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Analysis of blood for asphyxiant toxicants — Carbon monoxide and hydrogen cyanide

Analyse du sang pour substances toxiques asphyxiantes — Monoxyde de carbone et acide cyanhydrique

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 27368 was prepared by Technical Committee ISO/TC 92, *Fire safety*, Subcommittee SC 3, *Fire threat to people and environment*.

Introduction

Carbon monoxide (CO) and hydrogen cyanide (HCN) are two of the primary toxic combustion gases present in fire atmospheres. Upon burning, carbon-containing substances generate CO, whereas nitrogen-containing substances also produce HCN. Since structures surrounding human beings are composed of polymeric materials containing carbon and nitrogen elements as their constituents, these materials generate CO and HCN upon burning and fire victims are exposed to these gases by inhaling smoke. Although ISO 19701 documents methods for the analysis of CO and HCN in fire effluents, the actual toxic insult to exposed persons can be assessed only by the analysis of the fire casualties' blood for CO as carboxyhaemoglobin (COHb) and HCN as cyanide ion (CN^-). These analytical findings are useful for

— estimating life-threatening characteristics of fire atmospheres,

— evaluating the degree of toxicity caused by smoke inhalation in fire victims,

— determining the cause and manner of death of fire victims,

— improving understanding of the direct causes of fire injury and death,

— enhancing understanding of acute and delayed adverse effects of smoke on fire casualties,

— administering immediate treatment for smoke poisoning and monitoring delayed adverse effects of smoke,

— choosing appropriate emergency, long-term and/or follow-up treatments for surviving fire casualties,

— setting priorities for emergency treatment of multiple fire casualties,

— establishing relationships between the concentrations of CO and HCN in a fire atmosphere, blood COHb and CN^- levels, and the degree of toxicity and performance impairment,

— achieving correlations between concentrations of the two gases in fire atmospheres and of COHb and CN^- in blood in order to improve tenability models,

— identifying deficiencies with materials, products, assemblies, structures and escape routes, and

— improving forensic toxicology analytical processes and procedures.

Compliance with this International Standard can help ensure a consistent data set for use in a variety of fields such as

a) fire statistics, which themselves are frequently used to develop regulatory policy,

b) international collaboration on improved design, materials and use of habitable structures, and,

c) ultimately, improvement of international relations and trades.

Such compliance can further assist in developing better and safer fire-safety instruments and structures (residential and commercial buildings; locomotive passenger vans, automobiles, aerospace vehicles and other vehicular structures).

Various different methods are currently used for obtaining blood analysis data for these two fire toxicants and the lack of standardized procedures can result in a wide variation of interpretation. It is, therefore, proposed to set out best-practice, standardized procedures for blood sample collection, sample storage, sample processing/preparation, sample treatment and transfer to analytical instrumentation, analytical instrumentation

and techniques, data presentation and reporting, and guidance for data interpretation. The analytical methods included herein are based upon their suitability for performing an analysis on ante-mortem and post-mortem blood samples from fire victims and are commonly used in forensic toxicological analytical operations.

This International Standard is structured as follows.

— Clause 1 describes the scope of this International Standard.

— Clause 2 cites the normative references.

— Clause 3 provides terms and their definitions.

— Clause 4 lists symbols and abbreviated terms.

— Clause 5 provides a general description of collecting, storing and analysing blood samples.

— Clause 6 covers the quality of materials used during an analysis.

— Clause 7 summarizes common quality analytical elements.

— Clause 8 describes analytical methods for measuring CO as COHb.

— Clause 9 delineates analytical methods for measuring HCN as CN^- in blood.

— Annex A (normative) lists the information crucial for reporting blood analysis results.

— Annex B (informative) outlines additional aspects of analytical methods.

— Annex C (informative) discusses the interpretation of results, including the interactive effects of CO and HCN.

— The bibliography includes references cited in this International Standard.

Analysis of blood for asphyxiant toxicants — Carbon monoxide and hydrogen cyanide

SAFETY PRECAUTIONS — Due consideration shall be given to the fact that both the blood samples for the analyses of asphyxiant toxicants, carbon monoxide (CO) and hydrogen cyanide (HCN), and many of the reagents used for their analyses can be biohazardous and/or toxic and can thereby pose serious health hazards. It is recommended that the collection of blood samples from fire victims be performed by medical practitioners and in accordance with best practices established by the medical authorities in the area. Additionally, it is assumed that the procedures described herein are carried out by suitably qualified professional personnel, adequately trained in the hazards and risks associated with the handling of biological samples and such analyses and aware of any safety regulations that can be in effect. Consideration shall also be given to the safe and ecologically acceptable disposal of all biological samples and chemicals used for analyses. This can require extensive and specific treatment prior to release of the waste into the environment. Again, it is assumed in this International Standard that the personnel responsible for the safe disposal of such bio-samples and reagents are suitably qualified and trained in these procedures and techniques and are aware of the regulations that can be in force.

