

# Solubility of Haloether Anesthetics in Human and Animal Blood

Joao H. N. Soares, D.V.M.,\* Robert J. Brosnan, D.V.M., Ph.D.,† Fabíola B. Fukushima, D.V.M.,‡ Joanne Hodges, D.V.M.,§ Hong Liu, M.D.||

## ABSTRACT

**Background:** Anesthetic blood solubility predicts pharmacokinetics for inhaled agents and is essential for determination of blood anesthetic concentrations from end-tidal gas concentrations using Henry's Law. Though used to model anesthetic effects in humans, there are limited interspecies solubility comparisons that include modern haloethers. This study aimed to measure hematocrit-adjusted blood:gas anesthetic partition coefficients ( $\lambda_{B:G}$ ) for desflurane, sevoflurane, isoflurane, and methoxyflurane in humans and animals.

**Methods:** Whole blood was collected from 20 rats, 8 horses, and 4 each of cats, cattle, humans, dogs, goats, pigs, rabbits, and sheep. Plasma or cell volume was removed to adjust all samples to a packed cell volume of 40%. A single-agent calibration gas headspace was added to blood in a glass syringe and was mixed and equilibrated at 37°C for 2 h. Agent concentrations in the calibration gas and syringe headspace were measured using gas chromatography. Anesthetic solubility in saline, citrate-phosphate-dextrose-adenine, and olive oil were similarly measured.

**Results:** Except for goats, all animal species had at least one  $\lambda_{B:G}$  measurement that differed significantly from humans. For each agent,  $\lambda_{B:G}$  positively correlated with serum triglyceride concentrations, but this only explained 25% of interspecies variability. Desflurane was significantly less soluble in

## What We Already Know about This Topic

- Accurate volatile anesthetic blood-gas partition coefficients are needed to calculate blood concentrations from end-tidal concentrations measured at equilibrium
- The numerous factors that affect blood-gas partition coefficients could include species

## What This Article Tells Us That Is New

- Many blood-gas partition coefficients for desflurane, sevoflurane, isoflurane, and methoxyflurane measured in the blood of nine common species differed from those measured in human blood
- These differences could be because of species-related differences in both triglyceride concentration and binding to hemoglobin, plasma protein, and red cell membranes

blood than sevoflurane in some species (*e.g.*, humans) but not in others (*e.g.*, rabbits).

**Conclusions:** Anesthetic partition coefficients differ significantly between humans and most animals for haloether anesthetics. Because of their similar  $\lambda_{B:G}$  values, goats may be a better animal model for inhaled anesthetic pharmacokinetics in people.

**T**HE partition coefficient (partition ratio,  $\lambda$ ) describes the molar distribution ratio of a compound at equilibrium between two immiscible fluids.<sup>1</sup> For volatile and gaseous anesthetics,  $\lambda$  can be expressed as the concentration in saline or organic phase (such as blood, oil, or tissue) *versus* the gas phase at body temperature and 1 atmosphere pressure.

Accurate anesthetic partition coefficient measurements are important for several reasons. Agent concentrations can be measured easily and quickly from end-tidal gas samples. Under ideal gas conditions<sup>2</sup> and for a constant activity coefficient, Henry's Law states that the ratio of anesthetic vapor concentration within a liquid to the anesthetic vapor partial pressure is constant.<sup>3,4</sup> Because vapor concentration in the gas phase equals the vapor partial pressure divided by barometric pressure, the vapor concentration ratio between liquid and gas phases is also constant. Accurate Ostwald partition coefficients at body temperature are thus essential to correct calculations of blood and tissue anesthetic concentrations at equilibrium from measurements of end-tidal anesthetic gas concentrations.

Accurate solubility measurements allow for prediction of anesthetic potency. Conventional anesthetics generally obey

\* Resident, Veterinary Anesthesiology, § Associate Veterinarian, Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, Davis, California. † Associate Professor, ‡ Visiting Scholar, Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis. || Associate Professor, Anesthesiology and Pain Medicine, University of California Davis Medical Center, Sacramento, California.

Received from the Department of Surgical and Radiological Sciences, University of California, Davis, Davis, California. Submitted for publication November 10, 2011. Accepted for publication March 2, 2012. Supported by grant No. GM092821-02 from the National Institutes of General Medical Sciences, National Institutes of Health, Bethesda, Maryland, and by the Center for Equine Health at the University of California, Davis, California.

Address correspondence to Dr. Brosnan: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, One Shields Avenue, Davis, California 95616. rjbrosnan@ucdavis.edu. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

Copyright © 2012, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2012; 117:48–55

the Meyer-Overton hypothesis; the anesthetic  $EC_{50}$  or minimum alveolar concentration (MAC) correlates inversely with the oil:gas partition coefficient ( $\lambda_{O:G}$ ).<sup>5</sup> Among conventional and experimental anesthetics with greater range of water solubilities, the Meyer-Overton Constant, defined as  $MAC \times \lambda_{O:G}$ , correlates inversely with the saline:gas partition coefficient ( $\lambda_{S:G}$ ).<sup>6</sup> In addition to pharmacodynamics, partition coefficients are important to modeling inhaled anesthetic pharmacokinetics. Uptake from the lung into blood and from the blood into tissues is directly proportional to the blood:gas anesthetic partition coefficient ( $\lambda_{B:G}$ ). Agents with a high  $\lambda_{B:G}$  exhibit a slow rate-of-rise of the alveolar anesthetic partial pressure, resulting in a slow rate-of-rise in anesthetic partial pressure in the central nervous system and slow onset kinetics. During recovery, anesthetics with a high  $\lambda_{B:G}$  or  $\lambda_{O:G}$  will exhibit slow elimination kinetics because arterial-to-alveolar partial pressure diffusion gradients are lower for a given blood anesthetic concentration and because greater anesthetic solubility will increase the total quantity of anesthetic dissolved in tissues.<sup>7</sup>

The  $\lambda_{B:G}$  varies with temperature,<sup>8–11</sup> hematocrit,<sup>12,13</sup> plasma protein,<sup>13,14</sup> osmolarity,<sup>15</sup> and lipid concentration.<sup>13,14,16,17</sup> However,  $\lambda_{B:G}$  may vary between species as well. Although anesthetic partition coefficients have been measured in several species, it is difficult to know to what extent different values in animals and humans are because of methodology *versus* actual solubility differences. Furthermore, data for contemporary agents is entirely lacking in some animal species of veterinary and research importance.

