

CHAPTER 6

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Toxicology of air pollutants (animal studies)

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Introduction

6.1. To assess the possible role of air pollution in allergic disease it is important to have a good understanding of their biological effects on tissue. These effects will depend on the species and the organ exposed, the design of the experiment, the pollutant, whether exposure is acute or more long-term and the concentration.

6.2. Animal experiments have the advantage of being able to analyse tissue-damaging effects in detail through autopsy studies but the disadvantage that findings may not be relevant to humans because of species differences. Many of the animal toxicology studies have been weakened by using very high exposures which are of limited relevance to human exposures under normal conditions.

6.3. There is a large body of work on the toxicology of air pollutants in animals. The work of direct relevance to allergen responses will be discussed in Chapter 10. The work discussed here will attempt to give a feel for the biological effects of air pollutants on tissue which may be of relevance to allergic disease in a broad sense.

Sulphur dioxide (SO₂)

Acute exposure studies (<24 hours)

6.4. SO₂ causes bronchoconstriction in all species tested if sufficiently high doses are given. Thus in guinea-pigs there is a dose-dependent increase in airways resistance which returns to normal within 1 hour after exposure to concentrations of less than 100 ppm. In dogs there is increased resistance to inhaled SO₂ in nose and lower airways [90]. In cats bronchoconstriction occurs as a result of smooth muscle contraction and is dependent on an intact vagal nerve.

6.5. There is some evidence that particulate aerosols potentiate effects of SO₂ possibly by promoting conversion

to sulphuric acid. Metal salts such as manganese and ultrafine aerosols from coal combustion appear particularly active. For example a 3-hour exposure of guinea-pigs to 1.7 ppm (5 mg/m³) ultrafine zinc oxide aerosol with 1 ppm SO₂ decreased lung volumes and gas transfer in addition to causing oedema and evidence of damage to epithelial and endothelial cells for up to 72 hours following a single exposure [91, 92].

Chronic exposure studies (>24 hours)

6.6. In rats with a daily exposure of 10 ppm for 18–67 days there was evidence of airway mucosal thickening, decreased mucus transport and impaired ciliary activity [93]. In rats exposed to ten periods of 6 hours at 50–300 ppm there was marked epithelial damage in the trachea with destruction of goblet cells and increased numbers of goblet cells in the lower airways 72 hours post-exposure at the highest dose [94]. The relevance of these studies to human disease is doubtful except perhaps to indicate that even massive doses of SO₂ for prolonged periods causes only modest tissue damage.

6.7. In dogs exposed to 1 ppm SO₂ for a year only slowing of tracheal mucus transport was noted [95] and in rats with a daily exposure of 0.1–20 ppm for a total of 70–170 hours there was delayed clearance of inert particles from the lung [96].

6.8. In monkeys and guinea-pigs after one year of exposure to 0.1–5 ppm no pathology was detected [97]. In guinea-pigs exposed to 0.13–5.72 ppm continuously for a year no adverse effects were observed [98] and in monkeys exposed to 0.14–1.28 ppm for 78 weeks of continuous exposure there were no adverse effects [99]. In dogs exposed to 0.5 ppm SO₂ and 0.1 mg/m³ sulphuric acid for 16 hours/day for 18 months there were no adverse effects [100].

6.9. The consistent finding therefore of toxicology studies in animals, in a range of species, is that acute exposure to SO₂ can cause a modest and transient bronchoconstriction, and in chronic exposure the tissue-damaging effects of SO₂ are limited and only seen at concentrations that far exceed those likely to be encountered in ambient air in the U.K.

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Acid aerosols

Acute exposure studies (<24 hours)

6.10. In donkeys exposed to 194–1364 $\mu\text{g}/\text{m}^3$ for 1 hour three out of four animals showed short-term slowing of removal of ferric oxide particles from the lung [101]. In this study SO_2 only affected clearance at 300 ppm.

