



## Measurement of carbon monoxide and cyanide in blood

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### Introduction

Carbon monoxide and hydrogen cyanide are often found in fire atmospheres. These gases can incapacitate rapidly and thus render the victim unable to escape from the fire. High concentrations of carbon monoxide and hydrogen cyanide found in the blood of fire victims indicate a degree of survival in the toxic fire atmosphere. Concentrations may be sufficiently high in the blood to attribute death to carbon monoxide or hydrogen cyanide poisoning. The measurement of these substances in blood is quite straightforward but changes after death may raise or lower the cyanide concentration in the blood significantly. It is the impact of these processes that renders the cyanide level so difficult to interpret in many situations. This uncertainty in cyanide concentration may be responsible for the lack of correlation between cyanide and carboxyhaemoglobin concentrations in the blood of fire victims.

Carbon monoxide deaths may also occur following inhalation of exhaust fumes and cyanide deaths may follow ingestion of cyanide salts. The concentrations in blood and tissue in these circumstances may greatly exceed those encountered in fire death situations.

### Carbon monoxide

Carbon monoxide binds reversibly to haemoglobin to form carboxyhaemoglobin. The concentration is expressed as the percentage of haemoglobin saturated with carbon monoxide. Because carbon monoxide binds about 200 times more strongly than oxygen, a low level of atmospheric carbon monoxide will produce, given time, a comparatively high proportion of carboxyhaemoglobin, possibly with fatal results due to tissue hypoxia.

Carboxyhaemoglobin is normally present in the blood as the result of haem catabolism. Usually this level is less than 1% saturation, but patients with haemolytic anaemia may have concentrations greater than 5%. Exogenous uptake of carbon monoxide is due almost exclusively to inhalation. Common

sources are cigarette smoke, vehicle exhaust fumes, poorly maintained heating appliances and toxic smoke. Occupational exposure may result from the absorption of methylene chloride (paint stripper) and its metabolism to carbon monoxide.

Carbon monoxide is excreted almost entirely through the lungs, the half life being four to five hours. This may be reduced to 50 minutes by inhaling 100% oxygen, and to 22 minutes by inhaling hyperbaric (2.5 atmospheres) oxygen.

### MEASUREMENT OF CARBON MONOXIDE IN BLOOD

Analysis of clinical blood samples should present no great difficulty provided that the method is sufficiently sensitive at low carboxyhaemoglobin saturations. Post mortem blood that is aged or putrefied may contain pigments that resist chemical conversion into components that are commonly measured by spectrophotometry; in these cases gas chromatography may be the only reliable method. The use of derivative spectrophotometry, however, reduces the effects of extraneous pigments and so makes the spectrophotometric approach more applicable to post mortem blood. Recent methods have used the Soret region where absorption is 10 times more intense than in the visible region, allowing smaller sample volumes to be used.<sup>1,2</sup>

Recommendation of spectrophotometric methods has proved a problem because my laboratory uses the IL 282 Co-oximeter and a gas chromatographic method. An evaluation of zero order and derivative spectrophotometric methods has recently been made, however,<sup>1</sup> and I have drawn heavily on this work. A gas chromatographic method is described because it provides greater sensitivity at low carboxyhaemoglobin saturations, and is the method of choice for aged and contaminated post mortem blood. Furthermore, it can be used to measure carboxyhaemoglobin concentrations in tissue in cases for which blood is not available.

### ZERO ORDER SPECTROPHOTOMETRY

Measurement of absorption is made at two

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wavelengths as only two components, carboxyhaemoglobin and reduced haemoglobin, are present in solution. Blood is mixed with diluent containing sodium dithionite which reduces both oxyhaemoglobin and methaemoglobin to reduced haemoglobin. Absorption is measured at 420 nm ( $\lambda$  max carboxyhaemoglobin) and 432 nm ( $\lambda$  max reduced haemoglobin). These wavelengths should be checked for the particular spectrophotometer in use by measuring the absorption spectra for 0% carboxyhaemoglobin and 100% carboxyhaemoglobin containing blood.

**Apparatus:**

A dual beam spectrophotometer with 1 cm cuvettes is required.

