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A Meta-Analysis of Physiological Changes during Water Immersion for Incorporation into Physiologically-Based Pharmacokinetic Models (PBPK) of Chemical Exposures in Occupational Divers

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STATEMENTS

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The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

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Executive summary:

Physiological changes that occur with water immersion have been previously characterized; however, these reviews rely on specific studies to support, illustrate and exemplify such changes. Among the large number of studies that have investigated the physiology of water immersion, nearly all are based on small numbers of research subjects tested under a wide range of experimental treatment conditions. Therefore, the possibility exists that the physiological changes reported by any single study are limited to only the specific conditions under which the study was conducted and for the limited number of subjects studied. Hence, a different experimental protocol or a different population of subjects could yield different, and, in fact inconsistent finding are commonplace. The objective of this investigation was to conduct a quantitative analysis of the literature on physiological changes associated with water immersion. The purpose was to systematically address the issue that water immersion studies have been performed under a wide range of experimental conditions, as described in the many published reports cited herein. The objective of this study was to enable construction of Physiologically-Based Pharmacokinetic (PBPK) models for chemical exposures resulting from occupational diving in contaminated waters. In this initial phase of our investigation we have conducted a systematic literature search that yielded approximately 185 published papers on the relationship between water immersion and physiological response. Each study was evaluated for applicable data on several key physiological parameters affecting chemical absorption, distribution, metabolism and elimination. Treatment conditions, subject meta-data and mean physiological data were manually extracted from each study. Extracted data were analyzed using a three level meta-analysis approach that incorporates all suitable experimental studies to develop a statistical model applicable to the full range of conditions encountered by occupational divers. Our quantitative approach accounts for statistical heterogeneity in physiological endpoint measurements from all included studies, and within-study correlations attributable to repeated measurement of the same subjects under different treatment conditions. The described model is applied to an analysis of cardiac output but is applicable to a range of physiological endpoints including pulmonary functions, renal clearance, blood volume changes and regional tissue blood flow. Our model demonstrates that, physical exertion, immersion depth and age are the principal determinants of cardiac output, although it is expected each physiological endpoint is subject to regulation by a different combination of variables.

1. Introduction:

Navy divers perform diverse tasks in underwater environments that can be physically and cognitively demanding. During some activities, chemical contamination may be present in the divers' working environment. These hazards were well characterized by the Environmental Protection Agency in their 1985 report on diving operations in contaminated water (Traver 1985). Human health and performance can be compromised via the action of water contaminants on target organs and systems with high levels of exposures that exceed safe exposure limits. In this regard, occupational SCUBA diving activities can result in chemical exposures by multiple routes. Oral exposures can occur during diving operations through inadvertent swallowing of water during immersion. Inhalation exposures to aerosols or volatile chemicals can occur when conducing surface operations on small water craft, during head out of water immersion when not breathing tanked air, or with certain types of air supply systems (i.e., free flow respirator). However, the greatest exposure uncertainty may be dermal exposures since standard open neoprene wet suits are designed to be flooded during diving which allows an infinite dose of chemical contaminants to remain in contact with hydrated skin. Such wet suits typically provide only thermal protection but do not act as a barrier between the diver and the water (Traver 1985). The diver wearing a wet suit during dive activities is in intimate contact with his aquatic environment, which includes exposure to the skin, external auditory canals, nasal passages, and oral cavity.

The conditions associated with diving, such as pressure, buoyancy, cold or warm water temperatures and physical exertion can impact absorption, distribution, metabolism, and elimination (ADME) of xenobiotics, altering the internal dosimetry and thus, toxicity (or efficacy) at the target site. As a result, human health exposure criteria derived for general populations may not adequately reflect divers' exposure or health risks. A physiologically based pharmacokinetic (PBPK) modeling approach to exposure assessment is proposed to address the need and better characterize the impact of diving-related stressors on target tissue dosimetry of compounds to which occupational divers are exposed. In traditional pharmacokinetic models, where population-based kinetics are derived by "fitting" the model to a single data set, the derived parameters have limited correspondence to real-world physiological and biochemical processes. Furthermore, fitted parameters are not generalizable across different compounds, or in situations where multiple physiological parameters are altered concurrently. PBPK modeling is an approach well-suited to addressing these challenges because PBPK modeling relies on measurable physiological parameters such as cardiac output, blood flow rates, relative solubility (tissue vs. blood), and biotransformation to predict internal tissue level exposures.

In order to develop PBPK models that accurately describe internal chemical exposures resulting from waterborne occupational and recreational exposures, it is necessary to accurately characterize the magnitude of the effect that water immersion has on key human physiological parameters. There is currently an enormous body of literature on the effects of water immersion on human physiology. Endpoints commonly measured include heart rate, systolic and diastolic blood pressure, cardiac output/index, respiratory rate, inspiratory and expiratory volume, Oxygen consumption (VO_2), and many other parameters. Although it is known that water immersion affects many of these parameters, the extent to which these endpoints are impacted is largely dependent on experimental design and treatment conditions. Across the breadth of studies available in the published literature, there are a wide variety of experimental variables that either directly or indirectly affect the measured effect on

physiological parameters. Experimental variables that commonly differ across studies include the depth of water immersion (from knee level to fully immerse), duration of immersion, body position during immersion, water temperature and physical activity, type of ventilation (SCUBA, breath holding, snorkel, head out of water, etc.). Subject-related variables include age, gender, body mass index and diving experience, among others. Due to the large array of experimental conditions, there is a high degree of variability in the physiological outcomes reported across studies.

