Replies and answers to reviews regarding manuscript “Measuring molecular singlet oxygen ($^1O_2^*$) from atmospheric photosensitizers: Intercomparison of techniques, irradiation setups, data analysis and protocol recommendations”

Reply Legend:
Black: reviewer comment
Blue: author response
Blue + italics: text modified

Reviewer 2:

This work investigates key aspects of photochemical experiments such as repeatability and reliability of measurements across multiple light sources and actinometry methods for one of the most critical photochemical oxidants, singlet oxygen. Quantum yields for singlet oxygen were measured using both chemical probe experiments and phosphorescence measurements. Results suggest that reaction conditions and the selection of the light source can have strong impacts on observed, and hence predicted, levels in atmospheric scenarios such as wildfire aerosol emissions. This work is thorough and makes clear the importance of inter-lab comparisons across multiple approaches. The considerations for singlet oxygen assessment are valuable.

We thank the reviewer for their praise of our work.

However, the presentation quality is frequently problematic, particularly if this work is to be generally helpful to researchers with a wide range of backgrounds. At times poor wording and detailed lines of logic that omit key points may give rise to misapplication of the important points this manuscript seeks to demonstrate. I recommend this manuscript for publication, but not without such numerous corrections, listed below, as to amount to major revisions, whether in effort or importance. This work seems to be focused on getting the details right, which is absolutely commendable, yet the quality of presentation can work against this.

We thank the reviewer for their fair comments and have worked diligently to improve the presentation of the information throughout the manuscript, including all the comments listed below.

General Comments

Some of the applicability to a wider range of systems seems limited, in terms of the recommendations.

How can the suggestion "less than 1% of photosensitizers" be applied to more complex systems when the sample itself includes photosensitizer species? The production rate of singlet oxygen would also seem to be an important metric. Perhaps the authors can also suggest ways to diagnose whether quenching of singlet oxygen is a problem in more complex samples. Much more could be said in the final recommendations as to the utility of relative yield measurements in complex systems.

The suggestion of less than 1% of photosensitizers was not an accurate description of what we were trying to convey. Rather, our intent was to limit the probe concentration to account for less 1% of the total $^1O_2^*$ loss. The main text has been updated to read: *"2.* ***Chemical probe concentration:*** *We recommend using concentrations of furfuryl alcohol such that the reaction of* $^1O_2$* *with the probe accounts for less than 1% of the total* $^1O_2$* *loss, ensuring that the probe does not perturb steady-state* $^1O_2$* *concentrations. This condition can be evaluated by calculating the fraction of* $^1O_2$* *lost to FFA relative to other sinks (Sec. S12), which corresponds to [FFA]0 < 27 μM (at 25 °C)."*

The following text has also been added to section 3.3.6 to address the point of determining if quenching of singlet oxygen is a problem in more complex samples:

"Phosphorescence measurements can also be used to diagnose whether quenching of $^1O_2$* *is significant in complex samples (Madhiyan and Moor, 2026). Although not conducted here due to the use of single molecule sensitizers,* $^1O_2$* *can be generated using a known sensitizer at a wavelength outside the absorbance range of the sample matrix and the phosphorescence signal at 1270 nm compared in the presence and absence of the sample. A reduction in the signal when the sample is present indicates additional quenching of* $^1O_2$* *and this approach is complementary to chemical probe methods. For example, in systems that do not absorb above 500 nm, Rose Bengal can be excited at 550 nm to generate* $^1O_2$* *and evaluate quenching by the sample matrix."*

Can more be said about the universality of furfuryl alcohol as a chemical probe and what other chemical probes would be useful in some applications? Are there limitations, perhaps chemical compatibility to consider?

We thank the reviewer for this valuable comment. Text has been added to Section 3.3.1 to address the universality of furfuryl alcohol as a probe and potential alternatives:

"Furfuryl alcohol is widely used as a chemical probe for $^1O_2^*$ *due to its well characterized and selective reactivity (Haag et al., 1984; Appiani et al., 2017). Recent work has expanded the family of furan-based probes. For example, Arciva et al. (2025) reported singlet oxygen reaction kinetics for 17 furan derivatives, highlighting that alternative probes may be selected for specific*

experimental constraints. For example, less volatile probes such as 2-methylfuran-3,4-dicarboxylic acid may be advantageous in open systems where volatility is a concern, although this was not an issue for the capped solutions used here. To the best of our knowledge, chemical compatibility has not been systematically explored as a limitation of FFA as a probe for $^1O_2^*$*."*

Sunlight itself is not a single, constant light source, as it varies with solar zenith angle. Using a solar simulator to reproduce sunlight is certainly a good approach, but it is still a single light source that does not apply to all solar conditions.

