Ozone Gas: Scientific Justification and Practical Guidelines for Improvised Disinfection using Consumer-Grade Ozone Generators and Plastic Storage Boxes.

Robert Dennis Ph.D.   UNC and NC State Joint Department of Biomedical Engineering

Avery Cashion Ph.D.   Sandia National Laboratories. Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525 SAND2020-3866 J

Steven Emanuel MSE   UNC and NC State Joint Department of Biomedical Engineering

Devin Hubbard Ph.D.   UNC and NC State Joint Department of Biomedical Engineering

We describe direct measurements of ozone concentration achievable in small enclosed containers (plastic storage boxes) for use as improvised decontamination systems for small articles such as disposable PPE (N95 masks, nitrile gloves, etc.), clothing, mail and small packages, food, and other miscellaneous articles. The emphasis is on the reliable and sustained generation of ozone gas concentrations of sufficient concentration and duration to create an effective virucidal environment to achieve more than 95% to 99% viral inactivation, based upon the data already published in the peer-review literature on this topic.

The suggestion that ozone be used to inactivate virus is certainly not a new idea. Our objective in this report is to make clear that the necessary levels of ozone can be improvised using simple, easy-to-use, inexpensive, and widely available supplies, and that there is every theoretical and experimental reason to believe that this approach is as highly effective in viral inactivation by ozone as are the far more expensive, complex, cumbersome, and less available equivalent ozone (and other) disinfectant systems that have themselves become unavailable during times of pandemic crisis.

Using multiple types of readily available commercial ozone generators, concentration in the tested improvised enclosure is tracked over time to assess ozone charging and decay rates, and the ozone quenching effects of items placed in the box. Generator performance is compared against published ozone dosage values for virucidal and antimicrobial activity. Bubbler and box-fan-type ozone generators were found to be effective at achieving and maintaining target concentrations of 10ppm ozone or higher, whereas automotive cigarette lighter and universal serial bus type plug in "air freshener" ozone generators could not achieve the target concentrations in these experiments. Calculations and practical guidelines for assembly and effective use of an ozone box for improvised decontamination are offered.

The majority of this report is directed toward the scientific justification and rationale for this approach. The end of the document summarizes the findings and offers simplified designs for the construction and use of ozone boxes as an improvised method of disinfection.

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Caveats and Cautions

This paper discusses the potential use of ozone as an improvised virucidal strategy during a period of national crisis and severe shortages of protective supplies and sanitizing agents. Before undertaking any such action, consider that this strategy is not approved by the FDA, and that safety and efficacy has not been established by any federal agency. Ozone is a lung irritant and can be dangerous to humans, animals, and plants. Caution must be taken to avoid ozone exposure to humans, animals, or plants that exceed the Environmental Protection Agency National Ambient Air Quality Standards.

FDA Reminds Patients that Devices Claiming to Clean, Disinfect or Sanitize CPAP Machines Using Ozone Gas or UV Light Have Not Been FDA Authorized.

"The FDA has identified several manufacturers that are marketing ozone gas or UV light-based products claiming to clean, disinfect or sanitize CPAP devices and accessories in the home," said William H. Maisel, M.D., M.P.H, director of the Office of Product Evaluation and Quality in the FDA's Center for Devices and Radiological Health. "Exposure to high levels of ozone gas may worsen a patients' existing chronic respiratory diseases or increase the chance of a respiratory infection. UV light-based products could cause burns, eye damage or increase the risk of skin cancer due to over exposure. The FDA has contacted manufacturers of products making these claims and asked them to submit data demonstrating their safety and effectiveness."

The official safety communication from the FDA:

Potential Risks Associated With The Use of Ozone and Ultraviolet (UV) Light Products for Cleaning CPAP Machines and Accessories: FDA Safety Communication.

It is also important to note that occupational exposure to ozone is regulated by OSHA (enforceable) and NIOSH (as guidelines). For links to these resources, please see the References section at the end of this manuscript.

Introduction

Ozone (O_3) is an extremely potent oxidizer and has been used on a large industrial scale to kill microorganisms, mostly in waste water treatment and agricultural processing, while mobile, powerful ozone generators have been shown to greatly inactivate virus in enclosed spaces (1). In particular, ozone is an extremely effective germicide against viruses and bacteria (2-10, 11). Ozone acts to destroy virus particles through oxidation. Virus particles, unlike many other microorganisms, are unable to repair oxidative damage, and therefore would be expected to be more susceptible to oxidative antimicrobial action than, for example, fungi or bacteria or protozoa, or any eukaryotic organism.

A member of the *Coronaviridae* family, SARS-CoV-2 (responsible for COVID-19) is an enveloped virus which makes it particularly susceptible to destruction by disinfectants (2,12,11) including ozone. For enveloped viruses, ozone readily oxidizes the viral envelope, changing its structure or destroying it and rendering it inactive (2, 4, 11). Other virus families are destroyed by direct damage by ozone to capsid proteins and nucleic acids (2-4, 13).

Ozone may not be employed widely as a point-of-use virucide because of environmental/occupational hazard concerns, or concerns that ozone may quickly destroy substrate materials (such as disposable gloves or facemasks), or simply because there is not a large body of published data on viral inactivation kinetics, and therefore the very aggressive action of ozone on viral particles is not fully appreciated. In a parallel set of experiments, we report (elsewhere) the excellent durability of polypropylene filter fibers when exposed to virucidal doses of ozone, and the

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paucity of viral inactivation kinetics data in the literature may also have an explanation.

Detailed viral inactivation kinetics have not been extensively published, in part because they are difficult to measure because viral inactivation happens so quickly in the presence of ozone (14 - 16). Comparing viral inactivation in water by chlorine versus ozone, Katzenelson et al. note "Ozone is shown to have a much more rapid virucidal effect [than chlorine]", but they were unable to quantify the viral inactivation kinetics due to the limitation of the speed (8 second sampling interval) at which the inactivation kinetics could be measured (14). Five years later, with improved sampling speed using a fast-flow mixer and a sampling time point accuracy improved to 0.01 seconds (15), Katzenelson et al. were able to report the previously unobservable rapid two-phase viral inactivation kinetics, noting that "[within] 0.2 to 1.0 seconds, 95 to 99% of the virus was inactivated, depending on the ozone concentration (which ranged from 0.1 to 2.0 mg/liter)." Four decades later, the situation has apparently not improved markedly. In 2018, Wolf et al. state "Ozone is an effective disinfectant against all types of waterborne pathogens. However, accurate and quantitative kinetic data regarding virus inactivation by ozone are scarce, because of the experimental challenges associated with the high reactivity of ozone toward viruses." These reported measurements of inactivation kinetics were made in water, but it is plausible that comprehensive viral inactivation kinetics for viruses in both air and water are not widely reported simply because such measurements are exceedingly difficult owing to the rapid rate at which ozone inactivates virus. Ironically, it may be that ozone has received less scientific attention as a virucide precisely because it is far more rapid and effective, and therefore harder to measure, than many alternative antimicrobial agents.

Ozone is considered hazardous and occupational exposure is limited by OSHA, and therefore it must be handled with caution (see links in REFERENCES). However, consumer-grade ozone generators are widely available and are in common domestic use for deodorizing garages, basements and automobiles, hotel rooms, reducing the odor of garbage bins, deodorizing clothing, and sanitizing food and water. Ozone is produced naturally by lightning and incidentally by electrical sparks, and does not produce lingering toxic environmental pollutants. The byproduct of ozone breakdown is oxygen (O_2). The half-life of ozone at room temperature ($\sim$ 25C) in water is on the order of 15 to 20 minutes, and at most 2 to 3 days in air (17).

Persistent, long-term exposure to high concentrations of ozone in living and working spaces is to be avoided. However, when used responsibly in an open-air environment, the practical ease of use and efficiency of ozone as a virucide make it a very logical alternative for the disinfection of common household items, mail, packages, and food and, in emergencies, otherwise disposable protective equipment such as N95 face masks, gloves, and other items. Ozone will degrade many materials (18-22), so caution and good sense are essential. But the virucidal action of ozone is potent enough that its action as a disinfectant occurs much more rapidly than its action in the degradation of most materials. The fact that ozone gas is a dry virucide makes it particularly effective option for porous, fibrous, or textured materials. Ozone reaches the crevices and shadows that wipe disinfectants or ultraviolet sterilization cannot. Ozone is very reactive and generally does not store or transport well, so it is usually produced at the point-of-use, and at the time that it is needed. It is however, remarkably simple and inexpensive to produce, requiring only a source of electricity and regular atmosphere. Therefore, unlike most other disinfectant technologies, once an adequate ozone generator is available, it can be manufactured quickly and inexpensively, whenever needed, without consuming any further supplies or ingredients.

Ozone can be created by several natural and artificial means, including the interaction of ultra-violet light with oxygen. However, the most practical method for its production as a point-of-use disinfectant gas in adequately high concentrations (10 to 20 ppm) is by corona discharge. This typically requires high voltages, on the order of kV, but very low electrical current because the corona is generated around a dielectric plate, so very little actual current is conducted in the process. Water can be ozonated simply by bubbling ozone into the water followed by immediate use. Using a spray bottle, for example, this method may be useful for the viral inactivation of

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surfaces such as tabletops and doorknobs that could not be easily placed into a small ozonation chamber as we describe.

