Published in final edited form as:
Int J Cancer. 2008 December 1; 123(11): 2497–2502. doi:10.1002/ijc.23836.

PRENEOPLASTIC AND NEOPLASTIC LESIONS IN THE LUNG, LIVER, AND URINARY TRACT OF MICE EXPOSED TO ENVIRONMENTAL CIGARETTE SMOKE AND UV LIGHT SINCE BIRTH

Francesco D'Agostini[1], **Roumen Balansky**[1,2], **Vernon E. Steele**[3], **Gancho Ganchev**[2], **Carlo Pesce**[4], and **Silvio De Flora**[1,*]

[1]Department of Health Sciences, University of Genoa, Genoa, Italy

[2]National Center of Oncology, Sofia, Bulgaria

[3]National Cancer Institute, Rockville, MD, USA

[4]Department of Biophysical, Medical and Odontostomatological Sciences and Technologies, University of Genoa, Genoa, Italy

Abstract

It is difficult to reproduce the carcinogenicity of cigarette smoke (CS) in animal models. Recently, we showed that exposure of mice to mainstream CS (MCS) for 120 days, starting immediately after birth, resulted in an early and potent carcinogenic response. In parallel, we implemented studies evaluating intermediate biomarkers and tumors in mice exposed to environmental CS (ECS). To this purpose, we used 263 newborn CD-1 mice born from 27 dams. The whole-body exposure to ECS for 120 days, starting within 12 h after birth, resulted in an early appearance of preneoplastic lesions in lung, which however tended to attenuate after discontinuing exposure. When the experiment was stopped, after 330 days, the number of lung adenomas was higher in ECS-exposed mice as compared with sham-exposed mice, but such increase was statistically significant only in mice co-exposed to smoke and halogen light mimicking solar irradiation. Moreover, exposure to ECS produced extensive histopathological changes, mainly parenchymatous degeneration, in liver. The alterations produced in both lung and liver require that exposure to ECS starts immediately after birth, no effect being observed when exposure started 8 days later. In contrast, induction by ECS of alterations in the urinary tract, such as microadenomas and adenomas in renal pelvis and kidney, papillary hyperplasia of urothelium, and urinary bladder papillomas, were unrelated to the exposure time after birth. The results obtained with ECS cannot be directly compared to those previously obtained with MCS, since the latter involved shorter daily exposures to more massive CS doses.

Keywords

environmental cigarette smoke; UV light; neonatal mice; tumorigenicity

Environmental cigarette smoke (ECS), or secondhand cigarette smoke, is a mixture of sidestream smoke (SCS), released from the smouldering distal part of the cigarette, and that portion of mainstream cigarette smoke (MCS) that is exhaled by active smokers. ECS is

*Correspondence to: Department of Health Sciences, University of Genoa, Via A. Pastore 1, I-16132 Genoa, Italy. Fax: +39 010 3538504. E-mail: sdf@unige.it.

diluted with ambient air and undergoes ageing processes. Its inhalation results into involuntary or passive smoking, which was evaluated to be carcinogenic to humans and categorized by IARC in Group 1.1 SCS condensates were shown to be more potently carcinogenic than MCS condensates in mice when applied topically on the skin,2 and SCS condensates produced a dose-dependent increase in lung tumors in rats following implantation into the lungs.3 Witschi *et al*.4 developed a medium-term bioassay involving the whole-body exposure of A/J mice or other mouse strains to ECS for 5 months, followed by recovery in filtered air for an additional 4 months. As reviewed by Witschi *et al*.,5 18 studies performed in 4 laboratories, including ours,4-9 confirmed that there is a significant increase of lung tumors under these exposure conditions. However, the yield of lung tumors, which are detectable on the lung surface, is low. ECS tumorigenicity in this model was mainly ascribed to its gas phase and mainly to 1,3-butadiene.10,11

Recently, we provided evidence that MCS, which has been shown to be moderately carcinogenic in lifetime studies in both rats12 and mice,13 elicits a potent carcinogenic response in Swiss albino mice when exposure starts at birth and continues during the first 120 days of life.14 The carcinogenic response is characterized by (*a*) a short latency time, with the earliest lung tumors detectable after 75 days only; (*b*) a high incidence of preneoplastic and neoplastic lesions, detectable at the microscopical examination of lung sections, with an overall incidence of 78.3% tumor-bearing mice after 181-230 days; (*c*) a high multiplicity of benign lung tumors (6.1 and 13.6 tumors per mouse in males and females, respectively); (*d*) occurrence of malignant lung tumors in the 18.4% of mice within the first 210 days of life; and (*e*) occurrence of malignant tumors in the urinary tract and liver, some of which may have a metastatic origin from the lung.14 The potent carcinogenicity of MCS when exposure starts at birth is being fully confirmed by the preliminary results of further studies, also using other mouse strains and exposure times (R. Balansky *et al*., studies in progress).

The high susceptibility of neonatal mice to carcinogens has been demonstrated in several studies performed during the last 50 years,15 and can be ascribed to a variety of mechanisms, including the fact that, as shown by our studies in mice,16 evident DNA alterations occur immediately after birth in the lungs. In addition, a number of genes are overexpressed as a consequence of the oxidative stress occurring at birth in mouse lung.16,17 The carcinogens investigated in the neonatal mouse tumorigenicity bioassay also included 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone18 and benzo(*a*)pyrene [B(*a*)P],19-22 which are typical carcinogenic components of cigarette smoke.23

These premises prompted us to evaluate whether the whole-body exposure of mice to ECS during the first 120 days of life may result in the induction of tumors. A further goal of the present study was to investigate the possible influence of exposure to the UV-A and UV-B-containing light emitted by halogen quartz bulbs on responsiveness to ECS. In fact, our previous studies in hairless mice have shown that this light causes molecular, biochemical, and cytogenetic alterations not only in skin but also in distant organs, also including lung. Moreover, the light synergizes with ECS in producing alterations in the respiratory tract.24,25 The results herein reported provide evidence that ECS induces a moderate increase of lung tumors, which is more evident when exposure to ECS is combined with exposure to light. Moreover, exposure to ECS resulted in the formation of preneoplastic and neoplastic lesions in mouse liver and urinary tract.

