Ann. Ist. Super. Sanità, vol. 30, n. 2 (1994), pp. 151-156

Acute and chronic sulphur dioxide (SO_2) exposure: an overview of its effects on humans and laboratory animals

Simona PETRUZZI, Barbara MUSI (*) and Giorgio BIGNAMI

Laboratorio di Fisiopatologia di Organo e di Sistema, Istituto Superiore di Sanità, Rome, Italy

Summary. - Sulphur dioxide (SO_2) is a common air pollutant found both in indoor and outdoor environments. Studies of controlled human exposure as well as epidemiological and animal investigations have documented several short- and long-term effects of SO_2 exposure on the respiratory and other systems. Exercise, duration and other exposure factors may potentiate the pollutant's effects, especially in sensitive individuals such as children and asthmatics. Early postnatal somatic and behavioural alterations have been shown after maternal SO_2 exposure, during pregnancy and neonatal exposure. Such exposure should be considered as a complex toxic hazard which may interfere with the developmental processes in the offspring.

Key words: sulphur dioxide toxicity, pregnancy, neonatal and postnatal development, humans, rodents.

Riassunto *(Esposizione acuta e cronica a diossido di zolfo: una rassegna degli effetti sull'uomo e su alcune specie di animali)* - Il diossido di zolfo (SO_2) è uno degli agenti inquinanti più diffusi sia nell'ambiente esterno sia nei luoghi chiusi. Da studi condotti tramite esposizione controllata di soggetti umani e animali, e da indagini epidemiologiche è emerso che molti fattori, quali la durata del periodo di esposizione e l'esercizio fisico, possono potenziare gli effetti nocivi di tale sostanza, specialmente in soggetti sensibili quali i bambini e gli asmatici. Nella presente rassegna sono inoltre discusse le alterazioni somatiche e comportamentali che si verificano nelle prime fasi postnatali a seguito di esposizione pre o postnatale a SO_2. Tale esposizione appare suscettibile di interferire con i normali processi di sviluppo della prole.

Parole chiave: tossicità da diossido di zolfo, gravidanza, sviluppo neonatale e postnatale, uomo, roditori.

(*) *Present address:* Gambo Lundia AB, Regulatory Affairs & Preclinical Research Emdalavagen, Lund, Sweden

Introduction

Sulphur dioxide (SO_2) is a non inflammable, non explosive, irritant colourless gas. It is a product of the burning of fossil fuels, industrial processes, and motor vehicle operation. SO_2 is a common air pollutant in areas surrounding coal-fired power plants, smelters, sulphur acid factories and other industries as well as in densely populated areas [1]. Most people can smell SO_2 at concentrations from 0.3 to 1 ppm in air; at concentrations above 3 ppm, the gas has a pungent, irritating odour.

In the USA, 2 ppm of SO_2 is the industrial maximal average value permitted for an 8-h day in a 40-h working week [2]; this limit also applies to the greatest majority of developed countries, including those of the European Union. As concerns the external environment, the 1-h maximal value of SO_2 concentration recommended by WHO in Europe for the protection of public health is 350 $\mu g/m^3$ (0.03 ppm), with further reductions as a function of the concentration of suspended particulates [3]. Italian regulations indicate an "attention level" of 125 $\mu g/m^3$ (0.05 ppm) and an "alarm level" twice as high; both are defined as 24-h averages of the averages of within-hour measurements and subject to the condition that the concentrations of suspended particulates also be increased above specific thresholds (90 and 180 $\mu g/m^3$, respectively.)

As concerns the trends over time of SO_2 emissions, the values have shown a decrease of only 4% between 1985 and 1989 in the USA where it is estimated that about 0.9 million people are exposed to SO_2 annually [4]. At the present time, information derived from mineralogical research on regional sulphate deposition in successive years in the UK, is used for making previsions on the effects of the measures taken to reduce SO_2 emissions by 73% from their 1980 levels, in line with the United Nations Economic Commission for Europe's sulphur protocol negotiations [5].

