# Short communication

## Pulmonary oxygen toxicity breath markers after heliox diving to 81 metres

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**Abstract**

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Pulmonary oxygen toxicity (POT), an adverse reaction to an elevated partial pressure of oxygen in the lungs, can develop as a result of prolonged hyperbaric hyperoxic conditions. Initially starting with tracheal discomfort, it results in pulmonary symptoms and ultimately lung fibrosis. Previous studies identified several volatile organic compounds (VOCs) in exhaled breath indicative of POT after various wet and dry hyperbaric hypoxic exposures, predominantly in laboratory settings. This study examined VOCs after exposures to 81 metres of seawater by three navy divers during operational heliox diving. Univariate testing did not yield significant results. However, targeted multivariate analysis of POT-associated VOCs identified significant ($P = 0.004$) changes of dodecane, tetradecane, octane, methylcyclohexane, and butyl acetate during the 4 h post-dive sampling period. No airway symptoms or discomfort were reported. This study demonstrates that breath sampling can be performed in the field, and VOCs indicative of oxygen toxicity are exhaled without clinical symptoms of POT, strengthening the belief that POT develops on a subclinical-to-symptomatic spectrum. However, this study was performed during an actual diving operation and therefore various confounders were introduced, which were excluded in previous laboratory studies. Future studies could focus on optimising sampling protocols for field use to ensure uniformity and reproducibility, and on establishing dose-response relationships.

### Introduction

Pulmonary oxygen toxicity (POT) is a significant risk in oxygen diving and prolonged hyperbaric oxygen treatment, and has damaging effects on the alveolar epithelium caused by reactive oxygen species. First described in 1899, it may lead to destruction of cellular membrane lipid bilayers and DNA damage in nuclei.[1,2] This initiates immune responses, apoptosis, and subsequent tracheobronchitis characterised by coughing and retrosternal discomfort.[3,4] In more severe cases, acute respiratory distress syndrome can develop, and prolonged exposure may result in pulmonary fibrosis.[3,4] A partial pressure of oxygen ($PO_2$) of ≥ 51 kPa (0.5 atmospheres absolute [atm abs]) can cause POT after several hours of continuous exposure, and symptoms tend to manifest more rapidly with higher $PO_2$ levels.[5]

Special operations forces using rebreathers with 100% oxygen are susceptible to POT during long-range diving missions, which can last for several hours. Deep diving missions performed by mine-clearing divers also pose risks due to increased $PO_2$ levels in gas mixtures. To quantify hyperoxic exposure and stratify the risk of POT, the 'units of pulmonary toxic dose' (UPTD) measure has been developed (1 UPTD = 1 minute breathing 100% oxygen at 1 atm abs). Limits are based on the reduction in pulmonary vital capacity following excessive exposure to oxygen during hyperbaric chamber dives.[6] Currently, 615 UPTD is considered the maximum exposure for a single dive, corresponding with a 2% reduction in vital capacity in 50% of the population.[4]

The Royal Netherlands Navy has investigated volatile organic compounds (VOCs) in exhaled breath to identify

potential biomarkers of POT.[7–12] These laboratory studies conducted after shallow hyperoxic in-water dives and hyperbaric oxygen treatment exposures identified predominantly increased levels of alkanes and aldehydes. The current study aimed to collect breath samples after operational deep 16/84 heliox dives (16% oxygen and 84% helium gas mixture) to investigate if changes in VOCs under these specific diving circumstances align with the compounds identified in laboratory dives.