MATERIAL AND METHODS

Mice and experimental groups

A total of 27 pregnant Swiss albino CD-1 mice were purchased from Harlan Italy (San Pietro al Natisone, Udine, Italy). Within 12 h after birth, the neonatal mice and their dams were divided into the following experimental groups:

Group A, sham-exposed mice, kept in filtered air (6 dams and 62 newborns);

Group B, mice exposed to ECS for 120 days, starting within 12 h after birth (7 dams and 75 newborns);

Group C, mice exposed to ECS for 120 days, starting 8 days after birth (2 dams and 24 newborns);

Group D, mice exposed to light for 120 days, starting within 12 h after birth (5 dams and 39 newborns);

Group E, mice exposed to both ECS and light for 120 days, starting within 12 h after birth (5 dams and 50 newborns);

Group F, mice receiving a single subcutaneous injection of B(*a*)P within 12 h after birth (2 dams and 13 newborns).

The mice were housed in Makrolon cages on sawdust bedding, and maintained on standard rodent chow and tap water *ad libitum*. All mice were kept in ventilated cabinets with filtered air, excepting during the periods of exposure to either ECS and/or light. The environmental temperature was 23 ± 2°C, with a relative humidity of 55% and a 12 h day-night cycle. The experiments were licensed by the Italian Ministry of Health. Housing and treatments of mice were in accordance with National Institutes of Health (USA), Italian, and our institutional guidelines.

Treatments

B(*a*)P (Sigma Chemical Co., St. Louis, MO) was dissolved in olive oil, and 0.025 ml, containing 2 mg B(*a*)P, were injected subcutaneously in the interscapular region of each neonatal mouse.

A whole-body exposure of mice to ECS was achieved by using a smoking machine (model TE-10c, Teague Enterprises, Davis, CA), adjusted to produce a combination of SCS (89%) and MCS (11%), mimicking a high-dose exposure to ECS. ECS was generated by burning 5 Kentucky 2R4F reference cigarettes with filter (Tobacco Research Institute, University of Kentucky, Lexington, KY) at one time, for a total of 120 cigarettes burnt daily. These cigarettes have a declared content of 9.2 mg tar and 0.8 mg nicotine each, with a 23 mm butt remaining after smoking. Under these conditions, an average of 63.3 mg/m^3 total suspended particulate was achieved in the 4 exposure chambers. Exposure was daily, 6 h/day divided into two rounds with a 3-h interval.

Exposure of mice to UV-A and UV-B-containing light was achieved by using halogen quartz bulbs (12V, 50 W) incorporated into dichroic spot light lamps (Leuci, File, Lecco, Italy). The lamps were covered with filters cutting UV-C light (WG 280, Schott Optics Division, Mainz, Germany). A distance of approximately 50 cm from the back of mice accounted for an illuminance of 10,000 lux. Exposure was daily, 6 h/day.

The following schedule was used in the mice exposed to both ECS and light: 3 h ECS, 3 h light, 3 h ECS, and 3 h light. Light and ECS were not simultaneously applied both because

the illumination system cannot be accomodated in the ECS exposure chambers and because we cannot rule out alterations of ECS components by direct exposure to light.26

Interim and terminal sacrifices, and evaluation of preneoplastc and neoplastic lesions

The mice were weighed at periodical intervals, at the times reported in Table I. When the infant mice became post-weanling, approximately 35 days after birth, they were housed separately according to genders. All dams and subgroups of 10 post-weanling mice (5 males and 5 females) from each one of Groups A, B, D and E were sacrificed, and their biological samples were used for evaluating intermediate biomarkers in the respiratory tract and hematopoietic system27 and in the cardiocirculatory system.28 Moreover, *interim* sacrifices were performed at periodical intervals during the first 270 days, according to the schedule reported in Table II, in order to generate a follow-up of histopathological lesions in the respiratory tract. All B(*a*)P-treated mice were sacrificed after 120 days.

The experiment was terminated 330 days after birth and start of exposures. All 141 mice surviving at that time were sacrificed. *Interim* and terminal sacrifices of mice were performed by deep anesthesia with diethyl ether and killing by cervical dislocation. A complete necropsy was performed. The lungs were immersed in formalin for a better visualization of surface tumors, with the aid of a stereomicroscope, and then embedded in paraffin. The lungs, liver, kidneys, urinary bladder, and all other organs with suspected macroscopically visible lesions were subjected to standard histopathological analyses. From each lung, one section every 200 μm, for a total of 15-20 sections per lung, was analyzed microscopically. From each kidney, 3 standardized sections were analyzed microscopically. From each liver, 2-3 sections were analyzed microscopically. From each urinary bladder, depending on its size, 2-4 sections were analyzed microscopically.

Please see our previous paper14 for photographs showing the appearance of typical histopathological alterations in smoke-exposed mice.

Statistical analyses

The yield of tumors and other lesions was expressed in terms of incidence and, in case of multiple tumors, of multiplicity. Body weights and multiplicity data were expressed as means ± SE of the mice composing each experimental group, and comparisons between groups were made by Student's *t*-test for unpaired data. Comparisons between groups regarding survival and incidence were made by χ^2 analysis.