Efforts to define the nature of the physiological impact of SO_2 in humans have relied upon model studies in which animals are exposed to SO_2 under controlled conditions. SO_2 effects on the respiratory system have been described in several studies [6-13]. SO_2 is highly soluble in water, and during ordinary nasal breathing most of the inhaled SO_2 is absorbed in the upper airways [14]. The sulphur from inhaled SO_2 is known to enter the blood within minutes from the onset of exposure [15]. The rate of transfer of the bulk of the sulphur from the site

of absorption to the circulation is sufficient to sustain whole blood levels for many hours after the exposure is terminated [16]. The greatest part of inhaled sulphur is contained in the plasma and is associated with α-globulins. Most of the urinary sulphur is in the form of inorganic sulphate [16]. Apparently, no data are available concerning transplacentar transfer or passage into the milk of sulfur from inhaled SO_2.

Clinical studies of the acute effects of SO_2 have shown that reversible changes in lung function occur at levels of 1.0 ppm or higher [17]. However, other studies suggest that significant changes in airflow resistance may occur, already at a concentration of 0.25 ppm or lower, in sensitive subjects such as asthmatics, during mild to moderate activity [18, 19]. Prolonged exposure to SO_2 is one factor that might contribute to airway inflammation and bronchial hyperreactivity, thereby predisposing to episodes of asthma in children [20].

Sulphure dioxide can be oxidized to sulphate particles via a variety of reactions, some of which can be catalyzed by transition metals such as iron and manganese. Thus, SO_2 inhalation often occurs in the presence of sulphate particles. The water solubility of SO_2 accounts for the uptake of the gas in the upper airways while the adsorption of SO_2 to aerosols leads to enhanced transport of the gas to the deep lung [21].

Sulphite (SO_3-) and bisulphite anions (HSO_3-) are produced by hydration of SO_2. Sulphite/bisulphite salts are used extensively as preservatives in foods, beverages and pharmaceuticals; in Europe, the acceptable daily intake of sulphite over a lifetime is 0.7 mg/kg body weight. Gunnison [22] reported that daily intake of sulphite due to inhalation of atmospheric SO_2 at 0.14 ppm, is approximately 25 times less than that due to drinking 250 ml of wine containing 5 mM sulphite.

The data from experiments designed to evaluate the mammalian toxicity of ingested sulphites indicate that apart from the indirect toxicity resulting from a deficiency of dietary thiamine [22-24], and the direct irritant effect on the gastro-intestinal tract at relatively high intake levels (13 mmol/kg) [25], no serious adverse effects are produced by chronic exposure [22]. This suggests that the direct effects of SO_2 on the pulmonary system be of much greater significance than the absolute amount of SO_2 absorbed.

Epidemiological studies suggest that at relatively high ambient concentrations, SO_2 exposure may be associated with increased morbidity [26-29], and mortality [28] particularly in subjects with cardiopulmonary diseases.

Experimental studies of SO_2 effects on human subjects

Several studies have shown that SO_2 and sodium sulphite (Na_2SO_3), a known metabolite in blood after respiratory exposure to SO_2, can inhibit the growth of established cell lines [30], depress DNA synthesis and produce chromosomal abnormalities in human lymphocytes [31-33]. Recently, it has also been reported [34] that the workers of a sulphate factory exposed to low concentrations of SO_2 showed a significantly increased frequency of chromosomal aberrations. Altogether these results point to a clastogenic and genotoxic risk of SO_2 exposure.

Healthy human subjects exposed to 0.75 ppm SO_2 for two hours showed minor changes in various pulmonary function parameters. Such changes appeared to be reversible and do not suggest a significant health hazard to normal individuals exposed to SO_2 under these conditions [14]. Negative findings in healthy subjects have also been reported by other studies at low concentrations (0.4 ppm SO_2) [35]. On the other hand, more prolonged exposure (up to 6 hours) to a 1 ppm SO_2 concentration, produced a progressive decrease in the forced respiratory flow [36]. Exposure to higher concentrations of SO_2 (5 ppm for 2 hours), increased the bronchial clearance in healthy, non-smoking adults during intermittent exercise.

Other studies aimed at characterizing the acute effects of SO_2 have indicated that significant changes in airflow resistance may occur at exposure levels of 0.25 ppm or lower in sensitive individuals such as asthmatics when engaged in mild to moderate physical activity [18, 19]. The airway response to SO_2 in asthmatics is dose-related and besides the level of exercise, also the route of inhalation (nose versus mouth), and possibly individual reactivity to airway exposure are important determinants of the severity of this effect [17].

