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## Hydrogen and oxygen isotope values in hydrogen peroxide

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Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a widely used oxidizer with many commercial applications; unfortunately, it also has terrorist-related uses. We analyzed 97 hydrogen peroxide solutions representing four grades purchased across the United States and in Mexico. As expected, the range of hydrogen ( $\delta^2\text{H}$ , 230‰) and oxygen ( $\delta^{18}\text{O}$ , 24‰) isotope values of the H<sub>2</sub>O<sub>2</sub> solutions was large, reflecting the broad isotopic range of dilution waters. This resulted in predictable linear relationships of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of H<sub>2</sub>O<sub>2</sub> solutions that were near parallel to the Meteoric Water Line (MWL), offset by the concentration of H<sub>2</sub>O<sub>2</sub> in the solution. By grade, dilute (3 to 35%) H<sub>2</sub>O<sub>2</sub> solutions were not statistically different in slope. Although the  $\delta^2\text{H}$  values of manufactured H<sub>2</sub>O<sub>2</sub> could be different from those of water, rapid H<sub>2</sub>O<sub>2</sub>–H<sub>2</sub>O exchange of H atoms eliminated any distinct isotope signal. We developed a method to measure the  $\delta^{18}\text{O}$  value of H<sub>2</sub>O<sub>2</sub> independent of dilution water by directly measuring O<sub>2</sub> gas generated from a catalase-induced disproportionation reaction. We predicted that the  $\delta^{18}\text{O}$  values of H<sub>2</sub>O<sub>2</sub> would be similar to that of atmospheric oxygen (+23.5‰), the predominant source of oxygen in the most common H<sub>2</sub>O<sub>2</sub> manufacturing process (median disproportionated  $\delta^{18}\text{O}$  = 23.8‰). The predictable H–O relationships in H<sub>2</sub>O<sub>2</sub> solutions make it possible to distinguish commercial dilutions from clandestine concentration practices. Future applications of this work include synthesis studies that investigate the chemical link between H<sub>2</sub>O<sub>2</sub> reagents and peroxide-based explosive products, which may assist law enforcement in criminal investigations. Copyright © 2011 John Wiley & Sons, Ltd.

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a widely available oxidizer with many consumer and industrial applications. As a dilute solution (distributed as w/v or w/w) in water, hydrogen peroxide is unregulated and sold directly to consumers for many uses such as for general antiseptics (3%) or in hair bleach (6 to 12%). Reagent-grade H<sub>2</sub>O<sub>2</sub> (20 to 35%) is available through scientific suppliers whereas commercial-grade H<sub>2</sub>O<sub>2</sub> (30 to 70%) which is used in the paper/pulp, metals and electronics industries, is regulated and not available to the general public.<sup>[1]</sup>

Hydrogen peroxide is also illicitly used in the manufacture of peroxide-based explosives such as triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD) or simple mixtures containing H<sub>2</sub>O<sub>2</sub> and a carbon source. In 2001, Richard Reid was convicted of attempting to detonate an explosive device with a TATP initiator aboard an aircraft.<sup>[2]</sup> The July 2005 London transport system bombings were attributed to TATP<sup>[3]</sup> as was the attempt to carry peroxide-based explosive devices aboard aircraft flying direct routes between the United States and the United Kingdom.<sup>[4]</sup> More recently, in 2009, an individual was arrested in an alleged plot to detonate peroxide-based explosives on the New York City subway system.<sup>[5]</sup> As the use of peroxide-based explosives in improvised explosive devices becomes

more prevalent, law enforcement agencies will require the capacity to link the hydrogen peroxide reagent to a clandestine laboratory and to the explosive itself. Current forensic analytical techniques have focused on the rapid, positive chemical identification of peroxide-based explosive materials and post-blast residues using liquid chromatography/mass spectrometry (LC/MS),<sup>[6]</sup> gas chromatography/mass spectrometry (GC/MS),<sup>[7–9]</sup> infrared (IR) spectroscopy,<sup>[10]</sup> or desorption electrospray ionization (DESI).<sup>[11,12]</sup> What these current methods lack, however, is the ability to distinguish one specimen of H<sub>2</sub>O<sub>2</sub> from another.<sup>[13]</sup>

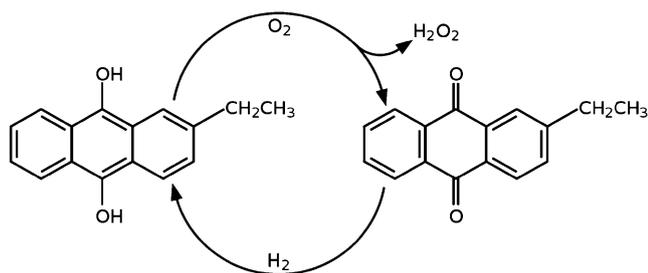
Hydrogen peroxide is manufactured almost exclusively through the catalytic ‘anthraquinone process’, a cyclic reaction where atmospheric air is bubbled through 2-ethyl-9,10-dihydroxyanthracene, producing H<sub>2</sub>O<sub>2</sub> (Fig. 1). The 2-ethylanthraquinone oxidation product is reduced back to 2-ethyl-9,10-dihydroxyanthracene with the addition of hydrogen gas (H<sub>2</sub>).<sup>[14]</sup>

We hypothesized that stable isotope ratio analyses would be a productive approach for distinguishing among H<sub>2</sub>O<sub>2</sub> solutions that were chemically alike but originated from different sources. Hydrogen atoms in water and hydrogen peroxide are expected to rapidly exchange with each other in solution, but the oxygen atoms in hydrogen peroxide should not exchange with oxygen atoms in water.

