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Long-term canine exposure studies with ambient air pollutants

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Long-term canine exposure studies with ambient air pollutants. J. Heyder, S. Takenaka. ©ERS Journals Ltd 1996.

ABSTRACT: Dogs are often the species of choice as an experimental model for the study of pulmonary responses to long-term exposure to air pollutants in chambers simulating environmental or occupational exposure in man. Their lungs bear a reasonable resemblance to human lungs, they are large enough to allow serial measurements of pulmonary responses, and they live long enough to ensure that findings are not confounded by aging.

Several long-term canine exposure studies with ambient air pollutants have been performed since 1957: seven studies with gaseous and particulate sulphur (IV); three studies with nitrogen oxides; three studies with ozone; two studies with acidic particles; three studies with mixtures of sulphurous pollutants that might have resembled the 1952 London smog; and one study in which raw and ultra violet (UV)-irradiated motor vehicle exhaust and sulphurous pollutants were used. The findings support the hypothesis that long-term exposure to air pollutants at ambient levels might cause bronchitic lesions (sulphur oxide), emphysematous lesions (nitrogen dioxide) or fibrotic lesions (ozone). None of the studies showed an indication of synergistic effects.

To improve our understanding of pulmonary responses initiated by the inhalation of pollutants over long periods of time, new concepts are needed. Investigators should consider studies with canine models of cardiopulmonary diseases, the application of novel immunological and molecular biology techniques, the phenomena of tolerance and adaptation to inhaled air pollution, and exposure atmospheres with increasing complexity, including fine and ultrafine particles.

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There is increasing evidence of an association between air pollution and the exacerbation and development of respiratory diseases. However, more data on exposure-response relationships and underlying mechanisms are needed to test the hypothesis of causality. The collection of these data requires integrated lines of investigation: long-term animal studies and *in vitro* studies with well-defined exposure atmospheres composed of single pollutants or mixtures of pollutants. These studies must be complemented by short-term human exposure studies. Together, these research efforts must address the following questions: 1) Which pollutants or combinations of pollutants should be considered pneumotoxins? 2) What dose of these pollutants should be considered harmful? 3) Which individuals are at risk? and 4) Is it feasible to extrapolate pulmonary responses to pollutants observed in laboratory animals to human lungs?

It is, therefore, very important to select the appropriate species for studies with laboratory animals. Progressive pulmonary responses observed in these animals should mimic the slowly developing, harmful effects to low levels of air pollution seen in humans.

Frequently, the species used are small (*e.g.* rat, mouse, hamster, guinea-pig, rabbit); especially for cross-sectional studies involving large groups of animals exposed to different levels of pollutants [1–13]. However, these animals are too small to be used in longitudinal studies, in which pulmonary responses of individuals during long-term exposure are of interest in order to simulate environmental or occupational long-term exposure of humans.

Monkeys and cats have been used to study morphological alterations initiated by inhaled pollutants [3, 4, 14–16]. Although monkeys are closer to humans than cats, they are difficult to handle and long-term studies are costly and labour intensive. Cats can also be difficult to handle. Therefore, dogs are often the species of choice for long-term exposure studies with air pollutants. They are easy to handle, their lungs bear a reasonable resemblance to human lungs, they are large enough to allow serial measurements of pulmonary responses over the course of a long-term exposure, and they live long enough

Previously published reviews of this series

1. Sandström T. Respiratory effects of air pollutants: experimental studies in humans. *Eur Respir J* 1995; 8: 976–995.
2. Chitano P, Hosselet JJ, Mapp CE, Fabbri LM. Effect of oxidant air pollutants on the respiratory system: insights from experimental animal research. *Eur Respir J* 1995; 8: 1357–1371.

to ensure that findings are not confounded by aging of the animals.

There is now sufficient evidence from long-term animal studies to conclude that exposure to high levels of sulphur dioxide results in bronchitic lesions [17–21], high levels of nitrogen dioxide produce emphysematous lesions, and ozone induces fibrotic lesions [22]. To better understand the pulmonary responses to these and other air pollutants, especially when present at lower, more ambient levels, a number of long-term studies have been conducted using dogs exposed in chambers [23–52]. The specific pollutants under investigation have been ozone, nitrogen oxides, gaseous and particulate sulphur(IV) S(IV), acidic particles, and motor vehicle exhaust. This review is focused on the results from these studies and is part of the series "Respiratory Effects of Air Pollution" [22, 53, 54]. Response of canine lungs to inhaled cigarette smoke [55–59], or inhaled radionuclides [59–61] are not considered in this review.

Sources, ambient levels, air quality standards and respiratory responses to the pollutants listed above are discussed and summarized in a number of documents [53, 62–69].

Characteristics of the canine respiratory system

Among canine strains, the beagle is the most widely-used for respiratory toxicology studies. The structural and functional characteristics of its respiratory system are well-documented, with the current database dating back to 1970, when it was inaugurated by ANDERSEN [70]. In this chapter, the beagle's respiratory characteristics are summarized and compared to those of the human and rat.

Mean values of selected lung function parameters are listed in table 1 [71]. Nasal, airway and acinar structures are each described below.

