CHAPTER 10. USING EXPERIMENTAL DATA TO EVALUATE THE CARCINOGENICITY OF MIXTURES IN AIR POLLUTION

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It is well recognized that the *air pollution* encountered by humans is a mixture of pollutants that varies from place to place and with time. Acknowledgement of the full complexity of the mixture is an appropriate starting point to consider approaches to research the carcinogenicity of air pollution and evaluate the evidence. Current regulatory and research strategies tend to disregard the full complexity of the mixture. A small number of widespread pollutant species or classes that are known or thought to be of key health importance have been given highest priority for regulatory attention. In response, most research efforts have been directed towards these few pollutant groups. This situation is quite understandable; however, to move forward, it must first be recognized that the *multipollutant dilemma* (Mauderly *et al.*, 2010) extends far beyond interactions among a few widely monitored species. In short, the carcinogenic hazard of air pollution cannot be understood by considering only those few pollutant species that are measured routinely and for which epidemiological evidence is available.

In the USA, for example, most regulatory and research energy is focused on the six *criteria pollutants* (carbon monoxide, lead, nitrogen dioxide, ozone, particulate matter [PM], and sulfur dioxide) named in the Clean Air Act (as amended in 1990), which leaves few resources to be directed towards the 33 *urban air toxics* designated as most important among the 188 *hazardous air pollutants* (HAPs) (EPA, 1999, 2001a, 2004), and fewer yet are directed towards the remaining 155 HAPs. HAPs exist in the particulate, vapour, and gas phases, and many are listed because of their known or suspected carcinogenicity. Very little attention is given to

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the myriad other natural and anthropogenic air contaminants that are not on these lists.

Air pollution is not only complex but also a mixture of mixtures that can vary considerably with time and location. A single example suffices to demonstrate the point. Diesel engine emissions constitute one of the embedded mixtures that receive considerable regulatory and research attention. There has been a tendency to treat diesel emissions as if they were a single material of some relatively constant composition. Very few health studies have characterized the exposure material beyond a few physical–chemical species, and many have reported only the PM mass concentration. Two reports described in detail the exposure atmospheres that resulted from dilution of emissions from two different contemporary engines operated under different conditions and not equipped with emission reduction technologies. Both listed more than 100 physical–chemical parameters, acknowledged falling short of full speciation, and demonstrated that PM was only a small portion of the emitted mass. In one case (McDonald *et al.*, 2004a) the mass concentration of volatile organic carbon species was approximately equal to the mass concentration of PM, and in the other (McDonald *et al.*, 2004b) PM constituted only 1% of the total mass of measured emissions and was 5-fold less than the mass of volatile organic carbon species. A third report described the marked change in the composition of emissions from one of the engines that was equipped with a catalysed PM trap and burned a different fuel (McDonald *et al.*, 2004c), demonstrating that diesel emissions have become even more heterogeneous during the current period of phase-in of progressive emission controls. Impacts of the striking evolution of the composition of diesel emissions on estimates of carcinogenic hazard from this source category were recently reviewed (McClellan *et al.*, 2012). The diesel example illustrates challenges that international differences in source technologies and changes in source emissions with time present to summarizing carcinogenic hazards from source-based *embedded* air pollution mixtures.

Déjà vu: does air pollution present a unique *mixture* dilemma?

Perhaps not. The challenges presented by the experimental assessment of the carcinogenic hazards of air pollution are no different, for the most part, from those encountered in the assessment of the carcinogenic hazard of any physically and chemically complex mixture of known and potential mutagens, carcinogens, and promoting agents. The International Agency for Research on Cancer has dealt with complex mixtures in the past (e.g. tobacco smoke and diesel emissions, both of which are components of ambient air pollution) and is familiar with the difficulties involved in designing informative experiments and interpreting experimental evidence. The research challenges of selecting the material to study, the biological test system and response indicators, and the experimental design are considerable, but fundamentally no different for air pollution than for other complex mixtures of varying composition. The biological research tools, which range from chronic inhalation bioassays of whole mixtures to dosing cultured cells with specific chemical fractions, are no different for air pollution than for other mixtures (and are not reviewed in detail here). Accordingly, the interpretive challenges associated with these issues are similar for air pollution and other mixtures. This commonality, however, does not mean that the answers are straightforward; these fundamental issues have not been fully resolved for any mixture. The two main lines of evidence for the carcinogenicity of air pollution are conceptually similar to those for the carcinogenicity of other mixtures. Epidemiology has demonstrated associations between common pollutants and cancer (e.g. fine PM and sulfur oxides) (Pope *et al.*, 2002), and

there is experimental evidence for the mutagenicity or carcinogenicity of individual compounds or components of air pollution (e.g. DeMarini, *et al.*, 2000; Claxton *et al.*, 2004). Similarly, there is suggestive epidemiological evidence for the carcinogenicity of engine emissions, and some individual components of emissions are proven carcinogens or mutagens (EPA, 2001b). These two lines of evidence suggest that there are two principal types of source material for studies of the carcinogenicity of air pollution and other mixtures: the mixture and components of the mixture.