RESULTS

Survival and body weights

Table I shows the body weights of the mice belonging to Groups A–E. The data are reported for mixed genders in weanling mice and are distinguished according to gender after weaning. At each time, the number of mice composing each group is reported between brackets. The decreases in the number of mice were due either to *interim* sacrifices or to spontaneous deaths. In particular, in the interval between day 29 and day 40, 10 mice (5 males and 5 females) per group were sacrificed from Groups A, B, D and E in order to perform studies evaluating intermediate biomarkers.27,28 Moreover, from day 35 to day 270, a total of 74 mice were sacrificed at monthly intervals in order to evaluate the time course of histopathological alterations in the respiratory tract (see Table II). Only 12 mice died spontaneously, including 4 sham-exposed mice (6.5% of the initial number), 1 mouse exposed to ECS for 120 days, starting at birth (1.3%), 5 mice exposed to ECS for 120 days, starting 8 days after birth (20.8%), and 2 mice exposed to both ECS and light for 120 days, starting at birth (4.0%). The spontaneous mortality in ECS-exposed mice did not differ

significantly from mortality in the sham-exposed mice. No spontaneous death were observed in the mice exposed only to light for 4 months, starting at birth.

Since the earliest measurement (8 days), the body weights were significantly decreased in the mice exposed for 120 days to either ECS or ECS + light, starting at birth. Since the second measurement (18 days), a similar loss of weight was recorded in the mice exposed to ECS for 120 days, starting 8 days after birth. The maximum ECS-related loss of weight was observed after 29 days in both males (−23.4%) and females (−21.9%), and thereafter was around 10% in both genders. In all ECS-exposed mice, the body weight returned to the sham values after 120 days, when exposure was discontinued. Surprisingly, the body weight was increased in the mice exposed to the light only. This effect was maximum after 18 days in mixed gender mice (+28.2%), and was statistically significant in females after 29-90 days (approximately +10%) and in both males and females after 120 days, with average increases from 10 to 20%, even after that exposure to light had been discontinued.

The body weights of B(*a*)P-treated mice (Group F) were significantly decreased until day 40, after which they were no longer significantly different from sham-exposed mice (data not shown).

Preneoplastic and neoplastic lesions in the respiratory tract

In the 13 mice treated with a single injection of B(*a*)P at birth and sacrificed after 120 days, a total of 11 adenomas were detected. Eight of them were detectable on the lung surface, while 3 were detected at the microscopic analysis of lung sections. The overall incidences were 1/5 (20%) in males, 4/8 (50%) in females, and 5/13 (38%) in males plus females. Multiplicities (means ± SE) were 0.4 ± 0.40, 1.1 ± 0.48, and 0.9 ± 0.34, respectively. By comparison, no lung tumor was detected in the 16 sham-exposed mice sacrificed from day 35 to day 240.

Table II reports the time-related histopathological alterations observed in the respiratory tract of those mice that were sacrificed at periodical intervals during the first 270 days of the study. Signs of hyperplasia of the alveolar wall and of the bronchial epithelium were already detectable, 35 days only after birth, when the mice became post-weanling, in the majority of the 20 investigated mice exposed to either ECS or ECS + light. Similar alterations were observed in the 8 ECS-exposed mice sacrificed after 60 and 90 days, whereas no change was detectable in the corresponding 8 controls. After 120 days, multiple lung microadenomas were detected in lung sections from the majority of 8 mice exposed to either ECS or ECS + light. However, from this time onwards, when exposure to ECS and light had been discontinued, lung tumors were no longer detectable in 12 ECS-exposed mice, 10 mice exposed to both ECS and light, and 8 control mice. The only exceptions were one adenoma on the lung surface of an ECS-exposed mouse sacrificed after 240 days and one microadenoma in a lung section from a mouse exposed to both ECS and light, sacrificed after 270 days.

Table III shows incidence data for bronchial hyperplasias, alveolar hyperplasias and lung adenomas, and Table IV shows multiplicity data for lung adenomas in the 141 mice sacrificed 330 days after birth, when the experiment was stopped. At that time, the incidences of bronchial and alveolar hyperplasias were significantly higher in both male and female mice exposed to either ECS or ECS + light during the first 120 days of life, starting at birth, as compared with the corresponding sham-exposed mice. When exposure to ECS started 8 days after birth, a significant increase of bronchial hyperplasia incidence was only observed in female mice.

A total of 21 adenomas were detected in the 141 mice belonging to the 5 experimental groups. All of them were detected on the lung surface and were confirmed at histopathological analysis, while no tumor was detected internally in lung sections. Compared to sham-exposed mice, there was no change in the incidence or in the multiplicity of lung adenomas either in light-exposed mice or in mice exposed to ECS starting 8 days after birth. In the mice exposed to ECS since birth there was about a doubling of lung adenoma yield in both males and females, but these differences were not statistically significant. A more evident increase of both incidence (Table III) and multiplicity (Table IV) of lung adenomas, which became statistically significant by combining the two genders, was observed in mice exposed to both ECS and light.

Preneoplastic and neoplastic lesions in other organs

As shown in Table III, histopathological alterations were also detected outside the respiratory tract, especially in the liver and urinary tract of the mice sacrificed after 330 days. In particular, exposure to ECS since birth produced a variety of alterations in the liver and characteristically induced a very high incidence of parenchymatous degeneration, especially in males. A similar effect was observed when the mice were exposed to both ECS and light since birth, while it was completely lost when exposure to ECS started 8 days after birth. In contrast, both papillary hyperplasia of the bladder urothelium and bladder papillomas were even more potently induced by ECS when exposure started 8 days after birth. The ECS-related increase of both incidence (Table III) and multiplicity (Table IV) of kidney microadenomas and adenomas was independent of the start of exposure. It is noteworthy that no induction of kidney microadenomas was observed when exposure to ECS was combined with exposure to light.

DISCUSSION

The results obtained in the present study show that the whole-body exposure of mice to ECS during the first 4 months of life results in an early appearance of preneoplastic lesions in the lung at the end of the period of exposure. However, these changes did not progress further to formation of multiple lung tumors, and the yield of lung adenomas was low even when the mice were kept in filtered air for 7 additional months in order to allow a better growth of the tumors induced during the first 4 months. Similarly to the situation in humans, it is conceivable that, at least under our experimental conditions, it is necessary to maintain the promoting stimulus of ECS components for a full development of tumors. For this reason, we started new studies in which the neonatal mice will be continuatively exposed to ECS throughout the whole duration of the experiment.