The aims of this study were to validate a chromatography method by comparing measurements of  $\lambda_{S:G}$  and  $\lambda_{O:G}$  for desflurane, isoflurane, methoxyflurane, and sevoflurane with previously published values and then to use this same method and equipment to determine  $\lambda_{B:G}$  of these anesthetics for humans, cats, cattle, dogs, goats, horses, pigs, rabbits, rats, and sheep.

## Materials and Methods

### Blood Collection and Preparation

A 120 ml sample of human blood was drawn aseptically and with the written, informed consent from four healthy and awake adult volunteers with the approval of the Institutional Review Board at the University of California, Davis, California. Samples were stored in separate sterile bags (Teruflex; Terumo Corporation, Tokyo, Japan) with 1 volume of citrate-phosphate-dextrose-adenine (CPDA-1) per 9 volumes of blood (13 ml CPDA-1 per 120 ml of blood). Animal phlebotomies were performed with the approval of the Institutional Animal Care and Use Committee at the University of California, Davis, California. Samples comprising less than 20% of estimated blood volumes were aseptically collected without sedation in four cattle, four dogs, four goats, eight horses, and four sheep, and stored in separate sterile 10% CPDA-1 bags as described previously for human blood. Four cats and four pigs were anesthetized with ketamine (10

mg/kg intramuscularly) and midazolam (0.5 mg/kg intramuscularly) for aseptic collection of less than 20% of estimated blood volumes into separate CPDA-1 bags; all animals subsequently recovered uneventfully. In four rabbits and 20 rats anesthetized with ketamine (40 mg/kg and 100 mg/kg, respectively, intraperitoneally) and midazolam (2 mg/kg and 5 mg/kg, respectively, intraperitoneally), a mid-line laparotomy was performed to expose and catheterize the abdominal aorta through which blood was collected into CPDA-1 bags until animals were exsanguinated. Because of their small size, blood from five rats was pooled into each collection bag to yield four separate and independent samples containing approximately 50 ml of blood each.

Packed cell volumes (%) were measured in centrifuged microhematocrit tubes, and plasma protein (g/dL) was estimated using a handheld refractometer (Model 10423; Leica Microsystems Inc., Buffalo Grove, IL). Hematocrit modestly affects anesthetic solubility.<sup>12</sup> Intra- and interspecies hemoglobin variability could thus increase solubility measurement variability; it could make it more difficult to determine whether solubility differences were because of particular blood protein and/or lipid compositions or simply because of hemoglobin concentration differences between species. Thus, packed cell volumes in all samples were adjusted to 36–42% by either withdrawing plasma or blood cells from bags. Potassium, sodium, and glucose were measured in whole blood using an automated analyzer (ABL800 FLEX; Radiometer America, Westlake, OH), and the sum of the glucose concentration plus two times the cation concentrations in mM was used to estimate plasma osmolarity. To measure triglyceride concentrations, 40  $\mu$ l of a saturated  $CaCl_2$  solution plus 40  $\mu$ l of a saturated  $MgCl_2$  solution were added to a 1 ml aliquot of blood from the CPDA-1 bag, which was subsequently centrifuged. The plasma was removed and analyzed using an automated colorimetric assay (Cobas C501; Roche Diagnostics, Indianapolis, IN). The accuracy of colorimetric triglyceride measurements from anticoagulated blood was validated using paired blood samples from horses and cattle with or without CPDA-1; differences between measurements were all within 10% of each other.

### Ostwald Partition Coefficient Measurements

Solubility of desflurane (Suprane, Baxter, Deerfield, IL), sevoflurane (SevoFlo, Abbot Laboratories, Abbot Park, IL), isoflurane (Attane, Piramal Critical Care, Boise, ID), and methoxyflurane (Penthrox, Medical Developments International, Springvale, VIC, Australia) were measured in 0.9% NaCl (USP, Baxter), olive oil (Bertoli Extra Light, Unilever, Englewood Cliffs, NJ), and CPDA-1 (Terumo Corporation, Tokyo, Japan). Blood solubility was measured within 1 week of collection, and saline anesthetic solubility was simultaneously measured again with the same agent as a process validation control. All studies were conducted in Davis, CA, which is located approximately 16 m above sea level.

Three drops of pure silicone oil (Fluke, American Fork, UT) were used to coat the plungers of noninterchangeable, graduated 100 ml glass syringes and were shown in a pilot study not to affect anesthetic solubility measurements in saline. The syringe chamber was sealed with a nylon stopcock, 10–40 ml of liquid (saline, oil, CPDA-1, or blood) was added, and the syringes were incubated in a temperature-calibrated oven at 37°C for 30 min. The precise volume of liquid in the syringe was measured using calibrated scales and previously determined densities for each study liquid.

A 20–40 ml volume of either a desflurane (1.080%), sevoflurane (2.610%), isoflurane (1.207%), or methoxyflurane (0.288%) calibration gas standard was anaerobically added using gravimetrically calibrated glass syringes to each liquid-containing 100 ml syringe, thus creating a headspace with a precisely known volume. To minimize the effects of tiny measurement error on calculations of partition coefficients, a higher ratio of liquid volume ( $V_L$ ) to gas volume ( $V_G$ ) in the syringe was used for desflurane and sevoflurane, the less soluble agents. Conversely, a lower  $V_L:V_G$  ratio in the syringe was used for the more soluble agents isoflurane and methoxyflurane.