Effects of SO_2 on laboratory animals

Effects on pulmonary functions

The effects of acute, subacute and chronic SO_2 exposure in adult animals are well documented and concern primarily pulmonary irritation [37-39]. Four-hours exposure to a pollutant combination consisting of 5 ppm SO_2 and 1.5 g/m^3 of sulphate aerosol did not produce any significant alteration in the clearance rate of the inhaled radioactive tracer particles from the lungs of adult rats [21]. After more extended exposure (1 ppm, 7 hours/day, 5 days/week for 5 weeks), lung clearance was markedly depressed in adult rats, while no change was observed in rats exposed at the same concentration for 2-4 weeks [40]. On the other hand, Wolf *et al.* [41] reported minimal effects of SO_2 exposure in rats (5 ppm for 4 weeks). In this study, no histological lesions were found in the lung or the nose after the termination of exposure. Therefore, the authors suggest an adaptation to the irritant effects of SO_2 during the 4-weeks exposure period.

As concerns haematological effects, adult rats exposed to 0.87 ppm SO_2 for 24 hours, showed higher haematocrit levels than controls [42].

A study using guinea-pigs failed to show changes in either pulmonary function, haematological parameters, body weight or survival after exposure to 0.13, 1.01, or 5.72 ppm SO_2 for 12 months [7].

In a study using adult mongrel dogs, subjects exposed to 50 ppm SO_2 for 5 to 11 months developed mucous hyper-secretion and airway obstruction, whereas those exposed to 15 ppm SO_2 showed minimal changes compared with control animals [43] (for higher concentrations see next section).

Pathology

Histopathologic studies of animals exposed to SO_2 have failed to show any tissue injury except at concentrations far in excess of those normally encountered in urban or industrial atmosphere.

Mice exposed to 10 ppm of SO_2 for periods up to 72 hours showed lesions in the nasomaxillary turbinates after 24 hours of exposure consisting of edema, necrosis and desquamation of the respiratory and olfactory epithelium [44].

Adult rats exposed to a very high SO_2 concentration (800 ppm for 8 hours) showed a gradient of decreasing damage in peripheral direction in the tracheobronchial tree. The trachea epithelium showed the most severe lesions represented by presence of groups of detached cells and necrotic cells, with disappearance of cilia and goblet cells. The entire apical surface was marked by short protrusions [12].

Chronic exposure of dogs at a high SO_2 concentration (200 ppm) produced a histological picture of chronic bronchitis which accounted for the observed alterations in pulmonary functions [45, 46].

In a study using long-term continuous exposure (24 hours a day, seven days a week for 78 weeks), cynomolgus monkeys *(M. irsutus)* did not show any effect at concentrations ranging from 0.14 to 1.28 ppm. By contrast, an overexposure to 4.69 ppm caused, after the initial 30 weeks, several histopathological alterations such as accumulation of moderate amounts of proteinaceus materials, presence of macrophages and multinucleated giant cells in the alveoli, and hyperplasia of pneumocytes and bronchiolar epithelium [47].

The mechanisms by which SO_2 produces its noxious effects include reactions with various biomolecules such as enzymes, coenzymes and nucleic acids (RNA DNA and associated proteins). Specifically, sulphite inhibits the *in vitro* activity of several enzymes with either NAD, FAD or FMN cofactors by adding to the active site of the cofactor [48, 49]; moreover, it affects the function of other enzymes that are involved in glucose metabolism (α-glucan phosphorilase, 2,3-diphosphogliceric acid phosphatase) by competitive inhibition with respect to the natural enzyme substrates [50]. Finally, the alterations of DNA and RNA can be produced by free radicals reactions [51].

Prenatal and postnatal effects of SO_2 exposure

In contrast with the extensive literature concerning the toxicity of SO_2 in adult subjects, only few studies have been aimed at assessing the effects of SO_2 exposure during pregnancy and the neonatal period on the somatic and neurobehavioural development of the offspring. As concerns the effects of high SO_2 concentrations, Singh reported that prenatal exposure of mice (65 or 125 ppm from pregnancy day 7 to 18), significantly decreased mean fetal weight, while the incidence of general and spinal haematomas in live foetuses was increased from 32 ppm upwards [52]. On the other side, the pups exposed to the highest concentration (250 ppm) were significantly heavier than control animals. SO_2 exposure did not produce any apparent signs of maternal toxicity during the gestation period nor significant differences in the number of dead or resorbed foetuses; few indications, however, are given concerning the procedures used to assess the effects on the mothers. Such an apparent lack of maternal toxicity is difficult to interprete on the basis of the results obtained in adult rats which have been reviewed in a previous section. In fact, the concentrations in Singh's study were very high, but the duration of exposure was brief. In any event, it cannot be excluded that pregnancy give at least partial protection from SO_2 toxicity via hormonal or other mechanisms.