**Reagents:**

- 1 0.01 mol/l TRIS (hydroxymethyl) amino methane (THAM) 99–99.5%, (Sigma Chemical Co, Poole BH17 7BR).
- 2 Sodium dithionite, about 80% (Sigma Chemical Co).
- 3 100% carbon monoxide (Air Products, Walton-on-Thames).
- 4 Diluent: dissolve 2.5 mg/ml sodium dithionite in 0.01 mol/l THAM just before use.

**Procedure:**

- 1 Add 10  $\mu$ l of blood to 20 ml of diluent, mix by gentle inversion.
- 2 Record the absorption spectrum at 420 and 432 nm within 2–3 minutes, using diluent as blank.
- 3 All specimens and controls are run in duplicate.

**Calculation:**

The following relation has been derived.<sup>2</sup>

$$\% \text{COHb} = 100 \times \frac{1 - (\text{Ar} \times F_1)}{[\text{Ar}(F_2 - F_1) - F_3] + 1}$$

Where

$$\text{Ar} = \frac{\text{Abs}_{420}}{\text{Abs}_{432}} \quad F_1 = 1.333 \quad F_2 = 0.4787 \quad F_3 = 1.9939$$

The values for  $F_1$ ,  $F_2$ , and  $F_3$  have been obtained from previous work.<sup>3</sup> It is recommended that these values are calculated for the instrument being used for the most accurate results.

**Calibration:**

Fresh blood obtained from a non-smoker is decarboxylated by passing laboratory air through it for 30 minutes (21% oxygen in nitrogen; Air Products). The blood may be exposed to ultraviolet light to encourage dissociation of carboxyhaemoglobin.

**Procedure:**

- 1 Add 10  $\mu$ l of negative blood to 20 ml diluent, mix by gentle inversion.
- 2 Record the absorption spectrum at 420 and 432 nm within 2–3 minutes.
- 3 Take 5 ml diluted blood and bubble carbon monoxide for 30 minutes. (Must be done in a fume cupboard).
- 4 Record the absorption spectrum (at 420 and 432 nm)

$$F_1 = \frac{\text{Abs}_{432}^{\text{Hb}}}{\text{Abs}_{420}^{\text{Hb}}} \quad F_2 = \frac{\text{Abs}_{432}^{\text{COHb}}}{\text{Abs}_{420}^{\text{Hb}}} \quad F_3 = \frac{\text{Abs}_{420}^{\text{COHb}}}{\text{Abs}_{420}^{\text{Hb}}}$$

Special precautions to shield haemolysates in diluting solutions from air are not required because the absorptions are measured within 2–3 minutes. The method has been applied to clinical samples<sup>2</sup> and fresh post mortem blood.<sup>1</sup> The method is weak for measuring low carboxyhaemoglobin concentrations, but has the advantages of speed and ease of performance. No equipment other than a modern dual beam spectrophotometer is required.

**SECOND DERIVATIVE SPECTROPHOTOMETRY**

Dilution of blood is carried out, as in the previous method. The absolute derivative value is recorded at 420 nm, a portion of diluted blood is then saturated with carbon monoxide, and the absolute derivative value is recorded again. Comparison of the two values gives the percentage saturation of carboxyhaemoglobin.

**Apparatus:**

A dual beam spectrophotometer capable of recording derivative spectra with 1 cm cuvettes is required.

**Procedure:**

Reagents have been described in the previous method.

- 1 Add 10  $\mu$ l of blood to 20 ml of diluent, mix by gentle inversion.
- 2 Record the second derivative absorption spectrum from 400 to 450 nm within 2–3 minutes. Use diluent as blank.
- 3 Saturate 5 ml diluted blood by bubbling carbon monoxide gas for 30 minutes in a fume cupboard.
- 4 Record the second derivative absorption spectrum from 400 to 450 nm using diluent as blank.
- 5 All specimens and controls are run in duplicate.

**Calculation:**

$$\% \text{COHb} = \frac{\text{Absolute derivative value at 420 nm for specimen}}{\text{Absolute derivative value at 420 nm for saturated specimen}} \times \frac{100}{1}$$

In this method only the absorption from carboxyhaemoglobin is measured. This absorption is sharper in the second derivative spectrum than in the zero order spectrum. The method is similar to that previously described and requires a spectrophotometer of little more complexity. The method is rapid and has been used for clinical and fresh post mortem blood.