## Methods

Three divers of the Royal Netherlands Navy made heliox dives to 81 metres of seawater (msw; equal to 816 kPa) in a Norwegian fjord using surface oxygen decompression. The dive profile was based on the Defence R&D Canada (DRDC) Helium-Oxygen Diving Table 8, using nitrox 32.5% instead of air as decompression gas[13] (Figure 1). The total dive and decompression time was 62 min, with calculated oxidative stress equal to 119 UPTD. The participating divers were all healthy, non-smoking volunteers and fit-for-diving according to the Netherlands Ministry of Defense's fitness requirements for diving. Informed consent was obtained before sampling. The sampling protocol was approved by the Medical Ethical Committee of the Amsterdam Academic Medical Center (ref. W18_424 # 21.083) and, together with the gas chromatography-mass spectrometry (GC-MS) analysis, was identical to those used in previous studies.[7,8,10,11] After diving and during sampling, the subjects were questioned whether they had experienced any pulmonary or other physical symptoms.

Four breath samples were collected per subject per dive: one pre-dive sample just before submerging, and at 30 min, 2 h, and 4 h after completing the surface decompression. In short, the subjects breathed for 5 min through an inspiratory VOC filter (Honeywell, Charlotte, NC, USA) to minimise environmental contamination. Thereafter, the subjects exhaled into a non-elastic balloon (Globos Nordic, Naestved, Denmark), from which 500 mL air was pumped (Gastec, Kanagawa, Japan) through a sampling tube (Tenax GR 60/80; Camsco, Houston, TX, USA). After sampling, the tubes were stored for several days at ~8°C until GC-MS analysis (GC-MS QP2010; Shimadzu, Japan, TD100; Markes, Sacramento, CA, USA) was performed in the laboratory of Amsterdam UMC. All divers were required to have a surface interval of at least 24 h between dives to minimise inert gas build-up and reduce the chance of decompression sickness. To minimise contamination from food, the subjects had a daily uniform diet and were not allowed to eat or drink within 1 h before sampling, except for water. The samples were analysed using GC-MS and compounds were identified using the NIST library.[14]

### STATISTICAL ANALYSIS

The data were statistically processed using R statistical software (v4.1.2; R Core team 2021) together with R-packages pROC (v1.18.0), Skillings.Mack (v1.10), and MixOmics (v6.18.1). Wilcoxon signed-rank and Skillings-Mack tests were conducted to select and identify untargeted ion fragments that varied significantly and to test fluctuations of individual molecules over time. Targeted sparse partial least squares discriminant analysis (sPLSDA) modeling for two components and Kruskal-Wallis rank sum testing were employed to identify and test previously discovered VOCs linked to POT from the VAPOR library.[15]

## Results

Twenty-seven exhaled breath samples were collected in eight dives. Complete series of four samples were collected in five dives, while two dives lacked the 4 h post-dive measurement (Figure 2). One dive consisted only of the pre-dive sample and thus was discarded. No symptoms of POT were reported by the subjects.

To determine if the consecutive dive days influenced the measurements, partial least squares discriminant analysis was performed of the sampling series. This demonstrated no individual dependencies of the sampling series between successive days. Thus, consecutive days of diving for a single diver could be statistically regarded as independent dives instead of repetitive dives.

Untargeted analysis did not yield significant findings between pre-dive and post-dive measurements. However, sPLSDA modeling based on previously identified VOCs of interest from the VAPOR library[15] found a significant ($P = 0.004$) change of component 1, consisting of dodecane, tetradecane, octane, methylcyclohexane, and butyl acetate (Table 1). When plotted according to the sampling timepoint, component 1 demonstrated a rise of sPLSDA values from pre-dive to 2 h post-dive, followed by normalisation of the signal intensity at 4 h post-dive (Figure 3).

## Discussion

To our knowledge, this is the first study to analyse exhaled VOCs after an operational heliox dive to 81 msw followed by surface oxygen decompression. As can be seen in Figure 1, the largest oxygen exposure in diving using surface decompression procedures comes from breathing 100% oxygen in the hyperbaric chamber during the surface phase. The surface decompression results in 80 UPTD, whereas the in-water stage is 32 UPTD. However, the effect of submersion during an in-water dive on POT is not fully understood and the UPTD was developed using dry hyperbaric exposures; thus, it could over- or underestimate in-water hyperoxic stresses. Nevertheless, it seems reasonable to assume that the surface decompression had the largest impact on the lungs.

