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CHARACTERIZATION OF AEROSOLS PRODUCED DURING TOTAL HIP REPLACEMENT SURGERY IN DOGS WITH ^{51}Cr -LABELED BLOOD

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Abstract—There is increasing concern over the potential inhalation hazard to health care workers from blood-borne pathogens. Previous studies have demonstrated that inhalable, blood-associated aerosols are produced during orthopedic surgery. However, the identification and quantitative estimation of blood-associated aerosols have been based upon simple "dipstick" analysis of the collected samples. In order to confirm the presence of these blood-associated aerosols and to estimate the amount produced, the red blood cells of five dogs were radiolabeled with ^{51}Cr before aerosol samples were taken during total hip replacement procedures. Various aerosol sampling devices including a Marple personal cascade impactor, two Lovelace multi-jet impactors, and air filters were used. The Marple personal cascade impactor was worn by the surgeon. One Lovelace multi-jet impactor was sampled near the surgical site, while the other Lovelace impactor and the filter samples were taken through a probe located near the surgical site. The samples were subjected to gravimetric analyses, and blood contents were assessed by Chemstrip 9 analysis and by radioactivity counting. Results confirmed that blood-associated aerosols were produced during orthopedic surgery. The time-averaged mass concentration near the surgical site, as measured by the personal impactor, was 0.37 mg m^{-3} ; of that amount, $6.5 \text{ }\mu\text{g m}^{-3}$ (1.8% of the total mass concentration) was attributed to red blood cells (RBCs). The estimated number of RBCs or hemoglobin that might be inhaled by a surgeon without any respiratory protection during the course of an orthopedic surgery was about 2.9×10^5 RBCs or $8.7 \mu\text{g}$ of hemoglobin. About 60% of the RBCs were associated with particles larger than $10 \mu\text{m}$ in aerodynamic diameter, and about 8% of the RBCs were associated with particles less than $0.5 \mu\text{m}$. The number ratio between the RBCs and lymphocytes for humans is about 2200:1; thus, the estimated number of lymphocytes that might be inhaled by a surgeon without any respiratory protection during the course of an orthopedic surgery would be less than 135. To assess the significance of our finding on the potential risk to health care workers will require further studies of the relationship between pathogens and particle sizes and the viability of pathogens associated with these blood-associated aerosols.

1. INTRODUCTION

Recent information on the production of blood-containing aerosols during surgical procedures, particularly during orthopedic surgery, has added to concerns about the hazards of working with HIV-positive patients (Jewett, 1990; Johnson and Robinson, 1991). These concerns are not based on laboratory findings or documented transmission via inhalation in orthopedic surgery (Tokars *et al.*, 1992; Petersen, 1980). Heinsohn *et al.* (1991) and Jewett *et al.* (1992) have demonstrated under laboratory conditions using bovine tissue that inhalable aerosols are generated by the power tools used in orthopedic surgery. Johnson and Robinson (1991) in their T-cell culture infectivity study demonstrated that HIV-1 can remain viable and may be recovered from aerosols created by surgical power tools. However, these aerosols were produced by using power tools directly on HIV infected blood in a laboratory, and whether similar aerosols could be produced by use of power tools on patients in an operating room is not known.

In a recent study to assess the amount of blood in aerosols potentially inhaled by surgeons during operations, Heinsohn and Jewett (1993) reported that a mean concentration of $1.4 \mu\text{g}$ of hemoglobin (Hgb) per cubic meter of air was detected. In a similar aerosol sampling study at a hospital (Yeh *et al.*, 1995), inhalable blood-associated aerosols were also detected during