Nose

The structure of the canine nose is more complex than the human nose because of its olfactory function. The nasal turbinates (nasoturbinate, maxilloturbinate and ethmoturbinate), covered by mucosa, occupy the major portion of the nasal cavity. They are characterized by a complex folding and branching pattern. The structure of the canine and human nasal and oral cavities allows oronasal breathing. In contrast, most small laboratory animals are obliged to breathe nasally.

Table 1. – Lung function characteristics [71]

	Beagle	Human	Rat
Tidal volume cm^3	193	500	1.4
f_R breaths·min^{-1}	19	12	115
Minute volume $cm^3 \cdot min^{-1}$	3700	6000	160
Lung compliance $cm^3 \cdot Pa^{-1}$	0.23	0.008	0.02
Total lung capacity cm^3	2.3	6	0.008

f_R: respiratory frequency.

Lung

Beagle lungs have seven lobes. The left lung is composed of cranial-middle and caudal lobes; the right lung of cranial, middle, caudal and accessory lobes. In comparison, human lungs have five lobes (left: upper and lower lobes; right: upper, middle and lower lobes) as do rat lungs (left lobe; right: cranial, middle, caudal and accessory lobes).

The comparative subgross anatomy of lungs is well-documented [72–74]. Dog and rat lungs have a thin pleural wall and little, if any, interlobular connective tissue. In contrast, human lungs have a thick pleural wall and extensive interlobular connective tissues.

Conducting airways

Airway branching patterns are shown in figure 1. This pattern is monopodial in beagle and rat lungs, but dichotomous in human lungs. Between the trachea and the most peripheral alveolus, canine lungs have 30 airway generations, whereas rat and human lungs have only 24.

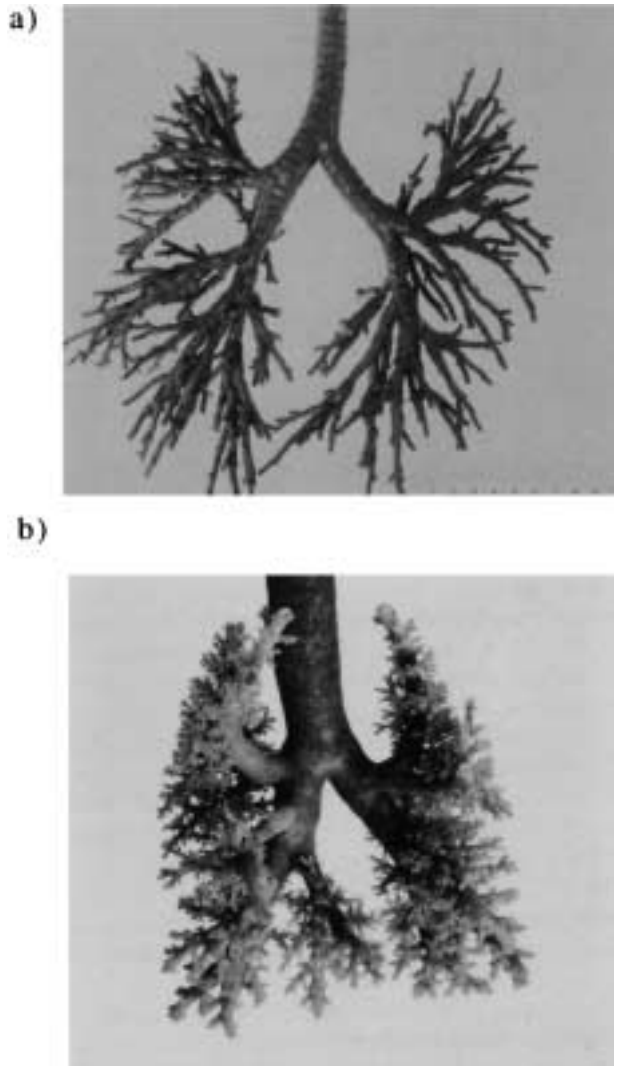

Fig. 1. – Tracheobronchial replica casts from: a) human lungs (courtesy of Dr Phalen); and b) beagle lungs (courtesy of Dr Schulz).

Table 2. – Typical pathway lung models [75]

