Environmental Health Perspectives
Vol. 17, pp. 253–285, 1976

Recent Advances in Investigations of Toxicity of Automotive Exhaust

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The influence of auto exhaust on man's health is difficult to gauge considering the intricacy of human environmental urban stresses and particularly of other air polluting (industrial, domestic) emissions. Epidemiological surveys made in road tunnel employees and in traffic officers have not demonstrated specific effects and have often been complicated by cigarette smoking as a factor. Long-term animal experiments run mostly on small rodents give evidence of little effect of the pathological actions of dilutions such as those encountered in high polluted cities. However the acute toxicity of gasoline exhaust emission is well known and mostly due to carbon monoxide. Considering the different types of cycles and operating conditions of vehicles (gasoline and diesel), auto exhaust gases constitute no more a chemical entity than they show, a definite toxicity. A great number of substances that they contain (nitrogen oxides, aldehydes, antiknock additives, heavy metals, possible catalysts are highly toxic as shown by *in vivo* and *in vitro* (mutagenic) tests. Interactions of the components are for the moment ignored or poorly understood. Besides, the evolution of the physicochemical properties and natures of the auto exhaust emission in the gaseous biotope of man under determined conditions of ultraviolet irradiation, temperature, and hygrometry provoke the formation of secondary products such as oxidants and ozone. Several experiments show clearly that irradiation increases the toxicity of auto exhaust significantly. For these reasons, geographical, meteorological, and chronological (circadian and seasonal) factors should be taken into consideration, especially with regard to emission standards.

Introduction

A few years after World War II, the formation of photochemical oxidant smog in Los Angeles County, reducing visibility, destroying vegetation and irritating inhabitants' eyes and noses, was partly attributed to solar irradiation of a mixture of nitrogen oxide and hydrocarbons emitted by motor vehicles in a poorly ventilated atmosphere (*1*, *2*). In 1959 the American Public Health Service created an automotive exhaust facility in Cincinnati and a Technical Task Group on automotive exhaust research. The Motor Vehicle Exhaust Act (U.S. Public Law 86 493), June 1960, stimulated medical and biological research, and in particular permitted recruitment of personnel and the construction of chambers for experimental exposure to irradiated automotive gas. In June 1962 the Surgeon General made a report to the U.S. Congress concerning "motor vehicles, air pollution, and health" (*3*). As the highest levels of automotive air pollution were found in Los Angeles, California became the pioneer state for vehicle exhaust emission standards. Oxidant air pollution has since been found in other cities of the United States (*4*) and in Toronto (*5*), but of much lower intensity. Generally speaking, conditions of solar irradiation, air stagnation and vehicle traffic in Europe are not considered favorable to the creation of such photochemical reactions (*6*), but oxidant levels of the Los Angeles type were detected in the Rotterdam area in 1968 (*7*). In 1969, the World Health Organization published a report on urban air pollution, especially by motor vehicles (*8*).

A strong relationship between urban air pollution and the density of motor traffic has been

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shown to exist in cities in every country all over the world. More specifically, atmospheric carbon monoxide, nitrous oxide, hydrocarbon and lead content correlates with the number of cars on city streets, and definite circadian variations are produced by the amount of traffic (*9*). Fuel rationing after the Suez affair permitted Lawther and Commins (*10*) to show unusually low concentrations of NO_2 and CO in the streets of the City of London; a Paris subway strike enabled Chovin et al. (*11*) to point out considerable increase in atmospheric CO due to the higher number of cars on the Paris streets.

This report reviews toxicological studies of the effects on men and animals of raw and irradiated exhaust and their principal components. The contribution made by such air polluting emissions to the etiology of bronchitis and lung cancer must be considered in order to decide their possible influence on human health. These two diseases have multifactorial etiology, including personal air pollution (cigarette) smoking), occupational exposure, and infectious and genetic predisposition (*12–16*). High concentration of exhaust pollution in cities is also related to the urban factor, not yet defined (*10, 17–20*), which many epidemiological surveys have proved aggravating to pulmonary and circulatory diseases and which is a baffling complex of physiological, alimentary, thermal, and respiratory stresses and the influence of crowding and noise.

Automotive emission makes an important contribution to air pollution (*21*), varying essentially with the intensity of traffic, meteorological factors (wind, sun irradiation) and the type and quantity of other air pollutants. Despite the immense complexity of automobile exhaust and its great diversity due to variations in fuel composition, types of engines (automotive or diesel) and operating conditions, a certain number of common toxic components have been identified in these emissions.

Table 1 presents a list of the principal components of auto exhaust in automotive gasoline

Table 1. Summary of automotive exhaust levels.

Gases and particles	Gasoline exhaust	Diesel exhaust	Urban polluted air	Biological activity threshold	Air quality standards
Oxygen, %	1–14	1–20	20.9	16–12	
Nitrogen, %	76–90		78.06		
Hydrogen, %	2–6	0.05–8			
Carbon dioxide, %	5–15	1–14	0.03–0.04	5	0.5
Carbon monoxide	2–6%	0–0.1%	2–50 ppm	200–300 ppm	30–120 ppm
NO_x, ppm	30–4000	30–2000	0.001–0.15		3–10
NO_2, ppm		0.5–40	0.02–0.08	0.5–5	0.2–0.5
Photochemical oxidants, ppm				0.13	0.015
Ozone, ppm			0–0.2	0.1–0.5	
PAN, ppm [a]			0.01	0.1	0.03
SO_2, ppm	0–80	100–300	0.2–1.6	1–5	0.03–0.1
Total aldehydes, ppm	40–300	10–120	0.2–1.2	0.06–0.1	
Formaldehyde, ppm	10–300	5–30	0.05–0.12	0.5–16	
Acrolein, ppm			0.01	0.2–5	0.01
Total hydrocarbons	0.03–1.5%	0.01–0.10%	2–15 ppm	1–20%	0.24 ppm
Methane, ppm	200–800		1.0–1.5		1000
Benzopyrene	1–10 $\mu g/m^3$			0.01–100 $\mu g/1000\ m^3$	
Lead	70–80% of the lead in gasoline	0	0.4–10 $\mu g/m^3$		15.50 $\mu g/m^3$
Oils, mg/m^3		200–900		1200	
Particles	0.2–3 mg/g of burned gasoline	150–450 mg/m^3	50–100 $\mu g/m^3$		60–75 $\mu g/m^3$
Cadmium, $\mu g/m^3$			0.0004–0.26	200	
Nickel, $\mu g/m^3$			0.001–0.12	1000	
Platinum, $\mu g/m^3$				2	

[a] Peroxyacetyl nitrate.

and diesel motor emissions, with their "toxic" concentrations. The fourth column shows their concentrations in polluted urban air (New York City, U.S. towns, Paris, London). The range is generally indicated by two numbers, the mean by one. The figures of gas concentrations must be taken mainly as indices, because of the tremendous differences in operating conditions, which widely change the concentrations of emitted gas. Further details concerning the data presented in Table 1 appear in the literature (*22–28*). Also shown are biological activity thresholds in mammals taken from different sources in the biological and medical literature (*29–32*), and air quality criteria monographs (*5, 6, 33–38*). Table 2 gives a conversion table from units of volume to other units (mass).

Table 2. Conversion table of units of volume (at 25°C, 760 torr) to units of mass.

	1 ppm equivalent to
Carbon monoxide	1.145 mg/m³
Nitrogen oxides (NO_x)	2 mg/m³
Nitrogen monoxide (NO)	1.230 μg/m³
Nitrogen dioxide (NO_2)	1.880 μg/m³
Ozone	1.960 μg/m³
PAN	4.91 μg/m³
Sulfur dioxide	2.65 μg/m³
Formaldehyde	1.230 μg/m³
Acrolein	2.690 μg/m³
Hydrocarbons	660 μg/m³
Methane	0.7 mg/m³

Principal Investigations of Toxicity of Auto Exhaust

Principal methods used for investigating the biomedical effects of motor vehicle air pollution are epidemiological studies of those occupationally or geographically exposed and experiments on men and animals. In addition, particular models (cells or tissues) have been assayed for special problems such as mutagenicity.

Epidemiological Studies

As carbon monoxide is a "sentinel" of automobile gas emission, the intensity of exposure to automotive air pollution can be roughly quantified by the carbon monoxide content of the atmosphere which, as the consequence of its affinity for hemoglobin, results in blood carboxyhemoglobin variations. The partial pressure of CO, its diffusion coefficient through the alveolocapillary membrane, pulmonary ventilation and cardiac output determine its penetration into pulmonary blood. Douglas et al. (*39*), Forbes et al. (*40*), and more recently Coburn et al. (*41*) developed equations to predict the increase of CO Hb in man according to the carbon monoxide concentration in inspired air and duration of exposure. Coburn's formula was verified physiologically in volunteers for concentrations up to 1000 ppm (*42*). Measurement of carbon monoxide in expired air and levels of blood carboxyhemoglobin substantiate exposure of men to automotive air pollution (*43–51*).

Lead measurement in human blood, urine or brain now provides an additional index of exposure to automobile air pollution (*9, 52*).

Epidemiological surveys have been made on different populations particularly exposed to this kind of air pollution: traffic officers, road tunnel employees, carpark workers and drivers, mainly volunteers. Recently published statistics of morbidity in California populations will also be presented, as they appear to be exposed to a particular type of air pollution as a result of the effects of sun irradiation on automotive gas emission.

Road Tunnels. Waller, Commins, and Lawther (*53*) reported eye irritation provoked by the atmosphere of two London road tunnels during periods of high traffic density, but did not then undertake medical investigations.

In 1963 Speizer and Ferris (*54*) reported the results of a questionnaire on respiratory symptoms and measurement of vital capacity, 1-sec forced expiration volume (FEV_1) in toll guards and sergeants of the Sumner Tunnel in Boston. This tunnel is 6000 ft long; approximately 35,000 vehicles use it daily; during peak commuter-traffic, the mean hourly CO concentration was over 100 ppm, and the Pb level was 30.9 g/m³ air. Fifty toll guards were in the tunnel for 2 hr at a time with 2-hr breaks, or at the toll booths for 8-hr periods. They usually spent 2 weeks in the tunnel and 6 weeks at the toll plaza. A group of five sergeants in charge of each 8-hr shift spent most of their time either at the toll booths or in the tunnel. Estimates of combined exposure for these two groups were 30 hr/week at the toll plaza and 5 hr/week in the tunnel. Chronic nonspecific respiratory disease and chest colds were more

prevalent in men who had worked more than 10 yr in the tunnel than in those who had worked only 10 yr or less. Both groups appeared to have essentially the same smoking habits. Mean vital capacity and FEV showed no change in affected subjects, but the mean maximal expiratory flow was less. This epidemiological investigation was made on a small number of people and did not take into consideration a control group of same origin, but living in conditions other than exposure to the polluted atmosphere in the tunnel.

A recent American study by Ayres, et al. in 1973 (*9*) compared two groups taken from a total population of 440 New York males, one of tunnel employees working both in tunnels and toll plaza areas, and one of Triborough Bridge attendants working in the toll plaza area. Standard ventilatory tests performed include: forced vital capacity (FVC), forced expiratory volume at 1 sec (FEV_1) and maximal expiratory flow rate (MEFR) and expiratory flow rate (MMFR). Carbon monoxide was determined in the terminal 200 ml of expired air after a 15-sec period of breath-holding. Chest roentgenograms, exercise ECG, and serum lead were also determined; a few measurements of red cells 2, 3-diphosphoglycerate, and methemoglobin were made. Very detailed analyses of air in a toll booth at Queens Midtown tunnel gave a monthly average of 63 ppm CO, 7.9 ppm total hydrocarbons, 0 total oxides, 1.38 ppm NO_x, 0.07 ppm NO_2, 0.05 ppm aldehydes, 0.003 ppm acrolein, 30.9μg/m^3 Pb, 0 μg/m^3 Ni, 64 μg/m^3 respirable particles, and 1.7 unit haze coefficient. Both MEFR and MMFR were lower in 175 men who had worked more than 10 yr than in 67 men who had worked less than 10 yr. The two maximum expiratory flow rates were less in the tunnels than in the bridge employees. Concerning reespiratory symptoms, the Medical Research Council questionnaire shows more coughs and chest illness in tunnel employees than in bridge workers; however, the difference was significant only in nonsmokers. No difference between the two groups was observed in exercise ECG, frequency of heart attacks, hemoglobin concentration, and hematocrit. Carbon monoxide and serum lead were higher in tunnel employees than in bridge workers. Increase in methemoglobin but no change in 2, 3-diphosphoglycerate was reported in a subgroup of tunnel workers. Almost all bridge and tunnel workers had increased closing volume when compared to a group of 30 nonsmoking employees in a lower Manhattan Hospital. The authors emphasize the fact that the average employee in the facilities of the New York Triborough Bridge and Tunnel Authority is exposed to twice the amount of air pollutants permitted by U.S. National Occupational Safety and Health Authorities when carbon monoxide is used as an index, and roughly five times the concentration of air pollutants fixed by the Federal Environmental Protection Agency's air quality recommendations. Air pollution in nonsmoking workers of Triborough Bridge (2.12% CO Hb) was close to air pollution for nonsmoking New Yorkers walking in the vicinity of Union Square (1.94% CO Hb). A nonsmoking bed-patient hospitalized on the third floor of a lower Manhattan hospital was less exposed to carbon monoxide pollution (1.56% CO Hb). In smokers, mean carboxyhemoglobin was 5.01% in the tunnel, 3.90% on the bridge and 3.84% in Union Square.

