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DETERMINATION OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE RESIDUES

TASK 12
FINAL REPORT
July 29, 1980

EPA Prime Contract No. 68-01-5915
MRI Project No. 4901-A12

Prepared for

U.S. Environmental Protection Agency
Office of Pesticides and Toxic Substances
Field Studies Branch
401 M Street, S.W.
Washington, D.C. 20460

Attn: Dr. Frederick Kutz, Project Officer
Ms. Sandra Strassman-Sundy, Task Manager

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DETERMINATION OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE RESIDUES

by

Duane Lakings
Wilma Subra
John Going

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PREFACE

This final report presents the results obtained on MRI Project No. 4901-A, Task 12 "Determination of Pentachlorophenol and Hexachlorobenzene Residues," for the Environmental Protection Agency (EPA Prime Contract No. 68-01-5915). This work was conducted through a subcontract with Gulf South Research Institute, Ms. Wilma Subra, Task Manager. This report was prepared by Dr. Duane B. Lakings, MRI Task Leader, and Ms. Wilma Subra.

MIDWEST RESEARCH INSTITUTE

John E. Going
Program Manager

Approved:

James L. Spigarelli, Director
Analytical Chemistry Department

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SECTION 1

INTRODUCTION

Between 1968 and 1971, the U.S. Army Lexington Blue Grass Depot received a large supply of wooden munition crates possibly treated with pentachlorophenol (PCP). A number of these crates, given or sold to residents of Madison County, Kentucky, were used in housing construction, paneling, etc. Thus, some area residents may have been exposed to high levels of PCP.

The Center for Disease Control (CDC), in response to requests by Congress and local medical authorities, initiated plans to investigate potential health problems associated with PCP exposure. A phase of the study was to determine and compare serum and urinary (unconjugated) PCP and serum hexachlorobenzene (HCB) levels in individuals exposed to PCP-treated ammunition crate wood in their home and in unexposed controls and to assess the levels of PCP and HCB in wood, air, and food product samples from the possible PCP-exposed residences and controls. The Environmental Protection Agency (EPA) through a contract with Midwest Research Institute (MRI) and a subcontract with Gulf South Research Institute (GSRI) assisted in the analysis of samples selected and collected by CDC.

The major goals of this study were:

1. Define and collect samples from a test group of approximately 30 individuals from 15 families having PCP-treated ammunition crate wood in their homes.

2. Identify and collect samples from a control group with no PCP-treated ammunition crate wood in the home. The control group was to be matched with the test group in number, approximate age, sex, and race. The residences of the control group were to be adjacent or near the homes of the test group.

3. Samples to be collected included serum and urine from each test and control individual and air, food products, and wood from the residences.

4. Analyze the collected samples as follows: serum - PCP and HCB; urine - unconjugated PCP; air samples - PCP and HCB; food products - PCP and HCB; and wood - PCP, HCB, and dioxins. The wood samples were analyzed at the EPA laboratory in Beltsville, Maryland.

The remainder of this report presents the summary, conclusions, recommendations, experimental protocol, results and discussion, and appendix.

SECTION 2

SUMMARY

A number of individuals who have been exposed to pentachlorophenol-treated ammunition crate wood obtained from the Blue Grass Army Depot, Lexington, Kentucky were sampled for serum and unconjugated urinary PCP levels and wood, air, and food product samples from selected residences were taken for PCP level determination. A control individual matched to each test case in age, sex, and residence location was also sampled. In addition, control residences were sampled to obtain background levels of PCP in wood, air, and food products. Also, two families who live in log homes treated with "penta" requested to be a part of the study and were designated as special cases. A total of 45 residences were sampled and resulted in 45 test case, 7 special case, and 45 control individual serum samples, 51 test case, 7 special case, and 47 control individual urine specimens, 18 test case, 2 special case, and 20 control individual residence air samples, 9 test case and 7 control individual residence food product samples, and 28 test case, 1 special case, and 21 control individual residence wood samples.

The serum, urine, air, and food product samples were analyzed for PCP at Gulf South Research Institute. The serum, air, and food products were also analyzed for HCB, a compound whose major metabolite is PCP. Urinary PCP levels were determined for the unconjugated compound. The wood samples were analyzed at the EPA Laboratory in Beltsville, Maryland.

The data for serum and unconjugated urinary PCP levels indicated that the test cases and control individuals had similar levels. Statistical evaluation of these data showed no increase in the PCP levels of the test cases and no correlation between the serum and unconjugated urinary PCP level.

However, the special cases, two familes who reside in log homes and do not have the ammunition crate wood present, had elevated serum and unconjugated urinary PCP levels in relation to the control individuals. The highest serum PCP levels were in the three special case children (ages 2, 4, and 9 years) and were 1,750, 1,680, and 910 ppb, respectively (compared to the normal control PCP serum level average of 24 ppb). The urinary excretion of unconjugated PCP in these three special cases was more than 20 times the average of the normal controls.

No HCB was detected in the serum of any test or special case or control individual.

The air samples obtained from test case and control residences had non-detectable (< 0.005 ng/l) levels of PCP and HCB. Air samples from one log home (special case residence) gave PCP levels of 0.2 and 0.38 ng/l. No air sample was obtained from the other log home.

Food products contained PCP levels ranging from not detectable (< 0.4 ppb) to 11.7 ppb. No correlation was made between the food products obtained from test case and control residences due to the limited number of samples analyzed. HCB was not detected in any food product analyzed. No food products were obtained from the special case residences.

The wood samples collected were analyzed for PCP and octachlorodibenzodioxin (OCDD) at the EPA laboratory in Beltsville, Maryland. Seven test case wood samples had detectable PCP (range 8.6 to 737 ppm) and eleven control wood samples had PCP (range 4.3 to 1,452 ppm). A wood sample from one special case log home also had PCP (836 ppm). No wood was obtained from the other special case log home. Only one wood sample had detectable OCDD and that level was at the detection limit of the analytical method (1 ppm). Data on the HCB levels in these wood samples will be reported later.

SECTION 3

CONCLUSIONS

The serum and unconjugated urinary levels of PCP in test cases and control individuals indicate that the residences with ammunition crate wood did not increase the PCP levels in the test case individuals. However, the two special case families, residing in log homes and not possessing ammunition crate wood, did have high serum and unconjugated urinary PCP levels. This increase was especially noted in the three children of the special case families where the serum PCP levels were 1,000 ppb or above. In an EPA report,[1] serum levels of PCP above 1,000 ppb were noted to cause toxic effects. Thus, toxicity from PCP is possible in the three young special case children.

The urine from the test cases, special cases, and control individuals was analyzed for unconjugated PCP, and no correlation between serum PCP and unconjugated urinary PCP levels existed for the test cases and control individuals. However, when selected urines were hydrolyzed, a 2 to 7 times increase in the PCP level was observed. This increase in PCP level for hydrolyzed urine does not affect the overall results since the serum PCP levels of the test cases and control individuals showed similar levels. The lack of correlation between the serum and unconjugated urinary PCP levels indicates that unconjugated urinary analysis cannot be employed alone to assess possible PCP exposure. However, the total urinary PCP excretion (conjugated and unconjugated) over a defined time period (24 hr collection) or collection period (early morning excretion) may provide correlation to serum PCP levels.

The results from the air sample analyses of PCP and HCB showed that the test case individuals were not being exposed to PCP in the air. The only air sample with detectable PCP was from a log home (special case), again indicating that the special case individuals were being subjected to PCP exposure.

The limited number of food products analyzed for PCP and HCB residues prevented correlation between the test case and control residences.

Also, no correlation was possible from the wood samples taken from test case and control residences and analyzed for PCP and OCDD. The one wood sample taken from a log home (special case) had a high level of PCP. Since the treatment of the log homes with penta (PCP) may have included the total dwelling, the residents of the log home may be exposed to much higher PCP levels than the test case individuals where only a limited amount of ammunition crate wood was present.

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[1] EPA Report No. 540-9-77-013, Recognition and Management of Pesticide Poisoning, D. P. Morgan, 2nd edition, page 25, printed by EPA.

SECTION 4

RECOMMENDATIONS

Additional studies on the PCP levels from test cases and control individuals are not required since similar PCP levels were found between these two groups indicating that the test case individuals are not carrying a body burden of PCP. However, the results from this study indicate that the special cases who reside in log homes are being subjected to high PCP exposure and additional studies on this group are recommended. These studies should be designed to define the health effects of chronic exposure to PCP and to determine the levels at which toxic effects to PCP become apparent. These data can be utilized to notify current log home residents of potentially adverse health effects to PCP exposure especially with regard to children and pregnant women.

Also, in future studies to assess possible exposure to PCP, or other toxic substances, the sampling protocol should be designed for the collection of a defined urine specimen, either a 24-hr collection or an early morning specimen, so that the urinary excretion can be better defined. The collected urine specimens should be analyzed for the unconjugated compounds and for possible metabolites such as the glucuronide or sulfate adduct so that the total urinary excretion (unconjugated and conjugated) of the compound can be determined. Prior to analyzing hydrolyzed urine specimens, studies to define the optimal hydrolysis conditions and the stability of the compound under these conditions should be conducted. By analyzing a defined urine specimen for total extretion of a compound and comparing the results to serum levels of the compound, a correlation between urinary excretion and serum levels may be obtained. This information may then be employed in other studies on the same compound using only defined urine specimens, a non-invasive technique which is usually more acceptable to the subjects being sampled, to determine the possible exposure and the exposure levels to the compound.

SECTION 5

STUDY PROTOCOL

The collection of human blood and urine specimens and air, wood, and food products samples was performed from January 28, 1980 through February 7, 1980. During that time period, the human specimens and other samples were collected from 45 residences in the Madison County area. Table 1 presents a list of the residences and Figure 1 indicates their location. From the 45 residences, blood and urine specimens were collected from 97 and 105 individuals, respectively. Blood specimens were not obtained from eight young children due to their age. A list of the individuals by age, sex and race, their Madison County Health Department code number, and the date of collection of the serum and urine samples are presented in Table 2. Air samples were collected from 38 residences, listed in Table 3, in the Madison County area. Food products were collected from five residences in the area and consisted of milk, eggs, pork, and butter. The residences and products collected from each are presented in Table 4.

Wood samples were collected from 42 residences in the Madison County area, and the samples sent to the U.S. EPA laboratory in Beltsville, Maryland for analysis. Table 5 presents a list of residences, their Madison County Health Department code numbers, the date collected, and a description of the wood sampled. The study area was revisited between February 11 and 14, 1980 to record the stenciled information on all ammunition boxes from the Blue Grass Depot. All families known to have arsenal wood in their home or out-buildings, i.e., storage sheds, barns, chicken coops, etc., were contacted, and appointments were made to visit those residences with stenciling apparent on the wood. An appointment was not scheduled for the residences with painted wood or those that had disposed of the wood after the initial visit of the collection team. All visible stenciling was recorded and 35 mm photographic slides taken of the accessible boards. The stenciling data recorded from the residences visited are presented in the Appendix. Stenciling blurred, worn off, or covered over by other boards are represented in the Appendix presentation as a question mark. The 35 mm slides are on file at MRI.

SAMPLE COLLECTION

General

The screening of test case volunteers and the selection of control volunteers were performed by the Center for Disease Control in cooperation with the Madison County Health Department and Kentucky State Department of Human Resources. The initial visit to each test and control home was made by Madison County Health Department personnel and GSRI personnel. Collection of human blood and urine specimens was performed by the health department personnel while wood, food product, and air sampling was performed by GSRI personnel.

TABLE 1. LISTING OF RESIDENCES SAMPLED FOR PENTACHLOROPHENOL IN THE MADISON COUNTY AREA, KENTUCKY

Residence number	Residence type	Residence number	Residence type
1	test case	23	control
2	control	24	control
3	test case	25	test case
4	control	26	control
5	test case	27	control
6	test case	28	control
7	control	29	test case
8	test case	30	control
9	special case	31	test case
10	test case	32	test case
11	test case	33	control
12	test case	34	control
13	control	35	test case
14	test case	36	test case
15	control	37	test case
16	control	38	test case
17	test case	39*	control*
18	test case	40	control
19	control	41	control
20	special case	42	test case
21	control	43	control
22	control	44	control
		45	control

Note: Numbers correspond to numbered locations on Madison County map (Figure 1).

* Control changed to test case after ammunition crate wood located in the residence.

Special cases are log home residences.

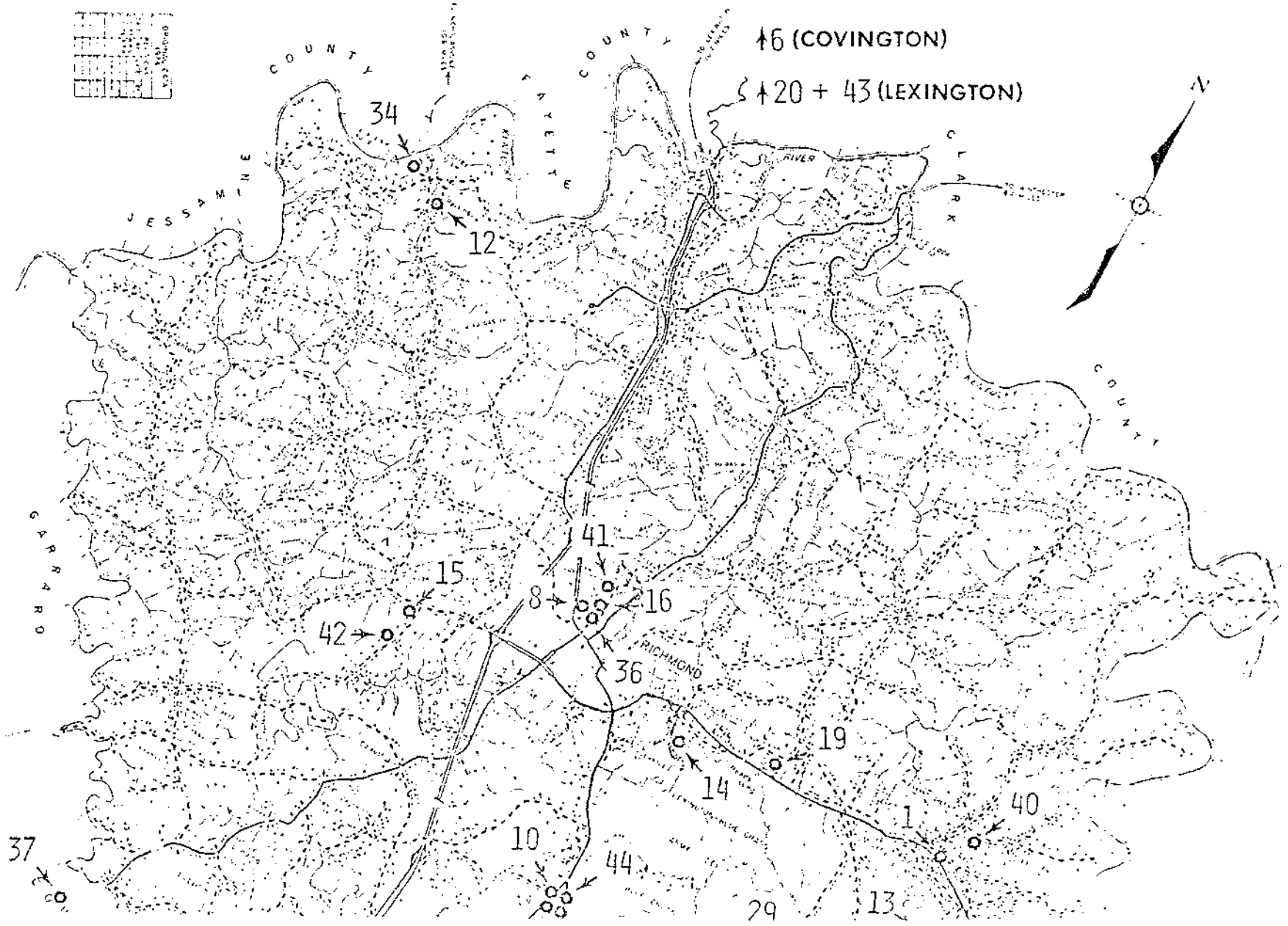

Figure 1. Location of residences sampled in Madison County, Kentucky.

