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VII. APPENDIX I

SAMPLING AND ANALYSIS OF CARBON MONOXIDE (CO)

Air Sampling Methods

Worker exposure to CO shall be measured with a portable, direct reading, hopcalite-type carbon monoxide meter calibrated against known concentrations of CO, or with gas detector tube units certified under Title 42 of the Code of Federal Regulations, Part 84. Samples shall be collected from the worker's breathing zone and a sufficient number shall be collected at random intervals throughout the workday so that a statistically accurate determination of compliance may be made as outlined in the following section.

Principles for Air Sampling for Carbon Monoxide (CO)

The characteristic manner in which CO proceeds from the pulmonary alveolar spaces, through the blood capillary membrane "barrier," into the plasma, through the red blood cell membrane, ultimately to combine with hemoglobin imposes certain requirements for air sampling if proper determination of compliance with the recommended standard is to be made. The rate of CO diffusion from the lung to hemoglobin depends upon the partition coefficient for CO between alveolar air and pulmonary blood. The magnitude of this coefficient is such as to delay transfer of CO to the circulating hemoglobin (Hb). This time delay makes it essential in any estimation of the carboxyhemoglobin level to know how long the exposure was experienced as well as the CO concentration during the exposure. Reference to Figure 2 shows that a continuous exposure of over eight hours' duration is required to attain maximum combination of CO with hemoglobin at the recommended standard of 35 ppm.

The sampling and analytical procedures recommended below will provide the necessary data to determine compliance with the recommended standard.

The methodology and equipment utilized to collect and analyze samples to determine concentrations of CO in the air or concentration of COHb in the exposed worker shall be subjected to a proficiency and/or calibration test program conducted by NIOSH or by another agency of the federal government under agreement with NIOSH for certification for such determinations.

Determination of Compliance

The following procedure shall be used to determine compliance with the eight-hour, time-weighted average standard based on a small number of instantaneous (grab) samples collected at random intervals during the workday.

Given: the results of n samples with a mean m and a range (difference between least and greatest) r. If, for from 3 to 10 samples, m is greater than the total of:

A. The standard

B. The percentage of systematic instrument error multiplied times the standard

C. $\frac{t \times r}{n}$

then the true average concentration exceeds the standard ($p < 0.05$). The value of t is taken from the following table:

n (number of samples)	t (student's "t" test value)
3	2.35
4	2.13
5	2.01
6	1.94
7	1.89
8	1.86
9	1.83
10	1.81

For a large number of samples the procedure given in Section 3-2.2.1 of National Bureau of Standards (NBS) handbook 91 shall be followed.

IX. APPENDIX II

ANALYSIS OF CARBOXYHEMOGLOBIN (COHb)

Analytical Methodology

An extensive review of the methodology covering the determination of carbon monoxide has been prepared by Maehly.[124] In an updating review of this area published in 1967, Feldstein[125] presents a critical review of procedures for the analysis of samples of combustion effluents, air, exhaled breath and blood. The methods involved range from sophisticated analytical procedures to less complicated colorimetric and gravimetric techniques.

Because carboxyhemoglobin constitutes the toxic product formed during carbon monoxide intoxication, and because the carboxyhemoglobin formed is quite stable, the analyst is presented with the opportunity of directly evaluating the toxic agent. In so doing, two facts are established: (1) proof that an exposure has occurred, and (2) quantitative proof the exposure has produced a particular toxic concentration in the body. Diffusion, chromatographic and gasometric methods all involve liberation of carbon monoxide from hemoglobin and either gasometric transfer operations or secondary titrations which are technically difficult for the microliter gas volumes involved. This limitation is even more critical when the effects of temperature and pressure on gas volumes are considered. Careful evaluation of the methods for direct measurement of carboxyhemoglobin has been reviewed in detail. Of these methods, three are considered to be feasible for consideration as the recommended method.

(a) The colorimetric method of Amenta[126] uses small blood samples and measures the absorbance of an ammonia-hemolyzed sample at three wave-length settings on the spectrophotometer: 560, 575, and 498 nm. In calculating the results, the absorbance of the mixture at 498 nm (an isosbestic point) is the denominator in the equation for oxyhemoglobin, carboxyhemoglobin, and the unknown. This mathematical manipulation gives a value corrected for the total hemoglobin concentration (i.e., the R factor). The values (R_{O_2} = 1.097, R_{CO} = 0.057) are then used for calculating percentage COHb by the ratio $R_{O_2}-R_X/R_{O_2}-R_{CO}$x100 = percentage COHb. The relatively small absorbance difference observed with this method reduces its precision and accuracy, especially in the low COHb range (ca. 10 percent).