Taxicab Drivers. Taxi drivers are particularly exposed to automobile air pollution. According to Stewart et al. (*43*) they have the highest CO Hb saturation observed in any urban occupational group. However, there do not appear to be recent epidemiological surveys of this group.

Californian Smog. In view of the importance of hydrocarbons and nitrous oxides emitted by vehicles in the production of California smog, it might be interesting to consider epidemiological surveys made on inhabitants of this region. Hechter and Goldsmith (*55*) examined mortality statistics in Los Angeles County between 1956 and 1958. Eliminating seasonal components by statistical means, they could detect no association between daily cardiac and respiratory deaths and oxidant or carbon monoxide concentrations. Three times as much eye discomfort was also reported in outdoor telephone workers in 1965 in Los Angeles than in San Francisco (*56*).

An epidemiological survey of 112 student nurses from November 1961 to May 1964, recently published (*57*) compared daily symptom reporting (eye and chest discomfort, coughs and headaches) and air pollution in Los Angeles. The air contained mean concentrations of 0.90 ppm photochemical oxidants, 15 ppm carbon monoxide, and 0.11 ppm NO_2. Of the participants in this study, 38% were smokers, 18% smoking more than 15 cigarettes per day.

Dose response relationships were obtained and thresholds of the different symptoms analyzed. The relative increase of these symptoms appeared at levels superior or equal to 0.40 ppm on highest oxidant days. Photochemical oxidant threshold levels were 0.05 ppm for headaches, 0.15 ppm for eye discomfort, 0.26 ppm for coughs, and 0.30 ppm for chest discomfort. Association between these four symptoms and photochemical oxidants were not explained by daily variations of the levels of carbon monoxide, nitrogen dioxide, or temperature.

An interesting study (*58*) was performed by Durham with 11,659 health records of student populations of seven California universities (five in Los Angeles and two in San Francisco areas) during the academic year 1970–1971 compared to eight air pollution variables and nine weather variables. A sophisticated statistical analysis accounts for the time series nature of the data, non-Gaussian frequency distribution of some variables, circaseptan rhythm, nonlinear relationships, and noncorrelations of variables, and eliminates excess variables. The mean air pollution, 2.6 ppm oxidants, 17.0 ppm NO_x, 7.0 ppm NO_2, 5.6 ppm CO, 1.3 ppm SO_2, 2.8 KM units particulate matter, was higher in Los Angeles than in San Francisco schools. Intervals were observed between air pollutant peak levels and the dates on which symptoms were first noticed. Asthma, eye irritation, headache, and hay fever showed highest correlation with pollution at zero and 1 day time lag. Bronchitic symptoms correlated with peak pollution, with time lags of 5 or 6 days. Pharyngitis, bronchitis, tonsillitis, colds, and sore throats (in that order) were the illnesses most closely related with air pollution, primarily with photochemical oxidants, sulfur dioxide and nitrogen dioxide. Factorial analysis of weather by date revealed four different factors. In one, characteristic of "good" weather (high temperatures, high barometric pressure, low relative humidity, no wind, no rain), bronchitis and pharyngitis were most strongly associated with high pollution. In another factor, characteristic of "bad" weather (cool, rainy and damp), consequently with almost no air pollution, highest associations of health variables occurred with pharyngitis and the common cold. A strong association of respiratory illnesses and pollution variables was found in Los Angeles but not in San Francisco schools. As a consequence, 16.7% difference in respiratory illness between these two areas suggests they were caused by excess air pollutant concentrations. Smokers (83% of the campus) had a much higher percentage of bronchitis than nonsmokers. Respiratory illnesses were more highly associated with pollutants among men (75% of the campus) than women. Although this was not a blind study, it uses a statistical comparison of meteorological factors and the delay in appearance of symptoms.

Human Exposure

In 1921–1922, Henderson et al. (*59, 60*) exposed themselves and several other men and women to the exhaust gas of a Ford car engine. At concentrations containing 200–300 ppm carbon monoxide they observed dizziness, headaches, fainting, and vomiting. They thought that carbon monoxide was the chief toxic substance of exhaust gas, and compared their results with data they had previously obtained in human beings breathing different dilutions of pure carbon monoxide, after which carbon monoxide was measured in blood and alveolar air. In these previous experiments they observed slight headaches for inhaled concentrations containing 600 ppm CO, distinct headaches for concentrations containing 800 ppm CO, and throbbing frontal headaches and Cheynes-Stokes breathing in one experiment which reached 1000 inhaled ppm CO, 780 ppm alveolar CO, and 38% Hb CO. However, the inaccurate Orsat method was used for measuring carbon monoxide in these pioneer experiments.

Sayers et al. (*61*) reported repeated daily exposure (7 hr/day for 68 days) to gasoline engine exhaust containing 200, 300, and 400 ppm of carbon monoxide. At 200 ppm, carboxyhemoglobin reached 25% in 5 or 6 hr, and almost half the subjects experienced frontal headaches. At 400 ppm almost all subjects suffered from headache within 4 hr.

Accidental and suicidal carbon monoxide intoxication by automobile exhaust gas in poorly ventilated enclosures (garages) have been reported, and it is generally admitted that the concentration of 4000 ppm which is often contained in undiluted exhaust is fatal in less than 1 hr (*62*). For this reason, and on account of medical ethics, human exposure is not conceivable.

The eyes of five men were exposed to irradiated automotive exhaust gas to try to determine the chemical factor responsible for eye irritation (*63*). A relationship was observed between hydrocarbons (15–208 pphm) and the speed at which reactants disappear, especially the rate of nitric oxide decrease.

A few observations have been made on men under poorly defined conditions. It is thought that nervous troubles (headaches, fatigue, sleepiness, insomnia) occur most frequently in drivers and people working in garages or living in a street with heavy automobile traffic (*64*). Lewis et al. (*65*) tested 16 subjects (between 18 and 28 years old) for auditory vigilance, addition, sentence comprehension, and digit copying while breathing polluted air pumped from the roadside, but whose pollutant components were not described; the marked decrease in mental efficiency they reported cannot, therefore, be reproduced experimentally. Aronow et al. (*66*) reported cardiopulmonary tests (bicycle volume and forced vital capacity, ECG) on 10 patients with angina pectoris driven 90 min by car on a Los Angeles freeway so as to breathe the heavy morning traffic-polluted air (21.5 ppm CO). Mean carboxyhemoglobin was 5.8% compared to 1.12% when breathing control air (5.3 ppm CO). Significant decrease in exercise performance was observed, and ischemic ST segment depression occurred in three of these ten patients while breathing freeway air.

After measuring diesel locomotive air pollution in a railroad tunnel, Katz, Rennie and Jegier (*67*) reported mainly slight eye and throat irritation but no effect on pulse and blood pressure for concentrations of 2.5 ppm CO, 0.5–2.5 ppm NO_2, 1.8–4.7 ppm NO_x, 0–0.13 ppm SO_2, 1.8–4.7 ppm formaldehyde, and 1000–1900 $\mu g/m^3$ particulate matter.

Animals: Acute Exposure

Respiratory physiology has recently developed very complex methods of investigation in man, but there is rarely histological data for verifying pathological changes in human subjects. Moreover, men cannot be submitted to long-term studies, and it does not seem reasonable to expose individuals with lung diseases, in controlled laboratory settings, to levels of pollutants which they encountered in Chicago or Los Angeles on certain selected days of the year (*68*). Despite scattering due to differences in species, experiments in animal exposure allow toxic levels to be defined, make possible long-term experiments under well defined physicochemical and ethological conditions and permit investigations of specifically affected target organs (*69*). Moreover, combined physiopathological factors in animals can be associated with exposure to air pollutants.

In 1921, Henderson et al. (*60*) exposed horses and men to high concentrations of exhaust fumes containing 3000–4000 ppm of carbon monoxide, and concluded that carbon monoxide is the most important toxic in gasoline engine exhaust. In 1967, the French National Institute of Health (INSERM) developed experimental investigations on the toxicity of air pollutants, especially carbon monoxide and automotive exhaust gas. Acute and chronic animal exposure was carried out on mice, rats, and guinea pigs. The French Technical Automobile, Motorcycle, and Cycle Union (UTAC) assisted in the installation for the experiment. A dilution of raw exhaust gas (Dauphine Renault) containing 2300 ppm CO, 2.2% CO_2, 18.8% O_2, 35 ppm NO_x, and <0.5 mg/l. hydrocarbon killed 26 out of 53 (49%) male SPF mice of CF strain and 1 out of 48 (2%) female mice of the same origin in 215 min at a temperature of 23°C. This is comparable to acute toxicity in the same strain of mice of mixtures containing 3300 ppm CO, which also showed the same difference of toxicity between sexes (*70*).

The use of the Konzett and Rössler technique enabled Mordelet-Dambrine, et al. (*37, 71*) to gauge the effects of automotive gas dilution and its gaseous components (CO, CO_2, NO_x) on the tracheal pressure of urethanized artificially ventilated guinea pigs. The dilutions of automotive exhaust contained concentrations of CO, CO_2, NO_x, NO_2 and oxygen as shown in Table 3. Table 4 presents variations of tracheal and blood pressure at these different dilutions; it will be noted that with increased concentrations of exhaust gas there is augmentation of tracheal pressure variations: bronchospasms, polyphasic changes, and superimposed muscular contractions (suppressed by injection of succinyl choline). As the concentration of exhaust dilutions increases, delay in appearance of variations in tracheal pressure diminishes. The threshold of action can be fixed at dilution

Table 3. Gas concentrations of different automobile exhaust gas dilution.

Exhaust dilution	CO_2, %	CO, %	NO_x, ppm	NO_2, ppm	O_2, %
A	0.17	0.1	—	—	20.6
B	0.5	0.1	3	1	20.3
C	0.7	0.1	75	1	20.1
D	1.0	0.5	2	0.1	19.4
E	1.0	1.0	—	—	18.9
F	1.3	0.1	15	5	19.7
G	2.0	0.2	8	—	18.4
H	3.0	0.5	15	4	15.7
I	5.0	0.5	50	10	13.6
J	5.75	1.0	80	—	14.4
K	6.0	1.0	45	7	11.8
L	12.0	3.0	100	15	2.8
M	12.5	5.0	—	—	6.9

D containing 1.0% CO_2, 5000 ppm CO, 2 ppm NO_x, and 0.1 ppm NO_2. This dilution corresponds roughly to ten times the ordinary peak of urban air pollution. In addition, the use of anesthesia and artificial ventilation greatly reduces the sensitivity of guinea pig response.

This experimental protocol can be used for studying the possibility of interactions between the gaseous components of autoexhaust. According to the experimenters, carbon dioxide appears to be one of the most physiologically active components.

In 1963–1964 Murphy of the Cincinnati Group (*72, 73*) reported acute effects on guinea pigs, rats, and mice of acute exposure to irradiated or nonirradiated auto exhaust. Aliquots of whole automobile exhaust continuously diluted with clean air were piped into glass, Teflon, stainless steel and aluminum animal exposure chambers. The diluted atmospheres were irradiated by fluorescent bulbs selected to simulate the spectrum and intensity of ultraviolet solar irradiation (3000–4500Å). Gas atmosphere analyses were performed for CO, total oxidants, NO_2, NO, formaldehyde, acrolein, and olefin. Guinea pigs were exposed through face masks attached to an exposure manifold and placed in body plethysmographs. Tidal volume, respiratory rate, and total respi-

Table 4. Automobile exhaust dilutions and changes of tracheal pressure and blood pressure of anesthetized guinea pigs.

Auto exhaust (dilution)[a]	Tracheal pressure changes								Muscular contractions	Blood pressure, mm Hg, at various times after inhalation (n)[b]		
	No. of animals	No. of inhalations	None	Bronchospasm	Increase	Decrease	Polyphasic	Delay, sec[b]		Initial	5 min	10 min
A	1	1	1	0	0	0	0		0	50 (1)	50 (1)	50 (1)
B	1	2	1	0	0	0	1	408 (1)	1	43 (1)	43 (1)	43 (1)
C	1	1	1	0	0	0	0		0	48 (1)	48 (1)	48 (1)
D	2	3	1	0	2	0	0	210 ± 254 (2)	0	53 ± 8 (3)	47 ± 3 (3)	48 ± 3 (3)
E	2	2	0	1	0	0	1	60 ± 0 (2)	0	55 ± 7 (2)	53 ± 4 (2)	
F	2	4	3	1	0	0	0	660 (1)	0	56 ± 15 (4)	53 ± 17 (4)	48 ± 15 (4)
G	1	1	0	0	0	0	1	180 (1)	0	25 (1)	30 (1)	
H	1	3	0	0	0	0	3	100 ± 46 (3)	1	52 ± 8 (3)	47 ± 6 (3)	48 ± 11 (2)
I	1	1	0	0	0	0	1	60 (1)	1	60 (1)		
J	1	1	0	0	0	0	1	36 (1)	1	60 (1)	50 (1)	40 (1)
K	1	1	0	0	0	0	1	60 (1)	1	45 (1)	60 (1)	60 (1)
L	1	1	0	0	0	0	1	12 (1)	1	45 (1)	20 (1)	
M	1	2	1	0	0	0	1	18 (1)	1	40 (1)	65 (1)	55 (1)

[a] See Table 3.