We expected differences between H<sub>2</sub>O<sub>2</sub> solutions. We specifically hypothesized that: (H1) the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of dilute hydrogen peroxide solutions should fall along a slope of approximately 8, similar to that of the Meteoric Water Line (MWL),<sup>[15]</sup> offset by the solution concentration; and (H2) the  $\delta^{18}\text{O}$  value of O from disproportionated

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**Figure 1.** Illustration of the catalytic production of hydrogen peroxide by the 'anthraquinone' process where 2-ethyl-9,10-dihydroxyanthracene is oxidized to 2-ethylanthraquinone (available through public domain<sup>[28]</sup>).

hydrogen peroxide should be +23.5‰, reflecting its atmospheric origin. Although we expected the slope values of the H<sub>2</sub>O<sub>2</sub> solution grades to be similar, we did expect intercept value differences. This is because the H<sub>2</sub> source will affect the δ<sup>2</sup>H value of H<sub>2</sub>O<sub>2</sub> in the reduction of 2-ethylanthraquinone to 2-ethyl-9,10-dihydroxyanthracene. Because the source of H<sub>2</sub> can vary based on manufacturer, location and supply we expect there may be a large possible range in δ<sup>2</sup>H values of concentrated H<sub>2</sub>O<sub>2</sub>.

The first unknown was, 'What are the magnitudes of hydrogen and oxygen isotope variations in hydrogen peroxide?' This is a challenge to answer because H<sub>2</sub>O<sub>2</sub> is available only as an aqueous solution and there are no simple procedures for fully isolating the water from H<sub>2</sub>O<sub>2</sub> prior to an isotopic measurement; nor is it feasible to produce anhydrous H<sub>2</sub>O<sub>2</sub>. Our approach was to make sequential measurements. First we measured the δ<sup>2</sup>H and δ<sup>18</sup>O values of a H<sub>2</sub>O<sub>2</sub> solution. This was followed by a disproportionation step, to measure the δ<sup>18</sup>O value of H<sub>2</sub>O<sub>2</sub>, independent of the dilution water. The disproportionation step facilitated the analysis of H<sub>2</sub>O<sub>2</sub> without exceeding the safe and practical limits of working with anhydrous solutions obtained through dehydration processes including; passively evaporating solutions,<sup>[16]</sup> heating,<sup>[17]</sup> fractional crystallization; chemical removal of water; or distillation.<sup>[1,16]</sup> Here the δ<sup>18</sup>O value of H<sub>2</sub>O<sub>2</sub> is measured as O<sub>2</sub>, the product of the disproportionation reaction, without subjecting the H<sub>2</sub>O<sub>2</sub> solution to any pre-processing or concentration.

We tested our two hypotheses through measurements of H<sub>2</sub>O<sub>2</sub> solutions obtained across North America that were of the four different grades available: pharmaceutical (3%); salon (6 to 12%); reagent (30 to 35%); and commercial (70%).

## EXPERIMENTAL

### Hydrogen peroxide solution collections

To quantify the natural variation in the δ<sup>2</sup>H and δ<sup>18</sup>O values of commercially available H<sub>2</sub>O<sub>2</sub>, an extensive United States survey was conducted. This survey was later expanded to include samples obtained in Mexico along the US/Mexico border. We surveyed four grades of H<sub>2</sub>O<sub>2</sub>: pharmaceutical (3%); salon (6 to 12%); reagent (30 to 35%); and commercial (70%) solutions. Pharmaceutical and salon-grade H<sub>2</sub>O<sub>2</sub>

solutions were obtained at grocery and beauty supply stores, or purchased by colleagues in regions of interest and returned by mail. Reagent-grade H<sub>2</sub>O<sub>2</sub> was purchased through scientific suppliers (Fisher Scientific, Pittsburgh, PA, USA; and VWR, Westchester, PA, USA). Commercial-grade H<sub>2</sub>O<sub>2</sub> was shipped directly from a single US manufacturer that provided H<sub>2</sub>O<sub>2</sub> produced in two US locations.

### Stable isotope ratio notation

Stable isotope abundances are expressed in 'delta' notation (δ) in parts per thousand (‰), relative to an international standard where:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \cdot 1000$$

and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of the rare to abundant isotope (e.g., <sup>18</sup>O/<sup>16</sup>O) in the sample and international standard, respectively. The international standard for both hydrogen and oxygen is Vienna Standard Mean Ocean Water (V-SMOW).