A new finding was that exposure to ECS causes histopathological alterations in extra-respiratory organs, especially in the form of parenchymatous dystrophy of the liver, microadenomas and adenomas in the renal pelvis and in kidney, and papillomas in the urinary bladder, which were absent in sham-exposed mice. Similar alterations, albeit less evident, were produced in the liver and urinary tract of MCS-exposed mice.14 It is likely that the liver of newborns is less efficient in carrying out detoxification of ECS and MCS, and in particular their glucuronidation. It has been demonstrated that, during the first weeks of postnatal life in mice, the levels of glutathione *S*-transferases are several fold lower than in adult animals.29

The multiorgan effects of ECS are consistent with the hypothesis that the pulmonary tumorigenicity of ECS in mice due to its gas phase and mainly to 1,3-butadiene10،11. Previous inhalation studies in both mice and rats showed that 1,3-butadiene induces tumors in multiple organs.30 However, the weak tumorigenicity of ECS contrasts with the evident alterations of a variety of intermediate biomarkers in several tissues of exposed mice

(reviewed in refs 31,32). Also in the mice belonging to the same experimental groups used in the present carcinogenicity study, sacrificed after 5 weeks of exposure to ECS, we observed a variety of significant alterations. These include cytogenetic damage in bone marrow and peripheral blood, formation of lipid peroxidation products in lung, increase of bulky DNA adducts and oxidative DNA damage in lung, heart and aorta, overexpression of *OGG1* gene in lung, and induction of apoptosis, proliferation and loss of Fhit protein in pulmonary alveolar macrophages and bronchial epithelial cells.27,28

By comparing the mice exposed to ECS during the first 5 weeks of life with their dams, exposed in the same cages, it was possible to conclude that young mice are more susceptible than adult mice to ECS-induced alterations of intermediate biomarkers, and especially to induction of oxidative DNA damage.27,28 In the present study, by comparing mice exposed to ECS for 120 days, starting either within 12 h after birth or 8 days later, it was evident that appearance in adult mice of both preneoplastic lesions in lung and parenchymatous dystrophy in liver requires that exposure to ECS starts shortly after birth. In contrast, the ECS-related increase of both incidence and multiplicity of kidney microadenomas and adenomas was independent of the start of exposure, and both papillary hyperplasia of the bladder urothelium and bladder papillomas were more frequently induced when exposure to ECS started 8 days after birth. These findings suggest that ECS-related alterations in the urinary tract are not linked to birth-related mechanisms.

Exposure of mice to the light alone did not produce any apparent histopathological alteration. However, the increase of lung tumor yield was more evident when the mice were co-exposed to ECS and light since birth. These data support our conclusion that there is a synergism between light and ECS in inducing DNA damage in the respiratory tract. Previous studies in hairless mice exposed to both light and ECS showed that, as compared with mice exposed to ECS only, there were further significant increases in bulky DNA adduct levels and upregulation of genes in lung and in micronucleus frequency in pulmonary alveolar macrophages.24,25 Also in the same CD-1 mice used in the present study, exposure to light induced oxidative DNA damage in lung, heart and aorta and synergized with ECS in enhancing oxidative DNA damage in lung as well as apoptosis in the bronchial epithelium. 27,28 The systemic genotoxic effects of the light emitted by UV-C-covered halogen lamps, simulating solar irradiation, have tentatively been ascribed to formation of long-lived mutagenic derivatives in the irradiated skin.24,25,27,28

In conclusion, exposure of mice to ECS during the first 4 months of life induced preneoplastic and neoplastic lesions, later in life, not only in lung but also in the liver and urinary tract. The weak tumorigenicity observed in the respiratory tract of ECS-exposed mice contrasts with the potent carcinogenic response of neonatal mice to MCS (14; R. Balansky *et al*., studies in progress). The results obtained in the studies with ECS and MCS cannot be compared, since the latter involved a daily exposure to an average 818 mg/m^3 total suspended particulate for 1 h, while the former involved a daily exposure to an average 63 mg/m^3 total suspended particulate for 6 h. Thus, the exposure to MCS was shorter but more intense than exposure to ECS. In any case, the view that ECS may be more potently carcinogenic than MCS needs to be re-evaluated. Future studies in neonatal mice exposed by inhalation will address this issue.

Acknowledgments

This study was supported by the U.S. National Cancer Institute contract N01-CN53301 and Bulgarian Ministry of Education and Science.

