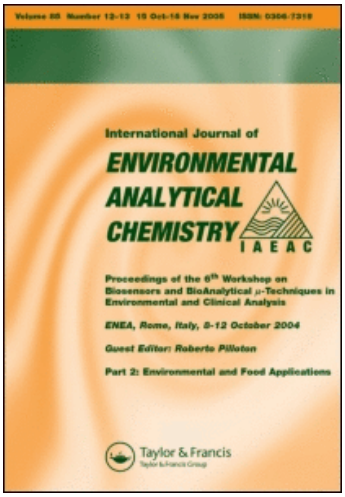

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Carcinogens and Analogs: Trace Analysis of Thirteen Compounds in Admixture in Wastewater and Human Urine

Charles R. Nony[a]; Malcolm C. Bowman[a]
[a] Department of Health, Education and Welfare Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas, U.S.A.

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Carcinogens and Analogs: Trace Analysis of Thirteen Compounds in Admixture in Wastewater and Human Urine†

CHARLES R. NONY and MALCOLM C. BOWMAN
Department of Health, Education and Welfare

Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079, U.S.A.

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A gas chromatographic method is described for determining traces of 13 carcinogens and related compounds (aromatic amines and estrogens) in admixture in wastewater and human urine. This method was developed for use in toxicological research for monitoring the safe disposal of wastewater and to signal any accidental exposure of personnel to hazardous test substances. Salient elements of the procedure are: extraction of phenolic and neutral residues from the acidified sample, liquid–liquid partitioning cleanup and separation of neutral from phenolic residues at pH 14 and 10.2, acid hydrolysis of the neutral component, subsequent alkalinization of the sample and extraction of the basic residues as the free amines, conversion of all residues to the corresponding pentafluoropropionyl (PFP) derivatives and quantification by electron-capture gas chromatography. Residues were detectable in wastewater and urine at the 0.1 and 1 ppb levels, respectively. Additional information is provided concerning partition values for all PFP derivatives in five solvent systems, structure verification of the derivatives by mass spectrometry and the adaption of this method to the monitoring of surfaces and air in potentially contaminated work areas.

KEY WORDS: Carcinogens, aromatic amines, estrogens, wastewater, human urine, electron-capture gas chromatography, trace analysis, derivatization.

INTRODUCTION

Long-term, low dose toxicological studies with large numbers of mice at our laboratory include tests with several chemicals known to cause, or suspected of causing, cancer in humans by occupational exposure.[1–4]

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Formulas of these compounds of current interest at our laboratory, some of their analogs and their abbreviations used in this paper are presented in Figure 1. The responsibility for controlling these substances begins when they enter the laboratory and ends only after their safe disposal. Two important factors relating to the control of test substances in nonclinical laboratory studies as proposed by the U.S. Department of Health, Education and Welfare, Food and Drug Administration[5] are: (1) to provide assurance that personnel and work areas remain free of contamination by the substances; and (2) to accomplish the safe disposal of contaminated experimental material.

Because there is no general agreement on the existence of a "threshold" level at which carcinogens cause cancer or at which other substances

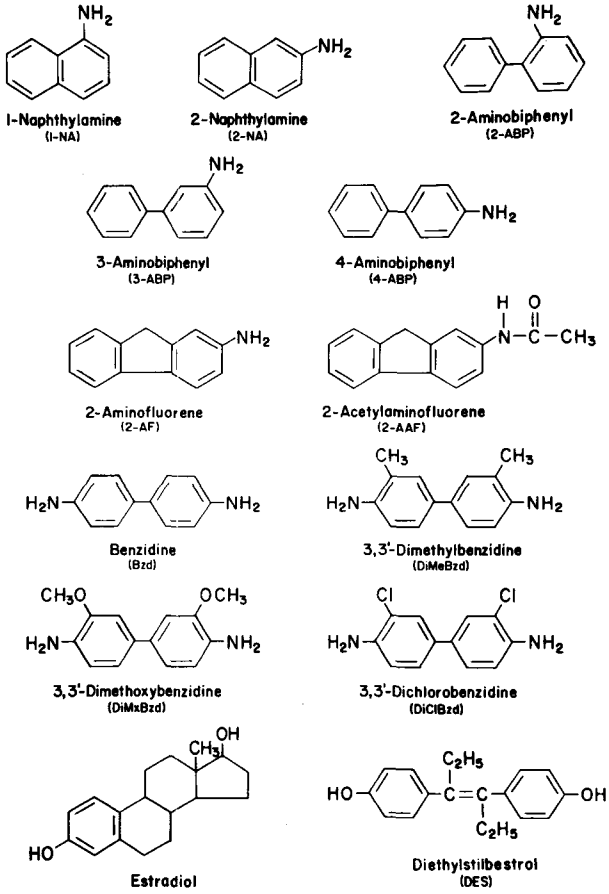

FIGURE 1 Formulas of 13 carcinogens and related compounds.

produce damage every effort must be made to ensure zero exposure of personnel to carcinogens by periodically monitoring samples of urine by using the most sensitive and specific analytical methods available.[6-9] In a similar manner, these methods are also used to routinely monitor wastewater that has been treated for the removal of all test chemicals[10] prior to sewage treatment and eventual discharge into the environment. Our present methods, based on spectrophotofluorescence (SPF), high-pressure liquid chromatography (HPLC) or electron-capture gas chromatography (EC–GC), have served us well in determining traces [low- or sub-ppb (ng/ml)] of a few chemicals analyzed separately. However, such methods will fail to detect, identify and quantify all of the compounds shown in Figure 1 when they are present in admixture.[11] Therefore, research was initiated to develop a method for screening wastewater and human urine for the presence of all of these substances at trace levels.

