**# 1**

**AUTHOR(s): AHMED, S. SULTAN., CHRISTOS B. MOSCHOS, MICHAEL M. LYONS, HENRY OLDEWURTEL, RICHARD J. COUMBISS, TIMOTHY J. REGAN, AND BESS JENKINS.**

**DATE: 1976**

**TITLE: CARDIOVASCULAR EFFECTS OF LONG-TERM CIGARETTE SMOKING AND NICOTINE ADMINISTRATION**

**CITATION: THE AMERICAN JOURANL OF CARDIOLOGY 37: 33-40(1976)**

**STUDY DESIGN:**

To compare the relative effects of long-term smoking and nicotine administration on the cardiovascular system, 18 month old beagles were prepared with a permanent tracheostomy. The were divided into three groups: I, seven control dogs; II, nine dogs that smoked seven cigarettes /day; and III, eight dogs that received an equivalent amount of nicotine. After a period of up to 22 months, the animals were catheterized under anesthesia for assessment of left ventricular function and volumes by indicator-dilution technique.

**FINDINGS/RESULTS:**

Heart rate, stroke volume, left ventricular end-diastolic pressure and volume and intraventricular conduction times did not differently significantly in the three groups. Left ventricular ejection fraction was 44 ± 3% in the control group, 35 ± 3 % in the dogs that smoked cigarettes and 27 ± 3 % in those given nicotine ($P < 0.01$)despite similar values for end-diastolic variable in the three groups. The first derivative of left ventricular pressure (dP/dt) normalized for pre and afterload was 2.4 ± 0.2 $cm/sec^{-1}$ in the control group, 1.41 ± 0.12 in the cigarette smoking group ($p<0.005$) and 1.34 ± 0.08 in nicotine group ($p < 0,01$). Mean aortic pressure was significantly elevated in both the smoking (127 ± 5 mm Hg) and nicotine (127 ± 10 mm Hg) groups, there was no significant correlation with the contractililtly indexes. Reduction of afterload to normal levels did not affect the abnormal ventricular performance. Hypertrophy, inflammation and abnormalities of cell ultrastructures were not present, and myocardial lipid and cation composition were normal. Since interstitial fibrosis was evident in both experimental groups, an alteration of elastic elements may be operative. These cardiovascular abnormalities appear to be predominantly dependent on the nicotine of cigarettes.

**CONCLUSIONS/COMMENTS:**

Since clinically evident heart disease was not observed in these animals, the conclusions of an epidemiologic study that cardiac risks of long-term smoking are only evidenced when associated with other risk factory may be well founded.

#2

**AUTHOR(s): AHMED, S. SULTAN., CHRISTOS B. MOSCHOS, HENRY OLDEWURTEL, and TIMOTHY J. REGAN**

**DATE: 1980**

**TITLE:** MYOCARDIAL EFFECTS OF LONG-TERM CIGARETTE SMOKING: RELATIVE ROLES OF CARBON MONOXIDE AND NICOTINE.

**CITATION: THE AMERICAN JOURNAL OF CARDIOLOGY 46:593-598 (1980)**

**STUDY DESIGN:**

Because controversy has existed as to whether nicotine or carbon monoxide is the major pathogenetic agent in cigarette smoking, 18 adult male beagles with chronic trachestomy were placed in three groups of six dogs each: Group 1 served as a control group; group2 received 7 cigarettes of low nicotine content (0.2 mg/cigarette), which effected a peak carboxyhemoglobin level approximating 5/100ml, similar to that of regular cigarettes; and group 3 received nicotine twice daily intramuscularly in an amount equivalent to seven cigarettes/day.

**FINDINGS/RESULTS:**

In the intact anesthetized state, heart rate, left ventricular end-diastolic pressure and volume (indicator dilution) did not differ among the three groups. To assess relative
myocardial wall stiffness, saline solution was infused into the left ventricular chamber. A significantly higher end-diastolic pressure and tensions were elicited in group 3, suggesting a decrease in left ventricular compliance. No such change was observed in either group 1 or 2. Only long-term nicotine use was associated with increased hydroxyproline content in the left ventricular myocardium, suggesting a basis for enhanced stiffness. An index of left ventricular contractility was derived from the peak rate of rise of left ventricular pressure (dP/dt), normalized for preload and afterload. Groups 1 and 2 showed no difference in this index (2.18 ± 0.3 versus 2.15 ± 0.18 muscle lengths/s per cm) at similar levels of aortic pressure, but the index was significantly lower (1.28 ± 0.12, $p < 0.01$) in group 3.

**CONCLUSIONS/COMMENTS:**

The effects of long-term cigarette use on the myocardium appear to be predominantly dependent on nicotine rather than on carbon monoxide.

#3
**AUTHOR(s): ANDO, KIYOSHI AND TOMOJI YANAGITA**

**DATE: 1981**

**TITLE: CIGARETTE SMOKING IN RHESUS MONKEYS**

**CITATION: PSYCHOPHARMACOLOGY 72: 117-127 (1981)**

**ABSTRACT:**

In the present pilot study, an attempt was made to shape and maintain cigarette smoking behavior in rhesus monkeys both with and without the simultaneous use of other reinforcers. Initially, 14 monkeys were trained to suck air and puff on cigarettes using sweetened liquid reinforcer. After smoking had been established, the sweetened liquid reinforcement was removed. Smoking without this reinforcement, referred to as ' voluntary smoking' was then observed during 20-h daily sessions. Of the 14 monkeys studied, 2 have engaged in voluntary smoking for 2 years or longer. The maximum figures recorded for any single 20-h session were 3,271 puffs (20 cigarettes) in one monkey and 16,384 puffs (47 cigarettes) in the other. Although the baseline variability of smoking by these monkeys was quite high, low-nicotine and nicotine free cigarettes seem to lead to a clear decrease in smoking. In 2 other monkeys that did not perform voluntary smoking, smoking was reestablished under a random-time or tandem schedule for sweetened liquid reinforcement. Within this situation (' Schedule-controlled smoking" ) schedule manipulations also led to changes in intake of cigarette smoke. The voluntary smoking model described in the present paper should be useful for studying the factors involved in initiating and maintaining smoking behavior and for studying the psychopharmacological effects of smoking, while the schedule-controlled smoking model should be useful for studying the physiological effects of smoking and for studying the relationship of smoking with various disease entities.

#4
**AUTHOR(s): AOYAMA, MITSUKO, HIROSHI FUJISE AND NORIHIDE TACHI**

**DATE: 1981**

**TITLE: EXPERIMENTAL STUDIES ON THE EFFECT OF CIGARETTE SMOKE ON THE PHYSIOLOGICAL GROWTH, LIFE-SPAN, AND SPERMATOGENESIS IN MALE MICE**

**CITATION: NAGOYA MEDICAL JOURNAL 25: 259-264 (1981)**

**STUDY DESIGN:**

45 male ddY mice used in this study were divided into three groups (each 15): Group A, Group B and the control. The smoke exposure experiments were performed in a chamber filled with smoke from 5 cigarettes (filter cigarette, Hi-lite Japanese brand) by the aid of an inhalation apparatus. Group A was exposed once a day for 50 minutes and group B for 25 minutes. The control group was manipulated in the similar manner for 25 minutes without smoke. The experiment was carried out for 360 consecutive days.

The levels of carbon monoxide measured in the chamber was usually 400-500 ppm, while carbon dioxide was ~5500-6000 ppm, and the smoke dust was ~ 120 mg/m$^3$. Tissue preparations for microscopical analysis were made from the testes of the mice which survived until the end of the experiment.

**FINDINGS/RESULTS:**

The effect of cigarette on the life span of the mice is indicated by their survival rates. Mice exposed for 50 minutes a day began to die from the 60$^{th}$ day of exposure, and the final survival rate was 13.3%. Mice exposed for 25 min. began to die also on the 60$^{th}$ day, and had a final survival rate of 20%. The control group on the other hand did not start to die until the 150$^{th}$ day, and the final survival rate was 53.3%. The death rate between the control and exposed groups was significantly different. Physiological increase in the body weight of the exposed mice was considerably more retarded than that of the control mice.

In the histological observations of the testes one of the two surviving mice in Group A on the 306$^{th}$ day of the experiment displayed azoospermia and two of the three surviving mice in Group B showed oligo- or azoospermia.

**CONCLUSIONS/COMMENTS:**

The marked inhibition of spermatogensis at the end of this experiment clearly indicates that a certain component in the smoke must have a toxic effect on spermatogensis.

#5
**AUTHOR(s): AUERBACH, OSCAR, AND LAWRENCE GARFINKEL**

**DATE: 1970**

**TITLE:** EFFECT OF CIGARETTE SMOKING ON DOGS. II. PULMONARY NEOPLASMS

**CITATION: ARCH ENVIRON HEALTH 21: 754-768 (1970)**

**STUDY DESIGN:**

Of 86 male beagle does trained to smoke through a tracheostoma, 12 dogs (group F) smoke filter-tip, 24 (group H) and 38 (group h) smoked non-filter cigarettes and 12 (group L), half as many non filter cigarettes. Eight dogs (group N) never smoked.

**FINDINGS/RESULTS:**

By day 875, none of the N dogs, 2 F dogs, 2 L dogs, 12 H dogs, and 12 h dogs had died, and the remaining N,F,L, and H dogs were killed. Noninvasive brochiolo-alveolar tumors were found in dogs of all five groups. Invasive bronchiolo-alveolar tumors were found only in H and h dogs; in two of 12 group h and group H dogs, respectively, which died, and eight of 12 group H dogs which were killed. One extended to and four into the pleura. Early invasive squamous cell carcinoma was found in bronchi of two of 12 group H dogs which were killed.

**CONCLUSIONS/COMMENTS:**

The smoking of cigarettes greatly increases the probability of development of noninvasive bronchiolo-alveolar tumors in male beagle dogs and that smoke from filter-tip cigarettes of the type used in this study is less potent in regard to tumor production than smoke from non-filter cigarettes of the type used.

No invasive tumors were found in the nonsmoking dogs, dogs smoking filter-tip cigarettes, and dogs which smoked half as many nonfilter cigarettes as were smoked by other smoking dogs. However, these types of tumors were found in 4 of 24 dogs that died after 626 to 753 days of smoking many nonfilter cigarettes and in eight of 12 dogs killed after 875 days of smoking nonfilter cigarettes. We conclude that the smoking of a large number of nonfilter cigarettes daily over two years can lead to the development of invasive bronchiolo-alveolar tumor in male beagles.

**# 6**
**AUTHOR(s):** Aviado, Domingo M. and Tetsuya watanabe

**DATE: 1974**

**TITLE:** FUNCTIONAL AND BIOCHEMICAL EFFECTS ON THE LUNG FOLLOWING INHALATION OF CIGARETTE SMOKE AND CONSTITUENTS. I. HIGH- AND LOW-NICOTINE CIGARETTES IN MICE

**CITATION: TOXICOLOGY AND APPLIED PHARMACOLOGY 30, 185-200 (1974)**

**STUDY DESIGN:**

Two strains of male mice (Swiss and ICR) were exposed to smoke twice a day, daily for either 5 or 10 weeks using a Walton horizontal smoke-exposure machine. Study was designed to examine the effects of the length of exposure (5 or 10 weeks), the nicotine content of the cigarettes (University of Kentucky 1R1 or 1A1 cigarettes), and the presence or exclusion of smoke particulate matter using Cambridge Filters on pulmonary function.

**RESULTS/FINDINGS:**

Daily inhalation of cigarette smoke for 5 or 10 wk elicited the following effects: (1) increase in pulmonary resistance; (2) decrease in functional residual capacity; (3) decrease in pulmonary compliance; (4) decrease in tidal volume; (5) no change in phospholipid content of the lung; and (6) increase in wet weight of the lung relative to body weight which was reduced. The increase in pulmonary resistance and the decrease in functional residual capacity were elicited by nonfiltered smoke as well as by the vapor phase, and their appearance was related to the nicotine content of the cigarettes and the duration of exposure. The decrease in pulmonary compliance was elicited by inhalation of nonfiltered smoke but not by the vapor phase. The decrease in tidal volume as well as the increase in pulmonary resistance, or bronchospasm, occurred more readily in ICT mice than in the Swiss mice. Both strains developed tolerance to bronchospasm after 10 wk of exposure. There was no increase in functional residual capacity and, hence, no functional sign of pulmonary emphysema in mice that had been exposed to cigarette smoke for 5 or 10 weeks.

**CONCLUSIONS/COMMENTS:**

The results indicate that the effects observed with pulmonary resistance and functional residual capacity are elicited by a combination of nicotine contained in particulate material and constituents of the vapor phase. The effect of smoke on pulmonary compliance indicates that the causative factor is in the particulate matter, probably nicotine, because the appearance of decreased compliance depended on the nicotine content of the cigarette. The ICR mice strain had a greater sensitivity to cigarette smoke compared to the Swiss strain. The phospholipid content of the lung remained unchanged in all of the mice, no matter the treatment. This suggest that chronic exposure to smoke does not reduce surfactant activity under the experimental conditions of this study.

#7
**AUTHOR(s):** AYRE, D.J., D. KEAST, AND J.M. PAPADIMITRIOU

**DATE: 1981**

**TITLE:** EFFECT OF TOBACCO SMOKE EXPOSURE ON SPLENIC ARCHITECTURE AND WEIGHT, DURING THE PRIMARY IMMUNE RESPONSE OF BALB/c MICE

**CITATION: Journal of Pathology 133:53-59 (1981)**

**STUDY DESIGN:**

Female mice BALB/c, 8-12 weeks of age were exposed once daily on weekdays to smoke of 30 cigarettes in a Hamburg II Small Animal Smoking Machine. High-tar, filtered cigarettes (16.0 mg tar, 1.1 mg nicotine/cigarette) were used in this study. Mice were exposed to smoke for 3days, or 18 or 28 weeks, prior to SRBC inoculation. Spleen weight changes were monitored 0, 1, 8 and 12 days post inoculation.

