Citizens for Alternatives to Animals Research & Experimentation
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February 4, 2020

Ann Schuchat, M.D.
Principal Deputy Director
Center for Disease Control and Prevention
Via email: Acs1@cdc.gov

Dear Dr. Schuchat:

I am writing on behalf of Citizens for Alternatives to Animal Research and Experimentation (CAARE), a national nonprofit organization dedicated to promoting research without using animals. This letter expresses the opinion of our board, staff, advisory board, consultants, and thousands of supporters.

We were very disturbed to learn that the Center for Disease Control (CDC) is planning to use animals to study the effects of vitamin E acetate and potentially other toxic compounds to investigate and mitigate the health crisis arising from e-cigarette or vaping associated lung injury (EVALI).

This was refenced multiple times in CDC communications or publications, including CDC's telebriefing and media release November 8 [1] and CDC report on November 15, 2019 in Morbidity and Mortality Weekly Report. [2]

This decision is totally out of step with the current trend by U.S. government regulatory agencies to reduce, and eventually eliminate animal testing by shifting to more relevant human test methods, which is based upon decades of inadequacies of animal tests to model human outcomes.

These deficiencies with animal tests are so widely acknowledged that the U.S. National Toxicology Program issued a "Roadmap to guide progress toward replacing animal use in toxicity testing" on January 30, 2018. The roadmap is the result of the partnership of sixteen federal agencies, asserting the need "to develop a strategic roadmap that offers a new framework for the safety testing of drugs and chemicals, which aims **to provide more human relevant toxicology data while reducing the use of animals."** [3] [Emphasis added]

Specifically, in the area of inhalation toxicology and respiratory pathology, there has been remarkable progress in developing and validating inhalation research methods without animals. These are detailed in a 2018 publication; "Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report." [4] As well, the entire edition of *Applied In Vitro Toxicology* in June 2018 was devoted to alternative testing methods for inhalation toxicology. [5] A total of 16 articles address non-animal technology currently available to replace substandard and painful inhalation experimentations with animals.

Copious studies spanning decades continue to demonstrate key differences in the anatomy and physiology of the respiratory tract of humans and other animals that significantly impact research outcomes, including the deposition, clearance and retention of inhaled substances. These include qualities of nasal tissue (epithelia) and airflow dynamics resulting from anatomical differences of both the upper and lower respiratory tract, which impact the dispersal of mucous secretions and products. [6]

These biological disparities make clear why testing on animal species will never provide accurate answers for human health challenges. They form the basis for the twenty-first century trend to move away from animal tests, as demonstrated by the U.S government's Tox21 program[7], initiated in 2008 to decrease reliance on animals for chemical safety testing. Tox21 was prompted by the National Research Council, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, [8] and inspired the 2018 Strategic Roadmap which furthers those goals.

CDC is advised to utilize any number of a broad range of available alternatives to inhalation studies on animals that include in vitro assays, in silico approaches, computational chemistry, adverse-outcome-pathway analysis, and a range of sophisticated tissue models that include 3D organoids and organs-on-chips to study the lung pathology and toxicant compounds associated with vaping injuries. Below we provide a brief overview.

AOP Inhalation Testing

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental economic organization with 36 member countries, which among other roles, promotes the replacement of animals in testing as well as sets international standards for reducing, replacing and refining animal tests. OECD is actively promoting Adverse Outcome Pathways for chemical screening tests.

OECD defines an Adverse Outcome Pathway as "an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning." [9]

A recent study carried out by the U.S. Army Engineer Research and Development Center's Environmental Laboratory successfully used AOP [10] to understand why people in South Korea began developing respiratory difficulties after chemical disinfectants were added to humidifiers.

The research team developed an AOP to clarify the connection of the toxicant to the adverse outcome of pulmonary fibrosis. Next, they conducted a systematic analysis to identify potential chemicals involved in this AOP, using the ToxCast database and deep learning artificial neural network models. They identified chemicals bearing a potential inhalation hazard and exposure hazards from the database that could be related to this AOP.

