

Possible Mutagenic Properties and Carcinogenic Action of the Irritant Gaseous Pollutants NO₂, O₃, and SO₂

by Giselher von Nieding*

Carcinogenic or cocarcinogenic effects of NO₂, O₃, and SO₂ have not been proven to date with sufficient reliability. However, nitrosamine formation after exposure to NO₂- or O₃- induced decrease in benzpyrene hydroxylase are potential hazards. A final reevaluation of a possible cocarcinogenic action of SO₂ requires further experimental studies.

Epidemiological studies would be of the greatest value in assessing the possibility of mutagenic properties and carcinogenic action of the irritant gaseous pollutants NO₂, O₃, and SO₂. To date, there has been only one such study (1), an investigation of the frequency of lung cancer in Los Angeles, where oxidant levels are high, as compared to the frequency in the San Francisco/San Diego area. However, considering the multiplicity of possible parameters in carcinogenesis and the relatively short period of exposure (1958-1963) to high oxidant levels, no detailed conclusions can be drawn from this study.

Experimental studies on the carcinogenic and mutagenic effects of an agent are naturally restricted to animals. The following is a brief account of what we know today from studies on animals exposed to NO₂, O₃, and SO₂.

Ever since Druckrey and Preussmann (2) postulated the formation of nitrosamines in the lung by reaction of NO and NO₂ with amines, a possible indirect carcinogenic action of oxides of nitrogen has been under discussion. Using *in vitro* experiments, Sander et al. (3) demonstrated that interactions of nitrite and amines lead to the production of nitrosamines in the stomach. However, this reaction requires an acidic medium; nitrosamine formation in a medium of neutral pH, as in the lung, has not been proven (4). It has been found that nitrite is formed in the lungs upon contact with NO_x and that nitrosamines are generated in lung

homogenates (5, 6). This evidence supports the hypothesis of Druckrey and Preussmann. The possibility of a mutagenic effect of NO_x may be suggested in the light of *in vitro* experiments showing that nitrite is a highly potent mutagen (7-10).

Brinkmann, Lamberts, and Veninga (11) first postulated radiomimetic properties of O₃ when comparing the effects of ultraviolet radiation and O₃ on human skin. This assumption has been supported by later investigators, and a review on this topic has been given by Veninga (12).

From the experimental results of Zelac et al. (13, 14) it is evident that O₃ has direct mutagenic properties. After 5 hr exposure of hamsters to 0.2 ppm O₃ (320 µg/m³), the frequency of chromosomal breaks increased in the lymphocytes. A comparison with the effect of 230 rad x-radiation indicated that at these concentrations O₃ was the more strongly acting agent. A combined exposure to O₃ and x-radiation did not show a synergistic effect. *In vitro* experiments on chick embryos (15) and human cell cultures (16) also showed the mutagenic property of O₃; the observed chromosome aberrations were apparently dose-dependent.

Formation of malignant tumors after long-term exposure to NO₂ or O₃ has not been observed. Henschler and Ross (17) and Ross and Henschler (18) exposed mice or hamsters to high concentrations of NO₂ (40 ppm; 76 mg/m³) for up to 16 months and did not see an increase in tumor rate. However, inflammation with cell proliferation in the terminal bronchioli—and thus a higher mitosis rate probably leading to a higher susceptibility to other carcinogens—was present in all animals.

*Bethanien Hospital, D-4130 Moers 1, West Germany.

Stupfel et al. (19) did not find an increased tumor rate in long-term NO_x exposures. On the other hand, when increased NO_x levels were combined with motor vehicle exhausts, an increased tumor rate was observed.

Formation of malignant tumors has not been observed in mice after long-term exposure to O₃ at levels of 2.5 ppm (4900 μg/m³/2 hr daily/5 weeks) (20) or 1 ppm (1960 μg/m³ for 15 months) (21). Exposure to ambient Los Angeles air was also without effect as far as the formation of malignant tumors was concerned (22, 23). An increased incidence of adenoma was found in the O₃ experiments. O₃ induced a decrease in benzpyrene hydroxylase in animals exposed to 0.75–1.0 ppm (1470–1960 μg/m³ for 3 or 1.5 hr) (1, 24–26) and caused delayed benzpyrene elimination (27).