	Dog			Human			Rat		
Generation No.	Tubes n	Length cm	Diameter cm	Tubes n	Length cm	Diameter cm	Tubes n	Length cm	Diameter cm
1 (trachea)	1	14.8	1.8	1	10.0	2.01	1	2.680	0.340
2 (main bronchus)	2	1.38	1.49	2	4.36	1.56	2	0.715	0.290
3	3	1.52	1.16	4	1.78	1.13	3	0.400	0.263
4	5	0.989	0.879	8	0.965	0.827	5	0.176	0.203
5	8	0.846	0.605	16	0.995	0.651	8	0.208	0.163
6	13	0.547	0.472	32	1.01	0.574	14	0.117	0.134
7	21	0.443	0.399	64	0.890	0.435	23	0.114	0.123
8	35	0.370	0.377	128	0.962	0.373	38	0.130	0.112
9	57	0.400	0.345	256	0.867	0.322	65	0.099	0.095
10	95	0.320	0.329	512	0.667	0.257	109	0.091	0.087
11	158	0.287	0.300	1024	0.556	0.198	184	0.096	0.078
12	262	0.256	0.264	2048	0.446	0.156	309	0.073	0.070
13	434	0.289	0.220	4096	0.358	0.118	521	0.075	0.058
14	721	0.191	0.191	8192	0.275	0.092	877	0.060	0.049
15	1195	0.162	0.176	16384	0.212	0.073	1477	0.055	0.036
16	1983	0.159	0.141	32768	0.168	0.060 A	2487	0.035	0.020 A
17	3290	0.134	0.114	65536	0.134	0.054	4974	0.029	0.017
18	5458	0.124	0.092	131072	0.120	0.050	9948	0.025	0.016
19	9054	0.103	0.074	262144	0.092	0.047	19896	0.022	0.015
20	15019	0.094	0.052 A	524288	0.080	0.045	39792	0.020	0.014
21	30038	0.081	0.044	1048576	0.070	0.044	79584	0.019	0.014
22	60076	0.071	0.036	2097152	0.063	0.044	159168	0.018	0.014
23	120152	0.063	0.031	4194304	0.057	0.043	318336	0.017	0.014
24	240304	0.055	0.027	8388608	0.053	0.043	636672	0.017	0.014
25	480608	0.049	0.025	3×10^8	0.025	0.030	3×10^7	0.0072	0.0086
26	961216	0.043	0.023						
27	1922432	0.038	0.022						
28	3844864	0.033	0.022						
29	7689728	0.029	0.021						
30	15379456	0.026	0.021						
31	8×10^8	0.0111	0.0133						

A: terminal bronchiole.

Typical pathway models of canine, human and rat lungs are listed in table 2 [75]. In the canine lung model, terminal bronchioles are represented by airway generation 20. However, morphological observations on lungs of six beagles (right caudal lobe) indicate that terminal bronchioles are actually associated with airway generation 25 [52].

Cell types, proliferation and differentiation in the mammalian airway epithelia are well-documented [76]. The density of cells differs among animal species and anatomical sites [77]. The cellular density in the bronchial tree of beagles (table 3) is similar to that of monkeys [16], and sheep [78]. Detailed data for human and rat lungs are not yet available.

The Clara cell becomes the major cell type in bronchioles. However, there are large differences among animal species in Clara cell morphology and distribution [79].

Submucosal glands are observed throughout the bronchial tree of beagles (table 4). In contrast to human and dog lungs, bronchial glands are not found in the lungs of small rodents. This interspecies variability is of great importance, since bronchial hyperplastic glands are typical indicators of chronic bronchitis.

Table 3. – Density of cellular components in the caudal lobe of the right beagle lung [52]

	Airway generation	
	5	20
Mucous goblet cell	0.129±0.047	0.181±0.028
Ciliated cell	0.387±0.018	0.385±0.033
Basal cell	0.274±0.021	0.282±0.025
Special type cell	0.025±0.019	0.024±0.013
Migratory cell	0.017±0.017	0.009±0.05
Small mucous granule cell and undifferentiated cell	0.169±0.034	0.125±0.044

Values are presented as mean±SD.

Table 4. – Volume fraction of submucosal glands in the bronchial wall of beagles [52]

Airway generation	Volume fraction
5	0.024±0.009
10	0.040±0.003
15	0.057±0.002
20	0.038±0.003

Values are presented as mean±SD.

Table 5. – Morphometric parameters of the acinar region [86]

	Dog	Human	Rat
Tissue volume cm^3	78	314	0.43
Epithelium			
Type I cells	16.5	32.5	0.08
Type II cells	5.6	32.1	0.04
Interstitium			
Cellular	12.9	54	0.07
Noncellular	22.8	98.3	0.13
Endothelium	17.6	42.6	0.09
Macrophages	2.5	54.7	0.02
Surface area m^2			
Epithelium			
Type I cells	51	89	0.39
Type II cells	1	7	0.02
Tissue thickness µm (harmonic mean)	0.45	0.75	0.38

Acinus

An acinus is composed of all airways distal to a terminal bronchiole and, thus, is served by a first-order respiratory bronchiole [80, 81]. In the acinus of beagle lungs, several generations of alveolated bronchioles (*i.e.* respiratory bronchioles) are detected; human acini have similar morphology. However, the lungs of small rodents have either no respiratory bronchioles or, at most, one generation [80]. The average number of alveolar pores is similar in a canine and a human alveolus (7.2 *vs* 6.1 pores per alveolus), but smaller (1.3 pores) in a rat alveolus [82]. Since the acinar region is the main target site for many air pollutants, these interspecies differences in acinar morphology must be considered when comparing pulmonary responses to inhaled pollutants among species.

The structure of the acinar region of beagle lungs is well-documented [83–87]. Morphometric parameters of the acinar region beyond the respiratory bronchioles are listed in table 5 [86].

