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THE HISTOPATHOLOGY OF RAT LUNG FOLLOWING SHORT TERM EXPOSURES TO MIXED OXIDES OF NITROGEN (NOx)

R. F. R. BROWN, W. E. CLIFFORD, T. C. MARRS AND R. A. COX

From the Procurement Executive, Ministry of Defence, Chemical Defence Establishment, Porton Down, Salisbury, Wilts SP4 0JQ

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Summary.—The histopathology of rat lung after exposure to high concentrations of mixed oxides of nitrogen (NOx) has been studied.

Considerable damage was observed, which initially took the form of 'thickening' and 'blebbing' of the alveolar epithelium and disruption of type II pneumocytes. These early changes were attributed to the direct effect of the oxidant action of NOx. There then followed a latent period of approximately 6 h after which the development of oedema of the interstitium and alveolar septum was observed. Clinical observations and the results from light and electron microscopical examination suggested that the lung damage caused by exposure to 518 parts/10^6 NOx for 5 min was greater than that caused by 1435 parts/10^6 for 1 min. This was not supported by the findings from light microscopy where similar damage was observed at both dose levels.

These results suggest that such exposures might pose a risk of lung damage to man.

THE OXIDES OF NITROGEN are a large and complex group of compounds which includes: nitrous oxide (N_2O), nitrogen peroxide (N_2O_2), nitric oxide (NO), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), nitrogen dioxide (NO_2) and dinitrogen pentoxide (N_2O_5). These compounds are easily interconverted by a variety of reactions and hence the component gases are difficult to assay. To overcome this problem the total oxides of nitrogen in an atmosphere, designated NOx, are determined by the summation of the concentrations of nitric oxide (NO) and nitrogen dioxide (NO_2) (National Air Quality Criteria Advisory Committee, 1971).

The toxic effects of inhaled NOx in both man and experimental animals has been extensively reviewed (Goldstein, 1975; Morrow, 1975; Guidotti, 1978). Most of these studies were concerned with the effects of NOx as atmospheric pollutants and describe toxic effects which were observed following long-term exposure to low concentrations (0·5–5 parts/10^6) of NOx. The effects described included pulmonary insufficiency and reduced resistance to infection. Histological investigations revealed alveolar collapse and emphysema as a common finding.

The literature describing effects following exposure to high concentrations of short durations is largely confined to clinical reports of accidental exposure. The evolution of NOx is associated with a wide variety of industrial or accidental situations; for example, fires involving plastics and polyurethane foams, the detonation of explosives, especially underground and without adequate ventilation, (Becklake *et al.*, 1957; Muller, 1969), from welding processes (Jones, Proudfoot and Hall, 1973) and in grain storage silos (Morrisey *et al.*, 1975). The most common finding was of a transient combination of upper airway irritation, cough, dyspnoea and chest tightness. A proportion of these patients subsequently developed pulmonary oedema and bronchiolitis obliterans

(Horvath *et al.*, 1978). Inevitably, due to the accidental nature of the exposure the concentration of NOx was not accurately determined in any of these situations.

In this study we have followed the changes in the structure of rat lung by optical and electron microscopy following exposure to high concentrations of NOx for short periods. By selection of suitable time intervals after exposure it has been possible to observe transient changes and record some of the morphological effects on the tissues and cells of the lung.

MATERIALS AND METHODS

Materials.—Male Porton strain rats, weight 200–250 g, were used and kept at an ambient temperature of 20° and humidity (40–60%) in the animal house.

Nitrogen dioxide (NO_2) were acquired from BOC International PLC.

Glutaraldehyde and the components of Spurr's low viscosity epoxy resin were supplied by TAAB Laboratories Equipment Ltd and all other chemicals were obtained from British Drug Houses Ltd. 'Euthatol', pentobaritone, was obtained from May & Baker, Ltd.

Exposure method.—The animals were exposed in a 55 l chamber. The test atmosphere was produced by mixing nitrogen dioxide (NO_2), generated by passing nitrogen through liquid nitrogen dioxide at 0°, with nitric oxide (NO) from a cylinder and metered by a rotameter. This mixture was then passed into an airstream of approximately 115 l/min.

The concentration of NOx was continuously measured by a chemiluminescent monitor. The mean concentration for each exposure was 1435 parts/10^6 for 1 min or 518 parts/10^6 for 5 min.

Each exposure consisted of 20 animals, which were randomly divided into groups of 4 and killed either immediately or at 6 h, 24 h, 2 days or 5 days following their exposure.

Controls, consisting of 2 groups of 5 animals, were exposed in the chamber to an airstream of 115 l/min for 5 min without the addition of nitrogen dioxide or nitric oxide. They were killed either immediately following exposure or at 5 days.

All animals were returned to the animal house immediately after their exposure and were observed daily and any toxic signs noted throughout the course of the experiment.

All animals were examined using histological techniques and the controls together with the animals killed at 0, 24 h and 5 days following exposure were observed using electron microscopy.

Post mortem and tissue selection.—The animals were killed by i.p. injection of 'Euthatal' (sodium pentobarbitone 200 mg/ml). The thorax was opened and the lungs rapidly removed. For electron microscopy a 3 mm thick slice was taken from the middle of the left lobe and placed in 3% glutaraldehyde in 0·1M cacodylate buffer pH 7·4 containing 3 mmol l^{-1} calcium chloride which had been cooled to 4°. The remaining portions of the left lobe were placed in 10% neutral buffered formalin for conventional histology.

Histological examination.—Tissue from the remainder of the left lobe was taken and after further fixation in 10% neutral buffered formalin was dehydrated and embedded in paraffin wax. Sections 3 μm thick were cut and stained with haematoxylin and eosin (Carleton and Drury, 1957).

Electron microscopy.—After fixation for 30 min the 3 mm slices were diced into approximately 1 mm × 1 mm cubes and further fixed in glutaraldehyde for 8 h. After transfer to 6% sucrose overnight they were rinsed in buffer and post fixed in 2% osmium tetroxide buffered to pH 7·4 for 2 h.

The tissues were then treated by a modification of the Wallis and Griffin method (1973) and finally embedded in Spurr's low viscosity epoxy resin (Spurr, 1969).