**FINDINGS/RESULTS:**

Mice exposed to tobacco smoke (TS) for 3 days or 18 or 28 weeks, prior to SRBC inoculation subsequently displayed less pronounced and/or `` shorter-lived'' splenomegaly than age matched controls. In addition mice exposed to TS for three days or 18 weeks displayed a reduction in both the magnitude and duration of the primary immune response as evidenced by the pattern of expansions of splenic white pulp and `` RNA-rich'' white pulp volumes. In contrast mice exposed to TS for 28 weeks, prior to inoculation, displayed white pulp and `` RNA-rich'' white pulp volumes similar to those of control mice.

**CONCLUSIONS/RESULTS:**

Cigarette exposed mice consistently exhibit a reduced splenomegaly following primary i.v. inoculation with SRBC. Similarly significant alteration of splenic architecture may be detected following both acute and chronic TS-exposure.

**#8**
**AUTHOR(s):** BAZIN, R., H. TURCOTTE, R. LAGACÉ AND M. BOUTET

**DATE: 1981**

**TITLE:** EFFETS CARDIOVASCULAIRES DE LA FUMÉE DE CIGARETTE CHEZ LE RAT

**CITATION: REV. CAND. BIOL 40: 263-276 (1981)**

**STUDY DESIGN:**

Seventy-two Sprague-Dawley rats were subjected to cigarette smoke for 1 day (acute effects), 2 weeks (sub-acute) and 15 weeks (chronic effects) and were sacrificed 5 minutes or 8 hours after smoking and the permeability of aortic endothelium and myocardial capillaries were examined using the diffusion tracer peroxidase.

**FINDINGS/RESULTS:**

Peroxydase was not generally present at the sub-endothelial level in the aortic endothelium of control animals and sham-smokers. However, in smokers, the increase in peroxidase permeability is proportional to the smoking period. Moreover, in these smokers we observed junctional and subendothelial vacuolar dilatations which correspond to degenerative morphologic changes. Cigarette smoke has a different effect on the right ventricle. Endothelial permeability of the myocardial capillaries increases in animals subjected to cigarette smoke for 1 day and those who smoked for 2 weeks sacrificed 5 minutes after smoking. This increase in permeability is not related to the significant morphologic changes observed in the myocardium and endothelial capillaries. However, the myocardial endothelial capillaries, the sub-endothelial space and the adjacent interstitial space were generally free of peroxidase in animals subjected to smoke for 15 weeks and those smoking 2 weeks sacrificed 8 hours later.

**CONCLUSIONS/COMMENTS:**

An adaptive phenomenon to the effects of cigarette smoke appears to exist in the myocardial endothelium capillaries contrary to that which is observed in the thoracic aorta.

**#9**

**AUTHOR(s): BENDDICT, WILLIAM F., ASHUTOSH BANERJEE, KEN K. KAGALINGAM, DAVID R. DANSIE, RICHARD E. KOURI AND CAROL J. HENRY**

**DATE: 1984**

**TITLE:** INCREASED SISTER-CHROMATID EXCHANGE IN BONE MARROW CELLS OF MICE EXPOSED TO WHOLE CIGARETTE SMOKE.

**CITATION: MUTATION RESEARCH 136 (1984) 73-80**

**STUDY DESIGN:**

BC3F1/Cum female mice were exposed nose-only to cigarette smoke using a large-capacity Smoke Exposure Machine (SEM II). Kentucky reference 2R1 and 3A1 cigarettes were used throughout these experiments, with the smoke being diluted to 10% (v/v) under the following conditions: 15 sec smoke, followed by 45 sec of air for each minute for 126 consecutive minutes on a daily basis for 1 week and up to 46 weeks. Control animals were both cage control and sham controls. Studies were scheduled so that all cytogenetic observations were made 2-3 days after the last smoke exposure.

**RESULTS/FINDINGS:**

Exposure of BC3F1/Cum mice to whole cigarette smoke significantly increased the number of SCE' s in bone marrow compared to sham-exposed controls. After exposure of mice to smoke for 1 week, the number of SCE' s was significantly ($p<0.05$) increased in 2R1 and 3A1 cigarette smoke exposed animals compared to sham-exposed animals, and this increase could be reproduced in two separate experiments. Differences between animals exposed to the two types of cigarette smoke were not significant. No increase in SCE' s was observed in the sham-exposed animals relative to the untreated, shelf-control animals.

Continued exposure to cigarette smoke for 4, 12, and 46 weeks showed similar significant increases in SCE in the smoke exposed mice. And again, no significant differences between mice exposed smoke from either cigarette type or between the sham-exposed and shelf-control animals were found at any of these time points. The persistence of smoke-induced increase in SCE' s was examined in two groups of mice, one exposed for 1 week and a second group exposed to smoke for 46 weeks. Significant increases in SCE' s persisted after cessation of smoke exposure for mice exposed to smoke for either 1 week or 46 weeks compared to sham-exposed mice.

**CONCLUSIONS/COMMENTS:**

This is the first demonstration of the induction of SCE' s in laboratory animals that have been exposed to smoke in vivo.

These data from this study suggest either that those cells damaged by smoke exposure are not eliminated but continue to function and turnover at normal rates, or that certain smoke constituents may be retained and continue to exert their effects.

**# 10**
**AUTHOR(s):** BERNFELD, PERER, F. HOMBURGER, AND E. SOTO

**DATE: 1983**

**TITLE:** SUBCHRONIC CIGARETTE SMOKE INHALATION STUDIES IN INBRED SYRIAN GOLDEN HAMSTERS THAT DEVELOP LARYNGEAL CARCINOMA UPON CHRONIC EXPOSURE.

**CITATION: JNCI 71 (1983) 619-623**

**STUDY DESIGN:**

Male BIO• 15.16 Syrian golden hamsters were exposed to smoke from each of three types of cigarettes, as well as sham smoking conditions, for total durations of 6,9,12, 15, and 20 weeks, using the modified reverse Walton smoking machine. Smoke exposure was intermittent: 27 sec of 22% cigarette smoke alternated with 33 sec of fresh air. Each hamster exposed to 12 minutes of this regimen, twice a day (4 hours between sessions), 7 days per week. The three types of experimental cigarettes employed were: 1) type A, a flue-cured, all tobacco cigarette found on the open market in the UK around 1975; 2) type B, a cigarette made of 100% Cytrel, and 3) a cigarette that consisted of 1:1 blend of the type A and B smoking materials. All cigarettes were filter-tipped.

**FINDINGS/RESULTS:**

The incidence and severity of laryngeal hyperplasia increased in these hamsters, a few (2) laryngeal papillomas appeared, alveolar macrophages became more frequent and aggregated, and hyperplasia of terminal bronchiolar epithelium occurred. This subchronic response of hamster to smoke markedly differed for the three types of cigarettes. Statistical evaluation of the data by log linear models proved these differences to be significant. At equal doses of smoke, the most severe response was caused by an all-tobacco cigarette. The weakest subchronic effects next to those seen in the negative control group, were elicited by smoke from a cellulose-derived tobacco supplement. The effects of smoke from a 1:1 blend of the two smoking materials were intermediate. The severity of the subchronic response of the respiratory tract paralled the extent of malignant transformations of the larynx previously observed in the same animal model with the same three types of cigarettes in chronic inhalation studies.

**CONCLUSIONS/COMMENTS:**

Although there is no evidence to assume that the short term phenomena observed were precancerous nature, the obvious parallelism between the subchronic and chronic response for the three types of cigarettes strongly suggest that the short term results may be of predictive nature for what will happen with a given type of cigarette upon chronic smoke exposure of hamsters.

**# 11**
**AUTHOR(s): BERNFELD, P., F. HOMBURGER, AND A.B. RUSSFIELD**

**DATE: 1974**

**TITLE: STRAIN DIFFERENCES IN THE RESPONSE OF INBRED SYRIAN HAMSTERS TO CIGARETTE SMOKE INHALATION**

**CITATION: JOURNAL OF THE NATIONAL CANCER INSTITUTE 53(1974) 1141-1151**

**STUDY DESIGN:**

Male hamsters, 102 from each of 2 inbred hamster lines (BIO 87.20 and BIO 15.16) were exposed to cigarette smoke (Kentucky reference 1R1) twice a day, 5 days/week for up to 100 weeks, in a modified Walton reverse-smoking machine. Sixty each of sham and cage control were used for each strain.

**RESULTS/FINDINGS:**

Smoke exposure for up to 100 weeks had no effect on mortality in either strain, but reduced body weight. Carboxyhemoglobin levels increased markedly immediately after each smoke exposure but returned to baseline levels in less than 24 hours. Serum triglyceride levels and virus profiles of smoke-exposed animals were unchanged. Chronic smoke exposure increased relative weight of the lungs and heart of both strains, but to different degrees. Over 90% of the smoke-exposed animals of both strains showed hyperplastic or neoplastic changes in the larynx. However, microinvasive cancer was nearly 5 times more frequent in the BIO15.16 strain than in the BIO87.20 strain. In the inbred line more susceptible laryngeal hyperplasia, 2 animals developed naso-pharyngeal tumors, one of which was malignant. Smoke exposure induced rare benign squamous papillomas in the air passages of both strains. The strain less susceptible to laryngeal hyperplasia exhibited more pulmonary adenomatosis, but its incidence was not significantly affected by smoke exposure. Clumping of pulmonary macrophages was proof that smoke had reached the lungs; 1 strain was more susceptible to this phenomenon than the other. In neither strain did smoke exposure affect the incidence of tumors arising outside the respiratory tract or the degenerative changes characteristic of aging hamsters.

**CONCLUSIONS/COMMENTS:**

The point of greatest practical importance to emerge from out work is the striking differences among various lines of hamsters with respect to susceptibility to acute toxic effects of smoke and to hyperplastic response of the larynx to smoke. Animals of the inbred BIO 15.16 line have both the highest resistance to smoke or nicotine toxicity and the greatest laryngeal susceptibility – qualities greatly increasing the sensitivity of the model.

#12
**AUTHOR(s): BERNFELD, P., F. HOMBURGER, E. Soto, and K.J. Pai**

**DATE: 1979**

**TITLE: CIGARETTE SMOKE INHALATION STUDIES IN INBRED SYRIAN GOLDEN HAMSTERS**

**CITATION: JOURNAL OF THE NATIONAL CANCER INSTITUTE 63(1979) 675-689**

**STUDY DESIGN:**

Male B1O' 15.16 Syrian golden hamsters were exposed to smoke from each of three types of cigarettes, as well as sham smoking conditions, for 59-80 weeks, using the modified reverse Walton smoking machine. Smoke exposure was intermittent: 27 sec of either 11 or 22% cigarette smoke alternated with 33 sec of fresh air. Each hamster exposed to 12 minutes of this regimen, twice a day (4 hours between sessions), 7 days per week. The three types of experimental cigarettes employed were: 1) all tobacco cigarette 2) a cigarette made of 100% Cytrel, and 3) a cigarette composed of 80% tobacco and 20% Cytrel, and 4) a cigarette made up of 50% tobacco and 50% Cytrel. All cigarettes were filter-tipped.

**RESULTS/FINDINGS:**

Invasive carcinoma of the larynx was induced in 36.8% of inbred Syrian golden hamsters from strain B1O' 15.16, susceptible to this type of cancer when exposed to smoke from reference filter cigarettes for 59-80 weeks. Nearly half (47.4%) showed laryngeal cancer, including noninvasive carcinoma and carcinoma in situ, which occurred at smoke concentrations of 22%. When the smoke concentration was reduced to 11%, the number of induced lesions was reduced proportionately. When a portion of tobacco was replaced in the cigarettes with Cytrel, a reduction of carcinogensis proportionate to the Cytrel content of the cigarette took place. Smoke from cigarettes containing only Cytrel and no tobacco induced no carcinomas under the conditions used. Other dose-related changes observed were laryngeal papillomas, laryngeal epithelial hyperplasia, tracheal epithelial hyperplasia, and metaplasia and accumulation of alveolar macrophages. Tar deposition in lungs and larynges was determined in a separate study by means of a marker, decacholrobiphenyl, added to the cigarettes. Admixture of Cytrel to cigarettes reduced tar deposition in the respiratory tract, which paralleled the decrease in the incidence of laryngeal carcinoma. However, the amounts of tar deposited in the larynx when 100% Cytrel was smoked were still significant, even though no carcinomas were observed.

**CONCLUSIONS/COMMENTS:**

Smoke from Cytrel cigarettes may be less carcinogenic than equal amounts of tobacco smoke.