Dosimetry of inhaled particles and gases

Dosimetry refers to the mass of a pollutant that reaches specific target sites in the respiratory system. The dose received at these sites can be expressed as the mass of the pollutant per unit epithelium surface area (surface dose), or per unit volume element of lung tissue (volume dose). It depends upon the interaction between the inhaled pollutant and the liquid layer lining the specific site. In the case of particle exposure, all particles that hit this layer adhere to it. However, in the case of exposure to a pollutant gas, adherence of molecules depends on the solubility of the gas in the lining layer. Inhaled sulphur dioxide is rapidly taken up by airway surfaces, so the proximal airways have to be considered specific target sites. Inhaled ozone or nitrogen dioxide is less rapidly taken up in the lungs. Therefore, the centriacinar region (junctions between respiratory bronchioles and alveolar ducts) is the specific target site for these gases.

Dosimetric models for inhaled particles and gases are not yet available. Therefore, surrogate parameters are used to estimate the dose received by the respiratory system. Frequently, the concentration of particles and gases in the inspired air and the exposure time are the only surrogate parameters considered. When used alone, these parameters present a very incomplete picture of dose. More accurate dosimetry also requires data on minute ventilation and the uptake efficiency of particle or gas at the target site. However, these latter two parameters are difficult to measure experimentally. Thus, they must often be estimated from literature values, guessed at, or omitted entirely from consideration. It is, therefore, necessary that current research continues to focus on experimental and theoretical concepts for estimating the effective dose at specific target sites in the human lungs and the lungs of laboratory animals.

In terms of intrapulmonary deposition pattern of particles, the lungs of beagles and humans are reasonably alike. Although canine airways branch monopodially and human airways branch dichotomously, particle deposition is similar [88, 89]. For example, both in canines and humans, bronchial deposition for particles smaller than 2 µm in diameter is rather low and increases with increasing particle size and with increasing penetration into the lungs, *i.e.* with increasing lung depth [90]. However, because the surface area of airways beyond the trachea increases rapidly with increasing lung depth, the surface dose of deposited particles declines correspondingly. Therefore, for accurate dosimetry, it must be recognized that despite the deposition of 0.5–5 µm particles being lowest in proximal airways, the resulting surface dose in these airways is actually highest and then decreases towards the lung periphery. It must also be appreciated that this particle deposition pattern will be changed by disease. For instance, in chronic bronchitis, deposition in conducting airways is enhanced [91].

Respiratory responses of canine lungs to inhaled ambient air pollutants

Environmentally-controlled chambers are employed in long-term exposure studies with dogs. The technologies utilized in these types of studies were recently discussed [92]. Typical exposure systems use airflow that is continuous, horizontal, unidirectional, and has low-turbulent displacement. Pollutants are injected continuously into the airflow to ensure uniform whole-body exposure. The dogs are both housed and exposed in the chambers. An optimum design combines longitudinal studies (serial physiological, biochemical and cytological investigations on exposed and unexposed animals) with cross-sectional studies (postmortem morphological investigations on exposed and unexposed animals).

A few studies with high levels of sulphur dioxide were performed to induce chronic bronchitis. In these cases, dogs inhaled this gas through cuffed tracheostomy tubes at a concentration of 200 parts per million (ppm) for up to 18 months [17–19], or at a concentration of 50 ppm for 11 months [20, 21].

Table 6. – Responses of the canine respiratory system to inhaled gaseous sulphur(IV) (sulphur dioxide) and particulate sulphur (IV) (neutral sulphite particles)

Reference	Animals	Exposure			Responses	
		Concentration	Particle size	Total period Weekly period Daily period	Assessment	Symptoms, findings
LULLING *et al*. 1968 [26] (longitudinal study)	13 male collies (6 unexposed, 7 exposed)	500 ppm SO_2 (660 mg S(IV)·m^{-3})		12 months 1–3 days·week^{-1} 1–4 h·day^{-1}	 Physiology, Morphology	Cough, dyspnoea, production of bronchial secretion Increase in airway resistance Hypertrophy of bronchial glands, centriacinar emphysema
US EPA study I						
LEWIS *et al*. 1969 [27] LEWIS *et al*. 1973 [28] (longitudinal study)	16 female beagles (8 unexposed, 8 exposed)	5 ppm SO_2 (6.6 mg S(IV)·m^{-3})		21 months 7 days·week^{-1} 21 h·day^{-1}	Physiology	Increase in airway resistance, decrease in pulmonary compliance
Philadelphia study						
CHAKRIN & SAUNDERS 1974 [34] SPICER *et al*. 1974 [35] LITT *et al*. 1976 [36] (longitudinal study)	20 male beagles (6 unexposed, 14 exposed)	550 ppm SO_2 (720 mg S(IV)·m^{-3})		5 months 2 days·week^{-1} 2 h·day^{-1}	 Rheology, Morphology	Excess of mucopurulent exudate Biphasic response of viscoelastic properties Increase in goblet cell number in small bronchi, hyperplasia of bronchial glands
HIRSCH *et al*. 1975 [37] (cross-sectional study)	12 beagles (4 unexposed, 8 exposed)	1 ppm SO_2 (1.3 mg S(IV)·m^{-3})		12 months 5 days·week^{-1} 3 h·day^{-1}	Clearance	Impairment of mucociliary activity
MALO *et al*. 1983 [41] (longitudinal study)	5 beagles, both sexes (5 exposed)	500 ppm SO_2 (660 mg S(IV)·m^{-3})		6 months 2 days·week^{-1} 2 h·day^{-1}	Physiology	Increase in airway responsiveness
GREENE *et al*. 1984 [42] (longitudinal study)	16 beagles, both sexes (4 unexposed, 12 exposed)	500 ppm SO_2 (660 mg S(IV)·m^{-3})		5 months 5 days·week^{-1} 2 h·day^{-1}	 Cytology Clearance Morphology	Mucoid nasal discharge, cough Persistent lung inflammation Impairment of tracheal mucous velocity Goblet cell hypertrophy, hyperplasia of bronchial glands
GSF study I						
HEYDER *et al*. 1992 [43] MAIER *et al*. 1992 [44] KREYLING *et al*. 1992 [45] SCHULZ *et al*. 1992 [46] TAKENAKA *et al*. 1992 [47] (longitudinal study)	11 male beagles (3 unexposed, 8 exposed)	1 mg Na_2SO_3·m^{-3}) 0.07 ppm SO_2 (0.3 mg S(IV)·m^{-3})	0.6 µm	10 months 7 days·week^{-1} 22.5 h·day^{-1}	Biochemistry/ cytology Clearance Physiology Morphology	Increase in alveolar-capillary permeability, decrease in formation of oxygen radicals, reduction in phagocytic capacity, lung inflammation Enhancement of macrophage-mediated particle transport Decrease in lung compliance, reduction in diffusion capacity Hyperplastic changes in the nasal cavity, enlargement of airspaces

