Chlorobenzenes* (1,2- and 1,4-Dichlorobenzene, 1,2,4-Trichlorobenzene)

Application	Determination in blood
Analytical principle	Gas chromatography
Completed in	December 1982

Summary

This is a sensitive and quick method for the determination of 1,2-dichlorobenzene; 1,4-dichlorobenzene; and 1,2,4-trichlorobenzene in blood. Extraction with n-heptane is followed by gas chromatographic separation either on packed or capillary columns and determination with an ECD.

Calibration standards are made up in n-heptane. This method permits a precise (relative standard deviations below 5.3%) and analytically accurate determination of chlorobenzenes in blood, as demonstrated by recovery rates in the 80–100% range.

1,2-Dichlorobenzene

Within-series imprecision:	Standard deviation (rel.)	s = 4.1–2.5%
	Prognostic range	u = 8.6–5.2%
	At concentrations ranging from 0.29–2.32 mg/L 1,2-dichlorobenzene in blood and where n = 20 determinations	
Inaccuracy:	Recovery rate	r = 80–95%
Detection limit:	0.05 mg/L 1,2-Dichlorobenzene in blood	

1,4-Dichlorobenzene

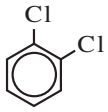
Within-series imprecision:	Standard deviation (rel.)	s = 5.3–2.3%
	Prognostic range	u = 11.1–4.8%
	At concentrations ranging from 0.25–1.96 mg/L 1,4-dichlorobenzene in blood and where n = 20 determinations	
Inaccuracy:	Recovery rate	r = 84–100%
Detection limit:	0.05 mg/L 1,4-Dichlorobenzene in blood	

* Additional chlorobenzenes can be determined if a capillary column is used for the gas chromatographic analysis.

1,2,4-Trichlorobenzene

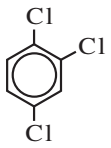
Within-series imprecision:	Standard deviation (rel.)	s = 2.6–2.3%
	Prognostic range	u = 5.4–4.8%
	At concentrations ranging from 0.06–0.45 mg/L 1,2,4-trichlorobenzene in blood and where n = 20 determinations	
Inaccuracy:	Recovery rate	r = 88–95%
Detection limit:	0.01 mg/L 1,2,4-Trichlorobenzene in blood	