Blocks were cut on a LKB Ultramicrotome Mk 3 using 55° glass knives. For orientation purposes, sections 1 μm thick were cut for optical microscopy, and stained for 20 min at at 60° in 1% toluidene blue in 1% aqueous borax. Sections were then cut for electron microscopy, 60–90 nm thick, mounted on copper grids and stained with 3% aqueous uranyl acetate for 10 min followed by alkaline lead citrate for 3 min (Venable and Coggeshall, 1965).

RESULTS

One animal, which had been exposed to 518 parts/10^6 NOx for 5 min died 2 days after the exposure; no tissues could be examined due to cannibalism.

Physical signs

Animals from both the exposures showed no significant signs for up to 6 h after the exposure.

1435 *parts*/10^6 *NOx for* 1 *min*

Twenty-four hours after exposure, slightly stertorous respirations were noted

in a few animals. This was not apparent at 2 days at which time the animals appeared normal. Five days after exposure no abnormalities could be detected.

518 parts/10^6 NOx for 5 min

Twenty-four hours after exposure all animals showed stertorous respirations which increased in severity up to 2 days post exposure. The animals were lethargic, with slight ruffling of fur and were eating and drinking very little. Five days after exposure some improvement in their condition was noted but the animals were still slightly lethargic.

Macroscopic appearances of the lung

The macroscopic appearance of the lungs from rats exposed to either 1435 parts/10^6 NOx for 1 min or 518 parts/10^6 NOx for 5 min showed isolated instances of slight petechial haemorrhages, pale discoloration and varying degrees of congestion. Froth exuded from the cut surfaces. These effects were more severe in animals which had been exposed to 518 parts/10^6 NOx for 5 min.

Histology

The structure of normal Porton strain rat lung has been described previously (Colgrave, Brown and Cox, 1979); no significant alterations in lung structure from these appearances could be detected in the controls, which had been killed immediately after their exposure to an airstream. Five days after exposure, however, slight and scattered alveolar collapse was observed in one animal and an area of

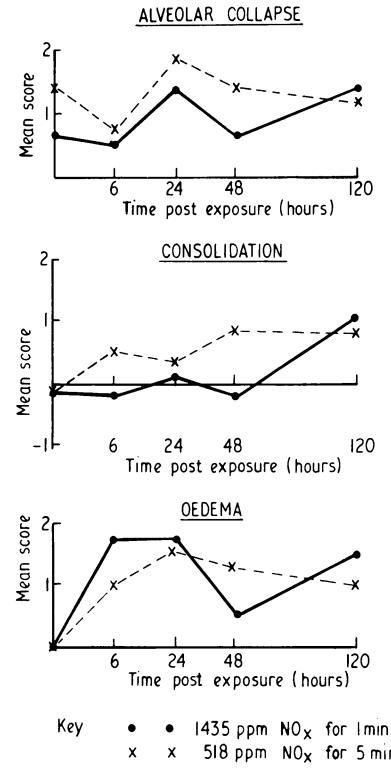


FIG. 1.—Plots of mean score for alveolar collapse, consolidation and oedema observed in the lungs of animals exposed to either 1435 ppm NOx for 1 min or 518 ppm NOx for 5 min.

TABLE I.—*Histological findings from animals exposed to an airstream (controls).*

Group/time of killing	Alveolar collapse	Consolidation	Oedema	Cellular infiltration	Bronchial exudate
Killed	0	0	0	0	0
immediately	0	0	0	0	0
post	0	0	0	0	0
exposure	0	0	0	0	0
	0	0	0	0	1
Killed	0	0	0	0	0
5 days	0	0	0	0	0
after	0	0	0	0	0
exposure	0	2	0	M2 L1	0
	1	0	0	0	0

Key: (Tables I, II, III) 0=not present; 1=slight, scattered; 2=mild; 3=moderate; M=macrophages; L=lymphocytes; P=polymorphonuclear leucocytes.

TABLE II.—*Histological findings from animals exposed to* 1435 *parts*/10^6 *NOx for* 1 *min*

Group/time of killing	Alveolar collapse	Consolidation	Oedema	Cellular infiltration	Bronchial exudate
Killed	0	0	0	0	2
immediately	0	0	0	0	0
post	2	0	0	0	2
exposure	1	0	1	0	0
Killed	1	0	3	0	0
6 h	1	0	1	M2	2
post	1	0	1	M1	0
exposure	0	0	2	L1	0
				M2	
Killed	2	0	2	M1	0
24 h	2	0	1	M2	0
post	2	1	2	P1	0
exposure				MS	
	0	0	2	M2	0
Killed	0	0	1	M1	1
2 days	1	0	0	M1	1
post	1	0	1	0	0
exposure	1	0	0	M1	0
Killed	2	2	2	M2	0
5 days				L1	
after				P1	
exposure	1	3	2	M2	0
				L2	
				P1	
	2	0	2	M2	2
	1	0	0	M1	0

consolidation with associated infiltration of macrophages and lymphocytes was seen in another (Table I).

1435 *parts*/10^6 *NOx for* 1 *min* (*Table II*)

Immediately after exposure no significant histopathological abnormalities were observed except for 2 animals which showed slight, scattered alveolar collapse and another in which slight, patchy oedema was seen. Two animals also showed a cellular bronchial exudate.

At 6 h (Fig. 2) the lungs of all animals showed peripheral oedema which varied in degree from slight and scattered to moderate. Alveolar collapse with associated infiltration of macrophages and lymphocytes was present in 3 out of 4 animals, one of which also showed a cellular bronchial exudate.

At 24 h, the oedema was more marked with alveolar collapse similar to that observed at 6 h. All animals showed macrophage infiltration, with intraalveolar macrophages predominating, and some alveolar cell damage was observed in one animal.

At 2 days the general picture was similar to that at 24 h although 2 animals did not show oedema, and one was free of macrophage invasion. Cellular bronchial exudate was present in the lungs of 2 out of 4 animals.

At 5 days, one animal showed a large area of lung consolidation; otherwise alveolar collapse, oedema and macrophage infiltration continued to be prominent together with polymorphonuclear and lymphocytic infiltration in the lungs of 2 animals. Cellular bronchial exudate was observed in the lungs of one animal.

518 *parts*/10^6 *NOx for* 5 *min* (*Table III*)

No significant histological abnormalities were seen in the lungs of any of the animals killed immediately following their exposure.

