In vitro toxicology assessments of different Optimum-Filter materials added to cigarette filters

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Context

Different materials developed by Optimum-Filter were added to cigarette filters that were manufactured either using conventional cavities or CelFx technology (Celanese). Initial trials were made on the comparative toxicology of these filters when attached to conventional cigarettes, using several different *in vitro* approaches. Data obtained were compared with data obtained from 3R4F reference cigarettes. In most tests, smoke was passed through a Cambridge pad: the tested material thus excluded the particulate phase of the smoke. The objective of this work was to determine if the new filters produce cigarette smoke that is less biologically damaging in these tests then smoke resulting from conventional filtration.

Free radical production

Cigarette smoke is well-known to contain large amounts of free radicals and reactive oxygen species (ROS), and cigarette smokers are well-known to have significantly larger incidences of oxidative stress-related disease compared with non-smokers.

1. Serum antioxidant capacity

Antioxidants play an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species. There are three categories of antioxidant species: enzyme systems (glutathione (GSH) reductase, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). Different antioxidants vary in their reducing power. Trolox (a water-soluble analog of Vitamin E) is used to standardize antioxidants, with all other antioxidants being measured in Trolox equivalents. Measurement of the combined non-enzymatic antioxidant capacity of biological fluids and other samples provides an indication of the overall capability to counteract ROS, resist oxidative damage, and combat oxidative stress-related diseases.

Two methods were used to measure serum antioxidant capacity: Randox and benzidine. In both cases, the smoke that had been passed through a Cambridge pad

was bubbled through control serum. The Randox method is a total antioxidant kit incorporating 2,2'-azino-bis(ethylbenzothiazoline-6sulfonic acid) (ABTS). The benzidine assay uses a peroxide-generating system (hydrogen peroxide and horseradish peroxidase, HRP) and a peroxide-sensitive chromogen (benzidine). Antioxidants present in the sample compete with the chromogen and hinder the generation of a detectable signal.

HRP

An initial "proof of concept" experiment showed that smoke increases free radical concentrations in serum, a change which could be modified by the inclusion of an Optimum-Filter material in the cavity filter.

Based on the proof of concept, additional experiments were performed using both whole smoke and vapor phase only. In general, Optimum-Filter materials included in either the cavity filter or in the CellFx matrix showed increased antioxidant capacity when compared with reference cigarettes. Increases were greater for the vapor phase (132%) than for whole smoke (up to 84%). These results indicate that smoke filtered through some of the Optimum-Filter materials contain significantly lower levels of oxidants as compared to smoke filtered through a conventional filter.

Randox

No experiments were performed with whole smoke. Data obtained with vapor phase only were more variable with the Randox technique than with the HRP technique; increases of up to 216% were noted for antioxidant capacity using Optimum-Filter materials when compared with the reference filters.

2. Saliva antioxidant capacity

This endpoint is a logical extension of the concept of the serum antioxidant capacity assays mentioned above and uses the same two assays (with some modifications). A total of 17 volunteers were used, consisting of a mix of male and female smokers and non-smokers aged 18-59 years. A slightly larger study (using 38 volunteers) was also performed, but this study was a screening study to select those filter configurations to be used in the evaluations mentioned above.

Saliva samples were taken before and after cigarette smoking. Two control filters (cellulose acetate and carbon) and two Optimum-Filter cigarettes were used. The two Optimum-Filters used were similar to those used in the serum work. Results for the two conventional filters showed significant decreases in the antioxidant status, whereas one of the Optimum-Filters exhibited a much lower decrease and the second Optimum-Filter

actually showed a slight increase. These results are broadly similar to those for the serum assays.

3. Lung epithelial and endothelial cells

Lung epithelial and endothelial cells play critical roles in the etiology of chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD), respectively. Smoke-induced cell death was evaluated using the human alveolar adenocarcinoma lung cell line A549, and with primary human umbilical vein endothelial cells (HUVEC).

Responses obtained included reductions in the smoke-induced death of A549 cells when the cigarettes filters were modified with several different Optimum-Filter materials. Similarly, the effects of cigarette smoke on cell death in HUVEC were reduced when cigarette filters were similarly modified. These results are consistent with the conclusion that filtering smoke through one or more different Optimum-Filters could reduce smoking-related COPD and CVD.

4. Inflammatory cytokine release

Exposure to cigarette smoke can induce inflammatory responses through the release of inflammatory cytokines, thought to come from macrophages. Interleukins 6 and 8 (IL-6 and IL-8) are commonly-induced cytokines, and both are thought to play crucial roles in the initiation and extension of an inflammatory response in the lung, a key precursor of smoking-related COPD.

The test system used to evaluate the resulting gas phase cigarette smoke was a three-dimensional (3-D) lung cell culture, which is more representative of what occurs *in vivo* than are simple 2-D systems. A variety of different cell cultures were exposed to cigarette smoke extracts from three different cigarette types (one reference and two filters containing Optimum-Filter materials). After a 48-h treatment, the inflammatory cytokines were measured in the supernatant media using a kit that detects both IL-6 and IL-8. Both cytokines were significantly reduced in both of the experimental cigarettes when compared with the control in aggregates containing macrophages.

Conclusion

The attenuations in biological activity shown here are highly promising in terms of potential harm reduction. In addition, these studies have identified the two or three most promising Optimum-Filter configurations.

Appendix – Qualifications of the Authors

Dr Chris Coggins is a Board-Certified Toxicologist, a U.S. qualification he has held since 1986. He has special expertise in respiratory toxicology, with a secondary interest in general toxicology. Dr Coggins is experienced in providing expert testimony in litigation concerning respiratory toxicology, with special interest in biomarkers of exposure. He is also a European Registered Toxicologist and is listed on the UK "Register of Toxicologists". Much of his recent work has been on the ingredients used in tobacco-burning and electronic cigarettes, with emphasis on the toxicology of these materials when presented by the inhalation route. He has published extensively on these issues and routinely acts as a reviewer for several different journals.

Dr Edward Sanders received a Ph.D. in organic chemistry from the University of Michigan in 1966. He joined Philip Morris in 1973 where he worked first in the US and then in Switzerland until his retirement in 2008. From that on, he worked as an independent consultant. During his career at PM, he held a large number of management positions and worked in several different scientific disciplines. Since 1996 his major scientific contributions have been in the domains of epidemiology and molecular biology. He has published over 30 scientific papers and has been awarded a significant number of patents.

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