Mixed-gas model for predicting decompression sickness in rats

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Lillo, R. S., and E. C. Parker. Mixed-gas model for predicting decompression sickness in rats. *J Appl Physiol* 89: 2107–2116, 2000.— A mixed-gas model for rats was developed to further explore the role of different gases in decompression and to provide a global model for possible future evaluation of its usefulness for human prediction. A Hill-equation dose-response model was fitted to over 5,000 rat dives by using the technique of maximum likelihood. These dives used various mixtures of He, N_2, Ar, and O_2 and had times at depth up to 2 h and varied decompression profiles. Results supported past findings, including *1*) differences among the gases in decompression risk (He $<$ N_2 $<$ Ar) and exchange rate (He $>$ Ar $\approx$ N_2), *2*) significant decompression risk of O_2, and *3*) increased risk of decompression sickness with heavier animals. New findings included asymmetrical gas exchange with gas washout often unexpectedly faster than uptake. Model success was demonstrated by the relatively small errors (and their random scatter) between model predictions and actual incidences. This mixed-gas model for prediction of decompression sickness in rats is the first such model for any animal species that covers such a broad range of gas mixtures and dive profiles.

diving; hyperbaric; inert gases; mathematical modeling

DURING THE LAST DECADE, DEVELOPMENT of human decompression procedures has been significantly aided by the use of probabilistic models for predicting decompression sickness (DCS; Ref. 25). By using techniques such as maximum likelihood, the probability of DCS can be expressed as a continuous function that includes variables such as depth, gas composition, and decompression profile. Unfortunately, although DCS is assumed to result from an excess of inert gas somewhere in the body, little is known about the properties of gases under high pressure in tissues (26) or about how bubbles develop and presumably lead to DCS (5, 23). Consequently, probabilistic modeling has generally been done empirically by fitting functions to a set of dives that allow risk prediction but have little, if any, physiological basis. As a result, most human predictive models do not extrapolate reliably to dive profiles much different from the original data, particularly to those that have high risk.

Use of animals offers the potential to improve prediction of DCS in humans. Large numbers of animal dives can be performed relatively easily and inexpensively over a broad range of DCS incidence rates. These data are often more suitable for modeling decompression risk than human data containing relatively few cases of DCS. However, much animal research has focused on investigating the pathophysiology of DCS or addressing basic scientific questions such as the role of bubbles in DCS rather than on helping to estimate human risk of DCS. Exceptions to this include the early use of goats to help develop decompression procedures for humans (4). A next major step is to develop a formal procedure for using animal decompression outcome to help predict human DCS.

Modeling efforts in our laboratory with small animals have demonstrated that prediction of risk of DCS can be improved by accounting for differences among gases in terms of *1*) "relative potency" (RP), which defines the level of decompression risk on a per-unit-pressure basis, and *2*) gas exchange rates (12–15). These previous models have generally dealt with specific types of dives such as saturation, gas switching, or dives with variable decompression. This report describes the next step of combining the different data sets and developing one general mixed-gas model for prediction of DCS in rats over a wide range of dive profiles with various mixtures of He, N_2, Ar, and O_2.

We believe that this is the first attempt to construct a model of such scope in terms of gases and types of dives. The rationale behind our work was that such a model should provide additional information about the role of different gases in decompression, thus increasing our understanding of the mechanisms of DCS. The hypothesis for this study was that such a model could be produced that would allow reliable predictions without significant bias with regard to the exact dive profile. This work was not done in anticipation of an increased use of mixed-gas diving in the US Navy. However, our work does provide a global model for

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possible future evaluation of its usefulness for human prediction. Recently, a similar effort using animal decompression outcome to predict human DCS was begun with sheep (2).

METHODS

Hill-equation dose-response models were fitted by the technique of maximum likelihood to a database of over 5,000 rat dives created by combining five sets of dives with very different profiles that were conducted over the last decade at the Naval Medical Research Institute (NMRI). The first four sets (*data sets 1–4*) had been analyzed separately earlier and previously reported (12–15); a fifth set (*data set 5*) represents new data that have not been modeled or described before. These dives, using various mixtures of He, N_2, Ar, and O_2, had times at depth up to 2 h and varied decompression profiles. A general mixed-gas model for prediction of decompression risk in rats was produced.

Animal Use

The experimental animal protocols for all the four past sets of experiments as well as for the new previously unreported data (*data set 5*) were reviewed and approved by the in-house Animal Care and Use Committee at NMRI before any experiments. The Committee used the animal use guidance required at the time of review by the Department of the Navy, which was the current version of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS publ. nos. (NIH) 78-23, 85-23, 86-23.

Animal numbers were one of many details carefully reviewed by the NMRI committee, and statistical justification was required to support all animal requests. In the case of decompression modeling, large numbers of dives are often required to produce parameter estimates with the precision needed to resolve differences in risk or gas exchange. Discussion regarding selection of the specific number of animals used is contained in some of the past reports (12–15). Similar studies involving large numbers of rodents continue to be approved at NMRI, as evidenced by the most recently conducted experiments comparing the decompression risk of He and H_2 (16).

Data Used for Modeling

The five sets of rat dives used in model development are summarized in Table 1.

Experimental Protocols

Our experimental procedures for conducting decompression experiments with rats have been described previously (12–15). Therefore, only a brief general discussion of the methods is provided here, followed by a more detailed description of procedures associated with *data set 5*, which has not been previously reported and thus needs to be described.

Male albino rats (*Rattus norvegicus*, Sprague-Dawley strain) weighing ~200–300 g were obtained from a local supplier and housed in the Institute's animal care facility for at least 1 wk before use. The animals were allowed access to food and water up until immediately before the dive began.

General diving procedures. Five animals were placed in a cylindrical cage, which was then loaded into a small-animal hyperbaric chamber. The chamber was compressed at a rate of 1.8 atmospheres absolute (ATA)/min to depth. Gas mixtures were made by serially adding air, O_2, or one of the inert gases (N_2, He, or Ar) during compression, with the precise amounts depending on the intended composition. For dives in which N_2 was to be absent in the dive mixture, the chamber was flushed with an appropriate amount of O_2 over a 5-min period before compression to remove all N_2 (13).

The composition of the chamber atmosphere was analyzed when final depth was first reached and at 10- to 15-min intervals thereafter at depth. A Beckman F3 paramagnetic O_2 analyzer and a Beckman 865 infrared CO_2 analyzer (Fullerton, CA) were used for O_2 and CO_2 analysis, respectively. The inert gases were measured by use of a UTI 100C mass spectrometer (Uthe Technology International, Sunnyvale, CA). Chamber temperature was kept at 28.0 ± 0.5°C throughout the exposure by means of a temperature controller (Yellow Springs Instrument, Yellow Springs, OH).

