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A New Monitoring Method Using Solid Sorbent Media for Evaluation of Airborne Cyclophosphamide and Other Antineoplastic Agents

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Cyclophosphamide is a known human carcinogen. In July 1999, in a report at a conference on cytotoxic drugs in Sweden, it was indicated that cyclophosphamide (CP) was not effectively controlled by high efficiency particulate air (HEPA) filters.(1) This then raised a concern that the existing air monitoring methods, which utilize polytetrafluoroethylene (a.k.a. PTFE, or Teflon) or glass fiber filters for evaluation of antineoplastics such as CP in air may also be ineffective for collection and quantification of such agents. It was decided that further evaluation of the existing filter method for monitoring antineoplastics in air be conducted. This evaluation determined that the filter method of monitoring was minimally effective for some antineoplastic agents, and that an alternate method of monitoring should be sought. The method subsequently developed utilizes a solid sorbent tube, Anasorb 708, a methacrylic acid polymer. Evaluation of this sorbent tube for adsorption and desorption properties found it had a greater than 90 percent recovery for both CP and ifosfamide. Other agents evaluated included 5-fluorouracil, doxorubicin, and paclitaxel. All three agents were able to be detected and measured by use of Anasorb 708 solid sorbent tube. Validation of the method was then conducted with air pulled through the tubes via attachment to an air manifold system at air flows ranging from 1.5 to ∼4.0 liters per minute for up to 24 hours. This evaluation did validate the Anasorb 708 tube as an effective media for collection of airborne concentrations of CP from less than 1 μg up to approximately 2 mg (2000 μg) per tube. This corresponds to a concentration range of approximately 0.7 $\mu g/m^3$ (0.0007 mg/m^3) to 0.7 mg/m^3 in a 5.76 m^3 volume of air. This method can provide accurate information on airborne concentrations of CP for purposes of conducting risk assessments or evaluation of risk management methods.

Keywords Antineoplastics, Air Monitoring Methods, Cyclophosphamide, Ifosfamide, 5-Fluorouracil, Doxorubicin HCl, Paclitaxel, Taxol, HPLC

Cyclophosphamide, one of the most frequently used antineoplastic agents in clinical treatment facilities, and a known human carcinogen (IARC class 1-A),(2,3) was determined to be a primary agent in need of further method development. Other agents were included in the study based on their potential health risks and frequency of use. These were ifosfamide, fluorouracil, doxorubicin, and paclitaxel.(4–15)

Because of its physical properties, it was previously thought that cyclophosphamide could only exist in air as a particulate. Typical monitoring consisted of using either 0.5-μm glass fiber filters or 0.45-μm polytetrafluoroethylene (or PTFE, Teflon) filters.(16–22) Samples were usually collected over periods of at least 40 hours, and frequently for more than 80 hours. Results generally were very low or below limits of detection, even when monitoring in areas where high volumes of cyclophosphamide were being used.(16,20) Further, the National Institutes of Health (NIH) recommended that work with hazardous drugs, which includes cyclophosphamide, be carried out in a biological safety cabinet (BSC), which is equipped with HEPA (filters) to control the agents.(21)

In July 1999, K.G. Schmidt gave a report at a conference on *Occupational Exposure to Cytotoxic Drugs* in Sweden.(1) He indicated that he found cyclophosphamide, and possibly other antineoplastic agents, are not effectively controlled by HEPA filters. Such filters are used for control of aerosol contaminants in class II, type A biological safety cabinets (BSCs).(21,23) These cabinets recirculate approximately 70 percent of cabinet air through HEPA filters into the cabinet, and the remainder is discharged through HEPA filters into the room. Schmidt speculated that the cyclophosphamide particles are probably captured by the HEPA filters as particles, but that it evaporates or sublimates

off the filter and returns to the local air through the BSC exhaust streams.

This observation is somewhat supported by Connors et al. recent work on surface contamination.[24] They have found several areas in cancer centers that have surface contamination from antineoplastic agents, which may be the result of contaminants in air settling onto solid surfaces. In addition, in a 1999 article by Sessink and Bos it was stated that "uptake of cyclophosphamide was even found in pharmacy technicians involved in the preparation of cytostatic drugs other than cyclophosphamide."[20] This would indicate they were likely being exposed by either airborne concentrations of cyclophosphamide or from surfaces contaminated with cyclophosphamide. Based on the observations of Schmidt, exposure was likely occurring by both mechanisms.

Because of these findings, it was concluded that if cyclophosphamide was not effectively collected and controlled by HEPA filters in BSCs, there may be a flaw in existing air monitoring methods, which utilize PTFE, or glass fiber filters for collection. If it were correct that cyclophosphamide evaporated or sublimated off the filters, it was likely that the filter monitoring method is not effective for monitoring cyclophosphamide, and an alternate monitoring method would need to be considered.

An evaluation of the effectiveness of the filter monitoring methods currently in use was conducted. Results indicated the filter monitoring method to be minimally effective for collection of cyclophosphamide. Based on this information, it was decided that a method using a solid sorbent might be more effective for collection of the cyclophosphamide as a vapor. Thus, steps were taken to develop a monitoring method in which solid sorbents would be used as an effective sample collection media for cyclophosphamide, as well as for some other commonly used antineoplastic agents. Because of their potential toxicity and common use in cancer therapy, ifosfamide, 5-fluorouracil, doxorubicin hydrochloride, and paclitaxel[4–15] were included in development of a monitoring method acceptable for cyclophosphamide.

MATERIALS AND METHODS

Selection of Air Monitoring Method

Methods available for consideration included use of an impinger to collect airborne contaminants in a solvent solution that could be directly injected into an analytical instrument, use of a filter collection method to collect airborne particles, or use of a solid sorbent to collect contaminants in the gas and/or vapor state. Use of impingers has a variety of drawbacks. For example, impingers can lose a portion of the collection solvent and are relatively easy to spill or to break. Therefore, it was decided to evaluate the other two types of air monitoring methods available, use of filters and use of solid sorbents for monitoring of airborne antineoplastics of interest.