With post mortem or aged blood the sodium dithionite must convert all methaemoglobin and oxyhaemoglobin to reduced haemoglobin and remove all oxygen from the diluent. The concentration and quality of the dithionite reagent is of critical importance. To ensure the integrity of the dithionite reagent it is recommended that sodium dithionite should be used from a tightly sealed container kept in a desiccator.

The powder should be weighed and diluted accurately for reproducible results. Dithionite should be exposed to air for as little time as possible before dilution and should be mixed in diluting solution and used immediately without excessive introduction of air at any stage during the analysis.

#### AUTOMATED DIFFERENTIAL SPECTROPHOTOMETRY

Instrumentation Laboratory (Warrington WA3 7PB) market a purpose-built instrument called the Co-oximeter. This is a convenient, rapid, and reliable method for determining carboxyhaemoglobin in clinical and all but the most grossly contaminated post mortem specimens.<sup>4</sup>

#### GAS CHROMATOGRAPHY

All gas chromatographic methods rely on the liberation of carbon monoxide from a given volume of lysed blood by mixing it with a releasing agent. The carbon monoxide is released into a headspace and the volume is measured chromatographically. The volume of carbon monoxide that would be released from the saturated specimen can be measured directly or calculated from the haemoglobin or total iron content of the specimen. The carbon monoxide released may be measured by thermal conductivity detection or by flame ionisation detection. The reduction of carbon monoxide to methane and its subsequent measurement by flame ionisation detection has the advantage of increased sensitivity, useful in the measurement of low saturations of carbon monoxide from small volumes of blood.<sup>5</sup> The method described here uses a thermal conductivity detector.<sup>6</sup>

#### Apparatus:

A gas chromatograph fitted with thermal conductivity detectors and a gas sampling loop is required. The columns are 2 m × 4.6 mm in diameter, Molecular Sieve 5A, 80/100 mesh. (Perkin Elmer Ltd Beaconsfield HP19 1QA). Helium carrier gas flow rate is 35 ml/minute, oven temperature is 100°C, and zone temperature is 125°C.

#### Reagents:

All reagents are BDH (Poole BH15 1TD) Analar grade unless otherwise stated.

- 1 Saponin (not AR): 10 mg/ml made up in water immediately before use.
- 2 Degassing reagent: 20 g anhydrous sodium carbonate and 20 g sodium hydrogen carbonate dissolved in 400 ml distilled water with stirring. Potassium ferricyanide (15 g) are added and the volume is made up to 550 ml. Finally 5 ml of triton X-100 are added. The reagent is kept in a brown bottle at room temperature and is stable for three months.
- 3 Standard carbon monoxide mixture: 8% carbon monoxide in nitrogen; PS standard calibration gas mixture (Phase Separations Ltd, Deeside, Clwyd CH5 2NU).

#### Procedure:

- 1 Blood or control (1 ml) is mixed with 1 ml saponin solution for 10 minutes.

- 2 The solution is centrifuged for 15 minutes at 3000 rpm.
- 3 Supernatant fluid (1 ml) is transferred to a 5 ml disposable plastic syringe leaving a 1 ml air space.
- 4 Degassing reagent (3 ml) are added to a second syringe.
- 5 The two syringes are connected together by a short length of tight fitting plastic tubing.
- 6 All the contents are transferred to one syringe.
- 7 The two syringes, still connected, are rotated on a rotary mixer for 10 minutes.
- 8 The 1 ml air space is transferred to one syringe and is carefully injected into a gas sampling loop for injection on to the gas chromatograph. Losses and deadspace at this stage must be minimised if accurate results are to be obtained.
- 9 The haemoglobin in the remaining supernatant fluid is measured by the cyanmethaemoglobin procedure.
- 10 All specimens and controls are run in duplicate.

Peaks elute in the order oxygen-nitrogen-carbon monoxide. The retention time for carbon monoxide is about 5 minutes. The separation between carbon monoxide and nitrogen decreases as moisture is absorbed by the column; optimal separation may be restored by heating the column at 250°C with reduced carrier gas flow overnight.