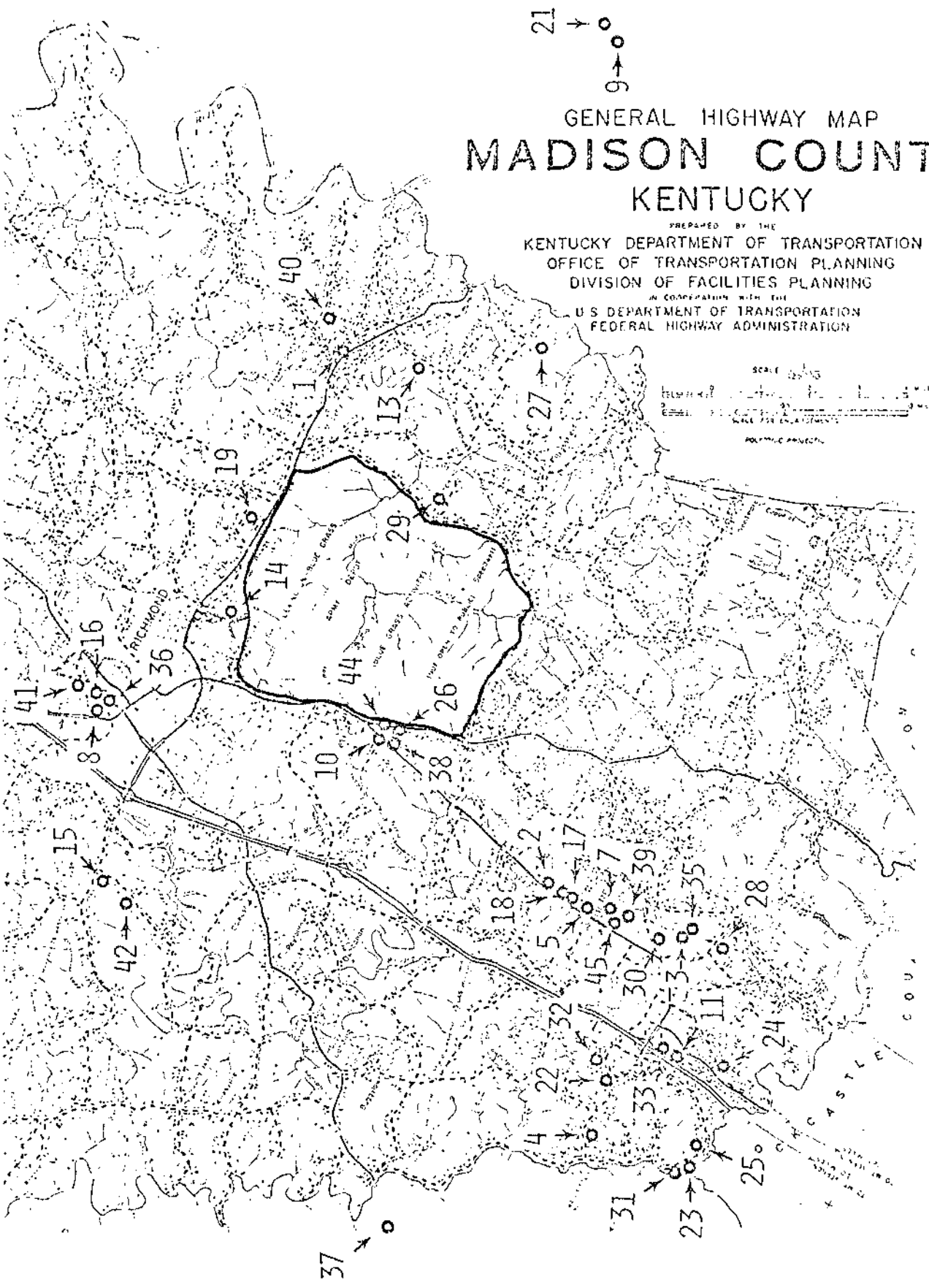

Figure 1 (concluded) (Blue Grass Army Depot outlined as shown)

TABLE 2. INDIVIDUALS OF MADISON COUNTY, KENTUCKY SAMPLED FOR PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN THEIR BLOOD AND URINE

Madison County Health Department number	Identification			Collection date	
	age	sex	race	serum	urine
Test cases					
100A	32	female	white	1/28/80	1/28/80
101A	62	female	white	1/28/80	1/29/80
102A	69	male	white	1/28/80	1/29/80
103A	11	female	white	1/28/80	1/28/80
104A	29	female	white	1/28/80	1/28/80
105A	47	male	white	1/29/80	1/30/80
106A	25	female	unknown	1/29/80	1/30/80
107A	11	male	white	a	1/30/80
108A	13	male	white	1/29/80	1/29/80
109A	49	female	white	1/29/80	1/30/80
110A	65	female	white	1/29/80	1/30/80
111A	20	female	white	1/29/80	2/1/80
112A	50	female	white	1/29/80	2/1/80
113A	56	male	white	1/29/80	2/1/80
114A	47	female	white	1/29/80	1/30/80
115A	14	female	white	1/29/80	1/30/80
116A	10	male	white	a	1/30/80
117A	05	female	white	a	1/30/80
118A	11	male	white	1/29/80	1/30/80
119A	26	male	white	1/30/80	1/31/80
120A	27	female	white	1/30/80	1/31/80
121A	33	female	white	2/1/80	2/1/80
122A	15	female	white	2/1/80	2/1/80
123A	12	female	white	2/1/80	2/1/80
124A	02	male	white	a	2/5/80
125A	55	female	white	2/5/80	2/2/80
200A	36	female	white	1/28/80	1/29/80
201A	14	male	white	1/28/80	1/29/80
202A	10	female	white	1/28/80	1/29/80
203A	57	female	white	1/29/80	1/30/80
204A	32	female	white	1/29/80	2/4/80
205A	25	female	white	1/29/80	2/5/80
213A	33	female	white	1/31/80	1/30/80
214A	08	female	white	a	1/31/80
215A	05	female	white	1/31/80	1/31/80
216A	56	male	white	1/31/80	2/1/80
217A	45	female	white	1/31/80	2/1/80
218A	15	female	white	1/31/80	2/1/80
219A	21	male	white	1/31/80	2/1/80
220A	17	male	white	1/31/80	2/1/80
221A	51	male	white	2/1/80	2/1/80

(continued)

TABLE 2 (continued)

Madison County Health Department number	Identification			Collection date	
	age	sex	race	serum	urine
202B	09	male	white	2/1/80	2/1/80
203B	49	female	white	1/30/80	1/31/80
204B	32	female	black	2/3/80	2/3/80
205B[c]	32	female	white	2/4/80	2/4/80
206B[c]	30	male	white	2/6/80	2/6/80
207B[c]	33	female	white	2/5/80	2/6/80
210B[c]	33	female	white	2/5/80	2/5/80
213B	28	female	white	2/5/80	2/6/80
214B	04	male	white	a	2/5/80
215B	06	male	white	2/5/80	2/6/80
216B	56	male	white	2/5/80	2/5/80
217B	55	female	white	2/5/80	2/5/80
218B	16	male	white	2/4/80	2/2/80
219B	32	male	black	2/3/80	2/3/80
220B	17	male	white	2/2/80	2/5/80
221B	65	male	white	2/5/80	2/5/80
222B	59	female	white	2/5/80	2/5/80
223B[b]	23	male	white	2/5/80	2/5/80
224B[b]	21	female	white	2/5/80	2/5/80
225B	54	male	white	2/2/80	2/3/80
226B	46	female	white	2/2/80	2/3/80
227B	29	female	white	2/5/80	2/5/80
228B	29	male	white	2/5/80	2/6/80

a Blood specimens not obtained from these young children.

b Control individual changed to test cases after ammunition crate wood located in their residences.

c Control individuals for special cases.

Note: None of the individuals were of hispanic origin.

TABLE 3. RESIDENCES OF MADISON COUNTY, KENTUCKY WHOSE AIR WAS SAMPLED FOR PENTACHLOROPHENOL AND HEXACHLOROBENZENE

Residence number and type	Madison County Health Department number	Collection Date	Description
1 - test case	I004	1/31/80	chicken house
2 - control	I019	2/1/80	dining room
3 - test case	I022	2/1/80	second floor
5 - test case	H006	1/29/80	basement
	H007	1/29/80	upstairs
7 - control	P0007	2/5/80	study
8 - test case	I002	1/30/80	kitchen
10 - test case	H010	1/30/80	kitchen cabinet under sink
11 - test case	H008	1/29/80	--[a]
12 - test case	I025	2/2/80	attic
13 - control	I050	2/5/80	living room
14 - test case	H001	1/29/80	upstairs bedroom
15 - control	I045	2/5/80	living room
16 - control	I003	1/30/80	--[a]
17 - test case	P0001	2/4/80	--[a]
18 - test case	P0002	2/5/80	--[a]
19 - control	I005	1/31/80	bedroom
20 - test case	I007	1/31/80	first floor
	I008	1/31/80	second floor
22 - control	I021	2/1/80	dining room
23 - control	I042	2/5/80	bedroom
24 - control	I040	2/5/80	study
25 - test case	I039	2/1/80	collected by resident
26 - control	I014	1/31/80	trailer
27 - control	I027	2/2/80	bedroom
28 - control	I033	2/2/80	sewing room
30 - control	I023	2/1/80	dining room
31 - test case	I031	2/2/80	library
32 - test case	H009	1/29/80	kitchen
33 - control	I016	2/1/80	den
34 - control	I035	2/3/80	kitchen
35 - test case	I015	1/31/80	closet in middle bedroom
36 - test case	I001	1/29/80	basement
39 - control[b]	I037	2/4/80	living room
40 - control	I047	2/5/80	hall pantry
41 - control	P0009	2/5/80	utility room
42 - test case	I006	1/31/80	stairway hall on second floor
43 - control	I052	2/6/80	den
44 - control	I029	2/2/80	living room
45 - control	P0005	2/4/80	--[a]

a No description of where air sample was taken.

b Changed from control residence to test residence after ammunition crate wood located in residence.

TABLE 4. FOOD PRODUCTS COLLECTED FROM VARIOUS RESIDENCES IN THE MADISON COUNTY, KENTUCKY AREA

Residence number and type	Madison County Health Department number	Collection Date	Description
1 - test case[a]	G022	1/31/80	egg white
	G021	1/31/80	egg yolk
	G023	1/31/80	egg shell
29 - test case[b]	G005	1/28/80	salted pork[d]
	G006	1/28/80	milk (raw)
	G007	1/28/80	egg yolk
	G008	1/28/80	egg white
13 - control[c]	I055	2/6/80	milk (pasteurized)
	I059	2/6/80	egg white
	I060	2/6/80	egg yolk
	I061	2/6/80	egg shell
32 - test case	G014	1/29/80	milk
	G015	1/29/80	butter[d]
40 - control[c]	I056	2/6/80	egg white
	I057	2/6/80	egg yolk
	I058	2/6/80	egg shell

a Chicken roost an ammunition crate.

b Chicken roost made from ammunition crate wood.

c Eggs store purchased.

d Items selected for sampling by sampling crew; no control items obtained.

TABLE 5. RESIDENCES IN THE MADISON COUNTY, KENTUCKY AREA FROM WHICH WOOD SAMPLES WERE COLLECTED

Residence number and type	Madison County Health Department number	Collection Date	Description
1 - test case	G024	1/31/80	ammunition crate chicken nest
2 - control	I018	2/1/80	old board in basement
3 - test case	I011	2/1/80	closet floor
4 - control	I054	2/6/80	scrap board
5 - test case	G001	1/28/80	beam in basement
7 - control	P0008	2/5/80	shelf in garage
8 - test case	G018	1/30/80	inside kitchen cabinet
10 - test case	G027	1/31/80	inside kitchen cabinet under sink
11 - test case	G016	1/30/80	outbuilding
11 - test case	G017	1/30/80	back porch
12 - test case	I026	2/2/80	attic
13 - control	I051	2/5/80	studs in new room
14 - test case	I049	2/5/80	toy box
15 - control	I046	2/5/80	studs in living room
16 - control	G019	1/30/80	toy train box in bedroom
17 - test case	P0004	2/5/80	bookshelf in bedroom
18 - test case	P0003	2/4/80	bookshelf in study
19 - control	G025	1/31/80	shelf board in closet
20 - special case	I009	1/30/80	second floor bath
22 - control	I020	2/1/80	outbuilding
23 - control	I043	2/5/80	firewood
23 - control	I044	2/5/80	ammunition box
24 - control	I041	2/5/80	firewood
25 - test case	I012	2/1/80	outside wood
25 - test case	I013	2/1/80	inside wood
26 - control	I010	1/31/80	dog house
27 - test case	I028	2/2/80	basement
28 - test case	I034	2/2/80	basement
29 - test case	G002	1/28/80	hog house
29 - test case	G003	1/28/80	cow and chicken house
29 - test case	G004	1/28/80	meat house
30 - control	I024	2/1/80	old board in basement
31 - test case	I032	2/2/80	subfloor
31 - test case	228A	2/2/80	deck floor
32 - test case	G011	1/29/80	ammunition box
32 - test case	G012	1/29/80	manger
32 - test case	G013	1/29/80	basement
33 - control	I017	2/1/80	workbench in basement
34 - control	I036	2/2/80	ammunition box
35 - test case	G026	1/31/80	closet

(continued)

TABLE 5 (continued)

Residence number and type	Madison County Health Department number	Date	Description
36 - test case	G010	1/29/80	subfloor
37 - test case	G009	1/28/80	ammunition crate shelf in kitchen
38 - test case	G020	1/30/80	pantry
39 - control[a]	I038	2/4/80	ammunition box
40 - control	I048	2/5/80	firewood
41 - control	P0010	2/5/80	shelf
42 - test case	G028	1/31/80	ammunition box
43 - control	I053	2/6/80	outside front door
44 - control	I030	2/2/80	outside wood
45 - control	P0006	2/4/80	board in garage

a Changed from control residence to test case residence after ammunition crate wood located in residence.

Blood

Blood specimens were drawn directly into red top Vacutainer collection tubes. The blood was allowed to clot and the serum separated by centrifugation. The serum was transferred into 15 ml pre-washed (soaked in sodium hydroxide followed by deionized water and acetone rinses) septum vials with pre-washed Pasture pipettes. The vials were sealed with Teflon lined septa and frozen.

Urine

Pre-washed wide mouth 60 ml amber bottles with Teflon lined lids were provided for the collection of urine specimens. The urine was transferred from the amber bottles into two pre-washed 15 ml septum vials with pre-washed Pasture pipettes. The vials were sealed with Teflon lined septa and frozen.

Air

Sampling of the air in the designated homes was performed using a Brailford air pump powered by three 12-volt and two 6-volt batteries. Air was drawn through two 500 mg portions of Tenax resin separated by glass wool and contained in 15 cm x 1.2 cm ID glass tubes for 6 to 8 hr at a flow rate of approximately 1 liter per minute. Following sampling, the glass tubes were wrapped in heavy duty aluminum foil and sealed in glass screw cap culture tubes with Teflon lined lids.

Food Products

Samples of food products were collected in 60 ml pre-washed amber wide-mouth bottles or heavy duty aluminum foil. Samples of meat and butter were sealed in the amber bottles with a Teflon lined lid and frozen. Samples of milk (raw or pasteurized) were transferred to pre-washed 15 ml septum vials using pre-washed Pasture pipettes, sealed with Teflon lined septa and frozen. Egg samples were cracked and the whites and yolks separated and collected in pre-washed amber 60 ml wide-mouth bottles. The egg shells were also placed in amber bottles. The bottles were sealed with Teflon lined lids and frozen.

Wood

Wood samples (10 to 20 g) were collected in pre-washed 60 ml wide-mouth amber bottles. The bottles were sealed with Teflon lined lids and the entire bottle wrapped in heavy duty aluminum foil and frozen. At each residence having arsenal wood, the data stenciled on the wood was recorded and 35 mm slides taken of accessible boards.

SAMPLE PREPARATION PROCEDURES

The procedures employed to prepare the samples for GC analysis of PCP and HCB levels are delineated in the following paragraphs. The serum and unconjugated urinary levels of PCP and the serum level of HCB were determined by the method outlined in "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Health Effects Research

Laboratory, Research Triangle Park, North Carolina, revised June 1977. The assay for hydrolyzed urine (unconjugated and conjugated PCP) was conducted using the method of Edgerton and Moseman.[2]

Serum and Urine

Serum and unconjugated urinary levels of PCP and serum HCB levels were determined by GC after extraction of the compounds from the sample matrix and acetylation of the phenolic group of PCP. The sample preparation procedure is as follows:

a. Quantitatively pipette 2.0 ml of serum or urine in a 15 ml culture tube with a Teflon-lined screw cap.

b. Add two drops of concentrated sulfuric acid to the sample and mix on a Vortex mixer for 30 sec.

c. Add 6.0 ml "nanograde" hexane (Mallinckrodt) to the sample and cap the vial.

d. Extract the mixture for 2 hr on a mechanical rotator at approximately 30 rpm.

e. Centrifuge the sample at 2,000 rpm for 10 min to break the emulsion.

f. Pipette 3.0 ml of the hexane layer to a 15 ml septum vial.

g. Add 2.0 ml 0.1 M sodium borate and 50 µl acetylation reagent (2 ml pyridine plus 0.8 ml acetic anhydride, prepared fresh daily).

h. Shake for 1 min and allow the phases to separate.

i. Transfer 1 ml of the hexane to a 1 ml autosampler vial for GC analysis.