Analytical Method

Analytical Equipment

Analytical methods reviewed included reverse-phase HPLC, GC, GC/mass spectrometry, and GC/mass spec–mass spec (tandem mass spectrometry).[16–22,24] The GC–tandem mass spec and the GC/mass spec have very high specificity and sensitivity, but they do have some restrictions. Complicated sample handling, often requiring derivatization, may be required in order to avoid the decomposition of agents like cyclophosphamide on the column.[16,21,22] Although the GC method was determined to have the sensitivity needed to measure low microgram concentrations of an antineoplastic, it does not have the versatility desired to simultaneously detect multiple agents having different chemical characteristics because of the derivatization step. Thus, it was decided that the reverse-phase HPLC would likely be the optimum method for simultaneous analysis of the five agents of interest.

A Bio-Rad reverse-phase HPLC equipped with a model 1790 programmable UV/VIS monitor was used. The system utilized a 500-μL injection loop and a Waters 150 $\times$ 4.6 mm Symmetry C18 column with 5.0 μm particles.[25]

Mobile Phase

A combination isocratic and gradient method was found acceptable for detection of all five agents with a single analysis. The first 20 min used a mobile phase of 22.75 percent acetonitrile in Milli-Q water, with the water buffered by potassium phosphate to a pH 6.0, and at a flow rate of 1.2 mL/min. This concentration of acetonitrile in water and associated flow rate was found to provide the separation of all but one agent peak in 20 min. This isocratic phase was followed by a 25-minute gradient phase to a 70 percent acetonitrile and buffered water to elute the last agent, paclitaxel. The gradient was then brought back to 22.75 percent over 5 more minutes, followed by a 10-minute period to completely return to the normal baseline. Gas formation from the acetonitrile was minimized by first degassing any solutions containing acetonitrile.

Preparation of Standards

The selectivity of the method was verified by the analysis of blank and spiked samples. Quantification (peak height) was carried out by reference to calibration curves constructed from the analysis of freshly prepared analytical standards. These analytical standards varied from containing an individual agent at a specific concentration to containing all five agents at select concentrations. The analytical standards used for calibration were prepared from the products indicated in Table I.

Each of these five agents was first prepared by dilution in a blend of one part methanol and one part Milli-Q water (some products had to be reconstituted first with distilled water before initial preparation as standards). Any subsequent dilutions to prepare standards of a desired concentration were obtained by blending with Milli-Q water.

TABLE I
Information on agents used to make standards

Brand name	Composition	Lot no.	Manufacturer
Cytoxan (for injection)	Cyclophosphamide (20 mg/mL)	Lot 9G22823	Mead Johnson (Bristol-Myers Squibb)
Ifex (for injection)	Ifosfamide (50 mg/mL)	Lot KCS99	Mead Johnson (Bristol-Myers Squibb)
Doxorubicin HCl	Doxorubicin HCl (2 mg/mL)	Lot 123200A	Novaplus
		Lot 93592	Bedford Labs
Adricil (for injection)	Fluorouracil (50 mg/mL)	Lot FFA221	Pharmacia & Upjohn
Paclitaxel (99.99%)	Paclitaxel	Not available	Sigma

Determination of UV Wavelength for Detector

Each of these agents (Table II) was scanned by spectrophotometry (Beckman DU-600) to determine their individual optimum UV wavelength range in nanometers (nm) for UV detection. It was learned that these agents were generally most detectable in the range between 190 and 200 nm.

Selection of Desorbing Solution

Typically, the solution used for desorbing chemical agents collected in the sorbents from either air or surface sampling media has been 50 percent methanol:50 percent water buffered to pH 6.0 by blending monobasic and dibasic potassium phosphate. Because of the variation in physical characteristics of the agents of interest in this study, especially polar aspects of each, various iterations of acetonitrile and methanol in water were tested for desorbing capabilities. It was determined that the most effective desorbent was a blend of 10 percent acetonitrile, 25 percent methanol, and 65 percent Milli-Q water. This blend assured optimum desorption of cyclophosphamide (CP) and ifosfamide (IF), and acceptable desorption of 5-fluorouracil (FU) doxorubicin (DX), and paclitaxel (Pac).

Evaluation of Overall Effectiveness of Monitoring Media

One important step in determining the effectiveness of a collection media for the monitoring of a contaminant in air is to evaluate the ability of the sorbent to retain the contaminant(s).

TABLE II
Optimum ultraviolet (UV) detection range for the agents of interest

Agent	UV wavelength (nm)
Fluorouracil	195 to 210
Ifosfamide	190 to 195
Cyclophosphamide	190 to 195
Doxorubicin	190 to 195, and 230 to 235
Paclitaxel	190 to 210

Note: Based on this information, the UV detector on the HPLC unit was set at 195 nm for detection.

Specifically, the ability of the collection material to retain the captured contaminant as air continues to be pulled through the sorbent after the contaminant is no longer present in the air.[26] Because of the potential for cyclophosphamide to sublimate off filter material, this was an important test for this study.

Air Manifold System

To conduct controlled testing of the various air monitoring methods available for the antineoplastic agent of interest, it was determined that a manifold design would be most effective. An air manifold was constructed using 15-inch length of 4″ PVC pipe, with a cap glued on one end and a screw-on cover at the opposite end. This pipe was then equipped with eight needle valves to control air flow at each air inlet port (see Figure 1).