ppm: parts per million; EPA: Environmental Protection Agency; GSF: National Research Center for Enviornment and Health.

Respiratory responses of canine lungs have been investigated for whole-body exposures to ozone, nitrogen oxides, S(IV) (*i.e.* sulphur dioxide and/or sulphite particles), acidic particles and motor vehicle exhaust. The corresponding canine exposure studies are listed and summarized in tables 6–10. They cover a wide range of exposure concentrations, exposure periods, and assessed respiratory responses. Most of the studies were performed with a single gaseous pollutant. Mixtures of pollutants have only been employed in four studies, in which several pollutants were given either simultaneously or successively.

Dogs were simultaneously exposed to SO_2 and H_2SO_4 droplets in both EPA studies, to simulate atmospheric conditions in which part of the ambient SO_2 is oxidized to SO_3 that ultimately reacts with water to form H_2SO_4 droplets. This mixture may have constituted the 1952 London fog, and it was suggested as early as 1954 that "it is possible that SO_2 dissolved as sulphuric acid in fog droplets appreciably reinforces the harmful effect of SO_2" [93]. Diluted exhaust from gasoline engines was used as the exposure atmosphere in the second EPA study.

The first GSF study attempted to simulate another ambient air pollution scenario. Ambient particles offer a huge surface area for interactions with SO_2 that can lead to either the direct formation of particulate S(IV) as sulphites or to SO_2 adsorption onto particles. Therefore, sulphite particles were used as surrogates for particle-associated S(IV). In the second GSF study, dogs were exposed to the sulphite particles in combination with acidic sodium bisulphate particles. Since it was recently recognized that the biologically active portions of acidic ambient compounds are H^+ ions [68], this exposure atmosphere may also resemble the pollution mixture of the 1952 London smog.

Although the database for long-term canine exposure studies is rather heterogeneous, there is sufficient coherence among the results to support the conclusions emerging from comparable studies with humans and small laboratory animals. In combination, the findings strengthen the proposition that prolonged exposure to high levels of air pollutants can cause irreversible lung injury and, ultimately, disease.

The studies also show that impaired lung function parameters indicative of parenchymal abnormalities (increased lung volumes; decreased compliance and diffusing capacity) correlate well with morphologically documented acinar airspace enlargement and, thus, emphysematous lesions. Furthermore, impaired lung function parameters indicative of airway abnormalities (increased airway resistance and decreased airway responsiveness) and

Table 7. – Responses of the canine respiratory system to inhaled nitrogen oxides

	Animals	Exposure		Responses	
		Concentration	Total period Weekly period Daily period	Assessment	Findings
US DHEW study II					
WAGNER *et al.* 1965 [24] (cross-sectional study)	26 mongrels		18 months 5 days·week^{-1} 6 h·day^{-1}		
	(6 unexposed)				
	(10 exposed)	1 ppm NO_2		Morphology	Enlargement of airspaces
	(10 exposed)	5 ppm NO_2		Morphology	Enlargement of airspaces
US Navy study I					
STEADMAN *et al.* 1966 [25] (cross-sectional study)	14 beagles	11 ppm NO_2	3 months 7 days·week^{-1} 24 h·day^{-1}	Mortality	No findings
US EPA study II					
LEWIS *et al.* 1974 [29] ORTHOEFER *et al.* 1976 [38] HYDE *et al.* 1978 [39] STARA *et al.* 1980 [40] (longitudinal study)	28 female beagles (12 unexposed)		61 months 7 days·week^{-1} 16 h·day^{-1}		
	(6 exposed)	0.6 ppm NO_2 0.3 ppm NO		Biochemistry	Increase in prolyl hydroxylase level (postmortem)
				Physiology	Reduction in diffusion capacity, decrease in peak expiratory flow
				Morphology	Enlargement of airspaces
	(10 exposed)	0.2 ppm NO_2 1.7 ppm NO		Biochemistry	Slight increase in prolyl hydroxylase level (postmortem)
				Physiology	No findings
				Morphology	Slight enlargement of airspaces

ppm: parts per million; DHEW: Department of Health, Education and Welfare (US); EPA: Environmental Protection Agency.