In a more recent study performed in the same laboratory [53], mice were exposed in the same period of pregnancy to either 0, 32, or 65 ppm. At both concentrations, the pups showed signs of impaired neuromuscular coordination; namely, a delayed righting reflex on postnatal day 1 (PND) and an increase of the negative geotaxis response on PND 10. The fact that neonatal body weight was reduced only at the higher concentration suggests that the neurobehavioural impairment is not necessarily the consequence of general somatic effects.

It must be underlined at this point that the results obtained in the study just mentioned cannot discriminate between direct effects of SO_2 exposure on the foetus and (i) indirect effects produced prenatally via changes in maternal functions (e.g. of a nutritional, metabolic, or endocrine kind) and (ii) postnatal maternal effects. Some information on the former point might be obtained by studies aimed at assessing whether SO_2 has depressant effects on food and water intake and body weight gain of pregnant animals such as those produced by another air pollutant (ozone) in pregnant rats and mice [54, 55]. On the other hand, the role of postnatal maternal effects

should be verified by assigning treated litters to foster dams not handled nor treated during pregnancy (see e. g. [56, 57]).

Postnatal SO_2 effects were studied by exposing mouse pups, along with their mothers, to either 1, 5, or 15 ppm SO_2 starting 3 days after birth. After two weeks, the young mice were weaned and further exposed for six weeks to the same SO_2 concentrations. Both in the mothers and the offspring SO_2 exposure at all concentrations reduced body weight and the average weight of various organs such as liver, kidneys, heart, brain, and spleen. Moreover, several haematological parameters were affected in the offspring, mostly at the highest concentrations [58]. In particular, 15 ppm SO_2 significantly increased the red blood cell count and the haematocrit value, whereas the white blood cell count was reduced at all concentrations. The mean red cell volume (MCV) was increased but this effect was larger at 1 ppm than at 5 ppm. The average red cell haemoglobin contents was also increased at 5 an 15 ppm, and the average red cell haemoglobin concentration decreased at 1 ppm and increased at 5 ppm.

Overall, the available data leave in doubt whether the sensitivity to SO_2 in the prenatal developmental phase is similar to, or higher than, that of adult animals. On the other hand, the postnatal exposure study points to a high sensitivity, resulting in impaired growth after relatively low SO_2 concentrations. This might be due to one or the other of several mechanisms, including an interference with dam-pup interactions that rely heavily on olfactory communication [59, 60]. In fact, the olfactory cues provided by the mouse pups are involved both in the onset of maternal behaviour at parturition [61] and in the control of important items of maternal care such as retrieving and licking [62]. Therefore, it could be expected that any treatment affecting olfactory functions be able to interfere with maternal care, resulting in an impairment of offspring development.

Finally, these data point to the need of longitudinal studies on children exposed to polluted air containing SO_2. For the moment, prenatal maternal and postnatal SO_2 exposure should be considered as a toxic hazard which may interfere with the normal developmental processes occurring to the offspring.

Acknowledgements

This study has been supported by the CNR-ENEL Project "Interaction of energy systems with human health and environment", Rome, Italy.

Received on 23 February 1994.
Accepted on 23 May 1994.

REFERENCES

1. STERN, A.C. 1976. The primary air pollutants. In: *Air pollution, air pollutants, their transformation and transport*. A.C. Stern (Ed.). Academic Press, New York. pp. 45-53.

2. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS. 1993/1994. *Threshold limit values for chemical substances and physical agents and biological exposure indices*. ACGIH, Cincinnati, Ohio.

3. WORLD HEALTH ORGANIZATION. 1987. *Air quality guidelines for Europe*. WHO, Regional Publications, Copenhagen (European Series, 23). pp. 338-359.

4. US ENVIRONMENTAL PROTECTION AGENCY. 1988. *National air quality and emissions trends report*. Research Triangle Park, NC (US EPA 450/4-88-001).

5. WALKER, G. 1994. Maps show gains of SO_2 emissions. *Nature* **368**: 175.

6. ALARIE, Y.C., KRUMM, A.A., BUSEY, W.M., URICH, C.E. & KANTZ, R.J. 1975. Long-term exposure to sulfur dioxide, sulfuric acid mist, fly ash, and their mixtures. Results of studies in monkeys and guinea pigs. *Arch. Environ. Health* **30**: 254-262.

