Peer review

Jiang, Dyanne. 2025. “Ground-Level Ozone’s Impact on Human Health in Terms of Respiratory Diseases Using Reactive Oxygen Species as an Inflammatory Marker.” *Journal of High School Science* 9 (3): 201–24. https://doi.org/10.64336/001c.142605.

The model needs to be more nuanced to reflect real world case scenarios. You will need to show agreement with conventional Cox proportional hazard models and introduce more complexity into your model. Stoichiometric calculations will increase the value of the paper and the necessity of the model. See my concerns below.

Model concerns

1.What are the results for this model? Where are they presented? I do not see any results whatsoever. Present a graph or table of O3 concentration versus deaths or lifespan. Is this the only result you can get from this simulation? See points below on making the simulation realistic and nuanced so that it agrees with conventional Cox proportional risk models.

2.Find another study (preferably multiple studies) such as this ((DOI: 10.1056/NEJMoa0803894), and present how your results are consistent (or not) with literature that uses standard Cox proportional hazard models to estimate deaths (or risk of increase in deaths) per unit increase of O3 concentration. Please perform a thorough search of the literature. If the results from your model are not consistent with those reported in the literature, explain the reason(s) for this inconsistency.

3.The cited reference # 5 in the paper actually states that “…On the whole, these results support the concept that much of the O3 toxicity is neutralized by the powerful antioxidant system of blood……”. Therefore, the model assumption of ROS increasing by 10X normal levels appears unjustified. Explain. You can still keep this number as a pure mathematical representation in the model but not use it to justify physiological mechanism deaths.

4.Going back to point 3, since O3 exposure accelerates death due to other respiratory diseases, you need to modify your model to consider this. For example, you will need to increase the rate at which your starting diseased population (but not the non-diseased population) approaches death (either by increasing the ROS accumulation rate or by increasing the death rate of affected neighbors). This will create a model that will be consistent with the mechanisms of death, and maybe you will find that this model agrees much better with existing Cox proportional hazard models (see point 2).

5.Going further, you will need some kind of exponential function that will increase this rate of death as a function of the diseased state. For example, if 5% of your population starts out diseased, then you will need to assign the degree of severity of the disease to that 5% by an exponential function.The rate at which ROS increases will be greater for persons who are severely diseased than for those who are moderately diseased (for the total 5% diseased population).

O3 concerns

1.Per wikipedia, WHO (2008) estimates that ground level O3 causes 22000 premature deaths in the EU. This is 3 premature deaths per 100,000 persons. CDC estimates 984 deaths per 100,000 persons for total disease and accidents. This means that O3 is responsible for 0.3% of the death rate per 100,000 persons (assuming independent effect from other diseases). Is this large enough to justify an independent effect study?

2.The EPA and OSHA have PEL of 70 and 100 ppb (averaged over 8 hours) for acceptable O3 levels. According to wikipedia, levels reach 100 ppb in polluted areas. This is still at or below PEL.

3.The epithelial lining fluid in the lung contains an 100 fold greater GSH concentration than in plasma, > 90% in the reduced state. It also contains other antioxidants such as catalase, SOD…etc. The stoichiometry of these antioxidants is more than sufficient to neutralize 100 ppb in 8 hours or 0.2 ppb per minute? Please provide basic stoichiometric calculations. It appears that the 100 ppb may be saturating the lung antioxidants. This is important since it will add to your claim of increase in deaths due to increase in O3. I have not seen such calculation in the public domain.

4.A relative risk of death from respiratory causes due to a 10 ppb increase was found to be 1.04 in this study (DOI: 10.1056/NEJMoa0803894), i.e. 4000 more persons per 100,000 people, which is significant. Cite this and other literature in the public domain to make your case stronger.