### Stable isotope ratio measurements

Hydrogen and oxygen isotope values of H<sub>2</sub>O<sub>2</sub> solutions were measured by continuous flow isotope ratio mass spectrometry (IRMS) on a Delta Plus XL mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a Thermo Scientific temperature conversion elemental analyzer (TC/EA) and a GC PAL autosampler (CTC Analytics, Zwingen, Switzerland). The TC/EA was modified by the addition of a sample injection port and was configured with reverse-flow helium carrier gas. Bulk hydrogen peroxide solutions were analyzed as close to their acquisition date as possible. Hydrogen peroxide solutions (0.4 mL) were prepped in borosilicate glass gas chromatography vials and capped with rubber, polytetrafluorethylene (PTFE)-lined septa for analysis. Solutions were pyrolyzed at 1400°C in the presence of carbon to produce H<sub>2</sub> and CO gases, which were then separated using a 5 Å molecular sieve GC column (1-m, 0.25"; Costech Analytical, Valencia, CA, USA). Bulk H<sub>2</sub>O<sub>2</sub> solutions were analyzed together with three water reference materials that had been previously calibrated to the V-SMOW scale. Measured isotope values were slope-intercept corrected with two of the reference materials. The standard deviation (±1 SD) of repeated measurements of the third quality control reference material was 1.6‰ for δ<sup>2</sup>H and 0.2‰ for δ<sup>18</sup>O.

### Disproportionation method

The δ<sup>18</sup>O value of H<sub>2</sub>O<sub>2</sub> in solution was measured by disproportionating H<sub>2</sub>O<sub>2</sub> to water and diatomic oxygen (O<sub>2</sub>) using catalase (Sigma, Catalog # C9322, St. Louis, MO, USA) and subsequently measuring the evolved O<sub>2</sub> gas on a Delta Plus XL mass spectrometer equipped with a TC/EA by manual injection. Hydrogen peroxide was introduced into an evacuated vial containing catalase. After disproportionation, the vial was subjected to freeze/thaw cycles and the evolved O<sub>2</sub> gas was analyzed. The disproportionation reaction is:



Direct injection of O<sub>2</sub> into a TC/EA-IRMS system was an atypical analytical procedure as the TC/EA was designed to accept a liquid or a solid sample, not a gas. However, an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer had previously been used successfully to measure  $\delta^{18}\text{O}$  values of O<sub>2</sub>.<sup>[18]</sup> To evaluate our ability to make unfractionated O<sub>2</sub> measurements using a TC/EA-IRMS system, we made manual injections using in-house compressed O<sub>2</sub> and observed a very small inter-sample variance ( $\delta^{18}\text{O} = 30.6 \pm 0.1\text{‰}$ ;  $n = 6$ ).

For unknown H<sub>2</sub>O<sub>2</sub> solutions, catalase was added in excess (~20 mg) to a 6-mL glass crimp-top vial and sealed with a PTFE septum-lined cap. The vial was completely evacuated via a needle pierce through the septum. A 2-mL sample of 3% H<sub>2</sub>O<sub>2</sub> was introduced into the evacuated vial by injection syringe and mixed by hand. Concentrated H<sub>2</sub>O<sub>2</sub> solutions were diluted to 3% with laboratory deionized (DI) water prior to disproportionation to maintain equivalent reaction volumes. Although we could identify no mechanism for rapid exchange,<sup>[19]</sup> we evaluated whether the oxygen in H<sub>2</sub>O<sub>2</sub> could exchange with the oxygen in (added) dilution water. We diluted 70% H<sub>2</sub>O<sub>2</sub> with two isotopically distinct waters ( $\delta^{18}\text{O} = -16.5\text{‰}$  and  $+14.5\text{‰}$ ) to 3% solutions, allowed them to equilibrate for 15 min and disproportionated them using the described method (all dilutions, *mean*  $\delta^{18}\text{O} = 23.7 \pm 0.1\text{‰}$ ;  $n = 3$ ).

To determine the time required to completely disproportionated H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>, we disproportionated six replicate vials of a single H<sub>2</sub>O<sub>2</sub> sample, stopping the reaction at 0, 8, 180, 300, 600 and 1200 s by the addition of sulfuric acid, which immediately denatured catalase. The evolved O<sub>2</sub> from each vial was measured and data are reported in Table 1. From this experiment, we determined that the disproportionation reaction should proceed for >300 s. For all unknown samples, the disproportionation time was set to 480 s.

The reacted vial was subjected to two freeze/thaw cycles by placing it upright on a block of dry ice until the water/catalase solution was frozen, then subsequently thawing it at room temperature. These freeze/thaw cycles burst the bubbles that formed during the disproportionation reaction. The vial was then frozen on dry ice a third and final time, effectively eliminating vapor pressure (from solution water and evolved product water) in the vial prior to O<sub>2</sub> sampling. We tested a 'blank' vial by adding catalase to a vial, evacuating the vial, adding water (but not H<sub>2</sub>O<sub>2</sub>), subjecting it to free/thaw cycles and then making (non-vented) manual injections on the TC/EA. No oxygen or nitrogen peaks were detected, indicating that the vial was leak-free and that freezing vials on dry ice was sufficient to eliminate water vapor as a source of oxygen in our measurements.