6.11. In rabbits exposed to 1 mg/ m^3 for 1 hour there were increased neutrophils in BAL 12–24 hours after exposure [102].

6.12. Rabbits were exposed for 3 hours to a combination of sulphuric acid (SA) 0–125 $\mu\text{g}/\text{m}^3$ and ozone 0–0.6 ppm. There were 16 groups of five rabbits and lungs were examined immediately after exposure. SA impaired phagocytosis, superoxide radical production and $\text{TNF}\alpha$ synthesis by alveolar macrophages at 75 and 125 $\mu\text{g}/\text{m}^3$. No very clear interactions with ozone were observed, with antagonism being the most obvious effect. There was no effect on cell numbers, differential cell count, viability or PGE_2 and PGF_2 concentrations [103].

6.13. Guinea-pigs are particularly sensitive to acid aerosols. 18–60 $\mu\text{g}/\text{m}^3$ for 8 hours was lethal as a result of bronchoconstriction and laryngeal spasm. Haemorrhage and some oedema was also detected after 72 hours of exposure [104] at lower concentrations. Bronchoconstriction was concentration-dependent and lung damage was dose-dependent. Guinea-pigs show greater increased airway resistance after exposure to SA compared to SO_2 for a given concentration of sulphur.

Chronic exposure studies

6.14. Donkeys exposed for 1 hour/day, 5 days week for 6 months to 100 $\mu\text{g}/\text{m}^3$ (0.3–0.6 μm aerosol) had variable changes in their lung clearance rates with abnormalities lasting for 3 months after exposure ceased. Two donkeys had increased and two donkeys had decreased clearance rates [105].

6.15. Rabbits exposed on a daily basis for 1 hour for 20 days to 250 $\mu\text{g}/\text{m}^3$ sulphuric acid also had variable changes in clearance with increased clearance during recovery phase. Increased BHR was noted as was increased number of goblet cells and airway narrowing. Changes persisted for more than 3 months [106].

Particulates, auto exhaust and synthetic smog

6.16. There are few acute exposure studies of relevance to allergic disease other than those discussed under sensitization (Chapter 10).

Chronic exposure studies

6.17. Eight groups of 12 Beagles were daily exposed to various combinations of gases including irradiated and non-irradiated auto exhaust as well as a no exposure group for 16 hours/day for 68 months. Dogs were killed 2 years later. Enlargement of air spaces and loss of interalveolar septa in proximal acinar regions were seen in dogs exposed to NO_2 and SO_2 . Hyperplasia of non-ciliated bronchiolar cells were seen in raw exhaust group [107]. All exposure groups had impaired lung function after exposure which deteriorated further after 2 years. Impairment suggested both airway and parenchymal disease.

Ozone (O_3)

6.18. Ozone is a powerful oxidant which can oxidize compounds directly or through generation of oxygen radicals. It oxidizes lipids, particularly polyunsaturated lipids of cell membranes and potentially membrane receptors by reacting with sulphhydryl groups of cysteine. O_3 can promote a biochemical cascade resulting in lipid peroxidation and formation of aldehydes which can react with amino acids, nucleic acids or proteins. O_3 interaction with cell membrane activates phospholipase C potentially leading to formation of leukotrienes and prostaglandins.

6.19. Sixty per cent of O_3 reaches the lower airways. Theoretical concentrations suggest respiratory bronchioles receive maximum doses of inhaled O_3 . Clara cells in bronchioles are thought to have an important role in removal of toxins. This role is dependent on intracellular glutathione and NADPH-dependent cytochrome p450 which are vulnerable to O_3 -mediated damage. Type II cells in alveoli perform similar functions to clara cells as well as producing surfactant. AM also have high levels of intracellular glutathione.

6.20. Acute exposure in the region of 4–10 ppm causes severe pulmonary oedema, but unlike NO_2 there is no evidence that it produces emphysema-like changes.