Animals were kept at depth for up to 2 h and then decompressed rapidly (<10 s to the surface) or slowly (1.8 ATA/min), with up to one stop lasting up to 40 min, back to surface. Rats were then observed for 30 min for signs of DCS, as described previously (13). Briefly, these signs consisted of walking difficulties, abnormal breathing patterns, forelimb

Table 1. *Summary of rat data used for modeling*

Data Set (Ref. No.)	Dive Profile	Gas Mixtures	Depth, ATA	Time at Depth, min	Decompression
1 (13)	Saturation	N_2-He-Ar-O_2 (20.9% O_2)	5.39–7.67	120	Rapid (<10 s) to surface
2 (12)	Variable time at depth	N_2-He-O_2 (20.9%) N_2-O_2 (variable %O_2) 1–3 ATA O_2	6.30 5.27–7.27	10–120 10–120	Rapid (<10 s) to surface
3 (15)	Gas switching at depth	Air, He-O_2, Ar-O_2 (all 20.9% O_2)	4.79–6.30	60	Wait 3–35 min at depth post-gas switch, then rapid (<10 s) to surface
4 (14)	N_2-O_2 decompression	N_2-O_2 (variable %O_2) 1–3 ATA O_2	6.27 or 7.27	60	Initial slow (1.8 ATA/min) to variable stopping depth for up to 20 min, followed by rapid ascent to surface
5 (unreported previously)	Inert gas decompression	N_2-He-Ar-O_2 (20.9% O_2)	6.45 or 9.33	60	Initial slow (1.8 ATA/min) to variable stopping depth for up to 40 min, followed by rapid ascent to surface
		Ar-O_2 (20.9% O_2)	4.79	10–120	Rapid (<10 s) to surface

ATA, atmospheres absolute.

and/or hindlimb paralysis, rolling in the cage, convulsions, and death. Animals were scored as having DCS only when one or more of these symptoms developed. For the gas-switching experiments (*data set 3*), the chamber atmosphere was switched to a different gas mixture at depth, 5–35 min allowed, and animals then decompressed rapidly to the surface. In terms of gas switching, *data set 3* is unique among the data used here.

Throughout the dive and postdecompression period, the animals were exercised by rotating the cage at a perimeter speed of ~3 m/min to ensure that all animals sustained a similar level of activity and to facilitate DCS scoring. After the 30-min postdive period, all surviving animals were euthanized by inhalation of CO_2. After death, animals were weighed on a triple-beam balance to the nearest gram.

Inert-gas decompression dives (data set 5). These dives involved various inert-gas mixtures (20.9% O_2) using N_2, He, and Ar. Most animals were held at depth for 1 h before being decompressed slowly (1.8 ATA/min) to a prescribed stopping depth and held there for up to 40 min. Animals were then rapidly (<10 s) decompressed to the surface. The exception to these profiles was one group of animals, dived on Ar-O_2, in which the time at depth was varied from 10 to 120 min before rapid decompression to the surface. These latter dives provided data from a type of profile that had been done previously with air and He-O_2 (*data set 2*) but not with Ar-O_2. The exact dive profiles that were performed are given in the leftmost column of Table 2.

Data Analysis

Hill-equation dose-response models predicting the probability of DCS in rats were fitted to the five data sets individually and in combination by the technique of maximum likelihood (8). The goal was to produce one general mixed-gas model based on all five data sets together. The model used here is adapted from models previously used with the individual *data sets 1–4* (12–15). Many of the details of modeling used here have been described previously but are repeated below, where necessary, for clarification.

Parameter values of the model were adjusted to maximize the log likelihood (LL) of the model by use of a modified Marquart nonlinear estimation algorithm (17). The likelihood ratio (LR) test was used to evaluate the significance of estimated parameters on the basis of improvement in fit (11). The shape of the likelihood surface near the converged parameters was used to estimate the precision of the parameter values. All DCS cases were treated equally, with no allowance in the model for grades in severity. The only accommodation for differences in response was to model all DCS cases (including fatalities) and then to repeat the modeling with only cases of death.

The dose-response model used for this analysis was the Hill equation

$$\text{Probability (DCS)} = \text{dose}^n/(\text{dose}^n + P_{50}^n) \qquad (1)$$

where P_{50} represents the dose at which there is a probability of 50% for the occurrence of DCS (or death), and the exponent (n) is the order of the Hill equation that controls the steepness of the central portion of the sigmoidal curve.

The dose in *Eq. 1* represents a measure of decompression stress and was defined in a manner similar to previous reports (12–15) on the basis of the traditional idea of total gas supersaturation. In the present experiments, the gases contributing to the decompression response are He, N_2, Ar, and O_2; CO_2 is ignored. Here, O_2 is treated as an inert gas, adding to the risk of DCS. Although other ways of modeling

a potential effect of O_2 exist, no one method appears to offer a clear advantage (19, 22). This issue is discussed further later in the text.

To allow for possible differences among gases, each partial pressure is multiplied by a RP value that weights each gas according to risk on a per-unit-pressure basis. The resulting equation for dose (in ATA) is

$$\text{dose} = [(\text{Pti}_{He} * \text{RP}_{He}) + (\text{Pti}_{N_2} * \text{RP}_{N_2})$$

$$+ (\text{Pti}_{Ar} * \text{RP}_{Ar}) + (\text{Pti}_{O_2} * \text{RP}_{O_2})] - 1 \qquad (2)$$

