Arh. hig. rada toksikol., **30** *(1979) suppl. pp. 613–620.*

PULMONARY FUNCTION IN MEN EXPOSED TO LOW LEVELS OF OZONE

L. FABBRI, C. MAPP, A. ROSSI, F. SARTO and A. TREVISAN
Institute of Occupational Health, University of Padua, Padua, Italy

ABSTRACT

The authors examined 31 workers employed in a chemical factory, exposed to ozone levels below 1 ppm (0.16–0.46) for about two years. The examination included: E.C.S.C. (European Carbon and Steel Community) questionnaire, spirography (FVC, $FEV_{1.0}$), MEFV ($\dot{V}_{max}$ 50% FVC and $\dot{V}_{max}$ 25% FVC) and acetylcholinesterase activity (AChE). We found significant differences in maximal expiratory flow rates between exposed workers and a control group from the same factory. These differences were found only among smokers.

Respiratory function was measured in 21 workers after 0,2,4,6 hours of a work shift; only five subjects showed a significant decrease of $\dot{V}_{max}$ 25% FVC.

AChE measured at the end of one work shift was reduced in five subjects and returned to normal values after three weeks of non exposure. This result confirms the inhibitory effect of ozone on AChE but it does not suggest that bronchoconstriction induced by ozone is mediated by the action of ozone upon AChE.

Our results show that chronic exposure to low levels of ozone causes impairment of small airways and confirm the validity of TLV of 0.1 ppm (ACGIH).

Ozone, one of the major air pollutants in urban areas, is believed to represent a significant health hazard to man. It acts upon the respiratory tract and, at high concentrations, causes irritation, impairment of respiratory function, pulmonary oedema, and hemorrhage. Studies of pulmonary function following an exposure to ozone at concentrations lower than 0.5 ppm have been reported: a significant increase of airway resistance[1,2], a decrease in maximum expiratory flow rates, in lung volumes and flow rates and a reduction in steady state diffusing capacity[2,8,10] have been observed in normal subjects after 1–2 hours of exposure to less than 0.5 ppm ozone. No meaningful changes in forced expiratory measures, lung volumes, or single breath N_2 indices, were found in asthmatics after controlled exposure to ozone at concentrations of approximately 0.2 ppm[13]. It has been suggested[15] that the bronchoconstriction induced by ozone might be mediated by the action of ozone upon acetylcholinesterase (AChE).

Requests for reprints should be addressed to: Dr Leonardo Fabbri, Istituto di Medicina del Lavoro, Via Facciolati 71, Padova 35100, Italia

Experimental animal studies on long-term effects of ozone report chronic pulmonary disease[6,16,19] and biochemical alterations[3]. Few studies are reported on long-term effects of occupational exposure to ozone[14,20]. These studies concern welders who are simultaneously exposed to coexisting pollutants.

In this study we investigated the long- and short-term effects of occupational exposure to less than 0.5 ppm ozone on respiratory function and on acetylcholinesterase.

SUBJECTS, MATERIALS AND METHODS

Population

Out of a total of 180 workers we examined 31 subjects (27 men and 4 women) who were employed in a factory producing plastic bags. They were exposed to ozone in the extrusion, stamping, and sealing work stations. A control group of the same factory matched for age, height, and sex was examined. The characteristics of the examined population are in Table 1.

TABLE 1
Mean values and standard deviations for morphometric characteristics and per cent of smokers of the exposed and control groups.

	Exposed group	Control group
No	31	31
Age (years)	28.4 ± 8.2	28.8 ± 8.2
Height (cm)	173.1 ± 5.8	172.3 ± 7.4
Weight (kg)	74.3 ± 8.5	73.3 ± 9.5
Smokers (%)	67.7	61.3

Conditions of working environment

The population examined was employed in a plastic processing factory producing polyethylene bags. Polyethylene granules are heated in a cylinder and forced through dyes which form plastic into tubes. The extruded tube, while still hot, is greatly expanded by air pressure, resulting in a tube of large diameter and a very thin wall. Simple bags are obtained by cutting and sealing one end. Before stamping the top surface of the polyethylene tube is treated with a high voltage electrical discharge in order to permit colour fixation. Ozone is produced during this process from oxygen. The machines are placed close to each other which allows ozone to diffuse all over the place.

