Immuno-based Personal Exposure Monitors

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Abstract

The feasibility of direct air monitoring using immuno-based collection systems is being investigated with the goal of developing personal exposure monitors that take advantage of the high specificity and sensitivity of immunochemical systems. A system is under development which will lead to compact, diffusion-based personal exposure monitors for specific target analytes. The interface problem of the aqueous based antibody system and the air medium has been overcome using semipermeable membrane tubes with a very high surface to volume ratio. An immuno-based collection and analysis system using a monoclonal antibody developed specifically for pentachlorophenol has been investigated. Similar systems are being developed for aldicarb and various nitroaromatics.

Introduction

The goal of this research is to develop personal exposure monitors (PEMs) that use either a polyclonal or a monoclonal antibody immobilized onto a silica support for collection and detection of a specific target analyte or compound class. Selectivity is based on the inherent characteristics of the antibody system used in the PEM device. The device should be applicable to short- and long-term monitoring and should allow the analysis to be performed immediately after sampling and in the field.

PEMs, both dynamic and passive, have long been used for assessing occupational exposure to hazardous materials. Recently, passive (diffusive) sample collection systems have become popular as PEMs, especially those that use sorbents. The well characterized diffusion rates, high sample capacity, and compact size of many of these sorbent-based diffusion samplers make them ideal for non-intrusive monitoring. Sorbents such as charcoal or Tenax which allow the PEM to collect an array of organic compounds are most commonly used. These compounds are then extracted and analyzed by gas chromatography or gas chromatography/mass spectrometry in a laboratory setting. This is an excellent approach when several components are of interest and the vapor is not characterized. However, this method is not particularly cost effective when only one or two compounds are to be monitored during a specific exposure scenario, such as a pesticide application or bag and drum operation. Only a limited number of compound-specific PEMs are currently available for this type of monitoring (i.e., formaldehyde and NO_x).

Immuno-based collection and detection systems have many attributes which enhance their appeal as PEMs. The antibodies are generally selective for a single compound or closely related class of compounds. This selectivity is an advantage for the isolation of target compounds. Also,

antibodies generally exhibit high binding constants for the target analytes, which means that their collection efficiencies are high. Finally, the antibodies can be easily regenerated to the appropriate labeled or active form and reused.

The antibody-based detection systems available exhibit good sensitivity, with detection limits often in the 10 pg to 100 pg range. Recent applications of amplification methods such as enzyme linked immunosorbent assays (ELISA) have led to these increases in sensitivity. The radioimmunoassay (RIA) procedures, previously used in many immunoassays, have been replaced with colorimetric methods which allow for rapid analysis of multiple samples using inexpensive colorimetric readers (or even visual comparison to standards in some cases). Most immunoassay formats are also relatively simple to use and are readily adaptable to field laboratories.

The limitation in using immunochemical techniques for air sampling is that antibodies are designed to work in aqueous systems. The few attempts to use "dry" antibodies have not been very successful. Studies have been carried out using antibodies immobilized to substrates such as polyethylene, Tenax, and indium [1,2]. The immobilized antibodies were then exposed directly to vapors without a wetting solution. Success was limited to those systems which developed a response in an aqueous solution after exposure. This limited success was most likely the result of the target analyte binding non-specifically to the protein covered surface and then binding specifically with the antibody after wetting. We have overcome this limitation by using a vapor permeable membrane as an interface.

The system we are investigating consists of a vapor-permeable membrane and a cavity that encapsulates the aqueous medium and the immobilized antibody. The membrane acts as the air-to-liquid interface, allowing vaporous analytes to diffuse into the aqueous medium. The ideal membrane should allow the molecules to pass freely and should not interact with the analyte and cause losses by nonselectively retaining the analyte. It should also provide a high surface-area-to-interstitial-volume ratio to keep the PEM small and the mass transport process rapid. Figure 1 shows a diagram of the PEM device. The analyte in vapor form diffuses through the porous membrane of a microdialysis tube. Once inside the tube, the analyte is captured by the antibody immobilized to the packing inside the tube. The capture results in the release of a labeled compound or an enzyme product (depending on the type of detection system) to the aqueous medium. At the end of the sampling period, the tube fittings are attached to a syringe or pump, the packing rinsed with solvent to remove the labeled compound or the enzyme product, and the rinsate analyzed to determine the concentration of the target analyte that diffused into the microdialysis tubing.

Information on the diffusion rates of selected compounds across the membranes is presented in this paper. Preliminary data on an antibody-based collection system for pentachlorophenol (PCP) are also presented.

Experimental

The membranes under evaluation are regenerated cellulose microdialysis tubing (Spectrum Medical). Typical tube dimensions are 50 μm in external diameter, 35 μm internal diameter, and 20 cm in length. Tubes are collected in bundles of 22 or 88 with pore sizes rated at 6,000 or 9,000 molecular weight cutoff (MWCO).