6.21. Ozone causes increased bronchial hyperresponsiveness in both humans and animals.

Acute exposure studies

6.22. In rats exposed to 1.0 ppm of O_3 for between 4–24 hours there was epithelial necrosis within 4 hours in terminal bronchioles and neutrophil influx which was maximal at 8–12 hours [108]. Neutrophil depletion did not affect epithelial damage. Isolated rat lungs exposed to 1.0–2.0 ppm O_3 for

3 hours had necrosis and sloughing of epithelial cells especially in larger bronchioles and bronchi [109].

6.23. Mice exposed to 2 ppm O_3 for 3 hours demonstrated neutrophil influx which was inhibited by indomethacin, colchicine or cyclophosphamide. These drugs had no effect on increased BAL protein content [110].

6.24. In baboons 0.5 ppm of ozone for 5 minutes increased respiratory resistance, an effect partially diminished by DSCG. There was no effect on prostaglandins in BAL [111].

6.25. Monkeys were exposed to 0.96 ppm for 8 hours with analysis at 1–168 hours post-exposure. There was a correlation between peak of epithelial damage in bronchi and increased numbers of airway eosinophils [112].

Chronic exposure studies (>24 hours)

6.26. Mice exposed to O_3 at a concentration of 2.5 ppm for 2 hours a day for 120 days had evidence of Clara cell hyperplasia which had not resolved by 120 days [113]. Similarly monkeys exposed to 0.4–0.64 ppm 8 hours/day for 3–12 months had Clara cell hyperplasia with no recovery after 3 months of clean air. Similar changes were seen with exposure to diesel exhaust and cigarette smoke [114].

6.27. Monkeys exposed to 0.8 ppm O_3 for 4–50 hours had hyperplasia of non-ciliated bronchiolar cells; partial resolution was seen by 7 days after exposure [115].

6.28. Rats exposed to 0.12–0.2 ppm for 2 days had an increase in protein content of BAL fluid [116], and in rats exposed to 0.8 ppm of O_3 for 3 days there was alveolar inflammation which returned to normal after 30 days [117].

6.29. Rats exposed to 0.95 ppm O_3 , 8 hours/day for 90 days had mild interstitial fibrosis and increased smooth muscle in alveolar ducts and respiratory bronchioles [118] and rats exposed to 0.12–0.25 ppm 12 hours/day for 42 days had mild interstitial inflammation with areas of increased collagen at highest concentration [119].

6.30. Rats exposed to 0.06 ppm with 1-hour day peak, 13 hours/day for 12 months showed reduced vitamin E (an anti-oxidant) in BAL. Vitamin E supplements reduced damage in rats exposed to 0.1 ppm compared to vitamin E-deficient mice [120].

6.31. In dogs exposed to 3 ppm for 20 minutes for 5 days, antioxidants inhibited development of bronchial hyperresponsiveness (BHR) without affecting neutrophil influx [121].

6.32. Indomethacin does not inhibit the increase in BHR after 0.4 ppm ozone for 2 hours [122], nor did thromboxane antagonists in dogs [123].

6.33. In rats exposed to 0.35–1.0 ppm for 2.25 hours for 5 days, adaptation of physiological response was observed, but not in the biochemical and morphological changes of epithelial damage and inflammation in the terminal bronchioles [124].

Nitrogen dioxide (NO_2)

6.34. NO_2 , like ozone, is an oxidizing agent and many of its activities are thought to be due to lipid peroxidation of cell membranes. Traditionally it is thought to be a 'deep lung irritant' acting primarily at the terminal bronchiolar/alveolar part of the lung rather than the main airways [125] although it clearly also has effects on the larger airways.

6.35. NO_2 produces pulmonary oedema if inhaled in very high concentrations.

6.36. NO_2 is poorly soluble and considered to be deposited mainly in the peripheral air spaces [126].

Acute exposure studies

6.37. Thirteen dogs were exposed to very high concentrations (200–400 ppm) for 1 hour: very modest falls in functional residual capacity and particle clearance were observed. Marked increase in BAL albumin and evidence of patchy oedema and inflammation on histology but no change in BAL cell counts. All abnormalities returned to normal within a few days [127].

