Technical Report for Ethylene Oxide-Based N95 FFR Decontamination and Reuse

Executive Summary
Ethylene oxide (EtO) decontamination is a widely used technique for sterilizing medical devices. The method is highly effective against a range of microorganisms including bacteria, viruses, and fungi. EtO can be used to decontaminate a wide variety of materials such as plastics and fabrics with no exposure to excessive heat, radiation, and moisture. Effective sterilization of N95 filtering facepiece respirators (FFRs) with EtO has also been reported. However, EtO is an inhalation-route human carcinogen, so chemical residue is a major unresolved concern for EtO decontamination. Long ventilation times are required to remove any residual EtO, and there is currently insufficient publicly-available data to determine a safe duration of ventilation for porous, multilayer respiratory devices such as N95 FFRs. Therefore, **EtO decontamination has not been proven safe for N95 FFR reprocessing and is therefore currently NOT recommended.** EtO has not been granted emergency use authorization (EUA) by the United States Food and Drug Administration (FDA) for decontamination of N95 respirators.

1. Overview
Ethylene oxide (EtO) decontamination is an established medical sterilization and decontamination method used to sterilize a wide array of medical devices for over 80 years (Freeman & Barwell, 1960). EtO is a highly cost-effective method with both antimicrobial and antiviral activity (Sidwell et al., 1969); (G. C. Mendes et al., 2011); (G. C. C. Mendes et al., 2007). EtO sterilization does not require the use of high heat and is compatible with a large variety of materials, including plastics and fabrics, with a broad spectrum of antimicrobial activity (American Chemistry Council, 2019). For this reason, approximately 50% of all sterile medical devices in the U.S. are sterilized with ethylene oxide; this includes gauze, surgically implantable devices, and surgical instruments (FDA, 2020).

Multiple academic groups have reported effective sterilization of N95 FFRs with EtO with fully-retained particle filtration, N95 fit, function and complete lack of odor (Viscusi et al., 2009), (Bergman et al., 2010), (CDCb, 2020), (Kumar et al., 2020). The technique has robust throughput, allowing a single large commercial facility to decontaminate as many as 100,000 N95 FFRs per week, as the company Medline has announced with the re-purposing of its Illinois plant (Crotti, 2020). The prohibitive factor for EtO reprocessing of N95 FFRs is the risk of chemical exposure and potential harm to the wearer caused by EtO residuals on reprocessed N95 FFRs. Furthermore, EtO gas is carcinogenic, flammable, and explosive, so decontamination processes must also be operated by well trained personnel (Shintani, 2017).
Alkylation is the mechanism of antimicrobial/antiviral action of ethylene oxide, which can cause toxicity in humans if residues remain on medical devices, requiring careful engineering controls and regulations (Salter et al., 2010). EtO is an inhalation-route human carcinogen (EPA, 2016). Due to the proximity of reused respirators to the user’s airway, residual EtO presents a major health risk. The allowable limits of ethylene oxide residues and by-products (ethylene chlorohydrin (ECH) and ethylene glycol (EG)) are specified in (ISO 10993-7:2008, 2016). These limits are established using health-based risk assessment studies (ISO 10993-7:2008, 2016). There is no standard method for determining residuals and each material has its own properties of residual deposition. Thus, it is important to have residuals tests conducted at the aeration phase of sterilization (Shintani, 2017).

Ethylene oxide is compatible with most materials such as metals, ceramics and most polymers (e.g. polyacetal, polyester, silicone). Ethylene oxide is less compatible with polymers that are sensitive to humidity, low temperature and high EtO gas concentration (Lerouge, 2010). Due to the toxicity and carcinogenicity of EtO, an aeration phase immediately following sterilization is crucial to remove residual EtO and by-products that may remain on reprocessed equipment. The duration of the aeration phase that follows the sterilization is material-dependent, ranging for a few hours for metals to several weeks for polymers that can rapidly absorb EtO but desorb much slower (Lerouge, 2010). The typical time for EtO sterilization of medical devices ranges from 12 hours to several days (Lerouge, 2010). By using a long ventilation or aeration process following the EtO sterilization, residual levels of EtO on an N95 FFR may be reduced to levels below those determined to be generally acceptable by OSHA and FDA. However, the United States Centers for Disease Control and Prevention (CDC) cautions that inner layers of an N95 respirator may retain residual EtO. Due to the health risks of EtO exposure, careful studies and controls are crucial prior to implementing EtO-based N95 FFR reprocessing.