The objective of this study was to quantitatively describe the physiological effects of water immersion across published studies. This is analysis is motivated by the need to parametrize physiologically-based pharmacokinetic models for chemical exposures during diving. The approach used to evaluate effect size is based on the full range of published literature and accounts for the moderating effects of experimental and individual variables as reported by each individual study. By incorporating all studies that stratify *a priori* established criteria for inclusion and exclusion, it was hypothesized that key experimental variables would be identified, and that the effect magnitude of each variable on each physiological endpoint could be estimated. These study parameters would be used for defining the physiological range for PBPK models applicable to real-world occupational diving scenarios.

2. Methods:

Literature search: We performed a comprehensive search for literature focused on identifying all retrievable primary literature data pertaining to the physiological effects of diving and water immersion. The literature search focused on identifying studies with partial to full-body water immersion as part of the overall study design. The search strategy included keyword searches of Medline and Scopus, and free form search of Google Scholar. In addition, tree searches were performed using several high quality review articles on diving physiology (e.g., Holmer 1992; Lin 1988; Pendergast and Lundgren 2009; Pendergast et al. 2011). Excluded studies were localized immersion studies (i.e., facial immersion or immersion of a single limb), hyperbaric chamber studies, simulated immersion studies (i.e., head-down tilt studies, “wet-suit” studies), studies that did not have a pre-immersion baseline control, and studies that did not provide variability of the treatments means (i.e., McArdle et al. 1976). Studies also needed to provide a clear discussion of the immersion methodology including immersion duration, depth of immersion, water temperature and composition of the study population. Additionally, breath-holding and free diving (diving without breathing equipment) studies were excluded, as well as studies where physiological effects were measured following immersion, such as at some point during the post-immersion recovery period.

The literature search focused on key physiological parameters relevant to physiologically-based pharmacokinetic modeling, principally for dermal and inhalation chemical exposures. Physiological parameters of greatest interest included cardiac output (QC), regional and tissue blood flow changes, ventilation rate changes (QP), glomerular filtration rate (GFR) changes, and effects on blood volume. For each of these physiological endpoints, across all evaluated studies, experimental results were extracted for each treatment condition. Additionally, since there is no standard protocol for conducting diving physiology studies, key study variables were also extracted to the extent that they were provided by the investigators. Experimental variables included the extent of immersion (depth), subject posture (sitting, standing, etc.), exertion effort (VO_2 max or watts) water temperature, duration of water immersion, cumulative experiment duration, and treatment duration (i.e., the period of immersion during which a

treatment condition (i.e. exercise) was imposed). Physiological studies were deemed suitable for analysis if the study included a control group for whom physiological endpoints were measured under dry, pre-immersion resting conditions.

The effect of water immersion on physiological parameters reported in study was based on mean values (± standard error) for each treatment condition. Mean effect size was based on the comparison of the mean treatment effect (for each treatment condition) to the corresponding mean pre-immersion values for the baseline control group (dry, resting, pre-immersion). For nearly all studies, evaluated physiological data for all treatment groups were available only as the mean ± standard deviation (or standard error) for each treatment group (or control); data from individual subjects was not typically available from most studies. For some studies, physiological endpoint data were conveniently provided in a tabulated format, however for a large number of studies the data required digital extraction from figures. Digital extraction was performed by copying figures from the electronic pdf documents using the Microsoft Snip software and uploading an image file to WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/). After setting axis scales, points (dot plots) or bar heights (bar plots) were quantified to three significant figures. Error bars were similarly obtained and the difference determined for calculation of the standard error or standard deviation, depending on the representation of the data. Digitally extracted figures and values have been retained for review and transparency. All study data and key study conditions were collected and tabulated in Microsoft Excel®, and analyzed in R statistical software using the Metafor package (Viechtbauer 2010).

Statistical analysis: The overall associations between water immersion and changes in physiology (i.e., the overall effect) were first estimated by fitting an overall three-level model to the data. This model consisted only of an intercept representing the overall effect. This overall "baseline" model consisted initially of only a random intercept using the rma.mv() function of the metaphor statistical package for statistical meta-regression (Viechtbauer 2010). The three-level meta-analysis model enabled comparison of diverse studies since it accounts for sampling variance of effect sizes at three levels in the model: level 1 accounts for variances within treatment groups; level 2 accounts for variance between effect sizes within a study; and level 3 accounts for variance between studies (Assink 2016; Van den Noortgate et al. 2013). This model permits effect sizes to vary between participants (level 1), outcomes (level 2), and studies (level 3). The three-level approach evaluates differences in outcomes within studies (within-study heterogeneity) and between studies (i.e., between-study heterogeneity). Evidence of significant heterogeneity in effect sizes is justification for incorporating one or more moderators into the model to test the extent to which experimental variables explain within-study or between-study heterogeneity.

