Overview information for

Benzo-(alpha) pyrene

BENZO-(ALPHA) PYRENE REFERENCES					
Author Name	Title	Journal	Volume	Page number(s)	Year
Scherer G, Scherer M, Rögner N, Pluym N.	Assessment of the exposure to polycyclic aromatic hydrocarbons in users of various tobacco/nicotine products by suitable urinary biomarkers.	Arch Toxicol.	Nov;96(11)	3113-3126	2022
Chen KM, Sun YW, Krebs NM, Sun D, Krzeminski J, Reinhart L, Gowda K, Amin S, Mallery S, Richie JP, El-Bayoumy K.	Detection of DNA adducts derived from the tobacco carcinogens, benzo[a]pyrene and dibenzo[def,p]chrysene in human oral buccal cells.	Carcinogenesis.	Sep 19;43(8)	746-753	2022
Li X, Luo Y, Jiang X, Zhang H, Zhu F, Hu S, Hou H, Hu Q, Pang Y.	Chemical Analysis and Simulated Pyrolysis of Tobacco Heating System 2.2 Compared to Conventional Cigarettes.	Nicotine Tob Res.	21(1)	111-118	2019
Lorkiewicz P, Riggs DW, Keith RJ, Conklin DJ, Xie Z, Sutaria S, Lynch B, Srivastava S, Bhatnagar A.	Comparison of Urinary Biomarkers of Exposure in Humans Using Electronic Cigarettes, Combustible Cigarettes, and Smokeless Tobacco.	Nicotine Tob Res.	21(9)	1228-1238	2019
Cai B, Li Z, Wang R, Geng Z, Shi Y, Xie S, Wang Z, Yang Z, Ren X.	Emission level of seven mainstream smoke toxicants from cigarette with variable tobacco leaf constituents.	Regul Toxicol Pharmacol.	103	181-188	2019
Weng MW, Lee HW, Park SH, Hu Y, Wang HT, Chen LC, Rom WN, Huang WC, Lepor H, Wu XR, Yang CS, Tang MS.	Aldehydes are the predominant forces inducing DNA damage and inhibiting DNA repair in tobacco smoke carcinogenesis.	Proc Natl Acad Sci U S A.	115(27)	E6152-E6161	2018
Zhang Y, Zou HY, Shi P, Yang Q, Tang LJ, Jiang JH, Wu HL, Yu RQ.	Determination of benzo[a]pyrene in cigarette mainstream smoke by using mid-infrared spectroscopy associated with a novel chemometric algorithm.	Anal Chim Acta.	902	Sep.43	2016

Thorne D, Crooks I, Hollings M, Seymour A, Meredith C, Gaca M.	The mutagenic assessment of an electronic-cigarette and reference cigarette smoke using the Ames assay in strains TA98 and TA100.	Mutat Res.	812	29-38	2016
Yershova K, Yuan JM, Wang R, Valentin L, Watson C, Gao YT, Hecht SS, Stepanov I.	Tobacco-specific N-nitrosamines and polycyclic aromatic hydrocarbons in cigarettes smoked by the participants of the Shanghai Cohort Study.	Int J Cancer.	139(6)	1261-9	2016
Wang H, Li X, Guo J, Peng B, Cui H, Liu K, Wang S, Qin Y, Sun P, Zhao L, Xie F, Liu H.	Distribution of toxic chemicals in particles of various sizes from mainstream cigarette smoke.	Inhal Toxicol.	28(2)	89-94	2016
Vu AT, Taylor KM, Holman MR, Ding YS, Hearn B, Watson CH.	Polycyclic Aromatic Hydrocarbons in the Mainstream Smoke of Popular U.S. Cigarettes.	Chem Res Toxicol.	28(8)	1616-26	2015
Machado Jde B, Chatkin JM, Zimmer AR, Goulart AP, Thiesen FV.	Cotinine and polycyclic aromatic hydrocarbons levels in the amniotic fluid and fetal cord at birth and in the urine from pregnant smokers.	PLoS One.	9(12):e116293.		2014
Ding YS, Ward J, Hammond D, Watson CH.	Mouth-level intake of benzo[a]pyrene from reduced nicotine cigarettes.	Int J Environ Res Public Health	11(11)	11898-914	2014
Zhang X, Hou H, Shi L, Liu Y, Wang A, Hu Q.	Novel method to analysis benzo[a]pyrene in filter by liquid chromatography/tandem mass spectrometry: application to assess mouth level benzo[a]pyrene exposure.	Rapid Commun Mass Spectrom.	28(13)	1468-72	2014
Dittrich David J; Fieblekorn Richard T; Bevan Michael J; Rushforth David; Murphy James J; Ashley Madeleine; McAdam Kevin G; Liu Chuan; Proctor Christopher J	Approaches for the design of reduced toxicant emission cigarettes.	SpringerPlus	3	374	2014

Slotkin Theodore A; Card Jennifer; Stadler Ashley; Seidler Frederic J; Levin Edward D	Effects of tobacco smoke on PC12 cell neurodifferentiation are distinct from those of nicotine or benzo[a]pyrene.	Neurotoxicology and teratology	43	19-24	2014
Siddique Shabana; Sadeu Jean C; Foster Warren G; Feng Yong-Lai; Zhu Jiping	In vitro exposure to cigarette smoke induces oxidative stress in follicular cells of F1 hybrid mice.	Journal of applied toxicology	34	224-6	2014
Hyodo T; Minagawa K; Inoue T; Fujimoto J; Minami N; Bito R; Mikita A	Estimation of mouth level exposure to smoke constituents of cigarettes with different tar levels using filter analysis.	Regulatory toxicology and pharmacology	67	486-98	2013
Garcia-Canton Carolina; Anadon Arturo; Meredith Clive	Genotoxicity evaluation of individual cigarette smoke toxicants using the in vitro γH2AX assay by high content screening.	Toxicology letters	223	Jul.81	2013
Yamamoto Mitsuko L; Chapman Aaron M; Schiestl Robert H	Effects of side-stream tobacco smoke and smoke extract on glutathione-and oxidative DNA damage repair-deficient mice and blood cells.	Mutation research	749	58-65	2013
Sadeu Jean Clair; Foster Warren G	The cigarette smoke constituent benzo[a]pyrene disrupts metabolic enzyme, and apoptosis pathway member gene expression in ovarian follicles.	Reproductive toxicology	40	Sep.52	2013
Jaiswal Aruna S; Panda Harekrushna; Pampo Christine A; Siemann Dietmar W; Gairola C Gary; Hromas Robert; Narayan Satya	Adenomatous polyposis coli-mediated accumulation of abasic DNA lesions lead to cigarette smoke condensate-induced neoplastic transformation of normal breast epithelial cells.	Neoplasia	15	454-60	2013
Piade J-J; Wajrock S; Jaccard G; Janeke G	Formation of mainstream cigarette smoke constituents prioritized by the World Health Organization--yield patterns observed in market surveys, clustering and inverse correlations.	Food and chemical toxicology	55	329-47	2013

Ashraf, M. Ejaz, S. Kim, B. Lim, C.W. Nawaz, M.	Cigarette smoke condensate and total particulate matter severely disrupts physiological angiogenesis	Food and Chemical Toxicology	47(3)	601-614	2009
Ashraf, M. Ejaz, S. Kim, B. Lim, C.W. Nawaz, M.	Anti-angiogenic and teratological activities associated with exposure to total particulate matter from commercial cigarettes	Food and Chemical Toxicology	47(2)	368-376	2009
Berger, A. Hamm, J.T. Misra, M. Mitchell, M. Vulimiri, S.V.	Effects of Mainstream Cigarette Smoke on the Global Metabolome of Human Lung Epithelial Cells	Chem. Res. Toxicol.	22	492-503	2009
Berndt-Weis, L. Douglas, G.R. Halappanavar, S. Stampfli, M.R. Williams, A. Yauk, C.L.	Toxicogenomic Analysis of Mainstream Tobacco Smoke-Exposed Mice Reveals Repression of Plasminogen Activator Inhibitor-1 Gene in Heart	Inhalation Toxicology	21(1-4)	78-85	2009
Bhattacharya, A. Chakrabarti, G. Das, A.	Cigarette Smoke Extract Induces Disruption of Structure and Function of Tubulin-Microtubule in Lung Epithelium Cells and in Vitro	Chem. Res. Toxicol.	22	446-459	2009
Chen, C.-C. Chen, R.-M. Pan, W.-C. Shen, Y.-C. Ueng, Y.-F.	Suppressive effect of tobacco smoke extracts on oral P-glycoprotein function and its impact in smoke-induced insult to oral epidermal cells	Toxicology Letters	185	116-123	2009
Chen, C.-C. Chen, R.-M. Pan, W.-C. Shen, Y.-C. Ueng, Y.-F.	Suppressive effect of tobacco smoke extracts on oral P-glycoprotein function and its impact in smoke-induced insult to oral epidermal cells	Toxicology Letters	185	116-123	2009
Chen, H.-W. Ku, H.-J. Lii, C.-K. Wang, T.-S.	Cigarette Smoke Extract Induces Expression of Cell Adhesion Molecules in HUVEC via Actin Filament Reorganization	Environmental and Molecular Mutagenesis	50	96-104	2009

Kim, I.k. Kim, K.-N. Kim, M.-K. Lee, J. Sohn, S.-H.	Effect of tobacco compounds on gene expression profiles in human epithelial cells	Environmental Toxicology and Pharmacology	27	111-119	2009
Kumaravel, T.S. Massey, E.D. McEwan, M. Thorne, D. Wilson, J.	Measurement of oxidative DNA damage induced by mainstream cigarette smoke in cultured NCI-H292 human pulmonary carcinoma cells	Mutation Research	673	08.Mar	2009
Al-Mugotir, M. Bitterman, P. Fang, Q. Kim, H. Kobayashi, T. Liu, X. Mao, L. Rennard, S. Togo, S. Wang, X.	NF-kappaB mediates the survival of human bronchial epithelial cells exposed to cigarette smoke extract	Respiratory Research	9(66)	11.Jan	2008
Aufderheide, M. Gressmann, H.	Mutagenicity of native cigarette mainstream smoke and its gas/vapour phase by use of different tester strains and cigarettes in a modified Ames assay	Mutation Research	656	82-87	2008
Carter, J.D. Crissman, K. Dailey, L.A. Foronjy, R.F. Ghio, A.J. Hilborn, E.D. Pinkerton, K.E. Richards, J.H. Stonehuerner, J. Uyeminami, D.	Particulate Matter in Cigarette Smoke Alters Iron Homeostasis to Produce a Biological Effect	Am J Respir Crit Care Med	178	1130-1138	2008
Casola, A. Castro, S.M. Garofalo, R.P. Guerrero-Plata, A. Kolli, D.	Cigarette Smoke Condensate Enhances Respiratory Syncytial Virus-Induced Chemokine Release by Modulating NF-kappa B and Interferon Regulatory Factor Activation	Toxicological Sciences	106(2)	509-518	2008

Coggins, C.R.E. Friedrichs, B. Gerstenberg, B. Gomm, W. Meurrens, K. Patskan, G.J. Podraza, K.F. Schnell, P. Stabbert, R. Terpstra, P. Veltel, D. Weber, S.	Toxicological Comparisons of Three Styles of a Commercial U.S. Cigarette (Marlboro) with the 1 R4F Reference Cigarette	Inhalation Toxicology	20(5-8)	695-721	2008
DeMarini, D.M. Gudi, R. Kehl, M. Kirby, P.E. Polzin, G. Rao, M. Recio, L. Richter, P.A. Szkudlinska, A.	Genotoxicity of 10 cigarette smoke condensates in four test systems: Comparisons between assays and condensates	Mutation Research	650	15-29	2008
Edmiston, J. Flora, J.W. Langston, T.B. Li, G. McKinney, W. Rana, G.S.J.B. Secrist, R.	Identification of in vitro differential cell secretions due to cigarette smoke condensate exposure using nanoflow capillary liquid chromatography and high-resolution mass spectrometry	Anal Bioanal Chem	391	2845-2856	2008
Edmiston, J.S. Kane, D.B. McKinney, W.J. Oldham, M.J. Scian, M.J.	Characterization of a Whole Smoke In Vitro Exposure System (Burghart Mimic Smoker-01)	Inhalation Toxicology	XX	10.Jan	2008
Edvinsson, L. Xu, C.-B. Zhang, W. Zhang, Y. Zheng, J.-P.	Lipid-Soluble Smoke Particles Upregulate Vascular Smooth Muscle ETB Receptors via Activation of Mitogen-Activating Protein Kinases and NF-kappaB Pathways	Toxicological Sciences	106(2)	546-555	2008
Kaushik, G. Kaushik, T. Khanduja, K.L. Khanduja, S. Pathak, C.M.	Cigarette smoke condensate promotes cell proliferation through disturbance in cellular redox homeostasis of transformed lung epithelial type-II cells	Cancer Letters	270	120-131	2008
Klus, H. Muller, L. Thielen, A.	Tobacco smoke: Unraveling a controversial subject	Experimental and Toxicologic Pathology	60(2-3)	141-156	2008

Ambrose, V. Dickson, S.J. Fowles, J. Hampton, S. Lea, R.A. Miller, J.H. Truman, P.	Tobacco particulate matter is more potent than nicotine at upregulating nicotinic receptors on SH-SY5Y cells	Nicotine & Tobacco Research	9(8)	793-799	2007
Aufderheide, M. Gressmann, H.	A modified Ames assay reveals the mutagenicity of native cigarette mainstream smoke and its gas vapour phase	Experimental and Toxicologic Pathology	58	383-392	2007
Avunduk, M.C. Civelek, S. Guven, C. Isik, A.C.U. Yardimci, S.	Morphologic Alteration Induced by Short-Term Smoke Exposure in Rats	ORL	69	13-17	2007
Baty, C.J. Choi, A.M.K. Guo, F. Karlsson, J.M. Kauffman, H.F. Kim, H.P. Lee, J.S. Liu, F. Postma, D.S. Ryter, S.W. Slebos, D.-J. van der Toorn, M. Wang, X. Watkins, S.C.	Mitochondrial Localization and Function of Heme Oxygenase-1 in Cigarette Smoke-Induced Cell Death	Am J Respir Cell Mol Biol	36	409-417	2007
Bergren, D.R.	Tobacco Smoke is an Adjuvant for Maintained Airway Sensitization in Guinea Pigs	Journal of Asthma	44	723-728	2007
Choi, A.M.K. Dong, Y. Feghali-Bostwick, C.A. Li, C. Matthay, M.A. Ning, W. Sun, J.	Cigarette Smoke Stimulates Matrix Metalloproteinase-2 Activity via EGR-1 in Human Lung Fibroblasts	Am J Respir Cell Mol Biol	36	480-490	2007
Churg, A. Kerstjens, H.A.M. Postma, D.S. Timens, W. Whittaker, P. Wright, J.L.	Airway Remodeling in the Smoke Exposed Guinea Pig Model	Inhalation Toxicology	19	915-923	2007
Coggins, C.R.E.	An Updated Review of Inhalation Studies with Cigarette Smoke in Laboratory Animals	International Journal of Toxicology	26	331-338	2007

Geerlings, M. Hylkema, M.N. Kerstjens, H.A. Luinge, M.A. Melgert, B.N. Postma, D.S. Schouten, J.P. Timens, W.	Effects of 4 months of smoking in mice with ovalbumin-induced airway inflammation	Clinical and Experimental Allergy	37	1798-1808	2007
Hayes, J.R. Meckley, D.R. Nelson, P.R. Stavanja, M.S. Swauger, J.E. Van Kampen, K.R.	Effect of a flue-curing process that reduces tobacco specific nitrosamines on the tumor promotion in SENCAR mice by cigarette smoke condensate	Food and Chemical Toxicology	45	419-430	2007
Hitchman, K. Kerepesi, L. Lu, B. Meng, Q.R. Wisse, L.	Cytotoxicity and Gene Expression Profiles in Cell Cultures Exposed to Whole Smoke from Three Types of Cigarettes	Toxicological Sciences	98(2)	469-478	2007
Ho, C.-Y. Lan, M.-Y. Lee, T.-C. Yang, A.-H.	Cigarette smoke extract induces cytotoxicity on human nasal epithelial cells	Am J Rhinol	21	218-223	2007
Hu, J. Tian, F. Xu, Y.-j. Zhang, Z.-x.	Effect of cigarette smoke extract on proliferation of rat pulmonary artery smooth muscle cells and the relevant roles of protein kinase C	Chin Med J	120(17)	1523-1528	2007
Iann, M.C. Johnson, M. Martin, M.B. Reiter, R. Richards, J.K. Shah, M.S. Singh, B. Stoica, A. Wang, A.	Effects of Tobacco Smoke Condensate on Estrogen Receptor-a Gene Expression and Activity	Endocrinology	148(10)	4676-4686	2007
Lauterbach, J.H. Momin, R.A. Rickert, W.S. Trivedi, A.H. Wright, W.G.	Effect of Smoking Conditions and Methods of Collection on the Mutagenicity and Cytotoxicity of Cigarette Mainstream Smoke	Toxicological Sciences	96(2)	285-293	2007
Meurrens K, Ruf S, Ross G, Schleef R, von Holt K, Schluter K-D	Smoking accelerates the progression of hypertension-induced mycocardial hypertrophy to heart failure in spontaneously hypertensive rats.	Cardiovascular Research	76	311-322	2007

Ayres, P.H. Curtin, G.M. Meckley, D.R. Nelson, P.R. Stavanja, M.S. Swauger, J.E.	DBA/2 mouse skin is unresponsive to dermal tumor promotion by cigarette smoke condensate	Experimental and Toxicologic Pathology	58	125-132	2006
Baginski, T.K. Dabbagh, K. Satjawatcharaphong, C. Swinney, D.C.	Cigarette Smoke Synergistically Enhances Respiratory Mucin Induction by Proinflammatory Stimuli	Am J Respir Cell Mol Biol	35	165-174	2006
Bauter, M.R. Chida, A.S. Kilty, I. Maggirwar, S.B. Rahman, I. Seweryniak, K. Shafiq, N. Yang, S.-R.	Cigarette smoke induces proinflammatory cytokine release by activation of NF-kB and posttranslational modifications of histone deacetylase in macrophages	Am J Physiol Lung Cell Mol Physiol	291	46-57	2006
Borlak, J. Gebel, S. Gerstmayer, B. Kuhl, P. Meurrens, K. Muller, T.	The Kinetics of Transcriptomic Changes Induced by Cigarette Smoke in Rat Lungs Reveals a Specific Program of Defense, Inflammation, and Circadian Clock Gene Expression	Toxicological Sciences	93(2)	422-431	2006
Brys, A.M. Gideon, K.M. Harbo, S.J. Jones, R. Lee, M.K. Meng, Q.R. Renne, R.A.	Gene Expression Profiling in Lung Tissues from Mice Exposed to Cigarette Smoke, Lipopolysaccharide, or Smoke Plus Lipopolysaccharide by Inhalation	Inhalation Toxicology	18	555-568	2006
Diekmann, J. Muller, B.L. Tewes, F.J. Wooten, J.B.	Involvement of Semiquinone Radicals in the in Vitro Cytotoxiticity of Cigarette Mainstream Smoke	Chem. Res. Toxicol.	19	1602-1610	2006
Espiritu, I. Maronpot, R.R. Witschi, H.	Lung tumors in 2 year old strain A/J mice exposed for 6 months to tobacco smoke	Cancer Letters	241	64-68	2006
Feng, Z. Hu, W. Hu, Y. Tang, M.-s.	Acrolein is a major cigarette-related lung cancer agent: Preferential binding at p53 mutational hotspots and inhibition of DNA repair	PNAS (Proceedings of the National Academy of Sciences)	103(42)	15404-15409	2006

Friedrichs, B. Van Miert, E. Vanscheeuwijck, P.	LUNG INFLAMMATION IN RATS FOLLOWING SUBCHRONIC EXPOSURE TO CIGARETTE MAINSTREAM SMOKE	Experimental Lung Research	32	151-179	2006
Fukano, Y. Yoshida, T. Yoshimura, H.	Heme oxygenase-1 gene expression in human alveolar epithelial cells (A549) following exposure to whole cigarette smoke on a direct in vitro exposure system	Experimental and Toxicologic Pathology	57	411-418	2006
Hayakawa, K. Hiramatsu, N. Kasai, A. Kitamura, M. Maeda, S. Yao, J.	High Levels of Dioxin-Like Potential in Cigarette Smoke Evidenced by In vitro and In vivo Biosensing	Cancer Res	66(14)	7143-7150	2006
Hur, G.Y. In, K.H. Jung, H.C. Jung, K.H. Kang, E.J. Kang, K.H. Kim, J.H. Lee, S.Y. Lee, S.Y. Shim, J.J. Shin, C. Yoo, S.H.	Peroxisome proliferator-activated receptor-y inhibits cigarette smoke solutioninduced mucin production in human airway epithelial (NCI-H292) cells	Am J Physiol Lung Cell Mol Physiol	291	84-90	2006
Jumarie, C. Morin, A. Porter, A. Prefontaine, D.	In vitro bioactivity of combustion products from 12 tobacco constituents	Food and Chemical Toxicology	44	724-738	2006
Kropotov, A. Krutilina, R. Leutenegger, C. Pleskach, N. Serikov, V.B. Suh, J.H. Tomilin, N.V.	Cigarette Smoke Extract Inhibits Expression of Peroxiredoxin V and Increases Airway Epithelial Permeability	Inhalation Toxicology	18	79-92	2006
Lin, S. Talbot, P. Wu, M. Yu, R.	Cigarette Smoke Toxicants Alter Growth and Survival of Cultured Mammalian	Toxicological Sciences	93(1)	82-95	2006
Mendes, E. Randhawa, K. Wanner, A.	Acute Effect of Cigarette Smoke and Nicotine on Airway Blood Flow and Airflow in Healthy Smokers	Lung	184	363-368	2006

Renne RA, Yoshimura H, Yoshino K, Lulham G, Minamisawa S, Tribukait A, Dietz DD, Lee KM & Westerberg RB	Effects of flavouring and casing ingredients on the toxicity of mainstream cigarette smoke in rats.	Inhalation Toxicology	18	685-706	2006
Aliaga, C. Dennis, P.A. Desai, D. El-Bayoumy, K. Fiala, E.S. Reinhardt, J. Seibert, E. Sodum, R.S. Sohn, O.S. Tsurutani, J. Wang, C.-X.	Induction of preneoplastic lung lesions in guinea pigs by cigarette smoke inhalation and their exacerbation by high dietary levels of vitamins C and E	Carcinogenesis	26(3)	605-612	2005
Arts, J.H.E. Buttner, A. Haussmann, H.-J. Stinn, W.	Cigarette Mainstream Smoke and Gas Phase-Depleted Particulate Phase Enhance Lung Tumorigenicity in the A/J Mouse	SOT Conference, New Orleans, 2005	Poster NewOrleans 05	2	2005
Barr, E.B. Belinsky, S.A. Finch, G.L. Gigliotti, A.P. Grimes, M.J. Hahn, F.F. Hobbs, C.H. Hutt, J.A. March, T.H. Mauderly, J.L. Seilkop, S.K. Vuillemenot, B. R.	Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways	Carcinogenesis	26(11)	1999-2009	2005
Bartalesi, B. Cavarra, E. Fineschi, S. Lucattelli, M. Lungarella, G. Lunghi, B. Martorana, P.A.	Different lung responses to cigarette smoke in two strains of mice sensitive to oxidants	Eur Respir J	25 (1)	15-22	2005
Bombick, B.R. Doolittle, D.J. Fields, W.R.	Gene Expression in Bronchial Epithelial Cells Following In Vitro Exposure to Cigarette Smoke Condensate	CORESTA 2005	SSPT23	16.Jan	2005

Bottlaender, M. Coulon, C. Dolle, F. Ottaviani, M. Syrota, A. Valette, H.	Acute inhibitor of Cardiac Monoamines Oxidase A after Tobacco Smoke Inhalation: Validation Study of [11C] Befloxatone in Rats Followed by a Positron Emission Tomography Application in Baboons.	The Journal of Pharmacology and Experimental Therapeutics	314(1)	431-436	2005
Boyle, J.O. Dannenberg, A.J. Duffield-Lillico, A.J. Gross, N.D. Morrow, J.D. Moskowitz, C.S. Subbaramaiah, K. Williams, M.K.	Levels of Prostaglandin E Metabolite, the Major Urinary Metabolite of Prostaglandin EZ, Are Increased in Smokers	Clin Cancer Res.	11(16)	6087-6093	2005
Chaudhuri, S.P. John, L. Pillai, B. Sharma, G.	Cigarette smoke extract induces changes in growth and gene expression of Saccharomyces cerevisiae	Biochemical and Biophysical Research Communications	338	1578-1586	2005
Chen, K. Chen, L. Gao, S. Li, W. Rich, C.B. Stone, P. Zhao, Y.	Transcriptional and Posttranscriptional Inhibition of Lysyl Oxidase Expression by Cigarette Smoke Condensate in Cultured Rat Fetal Lung Fibroblasts	Toxicological Sciences	87(1)	197-203	2005
Dawe, D.E. Fattouh, R. Inman, M.D. Jordana, M. Pouladi, M.A. Richards, C.D. Robbins, C.S. Stampfli, M.R. Vujicic, N.	Mainstream Cigarette Smoke Exposure Attenuates Airway Immune Inflammatory Responses to Surrogate and Common Environmental Allergens in Mice, Despite Evidence of Increased Systemic	Journal of Immunology	175	2834-2842	2005
Ejaz, S. Seok, K.B. Woong, L.C.	Toxicological Effects of Mainstream Whole Smoke Solutions on Embryonic Movements of the Developing Embryo	Drug and Chemical Toxicology	1	14.Jan	2005
Gottschalk, R.W.H. Kleinjans, J.C.S. Moonen, E.J. van Delft, J.H.M. van Herwijnen, M.H. van Leeuwen, D.M.	Differential Gene Expression in Human Peripheral Blood Mononuclear Cells Induced by Cigarette Smoke and Its Constituents	Toxicological Sciences	86(1)	200-210	2005

Hamm, J.T. Misra, M. Morrissey, R.L. Rajendran, N. Yee, S.F.	Focal Proliferative Lesions in A/J Mouse Lung Following 5-Month Exposure to Mainstream Cigarette Smoke	SOT Conference, New Orleans, 2005	PosterNew Orleans 05	2	2005
Hartman, H. Holloway, G. Millet, S. Sheabar, F. Ye, J.	Evaluation of Cigarette Smoke Cytotoxicity Under In Vitro Conditions	SOT Conference, New Orleans, 2005	SOT Poster March 05	1	2005
Haussmann, H.-J. Meurrens, K. Ruf, S. Schleef, R. Schluter, K.-D. von Holt, K.	Influence of Sub-Chronic Cigarette Smoke Exposure on the Progession of Myocardial Hypertrophy in Spontaneously Hypertensive Rats (SHR)	SOT Conference, New Orleans, 2005	Poster NewOrleans 05	2	2005
Heuer, L.S. Kaul, R. Pinkerton, K.E. Thao, N.N. Wenman, W.M. Wiedeman, J.A.	Tobacco smoke induces a persistent, but recoverable state in Chlamydia pneumoniae infection of human endothelial cells	Microbial Pathogenesis	39	197-204	2005
Hirano, Y. Ishii, H. Kobayashi, M. Kubo, S. Masunaga, Y. Shimizu, Y. Takahashi, K.	Cytokine and chemokine expression in cigarette smoke-induced lung injury in guinea pigs	Eur Respir J	26	993-1001	2005
Hoffmann, H. Hogel, J. Isner, C. Speit, G.	Genetic polymorphisms and the effect of cigarrette smoking in the comet assay	Mutagenesis	20(3)	359-364	2005
Witschi, H.	Carcinogenic Activity of Cigarette Smoke Gas Phase and Its Modulation by Beta-Carotene and N-Acetylcysteine	Toxicological Sciences	84	81-87	2005
Zhong, K.	Effect of Smoking on the Volatile SulfuretedCompounds (VSCs)inOral Cavity	CORESTA 2005	SSPT37	16.Jan	2005
Ahmad, A. Allen, R.H. Panayiotidis, M.I. Stabler, S.P. White, C.W.	Cigarette smoke extract increases S-adenosylmethionine and cystathionine in human lung epithelial-like (A549) cells	Chemico-Biological Interactions	147	87-97	2004

Aufderheide, M. Knebel, J.W. Ritter, D.	Comparative Assessment of Toxicities of Mainstream Smoke from Commercial Cigarettes	Inhalation Toxicology	16	691-700	2004
Aufderheide, M. Mohr, U.	The use of the modified CULTEX system for the direct exposure of bacteria to mainstream cigarette smoke	Coresta Congress, Kyoto, Japan		08.Jan	2004
Ayres, P.H. Curtin, G.M. Higuchi, M.A. Mosberg, A.T. Swauger, J.E.	Lung Tumorigenicity in A/J and rasH2 Transgenic Mice Following Mainstream Tobacco Smoke Inhalation	Toxicological Sciences	81	26-34	2004
Ayres, P.H. Higuchi, M.A. Sagartz, J.W. Shreve, W.K.	Comparative Subchronic Inhalation Study of Smoke From the 1R4F and 2R4F Reference Cigarettes	Inhalation Toxicology	16	20.Jan	2004
Baker RR, Massey ED & Smith G	An overview of the effects of tobacco ingredients on smoke chemistry and toxicity	Food and Chemical Toxicology	42	53-83	2004
Barakat, A.I. Douglas, G.R. Mariano, N.F. Soghomonians, A. Thirkill, T.L.	Effect of Aqueous Tobacco Smoke Extract and Shear Stress on PECAM-1 Expression and Cell Motility in Human Uterine Endothelial Cells	Toxicological Sciences	81	408-418	2004
Barankin, B. Bird, G. Freiman, A. Lauzon, G.J. Metelitsa, A.I.	Cutaneous Effects of Smoking	Journal of Cutaneous Medicine and Surgery	8(6)	415-413	2004
Barr, E.B. Bechtold, W. E. Belinsky, S.A. Finch, G.L. Gigliotti, A.P. Hahn, F.F. Hobbs, C.A. March, T.H. Mauderly, J.L. Seilkop, S.K.	Chronic Inhalation Exposure to Mainstream Cigarette Smoke Increases Lung and Nasal Tumor Incidence in Rats	Toxicological Sciences	81	280-292	2004
Bosio, A. Gebel, S. Gerstmayer, B. Haussmann, H.-J. Muller, T. Van Miert, E.	Gene expression profiling in respiratory tissues from rats exposed to mainstream cigarette smoke	Carcinogenesis	25 no 2	169-178	2004

Carchman, R.A. Gaworski, C.L. Meisgen, T.J. Podraza, K.F. Reininghaus, W. Roemer, E. Rustemeier, K. Stabbert, R. Veltel, D.J.	Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions	Toxicology	195	31-52	2004
Chaung, Y.H. Chen, H.W. Chien, M.L. Lii, C.K. Wang, T.S.	Extracts from cigarette smoke induce DNA damage and cell adhesion molecule expression through different pathways	Chem.-Biol. Interactions	150	233-241	2004
Chen, D. Hayata, I. Minamihisamatsu, M. Morishima, H. Sugahara, T. Wang, C. Wei, L. Yuan, Y. Zhang, W.	Effect of Smoking on Chromosomes Compared with That of Radiation in the Residents of a High-Background Radiation Area in China	J. Radiat. Res.	45 (3)	441-446	2004
Curtin, G.M. Hanausek, M. Mosberg, A.T. Slaga, T.J. Walaszek, Z.	Short-Term In Vitro and In Vivo Analyses for Assessing the Tumor-Promoting Potentials of Cigarette Smoke Condensates	Toxicological Sciences	81	14-25	2004
DeMarini, D.M.	Genotoxicity of tobacco smoke and tobacco smoke condensate: a review	Mutation Research	567	447-474	2004
Dolas, S.S. Maru, G. B. Pakhale, S.S. Thapliyal, R.	Evaluation of DNA Damage in Mice Topically Exposed to Total Particulate Matter from Mainstream and Sidestream Smoke from Cigarettes and Bidis	Mutagenesis	19	413-421	2004
Fuciarelli, A.F. Lee, K.-M. McKinney, W.J. Obot, C. J. Renne, R.A.	Characterization of Mainstream Cigarette Smoke-Induced Biomarker Responses in ICR and C576I/6 Mice	Inhalation Toxicology	16	701-719	2004
Garg, R. Hansch, C. Martin, P. Perfetti, T.A. Smith, C.J.	Percutaneous penetration enhancers in cigarette mainstream smoke	Food and Chemical Toxicology	42	15.Sep	2004

Geerlings, M. Hylkema, M.N. Kerstjens, H.A.M. Klok, P. A. Luinge, M.A. Melgert, B.N. Postma, D.S. Timens, W. van der Strate, B.W.A.	Short-Term Smoke Exposure Attenuates Ovalbumin-Induced Airway Inflammation In Allergic Mice	Am J Respir Cell Mol Biol	30	880-885	2004
Goldstraw, P. Pastorino, U. Ratcliffe, C. Tetley, T.D. Vanden Bon, E.J. Witherden, I.R.	Primary Human Alveolar Type II Epithelial Cell Chemokine Release Effects of Cigarette Smoke and Neutrophil Elastase	Am J Respir Cell Mol Biol	30	500-509	2004
Hayes, J.R. Meckley, D.R. Mosberg, A.T. Swauger, J.E. Van Kampen, K.R.	A responsive, sensitive, and reproducible dermal tumor promotion assay for the comparative evaluation of cigarette smoke condensates	Regulatory Toxicology and Pharmacology	39	135-149	2004
Hirose, M. Lee, I.S. Mori, Y. Nishikawa, A. Tanaka, T.	Cigarette Smoking, Metabolic Activation and Carcinogenesis	Current Drug Metabolism	5 (5)	363-373	2004
Intorp, M. Roper, W. Wieczorek, R.	HOFFMANN ANALYTES AND ClGARETTE SMOKE IN VITRO TOXICITY REVISITED - HOW DO THE DATA COMPARE?	Coresta Congress, Kyoto, Japan		08.Jan	2004
Samet, J.M.	Adverse effects of smoke exposure on the upper airway	Tob. Control	13 sup.1	57-60	2004
Abbott, B.D. Rogers, J.M.	Screening for Developmental Toxicity of Tobacco Smoke Constituents	Toxicological Sciences	75	227-228	2003
Aggarwal, B.B. Gairola, C.G. Potdar, P. Shishodia, S.	Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-kB activation through inhibition of IkBa kinase in human lung epithelial cells:correlation with suppression of COX-2, ...	Carcinogenesis	24(7)	1269-1279	2003

Andreoli, C. Gigante, D. Nunziata, A.	A review of in vitro methods to assess the biological activity of tobacco smoke with the aim of reducing the toxicity of smoke	Toxicology in Vitro	17	587-594	2003
Aoshiba, K. Koinuma, M. Nagai, A. Yokohori, N.	IMMUNOHISTOCHEMICAL EVALUATION OF OXIDATIVE STRESS IN MURINE LUNGS AFTER CIGARETTE SMOKE EXPOSURE	Inhalation Toxicology	15	1029-1038	2003
Arey, J. Iv, M. Riveles, K. Talbot, P.	Pyridines in cigarette smoke inhibit hamster oviductal functioning in picomolar doses	Reproductive Toxicology	17	191-202	2003
Aufderheide, M. Knebel, J.W. Ritter, D.	EXPOSURE OF HUMAN LUNG CELLS TO INHALABLE SUBSTANCES: A NOVEL TEST STRATEGY INVOLVING CLEAN AIR EXPOSURE PERIODS...	Inhalation Toxicology	15	67-84	2003
Ayres, P.H. Kinsler, S. Mosberg, A.T. Pence, D.H. Shreve, W.K.	RAT SUBCHRONIC INHALATION STUDY OF SMOKE FROM CIGARETTES CONTAINING FLUE-CURED TOBACCO CURED EITHER BY DIRECT-FIRED OR HEAT-EXCHANGER CURING PROCESSES	Inhalation Toxicology	15	819-854	2003
Bagnasco, M. Balansky, R.M. Bennicelli, C. Camoirano, A. Cartiglia, C. D'Agostini, F. De Flora, S. Izzotti, A. Longobardi, M.G. Lubet, R.A. Tampa, E.	Modulation of cigarette smoke-related end-points in mutagenesis and carcinogenesis	Mutation Research	523-524	237-252	2003
Balansky, R.M. Bennicelli, C. Camoirano, A. D'Agostini, F. De Flora, S. Izzotti, A. Lubet, R.A. Wang, Y. You, M. Zhang, Z.Q.	Molecular Alterations and Lung Tumors in 53 Mutant Mice Exposed to Cigarette Smoke	Cancer Res	63	793-800	2003