orthopedic surgeries. In both studies, the identification and estimation of blood-associated aerosols were based upon either the Hemastix (Miles Inc., Elkhart, IN) or Chemstrip 9 (Boehringer Mannheim Diagnostics, Indianapolis, IN) analysis of the samples obtained. Both Hemastix and Chemstrip 9 are "dipstick" measurement analyses that can respond to both Hgb and myoglobin, but cannot differentiate between the two. Myoglobin is distributed in smooth muscle, skeletal muscle, and myocardium. On the average, muscle in an adult human male contains about 700 mg myoglobin per 100 g wet weight (Mountcastle, 1968). HIV is carried primarily through the lymphocytes in the blood and has been isolated from serum (Michaelis and Levy, 1987). Hepatitis B virus (HBV) is also transmitted by infected blood, including serum (Lutwick, 1990). Thus, myoglobin in the muscle is probably not a good measure of the potential risk from such blood-borne pathogens. During surgery in which power tools are used, the aerosols produced might contain both muscle tissue and blood. Therefore, the positive response on a Hemastix or Chemstrip 9 might be due to myoglobin, and its presence would bias the risk estimates. Additionally, the results from Hemastix and Chemstrip 9 are only semi-quantitative, because the responses are classified into four discrete categories, based on changes in color.

This study was designed to more accurately characterize the blood-associated aerosols that might be produced during orthopedic surgical procedures. We used an animal model in which only the red blood cells (RBCs) were radiolabeled with ^{51}Cr to eliminate the potential signal bias from myoglobin. The dog was selected for this study because its size permitted the same orthopedic surgical procedures as those done in humans. The total hip replacement procedure was chosen because it is a common procedure in humans, it is a developed procedure in dogs, and prostheses are available.

2. METHODS

Five adult Beagle dogs (10–13 kg body weight) from the Institute's colony were used in this study. Until the day before the surgery, the dogs were housed in kennel buildings with indoor and outdoor runs. The dogs were fed 350 g of dry kibble dog food (Wayne Mini Lab Dog Diet) once a day, and water was available at all times from an automatic watering device. The dog was moved from the kennel to the veterinary hospital the day before the surgery, and food was withheld the morning of the procedure.

2.1. ^{51}Cr labeling of blood and administration to dogs

The most relevant biological elements that could be labeled would be the white blood cells (WBCs) that normally would carry the HIV when infected. However, because of the relatively small number of circulating WBCs, labeling these cells would not provide sufficient detection sensitivity for measurement of blood-containing aerosols. Therefore, a better strategy was to radiolabel the RBCs, for which the RBC to WBC ratio is around 700 for Beagle dogs (Lowseth *et al.*, 1990).

Cells were labeled using the method of Owen (1959). This procedure is based on the fact that hexavalent sodium chromate readily penetrates the RBC membrane and establishes a stable bond with the intracellular Hgb, whereas reduced trivalent ^{51}Cr does not penetrate the RBC. The labeling was achieved by incubating $^{51}\text{Cr(VI)}$ as sodium chromate with fresh or refrigerated whole blood; reduced Cr(III) was removed by multiple washings and centrifugations. Labeling efficiencies were about 90%. Following radiolabeling and purification, the blood was transported to the surgical suite and intravenously infused into the anesthetized dog. In order to obtain adequate detection sensitivity on aerosol samples, 500 mCi (19 GBq) of ^{51}Cr per dog were used.

Because of the relatively large amount of ^{51}Cr used, all attempts were made to maintain the concentration of stable Cr below $5 \mu\text{g ml}^{-1}$ RBC by purchasing the highest specific activity ^{51}Cr available (e.g., up to 370 mCi mg^{-1} chromium). This level of Cr concentration ($5 \mu\text{g ml}^{-1}$ RBC) has been shown to result in toxicity to the RBC (Owen, 1959). To maximize the ^{51}Cr concentration in blood *in vivo*, the labeled blood volume used was 300 ml.

The volume of blood required for the ^{51}Cr labeling was obtained from a combination of blood taken from the subject dog and supplemented with blood obtained from other donor dogs. This supplementation was required because not more than 40% of the blood volume (300 ml) can be removed without inducing hypovolemic shock. Small samples of blood from potential donor dogs and from the subject dog obtained prior to surgery were mixed and evaluated microscopically for evidence of agglutination. No cross-reactivity among blood samples was found during this study.