There are additional issues on *mixed exposure* that are also conceptually similar for air pollution and other mixtures. The ratio of components could be important to the carcinogenicity of the mixture. A first-order index of hazard might be developed by adding the products of the concentrations and relative potencies of the known carcinogenic components. However, it must be remembered that non-carcinogenic components also probably influence the carcinogenicity of the mixture, such as by acting as promoters (Madden *et al.*, 2000) or by creating mutagenic reaction products (Finlayson-Pitts and Pitts, 1997). The extent to which air pollution acts as a *complete* carcinogen undoubtedly depends on this factor. Moreover, issues on mixed exposure range beyond simply evaluating exposures to mixtures (reviewed in NIOSH, 2004). For example, the combination of exposure to ambient air pollutants and other factors (e.g. indoor air pollution, occupational carcinogens, and diet) could influence the relationship between ambient air pollution and cancer. It is also possible that the sequence of exposures to different pollutants, or to air pollution and other factors, may influence the risk of cancer.

Air pollution presents one dilemma that, while not strictly unique, is especially important in this case. Assuming that by *air pollution* we mean the complete mixture of ambient air contaminants, it must be recognized that there are very few complete physical–chemical characterizations of air pollution in any location, and certainly not in many locations or at many times. It can be assumed with confidence that cancer hazard is not limited to the species that are measured routinely. In contrast to at least limited detailed characterizations of embedded mixtures such as diesel emissions (cited above) and tobacco smoke (e.g. Guerin, 1987; Guerin *et al.*, 1987; IARC, 2004), there are few, if any, exhaustive characterizations of ambient air. There have been a few detailed characterizations of air pollution in the conduct of individual studies (e.g. Klemm *et al.*, 2004) and in monitoring programmes designed for that purpose (e.g. the United States Environmental Protection Agency PM Supersites Program, www.epa.gov/ttn/amtic/-supersites.html), but the scope of such data is small. This gap impairs the ability to select representative ambient sites and times to use for real-time exposures, to model air pollution mixtures in the laboratory, and to place the composition of air pollution mixtures into context regarding the composition of the mixtures for which we have the greatest body of experimental data.

Pathways and pitfalls: what can we learn from prior use of experimental tools to assess carcinogenic hazards of complex mixtures?

It should be instructive to consider the experience gathered to date with using experimental data to evaluate the carcinogenicity of complex mixtures. A complete review is not attempted here, but two cases – cigarette smoke and diesel emissions – are offered as illustrative examples. These examples are examined by considering the different approaches (exposure and biological response models) that have been used and their outcomes. There is little reason *a priori* to expect

better or worse success in using these approaches for air pollution.

There is epidemiological evidence for the carcinogenicity of both cigarette smoke and diesel emissions, but with very different degrees of certainty. Clearly a strong epidemiological link exists between cancer and tobacco smoke (reviewed in IARC, 2004), and although there is also a large body of experimental data that confirm the mutagenicity and carcinogenicity of tobacco smoke components, experimental data are not needed to confirm the cancer hazard or to assist in estimating risk. However, experimental data continue to be sought from cellular and animal models to understand carcinogenic mechanisms, to improve early detection and chemoprevention, and to develop *safer* smoking alternatives. In contrast, the epidemiological evidence for the cancer risk in humans from diesel emissions remains suggestive but uncertain. This is in large part due to the lack of exposure data (reviewed in IARC, 1989; EPA, 2001b; Bunn *et al.*, 2002). As for tobacco smoke, there is a large body of data that demonstrate the mutagenicity and carcinogenicity of components of historic diesel emissions, primarily soot-borne organic compounds. Experimental data were initially sought to confirm a cancer hazard and, considering the uncertainty of the epidemiological database, to assist in estimating the risk of cancer in humans (CalEPA, 1988). Experimental data continue to be sought to understand carcinogenic mechanisms and to compare the hazards of different emissions.