In performing the meta-analysis, we chose to apply a t-distribution adjustment of Knapp and Hartung (2003) rather than the default Z-distribution which has been shown to increase in the number of unjustified significant results (Assink 2016). Hence, the test statistic of individual coefficients is based on the t-distribution with k (number of effect sizes) – p (total number of coefficients in the model including the intercept) degrees of freedom. Restricted Maximum Likelihood estimation method (REML) was used for estimating the parameters in the model (Viechtbauer 2010).

For all evaluated studies, endpoint measurements were extracted from the published literature as mean +/- SEM (or percent change +/- % SEM) for each treatment condition and baseline control (Level 1 variance). Most studies evaluated several physiological endpoints under multiple experimental conditions. The effects of different treatment conditions on the measured physiological outcome are

accounted for in the model as moderating factors (mods). Passing one or more moderator to the model allows evaluation of the extent to which each experimental variable explains within-study or between-study heterogeneity by postulating a multivariate model. Based on this approach, it is possible to identify the relative effect attributable to each study, which are not otherwise comparable. Moderator analysis was performed using a stepwise approach that sequentially added one moderator at a time to the model. At each step, each potential moderator was individually tested in the model and the most significant moderator ($p<0.05$) that reduced residual heterogeneity was added to the model. This stepwise process was continued until no additional moderator contributed significantly to the explanation effect heterogeneity.

Nearly all the studies from which data was extracted were multiple-treatment, multiple endpoint designs, with most studies consisting of multiple treatment comparisons that ranged from only one relevant treatment condition to several relevant treatment combinations (e.g., seated resting baseline control (n=7) compared to seated resting immersed to the neck, 34^{o}C water (n=7)), or treatment conditions (i.e., a study comparing resting and physical exertion at two water temperatures, on land and immersed). These moderating conditions varied considerably across studies. For cardiac output the measurement metric (L/min) was consistent across all studies. For this reason the unstandardized effect size was used for analysis (measure="MD" for the escalc () function in the metfor package). For other outcomes that are measured using multiple metrics on different scales (kidney function, ventilation), standardized mean effect (measure = “SMD”) may be more suitable. Standardized mean variance does have the convenience that it is unit-less and therefore can accommodate tests using different scales, and is calculated as (mean group1i – mean group2i)/spi, where spi is the pooled standard deviation of the two groups.

In nearly all studies, subjects served in both the control and treatment groups, and typically participated in multiple treatment groups. Additionally, each study compared multiple different treatment groups to a single baseline control group. This study design is expected to introduce substantial study-level correlation bias. Therefore, sampling errors of the observed outcomes/effects within a study were *not* considered to be independent. To account for this within-study correlation structure, a variance-covariance matrix was constructed and used to control study-level correlations.

3. Results

Datasets: The literature search, after refinement and manual curation, yielded roughly ~200 research and review papers on the physiological effects of water immersion. Since our motivation is to define key physiological changes that affect pharmacokinetic disposition in the human body, the physiological endpoints of greatest interest included cardiac output (QC), respiratory ventilation, regional blood flow, kidney function and skin perfusion. Each of these physiological endpoints is directly incorporated into the PBPK model as a parameter affecting chemical absorption, metabolism, distribution and elimination. Each of these parameters were measured using a different metric, and in most cases multiple metrics have been used (Table 1) such that each dataset extracted from the literature has a different composition.

Table 1. Summary of endpoint datasets and data extraction status

Parameter	Number of identified studies	Total number of treatment conditions	Measured parameters	Extraction status
Cardiac output	34	190	• Cardiac output	Completed
Ventilation	37	~194	• Breaths/minutes (rate) • Tidal Volume • Minute Ventilation • Inspiratory time • Expiratory time • Duty cycle	Complete[1]
Kidney function	27	> 127	• Glomerular filtration rate • Urine flow • p-Aminohippuric acid clearance	Near Complete[2]
Regional blood flow	23	> 60	• Doppler • Vascular conductance (brachial, subcutaneous, cutaneous) • Isotope washout • Xenon clearance • Lidocaine clearance (Liver)	Ongoing[3]
Blood Volume	(2)	(23)	% change	Incomplete[4]

1. Data requires minor formatting for computational analysis; 2 additional studies require digital data extraction from pdf figures.
2. Data file requires minor formatting for computational analysis; 4 studies remain that require digital extraction.
3. Four studies remain that require digital extraction from pdf figure.
4. Studies are being prioritized for data extraction.