Practical Considerations

When considering the use of ozone gas as a disinfectant, several questions must be answered:

What is the required ozone gas concentration? This is a somewhat difficult question to answer. Specific numbers are difficult to find for gaseous ozone decontamination as the majority of the ozone virucidal studies are conducted with viruses in aqueous suspension with ozone introduced through bubbles or surface dissolution. A review of the existing scientific literature reveals that the virucidal action of ozone is extremely rapid and potent, however requisite gaseous ozone dosages are limited to only a few studies. We base the target dosage on four important studies of gaseous ozone inactivation of **a.** viruses (1, 2, 11) and **b.** other microorganisms (9) on solid surfacessurfaces that report quantitative results at practical dosage and humidity for improvised disinfection chambers . Tseng et al. investigated four different types of bacteriophage to represent a broad test of viral families. Enveloped viruses (like coronaviruses) were represented by the Φ6 double stranded RNA bacteriophage which, as expected, showed the highest susceptibility to ozone inactivation. Other viruses tested were a double stranded DNA (T7 phage), a single stranded RNA (MS2 phage), and a single stranded DNA (Φx174). The second study likewise tested four different types of germ (E. coli, Yeast, B. Subtilis, and P. Citrinum). All were effectively inactivated by ozone in varying doses. Hudson et al. demonstrate effective viral inactivation by gaseous ozone exposure on 12 viruses and report quantitative results at ambient humidity on inactivation of Herpes Simplex Virus (HSV), Influenza virus, and rhinovirus on substrates of plastic, glass, and stainless steel (1) in a chamber. Results from this study showed some variance in required ozone dose with substrate material. All of these studies demonstrate that microorganisms died significantly more rapidly with increased humidity. Specific calculations for ozone dosage based on these studies are shown in METHODS. The main take-away is this: higher concentrations and/or durations of ozone result in greater rates of virus inactivation and other microorganism destruction. There is some suggestion that viral inactivation is increased more than linearly by increasing ozone dose. That is, exponential decrease of active virus with increasing ozone dose approaching zero asymptotically. (2,9,11)

What is the required exposure time at the required concentration? Similar to the preceding question, the available literature is somewhat sparse. However, it is clear that longer exposure times lead to greater viral inactivation. Studies (2, 9, 11) show that the important factor for inactivation of viruses and other microorganisms is the total ozone dose which is calculated as the product of exposure time and concentration. Low concentrations for longer duration achieve the same results as high concentrations for short duration in these studies. Literature supports that the primary mechanisms of viral deactivation are through envelope oxidation, capsid protein damage, and nucleic acid damage (2, 4, 11, 13). Assuming that the susceptibility of envelopes, capsids, and nucleic acids to ozone does not vary from virus to virus, the primary contributor to deactivation time is likely related to the susceptibility of the viral capsid to oxidative stresses. We can use these findings to inform concentrations and durations for an ozone sterilization chamber. As shown in the Methods section, we target ozone dosages based on this literature but with higher concentrations and lower durations. This approach is reasonable but it is important to note that increasing concentration outside of the bounds of the literature is extrapolative, which is less certain than interpolative techniques. At very high levels of deactivation, the number of remaining particles will have been greatly reduced, so that it would not, for example, be possible to achieve an inactivation of "double" if the number inactivated is already 90%, since the upper limit is 100% deactivation. Nonetheless, taking this into account, exposure times, when increased, necessarily increase deactivation of viruses.

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Is the virucidal action specific or general among virus types? Once again, the existing literature does not cover all virus types, but it does report the virucidal effects on virus strains that are considered "difficult to kill" as defined by the EPA (23), as well as virus strains considered to be particularly dangerous to humans, such as poliovirus. In all cases and for all virus types tested that we were able to find in the literature, ozone appears to be highly effective. Results from two studies (2, 11) suggest that virucidal action of ozone corresponds inversely with the complexity of the capsid in non-enveloped viruses. This result makes sense from a perspective that more "armored" viruses survive longer from oxidation attacks. An important caveat is that enveloped viruses have been shown to be more sensitive to ozone than any other virus type, possibly due to oxidation of lipids in the fragile envelope. Studies conducted on Poliovirus 1 in water indicate that the primary mechanism of deactivation is through RNA damage (13)—further supporting the hypothesis that non-specific deactivation time depends on the complexity and susceptibility of the capsid to oxidative degradation. We think it likely, therefore, that the action of ozone on virus particles is principally non-specific in the sense that with sufficient dose it should be equally effective, more or less, regardless of viral strain.

Can direct measurements for the necessary ozone concentration and exposure time be made, specifically for the current virus in question (SARS-CoV-2 which causes the disease COVID-19)? Certainly, yes, this can and should be done. But it is beyond the scope of this report, and would require a period of time for adequate research that extends far beyond the horizon for the current crisis.

Does existing literature support the hypothesis that gaseous ozone will inactivate SARS-CoV-2?

We have found data on sixteen virus types tested for gaseous ozone disinfection on solid surfaces (1, 2, 9, 11) in the literature, and there are many more publications (3, 4, 5, 8,10, 14-16) that describe efficacy against various viruses in aqueous suspensions. Zhang et al. reportthat dissolved ozone solutions inactivate SARS-CoV-1 (10), commonly known as the SARS virus, which is very similar in structure to the SARS-CoV-2 virus causing the current COVID-19 pandemic. Hudson et al. demonstrate inactivation efficacy of ozone on Murine coronavirus (MCV), which was used at the time as a surrogate for SARS-CoV-1 (1). Literature suggests that ozone attacks capsid proteins in non-enveloped viruses and most readily attacks enveloped viruses (2, 4, 11). As SARS-CoV-2 is an enveloped virus, literature supports the hypothesis that ozone will be effective in its inactivation. It is important to note that we have not found a single study that shows a virus that was not inactivated by ozone. Lack of literature is of course not proof that ozone inactivates all viruses but the current evidence suggests it will effectively inactivate SARS-CoV-2.

Given the lack of specific information, can reasonable estimates for the necessary ozone concentration and exposure time be made? We endeavor to do that in this paper, based on our interpretation of the existing literature. These will be estimates and extrapolations, offered without proof or guarantee, but they are our best scientific estimates based on the available information.

Is it practical to make the required, estimated ozone gas concentration? The short answer is YES. The remainder of this paper will be directed to a clear explanation of the method, and considerations related to achieving and sustaining the required estimated ozone concentration level within a practical-sized decontamination chamber using inexpensive and readily-available components.

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Ozone is highly destructive. It aggressively destroys materials such as latex. For this reason, is it even possible to use ozone gas as a disinfectant for disposable PPE such as gloves or N95 facemasks? This valid concern was voiced by more than a dozen colleagues, but was given without any supporting data. The strategy in this report is to develop a method to deliver a sufficient dose of ozone for a time period that will destroy viruses, but have minimal degradation on the substrate materials. Many materials are resistant to ozone degradation. For example, a brief decontamination cycle may be designed that will inactivate most viruses while causing only limited damage to the filtration material of an N95 mask. This means that, using a carefully-selected combination of ozone concentration and exposure time, viral particles may be inactivated while doing minimal damage to the filter material of the disposable mask.

Latex and other natural rubber products are known to be some of the most susceptible materials to ozone degradation (18-22). In a study of ozone degradation on unlubricated latex condoms (22), scanning electron microscope (SEM) evaluation, burst pressure, and burst volume were tracked against ozone dose. At a concentration of approximately 0.3 ppm, surface degradation was observable using SEM analysis at 18-hour exposure. At 24-hour exposure, burst pressure and burst volume of all condoms in the study passed the world health organization (WHO) quality specifications (24) in accordance with international standards protocol (ISO 4074). Beyond 24-hour exposure, the samples did not all pass specification. From this study, the total ozone dose at which some samples began to fail WHO standards is approximately 432 min[ppm], about 4 times the target dose for 99% virus inactivation calculated in the METHODS section below. An incomplete, though logical interpretation of this study is that thin, unprotected latex materials can be effectively sterilized 3 times using the lower of the two ozone doses suggested in this paper or once with the higher dose without unacceptable performance degradation. As latex is well known to be among the most ozone susceptible materials used in most products, it is logical to hypothesize that most items would remain undegraded for a larger number of ozone decontamination cycles (more than 3 cycles) as described in this document.

In a separate study, the durability of N95 mask material (nonwoven polypropylene fiber mat) has been tested using this apparatus (manuscript in preparation), with this report serving largely as a detailed description of the ozone exposure methods for that study. Briefly and for convenience, prior to the publication of those results: Non-woven polypropylene material, the same as used in an N95 mask, was exposed using the ozone chamber described herein at ozone concentrations of 10 and 20 ppm, for durations of 10, 20, and 60 minutes. Microscopic examination revealed *no visible damage to the fibers*, and a forced-flow filtration efficiency experiment demonstrated conclusively that after these exposures there was n*o loss of filtration efficiency and no sign of degradation to the filter material*, as per the NIOSH specification for N95 mask material. INTERPRETATION: Polypropylene mask material is expected to withstand many disinfection cycles at ozone concentrations well in excess of the concentration and duration necessary for significant (greater than 95% to 99%) viral inactivation. Other components of the disposable N95 mask may deteriorate when so exposed, but filtration effectiveness will not be the limiting factor. Undesired byproducts of ozone exposure were not observed, but were not specifically tested for in this study, so the presence of undesirable byproducts of ozone exposure should also be considered when contemplating the use of ozone as a disinfectant for items such as food and disposable PPE. Also, the ozone used in this study was produced from atmosphere, not from a source of enriched or medical-grade oxygen, so other gaseous compounds will also be created by the corona discharge process, and therefore the potential health impacts of these should also be considered. Keep in mind: this is intended as an improvised expedient for rapid and scalable virucidal disinfection, not an optimal long-term solution.