One of the study leaders, Dr. Lyle Burgoon, describes their rationale in using AOP: "In the ideal world, we would have used experiments on humans to test whether the chemicals were detrimental to people. For obvious reasons, it would be very difficult to use people, so we then might turn to animals, but using animals is costly and raises many ethical questions." [11]

In vitro models

A growing number of biotech companies have developed prepared models and systems, many of which can be customized, that demonstrate highly efficient human-predictive testing for inhalation studies. Below we discuss several of the more important ones available. It should be noted that these examples are by no means comprehensive but illustrate the number, sophistication and variety of available methods to replace ill-performing animal tests.

MucilAir

MucilAir™ is a commercially available 3-dimensional in vitro tissue model, available through Epithelix. It is made up of human respiratory epithelial cells at the air-liquid interface on a porous membrane. The cells are sourced from surgical operations and differentiated to the required type (nasal or tracheal, for example) through cellular reprogramming. An extensive analysis shows that MucilAir™ cells maintain normal biochemical processes for at least six months. [12]

Company literature explains that Epithelix exports its tissue models to a wide range of customers "not limited to the drug development and testing industry. Its models can be used to assess the toxicity of any compounds that can be breathed in and therefore gain access to the respiratory tract – from airborne pollutants and nanoparticles, to smoke particles, deodorants and air fresheners." [13]

A 2018 study analyzed MucilAir's ability to study pulmonary absorption in vitro. The study, "Establishment of a Human 3-D Tissue-Based Assay for Upper Respiratory Tract Absorption," validated the capability of MucilAil to closely replicate "the morphology and function of native human tissues. Therefore, the *in vitro* system is much more relevant in predicting human response." [14]

MatTek EpiAirway

EpiAirway™ is a 3D tissue model available through biotech company MatTak Corporation. Produced from human-derived tracheal/bronchial epithelial cells, the ready-to-use model recapitulates the in vivo phenotypes of barrier, mucociliary responses, infection, toxicity responses and disease. [15]

EpiAirway exhibits human relevant tissue structure and cellular morphology with high uniformity and reproducibility, allowing for human relevant exposure to test materials. EpiAirway's ability to model human inhalation tests has been validated and described in a publication [16] which exposed test chemicals to EpiAirway for 3 hours. Fifty-nine chemicals covering a broad range of toxicity classes, chemical structures, and physical properties were evaluated, demonstrating that "the EpiAirway test is a promising alternative to the currently accepted animal tests for acute inhalation toxicity."

Vitrocell

Cigarette-smoking machines and robots have been developed by Vitrocell® for in vitro inhalation toxicology to study the impacts of gases, environmental atmospheres, nanoparticles and complex mixtures on lung cells. The machines are able to generate whole smoke, gas phase and side stream smoke, as well as adapt to electronic cigarettes via special e-cigarette product packages. Designed for research and development, the machines offer in vitro exposure models as well as routine testing capabilities.[17]

A study using the smoking machine VC1 successfully demonstrated Vitrocell VC1 aerosol generation and delivery across multiple nicotine product categories, as characterized using nicotine as a dosimetry marker. The data suggested that VC1 reproducibly generated and delivered tobacco products and next generation products (NGP) aerosols "for future in vitro assessment and matches the performance of reported exposure systems" [18]

Vitrocell® has successfully collaborated with clients from leading research institutes, contract research organizations, regulatory authorities and industrial laboratories across the world.

Organs-on-chips

The potential for organs-on-chips to elucidate precise mechanisms of intricate physiology makes them a perfect methodology for assessing lung damage due to vitamin E acetate and other compounds inhaled through e-cigarettes. Because the chips are miniaturized recreations of physiologic systems on a transparent chip, scientists can literally view biological interactions.

For example, research at the Charles Stark Draper Laboratory in the U.S. has demonstrated that using its organ chips, scientists can observe neutrophils penetrate cells and tissues in real time, indicative of an inflammatory response. Animal testing cannot show this. [19]

At the annual meeting of the American Association for the Advancement of Science in 2018, organs-on-chips featured prominently as one of the brightest advancements poised to supersede animal models. Robert Urban PhD, head of Johnson & Johnson Innovation told conference attendees how using organ chips make it possible **"to demonstrate how the molecular biology of toxicology is actually taking place. You can understand pharmacokinetics and other features you can't understand from animals. This is a timely addition and hopefully a replacement for laborious, ill-predictive animal models."** [20] [Emphasis added]

Below we describe a number of companies that have developed lung organ chips with complex, specialized functions, some of which have been validated to perform better than animal tests.