2. State of Federal Guidance

The United States Food and Drug Administration (FDA) regulates, monitors and sets standards for the safety of medical drugs and devices in the United States. The FDA has established regulatory authority over medical devices sterilized by ethylene oxide. To provide guidance for the EtO sterilization process, two voluntary consensus standards describe how to develop and control EtO decontamination processes, as well as validate that residual levels are acceptable (FDA, 2020):


Specifically, the exposure limit in general for a medical device used for less than 24 hours is 4 mg per device. Although the FDA has approved the use of EtO for a large variety of
medical equipment sterilization, to date, the FDA has not approved any method using EtO for N95 FFR decontamination.

In April 2020, OSHA released a recommendation against immediate use of EtO, stating that direct studies had not specifically demonstrated that residual levels were below the permissible EtO levels (OSHA permissible exposure limits: 1 ppm airborne EtO in the workplace and an action level of 0.5 ppm as an eight-hour time weighted average – 29 CFR § 1910.1047). These studies are pending or not public. Although EtO is a robust decontamination method, concerns of health risks caused by EtO residuals on N95 FFRs have not yet been resolved. Further testing on processes by specific vendors is required to quantify the toxicity hazard for a given process.

At the time of this publication in July 2020, the CDC stated EtO method was “promising but may be harmful to the wearer” and outlined concerns with throughput and health risks to wearer from even low levels of residual EtO gases as EtO is carcinogenic and teratogenic (CDCb, 2020). Similar to OSHA above, they called for further studies to ensure no off-gassing into the breathing zone of the wearer, citing a number of references regarding chronic occupational health risks including risk of neurologic damage, carcinogenesis, and teratogenesis that might occur at EtO plants. Medline has applied for emergency use authorization (EUA) for ethylene oxide decontamination of N95 respirators however as of July 24, 2020 the FDA has not approved an EUA for ethylene oxide (FDA, EUA, 2020).

3. Mode of Action

Ethylene oxide is a three-ringed epoxy compound with the molecular formula \( \text{H}_2\text{COCH}_2 \) with a molecular weight of 44 (see structure on right). At room temperature and atmospheric pressure, ethylene oxide is a colourless, highly reactive, and flammable gas. It is reactive in both the liquid and vapour phases (Liteplo & Meek, 2003).

Mechanistically, EtO is an alkylating agent that leads to microbial (bacterial, fungal, and viral) inactivation. Alkylation of nucleic acids and proteins leads to their denaturation, causing microbial inactivation. The chemical groups that undergo alkylation (such as sulfhydryl, hydroxyl, amino, and carboxyl groups) are not present in most medical devices and EtO does not affect their molecular structure. EtO does not require metabolic activation and is highly diffusible, both properties that support its ease of use (G. C. C. Mendes et al., 2007). Medical devices made from certain polymers (plastic or resin), metals, or glass, or that have multiple layers of packaging or hard-to-reach places (for example, catheters) are commonly sterilized with ethylene oxide (FDA, 2020).

Ethylene oxide sterilization can be divided into 3 stages: (1) preconditioning and humidification, (2) in-chamber processing (EtO gas introduction, exposure, evacuation), and (3) aeration. The aeration process is the most time-intensive and proprietary methods are used to increase removal of gas residues. Exact processes vary by vendor, but generally include vacuum and air flushes, heated aeration, and spacing of devices to limit EtO residuals. The three stages of ethylene oxide sterilization can be performed within a chamber or multiple chambers (CDC, 2019; Shintani, 2017).
4. **SARS-CoV-2 and Other Pathogen Inactivation**

Ethylene oxide is a sterilizing agent that has broad virucidal activity. EtO penetrates fabrics and is expected to create a uniform decontamination of all surfaces. Furthermore, it has been shown to destroy a variety of viruses, bacteria, and other pathogens (Kumar et al., 2020); (Sidwell et al., 1969), including pathogens known to be more resistant to decontamination than SARS-CoV-2 (e.g., bacterial spores). Data on the antiviral and microbial activity of EtO are shown in Table 1.

