

## A biological test for environmental toxins

**Every day, we breathe in gas emitted by waste incineration plants, evaporation from chemicals in wooden furniture and particulate matter from car exhausts or office equipment. Scientists in Dr. Richard Gminski's group at the Institute of Environmental Medicine and Hospital Hygiene (IUK) at the Freiburg University Medical Centre are investigating the components that can cause damage to body cells. The researchers are using living human cells to test what happens when airborne substances are inhaled and taken up by the human body. Molecular biology experiments shed light on the effect of the toxic gases or fine dust on human DNA.**



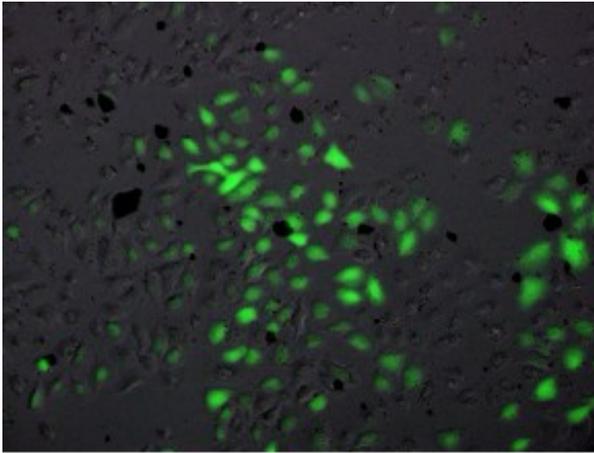
A laser printer being tested in the emission test chamber.  
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Are laser printers environmentally friendly for office staff? The so-called "toner study" carried out in 2009 by the Institute of Environmental Medicine and Hospital Hygiene (IUK) under the leadership of Director Prof. Dr. med. Volker Mersch-Sundermann at the Freiburg University Medical Centre showed that office equipment emits hundreds of potentially toxic chemicals. Office equipment is only one of many sources of airborne toxins to which we are exposed every day. Wood preservatives, pesticides, glue in wooden plates, nanoparticles in car exhaust gases, cigarette smoke - all these substances can enter our lungs and potentially penetrate deeper body areas." We know some of the compounds that are contained in these aerosols," said Dr. Richard Gminski from the Institute of Indoor-air Toxicology at the IUK. "We know that some are toxic and some are non-toxic. But we know very little about the effects airborne compound mixtures may have on human health."

### **Comet-like effects**

In order to find out more, the IUK researchers exposed human cells to the same environmental conditions to which we are exposed in our daily life. They developed an emission test chamber and

an exposition unit. The first is a closed chamber with constant temperature, air humidity and constant pressure. The chamber can be used to test any equipment that emits harmful chemical compounds or dust: laser printers, wooden plates, furniture that has been glued together. Tubes transfer the emitted gases to an exposition unit, which is the core of the chamber. In the exposition chamber, human lung cells or cells isolated from human blood are attached to Teflon membranes. The cells come into contact with the medium below and above, they are exposed to airborne pollutants contained in the emission test chamber. "This is designed to resemble the real-life situation in which we inhale air pollutants into the lung," said Gminski. "What we are looking for is how the cells react to the different airborne pollutants."



Free oxygen radicals are produced in cells treated with a gas mixture containing nanoparticles; these radicals can be visualised under the fluorescence microscope using a green fluorescent dye.

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In principle, all the different types of environmental influences can be simulated in the chamber. In addition to a laser printer in action or construction material emitting toxic compounds, Gminski and his team have also begun to investigate natural and artificial nanoparticles. These extremely small particles, which often contain metals and carbon, are found in many modern products, including food, clothing, sunscreens, paints or as catalysts in combustion engines. The particles are small enough to enter the tiny pulmonary alveoli where they can lead to inflammation or other changes that have not yet been examined. Some of the nanoparticles enter the blood vessels, from where they spread to other organs in the human body. In experiments carried out at the IUK in which lung cells were exposed to nanoparticles, the particles migrated as far as the cell nuclei where they caused DNA damage. When they carry out experiments in the chamber, the Freiburg researchers generally expose the cells to air enriched with nanoparticles for a period of an hour. The objective is to ascertain how many cells survive this procedure. In addition, the researchers look into what happens inside the cells, and have discovered that this leads to comet-like effects on the DNA level.

One of the tests carried out by Gminski and his team is the so-called comet test which shows the researchers whether air pollutant mixtures lead to DNA breakage. This experiment involves placing the cells in agarose, dissolving their membranes and exposing the cells to an electrical field, a process known as electrophoresis. During electrophoretic separation, the negatively charged DNA migrates to the plus pole, during which the DNA fragments become separated from each other, and are rearranged according to their size. Smaller fragments can cover a longer distance in a given time than larger fragments. Damaged, fragmented DNA is able to leave the cell nucleus. Under the microscope, the damaged cells, which are stained with fluorescent dyes such as

ethidium bromide, appear to have a tail of DNA fragments. "A cell, or rather its nucleus, once damaged by certain pollutants, looks like a comet," said Gminski. "Undamaged nuclei look like fluorescent spheres." In addition to the comet test, the scientists use another test to determine whether pollutants induce chromosomal damage in human lung cells. This test is known as a micronucleus test. The researchers also measure whether the emissions of the objects under investigation lead to the creation of free radicals (in particular oxygen radicals) in the cells.

### **Allergic reactions**

It will soon be known whether emissions from active office devices have a damaging effect on human cells. In addition to experiments in the emission test chamber, Gminski and his team also carry out human studies. One such test involved controlled exposure experiments on humans in order to assess the potential health risk posed by wood or wood material emissions. They also exposed so-called peripheral blood mononuclear cells (PBMC) of humans to the waste gas of the emission chamber and determined the type and quantity of second messenger substances produced by these cells when they were exposed to the gas. Second messengers include cytokines that are produced by certain blood cells in order to alert the immune system to the intrusion of unwanted foreign substances. "The presence and composition of the cytokines we found tells us whether certain emissions have an inflammatory or allergenic effect on human cells," said Gminski. For example, formaldehyde, which is found in cigarette smoke and wood glue, and whose carcinogenic effect is well known, often leads to allergic reactions and is characterised by a specific cytokine combination.

Besides basic research, industry might in future also benefit from the work at the IUK. "We offer our test facilities to numerous producers," said Gminski. "In the emission test chamber, we can for example test whether a company's product or its emissions are a risk to human health or not. We can also tell them how to optimise the product in order to guarantee its safe application." This is environmental toxicology research for the benefit of human beings.

### **Further information:**

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## Environmental medicine and biosensor technology