At 6 h slight, scattered to mild alveolar collapse with associated consolidation was seen in the lungs of 2 animals; peripheral

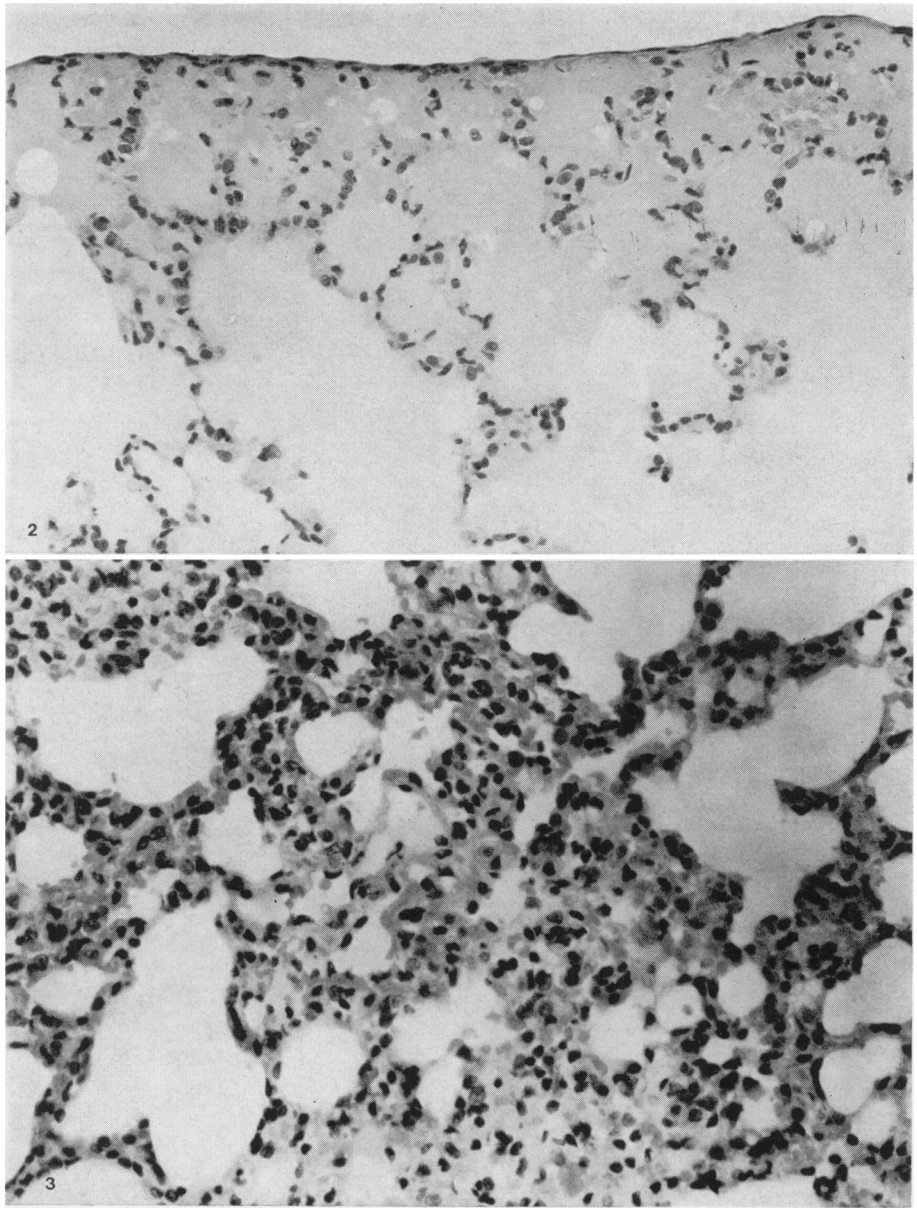


FIG. 2.—1435 parts/10^6 NOx, 6 h after exposure. Histological section of rat lung showing generalized peripheral oedema. × 144.

FIG. 3.—518 parts/10^6 NOx, 2 days after exposure. Histological section of rat lung showing areas of alveolar collapse, alveolar oedema and inflammatory cell invasion. × 328.

oedema was also evident. Macrophage infiltration was observed in the lungs of a further 2 animals, one of which also showed cellular bronchial exudate.

At 24 h, the lungs of one animal showed more generalised oedema but in 2 others it was slight and scattered with a variable number of macrophages present. In other respects the appearance was similar to that seen at 6 h.

