Evaluation and Applications of the Power-Prep " Universal" Automated Clean-up System for PCDDs, PCDFs, cPCBs, PCB Congeners, and Chlorinated Pesticides in Biological Samples.

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Introduction

Over the past several years at the Centers for Disease Control and Prevention (CDC) the demand for high throughput and high quality laboratory measurements for toxic environmental contaminants in human populations has increased dramatically. The increased demand is due in part to evidence from animal studies that organochlorine compounds can cause cancer and have adverse effects on the endocrine, immune, and nervous system (1). The weak link in preventing environmental disease caused by most toxicants is risk assessment (2). A body burden measurement reduces misclassification and greatly increases the probability of finding an association (if one exists) between exposure to an environmental toxicant and any potential human health effects.

In epidemiological studies where only one sample is available from each individual, it is desirable to analyze for as many compounds as possible. While attempting to increase the number of analytes, we have also investigated methods that decrease the time required for sample cleanup and quantitation by mass spectrometry. We report further improvements in and application of our rapid "universal" automated cleanup system to a variety of biological sample types.

Experimental Methods

Previously, we reported the development of a C18 solid phase extraction (SPE) procedure for serum to replace liquid-liquid extraction (3) and the evaluation of Fluid Management Systems', Inc. (FMS) Dioxin-Prep System, for parallel processing and automated sample cleanup (4). The SPE technique is used to process 10 samples at a time and the size of the SPE columns is selected to accommodate different amounts of serum, from 1 to 100 g. High-flow rate SPE columns are

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available for serum samples greater than 50 mL. Two Dioxin-Prep SystemsTM can be run in tandem to process up to 10 serum extracts in about an hour. During the automated cleanup procedure, two solvent fractions can be collected. The first fraction contains ortho-substituted polychlorinated biphenyls (PCBs) and chlorinated pesticides. The second fraction contains polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and coplanar biphenyls (cPCBs). Concurrently, we developed a new high-resolution gas chromatography (HRGC)/isotope-dilution high-resolution mass spectrometry (ID-HRMS) method for the quantification of the ortho-substituted PCBs and chlorinated pesticides (4). PCDDs/PCDFs/cPCBs are quantified as reported earlier (5). In combination, these methods are able to measure 73 organochlorine compounds in a single human serum sample.

We report here an evaluation and applications of a newly redesigned automated sample-cleanup apparatus. Shown in Figure 1 is a photograph of the Fluid Management Systems, Inc, (FMS) Power-Prep SystemTM. Each of the five chromatography panels consists of a valve module, a valve drive module, and a pump module. The standard 'dioxin' configuration uses disposable silica (acid, base, and neutral mix), basic alumina, and AX-21 carbon columns, also manufactured by FMS. These columns are made of Teflon and individually sealed in Mylar packaging. We have observed that columns, including alumina, have a shelf-life of at least one year.

Results and Discussion

The Power-Prep System is more versatile and rugged than its predecessor, the Dioxin-Prep System. It is computer controlled and can be programmed using Windows 95 based software by its operator in many different configurations to perform alternative methods. The operator can select the type of columns and solvents required, as well as the volumes, flow rates and direction of solvent flow. Recommended flow rates range from 5 to 15 mL/min and there are no restrictions on solvent volumes. For example: (a) using neutral silica and carbon columns we programmed the system to collect fractions in the forward direction containing PCBs and pesticides from extracts of 1 g serum samples; and (b) in the standard 'dioxin' configuration, PCDDs/PCDFs/cPCBs are eluted in the forward direction from the alumina columns onto carbon columns, and fractions collected in the reverse direction from the carbon columns.

Standard 'dioxin' silica columns can handle up to approximately 1 g of lipid in a sample extract, sufficient for serum samples up to 100-150 mL. However, the Power-Prep can be adapted to handle sample extracts containing larger amounts of lipid. In a previous report we described the preparation of larger silica columns that can accommodate up to 10 g of lipid (5). These Ace Glass columns (50 mm i.d. x 600 mm, with Teflon end-fittings adapters), packed with acid, base, and neutral silica, can be attached directly to the sample inlets of the Power-Prep and the solvent pumped onto the standard 'dioxin' columns. Subsequent steps in the cleanup program are identical to those described for serum. Using this 'large-column' technique, we have successfully analyzed a variety of biological samples including fish, milk, liver, adipose tissue, and other organ tissues. Typically, recoveries of analytes range between 60-80%, including sample extraction.

The most noteworthy changes in the redesigned Power-PrepTM are in its hardware. The five chromatography panels are designed to run in

parallel, however, each panel can be operated individually, or in any combination. As can be seen in Figure 1, all panel components are modular. Any module of a panel (pump, valve or valve drive) can be quickly removed for maintenance for repair, minimizing downtime. Valves in the valve module can be changed in a matter of minutes. Also, an important feature in the valve module are the patent-pending six-way valves with flexible Teflon diaphragms that reduce foreign particle related valve failures. A pressure sensor on each pump module automatically shuts the system off if the pressure exceeds restarted to complete the run. Two or more Power-PrepTM Systems can be run in tandem to double or triple sample throughput if needed.

Solvent fractions containing the analytes of interest are collected directly into 50 or 200 mL Zymark evaporation tubes used with the Zymark TurboVapR II Concentration Workstation for 'blowdown' to replace older more time-consuming rotary evaporation technology.

We are also currently investigating the possibility of expanding our cleanup method to include polychlorinated naphthalenes (PCNs) and toxaphenes (chlorinated bornances) in the same sample extract by collecting additional solvent fractions.gcms-2.tif

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Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

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