Table 8. – Responses of the canine respiratory system to inhaled ozone

	Animals	Exposure		Responses		
		Concentration	Total period Weekly period Daily period	Assessment	Findings	
US DHEW Study I						
Stockinger *et al*. 1957 [23] (cross-sectional study)	6 unspecified dogs (2 unexposed, 4 exposed)	1 ppm ozone	14 months 5 days·week^{-1} 6 h·day^{-1}	Morphology	No findings	
US Navy study II						
Jones *et al*. 1970 [31] (cross-sectional study)	26 male beagles (8 unexposed, 18 exposed)	1.5 ppm ozone	3 months 7 days·week^{-1} 24 h·day^{-1}	Mortality Morphology	No findings Nonspecific inflammatory changes	
US EPA study III						
Freeman *et al*. 1973 [32] Stephens *et al*. 1973 [33] (cross-sectional study)	48 female beagles (8 unexposed)		18 months 7 days·week^{-1}			
	(8 exposed)	1 ppm ozone	8 h·day^{-1}	Morphology	Accumulation of alveolar macrophages, deposition of fibrous elements	+1 +1
	(8 exposed)	1 ppm ozone	16 h·day^{-1}	Morphology	Accumulation of alveolar macrophages, deposition of fibrous elements	+2 +2
	(8 exposed)	1 ppm ozone	24 h·day^{-1}	Morphology	Accumulation of alveolar macrophages, deposition of fibrous elements, thickening of bronchiolar walls	+3 +2 +1
	(8 exposed)	2 ppm ozone	8 h·day^{-1}	Morphology	Accumulation of alveolar macrophages, deposition of fibrous elements, thickening of bronchiolar walls, squamous metaplasia	+3 +3 +2 +1
	(8 exposed)	3 ppm ozone	8 h·day^{-1}	Morphology	Accumulation of alveolar macrophages, deposition of fibrous elements, thickening of bronchiolar walls, squamous metaplasia	+4 +4 +3 +2

For abbreviations see legend to table 7.

Table 9. – Responses of the canine respiratory system to inhaled acidic particles

	Animals	Exposure			Responses	
		Concentration	Particle size	Total period Weekly period Daily period	Assessment	Findings
US EPA study I LEWIS *et al.* 1969 [27] LEWIS *et al.* 1973 [28] (longitudinal study)	16 female beagles (8 unexposed, 8 exposed)	0.9 mg $H_2SO4 \cdot m^{-3}$	Unknown	21 months 7 days·week^{-1} 21 h·day^{-1}	Physiology	Reduction in diffusion capacity, reduction in pulmonary compliance
GSF study III **in progress** (longitudinal study)	16 male beagles (8 unexposed, 8 exposed)	5.4 mg $NaHSO_4 \cdot m^{-3}$ (15 µmol $H^+ \cdot m^{-3}$)	1.1 µm	12 months 7 days·weeks^{-1} 6 h·day^{-1}	Biochemistry/ cytology Clearance Physiology Morphology	

For abbreviations see legend to table 6.

impaired mucociliary transport also correlate well with morphological observations (hypertrophy and hyperplasia of bronchial glands and nonciliated bronchiolar cells) and, thus, bronchitic lesions. In none of the studies was there an indication of allergic responses.

Sulphur(IV)

Sulphur(IV), the IV-valent sulphur, is the reactive molecule in sulphur oxide moieties. In long-term canine studies (table 6), its concentration has been varied by more than three orders of magnitude (0.3–720 mg·m^{-3}). At high levels of exposure, an increased prevalence both of chronic bronchitis [26, 34–36, 41, 42] and emphysematous lesions has been observed [26]. At lower levels of exposure, respiratory responses were similar to those observed at high levels, but they occurred later. For instance, GREENE *et al.* [42] reported persistent lung inflammation during exposure to 660 mg·m^{-3}, whereas, at the lowest exposure level, an inflammatory response was detected only late in the study [44]. In this GSF study, no indication of chronic bronchitis was observed, but there were functional and morphometrical indications of the early stage of emphysema, which resulted from delivery of particulate S(IV) to the lung periphery.

The S(IV)-concentration required to cause responses in the canine lungs is lower than that required to cause the same response in rat lungs [9]. This difference can be due to either a lower dose received by the canine lungs or a higher susceptibility of canine lung tissue in comparison to rat lung tissue.

Nitrogen oxides

NO_2-induced respiratory responses lead to emphysematous lesions in canine lungs (table 7). The findings of the second EPA study suggest that these lesions are dose-related, and nitrogen dioxide is a pneumotoxin of stronger potency than nitrogen oxide. The presence of fibrotic lesions is suggested by an increase in the intrapulmonary levels of prolyl hydroxylase, which is also dose-dependent.