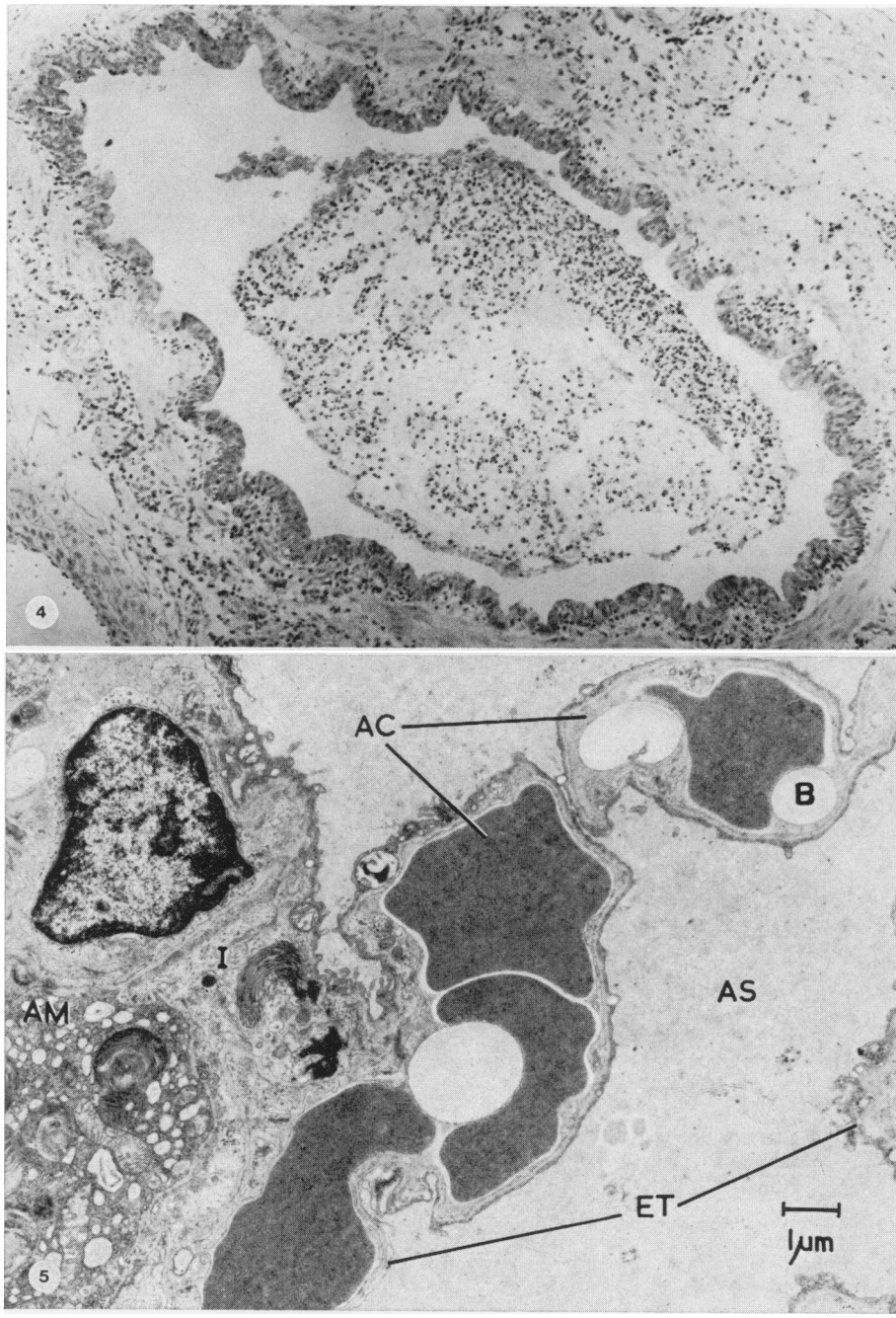


FIG. 4.—518 parts/10^6 NOx for 5 min, 5 days after exposure. Histological section of rat lung showing a bronchus with eosinophilic fluid and inflammatory cells in the lumen. × 328.

FIG. 5.—1435 parts/10^6 NOx for 1 min, 1 day after exposure. Alveolar capillaries showing areas of thickening of the epithelium and 'ballooning' of the endothelium. The alveolar space is filled with electron dense fluid and debris. × 7376. AC, Alveolar capillaries; ET, Thickening of epithelium; B, 'Ballooning' of endothelium; AM, Type II (surfactant-producing) pneumocyte; I, Interstitium; AS, Alveolar space.

TABLE III.—*Histological findings from animals exposed to* 518 *parts*/10^6 *NOx for* 5 *min*

Group/time of killing	Alveolar collapse	Consolidation	Oedema	Cellular infiltration	Bronchial exudate
Killed	1	0	0	0	0
immediately	2	0	0	0	2
post	0	0	0	0	0
exposure	1	0	0	0	0
Killed	0	2	2	L1	0
6 h	2	1	1	M2	0
post	0	0	0	0	0
exposure	1	0	1	M1	0
Killed	2	0	0	M1	0
24 h	2	0	3	M2	0
post	2	0	1	0	0
exposure	2	2	2	M3 L2 P1	2
Killed	1	0	1	M1 P1	0
2 days post	1	0	1	M1	0
exposure	2	2	1	M1	0
	2	2	0	M3 L2 P1	2
Killed	1	0	1	M1	0
5 days post	2	0	0	M1 L1	2
exposure	1	3	2	M2 L1	1

At 2 days (Fig. 3) the lungs of all animals showed some degree of alveolar collapse and oedema, with the lungs of 2 animals also showing areas of consolidation. A mixed inflammatory cell invasion with macrophages predominating was also evident. A cellular bronchial exudate was observed in one animal.

At 5 days observations were made on the lungs of the remaining 3 animals. The pathology was essentially the same as at 2 days but the extent of the oedema was more variable. Macrophage and/or lymphocyte invasion with cellular bronchial exudate (Fig. 4) was present in the lungs of 2 of the 3 animals.

To reveal any trends in lung pathology the pathologist's assessment was expressed on a severity scale using the following scoring method:

0 = no lesion detected
1 = slight and scattered
2 = mild
3 = moderate

The results are shown in Tables I–III.

'Mean scores' for alveolar collapse, consolidation and oedema were then plotted against time (Fig. 1). The graphs showed the following features:

1. Alveolar collapse occurred immediately after exposure and did not regress during the ensuing days.
2. Consolidation did not become significant until 2–5 days after the exposure.
3. There was a delay of approximately 6 h before the onset of oedema, which then remained evident throughout the course of the experiment.
4. Alveolar collapse and consolidation in the lungs of animals exposed to 518 parts/10^6 NOx for 5 min was greater than that observed in the lungs of animals exposed to 1435 parts/10^6 NOx for 1 min (from 6 h to 2 days after exposure). At 5 days after exposure the degree of damage found was similar for both dose levels. In the case of oedema, however, no difference was detected

TABLE IV.—*Summary of electron microscopic findings from control animals*

Time post exposure	Observation						
	Epith	End bl	Int oe	Alv fl	Art va	Ven va	Mac
Control (immediately after exposure)	+	−	−	−	−	−	−
	−	−	−	−	−	−	−
	−	−	−	−	−	−	−
	−	+	−	−	−	−	−
	−	+	−	−	−	−	−
Control (5 days after exposure)	+	+	−	−	−	−	−
	+	+	−	−	−	−	−
	−	−	−	−	−	−	−
	−	−	−	−	−	−	−
	−	−	−	−	−	−	−

Key: Epith, Epithelial thickening; End bl, Endothelial ballooning; Int oe, Interstitial oedema; Alv fl, Fluid in alveolar space; Art va, Arteriole endothelial vacuolation; Ven va, Venule endothelial vacuolation; Mac, Type II pneumocyte disruption; + = Present; − = Absent; EM sampling does not allow degree scoring.

TABLE V.—*Summary of electron microscopic findings from animals exposed to* 1435 *parts*/10^6 *NOx for* 1 *min*

Time post exposure	Observation						
	Epith	End bl	Int oe	Alv fl	Art va	Ven va	Mac
Immediately after exposure	−	+	−	+	−	−	+
	−	−	−	+	−	−	+
	+	−	−	−	−	−	+
	+	−	−	+	−	−	+
24 h after exposure	+	+	+	+	−	−	+
	+	+	+	+	−	−	+
	+	−	−	+	−	−	+
	+	−	−	+	−	−	+
5 days after exposure	+	−	+	+	−	−	+
	+	+	+	+	−	−	+
	+	−	−	+	−	−	+
	+	+	−	+	−	−	+

See Table IV for key.