*Cardiac output analysis***:** The cardiac output dataset (QC) is the largest and most complete among the extracted datasets (Table 1). It is also the most straightforward to interpret because all studies determined cardiac output on the same scale, which is in units of L/min. Therefore, this dataset is used as the basis for method development, which is directly applicable to remaining physiological parameters. Hence, for cardiac output, it was reasonable to use mean effect size rather than standardized mean effect size for our analysis. Consequently, the effect size observed for cardiac output with water immersion and other treatment variables (moderators) are directly interpretable in units of L/min rather than a standardized unit-less ratio. The cardiac output dataset is summarized in Table 2.

Table 2. Summary of studies for cardiac output	
Parameter	Value
Number of studies	34
Number of test conditions	190
Number of participants/study	9.1
Number males & females	298 M & 38 F
Mean age (years)*	30
Height (meters)*	1.8
Weight (kg)*	74.36

Body Mass Index (BMI)*	23.5
Age, height, weight and BMI were provided as the study mean values for most studies. Values provided here are the weighted means across all studies.	

From each published study treatment conditions and subject demographic data were extracted from the narrative text. Experimental conditions (for all physiological endpoints) available for all evaluated studies include immersion level, posture, exertion level, length of immersion, length of treatment (i.e., duration of exercise), and water temperature. Due to study-dependent variation in how authors describe immersion depth, posture and exertion, standardized categories were used to bin (group) the conditions so that comparisons could be made, as described below.

Exertion describes the level of exercise performed by the subject during the experiment. Across all experiments the physiological endpoint (i.e., cardiac output, respiratory rate) during exercise was compared to a baseline control. The baseline control was the physiological endpoint measured at rest prior to water immersion, initiation of exercise or other treatment condition. Exertion was given by all studies in units of Watts or VO_{2max}, or in some cases both, which facilitated interconversion among studies. Study designs for evaluating the effect of exertion varied considerably across studies. In some studies, exercise was performed under both dry and immersed conditions, while in other studies exercise was only performed during immersion. The duration of exertion and rest periods between bouts of exercise (when included) also varied across studies. For comparing exertion levels among studies, we applied an ordinal variable (category) relating equivalent exertion levels in watts and VO_{2max} (Table 3). The distribution of studies and subjects for each exertion level is also provided.

Table 3. Exertion levels

VO_2% of max	Watts	Category	Number of tests	Total subjects tested[1]
Rest	0	1	127	980
≤ 20%	0 to < 50	2	12	110
21 to ≤ 40%	50 to < 100	3	13	182
41 to ≤ 60%	100 to < 150	4	23	265
61 to ≤ 80%	150 to < 200	5	13	150
81 to 100%	≥ 200	6	2	40

1. In several studies subjects, were tested at rest under several different experimental conditions.

The immersion parameter describes the depth of immersion used in each treatment condition. Most of the studies used a single level of water immersion and altered other test conditions, such as water temperature or exertion effort, although a few studies tested different levels of immersion. Across all studies, the most common depth of immersion was approximately neck depth, which was variously described as “head-out of water”, “to the neck”, “sternal notch”, “sternoclavicular notch”, or “suprasternal notch”. Across all studies, 21 different immersion depths were that ranged from none to 30 meters submerged (Table 4). These depth descriptions were consolidated into seven immersion levels based on physiological equivalence, and then further grouped into three levels of none, to the chest, and fully immersed (Table 4).

Table 4. Immersion depth					
Immersion extent/level	Grouped	Number of subjects	Consolidated	Number of tests	Total subjects tested[1]
Submerged 30 m	Depth	7	Immersed	71	558
Submerged 20 m					
Submerged 6-10 m					
Submerged 1-5 m					
Submerged 50 cm	Immersed	5			
Fully immersed					
Chin	Neck/ Head out	59			
Head-out					
Neck					
First thoracic vertebrae	upper Chest	61	Chest	91	841
Shoulders					
Acromion					
(Supra)sternal notch					
Sternoclavicular notch					
Right atrium	lower Chest	30			
4th intercostal space					
Chest level					
Midchest					
Xiphoid					
Hips/waist	Below Chest	2	None	28	328
Air/dry	Control	26			
1. In several studies, subjects were tested at rest under several different experimental conditions.					

Posture describes the position of the subject at rest and during immersion. Typically, each study only evaluated one or two postures with the most common experimental position being the seated position (Table 5). The posture category was simplified for analysis by combining the Prone and Prone-on-side groups, standing and upright, and reclining and seated groups. The combining of groups was based on presumed similarity among combined positions rather than numbers of tests. The imbalance among posture group sizes is a challenge for analyzing this variable.

Table 5. Subject posture by test condition		
Position	Number of tests	Total subjects tested
Prone	9	67
Prone-on-side	4	48
Reclining	13	72
Seated	151	1438
Standing	9	73
Upright	4	29
Total	190	1727

Physiological effects across studies: The effects of water immersion on physiological endpoints was extracted from published literature for ventilation, kidney function, blood volume and regional blood flow, skin perfusion (for dermal absorption), and cardiac output. The decision to focus on cardiac output for the following analysis was motivated by several factors: First, cardiac output (QC) is a key parameter used in PBPK models to estimate tissue blood flows; second, cardiac output is among the most commonly measured endpoints (along with blood pressure and heart rate) which provides a robust dataset; third, human cardiac output is a well studied metric with human measurements falling within an expected range, so there was little need for relating different measurement scales among studies; this is not the case for endpoints like ventilation and kidney function where investigators have the option to measure function with different techniques or measure different functions (e.g., respiratory rate, tidal volume).