Hydrolysis of Urine

The sample preparation procedure described above for urinary PCP levels measured the unconjugated or free levels of PC. Normally, compounds with polar groups like the phenolic group of PCP are not excreted in the urine as the parent compound but are first metabolized in the liver. For PCP, the metabolism would most likely consist of conjugation to form the glucuronide or sulfate adduct. The conjugated PCP would be efficiently removed from the blood by the kidney; thus, very little of the metabolized PCP would be present in the blood. Due to the relative nonpolarity of PCP and the ability of the kidney to readsorb (through passive diffusion) nonpolar compounds back into the blood, very little unconjugated PCP would be expected to be present in the urine.

[2] Edgerton, T. R., and R. F. Moseman, "Determination of Pentachlorophenol in Urine: The Importance of Hydrolysis." J. Agric. Food Chem., Vol. 27, No. 1, 197-199 (1979).

Also, after the conjugated PCP is in the urine (bladder) or after excretion, a limited amount of hydrolysis may occur which would be detected as unconjugated PCP in the urine. The kinetics of hydrolysis would vary between humans, thus providing widely different levels of unconjugated compound in the urine. To obtain the true rate of urinary excretion of a compound, the urine must be analyzed for both the conjugated and unconjugated compound. If the conjugate is the glucuronide or sulfate adduct (which are the most likely adducts for PCP), hydrolysis to the free compound can be accomplished by enzymatic (aryl sulfatase, β-glucuronidase) or acid hydrolysis. The analysis of a hydrolyzed urine provides a measure of the conjugated and unconjugated level of a compound.

Analysis of the human urine specimens for unconjugated PCP was requested. A short study was conducted to determine the total (conjugated and unconjugated) urinary PCP levels. Selected urine specimens were first subjected to acid hydrolysis followed by hexane extraction. The sample preparation procedure was as follows:

a. Quantitatively pipette 5.0 ml of urine into a culture tube with a Teflon-lined screw cap.

b. Add 1.25 ml concentrated hydrochloric acid and seal the vial.

c. Heat the sample at 100°C for 1 hr with frequent shaking.

d. Cool to room temperature.

e. Adjust the pH to 11 to 12 with 1 N sodium hydroxide.

f. Add 5.0 ml "nanograde" hexane.

g. Seal the tube and extract for 1 hr on a mechanical rotator.

h. Centrifuge at 2,000 rpm for 10 min.

i. Draw off the hexane layer and discard.

j. Adjust to pH 1 with concentrated sulfuric acid.

k. Add 2 ml "nanograde" hexane, seal the tube, and extract for 1 hr on a mechanical rotator.

l. Centrifuge at 2,000 rpm for 10 min.

m. Pipette 1.0 ml of the hexane layer to a 15 ml septum vial.

n. Derivatize the sample using the same procedure outlined for serum and urine sample.

Air Samples

The front and back section of a Tenax tube were emptied into a 15 ml centrifuge tube and 5 ml of nanograde hexane added volumetrically. The tube was sealed and extracted on a mechanical rotator for 2 hr. The contents of the tube were centrifuged for 10 min and 1.5 ml of the hexane layer transferred to a 15 ml septum vial for derivatization. The derivatization procedure was the same as described for serum and urine samples.

Food Products

Two milliliters or 2 g (ground in a mortar and pestle) of food product were transferred to a 15 ml glass stoppered centrifuge tube. Two drops of concentrated sulfuric acid were added to the tube and the contents mixed for 30 sec on a Vortex mixer. Six milliliters of nanograde hexane was added volumetrically to the sample, the tube sealed, and extracted and derivatized by the method presented for serum and urine.

Wood

The wood samples were shipped on dry ice to the U.S. EPA laboratory in Beltsville, Maryland for analysis of PCP, HCB, and octachlorodibenzodioxin (OCDD).

ANALYTICAL METHODS

The levels of PCP and HCB in the various samples collected from individuals and their residences were determined by gas chromatography (GC) with electron capture (EC) detection. Samples which gave a positive GC response for PCP or HCB were subjected to gas chromatography-mass spectrometry (GC-MS) confirmation.

Gas Chromatography

The GC conditions employed for the quantitative determination of PCP and HCB in the various collected samples were as follows:

a. Instrument: Hewlett Packard 5840
b. Column: 1.5% OV-17/1.95% QF-1 on 100/120 mesh Supelcoport packed in a 6 ft glass column, 2 mm ID.
c. Injector Temperature: 250°C
d. Column Temperature: 165°C
e. Detector Temperature: 325°C
f. Carrier Gas: Helium
g. Flow Rate: 30 ml/min
h. Detector: Electron capture, ^{63}Ni.
i. Injection: 6 µl with an HP 7671 autosampler.

These parameters were employed for all samples analyzed. Those samples showing a positive PCP or HCB response were reanalyzed on a 1.5% OV-1/2.4% OV-225 on 80/100 mesh Chromosorb W, 6 ft x 2 mm ID. If the chromatographic peaks in the sample had the proper retention times for PCP or HCB in the two chromatographic systems, partial confirmation of the peak could be made.

Gas Chromatography-Mass Spectrometry Confirmation

The samples which had shown positive GC peaks for PCP or HCB in two chromatographic systems were confirmed by GC-MS using selected ion monitoring (SIM) techniques. GC-MS parameters employed to confirm PCP were as follows:

a. Instrument: Hewlett Packard 5985 GC-MS
b. Column: 1.5% OV-17/1.95% QF-1 on 100/120 mesh Supelcoport packed in a 6 ft glass column, 2 mm ID
c. Injector Temperature: 250°C
d. Column Temperature: 165°C
e. Carrier Gas: Helium
f. Flow Rate: 30 ml/min
g. Transfer Line and Jet Separator Temperature: 275°C
h. Ionization Voltage: 70 eV
i. Ions Monitored: m/e 263.8, 267.8, and 305.8
j. Injection: 5 µl

QUALITY CONTROL PROCEDURES

Analytical Method Validation and Method of Calculation

The EC-GC analytical method for the determination of PCP and HCB in the various matrices was evaluated by analyzing reference solutions of PCP and HCB. A linearity study was conducted by adding known levels of PCP and HCB (ranging from about 4 to 2,000 ng of each) to 5 ml hexane, derivatizing, and injecting 5 µl. The results of this study are given in Table 6. These data indicate a linear response to PCP and HCB for a concentration range of 0.8 to 400 ng/ml. This range is equivalent to a concentration range in extracted samples of 2.4 to 1,250 ppb if a 2 ml or 2 g sample is taken. The sensitivity limit of the technique was 0.4 ng/ml for both PCP and HCB.

During sample analysis, a reference solution of PCP (13.3 ng/ml) and HCB (12.9 ng/ml) was analyzed prior to samples and after every fifth sample. The response factor (peak area/ng injected) was calculated for each reference solution injected with a sample set, and the average response factor determined. This value was then employed to calculate the PCP and HCB levels in the samples. The equations for these calculations are:

1. $$\text{Response factor} = \frac{\text{peak area}}{\mu\text{l injected}} \times \frac{\text{total volume } (\mu\text{l})}{\text{ng compound}}$$

2. $$\text{ng compound/extract} = \frac{\text{peak area}}{\mu\text{l injected}} \times \frac{\text{total volume } (\mu\text{l})}{\text{response factor}}$$

3. $$\text{ppb compound} = \frac{\text{ng compound/extract}}{\text{weight sample (g)}}$$

The response factors for the reference solutions analyzed during the linearity study were calcualted and are shown on Table 6. A representative GC-EC chromatogram for the determination of PCP and HCB is presented in Figure 2.

TABLE 6. LINEARITY OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE ANALYSIS BY GC-EC

Reference solution no.	ng Added		Peak area		Peak area / ng injected	
	PCP	HCB	PCP	HCB	PCP	HCB
			(x 10^{-3})		(x 10^{-6})	
1	-	-	0	0	0	0
2	3.98	3.87	10.0	12.5	2.510	3.230
3	7.96	7.74	11.0	22.4	1.380[a]	2.895
4	23.9	23.2	57.0	80.9	2.385	3.485
5	79.6	77.3	181.4	249.3	2.280	3.225
6	159.2	154.6	373.1	488.2	2.345	3.160
7	199	193	472.1	591.3	2.370	3.065
8	398	386	981.4	1,139	2.465	2.950
9	796	772	2,083	2.196	2.615	2.845
10	1,990	1,930	5,254	5,146	2.635	2.665

Linear regression:

PCP - Y (peak area) = 2,650 x (ng) - 28,250
Correlation coefficient - 0.999

HCB - Y (peak area) = 2,670 x (ng) + 45,160
Correlation coefficient - 0.999

Response factor (peak area/ng injected)

PCP - average, 2.450 x 10^6, S.D. ± 0.13 x 10^6

HCB - average, 3.055 x 10^6, S.D. ± 0.245 x 10^6

Sensitivity limits: PCP - 0.4 ng/ml
HCB - 0.4 ng/ml

a Value not used for linearity or response factor calculations.

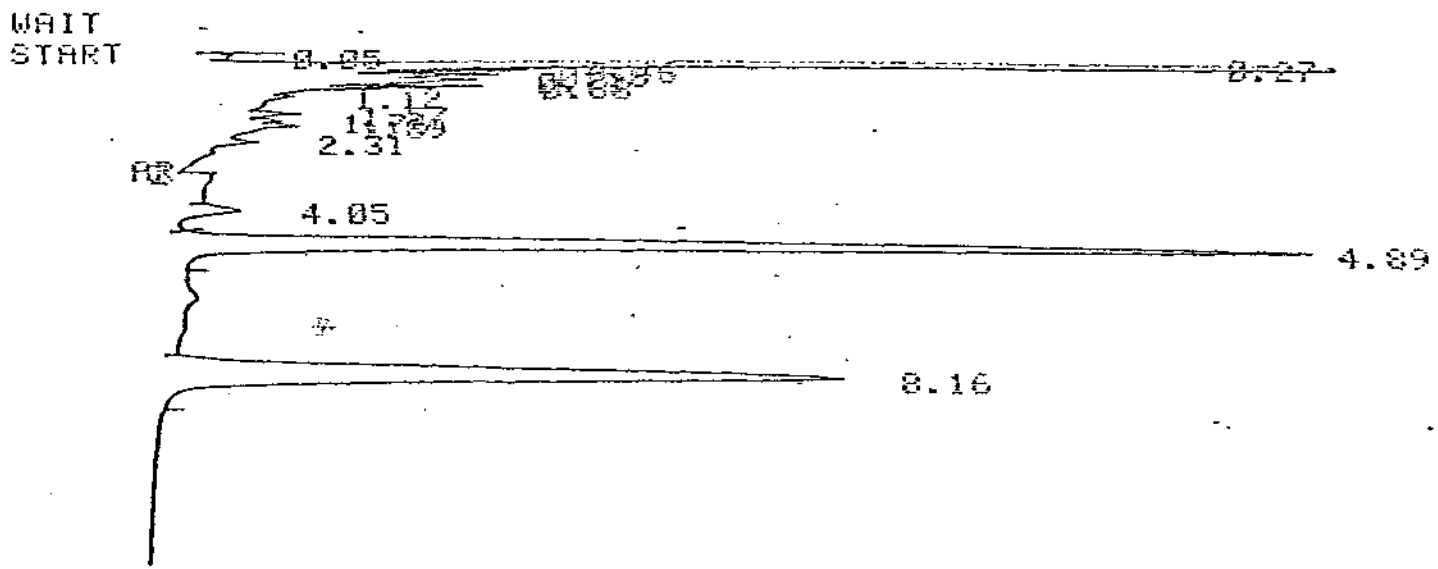

Figure 2. Pentachlorophenol standard on 1.5% OV-17/1.95% QF-1.

RRT (relative retention time): Pentachlorophenol - 8.16 min, 13.3 pg/µl
Hexachlorobenzene - 4.89 min, 12.9 pg/µl

Sample Analysis Quality Control

The quality control for the determination of PCP and HCB in the various samples consisted of analyzing duplicate aliquots of a sample for every 10 samples analyzed and spiking pooled samples with known levels of PCP and HCB. For those samples with detectable PCP or HCB, the sample was reanalyzed using a second GC column and if the retention times on the two systems agreed that the peak detected was PCP or HCB, tentative confirmation was made. Final confirmation of PCP or HCB being present in a sample was made by SIM-GC-MS.

For serum specimen PCP and HCB determinations, the precision and accuracy of the analytical results were assessed by analyzing duplicate aliquots of one sample and a pooled serum sample spiked with 40 ppb PCP and 39 ppb HCB with every 10 samples analyzed. A similar protocol was employed for urinary unconjugated PCP level precision and accuracy evaluations.

Food product samples of sufficient size were spiked with PCP and HCB to determine recovery values for samples carried through the analytical procedure. No duplicate food product samples were analyzed.

Analytical Procedure Quality Control

The methodology employed for the determination of PCP and HCB in human specimens was evaluated. For serum sample analysis, the extraction procedure was studied to determine if the recovery was higher with two 3-ml hexane extractions instead of a single 6-ml extraction. A short study was conducted to evaluate the effects of urinary sediments on the recovery of unconjugated PCP. Urine specimens with sediment were centrifuged, divided equally into a clear sample and a sediment sample, extracted, derivatized, and analyzed.

Field Sampling Quality Control

The stability of PCP and HCB in samples collected in Madison County, shipped to the analytical laboratory, and stored until assay was evaluated by field spiking studies. Field spikes for serum and urine specimens were prepared by adding a known level of PCP and HCB to collection vials, transporting the vials to the field, and adding 5 ml serum or 10 ml urine to the vials in the field. These spiked samples were then treated as collected specimens. A simulated field spiking study was conducted in the laboratory to confirm the results of the field spikes. For air samples, the collection tubes were field spiked with a hexane solution containing PCP and HCB. Two tubes were sealed immediately after spiking and two had air drawn through prior to sealing. In addition, the same procedure was used to prepare a set of laboratory spiked air samples.

SECTION 6

RESULTS AND DISCUSSION

SERUM PCP AND HCB DETERMINATIONS

Serum Analysis

The results for the GC-EC determination of PCP and HCB serum levels in 45 test case, 7 special case, and 45 control individuals are given in Tables 7 and 8. The test case individuals had an average serum PCP level of 21.0 ppb, a standard deviation (SD) of ± 11.3, a relative standard deviation (RSD) of 54%, and a range of 2.2 to 54.6 ppb. Five test case individuals, young children ages 2 to 11 years, could not be sampled for serum. The control individuals had an average serum PCP level of 24.0 ppb, with a SD and RSD of ± 12.5 and 52%, respectively, and a range of 4.5 to 67.9 ppb. Two control individuals, young children ages 2 and 4 years, did not have blood taken because they were controls for the test case individuals who could not be sampled. No HCB was detected in the serum of either the test cases or control individuals.

The individuals from the two special case families, who reside in log homes, had elevated serum PCP levels. Special cases 206A and 207A reside in a log home which they had treated with "penta," and special case 207A had a serum PCP level of 95.8 ppb which is 4 times the average PCP serum level for the control individuals, while special case 206A had a serum PCP level (47.6 ppb) that was 2 times the control serum PCP average level. Special case 208A, 209A, 210A, 211A, and 212A live in a commercially purchased log home. The serum PCP level for each of these special cases and the number of times above the control individual average were: 208A - 910 ppb (38X), 209A - 710 ppb (30X), 210A - 580 ppb (24X), 211A - 1,750 ppb (73X), and 212A - 1,680 ppb (70X), respectively. Special attention is made to the fact that the highest PCP serum levels occurred in the three children special cases (209A, 211A, and 212A). Representative GC-EC chromatograms of special cases 209A, 210A, and 211A on the 1.5% OV-17 and 1.95 QF-1 column are presented in Figures 3, 4, and 5, respectively. No HCB was detected in the serum of any of the special cases.

Confirmation of PCP Serum Analysis

The chromatographic peak designated as PCP on the OV-17/QF-1 column for the special case individuals was tentatively confirmed as PCP by reanalysis on a 1.5% OV-1 and 2.4% OV-225 column. Figures 6, 7, and 8 present chromatograms obtained for serum PCP confirmation for special cases 209A, 210A, and 211A on the OV-1/OV-225 system. Final confirmation of the observed GC peak being PCP was made by SIM-GC-MS. Figure 9 presents the GC-MS confirmation for special cases 209A, 210A, 211A, and 212A. The areas of the three m/e ions monitored were summed and are shown as a single peak.