TABLE VI.—*Summary of electron mircoscopic findings from animals exposed to* 518 *parts*/10^6 *NOx for* 5 *min*

Time post exposure	Observation						
	Epith	End bl	Int oe	Alv fl	Art va	Ven va	Mac
Immediately after exposure	+	+	−	−	−	−	+
	+	+	−	+	−	−	+
	+	+	−	−	+	−	+
	+	−	−	−	+	−	+
24 h after exposure	+	+	+	+	+	+	+
	+	+	+	+	+	+	+
	+	+	+	+	−	−	+
	+	+	+	+	−	−	+
5 days after exposure	+	+	−	−	−	−	+
	+	+	+	−	−	−	+
	+	+	+	+	−	−	+
	← Animal died →						

See Table IV for key.

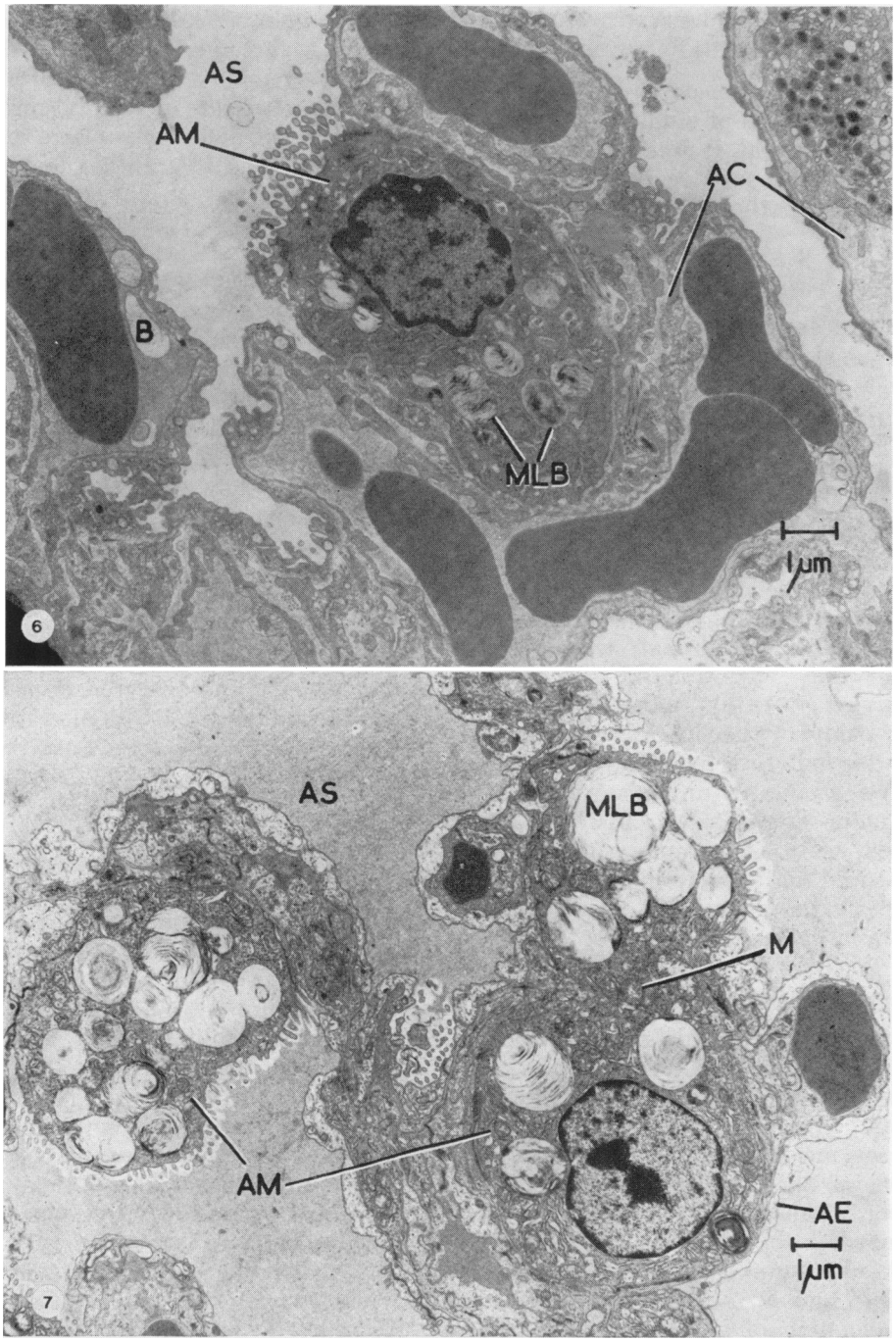


FIG. 6.—Control—airstream for 5 min, 5 days after exposure. Electron micrograph of rat lung showing a type II (surfactant producing) pneumocyte. × 7376. AM, Type II (surfactant producing) pneumocyte; AC, Alveolar capillaries; AS, Alveolar space; B, 'Ballooning' of endothelium; MLB, Mitochondrial lamellated bodies.

FIG. 7.—518 parts/10^6 for 5 min, immediately after exposure. Type II (surfactant producing) pneumocyte. The mitochondrial lamellated bodies are disrupted with loss of integral structure and the mitochondria appear swollen. There is alveolar oedema with thickening of the alveolar epithelium. × 6560. AM, Type II (surfactant producing) pneumocyte; MLB, Mitochondrial lamellated bodies; M, Mitochondria; AE, Alveolar epithelium; AS, Alveolar space.

since the levels observed were similar at all time intervals following exposure.

Because of these inconsistencies and also small numbers (4) of animals observed at each time interval, it was not possible to say which exposure regime caused the more severe pathological damage to the lung.

Electron microscopy

The appearance of the control rat lungs under the electron microscope was similar to that previously described (Colgrave *et al.*, 1979). In the group killed 5 days after their exposure to an airstream there was an increase in the incidence of both epithelial 'blebbing' and endothelial 'ballooning' as compared with those animals which were killed immediately after their exposure. These minor changes in ultrastructure are thought to be associated with stress to which the animals are subjected during the experimental procedures (Colgrave *et al.*, 1979) (Table IV).

