



### K49 *In Vitro* Adsorption of Carbon Monoxide and Hydrogen Cyanide in Pooled Blood

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After attending this presentation, attendees will be able to apply the findings of this study to the interpretation of results of blood carboxyhemoglobin (COHb) and cyanide (CN<sup>-</sup>) analyses.

This presentation will impact the forensic science community by informing those who investigate accidents associated with fires of the effect that an atmosphere containing primary combustion gases—that is, carbon monoxide (CO) and hydrogen cyanide (HCN)—will have on postmortem blood from open wounds of victims.

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) assists in the investigation of fatal aircraft accidents by conducting toxicological analyses of specimens received from victims of the accidents. One aspect of the analyses is the determination for the presence of primary combustion gases in blood specimens. Combined with the crash site investigation, autopsy and pathology findings, and toxicological results, the investigators could determine whether the crew members were incapacitated by engine CO leaks into the cabin area, whether they survived the crash and were overcome by inhaling CO and HCN from aircraft fires, whether and/or the victims died on impact or came to a rapid death from the intense heat of the fire without inhaling these gases.

Because of the violent impacts involved in crashes, victims quite often suffer large open wounds near sites on the body from where autopsy whole blood is collected. Many aircraft crashes result in fire, which in turn, fill the atmosphere of the victims with smoke (CO and HCN). It is important to determine whether pooled blood in those open wounds may have adsorbed CO and HCN after death and could erroneously lead investigators to determine that the presence of COHb and CN<sup>-</sup> in whole blood was the result of breathing in primary combustion gases.

A chamber was set up in the CAMI laboratory to determine whether CO and HCN may be adsorbed in undisturbed, pooled whole blood. To determine *in vitro* CO adsorption, a large laboratory desiccator was used as the chamber. A light film of silicone grease was applied to the valve and the rim of the lid and chamber. A female Luer-Lok fitting was affixed to the arm of the valve by use of a small piece of Tygon tubing. To facilitate air movement in the chamber, a large cross-shaped magnetic stirring bar was placed at the bottom of the chamber, which was rotated with a magnetic stirring plate. A ceramic plate with numerous rows of holes was placed above the stirring bar. Setting on it was a shallow open dish containing 4 mL of whole human blood that had been treated with sodium heparin. A 100-cc valved Luer gas syringe was used to evacuate air from the chamber and introduce pure CO into it to achieve desired concentrations. Prior to the setup, the volume of the chamber was determined by measuring the amount of water required to displace all the air in the chamber and lid, after taking into account the volumes of the blood sample and the items used in the desiccator. The chamber volume

was determined to be 9038 cc. Various concentrations and lengths of CO exposure to the pooled blood were conducted. COHb concentrations were determined spectrophotometrically.

The apparatus was modified slightly for the determination of *in vitro* HCN adsorption by using an additional open dish containing a 5- mL beaker having a weighed amount of sodium cyanide (NaCN). The Ideal Gas Law was used to determine the amount of NaCN required to achieve the desired concentrations of HCN in the chamber. To conduct the experiment, 4 mL of heparin-treated, whole human blood was used in the second dish. With the lid of the chamber partially opened, 1 mL of concentrated sulfuric acid was added to the beaker containing the NaCN; then the chamber lid was immediately closed. The volume of the chamber was determined to be 8981 cc, after taking into account the volumes of the blood sample, sulfuric acid, and the items used in the desiccator. Two concentrations and various lengths of HCN exposure to the pooled blood were conducted. CN<sup>-</sup> concentrations were determined colorometrically by microdiffusion; then, positives were quantitated spectrophotometrically.

No significant amount of COHb was detected in the whole blood of the experiment after exposure to CO at 5532, 8298, 11064, 22129, and 33193 ppm for 30- and 60-minute exposure times. However, CN<sup>-</sup> concentrations in whole blood increased with exposure to an atmosphere containing HCN at 100 and 200 ppm each at 15, 30, 45, and 60 minutes of exposure times. The CN<sup>-</sup> concentration in blood ranged from 1.55 to 5.01 µg/mL.

Therefore, there is a potential for blood CN<sup>-</sup> levels to increase by the adsorption of atmospheric HCN present in the smoke. This study also demonstrated that the COHb in pooled blood exposed to an atmosphere containing CO within the parameters of this experiment would not alter the integrity of



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postmortem blood at an aircraft crash site. This selective adsorption is consistent with the solubility of HCN and insolubility of CO in water. These findings suggest that the COHb and CN<sup>-</sup> levels should be carefully interpreted in view of the potential for selective presence of these primary combustion gases in blood.

**Carbon Monoxide, Hydrogen Cyanide, Blood**