TABLE 7. LEVELS OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN THE SERUM AND URINE (UNCONJUGATED) OF TEST CASES

Madison County Health Department number	Identification			Serum		Urine PCP (ppb)[a] (unconjugated)
	age	sex	race	(ppb)[a]	(ppb)[a]	
100A	32	female	white	40.0	N.D.[b]	2.4
101A	62	female	white	12.0	N.D.	1.1
102A	69	male	white	21.9	N.D.	1.2
103A	11	female	white	29.7	N.D.	1.4
104A	29	female	white	54.6	N.D.	0.6
105A	47	male	white	17.1	N.D.	1.9
106A	25	female	unknown	13.8	N.D.	1.3
107A	11	male	white	c	c	2.0
108A	13	male	white	25.3	N.D.	1.3
109A	49	female	white	9.9	N.D.	1.2
110A	65	female	white	17.1	N.D.	3.6
111A	20	female	white	2.2	N.D.	3.8
112A	50	female	white	15.2	N.D.	1.2
113A	56	male	white	13.5	N.D.	1.5
114A	47	female	white	19.9	N.D.	1.0
115A	14	female	white	27.8	N.D.	1.5
116A	10	male	white	c	c	1.7
117A	05	female	white	c	c	1.1
118A	11	male	white	22.1	N.D.	0.9
119A	26	male	white	27.3	N.D.	1.7
120A	27	female	white	33.1	N.D.	0.7
121A	33	female	white	7.1	N.D.	1.2
122A	15	female	white	8.1	N.D.	2.6
123A	12	female	white	28.7	N.D.	1.0
124A	02	male	white	c	c	0.9
125A	55	female	white	10.6	N.D.	2.8
200A	36	female	white	15.5	N.D.	0.9
201A	14	male	white	21.8	N.D.	0.8
202A	10	female	white	22.6	N.D.	1.5
203A	57	female	white	20.6	1.0	1.4
204A	32	female	white	14.2	N.D.	1.1
205A	25	female	white	38.0	N.D.	4.0
213A	33	female	white	10.6	N.D.	7.2
214A	08	female	white	c	c	2.3
215A	05	female	white	53.9	N.D.	4.0
216A	56	male	white	13.7	N.D.	2.4
217A	45	female	white	16.3	N.D.	1.6
218A	15	female	white	21.2	N.D.	1.0
219A	21	male	white	9.9	N.D.	2.2
220A	17	male	white	28.7	N.D.	0.7
221A	51	male	white	23.4	N.D.	1.6
222A	49	female	white	13.4	N.D.	1.6

(continued)

TABLE 7 (continued)

Madison County Health Department number	Identification age	sex	race	Serum (ppb)[a]	Serum (ppb)[a]	Urine PCP (ppb)[a] (unconjugated)
223A	16	female	white	21.1	N.D.	1.2
224A	22	male	white	16.5	N.D.	1.3
225A	49	male	white	11.0	N.D.	1.5
226A	49	female	white	13.6	N.D.	2.0
227A	29	female	white	30.5	N.D.	2.8
228A	39	male	white	40.6	N.D.	5.0
117B[d]	08	female	white	c	c	1.5
121B[d]	38	female	white	16.9	N.D.	0.6
224B[d]	21	female	white	22.4	N.D.	1.6
Number				45	-	51
Average				21.0	-	1.8
Standard deviation				± 11.5	-	± 1.2
Relative standard deviation				54%	-	68%
Special Cases - Log Home Residents						
206A	29	male	white	47.6	N.D.	5.1
207A	28	female	white	95.8	N.D.	1.7
208A	09	male	white	910	N.D.	54.7
209A	34	male	white	710	N.D.	46.8
210A	29	female	white	580	N.D.	50.8
211A	02	female	white	1,750	N.D.	216
212A	04	female	white	1,680	N.D.	51.2

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml; HCB, 0.4 ng/ml.

b N.D. - none detected, < 0.4 ppb.

c Sample not collected.

d Control subjects changed to test cases after PCP treated amunition crate wood located in their residence.

TABLE 8. LEVELS OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN THE SERUM AND URINE (UNCONJUGATED) OF CONTROLS

Madison County Health Department number	Identification			Serum		Urine PCP (ppb)[a] (unconjugated)
	age	sex	race	(ppb)[a]	(ppb)[a]	
100B	28	female	white	25.0	N.D.[b]	1.3
101B	44	female	white	25.8	N.D.	4.3
102B	44	male	white	16.9	N.D.	1.9
103B	17	female	white	20.5	N.D.	1.6
104B	26	female	white	49.8	N.D.	2.2
105B	58	male	white	67.9	N.D.	2.9
106B	30	female	white	21.0	N.D.	0.9
107B	09	female	white	30.8	N.D.	1.9
108B	13	male	white	40.0	N.D.	3.1
109B	51	female	white	29.4	N.D.	1.6
110B	57	female	white	29.0	N.D.	2.2
111B	15	female	white	31.6	N.D.	3.3
112B	48	female	white	42.2	N.D.	2.0
113B	51	male	white	9.5	N.D.	2.5
114B	31	female	unknown	10.0	N.D.	1.2
115B	12	female	white	11.7	N.D.	2.2
116B	10	female	white	17.6	N.D.	0.9
118B	09	female	white	33.5	N.D.	3.5
119B	36	male	white	6.3	N.D.	1.7
120B	33	female	white	4.4	N.D.	1.2
122B	14	female	white	26.2	N.D.	1.2
123B	15	male	white	7.4	N.D.	1.2
124B	02	female	black	c	c	1.2
200B	29	female	white	23.0	N.D.	3.4
201B	16	female	white	35.4	N.D.	4.7
202B	09	male	white	35.8	N.D.	1.7
203B	49	female	white	13.7	N.D.	2.0
204B	32	female	black	24.2	N.D.	4.1
205B[d]	32	female	white	19.2	N.D.	1.2
206B[d]	30	male	white	11.6	N.D.	1.2
207B[d]	33	female	white	20.6	N.D.	2.4
210B[d]	33	female	white	22.0	N.D.	0.4
213B	28	female	white	38.4	N.D.	1.5
214B	04	male	white	c	c	1.4
215B	06	male	white	38.1	N.D.	3.1
216B	56	male	white	29.3	N.D.	2.8
217B	55	female	white	14.6	N.D.	0.7
218B	16	male	white	22.8	N.D.	4.2
219B	32	male	black	18.4	N.D.	1.0
220B	17	male	white	4.5	N.D.	2.3

(continued)

TABLE 8 (continued)

Madison County Health Department number	Identification age	sex	race	Serum (ppb)[a]	Serum (ppb)[a]	Urine PCP (ppb)[a] (unconjugated)
221B	65	male	white	17.2	N.D.	1.3
222B	59	female	white	13.2	N.D.	2.2
223B	23	male	white	21.7	N.D.	1.4
225B	54	male	white	15.1	N.D.	11.0
226B	46	female	white	19.8	N.D.	2.0
227B	29	female	white	23.7	N.D.	2.6
228B	29	male	white	29.5	N.D.	2.4
Number[e]				45	-	47
Average				24.0	-	2.3
Standard deviation				± 12.5	-	± 1.6
Relative standard deviation				52%	-	72%

a ppb - parts per billion (ng/ml), sensitivity - 0.4, ng/ml; HCD, 0.4 ng/ml.

b N.D. - none detected, < 0.4 ppb.

c Sample not available.

d Control individuals for special cases.

e Number - number, average, standard deviation, and relative standard deviation includes controls for special cases.

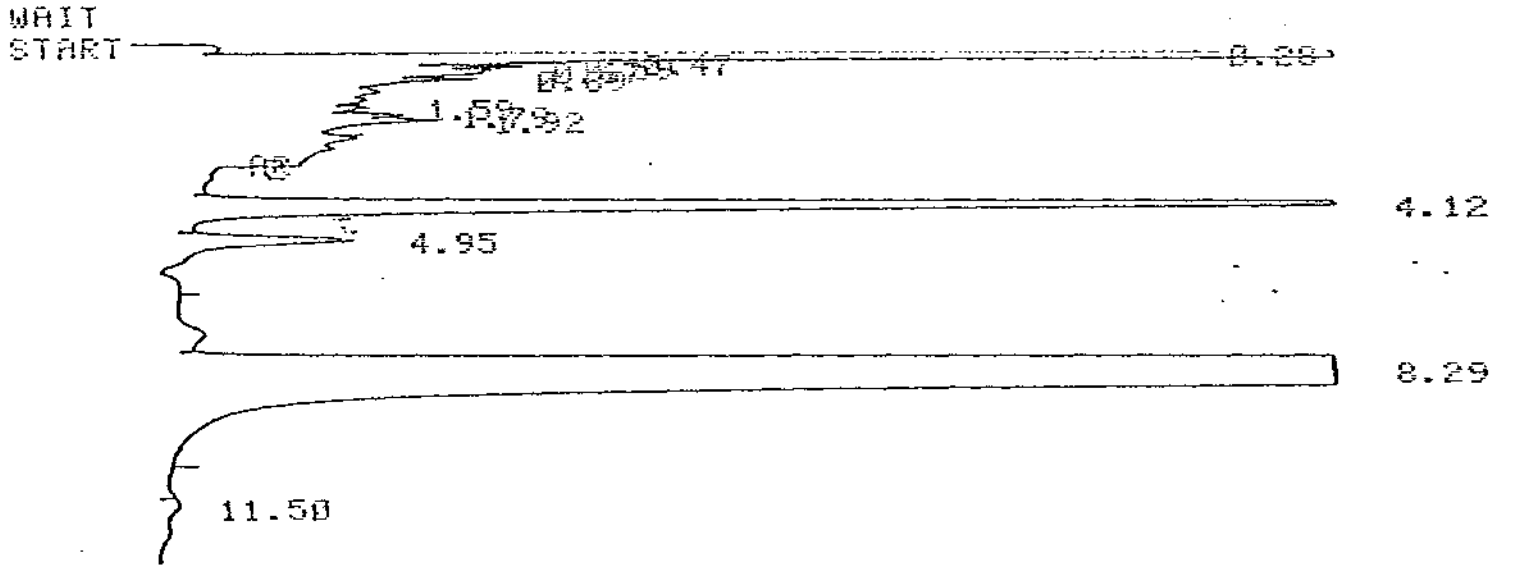

Figure 3. Serum sample of individual 209A on 1.5% OV-17/1.95% QF-1.

RRT: Pentachlorophenol - 8.29 min, 710 ppb

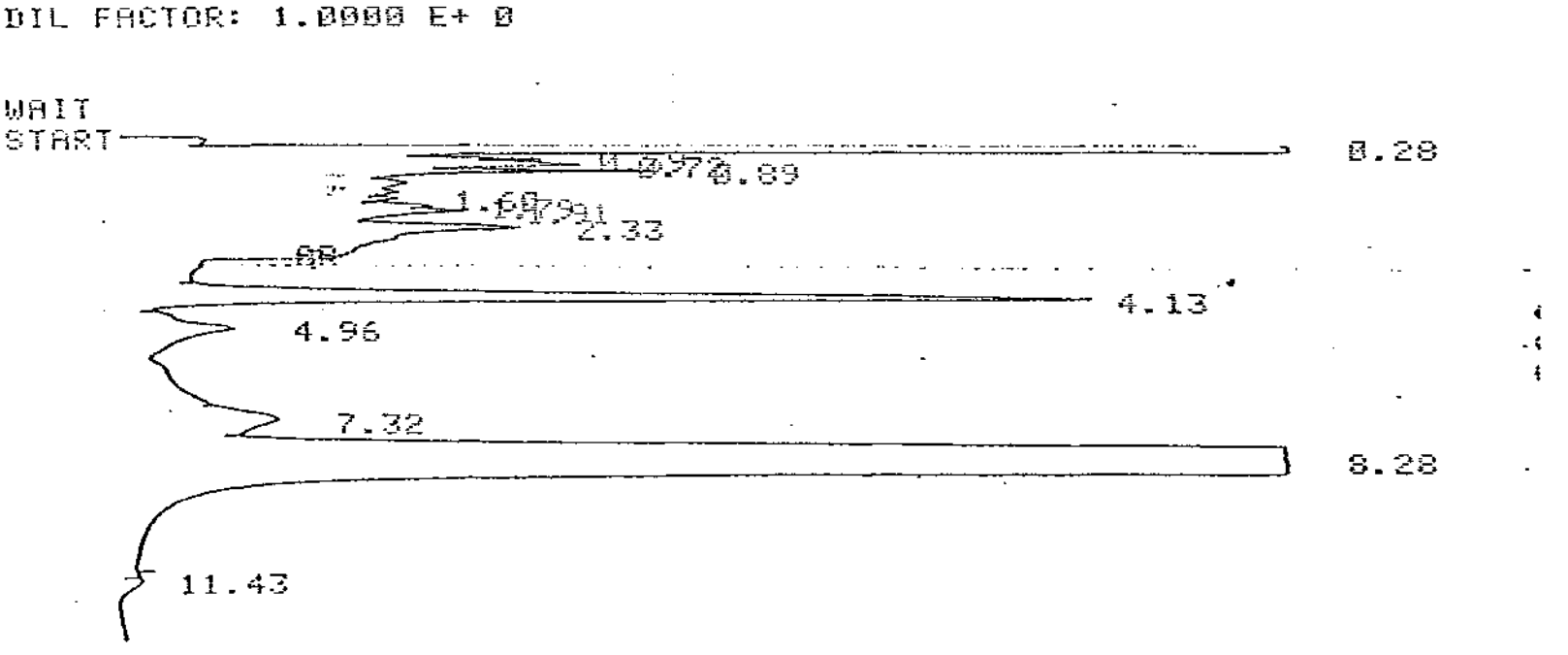

Figure 4. Serum sample of individual 210A on 1.5% OV-17/1.95% QF-1.

RRT: Pentachlorophenol 8.28 min, 580 ppb

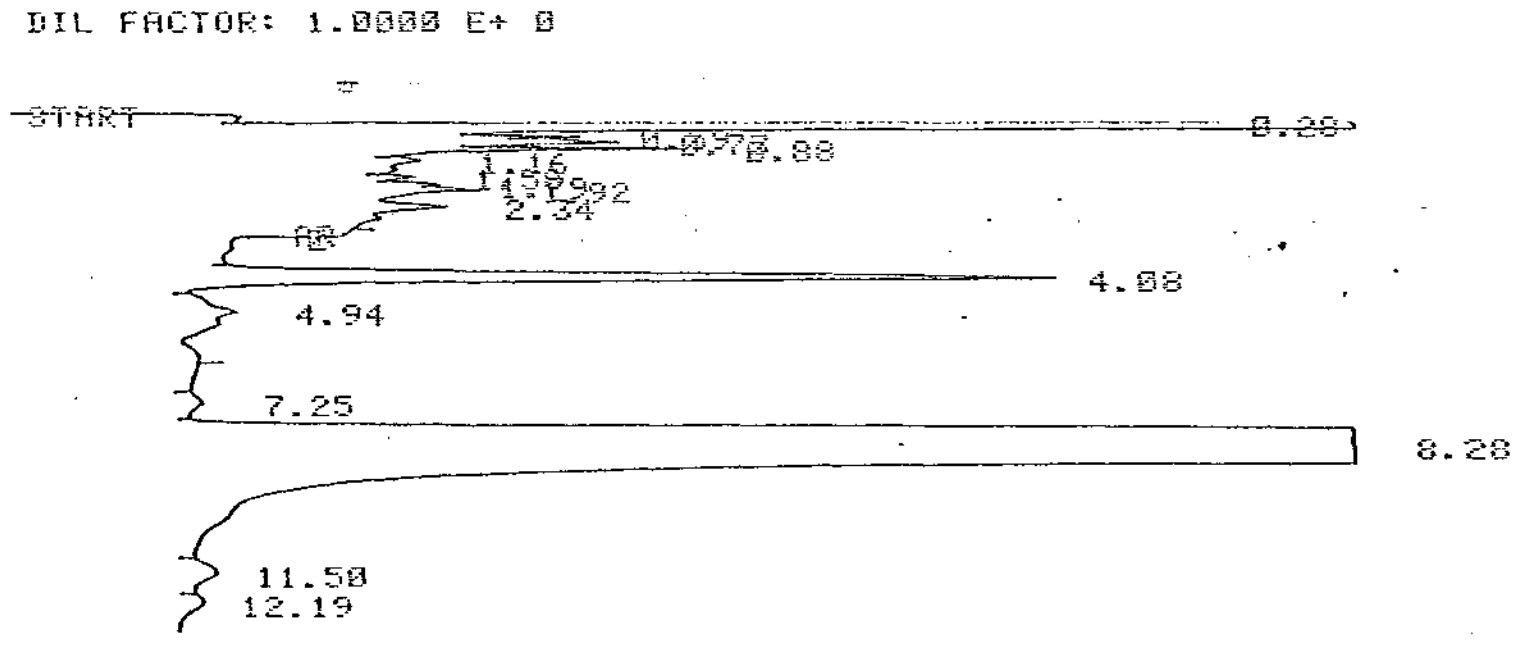

Figure 5. Serum sample of individual 211A on 1.5% OV-17/1.9% QF-1.