Chronic inhalation bioassays of cancer using conventional strains of rodents

For both cigarette smoke and diesel emissions, there is a long history of attempts to define carcinogenic hazard by chronic inhalation bioassays. The outcomes and interpretive challenges, however, have differed markedly between the two mixtures. Until recently, the many attempts to produce statistically significant increases in the incidence of lung tumours in rodents exposed chronically to tobacco smoke failed (reviewed in Mauderly *et al.*, 2004). Probable reasons include the failure of intermittent, nose-only, puff-by-puff exposures to achieve lung doses that model those incurred by human heavy smokers and the statistical weakness of small treatment groups. Using larger treatment groups and a conventional whole-body exposure approach that was estimated to model lung doses received by smokers of more than three packs per day produced significant increases in the incidence of lung tumours in both Fischer 344 rats (Mauderly *et al.*, 2004) and B6C3F1 mice (Hutt *et al.*, 2005). Moreover, the studies demonstrated genetic changes in lung tumour cells of rodents that mirror those of cancers in human smokers. Thus, conventional (i.e. not selected for genetic susceptibility) strains of rats and mice can model the human carcinogenicity of cigarette smoke.

In contrast, the first wave of studies on diesel emissions demonstrated conclusively by the mid-1980s that chronic whole-body inhalation exposures of conventional strains of rats (Fischer 344 and Wistar) to extreme concentrations (2.2–7.0 mg/m^3 PM) of fresh emissions produced significant dose-related increases in the incidence of lung tumours and accompanying DNA adducts (reviewed in Mauderly, 1999). Identical exposures of Syrian hamsters or standard strains of mice were not carcinogenic. However, a second wave of studies demonstrated that the response in rats was not related to organic mutagens; *clean* carbon black, and even titanium dioxide, caused the same response (including the adducts) with the same exposure–response slope (Heinrich *et al.*, 1995; Nikula *et al.*, 1995). Moreover, the lung tissue responses to heavy exposures to poorly soluble PM were found to differ between rats and primates (Nikula *et al.*, 1997). This experience led to awareness of the rat-specific particle *lung overload* phenomenon (Mauderly and McCunney, 1996) and an

improved understanding of the utility of the rat for evaluating cancer hazard from PM and mixtures that contain PM (Mauderly, 1997). Finally, analysis of the results of multiple studies revealed a threshold for significant increases in the incidence of lung tumours in rats that was much higher than environmental exposures to diesel emissions (Mauderly, 1999; Valberg and Crouch, 1999). Recent technology on-road emissions contain very little PM, often in lower concentration than in ambient air (McClellan *et al.*, 2012). These findings do not prove that there is no risk of cancer to humans from environmental exposures to diesel emissions; rather, they demonstrate some of the potential complexities and important precautions for high-dose studies of poorly soluble PM.

The experiences with chronic inhalation bioassays of cancer of tobacco smoke and diesel emissions provide lessons that are relevant to the use of such approaches for complex mixtures of air pollutants. The approach may be more sensitive for some physical–chemical species than for others, but this is not readily predicted in advance. The general approach remains a standard for assessing lung cancer hazard, assuming that suitable exposure atmospheres can be identified, but careful attention must be given to the experimental design. Advantage is gained by using multiple species, multiple exposure concentrations, sufficient group sizes, and measures of genetic alterations, and by avoiding unrealistic exposure concentrations. The selection of exposure concentrations has been reviewed, and guidance is available (Lewis *et al.*, 1989; Haseman and Lockhart, 1994).

Subchronic inhalation assays using genetically susceptible rodents

It would be desirable to have a sensitive bioassay that induced lung tumours but did not require near-lifetime exposures or large numbers of animals. Lung adenoma-prone mice have been used in attempts to develop such an assay, in which the increase in incidence and multiplicity of lung adenomas (nearly all benign) is examined in mice exposed subchronically (typically for 3–6 months) and then held for a few months after exposure. This assay is responsive to chemical mutagens and carcinogens (Stoner and Shimkin, 1982) and thus might be considered for use with atmospheres of complex air pollution.

This *tumorigenicity* assay has been used as an index of the cancer hazard of both cigarette smoke and diesel emissions. The experience with cigarette smoke has been variable. Increased incidences of adenomas have been produced in A/J, Balb/c, and SWR mice by simulated environmental cigarette smoke (D'Agostini *et al.*, 2001; Witschi *et al.*, 2002), but attempts using simulated mainstream smoke did not produce increases (Finch *et al.*, 1996; D'Agostini *et al.*, 2001). In a later study (Reed *et al.*, 2004), A/J mice were exposed for 6 hours per day on 7 days per week for 6 months to old technology diesel emissions at multiple concentrations up to 1000 mg/m^3 PM and held for 6 months without exposure before lung adenomas were assessed. This protocol produced no dose-related increase in the incidence of adenomas, although the PM extracts had characteristic direct-acting mutagenicity in the *Salmonella* reverse mutation assay. Even if the results from this assay were consistent, there would be considerable uncertainty in extrapolating from tumorigenicity in genetically susceptible mice to human lung cancer hazard. However, the variable results with cigarette smoke and lack of response to high concentrations of old technology diesel emissions do not provide encouragement for using this approach to evaluate the carcinogenic hazard of air pollution.