[b] Values are mean ± S.D. Numbers in parentheses are n.

ratory flow resistance (lung and chest wall) were measured without anesthesia by technique derived from Mead (*74*). Definite effects were obtained for concentrations of pollutants superior to those in urban atmospheres. Increase in total expiratory flow, accompanied by a decrease in respiratory rate and a small increase in tidal volume, were much more marked at an auto exhaust dilution of 1/1000 with irradiated than with raw autoexhaust. These effects were observed at all exposure times, which lasted from 2 to 4 hr. Most of these effects rapidly regressed to preexposure level when the animals were returned to clean air. Although these investigations did not identify the specific agents responsible for all the pulmonary effects observed, the experimenters thought they best correlated with the aldehyde content of the exhaust, unmeasured substances formed by photochemical reactions, and carbon monoxide. Table 5 shows the gaseous components measured in exhaust-contaminated atmospheres used in these experiments (*73*). Spontaneous running activity of mice measured in an ac-

Table 5. Chemical agents in exhaust-contaminated atmospheres.

	Experiment	Dilution ratio (air/exhaust)	Concentrations							
			CO, ppm	Total oxidants, ppm	NO_2, ppm	NO, ppm	HCHO, ppm	Acrolein, ppm	Olefin, μg/l.	HC/NO_x ratio [a]
A	Irradiated	150	290	0.78	5.5	1.0	1.93	0.17	8.9	—
B	Irradiated	155	310	0.80	2.66	0.21	2.42	0.20	12.9	4.0
C	Not irradiated	160	300	0.02	1.58	4.27	0.12	0.07	17.8	3.5
D	Irradiated	360	86	0.82	1.64	0.16	1.11	0.11	3.2	2.9
E	Irradiated	330	95	0.57	2.23	0.20	1.02	0.09	1.6	2.5
F	Irradiated	407	85	0.45	2.95	0.59	1.39	0.10	1.53	2.1
G	Not irradiated	375	85	0.00	0.38	2.58	0.38	0.02	5.37	2.6
H	Irradiated	1,350	34	0.35	0.49 [b]	0.25 [b]	0.54 [b]	—	0.57 [b]	2.4
I	Irradiated	935	47	0.33	0.43	0.17 [b]	0.39 [b]	—	1.22 [b]	2.9

[a] HC : hydrocarbons.

[b] Analysis at manifold was not obtained and figure refers to a representative analysis at the irradiation chamber. CO concentration calculated as ppm raw exhaust/dilution ratio.

Table 6. Effect of exhaust exposure on spontaneous running of mice.

Exhaust	Experiment	Preexposure day base [a]			
		No. of animals	Mean change (all animals), %	Animals with decrease/total animals tested	Mean decrease of animals affected, %
A	Irradiated	4	-80.3	4/4	-80.3
B	Irradiated	4	-44.7	3/4	-74.8
C	Not irradiated	5	-30.2	4/5	-38.6
D	Irradiated	5	-58.6	5/5	-58.6
E	Irradiated	5	-8.9	3/5	-33.6
F	Irradiated	5	-17.5	3/5	-37.8
G	Not irradiated	5	-12.7	4/5	-22.6
H	Irradiated	5	-26.6	5/5	-26.6
I	Irradiated	5	+22.4	1/5	-6.4

[a] All calculations are based on the activity of the day preceding exhaust exposure when animals were breathing clean air.

Table 7. Tests for biochemical effects in rats exposed to irradiated auto exhaust.

Assay	Dilution = 1:150 [a]		Dilution = 1:36 [b]	
	Exhaust	Air	Exhaust	Air
Alkaline phosphatase				
Lung, μg P/mg-hr (wet)	4.7 ± 0.34	4.9 ± 1.35	9.2 ± 0.28	9.9 ± 0.38
Serum, μg P/100 ml-hr	34.7 ± 4.71	26.0 ± 0.16	44.2 ± 2.75	44.0 ± 2.46
Glutamic oxaloacetic transaminase (20 min)				
Lung, μg pyruvate/mg (wet)	14.3 ± 1.13	17.2 ± 1.61	19.7 ± 0.63	20.2 ± 0.56
Serum μg pyruvate/mg-100 ml	238.7 ± 8.45	221.0 ± 9.02	166.0 ± 6.65	160.0 ± 7.65
Cholinesterase				
Lung, μl CO_2/100 mg (wet) (10 min)	47.6 ± 3.80	54.9 ± 3.21	—	—
Serum μl CO_2/ml (10 min)	164.0 ± 1.98	148.9 ± 5.20	—	—
Oxygen consumption of surviving lung slices μl O_2/mg-hr (dry)	6.0 ± 0.52	6.1 ± 0.30	—	—

[a] Mean value ± standard error of the mean for 3 animals in each group.

[b] Mean value ± standard error of the mean for 20 animals in each group.

tivity wheel decreased at almost all dilutions tested, but more with irradiated than raw auto exhaust (Table 6). Biochemical measurements (Table 7) on lungs and sera of rats killed during the first hour following a 6-hr exposure period showed little or no alteration at concentrations of 3 to 8 times current community levels as regards alkaline phosphatase, glutamic oxalacetic transaminase, and cholinesterase. Oxygen consumption of surviving lung slices was unchanged by exposure to a 1:150 dilution. Carboxyhemoglobin levels in exhaust-exposed animals increased approximately in proportion to the carbon monoxide concentration of the autoexhaust; however, for the same concentration of atmospheric CO, carboxyhemoglobin was higher in irradiated than non-irradiated gas. α-Naphthylthiourea (ANTU) was administered to male mice 30 min before being placed for 6 hr in chambers ventilated with clean or exhaust-contaminated air. After exposure to exhaust dilutions, mortality from pulmonary edema and pleural effusion was higher in all experiments but one (G).

A 4-hr exposure of mice to high dilutions of light-irradiated automobile exhaust (100 ppm CO, 0.35–0.67 ppm oxidants; 0.3–1.0 ppm NO_2; 0.06–1.9 ppm NO) increases mortality provoked by inhalation of a bacterial aerosol of streptococcus (Lancefield C). Table 8 shows these findings reported by Coffin and Blommer (*75*) of the Cincinnati group, experimenting on a total of 1120 female mice. The results are significant in 14 out of 22 experiments, and the threshold of effects can be fixed at dilutions containing 50 ppm of carbon monoxide. The authors compared these results with previous experiments in which increased infection was shown after exposure to 3.5 ppm nitrogen oxide (*76*) and concentrations of ozone greater than 0.08–1 ppm (*77*).

A study by Mettier et al. (*78*) of the localized effect on intact and de-epithialized rabbit cornea reported negative results after 4 hr exposure to a dilution of irradiated engine exhaust–gas mixture containing 50 ppm of carbon monoxide.

Animals: Chronic Exposure

Three types of animal exposure are to be found in the literature: exposure of laboratory mammals in chambers ventilated with air contaminated by raw or irradiated exhaust, emitted by car engines, exposure to "synthetic" auto exhaust, and field exposure to natural air pollution monitored by near-by stations. The first method necessitates good chemical and engineering support, as developed by the Cincinnati group (*79*). The last method needs a good network of chemical air pollution and meteorological factors, as in the Los Angeles basin. In addition, a variety of biological tests and surveys plus advanced statistical methods and a good deal of patience are the prerequisite for such types of research, which in the case of lifelong investigations on short-lived animals such as rats or mice requires 2–5 yr. These conditions limit the number and validity of this kind of experiment.

Table 8. Mortality from streptococal pneumonia in mice subjected to irradiated exhaust.

Concentration				Mice			Infective control			Significance	
CO, ppm	Oxidant, ppm	NO_2, ppm	NO, ppm	No.	No. dead	Mortality, %	No.	No. dead	Mortality, %	% Change	Probability p
100	0.67	0.8	0.15	20	5	25	20	0	0	25	0.024
100	0.65	1	0.03	20	13	65	20	5	25	40	0.012
100	0.63	0.4	0.02	20	17	85	20	1	5	80	<0.001
100	0.6	0.5	0.03	20	11	55	20	1	5	50	<0.001
100	0.59	1	1.9	20	11	55	20	1	5	50	<0.001
100	0.53	0.7	0.05	20	8	40	20	5	25	15	0.25
100	0.52	0.85	0.06	20	12	60	20	1	5	55	<0.001
100	0.49	—	—	20	7	35	20	4	20	15	0.24
100	0.48	0.5	—	20	14	70	20	3	15	55	<0.001
100	0.35	0.8	—	20	9	45	20	2	10	35	0.015
75	0.41	—	—	30	16	53	30	2	7	46	<0.001
50	0.29	0.55	—	30	13	43	30	2	7	36	<0.001
50	0.28	—	—	30	18	60	30	4	13	47	<0.001
50	0.26	—	—	30	21	70	30	3	10	60	<0.001
25	0.16	0.2	0	30	12	40	30	2	7	33	0.002
25	0.15	0.3	0	30	11	37	30	3	10	27	0.015
25	0.14	0.25	0	30	2	7	30	0	0	7	0.25
25	0.12	—	—	30	4	13	30	2	7	6	0.34
12	0.08	0.16	0	30	4	13	30	1	3	10	0.18
12	0.08	0.27	0	30	3	10	30	1	3	7	0.31
12	0.08	0.14	0	30	1	3	30	2	7	-4	0.5
12	0.08	0.1	0	30	1	3	30	1	3	0	0.75

Exposure Chambers for Auto Exhaust. Between 1966 and 1972 the Cincinnati group published the results of several investigations on the effects of long-term exposure of various mammals—mice, rats, guinea pigs, and dogs—to different dilutions of irradiated or nonirradiated auto exhaust atmosphere. In 1966 Huetter et al. (*80*) experimented on 2016 mice, 126 rats, 72 hamsters, and 99 guinea pigs, with a total of 96 animal exposure chambers containing four concentrations of irradiated auto exhaust (Table 9). During the first study, after 2 weeks exposure there was an outbreak of pneumonia in guinea pigs. Gross lung diagnosis indicated that pneumonia was more severe and resulted in higher mortality in animals exposed to irradiated exhaust. After 15 months exposure, a random sample of three strains of mice, LAF_1, A/j, and C 57/B, showed positive correlation between increasing concentrations of auto exhaust atmosphere and the presence of macrophage cells containing dark particulate material in the cytoplasm. After 14 months exposure, a significant increase in white blood cells was observed in all strains of mice submitted to the high concentration of irradiated exhaust. No statistical effect was noted concerning alveolar cell adenomas, oxygen consumption, or respiratory rates of these mice after 14 months exposure. Both raw and irradiated auto exhaust modified spontaneous mouse activity according to duration of the exposure. However, increase in bone lead concentration in mice and decrease in all lipid lung fractions in rats were observed in the raw auto exhaust atmosphere (dilution 4, with 16 $\mu g/m^3$ of lead). Guinea pig pulmonary function tested at 16-week intervals for a period of 20 months outside the auto exhaust atmosphere appeared to be normal. With regard to reproduction, there was marked decrease in the number of litters, total number of mice born, and total number of infant survivals for female mice receiving

the highest concentrations of irradiated auto exhaust (dilutions 3 and 4).

Table 9. Irradiated auto exhaust dilutions.[a]

	CO, ppm	Hydrocarbons (as CH_4), ppm	O_3, ppm	NO, ppm	NO_2, ppm
Dilution 1	20	6	0.04	0.4	0.15
Dilution 2	50	18	0.1	1.2	0.6
Dilution 3	60	20	0.12	1.3	0.7
Dilution 4	100	36	0.2	1.8	0.9

[a] Obtained by correcting Table 1 of Hueter et al. (*80*) with data of Table 2. Raw auto exhaust contains the same concentrations of CO and hydrocarbons as the irradiated auto exhaust but not the other components (O_3, NO, NO_2).