The manifold was connected to a vacuum pump able to pull up to 30 L/min total air volume through the eight ports on the manifold. A Dry-Cal calibration unit (Bios International Corp., Butler, NJ) was used to measure air flow rates at each of the needle valve ports on the manifold. Once the air flows were known for each inlet, the sampling train to be evaluated was attached to the port. After the attachment of the sample train, the air flows were re-measured at the inlet of the train. Air flow rates at all manifold inlets used were measured after any adjustment to any inlet and before initiation of the testing. A final measurement was made immediately prior to removal of the sample trains from the manifold ports. The initial and final measurements were averaged, then multiplied by the time in minutes to obtain the total volume of air moved through the sample train. Vinyl tubing was used to connect sorbent tubes or filters to the manifold as applicable.

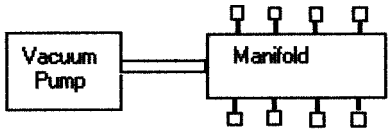

FIGURE 1
Air manifold system connected to a vacuum pump with inlets to the manifold equipped with needle valves for flow control of air through each sample test train.

TABLE III
Results from evaluation of PVC to determine stability of 2000 μg cyclophosphamide spiked onto the filter after air pulled through

Sample no.	Sample description	Air flow L/Min	Air vol. (m^3)	Cyclophosphamide mass recovered (μg)
1	PVC filter & support pad	2.16	2.19	1530
2	PVC filter, no support pad	2.02	2.07	1510
3	PVC support pad, no filter	2.36	2.41	1490
Anasorb 708 tubes after filters				
4	After filter—1	2.16	2.19	<0.2
5	After filter—2	2.02	2.07	<0.2
6	After filter—3	2.36	2.41	<0.2
Control		None	None	1980

Note: PVC filters: 37 mm diameter, 5.0 μm pore size, polyvinyl chloride filter circle.

Filter Method Evaluation

PVC Filter Evaluation

The first material tested as a possible monitoring media was a 37-mm, 5.0-μm PVC filter. The PVC filter was tested both with and without a support pad, and the support pad was tested without a PVC filter. In each case, the filter and/or support pad was spiked with 2.0-mg CP. An Anasorb 708 tube was connected downstream of the filter cassette and air was pulled through the sample train, typically for 16–17 hours. The results for both the filter analyses and Anasorb 708 analyses are found in Table III.

Results indicate approximately 500 μg of the 2000 μg spike was lost off the PVC filter and support pad, the filter without a support pad, and support pad without a filter after approximately two cubic meters of air pulled through each. Note that a control filter was spiked with 2000 μg, stored at ambient temperatures but with no air movement through it, and desorbed. Result yielded 1980 μg, or nearly 100 percent recovery. Because the cyclophosphamide lost from the PVC filters was not detected in the respective Anasorb 708 tubes, it was suspected that the lost cyclophosphamide either sublimated onto the sidewalls of connection tubing or sidewalls of filter cassette, or both. This was confirmed as indicated in Tables XIV through XVII.

PTFE (Teflon) Filter Evaluation

Tested next for potential use as monitoring media was a 37-mm, 0.45-μm PTFE filter placed in 37 mm SAN (styrene acrylonitrile) cassettes. Also evaluated were the cellulose support pads for the PTFE filters. These filters and support pads were each dosed with 2 mg of CP and evaluated. Approximately 5 m^3 of air (3350 cc/min for 1490 min) were pulled through the filter and/or support pads (see Table IV). Anasorb 708 tubes were placed after the PTFE filters to determine if the CP was being lost to the air exhausting from the filter cassette.

Results from this test found that the PTFE filter and the PTFE support pad without a filter both lost nearly 1700 μg from an initial loading of 2000 μg (2.0 mg) or greater than an 83 percent loss after 5 m^3 of air pulled through the PTFE filter cassette assembly. It was also noted that only a very small fraction of the cyclophosphamide loss was detected in the Anasorb 708 tubes downstream of the spiked filter assembly.

TABLE IV
Results from evaluation of PTFE filters and support pads to determine stability of 2000 μg cyclophosphamide spiked onto the filter after air pulled through

Sample no.	Sample description	Cyclophosphamide mass recovered (μg)
PTFE (Teflon) media tests		
1	PTFE filter & support pad—2.0 mg CP	335.4
2	PTFE support pad only—2.0 mg CP	324.9
3	Blank TEFLON support pad, no air	<0.2
4	Field blank Anasorb 708	<0.2
Analyses of Anasorb 708 after support pad spiked with 2.0 mg CP		
1	Anasorb 708, first tube after support pad	4.9
2	Anasorb 708, first tube after support pad	1.8
3	Anasorb 708, second tube after support pad	<0.2
Note: Field blank kept in admin. office for 24 hours with ends open		

Note: PTFE Filer: A 37-mm dia., 0.45-μm pore size PTFE (Teflon) filter circle.

TABLE V
Results from PTFE filters only to determine stability of 2000 μg cyclophosphamide spiked onto the filter after air pulled through

Sample no.	Sample description	Cyclophosphamide mass recovered (μg)
PTFE & Anasorb 708 media tests		
1	PTFE filter source #1—2.0 mg CP	209
2	Anasorb 708, first tube after filter source #1	2
3	PTFE filter source #2—2.0 mg CP	192
4	Anasorb 708, first tube after filter source #2	1

Note: PTFE filter: A 37-mm dia., 0.45-μm pore size PTFE (Teflon) filter circle.

Results in Table V indicate air pulled through the PTFE filter alone reduced the concentration from 2.0 mg cyclophosphamide to approximately 200 μg. This indicates a loss of cyclophosphamide off the PTFE filters only of approximately 90 percent due to air being pulled through the filter (see Table V). This is a greater loss than that off the filter and support pad or support pad only.