(b) The method of Harper[127] involves hemolysis of blood by distilled water, addition of a buffer (pH 7.2) and reduction of oxyhemoglobin by sodium hydrosulfite. The reduced hemoglobin is then converted to methemoglobin by the addition of potassium ferricyanide. The carboxyhemoglobin is converted to methemoglobin at a slower rate than reduced hemoglobin; therefore, the carboxyhemoglobin can be measured before appreciable conversion to methemoglobin takes place. The method requires a careful control of timed manipulations to obtain reproducible results.

(c) The method of Klendshoj, Feldstein and Sprague[128] is similar to and was published before Harper's method. In this method the oxyhemoglobin is reduced by the sodium hydrosulfite following hemolysis in dilute ammonia solution. The absorbances at 555 and 480 nm are compared to a diluted ammonia blank, and the percentage COHb is obtained from a previously constructed calibration curve. The absorbance difference between reduced hemoglobin and COHb represents a total difference 1.21 absorbance units. Methemoglobin does not interfere in the determination but the method cannot be used with hemolyzed samples. The precision and accuracy of the method are comparable with the Van Slyke gasometric method.[129] The sensitivity of the method is sufficient to detect 2 percent COHb with quantitative accuracy.

The method of Klendshoj, Feldstein and Sprague is recommended as the method of choice because it measures the physiologically active form of carbon monoxide directly with excellent sensitivity and accuracy in the area of biologic interest. In addition, it is a method free of difficult manipulations and does not require extraordinary laboratory equipment to obtain a precise and quantitative result.

Procedure

(a) Cleaning of glassware. All glassware shall be free of scratches and of any material that could potentially cause hemolysis. Rinsing in deionized water is usually sufficient.

(b) Collection and shipping of samples. Blood shall be collected using oxalated (or EDTA) evacuated test tubes. Blood for CO determination shall be drawn from a vein without stasis and shall be refrigerated immediately after collecting the sample. Samples shall be analyzed within 96 hours after collection. Samples may be shipped provided they reach the destination within 48 hours and are refrigerated upon arrival.

(c) Analysis of samples. One (1) ml of the oxalated blood is transferred to a 100 ml graduated flask and made up to volume with 0.4 percent ammonia. Three (3) ml of this solution is placed in a cuvette, ten (10) ml of sodium hydrosulfite is added, and read at once at 555 and 480 nm against 0.4 percent ammonia as a blank. The value of D555/D480 is calculated and the percentage of COHb is read from a prepared standard concentration-quotient curve.

Calibration and Standards

(a) Determination of Quotient D555/D480 for HbCO and reduced hemoglobin. Ten (10) ml of oxalated blood (or pooled samples) from sources known not to have been exposed to carbon monoxide are obtained. Five (5) ml are placed in each of two 250 ml separatory funnels. The air in one separatory funnel (A) is displaced with pure oxygen and tightly stoppered. The air in the second separatory funnel (B) is displaced with carbon monoxide and tightly stoppered. The two separatory funnels are rotated gently for 1/2 hour to ensure saturation with oxygen and CO respectively. A one (1) ml portion is diluted to 100 ml with 0.4 percent ammonia and analyzed according to procedure in Section (c) above. The quotient D555/D480 for reduced oxyhemoglobin should be approximately 3.15 ±0.05 and for "reduced" carboxyhemoglobin 1.94 ±0.05. Transfer and dilution should be performed as quickly as possible to avoid changes in the oxyhemoglobin concentrations.

(b) A calibration curve is constructed by mixing the following volumes of the blood from separatory funnels (A) and (B) from Section (a) and then performing an analysis as outlined in Section (c) above.

(A)	(B)	Percent COHb
1	0	0
0.9	0.1	10
0.8	0.2	20
0.5	0.5	50

A calibration curve of the percent COHb vs. the quotient D555/D480 is then plotted in linear fashion. Although the curve does not give a linear presentation over this range, readings between increments will give values correct to ±2 percent of the amount present.

Calculations

Concentrations shall be read directly from the curve or calculated from the following best fit formula of the curve:

$$\text{Conc. COHb(percent)} = mx + b$$

where m and b are determined by regression analysis of the ratios D555/D480 for all calibration standards and x is the sample D555/D480 ratio.