**# 13**
**AUTHOR(s): BERNFELD, E. Soto, and T.C. TSO**

**DATE: 1984**

**TITLE: THE EFFECTS OF TOBACCO MODIFICATION ON BIOLOGICAL RESPONSE TO 13-WEEK CIGARETTE SMOKE INHALATION IN INBRED SYRIAN HAMSTERS.**

**CITATION: JOURNAL OF THE NATIONAL CANCER INSTITUTE 63(1979) 675-689**

**STUDY DESIGN:**

Male BIO¹ 15.16 Syrian golden hamsters were exposed to maximally tolerated doses of smoke for 13 week from 12 types of investigational cigarettes, using the modified reverse Walton smoking machine. Smoke exposure was intermittent: 27 sec of 22% cigarette smoke alternated with 33 sec of fresh air. Each hamster exposed to 12 minutes of this regimen, twice a day (4 hours between sessions), 7 days per week. The experimental cigarettes used were as follows; 3 types of Bright and 3 types of Burley tobacco with varying leaf nicotine content (0.2,1.0 and 1.5 % for Bright) and (0.4, 1.0 and 1.5% leaf nicotine for Burley). Burley tobacco deproteinized with untreated Burley tobacco as control, maleic hydrazie field treated Bright tobacco (80 ppm HM), maleic hydrazied-spiked Bright tobacco (1000ppm MH) with hand-suckered Bright tobacco as control to the 2 MH treated tobaccos, and, as a positive control group, 2R1 Kentucky reference cigarettes.

**RESULTS/FINDINGS:**

Twice daily exposure for 13 weeks resulted in the following response: increased occurrence and aggregation of alveolar macrophages, increased occurrence of tracheal squamous metaplasia, and increased frequency and severity of laryngeal hyperplasia. The statistical significance of the changes was evaluated by means of loglinear models. The effect of varying the nicotine level in tobacco leaf between 0.2 and 1.5% caused no statistically significant changes in the subchronic response. Smoke from Bright tobacco with average tar yield of from 22.4 to 26.2 mg/cigarette caused significantly more alveolar macrophages and laryngeal hyperplasia but less tracheal squamous metaplasia than did smoke from Burley tobacco, with 9.3 to 10.5 mg tar per cigarette. Deporteiniaztion of tobacco did not change the response of the hamsters to the resulting smoke. Maleic hydrazide -field treatment of tobacco significantly reduced the alveolar macrophages, but spiking of tobacco with maleic hydrazide increased the response.

**CONCLUSIONS/COMMENTS:**

The present results suggest that smoke from low-nicotine cigarettes is not less tumorigenic in the hamster than that from high-nicotine cigarettes, that smoke from high-tar Bright is more carcinogenic than that from low-tar Burley tobacco, that deproteinization of tobacco does not affect the tumorigenicity of the resulting smoke, and that maleic hydrazide-field treatment of tobacco does not increase the tumorigenicity of the resulting smoke, whereas spiking of tobacco with maleic hydrazied might do so.

**# 14**
**AUTHOR(s): Boutet, M., M. BAZIN, H. TURCOTTE, R. LAGACÉ**

**DATE: 1980**

**TITLE: EFFECTS OF CIGARETTE SMOKE ON RAT THORACIC AORTA**

**CITATION: ARTERY 7(1):56-72 (1980)**

**STUDY DESIGN:**

Using the fine structural macromolecular tracer horseradish peroxidase the permeability of thoracic aorta was studied by light and electron microscopy in male Sprague-Dawley rate. Rats were exposed to cigarette smoke for 1 day (acute effect), 2 weeks (sub-acute effect) and 15 weeks (chronic effect), each animal smoking 2 cigarettes/day (25 mg tar, 1.5 mg nicotine), 5 days/week, each cigarette lasting for 8 minutes. Sham smokers were introduced into the smoking machine but not exposed to smoke.

**FINDINGS/RESULTS:**

Thoracic aorta sections from control rats and sham smokers were free of HRP reaction product. In smokers' thoracic aortas, permeability to peroxidase increased proportionately to the increase in length of the smoking period. Additionally, smokers' thoracic aortas exhibited focal vacuolar dilatations (subendothelial blebs) and an increase number of subendothelial macrophages.

**CONCLUSIONS/COMMENTS:**

Cigarette smoke has a progressive and chronic effect on rat endothelial permeability within thoracic aorta, consisting of increased permeability to the macromolecule peroxidase. Cigarette smoke may be associated with morphological changes characterized by vesicular dilatations of the subendothelial space, and by the accumulation of macrophages within the endothelial space.

**# 15**
**AUTHOR(s): brooks, wesley w., oscar h.l. bing, gary l. huber and walter h. abelmann**

**DATE: 1982**

**TITLE: EFFECTS OF CIGARETTE SMOKE ON RAT THORACIC AORTA**

**CITATION: ARCHIVES OF ENVIRONMENTAL HEALTH 37(2):93-97 (1982)**

**STUDY DESIGN:**

Eight male Charles River DC rats were subjected to tobacco smoke from Kentucky reference cigarettes (2R1) for periods of 10 min/hr for 5 hr/day for 180 days. Nineteen additional rats served as either sham-smoked controls or weight-matched, food deprived controls.

**RESULTS/FINDINGS:**

Rats exposed to tobacco smoke had a significant diminution n body and left ventricular weight compared to sham-smoked controls. When compared to food-deprived rats, no differences in weights were observed. Contraction mechanics were measured for each muscle at the peak of its length tension curve. No significant difference in cardiac muscle performance was found in rats exposed to tobacco smoke when compared to control animals with respect to contractile performance under oxygenated conditions, muscle performance during 60 min of hypoxia or subsequent reoxygenation, or sensitivity of mechanical per4formance to isoproterenol.

**CONCULSIONS/COMMENTS:**

Chronic cigarette smoke exposure did not alter the intrinsic mechanical performance of isolated rat ventricular muscle.

**# 16**
**AUTHOR(s): cHALMER, jANE, p.g. hOLT AND d. kEAST**

**DATE: 1975**

**TITLE: CELL-MEDIATED IMMUNE RESPONSES TO TRANSPLANTED TUMORS IN MICE CHRONICALLV EXPOSED TO CIGARETTE SMOKE**

**CITATION: JNCI 55(5) 1129-1134 (1975)**

**STUDV DESIGN:**

C57BL AND BABL/c mice were exposed to fresh cigarette smoke from king-size filter cigarettes (1.1 mg nicotine, 16 mg tar) for 7-8 minutes per day for varying periods up to 30 weeks before subcutaneous or intratracheal inoculation of viable tumor cells.

**RESULTS/FINDINGS:**

The growth rate of subcutaneous tumors in the mice exposed to smke were significantly higher than those of controls and more lung metastases were noted. Enhanced tumor growth rates in the respiratory tracts of smoke-exposed mice were evidenced by the markedly increased death rates in these animals after intratracheal inoculation of tumor cells. Increased tumor growth rates in mice that inhaled smoke were associated with depressed tumor-specific cytotoxic responses in both spleens and regional lymph nodes. Short-term exposure (10 weeks) or mice to cigarette smoke resulted in decreased tumor growth rates concomitant with enhanced cytotoxic responses.

**CONCLUSIONS/COMMENTS:**

The growth rat of subcutaneously established B16 melanoma was lower in animals that had undergone short-term exposure than in appropriate controls. Furthermore, spleen cell cytotoxicity developed more rapidly in the former than in their controls, suggesting that the lowered tumor growth rate may be due to enhanced cell-mediated immune responsiveness.

**# 17**
**AUTHOR(s): chameaud, j., r. perraud, j. chrÉtien, r. masse, and j. lafuma**

**DATE: 1982**

**TITLE: lung carcinogensis during in vivo cigarette smoking and radon daugher exposure in rats.**

**CITATION: RECENT ADVANCES IN CANCER RESEARCH 82: 11-20 (1982)**

**STUDY DESIGN:**

This study was designed to examine the synergistic effect of cigarette smoke with radon exposure. The doses of radon and its daughters chosen were 4,000, 500, and 100 work level months (WLM). The smoke concentration (nine [Gauloises Bleues brand] per 500 l air) was chosen so that the rats (Sprague-Dawley) were given 10-15 min inhalation sessions daily. The rats were exposed to some for 1 year, 4 days per week.

**RESULTS/FINDINGS:**

The synergistic action of cigarette smoke inhaled after exposure to cumulative doses of radon and its daughters of 4,000 WLM or of 500 WLM has been demonstrated in these two groups, since the number of cancers was significantly increased and the tumors were more invasive and metastatic. At 100 WLM one cancer and one suspicious adenomatosis were observed in smokers, as opposed to none in non-smokers. The synergistic effect could not be demonstrated at this exposure level. The combined effect of cigarette smoke and radon is not additive, since cancer was not found in rats exposed only to smoke. Blood carbon monoxide level in animals during smoke exposure was 0.6%. The histological type of cancer was not altered by smoke. The smoker and nonsmoker groups showed approximately 75% of epidermoid carcinoma and 20% of adenocarcinoma with a few bronchoalveolar and undifferentiated carcinomas.

**CONCLUSIONS/COMMENTS:**

In previous experiments covering a range of doses from 60 WLM to 12,000 WLM we established the shape of the dose-effect relationship for radon alone. In the present experiments the carcinogenic action of tobacco smoke associated to radom was clearly demonstrated.

**# 17**
**AUTHOR(s): Von Chevalier H.J. and W. Dontenwill**

**DATE: 1972**

**TITLE: Experimentelle Untersuchungen Über die ablagerung von inhalierten partikeln im kehlkopf von syrischen goldhamstern**

**CITATION: z. versuchstierk 14: 271-276 (1972)**

**SUMMARY:**

Syrian Golden hamsters were exposed to an aerosol of either crystal violet or methylene blue-solution or Sudan-red-B-powder (particle size 0.5-5 mm). Deposition of dyestuff in the respiratory tract is far more found at the base of the epiglottis than in the ventral meatus of the nose, the free margins of the vocal cords or the tracheal or bronchial bifurcations. Only in the region of the epiglottis staining is intensifying on continuous exposure.The extraordinary high particle deposition at the base of the epiglottis may lead back to the course of the inspiratory tract which impacts this localization. Alterations of the laryngeal mucosa due to inhalation generally occur at the base of the epiglottis.

**# 19**
**AUTHOR(s): coggins, c.r.e., c. musy and r. ventrone**

**DATE: 1982**

**TITLE: changes in the minute ventilation of rats exposed to different concentrations of cigarette smoke**

**CITATION: toxicology letters 11:181-185 (1982)**

**STUDY DESIGN:**

Male Sprague-Dawley rats were exposed on a Battelle Mk III smoking machine at smoke dilutions (3.8 and 7.6%) which resulted in concentrations of TPM at the rat nose of 1000 and 2000 mg/l respectively, for low- and high-dose groups.

Animals were exposed to a single 30 min period of smoke inhalation, 5 days per week. Measurements of tidal volume and breathing frequency were made after animals had been exposed for 8 weeks. The cigarette contained a flue-cured tobacco blend typical of that found in commercially available U.K. cigarettes. Breathing frequency and tidal volume were measured in 4 animals simultaneously during the exposure period and for 10 min prior to exposure.

**FINDINGS/RESULTS:**

Both tidal volume and breathing frequency were decreased in low-dose exposure to smoke, resulting in a 34% decrease in minute volume. At high-dose exposures (double the low-dose smoke concentration) tidal volumes showed a 23% increase over pre-exposure values with little chnages in breathing frequency, giving a net 20% increase in minute ventilation.

**CONCLUSIONS/COMMENTS:**

The results presented here indicate an apparently inverse dose-relationship in that the response at low-dose exposure was a reduction in minute ventilation, whereas a greater ' insult' resulted in increased ventilation. The authors suggest that possibly vapor phase constituents such as formaldehyde and acrolein might be involved the minute volume response and could possibly explain the inverse relationship observed in this study. The authors state that it is clear that the amount of TPM inhaled by the two groups are not in the same ratio as are the amounts of TPM presented. They therefore consider that depositions studies are pre-requisites when attempting to compare the responses produced in rodents by inhalation exposures.

**# 20**
**AUTHOR(s): coggins, and r. ventrone**

**DATE: 1986**

**TITLE: changes in the minute ventilation of rats exposed to the vapor phase of diluted cigarette smoke.**

**CITATION: THE toxicologIST (aBSTRACTS OF THE 25'' aNNIVERSARY mEETING VOL 6, no. 1, MARCH 1986**

**abstract:**

Cigarette smoke is composed of particulate matter, vapors/gases and semi-volatiles, that fraction of the whole smoke which passes through a Cambridge filter being defined as `` vapor phase'' . Exposures of rats to dilute cigarette smoke can result in substantial changes in breathing pattern, but it is not known which of the above components of whole smoke are responsible for these changes. Rats were exposed nose-only to diluted cigarette smoke, filtered in one group to remove the particulate matter. Comparisons were made with unfiltered smoke. Using whole-body plethysmography and pneumotachograph, estimates were made of breathing frequency and tidal volume during exposure to smoke and during air breaks before and after smoke exposure. Similar depressions (up to 37% when compared with air values) were noted in both filtered and unfiltered smoke groups, indicating that the particulate phase of the smoke is not involved in the response. Suggestions are made on the likely causation of the response, and reference is made to the importance of minute ventilation in inhalation studies with laboratory animals.

#22
**AUTHOR(s): coggins, c.r.e., r. IAM, AND K.T. MORGAN**

**DATE: 1982**

**TITLE: CHRONIC INHALTION STUDY IN RATS, USING CIGARETTES CONTAINING DIFFERENT AMOUNTS OF CYTREL TOBACCO SUPPLEMENT**

**CITATION: toxicology 22:287-296 (1982)**

**STUDY DESIGN:**

Male and female Sprague-Dawley rats were exposed to whole smoke diluted with air using Battelle Mk III smoking machines. Smoke dilutions of 1.47, 1.75, 1.94 and 5.41% were examined. Exposures were for 1 h/day, with a 15-min pause after the first ½ hr., 7 days/week for a total of 18 months. Cytrel was incorporated at 0, 25, 50 and 100% inclusion rates into cigarettes, the balance being a flue-cured tobacco blend typical of that found in commercial U.K. cigarettes. Two control groups were included in this experiment, sham controls, and room controls.