References

1. International Agency for Research on Cancer. Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of the carcinogenic risks to humans. Vol. 83. IARC; Lyon: 2004.
2. Mohtashamipur E, Mohtashamipur A, Germann PG, Ernst H, Norpoth K, Mohr U. Comparative carcinogenicity of cigarette mainstream and sidestream smoke condensates on the mouse skin. J Cancer Res Clin Oncol. 1990; 116:604–8. [PubMed: 2254379]
3. Grimmer G, Brune H, Dettbarn G, Naujack KW, Mohr U, Wenzel-Hartung R. Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream smoke of cigarettes evaluated by implantation into the lungs of rats. Cancer Lett. 1988; 43:173–7. [PubMed: 3203336]
4. Witschi H, Espiritu I, Peake JL, Wu K, Maronpot RR, Pinkerton KE. The carcinogenicity of environmental tobacco smoke. Carcinogenesis. 1997; 18:575–86. [PubMed: 9067559]
5. Witschi H. A/J mouse as a model for lung tumorigenesis caused by tobacco smoke: strengths and weaknesses. Exp Lung Res. 2005; 31:3–18. [PubMed: 15765916]
6. D'Agostini F, Balansky RM, Bennicelli C, Lubet RA, Kelloff GJ, De Flora S. Pilot studies evaluating the lung tumor yield in cigarette smoke-exposed mice. Int J Oncol. 2001; 18:607–15. [PubMed: 11179494]
7. De Flora S, Balansky RM, D'Agostini F, Izzotti A, Camoirano A, Bennicelli C, Zhang Z, Wang Y, Lubet RA, You M. Molecular alterations and lung tumors in p53 mutant mice exposed to cigarette smoke. Cancer Res. 2003; 63:793–800. [PubMed: 12591728]
8. Curtin GM, Higuchi MA, Ayres PH, Swauger JE, Mosberg AT. Lung tumorigenicity in A/J and rasH2 transgenic mice following mainstream tobacco smoke inhalation. Toxicol Sci. 2004; 81:26–34. [PubMed: 15159525]
9. Stinn W, Teredesai A, Kuhl P, Knorr-Wittmann C, Kindt R, Coggins C, Haussmann HJ. Mechanisms involved in A/J mouse lung tumorigenesis induced by inhalation of an environmental tobacco smoke surrogate. Inhal Toxicol. 2005; 17:263–76. [PubMed: 15814487]
10. Witschi H, Espiritu I, Maronpot RR, Pinkerton KE, Jones AD. The carcinogenic potential of the gas phase of environmental tobacco smoke. Carcinogenesis. 1997; 18:2035–42. [PubMed: 9395199]
11. Witschi H. The complexities of an apparently simple lung tumor model: The A/J mouse. Exp Toxicol Pathol. 2005; 57(Suppl 1):171–81. [PubMed: 16092725]
12. Mauderly JL, Gigliotti AP, Barr EB, Bechtold WE, Belinsky SA, Hahn FF, Hobbs CA, March TH, Seilkop SK, Finch GL. Chronic inhalation exposure to mainstream cigarette smoke increases lung and nasal tumor incidence in rats. Toxicol Sci. 2004; 81:280–92. [PubMed: 15213336]
13. Hutt JA, Vuillemenot BR, Barr EB, Grimes MJ, Hahn FF, Hobbs CH, March TH, Gigliotti AP, Seilkop SK, Finch GL, Mauderly JL, Belinsky SA. Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. Carcinogenesis. 2005; 26:1999–2009. [PubMed: 15944214]
14. Balansky R, Ganchev G, Iltcheva M, Steele VE, D'Agostini F, De Flora S. Potent carcinogenicity of cigarette smoke in mice exposed early in life. Carcinogenesis. 2007; 28:2236–43. [PubMed: 17522065]
15. Pietra G, Spencer K, Shubik P. Response of newly born mice to a chemical carcinogen. Nature. 1959; 183:1689. [PubMed: 13666865]
16. Izzotti A, Balansky RM, Camoirano A, Cartiglia C, Longobardi M, Tampa E, De Flora S. Birth-related genomic and transcriptional changes in mouse lung. Modulation by transplacental N-acetylcysteine. Mutat Res. 2003; 544:441–9. [PubMed: 14644347]
17. Bonner AE, Lemon WJ, Devereux TR, Lubet RA, You M. Molecular profiling of mouse lung tumors: association with tumor progression, lung development, and human lung adenocarcinomas. Oncogene. 2004; 23:1166–76. [PubMed: 14647414]
18. Beebe LE, Kim YE, Amin S, Riggs CW, Kovatch RM, Anderson LM. Comparison of transplacental and neonatal initiation of mouse lung and liver tumors by *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and promotability by a polychlorinated biphenyls mixture (Aroclor 1254). Carcinogenesis. 1993; 14:1545–48. [PubMed: 8353839]

19. Fujii K. Evaluation of the newborn mouse model for chemical tumorigenesis. Carcinogenesis. 1991; 12:1409–15. [PubMed: 1860162]
20. Yun TK. Usefulness of medium-term bioassay determining formations of pulmonary adenoma in NIH(GP) mice for finding anticarcinogenic agents from natural products. J Toxicol Sci. 1991; 16(Suppl 1):53–62. [PubMed: 1920544]
21. Yun TK, Kim SH, Lee YS. Trial of a new medium-term model using benzo(a)pyrene induced lung tumor in newborn mice. Anticancer Res. 1995; 15:839–45. [PubMed: 7645968]
22. Samarth RM, Panwar M, Kumar M, Kumar A. Protective effects of Mentha piperita Linn on benzo[a]pyrene-induced lung carcinogenicity and mutagenicity in Swiss albino mice. Mutagenesis. 2006; 21:61–6. [PubMed: 16399846]
23. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst. 1999; 91:1194–210. [PubMed: 10413421]
24. Balansky R, Izzotti A, D'Agostini F, Camoirano A, Bagnasco M, Lubet RA, De Flora S. Systemic genotoxic effects produced by light, and synergism with cigarette smoke in the respiratory tract of hairless mice. Carcinogenesis. 2003; 24:1525–32. [PubMed: 12844483]
25. Izzotti A, Cartiglia C, Longobardi M, Balansky R, D'Agostini F, Lubet RA, De Flora S. Alterations of gene expression in skin and lung of mice exposed to light and cigarette smoke. FASEB J. 2004; 18:1559–61. [PubMed: 15289447]
26. De Flora S, Camoirano A, Izzotti A, D'Agostini F, Bennicelli C. Photoactivation of mutagens. Carcinogenesis. 1989; 10:1089–97. [PubMed: 2655963]
27. De Flora S, D'Agostini F, Balansky R, Camoirano A, Cartiglia C, Longobardi M, Travaini G, Steele VE, Pesce C, Izzotti A. High susceptibility of neonatal mice to molecular, biochemical and cytogenetic alterations induced by environmental cigarette smoke and light. Mutat Res. 2007 Nov 17. Epub ahead of print.
28. Izzotti A, D'Agostini F, Balansky R, Pennisi TM, Steele VE, De Flora S. Exposure of mice to cigarette smoke and/or light causes DNA alterations in heart and aorta. 2008 Submitted for publication.
29. Shupe T, Sell S. Low hepatic glutathione S-transferase and increased hepatic DNA adduction contribute to increased tumorigenicity of aflatoxin B1 in newborn and partially hepatectomized mice. Toxicol Lett. 2004; 148:1–9. [PubMed: 15019083]
30. International Agency for Research on Cancer. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide (Part Two). IARC monographs on the evaluation of the carcinogenic risks to humans. Vol. 71. IARC; Lyon: 1999.
31. De Flora S, D'Agostini F, Balansky R, Camoirano A, Bennicelli C, Bagnasco M, Cartiglia C, Tampa E, Longobardi MG, Lubet RA, Izzotti A. Modulation of cigarette smoke-related endpoints in mutagenesis and carcinogenesis. Mutat Res. 2003:523–524. 237–52.
32. De Flora S, D'Agostini F, Izzotti A, Zanesi N, Croce C, Balansky R. Molecular and cytogenetical alterations induced by environmental cigarette smoke in mice heterozygous for *Fhit*. Cancer Res. 2007; 67:1001–6. [PubMed: 17283132]