Barnes, P.J. Buttery, L.D.K. Habib, M. Hoyt, J.C. Polak, J.M. Robbins, R.A. Springall, D.R.	CIGARETTE SMOKE DECREASES INDUCIBLE NITRIC OXIDE SYNTHASE IN LUNG EPITHELIAL CELLS	Experimental Lung Research	29	12	2003
Bosio, A. Bulles, H. Friedrichs, D. Gebel, S. Gerstmayer, B. Koeser, A. Schlage, W.K.	Gene Expression Profiling of Two 3T3 Cell lines Exposed to Cigarette Smoke Fractions Reveals Differences in the Induction of Xenobiotic-Metabolizing Enzymes	9th International Inhalation Symposium (June 11-14) Hannover Germany	June	1	2003
Budhiraja, R. Chalkley, R. Cooray, S. Hassour, P.M. Kayyali, U.S. Lanzillo, J.J. Pennella, C.M.	Upregulation of xanthine oxidase by tobacco smoke condensate in pulmonary endothelial cells	Toxicology and Applied Pharmacology	188	59-68	2003
Dybing, E. Fowles, J.	Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke	Tob. Control	12	424-430	2003
Fowler, C.J. Sandberg, M. Tiger, G.	Effects of Water-Soluble Cigarette Smoke Extracts Upon The Release of Beta-Hexosaminidase from RBL-2H3 Basophilic Leukaemia Cells In Response To Substance P Compound 48-80 Concanavalin A and Antigen	Inflammation Research	52	461-469	2003
Green, C.R. Rodgman, A.	Toxic Chemicals in Cigarette Mainstream Smoke – Hazard and Hoopla (part1)	Beiträge zur Tabakforschung	20 no 8 (part1)	481-545	2003
Green, C.R. Rodgman, A.	Toxic Chemicals in Cigarette Mainstream Smoke - Hazard and Hoopla (part 2)	Beiträge zur Tabakforschung	20 no 8 (part 2)	481-545	2003
Green, C.R. Rodgman, A.	Toxic Chemicals in Cigarette Mainstream Smoke - Hazard and Hoopla (part 3)	Beiträge zur Tabakforschung	20 no 8 (part 3)	481-545	2003

Ishihara, A. Kita, T. Nakashima, T. Nakatani, T.	EFFECTS OF EXPOSURE TO CIGARETTE SMOKE AT DIFFERENT DOSE LEVELS ON EXTENSOR DIGITORUM LONGUS MUSCLE FIBRES IN WISTAR-KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS	Clinical and Experimental Pharmacology and Physiology	30	671-677	2003
Ortug, C.	Scanning electron microscopic findings in respiratory nasal mucosa following cigarette smoke exposure in rats	Annals of Anatomy	185	207-210	2003
Altuntas, I. Dane, S. Gumustekin, K.	EFFECTS OF CIGARETTE SMOKING ON LIPID PEROXIDATION	Journal of Basic and Clinical Physiology and Pharmacology	13(1)	69-73	2002
Bosio, A. Gebel, S. Haussmann, H.-J. Janssen, U. Knorr, C. Muller, T.	Kinetics of gene expression profiling in Swiss 3T3 cells exposed to aqueous extracts of cigarette smoke	Carcinogenesis	23 no 5	741-748	2002
Carmines EL	Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach and review of results.	Food and Chemical Toxicology	40	77-91	2002
Coggins, C.R.E.	A MINIREVIEW OF CHRONIC ANIMAL INHALATION STUDIES WITH MAINSTREAM	Inhalation Toxicology	14	991-1002	2002
Degrell, P. Mazak, I. Molnar, G.A. Nagy, J. Vas, T. Wagner, L. Wagner, Z. Wittmann, I.	Cigarette Smoke and Its Formaldehyde Component Inhibit Bradykinin-Induced Calcium Increase in Pig Aortic Endothelial Cells	Endothelium	9	103-108	2002
Garg, R. Hansch, C. Morton, M.J. Perfetti, T.A. Rodgman, A. Selassie, C.D. Smith, C.J.	The Relative Toxicity of Substituted Phenols Reported in Cigarette Mainstream Smoke	Toxicological Sciences	69	265-278	2002

Haginaka, J. Kagota, S. Kunitomo, M. Yamaguchi, Y.	Participation of peroxynitrite in oxidative modification of LDL by aqueous extracts of cigarette smoke	FEBS Letters	512	218-222	2002
Krause, G. Scherer, G. Wolz, L.	The Comet Assay with MCL-5 Cells as an Indicator of Genotoxic Treatment with Chemicals and Cigarette Smoke Condensate	ATLA	30	331-339	2002
Paschke T, Scherer G & Heller W	Effects of ingredients on cigarette smoke composition and biological activity: A literature overview.	Beitrage zur Tabakforschung International	20	107-247	2002
RODGMAN A.	Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials and humectants.	Int.Contributions to Tobacco Research	20-4	279-300.,	2002
Rustemeier K Stabbert R Haussmann H J Roemer E L carmines E L	Evaluation of the potential effects if ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke.	Food and Chemical Toxicology	40	93-104	2002
Aoki, N. Hasumura, Y. Hioki, Y. Homori, M. Ishikawa, K. Kawaro, K. Maki, A. Yanagisawa, A. Yasumura, Y. Yoshiro, H.	Acute effects of cigarette smoking on platelet-dependent thrombin generation	European Heart Journal	22	56-61	2001
Coggins, C.R.E.	A Review of Chronic Inhalation Studies with Mainstream Cigarette Smoke, in Hamsters, Dogs, and Nonhuman Primates	Toxicologic Pathology	29	550-557	2001
Avalos, J.T. Bombick, B.R. Borgerding, M.F. Chepiga, T.A. Doolittle, D.J. Morton, M.J. Murphey, P.A. Swager, J.E.	A comparison of the mainstream smoke chemistry and mutagenicity.,of a representative sample of the US cigarette market with two Kentucky reference cigarettes (Kl R4F and Kl R5F)	Food and Chemical Toxicology	38	949-962	2000

Hansch, C. Smith, C.J.	The Relative Toxicity of Compounds in Mainstream Cigarette Smoke Condensate	Food and Chemical Toxicology	38	637-646	2000
Carchman, R.A. Gomm, W.A. Meisgen, T.J. Roemer, E. Tewes, F.J.	CIGARETTE PARAMETERS THAT INFLUENCE THE MUTA-GENICITY OF MAINSTREAM SMOKE CONDENSATE.	Toxicological Sciences	48	125	1999
Gaworski CL, Heck JD, Bennett MB & Wenk ML	Toxicologic evaluation of flavour ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice.	Toxicology	139	1-17	1999
Aufderheide, M. Jarck, P. Knebel, J.W. Koch, W. Lodding, H. Massey, E. Pohlmann, G. Windt, H.	Micronucleus induction in V79 cells after direct exposure to whole cigarette smoke	Mutagenesis	13(2)	145-149	1998
Gaworski CL, Dozier HM, Heck JD, Gerhart JM, Rajendran N, David RM, Brennecke LH & Morrissey R	Toxicologic evaluation of flavour ingredients added to cigarette tobacco: 13-week inhalation exposures in rats.	Inhalation Toxicology	10	357-381	1998
Ashida, K. Itoh, T. Kamisaki, Y. Kishimoto, Y. Nakamoto, K. Wada, K.	SUBSTANCES IN THE AQUEOUS FRACTION OF CIGARETTE SMOKE INHIBIT LIPID PEROXIDATION IN SYNAPTOSOMES OF RAT CEREBRAL CORTEX	Biochemistry and Molecular Biology International	42(1)	10.Jan	1997
Ayres, P.H. Bombick, D.W. Doolittle, D.J.	CYTOTOXICITY ASSESSMENT OF WHOLE SMOKE AND VAPOR PHASE OF MAINSTREAM AND SIDESTREAM CIGARETTE SMOKE FROM THREE KENTUCKY REFERENCE CIGARETTES	Toxicology Methods	7	177-190	1997
Ohshima, H. Yoshie, Y.	Synergistic induction of DNA strand breakage by cigarette tar and nitric oxide	Carcinogenesis	18(7)	1359-1363	1997

Kalra, V.K. Rattan, V. Shen, Y. Sultana, C.	Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes	American Journal of Physiology	270	1624-1633	1996
Doolittle, D.J. Fulp, C.W. Lee, C.K. Payne, V.M. Rees, D.C. Steele, R.H.	A comparison of the mutagenicity of mainstream cigarette smoke condensates from a representative sample of the U.S. cigarette market with a Kentucky reference cigarette (KlR4F)	Mutation Research	342	179-190	1995
Eiro, H.	EFFECTS OF CIGARETTE TOBACCO TAR BY GASTRIC ADMINISTRATION ON MURINE PULMONARY TUMORIGENESIS	Journal of Toxicologic Pathology	8	377-384	1995
Burns, A.R. Dunn, L.A. Hogg, J.C. Hosford, S.P. Walker, D.C.	Respiratory epithelial permeability after cigarette smoke exposure in guinea pigs	J Appl Physiol	66(5)	2109-2116	1989
Gonzalez-Rothi, R.J. Harris, J.O.	EFFECTS OF LOW-YIELD-CIGARETTE SMOKE INHALATION ON RAT LUNG MACROPHAGES	Journal of Toxicology and Environmental Health	17	221-228	1986
Hudgens, R.W. Sonnenfeld, G.	Effect of Sidestream and Mainstream Smoke Exposure on in Vitro Interferon - alpha/beta Production by L-929 Cells	Cancer Res	46	2779-2783	1986
Schmahl, D. Zeller, W.J.	Relevance of Gas and Particulate Phases of Tobacco Smoke for Lung Cancer Formation	Cancer Detection and Prevention	9	91-97	1986
Gairola, C.	Genetic effects of fresh cigarette smoke in Saccharomyces cerevisiae	Mutation Research	102	123-136	1982
Boyd, C.H. Dansie, D.R. Henry, C.J. Kouri, R.E. Rasmussen, R.E.	DNA Replication and Unscheduled DNA Synthesis in Lungs of Mice Exposed to Cigarette Smoke	Cancer Res	41	2583-2588	1981

Coggins, C.R.E. Fouillet, X.L.M. Lam, R. Morgan, K.T.	CIGARETTE SMOKE INDUCED PATHOLOGY OF THE RAT RESPIRATORY TRACT: A COMPARISON OF THE EFFECTS OF THE PARTICULATE AND VAPOUR PHASES	Toxicology	16	83-101	1980
Lam, R.	TRANSIENT EPITHELIAL LOSS IN RAT LARYNX AFTER ACUTE EXPOSURE TO TOBACCO SMOKE	Toxicology Letters	6	327-335	1980
Bernfeld, P. Homburger, F. Pai, K.J. Soto, E.	Cigarette Smoke Inhalation Studies in Inbred Syrian Golden Hamsters	J Natl Cancer Inst (JNCI)	63(3)	675-689	1979
Akin, F. J. Benner, J.F.	Induction of Aryl Hydrocarbon Hydroxylase in Rodent Lung by Cigarette Smoke : A Potential Short-Term Bioassay	Toxicology and Applied Pharmacology	36	331-337	1976
Ames, B.N. Kier, L. D. Yamasaki, E.	Detection of Mutagenic Activity in Cigarette Smoke Condensates	Proc. Natn. Acad. Sci. USA	71 no 10	4159-4163	1974
Leuchtenberger, C. Leuchtenberger, R. Zbinden, I.	Gas Vapour Phase Constituents and SH Reactivity of Cigarette Smoke Influence Lung Cultures	Nature	247	565-567	1974
Chevalier, H.-J. Dontenwill, W. Harke, H.-P. Lafrenz, U. Reckzeh, G. Schneider, B.	Investigations on the Effects of Chronic Cigarette-Smoke Inhalation in Syrian Golden Hamsters	J Natl Cancer Inst (JNCI)	51	1781-1832	1973
Dontenwill, W. Elmenhorst, H. Harke, H.-P. Misfeld, J. Reckzeh, G. Timm, J. Weber, K.H.	Experimentelle Untersuchungen über die tumorerzeugende Wirkung von Zigarettenrauch-Kondensaten an der Mäusehaut	Z. Krebsforsch.	73	285-304	1970
Leuchtenberger, C. Leuchtenberger, R.	Cytologic and Cytochemical Effects on Primary Mouse Kidney Tissue and Lung Organ Cultures after Exposure to Whole Fresh Smoke	Cancer Res	29	862-872	1969
Benedict, R. C. Stedman, R.L.	Complexity of Enzymatic Inhibition by Cigarette Smoke	Experientia	24 no 12	1205-1206	1968

Public Health England

Protecting and improving the nation's health

Polycyclic aromatic hydrocarbons (Benzo[a]pyrene)

Toxicological Overview

Key Points

Kinetics and metabolism

- PAHs are absorbed by all routes of exposure
- PAHs are distributed widely throughout the body, with fatty tissues tending to show higher amounts
- metabolites of PAH's are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion

Health effects of acute exposure

- few studies were identified that reported the effects of BaP alone in humans following acute inhalation, ingestion or dermal exposure

Health effects of chronic exposure

- chronic exposure to mixtures of PAHs in air have resulted in a range of respiratory effects, ischemic heart disease, chronic dermatitis, depressed immune system and cancer of the skin and lungs
- BaP amongst other PAHs is able to form DNA adducts which are likely key to their mutagenic potential
- complex mixtures of PAHs which include BaP are considered to be carcinogenic to humans

PHE publications gateway number: 2014790
Published: August 2018

Summary of Health Effects

PAHs typically occur in complex mixtures and not as individual compounds, BaP is considered to be one of the most toxic PAHs and has been extensively studied. For the general public, the main route of exposure to poly aromatic hydrocarbons (PAHs) is ingestion of food. For smokers, the contribution of smoking to total PAH exposure will be similar to that of food. Inhalation and skin absorption are the main routes of occupational exposure.

PAHs are rapidly distributed throughout the body following all routes of exposure; they may be detected in most tissues minutes to hours following exposure. Benzo[a]Pyrene (BaP) is metabolised by cytochrome P450 enzymes and epoxide hydrolase resulting in a number of metabolites being formed. These metabolites include the reactive epoxide BaP 7,8 diol-9,10-epoxide (BPDE), which is believed to be play a role in the carcinogenicity of BaP. Following metabolism PAHs are excreted in the urine and/or faeces depending on their molecular weight.

No data on the acute effects of BaP in humans were identified and few studies were reported in animals. Following acute oral exposure of rats to BaP, effects on the liver were observed.

Following chronic exposure to PAHs in an occupational setting a decrease in lung function was reported, as well as chest pain, respiratory irritation, cough, dermatitis and depressed immune system; although in most cases it was not possible to evaluate the contribution of BaP to such effects. Few adverse effects were observed in rats or hamsters exposed to BaP via inhalation. Following ingestion, myelotoxicity was observed in poor affinity aryl hydrocarbon-receptor (AhR) mice but not in high affinity mice. Hepatotoxicity was also reported.

BaP can cross the placenta; BaP-DNA adducts have been found in foetal cord blood and also in the sperm. Presence of these adducts has been associated with reduced sperm count in men and decreased motor development in infants. BaP was found to cause adverse developmental and reproductive effects in mice and rats. Dietary administration during gestation reduced fertility and fetal abnormalities whereas administration by gavage caused an increase in fetal death and decreased fertility.

Biomarkers of exposure to BaP are seen concurrently with biomarkers of genotoxic effect. The International Agency for Research on Cancer (IARC) has stated that BaP likely contributes to the genotoxic action of complex PAH mixtures that individuals may be exposed to occupationally.

IARC has classified BaP as carcinogenic to humans (Group 1). No epidemiological studies of exposure to BaP alone in man can be identified; however sufficient data exists for exposure to complex PAH mixtures containing BaP.

Introduction

PAHs are a large group of compounds consisting of hydrocarbons containing two or more benzene rings fused together or to other hydrocarbon rings. They are formed during the combustion of carbonaceous material at high temperatures [1]. PAHs typically occur in complex mixtures and not as individual compounds, the process generating the mixture may define its composition. BaP is considered to be one of the most toxic PAHs and has therefore been extensively studied; hence the majority of the data in this document refers to BaP. Unless otherwise specified, “PAHs” will refer to a mixture of PAH compounds (which may include BaP).

Kinetics and Metabolism

PAHs are lipophilic compounds that are readily absorbed from the lungs following inhalation, the gastrointestinal (GI) tract following ingestion and the skin following dermal exposure [2].

PAHs may adsorb onto particulate matter in air. In humans, it was reported that BaP measured in the lungs following inhalation of soot particles was much lower than expected. This may be due to the ability of the pulmonary epithelial cells to metabolise BaP thereby facilitating its absorption and clearance from the lungs [3]. Occupational studies have inferred that inhaled PAHs are absorbed, as urinary metabolites were present in workers exposed to PAHs [2]. The absorption of BaP following inhalation is highly dependent on the type of particles onto which it is adsorbed and the site of deposition in the respiratory tract [1]. Pulmonary absorption often occurs in parallel with mucociliary clearance, by which PAHs that are absorbed onto inhaled particulates are cleared out of the pulmonary tree and subsequently swallowed [3, 4].

PAHs are well absorbed in the GI tract by passive diffusion. The extent of absorption may vary depending of the bioavailability of particles, diet and PAH size (highest for smaller molecule PAHs) [1]. In rats, approximately 40% of absorption occurred through the GI tract following administration of 0.5 µg/kg BaP for 90 minutes directly into the duodenum; 38-58% absorption occurred following administration of BaP given by gavage or in the diet [2].

BaP is efficiently absorbed through the skin of animals [1]. Extensive skin absorption has been demonstrated in mice, as almost all of an applied dose of BaP appeared in the faeces following application to the skin [4]. Similarly, rapid absorption was demonstrated in rats, monkeys and guinea pigs [2].

PAHs are rapidly distributed throughout the body by all routes of exposure; they may be detected in most tissues minutes to hours following exposure [1]. Fatty tissues generally contain more PAHs than other tissues [1, 5]. However, PAHs do not accumulate in the body [1].

BaP can readily cross the placenta following oral, inhalation or dermal exposure. One study reported that when pregnant rats were exposed to BaP via inhalation, an increase in BaP and metabolites was measured in both maternal and fetal blood and tissues. Similarly, BaP

was measured in the fetus when rats were given oral BaP on day 21 of pregnancy [2]. BaP has been detected in human breast milk, particularly in smokers, however animal studies suggest that it does not accumulate in the offspring [6].

Cytochrome P450 enzymes (CYPs) and epoxide hydrolase are the main enzymes involved in PAH metabolism. Many studies have investigated the metabolism of PAHs in tissues and cells following ingestion of food containing PAHs, or inhalation or ingestion of environmental PAHs. The liver has the greatest capacity to metabolise PAHs followed by the lungs, intestinal mucosa, skin and kidneys. BaP stimulates its own metabolism by inducing CYP enzymes via the activation of the AhR. PAHs can also inhibit CYP enzymes [1].

PAHs are initially metabolised to several epoxides by CYP enzymes. The epoxides may spontaneously rearrange to phenols or are converted to dihydrodiols. PAHs are also metabolised to a number of quinones by CYPs. The dihydrodiols are further metabolised by CYPs to 4 optically active isoforms of dihydrodiol epoxides, notable amongst these is the anti-BaP 7,8 diol-9,10-epoxide (anti-BPDE). The stereoisomer (+) anti-BPDE is considered to be the most tumorigenic and predominant metabolite of BaP that forms DNA adducts in mammalian tissues. In addition, PAHs and their reactive metabolites undergo conjugation with sulphate, glutathione (GSH) and glucuronic acid [1, 7].

The route of exposure may influence the toxicity of PAHs. Following oral exposure the compound undergoes first-pass metabolism which reduces the levels of PAHs and metabolites that reach systemic circulation. Inhalation or dermal exposure may result in higher levels of PAHs reaching peripheral tissues as the compounds may bypass the first-pass effect of the liver [1, 2]. Genetic polymorphisms may affect the capacity of individuals to metabolise PAHs [1].

Studies suggest that metabolites of PAH’s are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion [7]. High molecular weight PAHs and their metabolites are mainly excreted via the faeces [1].

Sources and Route of Human Exposure

The major route of exposure to PAHs for non-smokers in the general population is food, with a minor contribution from inhalation of ambient air. For smokers, the contribution of smoking to total exposure is likely to be similar to that of food [8].

Food may become contaminated with PAHs from environmental sources, industrial preparation or during home cooking [8].Various foods such as vegetables, meat and fish have been shown to contain PAHs, but they are largely formed due to cooking at high temperatures such as charbroiling, grilling and frying. Smoked and barbequed food are particularly important sources of exposure, although the largest contributors to PAH intake are “cereals and cereal products” and “vegetable fats and oils” [3, 4, 9-11]. The maximum estimated daily intake of BaP for a 70 kg person is 6-8 ng/kg [8, 11]. After evaluating a recent food survey, the Food Standards Agency (FSA) concluded that PAHs were typically

found in low levels in food and that consumers do not need to change their eating habits [12].

In the past, metal production and agriculture were responsible for the majority of total BaP emissions in the UK. Likely effected by the Environmental Protection Act 1990 and the ban on burning agricultural stubble, total emissions in 2011 had reduced to roughly 1/20th (now 3 tonnes) of those in 1991. Natural sources in 2011 accounted from 47.2% of the total emissions, while residential and commercial sources contributed the greatest portion of the anthropogenic emissions at 76% [13].

Annual mean air concentrations of BaP in the UK are generally below the EU target value of 1 ng/m^3, with the vast majority of monitoring sites showing levels below the UK air quality objective of 0.25 ng/m^3. Locations with point sources (e.g. industrial instillations and domestic solid fuel burning) are an exception to this. The main sources of BaP in the UK are domestic coal and wood burning, outdoor fires and industrial processes [14, 15].

Indoor air may be contaminated with PAH's by infiltration of outdoor air or from indoor emissions, which include smoking, cooking, and heating with fuel stoves and fireplaces and to a lesser extent from incense and candle burning. Levels of BaP within the home appear to vary seasonally, with the highest concentrations found in winter. BaP levels in European homes were found to be between 0.01 to 0.65 ng/m^3 [1].

Mainstream tobacco smoke contains high concentrations of PAHs, levels in the range of 1-1.6 µg per cigarette have been measured and as such this represents a major source of exposure for smokers. BaP levels in sidestream smoke have been reported to range from 52-95 ng per cigarette, more than three times higher than that seen in mainstream smoke [5]. In a smoker's home more than 87% of total PAH's in air may be introduced by cigarette smoke; in a room heavily polluted with cigarette smoke, BaP levels may be as high as 22 ng/m^3 [1].

PAHs are commonly detected in surface waters, due to urban runoff and industrial activities [3]. Contamination of drinking water with PAHs is usually associated with coal tar linings of distribution pipes. However, drinking water contributes only a minor amount to the total intake of PAHs [4, 16].

PAHs are found in the majority of surface soils due to atmospheric deposition or urban runoff. Soils near industrial sources such as coal coking often contain high concentrations of PAHs [3, 9]. BaP in English soils comprises approximately 5-7% by weight of the total PAH content [17]. The British Geological Survey defined the normal background concentrations for BaP in England and Wales to be 3.6 mg/kg in urban areas and 0.5 mg/kg in all other areas [17, 18].

Occupational exposure is largely through inhalation and skin absorption. The greatest levels of occupational exposure to BaP are in aluminium production (up to 100 µg/m^3), with lesser exposure in roofing and paving (10-20 µg/m^3) and lesser still in coal processing, wood impregnation, chimney sweeping and in power plants (at or below 1µg/m^3) [5].

Health Effects of Acute/Single Exposure

Human data

Inhalation

No studies were identified that reported the effects of BaP in humans following acute inhalation exposure.

Ingestion

Data on acute oral toxicity of BaP in humans are not available.

Dermal/ocular exposure

No studies were identified that reported effects of BaP in humans following acute dermal exposure.

Animal and in-vitro data

Inhalation

No studies were identified that reported effects of BaP in animals following acute inhalation exposure.

Ingestion

Exposure of rats (intragastric administration) to 100 mg/kg bw/day BaP for four days increased relative liver weight by 27% and induced aldehyde dehydrogenase. Limited evidence suggested that acute ingestion of BaP (50-150 mg/kg bw/day for 4 days) does not cause adverse GI effects in rats, although enzyme activity was altered. It was suggested that more serious effects may occur at higher concentrations [2].

Dermal/ocular exposure

BaP applied dermally caused allergen specific contact hypersensitivity reactions in mice after acute applications of 120 µg. A dose dependent contact hypersensitivity response to dermal application of BaP has been observed in guinea pigs; two applications of 0.001% BaP over 2-3 weeks gave slight hypersensitivity while a 1% dose gave a more severe response [2].

Acute topical application of BaP (concentration and duration of exposure not stated) to the backs of shaved mice suppressed sebaceous glands, although it was not possible to determine if such effects were due to the solvent or BaP, as a control group was not used [2].

Health Effects of Chronic/Repeated Exposure

Human data

Inhalation

A large number of epidemiological studies have been carried out considering a variety of occupations in which the workforce is chronically exposed to a mixture of PAHs [4]. These studies have demonstrated that such exposures result in symptoms including respiratory distress, decreased ventilatory function, chest pain, chest and throat irritation, cough, haematemesis, chronic dermatitis, depressed immune system and cancer of the skin and lungs [2, 4]. It is not possible to determine with any certainty the contribution of individual PAHs to these effects [4].

One study investigated the respiratory effects of inhaled BaP in employees working in various areas of a rubber factory. The authors reported a decrease in ventilatory function following prolonged exposure, as assessed by duration of employment, the greatest effects being observed in workers that had the highest exposure to particulate matter and BaP. No attempt was made to identify other possible chemical exposures or to separate effects due to BaP or particulates [2].

Ischemic heart disease was observed to increase in a dose dependent manner in asphalt workers exposed to BaP. Mean exposure for the cohort was 273 ng/m^3 and exposure at or above this level was associated with a 1.64 fold greater risk of ischemic heart disease mortality compared to those exposed to below 68 ng/m^3 [1]

Ingestion

Data on chronic oral toxicity of BaP in humans are not available.

Dermal/ocular exposure

Few data are available pertaining to BaP alone.

Regressive verrucae (warts) were reported in humans following up to 120 applications of 1% BaP over a four month period [2].

Genotoxicity

The formation of DNA adducts is believed to be a key event in the mutagenicity and carcinogenicity of PAH's; adducts may lead to misrepair and result in mutations [1]. Anti-BPDE has been demonstrated to form DNA adducts in man and as such acts as a biomarker for exposure to BaP. Molecular epidemiological studies in individuals exposed to complex mixtures of PAHs have shown that BaP adducts are seen concomitantly with biomarkers of genotoxic effect. The observed effects include chromosomal aberrations, sister chromatid exchange, DNA damage and formation of 8-oxo-deoxyguanosine. These same markers of exposure and effect are also observed in experimental animals, with association. IARC considers that BaP contributes to the genotoxic effects seen in complex

PAH mixtures; with the anti-BPDE-DNA adduct being the most mechanistically relevant adduct [19].

Smoking and diet (major sources of BaP) have been highly correlated with levels of the anti-BPDE-DNA adduct. The metabolite responsible for these adducts is seen to cause a unique array of mutations in theTP53 tumour suppressor gene, in cancers associated with smoking [19].

Evidence suggests that inhalation exposure to BaP at levels over 1 ng/m^3 is predictive of greater genomic frequency of translocation, micronuclei and DNA fragmentation [1].

Carcinogenicity

No epidemiological studies on exposure to BaP alone are available for evaluation [19]. There is however extensive literature on the epidemiology of workforces exposed to complex mixtures of PAHs which include BaP. Studies include asphalt works, coke production plants, aluminium smelters and occupations where exposure to coal tar, coal tar pitches and soot occurs. Such studies clearly showed an elevated incidence of lung tumours following inhalation and skin tumours following chronic skin contact. It is difficult to assess with any confidence the contribution of BaP or any other individual PAH to such findings [3, 4, 20].

In the 2012 evaluation, IARC classified BaP as carcinogenic to humans (Group 1). It was concluded that BaP contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAHs mixtures. The robust animal evidence and consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification [19].

Estimated cumulative exposure to BaP of 100µg/m^3 (equivalent to 3.3 µg/m^3 for 30 years) in the aluminium smelting industry has been associated with a 2.68 fold increase in the incidence of lung cancer [1].

Reproductive and developmental toxicity

There is evidence to suggest that exposure to PAHs may cause developmental effects in humans. This is supported by evidence in animal developmental studies.

PAH-DNA adducts have been found in fetal cord and maternal blood after maternal exposure to PAHs in ambient air; in light of this the World Health Organisation states that prenatal exposure could increase cancer risk from PAHs [1].

Studies show a dose-response relationship between exposure to PAHs during pregnancy and effects related to intrauterine growth restriction. A study of neonates showed that those with increased levels of PAH-DNA adducts had significantly lower birth weight, length and head circumference [1].

High cord blood levels of BaP-DNA adducts has been associated with decreased birth weight and a reduction in postnatal weight [6].

An association between dietary BaP intake and decreased birth weight, decreased birth length and having a small for gestational age infant was found in women with low vitamin C intake [6].

There is evidence to suggest that BaP may cause developmental neurotoxic effects. Human studies of prenatal environmental PAH exposure (determined by personal air monitoring and measuring BaP-DNA adduct levels in cord blood) have reported neurodevelopmental effects including impaired cognitive ability, impaired neuromuscular function and increased attention problems and anxious/depressed behaviour following prenatal exposure [6]. Infants born close to a coal-fired power station in China had 0.32±0.14 BaP-DNA adducts per 10^8 nucleotides, this level was associated with a decreased motor development at age 2. A 0.1 unit increase in BaP DNA adducts per 10^8 nucleotides, at birth, was associated with a 2 fold greater chance of developmental delay at age 2. After closure of this plant, lower adduct levels were seen in the cord blood of a new cohort and was no longer associated with reduced motor development [1].

Evidence from human studies indicates that PAH or BaP exposure may cause reproductive toxicity in males and females. Studies in adult men exposed to PAH mixtures via occupational exposure or smoking have reported an association between higher levels of BaP-DNA adducts in sperm and male infertility [6, 21]. In a case control study in a Chinese population a strong association was reported between maternal blood BaP-DNA adducts and risk of miscarriage. In a study addressing the probability of conception in women undergoing IVF (in vitro fertilisation), follicular fluid BaP levels were significantly higher in women who did not conceive [6].

Animal and in-vitro data

Inhalation

Rats exposed to BaP dust via inhalation (7.7 mg/m^3, 2 hours per day, 5 days per week for 4 weeks) showed no treatment related lesions in the lungs or nasal cavities. No dose-response relationship could be demonstrated as only one concentration of BaP was tested [3]. In the same study, kidney sections were also examined and no adverse effects were noted [2, 4]. Similarly, male hamsters did not show any adverse effects following exposure via inhalation to 9.8 mg/m^3 or 44.8 mg/m^3 BaP for 4.5 hours per day, five days per week for 16 weeks [3].

Ingestion

Few data on chronic oral toxicity of BaP in animals are available. Daily oral administration of 120 mg/kg bw BaP to poor affinity AhR mice (DBA/2N) for one to four weeks caused deaths due to myelotoxicity, whereas high affinity mice (C57B1/6N) remained unaffected during the 6 month treatment. Hepatotoxicity, as well as effects on liver and kidney enzymes have also been reported at this concentration [3, 4].

Rats fed 1,100 mg/kg bw/day BaP in the diet for more than 100 days showed a decreased growth rate [3].

PAHs including BaP have been shown to promote atherosclerotic plaque formation in AhR responsive mice, chickens and pigeons [1].

Dermal Exposure

BaP (16, 32 or 64 g per application) was applied once a week for 29 weeks onto the skin of female mice. Dose-related epidermal thickening and a pronounced inflammatory response of the dermis, amongst other effects were reported in the first weeks of exposure in those administered the high dose, and subsequently in the lower dose groups [2].

Genotoxicity

Several PAHs are mutagenic and genotoxic and induce DNA adducts in vitro and in vivo [1, 11].

BaP has consistently been shown to be positive in in-vitro assays for point mutations in Salmonella and for chromosome damage in mammalian cells, in the presence of an exogenous source of metabolic activation. Indeed it is often used as a positive control in such assays [3].

An increase in the same biomarkers of genotoxic effect seen in man on exposure to complex PAH mixtures which may include BaP have also been seen in experimental animals exposed to BaP or anti-BPDE [19]. Such effects include point mutations, sister chromatic exchange, chromosomal aberrations, sperm abnormalities and somatic mutations. BaP induced mutations are notably found in tumour suppressor genes and proto-oncogenes [5].

There is strong evidence that the formation of DNA adducts by BaP is important in mouse lung tumorigenesis and that this mechanism and the formation of radical-cations by BaP is involved in mouse skin carcinogenesis [19].

There is some evidence for the role of BaP in bitumen-fume genotoxicity; in mice exposed to bitumen fume condensates, anti-BPDE–DNA adducts and BaP metabolites have been found in the lungs and urine respectively [7].

Carcinogenicity

IARC concluded that there is sufficient evidence that BaP is carcinogenic to experimental animals [19].

BaP applied directly to the skin of various strains of mice has been reported to induce malignant (and begin) skin tumours, predominantly squamous cell carcinomas. Oral administration of BaP to mice by gavage or diet has yielded an increase in tumour response in lymphoid and hematopoietic tissues and in several organs including the lung, liver oesophagus and tongue. An increase in mammary gland adenocarcinomas has been reported in rats administered BaP by gavage. Lifetime inhalation studies in hamsters gave dose-response related increases in papilloma’s and squamous cell-carcinomas in the upper respiratory tract and in the upper digestive tract [19].

Skin tumour development was not observed in AhR deficient mice, however squamous-cell carcinomas of the skin were present in the wild-type mice [19].

Reproductive and developmental toxicity

BaP exposure has been shown to have effects on the development of laboratory animals following prenatal exposure. Developmental effects reported include decreased number of pups, an increase in reabsorptions, reduced pup weight and malformations [1, 2, 6]. Studies in mice exposed to BaP via oral administration during gestation suggest that intrauterine growth restriction, stillbirths and malformations following exposure may be dependent upon the AhR status of the mother and offspring [1-3].

Evidence from animal laboratory studies indicates that gestational exposure to BaP can have an effect on the reproductive function of offspring. Decreases in testis weight, sperm production, testosterone levels and fertility have been reported in male rodents. Effects observed in female mice include decreases in ovary weight, numbers of follicles, corpora lutea and fertility [1, 6].

Persistent neurodevelopmental effects have been observed in rats and mice exposed to BaP via ingestion as neonates; such effects include deficits in learning and memory, anxiety-related behaviours, sensorimotor development and neuromuscular function [6].

Trans-placental exposure to BaP (with dibenzo[a,l]pyrene) has been shown to induce lung and livers tumours in mice [1].

Reproductive effects have been reported in adult laboratory animals exposed to BaP. Reductions in sperm count, motility and production have been reported in various strains of adult male rats and mice and across routes of exposure. Hormonal changes and histological changes in the testis have also been observed in male adult animals. Reduced fertility and reductions in ovary weight have been reported in female adult animals following oral or inhalation exposure to BaP [6].