The effects of preexposure of males and females to automobile exhaust with regard to conception, fetal development, fecundity and infant survival were defined by Lewis, Hueter, and Busch (*81*). Results appear in Table 10. A male I ♂ exposed to irradiated exhaust, or a male C ♂ preconditioned in clean air, was mated either with a female C ♀ preconditioned in clean air or I ♀ preconditioned in irradiated exhaust. The percentage of pregnant females, the number of implantation scars per female, the litter size per female, and finally the percentage of neonatal mortality is given. Two experiments (1 and 2) were performed at 15 day-intervals. In each experiment 150 virgin female mice of LAF_1 strain were preconditioned for 46 days either to clean filtered air or irradiated automobile exhaust (O_3 peak 1.0 ppm in experiment 1 and 0.4 ppm in experiment 2; NO_2 peak 1.0 ppm in experiment 1 and 1.5 ppm in experiment 2; NO peak 1.5 ppm in experiment 1 and 2 ppm in experiment 2; hydrocarbon peaks 40 ppm, carbon monoxide 100 ppm, in both experiments; 12 hr diurnal cycle established with a maximum from 0:00 to 14:00, minimum from 14:00 to 20:00 and another maximum from 20:00 to 0:00). At the age of 12–13 weeks these females were paired with 150 similarly preconditioned males. The exposure to clean or exhaust irradiated air was pursued during mating, gestation, parturition, and suckling periods. The exposure of males to irradiated autoexhaust before mating doubled the nonpregnancy average in females mated with them. This could be interpreted as a mutagenic effect on sperm cells. Fewer implantation scars and smaller litters at birth were noted when the male parent was preconditioned with irradiated auto exhaust and the female parent switched from clean to exhaust atmosphere or from exhaust to clean atmosphere, prior to mating. Mortality of nursling mice was significantly higher for litters exposed to irradiated auto exhaust than for those raised in clean air. No effect was noted on the development of the young, litters of all batches having the same body weight at day 12 and day

Table 10. Effects of irradiated automobile exhaust on reproduction of mice.[a]

	Matings of I♂			Matings of C♂		
	C♀	I♀	Avg.	C♀	I♀	Avg.
Pregnancy, %	87.1[b]			94.3		
Implantation scars per female	9.0	9.2	9.1[c]	9.7	9.6	9.6
Litter size per female	8.2	7.9	8.0[d]	8.6	8.9	8.7
Neonatal mortality, %						
Experiment 1	30.1	60.2[e]		17.8	70.0[e]	
Experiment 2	19.3[f]	30.8[f]		5.3	12.3	

[a] Data of Lewis et al. (*81*).

[b] Adverse effect on male reproductive function leading to decreased conception rate.

[c] Adverse effect on male reproductive function leading to decreased implantation of fertilized ova.

[d] Adverse effect on male reproductive function leading to decreased litter size.

[e] Direct adverse effect on neonatal survival.

[f] Adverse effect on male reproductive function leading to increased infant mortality.

21. The experimenters explain the difference in neonatal mortality between experiment 1 and experiment 2 by the difference in the amount of nesting material employed in the two experiments. More was used in the second experiment, and this could have lowered the concentration of oxidants breathed by the young burrowed down in their nests.

In the same research center, Lutmer et al. (*82*) reported the effect of exposure to nine different dilutions of auto exhaust on lead concentration in bones of male and female mice (A/j, C 57/B and LAF_1) (all bones except the spine). Mice exposed to low levels of raw auto exhaust in a cyclic diurnal pattern for 15 months had more lead in their bones than when exposed to equal or slightly lower concentrations of irradiated auto exhaust, even though the daily averages of atmospheric lead concentration were about the same in both cases (15 $\mu g/m_3$). The experimenters do not clearly explain this inconsistency except by possible dissimilarity in the physical or chemical forms of lead particles in irradiated and nonirradiated auto exhaust dilutions.

Studies on dogs have also been published. Two groups of twelve beagles were exposed for 18 months to raw auto exhaust (100 ppm CO; 24–30 ppm of hydrocarbon as methane; 0.1 ppm NO_2; 1.5–2.0 ppm NO) and another group to irradiated auto exhaust (100 ppm CO; 24–30 ppm of hydrocarbon as methane; 0.5–1.0 ppm NO_2; 0.1 ppm NO; 0.2–0.4 ppm oxidant as O_3). Nineteen control dogs breathed normal air. Tests of pulmonary function (transpulmonary pressure, respiratory air flow) performed outside the exposure chambers on dogs anesthetized with pentobarbital showed no effect of auto exhaust gas, irradiated or otherwise. Vaughan et al. (*83*) who published these negative results, pointed out that this pulmonary insensitivity of the dog may be due to the scrubber efficiency of the nose of this species of mammal, a peculiarity which has been referred to by other investigators (*84*). Pulmonary functional tests were repeated in the same beagles later, 36 and 61 months after beginning auto exhaust exposure (*85*) and a higher diffusing capacity was found in the dogs breathing auto exhaust dilutions. This was interpreted as increased pulmonary capillary blood volume induced to prevent hypoxia. Irradiated exhaust also increased expiratory resistance. In addition, it was noted that SO_2 (1.27 ppm) plus H_2SO_4 (0.09 ppm) added to raw exhaust provoked pulmonary hyperinflation. This indicates the importance of synergetic interactions of different air pollutants.

Possible long-term cardiovascular effects of exposure to auto exhaust was investigated in female beagles. Dogs were exposed for 5 yr for 16 hr daily (from 8:00 to 0:00): eleven to raw auto exhaust (CO=114.5 ppm; hydrocarbons as CH_4=15.7–19.6 ppm; NO_2=0.188 ppm; NO_2=0.188 ppm; NO=1.84–2.45 ppm), and eleven to irradiated auto exhaust (CO=114.5 ppm; hydrocarbons as CH_4=15.7–19.6 ppm; NO_2=0.94–1.88 ppm; NO=0.12 ppm; oxidants as O_3=0.393–0.785 ppm). Electrocardiograms taken outside the exposure chambers of dogs at rest and after exercise (swimming) were compared to the ECGs of 19 control dogs. Venous right ventricle, pulmonary, and systemic arterial pressure were also measured, but under pentobarbital anesthesia. Left axis deviation was observed in one control dog and bradycardia in one dog exposed to irradiated exhaust. Vectorcardiographic abnormalities were found in three dogs exposed to irradiated auto exhaust. Postexercise ECG abnormalities were observed in one dog out of 11 breathing SO_x (SO_2=0.14 ppm and H_2SO_4=0.10 ppm) added to the irradiated exhaust. Except for one dog exposed to irradiated exhaust which had statistically significant systemic arterial hypertension, no hemodynamic abnormalities were noted.

The work of the U.S. Environmental Toxicology Research Laboratory in Cincinnati is still continuing, and a report was recently presented by Stara and collaborators (*86*) of this Research Center concerning the toxicology of atmospheric pollutants resulting from fuel additives and emissions associated with the use of catalytic automobile converters. Two engines (Ford 1975 prototype and Chevrolet 1973) equipped with catalytic converters were used to compare effect on survival rates in infant rats and weight changes in lactating females. Subacute (7 days), rather than chronic experiments, showed the most severe effects in the groups exposed to irradiated and nonirradiated noncatalytic exhaust. After exposure for 5 days to exhaust without the catalyst, extensive histopathological lesions were found in adult hamsters and rats, consisting of subacute puru-

Table 12. Rats exposed for 10 weeks to auto exhaust (dilution B).

	Auto exhaust			Control chamber			Out of chamber		
	n	$\bar{x}$	σ	n	$\bar{x}$	σ	n	$\bar{x}$	σ
Organ weights									
Adrenals mg/rat	15	4.78	0.59	15	5.05	0.69	15	4.94	0.76
Brain, g/100 g body weight	15	0.474	0.024	15	0.459	0.026	14	0.471	0.051
Heart, g/100 g body weight	15	0.300	0.078	15	0.291	0.010	14	0.311	0.017
Lungs, g/100 g body weight	15	0.426	0.078	15	0.385	0.030	14	0.420	0.039
Water in organs									
Brain, g/100 g body weight	15	78.12	0.40	15	78.30	0.57	14	78.28	0.48
Heart, g/100 g body weight	15	77.36	0.43	15	77.27	0.49	14	77.37	0.50
Lung, g/100 g body weight	15	77.33	0.60	15	77.60	0.52	14	77.62	0.57
Blood tonometry									
pH without equilibration	15	7.40	0.09	14	7.41	0.07	15	7.34	0.06
pH 3.15% CO_2	15	7.43	0.06	14	7.44	0.03	15	7.41	0.05
pH 7.65% CO_2	15	7.25	0.03	14	7.26	0.03	15	7.24	0.05

rate, ventricular complex (QRS_{II}), electrocardiographic deflection, rat survival, or renal and aortic lesions resulting from aging, although the emphysematous process might be accelerated but not increased. Bouley, Prulière and Riotte (*90*) also studied animals exposed for 45–105 days to the preceding dilution of auto exhaust containing the highest concentration of NO_x (23 ppm) and observed no modification of several immunological tests in mice, but in rats they noted a decrease in the number of alveolar macrophages. Penetration of air pollutants into rat lungs and kidneys during exposure to auto exhaust dilution was tested by chemical qualitative analyses with Castaing's electronic microprobe (*91*). Calcium, iron, phosphorus, silicon, aluminum, magnesium, titanium, potassium, and traces of sulfur were detected in lungs and kidneys of rats exposed to clean air. These elements are the same as those found in the "black pigment" of human lungs (*92*). In addition to these elements, the lungs and kidneys of the rats discontinuously exposed during their entire lifetime to the dilution of exhaust gas containing 57 ppm CO, 0.37% CO_2, 23 ppm NO_x, 0.9 ppm NO_2, 2 ppm aldehydes, and 0.5 mg/l. hydrocarbon, and 8.5 $\mu g/m_3$ lead contained chlorine, manganese and zinc. Lead (partly as sulfate salt) was found constantly, mostly in heavily calcified regions of pulmonary tissue and femurs. Its concentration was five times that of control rats (E. Radford, personal communication, 1974).

Table 13. Blood chemistry for rats exposed for 5 months to auto exhaust (dilution A).

	Auto exhaust			Control chamber		
	n	$\bar{x}$	σ	n	$\bar{x}$	σ
Hemoglobin	15	168.5	14.4	15	166.8	18.5
Glucose, g/l.	15	0.98	0.14	15	0.96	0.14
Proteins, g/l.	15	67.9	3.1	15	66.0	3.9
Lipids, g/l.	15	5.76	1.48	15	5.98	2.39
Cholesterol, g/l.	15	1.29	0.42	15	1.74	0.60
Lactic acid, mg/l.	15	231	62	11	206	55
SGOT, mU/ml	15	57.9	6.2	14	54.8	13.4
SGPT, mU/ml	15	28.3	5.1	14	25.8	5.5
LDH, mU/ml	15	752	129	14	695	170

Table 14. Hematology for rats exposed to auto exhaust (dilution A) for 5 months.

	Auto exhaust			Control chamber		
	n	$\bar{x}$	σ	n	$\bar{x}$	σ
Hematocrit, %	15	39.06	2.43	13	39.84	2.71
Red blood cells $\times 10^{-3}$	15	7.789	0.899	13	8.055	0.718
Platelets $\times 10^{-3}$	15	0.816	0.126	13	0.828	0.206
White blood cells $\times 10^{-3}$	15	9.988	2.276	13	9.338	2.439
Leukocytes						
Neutrophiles	15	30.7	7.6	13	24.2	8.8
Lymphocytes	15	1.2	1.4	13	1.7	1.9
Eosinophiles	15	67.9	7.0	13	73.5	9.7
Monocytes	15	0.2	0.5	13	0.4	0.8

lent bronchiolitis and pneumonia. In the group exposed to catalyst-treated exhaust, no significant morphological changes were noted in either rat or hamster tissues. The concentrations of the pollutant components were not specified in the communication presented at the Paris International Symposium in 1974 (*87*).

A series of long-term exposures to nonirradiated automotive exhaust gas was carried out by a research group of the French National Institute of Health (INSERM) (*88, 89*). Pathogen-free Sprague Dawley male rats were exposed from 10 weeks to lifetime (2 yr) to exhaust gas containing 40–50 ppm CO, 0.065% CO_2, 0.2 ppm NO_x, 0.1 ppm aldehydes, and less than 0.5 mg/l. hydrocarbons. The exposure schedule was 6 hr/day, 5 days/week. One series of experiments was performed with dilutions of exhaust gas of the type to be found in polluted urban air (experiment A–1: 50 ppm CO; 0.2 ppm NO_x; 0.07% CO_2 0.1 ppm aldehydes; hydrocarbons < 0.5 mg/l.), and two series of experiments with much higher pollutant concentrations, especially the last one (experiment A–2: 84 ppm CO, 2 ppm NO_x, 0.08% CO_2, and < 0.5 mg/l. of hydrocarbons; experiment B: 58 ppm CO; 23 ppm NO_x, 0.08% CO_2 2 ppm aldehydes, hydrocarbons < 0.5 mg/l.). Each of these experiments was performed on groups of 15 or 30 rats exposed for periods varying from 10 weeks to 2 yr. Rats placed in an adjacent chamber ventilated with clean air at the same ventilation rate were used as controls. Body weight, continuous recording of CO_2 emission taken as an index of metabolism, ECG of unanesthetized animals outside the chamber, and avoidance training were also performed outside the chamber on some series of rats. In other series, organs (brain, heart, kidneys, liver, testes, suprarenals, skin) were weighed and water content determined; hematology, blood, pH and buffering capacity, biochemical blood analyses (glucose, lipids, proteins, cholesterol, lactic acid, transaminases and LDH), were carried out on blood obtained by decapitation. Tables 11–14 show most of the data obtained, which are negative except for increase of adrenal weight (Table 11) and neutrophils (Table 14). However, the dilution containing the highest concentration of NO_X (23 ppm), which is about 50–100 times that found in urban air pollution, produced definite biological effects: decreased body weight, diminution of sound-avoiding reflexes, and increase in the number of spontaneous tumors in relation to aging, none of which were pulmonary tumors. However, this dilution did not influence heart

Table 11. Rats exposed for 5 months to auto exhaust (dilution A).