All of the compounds tested may be classified according to their chemical properties as follows: (a) ten primary amines (basic), (b) one secondary amine (neutral), and (c) two estrogens (phenolic). This provided the basis for separating them into groups via acidification, alkalinization and solvent extraction. Because of the free hydrogen atoms present in all compounds, including the secondary amine (2-AAF) after acid hydrolysis and conversion to 2-AF, these compounds can be reacted with fluorinating agents[9,12-15] to produce derivatives that exhibit high sensitivity in assays using EC–GC. This paper describes procedures for extraction, separation, detection, identification and quantification of trace amounts of 13 chemical carcinogens and analogs in admixture in wastewater and human urine by EC–GC of their pentafluoropropionyl (PFP) derivatives.

EXPERIMENTAL

Test materials and other chemicals

The chemicals shown in Figure 1 were obtained from several suppliers as previously reported by Bowman,[6,7,9,15] Holder[8] and King,[14] who also described their properties and purity.

All solvents were pesticide grade and all reagents were CP grade. Sodium sulfate and glass wool were extracted with benzene for 40 hr in a Soxhlet apparatus and dried in an oven overnight at 130°C prior to use. All culture tubes were borosilicate glass and equipped with Teflon-lined screw caps. The 170-ml and 35-ml culture tubes were fabricated by Shamrock Scientific Glassware Co., Little Rock, AR.

Pentafluoropropionic anhydride (PFPA) (No. 65193) and heptafluorobutyric anhydride (HFBA) (No. 270085) were obtained from Pierce

Chemical Co., Rockford, IL. and Regis Chemical Co., Morton Grove, IL., respectively. A 100 μl Hamilton syringe, fitted with a Chaney adapter, was used to withdraw the derivatizing agent from the vial and deliver it into the reaction vessel. A fresh vial (1 g) was used for each group of samples. The trimethylamine (TMA) reagents (0.05 and 0.1 M in benzene), buffer solution (potassium monobasic phosphate, pH-6), sodium hydroxide (1 N) and sodium bicarbonate (1 M) were described by King *et al.*[14] and Bowman and Rushing.[9] The "keeper" solution was paraffin oil (20 mg/ml) in pentane.

Extraction and preparation of samples for analysis

Wastewater One hundred ml of the sample and 10 ml of concentrated HCl added to a 170-ml culture tube were shaken and allowed to stand for 5 min. Twenty ml of benzene were then added, the contents shaken for 1 min then the tube was centrifuged at 550 rpm for 10 min. The benzene layer was carefully withdrawn by using a syringe and cannula, percolated through a plug of sodium sulfate (25 mm diam. $\times$ 25 mm thick) and collected in a 100-ml round-bottom flask containing a glass bead and 0.5 ml of "keeper" solution. The extraction was repeated by using two additional 20-ml portions of benzene and the combined extracts, containing the phenolic and neutral compounds were evaporated just to dryness by using water pump vacuum and a 60°C water bath. The acidified aqueous phase was reserved for subsequent extraction of the basic compounds.

The flask containing the phenolic and neutral compounds was treated as described by Bowman and Nony[15] viz. 5 ml of benzene were used to transfer the residue to a 20-ml culture tube containing 4 ml of 1 N NaOH. The contents of the tube were shaken, centrifuged for 5 min at 1200 rpm and the benzene layer transferred to a 35-ml culture tube also containing 4 ml of 1 N NaOH. The contents of the second tube were shaken and centrifuged as described and the benzene layer was carefully withdrawn and percolated through a plug of sodium sulfate (18 mm diam. $\times$ 20 mm thick) and collected in a 50-ml round-bottom flask containing a glass bead and 0.5 ml of "keeper" solution. The flask and aqueous NaOH phases in both tubes were again sequentially washed and extracted in the same manner by using two additional 5-ml portions of benzene which were also percolated through a plug of sodium sulfate. The combined extracts containing the neutral fraction (2-AAF) were reserved for subsequent treatment. The contents of the 20-ml tube were transferred to the 35-ml tube and 10 ml of 1 M sodium bicarbonate, used to wash the 20-ml tube, were also added to the mixture. The mixture was then extracted three times with 15-ml portions of benzene; each extract was successively

percolated through a plug of sodium sulfate (25 mm diam. × 25 mm thick) and collected in a 100-ml round-bottom flask containing a boiling bead and 0.5 ml of "keeper" solution. The combined extracts containing the phenolic fraction were evaporated to dryness by using water pump vacuum and a 60°C water bath. The residue was reserved for subsequent derivatization with PFPA and assay by EC–GC.