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Methods

The first objective is to estimate the ozone gas concentration and exposure time that would be most practical for use in a simple system such as the one described herein. As partially described above, the practical ozone concentrations and durations we selected are based on four studies in which gaseous ozone was applied to viruses (1, 2, 11) and other microorganisms (9). In all four papers the authors study varying concentrations and/or durations, enabling calculation of an ozone "dosage" value simply by gaseous ozone concentration multiplied by time, yielding a dosage unit of min[mg/m^3] or min[ppm]. Two of these papers (2, 9) showed that inactivation rates were affected primarily by this product of ozone gas concentration multiplied by duration rather than by either independently; meaning that higher concentrations for shorter durations can have the same effect of lower concentrations for longer durations.

For the purposes of calculation, we will retain extra digits. However, this should not be taken to imply that a level of precision of three or four significant figures. As a practical matter, it is only the first digit or two that matters. For those unfamiliar with this scientific nuance, it means that, as an example, numbers reported as "113.59" should be taken to mean "about 110".

Tseng et al. provided dosage numbers for 99% viral inactivation of each virus type at 55% relative humidity. Tabulated below in Table 1.

Virus	Ozone dosage for 99% inactivation (min[mg/m^3])	Ozone Dosage for 99% inactivation min-ppm
ssDNA represented by (Φx174)	72	37
ssRNA represented by (MS2)	194	99
dsDNA represented by (T7)	223	114
Enveloped dsRNA represented by (Φ6)	58	30

Table 1. _Required ozone dosage for 99% viral inactivation as reported in Tseng et al. 2008._

We convert mg/m^3 concentrations to parts per million (ppm) for convenience. The equation for this conversion at 1 atm and 25° C is as follows:

$$C_{ppm} = \frac{24.45 C_{mg/m^3}}{M_{O_3}}$$

Where M$_{O3}$ is the molecular weight of ozone.

The dosage numbers in Table 1 become 36.68, 98.82, 113.59, and 29.54 min-ppm respectively.

The (Φ6) results should theoretically be the most similar to SARS-CoV-2 but we base our target dosage on the (T7) results to give a conservatively high dose. Either concentration or duration can be varied to achieve the required 113.59 min[ppm] for 99% reduction of the hardest virus to inactivate in the study. 11.36 minutes at 10ppm concentration is the baseline we chose for inactivating viruses.

In a separate study, Tseng et al measured requisite ozone dosage for 99% virus inactivation of the same viruses while airborne (15). The dosages required for 99% inactivation of airborne viruses were significantly lower: (Φx174), 1.58; (MS2), 2.60; (T7), 4.19; and (Φ6), 1.05 min[mg/m^3].

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Hudson et al. report viral inactivation data for various surfaces at 45% relative humidity which was represented graphically, and we visually approximate in Table 2.

Virus	Dosage for 99% inactivation on Plastic(min[ppm])	Dosage for 99% inactivation on Glass (min[ppm])	Dosage for 99% inactivation on Stainless steel (min[ppm])
Herpes Simplex Virus HSV	100	300	300
Influenza virus (H3N2)	200	100	300
Rhinovirus (RV)	300	300	30

Table 2. *Required ozone dosage for 99% viral inactivation as reported in Hudson et al. 2008.*

This data suggests that a dose of 300 min[ppm] would effectively inactivate 99% of all viruses tested on a variety of solid substrate surfaces.

Li et al. provide data on ozone germicidal effects on microorganisms other than viruses at 55% relative humidity. They do not provide the convenient 99% germicidal dosage values but we can still infer dosages from their 80% inactivation data tabulated in Table 3.

Microorganism	Destruction Rate	Ozone dosage (min[ppm])
E. Coli	80%	1.78 – 2.04
Yeast	80%	7.64 – 9.68
B. Subtilis	80%	73.86 – 76.41
P. citrinum	80%	30.56 – 61.13

Table 3.

An important consideration when calculating the requisite ozone dose is the significant effect of humidity. In all of four studies listed above, increased relative humidity caused required ozone dose for viral inactivation to decrease dramatically. Tseng et al report that an increase from 55% to 85% relative humidity required approximately half the ozone dose for 99% viral inactivation. Hudson et al report that using the same ozone dose, 38% humidity inactivated 80% of influenza virus and 70% humidity inactivated >99.99%. Interpreting results from different sections of the same report (1) suggests that increase from 38% to 45% relative humidity increases inactivation of the influenza virus from 80% to >99%. Thus, the higher doses for >99% inactivation shown in Table 2 may be in part due to the tests being run at lower humidity than the tests shown in Table 1..

We estimate from these studies that at 55% relative humidity or higher, that approximately 12 minutes at 10ppm (>113.59 min[ppm]) dosage value will exceed 99% reduction in all airborne and surface viruses and 80% reduction of all other microorganisms in the literature from which it is derived. Furthermore, we estimate that at 45% relative humidity or higher, a dose of 20ppm for 15 minutes (300 min[ppm]) is a practical dose likely to inactivate >99% of virions on a wide range of solid surfaces.

Ozone Sensor

The key item of equipment necessary to validate this approach is an accurate ozone sensor. The ozone sensor in this apparatus is the ULPSM-03 968-046 ozone gas sensor manufactured by Spec-Sensors, LLC. The published linear measurement range of this sensor is 0 to 20 ppm with a minimum detection limit of approximately 0.1ppm. The resolution is approximately 0.1 ppm with a measurement accuracy of ±2% of the reading.

A very simple circuit was constructed to interface the ozone sensor to provide power, buffer the sensor signal with a high input impedance operational amplifier, and measure the output. The

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sensor was powered by a locally regulated IC LDO regulator (MIC5213-3.0YC5), selected for its very low noise, at 3.0 Volts. The sensor outputs (Vref and Vgas) were each directly buffered through simple op-amp (LM324) voltage followers. The calibration constants were used as specified by the manufacturer, and certified and marked on each sensor. The differential voltage signal was carried through 1 meter of twisted pair 22 AWG solid copper wire, passed through a tight-fitting hole drilled into the ozone box, and filtered downstream using a capacitor stack (2200 uF aluminum electrolytic, x 5, to achieve 11,000 μF). The resulting signal was directly proportional to the ozone concentration but still contained "salt-and-pepper" noise, which was subsequently removed by means of a median filter of rank 10 after digital conversion.

Data were sampled at 10 S/second on a GW Instek 4-trace storage digital oscilloscope operating in "Strip Chart" mode. Ozone concentration was sampled for 1000 seconds for each test, for a total of 10,000 samples per data trace. Analog-to-digital conversion resolution was 0.008 V. Using a calibration factor of 38.06 ppm/V, this equates to an ozone concentration resolution of 0.305 ppm. Small signal sensor response was less than 10 seconds, and a linear response was evident up to at least 25 ppm, though the manufacturer guarantees linear operation within specifications only to 20 ppm. Zero offset in clean air drifted between +/- 0.040V, which equates to a random offset drift of approximately +/- 1.5 ppm. During sampling, ozone generation was begun during each trace at the 40 second time point (+/- 4 seconds), and is clearly evident by the immediate response in each trace to the increasing ozone concentration.

First-Pass Calculations for achieving the desired ozone concentration if an ozone sensor cannot be obtained

Most commercial ozone generators are rated by their ozone output rate in mg/hr. The simplest calculation to attempt to achieve target concentrations is as follows.

$$t_{min} = 117.9 * F * \frac{C_{ppm} * V_{m^3}}{R_{mg/hr}}$$

Where t_{min} is the time to run the generator in minutes, C_{ppm} is the desired concentration in ppm, V_{m3} is the volume of the chamber in m^3, $R_{mg/hr}$ is the ozone rate of the generator in mg/hr, and F is a multiplication factor dependent on the set-up.

117.9 is a conversion factor for (mg/m^3) to ppm and hours to minutes.

The multiplication factor F accounts for delays due to: startup time of the ozone generator, gas leakage out of the chamber, and quenching effects from oxidation of materials in the chamber. In a perfectly sealed empty box with nonreactive walls and an ozone generator withzero startup time, would be equal to 1. With the experimental setup in this paper, was approximately 10 for an empty chamber and 50 when loaded.

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Test Chambers: Ozone Decontamination Boxes

Two different boxes were used for this series of experiments. Both were Sterilite® brand, widely available in home and hardware stores. These plastic storage boxes were selected because the same box/lid/latch design was available in two different sizes:

Size	Volume	Dimensions	Manufacturer Part Number
Medium	24 Liter	41.3 cm x 28.6 cm x 29.5 cm	Sterilite® Latching Box number 1495
Large	61 Liter	60.3 cm x 40.6 cm x 34.3 cm	Sterilite® Latching Box number 1497

Table 4. *Specifications for containment boxes used in the report. www.sterilite.com*

The medium sized box (24 liter) was used for all experiments in this report, except where indicated. This container is very close in terms of volume to the 23-liter test chambers used for the ozone dosage studies (1,8). For reference, these earlier studies (1, 8) used an OZIPCS-V/SW ozone generator and measured ozone concentration using an ozone analyzer model 401, Advanced Pollution Instruments, San Diego, CA.

Ozone was introduced into the box through a flexible 3/16" ID (5/16" OD) silicone tube, thick wall (1/16") to prevent kinking, fitted through a 5/16" diameter hole drilled approximately in the center of one side of the plastic box. The tube fit snugly into the hole, and was pulled through the hole ~ 2" into the box.