1 Scope

This International Standard details analytical methods suitable for analysing the two primary toxic combustion gases, carbon monoxide (CO) and hydrogen cyanide (HCN), in blood samples collected from fire casualties. In blood, CO is measured as carboxyhaemoglobin (COHb) and HCN as cyanide ion (CN^-). Although numerous methods are reported in the literature for performing blood COHb and CN^- analyses, the analytical methods included herein are based upon their suitability for performing the analysis on ante-mortem and post-mortem blood samples from fire casualties. The analytical principle, analysis time, repeatability, reproducibility, robustness, effectiveness and instruments used are considered for those methods. Some of the methods described herein might not be suitable for analysing putrid or clotted blood. Burned (solid) blood can be analysed after homogenization.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 13344, *Estimation of the lethal toxic potency of fire effluents*

ISO/TS 13571, *Life-threatening components of fire — Guidelines for the estimation of time available for escape using fire data*

ISO 13943, *Fire safety — Vocabulary*

ISO 19701, *Methods for sampling and analysis of fire effluents*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 19701, ISO 13344, ISO/TS 13571, ISO 13943, ISO 3696, and the following apply.

3.1
analyte
substance that is being identified or determined in a specimen during an analysis

EXAMPLES COHb and CN^-.

3.2
analytical batch
set of aliquots taken out from the specimens associated with various cases (fire casualties) and from negative and positive blind controls for performing a particular type of analysis

3.3
asphyxiant
toxicant causing loss of consciousness and ultimately death resulting from hypoxic (deficiency-of-oxygen) effects, particularly on the central nervous and/or cardiovascular systems

3.4
blind controls
open controls but their identity is unknown to the analysts

See **open controls** (3.20).

3.5
calibrator
material that is based on, or traceable to, a reference preparation or material and whose values are determined by acceptable reference methods

3.6
carboxyhaemoglobin
compound formed when CO combines with haemoglobin

NOTE Haemoglobin has an affinity for binding to CO that is approximately 245 times higher than that for binding to oxygen; thereby the ability of haemoglobin to carry oxygen is seriously compromised during CO poisonings (see C.3.3 and Reference [73]).

3.7
Cheyne-Stokes respiration
breathing pattern characterized by rhythmic waxing and waning of the depth of respiration, with regularly recurring periods of breathing cessation

3.8
cutaneous blood vessels
blood vessels relating to, or affecting, the skin

3.9
cyanogenic glycosides
group of molecules containing a sugar moiety and a cyanide (CN) group

NOTE Cyanogenic glycoside can release the poisonous HCN gas if acted upon by some enzyme.

EXAMPLE Amygadlin from almond.

3.10
cyanomethaemoglobin
compound formed when CN^- combines with methaemoglobin

NOTE During the treatment of CN^- poisonings, haemoglobin is chemically converted to methaemoglobin, which easily binds with CN^-, producing cyanomethaemoglobin. The formation of cyanomethaemoglobin is an essential and critical step in the CN^- detoxification process (see Reference [71]).

3.11
cyanosis
bluish discoloration of the skin caused by the lack of oxygen in the blood

3.12
deoxyhaemoglobin
form of haemoglobin without oxygen, the predominant protein in the red blood cells

NOTE Haemoglobin forms an unstable, reversible bond with oxygen. The oxygen-bonded haemoglobin is known as oxyhaemoglobin. In the oxygen-unloaded form, it is called deoxyhaemoglobin and is purple-blue.

3.13
fire effluent
totality of gases and/or aerosols, including suspended particles, in the atmosphere resulting from combustion or pyrolysis

3.14
fractional toxic concentration
FTC
ratio of the percent of COHb in a blood sample to 70 % COHb (FTC_{COHb}) or of the concentration of CN^-, expressed in micrograms per millilitre, in a blood sample to 3,0 µg/mL CN^- (FTC_{CN^-})

NOTE It is considered that CO at 70 % COHb or HCN at 3,0 µg/mL CN^- individually can cause lethality. For an additive effect of a mixture of the two gases, FTC_{COHb} plus FTC_{CN^-} should be equal to unity. However, the above concept does not rule out other additive effects of these gases (see Clause C.5).

