LifeAire Systems

Creating Air for Life

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Ambient Air Quality Testing:

Volatile Organic Compounds - Location, Speciation and Concentration

Comprehensive Analysis, Report and Recommendations

[redacted]

VOC Canister Testing

[redacted]

CONFIDENTIAL INFORMATION

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1. INTRODUCTION

a. The Importance of Volatile Organic Compounds (VOCs) to the IVF Process

Volatile organic compounds (VOCs) are organic compounds with a high vapor pressure and thus, a high level of volatility [1]. They are a common airborne constituent of outside source air, recirculated air, HVAC components, equipment, personnel, and cleaning procedures. VOCs are ubiquitous and unique in their polarity, molecular weight and biochemical structure, and play a critical role in preimplantation toxicology and epigenetic processes [2].

Airborne VOCs are driven to reach thermodynamic equilibrium between the air phase and the water/cell culture media phase and are magnified in concentration as they partition from the air to the cell culture media. Once the VOCs have partitioned into the cell culture media, they become a permanent component of the media and cannot be removed. From the cell culture media, the VOCs partition into the cellular phase, inclusive of but not limited to, the gametes and embryos [3]. Once cellular, the individual VOCs exhibit known cytotoxic effects, potentially resulting in reduced blastocyst conversion, implantation, and clinical pregnancy rates [4-6]. Environmental factors cause physiological and cellular stress on developing embryos. These stresses lead to changes in gene regulation and/or expression which have imprinting and epigenetic effects, therefore it is critical to protect embryos and gametes from dangerous environmental contaminants [7].

b. Air Testing Performed

[redacted] is located at [redacted] Following conversations with the [redacted] team, LifeAire Systems was asked to conduct a comprehensive evaluation of VOCs. Sampling occurred in the IVF lab, with one sample occurring during the day and one at night. LifeAire Systems arranged the shipping of two canisters to [redacted] The canisters were set up by [redacted] personnel as instructed by LifeAire. Upon the completion of the sampling period, the canisters were shipped to an independent third-party testing and analysis laboratory for VOC identification, speciation, and concentration. All raw data of the analysis is available in a separate document and can be provided to the [redacted] team upon request. The sampling reported in this document was conducted on [redacted]

To perform a comprehensive evaluation of VOCs and to determine if there were localized point sources of VOCs, the US EPA TO-15 methodology was used. This is a broad-spectrum analysis with identification based upon gas chromatograph retention time and mass spectra. The third-party laboratory performing the analyses can identify up to 90,000 specific VOCs.

2. PROTOCOL USED FOR VOC ASSESSMENT – TO-15

a. Methods of Analysis for Volatile Organic Compounds:

The TO-15 sampling was performed by capturing one liter of air over an 8-hour period using a calibrated flow controller. The sample canisters are chemically clean, stainless-steel containers that have been evacuated to vacuum. The sampling canisters were obtained and shipped to [redacted] directly from Galson Laboratories in Syracuse, NY (third-party testing laboratory). A total of two (2) TO-15 canisters were placed: one in the day, one at night. This technique does not use a pump. At the end of the sampling period, the sample vessels were closed and packaged for shipment to Galson Laboratories to be analyzed.

The TO-14/TO-15 procedure is outlined in Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air- EPA 600/4-84-041, April 1984/1988. The instrument used was a Hewlett Packard Model 5989 GC/MS with an Entech 7000 cryogenic concentrator. A 100% dimethylpolysiloxane capillary column was used. Multiple blank samples were also run to verify the instrument baseline. All of the standards were less than the detection limit on the blank.

b. Quality Control for TO-15 Analyses:

All analyses were performed by the third-party laboratory using the following quality control procedures for the TO-15 analyses. Every 24 hours, the mass calibration on the mass spectrometer was checked with 4-Bromoflourobenzene. This compound was used to verify the accuracy of the generated spectra at several points on the mass spectra. The set-up calibration was performed by running the standards of approximately 60 common VOCs materials (TO-15) through the analytical instrumentation at five concentration ranges. The percentage of relative variation of all five standards is required to be less than 30% over the analytical range. After the mass spectrometer was tuned and set up calibration performed, a sample containing 50 nanograms of the following was run: 1, 1-dichloroethylene, benzene, toluene, chlorobenzene, and trichloroethylene. The determined concentrations must fall within 20% of the known values. Before analysis, a method blank was run to verify instrument stability and analytical integrity. Each sample was spiked with an internal standard. Recovery of these internal standards must be between 70% and 130%. Laboratory duplicates were run and all duplicates tested within +/- 20% of each other.

3. TEST RESULTS

a. TO-15 VOCs Identified – Location, Speciation, Concentration:

The tables listed below provide a summary of all VOCs present at ppb levels above detection specific to each testing area/time. All ppb by volume data was also converted to mass. The Cairo Consensus recommended total VOC level is less than 500 µg/m^3 [7].