After addition of the gas headspace, syringes were incubated in an oven for 2 h at 37°C and vigorously shaken every 15 min during the first hour. Anesthetic concentrations were measured using gas chromatography by direct headspace injection from a 0.25-ml sample loop onto a packed 183-cm, 0.32-cm 10% SF-96 column with a flame ionization detector (Clarus 500, Perkin Elmer, Waltham, MA). Chromatograph temperature and gas flow protocols, along with retention times for each agent, are listed in table 1. The detector signal was integrated over time using commercial software (TotalChrom, Perkin Elmer). Proportionality between the area under the curve and anesthetic concentration was verified using gas standards that encompassed the range of study measurements.

Liquid:gas partition coefficients ( $\lambda_{L:G}$ ) were calculated using Equation 1,

$$\lambda_{L:G} = \frac{V_G \times (C_0 - C_G)}{V_L \times C_G},$$

**Table 1.** Gas Chromatography Protocols and Retention Times for Methoxyflurane, Isoflurane, Sevoflurane, and Desflurane

	MOF	ISO	SEVO	DES
Temp (°C)				
Oven	120	100	100	100
Detector	125	150	150	150
Flow (ml/min)				
Air	350	350	350	350
H <sub>2</sub>	35	35	35	35
He	60	20	20	20
Retention times (min)	1.08	1.32	1.23	1.16

DES = desflurane; ISO = isoflurane; MOF = methoxyflurane; SEVO = sevoflurane.

where  $C_0$  and  $C_G$  are the respective concentrations (or areas under the chromatogram curves) of the calibration gas and the postequilibration headspace gas, and  $V_L$  and  $V_G$  are the respective volumes of liquid and gas in the syringe. Derivation of a similar relationship used to determine halothane  $\lambda_{L:G}$  *via* this headspace equilibration method has been published.<sup>18</sup>

### Statistical Analysis

Measurements were described using mean  $\pm$  SD. Anesthetic solubility comparisons between agents and species were made using a two-way ANOVA with Holm-Šidák<sup>19</sup> corrections for multiple two-tailed comparisons between agents within a species and for multiple two-tailed comparisons between humans and animals for each agent (v.11, SPSS, Chicago, IL). Pearson product-moments were used to assess correlation between solubility measurements for each agent and plasma osmolarity and serum triglycerides;  $P < 0.05$  defined statistical significance.

### Results

Measured blood parameters are presented in table 2, and solubilities for each of the haloethers are summarized in table 3. The saline- and oil-gas partition coefficients were similar among all agents except methoxyflurane, for which values in the present study were 5–8% lower for saline and 35–39% lower for oil. A log-log regression of human MAC for each agent<sup>20–23</sup> versus  $\lambda_{O:G}$  values in table 3 yielded a line described by the equation  $\log(\text{MAC}) = 1.043 \cdot \log(\lambda_{O:G}) + 2.113$ , with  $R^2 = 1.000$ .

Anesthetic solubility in blood differed significantly by agent and by species, as seen in table 3. In comparison with human blood, all anesthetics tended to be more soluble in blood from dogs, rats, and rabbits and less soluble in blood from cattle. Horse blood exhibited a mixed pattern, with greater desflurane solubility but less isoflurane solubility compared with human blood. Only goat blood approximated human  $\lambda_{B:G}$  for all of the anesthetics.

The rank order for increasing liquid phase solubility was: saline  $\approx$  CPDA-1 < blood < oil. For most species, the order of increasing  $\lambda_{B:G}$  was desflurane < sevoflurane < isoflurane < methoxyflurane. However, in rabbit blood, sevoflurane was actually slightly less soluble than desflurane, although this difference was not statistically significant.

Packed cell volumes and plasma osmolarities were similar between samples, but plasma protein and triglycerides varied considerably (table 2). Solubility for each agent positively correlated with triglyceride concentrations, but this only explained approximately 25% of the variability in  $\lambda_{B:G}$  values (table 4). Desflurane solubility also negatively correlated with plasma albumin concentration, but no statistically significant protein effects were detected for the other agents.

### Discussion

Haloether anesthetic  $\lambda_{B:G}$  were measured in humans and nine animal species of research and veterinary importance

**Table 2.** Species Demographic Data and Corresponding Measurements of Packed Cell Volume, Plasma Osmolarity, and Serum Triglycerides (Mean ± SD) in Blood Collected into Citrate-Phosphate-Dextrose-Adenine Bags

Sample	Age (yr)	Breed or Strain (N)	Sex (N)	Weight (kg)	PCV (%)	Protein (mg/dl)	Osmolarity (mOsm/l)	Triglycerides (mg/dl)
Cat	4.3 ± 0.8	Domestic Short Hair (4)	M (4)	6.5 ± 0.9	39.5 ± 2.7	6.5 ± 0.3	352 ± 9	242 ± 83
Cow	1.5–2.0	Angus (4)	F (4)	567 ± 38	37.0 ± 0.8	6.9 ± 0.2	335 ± 3	31 ± 3
Dog	4.2 ± 1.0	Labrador (2) Pitbull Terrier (1) Doberman Pinscher (1)	F (2) M (2)	34.1 ± 3.7	41.5 ± 1.3	5.9 ± 0.3	345 ± 3	83 ± 19
Goat	1.0 ± 0.0	Saanen (2) La Mancha (1) Alpine (1)	F (4)	60 ± 3	40.3 ± 1.7	6.2 ± 0.2	341 ± 3	41 ± 6
Horse	15.3 ± 2.4	Quarter Horse (3) Thoroughbred (2) Sella Français (1) American Paint Horse (1) Holsteiner (1)	F (3) M (5)	587 ± 32	38.9 ± 2.2	6.1 ± 0.3	323 ± 5	34 ± 9
Human	33.8 ± 5.0		F (2) M (2)	69 ± 29	39.0 ± 1.2	7.0 ± 0.4	328 ± 3	98 ± 35
Pig	0.3 ± 0.0	Yorkshire (4)	M (3) F (1)	44 ± 4	37.5 ± 1.7	5.3 ± 0.2	330 ± 3	69 ± 38
Rabbit	0.9 ± 0.0	New Zealand White (4)	M (4)	3.6 ± 0.4	39.5 ± 1.7	5.4 ± 0.2	324 ± 5	150 ± 80
Rat	1.0 ± 0.3	Brown Norway (20)	F (20)	0.37 ± 0.13	38.9 ± 2.3	6.0 ± 0.2	338 ± 13	164 ± 87
Sheep	0.3 ± 0.0	Suffolk (4)	F (4)	52 ± 1	36.0 ± 0.0	5.9 ± 0.3	354 ± 10	23 ± 3