At the bottom of the box, an array of seven small 1.5" square, 12 VDC box fans was placed in a co-linear wooden dowel bracket, with one fan placed at 90 degrees to the co-linear array, to circulate air inside the box during testing, in order to maintain a quasi-uniform ozone concentration throughout the box interior. It was thought that this would improve the accuracy of ozone measurements for experimental purposes, but is probably not required for the construction of a practical ozone decontamination box, because ozone gas diffuses readily and quickly throughout such a relatively small volume.
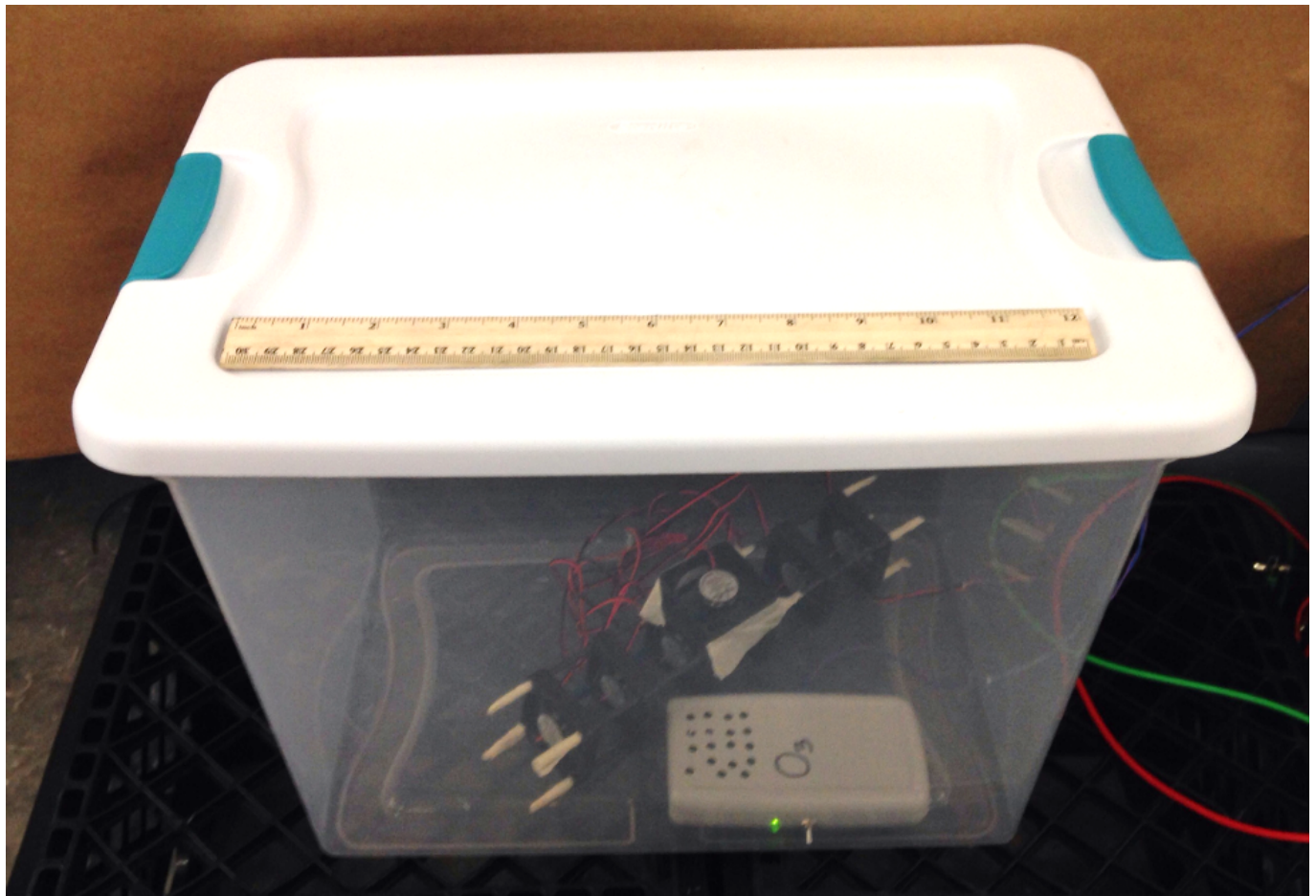
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Figure 1. *Test box (24-liter size) showing the box fan array and ozone sensor located in the bottom of the box. A 12-inch ruler is included for scale.*

Also placed at the bottom of the box was the ozone sensor and buffering circuit, assembled into a standard plastic electronics enclosure with a series of 3/16" diameter holes punched into the enclosure to allow air circulation into and around the ozone sensor element.

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Figure 2. *Ozone quenching effect of the materials being disinfected. For one experiment, materials were added into the box for a disinfection cycle to measure the effect of these materials on ozone concentration (quenching).*

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Simulated Disinfection Material for Ozone Quenching Measurements:

1 N95 mask

2 empty cardboard boxes: 220mm x 80 mm x 160mm each

= 35,200 + 70,400 + 25,600 = 131,200 mm^2 area for each box

1 pair nitrile gloves, 6 mil thickness, size XL

1 square foot of Household cleaning paper (Scott Towel)

A 12-inch ruler is included for scale (but was not used in the experiment)

These items were placed in the test box, allowing space around and between the objects as much as practically possible to facilitate ozone diffusion to all surfaces, and the lid was latched into place for the experiment.

General Test Procedure

Unless otherwise noted, the test conditions were set and recorded: box size of 24 or 61 liters, lid latched tightly, ozone generator selected, ozone sensor ON and stabilized for 30 minutes as per manufacturer recommendations, room temperature stabilized to 70 ˚F. Data collection was started, and at approximately 40 seconds, the ozone generator was turned ON and ozone was introduced to the box as described in each experiment until a concentration of 22 ppm was exceeded, at which time the ozone generator was turned OFF and data was collected for the remainder of the 1000 second test period.