The vapor chamber (Figure 2) consists of a 10 L stainless steel vessel with air sampling ports and a stirrer to ensure even vapor distribution within the chamber. Previous evaluations have indicated that the distribution is even throughout the chamber using this propeller mixer. The saturated vapor is created by placing the material (e.g., radiolabeled PCP) in the bottom of the chamber and allowing it to equilibrate until saturation is reached as determined by collecting periodic air samples on solid sorbents and analyzing them by gas chromatography (GC) or liquid scintillation counting (LSC).

PCP, 2,4-dinitrotoluene (DNT), and 2,4,6-trinitrotoluene (TNT) were obtained from Aldrich Scientific at 98% purity or better. The C^{14} radiolabeled materials were obtained from New England Nuclear. The radiochemical purity of each material was greater than 95%. The monoclonal PCP antibody was developed by WBAS, Inc. of Rockville, Maryland, and obtained through the U.S. Environmental Protection

Agency, Environmental Monitoring Systems Laboratory - Las Vegas. All other reagents were obtained from Sigma Chemical.

GC analysis of DNT and TNT was performed on a Varian 3700 equipped with an electron capture detector and a DB-5 30m, 0.25mm ID fused-silica open-tubular column. LSC was performed using a Packard 4170 Scintillation Counter.

The procedure used for the PCP vapor/liquid diffusion study is as follows. A 9-inch, 22-fiber bundle was masked off with Teflon tape to allow only 1.5 in. of fiber to be exposed to the atmosphere. The bundle was rinsed with ethanol and phosphate-buffered saline (PBS) to wash out the plasticizer according to the manufacturer's instructions. The bundle was filled with PBS, fitted into the cell holder, and placed into the ^{14}C-PCP vapor chamber. At 5-min intervals for 1 hr, 2 mL of fresh PBS were drawn through the bundle. The 2 mL of fluid, which contained the PCP samples during the interval, were transferred to a LSC vial. At the end of the 1-hr exposure time, the bundle was removed from the chamber and hooked up to a peristaltic pump and fraction collector. PBS was pumped through the bundle at a rate of 0.25 mL/min for 5 hr and the effluent collected in LSC vials. This post exposure study was performed to help identify and quantitate any hysteresis effect from PCP adsorbed to the tubing but not yet migrated into the filling solution. Quadruplicate vapor samples were taken from the exposure chamber while the bundles were being exposed and analyzed for PCP.

Preliminary experiments for nitroaromatics used water-filled dialysis tubing as the analyte collector in the chamber. These evaluations were performed in deionized water because it was unknown at this time what buffer system would be used. This allowed the determination of the analyte diffusion rates into the internal filling solution of the tubing. The bundles were not masked with Teflon tape as they were for PCP. This results in a larger sampling surface area for these evaluations than for the subsequent PCP evaluations. The microtubes were filled with deionized water after the manufacturer's extensive solvent rinsing procedure was performed. The filled tubes were placed into the chamber containing ^{14}C labeled DNT or TNT for 0, 5, 10, 20 and 60 minute periods, then removed, and the labeled analyte amounts contained in both the filling solution and the tubing material was determined by LSC.

The procedure for the diffusion studies using an antibody filling solution is as follows. The PCP antibody was suspended in a PBS-Tween 20 solution and injected into the microtubes. The tubes were then suspended for 15 minutes in the chamber containing the radiolabeled PCP vapor at 3 μg/L. Control tubes were also placed into the chamber, including one filled with PBS-Tween and another with Bovine Serum Albumin (BSA) protein suspended in PBS-Tween. The antibody-filled tubes were then dialyzed against deionized water for 4 hours to remove any unbound PCP and then analyzed.

Results

Table 1 lists the approximate equilibrium vapor concentration determined for the three analytes using solid sorbents to sample the vapor chamber. Repetitive samples were collected until the chamber concentration reached equilibrium. These values are not corrected for recovery efficiencies from the collection sorbents because previous work at MRI has indicated recoveries of better than 90% for these particular analytes.

Table 1. Chamber Vapor Concentrations

Analyte	Estimated Vapor Concentration (μg/L) from Solid Sorbent Collection	Confirmation Method
DNT	3	GC
TNT	100	GC
PCP	3	LSC

Figure 3 contains the results from an evaluation of the diffusion of DNT and TNT through the 6000 MWCO and 9000 MWCO dialysis microtubes.

The results for TNT and DNT are similar. The lower apparent diffusion rates for TNT are expected because TNT has a much lower equilibrium vapor concentration than DNT. The

plotted values are the average of two determinations at each time interval and have been corrected for surface area differences. Future work will couple this collection system with antibody-based detection systems.