Abbreviations

B(*a*)P	benzo(*a*)pyrene
ECS	environmental cigarette smoke
MCS	mainstream cigarette smoke
SCS	sidestream cigarette smoke

TABLE I

BODY WEIGHTS (G) OF CD-1 MICE (MEANS ± SE) AT VARIOUS TIMES AFTER BIRTH. THE NUMBER OF WEIGHED MICE COMPOSING EACH GROUP IS REPORTES BETWEEN BRACKETS

Time (days)	Gender	Treatment groups				
		(A) Sham	(B) ECS*	(C) ECS**	(D) Light*	(E) ECS+Light*
8	M + F	4.6 ± 0.10 (62)	4.3 ± 0.06^{a} (75)	4.6 ± 0.12 (24)	4.4 ± 0.13 (39)	3.8 ± 0.05^{c} (50)
18	M + F	7.1 ± 0.26 (62)	6.6 ± 0.12 (74)	6.3 ± 0.14^{a} (24)	9.1 ± 0.32^{f} (39)	6.1 ± 0.11^{b} (50)
29	M	22.2 ± 0.63 (34)	18.1 ± 0.40^{c} (48)	17.0 ± 0.55^{c} (15)	23.5 ± 0.76 (18)	17.0 ± 0.30^{c} (23)
	F	19.6 ± 0.43 (28)	16.1 ± 0.34^{c} (26)	15.7 ± 0.75^{c} (9)	21.1 ± 0.64 (21)	15.3 ± 0.36^{c} (27)
40	M	23.8 ± 0.51 (29)	21.2 ± 0.56^{b} (43)	20.7 ± 0.37^{c} (15)	24.5 ± 0.55 (13)	21.8 ± 0.44^{b} (18)
	F	21.1 ± 0.40 (23)	19.9 ± 0.41^{a} (21)	18.9 ± 0.38^{b} (9)	22.9 ± 0.35^{e} (16)	19.4 ± 0.61^{a} (22)
60	M	38.5 ± 0.57 (27)	35.8 ± 0.41^{c} (41)	34.9 ± 0.80^{c} (15)	39.1 ± 0.84 (13)	33.9 ± 0.67^{c} (18)
	F	29.8 ± 0.63 (21)	27.9 ± 0.56^{a} (19)	26.5 ± 0.73^{b} (9)	32.0 ± 0.69^{d} (16)	26.1 ± 0.63^{c} (22)
90	M	39.8 ± 0.62 (25)	36.1 ± 0.40^{c} (39)	35.5 ± 0.64^{c} (15)	41.2 ± 0.97 (13)	35.9 ± 0.68^{c} (18)
	F	30.7 ± 0.67 (19)	29.5 ± 0.52 (17)	27.7 ± 0.47^{b} (9)	33.8 ± 1.10^{d} (16)	27.8 ± 0.60^{b} (22)
120	M	41.8 ± 0.71 (25)	39.6 ± 0.49^{a} (39)	38.7 ± 0.66^{b} (15)	44.1 ± 0.90^{d} (13)	39.4 ± 0.71^{a} (18)
	F	32.5 ± 0.65 (18)	31.8 ± 0.59 (17)	29.8 ± 0.73^{a} (9)	36.0 ± 1.30^{d} (16)	29.7 ± 0.70^{b} (22)
150	M	40.3 ± 0.72 (23)	41.9 ± 0.55 (36)	39.9 ± 0.74 (14)	44.7 ± 0.96^{f} (13)	38.5 ± 0.84 (16)
	F	32.1 ± 0.50 (17)	33.0 ± 0.84 (15)	31.2 ± 0.80 (9)	36.2 ± 1.37^{e} (16)	30.9 ± 0.63 (20)
180	M	41.2 ± 0.76 (22)	42.9 ± 0.59 (34)	40.8 ± 0.74 (14)	45.9 ± 1.12^{e} (13)	39.6 ± 0.94 (16)
	F	32.9 ± 0.58 (17)	34.2 ± 0.96 (13)	31.6 ± 0.86 (9)	37.4 ± 1.41^{e} (16)	31.6 ± 0.68 (20)
210	M	42.1 ± 0.97 (18)	43.6 ± 0.66 (30)	41.7 ± 0.76 (14)	46.8 ± 1.24^{e} (13)	40.4 ± 0.98 (16)
	F	33.8 ± 0.64 (17)	35.1 ± 1.00 (13)	32.7 ± 0.93 (9)	38.5 ± 1.60^{e} (16)	32.3 ± 0.77 (20)
240	M	42.0 ± 0.89 (16)	43.9 ± 0.85 (26)	41.9 ± 0.96 (13)	47.5 ± 1.25^{e} (13)	40.2 ± 1.43 (14)
	F	35.2 ± 0.65 (15)	35.6 ± 1.02 (13)	32.9 ± 0.88 (9)	39.7 ± 1.43^{d} (16)	32.9 ± 1.21 (18)
270	M	41.9 ± 0.90 (16)	44.0 ± 0.75 (26)	42.1 ± 1.02 (12)	48.4 ± 0.79^{e} (13)	43.4 ± 1.07 (11)
	F	36.1 ± 0.70 (15)	36.4 ± 0.98 (13)	33.2 ± 0.96 (9)	41.3 ± 0.89^{e} (16)	33.9 ± 0.99 (15)