The study of the fine structure of the lung after exposure to NOx was confined to those groups of animals which were killed immediately, 1 day and 5 days after their exposure. A summary of electron microscope findings from each animal following exposure to both concentrations is shown in Tables V and VI.

1435 *parts*/10^6 *NOx for* 1 *min*

In animals from this group morphological alterations to the capillary epithelium were observed immediately following exposure. This took the form of swelling and occasional 'blebbing' with an apparent increase in the numbers of pinocytotic vesicles; the endothelium at this time was unchanged.

By 1 day after exposure the swelling and 'blebbing' had become more generalised; and ballooning of the endothelium into the lumen of the alveolar capillary was observed (Fig. 5).

At 5 days post exposure the level of damage to the epithelium was unchanged; there was, however, some improvement in the endothelium with only isolated incidences of 'ballooning' being detected.

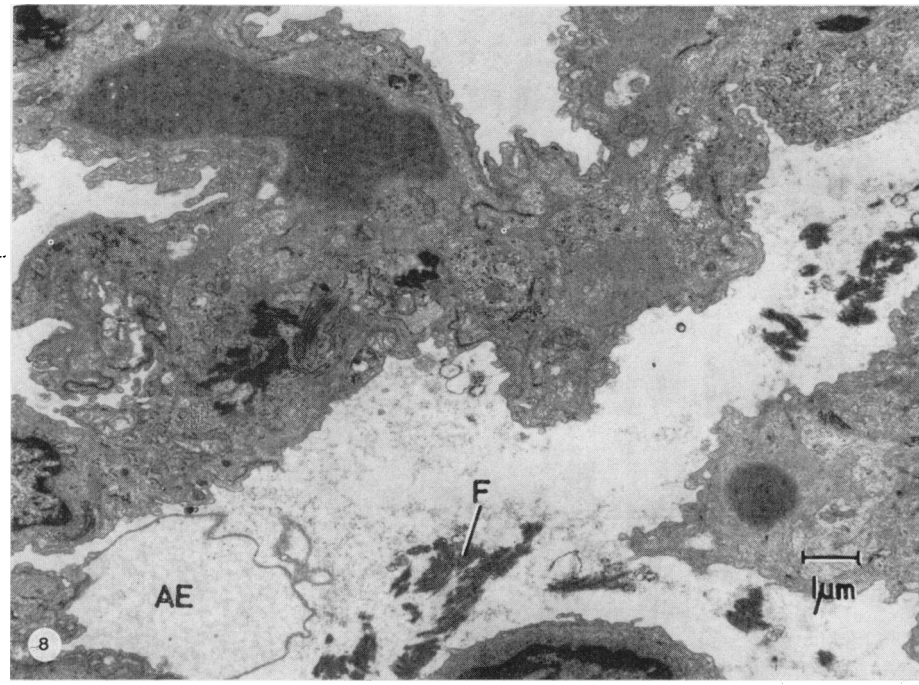
The other main finding was of changes to the ultrastructure of type II (surfactant producing) pneumocytes, which took the form of swelling, disruption and loss of integral structure of the mitochondrial lamellated bodies (Pattle *et al.*, 1974; Figs 6 and 7). The mitochondria also appeared swollen (Figs 5 and 7). The damage described was evident immediately after exposure and the cells remained unchanged in ultrastructure throughout the course of the experiment.

Alveolar oedema, characterized by dense granular fluid-filled alveolar spaces containing fibrin clots, was widespread by 1 day after exposure (Fig. 8). By 5 days the oedema had become isolated and patchy, with numerous free macrophages within the alveolar space. These had characteristic and numerous phagopolylysosomes within their cytoplasm (Fig. 9). Interstitial oedema was observed occasionally from one day onwards. The ultrastructure of bronchioles, arterioles and venules remained unchanged.

518 *parts*/10^6 *NOx for* 5 *min*

The morphological alterations to the alveolar capillary blood–air barrier were similar in nature to, but more severe than, those found in animals exposed to 1435 parts/10^6 NOx for 1 min. Immediately after exposure generalized epithelial separation and 'blebbing' of the epithelium with isolated 'ballooning' of the endothelium was present. Twenty-four hours after exposure widespread separation with 'blebbing' of the epithelium and occasional epithelial rupture was also observed. The appearances described remained unchanged at 5 days. Isolated sites of endothelial 'ballooning' were also evident at 24 h and 5 days.

Widespread damage to the type II (surfactant producing) pneumocytes was evident immediately after exposure. This was similar to that described in the previous exposure in that there was swelling, disruption and loss of integral



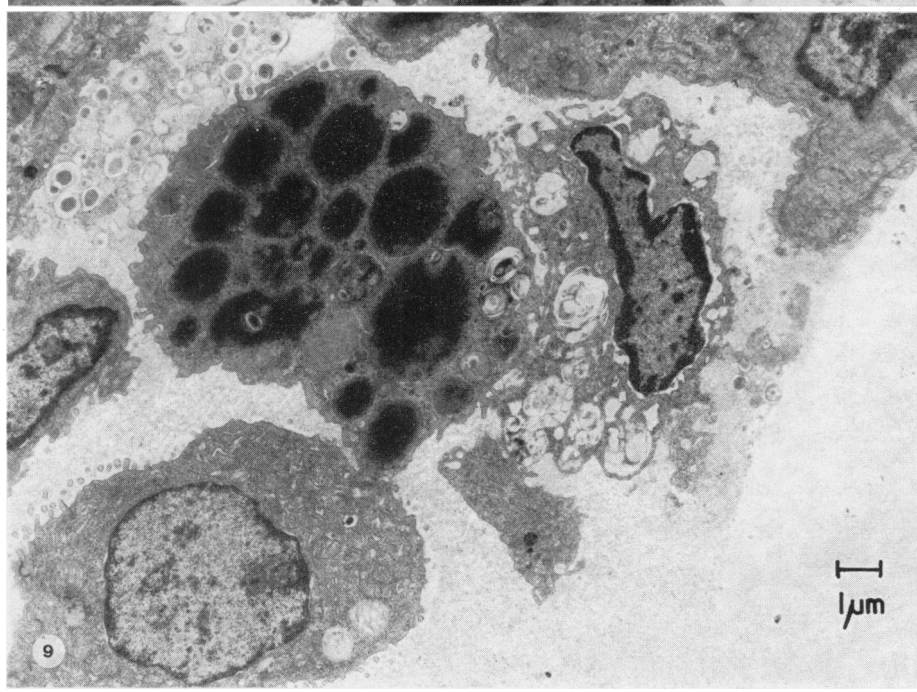


FIG. 8.—1435 parts/10^6 for 1 min 1 day after exposure. Widespread alveolar oedema with fibrin deposition in the alveolar space. × 7140. F, Fibrin; AE, 'Blebbing' of alveolar epithelium.