Chlorobenzenes



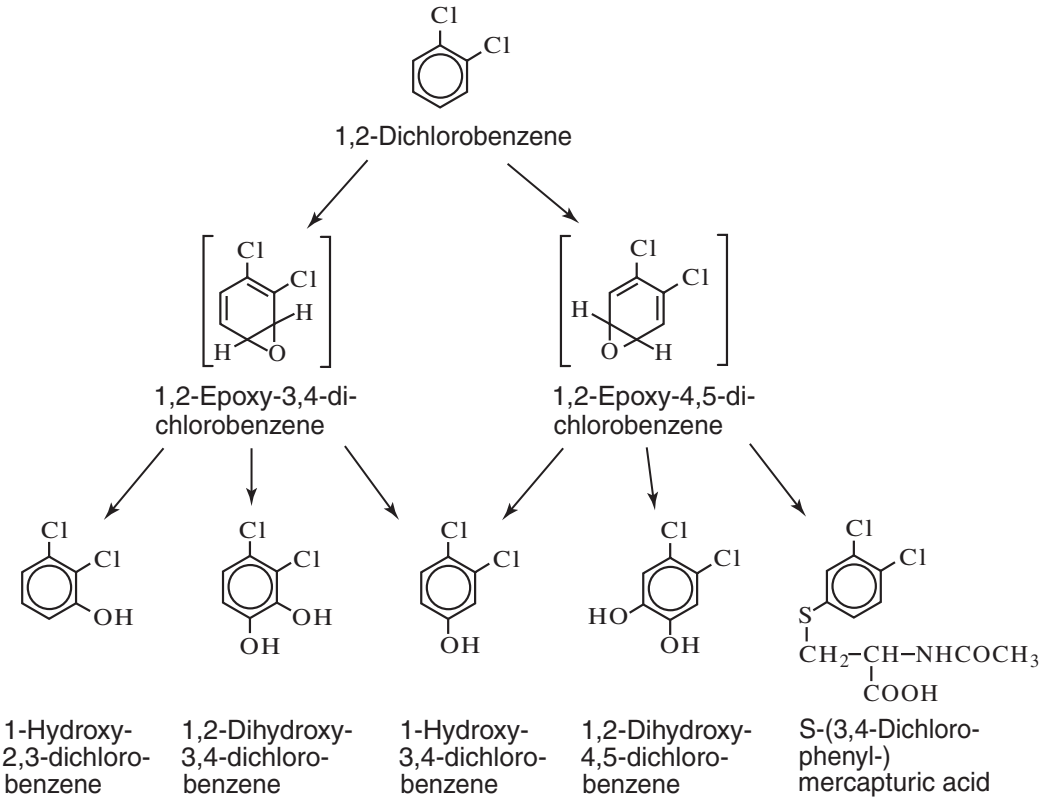
1,2-Dichlorobenzene

1,4-Dichlorobenzene



1,2,4-Trichlorobenzene

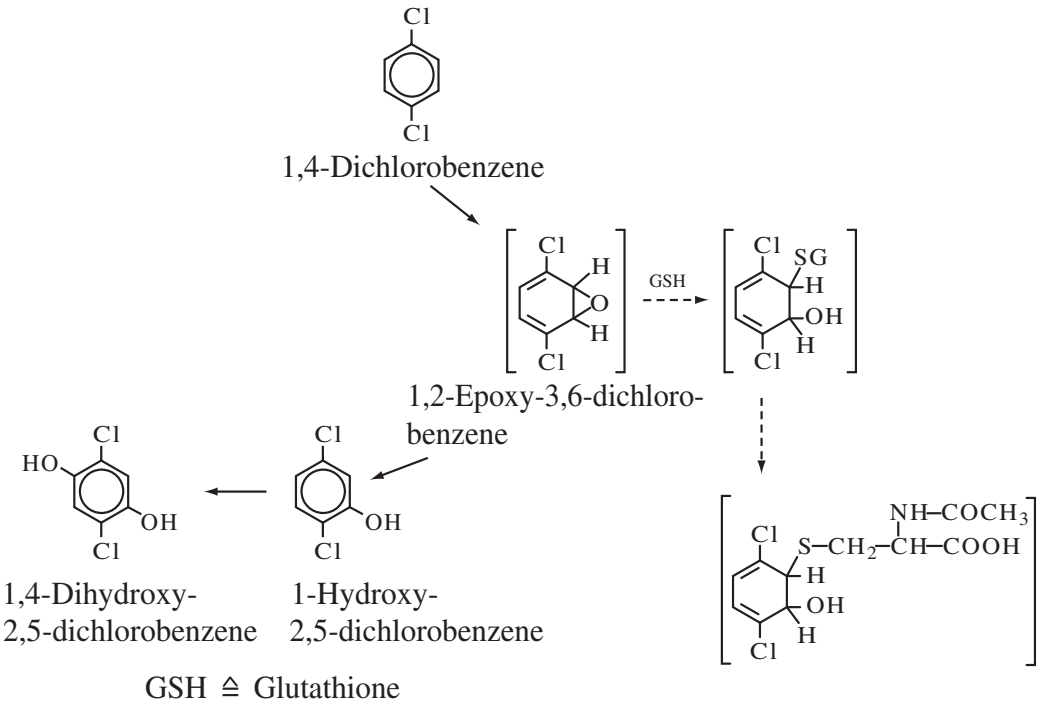
1,2-Dichlorobenzene is a liquid (bp 179.2°C, molar mass 147 g/mol) and an excellent solvent for waxes, resins, gums, oils, asphalts and fats. It serves as an intermediate in the manufacture of dyes. Its use as an insecticide (against termites) is limited because its p-isomer which is less toxic to humans may be used instead. The MAK (Maximum Concentration Value at the Workplace) for 1982 was 50 mL/m^3 (ppm) or 300 mg/m^3. In mammals it is metabolized according to the following pathway [1, 2]:



2,3-Dichlorophenol (1-hydroxy-2,3-dichlorobenzene), the primary metabolite of 1,2-dichlorobenzene, occurs in urine in glucuronide or sulfate form.