Non-inhalation in vivo and in vitro assays

An alternative to direct experimental evaluation of the carcinogenicity of air pollution as a complete mixture is to evaluate the carcinogenic

hazard of individual components of the mixture and thereby infer the cancer hazard of the mixture. A wide array of test systems is available to evaluate the mutagenicity, adduct-forming potential, clastogenicity, and carcinogenicity of components of air pollution and other complex mixtures. Examples are mutations in bacteria, mammalian cells, and intact animals, indices of chromosomal injury (e.g. sister chromatid exchange or micronuclei), indices of DNA injury (e.g. methylation or adduct formation), and tumour formation after instillation, injection, or implantation with extracted materials. No attempt is made to review these approaches in detail here as they all present the same set of fundamental advantages and disadvantages. Most of the common assays have been applied to components of both cigarette smoke (e.g. condensate) and diesel emissions (e.g. PM extract). For both mixtures, numerous assays have clearly demonstrated the presence of mutagenic, clastogenic, and carcinogenic components.

It has long since been demonstrated that components of air pollution are genotoxic. As an example, Seagrave *et al.* (2006) demonstrated marked differences in bacterial mutagenicity among ambient fine PM samples collected at different locations in the south-eastern USA. From such data, the plausibility of a cancer hazard from air pollution is already well established. Indeed, if demonstration of the plausibility of hazard without regard for dose or demonstration of carcinogenicity is sufficient, then little further work need be done.

There are several difficulties in using these approaches to estimate the actual human carcinogenic hazard presented by mixtures of air pollution. First, the relation between responses of these assays and cancer hazard for humans remains a perennial question, and the degree of confidence varies among the assays. Second, it is typically difficult to establish a relation between the cellular doses achieved in these assays and those incurred during real-world exposures of humans. Understanding the exposure–dose–response relationship is the key to extrapolating from hazard to human cancer risk. Third, the ability to extrapolate from the activity of individual components to the activity of the mixture is poor, even if we could assess the genotoxicity or carcinogenicity of each component of the mixture. The prevalent default assumption for estimating the cancer risk from mixtures is to assume that the cancer risks from individual components are additive (e.g. EPA, 1986, 1993). However, not only is it uncertain whether the activities of all genotoxic components are truly additive, it is also uncertain how the promoting activities of the many cytotoxic and inflammatory components of the air pollution mixture might influence the carcinogenicity of that mixture.

Identifying the culprit: what experimental designs can be used to disentangle the contributions of mixture components?

Assuming that a few *typical* air pollution mixtures could be defined and either located in the environment or reproduced for experimental study, the above approaches could be used to estimate the hazard of the mixtures. Reducing risk, however, requires knowledge of the components and sources that contribute most strongly to the hazard of the mixture. The fundamental approaches to evaluating the contributions of components to the effects of mixtures have been reviewed (Mauderly, 1993, 2004) and are summarized here. There are two fundamental approaches: study mixtures (top-down) and study components (bottom-up).

Top-down: the brute force approach

Clearly experiments cannot be conducted with every possible mixture of air pollutants; indeed, the number or nature of all air contaminants is not known. Studying realistically complex pollution mixtures requires selecting locations and times in the actual environment or simulating selected combinations of air contaminants in the laboratory. A study in the actual environment involves the challenges of the inability to predict detailed composition in advance, to hold composition constant for repeated exposures, or to concentrate all components identically to achieve (along with dilution) multiple exposure concentrations that exceed the highest ambient levels. Laboratory simulation necessarily falls short of the full spectrum of air contaminants and typically excludes many atmospheric reaction products. However, complex mixtures of key pollutants, such as representative combustion source emissions, can be (and have been) generated and studied experimentally.

There are two general *top-down* pathways for determining the causal components of complex mixtures. The most common is the physical–chemical dissection, or the *bio-effect directed fractionation* approach, in which the mixture is divided progressively and the fractions are tested until the most active ones are identified. A good example was the bio-effect directed fractionation of organic extracts of diesel PM to determine that certain nitro-aromatic compounds primarily drive the bacterial mutagenic activity (e.g. Schuetzle and Lewtas, 1986). This approach can potentially be used with any reproducible biological assay and is technically limited only by the ability to separate the different physical–chemical fractions of the mixture and satisfactorily expose the biological system. The study of concentrated ambient PM is a variant of this approach. With current methods, only the PM is concentrated, which prevents a study of the complete mixture in its original ratio of components.