FIG. 9.—1435 parts/10^6 for 1 min 5 days after exposure. Free alveolar macrophage with characteristic phagopolyribosomes in the cytoplasm. × 5584.

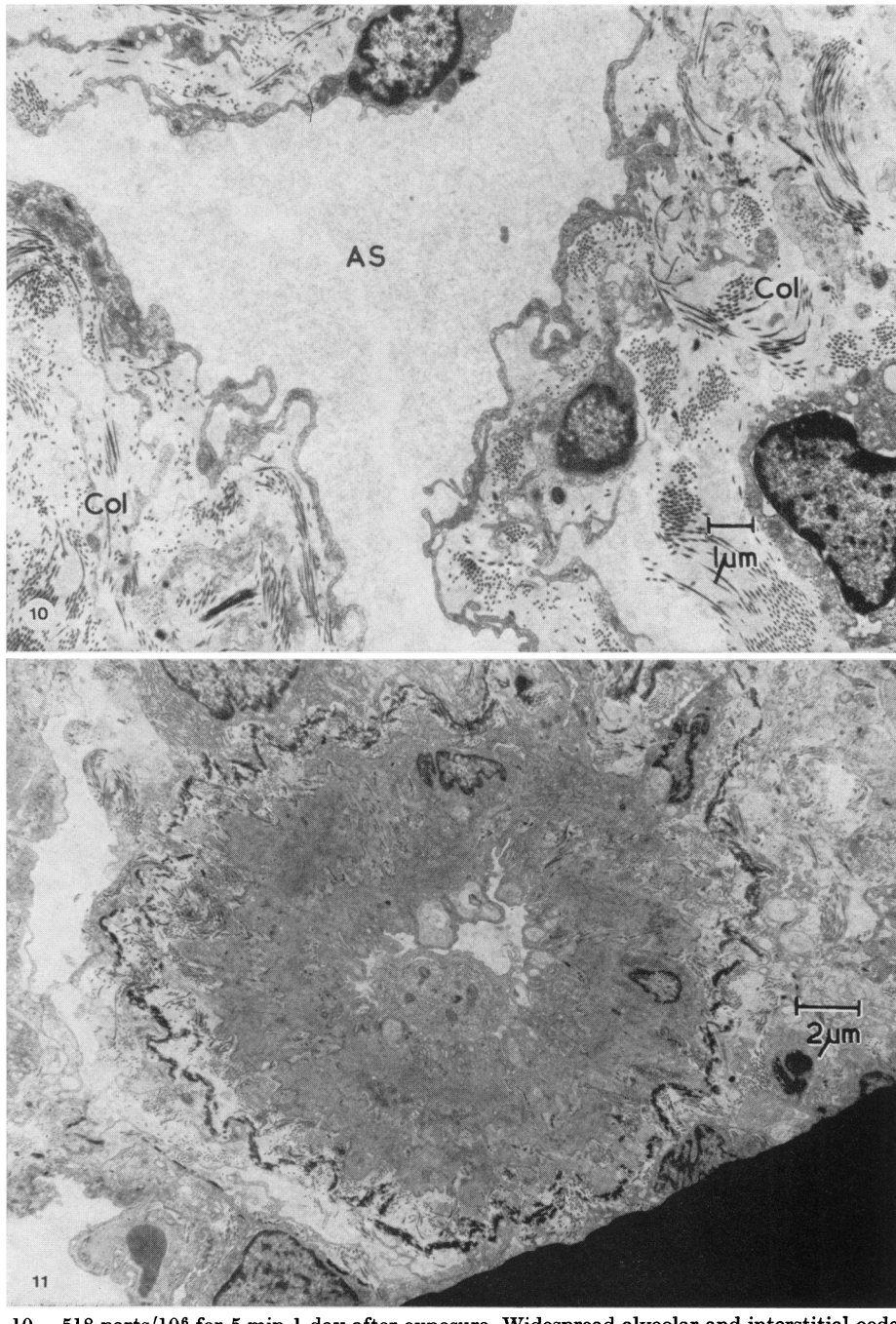


FIG. 10.—518 parts/10^6 for 5 min 1 day after exposure. Widespread alveolar and interstitial oedema with possible deposition of new collagen. × 6016. AS, Alveolar space; Col, Collagen.

FIG. 11.—518 parts/10^6 for 5 min 1 day after exposure. Arteriole showing 'ballooning' of the endothelium. × 4208.

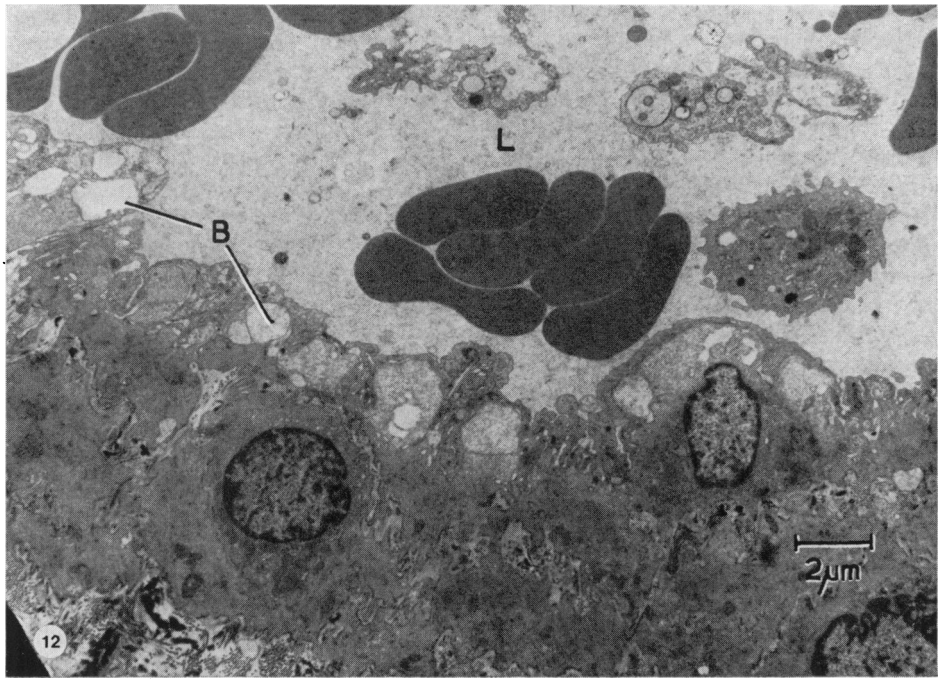


FIG. 12.—518 parts/10^6 NOx for 5 min 1 day after exposure. Venular wall showing 'ballooning' of the endothelium. × 5120. B, 'Ballooning'; L, Lumen of venule.

structure of mitochondrial lamellated bodies with swelling of the mitochondria.