Results

Ozone concentration was measured during the charging period and decay period in a decontamination chamber under the conditions specified for each figure below:
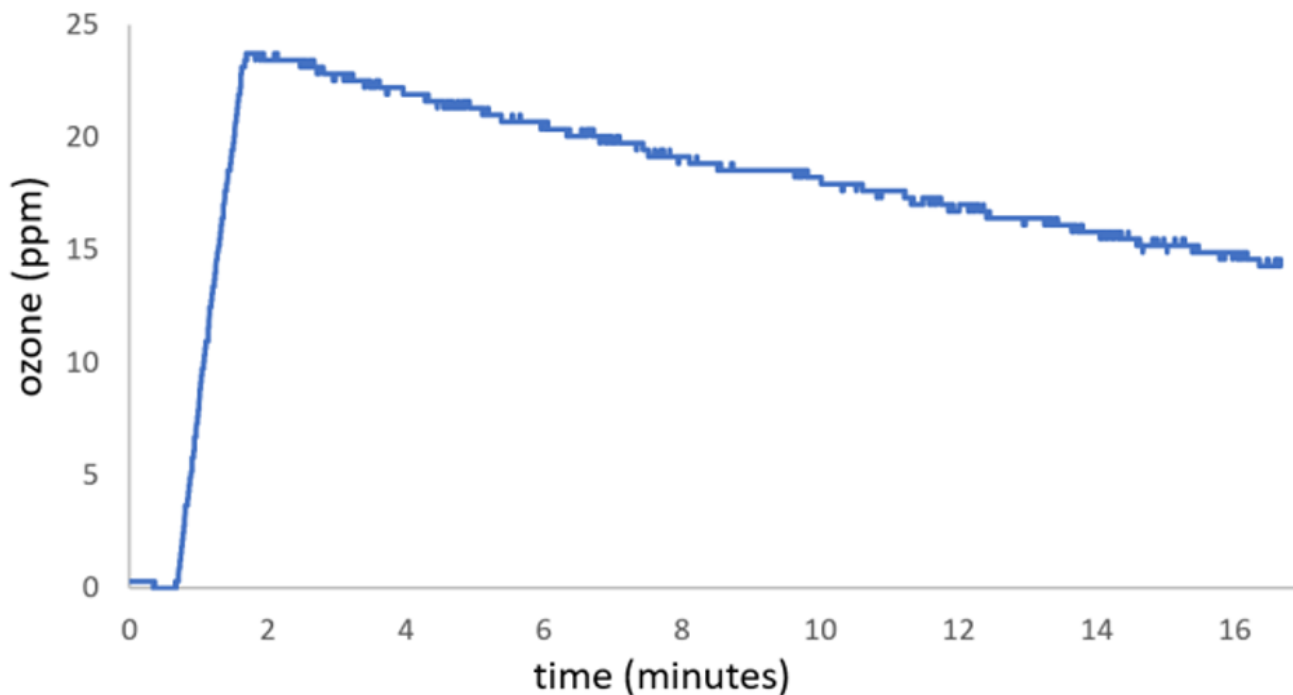
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Figure 3. *Time course of the ozone concentration, during charge and decay, in a 24L Sterilite storage box with the lid fitted tightly, and using an ozone bubbler specified to produce 600 mg/hr ozone ("A2Z"). Ozone concentration began to rise immediately when the generator was turned ON, and rose linearly to a level of approximately 24 ppm, at which point the ozone generator was shut OFF, and the ozone concentration was allowed to decay. Note that the box was empty, except for the ozone sensor and a mixing fan array that had already been through approximately 10 ozone charging cycles.*
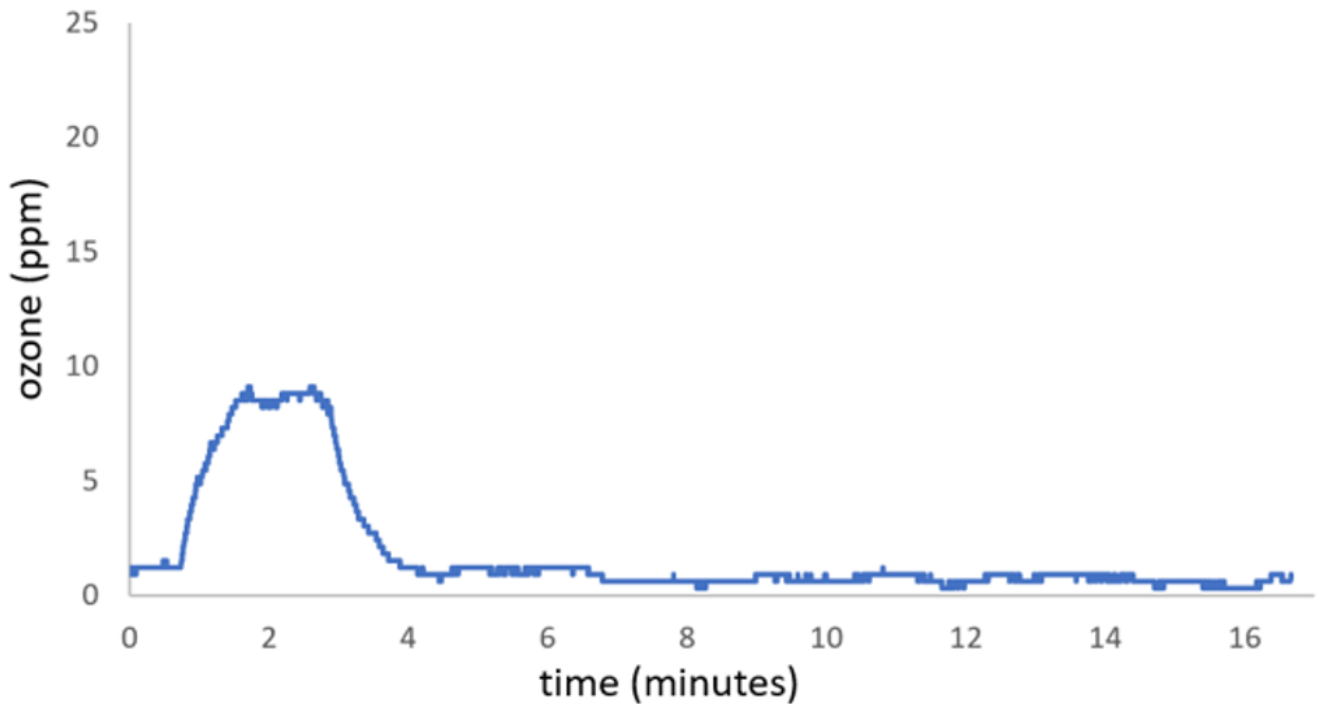
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Figure 4. *The same setup as the figure immediately above, but with the lid loosened and slid to the side slightly to allow a 1 cm gap on one side, allowing ozone to easily diffuse from the box. With such a large leak, we were unable to achieve the minimum desired ozone concentration of 10 ppm. The generator was able to maintain a concentration of approximately 9 ppm if left ON, but when switched OFF, the ozone concentration in the box quickly decayed to baseline. LESSON: lids should fit tightly and be placed properly.*
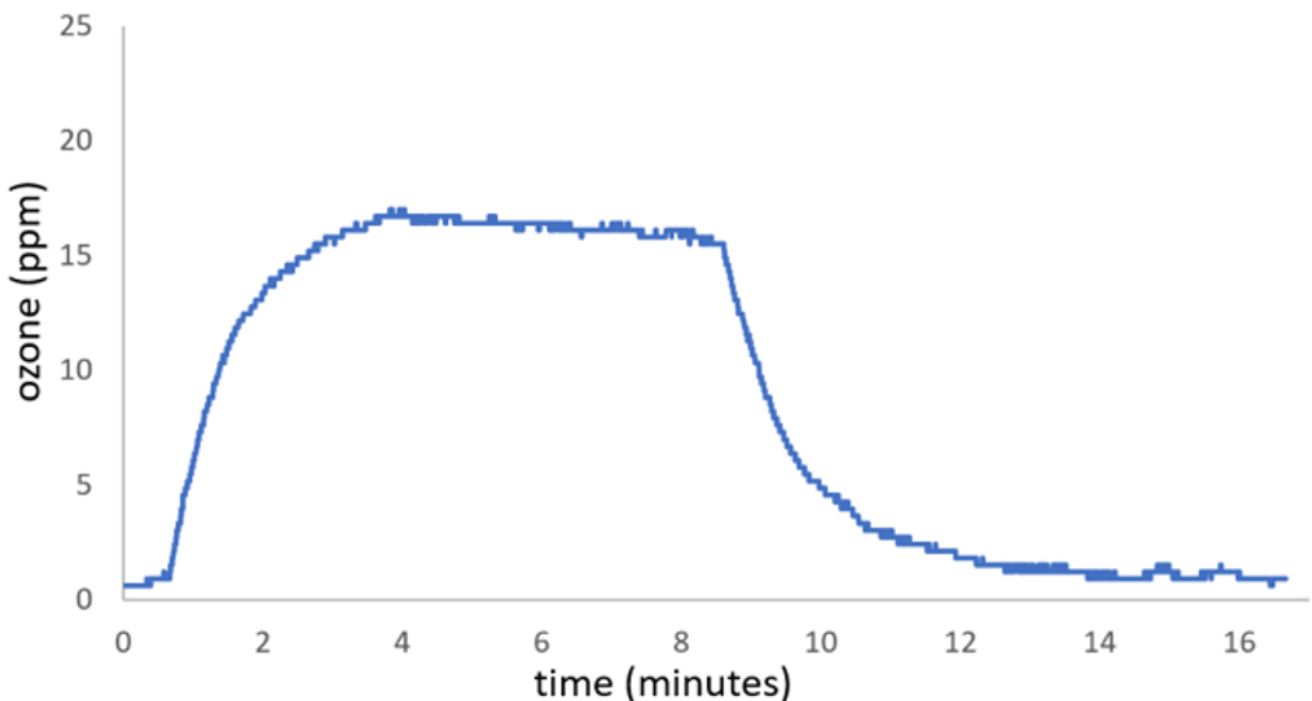

