

Annex D. Controlled Human Exposure Studies

Table D-1. Controlled human exposure studies.

Study	Subjects	Exposure	Findings
Adir et al. (1999, 001026)	15 healthy nonsmokers Gender: M Age: 22-34 yr	Inhaled Concentration: Not provided Exposure Duration: 3 min 45 s COHb Concentration: 4-6% COHb Analysis: CO-oximeter (IL-282) Exposures to CO and room air were separated by 1 mo, with the order of exposure randomly assigned.	Exposure to CO resulted in a decrease in postexposure exercise duration (Bruce protocol) relative to clean air exposure in 13 out of 15 subjects ($p = 0.0012$). Statistically significant decreases in METs were also reported following CO exposure ($p = 0.0001$). No CO-induced changes in HR, BP, ECG parameters, or myocardial perfusion were observed.
Bathoorn et al. (2007, 193963)	19 former smokers with COPD Gender: 18 M/1 F Age: 66-70 yr	Inhaled Concentration: 100 ppm (9 subjects) or 125 ppm (10 subjects) Exposure Duration: 2 h on each of 4 consecutive days COHb Concentration: 2.7% (following 4th day exposure) COHb Analysis: Not provided Exposures to CO and room air conducted were separated by at least 1 wk, using a randomized crossover design.	Following the 4th day of exposure, CO inhalation reduced sputum eosinophils relative to room air and also increased the provocative concentration of methacholine required to cause a 20% reduction in FEV ₁ . Neither of these effects were shown to reach statistical significance. No changes in sputum neutrophils, white blood cell counts or serum C-reactive protein (CRP) were observed. Although this study appears to demonstrate some evidence of an anti-inflammatory effect of CO among subjects with COPD, it must be noted that 2 of these patients experienced exacerbations of COPD during or following CO exposure, with 1 patient requiring hospitalization 2 mo after exposure (initial symptoms first experienced 1 wk postexposure).
Hanada et al. (2003, 193915)	20 healthy adults Gender: M Age: 26 ± 1 yr	Inhaled Concentration: Not provided Exposure Duration: 20 min COHb Concentration: 20-24% COHb Analysis: CO-oximeter (OSM-3) 15 subjects exposed for 20 min (10 min rest, 5 min handgrip exercise, 2 min postexercise ischemia, 3 min recovery) under the following 4 conditions: (1) normoxia (inspiratory O ₂ fraction 21.4%); (2) hypoxia (inspiratory O ₂ fraction 10.3%); (3) CO + normoxia; and (4) CO + hyperoxia (inspiratory O ₂ fraction 95.9%). Trials involving exposure to CO were conducted last in this sequence. Each of the 4 conditions was separated from the next by 20 min of rest. 5 subjects served as controls (4 consecutive 20 min periods of normoxia).	Blood oxygenation, BP, HR and respiratory rate were measured during exposure. Muscle sympathetic nerve activity (MSNA) and leg hemodynamics were evaluated in two subsets of the study group ($n = 8$ and 7 , respectively). Arterial oxygen saturation (pulse oximetry) was significantly lower, and resting HR and ventilation significantly higher during the period of hypoxia compared to the other periods; none of these measures were affected by exposure to CO. MSNA was shown to increase during hypoxia and CO exposure relative to normoxia. Neither hypoxia nor CO was found to affect leg blood flow or vasoconstriction.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated Science Assessments (ISAs) and the Integrated Risk Information System (IRIS).