RRT: Pentachlorophenol - 8.28 min, 1,750 ppb

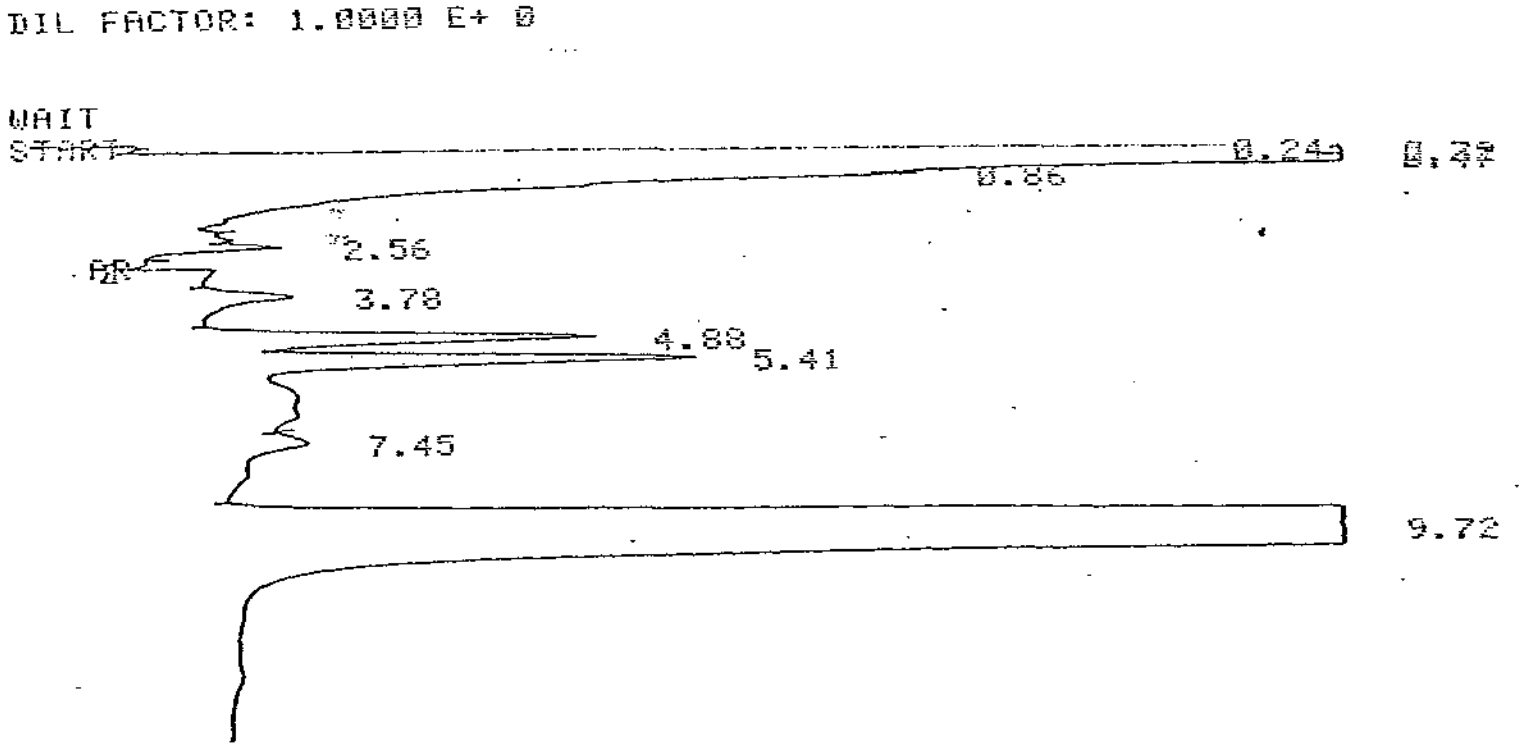

Figure 6. Serum sample of individual 209A on 1.5% OV-1/2.4% OV-225.

RRT: Pentachlorophenol - 9.72 min

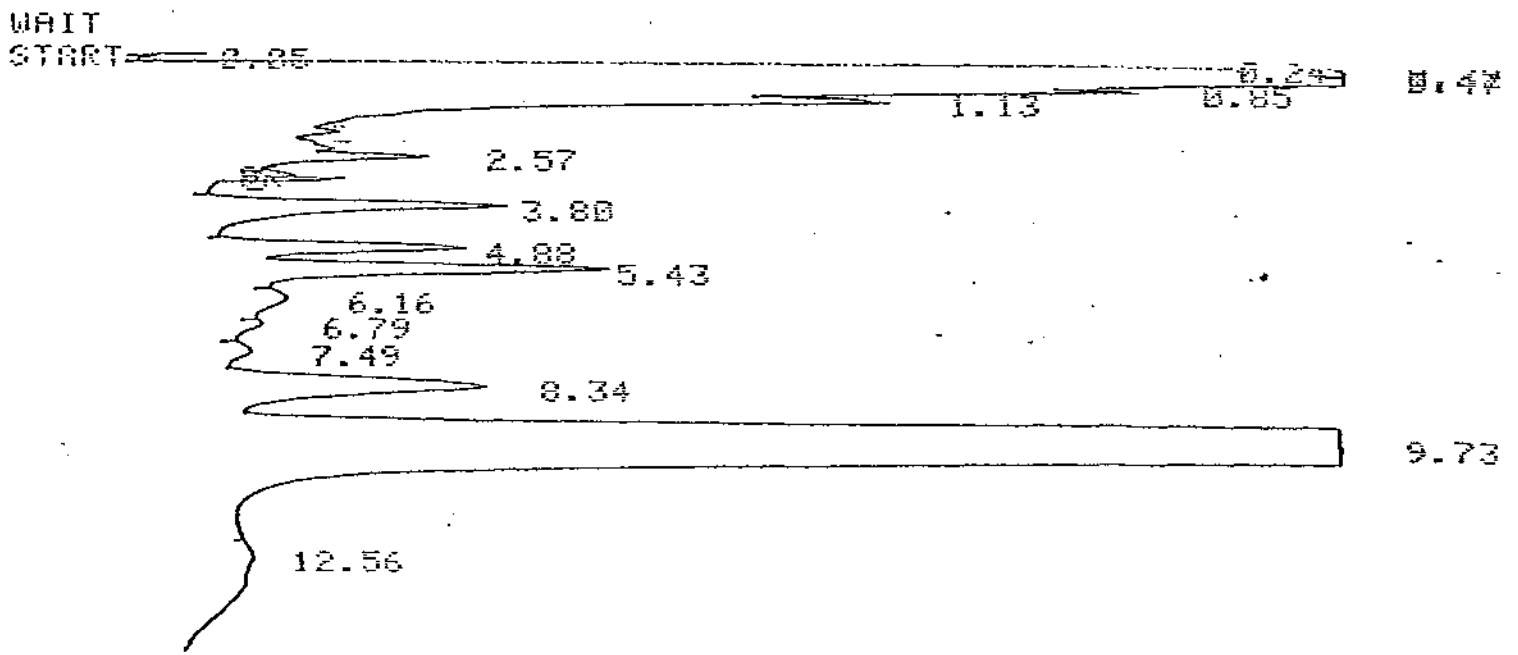

Figure 7. Serum sample of individual 210A on 1.5% OV-1/2.4% OV-225.

RRT: Pentachlorophenol - 9.73 min

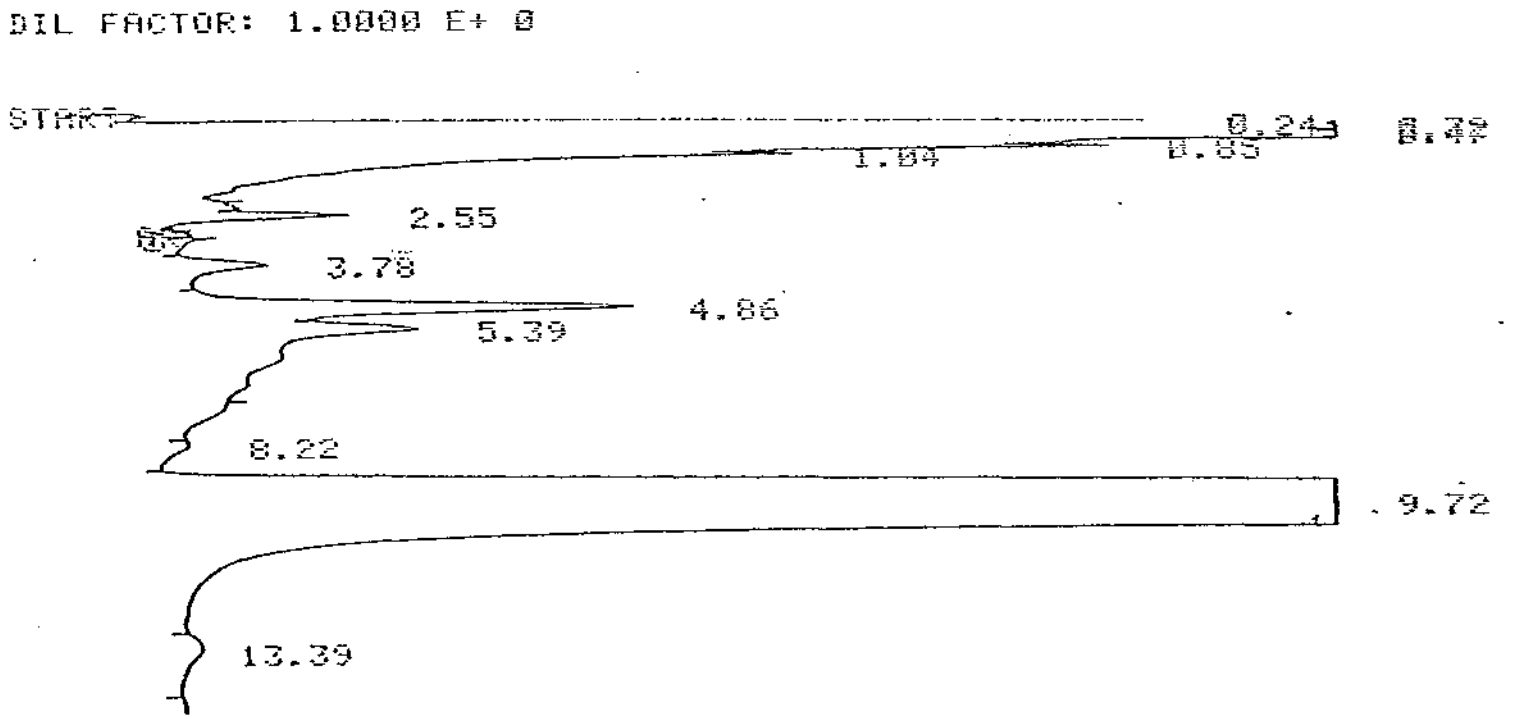

Figure 8. Serum sample of individual 211A on 1.5% OV-1/2.4% OV-225,

RRT: Pentachlorophenol - 9.72 min

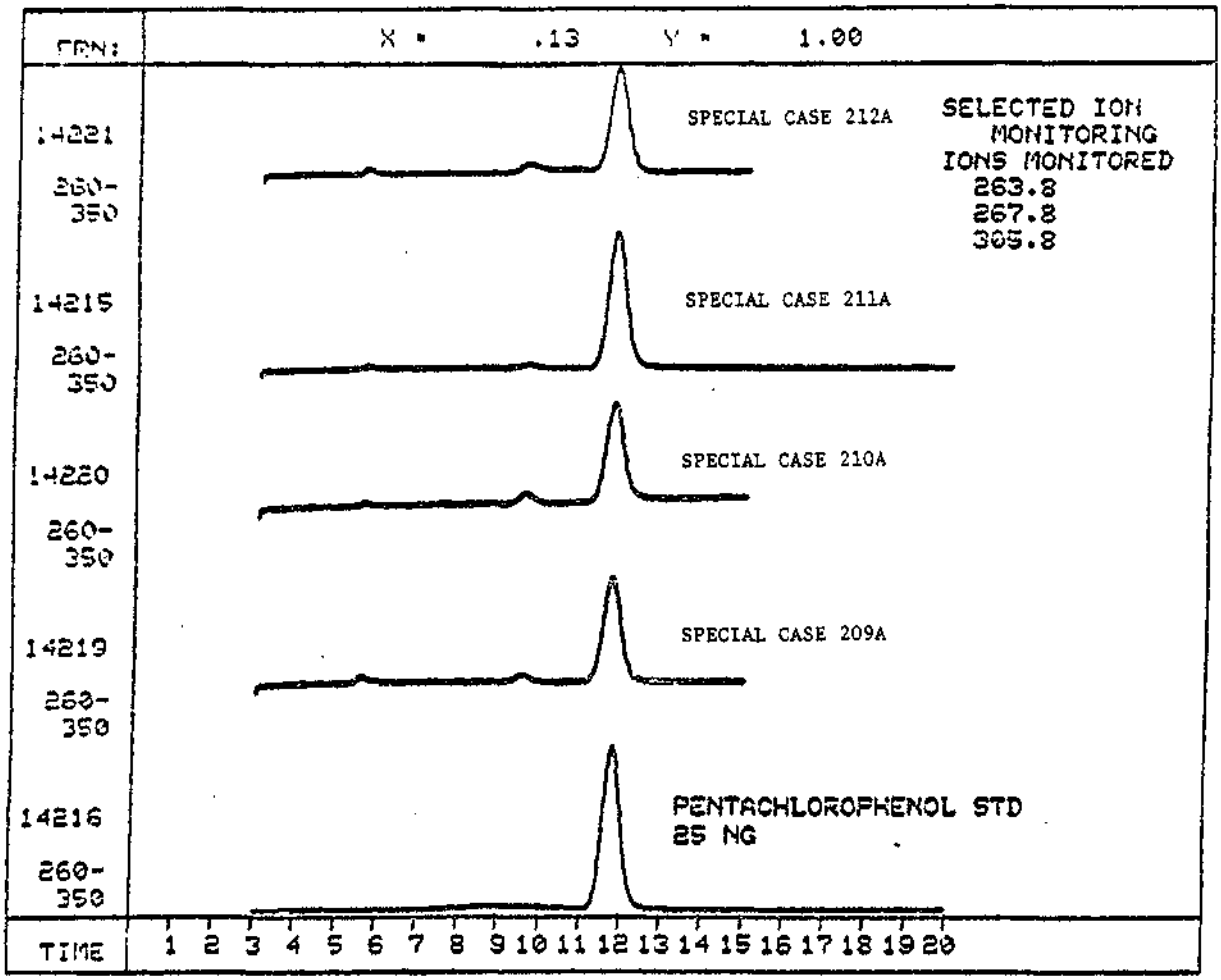

Figure 9. Mass spectrometry confirmation of serum of individuals 209A through 212A.

Quality Control - Sample Analysis, Precision and Accuracy

The precision and accuracy of the analytical data for PCP and HCB in serum was assessed by analyzing duplicate aliquots of a sample and spiked pooled serum aliquots. The duplicate analyses of PCP are given in Table 9. The standard deviation of pairs (S.D.P.) and relative standard deviation of pairs (R.S.D.P.) were ± 3.6 and 15.4%, respectively; and the average percent difference between pairs was 18.2 ± 18.9. Thus, the analytical method employed for serum PCP provided data within 20% of the actual value. Since no serum HCB was detected, evaluation of the method was not possible. The recovery of PCP and HCB in pooled serum samples (Table 10) averaged 78% and 91%, respectively, for 10 spiked samples. These data indicate that the analytical data have the necessary accuracy.

Quality Control - Sample Preparation Procedure

The sample preparation procedure of serum PCP and HCB levels was evaluated by extracting spiked serum with either one 6-ml or two 3-ml hexane aliquots. The results of this evaluation are summarized in Table 11 and show that the single extraction employed for this study was equivalent to a double extraction. Also, this study showed that the extraction was linear, i.e., similar recoveries were obtained for serum aliquots spiked with about 40 and 200 ppb of PCP and HCB.

UNCONJUGATED URINARY PCP DETERMINATION

Urine Analysis

The results for the GC-EC determination of unconjugated PCP urinary levels in 51 test case, 7 special case, and 47 control individuals are given in Tables 7 and 8. As was noted earlier for serum CP levels, the unconjugated PCP urinary levels for the test cases and control individuals were similar. The average, SD, RSD, and range for the test cases and control individuals unconjugated urinary PCP were: 1.8 and 2.3 ppb; ± 1.2 and ± 2.3; 68% and 72%; and 0.6 to 7.2 ppb and 0.4 to 11.0 ppb, respectively.

For five of the special case individuals, a substantial increase in unconjugated urinary PCP levels was noted. Special cases 206A and 207A had unconjugated urinary PCP levels of 5.1 and 1.7 ppb, respectively. While this level is within the range of the control individuals, the serum PCP level for these two special cases was elevated. Special cases 208A, 209A, 210A, 211A, and 212A, members of a single family who reside in a commercially purchased log home, all had elevated unconjugated PCP urinary levels. The levels and the number of times above the average unconjugated urinary PCP level for control individuals were: 208A - 54.7 ppb (24X), 209A - 46.8 ppb (20X), 210A - 50.8 ppb (22X), 211A - 216 ppb (94X), and 212A - 51.2 ppb (22X). As with the serum PCP levels for the special cases, the highest unconjugated urinary PCP was found in a child (211A), age 2 years. Representative GC-EC chromatograms for special cases 207A, 208A, and 209A on the OV-17/QF-1 column are shown in Figures 10, 11, and 12, respectively.