3.15
haemoglobin
biological substance in the red blood cells made up of iron and protein and involved in carrying oxygen to various parts of the body

NOTE Deoxyhaemoglobin or reduced haemoglobin is also referred as to haemoglobin.

3.16
isobestic point
wavelength at which the spectra of various species of a substance have the same absorbance

EXAMPLE The substance haemoglobin and its species oxyhaemoglobin and COHb.

3.17
methaemoglobin
particular type of transformed haemoglobin that is unable to bond with oxygen

NOTE Haemoglobin is converted to methaemoglobin by the oxidation of haemoglobin iron(II) (ferrous iron) into iron(III) (ferric iron). This oxidized form of haemoglobin is in firm union with water and is chemically unable to associate with oxygen; thus, it is ineffective for respiration. Large-scale conversion of haemoglobin to methaemoglobin can cause blueness of skin due to lack of oxygen.

3.18
methanation unit
unit capable of chemically converting CO into methane (CH_4) by using hydrogen in the presence of nickel as a catalyst

3.19
mydriasis
dilatation of the pupil

3.20
open controls
specimens prepared for the purpose of being used as a control and known to the analysts

3.21
oxyhaemoglobin
oxygen-bonded form of haemoglobin, the predominant protein in the red blood cells

NOTE Haemoglobin forms an unstable, reversible bond with oxygen. In its oxygen-loaded form, it is called oxyhaemoglobin and is bright red.

3.22
polymeric materials
materials composed of polymers

NOTE A polymer is a large molecule made up of many smaller repeating chemical units bonded together. These units are known as monomers. Some polymers are naturally occurring, while others are synthetically manufactured.

3.23
post-mortem interval
period after death

EXAMPLE Time between death and blood sample collection from a dead body.

3.24
putrefaction
decomposition of organic matter, especially protein, by microorganisms, resulting in the formation of substances of less complex constitution with the evolution of ammonia, hydrogen sulfide and other substances and, thus, in the production of foul-smelling matter

NOTE This process is usually characterized by the presence of malodorous smell.

3.25
pyocyaneous organisms
group of microorganisms capable of producing CN^-

3.26
reduced haemoglobin
haemoglobin in the red blood cells after the removal of oxygen from oxyhaemoglobin or after the reduction of iron(III) (ferric iron) in methaemoglobin to iron(II) (ferrous iron)

3.27
sulfaemoglobin
product formed by the action of hydrogen sulfide (or sulfides) on iron(III) (ferric iron) in methaemoglobin

NOTE This haemoglobin product is also known as sulfmethaemoglobin.

3.28
tachycardia
excessive rapidity in the action of the heart

3.29
tachypnea
excessive rapidity of respiration

3.30
thermostatization
process of automatic temperature regulation, especially wherein the expansive force of metals or gas acts directly upon the source of heat, ventilation or the like, or controls them indirectly by opening and closing an electric circuit

NOTE Derived from the term “thermostat”.

3.31
toxicants
poisonous substances capable of causing adverse, unwanted or undesired effect(s) on a living system

NOTE For the purpose of this International Standard, these substances are CO and HCN.

3.32
toxic insult
adverse, unwanted or undesired effect(s) on a living system due to, pertaining to, or of the nature of a poison

4 Symbols and abbreviated terms

A	Area
α	Absorbance
C	Concentration
CBI	1-Cyano-2-benzoisoindole or 1-cyano[*f*]benzoisoindole
ClCN	Cyanogen chloride
CN	Cyanide
CN^-	Cyanide ion
CO	Carbon monoxide
COHb	Carboxyhaemoglobin
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetate
F	Factor
FED(s)	Fractional effective dose(s)
FID	Flame ionization detector
FTC	Fractional toxic concentration
HCN	Hydrogen cyanide
HHb	Deoxyhaemoglobin
HPLC	High-performance liquid chromatograph

IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
MetHb	Methaemoglobin
MSD	Mass spectrometric detector
NDA	2,3-Naphthalenedialdehyde
NPD	Nitrogen phosphorus detector
OxyHb	Oxyhaemoglobin
R	Ratio
TCD	Thermal conductivity detector
tHb	Total haemoglobin
TIC	Total-ion chromatogram
V	Volume
w	Mass fraction

5 Blood samples

5.1 General

For the analyses of COHb and CN^-, blood from fire victims should be properly collected as soon as possible, preserved, stored and analysed as quickly as possible. See also C.3.1 and C.4.1.