2.2. *Surgical procedure*

The total hip replacement procedure used in the dogs was similar to that used in humans (Yeh *et al.*, 1995). Briefly, the procedure involved (1) opening the surgical site and exposing the hip joint, using a scalpel and electrocautery; (2) cutting off the head of the femur and reaming and enlarging the acetabulum to fit the acetabular prosthesis, using a bone drill, saw, and acetabular reamer; and (3) installing the prosthesis using a reamer, hammer, and cement, and closing the surgical site. Throughout the procedure, irrigation and suction were used intermittently to clean the site. Because of the amount of radioactivity used, personal protection (such as disposable clothing, gloves, and respirator) and radiation monitoring equipment were used, and a 6 mm thick lead shield was placed over the upper body of the dog to reduce the radiation doses to the operating room personnel. This shielding had no effect on the surgical procedure. At the end of the surgical procedure, while still under deep anesthesia, the dog was euthanized by exsanguination via cardiac puncture.

2.3. *Aerosol collection and characterization*

Because of the relatively large amount of radioactivity used in this study, the following factors were considered when the instruments were chosen for collecting and characterizing aerosol samples: (1) the instruments should be the same or similar to those used previously on humans in the hospital (Yeh *et al.*, 1995) and (2) the instruments should be easy to decontaminate so they could be used in the next experiment without having to wait for radioactive decay (half-life for ^{51}Cr is 27.8 d). Based on these considerations, the following instruments were chosen: (1) a Marple personal cascade (MPC) impactor (to be worn by the chief surgeon), (2) two Lovelace multi-jet (LMJ) cascade impactors, and (3) filter samplers. Both the MPC and LMJ impactors were well characterized and suitable for sampling the aerosols in the air with correction for inlet sampling efficiency and internal wall losses (Rubow *et al.*, 1987; Newton *et al.*, 1977, 1990). It should be noted, however, that neither of these impactors have been tested as inhalable aerosol samplers. The sampling devices were cleaned and decontaminated for each of the five hip replacements.

To mimic the configurations used in a previous field study at a hospital where an aerosol sampling probe was used for sampling aerosols by an impactor and a filter sampler (Yeh *et al.*, 1995), the aerosol sampling probe was attached to the top of the lead shield that covered the torso of the dog. During each of the five surgical procedures, two consecutive filter samples and a LMJ cascade impactor sample were obtained through this probe via a 10 l cylindrical aerosol chamber. The distance of the probe from the surgical site was 15–25 cm. One additional LMJ cascade impactor was placed next to the probe on top of the lead shield. Figure 1 shows the arrangement of these samplers. The MPC impactor was worn by the chief surgeon to sample breathing zone aerosols. The sampling flow rates were 2 l min^{-1} for the MPC impactor, 20 l min^{-1} for the filter sampler, and 14 l min^{-1} for the LMJ cascade impactors. Zefluor filters with $1.0\text{ }\mu\text{m}$ pore size were used in the filter samplers as well as the backup filters in the MPC impactor and LMJ cascade impactors.

2.4. *Data analyses and estimation of blood content collected on the samples*

At the conclusion of each surgical procedure, the MPC impactor, the LMJ cascade impactors, and the filter samplers were disassembled. The impactor substrates and filters

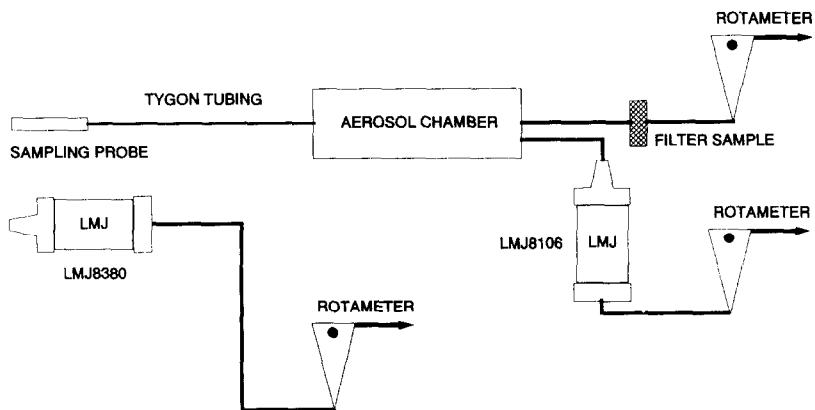


Fig. 1. Schematic diagram showing the arrangement of two LMJ impactors (one placed near the surgical site, parallel to the sampling probe, and the other one sampling through an aerosol probe and a chamber), a sampling probe, an aerosol chamber, and the filter sampler.