Emulate
Emulate Inc. is at the forefront of developing highly specialized organ chips to recreate intricate human biology. The company was formed in 2014 as a commercial offshoot of Harvard's Wyss Institute to facilitate distribution of Wyss's Organs-on-Chips to pharmaceutical and medical product companies. The Wyss Institute is one of the leading pioneers of organ-on-chip technology and developed the first human lung-on-a-chip in 2010. [21]

Emulate's scientists have developed two distinct lung chips to address the differentiated role of the specific areas of the lung. [22]

The Alveolus Lung-Chip recapitulates the fundamental functions of gas exchange and absorption that occur within the delicate alveoli. It provides a human relevant platform to study nanoparticle absorption and toxicity, and adverse drug effects that attack the lung, such as pulmonary edema and pulmonary thrombosis.

The Airway Lung-Chip models the epithelium that conducts air to the alveolus and is suitable for studying inflammation and the physiology of human small airway diseases such as asthma and chronic obstructive pulmonary disease (COPD).

Alveolix
AlveoliX's bioinspired lung-on-chip technologies recreate the microenvironment of the human lung by including a micro diaphragm. The ultra-thin air-blood barrier in human lungs is constantly subjected to the rhythmic pulse of the diaphragm. The negative pressure created by the diaphragm impacts even the most delicate lung structure where gas exchange takes place, the alveolar sacs, made up of epithelial cells. The cyclic stretching that results from the diaphragm's movements affect alveolar stability, tissue stiffness and cellular proliferation.

In the lung-on-chip model an external electro-pneumatic pump "induces a three-dimensional mechanical stress to the bioartificial alveolar membrane, on which lung cells are cultured", thereby mimicking in great detail the human lung.
This technology serves as a model for the alveolar barrier and can be used to assess inhalation toxicity and test for the effects of drugs.[23]

TissUse
TissUse has developed a proprietary "human-on-chip" technology that closely simulates the activity of multiple human organs. This miniaturized construct allows for the testing of chemicals and their metabolism at a systemic level, as opposed to a singular organ. Various combinations of organ models can be created, including lungs, to carry out chemical, cosmetic, food safety and pharmaceutical testing. Organ channels are connected by microfluidic channels and a connected pump allows for nutrients and oxygen to flow similarly to the human body.

TissUse offers contracts that be customized and crafted to suit the individual needs of a customer, depending on what is being tested. Different organ combinations may be selected and the chip design can be produced quickly due to its proprietary prototyping procedure. Given its

adaptability and production speed and the fact that it so closely simulates human biology, TissUse technology could be used to test e-cigarettes' effect on lung and other organs.[24]

Concluding remarks

Twenty-first century science has demonstrated repeatedly that animal data does not extrapolate with any confidence to human biology and medicine. This has resulted in the current trend by U.S. regulatory agencies to replace animal tests with human-relevant data, as exemplified by the Strategic Roadmap.

There is also an increasing focus on the ethical quandary posed by using animals. New and ongoing information on the high level of sentience of many animal species has shed light on the urgency to replace them in such painful tests. Their capacity to suffer greatly, particularly in experiments that induce discomfort associated with the sensitive and vital function of breathing, must be taken into account.

Given the severe limitations of animal tests, the availability of new technologies that perform better, and the implementation of the Strategic Roadmap to utilize more relevant human test methods, it is unjustifiable – indeed unconscionable – for the CDC to call for the use of animal tests to address the causes or treatments of lung injury due to e-cigarettes or vaping.

While the excuse for using animals is often that existing non-animal methods may not be perfect, animal tests are most certainly not perfect. By using combinations of these advanced and human relevant alternative methods, CDC can arrive at the important goal of more efficient human-predictive testing to understand the threats associated with e-cigarettes and vaping.

We strongly urge CDC to eschew all animal tests to study EVALI and other human health issues. We appreciate your attention to this letter and hope and trust that you will give it your utmost consideration.

Sincerely,

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