	Exhaust gas			Control chamber		
	n	$\bar{x}$	σ	n	$\bar{x}$	σ
Organ weight						
Adrenals, mg/rat	11	6.29	1.03	12	5.67	0.53
Brain, g/100 g body weight	15	0.425	0.015	15	0.397	0.086
Heart, g/100 g body weight	15	0.305	0.014	15	0.304	0.020
Kidneys, g/100 g body weight	15	0.791	0.053	15	0.795	0.079
Lungs, g/100 g body weight	15	0.446	0.047	15	0.433	0.054
Liver, g/100 g body weight	15	4.022	0.363	15	3.908	0.540
Spleen, g/100 g body weight	15	0.163	0.014	15	0.164	0.024
Testes, g/100 g body weight	15	0.712	0.073	15	0.706	0.171
Skin, g/100 g body weight	15	12.167	0.438	15	12.423	0.829
Carcass, g/100 g body weight	15	48.97	1.95	15	49.15	1.90
Water in organs						
Brain, %	15	78.25	0.85	15	77.99	0.50
Heart, %	14	77.91	0.40	15	78.06	0.66
Kidneys, %	15	77.60	0.65	14	77.24	0.65
Lungs, %	14	77.65	0.92	15	77.82	1.36
Liver, %	15	70.24	0.35	14	70.28	0.68
Spleen, %	15	77.97	0.25	14	77.72	0.48
Testes, %	15	86.81	0.18	14	86.81	0.17
Skin, %	15	54.70	1.92	15	54.00	2.06

Exposure to Synthetic Auto Exhaust. Considering the great variability in the composition of auto exhaust, attempts were made as early as 1956 to expose animals to synthetic auto exhaust. Kotin and Falk (*93, 94*), utilizing ozonized gasoline (1.0–3.8 ppm of ozone), noted increase of lung tumor incidence in strain A and C_{57} black mice when exposure to ozonized gasoline was combined with influenza virus (*94, 95*). Evaporating leadfree gasoline into a stream of oxygen and ozone after ultraviolet irradiation, Nettesheim and Szakal (*96*) obtained a maximum concentration of 1 ppm O_3 and 24–30 mg/m^3 gasoline, which provoked epithelial atrophy of the terminal bronchioles of mice exposed to the mixture for 15–18 months. Some histological lesions were observed in hamsters inhaling a concentration of this mixture containing 1 ppm O_3 and 105–110 mg/m^3 gasoline.

Animal Field Experimentation. Proposals have been made to study the reactions to urban air pollution of various animal species in zoos in order to obtain adequate animal models.

Urban (*97*) set up a study of long-term biological effects of air pollution on a flock of chickens exposed to ambient air, compared with a flock breathing charcoal-filtered air in an adjacent room. After 2 yr, records of food consumption, weight, egg production, fertility, and hatching quality of eggs and morbidity and mortality of the birds did not demonstrate any significant difference between the two flocks that could be attributed to polluted air.

Several authors have attempted to correlate naturally occurring canine pulmonary diseases with variations in ambient air quality. Results have generally been inconclusive (*98, 99*). However, Reif and Cohen (*100*) recently compared 1007 pet dogs living in Philadelphia and the surrounding country, and observed chest x-ray differences in aged city dogs whose lungs contained areas of black pigment.

Several experimentations on mammals were performed by the University of Southern California School of Medicine at Los Angeles (*101*). In 1965, Swann et al. (*102*) published results of studies made of three groups of 40 guinea pigs placed in three widely separated-air-polluted areas in Los Angeles. A sheltered group of 40 guinea pigs placed in a room supplied with filtered air was used as a control. Pulmonary resistance, measured monthly by plethysmography, showed no difference between guinea pigs living in polluted ambient air and those kept in filtered air. During the second year of their life span, mortality of animals living in ambient air was slightly, but not significantly greater than that of those living in filtered air. Additional measurements of pulmonary resistance were made on some of the guinea pigs on days with unusual weather conditions or smog during 1963 (oxidants, 0.2–0.9 ppm; CO, 40–72 ppm; hydrocarbons, 16–40 ppm; nitrogen oxides, 1.2–3.1 ppm). When these pulmonary resistances were compared with routine monthly measurements on the same animals, significant increases in resistance were found at oxidant levels of approximately 0.30 ppm or more. Low temperature with high relative humidity was also associated with increased pulmonary resistance. Aging increased pulmonary resistance of animals in all groups, but the effects of breathing polluted ambient air were the same in old and young guinea pigs. There were great individual differences in sensitivity to smog and in recuperation after the pollution episode of the variations measured by plethysmography. Some animals died during these smog episodes, and autopsy revealed that most of them had pneumonia or moderate pulmonary edema. The monthly mortality rate in older animals (over 22 months old) during the 2-month period following the pollution episode was almost double the average. No deaths were observed in younger guinea pigs during these two months. An electron micrograph survey of pulmonary alveolar tissue made by Bils (*103*) in small groups of 4–5 mice placed in the same areas in Los Angeles and killed during and after periods of heavy smog (more than 0.4 ppm oxidants) showed alteration of mitochondria, disruption of cytoplasm and lamellar inclusions. As the air irritants subsided, related cellular processes fell back to normal. Wayne and Chambers (*104*) observed increased incidence of pulmonary adenomas in certain strains of inbred mice exposed throughout their lives to ambient atmosphere in Los Angeles, but this was not confirmed. As a matter of fact, a study of 7000 mice of three inbred strains (A, A/j, and C_{57} black) exposed under the same conditions for 5 yr, showed no increased pulmonary neoplasia, but revealed increased susceptibility to endemic bacterial pneumonitis (*105*). In the same series

of animal field experimentations in the Los Angeles area, a study (*106*) was made of pathogen-free Sprague-Dawley male rats exposed during their natural life span in the four air-pollution sites (153 rats) and in a control room ventilated with filtered ambient atmosphere (92 rats). There was no significant difference in life span or bodyweight at death between rats exposed to polluted air and controls. The polluted ambient Los Angeles atmosphere (ozone concentration peak between 0.3 and 0.5 ppm) did not cause any discernible histological effect on lung tissue, but the incidence of chronic nephritis was significantly greater in senile male rats exposed to ambient air than in senile control rats. Neoplasms were found in 24 of the 153 rats exposed to the ambient Los Angeles air (15%) and 9 of the 92 breathing filtered air (9%); the difference between the two groups was not statistically significant.

Cellular Tests

Although *in vitro* experiments are difficult to extrapolate to reactions in a whole organism, they can nevertheless reveal fundamental mechanisms. Rounds, Awa and Pomerat (*107*) exposed Chang strain of human conjunctival cells to an atmosphere of undiluted raw exhaust gas. This resulted in a decrease of the total mitotic index and decrease of abnormal mitotic figures. When submitted to five different dilutions of auto exhaust (from undiluted to 1:100) treated by chloroform, which extracted mostly hydrocarbons, this culture also gave similar responses in mitotic figures. Different bacterial species were exposed to raw irradiated automotive exhaust in the U.S. Division of Air Pollution Laboratories (*3*). The growth of *Escherichia coli* was more inhibited by irradiated than nonirradiated exhaust. However, samples of ambient air collected in down-town Los Angeles during periods of little or no smog did not inhibit growth of bacteria.

Series of experiments were made with raw exhaust gas extracts in paraffin without substantiated chemical measurements. A single application of this paraffin extract caused alterations in mitotic abnormalities of chick fibroblast cultures (*108*), cultures of pulmonary tissue from chick embryos (*109*), and inhibition of corneal epithelial cell proliferation which had been induced by grafting a fragment of pulmonary tissue in the anterior chamber of the mouse eye (*110*).

More recently, Bouley et al. (*111*) confirming the previously observed inhibition of growth of *Escherichia coli* by diluted raw exhaust, attributed this reaction to its aldehyde content.

Active Biological Components of Automobile Exhaust

The worldwide effects of automotive exhaust are tangible, but in view of the tremendous quantitative and even qualitative variations of the gaseous, liquid, and solid components, and their possible physiocochemical and biological interactions, the scientific approach should be fundamentally analytical, taking each component of exhaust into consideration. Although there are almost infinite possibilities of chemical substances produced by automobile fuel combustion, toxicity studies exist for only a few of them. However, considering the extent of available data, a brief review only will be given here; further details can be found in the references.

Carbon Monoxide

The biological effects of carbon monoxide have been the subject of many studies since that of Bernard in 1875 (*112*). Physiological reviews (*113, 114*), a bibliography (*115*), the reports of a specialized conference of the New York Academy of Sciences (*116*), and a recent monograph of the U.S. Department of Health, Education and Welfare (*33*) have analyzed most of its toxic properties, and it is not our purpose to compile them here. According to the conclusion of the latter monograph (*33*) have analyzed most of its toxic properties, and it is not our purpose to compile them here. According to the conclusion of the latter monograph (*33*), exposure of nonsmoking men to a concentration of 30 ppm (35 mg/m^3), which is a level commonly resulting from urban air pollution by automotive traffic, gives 4% CO Hb in 4 hr and 5% CO Hb in 8–12 hr. Even more than carbon monoxide inhaled when breathing polluted air, tobacco-smoking presents a serious threat to man. Tobacco smoke contains 2–6% carbon monoxide, and anyone smoking 20 cigarettes a day could have 3–6%

CO Hb in his blood, a very heavy smoker as much as 10% CO Hb.

The level of 5% CO Hb in the blood is generally considered to affect psychological and psychomotor reactions of normal subjects (*117–119*). It has recently been observed that a 3.4% increase of CO Hb level would be sufficient to prejudice driving skills (*120*). Changes in cardiac output and myocardial arteriovenous oxygen have been measured in men after 12% carboxyhemoglobin saturation (*121*). This could be due to intensification of the venoarterial shunt effect, so that cardiopulmonary patients would be sensitive to a CO Hb blood level as low as 6%. This explains why higher risks of cardiac infarct are among the injuries to health attributed to tobacco via carbon monoxide.

Teratogenetic effects have been shown in chick embryos when eggs are exposed to 650 ppm CO during the first 18 days' incubation (*122*). A carbon monoxide level high enough to have such an effect is not encountered in polluted urban atmosphere, but lower birth weight of newborn has been observed when mothers are heavy smokers.

It is doubtful whether the absence of physiopathological reactions in smokers can be considered an adaptative phenomenon in man. Adaptation is very difficult to define. In the case of long-term exposure to carbon monoxide, adaptation could be considered absence of reactions, in particular no change in hematocrit, hemoglobin, and red cells. This has been demonstrated in long-term exposure of animals to concentrations of 50–100 ppm (*123, 124*).

Carbon Dioxide

Carbon dioxide emitted into the atmosphere by volcanos and biological activities of animals and plants is dissolved and/or buffered in the ocean as CO_2, bicarbonate, or carbonates, so that the atmosphere always contains 0.03% of this gas (*125*). Moreover, physiological action of carbon dioxide is obtained at a concentration of 3% in man, and death occurs in mice at concentrations of 50% (*38*). This eliminates the potential danger of the 5–14% carbon dioxide found in undiluted auto and diesel exhaust. More details of the numerous biological properties of carbon dioxide have been reviewed recently (*126*). McEnroe reported a rather amusing ecological phenomenon (*127*) concerning the effect on adult tick distribution (*Acarine ixodidae*) by increased pCO_2 along roads in Falmouth (Massachusetts).

Hydrocarbons

Experimental data of exposure of animals and humans to various hydrocarbon compounds were reviewed in a monograph of the U.S. Department of Health; Education and Welfare (*34*). Although Russian experimenters (*128*) report related experimental effects of very low concentrations, it is generally admitted that, to be biologically reactive, concentrations of aliphatic and alicyclic hydrocarbons must rise to more than 500 ppm, which is 100 and 1000 more than the levels found in ambient atmosphere. Concentrations over 1% produce narcosis and convulsions, supposedly due to hypoxic effect. Aromatic hydrocarbons are biochemically and biologically active. Eye irritation provoked by hydrocarbons has been attributed to their chemical structure (*129*).