Table 2 shows the values of ozone measured at three working stations during different shifts. The concentrations of ozone were measured by the buffered potassium iodide method[11].

TABLE 2
Air concentrations of ozone at the various work stations (ppm and mg/m^3).

	Ozone air concentration	
	mg/m^3	ppm
Extrusion	0.92	0.46
Stamping	0.60	0.30
Sealing 1st	0.42	0.21
Sealing 2nd	0.32	0.16

Definition of respiratory symptoms and measurements of lung function

The E.C.S.C. questionnaire for chronic bronchitis and emphysema was used to record respiratory symptoms, smoking habits, past history of chest diseases, and a detailed occupational history. Chronic bronchitis was defined as cough and phlegm for a minimum of three months in the year and for not less than two successive years.

In all subjects forced vital capacity (FVC), forced expiratory volume in one second ($FEV_{1.0}$) and maximal flows at 50% and 25% of FVC were measured by means of an OHIO 840 dry spirometer connected to an X-Y recorder (Honeywell 530) plotting volume with time and to another X-Y recorder (Hewlett-Packard 7045 A) to plot flow versus volume.

Acute changes in lung volumes and flow rates were measured at 0,2,4,6 hours of a work shift in the exposed group.

Acetylcholinesterase

AChE was assayed in red blood cells (RBC) of 22 exposed subjects, according to the method of De La Huerga and co-workers[4] with acetylcholine bromide (BDH, Poole) as a substrate. The activity is expressed in μM of hydrolised acetylcholine per hour per milliliter of RBC. The normal values in our laboratory range between 380 and 760 μM/ml of RBC.

The measurements of AChE were conducted in blood samples collected at the end of a work shift; a second measurement was carried out at the end of a three-week holiday before workers returned to work.

RESULTS

Table 3 shows the mean values of lung function tests and the prevalence rates of chronic bronchitis in the exposed and control groups; significant lower mean values of flow rates were found in the exposed group. Flow rates were significantly lower in the exposed smokers, but there was no difference in lung function between the non-smokers of the two groups (Tables 4 and 5).

TABLE 3
Mean and standard deviations for pulmonary function measurements and prevalence of chronic bronchitis of the exposed and control groups.

	Exposed group	Control group
FVC (litres)	5.0 ± 0.6	5.0 ± 0.7
$FEV_{1.0}$ (litres)	4.1 ± 0.5	4.2 ± 0.6
$\dot{V}_{max}$ 50 (l/sec)	5.4 ± 1.3**	6.1 ± 1.3
$\dot{V}_{max}$ 25 (l/sec)	2.4 ± 1.0*	2.9 ± 0.8
Chronic bronchitis (%)	6.5	6.5

* = $p < 0.05$; ** = $p < 0.01$

TABLE 4
Mean values and standard deviations for $\dot{V}_{max}$ 50 of smokers and non smokers of the exposed and control groups.

	Exposed group	Control group
Non smokers	(10) 6.0 ± 1.4	(12) 6.0 ± 0.8
Smokers	(21) 5.0 ± 1.0	(19) 6.2 ± 1.5**

** = $p < 0.01$

TABLE 5
Mean values and standard deviations for $\dot{V}_{max}$ 25 of smokers and non smokers of the exposed and control groups.

	Exposed group	Control group
Non smokers	(10) 3.0 ± 1.1	(12) 3.0 ± 0.9
Smokers	(21) 2.2 ± 0.7	(19) 2.8 ± 0.8*

* = $p < 0.05$

TABLE 6
Mean values and standard deviations for $\dot{V}_{max}$ 50 of the exposed and control groups classified according to the length of exposure.