Contaminant	Lab: Day Concentration ppbv	Concentration µg/m^3
Acetone	58	140
Ethyl Acetate	0.9	3.2
Isopropyl Alcohol	**1600**	**4000**
Methyl Ethyl Ketone	2.3	6.7
Pentane	14	40
Toluene	1.8	6.7
Butane	14	32
Total	**1691.0**	**4228.6**

Contaminant	Lab: Night Concentration ppbv	Concentration µg/m^3
1,2,4-Trimethylbenzene	1.8	9.1
Acetone	56	130
Cyclohexane	1.1	3.8
Ethyl Acetate	46	17
Ethylbenzene	1.2	5.3
Hexane	1.2	4.1
Isopropyl Alcohol	**950**	**2300**
Methyl Ethyl Ketone	1.4	4.1
Pentane	86	250
Toluene	17	63
m,p-Xylene	5.0	22
Butane	9.0	24
o-Xylene	1.9	8.4
Total	**1137.1**	**2840.8**

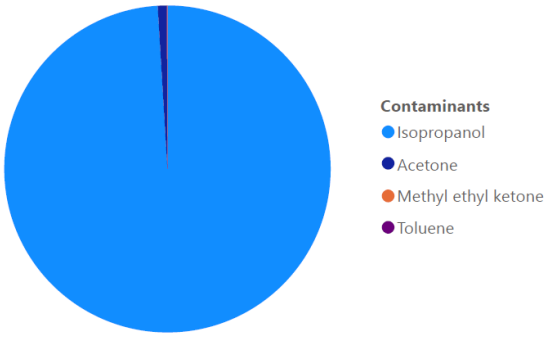
4. EMBRYONIC BURDEN AND TOXICITY (EBTOX) SCALE

The embryonic burden and toxicity (EBTOX) scale was developed by LifeAire and provides an indication of toxicity for specific VOCs. It utilizes the toxicity cofactors and the equilibrium modeling described in Fox's 2022 RBMO publication [3]. The EBTOX score is calculated by comparing the modelled toxic air phase concentration of each VOC to the measured air phase concentration. Any EBTOX score greater than 1 indicates substantial toxicity.

Note that the EBTOX scale is based upon toxicity studies from 23 VOCs that are commonly found in IVF facilities [3]. There exists insufficient data to appropriately predict the embryotoxicity of VOCs that fall outside of this scope, and therefore they are not currently included in the EBTOX contributions to toxicity. However, this does not exclude them from the possibility of presenting a serious chemical burden to exposed embryos. As a rule of thumb, it is best to remediate all VOCs to their lowest possible concentrations, especially when the potential for toxicity is unclear.

As shown in the tables and graphs on the following pages, of the previously modeled VOCs, isopropanol presents the most significant toxicity contributions at the concentrations detected in the lab. In both the daytime and nighttime samples, IPA has an EBTOX score much higher than 1, indicating that the concentrations measured in all samples are indeed embryotoxic. This high IPA concentration throughout is coupled with the presence of several other embryotoxic agents, including acetone, methyl ethyl ketone, and toluene, among others. With the extremely high concentrations of IPA as well as the various other VOCs being detected in this space, it is important that the appropriate sources may be identified and comprehensively remediated to prevent unforeseen risks to embryo health.

Day:

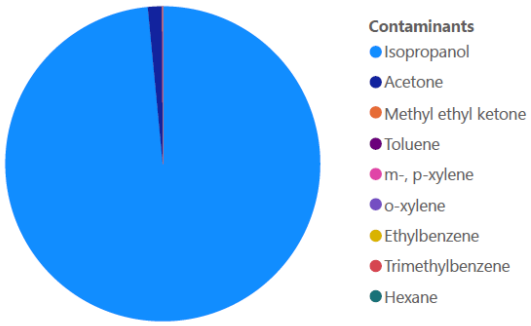


EBTOX Scores

Contaminants	EBTOX Score
Isopropanol	52.53
Acetone	0.48
Methyl ethyl ketone	0.02
Toluene	0.00

Contaminants	% Contribution to Toxicity
Acetone	0.91
Isopropanol	99.05
Methyl ethyl ketone	0.04
Toluene	0.00

Night:



EBTOX Scores

Contaminants	EBTOX Score
Isopropanol	30.21
Acetone	0.45
Methyl ethyl ketone	0.01
Toluene	0.00
m-, p-xylene	0.00
o-xylene	0.00
Ethylbenzene	0.00
Trimethylbenzene	0.00
Hexane	0.00

Contaminants	% Contribution to Toxicity
Acetone	1.46
Ethylbenzene	0.00
Hexane	0.00
Isopropanol	98.48
m-, p-xylene	0.00
Methyl ethyl ketone	0.04
o-xylene	0.00
Toluene	0.01
Trimethylbenzene	0.00

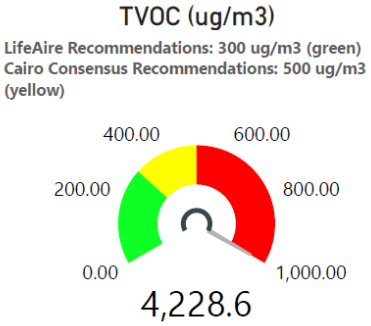
5. SUMMARY OF DATA

All testing occurred on [redacted] and was performed by [redacted] personnel.