were removed, and the samples were weighted using a Cahn C-31 microbalance (1 μg sensitivity) (Cahn Instruments, Inc., Cerritos, CA). After each sample was weighted, the ^{51}Cr activity was assayed using the Beckman 8000 automated gamma counter (Beckman Instrument Company, Fullerton, CA). Because of the low activity in the samples, a 500 min counting time was used for most samples. These counting data of aerosol samples represented aerosolized RBC samples. A blood sample taken from the labeled subject dog for each experiment was used to establish the converting factor between the "radioactivity count" and "number of RBCs". This was done by counting the radioactivity, followed by RBC counting (using Cell-Dyn 1600, Abbott Diagnostics, Irving, TX). Using the measured converting factor, the number of RBCs collected on each impactor substrate was estimated from the ^{51}Cr counting data, resulting in particle-size-specific quantities of RBCs. After counting, each filter and impactor substrate sample was washed with 20 μl of distilled water, and the Hgb content in each sample was qualified using Chemstrip 9.

3. RESULTS AND DISCUSSION

During the surgical procedures, the operating room personnel wore thermoluminescent dosimeters. The average dose (primarily from photons emitted by the ^{51}Cr) per surgical procedure registered by dosimeters positioned approximately mid-chest on these personnel was 20 mrem (2×10^{-4} Sv). Radiation doses to the hands per surgical procedure was about 43 mrem (4.3×10^{-4} Sv) for the chief surgeon who had the highest doses to the hands among the operating room personnel. These doses were small compared to the permissible annual limit of 5000 mrem (0.05 Sv) allowed by the U.S. regulatory agencies.

Because of the nature of the instruments used in this series of experiments, the aerosol size distribution data obtained represented the time-averaged information throughout the surgical procedures. Similar to observation during surgeries in humans (Yeh *et al.* 1995), the aerosol size distributions varied from experiment to experiment due to slight variations in procedure during surgery, where the activities changed all the time and were not always predictable: different tools were used, surgeons or nurses were changing locations from time to time, and the aerosols were generated in a discrete time-space "puffs-like" fashion. However, the shapes of the time-averaged particle size distributions were similar among the five dogs. Figures 2 and 3 show the results obtained from the MPC impactor worn by the chief surgeon. Figure 2 shows a bar graph of the collected mass and the estimated number of RBCs vs particle size. Also included are the results analyzed by Chemstrip 9 on each stage substrate for quantifying blood content. The estimated number of RBCs was obtained from the radioactivity of ^{51}Cr ; therefore, the estimated number of RBCs is proportional to the radioactivity within a given experiment. In spite of the qualitative and somewhat subjective

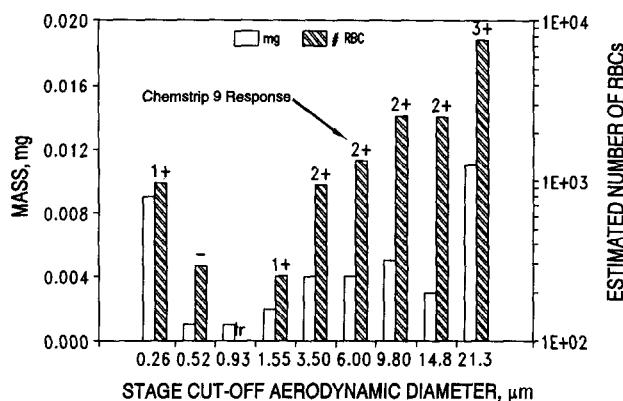


Fig. 2. Bar graph of aerosol mass and estimated number of RBCs collected on each stage of a MPC impactor during surgery on a dog. RBC number was estimated from activity of ^{51}Cr -labeled RBCs. (^{51}Cr -labeled dog: Run #1) (The amount of hemoglobin detected on each stage by Chemstrip 9: negative = 0 erythrocytes μl^{-1} , tr = trace ≈ 5 ery μl^{-1} , 1+ ≈ 10 ery μl^{-1} , 2+ ≈ 50 ery μl^{-1} , 3+ ≈ 250 ery μl^{-1} . Sample size = 20 μl).