TABLE 9. DUPLICATE ANALYSES OF SERUM SAMPLES FOR PENTACHLOROPHENOL

Madison County Health Department Number	Pentachlorophenol (ppb)[a] Replicate A	Replicate B	Average	(A-B)	%[b]
100A	36.8	41.1	39.0	4.3	11.0
109A	11.4	8.6	9.9	2.8	28.0
202A	22.0	23.3	22.6	1.3	5.8
213A	9.1	12.0	10.6	2.9	27.4
219A	9.6	10.2	9.9	0.6	6.1
226A	13.9	13.4	13.6	0.5	3.7
104B	49.5	50.1	49.8	0.6	1.2
108B	40.0	40.1	40.0	0.1	0.2
111B	27.5	35.7	31.6	8.2	26.0
203B	16.9	10.5	13.7	6.4	46.7
210B	21.4	22.5	22.0	1.1	5.0
218B	29.3	16.2	22.8	13.1	57.5
Avg A and B[c]	23.7			Avg[d]	18.2
S.D.P.[e]	± 3.6			S.D.[f]	± 18.9
R.S.D.P.[g]	15.4%				

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml.

b % - Percent difference between A and B values.

$$\% = \left(A-B\right) \Big/ \frac{(A+B)}{2} \times 100$$

c Avg A and B - Average of all A and B values.

d Avg - Average of percent values.

e S.D.P. - Standard deviation of pairs.

$$S.D.P. = \left(\Sigma (A-B)^2 / 2xP\right)^{1\ 2}$$

P = Number of pairs

f S.D. - Standard deviation.

$$S.D. = \left(\Sigma (\bar{x}-x)^2 / 2xN\right)^{1\ 2}$$

N = Number of pairs

g R.S.D.P. - Relative standard deviation of pairs.

R.S.D.P. = S.D.P./Avg A and B x 100

TABLE 10. RECOVERY OF PENTACHLOROPHENOL AND HEXACHLOROBENZENE ADDED TO POOLED SERUM ALIQUOTS

Pooled serum number	Level added (ppb[a]) PCP	HCB	Level found (ppb[a]) PCP	HCB	% Recovery[b] PCP	HCB
1	40	39	55.1	37.4	87	96
2	40	39	51.8	33.1	79	85
3	40	39	54.2	31.1	85	80
4	40	39	46.0	32.2	64	83
5	40	39	53.6	37.8	83	97
6	40	39	50.4	36.2	75	93
7	40	39	51.6	38.3	78	98
8	40	39	52.3	35.4	80	91
9	40	39	53.7	38.7	84	99
10	40	39	47.9	35.6	69	91
-	0	0	20.3	N.D.[c]		
				Avg	78	91
				S.D.	± 7	± 7

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml; HCB, 0.4 ng/ml.

b $$\% \text{ Recovery} = \frac{\text{ppb spiked - ppb unspiked}}{\text{ppb added}} \times 100$$

c N.D. - not detected, < 0.4 ng/ml.

TABLE 11. RECOVERY VALUES FOR PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN SERUM BY TWO EXTRACTION METHODS

Sample	Pentachlorophenol			Hexachlorobenzene		
	Level spiked (ppb[a])	Level recovery (ppb)	Recovery (%)	Level spiked (ppb)	Level recovery (ppb)	Recovery (%)
Extraction performed with one 6-ml aliquot of hexane:						
Serum spiked	40	50.7	86	38.8	34.5	86
Serum spiked	40	46.2	75	38.8	35.7	89
Serum spiked	200	185	84	194	155	78
Serum spiked	200	170	77	194	145	73
Serum blank	-	16.2		-	N.D.	
Serum blank	-	16.5		-	N.D.	
Extraction performed with two 3-ml aliquots of hexane:						
Serum spiked	40	52.8	84	38.8	42.3	106
Serum spiked	40	50.1	77	38.8	33.6	84
Serum spiked	200	178	79	194	167	84
Serum spiked	200	185	83	194	168	84
Serum blank	-	19.7		-	N.D.	
Serum blank	-	18.8		-	N.D.	

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml; HCB, 0.4 ng/ml.

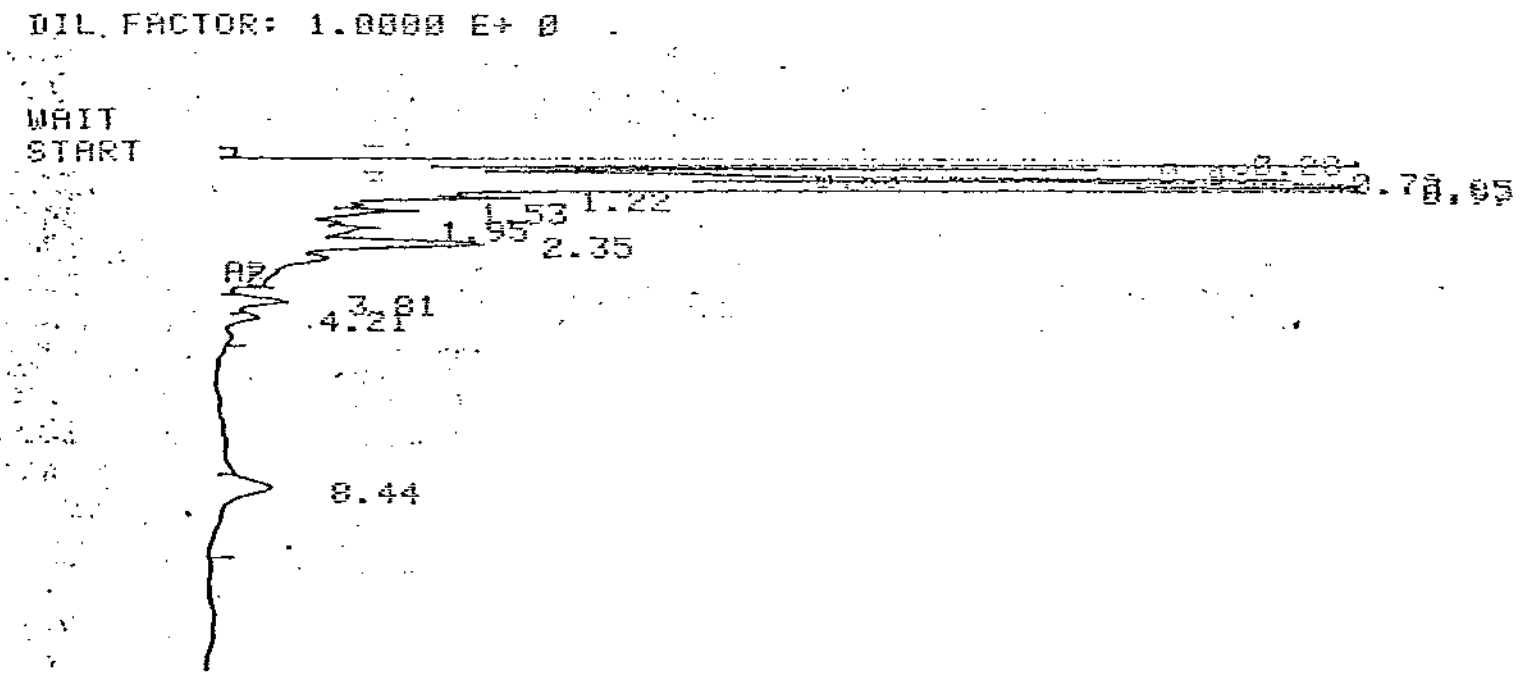

Figure 10. Urine sample (unconjugated) of individual 207A on 1.5% OV-17/1.95% QF-1

RRT: Pentachlorophenol 8.44 min, 1.7 ppb

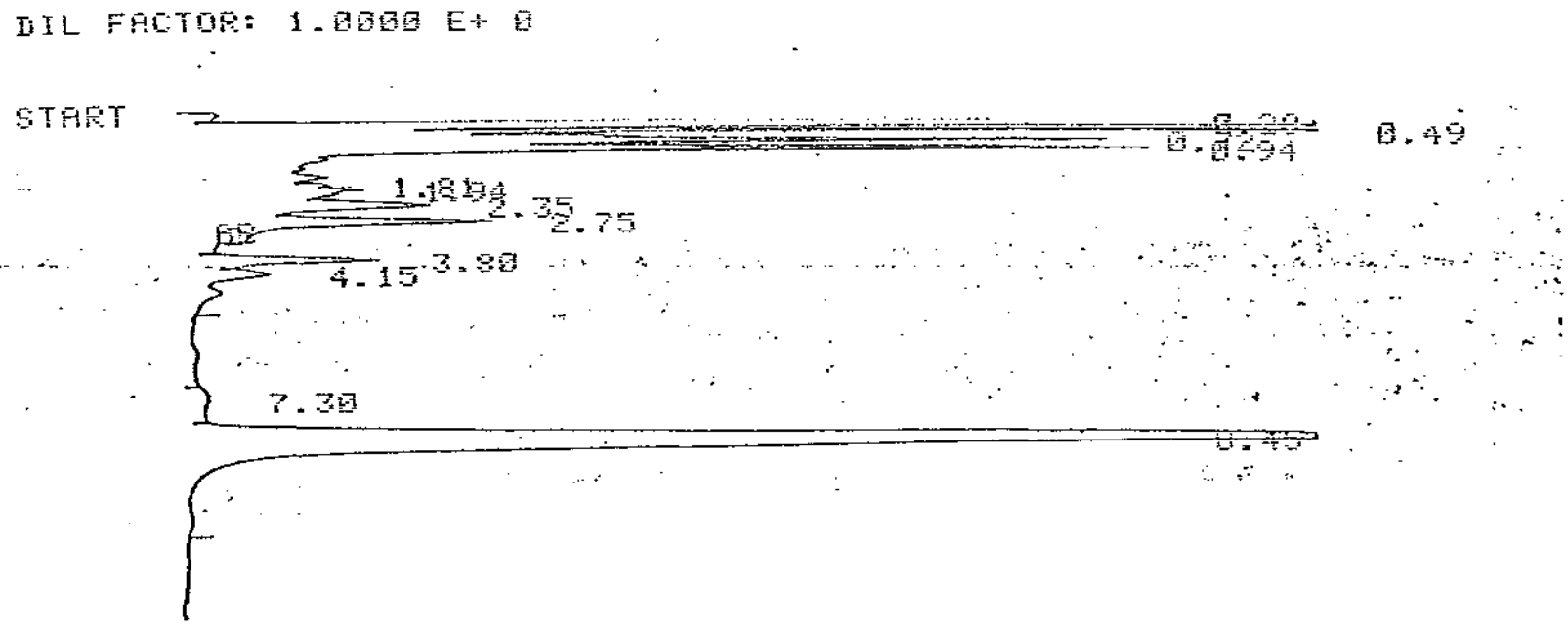

Figure 11. Urine sample (unconjugated) of individual 208A on 1.5% OV-17/1.95% QF-1.

RRT: Pentachlorophenol 8.45 min, 54.7 ppb

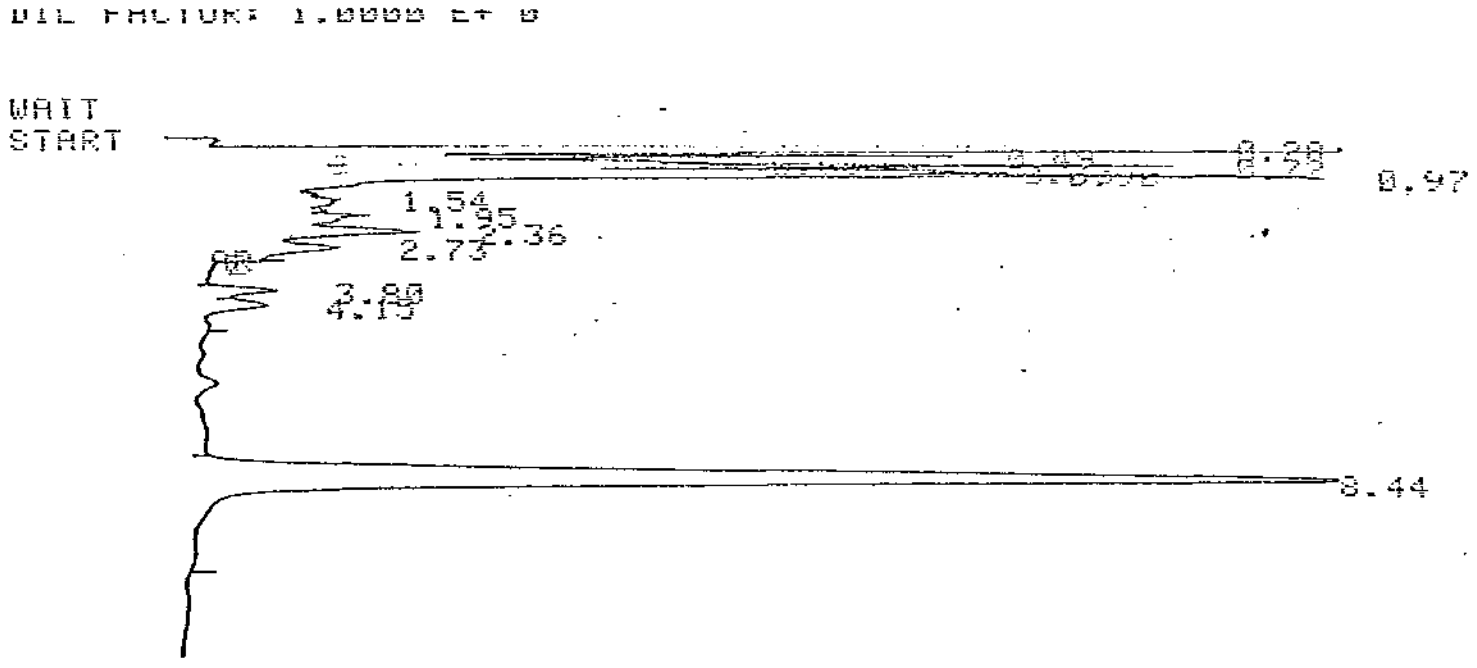

Figure 12. Urine sample (unconjugated) of individual 209A on 1.5% OV-17/1.95% QF-1

RRT: Pentachlorophenol - 8.44 min, 46.8 ppb

Confirmation of Unconjugated PCP Urinary Analysis

The unconjugated PCP in the urine specimens from the special cases was tentatively confirmed by reanalysis on the OV-1/OV-225 column. Final confirmation of the detected peak being PCP was made by SIM-GC-MS. Selected ion monitoring traces for special cases 206A through 212A and test case 213A are shown in Figures 13, 14, and 15. The low level of PCP in special cases 206A and 207A prevented GC-MS confirmation. The other special cases and test case 213A were confirmed as having unconjugated PCP in their urine.

Quality Control - Sample Analysis, Precision and Accuracy

The precision and accuracy of the determination of unconjugated PCP in urine was assessed through duplicate determinations of selected samples and the recovery of PCP added to a pooled urine sample. The results of the duplicate analyses are given in Table 12; no statistical evaluation of these duplicates was made because the low level of unconjugated PCP would cause large errors for small differences between analyses. However, these data do show that similar values for PCP were obtained for duplicate determinations. The accuracy of the unconjugated urinary PCP levels was determined by analyzing spiked pooled urine aliquots. The results are given in Table 13 and show an average PCP recovery of 97% with a SD of ± 7. Thus, quantitative recovery of PCP added to urine was obtained.

Quality Control - Sample Preparation Procedure

Selected urine specimens contained sediment which may have adsorbed unconjugated PCP; and thus, uniform sampling of urine may not have been possible. A study was designed to evaluate the effect of urinary sediment on the determination of unconjugated PCP levels. Urine samples containing sediment were centrifuged and equal aliquots taken, one with and one without the sediment. The results are given in Table 12, relicate column 3 for the sediment containing samples and replicate column 4 for the supernatant samples. These data show little difference between the unconjugated PCP levels in the separated urines, thus indicating that the urinary sediment did not affect the levels for unconjugated urinary PCP.

FIELD SPIKE EXPERIMENTS FOR SERUM PCP AND HCB AND URINARY PCP

The sampling, sample handling, storage, and shipment for serum and urine specimens was evaluated by conducting field spiking experiments. Pre-washed sample vials were spiked with a solution of PCP and HCB in hexane in the laboratory and shipped to the field with the sample vials. In the field, 10 ml of urine or 5 ml of serum were added to the vials, the vials sealed, and treated as samples. The results of the field spikes of urine and serum are presented in Table 14. Recovery values for PCP ranged from 61 to 88% in serum and 34 to 60% in urine. HCB was only detected in one serum and one urine field spike with recovery values of 67 and 27%, respectively. Due to the lack of recovery of HCB and the low recovery of PCP, a laboratory simulation of field spikes was performed. The vials were spiked and stored at room temperature 6 days prior to the addition of serum or urine. At that time, the serum and urine samples were added to the spiked bottles, and a similar set of serum and urine samples were added to septum bottles and these samples spiked.