A recent study on deep heliox dives with surface decompression showed a temporary decrease in spirometric parameters after diving to 80 msw, normalising within 24

**Figure 1**

Dive profile of the 81 msw heliox 16/84 (16% oxygen, 84% helium) dive, followed in-water decompression with enriched air nitrox (EANx) containing 32.5% oxygen) and 100% oxygen, and surface oxygen decompression to 121 kPa in a hyperbaric chamber

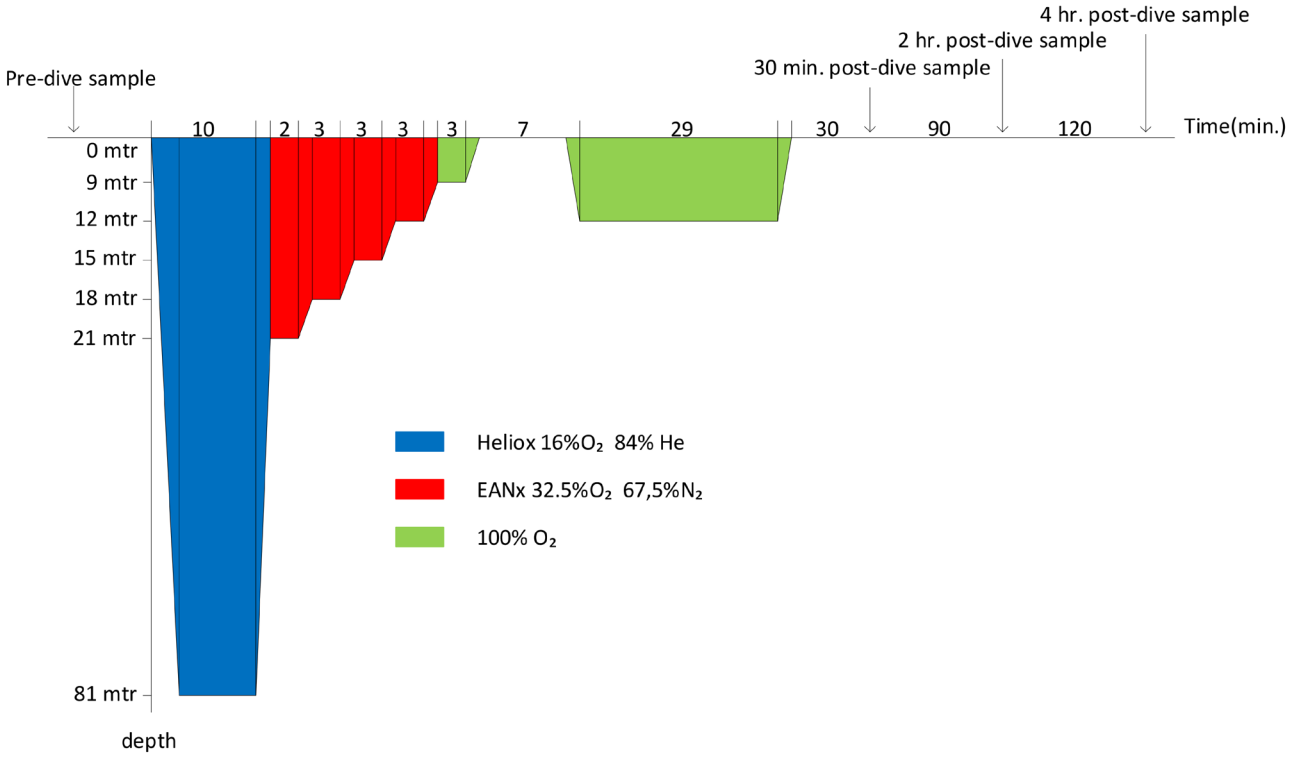



**Figure 2**

Overview of the sampling results

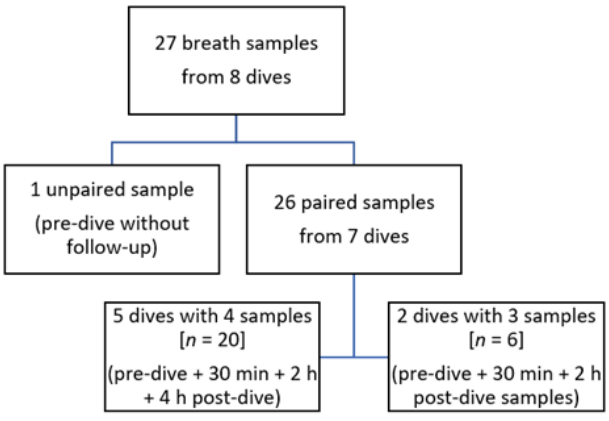



**Table 1**

Compounds identified by targeted analysis of known POT-associated VOCs from the VAPOR library;[15] CAS-no. – Chemical Abstract Services identification number[16]

| Compound | CAS-no. | Molecular weight | Ref |
|---|---|---|---|
| Dodecane | 112-40-3 | 170 | 6,7,9 |