Study	Subjects	Exposure	Findings
Kizakevich et al. (2000, 052691)	16 healthy nonsmokers Gender: M Age: 18-29 yr	Inhaled Concentration: Initial short term (4-6 min) exposure to 1,000 or 3,000 pap, followed by exposures to 27, 55, 83, or 100 ppm to maintain COHb concentration. Exposure Duration: 4-6 min at 1,000 or 3,000 pap, followed by 20 min at 27, 55, 83, or 100 ppm. Target COHb Concentrations: 5, 10, 15, and 20% COHb Analysis: CO-oximeter (IL-282) Subjects exposed on 4 separate days to increasing CO concentrations during either upper-body exercise (hand-crank) or lower-body exercise (treadmill). Targeted COHb concentrations were initially attained using short-term (4-6 min) exposures to CO at concentrations of 1,000 or 3,000 ppm. Chamber exposures were then conducted at CO concentrations required to maintain COHb levels of <2% (room air), 5% (27 ppm), 10% (55 ppm), 15% (83 ppm), and 20% (100 ppm).	At all levels of upper- and lower-body exercise, exposures to CO resulted in increases in HR, cardiac output, and cardiac contractility relative to clean-air exposures. Increases in HR reached statistical significance at COHb concentrations \geq 5%, and increases in both cardiac output and cardiac contractility reached statistical significance at COHb concentrations \geq 10%. CO exposure during exercise was not observed to cause ventricular arrhythmias or affect ECG wave shape (no evidence of ST-segment depression) at COHb concentrations \leq 20%.
Mayr et al. (2005, 193984)	13 healthy nonsmokers Gender: M Age: 18-38 yr	Inhaled Concentration: 500 ppm Exposure Duration: 1 h COHb Concentration: 7% COHb Analysis: CO-oximeter (AVL 912) Subjects exposed to both CO and clean air with exposures separated by a 6-wk period. Immediately following exposure, subjects were administered an intravenous bolus dose (2 ng/kg) of lipopolysaccharide (LPS).	Infusion of LPS significantly increased plasma concentrations of TNF- α , CRP, IL-6, and IL-8, with no difference in the inflammatory response between clean-air and CO exposures.
Morse et al. (2008, 097980)	12 healthy nonsmokers Gender: M Age: 25 \pm 2.9 yr	Inhaled Concentration: 3,000 ppm Exposure Duration: 3-8 min COHb Concentration: 6.2% COHb Analysis: Electrochemical sensor (Smokerlyzer) measuring CO in exhaled breath Exposures conducted on 2 separate occasions to room air (6 min) and CO. Subjects were exposed to CO until COHb reached 6% (3- to 8-min exposures).	Leg strength and muscle fatigue were evaluated immediately following exposure. CO exposure did not affect muscle strength (maximal voluntary isometric contraction) but did cause a statistically significant increase in muscle fatigue ($p < 0.05$).
Ren et al. (2001, 193850)	12 healthy adults (10 nonsmokers and 1 smoker) Gender: 9 M/3 F Age: 20-32 yr	Inhaled Concentration: 0.4% (4,000 ppm) Exposure Duration: 10-30 min at 0.4% followed by ~ 8-h with periodic exposure to maintain COHb concentration COHb Concentration: 10% COHb Analysis: Not provided Each subject underwent 4 different 8-h experimental protocols: (1) isocapnic hypoxia (end-tidal PO ₂ held at 55 mmHg); (2) withdrawal of 500 mL of venous blood at the start of an 8-h period; (3) CO exposure at a concentration required to maintain a COHb level of 10%; and (4) a control exposure where subjects breathed room air with no intervention.	A statistically significant increase in ventilation was observed following hypoxia, but no such increase was found following any of the other 3 protocols, including exposure to CO. One subject felt faint during the blood withdrawal protocol and did not complete the study.

Study	Subjects	Exposure	Findings
Resch et al. (2005, 193853)	15 healthy nonsmokers Gender: M Age: 27 ± 4 yr	Inhaled Concentration: 500 ppm Exposure Duration: 1 h COHb Concentration: ~ 10% COHb Analysis: CO-oximeter (AVL 912) Exposures to CO and synthetic air control were separated by a period of at least 1 wk.	COHb levels averaged 5.6% after 30 min and 9.4% after 60 min of exposure. Statistically significant increases in retinal blood flow, retinal vessel diameter, and choroidal blood flow were observed with CO exposure relative to synthetic air at both time points. Exposure to CO did not affect oxygen saturation of arterial blood.
Vesely et al. (2004, 194000)	10 healthy nonsmokers Gender: M Age: 22-52 yr	Inhaled Concentration: 1,200 ppm Exposure Duration: 30-45 min COHb Concentration: 10% COHb Analysis: CO-oximeter (OSM-3) Prior to and following exposure, subjects performed hypoxic and hyperoxic rebreathing tests. Four subjects were exposed to hypoxic conditions first, while 6 subjects were exposed to hyperoxic conditions first, both prior to and following CO exposure.	Ventilation rate was observed to significantly increase during hypoxic rebreathing relative to hyperoxic rebreathing. However, exposure to CO had no effect on ventilation under either hypoxic or hyperoxic conditions. The authors concluded that exposure to low levels of CO does not significantly affect chemoreflex sensitivity of the CO ₂ -induced stimulation of ventilation.
Zevin et al. (2001, 021120)	12 healthy smokers Gender: M Age: 27-47 yr	Inhaled Concentration: 1,200-1,500 ppm Exposure Duration: 10 min each h, 16 h each day, over 7 days COHb Concentration: 5-6% COHb Analysis: CO-oximeter (Ciba Corning 2500) Exposures were conducted over 21 consecutive days under 3 different protocols, with each protocol lasting 7 days. In 1 protocol, subjects smoked 20 cigarettes per day, 1 every 45 min. In the other 2 protocols, every 45 min (20 times per day) subjects breathed either air or CO from a 1-liter bag once per min for 10 min at a time. Subjects completed all 3 protocols, with 6 subjects exposed sequentially to CO, smoking, then air, and the other 6 exposed sequentially to air, smoking, then CO.	COHb levels were similar during smoking and exposure to CO, with average concentrations of 6% and 5%, respectively. Blood was drawn on day 4 of each exposure and analyzed for CRP, plasma platelet factor 4, and white blood cell count. Plasma levels of CRP and platelet factor 4 were significantly elevated with smoking but not with CO exposure, relative to air control. HR and BP were evaluated on day 3 of each protocol. Cigarette smoke but not CO was observed to significantly increase HR, while no difference in BP was observed between any of the 3 exposures.

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