After analyses of the spiked PTFE filters and/or support pads and the Anasorb 708 collection media, it appeared that a significant amount of cyclophosphamide mass had been removed from the filter. There was only a minimal amount of cyclophosphamide mass collected on the Anasorb 708 media downstream of the spiked filters. For purposes of mass balance, that is, to identify where the cyclophosphamide had moved to, the filter cassettes were rinsed, and the rinse was analyzed. Results from analyses of the rinse found a significant amount of the original cyclophosphamide mass was on the interior walls of the cassettes (average of 175 μg cyclophosphamide). Since filters were found to be inefficient collection media, solid sorbents were selected for evaluation.

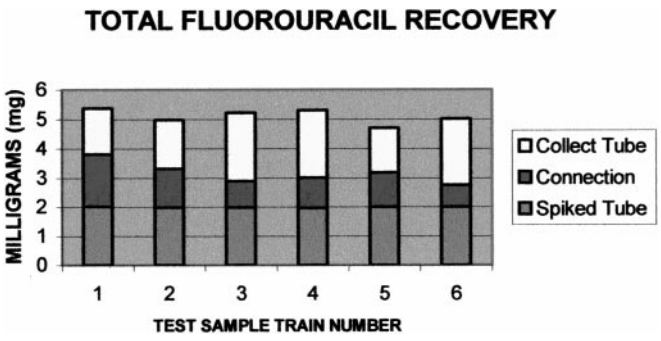

FIGURE 2
Recovery of fluorouracil from different sample train components.

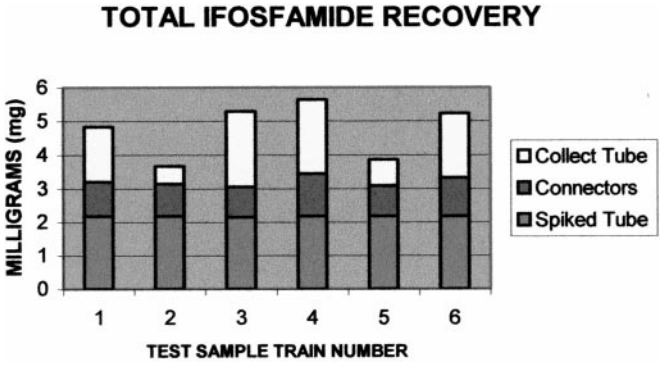

FIGURE 3
Recovery of ifosfamide from different sample train components.

Methods for Evaluation of Solid Sorbents

The first step in evaluation of solid sorbents was to select an acceptable method for determining the optimum sorbent. The method used in this study for evaluation of the sorbents was generally based on procedures developed in the 1970s by the National Institute for Occupational Safety and Health (NIOSH).[25,26] In this method, air spiked with contaminants of interest is drawn through a sample tube containing a sorbent material. After the sorbent potentially traps the contaminants, it is later desorbed and analyzed to determine acceptability, both in recovery and sensitivity. Development of a method using a solid sorbent for air monitoring has a number of advantages. Lightweight pumps and sample tubes are relatively cheap and user-acceptable. Increasing the flow rate or time of sampling can increase sensitivity. Many different chemicals can be sampled using a single sorbent, and a time-weighted average (TWA) is possible over relatively short periods (e.g., 8 hours).[26,27] The next step was selecting sorbents to test.

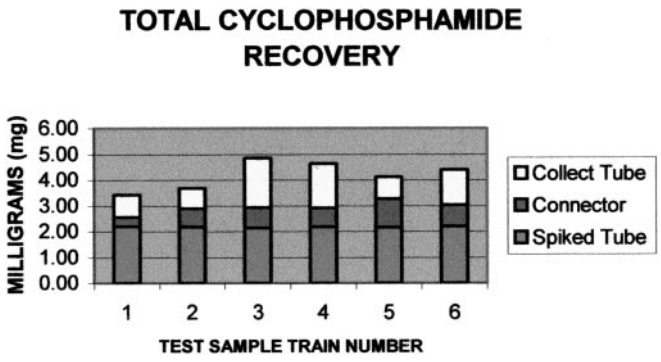

FIGURE 4
Recovery of cyclophosphamide from different sample train components.

Selection of Sorbent

The initial selection of sorbents to be evaluated was focused on monitoring of cyclophosphamide. This was because cyclophosphamide is a known human carcinogen; is widely used, and in relatively large quantities; and has therefore been evaluated as a marker of antineoplastic agent contamination.[28,29] However, because of the interest in also being able to detect and measure other contaminants of interest, it is important to identify a sorbent that has the capability for adsorption and subsequent desorption of other agents in addition to cyclophosphamide.

In 1996, Stanetzek et al.[30] evaluated several adsorbents for specific breakthrough and retention volumes, and for their respective adsorption enthalpies for various organic compounds. Adsorption enthalpy is a measure of the strength of the adsorptive interactions with the adsorbent surface.

One of the organic chemicals evaluated in the study by Stanetzek, which has a chemical structure somewhat similar to cyclophosphamide, was cyclohexylamine. Cyclohexylamine ($C_6H_{13}N$) has a molecular weight of 99.2 and is a polar chemical.[9,30] Cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P[H_2O]$) has a molecular weight of 279.1 and is also polar.[12] Although the molecular weights are different, the structures are similar in that each only has a single ring, and both have nitrogen molecules attached to the ring.

Cyclophosphamide, being a polar material, requires a sorbent having both favorable adsorption and desorption properties. Since the interest also is in simultaneous monitoring of other antineoplastic agents, some of which are nonpolar, it was also necessary to try to identify a sample media that had characteristics acceptable for effective sampling and desorption of nonpolar as well as polar agents.[31]

Reviewing the characteristics related to specific retention and breakthrough volumes for a variety of solute and adsorbent combinations, three solid sorbents appeared to be candidates for screening. These are Anasorb 708 (Amberlite XAD-8), Porapak R, and activated carbon.