Months of exposure	Exposed group	Control group
< 12	(5) 5.8 ± 0.9	(9) 6.0 ± 1.5
13—24	(15) 5.5 ± 1.5	(11) 6.4 ± 1.0
> 24	(11) 5.2 ± 0.9	(11) 5.9 ± 1.2

Tables 6 and 7 show the mean values of $\dot{V}_{max}$ 50 and $\dot{V}_{max}$ 25 in the two groups classified according to length of exposure. Even if not statistically significant and without any difference in age, height and smoking habits, there is a decrease in flow rates with the increase in the length of exposure.

TABLE 7.
Mean values and standard deviations for $\dot{V}_{max}$ 25 of the exposed and control groups classified according to the length of exposure.

Months of exposure	Exposed group	Control group
<12	(5) 2.9±0.8	(9) 2.7±0.9
13—24	(15) 2.4±1.2	(11) 3.1+1.0
>24	(11) 2.3±0.5	(11) 2.8±0.7

TABLE 8
Changes in $FEV_{1.0}$ and maximal expiratory flow rates in 21 subjects of the exposed group during the work shift.

Work shift (hours)	FEV (litres)	$\dot{V}_{max}$ 50 (l/sec)	$\dot{V}_{max}$ 25 (l/sec)
0	4.0±0.5	5.3±1.3	2.4±0.7
2	4.1±0.6	5.0±1.3	2.4±0.7
4	4.0±0.6	5.1±1.6	2.3±0.8
6	4.0±0.6	4.8±1.5	2.3±0.7

Table 8 illustrates changes in lung function in the exposed group at 0, 2, 4, 6 hours of a work shift. Neither $FEV_{1.0}$ nor flow rates show significant mean changes. Individual values of $\dot{V}_{max}$ 25 (Figure 1) show a significant decrease (more than 20%) in five subjects: all of them at the 6th hour; four at the 4th and only two at the 2nd hour. Other three subjects presented a significant decrease after two hours of exposure but not after four and six hours.

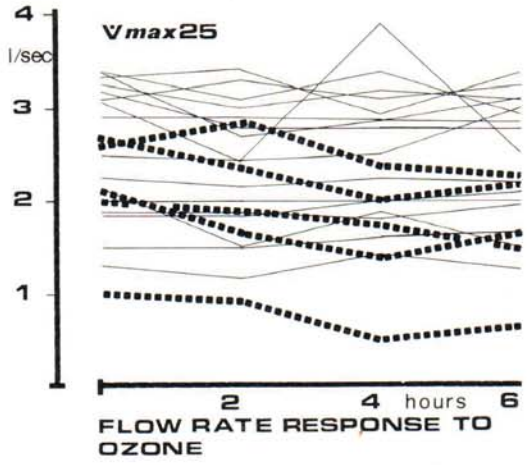

FIG. 1 – Individual values of $\dot{V}_{max}$ 25 at the beginning and at 2, 4, 6, hours of the work shift in 21 examined subjects of the exposed group.

Individual and mean values of AChE for 22 examined exposed subjects are reported in Figure 2: only in five exposed subjects a slight enzyme inhibition was found after a work shift. At the second determination (after 15 days of non exposure) mean value (500 ± 122 μM/ml of RBC) was not different from previous (502 ± 115 μM/ml of RBC), but no subject showed values of AChE