Apparatus

(a) Spectrophotometer with a band pass of 5 nm or less (2 nm is preferable)

(b) 1 cm path length cuvettes, volume - 3 ml

(c) 1 ml pipettes

(d) 100 ml graduated cylinders

(e) 1000 ml volumetric flask

(f) Spatula capable of transferring 10 mg solid reagent

(g) 250 ml separatory funnels

(h) Evacuated test tubes (10 ml) containing approximately 250 mg potassium oxalate

Reagents

(a) Cylinder of pure oxygen (medical or aviators breathing grade specification)

(b) Cylinder of pure carbon monoxide (certified 99 percent purity)

(c) Purified analytical grade sodium hydrosulfite ($Na_2S_2O_4$) (Sodium Dithionite)

(d) Concentrated ammonia (28%)

(e) 0.4% ammonia solution. Dilute 15 ml of conc. NH_3(28%) to 1 liter

Principle of the Method

Oxyhemoglobin in oxalated blood is completely reduced in the presence of small amounts of sodium hydrosulfite, whereas carboxyhemoglobin is not affected. The spectral absorbence curves of oxyhemoglobin (O_2Hb) and carboxyhemoglobin (COHb) are different. The ratio of the absorbance at 555 nm and 480 nm is directly proportional to the percent COHb in the blood.

Range and Sensitivity

For 1 ml of oxalated blood the range is 0 to 100 percent saturation COHb. Sensitivity is 0.5 percent saturation.

Interferences

Hemolyzed blood contains pigments arising from the breakdown of hemoglobin and will cause interference in the method. Bile pigments may also interfere.

Precision and Accuracy

The difference in concentrations obtained by this procedure and compared to results obtained on the same samples analyzed by the Van Slyke method are not significant at the 5 percent level [t-Value = 1.81 (14 deg. of Freedom)]. The average difference is 0.47 percent with a standard error of the mean of 0.26.

Advantages and Disadvantages of the Method

(a) The method is relatively fast, can be automated quite easily, requires few reagents, extensive training is not required, and does not require expensive analytical instruments nor reagents to obtain acceptable results.

(b) The hydrosulfite reagent is not very stable. Care must be exercised in collecting and storing the blood to prevent hemolysis. Pure air (or oxygen) and carbon monoxide gas must be available to prepare calibration standards. Interferences with the spectral absorption of COHb are possible, as aforementioned, especially if hemolysis occurs.

X. APPENDIX III

MATERIAL SAFETY DATA SHEET

The following items of information which are applicable to any tank or other device which contains or emits carbon monoxide shall be provided in the appropriate section of the Material Safety Data Sheet or approved form. If a specific item of information is inapplicable (i.e., flash point) initials "n.a." not applicable should be inserted.

(i) The produce designation in the upper left hand corner of both front and back to facilitate filing and retrieval. Print in upper case letters in as large print possible.

(ii) Section I. Name and Source.

(A) The name, address and telephone number of the manufacturer or supplier of the product.

(B) The trade name and synonyms for a mixture of chemicals, a basic structural material, or for a process material; and the trade name and synonyms, chemical name and synonyms, chemical family, and formula for a single chemical.

(iii) Section II. Hazardous Ingredients.

(A) Chemical or widely recognized common name of all hazardous ingredients

(B) The approximate percentage by weight or volume (indicate basis) which each hazardous ingredient of the mixture bears to the whole mixture. This may be indicated as a range of maximum amount, i.e., 10-20 percent V; 10 percent max. W.

(C) Basis for toxicity of each hazardous material (e.g., established OSHA standard), in appropriate units and/or LD_{50}, showing amount and mode of exposure and species or LC_{50} showing concentration, duration and species.

(iv) Section III. Physical Data.

(A) Physical properties of the total product including boiling point and melting point in degrees Fahrenheit; vapor pressure, in millimeters of mercury, vapor density of gas or vapor (air = 1), solubility in water, in parts per hundred parts of water by weight; specific gravity (water = 1); percentage volatile (indicate if by weight or volume) at 70° Fahrenheit; evaporation rate for liquids (indicate whether butyl acetate or ether = 1); and appearance and odor.

(v) Section IV. Fire and Explosion Hazard Data.

(A) Fire and explosion hazard data about a single chemical or a mixture of chemicals, including flash point, in degrees Fahrenheit; flammable limits, in percent by volume in air; suitable extinguishing media or agents; special fire-fighting procedures; and unusual fire and explosion hazard information.