(Kumar et al., 2020) used 4 different FFRs (3M 1860, 3M 1870 and VFlex 1804 and AO Safety 1054S), inoculated with Vesicular stomatitis virus (VSV) as a surrogate for SARS-CoV-2. Following the EtO decontamination cycle, the authors observed a reduction of greater than 6-log of infectious virus.

(Sidwell et al., 1969) developed a quantitative method for evaluating the virucidal activity for ethylene gas. The authors used decontamination of 1 mL of virus solution pipetted on wool gabardine, using 4 different virus strains: Herpes simplex, vaccinia, parainfluenza, and polio. Using a single EtO cycle at either 29°C for 180 min or 60°C for 48 min, titer reductions and swatch cytotoxicity to varied with the virus with reductions from 2.7 to 5 log for Herpes simplex, from 4 to 6.1 log for Vaccina, from 1.8 to 4.9 for Parainfluenza and from 4.9 to 7.7 log for Polio (Sidwell et al. 1969).

5. **Integrity of N95 Filtering Facepiece Respirators**

EtO has broad applications in decontamination of medical products and medical devices due to its effectiveness at lower temperatures and its general compatibility with common equipment including gowns, gauze, various resins, and medical devices. Multiple N95 FFR models have been shown to maintain filtration efficiency as well as form and fit after EtO decontamination cycles (Table 2). The specific analysis of form and fit factor is vital, as improper fit leads to a decreased protection factor (Reponen et al., 2011).

(Viscusi et al., 2009) report that EtO decontamination on 6 different N95 FFRs using a single cycle of 1 h EtO exposure followed by 4 h of aeration both had no effect on filter aerosol penetration, filter airflow resistance, or physical appearance of the N95 FFRs. Viscusi et al. (2009) used a 1h EtO exposure followed by a 4 h aeration cycle and 0.14 m³ chamber volume. The authors believed that a 4 h elimination phase would be sufficient to eliminate residuals on the N95 FFRs, though experiments were not carried out to validate this. More recent studies have examined significantly longer aeration times.

Another study by (Bergman et al., 2010) expands on the (Viscusi et al., 2009) work by looking at three cycles of EtO decontamination. (Bergman et al., 2010) report that the N95 FFRs retained their standard filter aerosol penetration of <4.01% even after three EtO treatment cycles. In this paper, they repeated the decontamination method on each N95 FFR in triplicate and increased the cycles of decontamination from one to three. Their evaluation showed no decrease in filter aerosol penetration and filter airflow resistance, as well as no changes in physical appearance and lack of any residual odor.
As mentioned in the previous section, in the study by (Kumar et al., 2020) using 4 different N95 FFRs subjected to 1 and 3 decontamination cycles of EtO, the authors observed no reduction in quantitative fit (using TSI PortaCount 8038+) during both normal and deep breathing exercises. Kumar et al. (2020) noted that although the N95 can be effectively decontaminated using ethylene oxide, more advanced studies are needed to ensure no ethylene oxide residues remain on the respirators.

6. Data Summary Tables

Table 1. Impact of Ethylene oxide on viruses.

<table>
<thead>
<tr>
<th>Author</th>
<th>Method of Challenge</th>
<th>Dose</th>
<th>Cycles of EtO</th>
<th>Challenge organism</th>
<th>Effectiveness (log reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Spotting on outer N95 surface (3 spots)</td>
<td>6.75 log TCID50 (VSV)</td>
<td>1 and 3 cycles</td>
<td>Vesicular stomatitis virus, Indiana serotype (VSV)</td>
<td>&gt;6 log reduction of infectious VSV particles.</td>
</tr>
<tr>
<td>B</td>
<td>1 mL of virus solution pipetted on wool gabardine</td>
<td>$10^6 - 10^8$ CCID50</td>
<td>1 cycle: either 29°C for 180min or 60°C for 48min</td>
<td>Herpes simplex, vaccinia, parainfluenza, and polio</td>
<td>Titer reductions (using either cycle): Herpes simplex: from 2.7 to 5 log Vaccinia: from 4.0 to 6.1 log Parainfluenza: from 1.8 to 4.9 log Polio: from 4.9 to 7.7 log</td>
</tr>
</tbody>
</table>

A: (Kumar et al., 2020), B: (Sidwell et al., 1969)