An alternative approach is to conduct identical evaluations of different mixtures and use multivariate statistical analysis to determine the components that co-vary most closely with the target biological response. This *mathematical dissection* strategy is amenable to any database that encompasses mixtures that have sufficient differences in both composition and toxicity. For example, combined principal component analysis and partial least-squares regression were applied to a database on the bacterial mutagenicity and lung toxicity of several combined PM and semivolatile organic compounds from gasoline and diesel emissions (McDonald *et al.*, 2004c). This study demonstrated that certain, but not total, nitro-aromatic compounds co-varied most closely with mutagenicity (which was previously known and served to validate the approach) and that engine oil tracers co-varied most closely with inflammation of the lung (which was not previously known). In addition, multivariate analysis of PM components was used to determine that silica (assumed to arise from street dust) co-varied most closely with electrocardiographic changes in dogs exposed to concentrated PM (Wellenius *et al.*, 2003). On a somewhat larger scale, the National Environmental Respiratory Center programme (http://www.nercenter.org) (McDonald *et al.*, 2004b; Reed *et al.*, 2004) followed this strategy by building a detailed database on the composition of several complex source emissions (e.g. diesel and gasoline emissions, wood smoke, road dust, and coal emissions) and a range of respiratory and cardiovascular responses. The identification of key components as the putative causes of pro-atherosclerotic vascular responses in mice (Seilkop *et al.*, 2012) suggests that this general strategy may have utility for identifying key carcinogens in highly complex air pollution mixtures.

Bottom-up: paralysis by permutation

Interactions between mixture components to cause biological effects can also be explored using a factorial approach (i.e. effects of A, effects of B, effects of A + B). For example, this approach was used by Anderson *et al.* (1992) to examine the relative contributions of aerosolized carbon and sulfuric acid to the effects of the combined materials on the respiratory function of asthmatics. It was also used by Kleinman *et al.* (2000) to examine interactions between ozone, carbon black, and ammonium bisulfate that cause changes in lung inflammation, cell division, and collagen in rats, and this approach requires confidence that the key components have been identified. More importantly, although this approach is very useful for testing hypotheses about interactions between a few components, the number of permutations of combinations of exposure becomes overwhelmingly large beyond three components.

Choosing the *right stuff*: how do we select the exposure atmosphere?

Assuming that a suitable biological system and experimental design can be identified, selecting the exposure atmosphere is a critical issue. It is important to recognize that there is no *correct* air pollution mixture. The three basic choices are to (i) use actual ambient air at some location and over some time period and accept the inherent variations in composition and concentration; (ii) generate in the laboratory a simpler, but still complex, mixture of pollutants in some *average* ratio; or (iii) generate in the laboratory complex source emissions that are important components (embedded mixtures) of ambient pollution. The second and third approaches are commonly used, and examples are given above.

Only a few experimental exposures to ambient air have used the natural ratio of components. For example, Moss *et al.* (2001) exposed rats directly to air in Mexico City for up to 49 days and evaluated respiratory tissues; no histopathology was found compared with rats exposed to clean air. More relevant to carcinogenicity, Soares *et al.* (2003) exposed mice for 120 days to air in Sao Paulo, Brazil, and found greater frequencies of micronuclei in circulating blood in those mice than in mice exposed in a location with less pollution. Somers *et al.* (2004) exposed mice to ambient air with and without filtration and found that the PM fraction was chiefly responsible for the induction of heritable mutations. Although this approach is inherently challenging, these studies demonstrate that it is possible to expose animals directly to ambient air pollution, and the study of Somers *et al.* provides direct evidence of a clastogenic effect of air pollution. Exposures to ambient air cannot be controlled beyond selecting location, time, and dilution. Relating effects to the composition of the mixture is dependent on the level of characterization of the exposure, including both the number of analytes and the frequency of analysis.

Conclusions: what to do now?

