Artificially Generated Cigarette Smoke – a Model for Studying Complex Atmospheres

Many pollutants known to originate from environmental and industrial sources, such as benzene, formaldehyde, lead or arsenic, also occur in cigarette smoke. Among the approx. 4,800 substances that have as yet been identified in tobacco smoke, there are 69 recognized carcinogens and a large number of toxic substances, some of them highly reactive. Cigarette smoke is an aerosol composed of a gaseous phase and a particle phase, the gaseous phase being defined indiscriminately as the fraction of the smoke aerosol which passes through a Cambridge glass filter. The particle phase is the fraction captured by the glass fiber filter (condensate). Toxicological evaluations of such aerosols so far have considered primarily condensates and extracts, while clarification of the effects of short-lived combustion products or of the gaseous phase has remained insatisfactory. Since cigarette smoke can be artificially generated under controlled conditions, it represents an ideal model atmosphere for the establishment of a biological in vitro system allowing to investigate native mixtures of substances.

Model for investigating complex atmospheres
»Two requirements must be fulfilled to allow for investigation of complex...
Details of the smoking robot, such as leak detection functions, automatic loading of the rotary holder, ignition, precision pump, and butt extractor

mixtures resulting from high-temperature processes, such as cigarette smoke: a sensitive biological testing system and the technical prerequisites for investigating test atmospheres, explains Prof. Dr. Michaela Aufderheide, Head of the Department of In Vitro Toxicology. Cultured human lung cells are used as indicators (see News Report June 2003, p. 3). This has the advantage that investigations can be performed in vitro, thus allowing for a reduction of animal experiments.

Robot generates cigarette smoke
Cigarette smoke seemed to be a suitable test atmosphere for the experiments, as it can be artificially generated in unlimited amounts. This allows us to characterize many different cellular reactions, says Aufderheide. To match the real-life smoking situation as closely as possible, we use a robot that has been specially designed for in vitro experiments.

Using the robot VC10 (Vitrocell), which meets current DIN/ISO requirements, it takes ten seconds for the smoke to reach the cells that are exposed on membranes contained in a special exposure unit CULTEX®. The smoke thus gets directly into contact with the cells and acts upon them. The robot allows for many variants of the experiment to be tested, since it can be flexibly adjusted to different smoking profiles and smoking regimens. It is thus possible to create the conditions to which a subject is exposed while smoking a cigarette.

The investigations aim to make available an in vitro method which can be used to characterize native aerosols. For one study, different types of cigarettes were provided by the Joint Research Centre of the European Commission in Ispra (Institute for Health and Consumer Protection, Physical and Chemical Exposure Unit, Ispra, Italy) and the efficiency of such a method to describe the biological effect of whole smoke and of the gaseous phase was proven (Ritter et al., Inhalation Toxicology 16: 691-700, 2004).

In vitro testing system suitable also for other pollutant analyses
With these investigations, we have set the stage for using such a validated in vitro system also in studies on complex environmental atmospheres. We are thus in a position to conduct targeted indoor and outdoor studies in the future, Aufderheide summarizes.

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Herbert Herxheimer Promoter’s Prize 2004
On September 15, Christina Nassenstein, Norbert Krug, Armin Braun and Veit Erpenbeck of the Fraunhofer ITEM were awarded the Herbert Herxheimer Promoter’s Prize during the first common congress of the Medical Association of German Allergologists (Ärzteverband Deutscher Allergologen, ADA), the German Society of Allergology and Clinical Immunology (Deutsche Gesellschaft für Allergologie und klinische Immunologie, DGAI) and the German Society of Pediatric Allergology and Environmental Medicine (Gesellschaft für Pädiatrische Allergologie und Umweltmedizin, GPA) in Aachen. They received the prize for their work on »The Neurotrophins Nerve Growth Factor, Brain-Derived Neurotrophic Factor, Neurotrophin-3 and Neurotrophin-4 are Survival and Activation Factors for Eosinophils in Patients with Allergic Bronchial Asthma«.
Simulation Model to Predict Exposure to Biocides
ITEM scientists have developed a method for determining inhalational and dermal exposures during biocide spraying at workplaces.

There are about 15,000 biocidal products currently available on the European Market. When treating large areas, biocides (pest control agents) are normally applied by spraying or nebulizing, generating complex aerosols. To allow for the inhalational and dermal exposures resulting from such applications to be determined and predicted was the aim of this research project.

Test measurements in model rooms and verification in field experiments Measurements were performed at the Fraunhofer ITEM to determine the operator’s exposure after application of biocidal products with commonly used spraying devices. Inhalation exposure was determined with the Respicon (a personal particle sampler developed at the Fraunhofer ITEM), while exposure pads were used to measure the potential dermal exposure. Furthermore, the droplet size spectra of the aerosols generated by the different spraying devices were determined.