Evaluation of Selected Solid Sorbents

Evaluation of the sorbents was a multi-phase activity. The first phase was to determine the ability for the antineoplastics of interest, especially CP, to be desorbed from the sorbent. This phase would likely determine which, if any, of the first three solid sorbents selected for evaluation would be acceptable. The next phase was to evaluate the collection ability of the sorbent found able to be acceptably desorbed. After lab evaluation, the method was field evaluated in a cancer infusion therapy area to determine its acceptability (see discussion section).

Sorbents Selected for Testing

As indicated earlier, three sorbents were selected for testing (SKC, Inc., Eighty Four, PA). Specific information on each sorbent is indicated in Table VII.

Initially, tests were conducted on each sorbent at various concentrations of antineoplastic agents to obtain a rough indication of the ability for adsorption and desorption for each agent. Results from this spiking and desorbing of the sorbents at varying concentrations are shown in Tables IV–VI.

Evaluation of Selected Sorbents for Desorption Acceptability

Procedures for evaluating each of the sorbents began with spiking each sorbent with a specific concentration of CP. The

TABLE VI
Properties of candidate sorbents for cyclophosphamide monitoring[A]

Sorbent name	Amberlite XAD-8 (Anasorb 708)	Porapak R	Activated carbon
Specific surface area (m^2/g)	140	450–600	1000
Average pore diameter (Å)	250	76	Not obtainable
Monomer composition	Methacrylic acid polymer	N-vinyl pyrrolidine polymer	Carbon (coconut charcoal)
Adsorption enthalpy (ΔH) (re: cyclohexylamine)	70.5-kJ/mol	55-kJ/mol	No values
Retention (V_g) & breakthrough volumes (V_D), & specific breakthrough volume (re: cyclohexylamine)	560 V_g(V_D) 310 (L/g)	330 V_g 330 (L/g)	No values Decomposition possible
Mesh size	Not available	50/80	Not available

[A]Ref: Stanetzek et al., 1996.[21]

Note: V_g = Retention volume (retention time of solute molecule [t_R or min/gram] multiplied by the mobile phase flow rate [F or L/min], or L/g).

V_D = Breakthrough volume (breakthrough time of solute molecule, which is when it is first detectable at the column outlet [t_D or min/gram] multiplied by the mobile phase flow rate [F or L/min], or L/g).

L/g = Specific breakthrough volume.

TABLE VII
Information on each of the three solid sorbents in sampling tubes tested

Sorbent name	Exp. date	Tube size (mm)	Sorbent quantity (mg)	Catalog number
Amberlite XAD-8 (Anasorb 708 or Chromasorb 108)	4/04	6 × 70	100 (Lot 1124)	226-30-08
Porapak R	4/04	6 × 70	35/70 (Lot 896)	226-59-04
Anasorb CSC (coconut charcoal)	8/04	8 × 110	350/350/350 (Lot 2000)	226-09-02

Note: All sorbents purchased from SKC South, Appomattox, VA. (SKC, Inc. Headquarters, Eighty Four, PA.)

TABLE VIII
Desorption results for activated carbon after spiking with agents

		Mass recovered (μg)				
Sample no.	Sample description	FU	IF	CP	DX	Pac
1	Blank activated carbon tube	<0.2	<0.2	<0.2	<0.5	<0.2
2	5.0 μg IF, CP, DX; 2.5 μg FU, Pac	<0.2	<0.2	<0.2	<0.5	3.5
3	33.3 μg IF; 13.3 CP, DX; 1.33 μg Pac	<0.2	<0.2	<0.2	<0.5	2.1

Note: Fluorouracil (FU) results are hidden in solvent peak.
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide, DX = Doxorubicin HCl, Pac = Paclitaxel.

TABLE IX
Desorption results for Porapak R tubes

		Mass recovered (μg)				
Sample no.	Sample description	FU	IF	CP	DX	Pac
1	Blank Porapak R tube	<0.2	<0.2	<0.2	<0.5	<0.2
2	5.0 μg/mL IF, CP, DX; 2.5 FU, Pac	<0.2	<0.2	<0.2	<0.5	<0.2
3	5.0 μg/mL IF, CP, DX; 2.5 FU, Pac	<0.2	<0.2	<0.2	<0.5	<0.2
4	25 μg/mL IF, FU; 10 CP, DX, 1 Pac	<0.2	30.9	15.3	2.15	1.5

Note: Fluorouracil peak hidden in solvent peak.
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide, DX = Doxorubicin HCl, Pac = Paclitaxel.

TABLE X
Results for Anasorb 708 (Chromosorb 108, XAD-8)

		Mass recovered (μg)				
Sample no.	Sample description	FU	IF	CP	DX	Pac
1	Blank Anasorb 708 tube	<0.2	<0.2	<0.2	<0.5	<0.2
2	1.0 μg IF, CP, DX; 0.5 μg FU, Pac	<0.2	1.5	1.3	0.2	0.7
3	1.25 μg IF, CP, DX, FU; 0.626 μg Pac	<0.2	1.1	0.9	0.1	2.1

Note: Fluorouracil results were hidden in solvent peak.
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide, DX = Doxorubicin HCl, Pac = Paclitaxel.