References

1. World Health Organisation (WHO), WHO guidelines for indoor air quality: selected pollutants. 2010, WHO Regional Office for Europe.
2. Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for polycyclic aromatic hydrocarbons (PAH). 1995, US department of Health and Human Services: Atlanta, US.
3. International Programme on Chemical Safety (IPCS), Selected non-heterocyclic policyclic aromatic hydrocarbons. Environmental Health Criteria 202. 1998, WHO: Geneva.
4. Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA), Contaminants in soil: Collation of toxicological data and intake values for humans. Benzo[a]pyrene. , in R&D Publications TOX 2. 2002, Environment Agency: Bristol.
5. International Agency for the Research on Cancer (IARC), Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2010, IARC: Lyon.
6. US EPA, Toxicological Review of Benzo[a]pyrene, I.R.I.S, Editor. 2017: Washington, DC.
7. International Agency for the Research on Cancer (IARC), Bitumens and Bitumen Emissions, and Some N- and S-Heterocyclic Polycyclic Aromatic Hydrocarbons, in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2013, IARC: Lyon.
8. European Food Safety Authority (EFSA), Findings of the EFSA Data Collection on Polycyclic Aromatic Hydrocarbons in Food, in A Report from the Unit of Data Collection and Exposure on a Request from the European Commission. 2008.
9. World Health Organisation (WHO), Air quality guidelines for Europe. WHO Regional Publications, European Series, No. 91. 2nd edition. 2000, WHO Regional Office for Europe: Copenhagen.
10. World Health Organisation (WHO), Polynuclear aromatic hydrocarbons in Drinking water. Background document for development of WHO Guidelines for Drinking water quality. 2003, WHO: Geneva.
11. European Food Safety Authority (EFSA), Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain. 2008. The EFSA Journal (2008) 724, 1-114.
12. Food Standards Agency (FSA), Polycyclic Aromatic Hydrocarbons In Cereals, Cereal Products, Vegetables, Vegetable Products And Traditionally Smoked Foods, in Food Survey Information Sheet. 2012.
13. National Physical Laboratory, Annual Report for 2012 on the UK PAH Monitoring and Analysis Network. Report to the Department of Environment, Food and Rural Affairs; the Department of Environment Northern Ireland; the Welsh Government and the Scottish Government. 2014.
14. DEFRA), Air Pollution in the UK 2015. 2016.
15. National Physical Laboratory (NPL), Annual report for 2015 on the UK PAH Monitoring and Analysis Network. 2016.
16. World Health Organisation (WHO), Guidelines for Drinking-Water Quality: Third edition. Vol 1. Recommendations. 1998, WHO: Geneva.
17. Department for Environment Food and Rural Affairs (DEFRA), Technical Guidance Sheet on normal levels of contaminants in English soils: Benzo[a]pyrene. 2012.
18. Department for Environment Food and Rural Affairs (DEFRA), Technical Guidance Sheet on normal levels of contaminants in Welsh soils: Benzo[a]pyrene. 2013.
19. International Agency for the Research on Cancer (IARC), Chemical Agents and Related Occupations, in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2012, IARC: Lyon.
20. International Agency for the Research on Cancer (IARC), Polynuclear aromatic compounds, part 1. Chemical, environmental and experimental data. Vol 32, in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1983, IARC: Lyon.
21. U.S. Environmental Protetcion Agency (U.S. EPA), Benzo(a)pyrene (BaP) TEACH Chemical Summary, in U.S. EPA, Toxicity and Exposure Assessment for Children's Health. 2007.

This document from the PHE Centre for Radiation, Chemical and Environmental Hazards reflects understanding and evaluation of the current scientific evidence as presented and referenced here.

First published: August 2018

For queries relating to this document, please contact chemcompendium@phe.gov.uk

For all other enquiries, please contact: phe.enquiries@phe.gov.uk

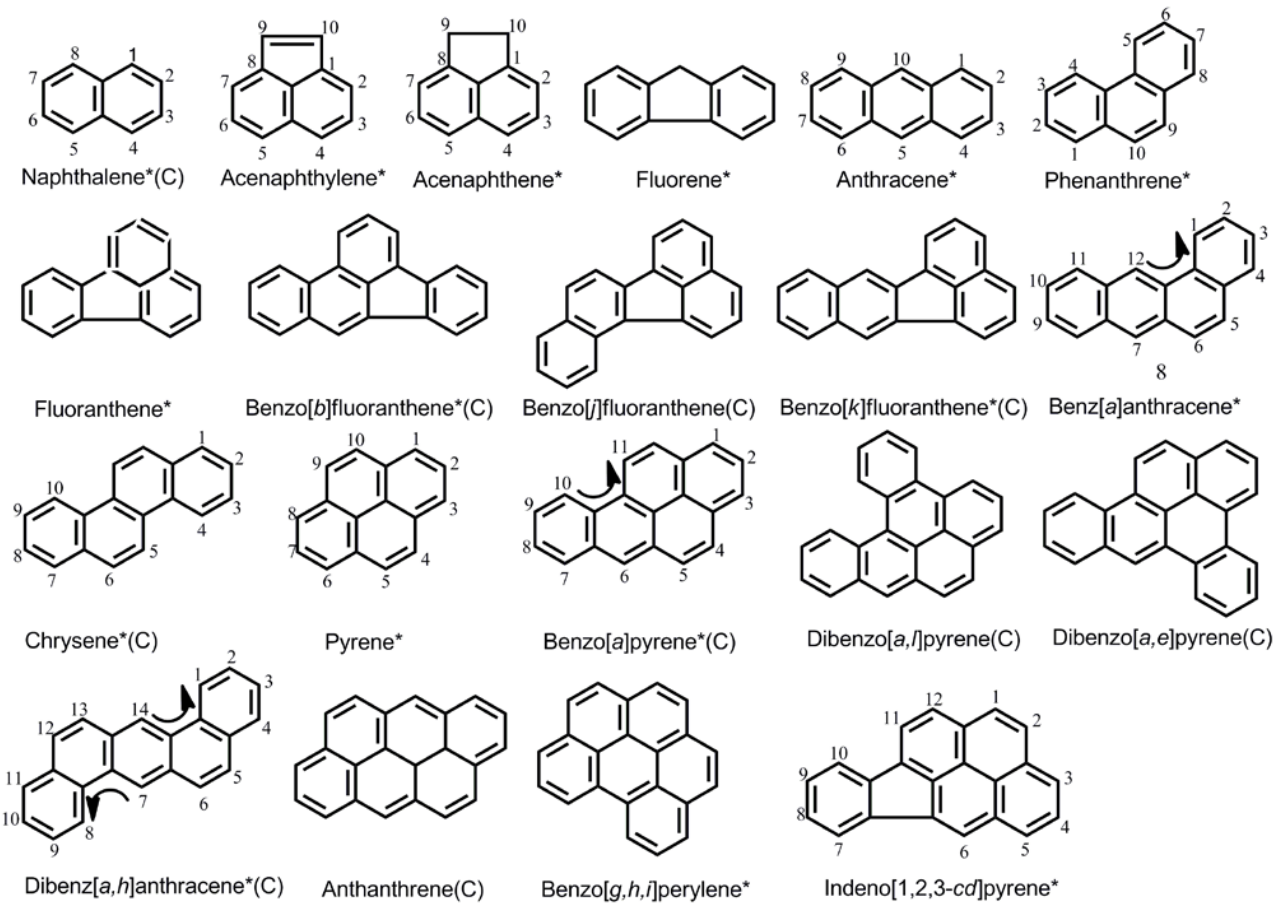
CHAPTER 7. POLYCYCLIC AROMATIC HYDROCARBONS IN AMBIENT AIR AND CANCER

Erik Dybing, Per E. Schwarze, Per Nafstad, Katarina Victorin, and Trevor M. Penning

Polycyclic aromatic hydrocarbons (PAHs), which are generated from the incomplete combustion of organic (carbonaceous) material, are ubiquitous contaminants in ambient air (IARC, 1983, 1984a, 1984b, 1985, 2010; WHO, 1998). Their occurrence in the air we breathe has been substantial during the past centuries due to emissions from industrial processes and energy production, motor vehicular traffic, incineration of refuse, and residential heating.

PAHs consist of two or more fused aromatic rings made up of carbon and hydrogen atoms. The ring systems can be present in multiple configurations and may be unsubstituted or substituted. PAHs range from semivolatile molecules to molecules with high boiling points. Thus, they may be found both in the gas and the particulate phase of ambient air or in mixtures of both phases. About 500 different PAHs have been detected in air, but often the measurements focus on benzo[*a*]pyrene (B[*a*]P) as a representative of the whole PAH family (WHO, 1998; Boström *et al.*, 2002). Many of the PAHs in ambient air are carcinogenic (IARC, 1983, 1984a, 1984b, 1985, 2010) (Figure 7.1), and a recent reassessment of their carcinogenic potential led to B[*a*]P being upgraded to a Group 1 *known human carcinogen* (IARC, 2010). Thus there is considerable concern about the relationship between PAH exposure in the ambient air and the potential to contribute to human cancer incidence. The United States Environmental Protection Agency (EPA) monitors 16 priority PAHs in air due to health concerns: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]-perylene, and dibenz[*a,h*]anthracene (in order of number of aromatic rings per structure) (Figure 7.1). Of particular note is that several PAHs (naphthalene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene and dibenzo[*a,l*]pyrene, and anthanthrene) have been found to be carcinogenic in experimental animals after inhalation or intratracheal ingestion, increasing concern about the levels of these carcinogens in ambient air (Figure 7.1).

Fig 7.1 PAHs in ambient air.



An asterisk denotes a United States Environmental Protection Agency priority pollutant. (C) indicates that the compound is carcinogenic by inhalation or intratracheal administration in experimental animals. Source: Park and Penning (2008); reproduced with permission from John Wiley & Sons.

PAH emissions in ambient air

A recent global atmospheric emission inventory of PAHs (Zhang and Tao, 2009) showed that the emission from the 16 priority PAHs listed by the EPA was 520 000 tonnes per year. Anthropogenic sources of total PAHs in ambient air emissions are greater than those that come from natural events such as forest fires and volcanic eruptions.

Apart from localized risk at or near the source of emission, PAHs can be dispersed regionally and intercontinentally through atmospheric long-range transport. For example, PAHs emitted from East Asia are transported to the west coast of the USA, and PAHs emitted in the Russian Federation influence atmospheric PAH concentrations in the Arctic (Zhang and Tao, 2009). The annual PAH emission from Asian countries is 290 000 tonnes (55% of the total); the amounts from China (114 000 tonnes per year) and India (90 000 tonnes per year) are the major contributors. The USA is the third largest emitter of PAHs, at 32 000 tonnes per year. By contrast, European countries account for only 9.5% of the total PAH emissions annually (Zhang and Tao, 2009). The contribution of the various anthropogenic sources of PAHs to the total emission

profile can vary by country and region. The global sources of PAH emissions are shown in Table 7.1, and the main sources of PAHs in six European countries are shown in Table 7.2.

The largest emission of PAHs globally comes from incomplete combustion of organic material, and the largest single source is from the combustion of biofuels. Biofuel is a single type of primary solid biomass (e.g. animal dung or peat) (Zhang and Tao, 2009). Burning biomass fuels such as wood on indoor open-pit stoves is common in developing areas, leading to harmful exposures to particulate matter < 2.5 μm in diameter ($PM_{2.5}$), carbon monoxide (CO), and PAHs, which can be significantly reduced by the introduction of modern stoves (Li *et al.*, 2011). Anthropogenic sources include PAHs that come from incomplete combustion processes (especially biofuels) and those that are made commercially, are by-products of industrial processes, or are generated from vehicle emissions, cooking, food preservation, and first- and second-hand cigarette smoke.

Anthropogenic sources of PAHs in ambient air

Commercial production

PAHs produced commercially include naphthalene, acenaphthene, phenanthrene, fluoranthene, and pyrene; however, only naphthalene is used directly without further processing, as a moth repellent.

Industrial processes

Many PAHs are released into the atmosphere during industrial processes such as coal coking and petroleum refining. It is estimated that coal coking was responsible for the release of thousands of tonnes of PAHs per year in different countries during the 1980s and early 1990s. Reduced coke production and technical improvements have led to reductions in PAH emissions from this source. Little is known about the composition of these PAH emissions (WHO, 1998). In petroleum refining, most of the emissions consist of smaller two- and three-ring compounds (94–99%, depending on the process studied) (IARC, 1989). Thus, the composition of PAHs from combustion (pyrogenic) versus the composition of PAHs from petroleum refining (petrogenic) can be widely different. Other industrial sources with significant PAH emissions are carbon black plants, wood preservation (creosote) plants, the asphalt and bitumen industry, aluminium production (Söderberg electrodes), iron and steel production, foundries, tyre production, power plants, waste incinerators, and stubble burning (WHO, 1998). Further restrictions may lead to lower PAH emissions from these industries (CORINAIR, 1997).

Estimation of the PAH emissions for six European countries indicates that the industrial sources contribute PAHs in the same range as mobile sources (Table 7.2; data from CORINAIR, 1997).

Residential sources

Domestic heating with oil and wood stoves leads to considerable PAH emissions in northern European countries, and especially in Scandinavia (Boström *et al.*, 2002). In Sweden, the emissions from wood-fired domestic heating are estimated to be about 100 tonnes per year, with minor contributions from oil combustion. Environmental tobacco smoke is also a considerable source of indoor air pollution and contamination within the home (Hoh *et al.*, 2012).

Motor vehicle emissions

The amount of PAHs released into the air from vehicles has been reduced considerably by the introduction of three-way converters. However, older diesel and gasoline cars with a

Table 7.1 Main sources of emission for the United States Environmental Protection Agency 16 priority PAHs in China, India, and the USA

Source	Global	China	India	USA
Biofuel	56.7%	66.4%	92.5%	9.1%
Wild fire	17.0%	0%	0%	3.3%
Consumer product use	6.9%	0.9%	0.6%	35.1%
Traffic oil	4.8%	2.0%	IS	23.0%
Domestic coal	3.7%	10.7%	1.3%	IS
Coke production	3.6%	14.4%	IS	IS
Petroleum refining	2.4%	1.0%	IS	8.7%
Waste incineration	1.9%	IS	IS	9.5%
Aluminium electrolysis	1.4%	IS	IS	1.9%
Open straw burning	IS	2.0%	3.2%	IS
Gasoline distribution	IS	IS	IS	3.0%
Aerospace industry	IS	IS	IS	2.5%
Other	1.5%		2.7%	3.9%
Tonnes in thousands	530	114	90	32

IS, insignificant.
Compiled from Zhang and Tao (2009).

catalytic converter of outmoded design have 5–10 times higher PAH emissions than modern cars. In addition, cold start at temperatures below the standardized cold start (23 °C), and especially at temperatures below 0 °C, results in a several-fold increase in PAH emissions. Several other technical variations lead to varying emissions, for example spark ignition engines (WHO, 1998). The total amounts of PAHs emitted from vehicles vary between countries; in the USA this can be as high as 6000 tonnes per year, and in six European countries the amount is about 400 tonnes per year (Table 7.1 and Table 7.2).

As might be expected, not all PAHs contribute equally to the emissions into ambient air. Table 7.3 lists a typical PAH profile in ambient air arising from different sources.

Human exposure

PAHs may be found in the gas and particulate phases (see Chapter 1). The levels given below frequently reflect the levels of discrete PAHs in the particulate phase and are often given as the sum of a limited number of PAH components. B[*a*]P is the traditional marker for PAH exposure. Several additional PAH components have been proposed as emission markers, for example fluoranthene, B[*a*]P, and benzo[*b*]fluoranthene. Boström *et al.* (2002) suggested the use of the following set of PAHs as emission and effect markers for monitoring air pollution: B[*a*]P, fluoranthene, phenanthrene, methylanthracenes/phenanthrenes, pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]-perylene, dibenz[*a*]anthracene, and dibenzo[*a,l*]pyrene. This list is quite similar to the 16 priority PAHs listed by the EPA (Figure 7.1). In some studies, the total PAH exposure is given as B[*a*]P toxic equivalency concentrations. In this approach, individual components are measured and ranked relative to B[*a*]P in terms of carcinogenicity. For example, chrysene has 1/1000th of the carcinogenicity of B[*a*]P and has a toxic equivalency concentration of 0.001. These calculations are used to estimate human health risk and can be used to calculate incremental lifetime cancer risk (ILCR). ILCR = exposure (μg/kg/day) × cancer slope factor (μg/kg/day). The ILCR is considered negligible when it is less than 1 in 10^5

Table 7.2 Main source sectors for PAHs in 1994 in six European countries (Austria, Denmark, Germany, Luxembourg, Norway, and the United Kingdom)

Sector	PAH emissions	
	Amount (tonnes per year)	Percentage of total
Combustion of energy and transformation industries	6.1	0.3
Non-industrial combustion plants plus wood burning	1120	60
Combustion in manufacturing industry	63	3.4
Production processes	248	13
Road transport	383	20
Other mobile sources	10	0.5
Waste incineration	30	1.6
Agriculture and forestry	1	< 0.1
Natural sources	8	0.4
Total (approximately)	**1900**	

Reproduced from Boström *et al.* (2002).

(less than 1 additional cancer case per 100 000 persons), and the cancer slope factor is based on the extrapolation of a dose–response curve for tumorigenicity seen at high dose in experimental animals.

Background levels of PAHs in remote locations have been measured between 0.01 ng/m^3 and 0.1 ng/m^3 for individual PAH components (WHO, 1998). In rural districts the levels were approximately 10 times higher, whereas in city streets levels may amount to 50 ng/m^3 or more of the more abundant individual PAHs (Boström *et al.*, 2002). Total PAHs in the centre of Stockholm, Sweden, ranged from below 100 ng/m^3 to 200 ng/m^3. The most abundant PAH was phenanthrene. In other cities higher levels of individual PAHs have been measured (WHO, 1998; Binková *et al.*, 2003). PAH was measured in the gas and particulate phase over summer and winter sampling periods in Kocaeli, Turkey. Σ_{13}PAH in the gas and particulate phases ranged from 6.2 ng/m^3 dibenz[*a*,*h*]anthracene to 98.6 ng/m^3 phenanthrene in the winter, and from 3.0 ng/m^3 benz[*a*]anthracene to 35.1 ng/m^3 phenanthrene in the summer. The most abundant PAH in both sampling periods was phenanthrene, followed by fluoranthene and pyrene. B[*a*]P toxic equivalency concentrations were found to be 3-fold higher in the winter months (Gaga *et al.*, 2012). A similar outcome was observed in a study of children aged 5–6 years (n = 260) in New York City when measurements were conducted in the heating and non-heating seasons (Jung *et al.*, 2010). In the United Kingdom, the Toxic Organic Micropollutants programme measured temporal trends in PAH in the atmosphere from 1991 to 2005 at six different sampling sites. Most showed a reduction in PAH levels and had concentrations that were lower than the new air quality standard of 0.25 ng/m^3. However, this value was exceeded in urban areas in the winter months (Meijer *et al.*, 2008).

Indoor PAH levels usually range from 1 ng/m^3 to 50 ng/m^3 due to tobacco smoke and residential heating with wood, coal, and other materials (WHO, 1998). Environmental tobacco smoke is a major contributor to air pollution and dust, and surfaces remain contaminated long after the smoking has ceased (called third-hand smoke). Measurement of PAHs in settled household dust in 132 homes showed that total PAHs were 990 ng/g in smoking households versus 756 ng/g in nonsmoking households, and when corrected

Table 7.3 Mean profiles of individual PAHs in ambient air (relative to benzo[*a*]pyrene = 1.0)

Compound	Point source	Near mobile source	Home heating	Transport	Geometric mean
Anthracene	5.5	7.6	1.0	1.8	2.9
Phenanthrene	38	200	39	43	60
Fluoranthene	14	48	12	13	18
Pyrene	9.3	28	11	7.1	12
Benz[*a*]anthracene	1.4	0.82	1.0	0.78	0.97
Perylene	0.33	0.25	0.22	0.24	0.26
Benzo[*e*]pyrene	1.5	1.3	1.6	1.4	1.4
Benzo[*g,h,i*]perylene	1.4	1.5	2.4	1.3	1.6
Indeno[1,2,3-*cd*]pyrene	1.5	1.3	1.5	1.4	1.4
Anthanthrene	0.19	0.15	0.13	0.20	0.17
Chrysene and triphenylene	3.0	2.7	3.5	2.9	3.0
Benzofluoranthene	3.6	2.9	3.6	4.4	3.6

Source: WHO (1998); reproduced with permission from the publisher.

for loading (dust/m^3), the fold change was greater than 2-fold (Hoh *et al.*, 2012).

PAHs in the ambient air can react with nitrates, hydroxyl radicals, or ozone, leading to the production of more water-soluble compounds. These compounds are rarely included in routine PAH measurements. However, nitro-PAHs have been detected on soot, and the formation of B[*a*]P-nitroquinone has been identified (Schauer *et al.*, 2004). Exposure levels of nine different nitroarenes resulting from diesel and gasoline exhaust have recently been reviewed by the International Agency for Research on Cancer; diesel exhaust was ranked as a Group 1 known human carcinogen (Benbrahim-Tallaa *et al.*, 2012).

Generally the mobile sources differ in their PAH profile, with the heavy diesel vehicles being characterized by lower-molecular-weight components than gasoline vehicles. However, per driven kilometre, total emissions from a gasoline-fuelled car are much lower than emissions from a diesel car. The three-way converter does not change the PAH profile of a gasoline-fuelled car significantly but reduces the total levels considerably. PAH levels vary with season, with higher levels being observed in the winter than in the summer. Data from Stockholm, Sweden, indicate that during the winter the levels of low-molecular-weight PAHs are increased compared with the summer (Prevedouros *et al.*, 2004).

Biomonitoring

Significant progress has been made in biomonitoring of human exposure to PAH. External dose can be measured using personalized air monitoring devices where PM is trapped on filters and then analysed for PAH content. Internal dose can be assessed by measuring blood and urinary biomarkers of exposure. Different analytes have been used as biomarkers of PAH exposure and effect. These include measuring PAH metabolites in the urine and intermediate biomarkers of effect (e.g. DNA and haemoglobin adducts). Analysis using urinary metabolites has given the most clear-cut results. Particulate pyrene is well correlated with total PAH in the breathing zone.

Urinary 1-hydroxypyrene may also reflect inter-individual variation in PAH metabolism. Occupational exposure has been found to lead to a 10–100 times greater urinary 1-hydroxypyrene content. Danish bus drivers excreted more 1-hydroxypyrene than mail carriers did, but outdoor working mail carriers had more

PAH metabolites in their urine than those working indoors, indicating the impact of outdoor air pollution (Hansen *et al.*, 2004). The use of 1-hydroxypyrene as a biomarker of PAH exposure has been criticized on the grounds that pyrene is not a carcinogenic PAH. This has led to the substitution of 3-hydroxy-B[*a*]P, but sensitive methods of detection have been a challenge. The detection of 3-hydroxy-B[*a*]P has also been criticized as a biomarker since this metabolite is not derived from any of the known pathways of B[*a*]P activation.

Measurements of urinary 1-hydroxypyrene-glucuronide, 2-naphthol, and malondialdehyde by synchronous fluorescence spectroscopy or high-performance liquid chromatography were used to evaluate seasonal and regional variations in PAH exposure and oxidative stress in Korean adults and women. Higher levels were found in individuals from industrialized areas and in the winter. Further elevation of 1-hydroxypyrene-glucuronide was observed in children exposed to environmental tobacco smoke (Yoon *et al.*, 2012). In a study in Chinese children from polluted and non-polluted areas, the levels of nine urinary monohydroxylated PAH metabolites and 8-oxo-2′-deoxyguanosine (8-oxo-dG) were compared. Children from the polluted area had a higher PAH burden than those from the non-polluted area, but no significant difference in 8-oxo-dG levels was noted (Fan *et al.*, 2012). The effect of involuntary tobacco smoke exposure on urinary levels of 23 monohydroxylated metabolites of PAH in 5060 subjects aged > 6 years was studied in the National Health and Nutrition Examination Survey (NHANES). After correcting for other confounders, significant increases in urinary 1-hydroxypyerene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyflourene, 1-hydroxypyrene, and 1-2-hydroxy-phenanthrene were observed. Increases of 1.1–1.4-fold for involuntary exposure were noted, which increased to 1.6–6.9-fold increases when children were actively exposed (Suwan-ampai *et al.*, 2009).

As there is compelling evidence for the conversion of PAH to diol-epoxides as an activation pathway (see below), there have been recent advances in measuring their corresponding tetraol hydrolysis products in humans. Progress has been made in developing stable isotope dilution liquid chromatographic mass spectrometric methods to detect phenanthrene tetraols (Hecht *et al.*, 2010; Zhong *et al.*, 2011). Phenanthrene contains a bay region and undergoes similar metabolic transformation to B[*a*]P to form diol-epoxides, which hydrolyse to tetraols. The detection of phenanthrene tetraols has also been criticized, since it is not a carcinogenic PAH. Recently, methods have been developed to measure urinary B[*a*]P tetraols with femtomole sensitivity (Hecht *et al.*, 2010), and these techniques can now be applied to biomonitoring studies.

Efforts have also been made to detect stable covalent diol-epoxide DNA and haemoglobin adducts in exposed humans. Repaired diol-epoxide DNA adducts in blood can be measured using ELISA and chemiluminescence-based methods, while unrepaired DNA adducts can be measured in lymphocytes by [^{32}P]-postlabelling methods. For example, (+)-7β,8α-dihydroxy-9α,10α-oxo-7,8,9,10-tetrahydro-B[*a*]P-N^2-deoxyguanosine [(+)-*anti*-B[*a*]PDE-N^2-dGuo] adducts have also been detected in human maternal and umbilical white blood cells after exposure to air pollution, using ELISA-based methods (Whyatt *et al.*,1998; Santella, 1999). Total DNA and B[*a*]P-like DNA adducts were measured by [^{32}P]-postlabelling in lymphocytes of nonsmoking policemen in Prague (n = 109) working 8 hour shifts. While there was no significant change in total DNA adducts, there was a marked increase in B[*a*]P-like DNA adducts correlated to personal exposure to PAHs collected on respirable particles (Topinka *et al.*, 2007). Diol-epoxide DNA adducts are

short-lived; therefore, attention has also focused on the development of methods to detect haemoglobin diol-epoxide adducts since the half-life of the red blood cell is 7–10 days (Day *et al.*, 1990).

Toxicokinetics, including metabolic activation

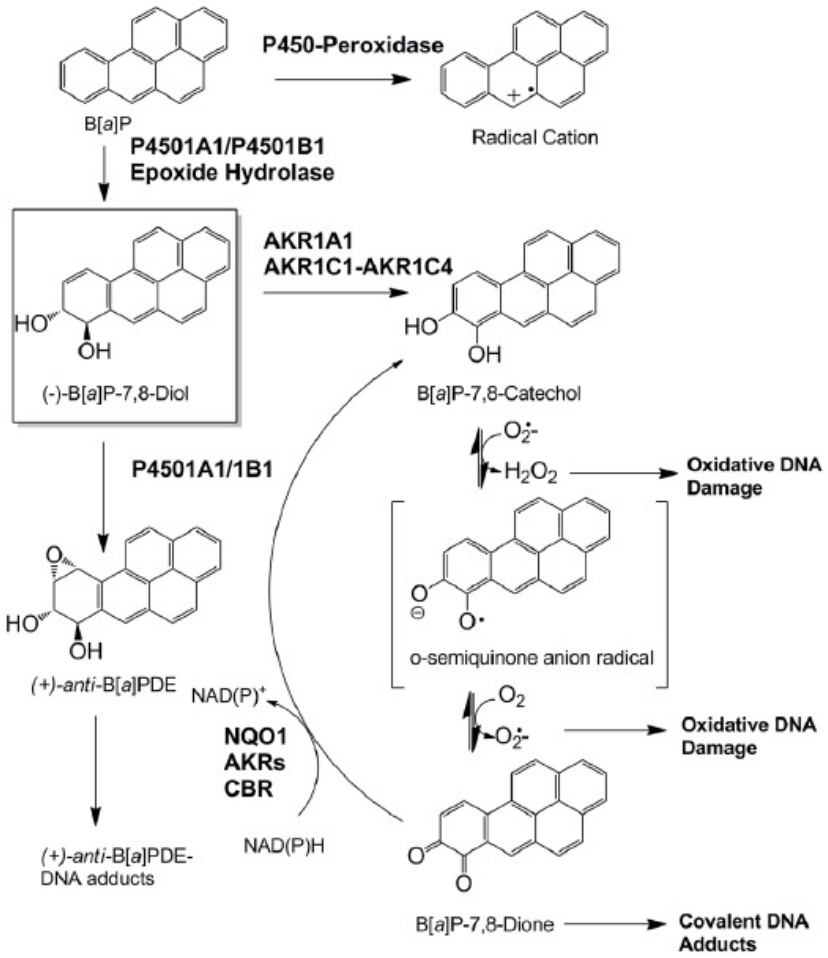
Parent PAHs have low chemical reactivity and must be metabolically activated to electrophilic intermediates to exert their carcinogenic effects (Sims and Grover, 1974; Conney 1982; Thakker *et al.*, 1985). Three pathways of PAH activation have been proposed in the literature and are best exemplified with B[*a*]P (Figure 7.2). In the first pathway, B[*a*]P is metabolically activated by either P450 peroxidase or another peroxidase by acting as a co-reductant of complex-1 (Fe^{V}). This leads to a radical cation on the most electron-deficient C6 atom, which is highly reactive and capable of forming unstable C8-guanine [8-(benzo[*a*]pyren-6-yl)guanine)], N7-guanine [7-benzo[*a*]pyren-6-yl)guanine], and N7-adenine [7-benzo[*a*]pyren-6-yl)adenine] depurinating DNA adducts (Cavalieri and Rogan, 1995). Evidence for this pathway comes from *in vitro* reactions with B[*a*]P, microsomes, and a peroxide substrate, which has led to the trapping of DNA adducts, as well as from mouse skin studies (Cavalieri *et al.*, 1990, 1991). Data exist that B[*a*]P and dibenzo[*a,l*]pyrene can exert their tumorigenicity through this mechanism in mouse skin and rat mammary gland (Cavalieri *et al.*, 1991, 2005) In addition, trace amounts of B[*a*]P-depurinating DNA adducts have been detected in the urine of smokers and in women exposed to household smoke (Casale *et al.*, 2001). However, apart from this single study, the evidence to support this mechanism due to inhalation exposure to PAH is not strong.

In the second pathway, B[*a*]P is metabolically activated to vicinal diol-epoxides (Jerina *et al.*, 1991) formed through a three-step process involving oxidation and hydrolysis reactions (Figure 7.2). In the first step, B[*a*]P is converted preferentially in the lung by the cytochrome P450 isozyme P4501B1 to the major (+)-7*R*,8*S*-epoxide and minor (–)-7*S*,8*R*-epoxide. In the second step, the 7*R*,8*R*-*trans*-dihydrodiol is predominately formed by the action of epoxide hydrolase. In the third step, diol-epoxide diastereomers are generated by another oxidation reaction via various P450 enzymes, including P4501B1 (Thakker *et al.*, 1985; Petruska *et al.*, 1992; Guengerich, 1993; Constantin *et al.*, 1994; Cavalieri and Rogan, 1995; Shimada *et al.*, 1999, 2001).

Diol-epoxides have been studied in various animal carcinogenicity models. It has been revealed that the diol-epoxides with the highest carcinogenic activity are in general the *anti*-diastereomers and especially the enantiomers with *R*-absolute configuration at the benzylic arene carbon (Thakker *et al.*, 1985; Glatt *et al.*, 1991). In studies of interactions of diol-epoxides with DNA, they demonstrate a high preference for the exocyclic amino group of deoxyguanosine and deoxyadenosine, where the major adduct derived from B[*a*]P is (+)-*anti*-B[*a*]PDE-N^2-dGuo (Jeffrey, 1985; Gräslund and Jernström, 1989; Jerina *et al.*, 1991; Geacintov *et al.*, 1997). This pathway of metabolic activation has been observed for many PAHs in ambient air, including 5-methylchrysene (Melikian *et al.*, 1983, Koehl *et al.*, 1996), benz[*a*]anthracene (Cooper *et al.*, 1980), benzo[*b*]fluoranthene (Ross *et al.*, 1992), B[*a*]P (as outlined above), dibenz[*a,h*]anthracene (Platt *et al.*, 1990), and dibenzo[*a,l*]pyrene (Luch *et al.*, 1997, 1999), in *in vitro* systems (cell extracts, microsomes, and cell culture systems), and in some cases in *in vivo* studies in animals and humans. For example, PAHs within airborne $PM_{2.5}$ produced DNA bulky stable adducts in human lung cell co-cultures (Abbas *et al.*, 2013).

In the third pathway, PAHs are metabolically activated to *o*-quinones by the action of aldo-keto reductases (AKRs) (Penning *et al.*, 1999; Penning, 2004). For B[*a*]P, the sequence involves

Fig 7.2 Pathways of PAH activation using benzo[*a*]pyrene as an example.



Source: Park and Penning (2008); reproduced with permission from John Wiley & Sons.

the NAD(P)$^+$-dependent oxidation of the 7*R*,8*R*-*trans*-dihydrodiol to a ketol catalysed by AKR1A1, AKR1C1–AKR1C4 (Figure 7.2). The ketol then spontaneously rearranges to a catechol, which undergoes air-oxidation to yield B[*a*]P-7,8-dione and reactive oxygen species (ROS) (Palackal *et al.*, 2001, 2002; Penning *et al.*, 1996). B[*a*]P-7,8-dione is both electrophilic (will react with DNA) and redox-active. In the presence of reducing equivalents and NQO1, AKRs themselves, and carbonyl reductase, the quinones can be reduced back to the corresponding catechols, and if they are not intercepted a futile redox cycle will ensue in which NADPH is depleted and ROS is amplified (Shultz *et al.*, 2011). This pathway of metabolic activation has been observed for several PAHs in ambient air, including phenanthrene, chrysene, 5-methyl-chrysene, benz[*a*]anthracene, and B[*a*]P in *in vitro* systems (recombinant enzymes) and cultures of human lung cells (Palackal *et al.*, 2001, 2002; Park *et al.*, 2008b).

Efforts have been made to assess the contribution of each of these pathways to the metabolic activation of B[*a*]P in human lung cells. Using a stable isotope dilution liquid chromatographic mass spectrometric method, signature

metabolites of each of the three pathways were measured: B[*a*]P-1,6-dione and B[*a*]-3,6-dione (radical cation metabolites), B[*a*]P-tetraol-1 (diol-epoxide metabolites), and B[*a*]P-7,8-dione (*o*-quinone metabolites) in human bronchoepithelial (H358) cells in the presence and absence of the aryl hydrocarbon receptor (AhR) agonist TCDD. It was found that each of the pathways contributed equally to B[*a*]P metabolism in the presence and absence of TCDD (Lu *et al.*, 2011).

The rate of absorption of PAHs from the tracheobronchial epithelium after inhalation exposure is determined by their high lipophilicity (Gerde *et al.*, 1993). For lipophilic carcinogens such as B[*a*]P, the delayed absorption in the airway mucosa is a result of slow passage through the airway epithelium, yielding a very high dose to these target cells. Because of the long retention time, the metabolic activation can be considerable even at low enzyme activities (Bond *et al.*, 1988).