The larger pore tubing (9000 MWCO) does exhibit faster diffusion than the smaller pore tubing (6000 MWCO). However, the magnitude of the difference is not large enough to justify the use of the larger pore tubing, which is more likely to lose water by evaporation over extended sampling periods.

The results from the diffusion rate studies of PCP are shown in Tables 2, 3, and Figure 4. Duplicate assays of each bundle type were performed. The results of assaying the PBS effluent while the bundles were in the exposure chamber are presented in Table 2, and representative plots of each bundle type are presented in Figure 4. Linear regression analysis was performed on the cumulative PCP passed through the membrane and into the PBS buffer as a function of time. The data indicate an average mass sampling rate of 14.3 ng/min for the 6000 MWCO bundle and 19.2 ng/min for the 9000 MWCO bundle. The effective sampling rate, calculated by division of the mass sampling rate by the PCP vapor concentration, averages 0.27 L/min for the 6000 MWCO bundle and 0.30 L/min for the 9000 MWCO bundle.

The correlation coefficients (Table 2) indicate good linearity for both bundle types with the MWCO 6000 bundles being greater than 0.970 and the 9000 being greater than 0.995. The x-intercept indicates the approximate delay time in which the vapor permeates into the bundles and into the PBS. These times are 6.7 min for the MWCO 6000 bundle and 2.9 min for the MWCO 9000 bundle.

The total amount of PCP that permeated the membrane during and after exposure is presented in Table 3. In the 5 hr after the bundles were removed from the chamber, 1049 and 709 ng of PCP continued to migrate through the MWCO 6000 bundle. Likewise, these values are 507 ng and 438 ng for the 9000 MWCO bundle. These results indicate a large fraction (46 percent for the 6000 bundle and 30 percent for the 9000 bundle) of the PCP takes a considerable amount of time to permeate through the membrane and into the buffer. However, this should not affect the usage since a waiting time of 1 hour can be inserted into the analysis scheme.

Representative plots of the postexposure permeation results for each bundle type are presented in Figure 5. These results indicate a very rapid PCP passthrough during the first 30 min, followed by a very slow accumulation during the next 4.5 hr. The slow permeation phase may be reaching an asymptotic limit, but it is impossible to estimate the limit from these data. A reasonable explanation for this phenomenon is that the fast permeation phase is the PCP diffusing through the aqueous portion of the open pores of the membranes, while the slow phase is the PCP diffusing through the regenerated cellulose portion of the membrane. The membrane may also become saturated with PCP at this vapor concentration.
To provide a mass balance, another type of exposure experiment was conducted in which the bundle was exposed while being completely static. A 9000 MWCO bundle was filled with PBS buffer and placed in the PCP exposure chamber. After 1 hr, the bundle was removed from the chamber and 10 mL of PBS buffer immediately washed through the bundle and assayed. Next, the buffer was pumped through the bundle and collected for 5 hr. At the end of the experiment, the bundle was sacrificed and assayed for PCP. This experiment detected 1400 ng PCP in the first of 10 mL of effluent, 376 ng in the postexposure effluent, and 61.5 ng in the bundles. The fraction of PCP permeating through the bundle was 20 percent of the total PCP sampled by the membrane. Only 3 percent of the PCP remained in the membrane indicating a low degree of permanent nonspecific adsorption. Therefore, while diffusion may be slow, very little of the analyte becomes permanently affixed to the tubing.

Table 4 contains the results from the preliminary evaluations of PCP-antibody loaded tubes. The tubes were exposed to the radiolabeled PCP vapor at 3 μg/L for 15 minutes in the static exposure chamber previously described. Tubes filled with the PBS-Tween solution and PBS-Tween/BSA were suspended in the chamber as controls. The purpose of this experiment was to demonstrate that the antibody-based collection system would irreversibly bind the target analyte for later

analysis. Such binding is important because analyte exposure may occur in an episodic manner and the retention of the analyte is key to accurately determining the exposure.

In assessing this limited data set, it is clear that the PCP-antibody is binding the PCP diffusing into the tube. This can be inferred, in a non-quantitative way, by comparing the amount of PCP retained in the PCP-antibody loaded tubes versus that retained in the control tubes after dialysis as determined by LSC. More definitive experiments are underway to quantify the relative retentions.

Conclusions

The above data indicate a high probability of success for the application of antibody-based PEMs. Monitoring limits are of course constrained by the detection capability of the antibody-based system. However, based on the reported limit for the PCP assay (1 ng) [3] and the diffusion measurements reported in this paper, the limit of detection for the PEM device should be from 1-5 ng of PCP. Based on a vapor concentration of 5 ppb (arbitrarily chosen as a representative air concentration for PCP), this would convert to a minimum exposure time around 20 minutes for the analyte to reach a detectable quantity within the PEM device.

