



Towards A Mouse Inhalation Model for Cigarette-Smoke-Induced Lung Tumorigenesis

Walter Stinn¹, Ansgar Buettner¹, Baerbel Friedrichs¹, Sonja Luetjen¹, Stephan Gebel¹, Rosemarie B. Lichtner², Frans van Overveld³, and Hans-Juergen Haussmann⁴
¹Philip Morris Research Laboratories, Cologne, Germany; ²Philip Morris International R&D, Neuchâtel, Switzerland; ³Philip Morris Research Laboratories, Leuven, Belgium; ⁴Toxicology Consultant, Roesrath, Germany.



Introduction

The smoking-related risk for lung cancer and other serious diseases is well established (US DHHS, 2004). However, established laboratory animal models for these diseases are not really available (Sohel et al., 2006; Cognigni, 2007). Such models are needed to unravel mechanistic and etiologic aspects of the disease, to investigate chemopreventive and early diagnostic means, and to evaluate risks from novel tobacco products (Haussmann, 2007). Some positive results in rodent lung cancer models with cigarette mainstream smoke (MS) inhalation were recently reported, but have not been reproduced to date (Mauderly et al., 2004; Hutt et al., 2005; Balinsky et al., 2007).

- 5- to 8-month inhalation exposure to an environmental tobacco smoke surrogate (ETSS)
- A required 4-month post-exposure period to allow the tumorigenic response to be seen
- Mouse strains known for their genetic susceptibility for developing lung adenocarcinoma mainly AJ, but also SWR/J
- Similar tumor response with ETSS whole smoke (WS) or its vapor phase (VP)

However, the majority of lung cancer cases are associated with active smoking, i.e. exposure to MS. Results of MS inhalation studies with this particular study design were not reproducible (Finn et al., 1990; D'Agostini et al., 2001; Cuthi et al., 2004; Hahn et al., 2007). This is considered to be due mainly to the variable and sub-optimal study designs employed.

Goal

Our goal is to establish a murine lung tumorigenesis model with cigarette mainstream smoke inhalation. This model should be biologically relevant, reproducible, and work without the need for a post-exposure period.

Approach

- Study 1:** Compare results for previous ETSS inhalation studies to MS inhalation
- Susceptible mouse strains (male AJ and SWR/J)
 - 5-month exposure + 4-month post-exposure periods
 - Whole smoke (WS), particulate phase (PP), and vapor phase (VP)
 - Dose dependence (0, 120, and 240 mg TPM/m³; low, WS, and high WS, respectively)
- Study 2:** Extend the MS exposure periods (4 post-exposure to long-term)
- Up to 18 months of exposure/post-exposure conditions
 - Evaluation of the need for a post-exposure period
 - Male AJ mice (18 to 36 mice per group) exposed to 0, 150, and 300 mg TPM/m³ (sham, MS-150, and MS-300, respectively)
 - Inclusion of mechanistic endpoints

Results, Study 1

Analytical characterization of test atmospheres:

- Efficient separation of PP and VP

Smoke Constituent	MS	VP	WS
Carbon monoxide	1.0	0.0	1.0
Acetone	0.0	0.0	0.0
Formaldehyde	0.0	0.0	0.0
Acrolein	0.0	0.0	0.0
Benzo(a)pyrene	0.0	0.0	0.0
Benzo(a)anthracene	0.0	0.0	0.0
Benzo(b)fluoranthene	0.0	0.0	0.0
Benzo(k)fluoranthene	0.0	0.0	0.0
Fluorene	0.0	0.0	0.0
Benzo(e)pyrene	0.0	0.0	0.0
Indeno(1,2,3-cd)pyrene	0.0	0.0	0.0
1,2,3,4-benzopyrene	0.0	0.0	0.0
1,2,3,4-dibenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7-hexabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8-heptabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9-octabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10-nonabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11-decabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12-undecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13-dodecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14-tridecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15-tetradecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16-pentadecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17-hexadecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18-heptadecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19-octadecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20-nonadecabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21-eicosa-benzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22-heneicosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23-tricosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24-tetracosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66-triacontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67-tetracontabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68-pentacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69-hexacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70-heptacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71-octacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72-nonacosabenzopyrene	0.0	0.0	0.0
1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,			