Table 2. *Summary of decompression results from data set 5*

Dive Profile (1 h time at depth)	Stop Time, min	Weight, g	DCS, %	Death, %	No. of Rats
Air	0	256 ± 10	90.0	75.0	20
9.33 ATA	5	256 ± 10	40.0	10.0	20
4.64 ATA stop	10	258 ± 10	45.0	25.0	20
He-O_2	0	254 ± 12	100.0	85.0	20
9.33 ATA	5	258 ± 12	20.0	15.0	20
4.64 ATA stop	10	259 ± 13	0.0	0.0	20
Ar-O_2	0	258 ± 14	95.0	95.0	20
6.45 ATA	5	255 ± 14	85.0	60.0	20
3.42 ATA stop	10	266 ± 13	50.0	35.0	20
	15	252 ± 15	30.0	0.0	20
	20	255 ± 10	10.0	0.0	20
Ar-O_2	0	264 ± 12	100.0	100.0	15
6.45 ATA	5	261 ± 15	85.0	75.0	20
4.64 ATA stop	10	258 ± 12	75.0	65.0	20
	20	264 ± 13	85.0	70.0	20
50% N_2-50% He-O_2	0	254 ± 11	93.3	86.7	15
9.33 ATA	1	247 ± 10	95.0	80.0	20
4.64 ATA stop	2	245 ± 9	90.0	75.0	20
	3	246 ± 8	85.0	50.0	20
	4	247 ± 10	35.0	10.0	20
	5	248 ± 9	30.0	0.0	30
	10	246 ± 8	10.0	0.0	10
	15	248 ± 8	0.0	0.0	15
	20	245 ± 8	0.0	0.0	10
50% N_2-50% Ar-O_2	0	253 ± 11	100.0	100.0	20
9.33 ATA	5	253 ± 8	100.0	100.0	20
4.64 ATA stop	10	244 ± 8	80.0	70.0	20
	15	251 ± 9	70.0	45.0	20
	20	248 ± 7	75.0	45.0	20
	25	251 ± 10	60.0	25.0	20
	30	250 ± 9	44.0	20.0	25
	40	257 ± 9	32.0	8.0	25
33% N_2-33% He-33% Ar-O_2	0	251 ± 8	100.0	100.0	20
9.33 ATA	5	244 ± 12	70.0	55.0	20
4.64 ATA stop	10	246 ± 8	40.0	25.0	20
	15	258 ± 14	40.0	5.0	20
	20	248 ± 11	35.0	20.0	20
	25	247 ± 9	0.0	0.0	20
	30	245 ± 8	15.0	0.0	20

	Bottom Time, min	Weight, g	DCS, %	Death, %	No. of Rats
Ar-O_2	10	250 ± 12	13.3	0.0	15
4.79 ATA	15	249 ± 10	33.3	26.7	15
Time at depth varied	20	252 ± 11	73.3	53.3	15
	25	242 ± 10	35.0	30.0	20
	30	247 ± 15	73.3	60.0	15
	40	251 ± 8	93.3	93.3	15
	45	249 ± 6	86.7	73.3	15
	60	245 ± 11	86.7	73.3	15
	90	246 ± 9	80.0	80.0	15
	120	245 ± 9	86.7	86.7	15

Values for weight are means ± SD. DCS, decompression sickness.

where Pti_{He}, Pti_{N_2}, Pti_{Ar}, and Pti_{O_2} are the tissue partial pressures (Pti) of He, N_2, Ar, and O_2, respectively (in ATA), in the animal immediately on reaching the surface. The parameters RP_{He}, RP_{N_2}, RP_{Ar}, and RP_{O_2} are the RP values for He, N_2, Ar, and O_2, respectively. The subtraction of 1 ATA defines the amount of supersaturation after decompression to the surface.

The Pti of He, N_2, Ar, and O_2 in the animal were calculated on the basis of single exponential kinetics, assuming a single compartment (i.e., whole rat) even though real tissues may need a more complex model (10, 18). After a step change in partial pressure of one gas at *time 0* (t_0), the Pti of the gas at *time t* can be described by the following equation for $t > t_0$

$$Pti = (Pam_i - Pti_0) * [1 - e^{-(t - t_0)TC_i}] + Pti_0 \qquad (3)$$

where Pam_i is the ambient partial pressure at *time t*, Pti_0 is the initial Pti, TC_i is the time constant (TC) affecting the rate of gas uptake or washout, and *i* is an index referring to each gas.

The calculation for Pti becomes more complicated during actual dives in which the ambient gas partial pressures change in a nonstepwise manner (e.g., during compression or decompression). The solution, as previously described in Ref. 16, was to treat each dive as a series of partial pressure ramps that describe the pressure history of the dive. Partial pressures of individual gases at the beginning and ending of each ramp were estimated by multiplying the chamber pressure by the percentage of each gas as measured. For calculation purposes, each ramp was subdivided into a number of smaller time intervals, and *Eq. 3* was used to successively compute Pti values at each interval along each ramp until decompression was completed. This procedures was performed for each of the gases that was being considered, in this case He, N_2, Ar, and O_2.

Separate TCs for the uptake and washout of each gas allowed for the possibility of asymmetry in gas kinetics. This was implemented by comparing Pti to Pam_i for each gas and adding the parameter ΔTC_i to TC_i when $Pti_0 > Pam_i$ in *Eq. 3* (i.e., during washout). This situation generally occurred some time after decompression began, although this was not the case for those instances in which the dive gas was switched to a different mixture before decompression.

Gas kinetics in *Eq. 3* assume that at equilibrium the partial pressures in the animal become equal to those in the chamber. This assumption avoids the difficult task of choosing a more complicated model on the basis of inadequate knowledge of tissue-blood relationships. Although *Eq. 3* models gas kinetics in the rat, it is important to emphasize that gas uptake and washout were not actually measured. Consequently, what is being modeled is the animal response to decompression and the effect of the estimated gas partial pressures on decompression risk. The TCs that are being estimated define the rates of change in processes that affect the probability of DCS or death, although interpretation in terms of gas kinetics is made in this report.

The partial pressures of the gases are reported in atmospheres absolute. To estimate the RP values, one of the potencies had to be fixed so that the other potencies could be calculated in relation to it. The RP_{N_2} value was arbitrarily set to 1.0 so that the P_{50} is expressed in terms of the partial pressure of N_2 in atmospheres absolute. The effect of this weighting calculation was to convert exposures of He, Ar, and O_2 into equivalent N_2 exposures.

Because rat weight has been shown to have a significant effect on the decompression outcome, a weight correction term for dose was included in the model, as previously done, with a power function (13). Animal weight (Wt), normalized to a weight (260 g) close to the average weight

of all animals (252 g), was raised to an exponent denoted as the weight factor (WtF). Consequently, the final expression for dose was

$$dose\ (Wt\ corrected) = dose\ (Wt/260)^{WtF} \qquad (4)$$

The modeling effort began with the individual data sets, which were then combined in all possible ways until the model was finally fitted to the combined group of all five sets, *data sets 1–5*. Parameter estimation began with the simplest possible model: P_{50}, *n*, and symmetrical gas kinetics with a single TC for uptake and washout for each gas. Each subsequent level of complexity of the model, with all possible combinations of estimated parameters, was evaluated by at least five different sets of starting parameter values to ensure that the maximum LL found was a global and not a local maximum. Parameter combinations that showed promising improvement to the fit were explored with many more starting values so that, overall, several thousand separate starting parameter sets were evaluated.