A single isolated area of alveolar oedema was seen in one animal immediately after exposure and in all animals oedema of both interstitium and alveolar septum had become widespread 24 h after exposure (Fig. 10). At 5 days patchy interstitial and alveolar oedema was still evident.

Vacuolation of the arteriolar endothelium was observed in 2 animals immediately after exposure. At 24 h the endothelia of both arterioles and venules contained large vacuoles which extended into the lumen of the vessels (Figs 11 and 12). This had disappeared at 5 days.

Immediately following exposure dense granular fluid was observed in the bronchiolar lumen of one animal; however, the bronchiolar epithelium was unchanged with the cells showing a normal ultrastructure with a full complement of undamaged cilia present.

DISCUSSION

The oxides of nitrogen are highly reactive compounds and are therefore expected to be toxic in biological systems. When inhaled, they react with water to form their corresponding acids. These acids attack the airway and alveolar surface by peroxidation of lung lipids, for example phosphatidyl ethanolamine (lecithin), a major constituent of biological membranes and pulmonary surfactant (Rowlands and Gause, 1971). The free radicals produced by this reaction are capable of further attack and oxidation of unsaturated fats and possible alteration of the characteristics of membranes resulting in for example, alveolar collapse (Guidotti, 1978). Denaturation of collagen and elastin and inactivation of enzymes has also been reported (Heuter and Fritzhaud, 1971).

The evolution of damage to the lung as described using histological techniques was similar whether the animals were

exposed to 1435 parts/10^6 for 1 min or 518 parts/10^6 NOx for 5 min. There was a latent period lasting between 1 h and 6 h during which there were few significant changes, followed by the development of pulmonary oedema with inflammation and considerable macrophage activity. Alveolar collapse occurred immediately after exposure and was evident throughout the course of the experiment. Lung consolidation was seen at later stages following exposure and was thought to be the result of secondary infection of oedematous regions of the lung. Shiel (1967) who observed pathological changes in dog lung following exposure to different concentrations of higher oxides of nitrogen during anaesthesia, reported that the pathological abnormalities were proportional to the concentration and duration of exposure. This could not be confirmed by the present experiment where only small numbers (4) of animals were exposed. Alveolar macrophage proliferation is a common non-specific tissue reaction to lung injury (Witschi, 1976).

The electron microscopic findings are in general agreement with the ultrastructure of the normal rat lung described by Low, (1953) and confirms that 'experimental stress' can produce significant increases in the incidence of epithelial 'thickening' and endothelial 'ballooning' (Colgrave *et al.*, 1979).

In animals killed immediately after their exposure to either 1435 parts/10^6 for 1 min or 518 parts/10^6 NOx for 5 min thickening of the epithelium and alteration to the structure of type II (surfactant producing) pneumocytes was the main finding. This would seem to be the direct effect of the oxidant action of NOx. Similar changes have been observed following low concentration/long term exposure to NO_2 and ozone (Bils, 1974). Exposure to 12 parts/10^6 NO_2 for 1 h resulted in similar changes together with mitochondrial swelling and increased pinocytosis (Dowell *et al.*, 1971). Dowell and colleagues also observed damage to the ciliated epithelium of the bronchiolar wall, but this was not confirmed by the present experiment. A latent period where few ultrastructural alterations to the alveolar septum were observed prior to the onset of both pulmonary and interstitial oedema is a common feature of both low concentration/long term and higher concentration/short term exposures (Guidotti, 1978; Morrow, 1975; Bils, 1974; Dowell *et al.*, 1971).

The clinical course of patients after accidental or industrial exposure where NOx was reported as a contributing factor are remarkably consistent from a variety of sources (Guidotti, 1978; Morrisey *et al.*, 1975). In most, the NOx concentration has not been ascertained but in the case of 'silo-fillers disease' concentrations of between 200 parts/10^6 and 4000 parts/10^6 have been reported. In other industrial situations the levels are thought to be much lower (Ramirez and Dowell, 1971). Immediately after their exposure, and if the concentration is high enough, patients may suffer hypoxaemia from asphyxiation, but commonly dyspnoea and bronchospasm, cough, diffuse weakness and often nausea and headache were experienced. Pulmonary oedema then developed after an interval which varied from a few hours to 2 days, and which was dose-dependent (Malatinsky, Kadlic and Kovacik, 1973). The pulmonary oedema was followed by the development of bronchitis and bronchiolitis together with varying degrees of bronchiolitis with associated obstruction, which resulted in permanent damage to the lung. Hatton *et al.* (1977) investigating 3 astronauts, who were exposed to NOx during the re-entry phase of their space flight, found that as well as respiratory symptoms their patients had abnormal chest X-rays and an increase in urinary hydroxylysine glycosides which indicated that considerable collagen degradation had occurred during their exposure.

The long term sequelae following NOx exposure are variable and are thought by Guidotti (1978) to relate to the extent of exposure and the severity of bronchiolitis

with associated obstruction. These findings are in general agreement with the results of the present experiment where, after a period of approximately 6 h, during which little alteration to the lung could be detected, there followed the development of pulmonary oedema. The duration of the experiment was not sufficient for the development of bronchiolitis to be detected.

Goldstein (1975) and Lawther (personal communication) drew attention to the many potentially significant differences between the respiratory anatomy of rats and other routine small laboratory rodents and man, and warned against too ready an extrapolation to man. Nevertheless, within the limitations of these experiments and bearing in mind the parallels between human accidental exposures and the findings of the present experiments, it is likely that exposure of men for short periods to concentrations of the order of those considered here will lead to lung tissue damage and the risk of development of pulmonary oedema.

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