PCV = packed cell volume.

using identical equipment and methodology. The consistency between partition coefficient measurements in saline, oil, and human blood measured in the present study and in published literature for contemporary agents served to validate these methods.

Although no longer widely used, methoxyflurane was included in this study as an example of a more soluble chloro-fluoroether for which interspecies  $\lambda$  differences might be magnified. The methoxyflurane  $\lambda_{O,G}$  measured here (table 3) was considerably lower than in previous studies.<sup>24,25</sup> This method used for measurement could simply be less accurate for vapors and media with high solubility, as evidenced by the large standard deviations in the methoxyflurane-oil measurement. However, it could also be because of technical limitations of previous methods. For instance, solubility measurements utilizing infrared spectrometry lack precision required to quantify large changes in methoxyflurane head-space concentration caused by its very high  $\lambda_{O,G}$ ;<sup>25</sup> and even with modern analyzers, anesthetic measurement accuracy can vary considerably.<sup>26</sup> Prior chromatographic methods with longer retention times<sup>1,27</sup> than in the protocol here (table 1) increase peak asymmetry and alter the relationship between peak height and the area under the chromatogram curve, thereby introducing a small error.<sup>28</sup> The present study also benefited from computerized unattenuated signal integration, high digital sampling rates, and high detector sensitivity, all of which increase measurement accuracy but were not as readily available during the times in which previous studies were performed.<sup>28</sup> Consequently, the Meyer-Overton log-log correlation between  $\lambda_{O,G}$  and anesthetic MAC in humans was slightly stronger using data from the present

study, as evidenced by an  $R^2$  of 1.000 versus 0.946 from a prior analysis.<sup>29</sup>

One limitation of the present study was the use of blood diluted in CPDA-1, which mimics banked but not fresh blood. Yu *et al.*<sup>30</sup> found that the  $\lambda_{B,G}$  of desflurane, isoflurane, and halothane were between 12 and 13% lower in banked blood than in fresh. Comparisons between these results and our data are somewhat difficult because the prior study did not state how long blood was stored or whether results were controlled for hematocrit, which would otherwise be decreased from hemodilution. Since the anesthetics are less soluble in anticoagulant than in whole blood (table 3), the addition of 1:9 CPDA-1: blood should reduce  $\lambda_{B,G}$  by 3.6–6.0%. However, since all blood samples contained the same volume fraction of anticoagulant, the relative results from interagent and species comparisons should remain unaffected.

Anesthetic  $\lambda_{B,G}$  varies for many anesthetics between humans and animals as well as between different animal species. However, one exception is the goat, for which  $\lambda_{B,G}$  was similar to humans for all agents in our study. For a given alveolar ventilation, cardiac output, and anesthetic partial pressure gradient, the rate of anesthetic uptake and elimination is determined by the agent  $\lambda_{B,G}$ .<sup>7</sup> Hence, goats may serve as the most suitable animal model for extrapolation of inhaled anesthetic pharmacokinetic data to humans.

The agents desflurane and sevoflurane had statistically indistinguishable  $\lambda_{B,G}$  values in some animal species (table 3). Although sample sizes in this study were relatively small, the measurement variability was likewise small. Even controlling for multiple comparisons, it was possible to detect statistically significant differences as low as 7% between

**Table 3.** The Partition Coefficients ( $\lambda_{S:G}$ ,  $\lambda_{O:G}$ , and  $\lambda_{B:G}$ ) for Desflurane, Sevoflurane, Isoflurane, and Methoxyflurane (Mean  $\pm$  SD) Measured at 37°C