#### Calculation:

The peak height (area) of the carbon monoxide is recorded for the specimen or control. This peak height is compared with the peak height of carbon monoxide in a standard gas mixture.

$$\frac{\text{Peak height of CO in sample}}{\text{Peak height of CO in standard}} \times \text{volume CO in standard} = \text{volume of CO in 0.5 ml sample}$$

To calculate the carbon monoxide capacity from haemoglobin depends on the knowledge that 1 molecule of haemoglobin combines with 4 molecules of carbon monoxide. Given 1 mole of gas occupies 22.4 litres at normal temperature and pressure and the molecular weight of haemoglobin is 64500:

64500 g haemoglobin combine with 4 × 22.4 = 89.6 litres carbon monoxide,

1 g haemoglobin combines with 1.39 ml carbon monoxide, therefore

1 ml fully carboxylated blood will release

$$\frac{\text{Hb (g/dl)} \times 1.39}{100} \text{ ml carbon monoxide}$$

$$\% \text{COHb} = \frac{\text{volume of carbon monoxide in 1ml sample}}{\text{volume of carbon monoxide in 1 ml fully carboxylated sample}} \times \frac{100}{1}$$

It is clear that the gas chromatographic procedure is not quick to perform and is therefore not suited to emergency situations. Specialised equipment is required which may not be routinely available in the clinical laboratory. For the analysis of old and grossly contaminated blood or in the analysis of tissue when blood is not available, the gas

Table 1 Normal (mean) concentrations

Authors	Method	n =	Non-smokers	n =	Smokers
McCredie & Jose <sup>7</sup>	GCTCD	70	0.8 (0.29)	23	4.13 (1.99)

Table 2 Concentrations in fire fatalities

Authors	Method	n =	Mean	Low	High
Dominguez <sup>8</sup>	GCFID	85	59	25	85
Mayes <sup>9</sup>	GCTCD	54	39.6	8	79

Table 3 Concentrations in car exhaust suicides

Author	Method	n =	Mean	Low	High
Mayes (unpublished)	IL282	10	71.5	60	82
	GCTCD	10	75.3	62	90

chromatographic procedure has to be done. For those engaged in forensic analysis, however, it is good laboratory practice to use two independent methods—in this case spectrophotometry and gas chromatography—for measuring an analyte.

#### CALIBRATION MATERIALS

The accuracy of the quantitative result depends on the quality of the calibration materials. Many methods depend on the preparation of blood containing 0% and 100% carboxyhaemoglobin. Fresh blood that has been diluted, haemolysed, and centrifuged may be effectively carboxylated by passing 100% carbon monoxide. If old blood is to be carboxylated it must be treated with sodium dithionite to convert methaemoglobin to haemoglobin because methaemoglobin will not bind carbon monoxide. For preparation of 0% carboxyhaemoglobin fresh blood from a non-smoker that has been diluted, haemolysed, and centrifuged may have laboratory air (21% oxygen in nitrogen) bubbled through it to remove carbon monoxide. The blood can be illuminated with ultraviolet light to encourage dissociation of carboxyhaemoglobin. Intermediate concentrations of carboxyhaemoglobin may be prepared by mixing appropriate volumes of 0% and 100% saturated blood. Care must be taken to minimise losses of carbon monoxide to the atmosphere from highly carboxylated blood by exposing it to the air for as little time as possible.

#### QUALITY CONTROL MATERIAL

Commercial materials are available from two sources.

- Instrumentation Laboratory (Warrington WA3 7PB). This company markets four solutions under the name Multi 4 Co-oximeter control: level 1 ranges 55 to 65% COHb; level 2 ranges from 1 to 7% COHb; level 3 ranges from 20 to 26% COHb; and level 4 ranges from 95 to 100% COHb.
- Dade Quanta Plus Whole Blood Gas Controls (Baxter Diagnostics Ltd) (available through B M Brown Ltd, Reading

RG7 4AB). Three levels are supplied; level 1 ranges from 11.9 to 15.9% COHb; level II ranges from 1.3 to 5.3% COHb; and level III ranges from 38.6 to 42.6% COHb.