| Tetradecane | 629-59-4 | 198 | 6,7,9 |
| Octane | 111-65-9 | 114 | 6,7,9 |
| Methylcyclohexane | 108-87-2 | 98 | 6,7,9–11 |
| Butyl acetate | 123-86-4 | 116 | 6,7,9,10 |

hours.[17] Although spirometry and exhaled breath analysis results showed little correlation in our previous studies, we feel it is important to note that these two modalities both show a transient response to deep helium diving and a full recovery afterwards.

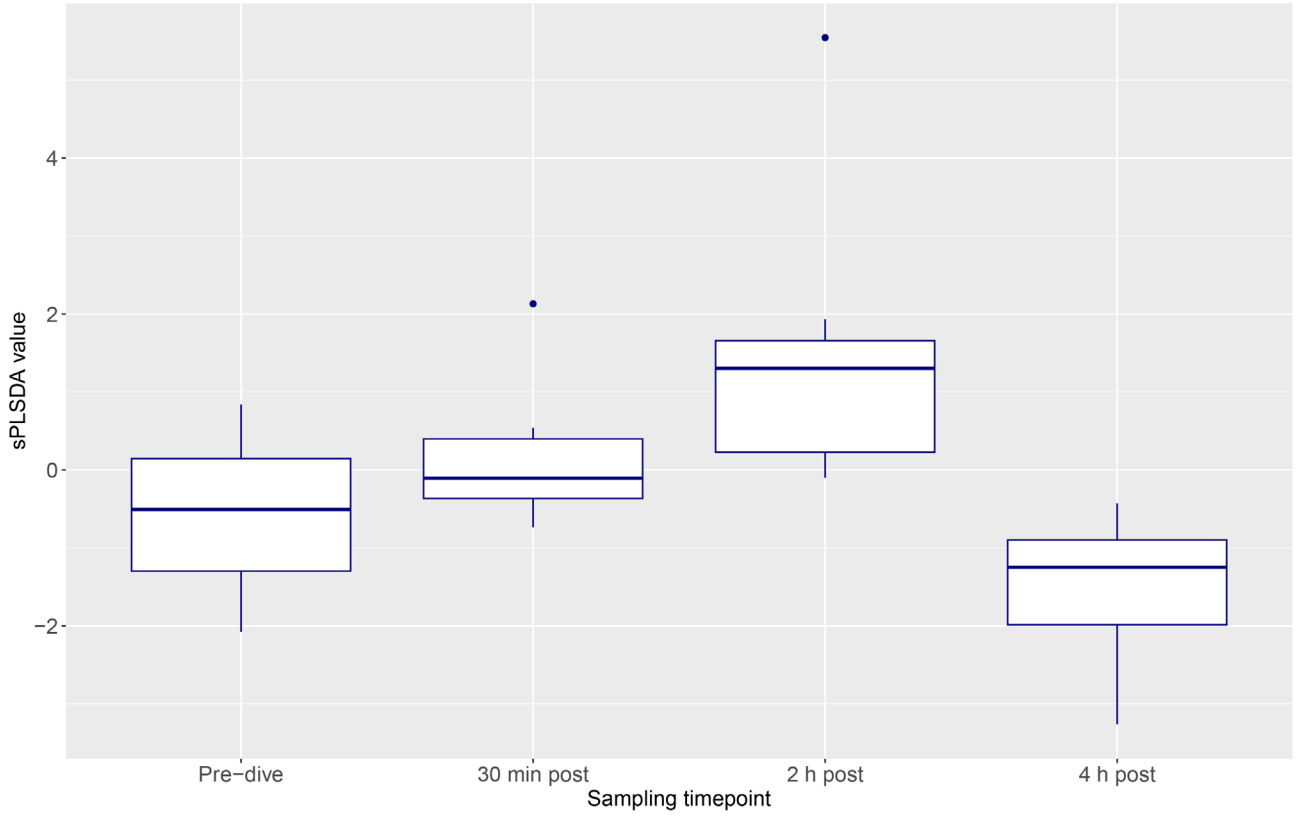
Similar to previous studies from our group, the change in VOC intensity was greatest in the 2 h post-dive samples, followed by a drop in intensity at the last measurement. However, this was only ascertained by targeted multivariate analysis of previously identified compounds from the VAPOR library, while untargeted testing was performed after previous hyperbaric exposures in laboratory settings.