Modes of action

Carcinogenic PAHs are generally positive in short-term tests for mutagenicity (Table 7.4), for example the bacterial *Salmonella* mutagenicity (Ames) assay and the *HPRT*-mammalian cell mutagenicity assay, provided a metabolic activation system is present (Malaveille *et al.*, 1977; MacLeod *et al.*, 1988; Chen *et al.*, 1990; Wei *et al.*, 1993). In the Ames assay, a rat liver S9 activation system is used; in the *HPRT* assay, recombinant P4501A1 and P4501B1 are co-expressed. The mutagenic species has been identified by comparing the mutagenic potency of different PAH metabolites, which demonstrates that of the known metabolites the diol-epoxides are the most potent mutagens (Malaveille *et al.*, 1977). Treatment of a plasmid containing *K-Ras* with the (+)-*anti*-B[*a*]PDE followed by transfection into NIH3T3 cells led to cell transformation with increased foci in soft agar. Rescue of the plasmid showed that there were single point mutations of the 12th and 61st codons, which could explain the transformation potential of the diol-epoxide. The dominant mutation observed was a G → T transversion, consistent with DNA-adduct formation on deoxyguanosine (Marshall *et al.*, 1984). One of the most compelling pieces of data has shown that by using ligation-mediated polymerase chain reaction, the (+)-*anti*-B[*a*]PDE preferentially forms DNA adducts in *hot spots* on the *p53* tumour suppressor gene, which is one of the most mutated genes in human lung cancer. These hot spots correspond to the same codons that are mutated in tumours obtained from humans with lung cancer. The dominant mutation observed was again a G → T transversion, consistent with DNA adduct formation on deoxyguanosine (Denissenko *et al.*, 1996; Hainaut and Pfeifer, 2001).

In a separate *in vitro* study, the mutagenic potency of (±)-*anti*-B[*a*]PDE and B[*a*]P-7,8-dione (AKR product) were compared in a yeast-reporter gene assay for *p53* mutation. It was found that B[*a*]P-7,8-dione was 80-fold more mutagenic than the diol-epoxide provided it was permitted to redox cycle (Yu *et al.*, 2002). In these experiments there was a linear correlation between (±)-*anti*-B[*a*]PDE mutagenicity and the formation of (+)-*anti*-B[*a*]PDE-N^2-dGuo adducts, and a linear correlation between B[*a*]P-7,8-dione mutagenicity and the formation of 8-oxo-dGuo adducts (Park *et al.*, 2008a). In addition, B[*a*]P-78-dione gave predominately G → T transversions, consistent with the base mispairing of 8-oxo-dGuo with adenine. The position of the point mutations within *p53* was quite random until there was biological selection for dominance, and then the spectrum of mutations was similar to that seen in lung cancer (Park *et al.*, 2008b). These data suggest that B[*a*]P-7,8-dione formed by AKRs has the potential to contribute to the carcinogenic mode of action of B[*a*]P.

Planar PAHs can induce their own metabolism. Compounds such as B[*a*]P can bind to the AhR (Nebert and Jensen, 1979; Nebert *et al.*,

Table 7.4 Genotoxicity of individual PAHs that are carcinogenic in experimental animals after inhalation or intratracheal instillation

Compound	Results
Anthanthrene	Positive, limited database
Benzo[*b*]fluoranthene	Positive
Benzo[*j*]fluoranthene	Positive
Benzo[*k*]fluoranthene	Positive
Benzo[*a*]pyrene	Positive
Chrysene	Positive
Dibenz[*a,h*]anthracene	Positive
Dibenzo[*a,i*]pyrene	Positive
Indeno[1,2,3-*cd*]pyrene	Positive
Naphthalene	Negative for gene mutations, positive for clastogenicity *in vitro*

Source: WHO (1998); reproduced with permission from the publisher.

1993, 2004). This leads to nuclear localization of the liganded AhR, where it can act as a transcription factor by binding to the xenobiotic response element to induce the *CYP1A1* and *CYP1B1* genes (Denison *et al.*, 1988a, 1988b, 1989), which will result in enhanced monoxygenation of the parent PAH. PAH metabolism leads to the production of electrophiles (e.g. quinones), which can activate the Nrf2-Keap 1 system. Nrf2 acts as a transcription factor and binds to the antioxidant response element to induce γ*GCS*, *NQO1* and *AKR1C1–AKR1C3*, and *AKR1B10* (Burczynski *et al.*, 1999; Jin and Penning 2007; Penning and Drury, 2007). Importantly, AKR1C1–AKR1C3 are involved in the metabolic activation of PAH *trans*-dihydrodiols to the electrophilic and redox active PAH *o*-quinones, which could further exacerbate PAH activation via induction of AKRs. The PAH *o*-quinones produced by this pathway are also ligands for the AhR (Burczynski and Penning, 2000). Thus, both the parent PAH and their downstream metabolites can lead to the metabolic activation of PAHs in ambient air.

PAHs may, in addition to initiating carcinogenesis via a genotoxic mechanism, exert promotional effects through various modes of action. Certain PAHs induce inflammatory processes (Casale *et al.*, 1997). The binding of PAHs to the AhR also leads to transcriptional upregulation of genes involved in growth as well as biotransformation and differentiation (Nebert *et al.*, 1993). Studies also indicate the ability of both PAHs and their metabolites to activate kinases involved in survival signalling, thus giving DNA-damaged cells a survival advantage (Burdick *et al.*, 2003). At higher concentrations some PAHs induce apoptosis (Solhaug *et al.*, 2004). In addition, PAHs show inhibitory effects on gap junctional intercellular communication (Upham *et al.*, 1996; Weis *et al.*, 1998).

Carcinogenicity studies in animals

Most investigations of PAH carcinogenesis by the respiratory route are intratracheal instillation studies (WHO, 1998). In all, 10 PAHs have been found to be carcinogenic in experimental animals after inhalation or intratracheal instillation (WHO, 1998; NTP, 2000) (Table 7.5). Only B[*a*]P and naphthalene have been studied by the inhalation route. In one inhalation study in hamsters, groups of 24 males were exposed to B[*a*]P condensed onto sodium chloride particles at concentrations of 2.2, 9.5, and 46.5 mg/m^3 for 4.5 hours per day, 7 days per week for the first 10 weeks, then for 3 hours per day for 2 years. Exposure was by nose breathing only. There were no tumours in the controls or in the low-exposure

group. In the other two groups, exposure-related tumours were found in the nasal cavity, larynx, trachea, pharynx, oesophagus, and forestomach, but not in the lung (Thyssen *et al.*, 1981). RIVM (1989) cites two other inhalation studies with B[*a*]P not found in the open literature: one in mice (Knizhnikow *et al.,* 1982; see RIVM, 1989) and one in rats with co-exposure with sulfur dioxide (Laskin *et al.,* 1970; see RIVM, 1989). In both studies malignant lung tumours were observed.

In recent bioassay inhalation studies with naphthalene, Fischer 344/N rats developed neuroblastomas of the nasal olfactory epithelium after being exposed in inhalation chambers to 0, 10, 30, or 60 ppm (80, 52, 157, or 314 mg/m^3) for 6 hours per day, on 5 days per week, for 105 weeks (NTP, 2000). The observed rates in males were 0/49, 0/49, 4/48, and 3/48, respectively, and in females 0/49, 2/49, 3/49, and 12/49, respectively. In addition, adenomas of the nasal respiratory epithelium were observed in 0/49, 06/49, 8/48, and 15/48 males and in 0/49, 0/49, 4/49, and 2/49 females, respectively. In the study with B6C3F$_1$ mice subjected to whole-body exposure of 0, 10, or 30 ppm (0, 52, or 157 mg/m^3) naphthalene in inhalation chambers for 6 hours per day, 5 days per week, for 104 weeks, a statistically significant increase in the incidence of bronchioloalveolar adenomas in high-dose female mice was observed (NTP, 2000). Increased incidences of bronchioloalveolar adenomas and carcinomas were observed in the male mice, but the increases were not statistically significant.

PAHs and their metabolites will also cause lung cancer in animals when administered by other routes. Classically, the newborn mouse model of lung cancer was used to rank the tumorigenicity of different B[*a*]P metabolites, given that the developing lung is more susceptible to carcinogen exposure. Studies such as these showed that the (+)-*anti*-B[*a*]PDE was the most potent lung tumorigen of the known B[*a*]P metabolites (Buening *et al.*, 1978; Kapitulnik *et al.*, 1978). Similarly, in the A/J mouse lung model of B[*a*]P-induced carcinogenesis, *anti*-B[*a*]PDE-DNA adducts were early lesions that could be detected in the initiation phase (Nesnow *et al.*, 1998).

Carcinogenesis experiments with mixtures containing PAHs have also been reported. Heinrich *et al.* (1994) exposed groups of 72 female Wistar rats to a coal tar/pitch aerosol containing either 20 or 46 µg/m^3 B[*a*]P for 17 hours per day, 5 days per week, for 10 or 20 months, followed by a clear air period of up to 20 or 10 months, respectively. The cumulative doses of inhaled B[*a*]P of the four exposure groups were 71, 143, 158, and 321 mg B[*a*]P/m^3 hours, and the corresponding lung tumour rates were 4.2%, 33.3%, 38.9%, and 97.2%, respectively, whereas there were no tumours in the control group. In similar experiments in which rats were exposed to coal tar/pitch vapour condensed on the surface of fine carbon black particles, the resulting lung tumour rate was about twice as high.

Pott and Heinrich (1990) have also performed a lifelong inhalation study with rats exposed to diesel exhaust. In this study, tumour rates similar to those in the study with pitch pyrolysis vapours were induced, although the PAH content (measured as B[*a*]P) was 100–1000 times lower. This result indicates that diesel exhaust contains other potent carcinogenic or tumour-promoting compounds besides unsubstituted PAHs.

Numerous carcinogenicity studies have been performed using dermal application and subcutaneous and intramuscular injection (for overview, see WHO, 1998). An oral gavage study with B[*a*]P revealed tumour development in the liver, forestomach, auditory canal, oral cavity, skin, and intestines in both sexes of rats, and additionally the kidney in males and the mammary gland and oesophagus in females (RIVM, 2001). However, no lung tumours were observed after this route of administration. In a feeding study of B[*a*]P in mice, tumours in the tongue,

Table 7.5 Carcinogenicity of individual PAHs in experimental animals after inhalation or intratracheal instillation

Compound	Carcinogenicity (weight of evidence)	Species	No. of studies with positive, negative, and questionable results		
			+	–	±
Anthanthrene	Positive	Mouse	1		
Anthracene	Negative	Rat		1	
Benzo[*b*]fluoranthene	Positive	Rat Hamster	1	1	
Benzo[*j*]fluoranthene	Positive	Rat	1		
Benzo[*k*]fluoranthene	Positive	Rat	1		
Benzo[*g,h,i*]perylene	Negative	Rat		1	
Benzo[*a*]pyrene	Positive	Mouse Rat Hamster	1 9 11	1	1
Benzo[*e*]pyrene	Negative	Rat		1	
Chrysene	Positive	Rat	1		
Dibenz[*a,h*]anthracene	Positive	Rat Hamster	1 1	1	
Dibenzo[*a,i*]pyrene	Positive	Hamster	2		
Indeno[1,2,3-*cd*]pyrene	Positive	Rat	1		
Naphthalene	Positive	Mouse Rat	1		2
Phenanthrene	Negative	Rat		1	
Pyrene	Negative	Hamster		1	

Source: WHO (1998); reproduced with permission from the publisher; IARC (2002).

oesophagus, forestomach, and larynx, but not lung, were observed (Culp *et al.*, 1998).

Carcinogenicity studies in humans

Occupational exposures

A review and meta-analysis on the association between occupational exposure to PAHs and lung cancer development in 39 cohorts found an average relative risk of 1.20 per 100 μg/m^3 years cumulative B[*a*]P (Armstrong *et al.*, 2004). For some occupations relative risks were considerably higher, but confidence intervals were very wide. For exposures in coke ovens, gas works, and aluminium industries, the risk is equivalent to a relative risk of 1.06 for a working lifetime of 40 years at 1 μg/m^3.

Ambient air exposures

Few studies have addressed the impact of exposure to PAHs in ambient air on human cancer. Studies using other exposure indicators (PM or NO_2) have shown associations between air pollution and lung cancer; however, no PAH exposure information was available (Pope *et al.*, 2002; Hoek *et al.*, 2002; Nafstad *et al.*, 2003). An analysis of the United States data on lung cancer, PM exposure, and older PAH and metal air concentration data, supports the plausibility that known chemical carcinogens may be responsible for the lung cancer attributed to $PM_{2.5}$ exposure in the American Cancer Society study (Harrison *et al.*, 2004). A study by Cordier *et al.* (2004) found an increased risk of childhood brain cancer associated with PAH exposure. Both paternal

preconception occupational PAH exposure and paternal smoking were associated with increased risks for childhood brain tumours.

Human susceptibility

PAHs are metabolically activated by phase I P450 isozymes (*CYP1A1*, *CYP1B1*) in combination with epoxide hydrolase (*EPHX*) and phase I AKR isozymes (*AKR1A1*, *AKR1C1-AKR1C4*) and are detoxified by phase II enzymes including *GSTs*, *UTGs*, *SULTs*, and *COMT*. In addition, bulky covalent diol-epoxide DNA adducts can be repaired by nucleotide excision repair proteins (*XPD* [helicase], *XPA*, and *XPC* [damage recognition]), and oxidative DNA lesions can be repaired by base excision repair enzymes (*hOGG1* and *APE*). Each of these genes is highly polymorphic in the human population. (A complete list of these variants is available at the NCBI database: http://www.ncbi.nlm.nih.gov/.) Many of these variants are non-synonymous single-nucleotide polymorphisms (nSNPs) that can affect enzyme activity. Combinations of these nSNPs rather than an individual SNP may affect human genetic susceptibility to PAH emissions in ambient air.

In a study of Prague policemen occupationally exposed to polluted air, B[*a*]P-like DNA adducts were detected and found to be positively associated with SNPs in *XPD* and *GSTM1* (Binková *et al.*, 2007). In another lung cancer case–control study, exposure to environmental tobacco smoke and polymorphisms in *CYP1B1 Leu(432)Val* was significantly associated with lung cancer susceptibility, with an odds ratio for at least one allele of 2.87 (95% confidence interval [CI], 1.63–5.07) (Wenzlaff *et al.*, 2005a). Combinations of the polymorphism in this phase I enzyme gene along with those selected from either phase II enzyme genes (*GSTM1* null, *GSTP1 Ile(105)Val*) or NADPH-quinone oxidoreductase (*NQO1*) *C(609)T*) were also evaluated. Here the combination of the *CYP1B1 Leu(432)Val* allele and the *NQO1 C(609)T* allele was associated with the highest risk of lung cancer (odds ratio [OR], 4.14; 95% CI, 1.60–10.74) (Wenzlaff *et al.*, 2005a). In the same study cohort, variants in *GSTM1*, *GSTT1*, and *GSTP1* were examined to determine whether there was an association of the genotype with lung cancer incidence in never-smokers. Individuals who had been exposed to household environmental tobacco smoke for > 20 years, and who were carriers of either the *GSTM1* null allele or the *GSTP1* Val allele, were at a 4-fold increased risk of developing lung cancer (OR, 4.56; 95% CI, 1.21–17.21) (Wenzlaff *et al.*, 2005b). In a lung cancer case–control study in China, women who were never-smokers were found to be at a significant increased risk of adenocarcinoma if they were carriers of the variants in the nucleotide excision repair variant *XRCC1* 399 Gln/Gln versus the Arg/Arg genotype (OR, 14.12; 95% CI, 2.14–92.95). The OR of lung adenocarcinoma for the *XRCC1* 399Gln allele with exposure to cooking oil smoke was 6.29 (95% CI, 1.99–19.85) (Li *et al.*, 2005). DNA integrity was investigated in 50 bus drivers, 20 garage men, and 50 controls in the Czech Republic and associated with variants in the base excision repair gene *hOGG1*. Carriers of at least one variant (Cys allele) had a higher degree of DNA damage (Bagryantseva *et al.*, 2010). To date, no molecular epidemiological study has been performed whereby combinations of polymorphic variants in phase I, phase II, and DNA repair genes have been pooled. However, based on the studies described, carriers of variants in all three classes of genes might be at higher risk of developing lung cancer from emissions of PAHs in ambient air.

Conclusions

PAHs generated from the incomplete combustion of organic material are ubiquitous contaminants in urban air. There are numerous unsubstituted PAHs (pyrogenic) and substituted PAHs (petrogenic). The pyrogenic PAHs may occur in the gas phase, particulate phase,

or mixtures of both phases. The major worldwide source is the combustion of biofuels, while other sources such as combustion plants, various industrial and production processes, road transport, and waste incineration can contribute. Total PAH levels in some urban areas are in the range of 100–200 ng/m^3 but may be even higher in more polluted areas and can show distinct seasonal variation. However, measurements of total PAHs are relatively scarce. B[*a*]P is the traditional marker for PAHs, but various other individual PAHs have also been proposed, such as fluoranthene, B[*a*]P, and benzo[*b*]fluoranthene. Biomarkers of exposure include 1-hydroxypyrene, 3-hydroxy-B[*a*]P, and tetraols, but DNA and protein adducts can also be measured as intermediate cancer biomarkers. The major disease end-point of interest is lung cancer, and approximately 10–15% of all lung cancer cases are seen in never-smokers. Parent PAHs must be metabolically activated to electrophilic intermediates (radical cations, vicinal diol-epoxides, and *o*-quinones) to act as lung carcinogens. All three routes have been observed in human lung cells. Various promotional effects of PAHs may contribute to their carcinogenic action. In all, 10 PAHs have been found to be carcinogenic in experimental animals after inhalation or intratracheal instillation. Naphthalene seems to be an exception compared with other carcinogenic PAHs as it appears to not be genotoxic. A meta-analysis of occupational cohort studies found a 20% increase in relative risk per 100 μg/m^3 years cumulative B[*a*]P exposure. Studies of ambient air pollution and cancer have demonstrated an association between carriers of polymorphic variants in phase I, phase II, and DNA repair enzyme genes.

References

Abbas I, Garcon G, Saint-Georges F *et al.* (2013). Polycyclic aromatic hydrocarbons within airborne particulate matter (PM 2.5) produced DNA bulky stable adducts in a human lung cell coculture model. *J Appl Toxicol*, 33: 109–119. PMID:21913209

Armstrong B, Hutchinson E, Unwin J, Fletcher T (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ Health Perspect*, 112: 970–978. doi:10.1289/ehp.6895 PMID:15198916

Bagryantseva Y, Novotna B, Rossner P Jr *et al.* (2010). Oxidative damage to biological macromolecules in Prague bus drivers and garagemen: impact of air pollution and genetic polymorphisms. *Toxicol Lett*, 199: 60–68. doi:10.1016/j.toxlet.2010.08.007 PMID:20723587

Benbrahim-Tallaa L, Baan RA, Grosse Y *et al.* (2012). International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes. *Lancet Oncol*, 13: 663–664. doi:10.1016/S1470-2045(12)70280-2 PMID:22946126

Binková B, Cerná M, Pastorková A *et al.* (2003). Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter seasons 2000–2001. *Mutat Res*, 525: 43–59. doi:10.1016/S0027-5107(02)00312-3 PMID:12650904

Binková B, Chvatalova I, Lnenickova Z *et al.* (2007). PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair gene polymorphisms. *Mutat Res*, 620: 49–61. doi:10.1016/j.mrfmmm.2007.02.022 PMID:17412371

Bond JA, Harkema JR, Russell VI (1988). Regional distribution of xenobiotic metabolizing enzymes in respiratory airways of dogs. *Drug Metab Dispos*, 16: 116–124. PMID:2894939

Boström CE, Gerde P, Hanberg A *et al.* (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect*, 110: Suppl 3: 451–488. doi:10.1289/ehp.02110s3451 PMID:12060843

Buening MK, Wislocki PG, Levin W *et al.* (1978). Tumorigenicity of the optical enantiomers of the diastereomeric benzo[*a*]pyrene 7,8-diol-9,10-epoxides in newborn mice: exceptional activity of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc Natl Acad Sci USA*, 75: 5358–5361. doi:10.1073/pnas.75.11.5358 PMID:281685

Burczynski ME, Lin HK, Penning TM (1999). Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: implications for the

alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. *Cancer Res*, 59: 607–614. PMID:9973208

Burczynski ME & Penning TM (2000). Genotoxic polycyclic aromatic hydrocarbon *ortho*-quinones generated by aldo-keto reductases induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. *Cancer Res*, 60: 908–915. PMID:10706104

Burdick AD, Davis JW 2nd, Liu KJ *et al.* (2003). Benzo(a) pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. *Cancer Res*, 63: 7825–7833. PMID:14633709

Casale GP, Higginbotham S, Johansson SL *et al.* (1997). Inflammatory response of mouse skin exposed to the very potent carcinogen dibenzo[*a,l*]pyrene: a model for tumor promotion. *Fundam Appl Toxicol*, 36: 71–78. doi:10.1006/faat.1997.2291 PMID:9073469

Casale GP, Singhal M, Bhattacharya S *et al.* (2001). Detection and quantification of depurinated benzo[*a*] pyrene-adducted DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem Res Toxicol*, 14: 192–201. doi:10.1021/tx000012y PMID:11258968

Cavalieri EL, Higginbotham S RamaKrishna NVS *et al.* (1991). Comparative dose-response tumorigenicity studies of dibenzo[*alpha,l*]pyrene versus 7,12-dimethylbenz[*alpha*]anthracene, benzo[*alpha*] pyrene and two dibenzo[*alpha,l*]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis*, 12:1939–1944. doi:10.1093/carcin/12.10.1939 PMID:1934274

Cavalieri EL & Rogan EG (1995). Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica*, 25: 677–688. doi:10.3109/00498259509061885 PMID:7483666

Cavalieri EL, Rogan EG, Devanesan PD *et al.* (1990). Binding of benzo[*a*]pyrene to DNA by cytochrome P-450 catalyzed one-electron oxidation in rat liver microsomes and nuclei. *Biochemistry*, 29: 4820–4827. doi:10.1021/bi00472a011 PMID:2364062

Cavalieri EL, Rogan EG, Li K-M *et al.* (2005). Identification and quantification of the depurinating DNA adducts formed in mouse skin treated with dibenzo[*a,l*]pyrene (DB[*a,l*]P) or its metabolites and in rat mammary gland treated with DB[a,l]P. *Chem Res Toxicol*, 18: 976–983. doi:10.1021/tx049682k PMID:15962932

Chen RH, Maher VM, McCormick JJ (1990). Effect of excision repair by diploid human fibroblasts on the kinds and locations of mutations induced by (+/-)-7β,8 α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in the coding region of the HPRT gene. *Proc Natl Acad Sci USA*, 87: 8680–8684. doi:10.1073/pnas.87.21.8680 PMID:2122466

Conney AH (1982). Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res*, 42: 4875–4917. PMID:6814745

Constantin D, Mehrotra K, Rahimtula A *et al.* (1994). Stimulatory effects of sulfur and nitrogen oxides on carcinogen activation in human polymorphonuclear leukocytes. *Environ Health Perspect*, 102: Suppl 4: 161–164. PMID:7821291

Cooper CS, Ribeiro O, Farmer PB *et al.* (1980). The metabolic activation of benz[*a*]anthracene in hamster embryo cells: evidence that diol-epoxides react with guanosine, deoxyguanosine and adenosine in nucleic acids. *Chem Biol Interact*, 32: 209–231. doi:10.1016/0009-2797(80)90079-4 PMID:6159115

Cordier S, Monfort C, Filippini G *et al.* (2004). Parental exposure to polycyclic aromatic hydrocarbons and the risk of childhood brain tumors: The SEARCH International Childhood Brain Tumor Study. *Am J Epidemiol*, 159: 1109–1116. doi:10.1093/aje/kwh154 PMID:15191928

CORINAIR (1997). *EMEP/CORINAIR Emission Inventory Guidebook - 2007.* Technical Report No. 16/2007. Copenhagen: European Environmental Agency.

Culp SJ, Gaylor DW, Sheldon WG *et al.* (1998). A comparison of the tumors induced by coal tar and benzo[a] pyrene in a 2-year bioassay. *Carcinogenesis*, 19: 117–124. doi:10.1093/carcin/19.1.117 PMID:9472702

Day BW, Naylor S, Gan LS *et al.* (1990). Molecular dosimetry of polycyclic aromatic hydrocarbon epoxides and diol epoxides via hemoglobin adducts. *Cancer Res*, 50: 4611–4618. PMID:2369737

Denison MS, Fisher JM, Whitlock JP Jr (1988b). Inducible, receptor-dependent protein-DNA interactions at a dioxin-responsive transcriptional enhancer. *Proc Natl Acad Sci USA*, 85: 2528–2532. doi:10.1073/pnas.85.8.2528 PMID:2833743

Denison MS, Fisher JM, Whitlock JP Jr (1989). Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. *J Biol Chem*, 264: 16478–16482. PMID:2550446

Denison MS, Fisher JM, Whitlock JP Jr (1988a). The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis. *J Biol Chem*, 263: 17221–17224. PMID:2846558

Denissenko MF, Pao A, Tang M, Pfeifer GP (1996). Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in P53. *Science*, 274: 430–432. doi:10.1126/science.274.5286.430 PMID:8832894

Fan R, Wang D, Mao C *et al.* (2012). Preliminary study of children's exposure to PAHs and its association with 8-hydroxy-2′-deoxyguanosine in Guangzhou, China. *Environ Int*, 42: 53–58. doi:10.1016/j.envint.2011.03.021 PMID:21511339

Gaga EO, Ari A, Döğeroğlu T *et al.* (2012). Atmospheric polycyclic aromatic hydrocarbons in an industrialized city, Kocaeli, Turkey: study of seasonal variations,

influence of meteorological parameters and health risk estimation. *J Environ Monit*, 14: 2219–2229. doi:10.1039/c2em30118k PMID:22699796

Geacintov NE, Cosman M, Hingerty BE *et al.* (1997). NMR solution structures of stereoisometric covalent polycyclic aromatic carcinogen-DNA adduct: principles, patterns, and diversity. *Chem Res Toxicol*, 10: 111–146. doi:10.1021/tx9601418 PMID:9049424

Gerde P, Muggenburg BA, Hoover MD, Henderson RF (1993). Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. I. The alveolar region. *Toxicol Appl Pharmacol*, 121: 313–318. doi:10.1006/taap.1993.1159 PMID:8346548

Glatt H, Piée A, Pauly K *et al.* (1991). *Fjord*- and bay-region diol-epoxides investigated for stability, SOS induction in Escherichia coli, and mutagenicity in *Salmonella typhimurium* and mammalian cells. *Cancer Res*, 51: 1659–1667. PMID:1900215

Gräslund A & Jernström B (1989). DNA-carcinogen interaction: covalent DNA-adducts of benzo(a)pyrene 7,8-dihydrodiol 9,10-epoxides studied by biochemical and biophysical techniques. *Q Rev Biophys*, 22: 1–37. PMID:2501820

Guengerich FP (1993). The 1992 Bernard B. Brodie Award Lecture. Bioactivation and detoxication of toxic and carcinogenic chemicals. *Drug Metab Dispos*, 21: 1–6. PMID:8095200

Hainaut P & Pfeifer GP (2001). Patterns of p53 G→T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis*, 22: 367–374. doi:10.1093/carcin/22.3.367 PMID:11238174

Hansen LD, Nestler C, Ringelberg D, Bajpai R (2004). Extended bioremediation of PAH/PCP contaminated soils from the POPILE wood treatment facility. *Chemosphere*, 54: 1481–1493. doi:10.1016/j.chemosphere.2003.09.046 PMID:14659950

Harrison RM, Smith DJT, Kibble AJ (2004). What is responsible for the carcinogenicity of $PM_{2.5}$? *Occup Environ Med*, 61: 799–805. doi:10.1136/oem.2003.010504 PMID:15377764

Hecht SS, Carmella SG, Villalta PW, Hochalter JB (2010). Analysis of phenanthrene and benzo[*a*]pyrene tetraol enantiomers in human urine: relevance to the bay region diol epoxide hypothesis of benzo[*a*]pyrene carcinogenesis and to biomarker studies. *Chem Res Toxicol*, 23: 900–908. doi:10.1021/tx9004538 PMID:20369855

Heinrich U, Roller M, Pott F (1994). Estimation of a lifetime unit lung cancer risk for benzo(*a*)pyrene based on tumour rates in rats exposed to coal tar/pitch condensation aerosol. *Toxicol Lett*, 72: 155–161. doi:10.1016/0378-4274(94)90023-X PMID:8202928

Hoek G, Brunekreef B, Goldbohm S *et al.* (2002). Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. *Lancet*, 360: 1203–1209. doi:10.1016/S0140-6736(02)11280-3 PMID:12401246

Hoh E, Hunt RN, Quintana PJ *et al.* (2012). Environmental tobacco smoke as a source of polycyclic aromatic hydrocarbons in settled household dust. *Environ Sci Technol*, 46: 4174–4183. doi:10.1021/es300267g PMID:22397504

IARC (1989). Occupational exposures in petroleum refining; crude oil and major petroleum fuels. *IARC Monogr Eval Carcinog Risks Hum*, 45: 1–322. PMID:2664246

IARC (1983). Polynuclear aromatic compounds, Part 1, chemical, environmental and experimental data. *IARC Monogr Eval Carcinog Risk Chem Hum*, 32: 1–453. PMID:6586639

IARC (1984). Polynuclear aromatic compounds, Part 3, industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding. *IARC Monogr Eval Carcinog Risk Chem Hum*, 34: 1–219.

IARC (1985). Polynuclear aromatic compounds, Part 4, bitumens, coal-tars and derived products, shale-oils and soots. *IARC Monogr Eval Carcinog Risk Chem Hum*, 35: 1–247. PMID:2991123

IARC (1984). Polynuclear aromatic hydrocarbons, Part 2, carbon blacks, mineral oils (lubricant base oils and derived products) and some nitroarenes. *IARC Monogr Eval Carcinog Risk Chem Hum*, 33: 1–222. PMID:6590450

IARC (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. *IARC Monogr Eval Carcinog Risks Hum*, 92: 1–853. PMID:21141735

IARC (2002). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum*, 82: 1–556. PMID:12687954

Jeffrey AM (1985). Polycyclic aromatic hydrocarbon-DNA adducts. Formation, detection, and characterization. In: Harvey RG, ed. *Polycyclic Hydrocarbons and Carcinogenesis*. Washington, DC: American Chemical Society, pp. 187–208.

Jerina DM, Chadha A, Cheh AM *et al.* (1991). Covalent bonding of bay-region diol epoxides to nucleic acids. *Adv Exp Med Biol*, 283: 533–553. doi:10.1007/978-1-4684-5877-0_70 PMID:2069024

Jin Y & Penning TM (2007). Aldo-keto reductases and bioactivation/detoxication. *Annu Rev Pharmacol Toxicol*, 47: 263–292. doi:10.1146/annurev.pharmtox.47.120505.105337 PMID:16970545

Jung KH, Yan B, Chillrud SN *et al.* (2010). Assessment of benzo(*a*)pyrene-equivalent carcinogenicity and mutagenicity of residential indoor versus outdoor polycyclic aromatic hydrocarbons exposing young children in New York City. *Int J Environ Res Public Health*, 7: 1889–1900. doi:10.3390/ijerph7051889 PMID:20622999

Kapitulnik J, Wislocki PG, Levin W *et al.* (1978). Tumorigenicity studies with diol-epoxides of benzo(a)

pyrene which indicate that (+/-)-*trans*-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a) pyrene is an ultimate carcinogen in newborn mice. *Cancer Res*, 38: 354–358. PMID:620406

Koehl W, Amin S, Staretz ME *et al.* (1996). Metabolism of 5-methylchrysene and 6-methylchrysene by human hepatic and pulmonary cytochrome P450 enzymes. *Cancer Res*, 56: 316–324. PMID:8542586

Li MC, Cui ZS, He QC, Zhou BS (2005). Association of genetic polymorphism in the DNA repair gene XRCC1 with susceptibility to lung cancer in non-smoking women [in Chinese]. *Zhonghua Zhong Liu Za Zhi*, 27: 713–716. PMID:16483478

Li Z, Sjödin A, Romanoff LC *et al.* (2011). Evaluation of exposure reduction to indoor air pollution in stove intervention projects in Peru by urinary biomonitoring of polycyclic aromatic hydrocarbon metabolites. *Environ Int*, 37: 1157–1163. doi:10.1016/j.envint.2011.03.024 PMID:21524795

Lu D, Harvey RG, Blair IA, Penning TM (2011). Quantitation of benzo[*a*]pyrene metabolic profiles in human bronchoalveolar (H358) cells by stable isotope dilution liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Chem Res Toxicol*, 24: 1905–1914. doi:10.1021/tx2002614 PMID:21962213

Luch A, Schober W, Soballa VJ *et al.* (1999). Metabolic activation of dibenzo[*a,l*]pyrene by human cyctochrome P450 1A1 and P450 1B1 expressed in V79 Chinese hamster. *Chem Res Toxicol*, 12: 353–364. doi:10.1021/tx980240g PMID:10207125

Luch A, Seidel A, Glatt H, Platt KL (1997). Metabolic activation of the (+)-*S,S*- and (-)-*R,R*-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydrodibenzo[*a,l*] pyrene: stereoselectivity, DNA adduct formation, and mutagenicity in Chinese hamster V79 cells. *Chem Res Toxicol*, 10: 1161–1170. doi:10.1021/tx970005i PMID:9348439

MacLeod MC, Adair G, Humphrey RM (1988). Differential efficiency of mutagenesis at three genetic loci in CHO cells by a benzo[a]pyrene diol epoxide. *Mutat Res*, 199: 243–254. PMID:3129654

Malaveille C, Kuroki T, Sims P *et al.* (1977). Mutagenicity of isomeric diol-epoxides of benzo[a]pyrene and benz[a] anthracene in *S. typhimurium* TA98 and TA100 and in V79 Chinese hamster cells. *Mutat Res*, 44: 313–326. doi:10.1016/0027-5107(77)90091-4 PMID:333280

Marshall CJ, Vousden KH, Phillips DH (1984). Activation of c-Ha-ras-1 proto-oncogene by *in vitro* modification with a chemical carcinogen, benzo(*a*)pyrene diol-epoxide. *Nature*, 310: 586–589. doi:10.1038/310586a0 PMID:6431299

Meijer SN, Sweetman AJ, Halsall CJ, Jones KC (2008). Temporal trends of polycyclic aromatic hydrocarbons in the U.K. atmosphere: 1991–2005. *Environ Sci Technol*, 42: 3213–3218. doi:10.1021/es702979d PMID:18522096

Melikian AA, LaVoie EJ, Hecht SS, Hoffmann D (1983). 5-Methylchrysene metabolism in mouse epidermis *in vivo*, diol epoxide–DNA adduct persistence, and diol epoxide reactivity with DNA as potential factors influencing the predominance of 5-methylchrysene-1,2-diol-3,4-epoxide–DNA adducts in mouse epidermis. *Carcinogenesis*, 4: 843–849. doi:10.1093/carcin/4.7.843 PMID:6872139

Nafstad P, Håheim LL, Oftedal B *et al.* (2003). Lung cancer and air pollution: a 27 year follow up of 16 209 Norwegian men. *Thorax*, 58: 1071–1076. doi:10.1136/thorax.58.12.1071 PMID:14645978

Nebert DW, Dalton TP, Okey AB, Gonzalez FJ (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem*, 279: 23847–23850. doi:10.1074/jbc.R400004200 PMID:15028720

Nebert DW & Jensen NM (1979). The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. *CRC Crit Rev Biochem*, 6: 401–437. doi:10.3109/10409237909105427 PMID:378536

Nebert DW, Puga A, Vasiliou V (1993). Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Ann N Y Acad Sci*, 685: 624–640. doi:10.1111/j.1749-6632.1993.tb35928.x PMID:8395783

Nesnow S, Mass MJ, Ross JA *et al.* (1998). Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatic hydrocarbons. *Environ Health Perspect*, 106: Suppl 6: 1337–1346. doi:10.1289/ehp.98106s61337 PMID:9860890

NTP (2000). *Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91–20–3) in F344/N Rats (Inhalation Studies)*. National Toxicology Program Technical Report No. 500; NIH Publ. No. 01-4434, Research Triangle Park, NC.