In both cases the controls are made from human haemoglobin in buffer. A full range of control material should always be run when analysis of specimens is carried out.

#### REFERENCE RANGES

It is clear that hypoxia caused by carbon monoxide displacing oxygen in haemoglobin, together with the Haldane effect (shift of oxygen dissociation curve to left, reducing ability of haemoglobin to release oxygen to tissues), is not the complete answer. Under certain circumstances a toxic mechanism operates at the cytochrome level. This may be responsible for the wide variation of carboxyhaemoglobin saturations found in fatalities. For normal concentrations see table 1. Concentrations for non-smokers range from 0 to 3% and those for smokers range from 3 to 10% saturation.

For concentrations in fire fatalities, see table 2. 10% saturation is a useful cutoff to separate fatalities who have not inhaled toxic smoke from those who have. The fatal threshold for inhalation of carbon monoxide in fire deaths has been taken to be 50% saturation, but fatalities occur with saturations ranging from 10 to 90%. Table 3 shows the concentrations found in cases of car exhaust suicide. These are higher than those in fire fatalities, suggesting that other agents in fire atmospheres have a role in bringing about death.

#### STORAGE OF SPECIMENS

Clinical specimens by their nature are analysed within a short time of receipt. It is always best to analyse post mortem specimens as soon as possible to reduce the effect of any changes in carboxyhaemoglobin concentrations during storage. Carbon monoxide can be lost from blood exposed to air, the loss depends on the area exposed and the volume of blood. Blood should be stored refrigerated in stoppered containers with a minimal headspace. If specimens are to be kept for some time they should be frozen.

#### EFFECTS OF PUTREFACTION

Experiments with dogs immersed in sea water for four days have shown that carboxyhaemoglobin concentrations are not substantially changed during decomposition. Other experiments with rats immersed in river water for one month have produced concentrations up to 19.8% carboxyhaemoglobin in blood. My experience in the analysis of badly putrefied blood is that production of carbon monoxide after death is uncommon.

#### PRESERVATION OF SPECIMENS

Clinical specimens should be collected into anticoagulant, either EDTA or heparin. To prevent the possible production of carbon monoxide in post mortem blood during storage it should be preserved with 1% sodium fluoride. Analysis of both anticoagulated and

preserved blood in forensic cases is useful in interpretation. The possibility of site dependence of carboxyhaemoglobin saturations should be considered. Blood specimens should be annotated as to their source and be obtained from extremities, if possible, to avoid the effects of contamination from the gut.

#### Measurement of cyanide in post mortem blood following inhalation of toxic smoke

Hydrogen cyanide gas is produced in toxic fire atmospheres by the combustion of nitrogen containing organic molecules. Fires with high temperatures and low concentrations of oxygen produce comparatively high concentrations of the gas. Inhalation of hydrogen cyanide presents the danger of a rapid "knock down" effect, preventing escape from the vicinity of the fire. Hydrogen cyanide exerts its toxic effect by disrupting electron transport in the mitochondrion; it inhibits cytochrome c oxidase, thus affecting cellular respiration.

Low concentrations of cyanide are found in the blood of normal people, mainly associated with the red cells. Its presence is due to normal metabolism, eating cyanide-producing food (for example, bitter almond kernels) and smoking cigarettes. The major method of metabolism is by enzyme transsulphuration to thiocyanate which is excreted in the urine. Thiocyanate has not proved useful as an indicator of transformed cyanide in investigations of fire deaths.<sup>10</sup>

#### ANALYSIS OF CYANIDE IN BLOOD

Methods use the fact that hydrogen cyanide is a weak acid ( $pK_a$  9.20). Thus acidification of blood (or tissue) will give off gaseous hydrogen cyanide which may be chromatographed directly or trapped in alkali to be derivatised later and quantitated by formation of a coloured complex.

#### SEPARATION OF HYDROGEN CYANIDE FROM BLOOD

A simple and effective method for releasing hydrogen cyanide from blood is by Conway Diffusion. (Conway Diffusion Cells are manufactured by Bel Art Products, available from Radleys Laboratory Equipment and Scientific Glassblowing Shire Hill, Saffron Waldon, Essex CB11 3AZ).