Ozone

There are only limited data concerning long-term canine exposure studies with ozone (table 8). To estimate the effects of inhaled ozone on function and structure of the respiratory system, the only available studies are with small laboratory animals [22], or nonhuman primates [15]. With ozone exposures, it is worthwhile noting that a constant product of concentration × exposure time does not always produce the same findings. The morphological scores indicate that the intensity of the morphological lesions increases with increasing concentration; the exposure time is less important [32].

Table 10. – Responses of the canine respiratory system to mixtures of air pollutants

	Animals	Exposure			Responses	
		Concentration	Particle size	Total period Weekly period Daily period	Assessment	Findings
US EPA study II						
Lewis *et al*. 1974 [29] Orthoefer *et al*. 1976 [38] Hyde *et al*. 1978 [39] Stara *et al*. 1980 [40] (longitudinal study)	47 female beagles (12 unexposed)			68 months 7 days·week^{-1} 16 h·day^{-1}		
	(10 exposed)	Auto exhaust: 98 ppm CO 28 ppm HC 0.05 ppm NO_2 1.5 ppm NO			Biochemistry Physiology Morphology	Slight increase in prolyl hydroxylase level (postmortem) Pulmonary hyperinflation Hyperplasia of nonciliated bronchiolar cells
	(5 exposed)	Irradiated auto exhaust: 95 ppm CO 24 ppm HC 1 ppm NO_2 0.2 ppm NO 0.2 ppm ozone			Biochemistry Physiology Morphology	Increase in prolyl hydroxylase level (postmortem) Increase in airway resistance, impairment in ventilatory distribution Slight hyperplasia of nonciliated bronchiolar cells
	(9 exposed)	0.1 mg H_2SO_4·m^{-3} 0.5 ppm SO_2 auto exhaust	0.5 µm		Biochemistry Physiology Morphology	Slight increase in prolyl hydroxylase level (postmortem) Pulmonary hyperinflation Hyperplasia of nonciliated bronchiolar cells
	(11 exposed)	0.1 mg H_2SO_4·m^{-3} 0.4 ppm SO_2 irradiated auto exhaust	0.5 µm		Biochemistry Physiology Morphology	Increase in prolyl hydroxylase level (postmortem) Increase in airway resistance Enlargement of air spaces
US EPA study I						
Lewis *et al*. 1969 [27] Lewis *et al*. 1973 [28] (longitudinal study)	16 female beagles (8 unexposed, 8 exposed)	0.9 mg H_2SO_4·m^{-3} 5 ppm SO_2	Unknown	21 months 7 days·week^{-1} 21 h·day^{-1}	Physiology	Reduction in diffusion capacity, reduction in residual and total lung volume, increase in airway resistance
US EPA study II						
Lewis *et al*. 1974 [29] Orthoefer *et al*. 1976 [38] Hyde *et al*. 1978 [39] Stara *et al*. 1980 [40] (longitudinal study)	20 female beagles (12 unexposed, 8 exposed)	0.1 mg H_2SO_4·m^{-3} 0.4 ppm SO_2	0.5 µm	68 months 7 days·week^{-1} 16 h·day^{-1}	Biochemistry Physiology Morphology	Slight increase in prolyl hydroxylase level (postmortem) No findings Enlargement of airspaces

Continued next page

Table 10. – continued....

	Animals	Exposure			Responses	
		Concentration	Particle size	Total period Weekly period Daily period	Assessment	Findings
GSF study II						
HEYDER *et al.* 1994 [48]	16 male beagles			13 months		
MAIER *et al.* (Submitted) [49]	(8 unexposed,			7 days·week^{-1}		
KREYLING *et al.* (Submitted) [50]	8 exposed)			22.5 h·day^{-1}		
SCHULZ *et al.* (Submitted) [51]						
TAKENAKA *et al.* (Submitted) [52] (longitudinal study)		5.4 mg $NaHSO_4 \cdot m^{-3}$ (15 µmol $H^+ \cdot m^{-3}$) + 1.4 mg $Na_2SO_3 \cdot m^{-3}$ 0.1 ppm SO_2 (0.43 mg $S(IV) \cdot m^{-3}$	1.1 µm 1.0 µm	6 h·day^{-1} 16.5 h·day^{-1}	Biochemistry/ cytology Clearance Physiology Morphology	Increase in alkaline phosphatase release Reduction in macrophage-mediated particle transport No findings Type II cell proliferation

For abbreviations see legend to table 6.

Acidic particles

The only long-term canine exposure study performed with acidic particles (table 9) offers little insight into respiratory responses caused by H^+ ions. One observation from physiological measurements is that these ions induce the lungs to produce bronchitic as well as emphysematous lesions.