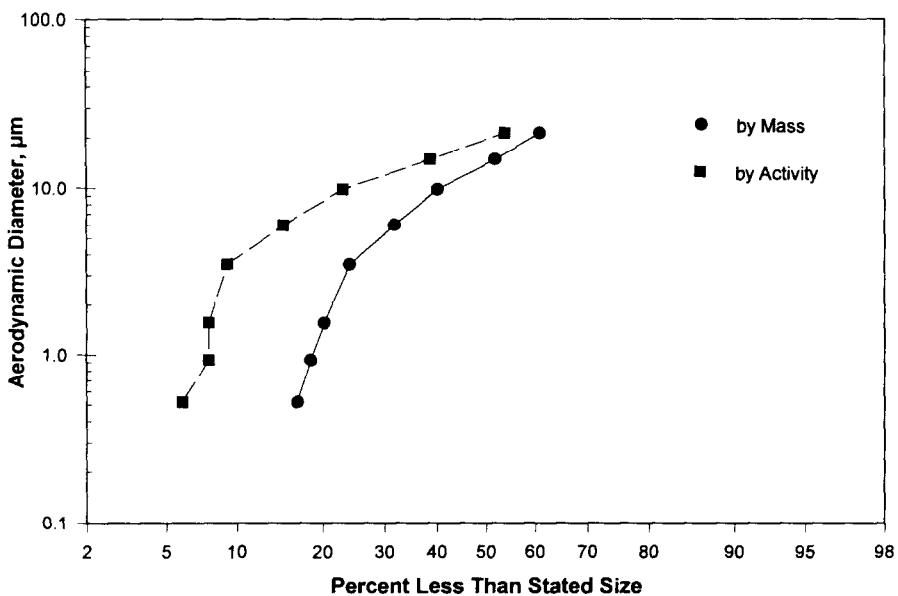


Fig. 3. Mass and activity cumulative distributions as determined by an MPC impactor.

nature of reading the color changes in the strip, the Chemstrip 9 results correlated fairly well with the radioactivity (and thus to the estimated number of RBCs). One should note that both the Chemstrip 9 and radioactivity analyses detected Hgb, not RBCs *per se*. The RBC has a diameter of about 8 μm and a thickness of about 2 μm with an equivalent volume diameter of about 5.8 μm . Therefore, those detected blood-associated aerosols with aerodynamic diameter less than about 6–7 μm would not consist of whole RBCs, but rather, would contain fragments of RBCs due to the use of surgical power tools, such as electrocautery and saw. Figure 3 shows the aerosol mass and activity (or RBCs) cumulative distributions, indicating that aerosols consisted of multimodal size distributions, probably due to different generation mechanisms of using various power tools during the surgical procedure. In general, the size distributions obtained by mass and by radioactivity correlated very well, despite the fact that the mass measurement was subjected to the uncertainty of evaporation from the blood-associated aerosols.

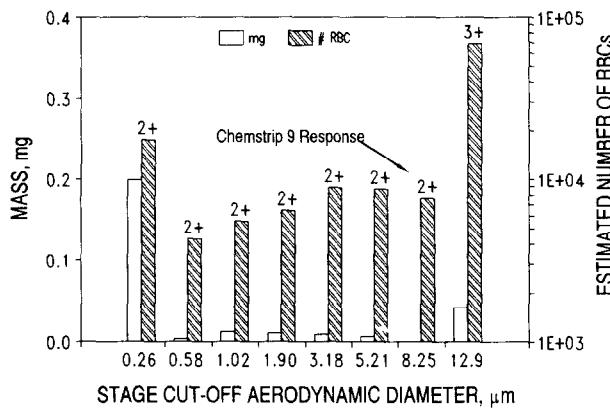


Fig. 4. Bar graph of aerosol mass and estimated number of RBCs collected on each stage of a LMJ cascade impactor during surgery on a dog. RBC number was estimated from activity of ^{51}Cr -labeled RBCs. (LMJ I.D. LMJ8380, ^{51}Cr -labeled dog; Run #1) (The amount of hemoglobin detected on each stage by Chemstrip 9: negative = 0 erythrocytes μl^{-1} , tr = trace ≈ 5 ery μl^{-1} , 1+ ≈ 10 ery μl^{-1} , 2+ ≈ 50 ery μl^{-1} , 3+ ≈ 250 ery μl^{-1} . Sample size = 20 μl).