The neutral fraction was evaporated to dryness as described and the residue transferred to a 20-ml culture tube by using three 1-ml portions of chloroform. The solvent in the tube was then evaporated to dryness in a tube heater (Kontes No. 720,000, Vineland, NJ) set at 40°C by using a gentle stream of dry nitrogen. The dry residue was hydrolyzed as described by Bowman and King[6] viz. by heating the sealed tube containing 4 ml of methanol and 2 ml of concentrated HCl in a tube heater set at 85°C for 2 hr. After the tube had cooled, 3 ml of water were added and the contents extracted three times with 7-ml portions of benzene. Each extract was carefully withdrawn and discarded by using a 10-ml syringe and cannula. Next, 4 ml of 10 N NaOH were added and the contents extracted three times with 7-ml portions of benzene. Each extract was successively percolated through a plug of sodium sulfate (18 mm diam. × 20 mm thick) and collected in a 50-ml round-bottom flask containing a boiling bead and 0.5 ml of "keeper" solution. The contents were evaporated just to dryness at 60°C by using water pump vacuum as described; the residue was transferred to an 8-ml culture tube by using three 1-ml portions of benzene which were also evaporated to dryness in a tube heater at 50°C by using a stream of dry nitrogen. The residue containing 2-AF, was reserved for subsequent derivatization with PFPA and assay by EC–GC.

The acidified aqueous phase (basic fraction), previously reserved for later treatment, was made strongly alkaline by adding 15 ml of 10 N NaOH and extracted three times with 20-ml portions of benzene. Each extract was percolated successively through a plug of sodium sulfate (25 mm diam. × 25 mm thick) and collected in a 100-ml round-bottom flask containing a boiling bead and 0.5 ml of "keeper" solution. The combined extracts were evaporated to dryness by using water pump vacuum and a 60°C water bath. The residue was transferred to an 8-ml culture tube by using three 1 ml portions of benzene and evaporated to dryness in a tube heater at 50°C with a stream of dry nitrogen. The residue was reserved for subsequent derivatization with PFPA and assay by EC–GC.

Human urine Two 50-ml portions of the sample were separately added to 75-ml culture tubes, each containing 5 g of NaCl; 5 ml of concentrated HCl were added to one tube (phenolic and neutral fractions) and 5 ml of 10 N NaOH were added to the other tube (basic fraction). After the tubes were shaken and allowed to stand for 5 min the contents were extracted

three times with 15-ml portions of benzene which were successively percolated through plugs of sodium sulfate (25 mm diam. × 25 mm thick) and collected in 100-ml round-bottom flasks containing a boiling bead and 0.5 ml of "keeper" solution. Each of these combined extracts was then treated exactly as described for wastewater.

Preparation of Derivative

Pentafluoropropionyl (PFP) and heptafluorobutyryl (HFB) derivatives of all compounds shown in Figure 1 (except 2-AAF) were prepared by our modification of the procedures reported by Walle and Ehrsson[12] and Ehrsson *et al.*[13] For derivatization of amines, TMA solution (0.5 ml, 0.05 M) was added to an 8-ml culture tube containing the compounds (10 μg or less) dissolved in exactly 1.5 ml of benzene (total benzene = 2.0 ml) and followed by the addition of 50 μl of either PFPA or HFBA reagent. The tube was immediately sealed, shaken, heated in a 50°C water bath for 20 min; cooled, and the reaction terminated by adding 2 ml of phosphate buffer (pH 6.0). The tube was shaken for 1 min, and after the phases had separated, the aqueous layer (bottom) was discarded. The extraction was repeated with an additional 2-ml portion of buffer; the tube was centrifuged for 1 min at 1000 rpm and the benzene layer (top) was either analyzed directly or appropriately diluted with benzene prior to analysis.

For derivatization of phenolic compounds, TMA solution (0.5 ml, 0.1 M) was added to a 50-ml round-bottom flask containing the compounds (10 μg or less) dissolved in exactly 0.5 ml of benzene (total benzene = 1.0 ml) and followed by the addition of 50 μl of either PFPA or HFBA reagent. The flask was immediately sealed with a glass stopper, the contents mixed by gentle swirling, and then allowed to stand at ambient temperature for 20 min. The reaction was terminated by adding 1 ml of phosphate buffer (pH 6.0) and gently swirling the contents. A 1-ml portion of benzene was then added, mixed with gentle swirling (total benzene = 2.0 ml), and the mixture transferred to an 8-ml culture tube to allow the phases to separate. The aqueous layer (bottom) was discarded, the benzene phase extracted with an additional 2-ml portion of buffer, centrifuged and analyzed as described for the amines.

Final residues from the extraction and cleanup procedures of the basic, neutral and phenolic fractions from wastewater or urine were dissolved in benzene (1.5 ml for amines; 0.5 ml for phenols) and derivatized by using TMA solution and PFPA reagents as described. For assays of wastewater containing amine or phenolic residues in the order of 0.10, 1.0 and 10 ppb, the entire extract (100-g equivalent) of the cleaned-up sample was de-

rivatized and 5-μl injections containing 250-, 25-, and 2.5-mg equivalents, respectively, were assayed by EC–GC. Likewise, for assays of urine containing residues in the order of 2 or 20 ppb, the entire extract (50-g equivalent) was derivatized and 12.5 and 1.25 mg equivalents, respectively, were analyzed.