The next step was to perform an LR test to determine whether the data sets were statistically combinable

$$LR = 2 * [LL_{1+2} - (LL_1 + LL_2)] \qquad (5)$$

where LL_1 and LL_2 are the LLs of the same model fitted to *data sets 1* and *2* separately, and LL_{1+2} is the LL fitted to the combined data sets. Data sets found by this criterion not to be combinable are thought to generally produce better predictions of decompression outcome when modeled separately. This issue will be discussed in more detail later.

In summary, this model *1*) predicts the probability of DCS in rats subjected to dives with mixtures of He, N_2, Ar, and O_2; *2*) assumes that the decompression response is dependent on the degree of supersaturation of these gases in the animal; and *3*) is used to estimate the parameters governing the location and shape of the dose-response curve, the values of RP, TC, and ΔTC for the individual gases, and the exponent correcting for animal weight.

RESULTS

Data Set 5

Table 2 presents results from *data set 5*, which is the only set that has not been reported previously. For a given gas mixture/depth profile, incidence of both DCS and death generally declined with increasing decompression time. For the Ar-O_2 dives in which time at depth was varied (last group shown in Table 2), the incidence of both DCS and death generally increased with time at depth until leveling out sometime after 30 min.

Summary of All Data (5 Data Sets)

Table 3 summarizes the decompression results from the 5,474 dives from rats that were used in this report.

Table 3. *Summary of decompression results from the 5 data sets used in the mixed-gas model*

Data Set	Weight, g	DCS, %	Death, %	No. of Rats
1	238 ± 13	60.3	46.4	1,404
2	258 ± 20	52.1	26.1	1,791
3	261 ± 20	57.8	33.6	896
4	252 ± 15	41.6	19.9	618
5	253 ± 12	57.3	41.6	765
Total	252 ± 19	54.7	34.0	5,474

Values for weight are means ± SD.

Table 4. *Parameter estimates for maximum likelihood models for DCS*

	Data Set 1	Data Set 2	Data Set 3	Data Set 4	Data Set 5	Data Sets 1, 2, 4, 5	Data Sets 1–5
No. of rats	1,404	1,791	896	618	765	4,578	5,474
P_{50}	3.40 ± 0.11	4.34 ± 0.15	3.01 ± 0.19	4.43 ± 0.38	3.04 ± 0.15	4.70 ± 0.09	4.67 ± 0.09
n	5.50 ± 0.60	6.79 ± 0.61	6.71 ± 0.87	3.38 ± 0.99	10.4 ± 1.1	6.51 ± 0.28	6.48 ± 0.25
WtF	1.69 ± 0.25	0.73 ± 0.12	0.71 ± 0.18	2.43 ± 0.81	0.37 ± 0.19	1.14 ± 0.08	1.07 ± 0.07
RP_{O_2}	0 F	0.91 ± 0.08	0 F	0.61 ± 0.22	0 F	0.95 ± 0.06	0.94 ± 0.06
RP_{He}	0.91 ± 0.02	0.88 ± 0.04	0.81 ± 0.04	0 F	0.77 ± 0.09	0.93 ± 0.01	0.91 ± 0.01
RP_{N_2}	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F
RP_{Ar}	1.42 ± 0.05	0 F	1.24 ± 0.04	0 F	1.05 ± 0.05	1.44 ± 0.04	1.46 ± 0.03
TC_{O_2}	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F
TC_{He}	0.00001 F	3.40 ± 1.39	15.9 ± 3.8	*	5.10 ± 2.06	4.30 ± 0.64	4.75 ± 0.46
TC_{N_2}	0.00001 F	18.8 ± 1.8	20.7 ± 3.6	11.7 ± 6.0	51.8 ± 9.5	14.7 ± 0.8	14.6 ± 0.7
TC_{Ar}	0.00001 F	*	15.9 ± 2.2	*	16.1 ± 3.5	10.7 ± 1.9	13.3 ± 1.2
ΔTC_{He}	*	*	-7.50 ± 2.96	*	*	*	*
ΔTC_{N_2}	*	*	*	*	-48.0 ± 9.4	*	*
ΔTC_{Ar}	*	*	*	*	*	*	-4.29 ± 1.51
$-LL$	813	1,037	436	364	338	2,626	3,080

Values are means $\pm$ SE. P_{50}, dose (ATA) producing 50% incidence; n, exponent of Hill equation; WtF, weight factor; RP, relative potency for each gas; TC, uptake time constant (min) for each gas; ΔTC, adjustment factor for gas washout (min); LL, log likelihood; F, fixed parameters. *Nonsignificant.

Overall incidence was 54.7% for DCS and 34.0% for death. Mean incidence for each of the five individual data sets ranged from 41.6 to 60.3% for DCS and 19.9 to 46.4% for death. Rat weights after diving ranged from 184 to 331 g with an overall mean $\pm$ SD of 252 $\pm$ 19 g; mean weights for the individual data sets are given in Table 3.

Model Parameters

Model parameters were estimated separately for DCS and death with the model described by *Eqs. 1–4*. Only parameters found to be significant at the 0.05 level are reported in Tables 4 and 5. For DCS, only *data sets 1* and *4* were found combinable by LR testing; for death, *data sets 2* and *5* and *data sets 4* and *5* were shown combinable. Thus, by this test, most of the data sets are best modeled separately. However, our experience has shown that it is often very difficult to pass the LR test despite apparent similarity of data. Given that the primary goal of this work was to produce one general mixed-gas model based on all the data, we proceeded with fitting the model to the combined five data sets. Potential problems related to combinability will be discussed later.

Reported results include those with the model fitted to all of the data combined as well as fitted separately to each of the five individual data sets. Because of the unique aspects of the gas-switching experiments (*data set 3*, Ref. 15), modeling results are also given for the combined data after exclusion of *data set 3* (noted as *data sets 1, 2, 4, 5* in Tables 4 and 5). Current parameter estimates agree closely with previously reported results for the individual data sets (12–15), with the exception of *data sets 1* and *2*, which had been fitted earlier with a somewhat different model. This would be expected but represents a good check of the new computer code and database structure that were written to allow analysis of the combined data. We note that the previous models included only one TC for both uptake and washout of each gas, thus forcing symmetrical gas exchange.