Resubmission Revisions

1. What are the results for the model? Where are they presented? I do not see any results whatsoever. Present a graph or table of O_3 concentration versus deaths or lifespan. Is this the only result you can get from this simulation? See points below on making the simulation realistic and nuanced so that it agrees with conventional Cox proportional risk models.
   I moved model development to the methodology section and redesigned my results section to include my quantitative results. A new table and figure was added to present my results, plotting ozone concentration against lifespan, and an exponential regressive line of best fit with a corresponding R^2 value was shown as well. A short paragraph was then written as an explanation of the visuals. The second paragraph of the discussion section contains an interpretation of the results to suggest more implications that can be derived from the conclusions of the model.
2. Find another study (preferably multiple studies) such as this (DOI: 10.1056/NEJMoa0803894), and present how your results are consistent (or not) with literature that uses standard Cox proportional hazard models to estimate deaths (or risk of increase in deaths) per unit increase of O3 concentration. Please perform a thorough search of the literature. If the results from your model are not consistent with those reported in the literature, explain the reason(s) for this inconsistency.
   I conducted a thorough literature review and found two studies involving Cox proportional hazard models that fully supported my conclusion. In the third paragraph of the discussion section, I compared my results with the results of the other two papers, stating any similarities and differences. There were no inconsistencies.
3. The cited reference #5 in the paper actually states that "…On the whole, these results support the concept that much of the O3 toxicity is neutralized by the powerful antioxidant system of blood…". Therefore, the model assumption of ROS increasing by 10X normal levels appears unjustified. Explain. You can still keep this number as a pure mathematical representation in the model but not use it to justify physiological mechanism deaths.
   Firstly, the only reference my paper had to reference #5 is in the third paragraph of the introduction that had the sole purpose of establishing that the focus of this study will be on the reaction of ozone with physiological saline. I did not refer to reference #5 for any other purpose. Secondly, I added a stoichiometry section (Equation 1 and Equation 2) in the introduction to prove that ozone toxicity will not be neutralized and become more damaging as people grow older and develop respiratory conditions. Thirdly, in the disease subsection in the methodology section, the second paragraph talks about how the 50% of physiological saline is the total amount of excessive ROS ever produced in a person, even if its already reacted and gone, not ROS accumulation or the currently existing amount of ROS within that individual.
4. Going back to point 3, since O3 exposure accelerates death due to other respiratory diseases, you need to modify your model to consider this. For example, you will need to increase the rate at which your starting diseased population (but not the non-diseased population) approaches death (either by increasing the ROS accumulation rate or by increasing the death rate of affected neighbors). This will create a model that will be consistent with the mechanisms of death, and maybe you will find that this model agrees much better with existing Cox proportional hazard models (see point 2).

I modified my model to have an additional agent representing the physiological saline of a person with pre-existing respiratory conditions. This new agent type approaches death much faster as it simulates a sickly person. Combined with the healthy person agent type, this model agrees with the Cox proportional hazard models as proved in the third paragraph of the discussion section.

5. Going further, you will need some kind of exponential function that will increase this rate of death as a function of the diseased state. For example, if 5% of your population starts out diseased, then you will need to assign the degree of severity of the disease to that 5% by an exponential function. The rate at which ROS increases will be greater for persons who are severely diseased than for those who are moderately diseased (for the total 5% diseased population).
   I developed a formula (Equation 4) to calculate an individual's lifespan by combining the lifespan of a healthy person (original physiological saline agent population) and a sickly person (new physiological saline agent population) proportionately. This equation allows the disease transmission rate between agents to no longer be constant but instead increase as people grow older and develop medical conditions.
6. Per Wikipedia, WHO (2008) estimates that ground level O3 causes 22000 premature deaths in the EU. This is 3 premature deaths per 100,000 persons. CDC estimates 984 deaths per 100,000 persons for total disease and accidents. This means that O3 is responsible for 0.3% of the death rate per 100,000 persons (assuming independent effect from other diseases). Is this large enough to justify an independent effect study?
   I changed the focus of my paper from the impact of ground-level ozone on the human body to the impact of ground-level ozone on existing respiratory diseases to make my research more significant.
7. The EPA and OSHA have PEL of 70 and 100 ppb (averaged over 8 hours) for acceptable O3 levels. According to Wikipedia, levels reach 100 ppb in polluted areas. This is still at or below PEL.
   Yes, this is true. However, this study explores how increasing ozone concentrations will decrease lifespan, even if it is not a staggering amount. It explores correlations and makes predictions for the future. For instance, 100 ppb will not decrease lifespan to a lethal length, but it does shorten longevity significantly, as shown in the results section of my paper.
8. The epithelial lining fluid in the lung contains a 100-fold greater GSH concentration than in plasma, > 90% in the reduced state. It also contains other antioxidants such as catalase, SOD… etc. The stoichiometry of these antioxidants is more than sufficient to neutralize 100 ppb in 8 hours or 0.2 ppb per minute? Please provide basic stoichiometric calculations. It appears that the 100 ppb may be saturating the lung antioxidants. This is important since it will add to your claim of increase in deaths due to increase in O3. I have not seen such a calculation in the public domain.
   I have added stoichiometric calculations in the introduction (Equation 1 and Equation 2) to prove that under the existence of a pre-existing respiratory disease and aging, GSH production is greatly reduced to the point where slight changes in exercise and diet could cause irreversible oxidative stress and ROS damage. I also added a new study in the literature review section about the role of GSH on immunity and inflammation relating to ROS.
9. A relative risk of death from respiratory causes due to a 10 ppb increase was found to be 1.04 in this study (DOI: 10.1056/NEJMoa0803894), i.e. 4000 more persons per 100,000 people, which is significant. Cite this and other literature in the public domain to make your case stronger.
   I have cited this and other literature in my paper to strengthen my case. I also changed the focus of my study to the impact of ground-level ozone on existing respiratory diseases instead of the impact of ground-level ozone on the human body.