5.2 Sample condition

Fresh blood samples can be easily obtained from live fire victims, but collecting quality blood samples from fire fatalities can frequently be challenging. This challenge is linked to the condition of the body, which is affected by the severity of burn, the time between the death and the discovery of the body (post-mortem interval), and the environmental factors, such as temperature and humidity. There are reports of the condition of blood, for example, fresh or putrid blood, having an impact on the outcome of the analyses. Therefore, the documentation of the history, condition and characteristics of the blood samples is crucial, and this information, along with the blood samples, should be submitted to the analytical laboratories performing analyses.

5.3 Sample collection

It is recommended that blood samples from fire casualties be preferably collected in 10 ml (or smaller size) sterile glass tubes containing heparin, or 20 mg of potassium oxalate and 100 mg of sodium fluoride, to prevent blood clotting and/or to preserve the specimens [1]. Some analytical methods use heparinized blood, while other methods can use blood treated with either heparin or potassium oxalate-sodium fluoride. The headspace in the tubes should be kept to a minimum and the tubes containing the blood samples should be airtight sealed to minimize dissociation of CO and HCN and to prevent any escape of these gases from the collected blood. Post-mortem blood samples can be collected from the heart, though no statistically significant difference has been observed between the COHb levels in post-mortem heart blood and peripheral blood specimens [2]. Regardless of the blood collection site, however, it is recommended that the sample collection site be mentioned in the documents submitted with the blood samples for analysis.

5.4 Sample storage

The blood specimens should be stored at 4 °C in the airtight, sealed containers to prevent the loss of CO, denaturation of haemoglobin and release of HCN [3],[4],[5],[6],[7],[8]. If it is necessary to store samples for a long period prior to analysis, then the samples should be frozen [3],[4],[5],[6],[9],[10],[11],[12],[13],[14].

5.5 Sample analysis

Analyses should be performed as quickly as possible after the collection of blood [9],[15],[16]. It is essential for the analysis of COHb to homogenize those blood samples that are not homogeneous. A similar recommendation has also been made for CN^- that autopsy blood should be homogenized before the analysis [17].

6 Materials

All reagents, solvents, gases, and chemicals used in analyses should be of analytical grade quality and of the highest available purity. Water used should be as defined in ISO 3696:1987, quality 3.

7 Common quality analytical elements

7.1 General

Forensic blood samples are precious. Depending upon the nature of the fire accident and condition of the fire victim, a blood sample might, or might not, have been submitted in a large amount for analyses. Once the blood samples are consumed during analyses, it might not be possible to obtain additional samples from the sample submitters. Therefore, it is customary in forensic toxicological operations to use samples submitted for analyses conservatively and cautiously.

Unless stated otherwise, all blind and open controls and calibrators used for analysis shall be prepared in human whole blood. It is important that blood be collected from healthy human subjects who are not smokers and are not exposed to CO. In other words, the collected human blood shall be free from CO and CN^-.

7.2 Qualitative, quantitative and confirmatory analyses

It is recommended that a qualitative analysis (screening) be performed initially on a portion (aliquot) of the blood sample collected from each victim. On the qualitatively positive (presumptive positive) samples, a quantitative analysis should be conducted. Although qualitative and quantitative analyses in some methods can be simultaneously conducted on the same aliquot, it is preferred that the quantitative analysis be performed on a different aliquot of the submitted sample than that which was used during the initial qualitative analysis.

Additionally, quantitative analytical results should be confirmed on a different aliquot of the blood sample by a second method based upon a analytical principle different from the method used during the first quantitative analysis. Such confirmatory analyses can be qualitative or quantitative.

7.3 Replicate analyses

If a sufficient amount of sample is not submitted, then a single analysis is obviously the option. Otherwise, it is recommended that the aliquot of a sample be analysed in duplicate for both qualitative and quantitative analyses. If one or both of the qualitative duplicate results is/are positive, then the sample should be analysed quantitatively.

The mean of the duplicate quantitative values should be reported, provided the duplicate analytical values do not differ by more than 10 % from the mean value. In the event that the duplicate values do not meet this difference criterion, the mean value should be rejected and a new aliquot of the sample should be reanalysed.

As mentioned in 7.2, positive findings should be qualitatively or quantitatively confirmed by a second analytical method using a different aliquot of the sample. For this second analysis, if the sample is not available in sufficient amount, a single analysis can be performed. Otherwise, the analysis should be conducted in duplicate and the mean of the two values should be calculated and evaluated to determine if the value meets the 10 % criterion. If the mean value meets the criterion, the value can be acceptable. Otherwise, the analysis can be accepted as a qualitative analytical finding, provided both duplicate analyses are positive. If the positive findings cannot be confirmed by a second analytical method, then the sample should be considered negative for the analytes.