Table 5. *Parameter estimates for maximum likelihood models for death*

	Data Set 1	Data Set 2	Data Set 3	Data Set 4	Data Set 5	Data Sets 1, 2, 4, 5	Data Sets 1–5
No. of rats	1,404	1,791	896	618	765	4,578	5,474
P_{50}	3.97 ± 0.09	4.26 ± 0.10	3.63 ± 0.18	5.05 ± 0.10	3.39 ± 0.15	4.61 ± 0.08	4.59 ± 0.08
n	6.41 ± 0.65	10.8 ± 1.0	6.34 ± 1.05	12.6 ± 1.8	11.4 ± 1.2	9.33 ± 0.42	8.91 ± 0.36
WtF	1.22 ± 0.21	0.73 ± 0.10	0.71 ± 0.24	0.66 ± 0.17	*	0.91 ± 0.07	0.84 ± 0.06
RP_{O_2}	0 F	0.46 ± 0.05	0 F	0.51 ± 0.08	0 F	0.50 ± 0.04	0.49 ± 0.04
RP_{He}	0.86 ± 0.02	0.82 ± 0.02	0.62 ± 0.06	0 F	0.79 ± 0.08	0.87 ± 0.01	0.84 ± 0.01
RP_{N_2}	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F	1.0 F
RP_{Ar}	1.56 ± 0.05	0 F	1.33 ± 0.05	0 F	1.11 ± 0.05	1.44 ± 0.03	1.46 ± 0.02
TC_{O_2}	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F	0.00001 F
TC_{He}	0.00001 F	5.07 ± 0.93	14.1 ± 6.6	*	6.29 ± 2.48	4.74 ± 1.04	4.69 ± 0.62
TC_{N_2}	0.00001 F	17.3 ± 1.2	21.6 ± 6.7	12.7 ± 3.2	2.47 ± 0.51	15.6 ± 1.0	15.9 ± 1.0
TC_{Ar}	0.00001 F	*	17.3 ± 4.0	*	12.3 ± 2.3	9.43 ± 1.16	16.1 ± 1.5
ΔTC_{He}	*	*	*	*	*	$+3.04 \pm 1.70$	$+3.58 \pm 1.34$
ΔTC_{N_2}	*	*	*	*	*	-9.65 ± 1.28	-9.45 ± 1.22
ΔTC_{Ar}	*	*	*	*	*	*	-8.67 ± 1.68
$-LL$	744	748	345	189	304	2,036	2,408

Values are means $\pm$ SE. *Nonsignificant.

P$_{50}$ values and exponents. For all the individual data sets except *data set 2*, P$_{50}$ values were greater for death than for DCS, reflecting the higher dose of decompression stress required to cause death. With the exception of *data set 3* (gas switching), exponents were also greater for death vs. DCS, indicating a steeper response slope for death. For the model fitted to the combined data, with and without *data set 3*, the P$_{50}$ was similar for DCS and death, although the exponent was greater for death.

Potencies. As was previously reported (12–15), large differences in potency for causing DCS or death were seen here among gases, although the magnitude of the differences varied somewhat with the specific data set. Helium potency was estimated for the individual data sets at ~10–20% less than that of N$_2$ for both DCS and death, although it was somewhat lower for *data set 3*. Ar potency for both responses was estimated to be up to ~55% greater than that of N$_2$, and O$_2$ was shown to introduce significant decompression risk with a potency up to 90% (DCS) or 50% (death) of that of N$_2$. Potencies for the combined data, with and without *data set 3*, agreed with the values estimated from individual data sets.

Predictive response curves (Fig. 1) based on the combined-data model illustrate both the differences in potencies among the inert gases (He < N$_2$ < Ar) and the model agreement with the saturation data (*data set 1*). As would be expected, the observed fit to the data is not nearly as good as that previously reported for a model developed for only the saturation data (13). We also emphasize that, although only a few data points are plotted for graphing purposes, the predictive curves are based on the model fitted to all the data. For example, the three data points for Ar in Fig. 1 represent all of the dives using Ar-O$_2$ from *data set 1*. We did not include the 90- and 120-min time at depth dives at 4.79 ATA from *data set 5* because these animals had mean weights considerably greater that those in *data set 1* and the 240 g used to generate the predictive curve. This weight difference in itself would have produced substantial deviation from the prediction, thus confounding the comparison in Fig. 1.

Gas exchange: Single data sets. For the individual data sets, gas uptake and washout rates were generally the same, although there were large differences among the three inert gases. The TC for He was estimated at ~3–6 min, although it was considerably greater for *data set 3*. If equilibration is considered to occur in four TCs, then He saturation or washout would occur in ~10–25 min. In contrast, gas exchange for N$_2$ and Ar was generally considerably slower, with saturation estimated at ~50–80 min. The major exception to these findings was *data set 5*, which included many long decompression dives that might be expected to improve the estimation of gas washout. Interestingly, for this data set, N$_2$ washout for DCS was estimated to be much faster than N$_2$ uptake. For prediction of death, N$_2$ uptake and washout for *data set 5* were symmetrical and much faster than with the other data. For all five individual data sets, the O$_2$ TC was
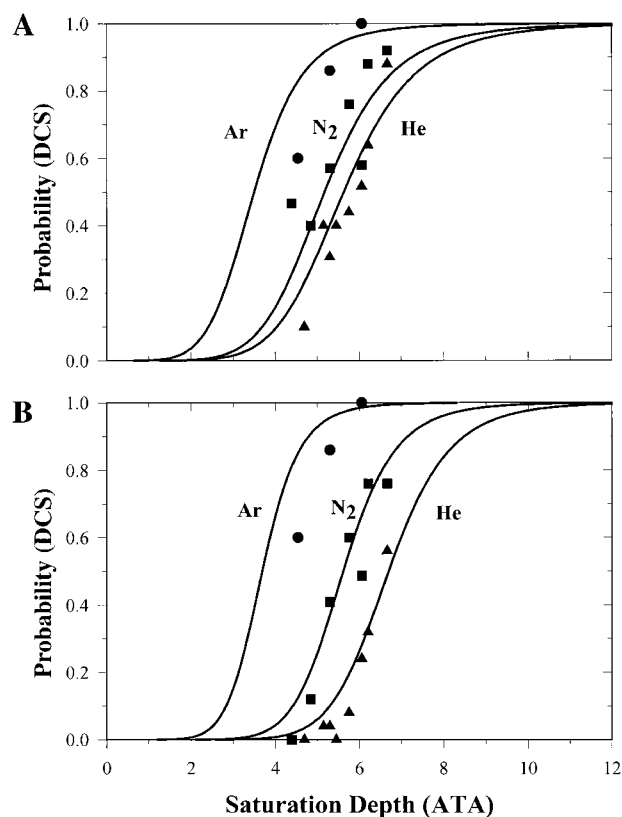

Fig. 1. Saturation dives. Probability of decompression sickness (DCS; *A*) and death (*B*) increases with saturation depth for He (▲), N$_2$ (■), and Ar (●); all single inert-gas mixtures with 20.9% O$_2$. Differences in the plots of the 3 gases reflect the differences in potency (relative decompression risk on a per-unit-pressure basis): He < N$_2$ < Ar. Decompression was rapid (<10 s) to the surface. Predictions were based on the model for the combined 5 data sets with rat weight set at 240 g, which was close to the mean weight (238 g) of the saturation dives. ATA, atmospheres absolute.

indistinguishable from zero and therefore was fixed at this value during model estimation (the TC for O$_2$ was actually fixed at 0.00001 to avoid the calculation problems associated with using zero). O$_2$ in the animal would thus be expected to equilibrate almost immediately with a change in chamber P$_{O_2}$.