Mixtures of pollutants

Under ambient conditions, people are always exposed to mixtures of pollutants. Therefore, controlled exposure studies with mixtures of pollutants (table 10) are closer to the "real world" than exposure studies using a single pollutant. However, it is controversial whether respiratory responses observed for single pollutants will be enhanced or reduced when these pollutants are inhaled as mixtures. Upon reviewing controlled human studies with air pollutants, SANDSTRÖM [53] concluded there is no potentiation of effects observed for pollutant mixtures in comparison to the separate constituents. In contrast, in a review of exposure studies with small laboratory animals, CHITANO *et al.* [22] concluded that synergistic effects are indeed observed with mixtures of nitrogen dioxide and ozone or mixtures of nitrogen oxide or ozone with other pollutants. No indication of synergistic effects was found in the long-term canine studies. However, respiratory responses due to the inhalation of mixtures of nitrogen dioxide and ozone were not studied in dogs.

Thus, it may be that synergism only occurs with exposure mixtures involving nitrogen dioxide and/or ozone; not for mixtures with other constituents. Synergism could also be characteristic of only certain animal species or pollutant interactions in the lungs over long but not short periods of time. Finally and importantly is the location of specific target sites. The specific target site both for nitrogen dioxide and ozone is the centriacinar region. Therefore, the likelihood of interactions between respiratory responses of these pollutants is much higher than that between pollutants deposited at different target sites, such as sulphur dioxide and sulphuric acid droplets. It is not surprising that respiratory responses produced by sulphur dioxide and sulphuric acid droplets in canine lungs appear to be additive rather than synergistic.

After dogs were chronically exposed to the exhaust of petrol engines (auto exhaust), nonciliated bronchiolar (Clara) cells were affected and fibrotic lesions were observed. These changes could suggest the onset of small airway disease. However, it is remarkable that a reduction, rather than an enhancement, of lesions was observed when dogs were exposed to ultra violet (UV)-irradiated auto exhaust, since the UV-irradiation produced appreciable concentrations of ozone and nitrogen dioxide. When UV-irradiated exhaust was ultimately combined with gaseous and particulate S(IV) exposure, the hyperplasia of nonciliated bronchiolar (Clara) cells disappeared, but there was emphysematous airspace enlargement without alveolar fenestrae [94].

After cessation of exposure, the dogs of the second EPA study were kept under clean air conditions for 3 yrs before necropsy. No recovery of lesions occurred during this "recovery" period.

The most notable findings concerning pollutant mixtures were reported from the second GSF study. The response pattern observed in this study was entirely different from that observed in the first study. In the first GSF study, beagles were exposed to particle-associated S(IV) and in the second study additionally to particle-associated H^+ ions. Since the particle size distributions in both studies and for both pollutants, S(IV) and H^+, were very similar, the specific target sites were the same. Nevertheless, most of the effects produced by the S(IV) particles disappeared when the dogs also inhaled particles carrying H^+ ions. Other effects also appeared, such as proliferation of type II cells and release of alkaline phosphatase by these cells. To fully understand these findings, a study is needed in which dogs are exposed solely to particle-associated H^+ ions. This study is in progress (table 9).

Limitations of canine exposure studies

In respiratory toxicology, there is a special need for investigations in humans, but such investigations are often impossible. Therefore, the only feasible way to address many air pollution questions is with laboratory animals. However, these animal studies must be undertaken with the caveat that extrapolation of findings from nonhuman observations to humans is subject to many sources of error, especially at unrealistically high exposure levels.

In addition to this general limitation, there are special considerations for long-term exposure studies with dogs. Firstly, ethical considerations, demanding labour requirements and high costs, limit studies to only a small number of animals. Secondly, the small sample size may require the use of sophisticated biostatistical techniques for analysis. Thirdly, dogs must be housed and exposed under conventional, hygienic conditions with frequent inspections to ensure that findings are not confounded by infectious diseases. Lastly, any spontaneously occurring functional and structural alterations must not be mistaken for exposure-related alterations, specifically fibrotic lesions in cranial lobes [47], or an increase in the number of eosinophils recoverable by bronchoalveolar lavage [95].

Future aspects

In epidemiological and controlled human exposure studies, susceptible populations have been identified, including infants, elderly individuals and individuals with underlying cardiopulmonary diseases. Healthy adults are considered to be much less, if at all, susceptible to inhaled pollutants at ambient levels. Nevertheless, most exposure studies with laboratory animals have been performed with healthy animals. Studies with animal models of cardiopulmonary diseases are, therefore, greatly needed.

Recently, it has been recognized that ambient particles might play a greater role in pollution-induced respiratory responses than previously thought [96–99]. Furthermore, fine or possibly ultrafine particles [100, 101] may be more toxic than coarse particles. The "physical" toxicity of these particles could even exceed their "chemical" toxicity. Therefore, it is beyond question, that there are tremendous research needs in the field of respiratory toxicology.

It must be re-emphasized that, in the "real world", humans are exposed to complex mixtures of ambient air pollutants, whereas most animal exposure studies have used only single pollutants. Therefore, long-term studies are needed with exposure atmospheres of increasing complexity. Eventually, it may be possible to simulate the complexity of the indoor and outdoor environment in controlled animal exposure studies, thereby enabling more relevant investigations of the interactions of pollutants in the lungs. Finally, it must be recognized that the roles of adaptation and tolerance have not been sufficiently addressed in air pollution exposure studies.

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