1,4-Dichlorobenzene is a white crystalline material, which sublimes at ordinary temperatures (mp 54 °C, bp 174.5 °C, molar mass 147 g/mol) and has a characteristic penetrating odor. It is used as an insecticide (especially against the clothes moth), a disinfectant and a deodorizer. The MAK for 1982 was 75 mL/m^3 (ppm) or 450 mg/m^3.

2,5-Dichlorophenol (1-hydroxy-2,5-dichlorobenzene), the primary metabolite of 1,4-dichlorobenzene, is excreted via the kidneys as the glucuronide or sulfate [2–4]:



Traces of 2,4-dichlorophenol are reported to be produced, too. The occurrence of the metabolites shown in brackets has not yet been verified [4].

Dichlorobenzene vapors cause irritation of the skin and mucous membranes, produce narcosis -and damage the nervous system.

1,2,4-Trichlorobenzene is a liquid (bp 212–213 °C, molar mass 181.45 g/mol) and is used as an insecticide. Its MAK for 1982 was 5 mL/m^3 (ppm) or 40 mg/m^3.

2,4,5-Trichlorophenol and 2,3,5-trichlorophenol (or 1-hydroxy-2,4,5-trichlorobenzene and 1-hydroxy-2,3,5-trichlorobenzene, respectively) have been identified as metabolites [5, 6]. 1,2,4-Trichlorobenzene is itself a metabolite of Lindane (γ-hexachlorocyclohexane). The toxicological data for 1,2,4-trichlorobenzene are summarized in [7].

1,2,4-Trichlorobenzene and 1,4-dichlorobenzene can initiate enzyme induction in the liver [8].

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Contents

* Additional chlorobenzenes can be determined if a capillary column is used for the gas chromatographic analysis.

1 General principles

For the analysis of 1,2-dichlorobenzene; 1,4-dichlorobenzene; and 1,2,4-trichlorobenzene the blood sample is extracted with n-heptane. The chlorobenzenes are then separated using a gas chromatograph and determined with the help of an electron-capture detector (ECD). The quantitative analysis is based on calibration standards in n-heptane.

2 Equipment, chemicals and solutions

2.1 Equipment

Gas chromatograph with electron capture detector (^{63}Ni), chart recorder or integrator
Stainless steel column: Length, 1.8 m; Inner diameter, 2.2 mm
Column packing: 5% DC 200 on Chromosorb W AW/DMCS, 80–100 mesh
or
Quartz capillary: Length, 30 m; Inner diameter, 0.33 mm; Stationary phase SE30, chemically bonded; Film thickness, 0.25 µm
10 µL Syringe for gas chromatography
Centrifuge
10 mL Graduated test tubes with ground-glass stoppers
Glass beads
10, 20 and 100 mL Volumetric flasks
0.1 and 0.5 mL Automatic pipettes (e.g., Eppendorf)
0.2 0.5, 1, 2, 4 and 5 mL Transfer pipettes for preparation of the calibration standards

2.2 Chemicals

Ethanol, p.a.
n-Heptane, p.a.
1,2-Dichlorobenzene, chem. pure
1,4-Dichlorobenzene, chem. pure
1,2,4-Trichlorobenzene, chem. pure
Argon/methane (90/10) or purified nitrogen gas (99.999%)

2.3 Calibration standards

Starting solution:
In a weighing bottle about 40 mg dichlorobenzene and 20 mg trichlorobenzene is weighed out exactly and transferred with ethanol to a 20 mL volumetric flask, which is

then filled to the mark with ethanol (2 g/L for 1,2-dichlorobenzene and 1,4-dichlorobenzene; 1 g/L for 1,2,4-trichlorobenzene).
Stock solution:
A 0.1 mL sample of the starting solution is pipetted into a 100 mL volumetric flask containing 50 mL n-heptane, and n-heptane is added to the mark (2 mg/L for 1,2-dichlorobenzene and 1,4-dichlorobenzene; 1 mg/L for 1,2,4-trichlorobenzene).
The following pipetting schedule provides a guide for the preparation of calibration standards containing the individual chlorobenzenes in n-heptane in a concentration range of 0.025–1.0 mg/L:

Stock solution	Calibration standard		
		Concentration of	
Volume	Final volume	1,2- and 1,4-Dichlorobenzene	1,2,4-Trichlorobenzene
mL	mL	mg/L	mg/L
0.5	20	0.05	0.025
1.0	20	0.10	0.050
2.0	20	0.20	0.10
2.0	10	0.40	0.20
4.0	10	0.80	0.40
5.0	10	1.00	0.50

3 Specimen collection and sample treatment

Venous blood is drawn with a disposable syringe containing a suitable amount of anticoagulant.