**Table 1.** Results ( $\delta^{18}\text{O}$ ) from experimental determination of the minimum H<sub>2</sub>O<sub>2</sub> disproportionation time

Disproportionation time (s)	Mean $\delta^{18}\text{O} \pm 1\text{SD}$ (‰)
0	18.3 $\pm$ 0.1
8	14.7 $\pm$ 0.2
180	22.6 $\pm$ 0.1
300	23.6 $\pm$ 0.1
600	23.5 $\pm$ 0.2
1200	22.9 $\pm$ 0.4

Diatom oxygen (50  $\mu\text{L}$ ) in the headspace of each frozen vial was manually injected using a 250- $\mu\text{L}$  gas-tight syringe (VICI, Baton Rouge, LA, USA) directly on the TC/EA. The samples were analyzed together with reference and quality control materials. Prior to each O<sub>2</sub> injection, the syringe was flushed three times with the sample. For sample injections, a 150- $\mu\text{L}$  sample was drawn from the headspace vial, and compressed to 50  $\mu\text{L}$ . The syringe was then vented and the O<sub>2</sub> immediately injected. Each gas sample was sequentially injected three times. Due to an observed memory effect, the first injection was discarded and an average value was reported for the second and third injections when the SD between them was <0.2‰. In instances where the SD was >0.2‰, the sample disproportionation was repeated. We evaluated whether the disproportionation method was reliable between vial preparations by repeatedly disproportionating a single sample of H<sub>2</sub>O<sub>2</sub> ( $n = 3$  replicate vials) and measuring the evolved O<sub>2</sub> gas ( $\delta^{18}\text{O} = 28.5 \pm 0.24\text{‰}$ ; 2–3 injections per vial,  $n = 7$ ).

Because multiple O<sub>2</sub> reference materials were not available for data corrections, these data were slope-intercept corrected using three carbon dioxide (CO<sub>2</sub>) reference gases (Oztech, Safford, AZ, USA) that had been previously calibrated to the Standard Mean Ocean Water (SMOW) scale. A single ultra-high purity (UHP) compressed O<sub>2</sub> gas sample (provided by the University of Utah, Salt Lake City, UT, USA) was measured as a secondary standard ( $\delta^{18}\text{O} = 26.1 \pm 0.24\text{‰}$ ;  $n = 17$ ) and a single H<sub>2</sub>O<sub>2</sub> sample was chosen for repeated disproportionation to evaluate the stability of the method over the course of several months ( $\delta^{18}\text{O} = 23.5 \pm 0.15\text{‰}$ ;  $n = 8$ ). Because the instrument method was manual with frequent reference material measurements, only 7–8 unknown, disproportionated H<sub>2</sub>O<sub>2</sub> samples could be measured in an 8-h period. To account for instrument drift over this period, we also injected three carbon monoxide peak pulses every 15–20 min or between each O<sub>2</sub> sample.

### Statistical analysis

Statistical analyses were conducted using Prism version 5.0c (GraphPad Software, Inc., La Jolla, CA, USA). To identify H<sub>2</sub>O<sub>2</sub> solution outliers, residuals of the H vs. O regressions for H<sub>2</sub>O<sub>2</sub> solutions were calculated using a linear regression and subsequently graphed by box plot in Kaleidagraph (Synergy Software Inc., Reading, PA, USA). Five H<sub>2</sub>O<sub>2</sub> solutions were thus eliminated from further statistical analysis: four 3% samples collected in the US and a single 30–35% sample. Differences in the hydrogen and oxygen isotope values of H<sub>2</sub>O<sub>2</sub> by solution grade were determined using the Kruskal-Wallis non-parametric analysis of variance (ANOVA) followed by a Dunn's multiple comparison post-hoc test to specifically identify which solutions were statistically different. Correlation coefficients between H and O in H<sub>2</sub>O<sub>2</sub> solutions were calculated using the Pearson correlation. The relationships between the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in H<sub>2</sub>O<sub>2</sub> solutions were analyzed using Deming linear regressions, an errors-in-variables linear model, with uncertainty defined as the precision of our secondary water reference material (SD of x error = 0.2‰; SD of y error = 1.6‰). The slope and intercept differences between H<sub>2</sub>O<sub>2</sub> solution grades were tested using an analysis of covariance (ANCOVA).

Oxygen isotope value outliers of O<sub>2</sub> gas from disproportionated H<sub>2</sub>O<sub>2</sub> were identified by box plot in Kaleidagraph. Two samples were eliminated from future O<sub>2</sub> statistical analyses: one each from the 3% US and the 3% Mexico categories. The oxygen isotope value differences between grades of disproportionated H<sub>2</sub>O<sub>2</sub> were determined by the Kruskal-Wallis non-parametric ANOVA and Dunn's multiple comparison test. For all statistical analyses,  $\alpha = 0.05$ .