Gas chromatographic assays

A Hewlett–Packard (Palo Alto, CA) Model 5750B instrument equipped with a ^{63}Ni electron-capture detector (Tracor, Inc., Austin, TX) and 125-cm glass columns (4 mm i.d.) containing 5% Dexsil 300, 5% OV–101 or 5% OV–17, all on Gas Chrom Q (80–100 mesh) conditioned at 270°C overnight prior to use, was operated isothermally at the various temperatures (Table I) with a nitrogen carrier flow of 160 ml/min. The detector, operated in the DC mode, was 300°C and the injection port was 20°C higher than the column oven. Lindane and heptachlor epoxide were used as reference standards to monitor the performance of the EC–GC system; all injections were made in 5 μl of benzene. Assays of the three fractions from wastewater or urine were performed on a column of Dexsil 300. Because of the wide differences in t_R values for the various PFP derivatives, the basic fraction was first analyzed at 165°C to quantify 2-NA and 4-ABP then at 220°C for the other amines. The neutral fraction was also assayed at 220°C and the phenolic fraction was assayed at 190°C for DES then at 220°C for estradiol. Derivatized samples of unknown residue content (except DES) were quantified by relating their peak heights to known amounts of the corresponding PFP derivatives. The DES residue content was quantified as described by King *et al.* (14) by relating the sum of the heights of the cis- and trans-peaks expressed as the trans-isomer (cis $\times$ 0.722 = trans) to known amounts of PFP derivatives of DES calculated in the same manner. Derivatized samples of unknown 2-AAF content were quantified by relating the peak height of the resulting PFP-2-AF to a known amount of PFP-2-AF and then expressing the results as 2-AAF (2-AF $\times$ 1.23 = 2-AAF).

Recovery experiments

Wastewater Triplicate samples (100 g) of wastewater in 170 ml culture tubes were separately spiked with 1 ml of methanol containing the appropriate amount of 10 selected carcinogens and analogs, i.e. (2-NA,4-ABP, 2-AF, 2-AAF, Bzd, DiClBzd, DiMeBzd, DiMxBzd, DES and Estradiol) to produce residues of 0, 0.10, 1.0 and 10 ppb. The tubes were sealed, mixed and allowed to stand overnight at 5°C prior to analysis.

TABLE I

Retention times of two electron-capturing derivatives of carcinogens and analogs on three chromatographic columns

Compound	Derivative[b]	Retention time (t_R, min) and oven temperature (°C) for Column Indicated[a]					
		Dexsil 300		OV-101		OV-17	
		°C	t_R	°C	t_R	°C	t_R
2-Aminobiphenyl (2-ABP)	PFP	165	1.65	155	2.20	165	1.90
	HFB	165	1.70	155	2.40	165	1.90
1-Naphthylamine (1-NA)	PFP	165	1.80	155	2.00	165	2.00
	HFB	165	1.95	155	2.25	165	1.95
2-Naphthylamine (2-NA)	PFP	165	2.30	155	2.45	165	2.50
	HFB	165	2.50	155	2.75	165	2.50
3-Aminobiphenyl (3-ABP)	PFP	165	4.50	155	4.80	165	4.90
	HFB	165	4.85	155	5.50	165	4.90
4-Aminobiphenyl (4-ABP)	PFP	165	5.05	155	5.40	165	6.10
	HFB	165	5.55	155	6.30	165	6.10
2-Aminofluorene (2-AF)	PFP	220	1.70	210	1.60	220	1.75
	HFB	220	1.80	210	1.80	220	1.70
Benzidene (Bzd)	PFP	220	3.20	210	3.00	220	2.75
	HFB	220	3.55	210	3.60	220	2.65

3,3′-Dichlorobenzidine (DiClBzd)	Underiv	220	6.60	210	4.80	220	11.85
	PFP	220	4.30	210	4.10	220	3.00
	HFB	220	4.70	210	5.20	220	2.90
3,3′-Dimethylbenzidine (DiMeBzd)	PFP	220	4.90	210	4.10	220	3.75
	HFB	220	5.50	210	5.20	220	3.60
3,3′-Dimethoxybenzidine (DiMxBzd)	PFP	220	8.25	210	6.90	220	6.00
	HFB	220	8.70	210	8.30	220	5.70
Diethylstilbestrol (DES)	PFP-cis	190	1.60	195	1.55	175	2.10
	HFB-cis	190	1.90	195	2.00	175	2.35
	PFP-trans	190	2.55	195	2.30	175	2.95
	HFB-trans	190	3.15	195	3.10	175	3.40
Estradiol	PFP	220	4.35	210	4.20	220	2.80
	HFB	220	5.15	210	5.45	220	2.95
Lindane (ref. std.)		165	4.15	155	3.70	165	6.40
		190	1.65	195	1.05	175	4.25
		220	0.85	210	0.85	220	1.00
Heptachlor epoxide (ref. std.)		165	11.50	155	11.90	165	—
		190	4.00	195	2.55	175	10.50
		220	1.75	210	1.75	220	2.20

[a]All columns were 125-cm glass (4 mm, i.d.) packed with 5% liquid phase on Gas Chrom Q (80–100 mesh); nitrogen carrier flowed at 160 ml/min.

[b]PFP and HFB are pentafluoropropionyl and heptafluorobutyryl derivative, respectively.

Human urine Two sets of triplicate 50 g samples of urine in 75 ml culture tubes were separately spiked with 0.5 ml of methanol containing the appropriate amounts of the 10 compounds used for wastewater to produce residues of 0, 2.0 and 20 ppb. The tubes were sealed, mixed and allowed to stand overnight at 5°C prior to analysis.