##### Reagents:

All reagents are Analar grade available from BDH.

- 1 Sulphuric acid: 10% v/v.
- 2 Sodium hydroxide: 0.1 mol/l.

##### Procedure:

- 1 The Bel Art Conway Diffusion Cells have an outer well which is filled with water to act as a seal with the plastic lid. Four drops of 10% sulphuric acid should be added to the outer well to prevent hydrogen cyanide dissolving in the water seal.
- 2 Blood (2 ml), standard or blank, are added to the middle well.

- 3 0.1 mol/l sodium hydroxide (2 ml) are added to the centre well.
- 4 Six drops of 10% sulphuric acid are added to the middle well, not directly into the blood, if possible.
- 5 The lid is placed on to the cell quickly.
- 6 The cell is rocked gently to mix the blood and acid.
- 7 Diffusion is allowed to proceed for 4 hours at room temperature.

#### COLOURIMETRIC PROCEDURE<sup>11</sup>

##### Apparatus:

A dual beam spectrophotometer reading at 580 nm with 1 cm cuvettes is required.

##### Reagents:

All reagents are Analar grade from BDH except chloramine T (Sigma Chemical Co) and barbituric acid (GPR grade).

- 1 Sodium dihydrogen orthophosphate solution 1 mol/l.
- 2 Chloramine T solution 0.25% w/v (keep refrigerated).
- 3 Pyridine-barbituric acid reagent. To a 50 ml volumetric flask add 3 g barbituric acid, 15 ml pyridine, and 3 ml concentrated hydrochloric acid. Dilute to 50 ml with water and mix with a magnetic stirrer until clear. Prepare this reagent in a fume cupboard, fresh each day.

##### Procedure:

- 1 Take 1 ml of alkaline solution from the centre well of the Conway Diffusion Cell.
- 2 Add 2 ml sodium dihydrogen orthophosphate solution.
- 3 Add 1 ml of chloramine T solution.
- 4 Vortex and stand for 3–4 minutes.
- 5 Add 3 ml pyridine-barbituric acid reagent.
- 6 Vortex and stand for 10 minutes.
- 7 Read the absorbance of the purple solution at 580 nm against a blank that has been taken through the whole procedure.
- 8 Standards of 0.25 to 5 mg/l are included. All determinations are done in duplicate. A graph of optical density against mg/l cyanide is plotted. The concentration of specimens is obtained from the calibration graph.

#### STANDARD SOLUTIONS

Commercial standard solutions are not available for control of cyanide analysis. Recovery of cyanide from whole blood spiked with cyanide is poor.<sup>12</sup> Standard solutions of potassium cyanide made up in aqueous alkaline solutions are stable for three months and can be used for quantitation. This is not a satisfactory situation and it is, of course, better to use a similar matrix for the standards. Transformation of cyanide seems to occur in the plasma, thus to reduce loss of recoverable cyanide in spiked blood the red cells may be separated from the plasma and the cells dissolved in an alkaline buffer.

##### Reagents:

Reagents other than blood are BDH Analar grade.

Table 4 Normal concentrations of cyanide in fatalities

Authors	Method	n =	Mean(mg/l)	Low	High
Anderson & Harland <sup>10</sup>	GCMS	19	0.08	0	0.22

Table 5 Cyanide concentrations in fire fatalities

Authors	Method	n =	Mean(mg/l)	Low	High
Anderson & Harland <sup>10</sup>	GCMS	139	1.24	0	6.43
Mayes <sup>9</sup>	Colourimetric	54	2.75	0.55	8.40

- 1 Fresh blood or recently expired blood bank blood.
- 2 Disodium hydrogen orthophosphate solution 0.01 mol/l pH adjusted to 11.5 with 10 mol/l sodium hydroxide.
- 3 Sodium hydroxide solution 10 mol/l.
- 4 Sodium chloride solution 0.9% w/v.
- 5 Potassium cyanide solution 2.4094 g/l equivalent to 1 g/l hydrogen cyanide. Always add 1 ml of 10 mol/l sodium hydroxide to the flask before adding the potassium cyanide and water.