Gas exchange: Combined data. In the DCS model for the combined five data sets, gas uptake rates were indistinguishable from washout rates for He and N$_2$ but not for Ar. With the exception of Ar, these results agree closely with the results from the individual data sets in terms of both symmetrical gas exchange and magnitude of the TCs. However, for death, significant differences in rate of gas uptake and washout were predicted for all three inert gases on the basis of the five-data-set model. Although the uptake rates for death were similar to those for DCS, He washout for death was approximately twice as slow as uptake, and N$_2$ and Ar washout were approximately twice as fast as uptake. These asymmetrical gas kinetics for death differ from the symmetrical gas exchange estimated for the separate data sets and are also observed when the data is modeled after *data set 3* is omitted.

Predictive curves illustrate both the differences in gas exchange rates among the three inert gases (Figs. 2 and 3) and the model agreement with the variable time-at-depth data (*data set 2*; Fig. 2). We note that, in Fig. 2, only one point for Ar is plotted. The Ar data from the additional variable-time-at-depth dives in *data set 5* were not included because these were done at 4.79 ATA vs. the 6.30 ATA used in Fig. 2. The 6.30 ATA was selected for display because all the data for He and N_2, in which time at depth was varied (*data set 2*), were at this depth. Consequently, most of the Ar data could not be used in Fig. 2, although, as we pointed out above, all the data were used to estimate model parameters.

Weight correction. Inclusion of a weight correction (the parameter WtF) in the model produced a significant improvement in fit for both DCS and death in all data sets except *data set 5*. However, the magnitude of WtF varied somewhat with the data set. A significant weight correction was also found with the combined data, with or without *data set 3*. This correction results in a greater probability of DCS and death in heavier animals.
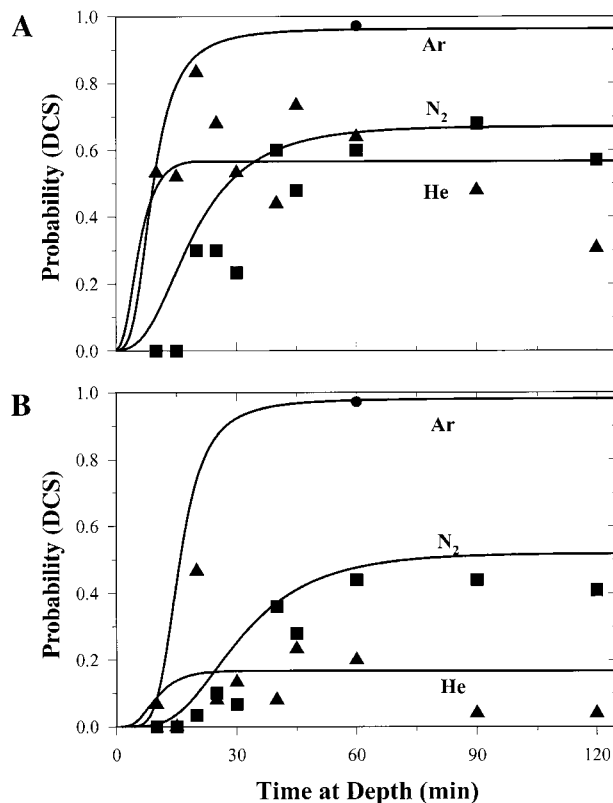

Fig. 2. Variable time at depth. Probability of DCS (*A*) and death (*B*) increases with time at depth for He (▲), N_2 (■), and Ar (●) for a 6.30-ATA dive; all single inert-gas mixtures with 20.9% O_2. Differences in the maximum probability each gas reaches at saturation reflect differences in the potencies of the 3 gases (He < N_2 < Ar). Helium reaches its asymptote faster than N_2 or Ar because of its faster gas uptake time constant. Decompression was rapid (<10 s) to the surface. Predictions were based on the model for the combined 5 data sets with rat weight set at 260 g, which was close to the mean weight (258 g) of the variable time-at-depth dives.
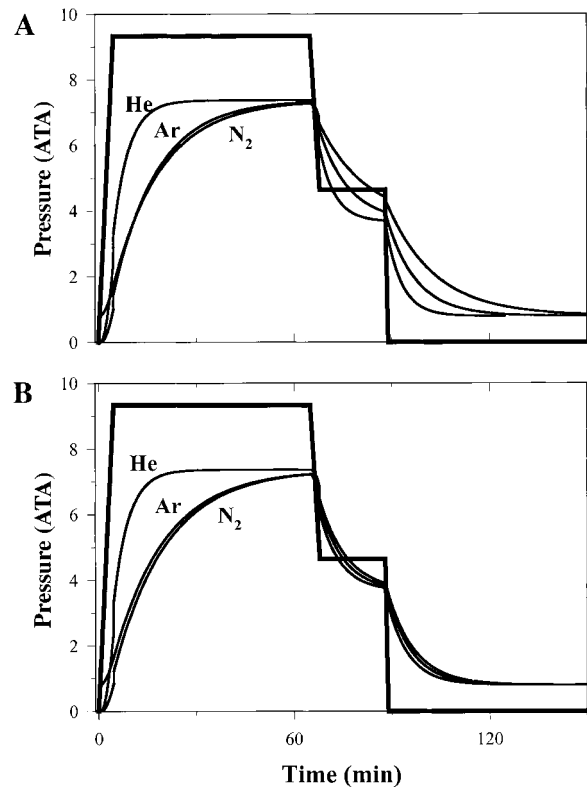

Fig. 3. Predicted gas pressures. Predicted partial pressure changes (thin lines) in rats during a 1-h dive at 9.3 ATA with 3 single inert-gas mixtures with 20.9% O_2. Initial ascent was done at 1.82 ATA/min to a stop at 4.64 ATA for 20 min, followed by rapid (<10 s) ascent to the surface. Partial pressure differences reflect differences in uptake and washout time constants. Thick lines represent total chamber pressure. Predictions for DCS (*A*) and death (*B*) were based on the model for the combined 5 data sets.