Time (days)	Gender	Treatment groups				
		(A) Sham	(B) ECS*	(C) ECS**	(D) Light*	(E) ECS+Light*
300	M	42.3 ± 0.87 (16)	44.9 ± 0.83 (25)	42.7 ± 0.70 (11)	49.0 ± 1.47[e] (13)	45.9 ± 1.63 (9)
	F	36.5 ± 0.76 (15)	37.9 ± 1.42 (13)	35.7 ± 0.98 (8)	43.4 ± 2.33[e] (16)	34.9 ± 1.19 (15)
330	M	42.5 ± 0.93 (16)	44.9 ± 0.83 (25)	42.9 ± 0.84 (11)	49.5 ± 1.45[e] (13)	45.5 ± 1.72 (9)
	F	36.2 ± 0.88 (15)	37.8 ± 1.48 (13)	33.4 ± 1.16 (8)	43.9 ± 2.35[e] (16)	36.2 ± 1.44 (15)

*Exposed for 120 days, starting within 12 h after birth.

**Exposed for 120 days, starting 8 days after birth.

[a] $P < 0.05$,

[b] $P < 0.01$,

[c] $P < 0.001$, significantly decreased as compared with the corresponding Sham.

[d] $P < 0.05$,

[e] $P < 0.01$,

[f] $P < 0.001$, significantly increased as compared with the corresponding Sham.

TABLE II

HISTOPATHOLOGICAL ALTERATIONS IN THE RESPIRATORY TRACT OF VARIOUSLY TREATED MICE, SACRIFICED AT VARYING TIME INTERVALS AFTER BIRTH (35 DAYS–9 MONTHS)

Time (days)	Treatment	Gender	No. of mice	No. of mice bearing the lesion		
				Alveolar hyperplasias	Bronchial hyperplasias	Lung adenomas or microadenomas
35	ECS	M	5	3	3	0
		F	5	3	3	0
	ECS + Light	M	5	4	4	0
		F	5	3	3	0
60	Controls	M	2	0	0	0
		F	2	0	0	0
	ECS	M	2	2	1	0
		F	2	2	1	0
90	Controls	M	2	0	0	0
		F	2	0	0	0
	ECS	M	2	2	2	0
		F	2	2	2	0
120	ECS	M	2	2	2	1[b]
		F	2	2	2	1[c]
	ECS + Light	M	2	2	2	2[d]
		F	2	2	2	2[e]
150	ECS	M	2	2	2	0
		F	2	2	2	0
180	Controls	M	4	0	0	0
	ECS	M	4	4	4	0
240	Controls	M	2	0	0	0
		F	2	0	0	0
	ECS	M	4	4	4	1[f]
	ECS + Light	M	2	1	1	0
		F	2	1	1	0
270	ECS + Light	M	3	1	1	0

Time (days)	Treatment	Gender	No. of mice	No. of mice bearing the lesion		
				Alveolar hyperplasias	Bronchial hyperplasias	Lung adenomas or microadenomas
		F	3	2	2	1[g]

[a] All treatments started at birth and were stopped after 4 months.

[b] 8 microadenomas;

[c] 1 microadenoma;

[d] 4 microadenomas each;

[e] 5 and 3 microadenomas, respectively;

[f] 1 surface adenoma;

[g] 1 microadenoma.

TABLE III

INCIDENCE OF HISTOPATHOLOGICAL ALTERATIONS IN CD–1 MICE EXPOSED TO EITHER ECS AND/OR LIGHT FOR 4 MONTHS AND SACRIFICED AFTER 7 ADDITIONAL MONTHS. THE NUMBER OF MALE (M) OR FEMALE (F) MICE WITHIN EACH GROUP IS REPORTED BETWEEN BRACKETS