There is no straightforward answer to the dilemmas faced in the experimental assessment of the carcinogenicity of air pollution. There is no single correct experimental approach, and the exposure material is extremely complex and variable. Certainly, experimental results have provided and will provide a basis for the plausibility of a risk of cancer by demonstrating both cancer hazards from individual pollutants and evidence of genotoxicity from exposures to ambient air. The former presents no great advance because it has been known for some time that many individual pollutants are mutagenic or carcinogenic, and most major contributing sources can probably be identified. That line of investigation could be pursued to identify the principal genotoxic components of ambient pollution, the relative hazards presented by

different pollution sources, and genotoxic interactions among pollutants. The results will not confirm or define actual cancer risk, but they will be useful, together with human exposure assessment and cancer epidemiology, in the overall assessment of risk and identification of its sources.

Chronic inhalation bioassays of ambient air can be conducted, but one has to be sceptical about their likely productivity because of their uncontrolled nature and the inability to exceed ambient exposure concentrations. It would be very challenging to conduct near-lifetime inhalation exposures of large numbers of animals to ambient air, but it is not impossible and may be valuable. Based on negative results from heavy exposures to source emissions that contain known mutagens and carcinogens (e.g. old technology diesel emissions at concentrations < 2 mg/m^3 PM), it is reasonable to assume this would be insufficient to cause increases in the incidence of lung tumours in standard strains of animals. The present high level of uncertainty in interpreting tumorigenicity results from genetically susceptible animals prevents giving substantial weight to results from their exposure to ambient air.

Overall, it must be decided whether there is value in the experimental demonstration of lung cancer in animals from chronic exposure to actual air pollution. If so, alternatives to designing a study of ambient air can then be explored, despite the challenges and limitations. If not, then there may not be a great need for further experimental work related solely to air pollution. It is known that components of air pollution are genotoxic and some are carcinogenic. The challenge therein, of course, is to determine whether to accept that evidence in view of the typically much lower long-term doses received from actual exposures to air pollution.

References

Anderson KR, Avol EL, Edwards SA *et al.* (1992). Controlled exposures of volunteers to respirable carbon and sulfuric acid aerosols. *J Air Waste Manage Assoc*, 42: 770–776. doi:10.1080/10473289.1992.10467028 PMID:1637548

Bunn WB 3rd, Valberg PA, Slavin TJ, Lapin CA (2002). What is new in diesel. *Int Arch Occup Environ Health*, 75: Suppl: S122–S132. doi:10.1007/s00420-002-0342-4 PMID:12397423

CalEPA (1988). *Health Risk Assessment for Diesel Exhaust*. Appendix III, Part B. *Proposed Identification of Diesel Exhaust as a Toxic Air Contaminant*. Office of Environmental Health Hazard Assessment. California Environmental Protection Agency. Available at http://www.arb.ca.gov/regact/diesltac/diesltac.htm

Claxton LD, Matthews PP, Warren SH (2004). The genotoxicity of ambient outdoor air, a review: *Salmonella* mutagenicity. *Mutat Res*, 567: 347–399. doi:10.1016/j.mrrev.2004.08.002 PMID:15572287

D'Agostini F, Balansky RM, Bennicelli C *et al.* (2001). Pilot studies evaluating the lung tumor yield in cigarette smoke-exposed mice. *Int J Oncol*, 18: 607–615. PMID:11179494

DeMarini DM, Shelton ML, Kohan MJ *et al.* (2000). Mutagenicity in lung of big Blue((R)) mice and induction of tandem-base substitutions in *Salmonella* by the air pollutant peroxyacetyl nitrate (PAN): predicted formation of intrastrand cross-links. *Mutat Res*, 457: 41–55. doi:10.1016/S0027-5107(00)00121-4 PMID:11106797

EPA (1986). Guidelines for the health risk assessment of chemical mixtures. [Washington, DC: United States Environmental Protection Agency.] *Fed Regist*, 52: 34014–34025.

EPA (2001b). *Health Assessment Air Pollution and Cancer Document for Diesel Exhaust*. Washington, DC: United States Environmental Protection Agency (EPA 600/8–90/057F).

EPA (1999). National Air Toxics Program: the integrated urban strategy. [Washington, DC: United States Environmental Protection Agency.] *Fed Regist*, 64: 38706–38740.

EPA (2001a). *National-Scale Air Toxics Assessment for 1996*. Washington, DC: United States Environmental Protection Agency (EPA 453/R-01–003).

EPA (2004). *The National-Scale Air Toxics Assessment*. Washington, DC: United States Environmental Protection Agency. Available at http://www.epa.gov/ttn/atw/nata

EPA (1993). *Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons*. Washington, DC: United States Environmental Protection Agency (EPA 600/R-93/089).