The size distribution of the sprayed droplets proved to be a key parameter in the inhalation process: The smaller the droplet diameter, the higher the inhalation exposure. The spraying direction turned out to be another relevant parameter: The highest potential dermal contamination of body surfaces resulted from overhead spraying. Subsequent field measurements taken in the sectors »food processing«, »private and public hygiene«, »veterinary hygiene«, »wood and masonry preservation« as well as »antifouling« confirmed the results of the model measurements.

Simulation model enables risk assessment for all sectors With the simulation model »Spray Expo« developed at the Fraunhofer ITEM, it is now possible to compute the behavior of the released droplets, allowing for a fairly accurate estimate of the inhalation exposure during a spraying operation and thus for risk assessment of a biocidal product.

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For further information, please refer to: www.item.fraunhofer.de/NR

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Priv.-Doz. Dr. med. Jens Hohlfeld, Head of the Department of »Clinical Allergy, Asthma and Inhalation Research« at the Fraunhofer ITEM, has been appointed to an extraordinary professorship by the Hannover Medical School.

Professor Dr. Clemens Dasenbrock, having held the position of Head of the Animal Laboratory at the Fraunhofer ITEM for many years, left the Institute on September 30, 2004, to work for Boehringer Ingelheim Pharma GmbH. Dr. Thomas Tillmann has been appointed Head of the Animal Laboratory, effective October 1, 2004.

Dr. Ilona Fleischhauer joined the Fraunhofer ITEM on January 1, 2004, as Head of the Quality Assurance Unit (QAU) in succession to Dr. Madhukar Ketkar, who retired. Having worked in the industry for many years, Dr. Fleischhauer has gained a lot of experience in preclinical and clinical quality assurance and in drug safety. At the Fraunhofer ITEM, she is responsible for implementing the Institute’s internal quality assurance program. A focus of her work is a close cooperation with the departments and working groups operating under GLP (Good Laboratory Practice) conditions, supporting them during GLP inspections and making sure that the Institute continues to be a GLP-certified testing institution acknowledged by the regulatory authorities. In addition, she is planning to further develop the GCP (Good Clinical Practice)-compliant quality assurance system in the »Clinical Inhalation« sector. By implementing the principles of GLP and GCP, the Fraunhofer ITEM as a contract research institution guarantees its customers a high quality standard in non-clinical and clinical trials.
Evaluation of Effects on Male Fertility

About one out of seven couples in Germany wishing to have children remains childless. Besides many other potential causes, exposure to chemicals is being discussed. In order to obtain further information in this regard, their effects are investigated in animal experiments. The questions arise, though, as to which parameters in toxicological tests are suited to ascertain a risk to human health, and how to extrapolate the results of animal experiments to humans. In seeking to answer these questions, the departments of »Reproductive Toxicology« and »Chemical Risk Assessment« at the Fraunhofer ITEM have reviewed the available literature on behalf of the German Federal Institute of Occupational Safety and Health in Dortmund.

Comparison of the different parameters applied in reproductive toxicology studies showed that histopathological investigations of the tests in many cases detected effects at lower doses than fertility investigations (number of pregnant females as well as number of offspring per female after mating with males that had been exposed to a chemical agent, see figure). One explanation is the fact that laboratory rodents, in contrast to humans, produce a vast excess of sperm compared to the amount necessary for successful fertilization (hyperspermatia). Damage of part of the sperm thus has no immediate impact on fertility. Furthermore, histopathological examinations are accurate and exhaustive if special techniques (»staging«) allowing to identify the different stages of spermatogenesis are used. They enable detection of effects already after a treatment duration which is shorter than a complete cycle of spermatogenesis (60-70 days, depending on species). Another sensitive and meaningful parameter is sperm motility, being of pivotal importance for sperm performance. Finally, the determination of weights of the prostate gland and the seminal vesicles provides clues to effects on fertility. All these parameters can be determined in repeated-dose investigations.

There were rather little data which allowed to compare the effects found in animal experiments and in humans. It became apparent, however, that a species extrapolation regarding the effects on male fertility is actually possible based on energy consumption. This corresponds to the approach used with other effects too.

If sensitive parameters could be established in 28- or 90-day studies also for female fertility in the future, costly and time-consuming generation studies would no longer be required for many substances. This is, however, not true whenever the specific topic of the investigation is the reproductive behavior.

Comparison of LOAELs for different endpoints of male reproductive toxicity compared in studies with rats after 4 weeks and 9 weeks of application (LOAEL body weight = 1). Figures in columns: number of studies analyzed. Figures above column: > 1: LOAEL lower than LOAEL body weight, i. e. higher sensitivity than body weight.

Where to meet us

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Download:
The complete study report »Extrapolation from Results of Animal Studies to Humans for the Endpoint Male Fertility« is available as a pdf file in the Internet under: http://www.baua.de/english/fors/fb03/fb984_e.pdf

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