Figure 5. *This is the same setup as the two figures immediately above, but in this case the lid of the box was opened only slightly, enough to allow a 3/16" ID thick-wall silicone tube to be introduced, through which ozone was pumped into the box. The ozone generator was able to exceed the minimum desired concentration of 10 ppm, but only achieved about 17 ppm, and was unable to easily achieve 20 ppm as it had when the lid was tightly and correctly fitted. LESSON: you can just flop the ozone tube into the box if you don't have a drill, but it will not work as well. Note also that, unlike the first figure above, the ozone concentration decay is very rapid even when the lid is only cracked open slightly.*
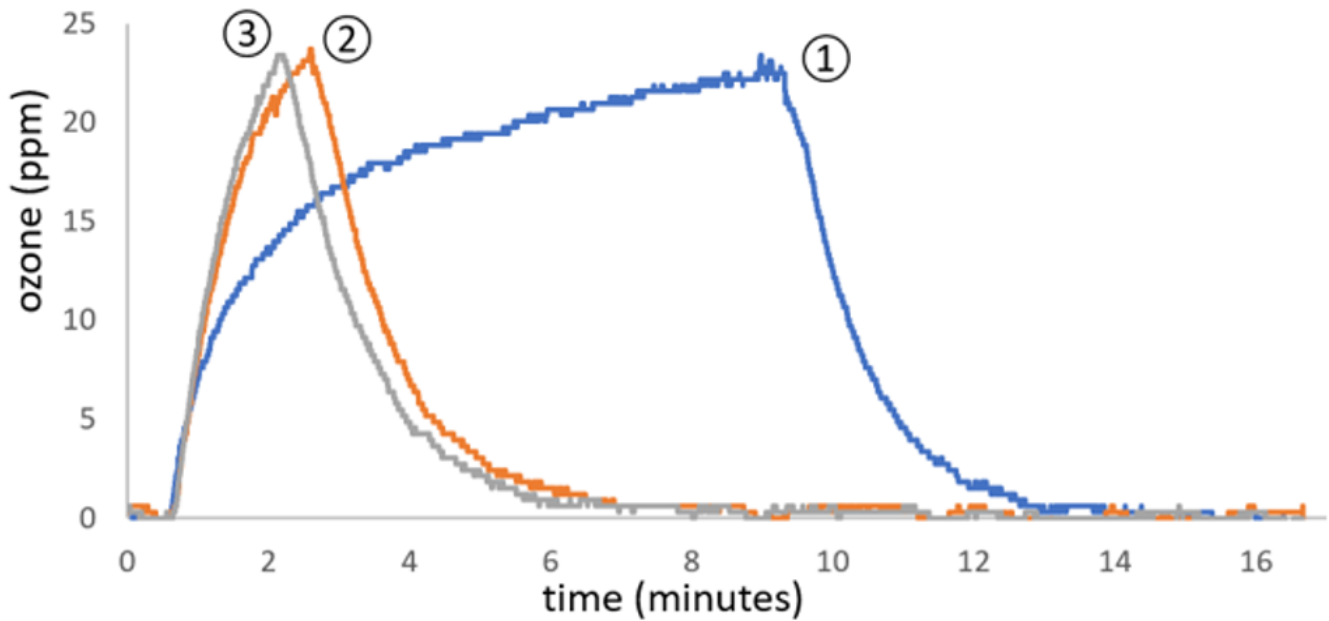
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Figure 6. *Effect of loading the box with ozone-quenching materials. This will simulate an actual decontamination cycle using dirty and porous materials, and also demonstrates the "wall effect", which reduces the half-life of ozone because it comes in contact with a surface (a wall). The ozone box was loaded with two small cardboard boxes (220mm x 80mm x 160mm each, total area ~ 1,300 cm^2 for each box), one pair of used nitrile gloves, and a used N95 face mask. The lid was fitted tightly and a decontamination cycle was performed using an ozone bubbler specified to produce 600 mg/hr ozone ("A2Z"). The first ozone concentration charging and decay cycle is shown in trace (1). It took nearly 10 minutes on the first decontamination cycle to achieve maximum ozone concentration, which was above 20 ppm. The lid of the decontamination chamber box was removed, and the remaining ozone was wafted out. The lid was replaced, followed immediately by a second (2), and then a third (3), decontamination cycle. The items were not removed, and each cycle followed immediately after the previous cycle. Note that ozone charging times are reduced with each cycle. This result demonstrates clearly that dirty, contaminated, and large surface area materials will initially exert a powerful quenching action on ozone. After the first decontamination, the ozone quenching action is very significantly reduced, with much smaller reductions in subsequent decontamination cycles. LESSON: when decontaminating dirty stuff, or a lot of stuff, decontaminate for longer and keep the ozone running longer, and consider decontaminating more than one cycle.*
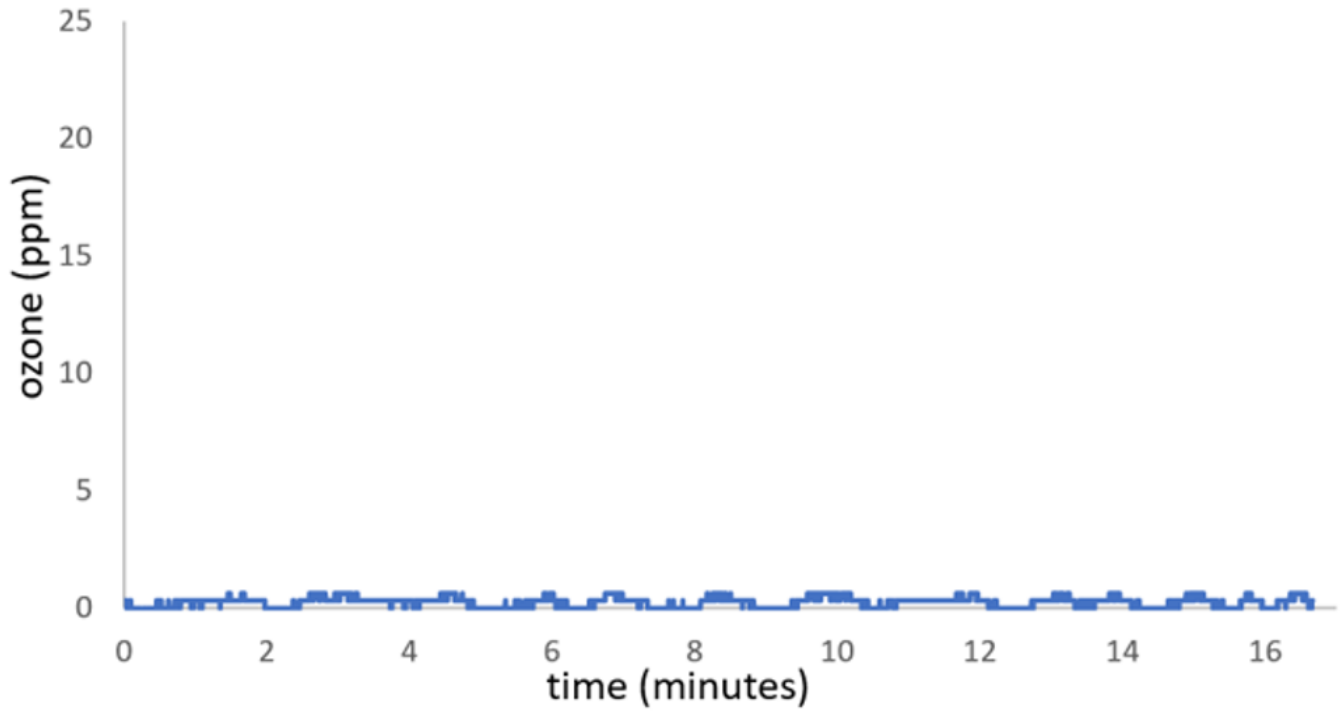
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Figure 7. *Demonstration of the effectiveness of an automobile cigarette lighter plug-in ozone generator. These may reduce odors in cars, but they do not achieve measurable levels of ozone, even in an otherwise small and sealed container. These are not advisable to use for the applications described in this document. LESSON: don't bother trying to use this type of ozone generator for this application.*
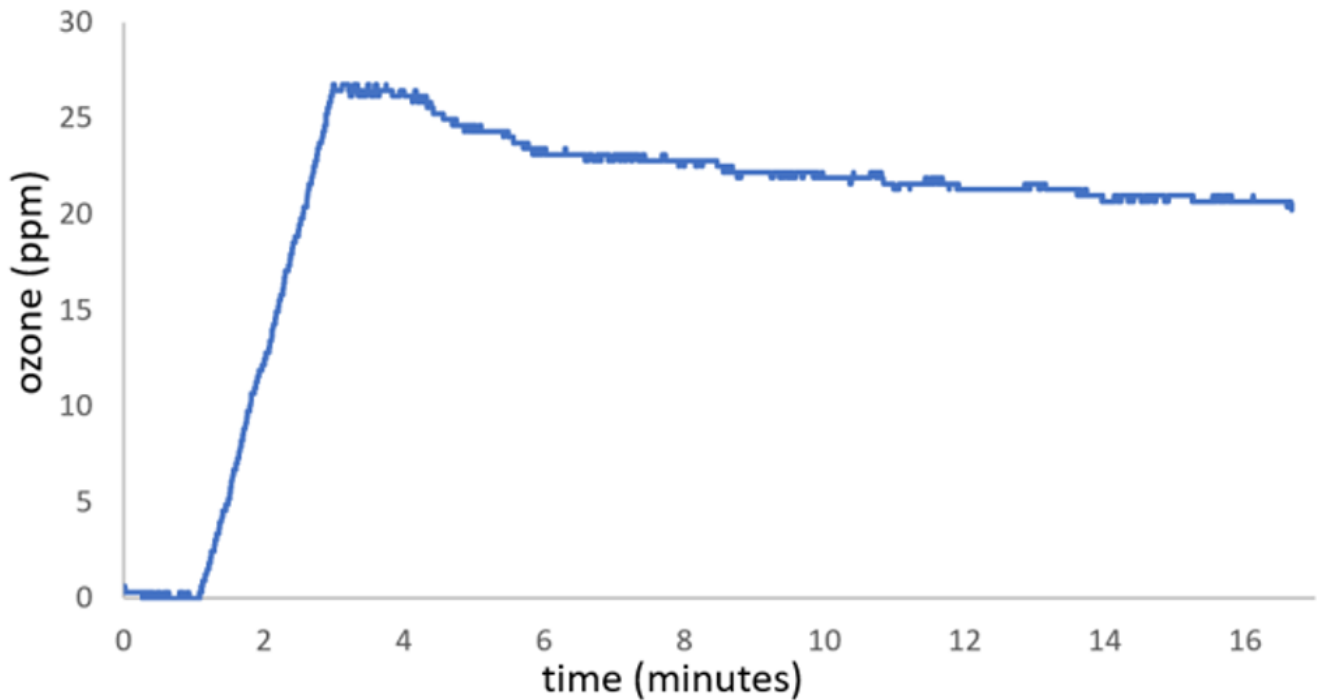