Palackal NT, Burczynski ME, Harvey RG, Penning TM (2001). The ubiquitous aldehyde reductase (AKR1A1) oxidizes proximate carcinogen *trans*-dihydrodiols to *o*-quinones: potential role in polycyclic aromatic hydrocarbon activation. *Biochemistry*, 40: 10901–10910. doi:10.1021/bi010872t PMID:11535067

Palackal NT, Lee S-H, Harvey RG *et al.* (2002). Activation of polycyclic aromatic hydrocarbon trans-dihydrodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. *J Biol Chem*, 277: 24799–24808. doi:10.1074/jbc.M112424200 PMID:11978787

Park J-H, Gelhaus S, Vedantam S *et al.* (2008a). The pattern of p53 mutations caused by PAH *o*-quinones is driven by 8-oxo-dGuo formation while the spectrum of mutations is determined by biological selection

for dominance. *Chem Res Toxicol*, 21: 1039–1049. doi:10.1021/tx700404a PMID:18489080

Park J-H, Mangal D, Tacka KA *et al.* (2008b). Evidence for the aldo-keto reductase pathway of polycyclic aromatic *trans*-dihydrodiol activation in human lung A549 cells. *Proc Natl Acad Sci USA*, 105: 6846–6851. doi:10.1073/pnas.0802776105 PMID:18474869

Park J-H, Penning TM (2008). Polyaromatic hydrocarbons. In: Stadler RH, Lineback DR, eds. *Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks*, Chapter 2. Hoboken, NJ: John Wiley & Sons, Inc. doi:10.1002/9780470430101.ch2h

Penning TM (2004). Aldo-keto reductases and formation of polycyclic aromatic hydrocarbon *o*-quinones. *Methods Enzymol*, 378: 31–67. doi:10.1016/S0076-6879(04)78003-9 PMID:15038957

Penning TM, Burczynski ME, Hung C-F *et al.* (1999). Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active *o*-quinones. *Chem Res Toxicol*, 12: 1–18. doi:10.1021/tx980143n PMID:9894013

Penning TM & Drury JE (2007). Human aldo-keto reductases: Function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys*, 464: 241–250. doi:10.1016/j.abb.2007.04.024 PMID:17537398

Penning TM, Ohnishi ST, Ohnishi T, Harvey RG (1996). Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon *trans*-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem Res Toxicol*, 9: 84–92. doi:10.1021/tx950055s PMID:8924621

Petruska JM, Mosebrook DR, Jakab GJ, Trush MA (1992). Myeloperoxidase-enhanced formation of (+-)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene-DNA adducts in lung tissue *in vitro*: a role of pulmonary inflammation in the bioactivation of a procarcinogen. *Carcinogenesis*, 13: 1075–1081. doi:10.1093/carcin/13.7.1075 PMID:1322250

Platt KL, Schollmeier M, Frank H, Oesch F (1990). Stereoselective metabolism of dibenz(*a,h*)anthracene to *trans*-dihydrodiols and their activation to bacterial mutagens. *Environ Health Perspect*, 88: 37–41. doi:10.1289/ehp.908837 PMID:2272331

Pope CA 3rd, Burnett RT, Thun MJ *et al.* (2002). Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA*, 287: 1132–1141. doi:10.1001/jama.287.9.1132 PMID:11879110

Pott F, Heinrich U (1990). Relative significance of different hydrocarbons for the carcinogenic potency of emission from various incomplete combustion processes. In: Vainio H, Sorsa M, McMichael AJ, eds. *Complex Mixtures and Cancer Risk*. Lyon: International Agency for Research on Cancer, pp. 288–297.

Prevedouros K, Brorström-Lundén E, Halsall CJ *et al.* (2004). Seasonal and long-term trends in atmospheric PAH concentrations: evidence and implications. *Environ Pollut*, 128: 17–27.PMID:14667717

RIVM (1989). *Integrated Criteria Document PAHs*. (RIVM Report No. 758474011). Bilthoven: National Institute of Public Health and the Environment.

RIVM (2001). *Tumorigenic Effects in Wistar Rats Orally Administered Benzo[a]pyrene for Two Years (Gavage Studies). Implications for Human Cancer Risks Associated with Oral Exposure to Polycyclic Aromatic Hydrocarbons* (RIVM Report No. 658603 010). Bilthoven: National Institute of Public Health and the Environment.

Ross JA, Nelson GB, Holden KL *et al.* (1992). DNA adducts and induction of sister chromatid exchanges in the rat following benzo[*b*]fluoranthene administration. *Carcinogenesis*, 13: 1731–1734. doi:10.1093/carcin/13.10.1731 PMID:1423831

Santella RM (1999). Immunological methods for detection of carcinogen-DNA damage in humans. *Cancer Epidemiol Biomarkers Prev*, 8: 733–739. PMID:10498391

Schauer C, Niessner R, Pöschl U (2004). Analysis of nitrated polycyclic aromatic hydrocarbons by liquid chromatography with fluorescence and mass spectrometry detection: air particulate matter, soot, and reaction product studies. *Anal Bioanal Chem*, 378: 725–736. doi:10.1007/s00216-003-2449-1 PMID:14704835

Shimada T, Gillam EMJ, Oda Y *et al.* (1999). Metabolism of benzo[*a*]pyrene to *trans*-7,8-dihydroxy-7, 8-dihydrobenzo[*a*]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase. *Chem Res Toxicol*, 12: 623–629. doi:10.1021/tx990028s PMID:10409402

Shimada T, Oda Y, Gillam EMJ *et al.* (2001). Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes P450 1A1 and P450 1B1 allelic variants and other human cytochromes P450 in *Salmonella typhimurium* NM2009. *Drug Metab Dispos*, 29: 1176–1182. PMID:11502724

Shultz CA, Quinn AM, Park JH *et al.* (2011). Specificity of human aldo-keto reductases, NAD(P)H:quinone oxidoreductase, and carbonyl reductases to redox-cycle polycyclic aromatic hydrocarbon diones and 4-hydroxyequilenin-*o*-quinone. *Chem Res Toxicol*, 24: 2153–2166. doi:10.1021/tx200294c PMID:21910479

Sims P & Grover PL (1974). Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. *Adv Cancer Res*, 20: 165–274. doi:10.1016/S0065-230X(08)60111-6 PMID:4617500

Solhaug A, Refsnes M, Låg M *et al.* (2004). Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells. *Carcinogenesis*, 25: 809–819. doi:10.1093/carcin/bgh069 PMID:14729587

Suwan-ampai P, Navas-Acien A, Strickland PT, Agnew J (2009). Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons

in the United States, 1999 to 2002. *Cancer Epidemiol Biomarkers Prev*, 18: 884–893. doi:10.1158/1055-9965.EPI-08-0939 PMID:19258471

Thakker DR, Yagi H, Levin W *et al.* (1985). Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: Anders MW, ed. *Bioactivation of Foreign Compounds*. New York: Academic Press, pp. 177–2424.

Thyssen J, Althoff J, Kimmerle G, Mohr U (1981). Inhalation studies with benzo[*a*]pyrene in Syrian golden hamsters. *J Natl Cancer Inst*, 66: 575–577. PMID:6937711

Topinka J, Sevastyanova O, Binková B *et al.* (2007). Biomarkers of air pollution exposure–a study of policemen in Prague. *Mutat Res*, 624: 9–17. doi:10.1016/j.mrfmmm.2007.02.032 PMID:17493640

Upham BL, Weis LM, Rummel AM *et al.* (1996). The effects of anthracene and methylated anthracenes on gap junctional intercellular communication in rat liver epithelial cells. *Fundam Appl Toxicol*, 34: 260–264. doi:10.1006/faat.1996.0195 PMID:8954755

Wei S-J, Chang RL, Bhachech N *et al.* (1993). Dose-dependent differences in the profile of mutations induced by (+)-7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene in the coding region of the hypoxanthine (guanine) phosphoribosyltransferase gene in Chinese hamster V-79 cells. *Cancer Res*, 53: 3294–3301. PMID:8324741

Weis LM, Rummel AM, Masten SJ *et al.* (1998). Bay or baylike regions of polycyclic aromatic hydrocarbons were potent inhibitors of Gap junctional intercellular communication. *Environ Health Perspect*, 106: 17–22. doi:10.1289/ehp.9810617 PMID:9417772

Wenzlaff AS, Cote ML, Bock CH *et al.* (2005a). CYP1A1 and CYP1B1 polymorphisms and risk of lung cancer among never smokers: a population-based study. *Carcinogenesis*, 26: 2207–2212. doi:10.1093/carcin/bgi191 PMID:16051642

Wenzlaff AS, Cote ML, Bock CH *et al.* (2005b). GSTM1, GSTT1 and GSTP1 polymorphisms, environmental tobacco smoke exposure and risk of lung cancer among never smokers: a population-based study. *Carcinogenesis*, 26: 395–401. doi:10.1093/carcin/bgh326 PMID:15528218

WHO (1998). Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons (Environmental Health Criteria 202). Geneva: World Health Organization.

Whyatt RM, Santella RM, Jedrychowski W *et al.* (1998). Relationship between ambient air pollution and DNA damage in Polish mothers and newborns. *Environ Health Perspect*, 106: Suppl 3: 821–826. PMID:9646044

Yoon HS, Lee KM, Lee KH *et al.* (2012). Polycyclic aromatic hydrocarbon (1-OHPG and 2-naphthol) and oxidative stress (malondialdehyde) biomarkers in urine among Korean adults and children. *Int J Hyg Environ Health*, 215: 458–464. doi:10.1016/j.ijheh.2012.02.007 PMID:22436105

Yu D, Berlin JA, Penning TM, Field J (2002). Reactive oxygen species generated by PAH *o*-quinones cause change-in-function mutations in p53. *Chem Res Toxicol*, 15: 832–842. doi:10.1021/tx010177m PMID:12067251

Zhang Y & Tao S (2009). Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmos Environ*, 43: 812–819. doi:10.1016/j.atmosenv.2008.10.050

Zhong Y, Wang J, Carmella SG *et al.* (2011). Metabolism of [D10]phenanthrene to tetraols in smokers for potential lung cancer susceptibility assessment: comparison of oral and inhalation routes of administration. *J Pharmacol Exp Ther*, 338: 353–361. doi:10.1124/jpet.111.181719 PMID:21515812

PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 7.

Polycyclic aromatic hydrocarbons and associated occupational exposures

Charles William Jameson

Properties of PAHs

Most polycyclic aromatic hydrocarbons (PAHs) with potential biological activity have been determined to have a molecular structure that ranges in size from two to six fused aromatic rings (IARC, 2010). The physicochemical properties of these PAHs that are critical to their biological activity vary greatly, because their molecular weights cover a vast range.

Aqueous solubility of PAHs decreases approximately logarithmically with increasing molecular mass (Johnsen et al., 2005). Two-ring PAHs, and, to a lesser extent, three-ring PAHs, dissolve in water; this makes them more readily available for biological uptake and degradation (Mackay and Callcott, 1998; Choi et al., 2010). Furthermore, two- to four-ring PAHs volatilize sufficiently to appear in the atmosphere predominantly in gaseous form, although the physical state of four-ring PAHs can depend on temperature (Atkinson and Arey, 1994; Srogi, 2007).

In contrast, PAHs with five or more rings have low solubility in water and low volatility. They therefore occur predominantly in solid form, bound to particulates in polluted air, soil, or sediment (Choi et al., 2010). In the solid state, these compounds are less accessible for biological uptake or degradation, which means that their persistence in the environment is increased (Johnsen et al., 2005; Haritash and Kaushik, 2009).

The properties that influence the biological activity of PAHs include their vapour pressure, their adsorption on surfaces of solid carrier particles, their absorption into liquid carriers, their lipid–water partition coefficient in tissues, and their limits of solubility in the lipid and aqueous phases of tissues. These properties are linked with the metabolic activation of PAHs, as well as their deposition and disposition.

PAHs share a similar mechanism of carcinogenic action in both humans and experimental animals.

This includes metabolic conversion to oxides and dihydrodiols, which in turn are oxidized to diol epoxides. These oxides and diol epoxides are the ultimate DNA-reactive metabolites of PAHs. The oxides form stable DNA adducts, and the diol epoxides form stable adducts but also unstable adducts (so-called depurinating adducts) with DNA through formation of electrophilic carbonium ions.

Occupational exposure to PAHs

Occupational exposure to PAHs occurs predominantly through inhalation and dermal contact. Industrial processes that involve the pyrolysis or combustion of coal and the production and use of coal-derived products, including coal tar and coal tar-derived products, are major sources of occupational exposure to PAHs. Workers at coal-tar production plants, coking plants, bitumen production plants, coal-gasification sites, smokehouses, aluminium production plants, coal-tarring facilities, and municipal waste incinerators are exposed to PAHs. Exposure may also result from inhalation of engine exhaust and from use of products that contain PAHs in a variety of other industries, such as mining, oil refining, metalworking, chemical production, transportation, and the electrical industry (Vanrooij et al., 1992).

Studies in Germany measured concentrations of PAHs in the breathing zone of chimney sweeps during "black work"; the PAHs in the air samples varied depending on the type of fuel burned (oil, oil/solid, or solid) (Knecht et al., 1989). Concentrations of PAHs in coal-tar products may range from less than 1% to 70% or more (ATSDR, 2002). Occupational exposure can lead to PAH body burdens among exposed workers that are considerably higher than those in the general population.

There is growing awareness that uptake of PAHs through the skin is substantial (Jongeneelen, 2001). Dermal uptake has been shown to contribute to the internal exposure of workers to PAHs; a study in the creosote industry found that the total internal dose of PAHs did not necessarily correlate with levels of inhalation exposure alone, and that dermal exposure contributed significantly (Vanrooij et al., 1992).

Classification of PAHs

The *IARC Monographs Programme* has reviewed experimental data for 60 individual PAHs (IARC, 2010). Of these 60 PAHs, one, benzo[*a*]pyrene, is classified as *carcinogenic to humans* (Group 1). Other PAHs reviewed by IARC include cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, and dibenzo[*a,l*]pyrene, which are classified as *probably carcinogenic to humans* (Group 2A), and benz[*j*]aceanthrylene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*c*]phenanthrene, chrysene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, indeno[1,2,3-*cd*]pyrene, and 5-methylchrysene, which are classified as *possibly carcinogenic to humans* (Group 2B). It should be noted that in the evaluations of benz[*j*]aceanthrylene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, cyclopenta[*cd*]pyrene, dibenzo[*a,h*]anthracene, and dibenzo[*a,l*]pyrene, the mechanistic data available for these compounds were critical for determining the overall evaluation for each one (IARC, 2010).

The remaining 45 PAHs reviewed by IARC were acenaphthene, acepyrene (3,4-dihydrocyclopenta[*cd*]pyrene), anthanthrene, anthracene, 11*H*-benz[*bc*]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]chrysene, benzo[*a*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4*H*-cyclopenta[*def*]chrysene, 5,6-cyclopenteno-1,2-benzanthracene, dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenzo[*a,e*]fluoranthene, 13*H*-dibenzo[*a,g*]fluorene, dibenzo[*h,rst*]pentaphene, dibenzo[*a,e*]pyrene, dibenzo[*e,l*]pyrene, 1,2-dihydroaceanthrylene, 1,4-dimethylphenanthrene, fluoranthene, fluorene, 1-methylchrysene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene, 6-methylchrysene, 2-methylfluoranthene, 3-methylfluoranthene, 1-methylphenanthrene, naphtho[1,2-*b*]fluoranthene, naphtho[2,1-*a*]fluoranthene, naphtho[2,3-*e*]pyrene, perylene, phenanthrene, picene, pyrene, and triphenylene. These compounds were determined to be *not classifiable as to their carcinogenicity to humans* (Group 3), because of limited or inadequate experimental evidence (IARC, 2010).

As noted above, benzo[*a*]pyrene is the only PAH classified by IARC in Group 1. A review of the data available for this PAH indicates that the complete sequence of steps in the metabolic activation pathway of benzo[*a*]pyrene to mutagenic and carcinogenic diol epoxides has been demonstrated in humans, in human tissues, and in experimental animals. After exposure, humans

Table 7.1. Group 1 agents associated with dermal exposures to polycyclic aromatic hydrocarbons (PAHs) that cause squamous cell carcinoma of the skin in humans and in rodents

Agent	Target organ		
	Humans	**Mice**	**Rats**
Benzo[*a*]pyrene	No data	Skin	Skin
Chimney sweep soots	Skin, including scrotum	Skin	No data
Coal tar	Skin, including scrotum	Skin	No data
Coal-tar pitch	Skin, including scrotum	Skin	No data
Mineral oils, untreated and mildly treated	Skin, including scrotum	Skin	No data
Shale oils	Skin, including scrotum	Skin	No data

metabolically activate benzo[*a*]pyrene to benzo[*a*]pyrene diol epoxides that form DNA adducts. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been measured in populations (e.g. coke-oven workers and chimney sweeps) exposed to PAH mixtures that contain benzo[*a*]pyrene. The reactive *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. Mutations (G → T transversions) in the K-*ras* proto-oncogene in lung tumours from mice treated with benzo[*a*]pyrene are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts. Similar mutations in the *KRAS* proto-oncogene and mutations in the tumour suppressor gene *TP53* were found in lung tumours from non-smokers exposed to PAH-rich products of coal combustion that are known to contain benzo[*a*]pyrene (as well as many other PAHs). In an in vitro study, the codons in *TP53* that are most frequently mutated in lung cancer in humans were shown to be targets for DNA adduct formation and mutation induced by benzo[*a*]pyrene. The strong and extensive experimental evidence for the carcinogenicity of benzo[*a*]pyrene in many animal species, supported by the consistent and coherent mechanistic evidence from studies in exposed humans and in experimentally exposed animals, and from in vitro studies in human and animal tissues and cells, provides biological plausibility to support the overall classification of benzo[*a*]pyrene as *carcinogenic to humans* (Group 1) (IARC, 2010, 2012).

Studies of cancer in humans

There are no epidemiological studies on human exposure to individual PAHs, because these chemicals never occur in isolation in the environment but are present as components of complex chemical mixtures. PAHs are very widespread environmental contaminants, because they are formed during incomplete combustion of materials such as coal, oil, gas, wood, or waste, or during pyrolysis of other organic materials, such as tobacco. Data on the carcinogenicity of PAHs to humans are available primarily from studies in occupational settings where workers are exposed to mixtures containing PAHs. It is difficult to ascertain the carcinogenicity of the component PAHs in these mixtures, because of potential chemical interactions and the presence of other carcinogenic substances.

Certain occupations associated with high exposure to PAHs have been classified by IARC as *carcinogenic to humans* (Group 1); these include coal gasification, coke production, coal-tar distillation, chimney sweeping, paving and roofing with coal-tar pitch, and work involving mineral oils, shale-oil production, and aluminium production. In most cases the classification is based on epidemiological studies of increased cancer incidence without reference to supporting evidence from bioassays in experimental animals. The roles of individual PAHs in the genesis of cancer observed in these occupations could not be defined (IARC, 2010).

Tumour site concordance

There are six IARC Group 1 agents that cause non-melanoma tumours of the skin (Rice, 2005). Five of these are related to occupations where PAH exposures are high and are believed to be the causative agents (Table 7.1). There is a precise correlation between carcinogenicity to human skin and carcinogenicity to mouse skin for these five

Table 7.2. Group 1 carcinogens associated with inhalation exposures to polycyclic aromatic hydrocarbons (PAHs) that cause lung cancer in humans and in rodents

Agent	Target organ	Route/target organ	
	Humans	**Mice**	**Rats**
Benzo[*a*]pyrene	No data	Intraperitoneal injection of and oral exposure to soot extracts/ lung	Intratracheal and intrapulmonary instillation of soot extracts/lung
Chimney sweep soots	Lung	No data	Intratracheal instillation of soot extracts/ lung
Coal-tar vapours from coke ovens	Lung	Inhalation/lung	Inhalation/lung
Soots and vapours from aluminium production	Lung, bladder	No data	No data

PAH-associated exposures when the complex mixtures isolated from the occupational environment are applied topically.

In 1775, Pott made the pioneering observation that cancer of the scrotum in chimney sweeps was an occupational disease resulting from direct contact with soot (Pott, 1775). All five established PAH-based chemical carcinogens for human skin to which exposures occur by direct dermal contact are complex mixtures: coal tar, coal-tar pitch, untreated and mildly treated mineral oils, shale oils, and soots. Because these mixtures contain PAHs, all have a genotoxic component to their mode of action in rodents. Most of the individual PAHs classified by IARC as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B) (listed above) are genotoxic and have been shown to cause skin cancer and/or be initiators of skin cancer in rodents (IARC, 1983, 2010).

Soots and vapours from coke production, aluminium production, and related industries also cause lung cancer in humans, but only extracts of soot and vapours from coke production have been tested in rodents by an appropriate route (Table 7.2). Both mice and rats developed lung tumours after inhalation of coal-tar vapours from coke ovens. Soot extracts caused lung tumours in rats after intratracheal instillation. There appears to be a good correlation between lung cancer in humans and in rodents for these two mixtures when studied by an appropriate route in mice and rats. All these complex mixtures have genotoxic activity, which is recognized to underlie their carcinogenic activity in the lung. In summary, many of the individual PAHs in these complex mixtures that have been classified by IARC as either *probably carcinogenic* or *possibly carcinogenic* to humans are also genotoxic and have been shown to cause lung tumours in rodents when administered by an appropriate route (IARC, 2010).

The various tissue compartments of the respiratory tract are metabolically active towards exogenous chemicals in both humans and experimental animals and are clearly capable of transforming many metabolism-dependent chemicals, including carcinogenic PAHs, to their chemically reactive metabolites (Rice, 2005). In the lung, metabolically active cell types include pulmonary macrophages as well as epithelial cells.

Benzo[*a*]pyrene is the only PAH that has been classified by IARC as *carcinogenic to humans* (Group 1). As indicated above, the basis for this classification is the extensive knowledge of the mechanism of carcinogenic action of benzo[*a*]pyrene in humans and experimental animals. None of the many remaining PAHs shown to be carcinogens in animals have been classified as an IARC Group 1 carcinogen, most likely because much less mechanistic information is available for these agents than for benzo[*a*]pyrene. These other PAHs are classified as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B). Most marked human occupational exposure to these compounds involves complex mixtures that contain more than one of these PAHs and that often contain other, non-PAH carcinogens. Therefore, the carcinogenic activity of these mixtures cannot confidently be ascribed to any one of their individual components.

References

Atkinson R, Arey J (1994). Atmospheric chemistry of gas-phase polycyclic aromatic hydrocarbons: formation of atmospheric mutagens. Environ Health Perspect. 102(Suppl 4):117–26. http://dx.doi.org/10.1289/ehp.94102s4117 PMID:7821285

ATSDR (2002). Toxicological profile for wood creosote, coal tar creosote, coal tar, coal tar pitch, and coal tar pitch volatiles. Atlanta (GA), USA: Agency for Toxic Substances and Disease Registry. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp85.pdf.

Choi H, Harrison R, Komulainen H, Delgado Saborit JM (2010). Polycyclic aromatic hydrocarbons. In: WHO guidelines for indoor air quality: selected pollutants. Geneva, Switzerland: World Health Organization. Available from: http://www.ncbi.nlm.nih.gov/books/NBK138709/.

Haritash AK, Kaushik CP (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater. 169(1–3):1–15. http://dx.doi.org/10.1016/j.jhazmat.2009.03.137 PMID:19442441

IARC (1983). Polynuclear aromatic compounds, Part 1, chemical, environmental and experimental data. IARC Monogr Eval Carcinog Risk Chem Hum. 32:1–453. Available from: http://publications.iarc.fr/50 PMID:6586639

IARC (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum. 92:1–853. Available from: http://publications.iarc.fr/110 PMID:21141735

IARC (2012). Chemical agents and related occupations. IARC Monogr Eval Carcinog Risks Hum. 100F:1–599. Available from: http://publications.iarc.fr/123 PMID:23189753

Johnsen AR, Wick LY, Harms H (2005). Principles of microbial PAH-degradation in soil. Environ Pollut. 133(1):71–84. http://dx.doi.org/10.1016/j.envpol.2004.04.015 PMID:15327858

Jongeneelen FJ (2001). Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann Occup Hyg. 45(1):3–13. http://dx.doi.org/10.1093/annhyg/45.1.3 PMID:11137694

Knecht U, Bolm-Audorff U, Woitowitz HJ (1989). Atmospheric concentrations of polycyclic aromatic hydrocarbons during chimney sweeping. Br J Ind Med. 46(7):479–82. PMID:2765421

Mackay D, Callcott D (1998). Partitioning and physical chemical properties of PAHs. In: Neilson A, editor. PAHs and related compounds. The handbook of environmental chemistry, Volume 3. Berlin, Germany: Springer-Verlag; pp. 325–45. http://dx.doi.org/10.1007/978-3-540-49697-7_8

Pott P (1775). Chirurgical observations. London, United Kingdom: Hawes, Clarke & Collins; p. 63.

Rice JM (2005). Human relevance of animal neoplasms: site concordance between humans and experimental animals for cancers caused by exposures to chemical carcinogens, review prepared for the International Programme on Chemical Safety. Geneva, Switzerland: World Health Organization.

Srogi K (2007). Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett. 5(4):169–95. http://dx.doi.org/10.1007/s10311-007-0095-0

Vanrooij JG, Bodelier-Bade MM, De Looff AJ, Dijkmans AP, Jongeneelen FJ (1992). Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminium workers. Med Lav. 83(5):519–29. PMID:1297067

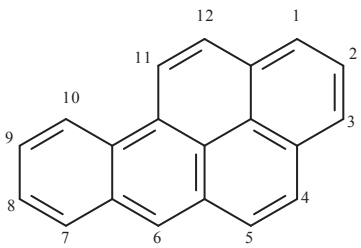
BENZO[*a*]PYRENE

Benzo[*a*]pyrene was considered by previous IARC Working Groups in 1972, 1983, and 2005 (IARC, 1973, 1983, 2010). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Services Reg. No.: 50-32-8
Chem. Abstr. Name: Benzo[*a*]pyrene
IUPAC Systematic Name: Benzo[*a*]pyrene
Synonyms: B*a*P; benzo[*def*]chrysene; 3,4-benzopyrene*; 6,7-benzopyrene*; benz[*a*]pyrene; 3,4-benz[*a*]pyrene*; 3,4-benzpyrene*; 4,5-benzpyrene*
(*alternative numbering conventions)



$C_{20}H_{12}$
Relative molecular mass: 252.31
Description: Yellowish plates, needles from benzene/methanol; crystals may be monoclinic or orthorhombic
Boiling-point: 310–312 °C at 10 mm Hg
Melting-point: 179–179.3 °C; 178.1 °C
Spectroscopy data: Ultraviolet/visual, infrared, fluorescence, mass and nuclear magnetic-resonance spectral data have been reported
Water solubility: 0.00162 mg/L at 25 °C; 0.0038 mg/L at 25 °C
log K_{ow} (octanol–water): 6.35
Henry's Law Constant: 0.034 Pa m^3/mol at 20 °C
From IARC (2010)

1.2 Occurrence and exposure

Benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants formed during incomplete combustion or pyrolysis of organic material. These substances are found in air, water, soils and sediments, generally at trace levels except near their sources. PAHs are present in some foods and in a few pharmaceutical products based on coal tar that are applied to the skin. Tobacco smoke contains high concentrations of PAHs (IARC, 2010).

1.2.1 Exposure of the general population

The general population can be exposed to benzo[*a*]pyrene through tobacco smoke, ambient air, water, soils, food and pharmaceutical products. Concentrations of benzo[*a*]pyrene in

sidestream cigarette smoke have been reported to range from 52 to 95 ng/cigarette — more than three times the concentration in mainstream smoke. Major sources of PAHs in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires. Average concentrations of individual PAHs in the ambient air in urban areas typically range from 1 to 30 ng/m^3; however, concentrations up to several tens of nanograms per cubic metre have been reported in road tunnels, or in large cities that make extensive use of coal or other biomass as residential heating fuel. Estimates of PAH intake from food vary widely, ranging from a few nanograms to a few micrograms per person per day. Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high-temperature processing); bread, cereals and grains (at least in part from gas/flame-drying of grains); and vegetables grown in contaminated soils, or in areas with surface contamination from atmospheric PAH fall-out (IARC, 2010).

1.2.2 Occupational exposure

Occupational exposure to PAHs occurs primarily through inhalation and via skin contact. Monitoring by means of ambient air-sampling or personal air-sampling at the workplace, to determine individual PAHs, sets of PAHs or surrogates (e.g. coal-tar pitch volatiles) has been used to characterize exposure via inhalation; more recently, biological monitoring methods have been applied to characterize the uptake of certain specific PAHs (e.g. benzo[*a*]pyrene) to be used as biomarkers of total exposure (IARC, 2010).

Industries where occupational exposure to benzo[*a*]pyrene has been measured and reported include: coal liquefaction, coal gasification, coke production and coke ovens, coal-tar distillation, roofing and paving (involving coal-tar pitch), wood impregnation/preservation with creosote, aluminium production (including anode manufacture), carbon-electrode manufacture, chimney sweeping, and power plants. Highest levels of exposure to PAHs are observed in aluminium production (Söderberg process) with values up to 100 µg/m^3. Mid-range levels are observed in roofing and paving (e.g. 10–20 µg/m^3) and the lowest concentrations (i.e. at or below 1µg/m^3) are observed in coal liquefaction, coal-tar distillation, wood impregnation, chimney sweeping and power plants (IARC, 2010).

2. Cancer in Humans

No epidemiological data on benzo[*a*]pyrene alone were available to the Working Group.

3. Cancer in Experimental Animals

Benzo[*a*]pyrene was considered by three previous Working Groups (IARC, 1973, 1983, 2010).

In *IARC Monograph* Volume 3 (IARC, 1973) it was concluded that benzo[*a*]pyrene produced tumours in all species tested (mouse, rat, hamster, guinea-pig, rabbit, duck, newt, monkey) for which data were reported following exposure by many different routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, intravenous). Benzo[*a*]pyrene had both a local and a systemic carcinogenic effect, was an initiator of skin carcinogenesis in mice, and was carcinogenic in single-dose studies and following prenatal and transplacental exposures.

In *IARC Monograph* Volume 32 (IARC, 1983) no evaluation was made of studies of carcinogenicity in experimental animals published since 1972, but it was concluded that there is *sufficient evidence* for the carcinogenicity of benzo[*a*]pyrene in experimental animals.

Carcinogenicity studies with administration of benzo[*a*]pyrene by multiple route of exposure, reported after the initial evaluations, were subsequently reviewed in *IARC Monograph* Volume 92 (IARC, 2010) and are summarized below (Table 3.1). See Table 3.2 for an overview of malignant tumours induced in different animal species.

3.1 Skin application

In several studies in which benzo[*a*]pyrene was applied to the skin of different strains of mice, benign (squamous cell papillomas and keratoacanthomas) and malignant (mainly squamous-cell carcinomas) skin tumours were observed (Van Duuren *et al.*, 1973; Cavalieri *et al.*, 1977, 1988a; Levin *et al.*, 1977; Habs *et al.*, 1980, 1984; Warshawsky & Barkley, 1987; Albert *et al.*, 1991; Andrews *et al.*, 1991; Warshawsky *et al.*, 1993). No skin-tumour development was seen in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice developed squamous-cell carcinomas of the skin (Shimizu *et al.*, 2000).

In a large number of initiation–promotion studies in mice, benzo[*a*]pyrene was active as an initiator (mainly of squamous-cell papillomas) when applied to the skin (IARC, 2010).

3.2 Subcutaneous injection

In subcutaneous injection studies of benzo[*a*]pyrene, malignant tumours (mainly fibrosarcomas) were observed at the injection site in mice (Kouri *et al.*, 1980; Rippe & Pott, 1989) and rats (Pott *et al.*, 1973a, b; Rippe & Pott, 1989). No fibrosarcomas were observed in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice did develop these tumours (Shimizu *et al.*, 2000).

In another study, male and female newborn Swiss mice that were given benzo[*a*]pyrene subcutaneously showed a significant increase in lung-adenoma incidence and multiplicity (Balansky *et al.*, 2007).

A single study in 12 strains of hamsters resulted in sarcomas at the site of injection in both sexes of all 12 strains (Homburger *et al.*, 1972).

3.3 Oral administration

After administration of benzo[*a*]pyrene by gavage or in the diet to mice of different strains (Sparnins *et al.*, 1986; Estensen & Wattenberg, 1993; Weyand *et al.*, 1995; Kroese *et al.*, 1997; Culp *et al.*, 1998; Hakura *et al.*, 1998; Badary *et al.*, 1999; Wijnhoven *et al.*, 2000; Estensen *et al.*, 2004), increased tumour responses were observed in lymphoid and haematopoeitic tissues and in several organs, including the lung, forestomach, liver, oesophagus and tongue.

Oral administration of benzo[*a*]pyrene to $XPA^{-/-}$ mice resulted in a significantly higher increase of lymphomas than that observed in similarly treated $XPA^{+/-}$ and $XPA^{+/+}$ mice (de Vries *et al.*, 1997). Benzo[*a*]pyrene given by gavage to $XPA^{-/-}/p53^{+/-}$ double-transgenic mice induced tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at higher incidences than in similarly treated single transgenic and wild-type counterparts. These cancer-prone $XPA^{-/-}$ or $XPA^{-/-}/p53^{+/-}$ mice also developed a high incidence of tumours (mainly of the forestomach) when fed benzo[*a*]pyrene in the diet (van Oostrom *et al.*, 1999; Hoogervorst *et al.*, 2003).

Oral administration of benzo[*a*]pyrene by gavage to rats resulted in an increased incidence of mammary gland adenocarcinomas (el-Bayoumy *et al.*, 1995).