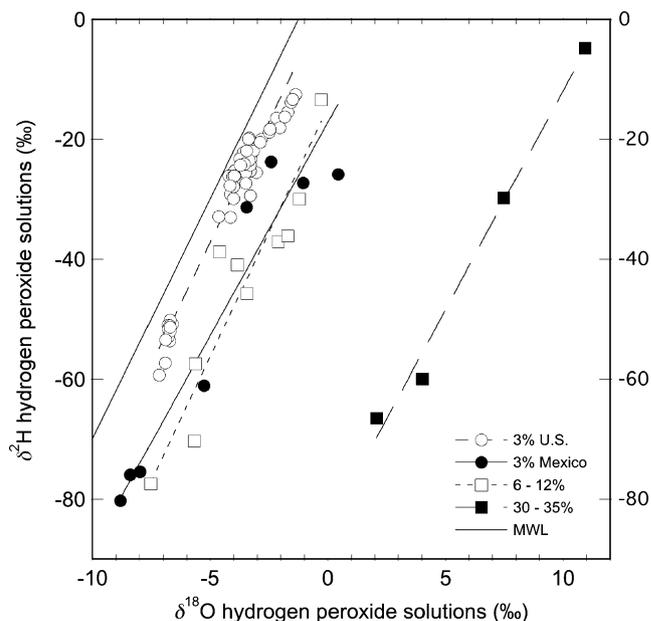
## RESULTS AND DISCUSSION

We collected 97 samples of H<sub>2</sub>O<sub>2</sub> from 53 US cities, representing 26 different states and from three cities in Mexico. As shown in Table 2, the US samples ( $n=89$ ) included four grades of commercially available H<sub>2</sub>O<sub>2</sub>: pharmaceutical (3%), salon (6–12%), reagent (30–35%), and commercial (70%), whereas the Mexico samples ( $n=8$ ) included only pharmaceutical grade solutions. Although obtaining a wide selection of marketed brands was not an intentional component of this analysis, 45 unique brands were ultimately sampled.

### H<sub>2</sub>O<sub>2</sub> solution isotope ratio results

There was measurable isotopic variation in the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of commercially available H<sub>2</sub>O<sub>2</sub> solutions. Figure 2 shows a plot of the hydrogen and oxygen isotope values of H<sub>2</sub>O<sub>2</sub> solutions separated by commercial grade and purchase country. Statistical trends for each class of surveyed samples are summarized in Table 3. For presentation, commercial grade solutions are not shown on Fig. 2 due to their extremely low  $\delta^2\text{H}$  values (–235‰ and –156‰) and high  $\delta^{18}\text{O}$  values (11.8‰ and 15.3‰). The H<sub>2</sub> used in H<sub>2</sub>O<sub>2</sub> manufacture may be a fossil fuel byproduct or generated by electrolysis (personal communication, Solvay: Houston, USA). These sources may have very different hydrogen isotope signatures and this may explain the large H variation between these two observations.

Statistically, there were significant stable isotope ratio differences between the H<sub>2</sub>O<sub>2</sub> grades with  $n \geq 4$  samples for both hydrogen ( $P < 0.0012$ , KW statistic 15.92) and oxygen isotope values ( $P < 0.0085$ , KW statistic = 11.69). The Dunn's multiple comparison test showed significant differences in hydrogen between 3% solutions purchased in the US and Mexico; and between 3% solutions and 6–12% solutions from the US. With respect to oxygen, there were significant differences between 3% (US) and 30–35% solutions; 3% (Mexico) and 30–35% solutions; and between 6–12% and



**Figure 2.** Cross plot of measured  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isotope values from surveyed hydrogen peroxide solutions purchased in the United States or in Mexico along the US/Mexico border delineated by solution grade. The Meteoric Water Line (MWL)<sup>[15]</sup> is included for reference. Commercial grade 70% solutions are not included on this figure.

30–35% solutions. We interpret these differences as being a function of the significantly larger fractions of H<sub>2</sub>O<sub>2</sub> (and less water) that are associated with increasingly concentrated H<sub>2</sub>O<sub>2</sub> solutions. The increasing proportions of H<sub>2</sub>O<sub>2</sub> reflect larger contributions of <sup>2</sup>H and <sup>18</sup>O inputs from H<sub>2</sub>O<sub>2</sub> that may be quite different from the dilution water <sup>2</sup>H and <sup>18</sup>O input.

Isotopic differences in dilution water from product to product and between purchase countries may explain some of the observed significant differences. For example, although the 3% solutions from the US and Mexico were identical by w/v composition, they were statistically different in H isotope ratio values. It is possible that the dilution water used during manufacture and throughout distribution is of a different origin in samples purchased (and probably manufactured) in the US from those purchased in Mexico.

The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of H<sub>2</sub>O<sub>2</sub> solutions were significantly correlated (see below; Fig. 2). The lines describing these relationships paralleled the MWL because they were compositionally mostly water. The hydrogen and oxygen isotopes in water are related due to kinetic fractionation events that occur when water vapor is evaporated or condensed.<sup>[20]</sup> Globally, these mechanisms result in variation in precipitation across landscapes, described by the MWL and the equation  $\delta^2\text{H} = 8 * \delta^{18}\text{O} + 10$ .<sup>[15]</sup> The Deming best-fit lines for the H<sub>2</sub>O<sub>2</sub> solutions that we measured were:

$$\delta^2\text{H}_{\text{H}_2\text{O}_2} = 8.1 * \delta^{18}\text{O}_{\text{H}_2\text{O}_2} + 3\text{‰}; r^2 = 0.9783; P < 0.0001; \text{ for } 3\% \text{ H}_2\text{O}_2 \text{ US}$$

$$\delta^2\text{H}_{\text{H}_2\text{O}_2} = 7.1 * \delta^{18}\text{O}_{\text{H}_2\text{O}_2} - 17\text{‰}; r^2 = 0.9538; P = 0.0002; \text{ for } 3\% \text{ H}_2\text{O}_2 \text{ Mexico}$$