	Desflurane	Sevoflurane	Isoflurane	Methoxyflurane
Human	0.498 $\pm$ 0.012 (0.424–0.522) <sup>8,30,49,50</sup>	0.639 $\pm$ 0.011 (0.60–0.686) <sup>8,51,52</sup>	1.32 $\pm$ 0.04 (1.25–1.46) <sup>10,12,50,53</sup>	14.3 $\pm$ 0.4 (13.0–16.0) <sup>9,10,25,54</sup>
Horse	0.537 $\pm$ 0.018*	0.648 $\pm$ 0.051 (0.47) <sup>55</sup>	1.13 $\pm$ 0.06* (0.92) <sup>55</sup>	13.0 $\pm$ 1.3 —
Cattle	0.442 $\pm$ 0.032 —	0.521 $\pm$ 0.016* —	1.22 $\pm$ 0.03 —	11.3 $\pm$ 0.7* —
Sheep	0.496 $\pm$ 0.056† —	0.557 $\pm$ 0.027* —	1.34 $\pm$ 0.09 (1.41) <sup>56</sup>	13.2 $\pm$ 0.9 (14.2) <sup>56</sup>
Goat	0.520 $\pm$ 0.017† —	0.564 $\pm$ 0.042 —	1.37 $\pm$ 0.06 —	13.0 $\pm$ 0.7 —
Pig	0.502 $\pm$ 0.054† (0.35†–0.40) <sup>57,58</sup>	0.521 $\pm$ 0.050 (0.45†–0.48) <sup>57,58</sup>	1.07 $\pm$ 0.05* (0.94)† <sup>58</sup>	11.1 $\pm$ 0.5* —
Dog	0.631 $\pm$ 0.015* —	0.664 $\pm$ 0.013 —	1.40 $\pm$ 0.16 (1.30) <sup>59</sup>	26.1 $\pm$ 4.0* —
Cat	0.583 $\pm$ 0.012*† —	0.593 $\pm$ 0.034 —	1.40 $\pm$ 0.08 —	26.4 $\pm$ 1.3* —
Rat	0.611 $\pm$ 0.027* (0.79) <sup>60</sup>	0.744 $\pm$ 0.019* (0.75–0.81) <sup>60–62</sup>	1.41 $\pm$ 0.09 (1.56–3.05) <sup>60,63,64</sup>	17.7 $\pm$ 1.9 (25.02) <sup>64</sup>
Rabbit	0.721 $\pm$ 0.052*† —	0.691 $\pm$ 0.030 —	1.37 $\pm$ 0.09 (1.59) <sup>3</sup>	25.0 $\pm$ 2.7* —
Saline	0.287 $\pm$ 0.003* (0.225–0.282) <sup>30,49,65</sup>	0.329 $\pm$ 0.007* (0.30–0.37) <sup>52,62,65</sup>	0.517 $\pm$ 0.014* (0.529–0.63) <sup>15,24,30,63,65</sup>	4.01 $\pm$ 0.17* (4.2–4.33) <sup>9,15,24</sup>
CPDA-1	0.290 $\pm$ 0.017*† —	0.315 $\pm$ 0.006* —	0.536 $\pm$ 0.060* —	3.05 $\pm$ 0.02* —
Olive oil	19.2 $\pm$ 0.4* (17.9–18.7) <sup>29,49</sup>	51.3 $\pm$ 1.5* (47.2–53.4) <sup>29,51,52</sup>	98.9 $\pm$ 3.1* (88.2–97.8) <sup>29,53,66</sup>	611 $\pm$ 77* (825–850) <sup>24,25</sup>

\* Partition coefficient values for an agent that are statistically different from measurements in human blood. † Within a species,  $\lambda$  was statistically different between agents, except as denoted between desflurane and sevoflurane. Published measurements at 37°C for each species are included in parentheses for comparison. ‡ Values denoted were measured at 38–38.5°C.

$\lambda_{B:G}$  = blood:gas anesthetic partition coefficient;  $\lambda_{O:G}$  = oil:gas partition coefficient;  $\lambda_{S:G}$  = saline:gas partition coefficient; CPDA-1 = citrate-phosphate-dextrose-adenine anticoagulant solution.

means. Furthermore, in contrast to humans, the  $\lambda_{B:G}$  in rabbits was actually greater for desflurane than for sevoflurane; this finding cannot be simply attributed to inadequate statistical power. When delivered at equal partial pressures, sevoflurane equilibration between the alveolar and central nervous system partial pressures may actually be slightly faster than for desflurane in rabbits. Yet since it is much less potent than sevoflurane as an anesthetic,<sup>31,32</sup> desflurane is typically administered at much higher concentrations, and these higher partial pressure gradients may still permit faster wash-in and washout kinetics.<sup>7</sup> A similar effect has been

demonstrated for desflurane and nitrous oxide in humans. Despite having approximately equal  $\lambda_{B:G}$  values, nitrous oxide has a much higher MAC than desflurane and is delivered at much higher concentrations, resulting in a faster rate of rise of the alveolar concentration for nitrous oxide than for desflurane.<sup>33</sup>

Although serum osmolality can affect  $\lambda_{B:G}$ ,<sup>15</sup> the range of values in this study were limited and not correlated with solubility. Hematocrit was adjusted for all species to approximately 40%, since changes in erythrocyte content could affect inhalants' blood solubility.<sup>12,13</sup> Hence  $\lambda_{B:G}$  differences cannot be because of hemoglobin quantity in this study. Red blood cells from rats and humans have different affinity for volatile organic compounds, such as toluene, chloroform and n-hexane, as well as for blood components, such as plasma proteins and hemoglobin.<sup>34</sup> Therefore, differences in both the blood protein concentration and the species-specific protein structure could explain differences in inhalant anesthetic  $\lambda_{B:G}$ . Four plausible explanations for  $\lambda_{B:G}$  variability remain: species differences in hemoglobin-anesthetic binding, cell membrane anesthetic solubility, plasma protein quantity and/or anesthetic binding, and plasma lipid quantity and/or solvent properties.

**Table 4.** Pearson Correlations between  $\lambda_{B:G}$  of Desflurane, Sevoflurane, Isoflurane, and Methoxyflurane with Plasma Osmolarity, Triglycerides, and Total Protein

Agent	Osmolarity	Triglycerides	Protein
Desflurane	–0.26	0.50*	–0.50*
Sevoflurane	–0.26	0.47*	–0.18
Isoflurane	0.36	0.54*	0.15
Methoxyflurane	0.32	0.48*	–0.10

\*  $P < 0.05$ .

$\lambda_{B:G}$  = blood:gas anesthetic partition coefficient.

