<u>Academic Research Case Study – AR 1</u>

A Non-Invasive Platform to Permit VOC Collection

and Determination from Murine Breath for a University Laboratory in the Netherlands

Development of Non-Invasive Platform to Permit VOC Collection and Determination from Murine Breath for a University Laboratory in the Netherlands

Problem Statement

Historically the collection of exhaled air from restrained rodents was limited by invasive methods with results impacted by anesthesia.

Procedures were commonly terminal preventing repeat occasion experiments.

Repeat occasion exhaled air collection was the specific target of University Investigators to assess breath Volatile Organic Compounds (VOCs) associated with metabolic activity in both healthy and disease states.

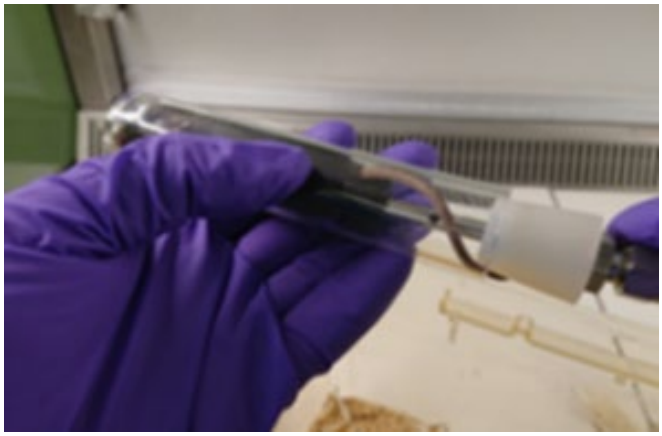
CH Technologies (USA) was asked to develop a system of murine conscious restraint with sealed tubes, directed flow air delivery and exhaust passed directly to adsorption media tubes.

System was required to minimize or eliminate the variability associated with materials of construction and the quality of the air supplied.

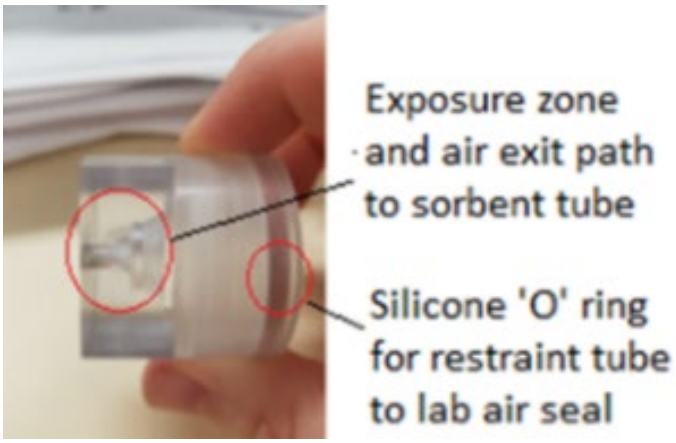
CH Technologies Developed Equipment

A platform was designed based around the CH Tech designed murine glass restraints fitted into acrylic holders each sealed with a silicone O-ring.

Air contact with the acrylic material was confined to the tip of the restraint with scavenging air directly exiting to ¼" by 3.5" stainless-steel sorbent holding tubes.