**Table 2.** Summary of H<sub>2</sub>O<sub>2</sub> samples obtained in this study by solution grade

H <sub>2</sub> O <sub>2</sub> %	H <sub>2</sub> O <sub>2</sub> Grade	n	Purchase country	Dilution sources (n)
3	Pharmaceutical	71	USA	unknown
3	Pharmaceutical	8	Mexico	unknown
6, 9, 12	Salon	11	USA	unknown
30, 35	Reagent	5	USA	unknown
70	Commercial	2	USA	1

**Table 3.** Summary statistics for surveyed bulk hydrogen peroxide solutions (including dilution water) by isotope value where (a)  $\delta^2\text{H}$  and (b)  $\delta^{18}\text{O}$

(a)	$\text{H}_2\text{O}_2$ (w/v)	n	$\delta^2\text{H}$ $\text{H}_2\text{O}_2$ solution (‰)				
			Minimum	Median	Maximum	Mean	$\pm 1\text{SD}$
	<b>3% (USA)</b>	67	-59	-25	-12	-29	12
	<b>3% (Mexico)</b>	8	-80	-46	-24	-50	25
	<b>6–12%</b>	11	-77	-39	-13	-44	18
	<b>30–35%</b>	4	-66	-45	-5	-40	29
	<b>70%</b>	2	-235	-196	-157	-196	56
(b)	$\text{H}_2\text{O}_2$ (w/v)	n	$\delta^{18}\text{O}$ $\text{H}_2\text{O}_2$ solution (‰)				
			Minimum	Median	Maximum	Mean	$\pm 1\text{SD}$
	<b>3% (USA)</b>	68	-7.2	-3.5	-1.4	-4.0	1.5
	<b>3% (Mexico)</b>	8	-8.8	-4.4	0.4	-4.6	3.5
	<b>6–12%</b>	10	-7.5	-3.6	-0.3	-3.6	2.3
	<b>30–35%</b>	4	2.1	5.8	10.9	6.1	3.9
	<b>70%</b>	2	11.8	13.6	15.3	13.6	2.5

$$\delta^2\text{H}_{\text{H}_2\text{O}_2} = 8.3 * \delta^{18}\text{O}_{\text{H}_2\text{O}_2} - 15\text{‰}; r^2 = 0.9323; P < 0.0001; \text{ for } 6\text{--}12\% \text{ H}_2\text{O}_2$$

$$\delta^2\text{H}_{\text{H}_2\text{O}_2} = 7.3 * \delta^{18}\text{O}_{\text{H}_2\text{O}_2} - 85\text{‰}; r^2 = 0.9937; P = 0.0063; \text{ for } 30\text{--}35\% \text{ H}_2\text{O}_2$$

The slope values of these equations were not statistically different (ANCOVA,  $F_{3, 81} = 1.528$ ,  $P = 0.2136$ ) from each other. The slope values were also similar to that of the MWL. Thus, the available data support hypothesis 1 that the slope of the  $\delta^2\text{H}$  vs.  $\delta^{18}\text{O}$  relationships in  $\text{H}_2\text{O}_2$  solutions approximates to 8, that of meteoric water.<sup>[15]</sup>

Because the slope values were not statistically different, we were able to compare intercepts. The intercept values were significantly different (ANCOVA,  $F_{3, 84} = 342.9$ ,  $P < 0.0001$ ) for  $\text{H}_2\text{O}_2$  solution comparisons by grade. As suggested by hypothesis 1 and our hypothesis statement regarding hydrogen, this result shows that H in  $\text{H}_2\text{O}_2$  (and not H in dilution water) drives the difference between  $\text{H}_2\text{O}_2$  solution grades.

Initially, it is difficult to constrain the interpretation of the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of bulk  $\text{H}_2\text{O}_2$ . Hydrogen peroxide is sold and shipped as a commodity. Thus, quantifying the additions of dilution water from  $\text{H}_2\text{O}_2$  as it travels from source-to-shelf is not easily done due to the inherent variation in commodity distribution networks and the proprietary nature of manufacture. The dilution water in commercially available  $\text{H}_2\text{O}_2$  solutions represents a significant component in the H-O measurement. Without the development of an alternative analytical method to measure  $\delta^{18}\text{O}$  values in  $\text{H}_2\text{O}_2$  independent of dilution water, it would remain difficult to draw conclusions about the actual isotope value of  $\text{H}_2\text{O}_2$ . In addition, the geographical information that is recorded in water may arise from multiple sources of dilution water. As a result of multiple (unknown) inputs, the power of bulk  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in  $\text{H}_2\text{O}_2$  could be limited to resolving differences between samples.

#### $\delta^{18}\text{O}$ values in $\text{H}_2\text{O}_2$ measured by disproportionation

The  $\delta^{18}\text{O}$  values of  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$  varied around the value expected for atmospheric diatomic oxygen.

The median value of oxygen gas from disproportionated  $\text{H}_2\text{O}_2$  for all grades was  $\delta^{18}\text{O} = 23.8 \pm 1.5\text{‰}$  ( $n = 93$ ) with a range between 16.3 and 27.5‰ (mean disproportionated  $\delta^{18}\text{O} = 23.6 \pm 1.5\text{‰}$ ). The  $\delta^{18}\text{O}$  value of the majority of  $\text{H}_2\text{O}_2$  samples that we measured strongly resembled that of the atmosphere, defined at 23.5‰,<sup>[21]</sup> providing strong support for hypothesis 2.