Overall, the US EPA TO-15 VOC air samples were very high with isopropyl alcohol (IPA), present in the highest concentration in both samples. The total VOC levels in both Day and Night samples are significantly above the Cairo Consensus recommended level of <500 µg/m^3 total volatile organic compounds (TVOCs) [7]. LifeAire further recommends total VOCs <300 µg/m^3, or ~100 ppb for optimal embryogenesis. While the identified levels are high, they can be comprehensively remediated.

The TVOC level for the daytime sample in the IVF lab was extremely high at 4228.6 µg/m^3. This is **8.46 times the Cairo Consensus maximum level and 14.1 times the LifeAire maximum recommended level of VOCs**. The TVOC level for the evening sample in the IVF lab was also extremely high at 2840.8 µg/m^3. This is **5.68 times the Cairo Consensus maximum and 9.47 times the LifeAire maximum recommendations**. In both samples, IPA was found in the highest concentration. The EBTOX score for IPA in the daytime sample in the IVF lab is 52.53. This is an alarmingly high score, showing the levels of IPA are **52.53 times the amount modeled to pose harmful toxicity endpoints to an embryo**, and IPA must therefore be aggressively remediated. The EBTOX score for IPA in the nighttime sample of the IVF lab is 30.21, showing the levels of IPA are **30.21 times the amount modeled to pose harmful toxicity endpoints to an embryo throughout the night**. Aside from IPA, there were a significant amount of different detectable VOC species, suggesting that the source is not isolated. Seven (7) VOCs were detected during the day, and thirteen (13) at night, with acetone and methyl ethyl ketone registering EBTOX scores within 0.01, demonstrating that they are present at levels that may not cause arrest of the embryo yet could have other non-lethal implications for development. The lab TVOC levels are extremely high and must be properly addressed, mainly because of elevated IPA yet also due to other VOCs.

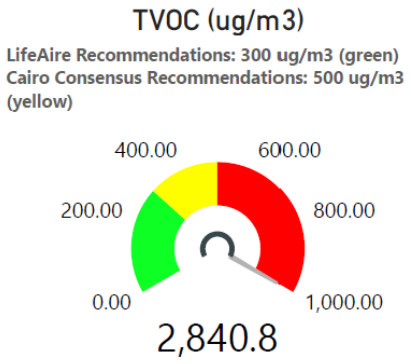
The day sample demonstrated extremely high VOC levels. At 4228.6 µg/m^3 (1691.0 ppb) this was approximately 1.48 times higher than the nighttime sample. IPA was found in the highest concentration at 4000 µg/m^3 (1600 ppb). Acetone, pentane, and butane follow. IPA's EBTOX Score in this sample is 52.53, which exceeds the toxicity threshold of 1 52-fold. Note that there is insufficient published, peer reviewed data to appropriately model the impacts of pentane and butane, and these VOCs may have further unknown impacts on the embryo in their elevated concentrations.



EBTOX Scores

Contaminants	EBTOX Score
Isopropanol	52.53
Acetone	0.48
Methyl ethyl ketone	0.02
Toluene	0.00

The night sample demonstrated slightly lower VOC levels (still well above recommendations) yet showed a different species profile from the other sample. Six additional VOCs were detected that did not register in the day: 1,2,4-Trimethylbenzene, cyclohexane, ethylbenzene, hexane, m,p-Xylene, and o-Xylene. Several of these could not be included in EBTOX scoring due to unknown toxicity thresholds in the context of IVF. IPA was still the VOC found in the highest concentration at 2300 µg/m^3 (950 ppb). IPA's EBTOX Score here is 30.21, exceeding the toxicity threshold of 1 30-fold. All other modeled compounds were below the individual threshold of 1, yet with thirteen different compounds appearing in the assay at this location, it is prudent to consider the possibility of mixture effects of these VOCs in imparting toxic burden on the embryo.