RESULTS AND DISCUSSION

A periodic analysis of the urine is one of the most convenient means of determining human exposure to test chemicals. Although partial metabolism of these substances is known to occur prior to excretion in the urine, the unaltered parent compound is also excreted in sufficient amounts to signal any appreciable exposure in the event that highly sensitive analytical procedures are employed. Therefore, in the absence of rapid and sensitive methods for a wide variety of metabolites of each of the test compounds shown in Figure 1, surveillance of personnel to detect accidental exposure to the test substances is based on periodic assays of the urine for traces of the parent compounds.

Recent development of methodology at our laboratory for assaying traces of DiClBzd,[9] DES[14] and estradiol[15] based on derivatization with fluorinated acid anhydrides led us to investigate the possibility of analyzing all of the compounds in the same manner. Indeed, it was found that all of these compounds, with the exception of 2-AFF, could be derivatized and analyzed with high sensitivity; 2-AAF, after hydrolysis to 2-AF[6] and subsequent derivatization could also be analyzed.

Both PFP and HFB derivatives of all of the compounds were prepared and subjected to GC analysis on columns of Dexsil 300, OV-101 and OV-17 by using a variety of isothermal operating conditions. Data concerning GC operations and retention times for all of these compounds as well as underivatized DiClBzd and reference standards of lindane and heptachlor epoxide are presented in Table I. DiClBzd was the only test substance that demonstrated appreciable electron-capturing properties without being subjected to derivatization; nevertheless, conversion of the compound to the PFP derivative enhanced its response about 300 fold which agrees with the value reported by Bowman and Rushing.[9] Although the PFP and HFB derivatives of the various compounds yielded about the same response, the use of PFP derivatives was adopted because the reagent was easier to use and generally produced fewer interference peaks. Data concerning t_R's of the PFP derivatives (Table I) indicate that Dexsil 300 is the column of choice for the analytical procedure because better separation is obtained. Although the present procedure for wastewater and urine employs derivatives on a column of Dexsil 300, the data

concerning both the PFP and HFB derivatives on all three column packings may be useful in confirmatory tests. Typical gas chromatograms of PFP derivatives of all of the compounds, underivatized DiClBzd and the reference standards assayed on the column of Dexsil 300 using various isothermal operating conditions are presented in Figures 2 and 3.

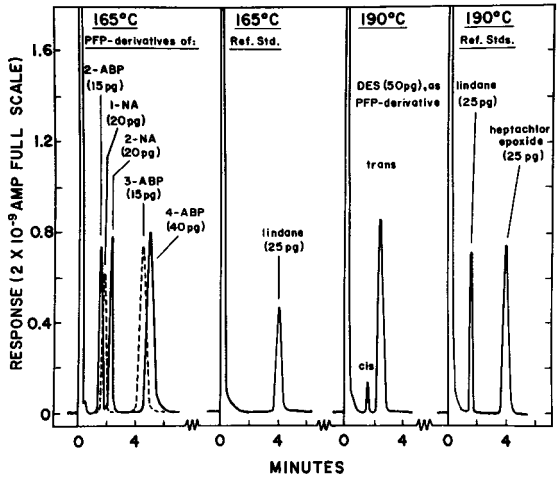

FIGURE 2 Electron-capture gas chromatograms of standards of PFP-derivatives of carcinogens, their analogs, and reference standards of lindane and heptachlor epoxide. All injections were in 5 μl of benzene.

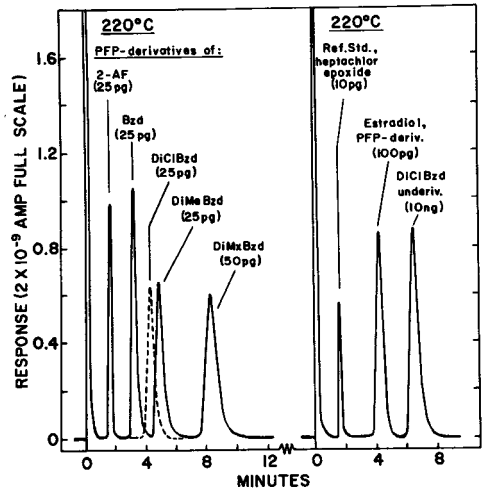

FIGURE 3 Electron-capture gas chromatograms of standards of PFP-derivatives of carcinogens, their analogs, a reference standard of heptachlor epoxide and underivatized DiClBzd. All injections were in 5 μl of benzene.

Excellent responses are obtained from injections of pg-amounts of the PFP derivatives.

The *p*-values (fraction of solute partitioning into the nonpolar phase of an equivolume immiscible binary solvent system) are useful for confirming the identity of unknown GC peaks where insufficient amounts are available for test by other means. Therefore, such values for all of the PFP derivatives in five solvent systems, determined as described by Bowman and Beroza,[16,17] are reported in Table II. Additional *p*-values were determined for all of the PFP derivatives in benzene versus the phosphate buffer (pH 6) used to terminate the derivatization reaction; all of the values were found to be 1.0 which indicated that no loss of the derivatives occurred during the process. Results from GC-mass spectrometric tests of the individual derivatives to determine the number of PFP groups added to each compound during derivatization are also presented in Table II. Bzd, its analogs and DES each contained two PFP groups while all of the other compounds contained one PFP group.

Stability studies employing periodic assays of benzene solutions (5 ng/ml) of the PFP derivatives stored in sealed tubes at 5°C indicated that all of the compounds were essentially stable during a 10-month period.