The data, even though preliminary, demonstrate the viability of using such PEMs. Studies carried out as part of this program have also indicated that the 6000 MWCO dialysis microtubing exhibits sufficient collection efficiency for other targetanalytes. Several antibody systems are under evaluation for use in PEM devices, and new systems will be evaluated as they become available. These early studies indicate that it will indeed be possible to apply antibodies to direct air monitoring systems through the use of microdialysis tubing as a semipermeable barrier which allows vapor diffusion without significant moisture loss.

References

1. Lukens, H.R., "Solid Substrate Immunological Assay for Monitoring Organic Environmental Contaminants," EPA Report No. 600/1-77-018, Environmental Health Effects Research Series, 1977.

2. Giaever, I., "The Antibody-Antigen Reaction - A Visual Observation," J. Immunology, 110, No 5 (1973) 144.

3. EPA Internal Report, "Evaluation of Westinghouse Bioanalytic Systems PCP Immunoassay," EPA/600/x-90/146, July 1990.

NOTICE: Although the research described in this paper has been supported by the United States Environmental Protection Agency, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TABLE 2. RESULTS OF UTILIZING MICRODIALYSIS TUBING FOR SAMPLING PCP VAPOR

	Linear Regression Analysis				
Bundle type	Correlation coefficient (r^2)	x-Intercept (min)	Slope (ng/min)	PCP vapor concentration (ng/L)	Effective sampling rate (L/min)
MWCO 6000	0.970	6.81	19.5	71.8	0.272
	0.972	6.69	14.8	55.4	0.267
MWCO 9000	0.996	3.08	20.5	58.3	0.351
	0.995	2.86	17.9	52.8	0.340

TABLE 3. DIFFUSION OF PCP THROUGH THE HOLLOW FIBER BUNDLES

Bundle Type	Amount of PCP diffused through membrane			
	With bundle in chamber		With bundle out of chamber	
	(ng)	(%)	(ng)	(%)
MWCO 6000	1135	52.0	1049	48.0
	857	54.7	709	45.3
MWCO 9000	1162	69.6	507	30.4
	1049	70.5	438	29.4

TABLE 4. COLLECTION OF PCP BY ANTIBODY SUSPENDED IN MICRODIALYSIS TUBES (15 min exposure time)

Filling Solution	PCP Amount (ng)[a]
PBS-Tween/PCP Antibody	184
	162
PBS-Tween	18
	29
PBS-Tween/BSA	39
	57