(vi) Section V. Health Hazard Data.

(A) Toxic level for total compound or mixture, relevant symptoms of exposure, skin and eye irritation properties, principal routes of absorption, effects of chronic (long-term) exposure and emergency and first-aid procedures.

(vii) Section VI. Reactivity Data.

(A) Chemical stability, incompatibility, hazardous decomposition products, and hazardous polymerization.

(viii) Section VII. Spill or Leak Procedures.

(A) Detailed procedures to be followed with emphasis on precautions to be taken in cleaning up and safe disposal of materials leaked or spilled. This includes proper labeling and disposal of containers containing residues, contaminated absorbants, etc.

(ix) Section VIII. Special Protection Information.

(A) Requirements for personal protective equipment, such as respirators, eye protection and protective clothing, and ventilation, such as local exhaust (at site of product use or application), general, or other special types.

(x) Section IX. Special Precautions.

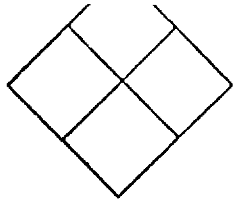
(A) Any other general precautionary information, such as personal protective equipment for exposure to the thermal decomposition products listed in Section VI, and to particulates formed by abrading a dry coating, such as by a power sanding disc.

(xi) The signature of the responsible person filling out the data sheet, his address, and the date on which it is filled out.

(xii) The NFPA 704M numerical hazard ratings as defined in Section (c)(5) following. The entry shall be made immediately to the right of the heading, "Material Safety Data Sheet" at the top of the page and within a diamond symbol preprinted on the forms.

DATA SHEET

Form Approved
Budget Bureau No.
Approval Expires
Form No. OSHA



SECTION I SOURCE AND NOMENCLATURE

MANUFACTURER'S NAME	EMERGENCY TELEPHONE NO.
ADDRESS (Number, Street, City, State, ZIP Code)	
TRADE NAME AND SYNONYMS	CHEMICAL FAMILY
CHEMICAL NAME AND SYNONYMS	FORMULA

SECTION II HAZARDOUS INGREDIENTS

BASIC MATERIAL	APPROXIMATE OR MAXIMUM % WT. OR VOL.	ESTABLISHED OSHA STANDARD	LD_{50}		LC_{50}	
			ORAL	PERCUT.	SPECIES	CONC.

SECTION III PHYSICAL DATA

BOILING POINT	°F.	VAPOR PRESSURE	mm Hg.
MELTING POINT	°F.	VAPOR DENSITY (Air=1)	
SPECIFIC GRAVITY (H_2O=1)		EVAPORATION RATE (________=1)	
SOLUBILITY IN WATER	Pts/100 pts H_2O	VOLATILE % Vol.	% Wt.
APPEARANCE AND ODOR			

SECTION IV FIRE AND EXPLOSION HAZARD DATA

FLASH POINT METHOD USED	FLAMMABLE (EXPLOSIVE) LIMITS	UPPER LOWER
EXTINGUISHING MEDIA		
SPECIAL FIRE FIGHTING PROCEDURES		
UNUSUAL FIRE AND EXPLOSION HAZARDS		

SECTION V HEALTH HAZARD DATA	
TOXIC LEVEL	CARCINOGENIC
PRINCIPAL ROUTES OF ABSORPTION	SKIN AND EYE IRRITATION
RELEVANT SYMPTOMS OF EXPOSURE	
EFFECTS OF CHRONIC EXPOSURE	
EMERGENCY AND FIRST AID PROCEDURES	

SECTION VI REACTIVITY DATA
CONDITIONS CONTRIBUTING TO INSTABILITY
CONDITIONS CONTRIBUTING TO HAZARDOUS POLYMERIZATION
INCOMPATIBILITY (Materials to Avoid)
HAZARDOUS DECOMPOSITION PRODUCTS

SECTION VII SPILL OR LEAK PROCEDURES
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED
WASTE DISPOSAL METHOD

SECTION VIII SPECIAL PROTECTION INFORMATION	
VENTILATION REQUIREMENTS LOCAL EXHAUST	PROTECTIVE EQUIPMENT (Specify Types) EYE
MECHANICAL (General)	GLOVES
SPECIAL	RESPIRATOR
OTHER PROTECTIVE EQUIPMENT	

SECTION IX SPECIAL PRECAUTIONS
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE
OTHER PRECAUTIONS

Signature ______________________ Address

Date ______________________