Organ	Sham			ECS*			ECS**			Light*			Light* + ECS*		
Histopatological alteration	**M (16)**	**F (15)**	**M+F (31)**	**M (25)**	**F (13)**	**M+F (38)**	**M (11)**	**F (8)**	**M+F (19)**	**M (13)**	**F (16)**	**M+F (29)**	**M (9)**	**F (15)**	**M+F (24)**
Lung															
Bronchial hyperplasia	3 (18.8%)	1 (6.7%)	4 (12.9%)	13 (52.0%)[a]	8 (61.5%)[c]	21 (55.3%)[c]	3 (27.3%)	3 (37.5%)[a]	6 (31.6%)	2 (15.4%)	3 (18.8%)	5 (17.2%)	6 (66.7%)[b]	9 (60.0%)[c]	15 (62.5%)[c]
Alveolar hyperplasia	2 (12.5%)	1 (6.7%)	3 (9.7%)	13 (52.0%)[b]	7 (53.8%)[b]	20 (52.6%)[c]	1 (9.1%)	2 (25.0%)	3 (15.8%)	1 (7.7%)	3 (18.8%)	4 (13.8%)	5 (55.6%)[a]	9 (60.0%)[b]	14 (58.3%)[c]
Adenomas	2 (12.5%)	1 (6.7%)	3 (9.7%)	5 (20.0%)	2 (15.4%)	7 (18.4%)	1 (9.1%)	0	1 (5.3%)	1 (7.7%)	2 (12.5%)	3 (10.3%)	3 (33.3%)	4 (26.7%)	7 (29.7%)[a]
Liver															
Parenchymatous degeneration	0	0	0	19 (76.0%)[c]	3 (23.1%)[a]	22 (57.9%)[c]	0	0	0	2 (15.4%)	0	2 (6.9%)	4 (44.4%)[b]	5 (33.3%)[a]	9 (34.6%)[c]
Fat dystrophy	0	0	0	1 (4.0%)	0	1 (2.6%)	0	0	0	0	0	0	1 (11.1%)	1 (6.7%)	2 (7.7%)
Proliferation of dark oval cells	0	0	0	0	1 (7.7%)	1 (2.6%)	0	0	0	0	0	0	0	0	0
Microadenomas	0	0	0	0	1 (7.7%)	1 (2.6%)	0	0	0	0	0	0	0	0	0
Renal pelvis															
Microadenomas	0	0	0	2 (8.0%)	0	2 (5.3%)	0	0	0	0	0	0	0	0	0
Adenomas	0	0	0	0	1 (7.7%)	1 (2.6%)	0	0	0	0	0	0	0	0	0
Kidney															
Focal hyperplasia	0	0	0	2 (7.7%)	0	2 (5.3%)	1 (9.1%)	0	1 (5.3%)	0	0	0	0	0	0
Cyst	0	0	0	2 (7.7%)	0	2 (5.3%)	0	0	0	0	0	0	0	0	0
Microadenomas	1 (6.3%)	0	1 (3.2%)	10 (40.0%)[a]	4 (30.8%)[a]	14 (36.8%)[c]	5 (45.5%)[a]	4 (50.0%)[a]	9 (47.3%)[c]	0	0	0	0	0	0
Adenomas	0	0	0	1 (4.0%)	2 (15.4%)	3 (7.9%)	1 (9.1%)	0	1 (5.3%)	0	0	0	0	2 (13.3%)	2 (7.7%)
Urinary bladder															

Organ	**Sham**			**ECS***			**ECS****			**Light***			**Light* + ECS***		
Histopatological alteration	**M (16)**	**F (15)**	**M+F (31)**	**M (25)**	**F (13)**	**M+F (38)**	**M (11)**	**F (8)**	**M+F (19)**	**M (13)**	**F (16)**	**M+F (29)**	**M (9)**	**F (15)**	**M+F (24)**
Papillary hyperplasia urothelium	3 (18.8%)	0	3 (10.3%)	12 (48.0%)[a]	0	12 (31.6%)[a]	9 (81.8%)[c]	3 (37.5%)[b]	12 (63.2%)[c]	1 (7.7%)	0	1 (3.4%)	2 (22.2%)	3 (20.0%)	5 (19.2%)
Papilloma	0	0	0	3 (12.0%)	0	3 (7.9%)	4 (36.1%)[a]	1 (12.5%)	5 (26.3%)[b]	0	0	0	3 (33.3%)[a]	3 (20.0%)	6 (23.1%)[b]
Uterus															
Hyperplasia of epithelium	–	1 (6.7%)	–	–	2 (15.4%)	–	–	0	–	–	0	–	–	1 (6.7%)	–
Ovary															
Ovarian or paraovarian cyst	–	2 (13.3%)	–	–	4 (30.8%)	–	–	4 (50.0%)	–	–	2 (12.5%)	–	–	3 (20.0%)	–

*Exposed for 4 months, starting within 12 h after birth.

**Exposed for 4 months, starting 8 days after birth.

[a]$P \leq 0.05$,

[b]$P \leq 0.01$,

[c]$P \leq 0.001$, as compared with the corresponding sham.

TABLE IV

MULTIPLICITY OF HISTOPATHOLOGICAL ALTERATIONS IN LUNG AND KIDNEY OF CD–1 MICE EXPOSED TO EITHER ECS AND/OR LIGHT FOR 4 MONTHS AND SACRIFICED AFTER 7 ADDITIONAL MONTHS. THE NUMBER OF MALE (M) OR FEMALE (F) MICE WITHIN EACH GROUP IS REPORTED BETWEEN BRACKETS

Organ	**Sham**			**ECS***			**ECS****			**Light***			**Light* + ECS***		
Histopatological alteration	**M (16)**	**F (15)**	**M+F (31)**	**M (25)**	**F (13)**	**M+F (38)**	**M (11)**	**F (8)**	**M+F (19)**	**M (13)**	**F (16)**	**M+F (29)**	**M (9)**	**F (15)**	**M+F (24)**
Lung															
Adenomas	0.1 ± 0.09	0.1 ± 0.07	0.1 ± 0.05	0.2 ± 0.08	0.2 ± 0.10	0.2 ± 0.06	0.1 ± 0.09	0	0.1 ± 0.05	0.1 ± 0.08	0.2 ± 0.14	0.1 ± 0.08	0.4 ± 0.24	0.3 ± 0.24	0.4 ± 0.13[a]
Kidney															
Microadenomas	0.1 ± 0.13	0	0.1 ± 0.07	2.1 ± 0.77[a]	2.1 ± 1.37	2.1 ± 0.68[a]	0.5 ± 0.16	2.0 ± 0.78[b]	1.1 ± 0.37[c]	0	0	0	0	0	0
Adenomas	0	0	0	0.3 ± 0.32	0.6 ± 0.57	0.9 ± 0.58	0.1 ± 0.09	0	0.1 ± 0.05	0	0	0	0	1.0 ± 0.81	0.6 ± 0.47

*Exposed for 4 months, starting within 12 h after birth.

**Exposed for 4 months, starting 8 days after birth.

[a] $P \leq 0.05$,

[b] $P \leq 0.01$,

[c] $P \leq 0.001$, as compared with the corresponding sham.