Additional changes:

- Provided a more critical analysis of the cited studies within the literature review section (Sections 2.1, 2.2)
- Added legends to applicable figures (Figures 1, 2, 3, 4)
- Explained more thoroughly the visual role of the ozone agent and the effect of the split parameter on its population (Section 3.3.5, paragraph 3)
- Projected the results onto real-life countries for further implication analysis (Section 5, paragraph 2)
- Added the exact disease transmission rates used to create the model and included how they affect the model (Section 3.3.4)
- Clarified the effects of the asexual breeding parameter on the Agent 1 population (Section 3.3.5, paragraph 1)
- Clarified the purpose of the disease tracker function and regional statistics function according to the new focus of the paper, especially by proposing formulas that would help confirm the validity of the results (Section 3.4)
- Updated research paper formatting according to Journal of High School Science guidelines

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Thank you for addressing my comments. The manuscript is very much improved from the earlier version. However, minor deficiencies remain. Please address the following comments

1.Table 1 needs standard deviations. Also mention how many times the simulation was run (for each ozone concentration).

2.Similarly, are the error bars in the Figure, standard deviations? The SD for all O3 concetrations seem equal. Please check.

3.I would like you to run a simulation with zero ozone concentration as the control. What is the predicted lifespan? If it is unreasonable, you may need to make changes to your model algorithm.

4.Under the limitations of the study, please include that the model is heuristic because values of the split energy threshold and breed energy;, among others; were input using trial-and-error to prevent algorithm breakdown.

5.The equations need paranthesis to properly present the heirarcy of operations. In equations 6 and 7, where does the number 1.81 originate from? Please describe.

6.I am assuming the numbers in equation 7 come from a figure. In this context, it will be *very instructive* for the reader to see graphs at 3 ozone concentrations of (say) 20, 60 and 100 ppb. Each of these 3 graphs should show the number or concentration of agent 1, agent 2, agent 3 and agent 4 (on the y axis) as a function of clicks (say at 10%, 20%, 30%…100% (29713 ticks representing 100%). These graphs will help explain your model lucidly and more convincingly than your static figures.

Resubmission Revisions

1. Table 1 needs standard deviations. Also mention how many times the simulation was run (for each ozone concentration).

   I have added standard deviations into Table 1. The last paragraph of the regional statistics section was moved to be the first paragraph of the results section, and also rewritten to highlight the number of trials executed.

2. Similarly, are the error bars in the Figure, standard deviations? The SD for all O3 concentrations seem equal. Please check.

   My error bars were incorrect in Figure 7. I have corrected them.

3. I would like you to run a simulation with zero ozone concentration as the control. What is the predicted lifespan? If it is unreasonable, you may need to make changes to your model algorithm.

The predicted lifespan of someone living in an environment with zero ozone concentration is approximately 114.33 years. This number is reasonable as it is less than the lifespan of Jeanne Calment of France, the human with the longest longevity in history, who lived for 122 years and 164 days. I added this new trial value to Table 1 and Figure 7, yielding a new line of best fit with an equation of $y=112.08\,e^{-0.008x}$ and an R^2 value of 0.995. I have made adjustments with the interpretation as well in the discussion section by updating to the new line of best fit equation and R^2 value, and inserting a new paragraph after the first paragraph.

4. Under the limitations of the study, please include that the model is heuristic because values of the split energy threshold and breed energy;, among others; were input using trial-and-error to prevent algorithm breakdown.

I added an extra portion at the end of the limitations paragraph in the discussion section warning about the heuristic nature of my model. Specifically, I highlighted the trial-and-error method I used to derive some of my parameters to prevent algorithm breakdown.

5. The equations need parentheses to properly present the hierarchy of operations. In equations 6 and 7, where does the number 1.81 originate from? Please describe.

I added parentheses to Equation 4 and Equation 5 to demonstrate the hierarchy of operations. In Equation 6 and Equation 7, I don't know why I originally decided to divide by 1.81 instead of multiplying by 0.55. The latter is much clearer and both yield approximately the same results. I have made the modification.