As previously mentioned,  $\text{H}_2\text{O}_2$  is largely produced through the 'anthraquinone process' where, for economic and safety reasons, atmospheric diatomic oxygen is the typical source of oxygen (personal communication, Solvay, Houston, TX, USA).<sup>[14,22,23]</sup> Although this study did not have the capacity to measure potential production leakiness and/or fractionation associated with the catalytic 'anthraquinone process', the mean value of 93 disproportionated  $\text{H}_2\text{O}_2$  solutions ( $\delta^{18}\text{O} = 23.6 \pm 1.5\text{‰}$ ) suggested that it is not unreasonable to assume that fractionation (or the net result of any fractionation) from the manufacturing process between atmospheric  $\text{O}_2$  and  $\text{H}_2\text{O}_2$  is minimal.

Table 4 shows statistical data for disproportionation measurements by solution grade. A Kruskal-Wallis non-parametric ANOVA comparing  $\text{H}_2\text{O}_2$  grades with  $n \geq 4$  samples was not statistically significant ( $P = 0.1048$ , KW statistic = 6.145). While we did not observe any significant differences in  $\delta^{18}\text{O}$  values among the  $\text{O}_2$  from  $\text{H}_2\text{O}_2$  grades, we did measure a  $>11\text{‰}$  range in the  $\delta^{18}\text{O}$  values for all samples and large isotopic variation within some sample grades. For example, three of the ten salon-grade  $\text{H}_2\text{O}_2$  samples had measured  $\delta^{18}\text{O}$  values of  $\text{O}_2$  that varied by more than 3 SD from the mean value of all the disproportionated  $\text{H}_2\text{O}_2$  samples (salon sub-group mean  $\delta^{18}\text{O} = 16.9 \pm 0.9\text{‰}$ ,  $n = 3$ ).

There may be multiple elements contributing to the observed  $\delta^{18}\text{O}$  variation in  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$ . Here, we suggest two: stability and process.  $\text{H}_2\text{O}_2$  is prone to spontaneous disproportionation even under normal storage conditions and we would expect that any disproportionation would generally favor the  $\text{H}_2^{16}\text{O}_2$  molecules, enriching the  $\delta^{18}\text{O}$  value of the remaining  $\text{H}_2\text{O}_2$ . Most commercially available  $\text{H}_2\text{O}_2$  has been stabilized by the addition of chelating agents that sequester metals and

**Table 4.**  $\delta^{18}\text{O}$  value summary statistics for disproportionated hydrogen peroxide

$\text{H}_2\text{O}_2$ (w/v)	$\delta^{18}\text{O}$ $\text{O}_2$ disproportionated $\text{H}_2\text{O}_2$ (‰)					
	n	Minimum	Median	Maximum	Mean	$\pm$ 1SD
<b>3% (USA)</b>	70	22.3	23.7	25.3	23.8	0.6
<b>3% (Mexico)</b>	7	21.7	24.2	26.5	24.2	1.4
<b>6–12%</b>	10	16.3	22.4	24.5	21.3	3.3
<b>30–35%</b>	4	22.8	23.9	25.7	24.1	1.4
<b>70%</b>	2	25.6	26.5	27.5	26.5	1.3

increase its shelf stability under normal storage conditions.<sup>[14]</sup> Unstabilized products are available and these solutions are probably subject to increased rates of spontaneous disproportionation. In this study, the 30–35% and 70% solutions were unstabilized. For example, three grades of  $\text{H}_2\text{O}_2$  (3% Mexico, 30–35% and 70%) had maximum  $\delta^{18}\text{O}$  values of  $\text{O}_2$  more than 2.0‰ higher than that of atmospheric  $\text{O}_2$  (Table 4).

Secondly, the process employed may explain variation in isotope ratios of  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$ . The isotope value measurements of the salon samples previously mentioned (salon sub-group *mean*  $\delta^{18}\text{O}$  =  $16.9 \pm 0.9\text{‰}$ ,  $n=3$ ) are consistent with alternative processes in the manufacture of some  $\text{H}_2\text{O}_2$  products having an impact on the  $\delta^{18}\text{O}$  value of the  $\text{H}_2\text{O}_2$ . For example, the source of  $\text{O}_2$  at some manufacturing plants may have been depleted relative to the atmosphere. Industrial sources of oxygen vary, but two common sources are cryogenic air separation and distillation or vacuum pressure swing adsorption (VPSA).<sup>[24]</sup> Alternatively, there may be a fractionation associated with manufacture; or samples may be produced by less common manufacturing processes, such as the 'direct synthesis' of  $\text{H}_2\text{O}_2$  by reacting  $\text{H}_2$  with  $\text{O}_2$  using a metal catalyst.<sup>[23,25]</sup> Although we suggest these processes as mechanisms that may introduce oxygen isotope variation in  $\text{H}_2\text{O}_2$ , the source of the variation that we observed is not known. Benson *et al.*<sup>[26]</sup> found measurable differences in the  $^{18}\text{O}$  value of TATP synthesized with different  $\text{H}_2\text{O}_2$  reagents. The range in  $\delta^{18}\text{O}$  values that we report here may begin to explain this variation.

