MedicalXpress

Ames test adapted successfully to screen complex aerosols

August 26 2014

The Ames test, a widely used method to determine whether a chemical has the potential to cause cancer, has been successfully adapted for use with cigarette smoke and other complex aerosols.

The traditional Ames test is not suitable for use with aerosols and gases, which means that in the past, the toxicity of cigarette smoke was tested using just the particulate extract from smoke and not the whole smoke, thereby giving an incomplete picture of the toxic profile. The particulate fraction is only a small part of the whole-smoke aerosol, which also comprises a vapour phase and contains more than 6000 chemicals, including volatile or insoluble components and short-lived products of combustion.

The Ames test works by observing how much a chemical causes bacteria used in the test to mutate. The standard test involves up to five strains of bacteria that mutate in different ways and are genetically modified to require an additional amino acid for growth. The bacteria are embedded in agar together with the test chemical and a small amount of the amino acid to enable them to grow and mutate. The mutagenicity of the test compound is proportional to the number of so-called revertant colonies present at the end of the test period, ie the number of colonies that have mutated (reverted) back to the original state and do not require the amino acid to survive.

Now, researchers at British American Tobacco and Covance Laboratories have shown that a modified Ames test can be used

successfully to assess the toxicological impact of mainstream cigarette-smoke.

The researchers modified the test by changing the bacterial strains used and the method by which they are exposed to the test chemical.

Two strains of *Salmonella typhimurium* and one of *Escherichia coli* were used—all accepted under current regulatory guidelines —in addition to two derivatives of S. typhimurium selected for increased sensitivity to nitroarenes and aromatic amines, known mutagens present in cigarette smoke.

The researchers further modified the test by spreading the bacteria on top of the agar to ensure they were directly exposed to the entire smoke aerosol—a more relevant exposure than submerging them. A commercially available aerosol system (Vitrocell VC 10 robot) was used to dilute mainstream cigarette-smoke into an adjustable constant flow of air that was fed over the bacteria. To help quantify smoke exposure, quartz-crystal microbalances, a newly emerging dosimetry tool, were embedded in a separate well to measure solid particles landing on the surface.

Following exposure to four concentrations of mainstream cigarette smoke, the researchers found concentration-related increases in the number of revertant colonies for the Salmonella strains, indicating that smoke causes (certain) mutations in a dose-dependent manner.

'We have demonstrated activity in a number of strains, some with greater sensitivity than others. We believe that this will offer a platform for exposure as well as more detail around the conditions needed for the assay to allow others to use the method in their own laboratory,' says BAT's Debbie Dillon, Senior Author. 'The method is currently illustrated using whole smoke but can be modified to test other aerosols and gases.'

The next step is to investigate additional Ames strains and incorporate the most effective into a regulator-acceptable test battery for cigarette smoke.

The Ames test, is widely used for the initial genotoxicity screening of pharmaceuticals and chemicals. The test is governed by international (OECD/ICH) guidelines, and is a core element of a preclinical data package expected by regulators for many consumer goods.

The research is published in *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*.

More information: DOI: 10.1016/j.mrgentox.2014.04.017

Provided by British American Tobacco