Effectiveness of the Model

The ability of the model, on the basis of all five data sets, to describe the wide range of dive profiles with various mixtures of He, N_2, Ar, and O_2 was examined. This was done by plotting the difference (residual), between model prediction and observed incidence vs. total decompression time for each different dive profile. All the data were used, with the observed values being the mean incidence rates of the specific dive profiles. A different symbol is used on the graphs for each of the five data sets (Fig. 4) and for each of the three inert gases (Fig. 5) to allow visual comparison. Animal weight was set at 260 g for all predictions. For *data set 3* in Fig. 4, total decompression time is calculated, for graphing purposes only, as starting immediately at the end of the 5-min switch to a different gas. Thus the profiles in this data set have total decompression times of 0, 10, 20, 30, or 40 min.

We emphasize that actual incidence data also have their own associated errors (not shown on graphs), which are based on the binomial distribution and are affected by the sample size. Although this data error will limit interpretation of the residuals, we point out that nearly all of the model predictions are within 40% of the observed incidence for DCS and within 30% for

death. The ability of the model to predict that well over such a range of gas mixtures and dive profiles is, in our opinion, quite remarkable and agrees with past results when only two gases (He and H_2) were being modeled (16). This degree of error is all the more surprising given the nearly 150-g range in rat weight and its relatively large effect on risk of DCS (~1% increase in DCS with each additional gram of rat weight; Ref. 13). More importantly, the scatter of points around zero illustrates that the model predicts DCS or death without significant bias and generally equally well regardless of total decompression time. The five symbols for the data sets and the three symbols for the inert gases also appear to be randomly distributed about zero, suggesting the absence of systematic model distortion with respect to data set or inert gas. Also important is the fact that the mean value of the residuals, for each of the individual data sets and gases, is ~0, with the actual means ranging from −0.05 to 0.01.

DISCUSSION

The mixed-gas model developed here for prediction of DCS in rats is the first such model for any animal species that covers such a broad range of gas mixtures
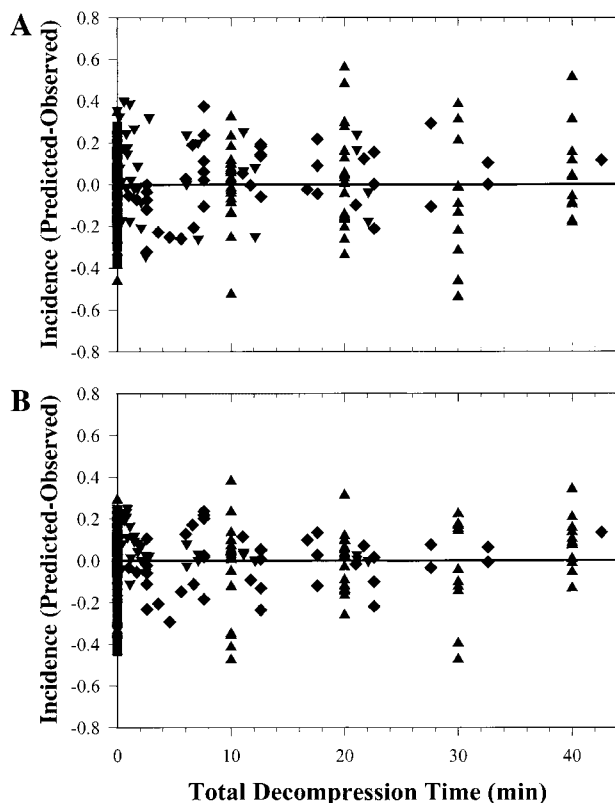
Fig. 4. Model error for the 5 data sets. Maximum likelihood models predict DCS (*A*) or death (*B*) without significant bias and generally equally well regardless of total decompression time and the specific data set. This is shown by plotting the difference between model prediction and observed incidence vs. total decompression time. Each of the 5 data sets is plotted with a different symbol: *data sets 1* (●), *2* (■), *3* (▲), *4* (▼), and *5* (◆). Predictions based on model for combined 5 data sets with rat weight set at 260 g. The observed values are the mean incidence rates of the specific dive profiles.
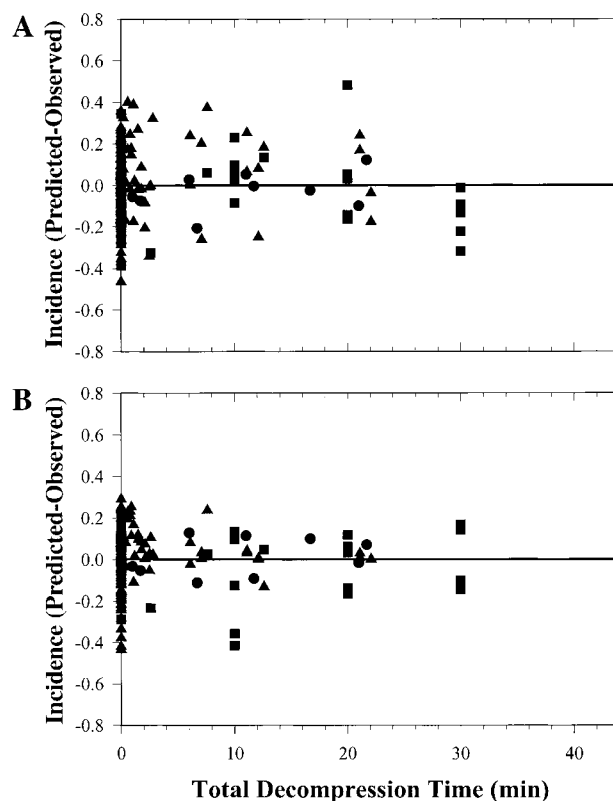
Fig. 5. Model error for the 3 inert gases. Similar to Fig. 4, maximum likelihood models predict DCS (*A*) or death (*B*) without significant bias and generally equally well regardless of total decompression time and the specific inert gas. This is shown by plotting the difference between model prediction and observed incidence vs. total decompression time. Only single inert-gas mixtures (20.9% O_2) are plotted: He (▲), N_2 (■), and Ar (●). Predictions were based on the model for all 5 data sets with rat weight set at 260 g. Observed values are the mean incidence rates of the specific dive profiles.

and dive profiles. The success of this model is demonstrated by *1*) the relatively small errors, in view of the variety of the data, between model predictions and actual incidences and *2*) the random distribution of these errors relative to total decompression time, gas mixture, and data set. Our results, based on over 5,000 dives, agree with many of the findings from past modeling of four subsets of these data (12–15), including prediction of *1*) differences among the gases in potency for causing DCS and exchange rate, *2*) significant decompression risk of O_2, and *3*) increased risk of DCS with heavier animals. New results include the prediction of asymmetrical gas exchange with gas washout often unexpectedly faster than gas uptake.