The danger of these low molecular weight hydrocarbons contained in auto exhaust does not lie in their chemical entity *per se* but in the fact that they are involved in the formation of photochemical smog, resulting in interactions between ultraviolet irradiation and nitrogen oxides which promote formation of oxidants far more biologically active than this kind of hydrocarbon, such as aldehydes, ketones, and peroxyacetyl nitrate.

The polycyclic hydrocarbons identified in the atmosphere of cities, resulting principally from heater and furnace emissions and also automotive, especially diesel exhaust, appeared potentially more dangerous to health. These hydrocarbons are mainly solid and are carried in the air on soot and particles by which they are absorbed. It is technically very difficult to measure their concentrations in urban air.

The U.S. Surgeon General's report of 1962 (*3*) indicates nine carcinogenic compounds in a list of polynuclear aromatics isolated in automotive exhaust particulates: chrysene, benz[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, benzo[j]fluoranthene, 11-H-benzo[b]fluoranthene, dibenz[a, h]anthracene, dibenzo[a, e]pyrene, and dibenzo[a, l]pyrene. The well-established relationship between cigarette smoking and pulmonary cancer has stimulated research into the role of air pollution as an etiological factor, as some urban air pollutants,

particularly polycyclic hydrocarbons, are similar to those found in cigarette smoke. Many investigations concerned benzo[a]pyrene, whose concentration in auto exhaust varies from 2.5 to 12.0 $\mu g/m^3$, depending on the cycle. Its air content in rural environment is approximately 0.5 $\mu g/1000\ m^3$, but has reached as much as 123–165 $\mu g/1000\ m^3$ in Vienna (Austria) during the winter months (*5, 130*). Relevant literature is extensive, especially as the carcinogenic effect of soot was the first to be demonstrated by Pott in the eighteenth century, and since the medical faculty now tends to attribute high carcinogenic risk to chemical environmental factors (*131, 132*).

Difficulties in representative air sampling, the 30 yr latent period necessary for carcinogenic action, and the incidence of tobacco smoking obscured the results of epidemiological surveys. However, reviewing recent epidemiological reports, Carnow and Meier (*133*) conclude that an increase of 1 $\mu g/m^3$ of benzo[a]pyrene in ambient air is responsible for a 5% increase in pulmonary cancer death rate.

Theories have been proposed establishing relationships between the electronic configurations of polycyclic hydrocarbons and their potential carcinogenicity (*134, 135*). However, basic research is still needed on the parts of their molecules which are still active after enzymatic action (such as benzopyrene hydroxylase) and concerning the targets aimed at. Animal experimentation interpreted benzo[a]pyrene as a cofactor in the cancer-forming process. Laskin, Kuschner, and Drew (*136*), for example, reported that 10 ppm SO_2 must be inhaled at the same time as 10 mg/m_3 benzopyrene to provoke squamous cell carcinomas in rat lungs.

Benzopyrene is mutagenic in *Drosophila, Neurospora, Entamoeba coli* and mice; it induces mitotic gene conversion in *Saccharomyces cerevisiae* (*137, 138*).

Here again it will be noted that effects are obtained only with enormous amounts of pollutants compared to those in polluted urban air. However, the risk must not be minimized, as was noted in the report of the U S. Surgeon General (*3*): "a person breathing the air of some U.S. cities over a year's time might inhale as much benzopyrene as from smoking two packs of cigarets daily." Since that time, and as a consequence of replacing coal combustion by other sources of energy, benzo[a]pyrene air concentration has decreased. The U.S. National Air Surveillance Network found 2.5 units of benzo[a]pyrene in urban areas in 1967, compared with 6.6 units in 1959 (*133*). In addition, penetration into the organism of these high molecular weight hydrocarbons is chiefly conditioned by the median mass diameter of the particles and the different mechanisms which determine their retention (*84, 139, 140*).

Though acute inhalation of high concentrations of gasoline vapors may cause severe pneumonia (*141–143*), the signs and symptoms of chronic exposure are not well defined (*144–146*). The toxicity of gasoline also depends on the lead additives it contains.

Aldehydes

Data on toxicology of aldehydes as air pollutants can be found in a monograph of the U.S. Department of Health, Education and Welfare (*34*). Formaldehyde is detectable by odor or optical chronaxy at concentrations of about 0.06 ppm. The threshold for eye irritation has been estimated at 0.01–1.0 ppm. The acrolein threshold for olfaction and eye irritation is 0.25 ppm. The irritant threshold for acetaldehyde is only 50 ppm. Much of the unpleasant smell and irritation of diesel and gasoline exhaust is due to their aldehydes content.

The upper airways appear very sensitive to these products, and *in vitro* ciliary activity of rat and sheep trachea is inhibited by small concentrations of aldehydes (*147–150*). This could inhibit local defenses against penetration of air particles into the lungs, though this approach might be rather teleogical. Concentrations of 0.20–1.20 ppm total aldehydes and 0.05–0.12 ppm formaldehyde seem to be physiologically active in the highly polluted Los Angeles air. However, this is far below the lethal doses resulting from acute inhalation: 150 ppm for acrolein in man, according to Champeix and Catilina (*151*), 16 ppm for formaldehyde in mice, guinea pigs, and rabbits, according to Salem and Cullumbine (*152*), 10,000–20,000 ppm for acetaldehyde in rats, according to Skog (*153*), and Smyth (*154*).

The mutagenicity of formaldehyde has been described in *Drosophila, Neurospora* and *Entamoeba coli* (*138*) and male mice (*137*). Nevertheless, it must be remembered that for-

maldehyde is used in enormous quantities (e.g. 3–4 billion pounds in the U.S. in 1965), generally in the form of aqueous solutions, so that the emission of aldehydes by auto exhaust might appear small in comparison.

Nitrogen Oxides

Most of the toxicological data concerning nitrogen oxides can be found in a monograph published by the U.S. Environmental Protection Agency (*35*). Several accidents have been reported in man acutely exposed to nitrogen dioxide: a level of 500 ppm provokes pulmonary edema and death, while 25–75 ppm induces bronchitis and bronchopneumonia. During occupational exposure of welders to atmospheres containing mixtures of nitrogen monoxide and nitrogen dioxide, decreased maximal breathing capacity and increased expiratory resistance accompanied by methemoglobinemia have been observed at peaks of 5 ppm NO_2.

In animals, a 3.5 ppm level of NO_2 increases mortality of mice exposed to *Klebsellia pneumoniae* (*155*); 0.5 ppm NO_2 is the threshold of increase in tracheal pressure of anesthetized guinea pig (*36*). Freeman et al. (*156*) detected histological lesions of bronchiolar epithelium of rats exposed during their entire life time to 2 ppm of nitrogen dioxide. Nitrous acid, which is obtained through hydration of nitrogen oxides, induces crosslinking of DNA strands and is a mutagenic agent (*137, 138*).

The maximum NO_x concentration found in the atmosphere of highly polluted cities, principally in California, is but little higher than the olfactory threshold, which has been estimated at 0.12 ppm.

Sulfur Oxides

Historically, sulfur oxide is the first gaseous air pollutant whose concentration was measured. Its pathological effects have been particularly well studied in Great Britain. During an acute smog episode in London in December 1952, the average SO_2 concentration reached 1.34 ppm, with a peak of "smoke" of 1200 $\mu g/m^3$, and was followed by 4,000 excess deaths. Lawther (*157, 158*) established a fully documented relationship between the bronchitis syndrome and this type of air pollution. The English Clean Air Act of 1956, limiting the number of sources of pollution (principally coal burnt in open fireplaces) in the City has freed London from this dangerous winter smog, and has apparently decreased the incidence of bronchial infections (*159*).

Acute toxicity in animals is obtained at quite high concentrations (50–16,000 ppm) of sulfur dioxide (*27, 38, 160*). This suggests that during these acute episodes there may have been an association with other pollutants which have not been measured, aggravation by meteorological factors, and also selection of elderly victims already suffering from cardiopulmonary diseases. In different animal species the variety of anatomical morphology, and presumably of mucous secretion mechanisms of the upper airways, plays a predominant part in the absorption of SO_2, as well as that of particles. Dogs, for instance, are not very sensitive to sulfur dioxide intoxication because of the "scrubber" effect of their long noses (*83*).

Man can detect the odor of concentrations of 1–3 ppm of sulfur dioxide.

SO_2 is emitted by coal- and sulfur-contaminated fuel heaters and power sources, and concentrations range from 0.2 to 1.6 ppm in polluted cities. Regarding motor vehicles, the risk of sulfur oxide pollution is limited to diesel engines, whose exhaust gases contain 100–300 ppm SO_2.

Oxygen

Auto exhaust gases containing between 1 and 14% oxygen are markedly hypoxic. Nevertheless, the amount of oxygen consumed by automotive engines does not decrease the oxygen content of ambient air, which appears to be quite stable. In any case, as it occurs at moderate altitude, hypoxia does not endanger human physiology.

Oxidants

A monograph of the U.S. Department of Health, Education and Welfare has recently summarized toxicological data concerning the two most important oxidants encountered in air pollution: ozone and peroxyacetyl nitrate (PAN) (*4*).

Ozone is the most toxic of known air pollutants. In man a concentration of 0.1 ppm provokes eye irritation, 0.6–0.8 ppm decreases diffusing capacity and other pulmonary functions, and 0.02–0.05 ppm is the olfactory threshold. Ozone odor fatigue develops readily.

In animals, LD_{50} in mice is 4 ppm (*38*). Freeman et al. (*161*) reported histological bronchiolar changes in dogs exposed to 1–3 ppm ozone for 8–24 hr/day for 18 months.

Peroxyacetyl nitrate toxicity is not so well known, but its median lethal concentration in mice has been estimated at 100 ppm (*162*).

Peaks of 0.2 ppm of ozone have been reported in Los Angeles, Denver, and Philadelphia, and it is certain that this oxidant is a real danger in polluted urban atmosphere. However, it results from electrical discharge and solar irradiation, as well as from photochemical reactions induced by ultraviolet irradiation of hydrocarbons and nitrogen oxides emitted by auto exhaust.

Metals

Gasoline and automobile engine parts contain small portions of metals which are emitted into the air, including platinum, palladium, and lead (*163*). Roadside soil and vegetation are contaminated by deposits of lead, cadmium, zinc, and nickel which have been ascribed to automobile traffic (*164*). Of these metals, a few could be a threat to human health: cadmium accumulates in the kidneys, and nickel is suspected of causing bronchial cancer (*32, 165*). Platinum toxicity should be reconsidered if this metal were to be widely used in catalytic treatment of auto exhaust. This subject was examined as part of a recent conference on mobile air emissions in attempts to evaluate potential exposures and toxicities of platinum and palladium (*163*). Lead, which is an additive to gasoline, constitutes the chief amount of metal in auto exhaust.

A recent WHO monograph (*166*) reviewed the danger of lead absorption by man. Recent symposia surveyed low level toxicity of heavy metals in the environment (*167, 168*) and summarized the behavioral, biological, biochemical, and developmental toxicities of lead in experimental animals and humans. Lead represents 0.01% of the surface soil of the earth, so that as an oligoelement of the human body it varies accordingly to geological factors. It is usually estimated that inhalation constitutes one third and ingestion two thirds of man's usual lead intake. As for carbon monoxide, a strong relationship has been established between lead and automobile traffic when leaded gasoline is used (*169*). Different concentrations of lead in the air ranging from 1.0 $\mu g/m^3$ (composite urban U.S.) to 44.5 $\mu g/m^3$ (Boston Sumner tunnel) correspond to human blood levels of 160 $\mu g/l$. (composite urban U.S.) to 300$\mu g/l$. (Boston Sumner Tunnel employees) (*170*). Populations geographically located near highways show higher lead concentrations than comparable samples taken at some distance from these automobile sources of lead emission. At more than 700 m (200 ft) from the highway, ambient air lead content reaches localized background levels (*171, 172*).

Lead content in soil is usually 20 $\mu g/kg$, but along heavily traveled roads it can reach as much as 403 mg/kg in the upper 5 cm of topsoil. From there it enters plant tissues and the bodies of animals grazing on polluted vegetation: in this way it contaminates the human food chain (*165, 167, 173–177*).

As previously outlined, blood lead reflects contact with lead but does not indicate the route of entrance, whether by inhalation or ingestion. Lead levels are also influenced by sex (blood lead levels in women are lower than those in men), tobacco smoking (smokers have a slightly higher level than nonsmokers) and age. Infants often have high blood lead levels for no clear reason, and they are also more susceptible to lead intoxication than adults. Moreover, lead penetrates into maternal milk.

Nothing positive has yet been reported on the pathology of lead inspired in air particles, except in cases of acute intoxication by fumes. However, lead in the New York Staten Island Zoo has been held responsible for morbidity and mortality of leopards, snakes, cats, primates, and even a great horned owl (*178*).