7. ANTWEILER, H. & BROCKHAUS, A. 1976. Respiratory frequency, flowrate and minute volume in non-anaesthetised guinea pigs during prolonged exposure to low concentrations of SO_2 and NO_2. *Ann. Occup. Hyg.* **19**: 13-16.

8. WITEK, T.J., SCHACHTER, E.N., BECK, G.J., CAIN, W.S., COLICE, G. & LEADERER, B.P. 1985. Respiratory symptoms associated with sulfur dioxide exposure. *Int. Arch. Occup. Environ. Health* **55**: 179-183.

9. STJERNBERG, N., EKLUND, A., NYSTRÖM, L., ROSENHALL, L., EMMELIN, A. & STRÖMQVIST, L.H. 1985. Prevalence of bronchial asthma and chronic bronchitis in a community in northern Sweden; relation to environmental and occupational exposure to sulfur dioxide. *Eur. J. Respir. Dis.* **6**: 41-49.

10. SANDSTRÖM, T., KOLMODIN-HEDMAN, B., STJERNBERG, N., ANDERSSON, M.C. & LÖFVENIUS, G. 1988. Challenge test for sulfur dioxide: symptom and lung function measurements. *Scand. J. Work Environ. Health* **14**: 77-79.

11. SKORNIK, W.A. & BRAIN, J.D. 1990. Effect of sulfur dioxide on pulmonary macrophage endocytosis at rest and during exercise. *Am. Rev. Respir. Dis.* **142**: 655-659.

12. STRATMANN, U., LEHMANN, R.R., STEINBACH, T. & WESSLING, G. 1991. Effect of sulfur dioxide inhalation on the respiratory tract of the rat. *Zbl. Hyg.* **192**: 324-335.

13. ABBEY, D.E., PETERSEN, F., MILLS, P.K. & BEESON, W.L. 1993. Long-term ambient concentrations of total suspended particulates, ozone, and sulfur dioxide and respiratory symptoms in a nonsmoking population. *Arch. Environ. Health* **48**: 33-46.

14. STACY, R.W., HOUSE, D., FRIEDMAN, M., HAZUCHA, M., GREEN, J., RAGGIO, L. & ROGER L.J. 1981. Effects of 0.75 ppm sulfur dioxide on pulmonary parameters of normal human subjects. *Arch. Environ. Health* **36**: 172-177.

15. BALCHUM, O. J., DYBICHI, J. & MENEELY, G.R. 1960. Pulmonary resistance and compliance with concurrent radioactive sulfur distribution in dogs breathing $^{35}SO_2$. *J. Appl. Physiol.* **15**: 62-66.

16. YOKOYAMA, E., YODER, R. E. & FRANK, N.R. 1971. Distribution of ^{35}S in the blood and its excretion in urine of dogs exposed to $^{35}SO_2$. *Arch. Environ. Health* **22**: 390-395.

17. SCHACHTER, E.N., WITEK, T.J., BECK, G.J., HOSEIN, H.R., COLICE, G., LEADERER, B.P. & CAIN, W. 1984. Airway effects of low concentrations of sulfur dioxide: dose-response characteristics. *Arch. Environ. Health* **39**: 34-42.

18. SHEPPARD, D., SAISHO, A., NADEL, J.A. & BOUSHEY, H.A. 1981. Exercise increases sulfur dioxide-induced bronchoconstriction in asthmatic subjects. *Am. Rev. Resp. Dis.* **123**: 486-491.

19. KIRKPATRICK, M.B., SHEPPARD, D., NADEL, J.A. & BOUSHEY, H.A. 1982. Effect of oronasal breathing route on the bronchoconstrictive response to sulfur dioxide in exercising asthmatic subjects. *Am. Rev. Respir. Dis.* **125**: 627-631.

20. TSENG, R.Y. & LI, C.K. 1990. Low level atmospheric sulfur dioxide and childhood asthma. *Ann. Allergy* **3**: 378-383.

21. MANNIX, R.C., PHALEN, R.F., KENOYER, J.L. & CROCKER, T.T. 1982. Effect of sulfure dioxide-sulfate exposure on rat respiratory tract clearance. *Am. Ind. Hyg. Assoc. J.* **43**: 679-685.

22. GUNNISON, A.F. 1981. Sulphite toxicity: a critical review of *in vitro* and *in vivo* data. *Food Cosmet. Toxicol.* **19**: 667-682.