The following text has been added to address this point:

"We note that solar irradiance is not constant in either intensity or spectral distribution, as it varies with solar zenith angle and season. Although irradiation sources that span wavelength ranges similar to natural sunlight can help reduce wavelength dependent quantum yield artifacts, it remains difficult to perfectly reproduce solar radiation under laboratory conditions."

More Specific Comments

Equation 1

While numerically the values may work out, this equation is very, very poorly expressed. The reference (Kaur 2019b) does this much more clearly, and the authors would do best to follow that example exactly.

Absorbance does not have units. It seems this should be absorption coefficient.

The summation over the wavelengths approximates the integration over wavelength, meaning that the 1/nm units are canceled via multiplication by $d\lambda$. Add a $\Delta\lambda$ in units of wavelength.

The goal of this paper is very clear validation, and these seemingly small shortcuts may quickly lead to poor application of the principles this manuscript is working to support.

The reviewer is correct, absorbance does not have units and was an oversight on our part to not change this to absorption coefficient. The equation has been changed to be absorption coefficient, and $\Delta\lambda$ was added. Thank you.

Equation 3

This equation is also problematic.

The final equivalence should be kobs*t, not just kobs.

The slope of the log plot is the observed rate constant.

This is correct, and we thank the reviewer for catching this typo. The equation has been corrected accordingly.

Equation 5

Absorbance does not have units, again, this should have absorption coefficient, not abs. The reviewer is correct. The equation has been changed to be absorption coefficient, not abs.

The screening factor should be more clearly defined, in particular its general purpose, which would include the reduction of light available to the photosensitizer. It would seem in the spirit of this manuscript to assume the audience is not strictly photochemists, but rather atmospheric chemists more broadly (or others) who need to consider the wider impacts of UV irradiation on their experiments.

We thank the reviewer for suggesting this important change. Text has been added to more clearly define the screening factor and why it is important: *"Internal light screening due to light absorption is the reduction of light intensity within a sample as photons are absorbed before they can reach the entire irradiated volume. The light screening of the sample depends on the light absorbance of the sample, the path length of the light through the sample, and the irradiance from the light source."*

Equation 8

All terms, especially the rate constants, must be clearly defined

We thank the reviewer for pointing out that not all terms in this equation were clearly defined. The text has been updated now to read:

"where Φ_{ISC} is the fraction of excited singlet photosensitizer molecules that undergo intersystem crossing to the excited triplet state, k^T_d is the deactivation of triplets, k_{O2} is the second-order rate constant for the physical quenching of $^3C^*$ with O_2, $k_{O2}\,[O_2]\,/\,k^T_d + k_{O2}\,[O_2]$ is the fraction of $^3C^*$ that is quenched by O_2, and $f\Delta$ is the fraction of the quenching that leads to the formation of $^1O_2^*$."

Table 2

The table should include a header row indicating “light absorbance equivalent hours,” this information should not be solely in the caption.

We thank the reviewer for this suggestion to improve clarity. Table 2 has been updated with a header row.

Table 3 and Section 3.3.6

The apparent low sensitivity of the phosphorescence method is noted, were higher concentrations of photosensitizer and/or oxygen saturation attempted?

Yes, 5 concentrations of the photosensitizer were conducted. The concentrations tested ranged in absorbance from 0.1 – 0.5. Oxygen saturation was not attempted in an effort to keep results as comparable to chemical probe experiments as possible. Text has been added to read:

“To address this, we tested five photosensitizer concentrations (absorbance 0.1–0.5), yet signals remained undetected. Oxygen saturation was intentionally avoided to maintain comparability with the ambient-air conditions of the chemical probe experiments.”

The authors should also further address, or at least specifically state for the reader, that 4-nitroanisole was measured to have a much higher yield than juglone using chemical probe measurements, yet phosphorescence measurements of 4-nitroanisole were apparently below detection limits while juglone produced singlet oxygen above phosphorescence detection limits.