**FINDINGS/RESULTS:**

The changes observed in the respiratory tracts of animals exposed for 18 months to smoke from all-tobacco cigarettes were remarkably similar to those induced by much shorter exposure periods. These results indicate that the changes induced are not progressive after about 3 months. It has been suggested that squamous metaplasia may be a pre-neoplastic lesion, but this lack of progression suggest that his is no the case at least under the conditions of the current study.

Responses for Cytrel blends at smoke concentrations higher than the concentration used for tobacco alone were no greater than those produced by all-tobacco cigarettes, and the incidence of metaplastic changes in both trachea and alveoli was in general decreased with increasing inclusion of Cytrel in the blend. In the present study the animals in the different groups were exposed to similar amount of PMWNF (particulate matter water and nicotine free (" tar" )), and yet markedly different grades of the same responses were seen, the decrease in response being approximately proportional to the level of Cytrel inclusion. Thus there is some evidence to show that in animals not only the quantity but also the quality of delivered PMWNF can influence the tissue response.

**CONCLUSIONS/COMMENTS:**

On the basis of this chronic rat inhalation test which shoed decreasing responses I the respiratory tract with increasing inclusions of Cytrel in the blend, and no novel effects attributable to Cytrel, we conclude that there should be no objection to blending Cytrel with tobacco in smoking products.

#23
**AUTHOR(s): COHEN, BEVERLY S., NAOMI H. HARLEY, AND T.C. TSO**

**DATE: 1985**

**TITLE: CLEARANCE OF POLONIUM-210 ENRICHED CIGARETTE SMOKE FROM THE RAT TRACHEA AND LUNG**

**CITATION: TOXICOLOGV AND APPLIED PHARMACOLOGV 79, 314-322 (1985)**

**STUDV DESIGN:**

Female Fischer rats were exposed (nose only) daily for 6 months to smoke (10% v/v) form cigarettes with 500 times the normal content of **Po. Control rats were exposed to smoke from 1R1 cigarettes. Animals were serially withdrawn and killed. After necropsy the trachea, major bronchi, larynx, and nasopharynx were examined for surface a activity by an etched track technique utilizing cellulose nitrate detectors.

**FINDINGS/RESULTS:**

Areas of accumulated activity were seen on samples of larynx from rats exposed to **Po-enriched cigarettes. No other local accumulations were seen on the airways. The lower lungs were analyzed radiochemically for **Po. The pulmonary burden increased from 4.3 pCi/g at 4 weeks after the start of smoking to 34.1 pCi/g at 6 months. The burden declined to about 1.2 pCi/g at 11 months or 5 months after cessation of exposure. Track analyses for the airways, alveolar tissue, larynx, and nasopharynx were conducted. The net track density seen at the larynx during the exposure period was much higher than for any of the other specimens. Many of the laryngeal specimens showed accumulations that were more than an order of magnitude greater than the densities seen on any other samples. Nasopharynx samples also showed high concentrations of activity during the exposure period. No areas of high local activity were seen when the airway surfaces were examined. Concentration gradients along individual airway samples were not observed. Following withdrawal from smoking, both short- and long-term clearance components were seen. The parameters which fit the postexposure data for clearance for the lung burden cannot fit the buildup during the exposure period.

**CONCLUSIONS:**

The measured pulmonary 210Po burden has a much steeper slope during the initial phase of smoking the enriched cigarettes than the model predicts with two compartments. At cessation of smoking, the measurements show a rapid decline followed by a longer half-life compartment. No combination of half-times which fit the removal phase will provide an adequate fit to the build-up phase.

#24
**AUTHOR(s): CROSS, F.T., R.F. PALMER, R.E. FILIPY, G.E. DAGLE, AND B.O. STUART**

**DATE: 1982**

**TITLE: CARCINOGENIC EFFECTS OF RADON DAUGHTERS, URANIUM ORE DUST AND CIGARETTE SMOKE IN BEAGLE DOGS**

**CITATION: HEALTH PHYSICS 42(1) 33-52 (1982)**

**STUDY DESIGN:**

Sixty-nine male and female beagle dogs were randomly assigned to 4 experimental groups for exposures: (1) Radon (105 ± 20 nCi/l), radon daughters (605 ± 169 WL), and uranium ore dust (12.9± 6.7 mg/m$^3$); (2) Radon, radon daughters, uranium ore dust and cigarette smoke; (3) cigarette smoke (1R1 cigarettes) (4) room air.

**FINDINGS/RESULTS:**

Chronic exposure to mixtures of these agents caused significant lifespan shortening when compared to the controls. Survival times of controls and smoke-exposed dogs were equivalent during the 4 to 5 year mean survival time of the dogs exposed to randon-daughter and ore-dust mixtures (with or without added cigarette smoke).

Animals with tumors of the respiratory tract generally had cumulative radon-daughter exposures exceeding 13,000 WLM, and their survival time was longer than the survival time of non-tumor bearing animals. Under the conditions of the experiment, exposure to cigarette smoke was found to have a mitigating effect on radon daughter-induced tumors.

Exposures to smoke from 10 cigarettes/d, 7 d/wk produced no significant respiratory tract lesions. However, exposure to 20 cigarettes/d, 7 d/wk resulted in pulmonary emphysema, fibrosis and chronic bronchitis and bronchiolitis.

Emphysema and fibrosis were much more prevalent and severe in the dogs exposed to mixtures which included radon daughters and uranium ore dust. These dogs also had adenomatous lesions which progressed to squamous metaplasia of aleveolar epithelium, epidermoid carcinoma and bronchioalveolar carcinoma. Pathologic changes in the airways of these dogs were most prominent in the nasal mucosa, and included a few squamous carcinomas in the nasal cavity.

**CONCLUSIONS/COMMENTS:**

The authors conclude that the beagle dog is a useful animal for modeling pulmonary lesions produced by uranium mine air contaminants. Tumors were produced at levels that did not greatly exceed some exposures reported for uranium miners. These tumors, found after approximately 50 months of exposure, might partially account for the absence of tumors in experiments where exposures were terminated before 50 months.

#25
**AUTHOR(s): DALBEY, WALDEN, PAUL NETTESHEIM, RICHARD GRIESEMER, JOHN E. CATON, AND MICHAEL R. GUERIN**

**DATE: 1980**

**TITLE: CHRONIC INHALATION OF CIGARETTE SMOKE BY F344 RATS**

**CITATION: JNCI 64(2) 383-390 (1980)**

**STUDY DESIGN:**

Female F344 rats were exposed by inhalation using the intermittent Maddox/ORNL smoking machine. Smoke was diluted to 10 % with air, National Cancer Institute Code 16 experimental cigarettes were used for all exposures. Eighty rats received lifetime exposure to 7 cigarettes/day, 5 days/week. Cage and sham controls were part of the study design.

**FINDINGS /RESULTS:**

Total pulmonary deposition of smoke particulates from a single cigarette was 0.25 mg in young rats. Rats were exposed to smoke for as long as 2.5 years, at which time 30% of the rats remained alive. Mortality of smoke-exposed animals was not different from that of the untreated or sham-exposed controls. Hyperplastic and metaplastic areas in the epithelium of the nasal turbinates, larynges, and tracheae of exposed animals were observed at death. The lungs accumulated pigmented macrophages, epithelial hyperplasia, fibrosis, and disrupted alveolar structure. Smoke exposure did not change the total number of tumor bearing animals relative to the controls: however, exposed rats has significantly fewer tumors in the hypophyses, hematopoietic-lymphoid systems, uteri, and ovaries but an increased number of tumors in the respiratory tracts and dermes. Only 1 of 93 (1%) control rats had a tumor (an alveologenic carcinoma) in the respiratory tract as opposed to 7 of 80 (9%) exposed animals(nasal tumors: 1 adenocarcinoma and 1 squamous cell carcinoma; pulmonary turmos: 5 adenomas, 2 alveologenic carcinomas, and 1 squamous carcinoma

**CONCLUSIONS:**

Studies with SPF F344 rats showed that significant damage could be induced in various parts of the respiratory tract by lifetime exposure to tobacco smoke. This damage consisted of hyperplastic and metaplastic epithelial lesions in the upper airways and of focal alveolitis and alveolar fibrosis in the lungs. The incidence of respiratory tract tumors was significantly elevated in smoke-exposed rats.

**# 26**
**AUTHOR(s): DAVIS, B.R., J.K. WHITEHEAD, M.E. GILL, P.N. LEE, A.D. BUTTERWORTH, AND F.J.C. ROE**

**DATE: 1975**

**TITLE: RESPONSE OF RAT LUG TO TOBACCO SMOKE CONDENSATE OR FRACTIONS DERIVED FROM IT ADMINISTERED REPEATEDLY BY INTRATRACHEAL INSTILLATION**

**CITATION: BRITISH J. CANCER 31: 453-461 (1975)**

**STUDV DESIGN:**

This study concerns the effects of cigarette smoke condensate (SWS) and of 6 different fractions derived from it administered by intra-tracheal instillation ininfusine. Condensate was collected from cigarettes specially manufactured from a composite blend of blue-cured tobacco, representing the major plain cigarette brands in the UK during 1967-1968. The fractions of whole smoke condensate used in these experiments were the polycyclic aromatic hydrocarbon rich materials separated by the application of procedures designed to concentrate them. The fractions were: Neutral (NF); G; L(G); L; (R+P)G and P(SG). Treatments for 10 of the groups was limited to 18 once-fortnightly instillations. In all other cases, one fortnightly instillations were continued throughout life.

**FINDINGS/RESULTS:**

The repeated intratracheal instillation of cigarette smoke condensate in rats at close to maximum tolerated dose levels failed to induce squamous neoplasms in the lungs although such treatment was associated with an increased incidence of cuboidal/columnar metaplasia (CCM) and squamous metaplasia (Sq.M) of alveolar epithelium.

With one exception, various fractions of SWS had no effect on lung tumor incidence though some were more effective than SWS in increasing the incidence of CCM and Sq.M.

The exceptional fraction, Fraction P, which contains most of the polycyclic aromatic hydrocarbons of smoke, gave rise to 4 squamous tumours of doubtful malignancy and one metastasizing squamous carcinoma among 3 groups of 18 animals exposed at 3 different dose levels.

**CONCLUSIONS/COMMENTS**:

The fact that no lung tumours arose in response to repeated intratracheal instillation of SWS at close to maximum tolerated doses provided no encouragement for the view that it might be possible to compare condensates derived from different tobaccos for carcinogenicity by the intratracheal instillation method in rats. However, the comparison of fractions seems still to remain a feasible proposition.

#27
**AUTHOR(s): DAVIS, B.R., J.K. WHITEHEAD, M.E. GILL, P.N. LEE, A.D. BUTTERWORTH, AND F.J.C. ROE**

**DATE: 1975**

**TITLE: RESPONSE OF RAT LUNG TO INHALED VAPOUR PHASE CONSTITUENTS(VP) OF TOBACCO SMOKE ALONE OR IN CONJUNCTION WITH SMOKE CONDENSATE OR FRACTIONS OF SMOKE CONDENSATE GIVEN BY INTRATRACHEAL INSTILLATION**

**CITATION: BRITISH J. CANCER 31: 462-468 (1975)**

**STUDY DESIGN:**

This study describes the effects of exposure to vapour phase of cigarette smoke on the lungs of rats and was designed to see whether exposure to VP alters the response of rat lung to the intratracheal instillation of cigarette smoke condensate or its fractions. Rats used were female Wistar, 13-14 weeks old at the start of the experiment. The rats were exposed to VP in an apparatus known as the `` Harrogate Smoker'' , with the smoke being passed through a Cambridge filter to give the VP. The rats were given cigarette smoke condensate in solid form without a vehicle once fortnightly by intratracheal instillation, at 3 dose levels with or without additional exposure to VP of smoke from 10 plain cigarettes once each week. Treatment continued for life. Six other groups were treated with one of 3 fractions (G, (R+P)G and P(SG)of condensate with or without VP.

**RESULTS/FINDINGS:**

Exposure to VP was associated with a significant reduction in body weight, but not significantly with the incidence or severity of any observed pathological change in the lungs.

A significant dose-related association was seen between SWS or its fractions and the incidence and degree of chronic respiratory disease (CRD), cuboidal or columnar metaplasia (CCM) and squamous metaplasia of alveolar epithelium (Sq.M) produced. No neoplasms were elicited. A significant correlation was found between the degrees of CCM and Sq.M in the 24 groups exposed to SWS or fractions.

**CONCLUSIONS/COMMENTS:**

The results provide convincing evidence that, under the conditions of the experiment, exposure to VP did not increase the incidence of any kind of neoplasm at any body site.

**# 28**
**AUTHOR(s): DAVIS, B.R., J.K. WHITEHEAD, M.E. GILL, P.N. LEE, A.D. BUTTERWORTH, AND F.J.C. ROE**

**DATE: 1975**

**TITLE: RESPONSE OF RAT LUNG TO INHALED TOBACCO SMOKE WITH OR WITHOUT PRIOR EXPOSURE TO 3,4-BENZPYRENE (BP) GIVEN BY INTRATRACHEAL INSTILLATION**

**CITATION: BRITISH J. CANCER 31: 469-484 (1975)**

**STUDY DESIGN:**

The main objective of the experiment was to see whether squamous cancers of the lung could be induced by repeated exposure of rats to tobacco smoke throughout their life-span using the Harrogate Smoker. A subsidiary objective was to see whether squamous cancer would arise in response t a single intratracheal dose of benzo(a)pyrene followed by life-long exposure. Female Wistar rats were exposed with the smoking machine adjusted to take one puff of 25 ml and 2 sec duration once very min into a chamber containing 100 ml air and to hold the 1:5 smoke air mixture in the chamber for a period of 15 sec. Rats were exposed to the smoke of one cigarette twice each day, 5 days/week for the whole of their lives. Two further groups were given a single dose of 3,4-benzpyrene (BP) by intratracheal instillation. One of these was then exposed to the smoke of 10 cigarette per week till death.