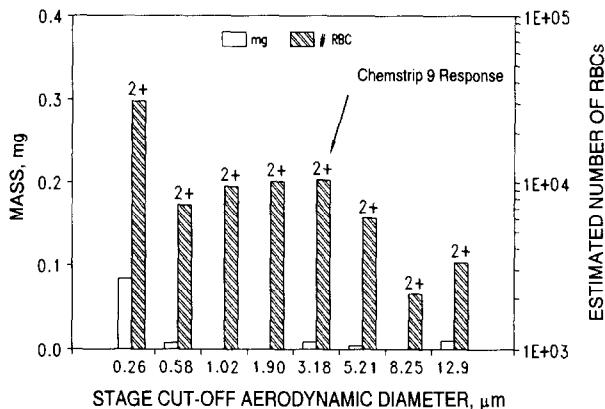


Fig. 5. Bar graph of aerosol mass and estimated number of RBCs collected on each stage of a LMJ cascade impactor during surgery on a dog. RBC number was estimated from activity of ^{51}Cr -labeled RBCs. (LMJ I.D. LMJ8106, ^{51}Cr -labeled dog; Run #1) (The amount of hemoglobin detected on each stage by Chemstrip 9: negative = 0 erythrocytes μl^{-1} , tr = trace ≈ 5 ery μl^{-1} , 1+ ≈ 10 ery μl^{-1} , 2+ ≈ 50 ery μl^{-1} , 3+ ≈ 250 ery μl^{-1} . Sample size = 20 μl).

Figures 4 and 5 allow comparison of the results from two LMJ impactors, one placed near the surgical site (LMJ8380), parallel to the aerosol sampling probe, and the other sampled through the sampling probe (LMJ8106) (Fig. 1). Although the bar graphs of the aerosol mass determined by the two LMJs were similar, the total aerosol mass concentration measured by LMJ8380 was much higher than that of the LMJ8106 (see Table 1). This may suggest that some losses occurred in the sampling line for the LMJ8106 (and filters that sampled through the aerosol chamber via a sampling probe). It may also suggest spatial nonuniformity of the aerosol concentration around the surgical area and within the surgical room. Again, the correlations between the Chemstrip 9 results and the estimated numbers of RBCs from radioactivity labeling were quite good, considering the discrete nature of the Chemstrip 9 reading.

Similar results were obtained for the other four experimental runs. The total average sample weight collected by the MPC impactors was 0.038 ± 0.021 mg (mean \pm S.D.; $n = 5$); the total estimated number of RBCs was $2.9 \times 10^4 \pm 1.5 \times 10^4$; and the sampled volume was 0.134 ± 0.020 m^3 . Table 1 summarizes all the data (after converting mass data to mass concentration format) obtained from the five dogs. This table shows the aerosol mass

Table 1. Aerosol mass and red blood cell (RBC) concentrations*, mean \pm S.D. (number of samples), measured by different instruments, during total hip replacement procedure with five dogs

Instrument	Mass concentration (mg m^{-3})	RBC concentration ($\# \text{m}^{-3}$)	Hgb concentration ($\mu\text{g m}^{-3}$)
Personal impactor	0.368 ± 0.203 (5)	$2.18 \times 10^5 \pm 1.40 \times 10^5$ (5)	6.54 ± 4.20 (5)
LMJ8380 [†]	0.382 ± 0.059 (5)	$1.72 \times 10^5 \pm 0.97 \times 10^5$ (4)	5.16 ± 2.91 (4)
LMJ8106 [‡]	0.122 ± 0.055 (5)	$7.53 \times 10^4 \pm 2.07 \times 10^4$ (5)	2.26 ± 0.62 (5)
Filters [‡]	0.134 ± 0.045 (5)	$6.34 \times 10^4 \pm 2.63 \times 10^4$ (5)	1.90 ± 0.79 (5)

*Estimated from measurement of ^{51}Cr -labeled blood activity.

[†]Near the surgical size.