23. FITZHUGH, O. G., KNUDSEN, L. F. & NELSON, A.A. 1946. The chronic toxicity of sulfites. *J. Pharmacol. Exp. Ther.* **86**: 37.

24. BHAGAT, B. & LOCKETT, M. F. 1964. The effect of sulphite in solid diets on the growth of rats. *Food Cosmet. Toxicol.* **2**:1.

25. TIL, H.P., FERON, V.J. & DE GROOT, A.P. 1972. the toxicity of sulphite. I. Long-term feeding and multigeneration studies in rats. *Food Cosmet. Toxicol.* **10**: 463.

26. CHIAROMONTE, L.T., BONGIORNO, J.R., BROWN, R. & LAANO, M.E. 1979. Air pollution and obstructive respiratory disease in children. *N. Y. State J. Med.* **70**: 394-398.

27. LAWATHER, P.J., WALLER, R.E. & HENDERSON, M. 1970. Air pollution and exacerbation of bronchitis. *Thorax* **24**: 525-539.

28. GLASSER, M., GREENBERG, L. & FIELD, F. 1967. Mortality and morbidity during a period of high levels of air pollution. *Arch. Environ. Health* **15**: 684-694.

29. ZEIDBERG, L.D., PRINDLE, R.A. & LANDAU, E. 1961. The Nashville Air Pollution Study. I. Sulfur dioxide and bronchial asthma. A preliminary report. *Am. Rev. Resp. Dis.* **84**: 489-503.

30. THOMPSON, J.R. & PACE, D.M. 1962. The effect of sulfur dioxide upon established cell lines cultured *in vitro*. *Can. J. Biochem. Physiol.* **40**: 207-217.

31. SCHNEIDER, L.K. & CALKINS, C.A. 1970. Sulfur dioxide induces lymphocites defects in human periferal blood cultures. *Environ. Res.* **3**: 473-483.

32. NORDENSON, I., BECKMAN, G., BECKMAN, L., ROSENHALL, L. & STJERNBERG, N. 1980. Is exposure to sulfur dioxide clastogenic? *Hereditas* **93**: 161-164.

33. BECKMAN, L. & NORDENSON, I. 1986. Interaction between some common genotoxic agents. *Hum. Hered.* **36**: 397-401.

34. MENG, Z. & ZHANG, L. 1990. Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of workers exposed to sulfur dioxide. *Mutat. Res.* **241**: 15-20.

35. KREISMAN, H., MITCHELL, C.A., HOSEIN, H.R. & BOUHUYUS, A. 1976. Effect of low concentrations of sulfur dioxide on respiratory function in man. *Lung* **154**: 25-34.

36. NEWHOUSE, M.T., DOLOVICH, M., OBMINSKI, G. & WOLFF, J. 1978. Effect of TLV levels of SO_2 and H_2SO_4 on bronchial clearance in exercising man. *Arch. Environ. Health* **33**: 24-32.

37. US ENVIRONMENTAL PROTECTION AGENCY. 1969. *Air quality criteria for sulfur oxides*. Research Triangle Park, NC (US EPA No. AP-50).

38. US ENVIRONMENTAL PROTECTION AGENCY. 1973. *Nationwide air pollution trends*. Research Triangle Park, NC (US EPA No. AP-115).

39. US ENVIRONMENTAL PROTECTION AGENCY. 1976. *National air quality and emission trends report*. Research Triangle Park, NC (US EPA No. 45/1-76-002).

40. FERIN, L. & LEACH, L.J. 1973. The effect of SO_2 on lung clearance of TiO_2 particles in rats. *Am. Ind. Hyg. Assoc. J.* **34**: 260-263.

41. WOLFF, R.K., GRIFFITH, W.C., HENDERSON, R.F., FLETCHER, F., HAHN, F.F., HARKEMA, J.R., REBAR, A.H., EDISON, A.F. & McCLELLAN, R.O. 1989. Effects of repeated inhalation exposures to 1-nitropyrene, benzo[α]pyrene, Ga_2O_3 particles, and SO_2 alone and in combinations on particle clearance, bronchioalveolar lavage fluid composition, and histopathology. *J. Toxicol. Environ. Health* **27**: 123-138.

42. BASKURT, O.K. 1988. Acute hematological and hemorheological effects of sulfur dioxide inhalation. *Arch. Environ. Health* **43**: 344-348.