Medical research has developed sensitive diagnostic criteria of human lead intoxication: anemia, coproporphyrinuria, δ-aminolevulinic acid (*d*-ALA) dehydrase urinary excretion, decrease of red blood cell *d*-ALA dehydrase, and measurement of lead in blood and hair. The difficulty in recognizing the threshold of air lead concentration is due to the numerous sources of lead intake (*179*), and the fact that 90% of the lead absorbed is known to be fixed and accumulated in bones. The long-term physiopathological significance of this accumulation is not yet well understood. Experiments on men and animals continuously inhaling high concentrations of lead (3.5–20 g/m^3) show increased blood lead and usually decreased red

blood cell *d*-ALA dehydrase, without further apparent modifications in health (*167, 180*).

Nevertheless, literature has reported possible human genetic effects, and lead acetate produces chromosome abnormalities *in vitro* (*180*).

In addition to lack of substantiated information on possible toxicity, the fact that it poisons chemical catalysis has caused various countries to decrease lead content of gasoline when possible. However, the eventual effects of the proposed metallic catalysts are not well known at present.

Odors and Subjective Nuisances

At the present time only subjective methods are able to detect odors. Ignorance of the chemical molecules responsible—aldehydes and oxidants are suspect—impedes a scientific approach (*181*).

Clouds of black smoke constitute a psychological nuisance for esthetic reasons which have psychophysiological value in human comfort and welfare. Particulates also screen solar ultraviolet irradiation.

Diesel exhausts discharging black, malodorous smoke have come under regulations forbidding this particularly objectionable aspect of air pollution (*182*).

Gasoline Additives Other Than Lead

Addition of catalysts usually reduces levels of carbon monoxide and total hydrocarbons, but increases the amount of particulates. The allergenic properties of platinum (*183, 184*) and even the toxicity of palladium might be suspect (*86*). Mutagenicity of other gasoline additives, such as β-propiolactone, dimethyl nitrosamine and trimethyl phosphate, has been demonstrated *in vitro*, mainly in *Neurospora* (*138*). However, information is lacking regarding the concentration of unreacted or biologically active products remaining in automotive exhaust after they have been submitted to the carburation of gasoline.

A subsidiary form of air pollution generated by automobiles is solid particles resulting from dispersion of road dust by movement of the wheels and brake and tire abrasion.

Factors Influencing Exhaust Gas Toxicity

The apparent variability in toxicity of a chemically well-defined inhaled gas must be emphasized. A simple experiment will illustrate this (*185, 186*): the lethality resulting from exposure to a mixture of 5.5% oxygen in 94.5% nitrogen should depend only on the pO_2 of the gas mixture. Inhalation of this gaseous mixture was repeated 28 times at 40-day intervals on a group of 100 SPF mice of the same strain and age. The percentage of mortality was 49.29 ± 18.53 ($\bar{x}$ and σ), range 6.48–77.04%. The variations depend on circadian factors, light, temperature, barometric pressure, and also biological factors, particularly sex and presumably genetics.

The physiopathological effects of air pollutants may possibly have even greater variability. The chemistry of air pollutants—Truhaut (*187*) speaks of a "soupe de pollutants"—is not definite as with a mixture of oxygen in nitrogen. The different physicochemical entities of auto exhaust components—gases, liquid or solid aerosols—determine their absorption into the airways (*84*). There are also many environmental factors (meteorology, altitude, presence of other pollutants, and individual factors such as age, sex, genetics, pathology, and possibly toxic addiction such as tobacco, all of which influence biological effects and consequently the toxicity of the different air pollutants. This would be the case for automotive exhaust components. Exaamples of these environmental and biological factors responsible for changes in the physiopathology of air pollution will be briefly reviewed.

Environmental Factors

Meteorology. Winds, rain and thermal changes depending on weather and also geographical conditions determine whether air pollutants remain or are dispersed, especially when in the form of aerosols. Increased mortality from respiratory diseases has been observed in downwind zones of Los Angeles (*188*). Local urban microclimates (*189*) and highway distribution must be investigated, rather than the global aspect of air pollution in the formation and evolution of local auto exhaust pollution. As was demonstrated in California (*2*) and other places, solar and ultraviolet irradiations can transform hydrocarbons into photooxidants.

As Durham's study also showed (*58*), since climate has an effect on man's pathology (*190*), it conditions not only the intensity of air pollution episodes, but also individual response to this type of environmental aggression (*191*). It must be remarked that most of the morbidity and mortality resulting from acute air pollution episodes (Meuse Valley, 1930; Donora, 1948; London, 1952) occurred in periods of damp and cold weather.

High altitude creates hypoxia in decreasing barometric pressure conditions, and thus constitutes a particular challenge in "high highway tunnel" engineering where carbon monoxide hypoxia results from automotive exhaust (*192*).

Rhythms of Automotive Exhaust Air Pollution. In the light of development of chronotoxicology (*193–196*) and demonstration (Fig. 1) of periodic circadian sensitivity (nycthemeral, daily) to carbon monoxide (*124*), it may be wondered whether the rhythm of circadian, circaseptan (weekly) and even circannual (seasonal) automative exhaust air pollution can have a cyclic effect on human health.

Circadian variations of street carbon monoxide result from the different intensities of automobile traffic (*10, 44, 197*). Figure 2, showing the average carbon monoxide air concentration and hourly car traffic count in midtown Manhattan in 1968, illustrate the 12-hr plateau of urban automobile activity in the city (*197*). Figure 3 illustrates a more subtle temporal evolution of circadian concentrations of carbon monoxide, nitrogen monoxide, nitrogen dioxide, and ozone found in the Los Angeles atmosphere (*35*).

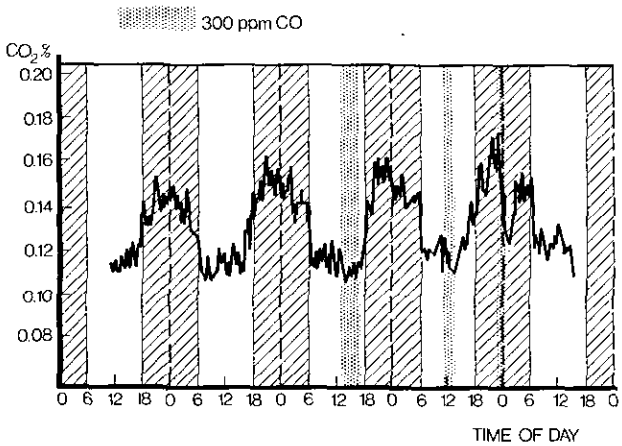

FIGURE 1. A group of 17 Sprague Dawley CFE male rats is synchronized by light (06:00–18:00) and dark (18:00–06:00) as shown by its circadian rhythm of CO_2 emission (continuously recorded). Concentrations of 300 ppm of carbon monoxide are given at different times of the light and dark period (dotted area). Only inhalation at 00:00 provokes decreased carbon dioxide emission. (Stupfel, unpublished data).

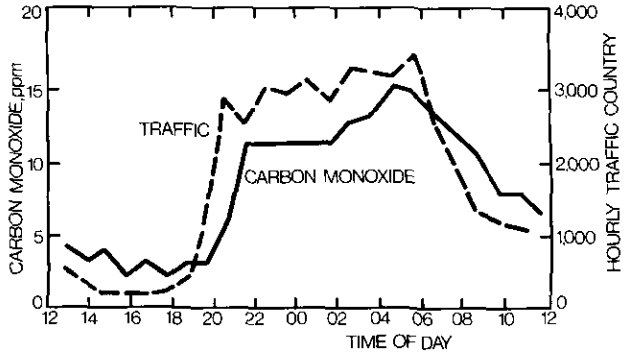

FIGURE 2. Hourly average carbon monoxide concentration and traffic count in mid-town Manhattan (*33*).

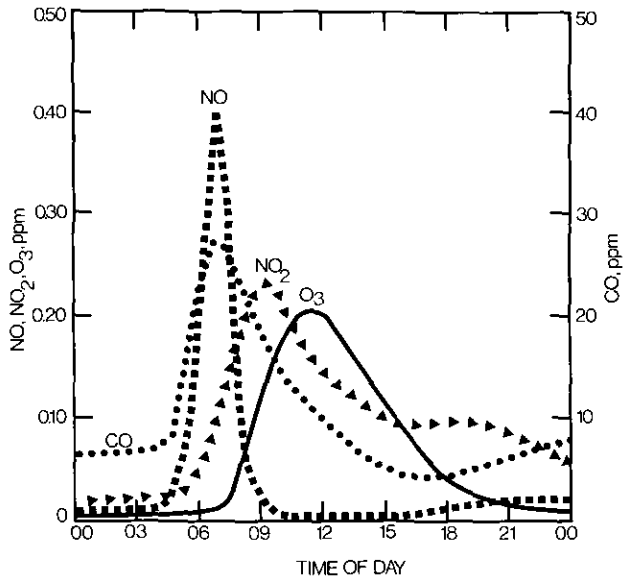

FIGURE 3. Average daily 1-hr concentrations of selected pollutants in Los Angeles, July 19, 1965 (*35*).

Circaseptan weekend air pollution rhythms result from social events regulating car traffic (Fig. 4) (*35*). The different proportion of diesel and gasoline vehicles on weekdays and

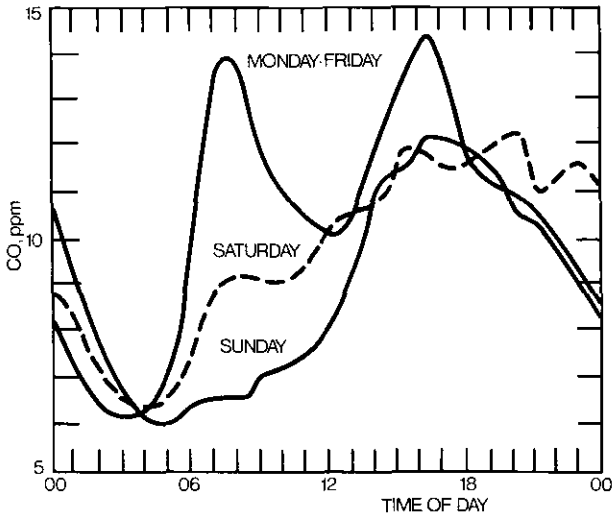

FIGURE 4. Diurnal variation of carbon monoxide levels on weekdays, Saturdays, and Sundays in Chicago, 1962–1964 (*35*).

at weekends provides another type of circaseptan rhythmn (*198*).

Seasons produce circannual rhythms of air contaminant concentrations. Pollen calendars (*199, 200*), heating sources in winter, and summer vacations rhythmically modify the contents of solid, liquid, and gaseous contaminants of the atmosphere, associated with cyclic seasonal meteorological variations, ultraviolet irradiation, clouds, wind, rain, fogs and vertical temperature gradients, all factors which condition origin and diffusion of air pollutants (*201–204*).

The existence of such external respiratory synchronizers raises the question whether they can influence rhythms already existing in living bodies, either from endogenous or exogenous sources. Fundamental bioenergetic rhythms, in fact, have been demonstrated in all living organisms (*193, 205, 206*) and these variations in energy are accompanied by changes in oxygen consumption, parallel to changes in human cardiac and respiratory rates (*207–211*). However, although circadian, circaseptan and circannual rhythms of air pollution are an accepted fact to-day, there is no clear parallel recognition of human circadian, circaseptan and circannual rhythms of pulmonary sensitivity.

Individual Factors

Age influences penetration and removal of air pollutants. Compared with adults, children have smaller airways which decrease the size of penetrating aerosols, and also higher oxygen consumption, which increases respiratory exchanges and hence the rapidity of toxic gas inhalation. In addition, as the walls of children's airways are thinner, they are consequently more fragile. Moreover, diseases in young children reflect their lack of immunity. The previously reported increase of lead in children's blood is also a source of investigation for hygienists. For all these reasons, infants appear particularly sensitive to air pollution, as revealed by various epidemiological inquiries (*212–215*).

Elderly people, especially those suffering from cardiorespiratory affections, are the chief victims of acute episodes of air pollution. Asthmatic people have been proposed as human "sentinels" for detecting air pollution, for they feel better when breathing in hypoallergenic chambers away from polluted environmental air (*216, 217*).

Sex differences in the pathological effects of tobacco and different air pollutants have been revealed in human epidemiological surveys (*58, 218*) and by air pollutant toxicological studies on animals (*38, 70*). It has been experimentally demonstrated that pregnancy in mice increases acute mortality resulting from carbon monoxide inhalation (*219*).

The importance of genetic factors has been pointed out in man as regards α-l-antitrypsin deficiency and bronchial sensitivity to tobacco (*12–14*), allergic asthma (*15, 16*) and experimental susceptibility of mouse to ozone (*220*).

Increases in metabolism resulting from physical work and exercise intensify cardiac output and respiratory ventilation and thus augment the rapidity with which air pollutants penetrate the airways and thence the organism. This could be taken as an example of the physiological conditions that considerably modify human behavior in an air polluted zone. High concentrations of oxidants have been observed to have a deleterious effect on athletic performance in cross-country track events in the Los Angeles area (*221*). Furthermore, when 20 young men—smokers and nonsmokers—were tested for maximal aerobic power in a 35°C environment, inhaling 50 ppm carbon monoxide reduced the work time (treadmill) of nonsmokers and elicited changes in the respiratory

patterns of both smokers and nonsmokers (*222*).