Table 3.1 Carcinogenicity studies of benzo[*a*]pyrene in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Skin application				
Mouse, Swiss ICR/Ha (F) 52 wk Van Duuren *et al.* (1973)	0 (untreated), 0 (vehicle control), 5 µg/animal, 3 × /wk, 52 wk 50/group	Skin T: 0/50, 0/50, 23/50 (46%; 13 P; 10 C)	+	NR (acetone)
Mouse, Swiss (F) 38–65 wk Cavalieri *et al.* (1977)	0 and 0.396 µmol [0.1 mg] per animal, twice/wk, 30 wk 40/group	Skin T: 0% [0/29], 78.9% [30/38] (7 P, 7 K, 36 C, 1 malignant Schwannoma)	+	99% (acetone)
Mouse, C57BL/6J (F) 60 wk Levin *et al.* (1977)	Experiment 1 and 2: 0 (DMSO/acetone), 0.02 [5.28 µg], 0.1 [26.43 µg], 0.4 [105.75 µg] µmol/animal, once/2 wk, 60 wk (high dose given in two paintings, 30 min apart) Experiment 3: 0 (acetone/NH_4OH), 0.025 [6.6 µg], 0.05 [13.21 µg], 0.1 [26.43 µg] µmol/animal, once/2 wk, 60 wk 30/group	Skin T (mainly SCC): Experiment 1–0%, 0%, 38% (13 T), 100% (44 T) Experiment 2–0%, 4% (1 T), 50% (15 T), 100% (40 T) Experiment 3– 0%, 7% (2 T), 59% (20 T), 91% (24 T)	+	NR (DMSO/acetone (1:3) or acetone/NH_4OH (1 000:1)) Effective number of animals not clearly specified At most, seven animals/group died prematurely without a skin tumour.
Mouse, NMRI (F) 63–109 wk Habs *et al.* (1984)	0, 2, 4 µg/animal, twice/wk 20/group	Skin T: 0/20, 9/20 (45%; 2 P, 7 C), 17/20 (85%; 17 C)	+	> 96% (acetone)
Mouse C3H/HeJ (M) 99 wk Warshawsky & Barkley (1987)	0 (untreated), 0 (vehicle control) or 12.5 µg/animal, twice/wk 50/group	Skin T: 0/50, 0/50, 48/50 (96%; 47 C, 1 P)	+	99.5% (acetone)
Mouse, Swiss (F) 42 wk Cavalieri *et al.* (1988a)	0, 0.1 [26.4 µg], 0.4 [105.7 µg] µmol/animal, twice/wk, 20 wk 30/group	Skin T incidence: 0/30, 26/29 (90%; SGA, 3 P, 23 SCC), 26/30 (90%; 2 P, 26 SCC)	+	Purified [NR] (acetone)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, C3H/fCum (M) Experiment 1: 15 mo Experiment 2: 18 mo Experiment 3: 18 mo Kouri *et al.* (1980)	Experiment 1: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or DMSO, Experiment 2: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or in DMSO, Experiment 3: 0 (trioctanoin/DMSO, 100:1), 0.9 µmol [0.23 mg] in trioctanoin/DMSO (100:1), 1 × 20 or 40/group	FibroS at injection site: *Experiment 1*–0/16, 0/20, 15/18 (83%), 12/19 (63%) *Experiment 2*–0/20, 0/18, 14/18 (78%), 7/19 (37%) *Experiment 3*–0/20, 36/40 (90%)	+	Pure (trioctanoin, DMSO)
Mouse, NR (F) 78 wk Rippe & Pott (1989)	0, 10, 100 µg/animal, 1 × NR/group	S at injection site: 1/30 (3%), 13/30 (43%), 20/30 (67%)	+	NR (tricaprylin)
Mouse, Swiss (newborn) (M, F) 75–200 d Balansky *et al.* (2007)	0 and 1.0 mg/animal, 1 × 12–15 M/group, 12–15 F/group	Lung A: M – 0/15, 9/12; F – 0/15, 11/12	$P < 0.001$	Pure (olive oil)
Rat, Wistar (F) ~530 d Pott *et al.* (1973a)	0, 33, 100, 300, 900, 2 700 µg/animal, 1 × 50/group	T (mainly fibroS) at injection site [incidence derived from dose–response curves]: 2/50 (~4%), 4/50 (~8%), 7/50 (~14%), 23/50 (~46%), 35/50 (~70%), 38/50 (~76%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 1 mg, 1 × NR/group	S at injection site: 0/24 (0%), 20/24 (83%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 15 mg, 1 × NR/group	S at injection site: 1/24 (4%), 19/24 (79%)	+	NR (DMSO)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian, RB (randomly bred), BIO inbred strains designated as: 1.5, 4.22, 4.24, 7.88, 12.14, 15.16, 45.5, 54.7, 82.73, 86.93, 87.20 (M, F) 53 wk Homburger *et al.* (1972)	500 µg/animal, 1 × 25 M/group, 25 F/group	FibroS at injection site: RB–M, 4/25 (16%); F, 6/23 (26%) 1.5–M, 5/25 (20%); F, 4/23 (17%) 4.22–M, 3/25 (12%); F, 8/25 (32%) 4.24–M, not tested; F, 9/25 (36%) 7.88–M, 13/25 (52%); F, 5/23 (23%) 12.14–M, 3/25 (12%); F, 9/22 (41%) 15.16–M, 9/25 (36%); F, 16/25 (64%) 45.5–M, 12/25 (48%); F, 7/15 (47%) 54.7:–M, 5/25 (20%); F, 5/25 (20%) 82.73–M, 4/21 (19%); F, 4/24 (17%) 86.93–M, 9/25 (36%); F, 8/25 (32%) 87.20–M, 16/25 (64%); F, 11/25 (42%)	+	NR (tricaprylin) No subcutaneous T observed in historical controls
Oral administration				
Mouse, A/J (F) 260 d Weyand *et al.* (1995)	0, 16, 98 ppm (total dose; 0, 11, 67 mg) in the diet 30/group	Lung T: 4/21 (19%; 4 A; 0.19 ± 0.09 A/animal), 9/25 (36%*; 7 A, 2 AdC; 0.48 ± 0.14 T/animal), 14/27 (52%*; 14 A; 0.59 ± 0.12 A/animal) Forestomach T: (0%) 0/21, (5/25) (20%; 3 P, 2 C; 0.24 ± 0.11** T/animal), 27/27 (100%**; 13 P, 14 C; 4.22 ± 0.41**)	*$P < 0.05$ **$P < 0.001$	NR (gel diet)
Mouse B6C3F1 (F) 2 yr Culp *et al.* (1998)	0 (acetone control diet), 5 ppm, 25 ppm, 100 ppm in the diet 48/group	Liver (A): 2/48 (4%), 7/48 (15%), 5/47 (11%), 0/45 (0%) Lung (A and/or C): 5/48 (10%), 0/48 (0%), 4/45 (9%), 0/48 (0%) Forestomach (P and/or C): 1/48 (2%), 3/47 (6%), 36/46 (78%****), 46/47 (98%****) Oesophagus (P and/or C): 0/48 (0%), 0/48 (0%), 2/45 (4%), 27/46 (59%**) Tongue (P and/or C): 0/48 (0%), 0/48 (0%), 2/46 (4%), 23/48 (48%***) Larynx (P and/or C): 0/35 (0%), 0/35 (0%), 3/34 (9%), 5/38 (13%*)	*$P < 0.014$ **$P < 0.0014$ ***$P < 0.0003$ ****$P < 0.00001$	98.5% (acetone)
Mouse, Swiss albino, inbred (F) 27 wk Badary *et al.* (1999)	0 and 1 mg/animal by gavage, twice/wk, 4 wk 10/group	0, 10/10 (100%) (forestomach P; multiplicity, 7.11 ± 1.05)	+	Highest purity grade (corn oil) Drinking-water contained 0.005% ethanol

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, $CSB^{-/-}$ or wild-type ($CSP^{\pm}$ or $CSB^{+/+}$) (M, F) 52 wk Wijnhoven *et al.* (2000)	0 and 13 mg/kg bw by gavage, 3 × / wk, 13 wk 6–18 M/group, 6–13 F/group	Wild-type: 5/27 (14 M, 13 F; 19%; 4 bronchiolo-alveolar A, 2 lymphoma), 17/29* (18 M, 11 F; 59%; 6 bronchiolo-alveolar A, 10 forestomach P, 2 forestomach SCC, 2 histiocytic S, 2 hepatocellular A, 1 intestinal AdC, 1 skin P) $CSB^{-/-}$: 0/13 (6 M, 7 F), 7/12** (6 M, 6 F; 58%; 2 bronchiolo-alveolar A, 2 uterine S, 1 forestomach SCC, 1 intestinal AdC, 1 skin histiocytic S)	*$P = 0.0023$ **$P = 0.0017$	NR (soya oil)
Rat, Crl:CD(SD)BR (F) 49 wk el-Bayoumy *et al.* (1995)	0 and 50 µmol/animal, once/wk, 8 wk by gavage 30/group	Mammary T incidence: 11/30 [37%] [incidence not clearly specified] (8 desmoplastic A, 2 A, 1 AdC), 29/30 (96.7%; 8 fibroA**, 17 desmoplastic A*, 7 A, 22 AdC**) Numbers of mammary T: controls, 14 desmoplastic A, 2 A, 1 AdC; treated animals, 14 fibroA*, 35 desmoplastic A, 11 A, 56 AdC**	*$P < 0.05$ **$P < 0.01$	99% (trioctanoin)
Intraperitoneal injection				
Mouse, B6C3F1; C3A/JF1 (M, F) 90 wk or lifetime Vesselinovitch *et al.* (1975a, b)	0, 75, 150 µg in 10 µL/g bw, 1 × at 1, 15, 42 d of age 30–63/group, 96–100 controls/group	B6C3F1 mice (all ages combined): Liver T (A and hepatocellular C)– M, 1/98 (1%), 69/162 (43%), 81/165 (49%); F, 0/96 (0%), 7/147 (5%), 10/126 (8%) Lung T (A and AdC)– M, 7/98 (7%), 57/162 (35%), 73/165 (44%); F, 2/90 (2%), 53/147 (36%), 50/126 (40%) Forestomach T (P and SCC)– M, 0/98 (0%), 39/162 (24%), 64/165 (39%); F, 0/96 (0%), 22/147 (15%), 40/126 (32%) Lymphoreticular T (mainly reticulum-cell S)– M, 2/98 (2%), 104/314 (33%) (high- and low-dose groups combined); F, 2/96 (2%), 148/281 (53%) (high-and low-dose groups combined)	+	NR (trioctanoin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Vesselinovitch *et al.* (1975a, b) Contd.		C3A/JF1 mice (all ages combined): Liver T (A and hepatocellular C)– M, 3/97 (3%), 30/148 (20%), 33/137 (24%); F, 0/100 (0%), 1.3% 2/126 (1.3%), 2/153 1.3%) Lung T (A and AdC)– M, 49/97 (49%), 1 438/148 (93%), 125/137 (91%); F, 26/100 (26%), 115/126 (91%), 141/153 (92%) Forestomach T (P and SCC)– M, 0/97 (0%), 18/148 (12%), 42/137 (31%); F, 0/100 (0%), 18/126 (14%), 31/153 (20%) Lymphoreticular T (mainly reticulum-cell S)– M, 0/97 (0%), 26/285 (9%); F, 2/100 (2%), 50/278 (18%) (high- and low-dose groups combined)	+	
Mouse, CD-1 (M, F) 1 yr Wislocki *et al.* (1986)	0 and 560 nmol [148 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 0, 8, 15) 37 M/group, 27 F/group	Liver T: M, 2/28 (7%; 2 A), 18/37* (49%; 11 A, 7 C*); F, no liver T found Lung T: M, 1/28 (4%; 1 A), 13/37** (35%; 13 A); F, 0/31, 13/27** (48%) (13 A) Malignant lymphoma: M, 1/28 (4%), 2/37 (5%); F, 1/31 (3%), 4/27 (15%)	*$P < 0.005$ **$P < 0.05$	> 99% (DMSO)
Mouse, CD-1 (M, F) 52 wk Lavoie *et al.* (1987)	0 and 1.1 µmol [290 µg] (total dose; given as 1/6, 2/6, 4/6 on PND 1, 8, 15) 17 M/group, 14–18 F/group	Liver T: M, 1/17 (6%; 1 H), 13/17* (76%; 9 hepatic A, 4 H); F, 0/18, 0/14 Lung A: M, 0/17, 14/17* (82%); F, 0/18, 9/14** (64%)	*$P < 0.005$ **[$P < 0.0005$]	> 99% (DMSO)
Mouse, Swiss-Webster BLU: Ha(ICR) (M, F) 26 wk Busby *et al.* (1989)	0 and 59.5 µg (total dose; given as 8.5, 17, 34 µg on PND 1, 8, 15) NR/group	Lung T: M, 12/91 (13%; 12 A, 1 AdC; 0.15 ± 0.04 T/mouse), 13/28 (46%; 13 A; 0.71 ± 0.19 A/mouse); F, 7/101 (7%; 7 A; 0.08 ± 0.03 A/mouse), 18/27 (67%; 18 A, 1 AdC; 1.19 ± 0.21 T/mouse)	+	> 99% (DMSO) statistics NR
Mouse, NR, newborn (M, F) 30 wk Rippe & Pott (1989)	0, 10, 100 µg/animal, 1 × NR/group	Lung T: 13% [5/38] (0.13 T/animal), 16% [5/31] (0.23 T/animal), 64% [21/33] (2.52 T/animal)	+	NR (saline solution + 1% gelatine + 0.4% Tween 20) Type of lung tumour NR; statistics NR
Mouse, A/J (F) 260 d Weyand *et al.* (1995)	0 (untreated), 0 (vehicle control), 1.79 mg/animal, 1 × 29–30/group	Lung T: 7/30 (23%; 7 A; 0.27 ± 0.12 A/animal), 11/30 (37%; 11 A; 0.43 ± 0.11 A/animal), 29/29* (100%; 27 A, 2 AdC; 15.8 ± 1.28** T/animal); forestomach T: 0/30 (0%), 0/30 (0%), 24/29** (83%; 15 P, 9 C; 1.83 ± 0.25** T/animal)	*$P < 0.05$ **$P < 0.001$	NR (tricaprylin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, B6C3F1 infant (M, F) 26 wk, 39 wk, 52 wk Rodriguez *et al.* (1997)	0 (untreated), 0 (vehicle controls), 125, 250, 375 µg/7 g bw, 1 × > 30 M/group, > 30 F/group	Liver T (M): At wk 26: 0/41, 0/58, 0/29, 0/25, 3/34 (9%; multiplicity, 1.0); at wk 39: 0/34, 0/59, 6/26 (23%; multiplicity, 1.0), 13/34 (38%; multiplicity, 1.9), 15/23 (65%; multiplicity, 1.9); at wk 52: 4/64 (6%; multiplicity, 1.0), 3/63 (5%; multiplicity, 1.0), 13/29 (45%; multiplicity, 1.8), 14/27 (52%; multiplicity, 2.2), 19/24 (79%; multiplicity, 2.5) No liver T in F	+	NR (corn oil) No forestomach tumours
Mouse, CD-1 (M) 12 mo Von Tungeln *et al.* (1999)	0, 100, 400 nmol [26, 111 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15) 24/group	Liver T: 3/20 (15%; 1 A, 2 C; 1.7 T/liver section), 5/21 (24%; 4 A, 1 C; 1.5 T/liver section), 9/20 (45%; 7 A*, 2 C; > 2.3 T/liver section) Lung T: 4/20 (20%; 4 A; 1.0 T/lung section), 1/21 (5%; 1 A; 1.0 T/lung section), 9/20 (45%; 7 A, 2 C; 1.9 T/lung section)	*P = 0.0234	> 99% (DMSO)
Rat, Wistar (F) ~112 wk Roller *et al.* (1992)	0 and 5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 3/41 (7.3%), 33/37 (89.2%)	+	NR (3:1 mixture of tricaprylin/ beeswax) Limited reporting
Rat, Wistar (F) ~116 wk Roller *et al.* (1992)	5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 19/38 (50%); historical controls, 11/369 (3%)	+	NR (saline solution) No control; limited reporting of tumour data
Inhalation				
Hamster, Syrian golden (M) Lifetime Thyssen *et al.* (1981)	0, 2.2, 9.5, 46.5 mg/m3, 4.5 h/d, 7 d/wk, 10 wk; thereafter 3 h/d, 7 d/wk (total average doses: 0, 29, 127, 383 mg/animal) 24/group (+ animals added during the study)	Respiratory tract T: (polyps, P, SCC)– 0/27, 0/27, 34.6% [9/26; 3 nasal, 8 laryngeal, 1 tracheal], 52% [13/25; 1 nasal, 13 laryngeal, 3 tracheal; no bronchogenic T] Upper digestive tract T: (polyps, P, SCC)– 0/27, 0/27, 26.9% [6/26; 6 pharyngeal, 1 forestomach], 56% [14/25; 14 pharyngeal, 2 oesophageal, 1 forestomach]	+	NR (0.1% saline solution); particle size, > 99% diameter 0.2–0.5 µm, > 80% diameter 0.2–0.3 µm Survival decreased for high dose-exposed animals (59 wk) *vs* other groups (96 wk).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Intrapulmonary injection				
Rat, OM (F) 64 (high-dose group)–133 wk (untreated controls) Deutsch-Wenzel *et al.* (1983)	0 (untreated), 0 (vehicle control), 0.1, 0.3, 1.0 mg/animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [10/35] (28.6%) (4 epidermoid C; 6 pleomorphic S), [23/35] (65.7%) (21 epidermoid C; 2 pleomorphic S), [33/35] (94.3%) (33 epidermoid C)	+	99.1% (1:1 mixture of beeswax and trioctanoin)
Rats, F344/NSlc (M) 104 wk Iwagawa *et al.* (1989)	0, 0.03, 0.1, 0.3, 1.0 mg/animal, 1 × NR/group	Lung T: 0/40, 1/29 (3%; 1 undifferentiated T), 7/30 (23%; 6 SCC, 1 undifferentiated T), 22/29 (76%; 20 SCC, 2 undifferentiated T), 9/13 (69%; 9 SCC)	+	NR (1:1 mixture of beeswax/tricaprylin)
Rat, Osborne-Mendel (F) 134 wk (low-dose group)–140 wk (vehicle controls) Wenzel-Hartung *et al.* (1990)	0 (untreated), 0 (vehicle control), 30, 100, 300 µg/animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [3/35] (8.6%; 3 SCC), [11/35] (31.4%; 11 SCC), [27/35] (77.1%; 27 SCC).	+	99.6% (beeswax/trioctanoin mixture of varying composition) SCC predominantly keratinized
Rat, F344/DuCrj (M) 100 wk Horikawa *et al.* (1991)	0, 50, 100, 200 µg/animal, 1 × 9–10/group	Lung T: 0/19, 0/10, 3/10 (30%; 2 SCC, 1 AdSC), 4/9 (44.4%; 3 SCC, 1 undifferentiated T)	+	NR (1:1 mixture of beeswax/tricaprylin)
Intratracheal administration				
Rat, Wistar-WU/Kisslegg (F) 124–126 wk Pott *et al.* (1987)	0 and 1 mg/animal, once/wk, 20 wk NR/group	Lung T: 0/40, 7/36 (19%; 1 A, 5 SCC, 1 mixed AdC/SCC)	+	NR (0.9% saline solution)
Rat, Sprague-Dawley (M, F) Controls, 131 wk; treated animals, 112 wk Steinhoff *et al.* (1991)	0 and 0 (physiological saline), 7 mg/kg bw/instillation (physiological saline with Tween 60), once/2 wk, 44 wk 20 or 50/group	M: 0/50, 0/50, 19/20 (95%; 19 malignant lung T) F: 0/50, 0/50, 19/20 (95%; 18 malignant, 1 benign lung T)	+	NR (physiological saline solution with or without Tween 60) Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 78 wk Feron (1972)	0 (only for M), 1 mg/animal, once/wk, 36 wk 35/group	Respiratory tract T/adenomatoid lesions: M–6/27 (22%; 1 tracheal P, 5 pulmonary adenomatoid lesion), 19/29 (66%; 1 tracheal P, 17 SCC, 26 pulmonary adenomatoid lesion, 5 A, 1 AdC, 1 SCC) F–22/27 (81%; 1 laryngeal SCC, 16 tracheal SCC, 2 bronchial A, 1 AdC, 21 pulmonary adenomatoid lesion, 8 A, 1 AdC)	+	> 99% (0.9% saline solution) No female controls; Statistics NR
Hamster, Syrian golden (M) 78 wk Feron *et al.* (1973)	0, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/animal, once/wk, 52 wk 30/group	Respiratory tract T: 0/29, 3/30 (10%; 3 tracheal P, 1 pulmonary A), 4/30 (13%; 1 tracheal P, 4 pulmonary A), 9/30 (30%; 5 tracheal P, 7 pulmonary A), 25/29 (86%; 2 tracheal polyp, 9 P, 5 SCC, 1 AdSC, 1 fibroS, 2 bronchial polyp, 1 P, 2 SCC, 1 AdSC), 26/28 (93%; 6 tracheal P, 11 SCC, 1 AdSC, 1 bronchial polyp, 2 P, 4 SCC, 2 AdSC, 4 AdC, 1 anaplastic C, 16 pulmonary A, 4 SCC, 3 AdSC, 1 AdC, 2 anaplastic C)	+	NR (0.9% saline solution)
Hamster, Syrian golden (M, F) M, 67–88 wk; F, 60–88 wk Henry *et al.* (1973)	0, 13.3–15.5 mg/animal, once/wk, 8 wk 50/group, 25 controls/group	Respiratory tract T: Controls– 1 tracheal polyp, 6 pulmonary bronchiolar adenomatoid lesions/47 animals Treated animals– 26/65 (40%; 1 nasal polyp; 6 laryngeal polyps, 1 P, 1 A, 1 AdC, 7 tracheal polyps, 1 AdC, 1 SCC, 1 fibroS, 2 bronchial AdC, 13 pulmonary bronchiolar adenomatoid lesion, 3 A, 5 AdC, 1 SCC, 2 anaplastic C, 1 mixed C, 1 myelogenous leukaemia, 1 neurofibroS) T at other sites: Controls– 1 renal A Treated animals– 3 blast-cell leukaemia, 2 adrenocortical A, 1 renal AdC, 1 oesophageal fibroS, 1 haemangioma	+	NR (0.5% gelatine in 0.9% saline solution) Tumour data for M and F combined; statistics NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 60 wk Kobayashi (1975)	0 and 1 mg/animal, once/wk, 30 wk 20–32 M/group, 20–28 F/group	Respiratory tract T: M–0/20, 11/26 (42.3%; 1 laryngeal polyp, 1 tracheal polyp, 1 P, 1 bronchial SCC, 9 lung A, 7 AdC, 3 SCC, 1 anaplastic C, 2 AdSC) F–0/20, 14/26 (53.8%; 1 laryngeal P, 2 tracheal polyps, 1 bronchial SCC, 10 lung A, 3 AdC, 1 SCC)	+	NR (0.9% saline)
Hamster, Syrian golden (M, F) 78 wk Kruysse & Feron (1976)	0 (untreated), 0 (vehicle controls), 1 mg/animal, once/2 wk, 52 wk 17 or 40/group	Respiratory tract T: M–0/40, 0/40, 13/14 (93%; 2 laryngeal P, 1 SCC, 4 tracheal P, 3 SCC, 1 anaplastic C, 1 S, 1 bronchial SCC, 1 AdC, 5 pulmonary A, 1 AdC) F–0/40, 0/40, 7/12 (58%; 2 tracheal P, 3 SCC, 1 bronchial P, 5 pulmonary A)	+	> 99% (saline solution)
Hamster, Syrian golden (M) 100 wk Sellakumar *et al.* (1976)	0 (untreated), 3 mg/animal, once/wk, 10 wk 48/group	Respiratory tract T: 0/48, 7/48 (15%; 2 laryngeal P, 4 tracheal P, 1 lung A) T at other sites: 6/48 (13%; 3 forestomach P, 2 lymphoma, 1 anaplastic C), 26/48 (54%; 21 forestomach P, 1 skin melanoma, 1 liver haemangioma, 1 adrenocorticoA, 3 adrenocorticoC)	+	> 99% (0.9% saline solution)
Hamster, Syrian golden (M, F) Experiment 1: up to 89 wk for M and 70 wk for F Experiment 2: up to 83 wk for M and 68 wk for F Ketkar *et al.* (1977)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/animal, 1 × 30/group	Respiratory tract T: Experiment 1– M 0/24, 3/30 (10%; 1 laryngeal P, 1 tracheal P, 1 lung S), 5/28 (18%; 1 laryngeal SCC, 1 tracheal P, 4 lung S), 4/27 (15%; 3 tracheal P, 1 lung A, 1 S) F 0/28, 3/29 (10%; 1 tracheal P, 2 lung A), 1/30 (3%; 1 lung A), 3/28 (13%; 1 laryngeal P, 2 lung A)	+	97% (0.9% saline solution or Tris buffer)
	Experiment 2: 0, 4, 8, 16 mg in Tris buffer/animal, 1 × 30/group	Experiment 2– M 0/27, 5/24 (21%; 1 tracheal P, 5 lung A), 13/25 (52%; 1 laryngeal P, 7 tracheal P, 4 lung A, 3 AdC), 8/27 (30%; 2 laryngeal P, 1 SCC, 3 tracheal P, 3 lung A) F 0/27, 3/27 (11%; 2 tracheal P, 1 lung AdC), 2/29 (7%; 2 tracheal P), 8/29 (28%; 1 laryngeal P, 4 tracheal P, 5 lung A)	+	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 81 wk Feron & Kruysse (1978)	0 (untreated), 0 (vehicle controls), 0.35, 0.7 mg/animal, once/wk, 52 wk 15 or 30/group	Respiratory tract T: M–0/30 (untreated and vehicle controls combined), 4/29 (14%; 2 tracheal P, 1 bronchial P, 2 pulmonary A), 19/30 (63%; 1 laryngeal P, 5 tracheal P, 1 SCC, 1 anaplastic C, 1 S, 2 bronchial P, 1 AdC, 11 pulmonary A, 2 AdC, 1 SCC, 1 anaplastic C) F–0/28 (untreated and vehicle controls combined), 3/27 (11%; 1 laryngeal P, 1 bronchial P, 1 pulmonary A), 7/24 (29%; 1 tracheal P, 2 SCC, 1 bronchial AdC, 5 pulmonary A)	+	> 99% (0.9% saline solution) Statistics NR
Hamster, Syrian golden (M, F) Average survival up to 41 wk for M and 35 wk for F Ketkar *et al.* (1978)	0, 0.1, 0.33, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: M–0/29, 5/26 (19%; 5 bronchiogenic A), 7/29 (24%; 5 tracheal P, 2 bronchiogenic A), 6/27 (22%; 5 tracheal P, 2 bronchiogenic A) F–0/30, 12/30 (40%; 1 tracheal P, 1 SCC, 10 bronchiogenic A), 10/28 (36%; 7 tracheal P, 5 bronchiogenic A, 1 SCC), 6/30 (20%; 3 tracheal P, 3 bronchiogenic A, 3 SCC)	+	97% (10% bovine serum albumin) Average survival time much lower in the high-dose group than in the other groups
Hamster, Syrian golden (M, F) Lifetime, up to 90 wk Stenbäck & Rowland (1978)	0, 3 mg large particles, 3 mg small particles/animal, once/wk, 18 wk 48 (M + F)/group	Respiratory tract T (M + F combined): 0/46, 31/47 (66%; 5 laryngeal P, 12 tracheal P, 20 SCC, 2 unspecified T, 2 bronchial P, 9 SCC, 3 A, 2 anaplastic C), 5/46 (11%; 1 laryngeal P, 1 SCC, 4 tracheal P)	+	99.4% (0.9% saline solution); particle size by weight: large-98% < 30 µm, 90% < 20 µm, 36% < 10 µm, 10% < 5 µm; small-98% < 10 µm, 79% < 5 µm, 5% < 1 µm
Hamster, Syrian golden (M) Average survival up to 88 wk Ketkar *et al.* (1979)	0 (untreated), 0 (vehicle controls), 0.125, 0.25, 0.5, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: 0/29, 0/28, 9/29 (31%; 2 laryngeal polyps/P, 1 tracheal P, 1 SCC, 2 lung A, 2 SCC, 5 AdC), 24/29 (83%; 1 nasal SCC, 2 laryngeal polyps/P, 4 tracheal P, 9 SCC, 5 lung A, 5 SCC, 11 AdC), 19/29 (66%; 1 laryngeal P, 2 SCC, 5 tracheal P, 11 SCC, 7 lung SCC, 2 AdC), 9/29 (31%; 1 laryngeal P, 1 SCC, 1 tracheal P, 5 SCC, 1 lung A, 4 SCC)	$P < 0.001$, all treated groups	97% (Tris buffer + 0.9% saline solution); particle size: majority < 10 µm but particles up to 80 µm also present Average survival in two highest-dose groups much lower than that in the other groups due to many early deaths from pulmonary lesions other than tumours

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 105 wk Feron *et al.* (1980)	0 (untreated), 0 (gelatine in 0.9% saline), 0.5, 1.0 mg fine particles, 0.5, 1.0 mg coarse particles, 1.0 mg wide-range particles/animal, once/wk, 52 wk 30–35/group	Respiratory tract T: M–0/29, 2/34 (6%; 2 laryngeal P), 7/34 (21%; 1 laryngeal P, 6 tracheal P, 1 lung A), 6/31 (19%; 2 laryngeal P, 1 tracheal P, 1 S,1 pulmonary A), 13/31 (42%; 2 laryngeal P, 3 tracheal P, 9 pulmonary A), 25/34 (74%; 2 laryngeal P, 9 tracheal P, 4 SCC, 2 S, 1 pulmonary A, 1 AdC), 23/34 (68%; 2 laryngeal P, 1 SCC, 6 tracheal P, 2 SCC, 1 bronchial P, 1 SCC, 13 pulmonary A, 2 AdC, 2 anaplastic C) F–0/28, 2/33 (6%; 1 tracheal P, 1 pulmonary A), 2/34 (6%; 1 bronchial P, 1 A), 5/32 (16%; 1 laryngeal P, 2 tracheal P, 3 pulmonary A), 9/32 (28%; 2 laryngeal P, 5 tracheal P, 6 pulmonary A), 19/32 (31%; 4 tracheal P, 1 SCC, 1 S, 1 bronchial P, 7 pulmonary A, 1 AdC), 11/34 (34%; 1 laryngeal P, 3 tracheal P, 2 bronchial P, 7 pulmonary A, 1 AdC)	+	NR; particles size by weight: fine, 77% < 5.2 µm, 60% < 3.9 µm; coarse, 77% < 42 µm, 3% < 16 µm; wide-range, 72% < 30 µm, 19% < 10 µm (gelatine in 0.9% saline solution) Statistics NR
Hamster, Syrian golden (M) 129 wk Godleski *et al.* (1984)	0 and 5 mg/animal, once/wk, 15 wk 80/group	Malignant T: 4/80 (5%; 1 multicentric undifferentiated lung C, 3 lymphoma), 25/80* (31%; 9 SCC, 2 undifferentiated C of the respiratory tract, 5 lymphoma, 1 SCC, 2 AdC of the gastrointestinal tract, 2 soft-tissue T, 1 hepatoma, 2 mouth SCC, 1 skin C)	*$P < 0.001$	> 99% (0.5% gelatine in 0.9% saline solution)
Intratracheal administration of combinations of benzo[*a*]pyrene and 'particles/fibres'				
Rat, Sprague-Dawley (M, F) Up to 130 wk Steinhoff *et al.* (1991)	0, (untreated), 0 (physiological saline), 10–40 mg/kg bw Bayferrox 130 (96.2% cubic α-Fe_2O_3), 10–40 mg/kg bw Bayferrox 920 (86.1% fibrous α-FeOOH), 7 mg/kg bw, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 130, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 920, ~once/2 wk, 44–~130 wk 20 or 50/group	Lung T: M–0/50, 0/50, 0/50, 0/50, 19 malignant T in 20 animals, 21 malignant and 1 benign T in 20 animals, 17 malignant and 1 benign T in 20 animals F–0/50, 0/50, 0/50, 1 malignant and 1 benign T in 50 animals, 18 malignant and 1 benign T in 20 animals, 16 malignant T in 20 animals, 17 malignant and 2 benign T in 20 animals	+	NR (physiological saline solution with or without Tween 60); Bayferrox 130, Bayferrox 920 Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 140 wk) Saffiotti *et al.* (1972)	Experiment 1 0, 50 mg ferric oxide, 5 mg + 45 mg ferric oxide, 12.5 mg + 37.5 mg ferric oxide/ animal, 1 × Experiment 2 (2 groups/dose level) 5 mg + 5 mg ferric oxide, 10 mg + 10 mg ferric oxide, 15 mg + 15 mg ferric oxide, once/wk, 15 wk 23–110 M/group, 18–107 F/group	Experiment 1: Respiratory tract T– M 0/45, 0/101, 3/92 (3%; 1 tracheal polyp, 1 P, 1 bronchial A), 3/27 (11%; 1 bronchial A, 1 bronchogenic SCC, 1 anaplastic C) F 0/44, 0/89, 4/97 (4%; 1 tracheal polyp, 1 P, 1 bronchiolar A, 1 AdC), 6/33 (18%; 1 bronchial P, 1 A, 2 bronchogenic SCC, 1 anaplastic C, 2 bronchiolar A) Forestomach P– M 5/45 (11%; 6 T), 5/101 (5%; 5 T), 15/92 (16%; 35 T), 8/27 (30%; 16 T) F 2/44 (5%; 2 T), 2/89 (2%; 3 T), 5/97 (5%; 5 T), 4/33 (12%; 6 T)	+	NR (0.9% saline solution); ferric oxide
		Experiment 2: Respiratory tract T (M + F combined)– 7/50 (14%; 2 tracheal P, 1 SCC, 1 bronchial P, 1 A, 2 SCC, 1 anaplastic C, 1 pulmonary SCC), 8/58 (14%; 2 tracheal polyps, 1 bronchial polyp, 2 SCC, 2 AdC, 2 pulmonary A, 2 AdC), 17/61 (28%; 2 tracheal polyps, 2 P, 5 SCC, 5 bronchial SCC, 1 pulmonary A, 1 SCC, 1 AdC, 1 anaplastic C), 25/60 (42%; 4 tracheal polyps, 3 P, 3 SCC, 4 anaplastic C, 1 bronchial P, 1 SCC, 2 anaplastic C, 4 AdC, 1 A, 2 pulmonary SCC, 2 anaplastic C, 6 A), 25/39 (64%; 1 tracheal P, 10 SCC, 1 anaplastic C, 3 bronchial P, 7 SCC, 11 anaplastic C, 2 AdC, 2 pulmonary SCC, 2 A), 35/55 (64%; 2 laryngeal SCC, 11 tracheal P, 1 polyp, 12 SCC, 1 carcinoS, 2 fibroS, 16 bronchial SCC, 10 anaplastic C, 6 AdC, 3 A, 2 pulmonary A) Forestomach T– M 8/22 (36%; 13 P, 1 SCC), 6/28 (21%; 9 P), 11/34 (32%; 28 P, 1 SCC), 11/30 (37%; 18 P), 5/22 (23%; 10 P), 1/28 (4%; 1 P) F 9/28 (32%; 14 P), 6/30 (20%; 9 P), 5/27 (19%; 20 P), 8/30 (27%; 10 P, 1 SCC), 5/17 (29%; 11 P), 3/27 (11%; 3 P)	+	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (F) Presumably lifetime Pott *et al.* (1973b)	340 µg in tricaprylin, 340 µg in Tween 60/saline solution, 340 µg in Tween 60/saline solution + 850 µg atmospheric dust/animal, 45 × within a period of 6.5 mo (total dose, ~15 mg; dust, 38 mg) 48/group	Respiratory tract T (benign and malignant T of the larynx, trachea or bronchi): 2/48 (4%), 14/48 (29%), 16/48 (33%)	+	NR (tricaprylin, Tween 60/saline solution); atmospheric dust from Bochum, Germany (particle size < 5 µm)
Hamster, Syrian (M, F) 100 wk Sellakumar *et al.* (1973)	0 (untreated), 3 mg + 3 mg ferric oxide, 3 mg + 6 mg ferric oxide, 3 mg + 9 mg ferric oxide, once/2 wk, 20 wk 36/group, 193 controls/group	Respiratory tract T (M + F combined): 0/193, 26/67 (39%; 3 laryngeal polyp, 3 P, 3 SCC, 7 tracheal polyp, 6 P, 2 SCC, 2 bronchial polyp, 5 SCC, 9 AdC, 1 anaplasic C, 7 lung A, 1 AdC), 28/64 (44%; 1 laryngeal polyp, 3 P, 6 SCC, 3 tracheal polyp, 9 P, 3 SCC, 3 bronchial polyp, 1 P, 4 SCC, 3 AdC, 1 anaplastic C, 7 lung A, 4 AdC), 26/66 (39%; 3 laryngeal polyp, 6 SCC, 6 tracheal polyp, 11 P, 1 SCC, 1 bronchial polyp, 1 P, 4 SCC, 4 AdC, 2 anaplastic C, 6 lung A, 6 AdC) Forestomach T: M–0/193 (M + F), 17/32 (53%; 37 P), 10/31 (32%; 16 P, 1 SCC), 6/35 (17%; 15 P) F–0/193 (M + F), 10/35 (29%; 30 P), 12/33 (36%; 25 P), 15/31 (48%; 33 P)	+	NR (0.9% saline solution); ferric oxide
Hamster, Syrian golden (M, F) Lifetime (up to 120 wk) Stenbäck *et al.* (1975)	0 (untreated), 2 mg + 1 mg magnesium oxide/animal, once/wk, 20 wk, 3 mg + 3 mg ferric oxide/animal, once/wk, 15 wk 48 or 90/group	Respiratory tract tumours (M + F combined): 0/89, 32/45 [71%] (11 laryngeal P, 3 SCC, 1 tracheal polyp, 20 P, 5 SCC, 1 AdC, 1 bronchial P, 3 A, 8 AdC, 9 SCC, 1 AdSC), 31/44 (70%; 10 laryngeal P, 4 SCC, 8 tracheal P, 12 SCC, 2 anaplastic C, 2 bronchial P, 4 A, 2 AdC, 17 SCC, 3 anaplastic C)	+	NR (0.2% saline solution); ferric oxide, magnesium oxide