**RESULTS/FINDINGS:**

Compared with untreated or sham exposed rats, exposure to smoke was associated with a significant reduction in incidence of mammary tumours. Exposure to smoke was associated with an increasing incidence of collections of macrophages laden with golden-brown pigment (GBM) and of areas of cuboidal or columnar metaplasia (CCM) or squamous metaplasia (Sq.M.) of alveolar epithelium. In the control rats there was virtually no GBM, a low incidence of CCM and Sw.M.. Four out of 406 smoke exposed rats which came to post mortem had squamous neoplasms in the lungs, 3 having lesions of doubtful malignancy and one having a squamous carcinoma. In contrast, no squamous neoplasms were seen in 197 control rats. This difference was not statistically significant.

The findings in rats given a single dose of BP were, in all the above respects similar to those in untreated rats, except that one developed a squamous carcinoma of the lung. The effects of s single dose of BP followed by smoke exposure were in general similar to those of smoke exposure only. Three rats on this treatments regimen developed squamous cancers of the lung. None of the treatments increased the incidence of adenomata of the lungs.

**CONCLUSIONS/COMMENTS:**

The experiments show that if rats are exposed to sufficient smoke for long enough then 2 kinds of lesions, viz. Aggregates of pigment laden marcophages and columnar or cuboidal metaplasia of alveolar epithelium are found in virtually every rat at death. In addition, lesions of a third kind, namely sqamous metaplasisa of alveolar epithelium, are found in some 30-40% of smoke exposed rats. The 2 kinds of metaplastic lesion may be useful as indices of biological activity.

**# 29**
**AUTHOR(s): DONTENWILL, WALTER P.**

**DATE: 1974**

**TITLE: TUMORIGENIC EFFECT OF CHRONIC CIGARETTE SMOKE INHALATION ON Syrian Golden Hamster**

**CITATION: Experimental Lung Cancer - Carcinogensis and Bioassays. Speinger-Verlag New York, 1974 (331-359)**

**STUDY DESIGN:**

Results of chronic inhalation experiments on Syrian golden hamsters were reported. 600 were used for their entire lifespans. Some animals received additional treatment with carcinogens (DMBA, asbestos and diethylnitrosamine). Effects of smoke from various types of cigarettes (standard cigarettes; reconst. Tobacco sheet from standard cigarette + 6.1% $NaNO_3$; reconst. tobacco from standard cigarettes; Black cigarettes; standard cigarette with acetate filter, std. Cigarette with cellulose filter; and standard cigarette with charcoal filter), and at different levels (30, 60 or 90 cigarettes per day). Some groups of animals were exposed to whole smoke, and others to vapor phase. Appropriate controls were incorporated into the study design.

**Results/findings:**

The mean survival time is distinctly reduced in those animals having received increased doses of smoke and in those animals having received an additional treatment of 9,10-dimethyl-1,2-benzanthracene (DMBA) when compared to the control animals. The highest decrease in body weight is observed in the group having received the highest amount of smoke; this loss in body weight is also evident in comparison with the controls. In alterations of the larynx: the optimal dose-response-relationship was achieved with a 10 min some exposure twice daily.
In general, animals that died early or animals that received cigarettes with lower activity have a greater tendency to stages of lower classification, e.g., stages 2 to 4. Cigarettes with lower activity clearly produced at a less frequent rate the stages of higher classification, e.g. stage 5 and stage 6 (carcinomas). Using $NaNO_3$ as additive or using reconstituted tobacco sheets or filters reduced condensate and thereby alterations, especially the incidence of stage 5 and 6 in the larynx. This is also true for the " black cigarette" , which showed a very low condensate yield due to the mixture used in this experiment. A significant reduction of the biological effect is also demonstrated by consideration of the different number of puffs and the period of smoke exposure. A more than additive effect was observed for the combined treatment with DMBA and smoke compared to treatment with DMBA alone or with smoke alone. The effect of combined treatment with smoke and diethylnitrosamine was surprising. The additional nitrosamine effect does not enhance the " smoke effect" . Following nitrosamine application, papillomas of a different morphological structure and localization in the larynx occurred than following smoke exposure alone. Cigarettes treated with a high dose of sodium nitrate showed no findings explicable as nitrosamine effect as it can be found in bronchi and trachea in animals treated with DENA and DMBA.

**CONCLUSIONS/COMMENTS:**

The method reported is suitable for comparing different smoke qualities as to their biological activity. Moreover, it gives us information about the cigarettes and enables, to select less harmful cigarettes, which is the main goal of our investigations.

**# 30**
**AUTHOR(s): DONTENWILL, WALTER P.**

**DATE: 1973**

**TITLE: THE EFFECT OF LONG-TERM CIGARETTE SMOKE EXPOSURE ON THE CARDIOVASCULAR SYSTEM OF SYRIAN GOLDEN HAMSTERS**

**CITATION: VIRCHOWS ARCH. ABT. A PATH. ANAT. 361 147-162 (1973)**

**SUMMARY:**

**Syrian Golden Hamsters were exposed to high doses of cigarette smoke throughout lives. Changes in the heart and cardiovascular system in smoke-exposed animals were compared with those in control animals. Inflammatory changes of the heart were predominantly observed in the valves, especially the mitral valves. Moreover, degenerative changes of the myocardium were found as well as a low incidence of myocardial infarction. Arteritis was observed both in the heart and in the large peripheral vessels; in some cases the arteritis ws severe. In a few cases, aneurysms were seen in the aorta. Vascular changes morphologically comparable to arteriosclerosis in man were not found. There were no significant differences between experimental animals and control animals in the**

incidence of vascular diseases.

#33
**AUTHOR(s): DONTENWILL, W., -J. CHEVALIER, H.-P. HARKE, U. LAFRENZ, G. RECKZEH, AND B. SCHNEIDER.**

**DATE: 1973**

**TITLE: INVESTIGATIONS ON THE EFFECTS OF CHRONIC CIGARETTE SMOKE INHALATION IN SYRIAN GOLDEN HAMSTERS.**

**CITATION: JNCI 51(6) 1781-1832 (1973)**

**STUDY DESIGN:**

Results on chronic inhalation experiments on male and female Syrian golden hamsters were reported. Of 4440 hamsters, 3610 were used for their entire lifespans; the remaining were exposed for no longer than 52 weeks. Some animals received additional treatment with carciongens (DMBA, Asbestos, DENA). Effects of smoke from various types of cigarettes (standard cigarettes; reconst. Tobacco sheet from standard cigarette + 6.1% $NaNO_3$; reconst. tobacco from standard cigarettes; Black cigarettes; standard cigarette with acetate filter, std. cigarette with cellulose filter; and standard cigarette with charcoal filter), and at different levels were evaluated.

**FINDINGS/RESULTS:**

Survival times and body and organ-weight development were correlated with treatment or morphologic changes. Experimental results demonstrated a dose-response relationship.
Results were as follows: 1) Changes induced by smoke exposure - striking differences were found between experimental groups. Alterations were most pronounced in the larynx and depended on duration of treatment and dosage; survival times were reduced, and loss body weight was dose dependent; the number of erythrocytes increased and hemoglobin rose. 2) Changes enhanced by smoke exposure – Incidence of `` smoke cells'' was greater; increase in ` adenomatoid lesions'' was slightly significant. 3) Effects of treatment with DMBA—The number of tumors increased in the oral cavity, pharynx, esophagus, stomach, trachea, liver, and ovary; the occurrence of ovarian cysts also increased. 4) Effects of nitrosamine treatment – Papillomas in the trachea and lower region of the larynx differed from those in animals exposed to smoke. 5) Changes not connected with treatment – Findings included stomach ulcers, gastritis, pulmonary emphysema, inflammation of the respiratory tract, generalized amyloidosis, testicular atrophy, formation of thromboses in heart and lungs, cardiomyopathy, inflammatory changes in the soft tissue and interstinal organs, bile-duct cysts, bile-duct proliferation; also tumors of the nasal cavity, skin, connective and supporting tissues, hematopoietic system, and adrenal glands, and biochemical and hematologic changes (apart from changes in erythrocytes and hemoglobin.

#34

**AUTHOR(S): FINCH, GREGORY L., FRISTEN J. NIKULA, STEVEN A. BELINSKY, EDWARD B. BARR, GARY D. STONER, JOHN F. LECHNER**

**DATE: 1996**

**TITLE: FAILURE OF CIGARETTE SMOKE TO INDUCE OR PROMOTE LUNG CANCER IN THE A/J MOUSE**

**CITATION: CANCER LETTERS 99 161-167 (1996)**

**STUDY DESIGN:** A 6-month bioassay in A/J mice was conducted to test the hypothesis that chronically inhaled mainstream cigarette smoke would either induce lung cancer or promote lung carcinogenicity induced by the tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3pyridyl)-1-butanone (NNK). Groups of 20 female A/J mice were exposed to filtered air (FA) or cigarette smoke (CS), injected with NNK, or exposed to both CS and NNK. At 7 weeks of age, mice were injected once with NNK; 3 days later, there were exposed to CS (Univ. of Ky. 1R3 cigarettes) for 6 h/day, 5 days/week for 25 weeks at a mean 248 mg total particulate matter/$m^3$ concentration. Animals were sacrificed 5 weeks after exposures ended for gross histological evaluation of lung lesions.

**FINDINGS/RESULTS:**

No significant differences in survival between exposure groups was observed. A biologically significant level of CS exposure was achieved as indicated by CS-induced body weight reductions, lung weight increases, and carboxyhemoglobin levels in blood of about 17%. Crude tumor incidences, as determined from gross observation of lung nodules, were similar between the CS-exposed and FA groups, and the NNK and CS+NNK groups. Incidences in either of these latter groups were greater than either the CS or FA groups. Furthermore, tumor multiplicity in tumor-bearing animals was not significantly different among any of the three groups (FA, NNK, CS+NNK) in which tumors were observed.

**CONCLUSIONS/COMMENTS:**

Cigarette smoke exposure neither induced lung tumors nor promoted NNK-induced tumors. Because the CS exposure concentration was probably near the maximally tolerable level, longer exposures should be evaluated to potentially establish a CS-induced model of lung carcinogenesis in the A/J mouse.

#35
**AUTHOR(S): FRASCA, JULIO M., OSCAR AUERBACH, HARRY W. CARTER, AND VERTA R. PARKS**

**DATE: 1983**

**TITLE: MORPHOLOGIC ALTERATIONS INDUCED BY SHORT-TERM CIGARETTE SMOKING**

**CITATION: AM. J. PATHOL 111:11-20 (1983)**

**STUDV DESIGN:**

12 Male beagle dogs that ranged in age from 1.7 to 2.1 years were exposed to 2-7 cigarettes (National Cancer Institute Code 26) daily for 2-4 months. One dogs was sacrificed after having smoked 172 cigarettes, one after 282 cigaretes and the others after 480 and 534 cigarettes respectively. 2 of the dogs were sham controls.

**RESULTS/FINDINGS:**

Examination of the lungs by scanning and transmission electron microscopy showed a range of response from the presence of numerous smoker' s marcophages to extensive alterations, including destruction and enlargement of alveolar ducts and varying degrees of enlargement of alveolar spaces. Interalveolar pores were enlarged, and marked fenestrations leading to destruction of the alveolar walls became apparent. These features were accompanied by interstittial fibrosis of the interalveolar septa. Light- and electron-microscopic examinations showed no evidence of bronchitis and/or bronchiolitis or of physical obstruction to the terminal airways in the early development of fibrosis and emphysema.

**CONCLUSIONS/COMMENTS:**
Pulmonary fibrosis and emphysema were produced in beagle dogs by their direct inhalation of cigarette smoke over a relatively short period of time.

#36
**AUTHOR(S): GAAFAR, HAZEM A. AND AHMAD H. AL-MANSOUR**

**DATE: 1982**

**TITLE: THE EFFECT OF CIGARETTE SMOKE ON TH TRACHEAL MUCOSA OF THE RABBIT**

**CITATION: JOURNAL OF LARYNGOLOGV AND OTOLOGY 96:943-950 (1982)**

**STUDV DESIGN:**

Twenty adult male rabbits, were exposed for 10 minutes to cigarette smoke twice/day for a period. Each exposure used 3 filter-tipped Egyptian cigarettes. Control animals were similarly treated, except the inhalation chamber was filled with air instead of smoke. Twenty four hours after the last exposure the animals were sacrificed and the trachea dissected and prepared for electron microscopy.

**RESULTS/FINDINGS:**

In this study irritation of the epithelial cells of the tracheal mucosa was noted by the sparse and abnormal ciliary pattern in these cells, together with oedema in their cytoplasm. The electron dense material observed in the cytoplasm of the basal cells could indicate an early transformation of these cells to goblet cells. The indefinite and ill-defined basal lamina in the smoking group could be due to the activity of basal cells. The rounded homogeneous bodies in the submucosa of the smoking group could represent either a degenerative change or a by-product stimulated by cigarette smoke but their significance is unclear.