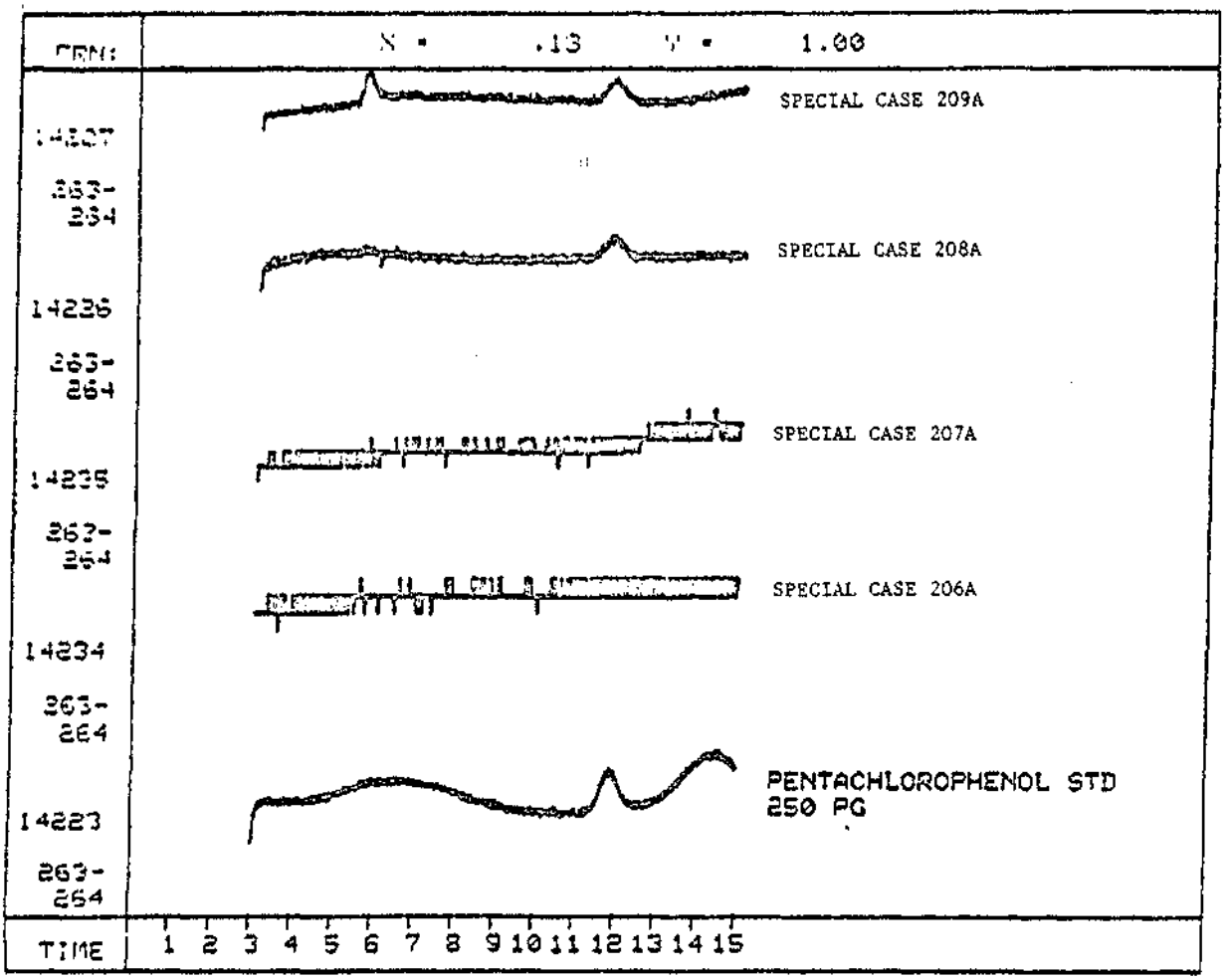

Figure 13. Mass spectrometry confirmation of urine of individuals 206A, 207A, 208A and 209A.

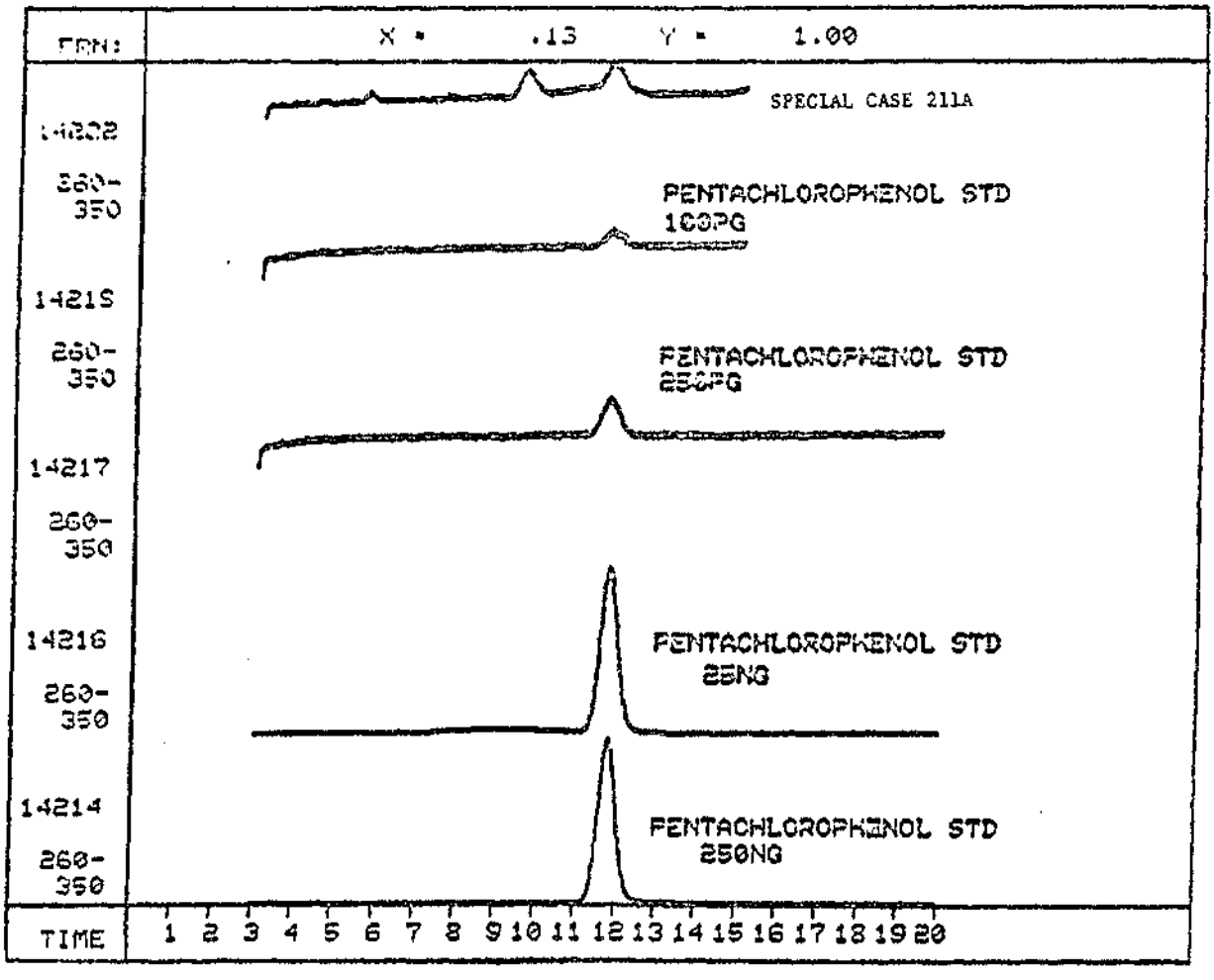

Figure 14. Mass spectrometry confirmation of urine of individual 211A.

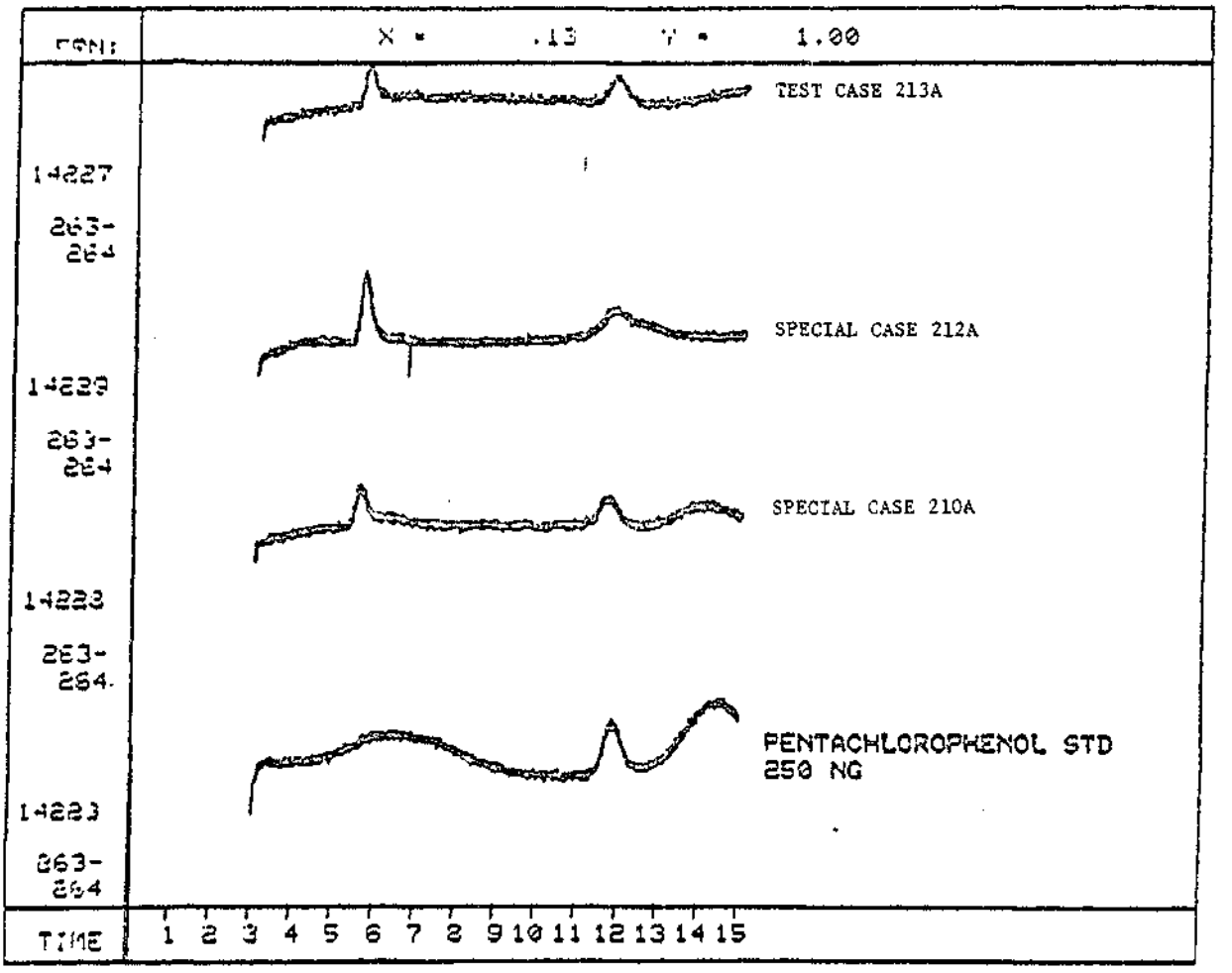

Figure 15. Mass spectrometry confirmation of urine of individuals 210A, 212A, and 213A.

TABLE 12. REPLICATE ANALYSES OF URINE SAMPLES FOR UNCONJUGATED PENTACHLOROPHENOL

Madison County Health Department number	Pentachlorophenol (ppb[a]) Replicate 1	2	3[b]	4[b]	Average
100A	2.7	2.2			2.4
101A	1.1	1.1			1.1
105A	1.0	2.5	2.4	1.7	1.9
110A	4.2	3.1			3.6
207A	2.2	1.0	1.8	1.7	1.7
225A	1.1	0.8	2.0	2.0	1.5
100B	1.2	1.4			1.3
107B	1.1	2.2	2.8	1.4	1.9
121B	0.6	0.5			0.6
200B	0.9	2.3	4.9	5.5	3.4
213B	1.6	1.4			1.5
214B	1.4	1.4			1.4

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml.

b Four milliliters of urine centrifuged and 2 ml of supernatant extracted as replicate 4 and the 2 ml of urine containing the sediment extracted as replicate 3.

TABLE 13. RECOVERY OF PENTACHLOROPHENOL ADDED TO POOLED URINE ALIQUOTS

Pooled urine number	Level added (ppb[a]) PCP	Level found (ppb[a]) PCP	% Recovery[b] PCP
1	40	35.1	86
2	40	42.3	104
3	40	37.2	92
4	40	35.6	88
5	40	39.6	98
6	40	41.8	103
7	40	42.2	104
8	40	38.5	95
9	40	38.7	95
10	40	42.3	104
Blank	0	0.6	
		Avg	97
		S.D.	± 7

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml.

b $\%\ \text{Recovery} = \frac{\text{ppb spiked - ppb unspiked (blank)}}{\text{ppb added}} \times 100$

TABLE 14. RECOVERY VALUES FOR PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN FIELD SPIKES OF SERUM AND URINE (UNCONJUGATED)

Sample	Pentachlorophenol			Hexachlorobenzene		
	Level spiked (ppb[a])	Level recovery (ppb)	Recovery (%)	Level spiked (ppb)	Level recovery (ppb)	Recovery (%)
Serum:						
Field blank #1	--	N.D.[b]		--	N.D.	
Field blank #2	--	N.D.		--	N.D.	
Field blank #3	--	N.D.		--	N.D.	
220A	16	30.8	76	15.5	N.D.	--
220A	--	18.7		--	N.D.	
101B	16	37.7	74	15.5	N.D.	--
101B	--	25.8		--	N.D.	
110B	16	41.6	79	15.5	N.D.	--
110B	--	29.0		--	N.D.	
106B	80	91.3	88	77.3	51.7	67
106B	--	21.0		--	N.D.	
112B	80	90.7	61	77.3	N.D.	--
112B	--	42.2		--	N.D.	
200B	80	79.4	71	77.3	N.D.	--
200B	--	23.0		--	N.D.	
Urine:						
Field blank #1	--	N.D.		--	N.D.	
Field blank #2	--	N.D.		--	N.D.	
Field blank #3	--	N.D.		--	N.D.	
108B	120	75.0	60	116	30.9	27
108B	--	3.1		--	N.D.	
202B	120	42.3	34	116	N.D.	--
202B	--	1.7		--	N.D.	

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml; HCB, 0.4 ng/ml.

b N.D. - none detected, < 0.4 ng/ml.

The spiked serum and urine samples were frozen and stored for 7 days prior to extraction. At the time of extraction, additional serum and urine samples were spiked directly in the extraction tubes. The results of the simulated field spikes are presented in Table 15. The recovery value of PCP increased from spikes in the vials to spikes directly into the serum and urine samples. The recovery of HCB in vials spiked prior to addition of the serum and urine was non-existant. However, recovery of HCB from the field spikes which consisted of spiking directly into the samples compared favorably with the extraction time spikes. The vials used to store the samples were sealed with Teflon-lined septa and the spiking volume was 10 µl. Thus, the loss of HCB and the low recovery of PCP in the field spikes and laboratory simulation may have been caused by irreversible adsorption of the compounds on the walls of the vials or in the septum material. Another possible explanation is that the 10 µl hexane evaporated leaving the dried material on the glass. When the physiological fluid was added, the compounds may not have been dissolved and thus were not extracted efficiently. The greater polarity of PCP compared to HCB and the partial recovery of PCP with no recovery of HCB when the compounds were added to vials prior to the physiological fluid would favor this rationalization. Since good recovery of PCP and HCB were obtained when spiked into the physiological fluid, the data on the serum and urinary levels should not have been compromised.

STATISTICAL EVALUATION OF SERUM AND UNCONJUGATED URINARY PCP LEVELS

The serum and unconjugated urinary PCP levels of the control individuals and test cases were statistically evaluated at the Center for Disease Control. For these evaluations, the residences of the log homes (test case 206A through 212A) and their corresponding control individuals (controls 206B, 207B, and 210B) were ommitted. Also, the three control individuals (controls 117B, 121B, and 224B) who were found to have ammunition crate wood in their residence were changed to test cases. The breakdown of the test cases, special cases, and control individuals by number, age, sex, and race is given in Table 16. A total of 51 test cases and 44 control individuals were evaluated. The statistical evaluation of the serum PCP levels and of the unconjugated urinary PCP levels is presented in Table 17. These data show little or no difference in the average serum or unconjugated urinary PCP levels of the test cases and the control individuals.