## LIMITATIONS

This was a field study in an operational setting and thus has several limitations. Available resources and time management options were restricted. The full series of four samples per dive could not be collected in all cases due to

**Figure 3**

The sparse partial least squares discriminant analysis (sPLSDA) values at each sampling timepoint for component 1, consisting of the POT-associated VOCs dodecane, tetradecane, octane, methylcyclohexane, and butyl acetate; the boxes summarize the central tendency (median) and spread (interquartile range) of the sPLSDA values, the whiskers extend to values within 1.5 times the interquartile range, with two individual outliers at the second and third sampling points. Kruskal-Wallis $Chi^2 = 13.317$, df = 3, $P = 0.004$




last-minute changes in plans and activities that had priority over this study, such as surface tending the next dive sortie. Another limitation is the small number of participating subjects. Due to operational dive team restrictions and smoking habits, only three divers were included in this study.

The dives were carried out by two divers per sortie, with subsequent dives starting after the surface decompression procedure of the previous pair of divers was completed. Consequently, samples were not collected at the same time of day for all divers, as in previous laboratory dives; one series of samples was collected in the morning and the next series was collected in the afternoon. This may have influenced VOC intensities because they fluctuate during the day.[18] This variability also applies to food ingestion. Although no food was consumed within 1 h before sampling, the morning divers had lunch in the 1 h food window between the third and fourth sampling timepoints, whereas the afternoon divers had lunch before the pre-dive sampling. Therefore, minimal influences of food cannot be excluded.

The small sample size ($n = 26$) and relatively short hyperoxic exposures (62 min) with a relatively low (119) UPTD may explain why the VOC changes were smaller compared to previous studies. Additionally, the exact role of helium in a hyperbaric environment is not fully understood, but several studies suggest that helium plays a protective role at the cellular level.[19,20]

## Conclusion

The findings of this study indicate a limited and reversible reaction to hyperoxia occurs after deep heliox diving using surface oxygen decompression, but no signs of prolonged pulmonary damage were observed. This strengthens the theorem that POT develops sub-clinically before first symptoms are experienced. This study also demonstrates that it is feasible to capture VOCs in operational settings for further analysis. Further studies should focus on optimising sampling protocols for field use to ensure uniformity and reproducibility, and on establishing dose-response relationships of POT biomarkers in breath after various hyperbaric hyperoxic exposures.

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