**CONCLUSIONS/COMMENTS:**

The effect of whole cigarette smoke on the tracheal mucosa of the rabbit has been studied by the electron microscope. The epithelium shows oedema and disturbed stratification. The cilia are sparse and some show clubbing. The basal cells are active. Large amorphous bodies and numerous vacuoles are found in the tunica propria. Some damage in the smooth muscle is found. The irritative effect of cigarette smoke on the epithelium is demonstrated.

#37
**AUTHOR(S): GARIOLA C. GARY, AND R.C. GUPTA**

**DATE: 1991**

**TITLE: CIGARETTE SMOKE-INDUCED DNA ADDUCTS IN THE RESPIRATORY AND NONRESPIRATORY TISSUES OF RATS**

**CITATION: ENVIRONMENTAL AND MOLECULAR MUTAGENSIS 17:253-257 (1991)**

**STUDY DESIGN:**

To determine how chronic exposure to cigarette smoke affects the distribution of DNA adducts in selected respiratory and nonrespiratory tissues, male Sprague-Dawley rats were divided into three groups: 1) room controls, 2) sham-treated and 3) smoke exposed. Rats in group 3 were exposed to fresh mainstream smoke from the University of Kentucky reference cigarettes (2R1), twice daily, 7 days/weeks in a nose-only exposure system for 32 consecutive weeks using a peristaltic pump smoke generation and delivery system. Blood carboxyhemoglobin, total particulate matter (TPM) intake and pulmonary aryl hydrocarbon hydroxylase values indicated effective exposure to animals to cigarette smoke. DNA was extracted from three respiratory (larynx, trachea, and lung) and three non-respiratory (liver, heart, and bladder) tissues and analyzed for DNA adducts by 32P-postlabeling assay under conditions capable of detecting low levels of diverse aromatic/hydrophobic adducts.

**RESULTS/FINDINGS:**

Data showed that the total DNA adducts in the lung, heart, trachea and larynx were increased by 10- to 20- fold in the smoke-exposed group. Five-fold increase was observed in the bladder tissue, but differences were not present in the liver DNA of the control and smoke-exposed group.

**CONCLUSIONS/COMMENTS:**

These data suggest selective formation of DNA adducts in the tissues.

#38
**AUTHOR(S): GIES, R.A., F.T. CROSS, AND G.E. DAGLE**

**DATE: 1987**

**TITLE: A HISTOLOGIC STUDY OF THE INFLUENCE OF CIGARETTE SMOKING IN SUPPRESSING Rn DAUGHTER CARCINOGENSIS IN DOGS**

**CITATION: HEALTH PHYSICS 53 (5) 527-529 (1987)**

**STUDY DESIGN:**

This is a follow up study which further investigates dogs exposed by inhalation to: $^{***}$Rn, $^{***}$Rn daughters, and U ore dust (Group 1); $^{***}$Rn, $^{***}$Rn daughters, and U ore dust and cigarette smoke (Group 2) to ascertain possible reasons for the unexpected decrease in tumoigenic response observed in the smoking dogs. Dogs in group 1 and 2 were exposed to 100 nCi $L^{-1}$ $^{***}$ Rn gas, 600 WL (working level) of $^{***}$ Rn daughters , and 13 mg $m^{-3}$ U ore dust for 4 h/day, 5 days/week, up to 4 ½ years. Mean Rn-daughter exposure levels were 13,000 WLM. Group 2 dogs were additionally exposed to smoke from 10 cigarettes/day in fractionated exposures before and after radon exposures, 7 days/week, continuing throughout their lifesapns. Lungs and tracheas of these dogs were examined to determine population or size differences in cells and glands involved in mucus production, as well as to measure differences in epithelial thickness between these two groups.

**RESULTS/FINDINGS:**

Overall there were no significant differences in the size and numbers of cells and glands involved in mucus production nor in the thickness of epithelium in the trachea, bronchi, and bronchioles of the lung, between the Rn- and dust-exposed dogs and the Rn- and -dust plus cigarette smoke exposed dogs (the latter having fewer respiratory carcinomas).

**CONLCUSIONS:**

The cause of the significant difference in numbers of respiratory carcinomas between the two groups may be the difference in the overall mucus production. More mucus could be produced by the same number or size of goblet cells or mucus glands.

#39
**AUTHOR(S): Griffith, R.B. AND S. STANDAFER**

**DATE: 1985**

**TITLE: SIMULTANEOUS MAINSTREAM-SIDESTREAM SMOKE EXPOSURE SYSTEMS II. THE RAT EXPOSURE SYSTEM**

**CITATION: TOXICOLOGY 35:13-24 (1985)**

**ABSTRACT:**
**This paper describes in detail the exposure system and monitoring procedures for rat exposures.**

A system for exposing rats to mainstream (MS) and sidestream (SS) smoke simultaneously from the same cigarette, and monitoring procedures, are described in detail. The equipment and procedures were used to expose Spargue-Dawley rats to mainstream smoke (2R1 cigarettes) and to target deliveries of 10, 25 or 50% of the total SS smoke for 17 weeks.
The estimated total particulate matter (TPM) dose was highly correlated with the increase in percent COHb for MS and SS smoke, but the COHb/TPM relationships were different for the 2 kinds of smoke. All SS SPM doses were much lower than the MS TPM dose, and the COHb/TPM ratio of SS smoke was much higher than for MS smoke. The TPM dose and percent COHB for SS smoke were highly correlated with the percent of SS sent to the exposure chambers. There were no significant differences in the total weight changes during the study for any of the smoke exposed groups, but weight changes during the 12 -17-week period for sidestream exposed groups were inversely correlated with the level of sidestream exposure.

#40

**AUTHOR(S): GUERIN, MICHAEL R., JAMES R. STOKELY, CECIL E. HIGGINGS, JACK H. MONEYHUN, AND ROBERT W. HOLMBERG**

**DATE: 1979**

**TITLE: INHALATION BIOASSAY CHEMISTRY – WALTON HORIZONTAL SMOKING MACHINE FOR INHALATION EXPOSURE OF RODENTS TO CIGARETTE SMOKE**

**CITATION: JNCI 63(2) 441-448 (1979)**

**ABSTRACT:**

Studies of experimental tobacco smoke carcinogensis have suffered from the lack of a conveniently available and well-characterized device for exposing animals to tobacco smoke for inhalation. The Walton Horizontal Smoking Machine, a commercially available system designed to expose up to 20 mice to smoke of a single cigarette, may fulfill this need. This system produced a uniform smoke aerosol of predictable concentraiton and appropriate composition for cigarettes with high delivery of nicotine (40 mg total particulate matter, 2.6 mg nicotine and 17cm³ carbon monoxide per cigarette) and low delivery of nicotine (30 mg total particulate matter, 0.3 mg nicotine, and 17 cm³ carbon monoxide). The exposure period is characterized by a decrease in the concentration of particulate matter and most gas phase constituents and an increase in particle size and in carbon dioxide concentration in the exposure chamber. C57BL and DBA/2Bd strains of mice were employed for the characterization of this machine.

#41
**AUTHOR(S): HAKIM, T.S., M. KING, C.G. WANG, AND M. COSIO**

**DATE: 1985**

**TITLE: EFFECT OF CHRONIC CIGARETTE SMOKE EXPOSURE ON PULMONARY VASOMOTION IN BEAGLE DOGS**

**CITATION: j. Appl. Physiol. 59(6); 1815-1822 (1985)**

**STUDV DESIGN:**

The arterial and venous occlusion technique was used to determine whether the site and magnitude of pulmonary vasoconstriction are altered by chronic cigarette smoke exposure.
12 control beagles and 5 beagles whole had smoke cigarettes (50 cigarettes/week for 40 weeks) were perfused in situ to measure the vascular pressure-flow relationship and the resistance of the three vascular segments with the arterial and venous occlusion technique.

**RESULTS/FINDINGS:**

In the control subjects the vascular resistance in the arterial, middle and venous segments was 23, 36, and 41% of the total, respectively. The segmental distribution of vascular resistance was not significantly different in the cigarette smoke exposed dogs, despite the fact that the absolute values were 30-40% less than that of the control group. The longitudinal distribution of resistance among the three vascular segments and their response to drugs were different in beagles than was previously found in mongrels. In all beagles the veins were considerably more reactive than arteries. Vasoconstriction with serotonin (5-HT)
prostaglandin **F..** (PGF.. ), norepinephrine, histamine, and methacholine(M) infusion occurred predominantly in the veins.
The effect of PGF.. and 5-HT was totally different than that previously observed in mongrels in which the constriction was predominantly in the arteries.

**CONCLUSIONS/COMMENTS:**

Chronic cigarette smoking reduced the basal pulmonary vascular resistance and attenuated the venoconstrictor response to 5-HT and M but potentiated the hypoxic pressor response of the microvessels.

#42

**AUTHOR(S): HAMMOND, E. CUYLER, OSCAR AUERBACH, DAVID KIRMAN, AND LAWRENCE GARFINKEL**

**DATE: 1970**

**TITLE: EFFECTS OF CIGARETTE SMOKING DOGS I. DESIGN OF EXPERIMENT, MORTALITY, AND FINDINGS IN LUNG PARENCHYMA**

**CITATION: ARCH. ENVIRON. HEALTH 21:740-753 (1970)**

**ABSTRACT:**

Tracheostomy was performed on 97 male beagles. All but eight (group N) were trained to smoke cigarettes over the first 56 days through tubing from a cigarette holder to the tracheostoma. Of the 89 smoking dogs, two died and one was withdrawn during this period. On day 57, the remaining 86 dogs were divided into four groups assigned to various smoking categories, some smoking filter-tip and others nonfilter cigarettes. Starting on day 876, all surviving dogs were killed and lung sections were examined microscopically. The lungs of the group N dogs were normal while histopathological changes were found in all smoking dogs. Greatest changes were in the lungs of dogs smoking nonfilter cigarettes most heavily.

**FINDINGS/RESULTS:**

Finding in the study indicate that smoking cigarettes equipped with efficient filters produces less pulmonary fibrosis and emphysema in male beagle dogs than smoking the same cigarettes with the filter removed, duration of smoking and number of cigarettes smoke per day being the same. Additionally in this study it was confirmed a greater degree of emphysema and fibrosis was produced by smoking a large number of nonfilter cigarettes than by smoking half that number.

**CONCULSIONS/COMMENTS:**

We conclude that findings in this study strongly suggest that smoking cigarettes with an efficient filter will produce less damage to the human lung parenchyma than smoking identical cigarettes without filters.

#43
**AUTHOR(S): HARADA, T., A. ENOMOTO, T. KITAZAWA, K. MAITA, AND Y. SHIRUASU**

**DATE: 1987**

**TITLE: ORAL LEUKOPLAKIA AND COSTOCHONDRAL HYPERPLASIA INDUCED BY DIETHYLNITROSAMINE IN HAMSTERS EXPOSED TO CIGARETTE SMOKE WITH OR WITHOUT DIETARY VITAMIN C.**

**CITATION: VET. PATHOL 24:257-264 (1987)**

**STUDY DESIGN:**

Male Syrian golden hamsters receiving 12 weekly subcutaneous injections of diethylnitrosamine (DEN) were subjected to cigarette smoke inhalation (twice a day, 5 days per week in a Hamburg II smoking machine) and fed a diet with or without 1% vitamin C supplement for a period of 58 weeks. Another group was sham-smoked control and was not fed vitamin C. Tissues of the oral cavity and costal cartilage were examined by light and./or scanning electron microscopy.

**FINDINGS/RESULTS:**

Oral leukoplakia and costochondral hyperplasia occurred with high frequency in all groups treated with DEN. Leukoplakic lesions were found in the palate, tongue, and pharynx; the early change was focal erosion with mild epithelial hyperplasia and inflammatory cell infiltration. Advanced lesions had marked mucosal thickening due to acanthosis, parakeratosis, hyperkeratosis, and submucosal infiltration of lymphocytes and plasma cells. Precancerous lesionswere noted in tongue and pharynx. Scanning electron microscopy of tongues revealed destruction of filiform papillae. The incidence of leukoplakic lesions was higher in smoke-exposed hamsteres than in controls, but the incidence in vitamin C-supplemented hamsters was low when compared with the smoke-exposed hamsters without vitamin C. Costochondral hyperplasia was initiated by thickening of the perichondrium followed by proliferation of chondocytes. Costochondral hyperplasia appeared earlier, and the incidence was higher in the vitamin C-supplemented hamsters. I could not be determined whether costochondral hyperplasia was the primary lesion induced by DEN or secondary change.

**CONCLUSIONS/COMMENTS:**

The significant increased incidence of the oral leukoplakic lesions in the smoke-exposed hamsters with DEN indicated that cigarette smoke exposure might promote the induction of oral leukoplakia by DEN. In this study, precancerous lesions which could be regarded as carcinoma in situ were occasionally seen, but the frequency was higher in the smoke-exposed hamsters than in the sham-smoked controls. This suggest the cigarette smoking may enhance the propensity of oral leukoplakic lesions to become malignant.

#44

**AUTHOR(S): RUSSO PATRIZIA, MAURO PALA, SILVIO PARODI, CARLA GHIARA, NICOLETTA FERRARI, AND GIORGIO VIDALI**

**DATE: 1984**

**TITLE: EFFECTS OF VITAMIN E ON LIVER DNA**

**CITATION: CANCER LETTERS, 25:163-170 (1984)**

**SUMMARY:**

The study was designed to investigate the effect of vitamin E on rat liver DNA using the alkaline elution technique. Vitamin E, both in the form of dl-a-tocopherol and dl-a-tocopheryl acetate, was capable of inducing an increased alkaline elution rate of liver DNA from rats (Sprague-Dawley) treated i.p. with the vitamin. This activity was clearly both dose and time-dependent. A statistically significant effect was observed at dosages (1.25-5.0 mg/kg) that are in the range of biological activity of the vitamin in the rat. Moreover, the effect was observed at dosages that are clearly not toxic. An increased alkaline elution rate of DNA is usually interpreted as suggestive of DNA damage, however recent observations seem to indicate that functional modifications of chromatin packaging can also affect the elution rate of DNA.