A 0.5 mL sample of venous blood is pipetted into a 10 mL test tube, containing 1 mL n-heptane and three small glass beads. This is shaken vigorously for 2 min and then centrifuged for about 3 min at 3000 min^{-1}.

4 Operational parameters for gas chromatography

4.1 Packed column

Column:	Material:	Stainless steel
	Length:	1.80 m
	Inner diameter:	2.2 mm
Column packing:	5% DC200 on Chromosorb W/AW-DMCS, 80–100 mesh	

Detector:	Electron-capture detector (ECD) with ^{63}Ni	
Temperature:	Column	130 °C
	Injection block	200 °C
	Detector	250 °C
Carrier gas:	90% argon + 10% methane	
	Flow rate: 30 mL/min	
Sample volume:	1 µL	

The following retention times can be used as a guide:

1,2-Dichlorobenzene	2.19 min
1,4-Dichlorobenzene	1.97 min
1,2,4-Trichlorobenzene	4.04 min

Figure 1 shows gas chromatograms from blood extracts using a packed column.

4.2 Capillary column

Capillary column:	Material:	Quartz
	Length:	30 m
	Inner diameter:	0.33 mm
	Stationary phase:	DB-1 (SE 30), chemically bonded; film thickness 0.25 µm
Detector:	Electron-capture detector (ECD) with ^{63}Ni	
Temperatures:	Column	120 °C
	Injection block	200 °C
	Detector	300 °C
Split:	1:20	
Carrier gas:	Purified nitrogen	
	Flow rate: 2 mL/min; make-up gas, 40 mL/min	
Sample volume:	1 µL	

The following retention times can be used as a guide:

Monochlorobenzene	3.15 min
1,2-Dichlorobenzene	4.50 min
1,4-Dichlorobenzene	4.30 min
1,2,4-Trichlorobenzene	6.85 min

Figure 2 gives an example of a gas chromatogram made with a capillary column.

5 Analytical determination

The instruments are set to the given operational parameters and 1 µL samples of each n-heptane extract are injected into the gas chromatograph.

6 Calibration

Calibration standards made up in n-heptane (see Sect. 2.3) are analyzed directly in the gas chromatograph. The area of the peak corresponding to the characteristic retention time of each chlorobenzene (see Sect. 4) is computed either with an integrator or by multiplying peak amplitude by its width at half amplitude. The value for peak area is then divided by the dilution factor 2 and these corrected values are plotted as a function of the concentration of the calibration standard to give a calibration curve. Examples of such calibration curves for the three chlorobenzenes are shown in Fig. 3.

7 Calculation of the analytical result

The peak area (amplitude times width at half amplitude or integrator computation) of the signal at the characteristic retention time of each chlorobenzene is computed and the corresponding concentration of chlorobenzene in blood (mg/L) is read off the appropriate calibration curve.

8 Reliability of the method

The following reliability criteria were determined using a packed column.

8.1 Precision

To determine the within-series imprecision, two blood samples, containing different concentrations of one chlorobenzene, were analyzed 20 times each. The standard deviations and prognostic ranges of these analyses are listed in Table 1 for each of the three chlorobenzenes tested.

8.2 Accuracy

Recovery experiments were performed to check the accuracy of the method. For each chlorobenzene five blood samples, containing different but known concentrations of that

chlorobenzene, were analyzed using the curves plotted from the calibration standards in n-heptane. The recovery rates for each chlorobenzene are given in Table 2.

8.3 Detection limit

The detection limits are about 0.05 mg/L for 1,2-dichlorobenzene and 1,4-dichlorobenzene in blood and about 0.01 mg/L for 1,2,4-trichlorobenzene.