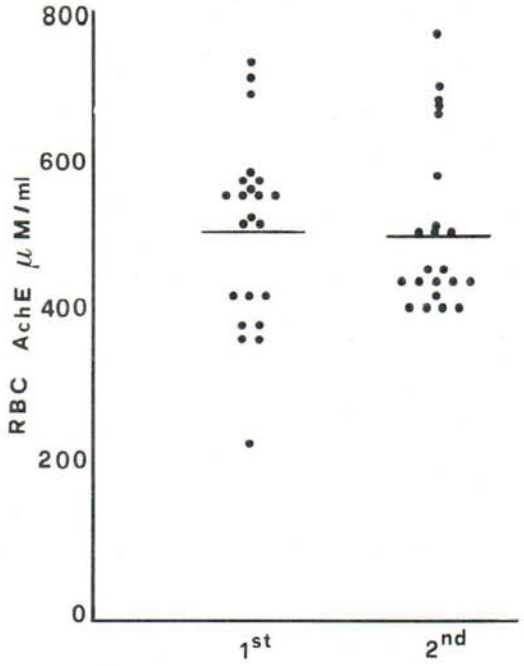

FIG. 2 – Individual and mean values of AChE in the exposed group after a work shift (E_1) and after 15 days of non exposure (E_2).

lower than normal limit. All the five subjects with low values of AChE were smokers and mean values of their lung function measurements were not different from those of exposed smokers. Only at the first determination we observed a slight not significant difference in AChE between smokers and non-smokers 477 ± 121 μM/ml against 560 ± 80 μM/ml).

DISCUSSION

Many occupational lung diseases appear dependent upon whether or not the workers smoke cigarettes. Some clear examples of such interactions are found in pneumoconioses[18] and lung cancer from asbestos[17]. Experimental animal studies demonstrated that the toxicity of ozone is increased if nitrogen oxides are present[5], and that even if morphologic responses to these two gases seem

comparable, there are important differences in their target. Whereas low levels of NO_2 affect the epithelium of the entire bronchiole, the main effect of ozone is shifted slightly more to the periphery to involve the epithelium of the terminal bronchiole and the entire alveolar duct[7]. The fact that smokers who inhale high concentrations of nitrogen oxides in tobacco smoke show an enhanced susceptibility to ozone has been confirmed by the results of the present study. A significant difference between the exposed and control groups has been found only in smokers and for $\dot{V}_{max}$ 50 and $\dot{V}_{max}$ 25 which indicate the main involvement of small airways.

In addition to its direct effects, ozone inhibits lung defence against infection, interfering with the phagocytic function of alveolar macrophage cells, and inhibiting lysozyme[1]. Furthermore it increases trypsin-protein esterase concentration[14].

The disease of small airways observed in the exposed group may be due to an inflammatory response to an additive interaction between nitrogen oxides of tobacco smoke and ozone.

Contrary to experimental studies on volunteers[2,8] we did not observe any short-term effect of ozone on lung function in the whole exposed group; but exposure cannot be considered continuous.

An "adaptation" was hypothesized to explain difference in sensitivity to ozone between inhabitants of high and low polluted areas[9].

In our study the five subjects who presented a significant decrease in $\dot{V}_{max}$ 25 were exposed for more than 12 months; this result cannot confirm the "adaptation hypothesis" suggested by Hackney and co-workers[9].

The hematological results of the present study may suggest an interaction of ozone and smoke in the inhibiton of AchE but cannot confirm it as the cause of bronchoconstriction.

CONCLUSIONS

1. Long-term exposure to ozone below 0.5 ppm impairs lung function only in smokers.

2. The function impairment indicates the main involvement of small airways.

3. Neither a significant short-term effect of ozone on respiratory function nor a relationship between ozone induced inhibition of AChE and bronchoconstriction can be confirmed by our study.

REFERENCES

1. *Alpert, S. M., Gardner, D. E., Hurst, D. J., Lewis, T. R., Coffin, D. L.* Effect of exposure to ozone on defensive mechanism of the lung. J. Appl. Physiol., **31** (1971) 247–252.

2. *Bates, D. V., Bell, G. M., Burnham, C. D., Hazucha, M., Mantha, J., Pengelly, L. D., Silverman, F.* Short-term effects of ozone on the lung. J. Appl. Physiol., **32** (1972) 176–181.

3. *Cross, C. E., De Lucia, A. J., Reddy, A. K., Hussain, M. Z., Chow, C. K., Mustafa, M. G.* Ozone interactions with lung tissue. Am. J. Med., **60** (1976) 929–935.