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 100 wk) Stenbäck & Rowland (1979)	0 (untreated), 0 (saline), 0 (gelatine in saline), 3 mg silicon dioxide in saline, 1.5 mg manganese dioxide in saline, 3 mg in saline, 3 mg in gelatine/saline, 3 mg + 3 mg silicon dioxide in saline, 1.5 mg + 1.5 mg manganese dioxide in saline/animal, once/wk, 20 wk 50/group	All T (M + F combined): 2/100 (2%; 2 lymphoma), 1/48 (2%; 2 forestomach P), 2/45 (4%; 2 lymphoma), 0/48 (0%), 2/48 (4%; 1 forestomach P, 1 lymphoma), 18/46 (39%; 1 laryngeal P, 1 SCC, 4 tracheal P, 15 forestomach P), 11/47 (23%; 2 tracheal P, 1 SCC, 3 bronchial SCC, 1 splenic haemangioma, 1 adrenal cortical A, 1 lymphoma, 2 forestomach SCC), 25/48 (52%; 1 laryngeal SCC, 8 tracheal P, 2 SCC, 3 bronchial SCC, 6 lung A, 3 AdC, 10 forestomach P, 1 thyroid A, 1 uterine fibroma, 1 A, 1 lymphoma), 20/48 (42%; 1 laryngeal P, 3 tracheal P, 1 SCC, 1 bronchial SCC, 24 forestomach P, 1 ovarian fibroma, 1 thyroid A, 2 forestomach SCC, 1 squamous-cell fibroma)	+	> 99% (saline, 0.5% gelatine in saline); manganese dioxide, silicon dioxide
Hamster, Syrian golden (M, F) 82 wk Reynders *et al.* (1985)	0 and 8 mg + 6 mg ferric oxide/animal, once/wk, 6 wk 35/group	Respiratory tract T: M–0/32, 12/24 (50%; 15 T: 3 laryngeal P, 1 tracheal P, 1 SCC, 2 bronchial polyp, 2 SCC, 1AdC, 3 pulmonary SCC, 1 AdSC, 1 AdC) F–0/35, 9/26 (35%; 12 T: 1 laryngeal P, 5 tracheal P, 2 bronchial polyp, 2 pulmonary SCC, 1 AdSC, 1 AdC)	+	NR (0.9% saline solution); ferric oxide
Buccal pouch				
Hamster, Syrian golden (M) Up to 40–44 wk with interim kills after 5, 20, and 24–32 wk Solt *et al.* (1987)	Painting of both buccal pouch with 0, 20 mM solution/animal, twice/wk, 20 wk 28/group, 20 controls/group	Forestomach P: 0/6, 8/10* (after 40–44 wk) Buccal pouch SCC: 0/6, 1/10 (after 40–44 wk)	*[$P < 0.01$]	NR (paraffin oil)
Intramammary or intramamillary administration				
Rat, Sprague-Dawley (F) 20 wk Cavalieri *et al.* (1988a)	0 and 0 (untreated contralateral mammary gland), 4 [1 mg], 16 µmol [4.2 mg] (5th right mammary gland), 1 × 20/group	Mammary gland T: [0/20] (0%), [0/20] (0%), [10/20] (50%; 6 AdC, 4 fibroS), [16/20] (80%; 8 AdC, 2 fibroA, 10 fibroS)	+	> 99% (no vehicle)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Rat, Sprague-Dawley (F) 45 wk Cavalieri *et al.* (1988a, b)	0 and 4 µmol [1 mg]/mammary gland (2nd, 3rd, 4th and 5th mammary gland on both sides injected), 1 × 20/group	Epithelial mammary T: [3/20] (15%; 3 fibroA), [14/20] (70%; 13 AdC, 3 fibroA); multiplicity: controls, 3/3 [1]; treated rats: AdC, 18/13 [1.4]; fibroA, 4/3 [1.3] Mesenchymal (mammary) T: [0/20] (0%), [11/20] (55%; 11 fibroS; multiplicity, 20/11 [1.8]) Skin T: [0/20] (0%), [9/20] (45%; 9 SCC; multiplicity, 11/9 [1.2])	+	> 99% (trioctanoin)
Rat, Sprague-Dawley (F) 24 wk Cavalieri *et al.* (1991)	0, 0.25 [66 µg], 1 µmol [264 µg]/mammary gland (the 2nd, 3rd, 4th and 5th on both sides), 1 × 20/group	Epithelial mammary gland T: 1/18 (6%; 1 fibroA), 1/20 (5%; 1 AdC), 0/20 (0%) Mesenchymal (mammary) T: 0/18 (0%), 6/20 (30%; 6 fibroS; multiplicity, 7/6), 8/20 (40%; 8 fibroS; multiplicity, 10/8) Skin T: 0/18 (0%), 0/20 (0%), 1/20 (5%; 1 SCC)	+	> 99% (trioctanoin) Statistics NR
Intracolonic instillation				
Mouse, Swiss albino (M, F) 120 wk Toth (1980)	0, 200, 2000 µg/g bw (total doses); control and high-dose group, 10 × / wk instillations of 0 and 200 µg, respectively; low-dose group, 1 instillation 50/group/sex	Malignant lymphoma: M–0/50, 6/50* (12%; 1 histiocytic, 4 lymphocytic, 1 mixed), 7/50** (14%; 2 histiocytic, 3 lymphocytic, 2 mixed) F–11/49 (22%; 5 histiocytic, 6 lymphocytic), 21/50*** (42%; 5 histiocytic, 16 lymphocytic), 18/49 (36%; 6 histiocytic, 8 lymphocytic, 4 mixed) Oesophagus T: M–no tumour F–0/49, 0/50, 5/49 (10%) Forestomach T: M–0/50, 2/50 (4%; 2 P), 10/50**** (20%; 9 P, 1 SCC) F–1/49 (2%; 1 SCC), 5/10 (20%; 3 P, 2 SCC), 11/49**** (22%; 9 P, 2 SCC)	*$P < 0.04$ **$P < 0.02$ ***$P < 0.053$ ****$P < 0.006$ *****$P < 0.0001$	98% (olive oil) Anal and skin tumours probably due to release of benzo[*a*]pyrene through the anal orifice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Toth (1980) Contd.		Anal T : M–0/50, 0/50, 7/50** (14%; 4 P, 3 SCC) F–0/49, 1/50 (2%; 1 P), 6/49* (12%; 1 P, 4 SCC, 1 K) Skin T : M–1/50 (2%; 1 K), 0/50, 13/50***** (26%; 5 P, 7 SCC, 1 K) F–0/49, 2/50 (4%; 2 SCC), 11/49**** (22%; 4 P, 5 SCC, 2 K)		
Mouse, C57Bl/6 (F) 18 mo Anderson *et al.* (1983)	0 (untreated, olive oil or β-naphthoflavone in olive oil), 1 mg/animal (in olive oil), once/wk, 14 wk 45–60/group	Forestomach P: 7/34 (21%; multiplicity, 1.1 ± 0.4), 17/18* (94%; multiplicity, 3.2 ± 2.3*) Peritoneal S: 0/40, 5/32* Lymphoma: 1/40 (2.5%), 9/32* (28%)	*$P < 0.05$	99% (olive oil, enzyme inducer β-naphthoflavone) No colon tumours found
Intravaginal application				
Mouse, C57B1 (F) 5 mo Näslund *et al.* (1987)	Cotton swab soaked in acetone (controls) or 1% solution of benzo[*a*]pyrene in acetone, twice/wk 10 or 76/group	0/10, 17/76 (22%; invasive cervical C)	+	NR (acetone)
Intrafetal injection				
Mouse, Swiss (M, F) 12 wk Rossi *et al.* (1983)	0, 0.4, 4.0, 9.9, 19.8 nmol [0, 0.1, 1, 2.6, 5.2 µg]/animal, 1 × 43–56/group	Lung A (M + F combined): 0/37, 1/39 (3%), 10/42 (25%), 10/38 (26%), 12/31 (39%)	+	> 99% (trioctanoin-acetone mixture (1:1))

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; bw, body weight; C, carcinoma; d, day or days; DMSO, dimethyl sulfoxide; F, female; H, hepatoma; K, keratocanthoma; M, male; min, minute or minutes; mo, month or months; NH_4OH, ammonium hydroxide; NR, not reported; P, papilloma; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; SGA, sebaceous gland adenoma; T, tumour; *vs*, versus; wk, week or weeks; yr, year or years

Table 3.2 Summary of reports of malignant tumours clearly induced in experimental animals by benzo[*a*]pyrene

Organ site/ species	Lung	Trachea	Larynx	Forestomach	Liver	Lymphoid tissue (lymphoma)	Sarcoma (injection site)	Skin	Mammary gland
Mouse	x			x	x	x	x	x	
Rat	x						x		x
Hamster	x	x	x	x			x		

3.4 Intraperitoneal injection

In a series of studies in newborn and adult mice, intraperitoneal injection of benzo[*a*]pyrene increased the incidence of liver (adenomas and carcinomas) and lung (adenomas and adenocarcinomas) tumours and, occasionally, forestomach (squamous cell papillomas and carcinomas) and lymphoreticular tumours (Vesselinovitch *et al.*, 1975a, b; Wislocki *et al.*, 1986; Lavoie *et al.*, 1987; Busby *et al.*, 1989; Rippe & Pott, 1989; Mass *et al.*, 1993; Nesnow *et al.*, 1995; Ross *et al.*, 1995; Weyand *et al.*, 1995; Rodriguez *et al.*, 1997; Von Tungeln *et al.*, 1999).

In one study in rats with a single intraperitoneal injection of benzo[*a*]pyrene, a high incidence of abdominal mesotheliomas and sarcomas was observed (Roller *et al.*, 1992).

3.5 Inhalation

In a lifetime inhalation study (Thyssen *et al.*, 1981) in male hamsters, benzo[*a*]pyrene induced dose-related increases in the incidence of papillomas and squamous-cell carcinomas in both the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach).

3.6 Intrapulmonary injection

Dose-related increases in the incidence of malignant lung tumours (mainly epidermoid and squamous-cell carcinomas and a few pleomorphic sarcomas) were found after injection of benzo[*a*]pyrene into the lung of rats (Deutsch-Wenzel *et al.*, 1983; Iwagawa *et al.*, 1989; Wenzel-Hartung *et al.*, 1990; Horikawa *et al.*, 1991).

3.7 Intratracheal administration

Intratracheal administration of benzo[*a*]pyrene alone or mixed with particulates and suspended in saline with or without suspendents resulted in benign and malignant respiratory tumours in mice (Heinrich *et al.*, 1986a), rats (Pott *et al.*, 1987; Steinhoff *et al.*, 1991) and in numerous studies in hamsters (IARC, 2010). This treatment also induced forestomach tumours in hamsters (Saffiotti *et al.*, 1972; Sellakumar *et al.*, 1973; Smith *et al.*, 1975a, b, Stenbäck & Rowland, 1979). Larger benzo[*a*]pyrene particles were generally more effective than smaller ones.

Mice that lack the nucleotide excision-repair gene *XPA* (*XPA*$^{-/-}$ mice) showed a stronger lung-tumour response after intratracheal instillation of benzo[*a*]pyrene than did their similarly treated *XPA*$^{+/+}$ and *XPA*$^{+/-}$ counterparts (Ide *et al.*, 2000).

3.8 Buccal pouch application

Repeated application of benzo[*a*]pyrene to the buccal pouch mucosa of male hamsters resulted in a high incidence of forestomach papillomas (Solt *et al.*, 1987).

3.9 Subcutaneous tracheal grafts transplantation

In one study conducted in rats transplanted with subcutaneous rat tracheal grafts exposed to beeswax pellets containing various amounts of benzo[*a*]pyrene, a high incidence of squamous-cell carcinomas was reported (Nettesheim *et al.*, 1977).

3.10 Intramammilary administration

In three studies in rats, benign and malignant mammary gland tumours developed after intrammilary injection of benzo[*a*]pyrene (Cavalieri *et al.*, 1988a, b, 1991).

3.11 Intracolonic instillation

In three experiments in mice, intracolonic instillation of benzo[*a*]pyrene induced lymphomas and a variety of benign and malignant tumours in various organs including the forestomach (Toth, 1980; Anderson *et al.*, 1983).

3.12 Intravaginal application

Intravaginal application of benzo[*a*]pyrene in mice produced invasive cervical carcinoma; no such tumours were seen in controls (Näslund *et al.*, 1987).

3.13 Intrafetal injection

In one study in male and female Swiss mice, intrafetal injection of benzo[*a*]pyrene produced lung adenomas (Rossi *et al.*, 1983).

4. Other Relevant Data

Benzo[*a*]pyrene is a carcinogen that induces tumours in many animal species. Some of the examples relevant for this review are: lung tumours in mice, rats, and hamsters; skin tumours in mice; liver tumours in mice; forestomach tumours in mice and hamsters; and mammary gland tumours in rats (Osborne & Crosby, 1987; IARC, 2010). In humans, occupational exposures to benzo[*a*]pyrene-containing mixtures have been associated with a series of cancers: coke production: lung; coal gasification: lung, bladder; paving and roofing: lung; coal tar distillation: skin; soots: lung, oesophagus, haematolymphatic system, skin; aluminum smelting: lung, bladder; tobacco smoking: lung, lip, oral cavity, pharynx, oesophagus, larynx, bladder (IARC, 1984, 1985, 1986, 2010).

Studies on the mechanisms of action of benzo[*a*]pyrene have been reviewed (Xue & Warshawsky, 2005; IARC, 2010).

4.1 Metabolism

Benzo[*a*]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide (Osborne & Crosby, 1987). Benzo[*a*]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[*a*]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[*a*]pyrene-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of benzo[*a*]pyrene.

Both CYPs and peroxidases (e.g. prostaglandin-H synthase) can oxidize benzo[*a*]pyrene. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1 (Eling *et al.*, 1986; Shimada, 2006). Cytochrome P450s are inducible by benzo[*a*]pyrene and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, leading to changes in gene transcription of CYPs and phase-II enzymes. Mice lacking the AhR receptor are refractory to benzo[*a*]pyrene-induced tumorigenesis (Shimizu *et al.*, 2000). Both CYPs and peroxidases can form radical cations by one-electron oxidation. These cations comprise another class of ultimate carcinogenic metabolites (Cavalieri & Rogan, 1995). Some polymorphisms in human CYPs and phase-II enzymes (glutathione *S*-transferases, uridine 5′-diphosphate glucuronosyltransferases and sulfotransferases modulate susceptibility to cancer (Shimada, 2006). In another metabolic pathway, benzo[*a*]pyrene-7,8-dihydrodiol is oxidized to benzo[*a*]pyrene-7,8-quinone by enzymes of the aldo-keto reductase (AKR1) family. Among these, gene polymorphisms that influence susceptibility have been identified. NAD(P)H: quinone oxidoreductase-1 (NQO1) catalyses the reduction of benzo[*a*]pyrene quinones to hydroquinones, which may be re-oxidized and generate reactive oxygen species. Polymorphisms in this gene have also been described (Penning & Drury, 2007; IARC, 2010).

The current understanding of mechanisms underlying benzo[*a*]pyrene-induced carcinogenesis in experimental animals is almost solely based on two complementary pathways: those of the diolepoxides and the radical cations. Each provides a different explanation for the effects observed in experimental animals in specific tissues.

4.2 Diolepoxide mechanism

The diolepoxide mechanism for benzo[*a*]pyrene features a sequence of metabolic transformations: benzo[*a*]pyrene → benzo[*a*]pyrene-7,8-oxide (by CYP1A1 and CYP1B1) → benzo[*a*]pyrene-7,8-diol (by epoxide hydrolase) → benzo[*a*]pyrene-7,8-diol-9,10-epoxides (by CYP1A1 and CYP1B1) (Xue & Warshawsky, 2005). Each class of metabolic intermediate has been shown to be genotoxic and carcinogenic (Osborne & Crosby, 1987). The stereochemistry of the metabolic transformation of benzo[*a*]pyrene to diols and diolepoxides is an important component of this mechanism of action. Due to the creation of chiral carbons during the metabolic conversions, many of the metabolic intermediates of benzo[*a*]pyrene have multiple streochemical forms (enantiomeric and diastereomeric). As the metabolism proceeds the complexity of the stereo-chemical forms increases, eventually leading to four benzo[*a*]pyrene-7,8-diol-9,10-epoxides [(+)- and (-)-*anti*, (+)- and (-)-*syn*]. Diolepoxides react with DNA, mainly with the purines, deoxyguanosine and deoxyadenosine, and each diolepoxide can form both *cis* and *trans* adducts thus giving a total of 16 possible benzo[*a*]pyrene-7,8-diol-9,10-epoxide DNA adducts. However, in most cases far fewer DNA adducts are actually observed. The most ubiquitous benzo[*a*]pyrene adduct detected in isolated mammalian DNA after metabolic conversion in metabolically competent mammalian cells in culture, or in mammals, is the N^2-deoxyguanosine adduct, (+)-N^2-10*S*-(7*R*,8*S*,9*R*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene)-yl)-2'-deoxyguanosine (BPDE-deoxyguanosine), derived from 7*R*,8*S*-dihydroxy-9*R*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide, or BPDE). This adduct was first fully identified after isolation from benzo[*a*]pyrene-treated human and bovine bronchial explants (Jeffrey *et al.*, 1977). This diolepoxide is considered to be an ultimate, DNA-reactive,

metabolite of benzo[*a*]pyrene (Osborne & Crosby, 1987). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide can form both stable and unstable (so-called 'depurinating') adducts with DNA, mediated by electrophilic carbonium ions (Chakravarti *et al.*, 2008). *In vivo*, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide produces stable adducts that were formed primarily with guanines in many species and organs (IARC, 2010).

Mice treated with benzo[*a*]pyrene had *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-N^2-deoxyguanosine adducts in their lung tissue, while the lung tumours induced by benzo[*a*]pyrene had G→T and G→A mutations in the K_i-*Ras* gene at codon 12 (Mass *et al.*, 1993). In mice treated with benzo[*a*]pyrene the major stable DNA adduct in the epidermis was the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine adduct (Melendez-Colon *et al.*, 1999). Skin tumours from benzo[*a*]pyrene-treated mice or in preneoplastic skin from benzo[*a*]pyrene-treated mice had G→T mutations in codon 13 and A→T mutations in codon 61 of the Ha-*Ras* gene (Chakravarti *et al.*, 2008).

Benzo[*a*]pyrene-induced skin tumours harboured G→T transversion mutations in the *Tp53* tumour-suppressor gene (Ruggeri *et al.*, 1993). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts occurred at guanine positions in codons 157, 248, and 273 of the *TP53* gene in *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-treated human HeLa cells. The same positions are the major mutational hotspots found in human lung cancers (Denissenko *et al.*, 1996).

4.3 Radical-cation mechanism

The radical-cation mechanism for benzo[*a*]pyrene has been studied exclusively in connection with mouse-skin tumorigenesis (Cavalieri & Rogan, 1995). One-electron oxidation of benzo[*a*]pyrene by CYPs or peroxidases creates a radical cation localized on carbon 6, as a consequence of the ionization potential and geometric configuration. In mouse skin, this radical cation gives rise to the formation of covalent adducts with guanine (at the C8 carbon and N7 nitrogen) and adenine (at the N7 nitrogen). These adducts are unstable and are thought to generate apurinic sites in mouse skin. However, only low levels of apurinic sites were measured in the epidermis of mice treated with benzo[*a*]pyrene (Melendez-Colon *et al.*, 1999) and no studies to date have shown an increase in the number of apurinic sites in lung tissues treated with benzo[*a*]pyrene. In two *in vivo* studies, rats treated intraperitoneally with benzo[*a*]pyrene were shown to excrete 7-(benzo[*a*]pyrene-6-yl)-*N7*-guanine in faeces and urine, while the same adduct was detected in lung tissue of mice treated intraperitoneally with benzo[*a*]pyrene (Rogan *et al.*, 1990; Banasiewicz *et al.*, 2004). Skin papillomas obtained from mice treated topically with benzo[*a*]pyrene showed mutations (at guanine and/or adenine) at codons 12, 13 and 61 in the Ha-*Ras* oncogene (Wei *et al.*, 1999). Similar studies in preneoplastic skin from benzo[*a*]pyrene-treated mice showed Ha-*Ras* mutations at codons 13 and 61 (Chakravarti *et al.*, 2008). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide can also form depurinating DNA adducts at guanine and adenine (at the N7 nitrogen). The distribution and chemical nature of the depurinating adducts (from both radical-cation and diolepoxide intermediates) in mouse skin and the distribution and chemical nature of the specific benzo[*a*]pyrene-induced mutations in mouse-skin papillomas have been reported (Chakravarti *et al.*, 2008).

4.4 Other activation mechanisms of benzo[*a*]pyrene

4.4.1 Meso-region mechanism

The mechanism of meso-region biomethylation and benzylic oxidation features biomethylation of benzo[*a*]pyrene to 6-methylbenzo[*a*]

pyrene, with *S*-adenosylmethione as the carbon donor (Flesher *et al.*, 1982). This process has been shown to occur *in vitro*, and *in vivo* in rat liver (Stansbury *et al.*, 1994). 6-Methylbenzo[*a*]pyrene is further metabolized by CYPs to 6-hydroxymethylbenzo[*a*]pyrene (Flesher *et al.*, 1997) and then conjugated to sulfate by 3′-phosphoadenosine-5′-phosphosulfate sulfotransferase to 6-[(sulfooxy)methyl]-benzo[*a*]pyrene. This reactive sulfate ester forms DNA adducts *in vivo* (Stansbury *et al.*, 1994). These benzo[*a*]pyrene-DNA adducts have only been measured in rat liver (Surh *et al.*, 1989), which is not a target for benzo[*a*]pyrene-induced carcinogenesis. There is no evidence to date that this mechanism operates in lung.

4.4.2 Mechanism via formation of ortho-quinone/ reactive oxygen species

This mechanism features enzymatic oxidation of benzo[*a*]pyrene-7,8-diol to the *ortho*-quinone, benzo[*a*]pyrene-7,8-quinone, by aldo-keto reductases (Mangal *et al.*, 2009). Benzo[*a*]pyrene-7,8-quinone can react with DNA to yield both stable and depurinating DNA adducts *in vitro* (McCoull *et al.*, 1999; Balu *et al.*, 2006) and can also undergo repetitive redox cycling which generates reactive oxygen species that damage DNA (Penning *et al.*, 1999). In human A549 lung-tumour cells benzo[*a*]pyrene-7,8-quinone increased the formation of 8-oxo-deoxyguanosine and DNA strand-breaks (Park *et al.*, 2008; Mangal *et al.*, 2009). In a yeast reporter-assay, benzo[*a*]pyrene-7,8-quinone (in the presence of redox cycling) induced 8-oxo-deoxyguanosine formation and G→T transversions in the *Tp53* tumour-suppressor gene. The mutational spectra induced in the yeast reporter-assay closely matched those seen in DNA from human lung tumours (Shen *et al.*, 2006). Benzo[*a*]pyrene-7,8-quinone inhibited the activity of protein kinase C in MCF-7 cell lysates suggesting an ability to alter cell signalling (Yu *et al.*, 2002). Rats treated with benzo[*a*]pyrene showed increased urinary concentrations of 8-oxo-deoxyguanosine, but lower levels in liver and lung tissues. This suggested that reactive oxygen species are generated during the CYP-dependent metabolism of benzo[*a*]pyrene, but induction of DNA-repair mechanisms may reduce these levels in target tissues (Briedé *et al.*, 2004). To date this mechanism has been studied only in in-vitro systems.

It is noted that formation of reactive oxygen species is not limited to the redox cycling of the *ortho*-quinone of benzo[*a*]pyrene (benzo[*a*]pyrene-7,8-quinone). There are several other sources of benzo[*a*]pyrene-induced reactive oxygen species. *In vivo*, both mice and rats metabolize benzo[*a*]pyrene to benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone and benzo[*a*]pyrene-6,12-quinone and these quinones enter into redox cycling and induce mutations (Osborne & Crosby, 1987; Joseph & Jaiswal, 1998). Many of the reactive intermediates of benzo[*a*]pyrene (oxides, diol-epoxides, radical cations) and quinone-generated reactive oxygen species can disrupt the balance of cellular oxidants and anti-oxidants by reducing the anti-oxidant levels thus leading to an imbalance and an excess of reactive oxygen species.

4.4.3 Aryl hydrocarbon-receptor mechanism

The AhR regulates the transcription of a series of genes including *Cyp1A1*, *Cyp1A2*, *Nqo1*, *Aldh3a1* (encoding aldehyde dehydrogenase 3A1), *UGT1a6* (uridine 5′-diphosphate-glucuronosyl transferase), and *Gsta1* (glutathione *S*-transferase A1). All these genes are activated by AhR-ligands, including benzo[*a*]pyrene, via the AhR-mediated aromatic hydrocarbon response element. The AhR plays a role in the response to oxidative stress in cell-cycle regulation and apoptosis. In addition, the CYP1A1/1A2-mediated metabolism generates oxidative stress (Nebert *et al.*, 2000). Mitochondrial hydrogen-peroxide production was induced by an AhR-ligand in

wild-type mice but not in *AhR*$^{-/-}$ knockout mice (Senft *et al.*, 2002). These mice were shown to be refractory to benzo[*a*]pyrene-induced carcinogenicity (Shimizu *et al.*, 2000). Benzo[*a*]pyrene induced oxidative stress in mouse lung (Rajendran *et al.*, 2008).

4.4.4 Immunosuppression mechanism

Benzo[*a*]pyrene induces immunosupression in adult mice by altering the cell-mediated responses (Wojdani & Alfred, 1984). Immune development in offspring is also altered following *in utero* exposure to benzo[*a*]pyrene (Urso & Gengozian, 1984). It is postulated that PAHs, including benzo[*a*]pyrene, act principally through their AhR-mediated CYP-derived metabolites (diolepoxides, quinones) to activate oxidative and electrophilic signalling pathways in lymphoid and nonlymphoid cells, including myeloid cells, epithelial cells, and other cell types. Furthermore, there is evidence that PAHs suppress immunity by p53-dependent pathways, by modulating signalling pathways in lymphocytes via non-genotoxic mechanisms, and by oxidative stress (Burchiel & Luster, 2001).

4.4.5 Epigenetic mechanisms

Benzo[*a*]pyrene and/or its metabolites have been shown to increase cell proliferation in several human cell lines, including terminally differentiated human bronchial squamous epithelial cells and in lung-cancer cells where increased expression of the *Cdc25B* gene (cell-division cycle 25B) was observed, along with reduced phosphorylation of Cdk1 (cyclin-dependent kinase 1) (Oguri *et al.*, 2003). Treatment with benzo[*a*]pyrene increased the number of human embryo lung-fibroblasts in the G1–S transition via the activation of c-Jun, through the p53-dependent PI-3K/Akt/ERK (phosphatidylinositol-3-kinase/protein kinase β/extracellular signal-regulated kinase) pathway (Jiao *et al.*, 2008).

Benzo[*a*]pyrene and/or its metabolites also affect apoptosis. Benzo[*a*]pyrene induced apoptosis in human MRC-5 lung fibroblasts via the JNK1/FasL (c-Jun N-terminal kinase 1/Fas Ligand) and JNK1/p53 signalling pathways (Chen *et al.*, 2005). Apoptosis induced by *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide in H460 human lung-cancer cells was associated with induction of Bak (BCL2-antagonist/killer) and with activation of caspase, but it was independent of Bcl-2 (Xiao *et al.*, 2007).

Altered DNA methylation has been reported to be associated with exposure to benzo[*a*]pyrene and/or its metabolites. After treatment of immortalized bronchial epithelial cells with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide, the concentration of cytosine-DNA methyltransferase-1 was increased and was associated with hypermethylation of the promoters of 5–10 genes, including members of the cadherin gene-family (Damiani *et al.*, 2008).

4.5 Human exposure to PAH-rich mixtures

4.5.1 Biomarkers of exposure and effect

Molecular-epidemiological studies of cancer associated with occupational and environmental exposures to PAH have provided biomarkers that may be used to estimate internal exposure as well as to inform about molecular mechanisms that may be relevant to cancer causation, particularly in defining the early events in the carcinogenesis process. Biomarkers can be detected in the target organ, in surrogate tissues, or in tumours. These can be categorized into *biomarkers of exposure*, which are generally specific to the PAH of concern (e.g. DNA or protein adducts), *biomarkers of effect* (e.g. genotoxic and cytogenetic effects, 8-oxo-deoxyguanosine, sister chromatid exchange (SCE), micronuclei, chromosomal aberrations, mutations in oncogenes, tumour-suppressor genes, or indicator genes),

and *biomarkers of susceptibility* (DNA-repair enzymes, e.g. XPA, XPC – *xeroderma pigmentosum* complementation groups A and C), bioactivation enzymes (e.g. CYPs), detoxification enzymes (e.g. GSTs), and mutagenic metabolites in urine (Kalina *et al.*, 1998; Pilger *et al.*, 2000; Simioli *et al.*, 2004; Raimondi *et al.*, 2005; Vineis & Husgafvel-Pursiainen, 2005; Matullo *et al.*, 2006; Farmer & Singh, 2008; Gyorffy *et al.*, 2008). Although biomarkers of effect and susceptibility are generally not unique to any specific PAH exposure, several these biomarkers may provide insight into the mechanism of carcinogenesis induced in humans by PAHs or PAH-rich exposures.

4.5.2 Exposure to benzo[a]pyrene and relationship with specific biomarkers

Biomarkers of exposure to complex mixtures that contain benzo[*a*]pyrene have been studied in populations exposed in industrial settings: coke production, coal-tar distillation, the aluminium industry, roofing and paving with coal-tar pitch, coal gasification, chimney sweeping, and iron and steel founding. Most if not all of these biomarkers are genotoxic markers. Populations of patients who undergo coal-tar therapy and groups exposed to combustion emissions, and tobacco smokers have also been evaluated. Studies on biomarkers of exposure are dominated by those focusing on the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct, the most commonly studied PAH-DNA adduct because of the availability of specific analytical methods and standards (Gyorffy *et al.*, 2008). In one study the depurinating adducts resulting from radical-cation formation, *viz.* 7-(benzo[*a*]pyrene-6-yl) guanine and 7-(benzo[*a*]pyrene-6-yl)adenine were found in the urine of women exposed to coal smoke (Casale *et al.*, 2001). Concomitantly, several biomarkers of effect have also been evaluated in these studies: chromosomal aberrations, sister chromatid exchange (Kalina *et al.*, 1998), DNA damage (measured by the comet assay) and 8-oxo-deoxyguanosine formation (Marczynski *et al.*, 2002). It is important to note that these genotoxic effects observed in humans in relation to exposure to benzo[*a*]pyrene-containing mixtures have also been observed in experimental studies where benzo[*a*]pyrene or *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide has been shown to induce sister chromatid exchange (Pal *et al.*, 1980; Brauze *et al.*, 1997), chromosomal aberrations, micronuclei (Kliesch *et al.*, 1982), DNA damage (Nesnow *et al.*, 2002), and 8-oxo-deoxyguanosine (Thaiparambil *et al.*, 2007). Tobacco smoke, dietary habits and indoor ambient air are also important sources of exposure to benzo[*a*]pyrene, which has been implicated as one of the components of tobacco smoke related to the induction of lung cancer in smokers (Watanabe *et al.*, 2009). In a large study of 585 smokers and nonsmokers, smoking and diet were highly correlated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct levels (Pavanello *et al.*, 2006). Several studies have demonstrated moderately increased levels of 8-oxo-deoxyguanosine from lungs, sperm, and leukocytes of smokers. Increased urinary excretion of 8-oxo-deoxyguanosine has also been reported (Hecht, 1999). In rats exposed to benzo[*a*]pyrene via oral, intratracheal and dermal routes, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts were formed in white blood cells independently of the exposure route and their numbers correlated with those found in lung DNA, suggesting that *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct levels in white blood cells may be used as a surrogate for pulmonary *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts (Godschalk *et al.*, 2000).

4.5.3 Relationship of biomarkers to human cancer

Mutations in *TP53* are common in lung cancers from smokers and less common in nonsmokers. These mutations are G→T transversions with hotspots in codons 157, 248 and 273 (Hainaut & Pfeifer, 2001; Pfeifer *et al.*, 2002) and they are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts. The active metabolite *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide causes a unique spectrum of *TP53* mutations distinct from those found in cancers that are not associated with smoking (Campling & el-Deiry, 2003). Similar G→T mutations have been reported in lung tumours from nonsmoking Chinese women whose tumours were associated with exposure to PAHs from smoke generated by burning smoky coal in unventilated homes. The mutations were clustered at the CpG rich codons 153–158 of the *TP53* gene, and at codons 249 and 273. The mutation spectrum was fully consistent with exposure to PAHs (DeMarini *et al.*, 2001).

4.6 Synthesis

Benzo[*a*]pyrene is metabolically activated to a series of reactive intermediates by CYP450 and related enzymes under control of the aryl-hydrocarbon receptor. There is strong evidence that the benzo[*a*]pyrene diolepoxide mechanism operates in mouse-lung tumorigenesis, while there is also strong evidence that both the radical-cation and the diolepoxide mechanisms are involved in mouse-skin carcinogenesis. The meso-region mechanism has been studied only in rat liver, while the mechanism that involves the formation of *ortho*-quinone/reactive oxygen species has only been studied *in vitro*, although reactive oxygen species can be formed *in vivo* by other benzo[*a*]pyrene-mediated mechanisms. All these pathways reflect genotoxic mechanisms, as they involve alterations to DNA. Benzo[*a*]pyrene is pleotropic and has the ability to affect many cell- and organ-based systems. Therefore, there are probably many modes of carcinogenic action operating to different extents *in vivo*. These include mechanisms that involve AhR, oxidative stress, immunotoxicity and epigenetic events.

Based on the best available, consistent and strong experimental and human mechanistic evidence it is concluded that benzo[*a*]pyrene contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAH mixtures that contain benzo[*a*]pyrene. The most commonly encountered – and most widely studied – mechanistically relevant DNA lesion is the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct. The formation of this adduct is consistent with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-associated genotoxic effects in surrogate tissues and with the mutation pattern in the *TP53* gene in lung tumours from humans exposed to PAH mixtures that contain benzo[*a*]pyrene. The fact that those PAH mixtures and benzo[*a*]pyrene itself induce genotoxic effects like sister chromatid exchange, chromosomal aberrations, micronuclei, DNA damage (comet assay) and 8-oxo-deoxyguanosine, supports the notion that benzo[*a*]pyrene contributes to human cancer.

5. Evaluation

There is *sufficient evidence* for the carcinogenicity of benzo[*a*]pyrene in experimental animals.

[No epidemiological data on benzo[*a*]pyrene alone were available to the Working Group.]

The genotoxic mechanism of action of benzo[*a*]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These anti-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts induce mutations in the K-*RAS* oncogene and the *TP53* tumour-suppressor gene in human lung tumours, and

in corresponding genes in mouse-lung tumours. Exposure to benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures also induce other genotoxic effects, including sister chromatid exchange, micronuclei, DNA damage and 8-oxo-deoxyguanosine, all of which can contribute to the carcinogenic effects of benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures in exposed humans.

Benzo[*a*]pyrene is *carcinogenic to humans (Group 1)*.

In making the overall evaluation, the Working Group took the following into consideration:

The strong and extensive experimental evidence for the carcinogenicity of benzo[*a*]pyrene in many animal species, supported by the consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification of benzo[*a*]pyrene as a human carcinogen (Group 1).