EBTOX Scores

Contaminants	EBTOX Score
Isopropanol	30.21
Acetone	0.45
Methyl ethyl ketone	0.01
Toluene	0.00
m-, p-xylene	0.00
o-xylene	0.00
Ethylbenzene	0.00
Trimethylbenzene	0.00
Hexane	0.00

There are many activities which occur within an IVF facility that contribute to the overall VOC load. For instance, cleaning products often contain IPA and are known to release VOCs into the atmosphere. Some of the VOCs present have not yet been extensively studied in the context of IVF, and with a lack of knowledge surrounding the impacts on embryogenesis that they may impart, LifeAire recommends thorough remediation. Typical laboratory VOC sources may include containerized gases, plasticware, sanitizers, building materials, or infiltration from outside sources [7].

The total VOC level was significantly elevated in the daytime sample as compared to the nighttime sample. However, the nighttime sample still showed total levels well above the LifeAire recommended level for optimal embryogenesis. This is an indication that the VOC load generated during daily operation is not being effectively remediated at the necessary rate by the current air purification system installed. Though the identified VOC levels are extremely high, they can be comprehensively remediated.

6. CONCLUSIONS, RECOMMENDATIONS AND SOLUTIONS

Overall, the US EPA TO-15 VOC air samples are elevated with high levels of IPA. The total VOC levels well exceed LifeAire recommendations of 300 µg/m^3 TVOCs for optimal embryogenesis.

The current AHU, HVAC and air filtration system are not delivering the levels of VOCs deemed appropriate for the support of an optimal *in vitro* culture environment for the human embryo within the [redacted] facility. Some VOC levels measured were significantly higher than the recommended level and exposure to these elevated VOC levels is known to be embryotoxic. VOCs can equilibrate from the air into the cell culture media and into the cells within a matter of minutes. Once cellular, the VOCs cannot be removed and are associated with copious embryotoxic mechanisms including disruption of the cellular membrane, an increase in intracellular calcium, impaired mitochondrial function, decreased spindle formation, decreased chromosome alignment, perturbation of DNA and RNA replication, increased DNA oxidative damage and increased DNA fragmentation.

After a thorough review of all data, a two-tiered approach is recommended towards the comprehensive remediation of embryotoxic VOCs and the delivery of an optimal solution: 1. engineering evaluation of the HVAC system and air flow serving the critical areas, and 2. use of a targeted air purification system. This approach will ensure environmental optimization of all critical areas for improved embryo culture and patient delivery.

a. Engineering/Design Evaluation:

The engineered solution will evaluate HVAC and TAB information specific to critical areas. This evaluation will examine all areas of the HVAC communication including source, return and plenum air. In collaboration with [redacted] engineers, LifeAire engineers can evaluate the flow of air relative to the process and personnel in the space, source of air per AHU, source of air per space and positive pressure relative to each critical area. This evaluation will help identify areas that can be improved specific to the embryotoxic VOC levels identified.

b. Systems' Solution:

Upon evaluation of all HVAC and mechanical information, LifeAire engineers can size the area to provide a systems' solution to purify all source and recirculated air to the critical space. The LifeAire System was designed based upon 15 years of clinical and air testing outcome data. This design achieves airborne metrics defined as optimal for the culture of the living cell. The system provides 24/7 real time kill and remediation of all airborne bacterial, viral, and chemical pathogens. It also drives all embryotoxic VOCs present in ambient air to below detection by using a targeted molecular media specifically designed for aggressive VOC remediation. The system has been tested in the most sensitive environment and produces no byproducts such as ozone or intermediate molecules. After a single pass, with reduced ACH, the LifeAire system delivers the required airborne metrics required for successful and consistent embryo culture by reducing all VOCs, viable and non-viable particulates to levels deemed optimal for the embryo culture. Current GMP and ISO metrics do not address the embryotoxic levels of VOCs and viable particulates. HEPA/ULPA filtration, laminar flow, air flow control, high ACH rates and current in-duct technology, alone, are not enough to comprehensively remediate all categories of airborne pathogens in the IVF environment. Aggressive management of the IVF environment must involve strategies for staff, surface, and comprehensive air disinfection during real time.

c. Summary of Phased Solution and Recommendations:

- Phase I. Comprehensive VOC Testing of [redacted]
- Phase II. Presentation of Data and Recommendations
- Phase III. Engineering Solutions Phase
- Phase IV. Systems Solution

The recommended solution includes four distinct phases. Phase I. included a comprehensive VOC evaluation of the [redacted] facility. This testing is described in this report and was completed on [redacted] Phase II. represents the presentation of all third-party analysis data, interpretation and recommendations. This report in conjunction with a follow up call will complete this phase. Phase III. includes a recommended engineering

solutions phase in which LifeAire engineers evaluate air flow and HVAC design to identify critical areas that can be improved specific to the mitigation of embryotoxic VOCs. Phase IV. represents the system solution phase which includes the sizing, manufacturing, and installation of a LifeAire System to comprehensively remediate embryotoxic VOCs, viable and non-viable particulates from all source and recirculated air within critical areas. The system solution phase will remove the embryotoxic variable of air from the environment and deliver the metrics deemed optimal for the human embryo.

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