As discussed earlier, gas kinetics were extrapolated here, as well as in our previous models, from rate constants estimated from changes in DCS risk rather than determined from direct measurement of gas uptake and washout. The simplistic approach we have taken here and in previous studies (12–15) avoids using more complicated models based on unproven assumptions about blood-tissue exchange. Experience has demonstrated that very different models can often predict the response to decompression equally well. For

example, in the case of rats, little effect on predictability has been observed when *1*) using alternate ways for correcting for rat weight or *2*) omitting the 1-ATA subtraction in the dose (13). A term for the small constant contribution of the metabolic gases (venous P_{O_2} and P_{CO_2} and water vapor pressure) to decompression risk has sometimes been included in past modeling efforts by others. However, its importance to predictive ability has not been clearly demonstrated. For instance, a recent probabilistic modeling exercise with human decompression data (21) has shown that, when the specific values chosen for the metabolic gases were varied, there was no significant effect on predictability of DCS.

Our approach of modeling the rat response and treating all gases, including O_2, as a potential risk factor without using a physiologically based model is consistent with our past work. Again as before, we did not incorporate any link to bubble formation or growth into our overpressure model. Although a dose-response relationship has been reported for human decompression and Doppler bubble detection (7), symptoms of DCS in humans have never been shown to be well correlated with Doppler measurements (3). Watt and Lin (24) agree to the poor correlation of intravascular bubbles and DCS in animals.

A major difference with the rat model developed here, compared with the earlier models, is that this model allows for asymmetrical gas kinetics, which were predicted for all three inert gases for death but only for Ar for DCS. Until about 20 years ago, inert-gas elimination during decompression had been assumed to simply be the reverse of inert-gas uptake, both of which could be described by the same exponential function. However, a number of studies have since suggested that gas washout may be slower with decompression, owing in part, at least, to cardiovascular changes or bubble development (6, 9). Indeed, we have estimated that washout of He and H_2 is over an order of magnitude slower than uptake in rats after very deep dives (20–50 ATA), although the explanation for this extreme slowing is unclear (16). The need for kinetics that reflect slower tissue washout has recently led to use of decompression models with a combination of both linear and exponential rate functions (21). Consequently, in the present model, for the combined data, the slower washout of He predicted for rat death, relative to uptake, was not surprising. However, the faster washout predicted for N_2 and Ar was unexpected and, to the best of our knowledge, unreported before.

Because asymmetry in gas exchange was generally not predicted for the individual data sets, the issue arises concerning data set combinability and its effect on parameter estimates of the model for the combined data, particularly gas exchange TCs. As was stated earlier, we had proceeded with modeling the combined five data sets, although LR testing had shown that most of the data was not combinable. Despite our opinion that the formalism of the LR test produces a very conservative test that is often very difficult to pass, an important question remains whether merging

of the data caused model distortion. Such distortion may have incorrectly led to prediction of asymmetry, including faster washout of some of the gases. We had concluded above that the random distribution of errors with respect to data set and inert gas suggested the absence of model distortion. We also indicated that all of the parameter estimates for the combined model, with the exception of the TCs, agreed well with the estimates for the individual data sets. However, the modeling differences with *data set 3*, with its relatively long He TC, and *data set 5*, with its asymmetrical N_2 kinetics (DCS) or relatively short N_2 TC (death), make these two data sets suspect for causing problems when combined with other data. Nevertheless, results argue against this. First, as discussed earlier, parameters for the combined model, with and without *data set 3*, agreed closely except for the Ar TC. Second, a similar comparison of the combined model, with and without *data set 5*, also produced good agreement, although this was not presented in Tables 4 and 5.

The most likely explanation for the predicted asymmetry of the combined model is that the merged data provided the range in dive profiles, not available in the individual data sets, necessary to resolve uptake and washout rates. This reasoning is supported by the narrow spectrum of dives within each of the data sets. Clearly data sets that were primarily designed to look at differences in gas uptake (*data set 2*) and gas washout (*data sets 4* and *5*) might be expected to be limited in their ability to estimate both aspects of gas exchange. *Data set 1* (saturation dives followed by rapid decompression) should provide little kinetic information, although *data set 3* (gas switching) might be expected to contain data helpful to describe both gas uptake and washout. However, we again stress that we are modeling the rat response to decompression and not gas kinetics, and we propose that the risk of DCS may become uncoupled from the gas load under certain circumstances, as suggested previously with regard to rats (16). Indeed, the comparison of the time course of predicted bubble evolution with predicted DCS risk in humans has indicated that gas-phase dynamics may not fully explain persistent DCS risk (1).

Until recently, only inert gases have been included in estimation of risk of human decompression; O_2 has generally been ignored. O_2 was assumed to be metabolized so rapidly during decompression that it had little effect on bubble formation and, presumably, on DCS. The present results support our previous findings (12, 14) that O_2 can substantially add to the risk of DCS in rats, although this effect diminishes very quickly during decompression. Recently, two very different probabilistic models have predicted that elevated P_{O_2} levels contribute to DCS risk in humans, although less than the equivalent amount of N_2 (19). One model treated O_2 as we did, as an inert gas adding to the decompression load, although a threshold was included that limited the contribution of O_2 to pressures above a certain level. The other model allowed the P_{O_2} to alter the N_2 washin-washout kinetics, acknowledging the ability of P_{O_2} to affect central and peripheral circulation. Inter-

estingly, both models predicted risk for various human profiles similarly, demonstrating the inability to distinguish these very different approaches to defining the role of O_2 in decompression. These results again emphasize the limitations of decompression modeling and raise the issue of often-unneeded model complexity. No further improvement in fit was seen in that study (19) when a combination of the two effects was employed. In another report (22), the effect of O_2 was implemented similarly into a bubble model for predicting human DCS after N_2 and/or He dives. The best fit in that case was obtained by including a combined O_2 effect via the direct contribution to the bubble as a gas and vasoconstriction induced by hyperoxia. Despite concerns raised in these and other studies that O_2 should not be used blindly without concern for potentially contributing to the risk of DCS, O_2 is still deemed very useful during decompression (19). The advantage of using high-O_2 mixtures was evident during the recent development of decompression procedures for the US Navy's upgraded 1.3-ATA P_{O_2} rebreather, in which significant reduction in decompression times was achieved (20).

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