We thank the reviewer for highlighting this point. It was an unexpected finding that requires more explanation. The discrepancy in quantum yield measurements from the two techniques is observed for all three nitroanisole compounds. To iterate this point, text has been added to read:

“Notably, for 4-nitroanisole, while a quantum yield of 8.83% was measured using the chemical probe method, no corresponding signal was detected via direct phosphorescence. The relative position of the methoxy and nitro groups are impacting the reactivity of these nitroanisoles, and warrants further experiments to understand why and how”.

Line 28 “potent but oxidant”. Please complete the sentence.

The word “but” was a typo, and has been removed from the sentence. Text now reads: *“generating singlet oxygen ($^1O_2^*$), a potent oxidant”*

55

Lack of reproducibility is vague here. Please revise to make this clearer.

Agreed. The text has been revised for clarity: *"The challenge of extrapolating laboratory measurements of* $^1O_2^*$ *to the atmospheric context is exacerbated by the current lack of reproducibility in environmental samples such as the Suwannee River fulvic acid samples (Partanen et al., 2020), making it difficult to distinguish between variability in* $^1O_2^*$ *measurements and true inconsistencies in reproducibility."*

95

UBC Setup : "…20 mg/L of lignin were used to make up" It would be clearer if you state something more like "20 mg/L of lignin were the concentrations". The same applies to the other setups.

Thank you for this suggestion to improve clarity. The text has been updated to read (note different concentrations for the different set ups, but generally reads the same):

"Experimental solutions contained 20 µM furfuryl alcohol, 1 mM of isopropanol and 10 µM of either perinaphthenone, Rose Bengal, or 30 µM juglone, or 40 mg/L of lignin."

185

What quencher?

Text has been updated to read: *"due to the use of isopropanol as a quencher"*

221

The use of parentheses to indicate the symbol for the relative quantum yield is confusing here.

Please instead use a set of commas to indicate this use of an appositive.

Commas have been used instead of parentheses. The text now reads: *"In contrast, the relative quantum yield of* $^1O^*_2$, Φ^r_{1O2*} , *…"*

364

The potential for screening of 2-NBA at the excitation wavelength due to the overlapping absorption of the product during actinometry should be mentioned. Also, it should be made clear that both actinometers should be utilized at low optical depth.

We thank the reviewer for highlighting these points. Text has been added starting on line 357: *"Additionally, both chemical actinometers should be used at low optical depth, and potential light screening by all chromophores, including reaction products, should be considered."*

426

It should be made clearer that “optimized” screening factors indicates reduction sample absorbance. Furthermore, the Ircelyon screening factor is 0.68, far from a value of 1, while this is a value that is reasonable to correct for, this deviation from 1 should be noted here.

We thank the reviewer for flagging this unclear statement. For the Ircelyon photoreactor, the irradiation pathlength through the sample is 3.5 cm leading to screening factors that deviate from 1. Text has been updated to address both points: *“In our case, the screening factors were optimized to be close to 1, by reducing sample absorbance, to specifically avoid screening (Tables S1-S4). However, in the Ircelyon photoreactor, the 3.5 cm irradiation pathlength through the sample produced screening factors that deviated from 1”*

448

It seems this is the “deactivation lifetime”. Please clarify.

The reviewer is correct, “deactivation lifetime” is the better term compared to just “lifetime” and has been changed in the text. Thank you.

470-480

Normalizing production yield to the rate of absorption is simply the definition of quantum yield. What is the purpose of this sentence? Is there something else implied here? The second paragraph simply states that the observed quantum yield with lignin and juglone was not consistent with the other photosensitizers. Indeed, the most likely source is some sort of wavelength dependence. These paragraphs could be condensed and edited to greatly increase clarity.

The reviewer is correct in that the purpose of the sentence and section was to highlight the fact that for photosensitizers perinaphthenone and Rose Bengal, we did not observe a wavelength dependent singlet oxygen quantum yield. For lignin and juglone we did observe wavelength dependent singlet oxygen quantum yields. The text has been condensed and edited to improve clarity of the point we are trying to convey:

“Rates of light absorbance and ${}^1O^*_2$ *steady-state concentrations differed by several orders of magnitude across the different laboratories (Fig. 3). These differences reflect variation in photon flux among the light sources, with higher intensity sources producing higher absorbance rates and correspondingly higher* ${}^1O^*_2$ *concentrations (Fig. 4 a,b). Despite these order of magnitude differences in Rabs and* $[{}^1O^*_2]_{SS}$*, the apparent quantum yield,* Φ_{1O*2} *, for Rose Bengal and for perinaphthenone were consistent and reproducible across photoreactors and aligned with*

literature values (Schmidt et al., 1994; Wilkinson et al., 1993) (Fig. 4c), indicating that these sensitizers singlet oxygen generation efficiency exhibit minimal dependence on the irradiation conditions explored here.