Across studies, there was a large degree of variation in cardiac output as a function of depth (Fig. 1). This variation was apparent when visualized as a function of mean cardiac output (raw QC for each experimental condition, Fig. 1A), as the difference in mean cardiac output (Fig. 1B), as the change in cardiac output as a percentage of baseline (Fig. 1C) and as the percent change (Fig 1D). These data together suggested that one or more experimental conditions (moderators) interact with immersion depth to affect cardiac output. One solution that could be used to simplify the analysis would be to discard all treatment conditions that elevate cardiac output, such as immersion, and analyze the relationship between immersion and resting cardiac output. However, this approach results in a substantial loss of information about what happens under the full range of conditions that would be expected during occupational diving operations, and information on how different variables interact. In order to better understand the relative impact of water immersion on cardiac output with respect to the full range of tested experimental conditions, treatment variables and mean subject characteristics were evaluated as moderators as part of a multi-level random effect meta-analysis.

Overall effects: We evaluated more than 30 independent primary studies that investigated effect sizes of different treatment conditions on cardiac output. Effect size is the mean difference between an outcome measurement in treatment subjects compared to the control group. We made the assumption that effect size may not be homogenous within studies, therefore, to deal with the possible dependency of effect sizes within studies, we implemented a three-level meta-analytic model. The multiple studies described above consist of three different variance components distributed over the levels of the model, which are: Level 1, sampling variance of the mean effect sizes at the level of each treatment (190 individual treatments for QC); Level 2, variance between effected sizes from within the same study; Levels 3, variance between studies. This model allows effect sizes to vary between participants (level 1), outcomes (level 2), and studies (level 3). The utility of this approach is that it allows examination of the differences in outcomes (heterogeneity) within studies and between studies. When heterogeneity is observed, moderator analyses can be conducted to evaluate which experimental variables help explain within-study and/or between-study heterogeneity. The effect of moderators can be used to inform parameterization of the PBPK model.