The chemical properties of the test substances provided a convenient means of separating them into basic, phenolic and neutral fractions. The analytical scheme used for the extraction, separation and analysis of all 13 compounds in admixture in wastewater is illustrated in Figure 4. Salient elements of the procedure after separation of the sample into three fractions are extraction of the phenolic residues with benzene (pH 10.2), hydrolysis of 2-AAF in the neutral fraction and extraction of the product (2-AF) as the free amine, extraction of the free amines from the basic fraction (pH 12), conversion of all residues to the PFP derivatives and analysis via EC–GC by using three isothermal operating conditions.

Typical gas chromatograms of derivatized fractions from untreated wastewater along with 1 ppb amounts of 10 of the compounds as PFP derivatives (superimposed) are presented in Figure 5. Resolution was excellent for eight of the 10 derivatives. Although baseline resolution was not achieved for DiClBzd and DiMeBzd, no problem was experienced in using peak height measurement to quantify residues as low as 0.1 ppb.

Results from triplicate assays of wastewater unspiked and spiked with 0.10, 1.0 and 10 ppb of 10 of the compounds in admixture are presented in Table III. Recoveries were generally good at the 10 ppb level but tend to drop significantly at 1 ppb. At 0.10 ppb, recoveries of 2-NA, Bzd and DiMxBzd were 11% or less; however, the fact that the compounds were detectable indicated that the procedure is useful even at this low level.

TABLE II

Partition values (p-values) and GC-mass spectrometric verification of PFP-derivatives of carcinogens and analogs

Compounds	PFP groups per molecule	Solvent system				
		Hexane-Acetonitrile	Hexane-40% Acetonitrile (60% water)	Heptane-90%Ethanol (10% water)	Isooctane-90% Acetone (10% water)	Isooctane-80% Acetone (20% water)
1-NA	1	0.038	0.63	0.11	0.46	0.62
2-NA	1	0.052	0.77	0.12	0.45	0.70
2-ABP	1	0.090	0.91	0.27	0.42	0.70
3-ABP	1	0.050	0.88	0.13	0.48	0.76
4-ABP	1	0.044	0.85	0.13	0.44	0.74
2-AF	1	0.046	0.86	0.14	0.56	0.73
Bzd	2	0.016	0.45	0.020	0.48	0.66
DiClBzd	2	0.042	1.00	0.14	0.59	0.78
DiMeBzd	2	0.031	0.52	0.046	0.50	0.66
DiMxBzd	2	0.044	0.98	0.22	0.69	0.86
DES-cis	2	0.40	0.97	[a]	0.91	0.98
DES-trans	2	0.44	0.96	[a]	0.91	0.99
Estradiol	1	0.50	0.99	0.88	0.90	0.97

[a]Instability of derivative in solvent system precluded analysis.

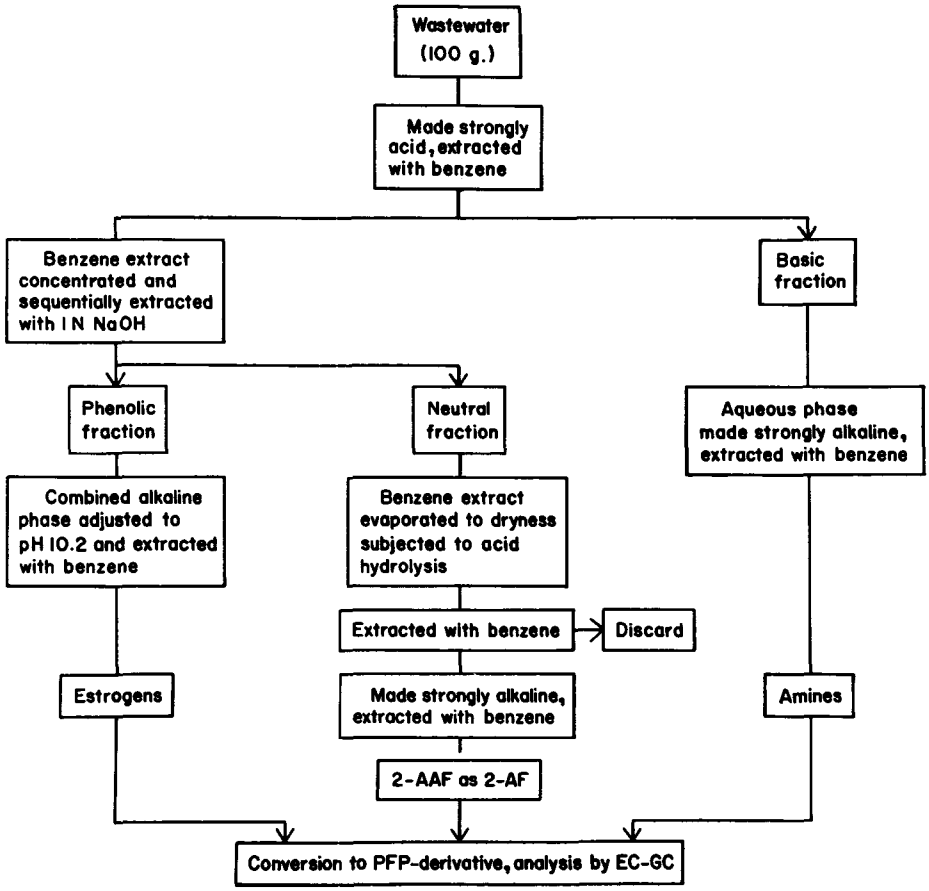

FIGURE 4 Scheme for extraction, separation and analysis of 13 carcinogens and related compounds in admixture in wastewater.