[a] Duplicate determinations by LSC; Exposure time of 15 minutes

FIGURE 1

Personal Exposure Monitor

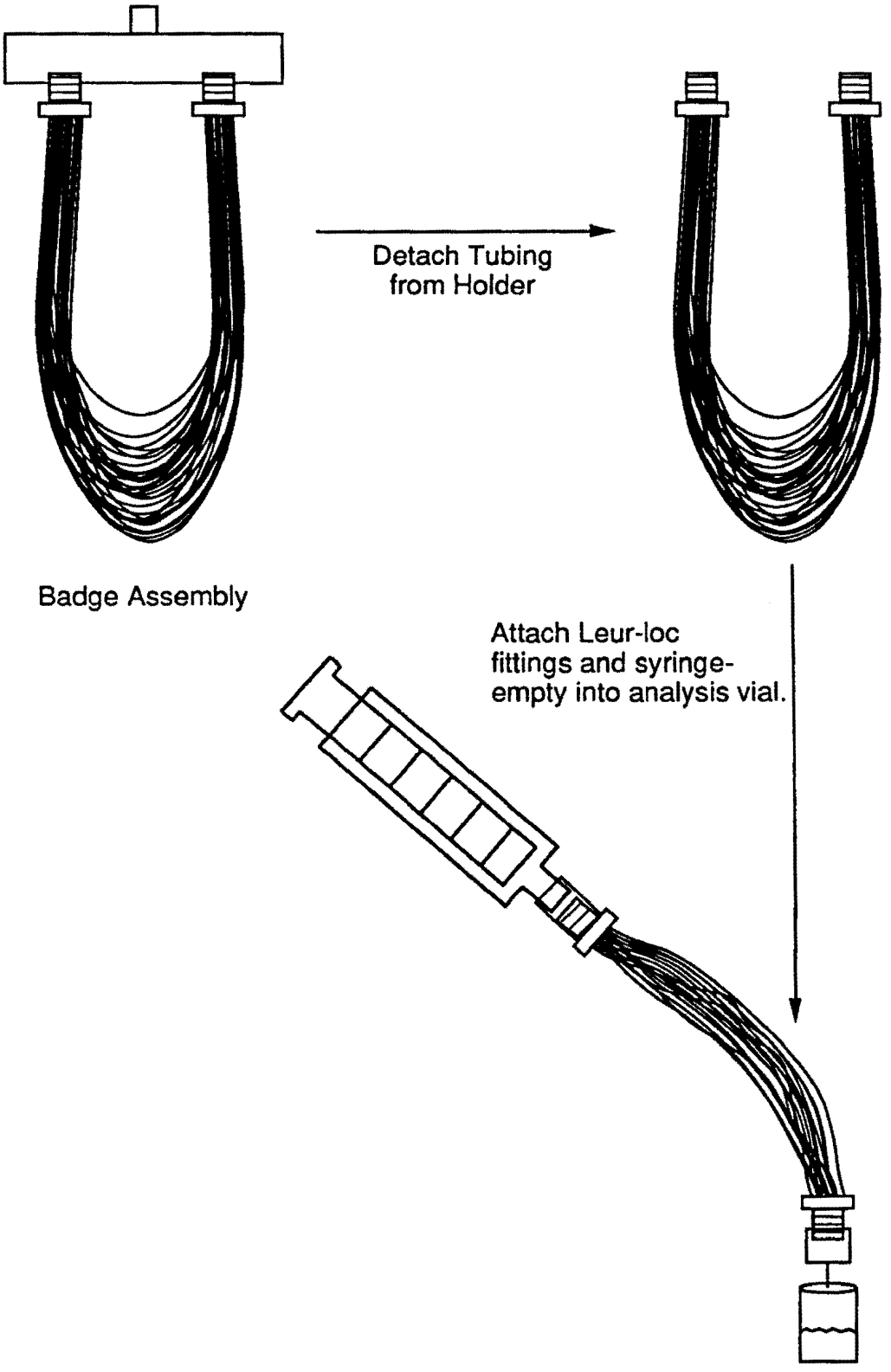


FIGURE 2

Exposure Chamber Used for Exposure Studies

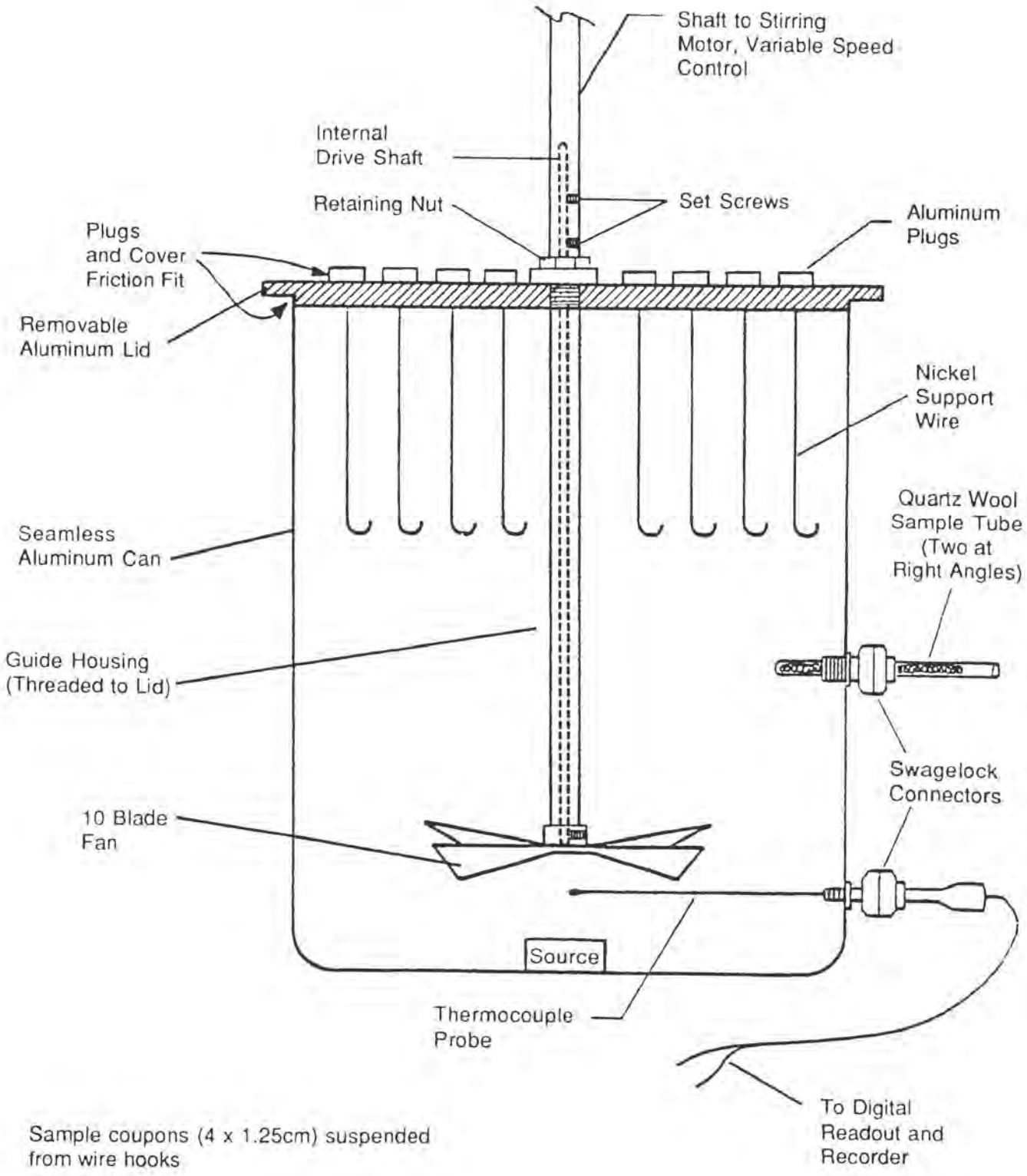


FIGURE 3

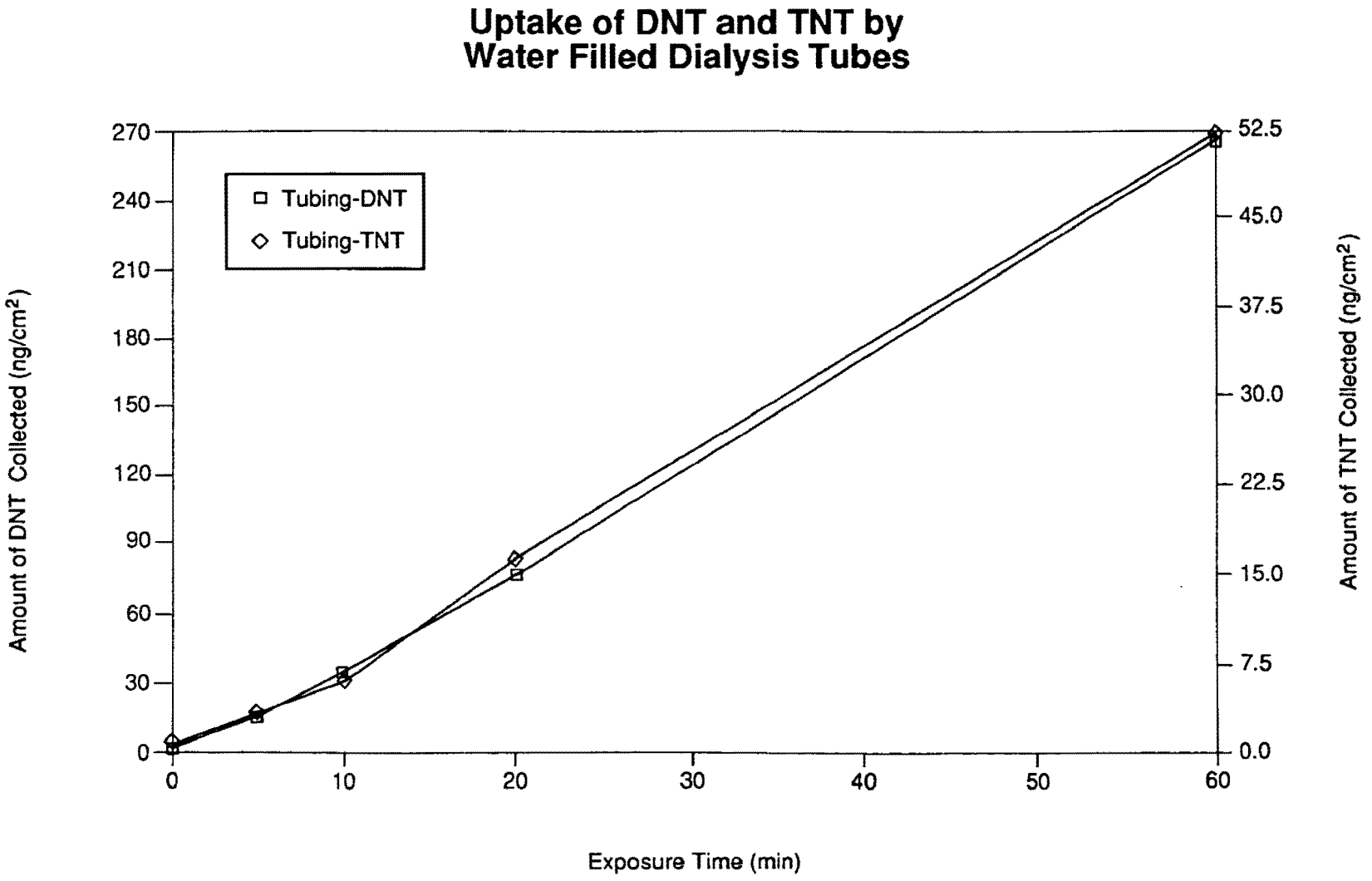

Uptake of DNT and TNT by
Water Filled Dialysis Tubes
Tubing-DNT
Tubing-TNT
Amount of DNT Collected (ng/cm²)
Amount of TNT Collected (ng/cm²)
Exposure Time (min)
0
30
60
90
120
150
180
210
240
270
0.0
7.5
15.0
22.5
30.0
37.5
45.0
52.5
0
10
20
30
40
50
60