Among diverse physiopathological factors that could influence the toxicity of air pollutants, infection is the only one that is well known, for it has been the subject of bacteriological experiments on animals. As already pointed out, nitrous oxides and irradiated auto exhaust dilutions increase mortality from experimental microbial infection (*75, 76*). The mechanism could be a decrease in pulmonary defences, especially by action on alveolar macrophages (*223–226*) or even exacerbation of microbial pathogenicity (*227*).

Although more studies are necessary, in all likelihood self-intoxication considerably increases the action of auto exhaust. In car accidents in which carbon monoxide from auto exhaust was suspected of causing modifications in neurophysiological reflexes, the driver has very often been smoking and drinking heavily (*228, 229*).

It has been clearly shown that cigarette smoking is an important etiological factor of bronchitis and pulmonary cancer in man. Most of the components of automotive exhaust are found in tobacco smoke (Table 15), but the major difference is that tobacco smoke is directly inhaled, while the components of auto exhaust are diluted in the atmosphere.

Table 15. Gaseous and particular components of automotive exhaust and tobacco smoke.

Gases and particles	Automotive exhaust	Tobacco smoke
N_2, %	76–90	67–71
Argon, %	1	1
O_2, %	1–14	12–14
CO_2, %	5–12	7–10
CO, %	2–6	2–4
NO_x, ppm	30–1500	150–600
SO_2, ppm	0–30	—
Acrolein, ppm		60
Formaldehyde, ppm	10–300	120
CH_4, ppm	200–800	2000–3000
Saturated hydrocarbons, ppm	500–5000	45,000
Unsaturated hydrocarbons, ppm	500–5000	12,000
Benzopyrene	1–10 $\mu g/m^3$	0.002–0.12 μg/cigarette
HCN, ppm	—	300–1500
Particles	0.2–3 mg/g burned gasoline	8.2%
Lead	70–80% of the lead in the motor fuel	

Interaction between cigarette smoking and auto exhaust air pollution is obvious as regards blood carboxyhemoglobin. The fixation of carbon monoxide on the blood of smokers is repeatedly increased with each cigarette smoked (Fig. 5), and the wash-out from the blood depends on the pCO of the ambient air (*230, 231*). It has also been generally observed that incidence of pulmonary symptoms resulting from occupational respiratory exposure to organic materials, as in byssinosis, is more frequent in smokers than in nonsmokers (*232, 233*). It might also be supposed, therefore, that tobacco-smoking increases reaction to particular auto exhaust materials suspended in the air. The fact that tobacco smoking is the most dangerous individual form of air pollution leads to reconsidering the conclusions of many previous epidemiological surveys on the effects of air pollution on bronchitis and pulmonary cancer which did not take the smoking factor into account. This factor is generally quantified by the number of cigarettes, and is more precisely estimated than the usual intermittent exposure to variable concentrations of air pollutants.

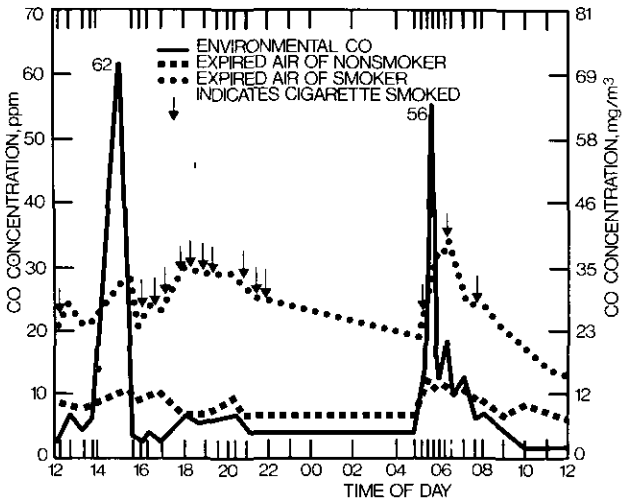

FIGURE 5. Carbon monoxide levels of environmental air and of expired air from smokers and nonsmokers, Los Angeles and Pasadena, August 1962 (*33*).

Thresholds of Biological Activity—Air Quality Standards

These few examples show the intricacy of environmental and individual cofactors in the physiopathological reactions of living systems (such as respiratory or neurophysiological reactions). Man's very specific reactivity is the result of genetic origins, motivation and social and economical backgrounds. In view of the rareness of chance observations in man during impressive episodes of acute air pollution and the toxicity of most of these air pollutants it is necessary to resort to animal experiments, which provide a very imperfect model, however, because of metabolic variations between species. Toxicologists therefore have to fix thresholds of biological activities and quality standards by such disparate data as monkey exposure experiments and human field studies (*68*). In addition, the lack of standardized methodology applicable to experimental and epidemiological research on harmful environmental factors hinders the evaluation and comparison of data obtained in different laboratories, and leads to repetition of incomplete results, hastily arrived at and too often apparently contradictory (*234*).

All this must be taken into account when examining Table 1. Biological activity thresholds correspond to concentrations of isolated gases or particles which can affect either men or laboratory animals. When two figures are given in the column on biological activity threshold in Table 1, the first corresponds to the minimum concentration physiologically detectable and the second to a value which includes a few days' pathological reactions. For example, 200 ppm of carbon monoxide after 1 hr provokes a HbCO concentration of 5% in a nonsmoker, which corresponds to the HbCO blood level of a man smoking one pack of cigarettes per day. No apparently detrimental effect is noted, except possible but contested impairment of visual function. When inhaled for 1 hr, a 300 ppm CO concentration results in 7% HbCO blood level, which could provoke myocardial changes in a coronary patient.

It is already very difficult to fix a definite threshold for the pollutants enumerated in this list, while it is quite impossible for respiratory allergens whereby individuals react to trace concentrations. This reaction depends more on genetic origins and previous physiopathological history than on definite concentrations.

Obviously, if all the toxic substances in Table 1 were inhaled simultaneously at the concentrations of threshold biological activity given, a dangerous toxic, and presumably mortal effect would result.

The data given in Table 1, air quality standards as defined mainly in the United States of America, generally correspond to one-tenth or even a much smaller fraction of the concentrations given in the adjacent column referring to biological activity thresholds. Air quality standards were determined mainly for the purpose of protecting human health, and also with a view to their possible economical application. This last point explains why fixed standards of air pollutant concentrations in the United States are higher in California than in other states of the union.

Conclusions

Auto exhaust gases have no more definite toxicity than chemical entity. American laboratory and field studies have demonstrated the interactions between their principal gaseous, liquid, and solid components and the environment in which they are emitted. Their physicochemical properties, nature and evolution depend on various atmospheric factors: temperature, humidity, insolation, cosmic rays, etc. Meteorology and geography, time of day and season of the year are determinant in the qualitative and quantitative equilibrium of their chemical components with other air pollutants produced by human activity.

Numerous studies have recently been made on the toxicity of the principal components of auto exhaust: carbon and nitrogen oxides, hydrocarbons, aldehydes, oxidants, and metals. The acute toxicity of these substances is much better known than subacute and chronic toxicity, and generally pathological action has been demonstrated at concentrations one hundred times those found in polluted urban atmospheres. Subtle toxicological interactions are possible that make it difficult to extrapolate toxicity data of separate substances to combinations of substances. The simplest and surest approach is to study dilutions of auto exhaust, but their diversity of composition due to the different types, cycles and operating conditions of vehicles (gasoline or diesel) and varieties of

fuel impede comparison and generalization of most experimental data obtained in this way.

Determination of acute and even subacute toxicity of auto exhaust is impossible in human beings, in view of health hazards and medical ethics. Unlike acute episodes of air pollution, acute auto exhaust accidents are rare and mainly suicidal. Odor, lacrymation, and intense physiological reactions disclose high concentration risks. Its insidiousness and high toxicity make carbon monoxide the most dangerous component, and it must be monitored everywhere potential danger from auto exhaust exists.

As it is practically impossible to isolate auto exhaust emissions from other air pollutants, no human epidemiological survey can be directly related to auto exhaust action. Californian epidemiological surveys of the effects of air pollution on human pathology (respiratory and cardiovascular) are of interest, for auto exhaust constitutes an important part of atmospheric pollution in this state. Studies analyzing health conditions of road tunnel employees and traffic officers are more specific. Long-term surveys of such populations have been performed only in important cities of the United States. Nowadays they do not give clearcut pathological results, except for nonspecific respiratory disorders, partly due to other sources of air pollution and tobacco smoking, which overwhelm the concentrations of auto exhaust emissions.

Experimental studies on laboratory animals —mice, rats, guinea pigs, and dogs—give the only reliable data about acute and long-term auto exhaust toxicity, since sufficiently high dilutions can be used, the concentrations of the different components controlled, and the time of exposure determined. Except for a few recent French experiments (INSERM), most of the published data come from United States laboratories (especially Cincinnati). Auto exhaust has greater toxic effects when irradiated (formation of oxidant components) than when raw. Nonspecific respiratory reactions, increased pulmonary infections and reproductive disorders (possibly mutagenesis) have been reported, but at concentrations generally one hundred times higher than those detected in the air of the most highly polluted cities (published data). Although possible carcinogenic action of such high concentrations cannot be completely excluded, they do not seem to affect animal longevity.

Recent human epidemiological air pollution surveys and animal experiments on gaseous toxicity, particularly carbon monoxide, emphasize the importance of associated environmental factors (meteorology, chronobiology, other toxic substances) and individual circumstances (age, sex, pathology, genetics). These cofactors explain the apparent variation in toxicity and the existence of very susceptible groups of individuals which certain authors have proposed considering as air pollution "sentinels."

As auto exhaust pollution is essentially urban, it does not affect wild animals, except in City zoos. Sheep and cattle grazing beside motorways with heavy traffic become contaminated by the lead content of the vegetation.

To counteract the effects of auto exhaust on human health more biological research is needed. To judge the effect of an air pollutant, its penetration into the organism must be demonstrated. This has been proved for carbon monoxide and metals, but for high weight molecules (hydrocarbons, carcinogens) and even gases (nitrous and sulfur oxides), the nature of the biologically active metabolites penetrating into the organism are unknown. Differences in metabolism (especially from one animal species to another) are highly probable and do not permit extrapolation to man. Excretion or retention of most auto exhaust components, particularly trace metals, should be elucidated. Target organs and long-term and mutagenic effects should be defined. Methodology of experimental and epidemiological research must be developed to provide sensitive and reliable tests for respiratory and even neurophysiological investigations. Planning, standardization of techniques, exchanges of results at the international level, and evaluation and comparison of data obtained in different laboratories are needed to avoid the repetition of incomplete results, hastily reached, and too often apparently contradictory. The physicochemical interaction between air pollutants and their by-products conditioned by diverse environmental factors, particularly meteorological ones, need further investigation for better measurements and monitoring in the field. As exposure to air pollution, even occupational, is also discontinuous in man, processes to quantify the degree of exposure (dosimetry) would be welcome.

Secondly, the incomplete state of air quality standards already referred to makes threshold

fixation more a matter of opinion than a scientific approach, which will be possible only when the mechanism of action of each component of air exhaust is fully understood, as is the case for carbon monoxide. Moreover, the example of Los Angeles illustrates the importance of geographical and weather incidence on local autoexhaust air pollution. European climates, traffic intensity, emission of other air pollutants and everything that concerns genetic origins, alimentation, and pathology of populations is sufficiently diverse to consider appropriate separate air quality criteria based on local considerations. Man's possibilities of adaptation are also difficult to predict. For example, the last world war showed that the calculated requirements of caloric energy in Europe could be reduced by 30% without resultant undernutrition. Truhaut's conclusions in 1964 (*235*) concerning air pollution standards are still valid: "The sometimes excessive measures of hygienists and toxicologists must be tempered by an appraisal of the negative social and economic consequences which may result from too rigid attitudes. As it is impossible actually to set air purity standards on the basis of an ideal criterion, namely, demonstrated harmless, certain calculated risks must be accepted, without however, overlooking the necessity of reducing emissions of potentially toxic air pollutants as far as possible."

Thirdly, there should be a preventive attitude towards the noxious effects of air pollutants on human health. Technical engineering procedures should be used to try to reduce the emission of the most dangerous compounds (carbon monoxide, oxidants, metals), but these modifications should involve introduction of other potential dangers; for example, some antiknock products and catalysts are possibly mutagens. Tobacco, alcohol, drugs, alimentary additives, and psychosociological stresses have been proved greater potential dangers to human welfare and health than auto exhaust. In any case, the automotive industry must participate in the improvement of the quality of life. The fact that in most American and European cities the burden of auto exhaust air pollution has been stabilized or even decreased is a great encouragement. In addition, air monitoring and medical surveys of high risk patients and people particularly exposed to auto exhaust must also be developed.

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