Finch GL, Nikula KJ, Belinsky SA *et al.* (1996). Failure of cigarette smoke to induce or promote lung cancer in the A/J mouse. *Cancer Lett*, 99: 161–167. doi:10.1016/0304-3835(95)04059-5 PMID:8616820

Finlayson-Pitts BJ & Pitts JN Jr (1997). Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles. *Science*, 276: 1045–1052. doi:10.1126/science.276.5315.1045 PMID:9148793

Guerin MR (1987). Formation and physicochemical nature of side-stream smoke. In: O'Neill IK, Brunnemann KD, Dodet B, Hoffmann D, eds. *Environmental Carcinogens: Methods of Analysis and Exposure Measurement, Passive Smoking*, Vol. 9. Lyon: International Agency for Research on Cancer, pp. 11–23.

Guerin MR, Higgins CE, Griest WH (1987). The analysis of the particulate and vapour phases of tobacco smoke. In: O'Neill IK, Brunnemann KD, Dodet B, Hoffmann D, eds. *Environmental Carcinogens: Methods of Analysis and Exposure Measurement, Passive Smoking*, Vol. 9. Lyon: International Agency for Research on Cancer, pp. 115–139.

Haseman JK & Lockhart A (1994). The relationship between use of the maximum tolerated dose and study sensitivity for detecting rodent carcinogenicity. *Fundam Appl Toxicol*, 22: 382–391. doi:10.1006/faat.1994.1043 PMID:8050633

Heinrich U, Fuhst R, Rittinghausen S *et al.* (1995). Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. *Inhal Toxicol*, 7: 533–556. doi:10.3109/08958379509015211

Hutt JA, Vuillemenot BR, Barr EB *et al.* (2005). Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. *Carcinogenesis*, 26: 1999–2009. doi:10.1093/carcin/bgi150 PMID:15944214

IARC (1989). Diesel and gasoline engine exhausts and some nitroarenes. *IARC Monogr Eval Carcinog Risks Hum*, 46: 1–458. PMID:2483415

IARC (2004). Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*, 83: 1–1438. PMID:15285078

Klemm RJ, Lipfert FW, Wyzga RE, Gust C (2004). Daily mortality and air pollution in Atlanta: two years of data from ARIES. *Inhal Toxicol*, 16: Suppl 1: 131–141. doi:10.1080/08958370490443213 PMID:15204801

Kleinman MT, Bufalino C, Rasmussen R *et al.* (2000). Toxicity of chemical components of ambient fine particulate matter (PM 2.5) inhaled by aged rats. *J Appl Toxicol*, 20: 357–364. doi:10.1002/1099-1263(200009/10)20:5<357::AID-JAT699>3.0.CO;2-6 PMID:11139166

Lewis TR, Morrow PE, McClellan RO *et al.* (1989). Establishing aerosol exposure concentrations for inhalation toxicity studies. *Toxicol Appl Pharmacol*, 99: 377–383. doi:10.1016/0041-008X(89)90147-6 PMID:2749728

Madden MC, Richards JH, Dailey LA *et al.* (2000). Effect of ozone on diesel exhaust particle toxicity in rat lung. *Toxicol Appl Pharmacol*, 168: 140–148. doi:10.1006/taap.2000.9024 PMID:11032769

Mauderly JL (1999). Diesel exhaust. In: Lippmann M, ed. *Environmental Toxicants: Human Exposures and Their Health Effects*, 2nd ed. New York: Wiley, pp. 193–241.

Mauderly JL (2004). Health effects of complex mixtures: where are we and where do we need to be? In: Heinrich U, ed. *Effects of Air Contaminants on the Respiratory Tract – Interpretations from Molecules to Meta Analysis.* Stuttgart: Fraunhofer IRB Verlag (INIS Monographs), pp. 43-52.

Mauderly JL (1997). Relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk. *Environ Health Perspect*, 105: Suppl 5: 1337–1346. PMID:9400748

Mauderly JL (1993). Toxicological approaches to complex mixtures. *Environ Health Perspect*, 101: Suppl 4: 155–165. doi:10.1093/annhyg/38.inhaled_parti_cles_VII.403 PMID:7515806

Mauderly JL, Burnett RT, Castillejos M *et al.* (2010). Is the air pollution heath research community prepared to support a multipollutant air quality management framework? *Inhal Toxicol*, 22: Suppl 1: 1–19. doi:10.3109/08958371003793846 PMID:20462389

Mauderly JL, Gigliotti AP, Barr EB *et al.* (2004). Chronic inhalation exposure to mainstream cigarette smoke increases lung and nasal tumor incidence in rats. *Toxicol Sci*, 81: 280–292. doi:10.1093/toxsci/kfh203 PMID:15213336

Mauderly JL, McCunney RJ (1996). *Particle Overload in the Rat Lung and Lung Cancer: Implications for Human Risk Assessment*. Washington, DC: Taylor & Francis.