FIGURE 4

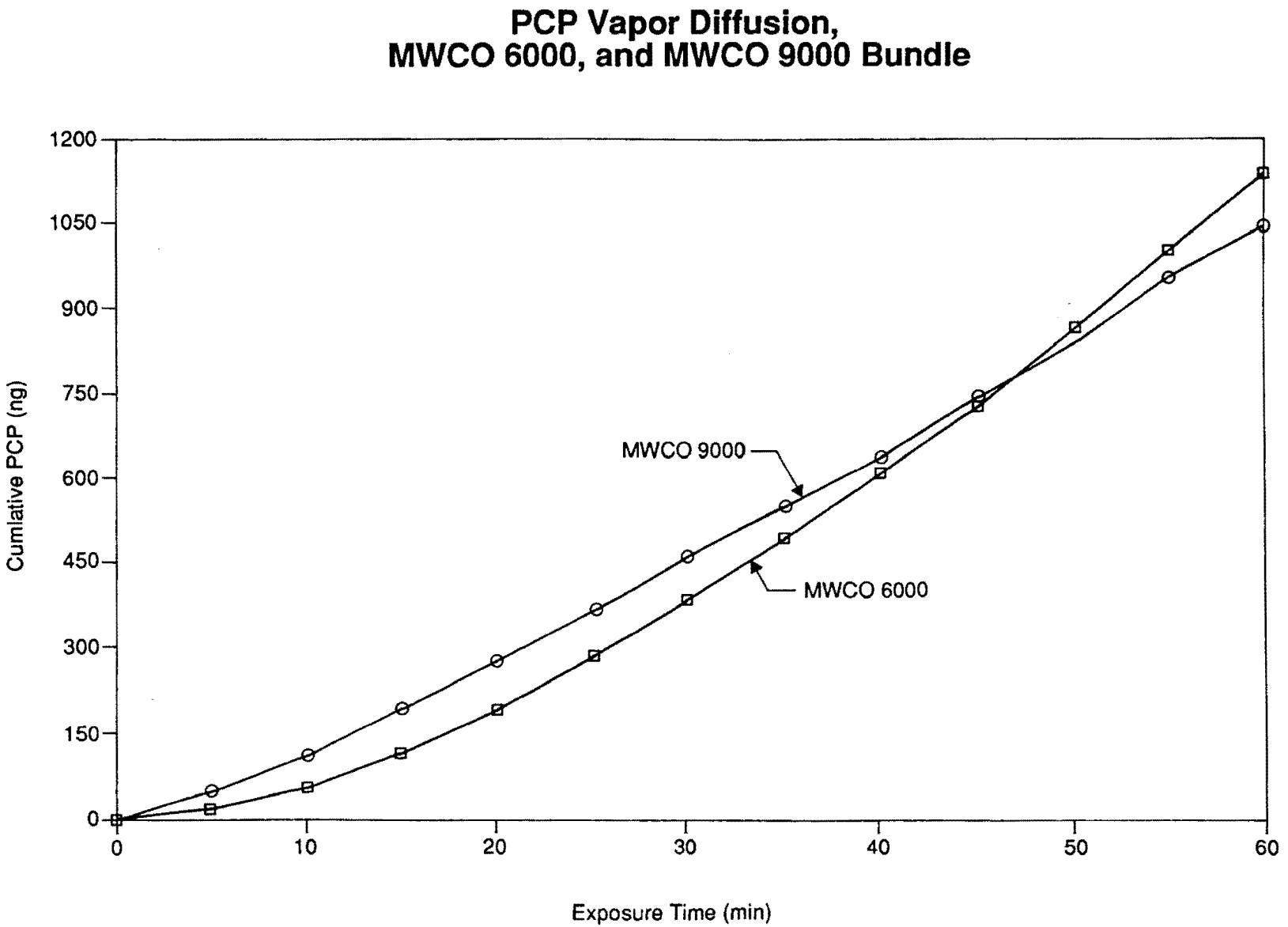
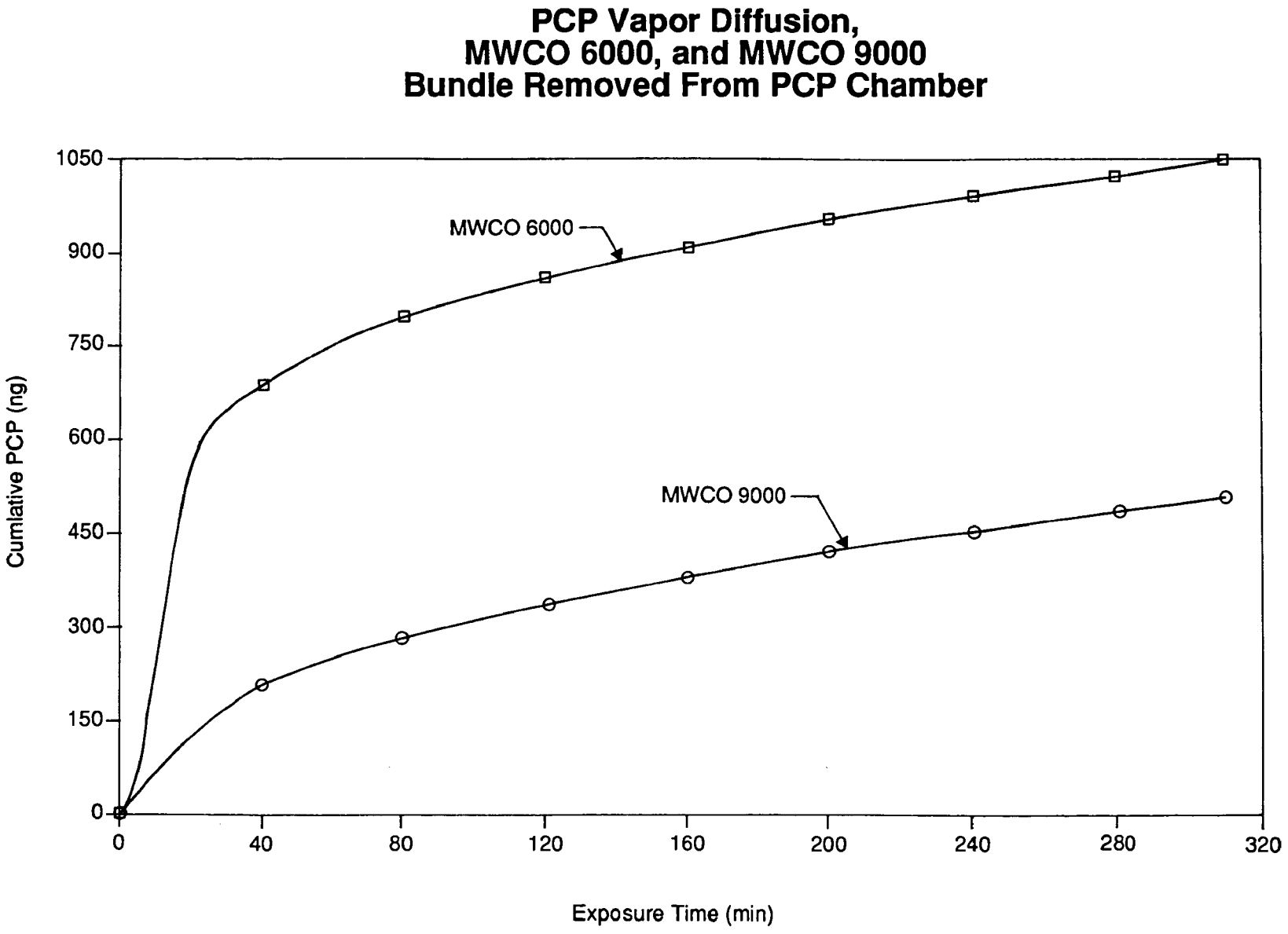

PCP Vapor Diffusion,
MWCO 6000, and MWCO 9000 Bundle
Cumlative PCP (ng)
1200
1050
900
750
600
450
300
150
0
0
10
20
30
40
50
60
Exposure Time (min)
MWCO 9000
MWCO 6000

FIGURE 5


PCP Vapor Diffusion,
MWCO 6000, and MWCO 9000
Bundle Removed From PCP Chamber
Cumlative PCP (ng)
1050
900
750
600
450
300
150
0
MWCO 6000
MWCO 9000
0
40
80
120
160
200
240
280
320
Exposure Time (min)

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