TABLE XI
Recovery evaluation results for five Anasorb 708 (Chromosorb 108) tubes

Sample no.	Mass recovered (μg)			
	IF	CP	DX	Pac
1	22.4	9.1	5.0	1.0
2	21.4	7.2	12.0	1.1
3	24.4	7.8	13.0	0.8
4	24.6	7.5	10.0	0.8
5	23.9	10.1	9.0	0.7
Average recovered (μg)	23.3	8.3	9.8	0.9
Average recovery (%)	93.2	83.3	98.0	87.0
S.D. (μg)	1.4	1.2	3.1	0.2
Coeff. of Variation (C.V., %)	5.9	14.4	31.8	19.2
Amount spiked (μg)	25.0	10.0	10.0	1.0

IF = Ifosfamide, CP = Cyclophosphamide, DX = Doxorubicin HCl, Pac = Paclitaxel.

spiked sorbent tubes were sealed (capped) and stored at ambient temperatures for at least 12 hours. The tubes were than cut open and the sorbent was put into a desorbing vial (a clear 10 mL glass microvial with a screw-on cap and septum [Kimble Glass, Inc., Art. No. 60710-10, Vineland, NJ]). Typically, 2 mL of desorbing solvent was injected into each vial for desorption of the contaminants. The solvent blend determined to have optimum desorption capabilities, based on trials of various blends, was a mixture of 10 percent acetonitrile, 25 percent methanol, and 65 percent Milli-Q water. After the solvent was added, the vial(s) were placed on an orbital shaker (Lab-line orbital shaker) and shaken for 30 min at 150 rpm. After shaking, the desorbate was removed via a syringe equipped with a syringe filter (a 13-mm Syringe Filter, 0.2-μm PVDF Filter Media, sterile and non-pyrogenic, Catalog No. 6791-1302, Whatman International, Ltd., Maidstone, England) and placed into a 15-mL disposable tube with a screw-on cap (disposable centrifuge tubes w/flat cap, non-pyrogenic polypropylene, catalog no. 430790, Corning Corp., Corning, NY). After desorbing, analysis of the desorbate was conducted by the analytical method developed earlier.

TABLE XIII
Flow rates, test period, and total volume of air for each of six sample sets

Sample no.	Air flow rate (L/min)	Sample time (min)	Sample vol. (m^3)
1	1.80	1330	2.21
2	1.66	1330	2.39
3	1.91	1435	2.75
4	1.88	1435	2.70
5	2.76	1435	3.96
6	2.37	1435	3.40

Note: Flow rates were chosen that would be comparable to those likely to be used in actual monitoring situations.

Activated Carbon

Activated carbon was evaluated first by injection of 10 μg of FU, IF, CP, and DX and 5 μg of Pac. After desorption in 2.0 mL of desorbent, the expected results would be 5.0 μg/mL IF, CP, DX; 2.5 FU, Pac if 100 percent desorption. When results at this concentration were found to be poor, the sorbent was tested at 100 μg for IF, 40 μg for CP and DX, and 4 μg of paclitaxel, but this time desorption was with 3 mL of desorbing solution. This would provide an expected concentration of 33.3 μg/mL IF; 13.3 CP, DX; 1.33 Pac.

Results for this concentration were also poor. It was noted that the FU could not be detected at either concentration due to its peak being hidden in the solvent peak at the beginning of the chromatogram. These results indicated an inability for activated carbon to be effectively desorbed for IF, CP, and DX, and possibly FU. Therefore, activated carbon was found to be unacceptable as a solid sorbent for monitoring the antineoplastic agents of interest in this study.

TABLE XII
Results from evaluation of Anasorb 708 spiked with 200 μg CP and air pulled through

Sample no.	Sample description (flow rate)	Air vol. (m^3)	Cyclophosphamide mass recovered (μg)
1	Spike #1 (1704 cc/minute)	2.13	161.5
2	Spike #2 (2541 cc/minute)	3.17	274.7
3	Spike #3 (2929 cc/minute)	3.65	263.9
4	Spike #4 (3186 cc/minute)	3.97	151.2
5	Spike #5 (1830 cc/minute)	2.28	138.3
6	Spike #6 (2061 cc/minute)	2.57	150.0
Average mass recovered (μg)			189.9
Standard deviation (μg)			62.0
Coefficient of variation (C.V., %)			32.6
Spiked quantity (μg)			200.0

Note: Period for pulling air through spiked tubes was 1247 minutes for each tube.

TABLE XIV
Analytical recovery results for fluorouracil from test sample train, 5.0 mg spike onto Anasorb 708 tubes

		Mass recovered (mg)			
Sample no.	Air vol. (m^3)	Initial	Connectings	Collectors	Total
1	2.21	2.0	1.8	1.6	5.4
2	2.39	2.0	1.3	1.7	5.0
3	2.75	2.0	0.9	2.3	5.2
4	2.69	2.0	1.0	2.3	5.3
5	3.96	2.0	1.2	1.5	4.7
6	3.40	2.0	0.8	2.2	5.0
Avg recovered (mg)		2.0	1.2	1.9	5.1
Avg recovery (%)		40	23	39	102
S.D. (mg)		<0.1	0.4	0.4	0.3
C.V. (%)		0.9	31.2	20.3	4.9

Note: Bias of 10.0%, precision (based on C.V. of 4.9%), and estimated accuracy of 19.8%.

Porapak R

Results from screening tests on Porapak R tubes for use in monitoring antineoplastic agents are shown in Table IX below. Like the activated carbon tubes, these tubes were tested blank (no spike), then with different concentrations of the antineoplastic agents of interest to determine desorption efficiency.

Anasorb 708

Results from the screening tests on Anasorb 708 (Chromasorb 108, XAD-8) were very good. Results indicated detection from the initial testing at spike concentrations of 1.0 μg/mL for IF, CP, and Pac. Results from the screening tests are shown in Table X.