Red blood cells are an important, and perhaps the major, carrier of inhalant anesthetics in blood.<sup>13,34</sup> Two major red blood cells components that can be responsible for  $\lambda_{B,G}$  variation within species are hemoglobin and lipids. Species differences in hemoglobin-oxygen binding affinity are well established,<sup>35,36</sup> and are in part the result of differences in hemoglobin molecular structure.<sup>37,38</sup> For example, bovine hemoglobin has a lower affinity for oxygen compared with human hemoglobin because of substitution of alanine by lysine in the N-terminal that increases hydrophilicity.<sup>36</sup> Substitution of histidine at position 2 with methionine in bovine hemoglobin  $\beta$ -chains increase affinity for oxygen even in the absence of 2,3-diphosphoglycerate by creating a hydrophobic pocket.<sup>36</sup> Volatile and gaseous anesthetics can also bind to hydrophobic sites of proteins,<sup>39</sup> such as occurs for xenon in equine hemoglobin.<sup>40</sup> Currently unknown hemoglobin variations may be present in other species as well, and create variability in the number and/or size of hydrophobic and amphipathic pockets suitable for anesthetic binding. Further study of species-specific hemoglobin ultrastructure may be useful to potentially explain the  $\lambda_{B,G}$  variability.

Erythrocyte membrane lipids vary quantitatively and qualitatively.<sup>41</sup> Mammalian erythrocyte membranes have higher concentrations of phospholipids and neutral lipids than gangliosides or glycolipids, although total lipid composition differs among species.<sup>41</sup> If the four haloethers used in this study follow the same pattern as xenon, which had different solubility for gangliosides, phospholipids, and neutral lipids,<sup>42</sup> variation in  $\lambda_{B,G}$  among species might be because of different lipid solvent properties of erythrocyte membranes.

Albumin is the most abundant protein in mammals, and its sequence homology among humans, cows, sheep, rats, horses, dogs, and rabbits is greater than 70%. However, because of albumin's importance in binding lipophilic molecules, any nonconservative structural changes could confer differences in drug-binding properties.<sup>43</sup> In the presence of normal concentrations of serum constituents, albumin transports three times more halothane than triglycerides,<sup>44</sup> and it is an important determinant of anesthetic solubility.<sup>45</sup> Albumin has two major drug-binding sites (I and II), and these can vary among species: Dog albumin has the same site II as humans, but rabbits and rats have the same site I.<sup>43</sup> Interestingly, inhalant anesthetics can bind to albumin in other hydrophobic pockets, and binding affinity and capacity may be affected by the number and size of these pockets. Bovine albumin has 3–5-fold more affinity than human albumin for halothane as a result of a leucine residue at position 135 (one of the putative albumin-binding sites) instead of a tryptophan, suggesting a role for aromatic amino acids in inhaled anesthetic binding.<sup>46,47</sup> Even within a given albumin species, affinity can differ between structural isomers, such as between isoflurane and enflurane,<sup>48</sup> and so relative affinities and effects on interspecies  $\lambda_{B,G}$  are not generalizable across all haloethers.

Plasma triglycerides showed a positive correlation with the  $\lambda_{B,G}$  of all studied agents. The species with lower plasma triglycerides tended to have lower  $\lambda_{B,G}$ ; this finding is consistent with intraspecies triglyceride solubility effects,<sup>14,17,18</sup> but only accounts for 25% of measured interspecies variability. The remaining variability in these data are probably explained by the effects of red blood cells and plasma proteins on the anesthetic solubility in blood.

In summary, Ostwald blood:gas partition coefficients for desflurane, sevoflurane, isoflurane, and methoxyflurane are different between humans and most animals. Use of species nonspecific  $\lambda_{B,G}$  values in pharmacokinetic models or in calculations of blood anesthetic concentrations from headspace or alveolar gas can introduce significant error.

The authors thank John Grayson, D.V.M., Ph.D., Veterinarian, School of Veterinary Medicine, University of California, Davis, California, and Julie Burges, B.S., Animal Health Technician Supervisor, Leontyne Hansing, B.S., Animal Health Technician, and Carlyle Hass, B.S., Clinical Laboratory Scientist, Pritchard Veterinary Medical Teaching Hospital, University of California, Davis.

## References

- Berthelot MP, Jungfleisch ÉC: Sur les lois qui président au partage d'un corps entre deux dissolvants (expériences). *Ann Chim Phys* 1872; 26:396–407
- Eger EI 2nd, Johnson BH: Do volatile anesthetics act as ideal gases? *Anesth Analg* 1979; 58:322–3
- Coburn CM, Eger EI 2nd: The partial pressure of isoflurane or halothane does not affect their solubility in rabbit blood or brain or human brain: Inhaled anesthetics obey Henry's Law. *Anesth Analg* 1986; 65:960–2
- Lockhart SH, Eger EI 2nd: Absence of abundant saturable binding sites for halothane or isoflurane in rabbit brain: Inhaled anesthetics obey Henry's law. *Anesth Analg* 1990; 71:70–2
- Overton CE: *Studies of Narcosis*. London: Chapman and Hall; 1991
- Fang Z, Ionescu P, Chortkoff BS, Kandel L, Sonner J, Laster MJ, Eger EI 2nd: Anesthetic potencies of n-alkanols: Results of additivity and solubility studies suggest a mechanism of action similar to that for conventional inhaled anesthetics. *Anesth Analg* 1997; 84:1042–8
- Eger EI 2nd: *Anesthetic Uptake and Action*. Baltimore: Williams & Wilkins; 1974
- Lockwood GG, Sapsed-Byrne SM, Smith MA: Effect of temperature on the solubility of desflurane, sevoflurane, enflurane and halothane in blood. *Br J Anaesth* 1997; 79:517–20
- Stoelting RK, Longshore RE: The effects of temperature on floxerone, halothane, and methoxyflurane blood-gas and cerebrospinal fluid-gas partition coefficients. *ANESTHESIOLOGY* 1972; 36:503–5
- Eger RR, Eger EI 2nd: Effect of temperature and age on the solubility of enflurane, halothane, isoflurane, and methoxyflurane in human blood. *Anesth Analg* 1985; 64:640–2
- Allott PR, Steward A, Flook V, Mapleson WW: Variation with temperature of the solubilities of inhaled anaesthetics in water, oil and biological media. *Br J Anaesth* 1973; 45:294–300
- Lerman J, Gregory GA, Eger EI 2nd: Hematocrit and the solubility of volatile anesthetics in blood. *Anesth Analg* 1984; 63:911–4
- Pang YC, Reid PE, Brooks DE: Solubility and distribution of halothane in human blood. *Br J Anaesth* 1980; 52:851–62
- Lerman J, Gregory GA, Willis MM, Eger EI 2nd: Age and