4. *De La Huerga, J., Petrus, E. A., Sherrick, L. C.* Detection of cholinesterase inhibiton. In: Sunderman F. W., Sunderman F. W., jr. eds. Laboratory diagnosis of diseases caused by toxic agents, Hilger, London. 1970, p. 171.

5. *Diggle, W. M., Cage, J. C.* The toxicity of ozone in the presence of oxides of nitrogen. Br. J. Ind. Med., **12** (1955) 60–64.

6. *Freeman, G., Stephens, R. J., Coffin, D. L., Stara, J. F.* Changes in dogs' lungs after long-term exposure to ozone. Arch. Environ. Health, **26** (1973) 209–216.

7. *Goldstein, E., Warshauer, D., Lippert, W., Tarkington, B.* Ozone and nitrogen dioxide exposure. Arch. Environ. Health, **28** (1974) 85–90.

8. *Hackney, J. D., Linn, W. S., Law, D. C., Karuza, S. K., Greenberg, H. G., Buckley, R. D., Pedersen, E. E.* Experimental studies on human health effects of air pollutants, 2-hour exposure to pollutant gases. Arch. Environ. Health, **30** (1975) 385–390.

9. *Hackney, J. D., Linn, W. S., Karuza, S. K., Buckley, R. D., Law, D. C., Bates, D. V., Hazucha, M., Pengelly, L. D., Silverman, F.* Effects of ozone exposure in Canadians and Southern Californians. Evidence for adaptation? Arch. Environ. Health, **32** (1977) 110–115.

10. *Hazucha, M., Silverman, F., Parent, C., Field, S., Bates, D. V.* Pulmonary function in man after short-term exposure to ozone. Arch. Environ. Health, **27** (1973) 183–188.

11. *Katz, M.* Inorganic gaseous pollutants. In: Stern A. C. ed. Air pollution, Washington, 1968, Vol. 2, p. 86.

12. *Kagawa, J., Toyama, T.* Effects of ozone and brief exercise on specific airway conductance in man. Arch. Environ. Health, **30** (1975) 36–39.

13. *Linn, W. S., Buckley, R. D., Spier, C. E., Blessey, R. L., Jones, M. P., Fisher, D. A., Hackney, J. D.* Health effects of ozone exposure in asthmatics. Am. Rev. Respir. Dis., **117** (1978) 835–843.

14. *Lotti, M., Mazzotta, M.* L'alfa l'-antitripsina, la STIC (serum trypsin inhibitory capacity) e la TPE-activity (trypsin-protein esterase activity) nel siero di un gruppo di saldatori. Med. Lavoro, **64** (1973) 437–443.

15. *P'an, A. Y. S., Jegier, Z.* The effect of sulphur dioxide and ozone on acetylcholinesterase. Arch. Environ. Health, **21** (1970) 499–501.

16. *Penha, P. D., Wertharuer, S.* Pulmonary lesion induced by long-term exposure to ozone. Arch. Environ. Health, **29** (1974) 282–289.

17. *Selikoff, I. J., Hammond, E. C., Churg, J.* Asbestos exposure, smoking, and neoplasia. J. Am. Med. Assoc., **204** (1968) 106–112.

18. *Slinis-Cremer, G. K., Walters, L. G., Sichel, H. S.* Chronic bronchitis in miners and non miners, An epidemiological survey of a community in the gold-mining area in the Transvaal. Br. J. Ind. Med., **24** (1967) 1–12.

19. *Stokinger, H. E., Wagner, W. D., Doborogorski, O. J.* Ozone toxicity studies: Part III, chronic injury to lungs of animals, following exposure at a low level. Arch. Ind. Health, **16** (1957) 514–522.

20. *Young, W. A., Shaw, D. B., Bates, D. V.* Pulmonary function in welders exposed to ozone. Arch. Environ. Health, **7** (1963) 337–340.