Further Evaluation of Anasorb 708

Based on these screening test results for Anasorb 708, it was decided to conduct spiking and analyses on a set of five tubes of this sorbent for a more accurate evaluation (see Table XI). Results from the five tube analyses found that the average recovery of cyclophosphamide, ifosfamide, doxorubicin, and paclitaxel from Anasorb 107 were quite acceptable, ranging from 83.3 percent for cyclophosphamide to 98 percent for doxorubicin. However, precision and accuracy were very poor for doxorubicin, marginal for cyclophosphamide and paclitaxel; ifosfamide data were acceptable. Fluorouracil was also added to each spiked tube at a concentration of 25 μg, but it was not detected. It is believed

TABLE XV
Analytical recovery results for ifosfamide from test sample train, 5.0 mg spike onto Anasorb 708 tubes

		Mass recovered (mg)			
Sample no.	Air vol. (m^3)	Initial	Connectings	Collectors	Total
1	2.21	2.2	1.0	1.6	4.8
2	2.39	2.2	1.0	0.5	3.7
3	2.75	2.2	0.9	2.2	5.3
4	2.69	2.2	1.3	2.2	5.7
5	3.96	2.2	0.9	0.8	3.9
6	3.40	2.2	1.1	1.9	5.2
Avg recovery (mg)		2.2	1.0	1.5	4.8
Avg recovery (%)		44	20	30	95
S.D. (mg)		<0.1	0.1	0.7	0.8
C.V. (%)		0.5	13.6	47.0	16.9

Note: Bias of 4.8%, precision (based on C.V. of 16.9%), and estimated accuracy of 38.7%.

TABLE XVI
Analytical recovery results for cyclophosphamide from sample train, 5.0 mg spike onto Anasorb 708 tubes

		Mass recovered (mg)			
Sample no.	Air vol. (m^3)	Initial	Connectings	Collectors	Total
1	2.21	2.2	0.4	0.9	3.4
2	2.39	2.2	0.7	0.8	3.7
3	2.75	2.1	0.8	1.9	4.9
4	2.69	2.2	0.8	1.7	4.6
5	3.96	2.2	1.1	0.8	4.1
6	3.40	2.2	0.8	1.4	4.4
Avg recovered (mg)		2.2	0.8	1.3	4.2
Avg recovered (%)		44	15	25	84
S.D. (mg)		<0.1	0.2	0.5	0.6
C.V. (%)		1.1	31.7	39.2	13.2

Note: Bias of 16.3%, precision (based on C.V. of 13.2%), and estimated accuracy of 42.7%.

the solvent peak on the chromatogram hid the fluorouracil peak. Because of the good average recovery for each agent, it was decided that this was the sorbent of choice for subsequent method development.

Results from the five samples above provide information on the accuracy and precision in desorption of ifosfamide and cyclophosphamide. The average recovery for ifosfamide at a concentration of 25 μg was 23.3 μg, or 93.2 percent average recovery. Bias was 7 percent, and precision, based on a C.V., was 5.9 percent. Accuracy, based on bias (100%—avg. recovery, or 8.6%) plus twice precision (2 $\times$ C.V.), was 20.4 percent. The average recovery for cyclophosphamide at a concentration of 10 μg was 8.3 μg, or 83.3 percent average recovery. Precision was estimated to be 14.4 percent, with a bias of 16.7 percent. Accuracy was estimated at 45.5 percent. Average recovery for doxorubicin was 98 percent, but the precision was 31.7 percent, and estimated accuracy was 65.5 percent.

Average recovery for paclitaxel was acceptable at 87 percent, but again, there was a fairly wide range of results. Precision for paclitaxel was estimated at 19.18 percent, and estimated accuracy was 51.36 percent. Fluorouracil could not be evaluated because its peak could not be separated from the solvent peak on the chromatogram at the concentration tested (25 μg).

Although the solid sorbent media were able to detect paclitaxel from the injected concentration, because of its physical characteristics it was determined that paclitaxel would not sublimate or evaporate. And since it would remain a particulate, continued use of the filter method would be a more accurate method for collection of paclitaxel in air. Based on this conclusion, paclitaxel was not included in subsequent tests for this method.

TABLE XVII
Analytical recovery results for doxorubicin from test train, 2.0 mg spike onto Anasorb 708 tubes

		Mass recovered (mg)			
Sample no.	Air vol. (m^3)	Initial	Connectings	Collectors	Total
1	2.21	0.8	0.2	<0.1	1.0
2	2.39	0.7	0.4	<0.1	1.1
3	2.75	0.4	<0.1	1.2	1.6
4	2.69	0.5	0.4	<0.1	0.9
5	3.96	1.0	0.3	<0.1	1.2
6	3.40	0.8	<0.1	0.5	1.3
Avg recovery (mg)		0.7	0.2	0.3	1.2
Avg recovery (%)		34.0	10.5	14.5	60.0
S.D. (mg)		0.2	0.2	0.5	.3
C.V. (%)		29.6	87.0	170.8	21.3

Note: Bias of 40.5%, precision (based on C.V. of 21.3%), and estimated accuracy of 83.1%.

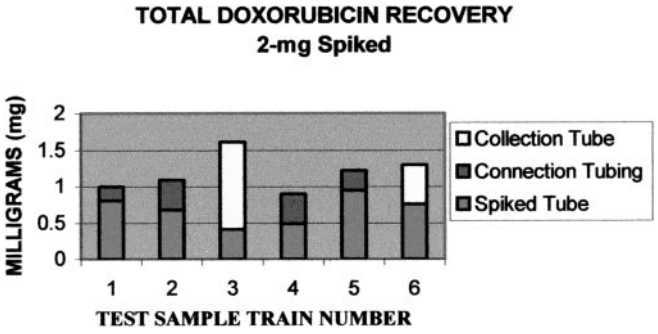

FIGURE 5
Recovery of doxorubicin from different sample train components.

Validation of Anasorb 708 for Monitoring Antineoplastics

Six Anasorb 708 tubes were spiked with 200 μg of cyclophosphamide. Air was then pulled through each of them, into and through another Anasorb 708 tube for downstream collection of any agent that might be released from the spiked tubes.