Table 2. Impact of EtO on N95 Performance

<table>
<thead>
<tr>
<th>Author</th>
<th>N95 FFRs</th>
<th>Dose</th>
<th># cycles</th>
<th>Time (min)</th>
<th>Filtration efficiency</th>
<th>Filtration Effectiveness</th>
<th>Respirator damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6 different N95 models (n = 3 per model)</td>
<td>725 mg/L 100% EtO gas at 55°C</td>
<td>1</td>
<td>1h treatment, 4h aeration</td>
<td>Unchanged from controls.</td>
<td>Unchanged percent filter aerosol penetration (%P) and mean initial airflow resistance to untreated (“as-received”) NIOSH-approved N95 FFRs.</td>
<td>No observed physical changes or odor.</td>
</tr>
<tr>
<td>B</td>
<td>6 different N95 models (n = 6 per model)</td>
<td>736 mg/L 100% EtO gas at 55°C</td>
<td>1 and 3</td>
<td>1h treatment, 12h aeration</td>
<td>Unchanged from controls.</td>
<td>%P &lt;4.01% and mean initial filter airflow resistance &lt; 17.6 mm H2O (both the same as untreated controls), at both 1 and 3 cycles.</td>
<td>No observed physical changes or odor.</td>
</tr>
<tr>
<td>C</td>
<td>4 different N95 models (n = 1 per model)</td>
<td>Not given</td>
<td>1 and 3</td>
<td>1h treatment, 12h aeration</td>
<td>Not tested.</td>
<td>-</td>
<td>Using PortaCount Result for structural integrity: All FFRs retained structural integrity for both normal &amp; deep breathing exercises.</td>
</tr>
</tbody>
</table>
7. Strategies

Ethylene oxide decontamination is a cost-effective, low-temperature sterilization process that can occur at various scales, including on-site modular units in hospitals or off-site on industrial scale sterilization facilities, of which there are numerous throughout the US (G. C. C. Mendes et al., 2007); (FDA, 2020); (G. C. C. Mendes et al., 2007). These industrial sites are used to sterilize heat-sensitive reusable medical devices, many of which are used in elective procedures. During the COVID-19 pandemic, elective medical and surgical procedures have been cancelled during times of case surge, which may temporarily increase EtO capacity for PPE decontamination.

Proper regulatory and chain of custody controls may allow a small clinic to collect N95 FFRs in an appropriate container and ship them to a central facility for decontamination and aeration. For example, Medline announced plans to use EtO decontamination to decontaminate as many as 100,000 N95 FFRs per week (Crotti, 2020). Many hospitals have modular EtO units onsite for sterilization purposes; these units are sold by various vendors (3M, 2020). In-hospital units may have significantly lower throughput and less robust vacuum based aeration than larger systems. EtO processes require extensive residual testing and regulatory oversight prior to use on N95 FFRs.

8. Primary Risks, Occupational Safety, Unknowns

The toxic effects of EtO are concentration-dependent and range from mild to severe. Long-term and occupational exposures to EtO have been linked to cancer resulting in its classification as a carcinogen in humans (IARC, 2012); (Liteplo & Meek, 2003). EtO alkylation of genomic DNA causes accumulation of mutations causing the induction of a variety of tumors, such as leukaemia, lymphoma, lung cancer (Liteplo & Meek, 2003). The CDC reiterated the risk of cognitive impairment, polyneuropathy, cataracts and other neurologic dysfunction with chronic occupational exposures to EtO in a 2008 review (Rutala & Weber, 2008).

Ethylene oxide is highly toxic, haemolytic, and mutagenic, and is an inhalation-route human carcinogen (Lerouge, 2010). These cautions are of particular concern when considering ethylene oxide for decontamination of respiratory devices. EtO is currently not recommended for decontamination of N95 FFRs due to the present lack of data on removal of carcinogenic residues following EtO processes.