In contrast, lignin and juglone exhibited deviations in quantum yields across photoreactors. Specifically, xenon lamp systems (UCD and Ircelyon) yielded lower Φ_{1O*2} values, whereas the UV bulb system (UBC) produced higher values. For juglone, this discrepancy was particularly pronounced, as the highest photon flux (UCD; Fig. 4a) corresponded to the lowest measured quantum yield (Fig. 4c, left). These results suggest that, unlike Rose Bengal and perinaphthenone, the apparent quantum yields of lignin and juglone are influenced by the spectral distribution of the light source, consistent with a wavelength dependent mechanism."

480 and elsewhere

It is more descriptive to describe wavelengths as "short" and "long", rather than low and high. Low and high are better adjectives for frequency.

Thank you for pointing this out. All descriptions of wavelength have been updated to be short and long rather than low and high.

493

Combinations of several narrowband excitations allow for the adjustments between changing light source wavelength profiles, while at the same time requiring many more experimental trials. As noted above a solar simulator set to one solar flux profile does not cover all atmospheric conditions either.

Thank you for highlighting the use of several narrowband excitations as a way to assess wavelength dependent quantum yields. The text has been updated to read:

"Due to wavelength dependencies, results obtained using narrow or single wavelength irradiation can be difficult to reliably extrapolate to the broader solar spectrum. Accordingly, using sunlight-mimicking irradiation sources, or combinations of several narrow band excitations are best recommended."

Section 3.3.7

The first paragraph is poorly worded to the point of confusion. The tedious explanation of how to determine the oxygen concentration in solution is not necessary, stating that the dissolved oxygen concentration was determined the atmospheric partial pressure and the Henry's law constant is sufficient, and adjustments to altitude can be noted.

The second paragraph tells us that the discrepancy between chemical and phosphorescence detection discussed in section 3.3.6 is now due to reaction with furfuryl alcohol. I understand the presentation of results in 3.3.6 and discussion in 3.3.7. Perhaps somehow these sections can be combined in a succinct way to make a clearer presentation.

Section 3.3.6 is intended to discuss the comparison between the probe method and direct phosphorescence for the measurement of 1O2*, whereas Section 3.3.7 is intended to discuss the role of dissolved oxygen. It's true that Section 3.3.7 also uses the phosphorescence measurements to make this point, and so although we don't think merging is effective in conveying our 2 messages, we have edited both sections for conciseness.

523

Why wasn't an equation noted? Instead "this calculation" is simply stated without clearly indicating what calculation was done. This is insufficient.

We thank the reviewer for flagging this unclear point. The text now reads:

"The calculation of $[O_2]$ in solution led to calculated $^3C^*$ deactivation pathways yields, $k_{O2}[O_2]$, that were 2.8 times larger than k_d^T in Vancouver, and 2.5 times larger in Calgary. "

524-525

If an equation was noted or referenced, it be immediately clear to any reader that lower oxygen concentration leads to a lower quantum yield for any photosensitizer. This statement suggests that something special is occurring here, when this is exactly as expected. Do the authors suggest that there is a unique reason why lower oxygen concentration leads to lower quantum yields?

We thank the reviewer for this clarification. We do not suggest a unique mechanism, and the decrease in quantum yield at lower oxygen concentrations follows directly from the relationship described in Eq. 9. The text has been revised to explicitly reference Eq. 9 to make this connection clearer to the reader.

535

This sentence does not really define photochemical “action spectra”. Typically, an action spectrum would refer to a chemical response as a function of wavelength, such as an OH photolysis yield. The rate of absorption doesn’t need a new definition or seem to quite fit the term “action spectrum.”

We thank the reviewer for pointing out this oversight on our part. The reviewer is correct, “action spectrum” inherently contains information of photochemical reactivity while our definition does not. All mentions of “action spectra” have been updated to read *“action spectra of light absorbance”.*

600

It should be noted that the solar spectrum is not constant in either intensity or wavelength dependence across solar zenith angles.

The following text has been added to address this point: *“The ideal photoreactor would have 1) an irradiance spectrum that spans a broad range of wavelengths, attempting to reproduce the solar spectrum, such as a xenon lamp”*