8.4 Sources of error

The following solvents caused no interference at concentrations of about 1 g/L blood: acetone, ethanol, ethyl ether, benzene, n-hexane, methanol, acetic acid ester and carbon disulfide. The following chlorinated hydrocarbons and phenols also did not interfere at concentrations of about 2 mg/L blood: trichloroethene; 1,2,2-trichloroethane; tetrachloroethene; 1,3-dichloropropene; tetrachloromethane; trichloromethane; dichloromethane; 1,1,1-trichloroethane; 1,1,2-trichloroethane; 1,1,2-trichloro-l,2,2-trifluoroethane; 2,6-dichlorophenol; 2,4-dichlorophenol; 2,3-dichlorophenol; 3,4-dichlorophenol, 2,4,6-trichlorophenol; 3,4,5-trichlorophenol; 2,4,5-trichlorophenol; 2,3,4,5-tetrachlorophenol; 2,3,4,6-tetrachlorophenol; pentachlorophenol; benzotrichloride.

The determination of 1,4-dichlorobenzene in the presence of benzyl chloride and 1,1,2,2-tetrachloroethane was not possible with the packed column used here.

9 Discussion of the method

There is no literature on the determination of 1,2-dichlorobenzene; 1,4-dichlorobenzene; and 1,2,4-trichlorobenzene in human blood. This simple-to-use method makes it possible to determine quickly the concentration of three chlorobenzenes in the blood of occupationally exposed persons. Additional chlorobenzenes can be separated and determined, using the flexible capillary column described here.
Instruments used:
Gas chromatograph 5710 A with ^{63}Ni-ECD and integrator 3350 A from Hewlett Packard

10 References

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[8] *G. P. Carbon* and *R. G. Tardiff:* Effect of chlorinated benzenes on the metabolism of foreign organic components. Toxicol. Appl. Pharmacol. *36,* 383 (1976).

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Table 1. Within-series imprecision of analyses of chlorobenzenes in blood.

Chlorobenzene	n	Concentration in blood mg/L	Standard deviation (rel.) %	Prognostic range %
1,2-Dichlorobenzene	20	0.289	4.1	8.6
		2.315	2.5	5.2
1,4-Dichlorobenzene	20	0.245	5.3	11.1
		1.960	2.3	4.8
1,2,4-Trichlorobenzene	20	0.056	2.6	5.4
		0.445	2.3	4.8

Table 2. Inaccuracy of analyses of chlorobenzenes in blood.

Chlorobenzene	Calculated value mg/L	Measured value mg/L	Recovery rate %
1,2-Dichlorobenzene	0.116	0.093	80
	0.232	0.220	95
	0.463	0.380	82
	0.926	0.824	89
	1.852	1.722	93
1,4-Dichorobenzene	0.098	0.082	84
	0.196	0.196	100
	0.392	0.332	85
	0.784	0.682	87
	1.568	1.380	88
1,2,4-Trichlorobenzene	0.045	0.040	89
	0.089	0.082	92
	0.178	0.166	93
	0.356	0.313	88
	0.712	0.676	95

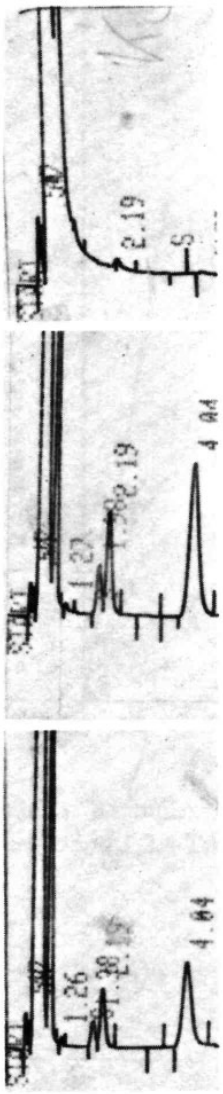

Fig. 1. Gas chromatograms of blood extracts using the packed column.
a) Control blood extract
b) Extract of a blood sample after addition of:
0.463 mg/L 1,4-Dichlorobenzene (A)
0.392 mg/L 1,2-Dichlorobenzene (B)
0.178 mg/L 1,2,4-Trichlorobenzene (C)
c) Extract of a blood sample after addition of:
0.232 mg/L 1,4-Dichlorobenzene (A)
0.196 mg/L 1,2-Dichlorobenzene (B)
0.089 mg/L 1,2,4-Trichlorobenzene (C)