Safety measures also need to be taken when decontamination personnel handle ethylene oxide to minimise any exposure to residues and byproducts (e.g. ethylene chlorohydrin, and the less toxic ethylene glycol (Lerouge, 2010)). The desorption of EtO depends on the material (Buben et al., 1999); (Gilding et al., 1980); (Lucas et al., 2003); see also Table 3). (Lucas et al., 2003) used the ANSI/AAMI/ISO (ISO 10993-7:2008, 2016) method to test for EtO residues on two different polyurethanes, Pellethane 2363-75D (PU 75D) and 2383-80A (PU 80A), and Nylon 66. Results of their study indicated variation in EtO residuals with the polymer (PU 75D and Nylon 66 had higher EtO residuals than PU 80A) but the
sterilization cycles had limited impact on the amount of EtO residuals. It is worth noting that the aforementioned studies use methodologies that might not be applicable to N95 FFRs. Furthermore, the significant material dependence of residual EtO indicates that the amount of residual EtO on the facepiece, straps, and nose foam of N95 FFRs following treatment may be model-dependent.

Studies related to EtO residuals in N95 FFRs are limited (see Table 3). (Salter et al., 2010) published an analysis on residual chemicals remaining on face masks following decontamination. Using 6 different N95 FFRs in triplicate, each model was exposed to EtO for 3h at 54°C, followed by 12 h of aeration. Although no residual EtO was detected, the EtO detection limit in this experiment (0.5 ppm) was higher than the NIOSH recommended exposure limit (<0.1 ppm EtO) (CDC, 2018). Additionally, traces of diacetone alcohol (DAA) and 2-hydroxyethyl acetate (HEA) were detected on the N95 FFRs, both at ≤3 times the signal-to-noise of the baseline. DAA is a Class II combustible liquid, yet the authors note that “an adjustment to the procedure or composition of the EO sterilant might eliminate this contaminant”. HEA is listed as a possible carcinogen and mutagen and was only detected on the elastic N95 FFR straps. Furthermore, both DAA and HEA were detected in trace amounts, so future studies must examine a quantitative concentration before clinical significance can be determined (Salter et al., 2010).

For EtO decontamination to be safe, proper processing is needed to prevent environmental release. Another safety concern is that ethylene oxide is extremely flammable (OSHA EtO MSDS) and could pose a safety risk to workers conducting decontamination. OSHA therefore has an established framework and workflows that are currently followed for sterilization of other medical equipment that would be applied to N95 FFR reuse. The EPA and other regulators have cited and shut down EtO facilities without engineering controls to prevent environmental release (Lim, 2019). Catalytic treatment modules convert EtO exhaust into carbon dioxide and water at >99.9% efficiency in ideal conditions (Wiser J., 2017).

Medical facilities follow EPA and OSHA specifications to reduce workplace EtO exposures and minimize potential health impact (EPA, 2016; OSHA, 2002). Ultimately, to ensure the safety of FFRs treated with ethylene oxide, rigorous testing must be done to ensure complete elimination of EtO and related breakdown products to null or below FDA standards.

Table 3. Data on Toxic residuals following EtO treatment of medical devices

<table>
<thead>
<tr>
<th>Author</th>
<th>Medical Device</th>
<th>EtO treatment</th>
<th>Toxic Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N95 FFRs</td>
<td>3 hours at 54°C, 12 hours of aeration</td>
<td>Traces of diacetone alcohol (DAA) and 2-hydroxyethyl acetate (HEA). No quantitative measurement, but both at ≤ 3 times the signal-to-noise of the baseline.</td>
</tr>
<tr>
<td>B</td>
<td>Variety of materials: PTFE, Polyester, Nylon, Silicone rubber, Biomer and Crystalline/glassy polymers (Acrylic)</td>
<td>30 mins at 55 °C, 2.5 hours aeration (at 0.5 mmHg and 90 °C)</td>
<td>All levels at 0ppm EtO (except for PVC at 5ppm). Crystalline/glassy polymers show high residue levels: up to 4500ppm</td>
</tr>
</tbody>
</table>
9. Conclusions

EtO has broad antimicrobial activity and could effectively decontaminate and sterilize medical devices including N95 FFRs without compromising function. Several small studies have demonstrated that N95 FFRs retain their filtration performance after at least 3 cycles of EtO decontamination. However, ethylene oxide is toxic, haemolytic, and mutagenic, and is classified as a human carcinogen by IARC and EPA. Special safety procedures must be taken to avoid any exposure to personnel that may be exposed to residuals or by products as a result of sterilization processes. Further independent data and FDA review would be needed to demonstrate the proper engineering controls result in safe N95 FFR decontamination, prevention of occupational exposures, and prevention of environmental releases.

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