- solubility of volatile anesthetics in blood. *ANESTHESIOLOGY* 1984; 61:139-43
15. Lerman J, Willis MM, Gregory GA, Eger EI 2nd: Osmolarity determines the solubility of anesthetics in aqueous solutions at 37 degrees C. *ANESTHESIOLOGY* 1983; 59:554-8
  16. Saraiva RA, Willis BA, Steward A, Lunn JN, Mapleson WW: Halothane solubility in human blood. *Br J Anaesth* 1977; 49:115-9
  17. Weaver BM, Webb AI: Tissue composition and halothane solubility in the horse. *Br J Anaesth* 1981; 53:487-93
  18. Larson CP Jr, Eger EI 2nd, Severinghaus JW: The solubility of halothane in blood and tissue homogenates. *ANESTHESIOLOGY* 1962; 23:349-55
  19. Ludbrook J: Multiple comparison procedures updated. *Clin Exp Pharmacol Physiol* 1998; 25:1032-7
  20. Saidman LJ, Eger EI 2nd, Munson ES, Babad AA, Muallem M: Minimum alveolar concentrations of methoxyflurane, halothane, ether and cyclopropane in man: Correlation with theories of anesthesia. *ANESTHESIOLOGY* 1967; 28:994-1002
  21. Stevens WD, Dolan WM, Gibbons RT, White A, Eger EI, Miller RD, DeJong RH, Elashoff RM: Minimum alveolar concentrations (MAC) of isoflurane with and without nitrous oxide in patients of various ages. *ANESTHESIOLOGY* 1975; 42:197-200
  22. Scheller MS, Saidman LJ, Partridge BL: MAC of sevoflurane in humans and the New Zealand white rabbit. *Can J Anaesth* 1988; 35:153-6
  23. Rampil IJ, Lockhart SH, Zwass MS, Peterson N, Yasuda N, Eger EI 2nd, Weiskopf RB, Damask MC: Clinical characteristics of desflurane in surgical patients: Minimum alveolar concentration. *ANESTHESIOLOGY* 1991; 74:429-33
  24. Koblin DD, Laster MJ, Ionescu P, Gong D, Eger EI 2nd, Halsey MJ, Hudlicky T: Polyhalogenated methyl ethyl ethers: Solubilities and anesthetic properties. *Anesth Analg* 1999; 88:1161-7
  25. Eger EI 2nd, Shargel R: The solubility of methoxyflurane in human blood and tissue homogenates. *ANESTHESIOLOGY* 1963; 24:625-7
  26. Hendrickx JF, Lemmens HJ, Carette R, De Wolf AM, Saidman LJ: Can modern infrared analyzers replace gas chromatography to measure anesthetic vapor concentrations? *BMC Anesthesiol* 2008; 8:2
  27. Carpenter RL, Eger EI 2nd, Johnson BH, Unadkat JD, Sheiner LB: Pharmacokinetics of inhaled anesthetics in humans: Measurements during and after the simultaneous administration of enflurane, halothane, isoflurane, methoxyflurane, and nitrous oxide. *Anesth Analg* 1986; 65:575-82
  28. Grob RL, Kaiser MA: Qualitative and quantitative analysis by gas chromatography. In: Grob RL, Barry EF, eds. *Modern Practice of Gas Chromatography*, 4th ed. Hoboken: John Wiley & Sons; 2004:403-460
  29. Taheri S, Halsey MJ, Liu J, Eger EI 2nd, Koblin DD, Laster MJ: What solvent best represents the site of action of inhaled anesthetics in humans, rats, and dogs? *Anesth Analg* 1991; 72:627-34
  30. Yu RG, Zhou JX, Liu J: Prediction of volatile anaesthetic solubility in blood and priming fluids for extracorporeal circulation. *Br J Anaesth* 2001; 86:338-44
  31. Scheller MS, Saidman LJ, Partridge BL: MAC of sevoflurane in humans and the New Zealand white rabbit. *Can J Anaesth* 1988; 35:153-6
  32. Doorley BM, Waters SJ, Terrell RC, Robinson JL: MAC of I-653 in beagle dogs and New Zealand white rabbits. *ANESTHESIOLOGY* 1988; 69:89-91
  33. Yasuda N, Lockhart SH, Eger EI 2nd, Weiskopf RB, Johnson BH, Freire BA, Fassoulaki A: Kinetics of desflurane, isoflurane, and halothane in humans. *ANESTHESIOLOGY* 1991; 74:489-98
  34. Lam CW, Galen TJ, Boyd JF, Pierson DL: Mechanism of transport and distribution of organic solvents in blood. *Toxicol Appl Pharmacol* 1990; 104:117-29
  35. Clerbaux T, Gustin P, Detry B, Cao ML, Frans A: Comparative study of the oxyhaemoglobin dissociation curve of four mammals: Man, dog, horse and cattle. *Comp Biochem Physiol Comp Physiol* 1993; 106:687-94
  36. Poyart C, Wajcman H, Kister J: Molecular adaptation of hemoglobin function in mammals. *Respir Physiol* 1992; 90:3-17
  37. Giardina B, Brix O, Clementi ME, Scatena R, Nicoletti B, Cicchetti R, Argentin G, Condo SG: Differences between horse and human haemoglobins in effects of organic and inorganic anions on oxygen binding. *Biochem J* 1990; 266:897-900
  38. Dresler SL, Runkel D, Stenzel P, Brimhall B, Jones RT: Multiplicity of the hemoglobin alpha chains in dogs and variations among related species. *Ann N Y Acad Sci* 1974; 241:411-5
  39. Eckenhoff RG, Johansson JS: Molecular interactions between inhaled anesthetics and proteins. *Pharmacol Rev* 1997; 49:343-67
  40. Schoenborn BP: Binding of xenon to horse haemoglobin. *Nature* 1965; 208:760-2
  41. Nelson GJ: Lipid composition of erythrocytes in various mammalian species. *Biochim Biophys Acta* 1967; 144:221-32
  42. Kwan E, Trevor A: The association of xenon with subcellular components of rat cerebral cortex. *Mol Pharmacol* 1969; 5:236-43
  43. Kosa T, Maruyama T, Otagiri M: Species differences of serum albumins: I. Drug binding sites. *Pharm Res* 1997; 14:1607-12
  44. Pang YC, Reid PE, Brooks DE: Solubility and distribution of halothane in human blood. *Br J Anaesth* 1980; 52:851-62
  45. Laasberg LH, Hedley-Whyte J: Halothane solubility in blood and solutions of plasma proteins: Effects of temperature, protein composition and hemoglobin concentration. *ANESTHESIOLOGY* 1970; 32:351-6
  46. Eckenhoff RG: Amino acid resolution of halothane binding sites in serum albumin. *J Biol Chem* 1996; 271:15521-6
  47. Johansson JS, Eckenhoff RG, Dutton PL: Binding of halothane to serum albumin demonstrated using tryptophan fluorescence. *ANESTHESIOLOGY* 1995; 83:316-24
  48. Liu R, Eckenhoff RG: Weak polar interactions confer albumin binding site selectivity for haloether anesthetics. *ANESTHESIOLOGY* 2005; 102:799-805
  49. Eger EI 2nd: Partition coefficients of I-653 in human blood, saline, and olive oil. *Anesth Analg* 1987; 66:971-3
  50. Zhou JX, Liu J: Dynamic changes in blood solubility of desflurane, isoflurane, and halothane during cardiac surgery. *J Cardiothorac Vasc Anesth* 2001; 15:555-9
  51. Wallin RF, Regan BM, Napoli MD, Stern IJ: Sevoflurane: A new inhalational anesthetic agent. *Anesth Analg* 1975; 54:758-66
  52. Strum DP, Eger EI 2nd: Partition coefficients for sevoflurane in human blood, saline, and olive oil. *Anesth Analg* 1987; 66:654-6
  53. Cromwell TH, Eger EI 2nd, Stevens WC, Dolan WM: Forane uptake, excretion, and blood solubility in man. *ANESTHESIOLOGY* 1971; 35:401-8
  54. Lerman J, Gregory GA, Eger EI 2nd: Hematocrit and the solubility of volatile anesthetics in blood. *Anesth Analg* 1984; 63:911-4
  55. Bergadano A, Lauber R, Zbinden A, Schatzmann U, Moens Y: Blood/gas partition coefficients of halothane, isoflurane and sevoflurane in horse blood. *Br J Anaesth* 2003; 91:276-8
  56. Lerman J, Gregory GA, Willis MM, Schmitt BI, Eger EI 2nd: Age and the solubility of volatile anesthetics in ovine tissues. *Anesth Analg* 1985; 64:1097-100
  57. Zhou JX, Liu J: Tissue solubility of four volatile anesthetics in