[‡]Sampled through an aerosol probe and a chamber.

concentration and estimated RBC (or activity) concentration obtained from the MPC impactor, two LMJ cascade impactors, and the filter samples. A relatively large standard deviation indicated large variability between the surgical procedures. Again, Table 1 shows that the data obtained through the probe (LMJ8106 and filters) had lower aerosol concentrations than those obtained by either the personal impactor or LMJ8380. However, the agreements between the personal impactor and LMJ8380, or between the LMJ8106 and the filters were very good.

The good correlation between the Chemstrip 9 response and the estimated number of RBCs based on radioactivity of ^{51}Cr indicated that the Chemstrip 9 response was primarily from blood-associated (Hgb) aerosols rather than from myoglobin, because only the Hgb was labeled with ^{51}Cr . Close examination of Hgb responses measured using the Chemstrip 9 revealed that the MPC impactor data were similar to those derived from the human orthopedic procedures (Yeh *et al.*, 1995). Thus, it would be reasonable to assume that the values of the RBCs estimated from the labeled dog study could be used to estimate the potential exposure level of blood-associated aerosols to orthopedic surgeons in hospitals. Again, previous studies indicated that nearly all samples from stages of the MPC impactor worn by the chief surgeon (and other surgeons) showed aerosols that contained RBCs (Yeh *et al.*, 1995). This finding suggests that blood-associated, inhalable aerosols were produced during the total hip replacements and other orthopedic surgical procedures with humans.

During our previous study, we observed that the aerosol concentration was higher during the early part of the surgery and decreased rapidly thereafter (Yeh *et al.*, 1995). Therefore, to compare this study to others, comparing the total aerosols sampled, normalized to the same sampling flow rate, may be the most practical approach. Heinsohn and Jewett (1993) reported a mean Hgb exposure concentration of $1.4 \mu\text{g m}^{-3}$ for primary surgeons from 14 cases of various types of procedures. Their average sampling time was 159 min with sampling flow rates at 2.0l min^{-1} . Thus, their total of sampled Hgb was $0.45 \mu\text{g}$. Our personal impactors were also sampled at 2.0l min^{-1} , and we obtained an average total of 2.9×10^4 RBC, or $0.87 \mu\text{g}$ or Hgb. Considering the qualitative nature of estimating Hgb (or RBCs) using Hemastix by Heinsohn and Jewett (1993), the data are in reasonable agreement, within a factor of two.

To estimate the number of RBCs that might be associated with the aerosols produced during orthopedic surgery and inhaled by surgeons, we assumed that the surgeon's respiratory minute volume was 20l min^{-1} (corresponding to light exercise or moderate work conditions), which was 10 times the flow rate of the MPC impactor (2.0l min^{-1}). Under this assumption, the estimated total number of RBCs that might be inhaled by a surgeon would be 2.9×10^5 (or $\approx 8.7 \mu\text{g}$ of Hgb).

If a disease is carried through infected lymphocytes, then the number of inhaled lymphocytes will be of significance. To determine the potential of the surgeon inhaling a lymphocyte, it is assumed that the ratio of RBCs and lymphocytes is about 2200:1 for humans (Wintrobe *et al.*, 1981). Therefore, the estimated number of lymphocytes that a surgeon might be exposed to is about 135 during the course of an orthopedic procedure. The

above estimated values are based on no respiratory protection. The use of any type of respiratory protection device would modify this estimate depending on the efficiency of the device as a function of particle size.

Close examination of personal impactor data indicated that about 60% of the RBCs were associated with particles larger than 10 μm , and about 8% of the RBCs were associated with particles less than 0.5 μm . Johnson and Robinson (1991) reported that no infectious HIV-1 was detected in aerosols generated by electrocautery, and the majority of particles less than 0.5 μm probably originated from the electrocautery. In assessing the potential inhalation risk from aerosols of blood-borne pathogens, the probability of blood-associated particles carrying pathogens should also be considered. From all of these considerations, the potential inhalation risk from aerosols produced during orthopedic surgery seems very low. One should note that the existing literature did not provide evidence that blood-borne pathogens, such as HIV or HBV, have been transferred by the inhalation route (Tokars *et al.*, 1992; Petersen, 1980). To ascertain the significance of our results, further studies are required to assess the amount and viability of pathogens associated with these blood-associated aerosols.

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