#45

**AUTHOR(S): HARADA, TAKAMORI, KEIZO MAITA, NOBUAKI NAKASHIMA, YOSHITSUGU ODANAKA, AND YASUHIKO SHIRASU**

**DATE: 1983**

**TITLE: QUANTITATIVE STUDIES OF BIOLOGICAL RESPONSES IN HAMSTERS EXPOSED TO TOBACCO SMOKE AND EFFECTS OF VITAMIN C SUPPLEMENT ON SMOKE INHALATION TOXICITY**

**CITATION: JPN. J. VET. SCI., 45(5), 613-626 (1983)**

**STUDY DESIGN:**

Male Syrian golden hamsters were exposed to smoke from 10, 20, or 30 cigarettes twice a day, 5 days a week, in a Hamburg II type smoking machine for quantitative evaluation of biological responses. An additional group received 1% dietary vitamin C supplement and was exposed to smoke from 30 cigarettes in the same manner as the other smoke exposed groups to study the effect of vitamin C on smoke inhalation toxicity. These hamsters were killed by design after 4,13, and 53 weeks of exposure.

**FINDING/RESULTS:**

The smoke exposed hamsters exhibited decreased body weight gain and food efficiency depending on the dose of cigarettes and showed various tobacco-related histological changes in the respiratory tract. Histometrical evaluation revealed that smoke exposure enhanced alveolar macrophage mobilization and thickening of the laryngeal mucosa relating to the dose of cigarettes and duration of exposure. While the vitamin C-supplemented group showed slightly improved body weight gain and food efficiency, significantly lower incidences of rhinitis, focal bronchial epithelial hyperplasia and bronchiolar adenomatoid lesion, and depressed alveolar macrophage mobilization as compared with those in the smoke-exposed group at the same dose of cigarettes.

**CONCLUSIONS/COMMENTS:**

These results indicate that measurement of alveolar macrophage count and thickness of the laryngeal mucosa may be most useful in rating the biological damage elicited by cigarette smoke in hamsters. In addition, it is assumed that vitamin C may have a protective effect in some part on smoke inhalation toxicity.

#46

**AUTHOR(S): HARADA, TAKAMORI, KEIZO MAITA, YOSHITSUGU ODANAKA, AND YASUHIKO SHIRASU**

**DATE: 1984**

**TITLE: EFFECTS OF QUERCETIN AND BUTYLATED HYDROXYTOLUENE ON CIGARETTE SMOKE INHALATION TOXICITY IN SYRIAN GOLDEN HAMSTERS**

**CITATION: JPN. J. VET. SCI., 46(4), 527-532 (1984)**

**STUDY DESIGN:**

Male Syrian golden hamsters were divided into 5 groups of 10 animals each: Group I was cage controls, group II were subjected to sham smoking, group III, IV, and V exposed to cigarette smoke for 6 minutes, twice a day and 5 days a week in a Hamburg II type smoking machine for a period of 13 weeks.. Group III was smoke exposed control for Groups IV and V. Groups IV and V were fed the basal diet mixed with quercetin and butylated hydroxytoluene (BHT) respectively.

**FINDINGS/RESULTS:**

In comparison with the smoke-exposed controls, the quercetin-supplemented hamsters showed slightly improved body weight gain and food efficiency and a significant inhibition of thickening of the laryngeal mucosa, whereas BHT treatment resulted in marked growth retardation and significant depletion of liver vitamin A level.

**CONCLUSIONS/COMMENTS:**

These results indicate that quercetin but not BHT may have some ameliorative effects on the biological damage elicited by cigarette smoke.

#47

**AUTHOR(S): HARRIS, R.J.C., G. NEGRONI, SUSAM LUDGATE, C.R. PICK, F.C. CHESTERMAN, AND B.J. MAIDMENT**

**DATE: 1974**

**TITLE: THE INCIDENCE OF LUNG TUMOUR IN C57BL MICE EXPOSED TO CIGARETTE SMOKE:AIR MIXTURES FOR PROLONGED PERIODS**

**CITATION: INT. J. CANCER 14, 130-136 (1974)**

**ABSTRACT/SUMMARY:**

C57Bl mice, allowed to breathe a cigarette smoke:air (vapor phase – they used a Cambridge filter prior to inhalation chamber) for short periods and at frequent intervals throughout their lives, develop a higher incidence of lung cancers, adenomas and carcinomas, than untreated control animals. Smoke from the cigarettes made from a flue-cured tobacco (T2) was more toxic and elicited cancers more quickly than that form cigarettes made from air-dried tobacco (T3) of the same crop. Filtered smoke:air from T2 and unfiltered smoke gave similar yields of lung tumours.

#48

**AUTHOR(S): HECHT, STEPHEN S., JOHN D. ADAMS, SATOSHI NUMOTO, AND DIETRICH HOFFMANN**

**DATE: 1983**

**TITLE: INDUCTION OF RESPIRATORY TRACT TUMORS IN SYRIAN GOLDEN HAMSTERS BY A SINGLE DOSE OF 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) AND THE EFFECT OF SMOKE INHALATION.**

**CITATION: CARCINOGENESIS 4(10) 1287-1290 (1983)**

**STUDY DESIGN:**

Experiment was designed to determine the effects on Syrian golden hamsters of a single s.c. dose of NNK, followed by treatment with smoke or sham smoking. Four groups of 10 male and female Syrian golden hamsters were given single s.c. injections of either 0.3 ml trioctanoin or of 0.3 ml of trioctanoin containing either 1.0 mg, 3.3 mg, or 10 mg of NNK. These hamsters were then exposed to diluted smoke of 30 cigarettes twice daily for 72 weeks. Four control groups received the same injections of NNK or trioctanoin but were treated by sham smoking.

**RESULTS/FINDINGS:**

Hamsters exposed to smoke generally have a longer survival rate than the sham smoke animals. The target tissues for NNK in the Syrian golden hamster were the lung, nasal mucosa, and trachea. No tumors were observed in these tissues in control animals. Basal cell hyperplasia was observed in 54% of the larynges of the hamsters exposed to smoke, compared with 9% in the sham exposed animals.
The incidence of lung tumors in the females treated with 3.3 mg of NNK and smoke was significantly higher ($p<0.01$) than in the females treated with 3.3 mg of NNK and sham smoking. No other significant differences in respiratory tract tumor incidences were observed between the hamsters treated with smoke or sham treated. The incidences of lung tumors in the males and females treated with 10 mg of NNK and smoke and in females treated with 3.3 mg of NNK and smoke were significant ($p<0,05$) compared with animals treated with trioctanoin. The total numbers of animals with respiratory tract tumors were significantly higher in several of the groups treated with NNK than in the corresponding groups treated with trioctanoin. The combined numbers of males and females with respiratory tract tumors were significantly higher in several of the groups treated with NNK and smoke compared with those treated with trioctanoin and smoke. The combined incidences of respiratory tract tumors in males andd females treated with 1.0 mg NNK and sham smoking or 10 mg NNK and sham smoking were also significantly higher than in the corresponding animals treated with trioctanoin.

**CONCLUSIONS/COMMENTS:**

These results demonstrate that even a single dose of NNK can induce respiratory tract tumors in Syrian golden hamsters. Smoke inhalation did not result in an increase in respiratory tract tumor incidence in most of the NNK treated groups.

#49

**AUTHOR(S): HECKMAN, CAROL A. AND WALDEN E. DALBEY**

**DATE: 1982**

**TITLE: PATHOGENESIS OF LESIONS INDUCED IN RAT LUNG BY CHRONIC TOBACCO SMOKE INHALATION**

**CITATION: JNCI 69(1) 117-128 (1982)**

**STUDY DESIGN:**

In this experiment, animals exposed in parallel with those for the lifetime study were killed at earlier intervals for assessment of morphologic lesions induced by smoke inhalation. SPF females F344 rats were exposed to tobacco smoke (from NCI Code 16 nonfiltered cigarettes) in the Maddox/ORNL smoking machine. Two final dose levels were used, 7 or 10 cigarettes/day, and the animals were killed at time intervals from 1 to 2 years after exposure began. Since mortality was high in the 10 cigarettes/day group, all the remaining animals in the group were killed at 1.5 years. Both untreated and sham-exposed groups were killed in parallel with the exposed animals.

**FINDINGS/RESULTS:**

Parallel lifetime exposures induced pulmonary tumors in 9% of the animals. In serially killed animals, four types of lesions were found: 1) perivascular or peribronchiolar accumulation of lymphoreticular cells, 2) fibrotic and cellular enlargement of peribronchiolar septa, 3) type II cell hyperplasia with septal fibrosis, and 4) air-space enlargement (emphysema). However, emphysema occurred only in animals exposed to a higher dose of tobacco smoke (10 cigarettes). Ultrastructural studies showed all of the focal lesions to be infiltrated by cells typical of the inflammatory response. The type II hyperplastic and peribronchiolar alveolar lesions involved larger portions of the parenchyma in fibrotic changes but differed in structure, location, and frequency. The incidence of the peribronchiolar alveolar lesions was temporally related to tumor incidence.

**CONCLUSIONS/COMMENTS:**

Peribronchiolar lesions were dependent on the duration of exposure and/or on the age of the exposed animals.

#50
**AUTHOR(S): HECKMAN, CAROL A. AND GERALD L. LEHMAN**

**DATE: 1985**

**TITLE: ULTRASTRUCTURE AND DISTRIBUTION OF INTRACELLULAR SPICULES IN RAT LUNG FOLLOWING CHRONIC TOBACCO SMOKE EXPOSURE**

**CITATION: JNCI 74(3) 647-657 (1985)**

**ABSTRACT:**

The present studies were undertaken to determine whether the development of proliferative lesions in the respiratory airways of smoke-exposed rats was preceded by ultrastructural alterations in the epithelium. Previous studies had shown that tobacco smoke exposures of 1-2 years duration induced only one major type of lesion involving the respiratory airways, i.e. fibrotic and cellular enlargement of peribronchiolar alveolar septa. The airway epithelium in these areas was metaplastic and in some of the lesions, the airway lining epithelium advanced out onto the surfaces of adjacent alveoli. Epithelial cells in these lesions frequently contained elongated cytoplasmic inclusions which were oriented with their long dimensions roughly in the same plane as the long axis of the cell. Macrophages contained similar but larger inclusions. Because the composition of the inclusions could be indicative of their origin, we subjected samples of treated and control lung tissues concurrently to transmission electron microscopy and energy-dispersive X-ray fluorescence spectrometry. Spectra from inclusions of macrophages indicated the presence of the elements sulfur, phosphorus, aluminum, silicon, and iron. Spectra from type II cells, however, which did not contain inclusions, showed a different elemental composition. The results suggested that spicules were present in epithelial cells throughout the airways. Minor lesions corresponding to " microinvasion" of epithelium into the lamina propria and of capillaries into the epithelial layer were also found in the trachea.

#51

**AUTHOR(S): HENRY, C.J., J.E.CATON, J.R. STOKELY, M.R. GUERIN, A. LOPEZ, M.D. AVERY, D.R. DANSIE, G.M. HENDERSON, T. GAYLE, C.E. WHITMIRE, AND R.E. KOURI**

**DATE: 1981**

**TITLE: DEPOSITION AND DISTRIBUTION OF THE TOTAL PARTICULATE MATTER OF CIGARETTE SMOKE INMICE USING A LARGE-CAPACITY SMOKE EXPOSURE SYSTEM.**

**CITATION: TOXICOLOGY AND APPLIED PHARMACOLOGY 58, 399-409 (1981)**

**ABSTRACT:**

A newly developed automatic smoke exposure machine (SEM II) was used to generate [$^{14}$C]dotriacontane-labeled University of Kentucky reference 2A1 or 2R1 cigarette smoke. The SEM II is a large-capacity (480 mice) dynamic smoke exposure system in which smoke is routed through the animal containment system as a continuously flowing stream. Mice are restrained about the neck in stock-like holders for " nose-only" exposure. Using standard smoke exposure conditions, the deposition and internal distribution of the total particulate matter (TPM) from cigarette smoke was determined in BC3F1/Cum male and female mice. Results show: (a) smoke exposure conditions can be varied so that deposition from 30 to 200 mg TPM/lung can be obtained, (b) 80-90% of the TPM deposition was found in the respiratory tissues, © the mouse to mouse variation for TPM deposition in pulmonary tissue was ~ 20%, (d) similar deposition and distribution of TPM was observed in male and female mice, and (e) deposition and distribution of TPM was not altered in mice exposed to smoke on a daily basis over a 6-month period of time.

#52

**AUTHOR(S): HENRY, C.J., DANSIE, K.K. KANAGALINGAM, AND R.E. KOURI**

**DATE: 1985**

**TITLE: CHRONIC INHALATION STUDIES IN MICE I. Facilities and Equipment for " Nose-Only" Exposure to Cigarette Smoke**

**CITATION: Beitrage zur Tabakforschung International 13(1): 37-53 (1985)**

**SUMMARY:**

Facilities and equipment are described for large-scale, long-term `` nose-only'' inhalation exposure of mice to whole cigarette smoke. Experimental procedures and equipment were designed to provide the mice with exposure conditions where [1] the lung was the major target organ for the smoke, [2] large quantities of fresh, whole cigarette smoke could be generated, [3] large numbers of animals could be exposed at one time, [4] routine, daily exposures could be given over a major portion of the lifetime of the animal, [5] monitoring and documentation of the quantity of smoke presented to the animals was provided during each exposure session, [6] safety systems were provided that assured exposure of the animals to smoke only under pre-set exposure conditions, and [7] cigarette smoke was generated under conditions where factors, such as cigarette type, smoke aerosol concentration and smoke particle size, were controlled.