References

Albert RE, Miller ML, Cody T *et al.* (1991). Benzo[a]pyrene-induced skin damage and tumor promotion in the mouse. *Carcinogenesis*, 12: 1273–1280. doi:10.1093/carcin/12.7.1273 PMID:2070493

Anderson LM, Priest LJ, Deschner EE, Budinger JM (1983). Carcinogenic effects of intracolonic benzo[a]pyrene in β-naphthoflavone-induced mice. *Cancer Letters*, 20: 117–123. doi:10.1016/0304-3835(83)90039-3 PMID:6321017

Andrews J, Halliday GM, Muller HK (1991). A role for prostaglandins in the suppression of cutaneous cellular immunity and tumour development in benzo[a]pyrene- but not dimethylbenz(a)anthracene-treated mice. *Clin Exp Immunol*, 85: 9–13. PMID:1906386

Badary OA, Al-Shabanah OA, Nagi MN *et al.* (1999). Inhibition of benzo[a]pyrene-induced forestomach carcinogenesis in mice by thymoquinone. *European Journal of Cancer Prevention*, 8: 435–440. doi:10.1097/00008469-199910000-00009 PMID:10548399

Balansky R, Ganchev G, Iltcheva M *et al.* (2007). Potent carcinogenicity of cigarette smoke in mice exposed early in life. *Carcinogenesis*, 28: 2236–2243. doi:10.1093/carcin/bgm122 PMID:17522065

Balu N, Padgett WT, Nelson GB *et al.* (2006). Benzo[a]pyrene-7,8-quinone-3′-mononucleotide adduct standards for 32P postlabeling analyses: detection of benzo[a]pyrene-7,8-quinone-calf thymus DNA adducts. *Anal Biochem*, 355: 213–223. doi:10.1016/j.ab.2006.05.023 PMID:16797471

Banasiewicz M, Nelson G, Swank A *et al.* (2004). Identification and quantitation of benzo[a]pyrene-derived DNA adducts formed at low adduction level in mice lung tissue. *Anal Biochem*, 334: 390–400. doi:10.1016/j.ab.2004.08.006 PMID:15494147

Brauze D, Wielgosz SM, Pawlak AL, Baer-Dubowska W (1997). Effect of the route of benzo[a]pyrene administration on sister chromatid exchange and DNA binding in bone marrow of mice differing with respect to cytochrome P450 1A1 induction. *Toxicol Lett*, 91: 211–217. doi:10.1016/S0378-4274(97)00024-6 PMID:9217241

Briedé JJ, Godschalk RW, Emans MT *et al.* (2004). In vitro and in vivo studies on oxygen free radical and DNA adduct formation in rat lung and liver during benzo[a]pyrene metabolism. *Free Radic Res*, 38: 995–1002. doi:10.1080/10715760400000976 PMID:15621718

Burchiel SW & Luster MI (2001). Signalling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. *Clin Immunol*, 98: 2–10. doi:10.1006/clim.2000.4934 PMID:11141320

Busby WF Jr, Stevens EK, Martin CN *et al.* (1989). Comparative lung tumorigenicity of parent and mononitro-polynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. *Toxicology and Applied Pharmacology*, 99: 555–563. doi:10.1016/0041-008X(89)90162-2 PMID:2749740

Campling BG & el-Deiry WS (2003). Clinical implications of p53 mutations in lung cancer. *Methods Mol Med*, 75: 53–77. PMID:12407735

Casale GP, Singhal M, Bhattacharya S *et al.* (2001). Detection and quantification of depurinated benzo[a]pyrene-adducted DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem Res Toxicol*, 14: 192–201. doi:10.1021/tx000012y PMID:11258968

Cavalieri E, Mailander P, Pelfrene A (1977). Carcinogenic activity of anthanthrene on mouse skin. *Zeitschrift fur Krebsforschung*, 89: 113–118. PMID:143140 doi:10.1007/BF00308512

Cavalieri E, Rogan E, Cremonesi P *et al.* (1988a). Tumorigenicity of 6-halogenated derivatives of benzo[a]pyrene in mouse skin and rat mammary gland. *Journal of Cancer Research and Clinical Oncology*, 114: 10–15. doi:10.1007/BF00390479 PMID:3350835

Cavalieri E, Rogan E, Sinha D (1988b). Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. *J. Cancer clin. Oncol*, 114: 3–9. PMID:3350839.

Cavalieri EL, Higginbotham S, RamaKrishna NVS *et al.* (1991). Comparative dose–response

tumorigenicity studies of dibenzo[a,l]pyrene versus 7,12-dimethylbenz[a]anthracene, benzo[a]pyrene and two dibenzo[a,l]pyrene dihydrols in mouse skin and rat mammary gland. *Carcinogenesis*, 12: 1939–1944. doi:10.1093/carcin/12.10.1939 PMID:1934274

Cavalieri EL & Rogan EG (1995). Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica*, 25: 677–688. doi:10.3109/00498259509061885 PMID:7483666

Chakravarti D, Venugopal D, Mailander PC *et al.* (2008). The role of polycyclic aromatic hydrocarbon-DNA adducts in inducing mutations in mouse skin. *Mutat Res*, 649: 161–178. PMID:17931959

Chen JH, Chou FP, Lin HH, Wang CJ (2005). Gaseous nitrogen oxide repressed benzo[a]pyrene-induced human lung fibroblast cell apoptosis via inhibiting JNK1 signals. *Arch Toxicol*, 79: 694–704. doi:10.1007/s00204-005-0001-0 PMID:16041517

Culp SJ, Gaylor DW, Sheldon WG *et al.* (1998). A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. *Carcinogenesis*, 19: 117–124. doi:10.1093/carcin/19.1.117 PMID:9472702

Damiani LA, Yingling CM, Leng S *et al.* (2008). Carcinogen-induced gene promoter hypermethylation is mediated by DNMT1 and causal for transformation of immortalized bronchial epithelial cells. *Cancer Res*, 68: 9005–9014. doi:10.1158/0008-5472.CAN-08-1276 PMID:18974146

de Vries A, van Oostrom CTM, Dortant PM *et al.* (1997). Spontaneous liver tumors and benzo[a]pyrene-induced lymphomas in XPA-deficient mice. *Molecular Carcinogenesis*, 19: 46–53. doi:10.1002/(SICI)1098-2744(199705)19:1<46::AID-MC7>3.0.CO;2-L PMID:9180928

DeMarini DM, Landi S, Tian D *et al.* (2001). Lung tumor KRAS and TP53 mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. *Cancer Res*, 61: 6679–6681. PMID:11559534

Denissenko MF, Pao A, Tang M, Pfeifer GP (1996). Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science*, 274: 430–432. doi:10.1126/science.274.5286.430 PMID:8832894

Deutsch-Wenzel RP, Brune H, Grimmer G *et al.* (1983). Experimental studies in rat lungs on the carcinogenicity and dose-response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. *J Natl Cancer Inst*, 71: 539–544. PMID:6577228

el-Bayoumy K, Chae Y-H, Upadhyaya P *et al.* (1995). Comparative tumorigenicity of benzo[a]pyrene, 1-nitropyrene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine administered by gavage to female CD rats. *Carcinogenesis*, 16: 431–434. doi:10.1093/carcin/16.2.431 PMID:7859378

Eling T, Curtis J, Battista J, Marnett LJ (1986). Oxidation of (+)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by mouse keratinocytes: evidence for peroxyl radical- and monoxygenase-dependent metabolism. *Carcinogenesis*, 7: 1957–1963. doi:10.1093/carcin/7.12.1957 PMID:2430728

Estensen RD, Jordan MM, Wiedmann TS *et al.* (2004). Effect of chemopreventive agents on separate stages of progression of benzo[alpha]pyrene induced lung tumors in A/J mice. *Carcinogenesis*, 25: 197–201. doi:10.1093/carcin/bgg196 PMID:14578161

Estensen RD & Wattenberg LW (1993). Studies of chemopreventive effects of myo-inositol on benzo[a]pyrene-induced neoplasia of the lung and forestomach of female A/J mice. *Carcinogenesis*, 14: 1975–1977. doi:10.1093/carcin/14.9.1975 PMID:8403228

Farmer PB & Singh R (2008). Use of DNA adducts to identify human health risk from exposure to hazardous environmental pollutants: the increasing role of mass spectrometry in assessing biologically effective doses of genotoxic carcinogens. *Mutat Res*, 659: 68–76. doi:10.1016/j.mrrev.2008.03.006 PMID:18468947

Feron VJ (1972). Respiratory tract tumors in hamsters after intratracheal instillations of benzo(a)pyrene alone and with furfural. *Cancer Res*, 32: 28–36. PMID:5007686

Feron VJ, de Jong D, Emmelot P (1973). Letter: Dose-response correlation for the induction of respiratory-tract tumours in Syrian golden hamsters by intratracheal instillations of benzo(a)pyrene. *Eur J Cancer*, 9: 387–390. doi:10.1016/0014-2964(73)90057-1 PMID:4746737

Feron VJ & Kruysse A (1978). Effects of exposure to furfural vapour in hamsters simultaneously treated with benzo[alpha] pyrene or diethylnitrosamine. *Toxicology*, 11: 127–144. doi:10.1016/S0300-483X(78)90889-2 PMID:715798

Feron VJ, van den Heuvel PD, Koëter HB, Beems RB (1980). Significance of particle size of benzo(a)pyrene for the induction of respiratory tract tumours in hamsters. *Int J Cancer*, 25: 301–307. doi:10.1002/ijc.2910250220 PMID:7390654

Flesher JW, Horn J, Lehner AF (1997). 6-sulfooxymethylbenzo[a]pyrene is an ultimate electrophilic and carcinogenic form of the intermediary metabolite 6-hydroxymethylbenzo[a]pyrene. *Biochem Biophys Res Commun*, 234: 554–558. doi:10.1006/bbrc.1997.6679 PMID:9175750

Flesher JW, Stansbury KH, Sydnor KL (1982). S-Adenosyl-L-methionine is a carbon donor in the conversion of benzo[alpha]pyrene to 6-hydroxymethylbenzo[alpha]pyrene by rat liver S-9. *Cancer Lett*, 16: 91–94. doi:10.1016/0304-3835(82)90095-7 PMID:6288234

Godleski JJ, Melnicoff MJ, Sadri S, Garbeil P (1984). Effects of inhaled ammonium sulfate on benzo[a]pyrene carcinogenesis. *J Toxicol Environ Health*, 14: 225–238. doi:10.1080/15287398409530575 PMID:6502734

Godschalk RW, Moonen EJ, Schilderman PA *et al.* (2000). Exposure-route-dependent DNA adduct formation by

polycyclic aromatic hydrocarbons. *Carcinogenesis*, 21: 87–92. doi:10.1093/carcin/21.1.87 PMID:10607738

Gyorffy E, Anna L, Kovács K *et al.* (2008). Correlation between biomarkers of human exposure to genotoxins with focus on carcinogen-DNA adducts. *Mutagenesis*, 23: 1–18. doi:10.1093/mutage/gem043 PMID:17989146

Habs M, Jahn SAA, Schmähl D (1984). Carcinogenic activity of condensate from coloquint seeds (Citrullus colocynthis) after chronic epicutaneous administration to mice. *J Cancer Res Clin Oncol*, 108: 154–156. doi:10.1007/BF00390988 PMID:6746706

Habs M, Schmähl D, Misfeld J (1980). Local carcinogenicity os some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. *Arch Geschwulstforsch*, 50: 266–274. PMID:7436704

Hainaut P & Pfeifer GP (2001). Patterns of p53 G–>T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis*, 22: 367–374. doi:10.1093/carcin/22.3.367 PMID:11238174

Hakura A, Tsutsui Y, Sonoda J *et al.* (1998). Comparison between in vivo mutagenicity and carcinogenicity in multiple organs by benzo[a]pyrene in the lacZ transgenic mouse (Muta Mouse). *Mutat Res*, 398: 123–130. PMID:9626972

Hecht SS (1999). Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst*, 91: 1194–1210. doi:10.1093/jnci/91.14.1194 PMID:10413421

Heinrich U, Pott F, Mohr U *et al.* (1986a). Lung tumours in rats and mice after inhalation of PAH-rich emissions. *Exp Pathol*, 29: 29–34. PMID:3699126

Henry MC, Port CD, Bates RR, Kaufman DG (1973). Respiratory tract tumors in hamsters induced by benzo(a)pyrene. *Cancer Res*, 33: 1585–1592. PMID:4721222

Homburger F, Hsueh S-S, Kerr CS, Russfield AB (1972). Inherited susceptibility of inbred strains of Syrian hamsters to induction of subcutaneous sarcomas and mammary and gastrointestinal carcinomas by subcutaneous and gastric administration of polynuclear hydrocarbons. *Cancer Res*, 32: 360–366. PMID:5058191

Hoogervorst EM, de Vries A, Beems RB *et al.* (2003). Combined oral benzo[a]pyrene and inhalatory ozone exposure have no effect on lung tumor development in DNA repair-deficient Xpa mice. *Carcinogenesis*, 24: 613–619. doi:10.1093/carcin/24.3.613 PMID:12663525

Horikawa K, Sera N, Otofuji T *et al.* (1991). Pulmonary carcinogenicity of 3,9- and 3,7-dinitrofluoranthene, 3-nitrofluoranthene and benzo[a]pyrene in F344 rats. *Carcinogenesis*, 12: 1003–1007. doi:10.1093/carcin/12.6.1003 PMID:2044179

IARC (1973). Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. *IARC Monogr Eval Carcinog Risk Chem Man*, 3: 1–271.

IARC (1983). Polynuclear aromatic compounds, Part 1, chemical, environmental and experimental data. *IARC Monogr Eval Carcinog Risk Chem Hum*, 32: 1–453. PMID:6586639

IARC (1984). Polynuclear aromatic compounds, Part 3, industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding. *IARC Monogr Eval Carcinog Risk Chem Hum*, 34: 1–219.

IARC (1985). Polynuclear aromatic compounds, Part 4, bitumens, coal-tars and derived products, shale-oils and soots. *IARC Monogr Eval Carcinog Risk Chem Hum*, 35: 1–247. PMID:2991123

IARC (1986). Tobacco smoking. *IARC Monogr Eval Carcinog Risk Chem Hum*, 38: 1–421.

IARC (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. *IARC Monogr Eval Carcinog Risks Hum*, 92: 1–853. PMID:21141735 PMID:18756632

Ide F, Iida N, Nakatsuru Y *et al.* (2000). Mice deficient in the nucleotide excision repair gene XPA have elevated sensitivity to benzo[a]pyrene induction of lung tumors. *Carcinogenesis*, 21: 1263–1265. doi:10.1093/carcin/21.6.1263 PMID:10837020

Iwagawa M, Maeda T, Izumi K *et al.* (1989). Comparative dose-response study on the pulmonary carcinogenicity of 1,6-dinitropyrene and benzo[a]pyrene in F344 rats. *Carcinogenesis*, 10: 1285–1290. doi:10.1093/carcin/10.7.1285 PMID:2736719

Jeffrey AM, Weinstein IB, Jennette KW *et al.* (1977). Structures of benzo(a)pyrene–nucleic acid adducts formed in human and bovine bronchial explants. *Nature*, 269: 348–350. doi:10.1038/269348a0 PMID:904688

Jiao S, Liu B, Gao A *et al.* (2008). Benzo(a)pyrene-caused increased G1-S transition requires the activation of c-Jun through p53-dependent PI-3K/Akt/ERK pathway in human embryo lung fibroblasts. *Toxicol Lett*, 178: 167–175. doi:10.1016/j.toxlet.2008.03.012 PMID:18448277

Joseph P & Jaiswal AK (1998). NAD(P)H:quinone oxidoreductase 1 reduces the mutagenicity of DNA caused by NADPH:P450 reductase-activated metabolites of benzo(a)pyrene quinones. *Br J Cancer*, 77: 709–719. PMID:9514048

Kalina I, Brezáni P, Gajdosová D *et al.* (1998). Cytogenetic monitoring in coke oven workers. *Mutat Res*, 417: 9–17. PMID:9729241

Ketkar M, Green U, Schneider P, Mohr U (1979). Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo[a]pyrene in a mixture of Tris buffer and saline in Syrian golden hamsters. *Cancer Lett*, 6: 279–284. doi:10.1016/S0304-3835(79)80046-4 PMID:436122

Ketkar M, Reznik G, Misfeld J, Mohr U (1977). Investigations on the carcinogenic burden by air pollution in man. The effect of a single dose of benzo(a)

pyrene in Syrian golden hamsters. *Cancer Lett*, 3: 231–235. doi:10.1016/S0304-3835(77)95980-8

Ketkar M, Reznik G, Schneider P, Mohr U (1978). Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo(a)pyrene in bovine serum albumin in Syrian hamsters. *Cancer Lett*, 4: 235–239. doi:10.1016/S0304-3835(78)94787-0 PMID:647664

Kliesch U, Roupova I, Adler ID (1982). Induction of chromosome damage in mouse bone marrow by benzo[a] pyrene. *Mutat Res*, 102: 265–273. doi:10.1016/0165-1218(82)90136-7 PMID:7144782

Kobayashi N (1975). Production of respiratory tract tumors in hamsters by benzo(a)pyrene. *Gann*, 66: 311–315. PMID:1181231

Kouri RE, Wood AW, Levin W *et al.* (1980). Carcinogenicity of benzo[a]pyrene and thirteen of its derivatives in C3H/fCum mice. *J Natl Cancer Inst*, 64: 617–623. PMID:6766516

Kroese ED, Dortant PM, van Steeg H *et al.* (1997). Use of E μ-PIM-1 transgenic mice short-term in vivo carcinogenicity testing: lymphoma induction by benzo[a] pyrene, but not by TPA. *Carcinogenesis*, 18: 975–980. doi:10.1093/carcin/18.5.975 PMID:9163683

Kruysse A & Feron VJ (1976). Repeated exposure to cyclopentenone vapour: long-term study in Syrian golden hamsters *Zentralbl Bakteriol*, [Orig.B]163: 5–6448–457. . PMID:1020535.

Lavoie EJ, Braley J, Rice JE, Rivenson A (1987). Tumorigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. *Cancer Letters*, 34: 15–20. doi:10.1016/0304-3835(87)90068-1 PMID:3802065

Levin W, Wood AW, Wislocki PG *et al.* (1977). Carcinogenicity of benzo ring derivatives of benzo[a] pyrene on mouse skin. *Cancer Research*, 37: 3357–3361. PMID:884679.

Mangal D, Vudathala D, Park JH *et al.* (2009). Analysis of 7,8-dihydro-8-oxo-2′-deoxyguanosine in cellular DNA during oxidative stress. *Chem Res Toxicol*, 22: 788–797. doi:10.1021/tx800343c PMID:19309085

Marczynski B, Rihs HP, Rossbach B *et al.* (2002). Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. *Carcinogenesis*, 23: 273–281. doi:10.1093/carcin/23.2.273 PMID:11872632

Mass MJ, Jeffers AJ, Ross JA *et al.* (1993). Ki-ras oncogene mutations in tumors and DNA adducts formed by benz[j]aceanthrylene and benzo[a]pyrene in the lungs of strain A/J mice. *Molecular Carcinogenesis*, 8: 186–192. doi:10.1002/mc.2940080309 PMID:8216737

Matullo G, Dunning AM, Guarrera S *et al.* (2006). DNA repair polymorphisms and cancer risk in non-smokers in a cohort study. *Carcinogenesis*, 27: 997–1007. doi:10.1093/carcin/bgi280 PMID:16308313

McCoull KD, Rindgen D, Blair IA, Penning TM (1999). Synthesis and characterization of polycyclic aromatic hydrocarbon o-quinone depurinating N7-guanine adducts. *Chem Res Toxicol*, 12: 237–246. doi:10.1021/tx980182z PMID:10077486

Melendez-Colon VJ, Luch A, Seidel A, Baird WM (1999). Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. *Carcinogenesis*, 20: 1885–1891. doi:10.1093/carcin/20.10.1885 PMID:10506100

Näslund I, Rubio CA, Auer GU (1987). Nuclear DNA changes during pathogenesis of squamous cell carcinoma of the cervix in 3,4-benzopyrene-treated mice. *Analytical and Quantitative Cytology*, 9: 411–418. PMID: 3675800.

Nebert DW, Roe AL, Dieter MZ *et al.* (2000). Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol*, 59: 65–85. doi:10.1016/S0006-2952(99)00310-X PMID:10605936

Nesnow S, Davis C, Nelson GB *et al.* (2002). Comparison of the genotoxic activities of the K-region dihydrodiol of benzo[a]pyrene with benzo[a]pyrene in mammalian cells: morphological cell transformation; DNA damage; and stable covalent DNA adducts. *Mutat Res*, 521: 91–102. PMID:12438007

Nesnow S, Ross JA, Stoner GD, Mass MJ (1995). Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. *Toxicology*, 105: 403–413. doi:10.1016/0300-483X(95)03238-B PMID:8571376

Nettesheim P, Griesemer RA, Martin DH, Caton JE Jr (1977). Induction of preneoplastic and neoplastic lesions in grafted rat tracheas continuously exposed to benzo[a]pyrene. *Cancer Research*, 37: 1271–1278. PMID: 856459.

Oguri T, Singh SV, Nemoto K, Lazo JS (2003). The carcinogen (7R,8S)-dihydroxy-(9S,10R)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene induces Cdc25B expression in human bronchial and lung cancer cells. *Cancer Res*, 63: 771–775. PMID:12591724

Osborne MR, Crosby NT (1987). *Benzopyrenes*. Cambridge Monographs on Cancer Research. Cambridge, England: Cambridge University Press

Pal K, Grover PL, Sims P (1980). The induction of sister-chromatid exchanges in Chinese hamster ovary cells by some epoxides and phenolic derivatives of benzo[a] pyrene. *Mutat Res*, 78: 193–199. doi:10.1016/0165-1218(80)90098-1 PMID:7393246

Park JH, Mangal D, Tacka KA *et al.* (2008). Evidence for the aldo-keto reductase pathway of polycyclic aromatic trans-dihydrodiol activation in human lung A549 cells. *Proc Natl Acad Sci U S A*, 105: 6846–6851. doi:10.1073/pnas.0802776105 PMID:18474869

Pavanello S, Pulliero A, Saia BO, Clonfero E (2006). Determinants of anti-benzo[a]pyrene diol epoxide-DNA adduct formation in lymphomonocytes of the general population. *Mutat Res*, 611: 54–63. PMID:16978913

Penning TM, Burczynski ME, Hung CF *et al.* (1999). Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. *Chem Res Toxicol*, 12: 1–18. doi:10.1021/tx980143n PMID:9894013

Penning TM & Drury JE (2007). Human aldo-keto reductases: Function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys*, 464: 241–250. doi:10.1016/j.abb.2007.04.024 PMID:17537398

Pfeifer GP, Denissenko MF, Olivier M *et al.* (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene*, 21: 7435–7451. doi:10.1038/sj.onc.1205803 PMID:12379884

Pilger A, Germadnik D, Schaffer A *et al.* (2000). 8-Hydroxydeoxyguanosine in leukocyte DNA and urine of quartz-exposed workers and patients with silicosis. *Int Arch Occup Environ Health*, 73: 305–310. doi:10.1007/s004200000117 PMID:10963413

Pott F, Brockhaus A, Huth F (1973a[Tests on the production of tumours in animal experiments with polycyclic aromatic hydrocarbons.] [in German]). *Zbl. Bakt. Hyg. Abt. Orig. B*, 157: 34–43.

Pott F, Tomingas R, Reiffer FJ (1973b). Experimental studies on the carcinogenicity and the retention of benzo[a]pyrene in application region after intratracheal and subcutaneous injection. *Zbl. Bakt. Hyg. I.Abt. Orig. B*, 158: 97–108.

Pott F, Ziem U, Reiffer F-J *et al.* (1987). Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. *Exp Pathol*, 32: 129–152. PMID:3436395

Raimondi S, Boffetta P, Anttila S *et al.* (2005). Metabolic gene polymorphisms and lung cancer risk in non-smokers. An update of the GSEC study. *Mutat Res*, 592: 45–57. PMID:16009381

Rajendran P, Ekambaram G, Sakthisekaran D (2008). Cytoprotective effect of mangiferin on benzo(a) pyrene-induced lung carcinogenesis in swiss albino mice. *Basic Clin Pharmacol Toxicol*, 103: 137–142. doi:10.1111/j.1742-7843.2008.00254.x PMID:18816296

Reynders JB, Immel HR, Scherrenberg PM *et al.* (1985). Respiratory tract tumors in hamsters after severe focal injury to the trachea and intratracheal instillation of benzo[a]pyrene. *Cancer Lett*, 29: 93–99. doi:10.1016/0304-3835(85)90128-4 PMID:4063958

Rippe RM, Pott D (1989). *Kanzerogenitätsuntersuchungen von Nitro-PAH (Nitroarenen) im Hinblick auf ihre Bedeutung für die krebserzeugende Wirkung von Dieselmotorabgas*. In: *Gesellschaft zur Förderung der Lufthygiene und Silikoseforschung*. Düsseldorf: Stefan W. Albers, pp. 65–89.

Rodriguez LV, Dunsford HA, Steinberg M *et al.* (1997). Carcinogenicity of benzo[a]pyrene and manufactured gas plant residues in infant mice. *Carcinogenesis*, 18: 127–135. doi:10.1093/carcin/18.1.127 PMID:9054599

Rogan EG, RamaKrishna NVS, Higginbotham S *et al.* (1990). Identification and quantitation of 7-(benzo[a] pyren-6-yl)guanine in the urine and feces of rats treated with benzo[a]pyrene. *Chem Res Toxicol*, 3: 441–444. doi:10.1021/tx00017a009 PMID:2133095

Roller M, Kamino K, Rosenbruch M (1992). *Carcinogenicity testing of bladder carcinogens and other organic compounds by the intraperitoneal and intravesicular route*. In: *Environmental Hygiene III*. Seemayer NH, Hadnagy W, editors. Berlin: Springer-Verlag, pp. 95–98.

Ross JA, Nelson GB, Wilson KH *et al.* (1995). Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res*, 55: 1039–1044. PMID:7866986

Rossi L, Barbieri O, Sanguineti M *et al.* (1983). Carcinogenic activity of benzo[a]pyrene and some of its synthetic derivatives by direct injection into the mouse fetus. *Carcinogenesis*, 4: 153–156. doi:10.1093/carcin/4.2.153 PMID:6297822

Ruggeri B, DiRado M, Zhang SY *et al.* (1993). Benzo[a] pyrene-induced murine skin tumors exhibit frequent and characteristic G to T mutations in the p53 gene. *Proc Natl Acad Sci U S A*, 90: 1013–1017. doi:10.1073/pnas.90.3.1013 PMID:8430068

Saffiotti U, Montesano R, Sellakumar AR *et al.* (1972). Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[a] pyrene and ferric oxide. *Cancer Res*, 32: 1073–1081. PMID:4336025

Sellakumar A, Stenbäck F, Rowland J (1976). Effects of different dusts on respiratory carcinogenesis in hamsters induced by benzo (a) pyrene and diethylnitrosamine. *Eur J Cancer*, 12: 313–319. doi:10.1016/0014-2964(76)90112-2 PMID:954792

Sellakumar AR, Montesano R, Saffiotti U, Kaufman DG (1973). Hamster respiratory carcinogenesis induced by benzo[a]pyrene and different dose levels of ferric oxide. *J Natl Cancer Inst*, 50: 507–510. PMID:4702121

Senft AP, Dalton TP, Nebert DW *et al.* (2002). Mitochondrial reactive oxygen production is dependent on the aromatic hydrocarbon receptor. *Free Radic Biol Med*, 33: 1268–1278. doi:10.1016/S0891-5849(02)01014-6 PMID:12398935

Shen YM, Troxel AB, Vedantam S *et al.* (2006). Comparison of p53 mutations induced by PAH o-quinones with those caused by anti-benzo[a]pyrene diol epoxide in vitro: role of reactive oxygen and biological selection. *Chem Res Toxicol*, 19: 1441–1450. doi:10.1021/tx0601206 PMID:17112231

Shimada T (2006). Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab*

Pharmacokinet, 21: 257–276. doi:10.2133/dmpk.21.257 PMID:16946553

Shimizu Y, Nakatsuru Y, Ichinose M *et al.* (2000). Benzo[a] pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 97: 779–782. doi:10.1073/pnas.97.2.779 PMID:10639156

Simioli P, Lupi S, Gregorio P *et al.* (2004). Non-smoking coke oven workers show an occupational PAH exposure-related increase in urinary mutagens. *Mutat Res*, 562: 103–110. PMID:15279833

Smith DM, Rogers AE, Herndon BJ, Newberne PM (1975a). Vitamin A (retinyl acetate) and benzo[a]pyrene-induced carcinogenesis in hamsters fed a commercial diet. *Cancer Res.*, 35: 11–16. PMID:162856

Smith DM, Rogers AE, Newberne PM (1975b). Vitamin A and benzo[a]pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. *Cancer Res*, 35: 1485–1488. PMID:1131819

Solt DB, Polverini PJ, Calderon L (1987). Carcinogenic response of hamster buccal pouch epithelium to 4 polycyclic aromatic hydrocarbons. *Journal of Oral Pathology*, 16: 294–302. doi:10.1111/j.1600-0714.1987.tb00697.x PMID:2445943

Sparnins VL, Mott AW, Barany G, Wattenberg LW (1986). Effects of allyl methyl trisulfide on glutathione S-transferase activity and BP-induced neoplasia in the mouse. *Nutrition and Cancer*, 8: 211–215. doi:10.1080/01635588609513895 PMID:3737423

Stansbury KH, Flesher JW, Gupta RC (1994). Mechanism of aralkyl-DNA adduct formation from benzo[a]pyrene in vivo. *Chem Res Toxicol*, 7: 254–259. doi:10.1021/tx00038a019 PMID:8199315

Steinhoff D, Mohr U, Hahnemann S (1991). Carcinogenesis studies with iron oxides. *Exp Pathol*, 43: 189–194. PMID:1797572

Stenbäck F & Rowland J (1978). Role of particle size in the formation of respiratory tract tumors induced by benzo(a)pyrene. *Eur J Cancer*, 14: 321–326. doi:10.1016/0014-2964(78)90200-1 PMID:656185

Stenbäck F & Rowland J (1979). Experimental respiratory carcinogenesis in hamsters: environmental, physicochemical and biological aspects. *Oncology*, 36: 63–71. doi:10.1159/000225320 PMID:223099

Stenbäck F, Sellakumar A, Shubik P (1975). Magnesium oxide as carrier dust in benzo(a)pyrene-induced lung carcino-genesis in Syrian hamsters. *J Natl Cancer Inst*, 54: 861–867. PMID:1127716

Surh YJ, Liem A, Miller EC, Miller JA (1989). Metabolic activation of the carcinogen 6-hydroxymethylbenzo[a] pyrene: formation of an electrophilic sulfuric acid ester and benzylic DNA adducts in rat liver in vivo and in reactions in vitro. *Carcinogenesis*, 10: 1519–1528. doi:10.1093/carcin/10.8.1519 PMID:2752526

Thaiparambil JT, Vadhanam MV, Srinivasan C *et al.* (2007). Time-dependent formation of 8-oxo-deoxyguanosine in the lungs of mice exposed to cigarette smoke. *Chem Res Toxicol*, 20: 1737–1740. doi:10.1021/tx700289g PMID:18031018

Thyssen J, Althoff J, Kimmerle G, Mohr U (1981). Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. *J Natl Cancer Inst*, 66: 575–577. PMID:6937711

Toth B (1980). Tumorigenesis by benzo[a]pyrene administered intracolonically. *Oncology*, 37: 77–82. doi:10.1159/000225408 PMID:7360483

Urso P & Gengozian N (1984). Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after in utero exposure to benzo[a]pyrene. *J Toxicol Environ Health*, 14: 569–584. doi:10.1080/15287398409530606 PMID:6239929

Van Duuren BL, Katz C, Goldschmidt BM (1973). Cocarcinogenic agents in tobacco carcinogenesis. *J Natl Cancer Inst*, 51: 703–705. PMID:4765384

van Oostrom CT, Boeve M, van Den Berg J *et al.* (1999). Effect of heterozygous loss of p53 on benzo[a]pyrene-induced mutations and tumors in DNA repair-deficient XPA mice. *Environ Mol Mutagen*, 34: 124–130. doi:10.1002/(SICI)1098-2280(1999)34:2/3<124::AID-EM11>3.0.CO;2-F PMID:10529736

Vesselinovitch SD, Kyriazis AP, Mihailovich N, Rao KVN (1975a). Factors influencing augmentation and/or acceleration of lymphoreticular tumors in mice by benzo[a]pyrene treatment. *Cancer Res*, 35: 1963–1969. PMID:1097103

Vesselinovitch SD, Kyriazis AP, Mihailovich N, Rao KVN (1975b). Conditions modifying development of tumors in mice at various sites by benzo[a]pyrene. *Cancer Res*, 35: 2948–2953. PMID:1182688

Vineis P & Husgafvel-Pursiainen K (2005). Air pollution and cancer: biomarker studies in human populations. *Carcinogenesis*, 26: 1846–1855. doi:10.1093/carcin/bgi216 PMID:16123121

Von Tungeln LS, Xia Q, Herreno-Saenz D *et al.* (1999). Tumorigenicity of nitropolycyclic aromatic hydrocarbons in the neonatal B6C3F1 mouse bioassay and characterization of ras mutations in liver tumors from treated mice. *Cancer Letters*, 146: 1–7. doi:10.1016/S0304-3835(99)00206-2 PMID:10656603

Warshawsky D & Barkley W (1987). Comparative carcinogenic potencies of 7H-dibenzo[c,g]carbazole, dibenz[a,j]acridine and benzo[a]pyrene in mouse skin. *Cancer Letters*, 37: 337–344. doi:10.1016/0304-3835(87)90119-4 PMID:3677065

Warshawsky D, Barkley W, Bingham E (1993). Factors affecting carcinogenic potential of mixtures. *Fundamental and Applied Toxicology*, 20: 376–382. doi:10.1006/faat.1993.1048 PMID:8504912

Watanabe KH, Djordjevic MV, Stellman SD *et al.* (2009). Incremental lifetime cancer risks computed for benzo[a] pyrene and two tobacco-specific N-nitrosamines in mainstream cigarette smoke compared with lung

cancer risks derived from epidemiologic data. *Regul Toxicol Pharmacol*, 55: 123–133. PMID:19540296

Wei SJ, Chang RL, Merkler KA *et al.* (1999). Dose-dependent mutation profile in the c-Ha-ras proto-oncogene of skin tumors in mice initiated with benzo[a] pyrene. *Carcinogenesis*, 20: 1689–1696. doi:10.1093/carcin/20.9.1689 PMID:10469612

Wenzel-Hartung R, Brune H, Grimmer G *et al.* (1990). Evaluation of the carcinogenic potency of 4 environmental polycyclic aromatic compounds following intrapulmonary application in rats. *Exp Pathol*, 40: 221–227. PMID:1711479

Weyand EH, Chen Y-C, Wu Y *et al.* (1995). Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[a]pyrene vs manufactured gas plant residue. *Chemical Research in Toxicology*, 8: 949–954. doi:10.1021/tx00049a008 PMID:8555410

Wijnhoven SWP, Kool HJM, van Oostrom CTM *et al.* (2000). The relationship between benzo[a]pyrene-induced mutagenesis and carcinogenesis in repair-deficient Cockayne syndrome group B mice. *Cancer Res*, 60: 5681–5687. PMID:11059760

Wislocki PG, Bagan ES, Lu AY *et al.* (1986). Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. *Carcinogenesis*, 7: 1317–1322. doi:10.1093/carcin/7.8.1317 PMID:3731386

Wojdani A & Alfred LJ (1984). Alterations in cell-mediated immune functions induced in mouse splenic lymphocytes by polycyclic aromatic hydrocarbons. *Cancer Res*, 44: 942–945. PMID:6420057

Xiao H, Rawal M, Hahm ER, Singh SV (2007). Benzo[a] pyrene-7,8-diol-9,10-epoxide causes caspase-mediated apoptosis in H460 human lung cancer cell line. *Cell Cycle*, 6: 2826–2834. PMID:17986867

Xue W & Warshawsky D (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicol Appl Pharmacol*, 206: 73–93. doi:10.1016/j.taap.2004.11.006 PMID:15963346

Yu D, Kazanietz MG, Harvey RG, Penning TM (2002). Polycyclic aromatic hydrocarbon o-quinones inhibit the activity of the catalytic fragment of protein kinase C. *Biochemistry*, 41: 11888–11894. doi:10.1021/bi020270p PMID:12269833