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Figure 8. *The use of a generic ozone bubbler, of the sort sold commercially for decontaminating fruit and vegetables and ozonating water. This was done using the same well-sealed 24 L Sterilite storage box as used above. This very inexpensive generic ozone "bubbler" generator (they had been given away as gifts to meeting participants at SOPMed 2019), performed very adequately and could be used to great effect for the viral inactivation applications described herein. An ozone concentration well in excess of 20 ppm was quickly and easily achieved, and much higher concentrations appear to have been possible, but could not be verified because of the upper limit of the ozone sensor that was available for this study. When turned OFF, the ozone concentration decayed slowly in the empty sealed box, as expected. LESSON: generic commercially-available ozone "bubblers" that produce ozone at least at the rate of 400 to 500 mg/hr appear to work well for the applications described in this document.*
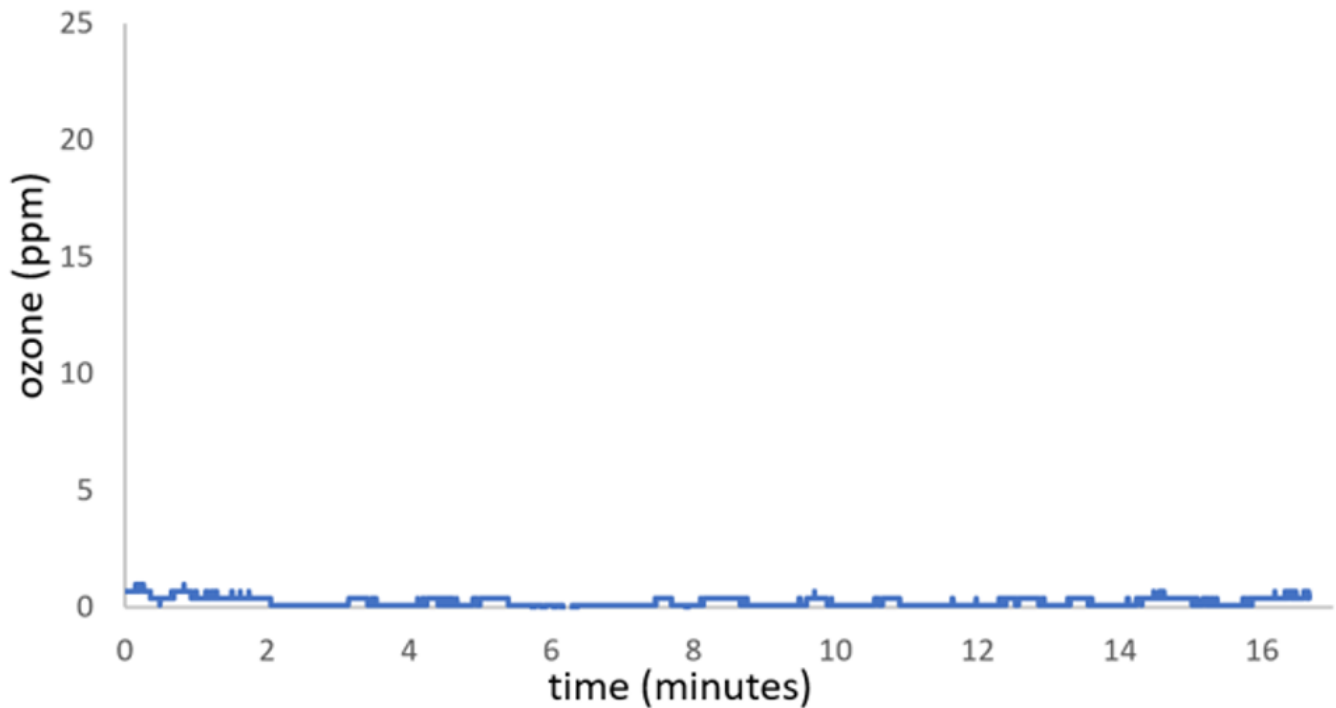
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Figure 9. *Demonstration of the use of a small, USB-powered room air freshener disk (see table of ozone generators tested). Though it was able to generate a distinct ozone odor when powered ON, as did the cigarette lighter ozone generator, when sealed into a chamber it was unable to generate measurable concentrations of ozone, though running continuously at full power, and therefore would be unsuitable for the viral inactivation applications described herein. LESSON: don't bother trying to use this type of ozone generator for this application.*
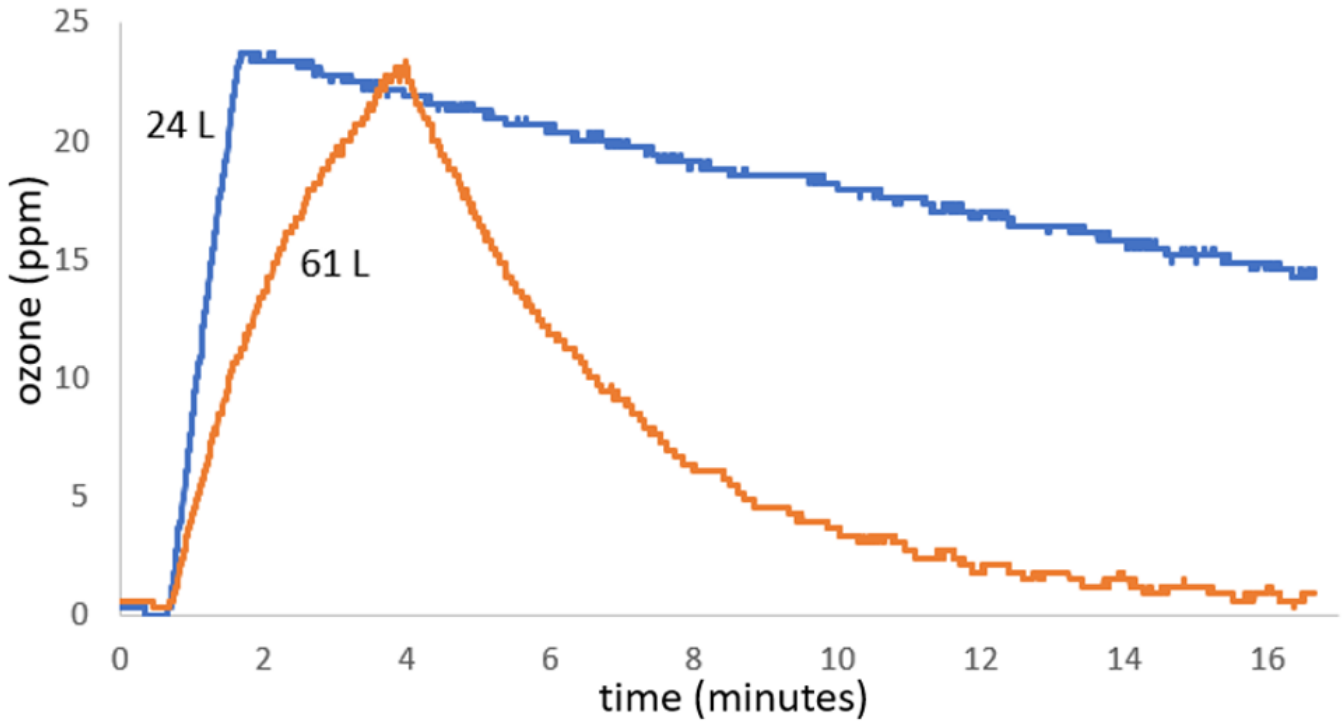
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Figure 10. *Charging a much larger decontamination box, in this case 61 liters, compared to the smaller box (24 L) used for all testing shown in prior graphs. This provides a useful comparison, because the boxes were of otherwise identical design, though the smaller one was 24 liters compared to 61 liters, and the ozone generator that was used was the same. As expected, the ozone charging time was much longer (141 seconds for 61 L, 48 seconds for 24 L), and less linear, suggesting ozone concentrations in the larger box could not reach as high as they might in the smaller box. Also, the decay rate was much faster, which may be due to the fact that the interior of the larger box was quenching ozone aggressively, and possibly that the larger box was somewhat leakier. LESSONS: Larger boxes can work, but they need more time to charge, would require more continuous ozone injection, and may benefit from a more powerful ozone generator.*
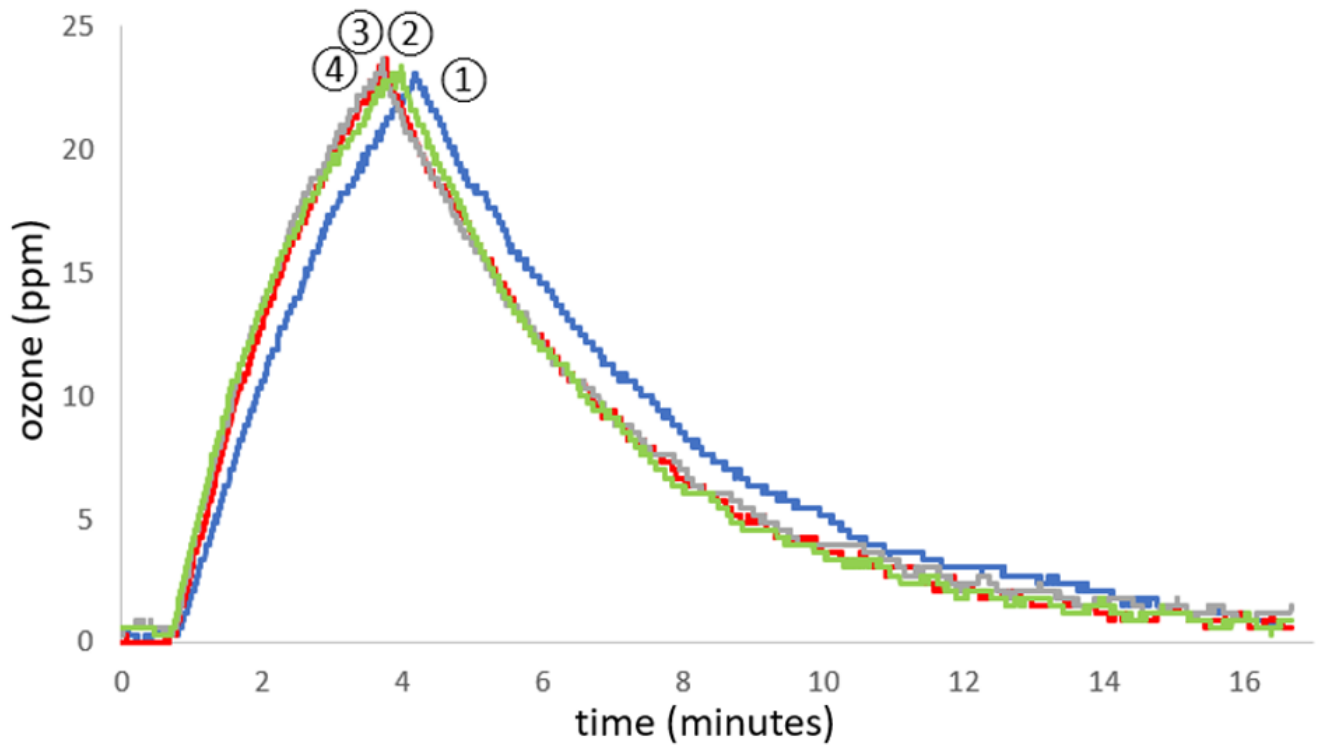
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Figure 11. _Showing the minimal quenching effects of a new, empty 61-liter box. Made of polypropylene, the box wall itself does not appear to have a significant quenching effect on ozone, as shown in four consecutive decontamination cycles._
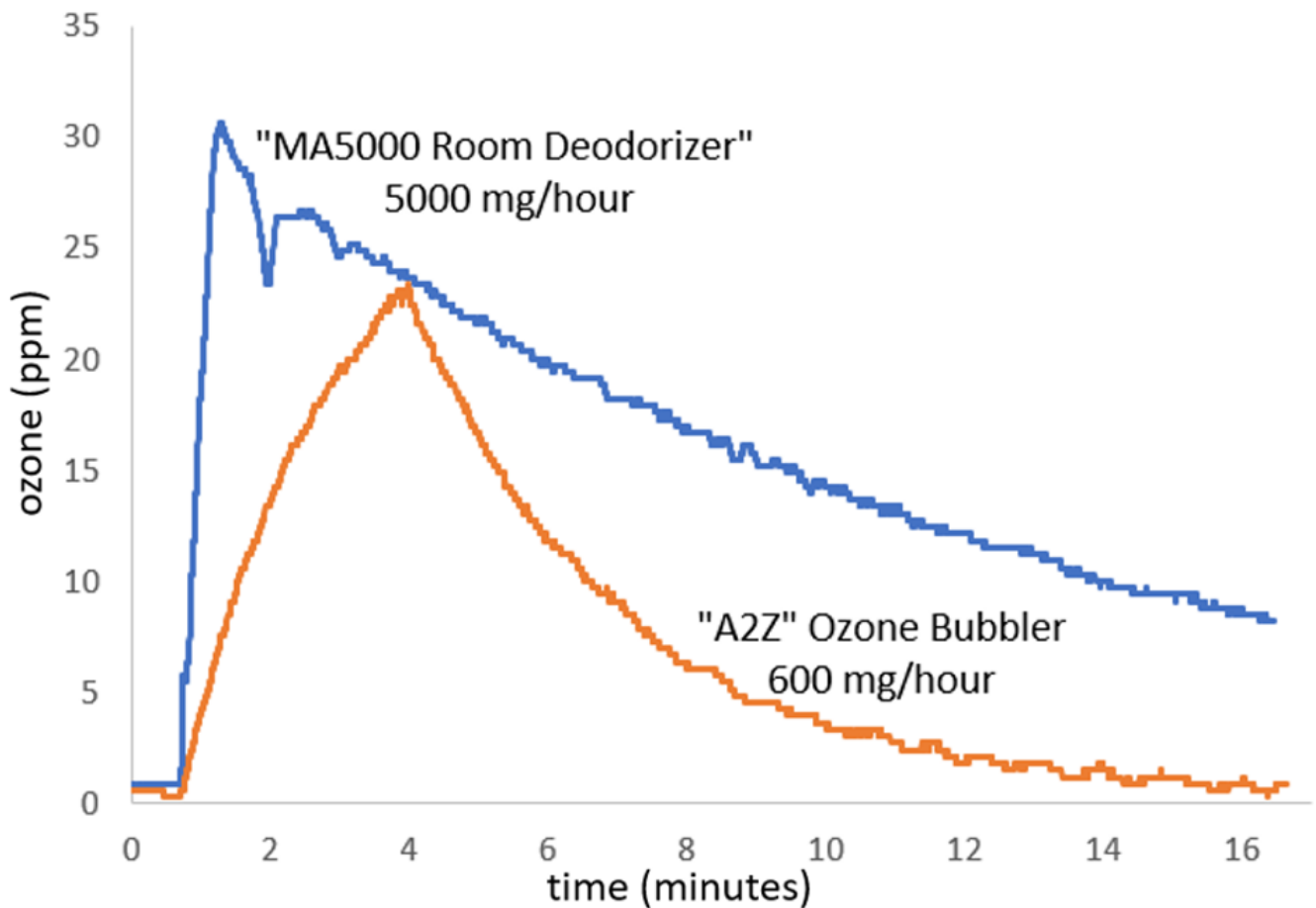
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Figure 12. *Comparison of a commercial, high output ozone generator (5000 mg/h) compared to a much lower ozone output "bubbler" (600 mg/h), as used in all previous experiments. The high-capacity generator was able to charge the 61-liter volume to 20 ppm in 19 seconds, whereas the lower capacity ozone generator required 141 seconds to achieve 20 ppm.*