*Procedure:*

- 1 Blood (10 ml) are mixed with 10 ml sodium chloride solution by inversion and centrifuged for 10 minutes at 2000 rpm.
- 2 The supernatant fluid is discarded.
- 3 Washing is repeated at least twice more.
- 4 0.01 mol/l sodium phosphate buffer, pH 11.5 (10 ml) are added to the washed red cells.
- 5 The solution is mixed for 5 minutes and centrifuged for 10 minutes at 2000 rpm.
- 6 The supernatant fluid is retained. It is most economical to prepare at least 6 × 10 ml of lysed cells for standard production simultaneously.

## WORKING CYANIDE SOLUTION

The stock potassium cyanide solution is diluted to produce a working cyanide solution. Dilute 10 ml stock solution to 100 ml with water. Add 100 µl of 10 mol/l sodium hydroxide solution to the flask before the addition of water to the stock solution. The working cyanide solution is made up fresh before use.

*Preparation of spiked standards:*

mg/l hydrogen cyanide	µl working standard added to 10 ml of prepared blood
0.25	25
0.5	50
1.0	100
2.0	250
5.0	500

These standards are stable when refrigerated for up to four weeks.

GAS CHROMATOGRAPHIC PROCEDURE<sup>13</sup>*Apparatus:*

A gas chromatograph fitted with a 2 m × 4.6 mm internal diameter column packed with Porapak Q 100/120 Mesh (Perkin Elmer, Beaconsfield HP19 1QA) is required. Oven temperature 165°C; detector/injector temper-

ature 250°C; carrier gas helium flow 30 ml/minute; nitrogen selective detection.

*Reagents:*

All reagents are BDH Analar grade.

1 Methyl cyanide (acetonitrile) internal standard. Stock solution 200 µl/l in water. Working internal standard: 20 ml stock solution diluted to 100 ml with water. Prepare fresh before analysis.

2 Orthophosphoric acid.

*Procedure:*

- 1 Blood (ml) standard or blank, is placed in a headspace phial, 10 ml capacity.
- 2 Aqueous methyl cyanide internal standard (1 ml) is added.
- 3 Phosphoric acid (100 µl) is added carefully to the side of the phial.
- 4 The phial is crimped closed with a PTFE lined rubber septum before the phosphoric acid reaches the diluted blood.
- 5 The phial is mixed and equilibrated for 30 minutes at 60°C.
- 6 Headspace (1 ml) is injected on to the column. The hydrogen cyanide elutes in about 1.5 minutes and the methyl cyanide elutes in about 4.5 minutes. The area or height ratio of hydrogen cyanide to methyl cyanide is plotted for the range of standards 0.25 to 5 mg/l. A straight line is obtained for standards in this range.

## REFERENCE RANGES

The fatal threshold for inhalation of hydrogen cyanide gas in fire fatalities is taken to be 2.70 mg/l. Table 4 shows the normal concentrations in fatalities.

The normal concentrations measured by Anderson and Harland were measured in post mortem blood from fatalities not involving fire. Table 5 shows the cyanide concentrations in fire fatalities.

## STORAGE AND PRESERVATION OF SPECIMENS

A prolonged period between death and post-mortem may result in a substantial reduction of cyanide<sup>14</sup> due to the following processes: evaporation; thiocyanate formation; reaction with aldehydes and ketones; hydrolysis to form ammonium formate; transformation to aminomalonnitrile.

These losses may continue during the storage of blood in the laboratory. Changes are minimised by storage at -20°C.<sup>15</sup> Formation of concentrations up to 150 mg/l in blood has been observed,<sup>16</sup> attributed to production by micro-organisms. This can be prevented by preservation of blood with 1% sodium fluoride. In my view concentrations greater than 10 mg/l of cyanide observed in toxic smoke inhalation fatalities are due largely to postmortem production.<sup>17</sup> Blood should be preserved with sodium fluoride and kept frozen until analysis is performed.

## EMERGENCY DETERMINATION OF BLOOD CYANIDE

The Conway Diffusion-Colourimetric method has been adapted<sup>18</sup> for emergency situations, using a diffusion time of 10 minutes. The

authors indicate that 50–60% of cyanide is recovered in this time and suggest that the result is suitable to determine the need for treatment.

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