McClellan RO, Hesterberg TW, Wall JC (2012). Evaluation of carcinogenic hazard of diesel engine exhaust needs to consider revolutionary changes in diesel technology. *Regul Toxicol Pharmacol*, 63: 225–258. doi:10.1016/j.yrtph.2012.04.005 PMID:22561182

McDonald JD, Barr EB, White RK (2004a). Design, characterization, and evaluation of a small-scale diesel exhaust exposure system. *Aerosol Sci Technol*, 38: 62–78. doi:10.1080/02786820490247623

McDonald JD, Barr EB, White RK *et al.* (2004b). Generation and characterization of four dilutions of diesel engine exhaust for a subchronic inhalation study. *Environ Sci Technol*, 38: 2513–2522. doi:10.1021/es035024v PMID:15180045

McDonald JD, Eide I, Seagrave JC *et al.* (2004c). Relationship between composition and toxicity of motor vehicle emission samples. *Environ Health Perspect*, 112: 1527–1538. doi:10.1289/ehp.6976 PMID:15531438

Moss OR, Gross EA, James RA *et al.* (2001). *Respiratory Tract Toxicity in Rats Exposed to Mexico City Air.*

Research Report 100. Cambridge, MA: Health Effects Institute.

Nikula KJ, Avila KJ, Griffith WC, Mauderly JL (1997). Lung tissue responses and sites of particle retention differ between rats and cynomolgus monkeys exposed chronically to diesel exhaust and coal dust. *Fundam Appl Toxicol*, 37: 37–53. doi:10.1006/faat.1997.2297 PMID:9193921

Nikula KJ, Snipes MB, Barr EB *et al.* (1995). Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. *Fundam Appl Toxicol*, 25: 80–94. doi:10.1006/faat.1995.1042 PMID:7541380

NIOSH (2004). *Mixed Exposures Research Agenda: A Report by the NORA Mixed Exposures Team*, (DHHS (NIOSH) Publication No. 2005–106). Research Triangle Park, NC: National Institute of Occupational Safety and Health.

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

Reed MD, Gigliotti AP, McDonald JD *et al.* (2004). Health effects of subchronic exposure to environmental levels of diesel exhaust. *Inhal Toxicol*, 16: 177–193. doi:10.1080/08958370490277146 PMID:15204765

Schuetzle D & Lewtas J (1986). Bioassay-directed chemical analysis in environmental research. *Anal Chem*, 58: 1060A–1075A. PMID:3532864

Seagrave JC, McDonald JD, Bedrick ES *et al.* (2006). Lung toxicity of ambient particulate matter from southeastern U.S. sites with different contributing sources: relationships between composition and effects. *Environ Health Perspect*, 114: 1387–1393. doi:10.1289/ehp.9234 PMID:16966093

Seilkop SK, Campen MJ, Lund AK *et al.* (2012). Identification of chemical components of combustion emissions that affect pro-atherosclerotic responses in mice. *Inhal Toxicol*, 24: 270–287. doi:10.3109/08958378.2012.667455 PMID:22486345

Soares SR, Bueno-Guimarães HM, Ferreira CM *et al.* (2003). Urban air pollution induces micronuclei in peripheral erythrocytes of mice in vivo. *Environ Res*, 92: 191–196. doi:10.1016/S0013-9351(02)00061-0 PMID:12804515

Somers CM, McCarry BE, Malek F, Quinn JS (2004). Reduction of particulate air pollution lowers the risk of heritable mutations in mice. *Science*, 304: 1008–1010. doi:10.1126/science.1095815 PMID:15143280

Stoner GD & Shimkin MB (1982). Strain A mouse lung tumor bioassay. *J Am Coll Toxicol*, 1: 145–169. doi:10.3109/10915818209013138

Valberg PA & Crouch EAC (1999). Meta-analysis of rat lung tumors from lifetime inhalation of diesel exhaust. *Environ Health Perspect*, 107: 693–699. doi:10.1289/ehp.99107693 PMID:10464067

Wellenius GA, Coull BA, Godleski JJ *et al.* (2003). Inhalation of concentrated ambient air particles exacerbates myocardial ischemia in conscious dogs. *Environ Health Perspect*, 111: 402–408. doi:10.1289/ehp.5775 PMID:12676590

Witschi H, Espiritu I, Dance ST, Miller MS (2002). A mouse lung tumor model of tobacco smoke carcinogenesis. *Toxicol Sci*, 68: 322–330. doi:10.1093/toxsci/68.2.322 PMID:12151628