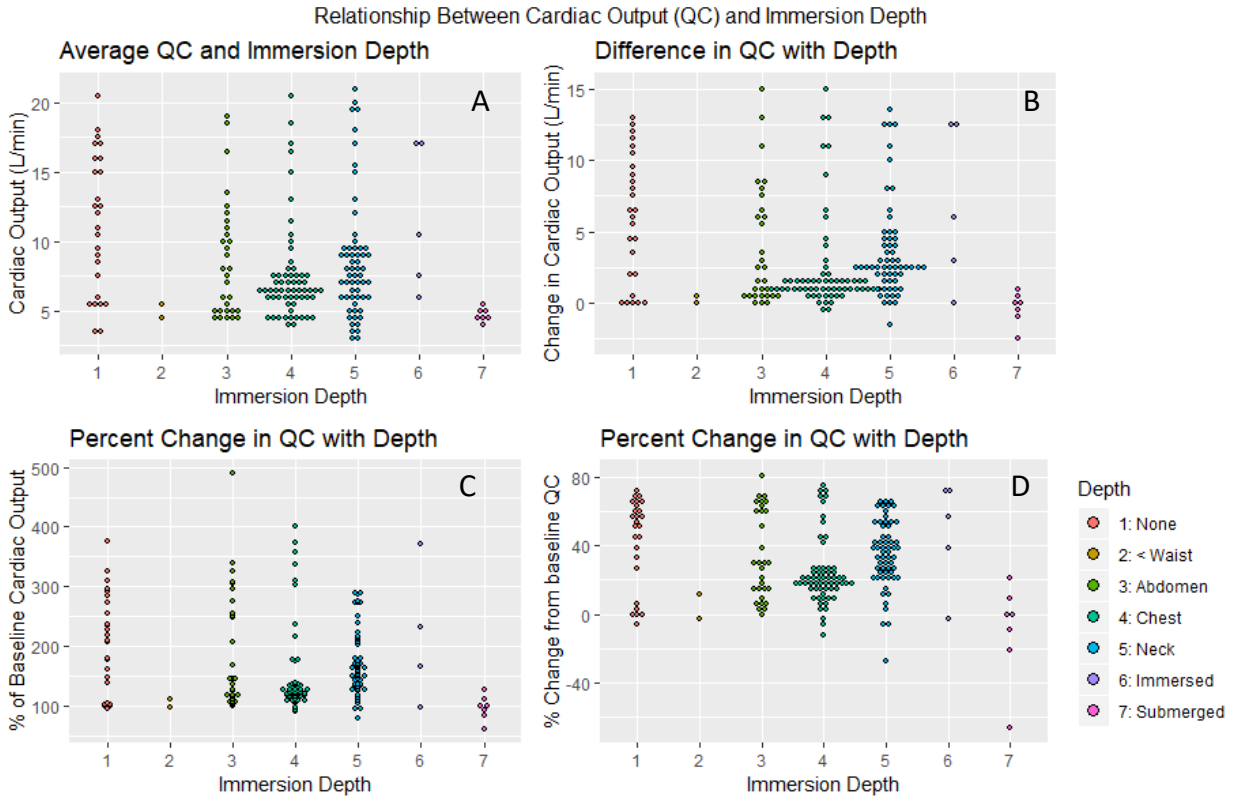

Figure 1. Evaluation of cardiac output (QC) changes with water immersion. Panel A is the mean QC for each study condition (standard error on each point is omitted). Panel B is the change in QC, which is the mean test QC – mean baseline QC for each experimental condition. Panel C is the percent change in QC from baseline, which is mean test QC/mean baseline QC for each tested condition. Panel D is the percent change in QC, which is the change in QC (as in panel B) /baseline QC. Depth of immersion is indicated on the x axis, and is described in the legend.

For cardiac output, the random intercept model comprised a dataset of 190 experimental test conditions from across 34 studies. Total heterogeneity was evaluated across all studies and treatments in the absence any moderating conditions. In this overall 'baseline' model, the total variance between effect sizes within studies (Level 2; σ^2_1) is 6.33. Likewise, between-study variance (Level 3; σ^2_2) was 7.06. As might be expected from inspection of the distribution of data in Fig. 1, the test for heterogeneity was highly significant ($p < 0.001$), meaning that at least one of the variance components is not equal zero and hence, the null hypothesis (H_0) of homogeneity is rejected. These results are not, however, our primary focus; rather, these results serve as a baseline model to investigate the degree to which moderators explain the observed heterogeneity (Assink 2016). The overall association between water immersion and cardiac output (equivalent to Cohen's *d*), without controlling for moderators, is 2.72 ± 0.52 (95% CI: 1.7601 to 3.8717), which was highly significant ($p<0.001$) (Table 6). According to the criteria formulated by Cohen (1988), d = 0.2, d = 0.5, and d = 0.8 are small, moderate, and large effects, respectively. Hence, for our meta-analysis, an overall effect of 2.186 is a very large effect overall.

It was of interest to determine the extent to which within study covariance contributes to total variance of the baseline model. As discussed above, nearly all the studies we evaluated used a study design where the same group of subjects was evaluated under all treatment conditions, and many of the studies compared effects under multiple treatment conditions to pre-immersion baseline

measurements. A comparison was made between two formulations of the overall 'baseline' model: one model was formulated without accounting for within model covariance, and the other formulated using a variance-covariance matrix. Comparison of the results of the tests for residual heterogeneity show that there is significant unexplained variance left between all effect sizes in the baseline model without adjustment:

Overall model without covariance adjustment: Q(df = 189) = 13034.52, p-val < .0001

Overall model accounting for covariance: Q(df = 189) = 6608.39, p-val < .0001

This comparison demonstrated two facts: first, the residual variance is reduced by approximately half when covariance is accounted for in the model; second, both models still have significant residual heterogeneity, which justifies further moderator-based analysis.

Moderator analysis for cardiac output was performed step-wise to identify the most significant variables that contribute to variance in cardiac output. This same approach is entirely applicable to the analysis of all remaining physiological endpoints. Without this meta-analysis, it is not possible to test which factors have the greatest impact on cardiac output since most studies are not directly comparable. Choosing to base conclusions on any one model assumes that the findings in that study are generalizable to all other studies, and that variance across studies is homogeneous. Rather, it is reasonable to assume that each physiological endpoint is likely to have different responses to different treatment combinations. The meta-analysis approach does not make assumptions about generalizability or homogeneity. Instead, it permits a systematic evaluation of how experimental variables interact. For cardiac output, the regression coefficients (betas (β)) can be directly interpreted as a one unit change in variable x that produces a concordant cardiac output change expressed in L/min.

The three-level meta-analysis model described above serves as the baseline for evaluation of moderators. The first moderator evaluated was water immersion. Immersion was evaluated in two ways: one was as a seven level ordinal variable, with level 1 representing no water immersion (dry), and level 7 representing full immersion up to 30 meters of depth (Table 4). The second approach aggregated these seven categories into three groups described as no immersion ("None"), immersion to chest levels ("Chest"; to the first thoracic vertebrae), and "Immersed" (from the neck up). When tested in the overall model, the Test of Moderators (Q_M) and regression coefficients exceeded the p-value 0.05 (based on an F distribution). The interpretation of this is that immersion alone was not a significant explanation for model variance regardless of whether the seven-level or three-level categorical variables were used. This was unexpected since immersion has been repeatedly associated with increased cardiac output. One potential explanation for this discrepancy could be that the distribution of cardiac output from the literature-extracted dataset is described by experimental moderators other than immersion. Accounting for this other more important moderator could help reveal the more modest effect of immersion.