6.38. Squirrel Monkeys exposed to 10–50 ppm NO_2 for 2 hours had evidence of epithelial damage and alveolar inflammation with frank oedema at 50 ppm.

6.39. Guinea-pigs exposed for 2–4 hours to 5–13 ppm of NO_2 had increased respiratory rate and decreased tidal volume. This returned to normal within 3 hours. Generally it appears that NO_2 at concentrations in the region of 10–50 ppm cause a decrease in tidal volume and in some species an increase in respiratory rate which is reversible in time proportionate to the length of exposure.

Chronic exposure studies (>24 hours)

6.40. Rats were exposed to 0.8–25 ppm for 16 weeks. At 0.8 ppm no effect was observed. At 2 ppm minor histological

changes in bronchiolar epithelium with reduced or absent cilia were seen. Changes were more marked with 4 ppm. After exposure to 10–25 ppm animals died of respiratory failure with 'emphysema-like lungs' with enlarged and fewer alveoli and damaged bronchiolar epithelium.

6.41. Beagle dogs were exposed for 6 months to 25 ppm NO_2 . There was a diffuse increase in collagen. One of six dogs developed bullous emphysema and the other five dogs scattered small bullae. When exposed to 50 ppm for 5 hours/day for 7 days there was destruction of airway epithelium at day 1 with loss of cilia and cell disintegration which was more pronounced in major bronchi than peripherally, but more rapid repair in large airways which was occurring by day 3. Decrease in airway diameter and increased mural thickness in terminal bronchioles.

6.42. Rats were exposed for 3 days, 6 hours a day to either 14.4 ppm NO_2 , 0.8 ppm O_3 , or both. There were increases in epithelial cell counts in BAL fluid and protein content with both NO_2 and O_3 alone. Increased neutrophil counts and a marked increase in epithelial counts and BAL protein were seen when both were given together [128].

6.43. Isolated human bronchial rings were exposed to either air, 1 ppm NO_2 or 2 ppm NO_2 : 2 ppm NO_2 increased the contractile response to carbachol, histamine and substance P [129].

Summary

6.44. Most of the animal work offers little, over and above human studies, in understanding the potential affects of air pollutants on allergic disease because of the study design or the very high concentrations of air pollutants used.

6.45. SO_2 can cause bronchoconstriction at concentrations depending on the species and conditions of challenge. The

mechanism of SO_2 -induced bronchoconstriction is unclear but may be mediated by a neural reflex.

6.46. There is little to no evidence from animal work that SO_2 causes airway inflammation or any significant tissue-damaging effects at concentrations close to those currently encountered in the U.K. under normal conditions.

6.47. Acid aerosols can cause bronchoconstriction with considerable variation in species sensitivity with guinea-pigs being particularly sensitive and dogs and monkeys insensitive.

6.48. There is evidence for acid aerosols causing impaired tracheobronchopulmonary clearance at high concentrations in animals but this is only at high concentrations and appears variable in effect.

6.49. The toxicology of particulates has been the least well studied of the air pollutants and little can be gleaned from the literature of relevance to asthma and allergic disease other than the studies referred to in Chapter 10.

6.50. Ozone is a highly toxic gas which causes marked airway inflammation in animals. A lower limit for the pro-inflammatory effects of ozone have not been established. Ozone causes bronchial hyperresponsiveness in animals by a mechanism that is likely to be closely related to its pro-inflammatory actions.

6.51. There is considerable variation in the physiological response to ozone. The extent to which this is also seen in the inflammatory effects is not clear from animal studies.

6.52. Nitrogen dioxide appears to be a generally less potent oxidant than ozone and while able to cause airway inflammation in animals this only is apparent at concentrations well above those currently encountered in the U.K. It appears to cause emphysema in long-term exposure studies.