Cell culture reactor – a mechanical lung as an alternative to animal experiments

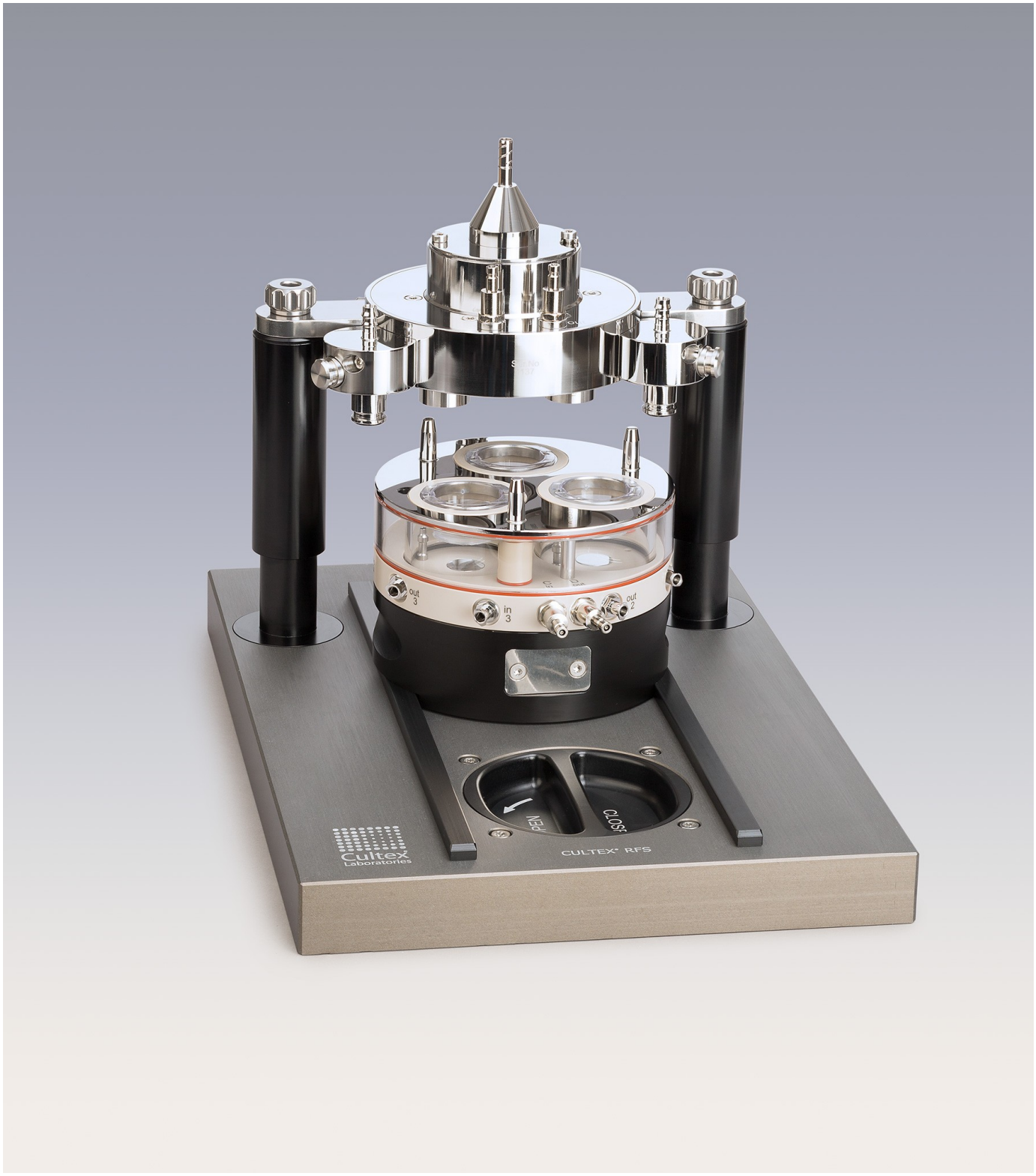
When people breathe, not only do they inhale oxygen, but they also ingest harmful substances into the lung. Animal experiments still need to be carried out in order to assess the potential effects of air-borne pollutants, but alternatives are being sought in order to reduce the number of animal tests. In cooperation with Cultex Laboratories GmbH, Askea Feinmechanik GmbH from Amtzell has developed a cell culture reactor for conducting in vitro inhalation tests without using animals. The device is able to test the effect of air pollutants on human or bacterial cells. For example, it can be used for examining harmful effects of substances in environmental or workplace atmospheres or for other toxicological examinations.