All moderators extracted from literature sources were evaluated individually in the baseline model. Of these variables, the only one that was statistically significant ($p < 0.001$) was exertion. This was not a surprise since exercise clearly has a strong effect on cardiac output, and in several studies,

subjects were asked to exercise at > 80% VO_{2max}. As a consequence, all levels of exertion (Table 3) were significant in the model.

Next, we evaluated each of the remaining experimental variables as part of a new model, that consisted of the baseline model with exertion as the sole moderator. Among all of the variables tested in this 'exertion' model, next most important variable was immersion depth, with both chest and fully immersed being significant (Table 6). In this regard, a one unit change in immersion depth resulted in roughly a 1 L/min change in cardiac output. Full immersion did not appear to have a larger effect than chest immersion. This result did not change when immersion depth was evaluated using the more refined seven-level categorization. It is possible that the small number of full immersion studies with greater depth (i.e., 5 to 30 m) was under-powered for identifying an effect at these depths, however these studies were also complicated by technical challenges of measuring stroke volume under experimental conditions.

Again, a new model was produced by the inclusion of both exertion level and immersion depth. This new model with two moderators was again interrogated with each of the remaining variables to discover whether there were other significant determinants of cardiac output. This analysis identified age as a third important negative determinant of cardiac output (Table 6). As a negative moderator, an increase in age is associated with a decrease in cardiac output. This negative relationship between age and cardiac output, especially as a consequence of exercise, is a well-established principal in cardiac physiology.

Continuing with the step-wise process for inclusion of moderators, each remaining experimental variable was again tested to identify if any others might explain another significant portion of cardiac output variance. This analysis did *not* identify any additional parameters that were significant contributors to the overall model. A subsequent analysis was performed to evaluate justifiable interactions between variables; however, no consistent interactions were observed, and it is therefore concluded that, for the experiments evaluated in this meta-analysis, the key factors contributing to the heterogeneity in the cardiac output dataset are exertion level, immersion depth and age.

Table 6. Results of step-wise meta-analysis of experimental moderators of cardiac output

Moderator (Study Variable)	Test for Residual Heterogeneity	Within Between	Coefficient ± SE
Intercept model	6608.39 ($p < 0.0001$)	σ^2_1 = 6.3280 Within σ^2_2 = 7.0606 Between	Intercept: 2.72 ± 0.52***
Immersion	6583.7612 ($p < 0.0001$)	σ^2_1 = 6.3223 Within σ^2_2 =7.2324 Between	Intercept: 3.08 ± 0.74 *** Chest: 0.02 ± 0.66 (NS) Immersed: -1.08 ± 0.80 (NS)
Exertion	2363.7678 ($p < 0.0001$)	σ^2_1 = 1.5028 Within σ^2_2 = 1.4594 Between	Intercept: 1.28 ± 0.26 *** Exertion 2: 1.38 ± 0.50 ** Exertion 3: 4.1 ± 0.47 *** Exertion 4: 5.90 ± 0.38 ***

			Exertion 5: 8.94 ± 0.47 *** Exertion 6: 11.74 ± 1.00 ***
Immersion Exertion	1993.1994 (p < 0.0001)	σ^2_1 = 1.2811 Within σ^2_2 = 1.5387 Between	Intercept: 0.02 ± 0.37 Exertion 2: 1.60 ± 0.47 *** Exertion 3: 4.46 ± 0.45 *** Exertion 4: 6.14 ± 0.36 *** Exertion 5: 9.29 ± 0.46 *** Exertion 6: 12.08 ± 0.93 *** Chest: 1.51 ± 0.32*** Immersed: 1.09 ± 0.39 **
Immersion Exertion Age_years	1968.61 (p < 0.0001	σ^2_1 = 1.24 Within σ^2_2 = 1.63 Between	Intercept: 1.39 ± 0.71 . Exertion 2: 1.62 ± 0.46 *** Exertion 3: 4.46 ± 0.44 *** Exertion 4: 6.17 ± 0.36 *** Exertion 5: 9.28 ± 0.45 *** Exertion 6: 12.08 ± 0.92 *** Chest: 1.50 ± 0.31 *** Immers: 1.08 ± 0.39 ** Age_years: -0.04 ± 0.02 *
Exertion levels correspond to levels in Table 4 Immersion levels ("Chest" and "Immersed") correspond to the consolidated levels in Table 5. Statistical significance levels are '***' is p-value < 0.001; '**' is p-value < 0.01; '*'is p-valued < 0.05; '.' Is p-value < 0.1 "Within" and "Between" are within studies (Level 2) and between studies (Level 3)			