#53
**AUTHOR(S): HENRY, Carol J., AND Richard E. KOURI**

**DATE: 1986**

**TITLE: CHRONIC INHALATION STUDIES IN MICE. II. EFFECTS OF LONG-TERM EXPOSRE TO 2R1 CIGARETTE SMOKE ON (C57BL/Cum X C3H/AnfCum)F1 MICE**

**CITATION: jnci 77(1) 203-212 (1986)**

**STUDV DESIGN:**

The experimental conditions in this chronic smoke inhalation study were designed to maximize the responses known to be involved from the short-term effects and to observe the results of long-term exposure to cigarette smoke. Standardized exposure conditions with Kentucky reference 2R1 cigarettes were used to expose 2,053 (C57BL/Cum X C3H/AnfCum)F1 female mice (nose only) to fresh, whole, cigarette smoke. In addition, 1,014 mice were sham-exposed, and 449 mice were held as shelf controls. The protocol entailed exposing mice to smoke (or sham-exposure) on a daily basis, 5 days/week for 110 weeks and observing remaining mice until death. A large number of animals was used so that the smoke generation and animal-holding systems could be tested and evaluated and yet provide significant numbers of animals for exposure to cigarette smoke for a major portion of their lifetime.

**FINDINGS/RESULTS:**

Deposition of smoke particulates was estimated to be about 125-200 mg TPM/lung/day. The only lung cancers observed were diagnosed as alveolar adenocarcinomas (ACC). A total of 19 of 978 smoke-exposed mice and 7 of 651 sham-exposed mice were observed with ACC. The difference between the smoke- and sham-exposed groups was not statistically significant at $P<0.05$, but the data suggested that the tumors occurred with a shorter latency in the smoke exposed group ($P=.10$) The data were analyzed by various methods, including analysis of subsets of the population of animals. A significant increase in the incidence of lung cancer was observed in one subset; however, this difference was not found in the population as a whole or as a results of any other analyses.

**CONCLUSIONS/COMMENTS:**

Under these exposure conditions, 2R1 cigarette smoke would seem to have weak carcinogenic activity in mouse lung tissue. Other changes associated with smoke exposure were increased incidence of pigmented alveolar macrophage accumulation, otitis media, and head and neck fibrosarcomas. However, the incidence of nephritis, hematopoietic cancers (e.g. leukemias, lymphosarcomas, and reticulum cell sarcomas), and pulmonary congestion was significantly higher in the sham-exposed animals.

#54

**AUTHOR(S): HOLT, PATRICK G., JANE E. CHALMER, LYNETTE M. ROBERTS, JOHN M. PAPADIMITRIOU, WAYNE R. THOMAS, AND DAVID KEAST**

**DATE: 1976**

**TITLE: LOW-TAR AND HIGH-TAR CIGARETTES - COMPARISON OF EFFECTS IN TWO STRAINS OF MICE**

**CITATION: ARCHIVES OF ENVIRONMENTAL HEALTH 31:258-265 (1976)**

**STUDV DESIGN:**

This study reported on the comparative effects of high-tar (HT) and low-tar (LT) cigarettes on a number of parameters relating to immunity in laboratory mice. C57/Black or BABL/c strains of mice were exposed to fresh cigarette smoke in a Hamburg II inhalation apparatus. Fresh smoke was mixed with air in the machine in the ratio of 1:7, the mice were exposed 7 to 8 minutes per day, 5 days/week for 26 weeks. The HT type cigarette contained 16.0 mg tar and 1.1 mg nicotine, while the LT type contained 6.0 mg tar and 0.3 mg nicotine per cigarette.

**FINDINGS/RESULTS:**

Mice exposed to HT cigarettes exhibited more marked alteration in humoral immune responsiveness, hematological profiles, and pulmonary pathologic findings than those exposed to LT cigarettes. However, cell mediated immune responsiveness to both bacterial and tumor-specific antigens was depressed similarly in animals exposed to HT or LT cigarettes. Furthermore, the growth rates of subcutaneously established tumors were enhanced similarly in the two groups, with respect to those in control animals.

**Conclusions/comments:**

The results of this study suggest that while the effects of LT cigarettes on the immune system are in some respects less severe than those of HT cigarettes, both types exert marked suppressive effects on T-lymphocyte function.

#55
**AUTHOR(S): HOMBURGER, FREDDY, PETER BERNFELD, AND A.B. RUSSFIELD**

**DATE: 1974**

**TITLE: CIGARETTE SMOKE INHALATION STUDIES IN INBRED SYRIAN HAMSTERS**

**CITATION: eXPERIMENTAL IUNG cANCER - cARCINOGENSIS AND bIOASSAYS. sPRINGER-vERLAG, NEW YORK, 320-330 (1974)**

**STUDV DESIGN:**

Groups of 102 inbred Syrian hamsters of the BIO 87.20 and BIO 15.16 strains were exposed twice daily, 5 days/week to 8 puffs from Kentucky 1R1 cigarettes of 2 second duration, generated every minute in a Walton-Morrissey Reverse Smoker. The smoke exposure to about 1 to 5 diluted smoke lasted 15 seconds and was followed by a 43 seconds exposure to fresh laboratory air. Smoke exposures lasted 45 to 90 weeks. 60 animals of each strain were used as cage controls and 60 of each strain were sham-controls. Histopathologic studies were performed after 45 to 90 weeks of treatment.

**FINDINGS/RESULTS:**

In hamsters exposed to smoke, macrophage clusters containing iron pigment were seen in the pulmonary parenchyma, especially frequently and early in 87.20 hamsters. 2 tumors were found in the nasopharynges of smoke-exposed BIO 15.16 animals, 1 fibrosarcoma and other cystadenoma. Dysplastic changes were seen in the larynges after approximately 40 week of smoke exposure and the total incidence of such changes in smoke-exposed animals was 40% and 13%, respectively. Incipiently invasive, but still very small, lesions were found in the larynges of smoke exposed animals of each strain after approximately 80 weeks of smoking. By 90 weeks, there were microinvasive carcinomas in 19% of the animals in the more susceptible BIO 15.16 line and 4% in the less susceptible BIO 87.20 line.
Chronic smoke-exposure had no effect on survival time in either strain. Smoke exposure did not increase proliferative changes outside of the respiratory tract or the non-neoplastic degenerative changes characteristic of aging hamsters.

**CONCLUSIONS/COMMENTS:**

The point of greatest practical importance to emerge from this work is the demonstration of striking strain differences among various lines of hamsters with respect to susceptibility to acute toxic effects of smoke and to hyperplastic response of the larynx to smoke.

#56

**AUTHOR(S): HUBER, G.L., P. DAVIES, G.R. ZWILLING, V.E. POCHAV, W.C. HINDS, H.A. NICHOLAS, V.K. MAHAJAN, M. HAYASHI, AND M.W. FIRST**

**DATE: 1981**

**TITLE: A MORPHOLOGIC AND PHYSIOLOGIC BIOASSAY FOR QUANTIFYING ALTERATIONS IN THE LUNG FOLLOWING EXPERIMENTAL CHRONIC INHALATION OF TOBACCO SMOKE – A REVIEW**

**CITATION: BULL. EUROP. PHYSIOPATH. RESP. 17:269-327 (1981)**

**SUMMARY:**

Despite prevailing hypotheses, derived by statistical relationships from epidemiologic data, associating tobacco cigarette smoking with chronic pulmonary disease, little or no cause and effect evidence has emerged to link tobacco directly to airway or parenchymal alterations in the lung. To study this apparent discrepancy, the effect of exposure for six months to whole tobacco smoke of high tar delivery on the airways and on the pulmonary parenchyma distal to the terminal airways was quantified in rats. Experimental smoke inhalations were performed three times per day, delivering a retained tobacco smoke dose estimated to be equivalent to approximately 1 ½ packs of unfiltered cigarettes per day in man. Airway quantitative morphologic studies were performed on lungs. Exposure to smoke did not increase the volume density or number of total airway secretory cells, but did increase the proportion of stainable secretory cells, in the airways of the experimental animals. The volume densities of the tissue component of lung parenchyma distal to the terminal airways of control and smoke-exposed lungs, respectively, were 0.13 and 0.11 ($p<0.01$); The volume densities of parenchymatous air space component of lung parenchyma were 0.87 and 0.89 ($p<0.01$); the surface densities of alveolar epithelium were 0.050 and 0.044 ($p<0.01$). These data represent a decrease of 21% in parenchymatous tissue and a 12% decrease in the alveolar surface area of the lungs of smoke exposed animals. Topographical analysis of the alveolar surface revealed that the numerical densities of type I pneumocytes and type II pneumocytes per volume of lungs were both significantly decreased following smoke exposure. Post-mortem physiologic measurements, including volume pressure studies, performed on these lungs revealed a loss of pulmonary elasticity, relatively large lung volumes per animal weight, and an increase in residual trapping of deflation volumes of smoke exposed animals. The effect to tobacco smoke on the airway and alveolar defenses in this model also is reviewed, especially since alterations in these defenses might be important in the pathogenesis of potentially adverse lung adaptations to smoke exposure. The experimental data demonstrate that an adaptive response within the airways occurred, with a physiologic correlate in loss of pulmonary elastic recoil, following experimental exposure to tobacco smoke of a high tar delivery for a six month duration.

#57

**AUTHOR(S): HUMPHREY, EDWARD W., STEPHEN L. EWING, JOHN V. WRIGLEY, WILLIAM F. NORTHRUP, III, THOMAS E. KERSTEN, JOHN E. MAYER, AND RICHARD L. VARCO**

**DATE: 1981**

**TITLE: THE PRODUCTION OF MALIGNANT TUMORS OF THE LUNG AND PLEURA IN DOGS FROM INTRATRACHEAL ASBESTOR INSTILLATION AND CIGARETTE SMOKING**

**CITATION: CANCER 47:1994-1999 (1981)**

**study design:**

Nine male beagle dogs used in this investigation were given yearly intratracheal instillations of corcidolite asbestos for periods up to three years. The maximum dose totalled 66 mg/kg. In addition seven of these dogs smoke nine high-tar unfiltered cigarettes per day, five days per week for six years.

**FINDINGS/RESULTS:**

A malignant pleural and/or peritoneal mesothelioma developed in six of these dogs, and adenocarcinomas of the lung developed in four, one of which had areas of squamous differentiation. The first animal died of a malignant tumor six years after the onset of exposure, and the last animal died eight years after the onset. Three of the seven dogs exposed to both asbestos and cigarette smoke did not develop a malignancy. One died at five years of multiple small bowel perforations, one at seven years of pneumonia, and one was killed at nine years. Both dogs that were exposed to asbestos alone did develop a malignant tumor.

**CONCLUSIONS/COMMENTS:**

Because of the effectiveness of this dose of asbestos in producing tumors, it is difficult to evaluate the role of cigarette smoke. If, as has been postulated, the synergism between cigarette smoke and asbestos is due to the impairment of bronchial clearing by smoking and subsequent retention of asbestos in the lung, this dose of asbestos may of itself have overwhelmed the clearing mechanism and so obscured such synergism.

#59

**AUTHOR(S): IWASAKI, MAKOTO, TAKANORI HARADA, TEIJI MIYAOKA, SHUJI TSUDA, AND YASUHIKO SHIRASU**

**DATE: 1981**

**TITLE: EFFECT OF MALEIC HYDRAZIDE ON CIGARETTE SMOKE INHALATION TOXICITV IN SYRIAN GOLEND HAMSTERS**

**CITATION: j. PESTICIDE SCI. 6, 17-24 (1981)**

**study design:**

Effects of maleic hydrazide (MH) on cigarette smoke inhalation toxicity were studied with male Syrian golden hamsters. The hamsters were divided into 5 groups consisting of 10 animals each. Three of the groups were exposed to smoke from reference cigarettes, cigarettes containing 1% sodium salt of MH (MH-Na), and cigarettes containing 1% diethanolamine salt of MH (MH-DE), respectively, twice a day for 8 minutes, 5 days/week for 26 weeks in a Hamburg II type smoking machine. The other two groups were served as sham-smoked and cage-held controls. Body weight and food consumption were measured weekly during the testing period. Hematological and blood biochemical analyses, organ weight measurements, and gross and histopathological observations were performed on all hamsters at the termination.

**RESULTS/FINDINGS:**

Decreased body weight gains, lower food efficiencies and higher blood glucose levels were observed in all the smoke-exposed groups. These changes were most evident in the group exposed to smoke from cigarettes with MH-DE; as they exhibited from mild to significant differences from other smoke-exposed groups. This group also showed a significant decrease in plasma protein and blood urea nitrogen levels as compared with the sham-smoked control. On the other hand, addition of MH-Na to cigarettes resulted in no significant differences in any result from the group exposed to the reference cigarette smoke. Histopathological examination revealed that cigarette smoke exposure to the hamsters enhanced alveolar macrophage mobilization and induced epithelial alterations in the respiratory tracts, although there were no significant differences in the incidence and severity of the lesions among the three smoke-exposed groups.

**CONCLUSIONS/COMMENTS:**

It is suggested that MH may have a mild promoting effect on the smoke inhalation toxicity to hamster in the form of its diethanolamine salt, but not sodium salt.