The possible correlation between the serum and unconjugated urinary PCP levels was evaluated by plotting the serum level versus the urinary level as shown in Figure 16. A correlation coefficient of 0.1206 (1.000 being a perfect fit) indicates that the serum PCP levels do not correlate with unconjugated urinary level. When the outliners, i.e., those values statistically outside the average value, were excluded as shown in Figure 17, a correlation coefficient of 0.3025 was obtained. Likewise, when the natural logarithm of the serum and unconjugated urinary PCP level were compared (Figure 18), no correlation (0.0823 correlation coefficient) in the values was obtained.

PCP LEVELS IN HYDROLYZED URINES

The normal means of urinary excretion of PCP would be as a conjugate, i.e., glucuronide or sulfate adduct through the phenolic group.

TABLE 15. RECOVERY VALUES FOR PENTACHLOROPHENOL AND HEXACHLOROBENZENE IN SIMULATED FIELD SPIKES

Sample	Pentachlorophenol			Hexachlorobenzene		
	Level spiked (ppb[a])	Level recovery (ppb)	Recovery (%)	Level spiked (ppb)	Level recovery (ppb)	Recovery (%)
Serum:						
Bottle spiked[b]	16	22	*[e]	15.5	N.D.[f]	--
Bottle spiked	16	22	*	15.5	N.D.	--
Serum spiked and frozen[c]	16	29	*	15.5	14	90
Serum spiked immediately before extraction[d]	40	59	*	38.8	36	93
Bottle spiked	80	60	*	77.3	N.D.	--
Bottle spiked	80	58	*	77.3	N.D.	--
Serum spiked and frozen	80	84.3	*	77.3	70	91
Serum spiked immediately before extraction	200	163	*	193	160	83
Urine:						
Bottle spiked	8	5.7	55	7.8	N.D.	--
Bottle spiked	8	6.6	66	7.8	N.D.	--
Urine spiked and frozen	8	8.7	92	7.8	6.5	83
Urine spiked and frozen	8	8.7	92	7.8	6.5	83
Urine spiked immediately before extraction	40	34	82	38.8	37	95
Urine spiked immediately before extraction	40	38	92	38.8	44	113
Bottle spiked	40	25	59	38.8	N.D.	--
Bottle spiked	40	34.2	82	38.8	N.D.	--
Urine spiked and frozen	40	39	94	38.8	19.2	50
Urine spiked and frozen	40	42	102	38.8	22	57
Urine spiked immediately before extraction	200	180	89	193	153	79

(continued)

TABLE 15 (continued)

Sample	Pentachlorophenol Level spiked (ppb[a])	Pentachlorophenol Level recovery (ppb)	Pentachlorophenol Recovery (%)	Hexachlorobenzene Level spiked (ppb)	Hexachlorobenzene Level recovery (ppb)	Hexachlorobenzene Recovery (%)
Urine spiked immediately before extraction	200	183	91	193	153	79
Urine blank	--	1.6		--	N.D.	
Urine blank	--	0.9		--	N.D.	

a ppb - parts per billion (ng/ml), sensitivity - PCP, 0.4 ng/ml; HCB, 0.4 ng/ml.

b Spiking solutions added to glass vials with Teflon-lined septa. Six days after spiking the bottles, urine or serum was added to the vials and the samples frozen. Seven days after adding the samples to the vials, the samples were extracted.

c Serum and urine samples added to clean glass bottles and the spiking solutions added directly into the samples. The samples were frozen and extracted seven days after the samples were spiked.

d Serum and urine samples spiked immediately prior to extraction.

e Recovery values could not be calculated due to loss of blank serum sample.

f N.D. - none detected, < 0.4 ng/ml.

TABLE 16. TEST CASES AND CONTROL INDIVIDUALS FOR THE PENTACHLOROPHENOL STUDY IN RICHMOND, KENTUCKY

	Cases	Controls	Special Cases	Special Case Control	Total
Age					
0-9	5	6	3	-	14
10-19	13	10	-	-	23
20-29	10	7	3	-	20
30-39	7	7	1	3	18
40-49	7	5			12
50-59	6	8			14
60+	3	1			4
Sex					
Male	18	17	3	1	39
Female	33	27	4	2	66
Race					
White, not hispanic	50	40	7	3	100
Black, not hispanic	0	3			3
Unknown	1	1			2
Total	51	44	7	3	105

TABLE 17. SERUM AND UNCONJUGATED URINARY PENTACHLOROPHENOL CONCENTRATION (ppb) FOR CASES AND CONTROLS

	Test Cases[a]	Controls[b]
Serum:		
Number	45	42
Range	2.20 - 54.6	4.32 - 67.9
Median	18.7	23.35
Arithmetic mean	20.99	24.19
Standard deviation (S.D.)	11.27	12.79
Geometric mean	18.19	20.70
Median log concentration	2.929	3.150
Mean log concentration	2.901	3.030
S.D. log concentration	0.572	0.610
Urinary (Unconjugated):		
Number	51	44
Range	0.6 - 6.6	0.7 - 11.0
Median	1.5	2.0
Arithmetic mean	1.83	2.34
Standard deviation (S.D.)	1.23	1.67
Geometric mean	1.56	2.00
Median log concentration	0.405	0.693
Mean log concentration	0.446	0.693[c]
S.D. log concentration	0.541	0.534

a Cases - does not include seven special cases; does include three control individuals later changed to test cases when ammunition crate wood was located in their residences.

b Controls - does not include three controls for the seven special cases.

c $p < 0.05$ 2-tailed unpaired t-test ($t = 2.24$, $df = 93$).

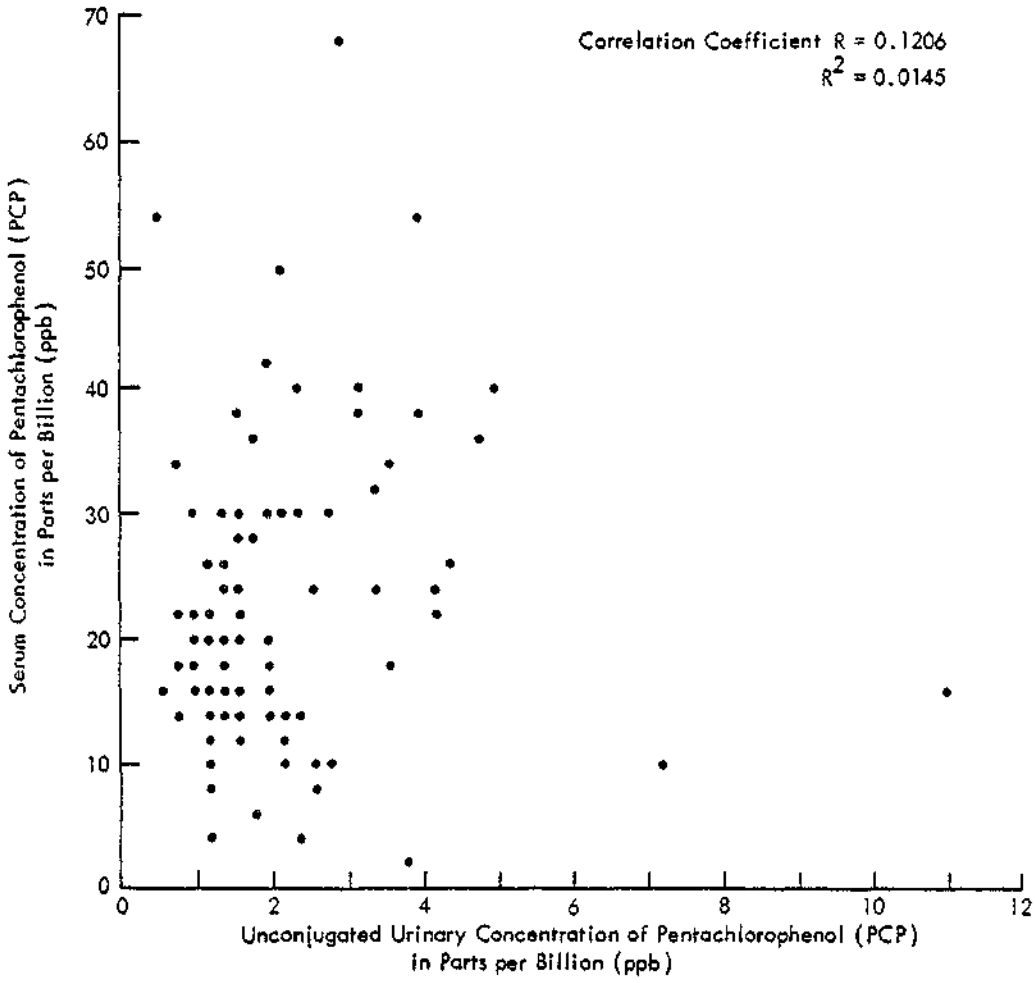

Figure 16. Correlation of serum and urinary (unconjugated) concentrations of pentachlorophenol.

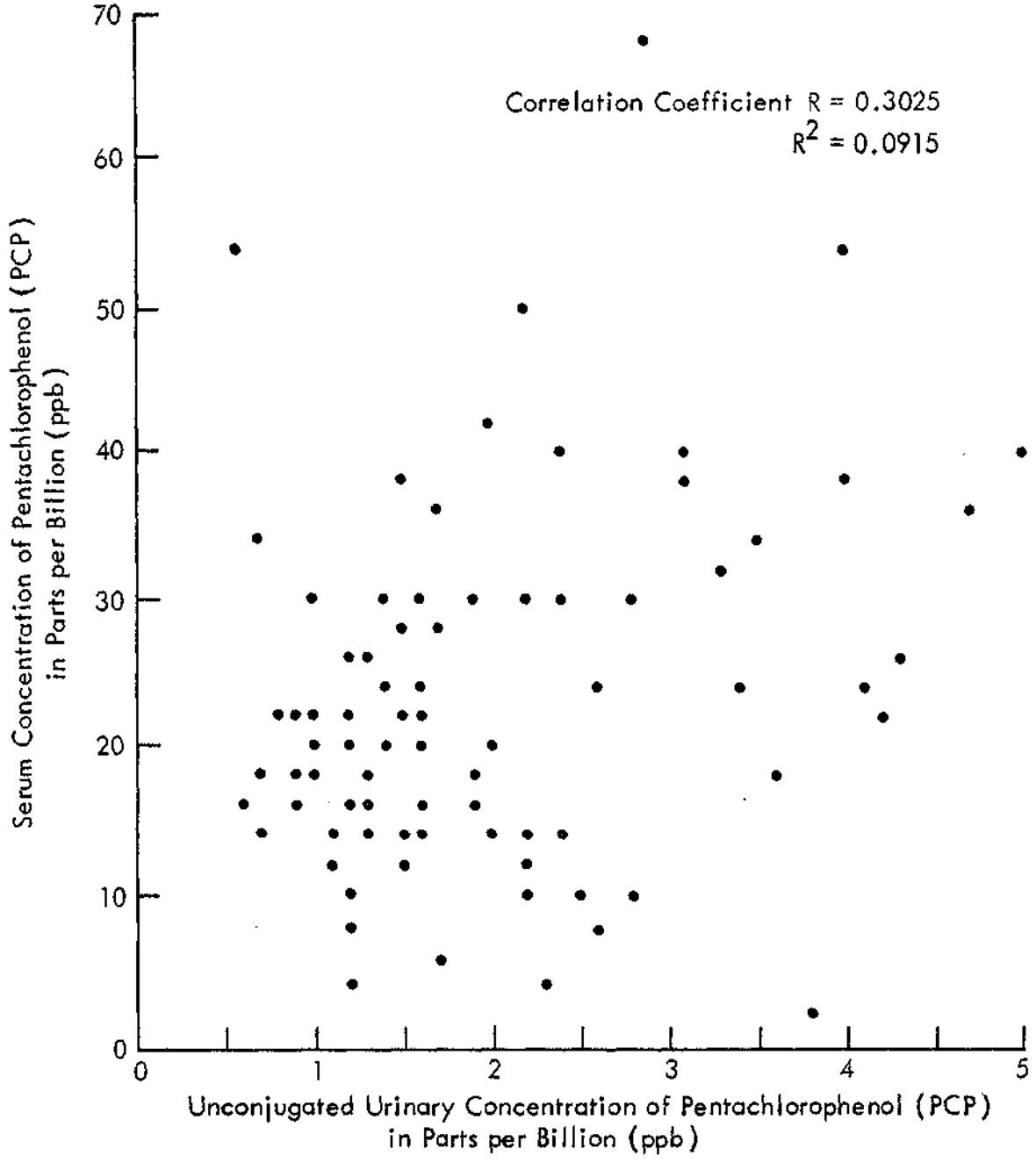

Figure 17. Correlation of serum and urinary (unconjugated) concentrations of pentachlorophenol excluding outliers.

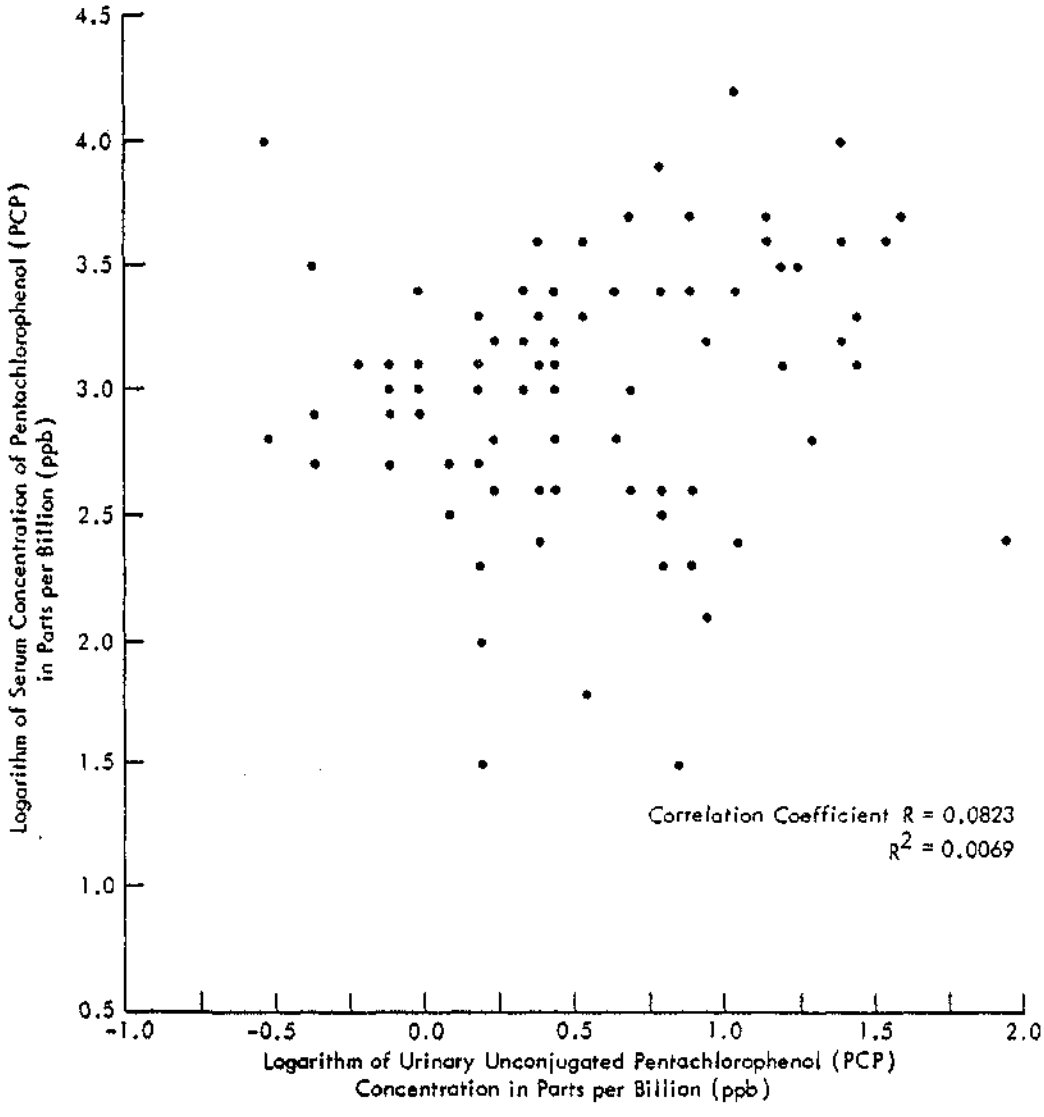

Figure 18. Correlation of logarithms of serum and urinary (unconjugated) concentrations of pentachlorophenol.