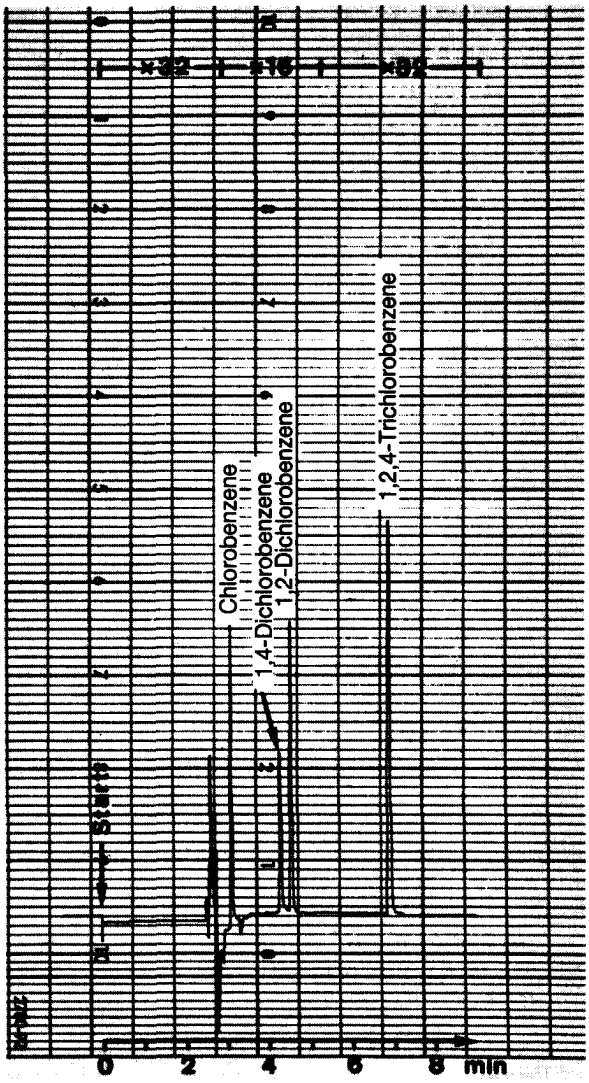

Fig. 2. Gas chromatogram of a blood extract using the capillary column.
Concentrations: 1.61 mg/L Chlorobenzene in blood
1.75 mg/L 1,2-Dichlorobenzene in blood
1.62 mg/L 1,4-Dichlorobenzene in blood
0.95 mg/L 1,2,4-Trichlorobenzene in blood

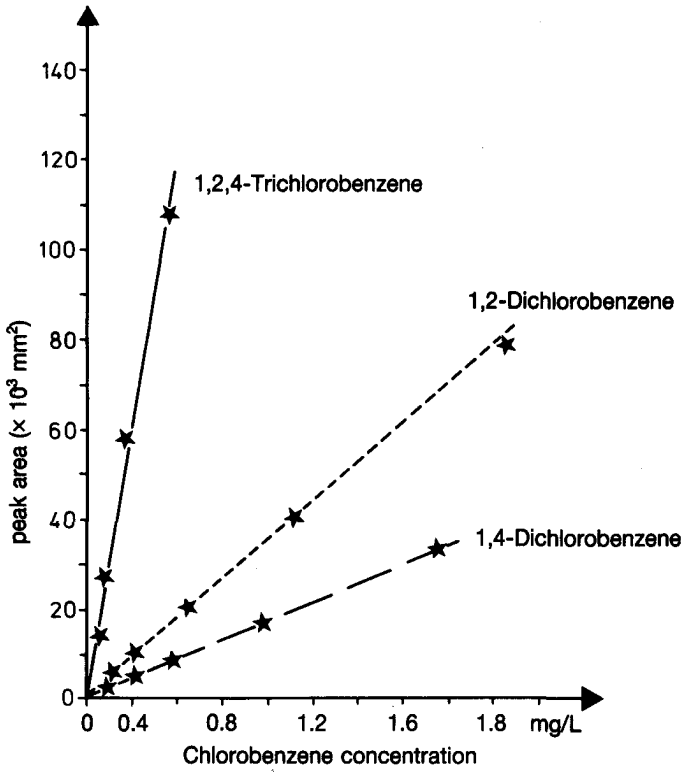

Fig. 3. Examples of calibration curves for the determination of 1,2-Dichlorobenzene; 1,4-Dichlorobenzene; and 1,2,4-Trichlorobenzene in blood.