6. I am assuming the numbers in equation 7 come from a figure. In this context, it will be very instructive for the reader to see graphs at 3 ozone concentrations of (say) 20, 60 and 100 ppb. Each of these 3 graphs should show the number or concentration of agent 1, agent 2, agent 3 and agent 4 (on the y axis) as a function of clicks (say at 10%, 20%, 30%…100% (29713 ticks representing 100%). These graphs will help explain your model lucidly and more convincingly than your static figures.

After careful consideration of this comment, I believe that adding a graph highlighting the concentrations of each agent type would not advance the study. First, as stated in the paper, Agent 3 dies out after starting off the initial infection. After the disease is successfully transmitted to enough Agent 2 and Agent 4 to keep the diseased population alive, the increase in the number of diseased agents itself simulates ozone exposure – the actual Agent 3 itself is no longer needed as a visual representation. If the Agent 3 population is displayed on a graph, the population would be seen to disappear after the first 100 ticks, even though the ozone exposure is still visibly demonstrated within the model, hence potentially confusing the reader. Second, Agent 1's diseased population soon becomes insignificant as the amount of diseased agents increase. At the beginning of the simulation when each individual diseased agent is important to play a role in spreading the infection, Agent 1's diseased agents are accounted for and can be visually seen. However, as the model continues to run, the small number of the diseased Agent 1 population will have a reduced significance and be lumped with the infected Agent 2 and Agent 4 populations. On a graph, the infected Agent 1 population will seem to disappear, though it is still visually and quantitatively expressed within the model. Thirdly, the death threshold of 55% percent as mentioned in the paper applies to the infected Agent 2 and Agent 4 populations in comparison to the total Agent 2 and Agent 4 populations, respectively, not the total agent population. Hence, no clear threshold will be seen if a graph is created with the latter in mind. Another method could be to graph the percentage of infected agents in the Agent 2 and Agent 4 populations on the same axis but separately on different trendlines. This could be used to see the infected percentage of one agent type while the other is at another infected percentage. However, the sum of the two percentages will not have the significance of adding up to 100%, and the graph overall will be arbitrarily created and fail to advance the study as the idea it displays of the 55% death threshold is already clearly stated in the paper. Lastly, if this

comment is referring to the non-diseased or total agent population of each type, the graphs created will prove to be less relevant because the conclusions of this study is drawn considering the percentage of diseased agents to healthy agents in each population, not the comparison of the populations of the different agent types with each other. Displaying the percentage of the entire population consisting of each agent type is irrelevant to the focus of the paper. As Figure 5 already displays the change in number of total infected agents compared to total healthy agents, which is sufficient to support the conclusions made by this study, adding an additional graph separating the diseased population and/or the total population of each agent type is not necessary to add value to this paper.

Additional changes:

- Changed the figure and table titles to include "± 2SD" in Table 1 and Figure 7
- Conducted more trials to ensure accurate results, as displayed in the slight shift in data values in Table 1

Thank you for addressing my comments. I have some minor comments that need to be addressed prior to acceptance

1.Please renumber references - not as superscripts - but as numbers enclosed in curved parenthesis. Do NOT hyperlink references to the references section.
2.The references section needs to be rewritten keeping the formatting requirements of the Journal in mind. Please follow the Journal's formatting requirements or deduce this information from published manuscripts. Formatting must be consistent for all references.
3.Re. availability of data and materials, can you deposit into the GitHub repository and then include a link to it in the manuscript please.
4.Where-ever you have used abbreviations, write the full form in the first instance where you use the abbreviation.
5.Make the abstract more concise by including essential information; not information that is going to be repeated in the text.

- **Resubmission Revisions**
- Please renumber references - not as superscripts - but as numbers enclosed in curved parenthesis. Do NOT hyperlink references to the references section.
  - I have corrected this.
- The references section needs to be rewritten keeping the formatting requirements of the Journal in mind. Please follow the Journal's formatting requirements or deduce this information from published manuscripts. Formatting must be consistent for all references.
  - I have corrected this.
- Re. availability of data and materials, can you deposit into the GitHub repository and then include a link to it in the manuscript please.
  - I have included a GitHub link in the appendix section at the end of the paper.
- Where-ever you have used abbreviations, write the full form in the first instance where you use the abbreviation.
  - I have made the necessary changes. The only instance that needed to be changed was "ROS" to "Reactive Oxygen Species" in the title.
- Make the abstract more concise by including essential information; not information that is going to be repeated in the text.
  I have reworked all five sections of the abstract to be more concise by rewording information and only keeping pieces that will benefit as a summary without repeating sections of the paper itself.

Thank you for addressing all my comments. Accepted.