A laboratory may choose to report the one of the two acceptable quantitative mean values deemed to be obtained from the most reliable analytical method. This decision can also be based upon the laboratory's standard operating procedures.

The total amount of sample required for the analyses is based on the selectivity and sensitivity of the methods adopted by a particular laboratory. It should also be considered that the submitted blood sample will be analysed in duplicate for blood COHb and for blood CN^-. Therefore, these factors should be carefully evaluated and considered by the sample collector, sample submitter and the laboratory receiving the sample and conducting the analyses.

7.4 Analytical batch

In addition to the aliquots of the blood samples from fire victims, each analytical batch shall contain at least two aliquots from blind controls: one from a negative blind control and the other from a positive blind control. In any batch, identity, origin and sequence of the aliquots in relation to the blood samples of the victims or of the blind controls shall not be known to the analysts performing the batch analysis. The analytical result of the negative blind control should be negative and, for the positive blind control, it should be within the limits of the target values established by the respective laboratories. If these two analytical criteria are not met, the batch can be rejected and a new analytical batch can be issued for analysis.

NOTE A negative blind control is a blood specimen free from CO and CN^-. A positive blind control is a blood specimen containing known amounts of COHb and CN^-.

7.5 Open controls

Along with the aliquots of a batch, one negative open control and at least one positive open control shall be processed and analysed by the analysts. Open controls should be known to the analysts. A single analysis is acceptable for open controls. Analytical results for negative open control shall be negative and, for positive open control, it shall be within ± 20 % of the target value established by the laboratory. If open control results do not meet these criteria, then a new analytical batch should be issued and the samples should be reanalysed.

7.6 Calibrators

The calibrators shall cover the linear range of the calibration curve. The analytical values of the samples shall fall between the lowest and the highest calibrators in the linear range of the curve.

8 Measurement of CO in blood as COHb

8.1 COHb by whole-blood oximeters

8.1.1 Principle

Oximeters are commonly self-contained instruments and include hardware and electronics. By means of these dedicated, special-purpose instruments, the percentage of COHb in suitably diluted whole-blood samples is measured by simultaneous automated differential visible spectrometry at various characteristic wavelengths.

8.1.2 Reagents and materials

The instrument vendors supply necessary reagents/materials, such as blood diluent solution, zeroing solution, cleaning agent solution, calibrators and other necessary reagents and supplies.

8.1.3 Apparatus

Examples of commercially available oximeters[1)] are CO-Oximeter (Instrumentation Laboratory, Inc., Lexington, MA) and AVOXimeter (A-VOX Systems, Inc., San Antonio, TX) [18],[19],[20].

NOTE These devices also measure whole-blood deoxyhaemoglobin (HHb), oxyhaemoglobin (OxyHb), and methaemoglobin (MetHb).

8.1.4 Sample

The amount of blood sample required for the analysis ranges from 100 µl to 400 µl. The recommended amount of the sample is 0,5 ml to 2 ml.

8.1.5 Procedure

Instrument manuals provide details of the analytical procedures. Analysis of the samples shall be performed following the instructions given in the manuals. Oximeters shall be calibrated as instructed by the manufacturers.

8.1.6 Calculation

Digital readout of percentage COHb is usually displayed by the instruments. Percentages of HHb, OxyHb and MetHb are also displayed. The percent mass fraction of COHb, w_{COHb}, is calculated by Equation (1):

$$w_{COHb} = \left(\frac{C_{COHb}}{C_{COHb} + C_{HHb} + C_{OxyHb} + C_{MetHb}} \right) \times 100 \quad (1)$$

where

C_{COHb} is the concentration of COHb;

C_{HHb} is the concentration of HHb;

C_{OxyHb} is the concentration of OxyHb;

C_{MetHb} is the concentration of MetHb.

NOTE The sum of the concentrations of COHb, HHb, OxyHb, and MetHb, expressed in grams per decilitre, is considered equal to the total haemoglobin (tHb), expressed in grams per decilitre.

8.1.7 Sensitivity

Oximeters are capable of measuring $w_{COHb} \geqslant 10$ % in fresh blood from live victims with an accuracy of 1 % to 2 %. The main difference between the results obtained from oximeter analyses of 23 blood samples and the analyses by gas chromatography and photometry analyses was 0,35 % [18],[21].

1) These are examples of suitable products available commercially. This information is given for the convenience of users of ISO 27368 and does not constitute an endorsement by ISO of these products.