Urine is assayed by using the same scheme as for wastewater except that the sample is initially divided into two 50 ml portions. One of the portions is made strongly alkaline for extraction and analysis of the basic fraction (amines); the other is made strongly acid for extraction and analysis of the phenolic (estrogens) and neutral (2-AAF) fractions. Typical electron-capture gas chromatograms of the derivatized fractions from untreated urine along with 10 ppb amounts of 10 of the compounds as PFP derivatives (superimposed) are presented in Figure 6. Results from triplicate assays of urine unspiked and spiked with 2.0 and 20 ppb of the 10 compounds are presented in Table IV. Recoveries at the 20 ppb level

were good; however, a marked decrease in recovery was found at the 2 ppb level. Also, background interferences from untreated urine as high as 2 ppb were observed in the case of DiMxBzd. Nevertheless, the procedure does serve as an excellent means of monitoring for residues of the compounds at levels of 1 to 2 ppb in the urine of our personnel.

It should be noted that no cleanup steps are used in the procedure for wastewater and urine; nevertheless, some cleanup was achieved by our method as a result of the extraction, separation and derivatization steps.

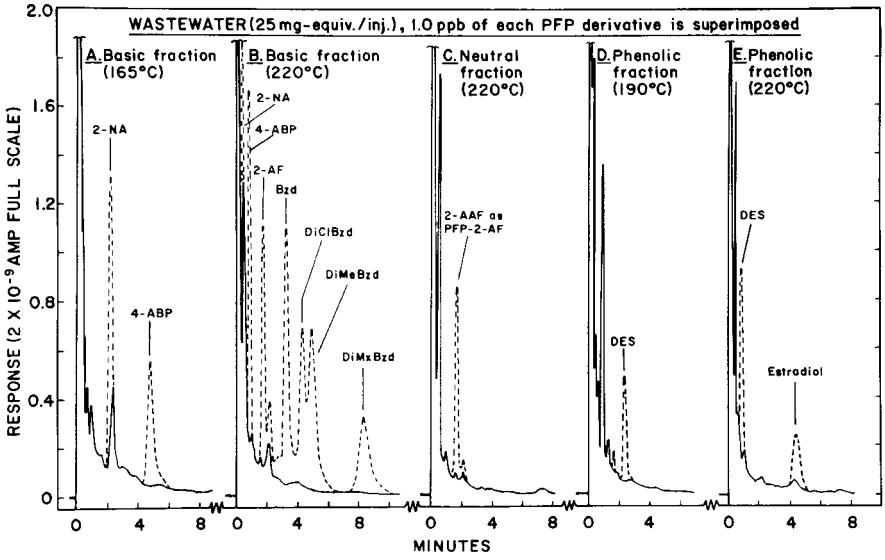

FIGURE 5 Electron-capture gas chromatograms of the three fractions from wastewater. Solid lines are 25-mg equivalents of untreated wastewater after derivatization; broken lines (superimposed) illustrate responses from 1-ppb amounts of the compounds assayed as PFP derivatives. All injections were in 5 μl of benzene.

Bowen[18] was also able to assay for certain aromatic amines at ppb levels in aqueous waste streams via flame ionization GC without sample treatment in many cases. Although extensive studies of clean-up procedures were conducted at our laboratory employing XAD-2 resin and Sephadex LH-20 prior to derivatization and alumina and silica gel before and after derivatization, no system could be devised that allowed good recoveries of all compounds at the levels tested. Therefore, it appears that further refinement of the present procedure to improve sensitivity and recovery should be directed towards the individual compound and substrate.

TABLE III

Analysis of wastewater unspiked and spiked with 0.10, 1.0 and 19 ppb of ten carcinogens and analogs in admixture

Compounds	Recovery ($\bar{X} \pm$SE)[a]						
	Unspiked	Spiked with 0.10 ppb		Spiked with 1.0 ppb		Spiked with 10 ppb	
	ppb	ppb	%	ppb	%	ppb	%
2-NA	0.123 ± 0.025	0.011 ± 0.004	11 ± 4	0.443 ± 0.039	44.3 ± 3.9	7.64 ± 0.60	76.4 ± 6.0
4-ABP	0.002 ± 0.001	0.040 ± 0.003	40 ± 3	0.657 ± 0.036	65.7 ± 3.6	8.52 ± 0.30	85.2 ± 3.0
2-AF	0.002 ± 0.000	0.022 ± 0.000	22 ± 0	0.555 ± 0.048	55.5 ± 4.8	8.17 ± 0.10	81.7 ± 1.0
2-AAF	0.002 ± 0.001	0.056 ± 0.006	56 ± 6	0.650 ± 0.042	65.0 ± 4.2	7.52 ± 0.06	75.2 ± 0.6
Bzd	0.002 ± 0.001	0.004 ± 0.003	4 ± 3	0.208 ± 0.009	20.8 ± 0.9	7.05 ± 0.33	70.5 ± 3.3
DiClBzd	0.003 ± 0.001	0.055 ± 0.004	55 ± 4	0.580 ± 0.064	58.0 ± 6.4	7.47 ± 0.08	74.7 ± 0.8
DiMeBzd	0.005 ± 0.002	0.028 ± 0.001	28 ± 1	0.583 ± 0.037	58.3 ± 3.7	8.39 ± 0.14	83.9 ± 1.4
DiMxBzd	0.003 ± 0.001	0.009 ± 0.001	9 ± 1	0.465 ± 0.048	46.5 ± 4.8	7.25 ± 0.10	72.5 ± 1.0
DES	0.016 ± 0.003	0.041 ± 0.005	41 ± 5	0.441 ± 0.000	44.1 ± 0.6	4.99 ± 0.24	49.9 ± 2.4
Estradiol	0.018 ± 0.006	0.078 ± 0.001	79 ± 1	0.839 ± 0.018	83.9 ± 1.8	8.91 ± 0.30	89.1 ± 3.0

