines, the recovery is roughly half of 2,3,7,8-TCDD, with seven chlorines, the recovery is in the 10-15% range, and finally with OCDD, the recovery drops below 1% indicating little specific binding. Thus, the recovery experiments indicate the antibody can accommodate some increase in the number of chlorines, but with decreased efficiency. While the correlation between the ELISA results and the IAC recoveries is good, there is one anomaly in the dibenzodioxin series. The binding of 1,2,3,4,7,8-HxCDD is better than 1,2,3,6,7,8-HxCDD with the latter isomer showing much better recovery from the IAC experiments. Better binding of the 1,2,3,6,7,8-HxCDD might be expected if access to the dioxin oxygen is involved in antibody binding.

In conclusion, the monoclonal IAC allowed the direct application of serum samples, and with dichloromethane extraction, gave suitable samples for HRGC-MS analysis. In order to retain all congeners, new antibodies need to be generated and the elution conditions explored. ELISA analysis gives a rough guide to the recovery of congeners from an IAC.

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