- fresh and frozen tissue specimens from swine. *Am J Vet Res* 2002; 63:74-7
58. Yasuda N, Targ AG, Eger EI 2nd, Johnson BH, Weiskopf RB: Pharmacokinetics of desflurane, sevoflurane, isoflurane, and halothane in pigs. *Anesth Analg* 1990; 71:340-8
  59. Zbinden AM, Frei FJ, Funk B, Thomson DA, Westenskow D: Determination of the partial pressure of halothane (or isoflurane) in blood. *Br J Anaesth* 1985; 57:796-802
  60. Holdcroft A, Bose D, Sapsed-Byrne SM, Ma D, Lockwood GG: Arterial to inspired partial pressure ratio of halothane, isoflurane, sevoflurane and desflurane in rats. *Br J Anaesth* 1999; 83:618-21
  61. Rao Y, Wang YL, Li H, Zhang W, Liu J: Effects of pregnancy on the solubility of halogenated volatile anaesthetics in rat blood and tissues. *Anaesth Intensive Care* 2008; 36:830-4
  62. Stern RC, Towler SC, White PF, Evers AS: Elimination kinetics of sevoflurane and halothane from blood, brain, and adipose tissue in the rat. *Anesth Analg* 1990; 71:658-64
  63. Gargas ML, Burgess RJ, Voisard DE, Cason GH, Andersen ME: Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol Appl Pharmacol* 1989; 98:87-99
  64. Fassoulaki A, Eger EI 2nd: Starvation increases the solubility of volatile anaesthetics in rat liver. *Br J Anaesth* 1986; 58:327-9
  65. Hönemann CW, Washington J, Hönemann MC, Nietgen GW, Durieux ME: Partition coefficients of volatile anesthetics in aqueous electrolyte solutions at various temperatures. *ANESTHESIOLOGY* 1998; 89:1032-5
  66. Koblin DD, Eger EI 2nd, Johnson BH, Collins P, Harper MH, Terrell RC, Speers L: Minimum alveolar concentrations and oil/gas partition coefficients of four anesthetic isomers. *ANESTHESIOLOGY* 1981; 54:314-7