CONCLUSIONS

The objective of this investigation was to use the full body of published literature to gain deeper insights on the physiological effects of water immersion. We have conducted a quantitative investigation of how water immersion affects key physiological parameters needed for PBPK modeling of chemical exposures during occupational diving. Several reviews have been written on the subject (e.g., Holmer 1992; Lin 1988; Pendergast and Lundgren 2009; Pendergast et al. 2011); however these reviews typically cite specific studies that illustrate the observed changes. Such changes are limited to only the conditions tested in the specific study cited. Consequently, a different experimental protocol or cohort of study subjects might yield different results. For this reason, the results of a single study (or a few studies) are not generalizable to real-world diving conditions. Here, we have used results compiled from over 185 studies to evaluate the effects of water immersion on cardiac output across a large range of experimental conditions. During the early stages of our investigation we recognized that selecting one or two studies that were "most representative" of occupational diving conditions was highly problematic because divers rarely work under ideal laboratory conditions. Rather, diving operations occur at many depths, in different water temperatures, and for different durations. Diving operations may be performed by either men or women of different sizes, ages and physical conditions. Diving activities may also involve water currents or hard physical activities resulting in exertion of substantial efforts. For these reasons, we determined that the most comprehensive approach was to build a statistical model that captured

the most critical parameters that determine the changes in physiological endpoints and describe the interplay of these conditions on the occupational diver.

One surprise from this analysis was that that there were very few studies of full submersion (head under water), at least for the cardiac output endpoint. Among the included studies, nearly all of them used some version of the “head out” of water design. Perhaps this is because cardiac output is calculated based on heart rate x stroke volume. Although heart rate is easily determined under just about any condition, measuring stroke volume involves imaging the heart, or a more invasive approach of cardiac catheterization. These measurements likely become more technically challenging when depth is increased and experimental conditions become less controlled. For example, full immersion requires a larger tank or pool, and a breathing apparatus of which there are many options. Furthermore, some subjects can have strong emotional responses to complete immersion, which directly impacts heart rate and therefore cardiac output estimates. Because we identified very little quantitative data on full ‘deep water’ immersion, extrapolation of the current model deeper divining scenarios has a high degree of uncertainty.

In our analysis, exertion had a much more significant effect on cardiac output than water immersion. For each level of exercise, there was a highly significant corresponding increase in cardiac output, as measured in L/min. In PBPK models, cardiac output is the central determinant of tissue blood flow estimates, which in turn is the critical factor for blood flow-limited diffusion of chemicals into tissues, and also renal and pulmonary clearance rates. Since diving activities require some minimal physical exertion, it is important to account for physical activity in the PBPK model. Our estimates of cardiac output, as predicted by the meta-analysis model, can be directly incorporated in the PBPK model to support predictions of chemical exposures.

One significant limitation of our dataset is the role of gender as a modulator of cardiac output, and other physiological endpoints. Relatively few of the studies we identified included women, and most of those used a mixed population without providing individual measurement mean results for men and women. Only a couple studies focused solely on women. This could be a significant data gap, and reflects the overall male bias in subject selection. In our analysis did not observe any evidence of a difference between males and females in the effect of water immersion on cardiac output, however it is entirely possible that the number of females tested was too small for the difference to be observed. In other words, it is likely that the meta-analysis is under powered for gender-related differences to be observed. For this reason, the changes in physiological endpoints observed for males will be assumed to also be applicable to females.

Immersion time has been reported to have an effect on cardiac output. It has been reported that translocation of blood into the thorax and elevation of plasma volume by shifting of extravascular fluid to the vascular compartment led to increased cardiac stroke volume and cardiac output (Pendergast et al. 2011). Our meta-analysis of the literature did not demonstrate that immersion time is a significant contributor to cardiac output. We evaluated immersion time both as an independent moderator, and as an interaction with immersion depth. We also evaluated immersion time as an interaction with exercise, since it has been previously reported that cardiac output is affected by three hours of immersion (Kame and Pendergast 1995); however, our analysis observed only a marginal effect with immersion time, and no clear relationship with exertion levels. Therefore, it does not appear that there is a need to account for the effect of immersion time on

cardiac output. However, shifting of fluids to the central compartment may impact regional tissue blood flows, particularly the liver, lung and kidney, which could be significant for chemical metabolism and clearance by these routes. This question will be addressed when this parameter is investigated in subsequent analyses.

In conclusion the key factors contributing to the heterogeneity of cardiac output data are within study variance, exertion levels, immersion depth and age. After accounting for these parameters, there still remains significant heterogeneity, which is not accounted for by any of the tested variables. It is likely that a substantial fraction of this variability is a result of technical or methodological differences among studies, study variables that were either not consistently reported in the published reports, or were not captured as part of our analysis. It should be emphasized that manual curation of the study variables that we did capture was a significant challenge and very labor intensive. In retrospect, our experience suggests that extraction of additional study variables would likely be of low yield since older studies tended to provide less experimental details and data variance (McArdle et al. 1976).

Appendix A

	Authors	Title	Journal
1	Pendergast DR, Lundgren CE.	The underwater environment: cardiopulmonary, thermal, and energetic demands.	J Appl Physiol (1985). 2009
2	Pendergast DR, Moon RE, Krasney JJ, Held HE, Zamparo P.	Human Physiology in an Aquatic Environment.	Compr Physiol. 2015
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