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List of Ozone Generators Tested

Bubbler type (with tubing)

Figure 13. *A2Z (water-bubbler type for sanitizing fruit and vegetables). Rated ozone production: 600 mg/hr with enriched oxygen input .Source: amazon.com. For performance, see Figures: 2, 3, 4, 5, 6, 10, 11, 12.*

Figure 14. *"A Fresh Thing" (water-bubbler type for water, oils, etc.). Rated ozone production: estimated 400 – 600 mg/hr. Source: http://www.afreshthing.com/ For performance, see Figure: 8.*

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Figure 15. *"O3 Pure" (water-bubbler type for water, oils, etc.). Rated ozone production: 600 – 650 mg/hr. Source: amazon.com. For performance, see Figure: not shown, but equivalent to A2Z.*

Room (commercial)

Figure 16. *"MA5000" (commercial, for whole-room use). Rated ozone production: 5000 mg/hr. Source: amazon.com. For performance, see Figure: 12.*

"Plug-in" Air freshener

Figure 17. *"FULOXTECH" (USB plug-in). Rated ozone production: 2 mg/hr. Source: amazon.com. For performance, see Figure: 9.*

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Figure 18. *"Car Air Purifier" (Cigarette lighter plug-in). Rated ozone production: 0.005 mg/m3 over a period of 36 hours. Source: amazon.com. For performance, see Figure: 7.*

These data are not intended to suggest that these products do not work as advertised. The data presented reflect only the performance of these products for the specific test conditions reported in this paper.

Practical Reccommendations

Although the measurements reported in this paper were taken under laboratory conditions to allow repeatability and accuracy, in practice these findings can be used under extremely simple and realistic conditions.

Building the system

1. **Select an ozone generator:** We suggest getting an ozone generator rated at least at 400 mg/hr, with the express purpose of generating ozone for disinfecting fruit and vegetables, or ozonating a large volume of air. The small, discrete, types do not generate enough ozone rapidly enough to build a practical improvised disinfection system that can be expected to perform well in 10 to 20 minutes.
2. **Select a box:** Convenient volumes for mail and small packages seem to be in the range of 40 to 80 liters. Smaller boxes such as the 24-liter box reported in this paper can be used if the intended use is for few and small objects. Be sure the box has a tight-fitting lid (it does not have to be air-tight, but should not have large gaps), but it should be easy to remove and replace.
3. **Decide how ozone will be introduced into the box:** If the ozone generator has an outlet tube, simply drill a tight-fitting hole in a convenient location on one side of the box, insert the tube, and pull it into the box. For the room-type ozone generators without an outlet tube, you will need to find a way to place it inside the box and allow it to operate without obstructing the inlet or output grill on the ozone generator, or you will need to fashion a duct system to allow the ozone generator to be placed outside the plastic box, while ducting the ozone into the box.
4. **Seal up holes and cracks:** You may need to drill extra holes in the box for power cables, etc. You can seal holes and cracks with a silicone sealant, or with simple tape if that is all that is available. Ozone may degrade these, so they should be checked occasionally and repaired if necessary.
5. **Location:** We suggest setting the improvised system up outside, to prevent generating excess ozone gas in living or working spaces. A convenient location near a door where objects can be placed before bring then into the home, or objects from the home/office can be taken outside briefly for disinfection, with an electrical outlet nearby, and propped up on a small table, has prove to be extremely convenient and useful, as we have been field testing these systems for approximately one month.
6. **Helpful options:** Placing bits of wood or plastic into the box will help lift items to allow

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airflow around all surfaces to be disinfected. Tools for lifting, grabbing, and carrying, such as tongs and gloves, can be kept in the box and used to pick up items such as packages and mail for placement into the box. These tools will get disinfected after every use if kept in the box.

Operating the system

1. **Power:** Set things up so that the ozone generator can be easily turned ON and OFF.
2. **Testing and prep:** Before use, we suggest you test the system by cycling it a few times. The smell of ozone should be obvious, but be careful not to inhale it deeply or for long periods. Several cycles of testing will also reduce the ozone quenching due to the box itself, which will improve performance.
3. **Operation:** As it is unlikely that an ozone sensor will be generally available, we advise conservative (a bit extra) use of ozone, but excess use is probably not necessary. As a very rough rule of thumb, we recommend running the ozone generator for at least enough time to charge the box volume to 10 – 20 ppm (See METHODS for a simple calculation). For the lower-capacity "bubbler" type ozone generators and a typical large plastic storage box, it may be necessary to allow 3 to 5 minutes to bring the box up to the desired concentration, and it may be necessary to keep the ozone generator ON to maintain the exposure for at least an additional 10 minutes, but this time could easily be extended to 30 minutes if difficult surfaces, such as stainless steel [1], are involved, or simply out of an abundance of caution. If a room-size commercial ozone generator is used, there is a risk that excessive ozone will accumulate in the limited volume of the box. Based on the data above, a room-sized ozone generator can easily charge even a large plastic storage box to well above 20 ppm in less than 30 seconds. It may be necessary to introduce the ozone only briefly, or in short bursts, if using a high-capacity ozone generator.

Summary of Findings

Ozone can be easily used to make an improvised disinfection box system using inexpensive and readily available components. The ozone concentrations necessary for effective virucidal inactivation remain sparsely reported, but have been estimated for this application, based upon extrapolations of the available scientific data. The required ozone concentrations of 10 to 20 ppm are easily achieved and maintained for the necessary period of at least 10 minutes by continuous or intermittent operation of the ozone generator. There are important safety and health factors to consider when using ozone, but otherwise we conclude that it can be used widely, ion a large scale, as an improvised disinfectant, specifically for inactivating viruses.

Statement of Conflict of Interest

The authors have no financial or commercial interest in any of the material presented in this report, and claim no intellectual priority or rights. This information is offered entirely in the public interest, without restriction or limitation.

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https://www.fda.gov/medical-devices/safety-communications/potential-risks-associated-use-ozone-and-ultraviolet-uv-light-products-cleaning-cpap-machines-and

https://www.cdc.gov/niosh/topics/ozone/default.html

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