[a]Mean and standard error from triplicate assays; spiked samples are corrected for background of unspiked samples.

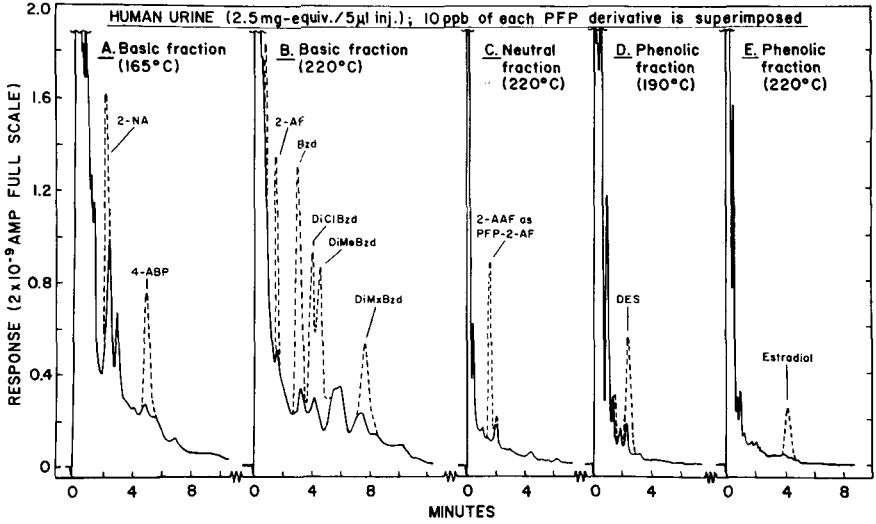

FIGURE 6 Electron-capture gas chromatograms of the three fractions from human urine. Solid lines are 2.5-mg equivalents of untreated urine after derivatization; broken lines (superimposed) illustrate responses of 10-ppb amounts of the compounds analyzed as PFP derivatives. All injections were in 5 μl of benzene.

TABLE IV

Analysis of human urine unspiked and spiked with 2.0 and 20 ppb of ten carcinogens and analogs in admixture

Compounds	Recovered ($\bar{X} \pm$ SE)[a]				
	Unspiked	Spiked with 2.0 ppb		Spiked with 20 ppb	
	ppb	ppb	%	ppb	%
2-NA	0.788 ± 0.038	0.637 ± 0.172	31.9 ± 8.6	13.3 ± 0.7	66.6 ± 3.3
4-ABP	1.06 ± 0.08	1.03 ± 0.08	51.3 ± 4.0	17.3 ± 0.2	86.5 ± 1.1
2-AF	0.515 ± 0.031	0.480 ± 0.040	24.1 ± 2.0	14.9 ± 0.2	74.2 ± 1.0
2-AAF	0.023 ± 0.005	1.16 ± 0.12	57.7 ± 5.9	15.9 ± 0.8	79.2 ± 3.8
Bzd	0.213 ± 0.023	0.907 ± 0.023	45.1 ± 1.2	14.1 ± 0.2	70.4 ± 0.9
DiClBzd	0.830 ± 0.038	1.41 ± 0.06	70.7 ± 2.8	19.9 ± 0.3	99.3 ± 1.4
DiMeBzd	0.215 ± 0.037	0.973 ± 0.012	48.6 ± 0.4	17.3 ± 0.3	86.4 ± 1.3
DiMxBzd	2.13 ± 0.00	0.953 ± 0.021	47.5 ± 1.0	18.6 ± 0.3	92.6 ± 1.6
DES	0.543 ± 0.014	0.876 ± 0.006	43.8 ± 0.2	11.1 ± 0.7	55.3 ± 3.5
Estradiol	0.730 ± 0.014	1.22 ± 0.02	60.8 ± 0.8	17.6 ± 0.1	88.0 ± 0.5

[a]Mean and standard error from triplicate assays; spiked samples are corrected for background of unspiked samples.

This method may easily be adapted to the monitoring of work areas (animal cages, floors, apparatus, air filters, etc.) suspected of being contaminated with the test chemicals. The procedure currently used at our laboratory[7] for sampling surfaces employs a kit consisting of a cotton applicator and a 5-ml culture tube containing a known volume of a suitable solvent. The applicator is saturated with the solvent and used to swab a specific area then the applicator is vigorously stirred in the solvent after each of several swabbings of the same area. In a similar manner fiberglass filters used to trap particulate matter from the air may be cut into small pieces and extracted with a suitable solvent. These extracts may then be screened for contaminants by slightly modifying the procedure described.

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