Prototype Restraint Tube

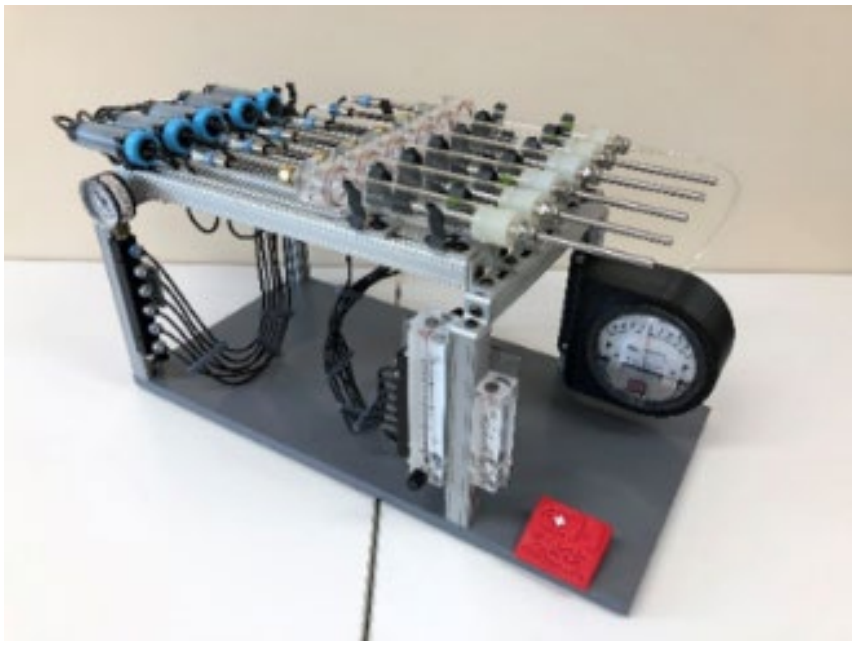


Prototype Acrylic Chamber

The final multi animal design included 6 racked restraint positions with airflow controls, a Jaeger Metabolic Analysis (JAMA) system to permit real-time CO_2 monitoring.

Air supply to restraints supplied by the lab house air system with HEPA and charcoal filters to minimize supply side VOCs and contamination.

Restraint airflow controllable using interchangeable chokes that could provide 75, 139, 185, 239 or 304 mL per minute from a manifold.

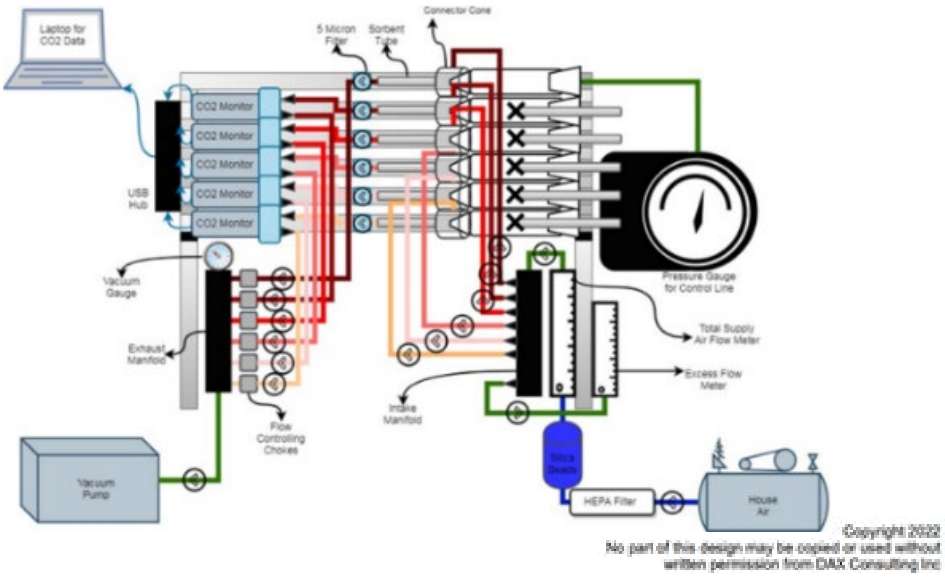


Final six tube rack system

Overall inlet airflow was monitored and controlled by variable area flowmeters.

After passing the nose of a restrained mice the air was drawn under negative pressure through the sorbent material to the CO_2 sensor, then to a manifold with a vacuum gauge before being taken to waste.

Components of the overall system were connected using Viton tubing



System Schematic

Research Outcome

A proof-of-concept validation study was performed by the University and published in The Journal of Breath Research.

The research conclusion was that the CH Tech produced platform permits non-invasive murine breath collection studies.

The absence of anesthesia minimizes contamination and influence of results by exogenous sources and future longitudinal animal studies are envisioned without a requirement for large numbers of terminal procedures.

Adaptation of the methods employed will permit similar longitudinal occasions of breath collection in other laboratory rodent species and expand understanding of physiological processes in both sickness and health.

Of particular interest in future work will be the potential identification of biomarkers of disease and disease progression.

CH TECHNOLOGIES (USA)

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