After pulling air through the spiked tubes into and through the collection tubes, all tubes were desorbed with 2.0 mL of desorbent each. Results for the spiked tubes after air was pulled through them are shown in Table XII. Cyclophosphamide was not detected in any of the downstream sample tubes, only in the spiked tubes.

The average recovery results from this testing of Anasorb 708 for effectiveness in collecting cyclophosphamide were good ($\sim$190 μg average recovery from 200 μg spike). However, the precision at a C.V. 32.6 and estimated accuracy of 75.3 was not as good as desired. This was attributed, at least in part, to the likelihood that the capacity of the 100 mg Anasorb 708 sorbent tube was greater than 200 μg, Thus, the mass of 200 μg cyclophosphamide injection did not exceed the capacity of the spiked tube to allow for excess cyclophosphamide to be released from the tube and be collected in downstream tubes. Based on this observation, it was decided to spike the Anasorb 708 tubes while the manifold system was operating, and with a larger mass of each agent of interest.

Anasorb 708 collection tubes were placed behind the tube to be spiked on the sample train. A syringe was used to inject the antineoplastic mass into the first set of tubes as air was being pulled through them. A total mass of 5.0 mg FU, IF, and CP, and 1.0 mg DX was added slowly to each Anasorb 708 tube to be spiked during the evaluation period. The collection and retention abilities of the combination of spiked source tubes, connecting tubing, and both collection tubes are shown for each agent in Tables XIV through XVII and in Figures 2 through 5. Table XIII indicates the average flow rate (cc/min), period of the test (min), and the total volume for each series of tubes and connecting tubing, otherwise referred to as the test sample train.

DISCUSSION AND CONCLUSIONS

Historically, based on the physical characteristics for agents such as CP, it was believed that these agents remained in particulate form; thus, the use of filters for monitoring would be most effective. However, the information obtained during the development of this method confirms the observations by Schmidt.[1] That is, CP may be captured by air filters, but then sublimate or evaporate off the filter. In such a case, even if diffusion of the CP particles occurs at the surface of the filter, once it sublimates, the filter surface area is miniscule compared to that of the surface of the solid sorbent. Consequently, collection of the now gaseous molecules by the filter is inefficient. This explains why monitoring results for cyclophosphamide from methods utilizing filters were generally very low or below detection limits, even when the monitoring periods were for relatively long periods of time (e.g., several days).

In addition to the filter method not being acceptable for air monitoring for CP, and possibly some of the other antineoplastics, e.g., ifosfamide and fluorouracil, this information also supports the observation that the HEPA filter is not an acceptable control for BSCs that return filtered air to the work area. Because of this, CP exposures to healthcare professionals are likely to have been occurring, even when results from long-term air monitoring by filter method of the work environment indicated no or very low exposures to be occurring.

This method was tested by monitoring in an infusion therapy clinics oncology pharmacy preparation hood (BSC). Results did identify levels at 0.35 μg/m^3, indicating monitoring of air concentrations for CP below 1.0 μg/m^3 was able to be achieved with a 24 h sample, as observed in lab tests. But, it is likely that these results were lower than actual CP in air concentrations due to the sampling having been compromised by the attachment of the filter cassette and filter ahead of the Anasorb 708 tube. The cyclophosphamide would likely go through the filter, but some could be attracted to the interior sidewalls of the filter cassette. Thus, a reduced concentration would reach the Anasorb 708 tube. It is important that there be no cassette or tubing ahead of the inlet to the Anasorb 708 tube when monitoring for cyclophosphamide, ifosfamide, or fluorouracil.

This study found that the method utilizing Anasorb 708 solid sorbent for air monitoring is accurate to measure ifosfamide and cyclophosphamide concentrations from less than 1.0 ug/mL to $>$1.0 mg/mL. This provides a monitoring range of $<$0.35 μg/m^3 to approximately 350 μg/m^3 when sampling is conducted for 24 hr at 2 L/min. Thus, the lower limit of detection for cyclophosphamide provides adequate sensitivity to detect concentrations of cyclophosphamide in air well below the recommended exposure limit of 0.001 mg/m^3 (1.0 μg/m^3). Further, it is also acceptable for monitoring fluorouracil and ifosfamide.

Results from analysis of the spiked Anasorb 708 tubes indicated the maximum capacity of cyclophosphamide to be a consistent 2.17 μg, with a standard deviation (S.D.) of 0.02 and a coefficient of variation (C.V.) of 1.08. This was observed with air volumes pulled through the tubes in the range from 2.2 to 3.9 m^3. This indicates very good precision and accuracy over a relatively wide range of air volume. Similar results were seen for fluorouracil and ifosfamide. Average tube capacity for fluorouracil was 1.99 mg, with an S.D. of 0.02 and C.V. of 0.88 percent.

Average tube capacity for ifosfamide was 2.18 mg, with an S.D. of 0.01 and C.V. of 0.54 percent.

This method may be acceptable for monitoring doxorubicin, but further evaluation is needed due to the relatively rapid decomposition of doxorubicin, especially when it is in contact with other chemical agents.

Based on desorption efficiency and capacity tests, this method has the sensitivity and precision to conduct assessments for airborne concentrations of not only cyclophosphamide, but also fluorouracil and ifosfamide wherever they may be used.[27] Further, high accuracy can be expected for cyclophosphamide, fluorouracil, and ifosfamide based on the consistency in the mass of each recovered (the capacity) from the spiked tubes in lieu of a wide range of total air volume pulled through each tube (from $\sim$2.2 m^3 to $\sim$4.0 m^3). This method can therefore be used to provide credible exposure information for use in epidemiological studies, risk assessment studies, risk management purposes, and establishment of compliance standards based on dependable information. Relative to risk management, availability of accurate monitoring data can also provide important information on the effectiveness of various engineering and administrative exposure controls available where cyclophosphamide is or may be handled.

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