Our experimental results have shown that the dilution water has no effect on the  $\delta^{18}\text{O}$  isotope value of  $\text{H}_2\text{O}_2$ . Therefore, the dilution of concentrated  $\text{H}_2\text{O}_2$  solutions for peroxide-based explosive synthesis will not affect the  $\delta^{18}\text{O}$  value of the  $\text{H}_2\text{O}_2$ . With the development of the disproportionation approach, we can use mass-balance to calculate the O isotope value of dilution water in bulk solutions. Due to H exchange between  $\text{H}_2\text{O}_2$  and  $\text{H}_2\text{O}$  in solution, this approach is not possible for hydrogen.

Previously, stable isotope ratio analyses of explosive reagents and products have been investigated for their potential to distinguish explosive materials such as ammonium nitrate, hexamethylenetetramine (hexamine), pentaerythritol tetranitrate (PETN),<sup>[26,27]</sup> TATP,<sup>[26]</sup> cyclotrimethylenenitramine (RDX),<sup>[13]</sup> and HMTD.<sup>[13,26,27]</sup> Each of these initial studies suggests that there are sufficient stable isotope value variations among explosive samples that this measurement approach could be useful in distinguishing chemically identical specimens from each other. In addition, there may be potential to use stable isotopes to study

post-blast residues when sufficient quantities of explosive can be recovered. The results of this study suggest that stable isotope value analyses of  $\text{H}_2\text{O}_2$  solutions may also provide information of forensic utility as was also suggested by Lock.<sup>[17]</sup> Furthermore, because the relationships between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in a  $\text{H}_2\text{O}_2$  solution are predictable once the  $\text{H}_2\text{O}_2$  concentration is known, it is possible to distinguish  $\text{H}_2\text{O}_2$  solutions that are the result of dilutions (commercial practices) versus concentration by evaporation (clandestine practices).

The development of the disproportionation approach provides a mechanism to measure a peroxide-based explosive reagent that was previously not possible. Quantifying the oxygen isotope values in  $\text{H}_2\text{O}_2$  will allow future studies to relate peroxide-based reagents to explosive products and possibly to clandestine processing procedures. This can be achieved because the oxygen in  $\text{H}_2\text{O}_2$  is the sole source of the oxygen in TATP. Investigating source and process may be accomplished through various means that include synthesis (e.g., reaction time or temperature), stoichiometric (e.g., limited reagent) and reagent manipulation (e.g., dilution and concentration) studies.

The  $\delta^{18}\text{O}$  measurements that we made were on unaltered purchased samples. In contrast, we may expect that a terrorist will manipulate the sample before attempting to use it in synthesizing a peroxide-based explosive. Most peroxide-based explosives are synthesized with a relatively concentrated  $\text{H}_2\text{O}_2$  solution, approximately 30%.<sup>[3,17]</sup> In that case, the majority of commercially available  $\text{H}_2\text{O}_2$  would require some degree of concentration. Isotope fractionation in  $\text{H}_2\text{O}_2$  may occur when the solution is subject to evapoconcentration.<sup>[20]</sup> The disproportionation approach provides a means of assessing how the illicit concentration of  $\text{H}_2\text{O}_2$  may affect the  $\delta^{18}\text{O}$  values of stabilized and unstabilized  $\text{H}_2\text{O}_2$ , which in turn may then be recorded in an explosive such as TATP.

Ultimately, stable isotopes may be used to characterize peroxide-based reagents and products, link a reagent to a product, a seized specimen to a specific laboratory, or in a broad sense, potentially to a geographical region. Future applications of these results may be of use to law enforcement and/or to prosecution in a court of law.

## CONCLUSIONS

This study reports  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for bulk hydrogen peroxide solutions and  $\delta^{18}\text{O}$  isotope values of  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$ . Hydrogen peroxide samples were

acquired at four grades: pharmaceutical (3%), salon (6–12%), reagent (30–35%) and commercial (70%). Key observations from  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values on bulk  $\text{H}_2\text{O}_2$  solutions were:

- there were large ranges in the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values bulk  $\text{H}_2\text{O}_2$  solution isotope values of 230‰ and 24‰, respectively, in purchased samples;
- the slopes of a plot of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in dilute (3 to 35%)  $\text{H}_2\text{O}_2$  solutions were not statistically different by grade and were similar in slope to the MWL because they are compositionally mostly water;
- the  $\delta^2\text{H}$  values from the  $\text{H}_2\text{O}_2$  (and not the water) drives the differences in  $\delta^2\text{H}$  values between bulk  $\text{H}_2\text{O}_2$  solution grades.

We developed a disproportionation approach to measure the  $\delta^{18}\text{O}$  values of  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$  independent of dilution water. Key observations from the  $\delta^{18}\text{O}$  value measurements in  $\text{H}_2\text{O}_2$  were:

- the  $\delta^{18}\text{O}$  values of  $\text{O}_2$  from disproportionated  $\text{H}_2\text{O}_2$  samples were similar to that of atmospheric diatomic oxygen, the predominant source of  $\text{O}_2$  used in  $\text{H}_2\text{O}_2$  manufacture;
- there was an 11‰ range in the  $\delta^{18}\text{O}$  values of  $\text{O}_2$  of survey samples, indicating that this approach could distinguish among  $\text{H}_2\text{O}_2$  samples;
- the O in  $\text{H}_2\text{O}_2$  did not exchange with the O in  $\text{H}_2\text{O}$ .

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