Previously, the effects of air-borne pollutants such as cigarette smoke, industrial welding dust or incinerator emissions on human health could only be tested to an inadequate extent. Animals had to be used for examining the effects of air pollutants on human health because traditional alternative test methods are unsuitable for these types of investigations.

In vitro toxicology testing involves the addition of potentially hazardous substances – filter extracts in the case of air pollutants, for example – to the cell culture medium. However, this is not appropriate for testing the effect of toxic substances on the lungs for two reasons: first, such an approach does not reflect natural exposure and second, the cell culture medium does not allow an accurate statement to be made on the effect of the air-borne particles.

“On the one hand, the cell culture medium might change the particle surface. On the other, it cannot be excluded that the pollutant dose to which the cells are exposed is falsified because very tiny particles are floating in the medium and prevented from reaching the cells,” explains Hermann Le Guin, managing director of Askea Feinmechanik GmbH. A new cell culture reactor CULTEX® RFS (CULTEX Radial Flow System), developed by Askea Feinmechanik GmbH and Cultex Laboratories GmbH, has the potential to improve the current situation.

Simulation of breathing using modern technology



Askea Feinmechanik GmbH's cell culture reactor can be used for the in vitro analysis of air pollutants.

The cell culture reactor consists of a basic module and a lid in a special frame to tightly seal in the reactor. The lid has a central channel through which air pollutants can be guided into the basic module. A controlled quantity of the air-particle mixture is guided into three gas chambers in the upper section of the basic module by way of three radially arranged tubes. Each chamber contains cells in small culture vials and a vacuum pump is used to generate an air flow through the cell culture reactor. The volume of the test atmosphere is regulated by a mass flow controller. "The radial distribution system enables the accurate distribution of the test substance to the three chambers and guarantees high reproducibility," explains Le Guin. During exposure, the main flow is

guided over the cells and subsequently out of the system. “The exact dose of the atmosphere to which the cells are exposed depends on the time of exposure, the initial particle concentration and the size of the particles present.”

The cell culture reactor was optimized using computational fluid dynamics (CFD) analysis, a recognized method for the approximate simulation of flow conditions. “CFD analysis allows the simulation of the natural flow path of the particles through CULTEX® RFS. The flow path depends on the diameter of the particles,” explains Le Guin. An additional module that provides the particles with an electrical charge by attaching ions was developed in order to be able to separate very tiny particles. Even nanoparticles can thus be distributed equally and brought into contact with the cells, thereby providing a realistic scenario for analyzing their biological effect.

Cell culture tests under conditions that are close to reality

The CULTEX® RFS and its flexible and modular adaptor system is suitable for testing a broad range of toxic substances on bacteria and on human and animal cells. In addition, the cell culture reactor can bring mono- and co-cultures of cells isolated from the human respiratory tract into contact with air pollutants at the air-fluid boundary under conditions that closely mimic the natural situation. This enables researchers to examine the potential effects of air pollutants on human health. The analyses are carried out by exposing cell cultures to gases, complex mixtures and particle-laden atmospheres such as dusty air. “A modified Ames test can be used to determine the mutagenic effect of complex substance mixtures with bacteria,” explains Le Guin. The Ames test is a widely used in vitro method for studying the mutagenic effect of complex substance mixtures such as cigarette smoke or drugs.

After exposure, the effect of the substances can be determined on the basis of cell vitality or cellular reactions using microscopic, biochemical and molecular biological methods. Acute and chronic effects of pollutants can be studied on the cellular level and compared to in vivo human data, providing information about the health hazards of fine dust, exhaust gases and cigarette smoke and assessing their role in the development of diseases of the respiratory tract.

Legal approval of the method still pending

Periphery devices such as a particle generator with elutriator lead to an optimal distribution of the particles; particles that are too big are removed before they come into contact with the cells.

The cell culture reactor CULTEX® RFS was awarded the Baden-Württemberg Innovation Prize in 2013 and its reproducibility, stability and informative value were assessed in a BMBF-funded project. Coordinated by Cultex Laboratories, a cooperative pre-evaluation study involving the German Armed Forces Institute of Toxicology and Pharmacology in Munich and the Institute of Pathology of Mainz University Hospital was also successfully completed in 2013. "Despite the excellent and reproducible results obtained by the three cooperation partners, it will still be some time before the method is legally approved as an alternative to animal methods. This is most likely due to the lack of acceptance of alternative methods and the lack of funding for projects involving the development and establishment of new methods of investigation. And this is true for federal funding programmes

and even more so for the industry," says Le Guin with regret.

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