43. SCANLON, P.D., SELTZER, J., INGRAM, R.H. Jr., REID, L. & DRAZEN, J.M. 1987. Chronic exposure to sulfur dioxide. Physiologic and histologic evaluation of dogs exposed to 50 or 15 ppm. *Am. Rev. Respir. Dis.* **135**: 831-839.

44. GIDDENS, W.E. & FAIRCHILD, G.A. 1972. Effects of sulfur dioxide on the nasal mucosa of mice. *Arch. Environ. Health* **25**: 166-173.

45. DRAZEN, J.M., O'CAIN, C.F. & INGRAM, R.H. 1982. Experimental induction of chronic bronchitis in dogs, effects on airway obstruction and responsiveness. *Am. Rev. Resp. Dis.* **126**: 75-79.

46. SELTZER, J., SCANLON, P.D., DRAZEN, J.M., INGRAM, R.H. & REID, L. 1984. Morphological correlation of physiologic changes due to SO_2-induced bronchitis in dogs: the role of inflammation. *Am. Rev. Resp. Dis.* **129**: 790-797.

47. ALARIE, Y., ULRICH, C. E., BUSEY, M.W., KRUMM, A.A. & MACFARLAND, N. 1972. Long-term continuous exposure to sulfur dioxide in cynomolgus monkeys. *Arch. Environ. Health* **24**: 115-128.

48. MULLER, F. & MASSEY, V. 1969. Flavin-sulfite complexes and their structures. *J. Biol. Chem.* **244**: 4007-4010.

49. SHIH, N.T. & PETERING, D.H. 1973. Model reactions for the study of the interaction of sulfur dioxide with mammalian organisms. *Biochem. Biophys. Res. Commun.* **55**: 1319-1323.

50. KAMOGAWA, A. & FUKUI, T. 1973. Inhibition of α–glucan phosphorilase by bisulfite competition at the phosphate binding site. *Biochem. Biophys. Acta* **302**: 158-162.

51. INOUE, M. HAYATSU, H. & TANOOKA, H. 1972. Concentration effect of bisulfite on the inactivation of transforming activity of DNA. *Chemico-Biol. Interactions* **5**: 85-91.

52. SINGH, J. 1982. Teratological evaluation of sulfure dioxide. *Proc. Inst. Environ. Sci.* **28**: 144-145.

53. SINGH, J. 1989. Neonatal development altered by maternal sulfur dioxide exposure. *Neurotoxicology* **10**: 523-528.

54. KAVLOCK, R.J., MEYER, E. & GRABOWSKY, C.T. 1980. Studies on the developmental toxicity of ozone: II. Postnatal effects. *Toxicol. Lett.* **5**: 3-9.

55. BIGNAMI, G., MUSI, B., DELL'OMO, G. & ALLEVA, E. Limited effects of ozone exposure during pregnancy on physical and neurobehavioural development of cd-1 mice (submitted to *Toxicology and Applied Pharmacology*)

56. CHIAROTTI, F., ALLEVA, E. & BIGNAMI, G. 1987. Problems of test choice and data analysis in behavioral teratology: the case of prenatal benzodiazepines. *Neurotoxicol. Teratol.* **9**: 179-186.

57. BIGNAMI, G., ALLEVA, E., CHIAROTTI, F. & LAVIOLA, G. 1992. Selective changes in mouse behavioral development after prenatal benzodiazepine exposure: a progress report. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **16**: 587-604.

58. SINGH, J. 1976. Effects of sulfur dioxide exposure on the post-natal development and hematological studies in mice. *Proc. Inst. Environ. Sci.* **22**: 14-18.

59. GANDELMAN, R., ZARROW, M.X., DENENBERG V.H. & MYERS, M. 1971. Olfactory bulb removal eliminates maternal behavior in the mouse. *Science* **171**: 210-211

60. VANDERBERGH, J. G. 1973. Effects of central and periferal anosmia on reproduction of female mice. *Physiol. Behav.* **10**: 257-261.

61. LONDEI, T., SEGALA, P. & LEONE V.G. 1989. Mouse pup urine as an infant signal. *Physiol. Behav.* **45**: 579-583.

62. CALAMANDREI, G., WILKINSON, L.S. & KEVERNE, E.B. 1992. Olfactory recognition of infants by laboratory mice: Role of noradrenergic mechanisms. *Physiol. Behav.* **52**: 901-907.