From the experimental results of Laskin et al. (28) a cocarcinogenic action of SO₂ may be suggested when combined with benzo[a]pyrene. Rats were exposed to 10 mg/m³ benzo[a]pyrene and about 10 mg/m³ SO₂ 1 hr/day, 5 days/week. After 98 weeks, two of 21 rats had developed squamous cell carcinoma. In another group exposed to 10 mg/m³ benzo[a]pyrene and 10 mg/m³ SO₂ for 1 hr/day with an additional exposure to 30 mg/m³ SO₂ 6 hr/day, 5 days/week, five out of 21 rats had developed squamous cell carcinoma after 98 weeks. However, from the comparison with a control group of only three animals exposed to pure air or sulfur dioxide only, no firm conclusions can be drawn.

In conclusion, although the carcinogenic or cocarcinogenic effects of NO₂ have not been proven to date, nitrosamine formation is a potential hazard. Furthermore, the effects of exposure to O₃ on the metabolic handling of benzpyrene must be kept in mind when discussing this pollutant's possible carcinogenicity. Concerning SO₂, there is only little evidence regarding a cocarcinogenic action; before a final evaluation can be made more experiments are necessary.

REFERENCES

- Buell, P., Dunn, I. E., and Breslow, L. Cancer of the lung and Los Angeles type air pollution: prospective study. *Cancer* 20: 2139 (1967).
- Druckrey, H., and Preussmann, R. Zur Entstehung carcinogener Nitrosamine am Beispiel des Tabakrauches. *Naturwiss.* 49: 498 (1962).
- Sander, J., et al. Untersuchungen *in vitro* über die Möglichkeit einer Bildung cancerogener Nitrosamine im Magen. *Arzneimittelforsch.* 21: 411 (1971).
- Wagner, H. M. A preparatory study for establishing criteria (dose/effect relationships) for nitrogen oxides. Commission of the European Communities, Report No. EUR 5436 e, 1976.
- Kaut, V., Švorcová, S., and Tušl, M. Determination of nitrite-ions in the lungs and in the blood of animals after inhalation of low concentrations of nitrogen oxides (in Czech). *Coll. Sci. Works Fac. Med. Charles Univ., Hradec Kralové (Suppl)* 13: 213 (1970).
- Kaut, V. Zur Frage der Entstehung von Nitrosaminen beim Einatmen. *Cs. Hyg.* 15: 213 (1970). (In Czech).
- Watson, J. D. *Molecular Biology of the Gene*. W. A. Benjamin, London–Amsterdam, 1976, pp. 255, 363.
- Zimmermann, F. K., and Schwaier, R. Induction of mitotic gene conversion with nitrous acid, 1-methyl-3-nitro-1-nitrosoguanidine and other alkylating agents in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 100: 63 (1967).
- Harper International Editions, New York, London, 1971, p. 862.
- Drake, J. W. *The Molecular Basis of Mutation*. Holden-Day Ed., San Francisco–London–Amsterdam, 1970.
- Brinkmann, R., Lamberts, H. B., and Veninga, T. S. Radiomimetic toxicity of ozonized air. *Lancet* 1: 133 (1964).
- Veninga, T. Toxicity of ozone in comparison with ionizing radiation. *Strahlenther.* 134: 469 (1967).
- Zelac, R. E., et al. Inhaled ozone as a mutagen. I. Chromosome aberrations induced in Chinese hamsters lymphocytes. *Environ. Res.* 4: 262 (1971).
- Zelac, R. E., et al. Inhaled ozone as a mutagen. II. Effect on the frequency of chromosome aberrations observed in irradiated Chinese hamsters. *Environ. Res.* 4: 325 (1971).
- Sachsenmaier, W., and Tjong-an, Tan. Effects of ozone upon mouse ascites tumor cells and upon chick fibroblasts in tissue culture. *Z. Krebsforsch.* 67: 113 (1965).
- Fetner, R. H. Ozone induced chromosome breakage in human cell cultures. *Nature* 194: 793 (1962).
- Henschler, D., and Ross, V. Zur Frage einer cancerogenen Wirkung inhalierter Stickstoffoxyde. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmacol.* 253: 495 (1966).
- Ross, V., and Henschler, D. Fehlen einer cancerogener Wirkung von nitrosen Gasen beim Goldhamster. *Experientia* 24: 55 (1968).
- Stupfel, M., et al. Lifelong exposure of SPF rats to automotive exhaust gas. *Arch. Environ. Health* 26: 264 (1974).
- Penha, P., et al. Ozone air pollutants and lung damage. *Intern. Med.* 41: 17 (1972).
- Stokinger, H. E. Effects of air pollution on animals. In: *Air Pollution*, A. Stern, Ed. Academic Press, New York, 1st ed., 1962, Vol. 1, Chap. 9; cited from 2nd ed., 1968.
- Gardner, M. B. Biological effects of urban air pollution. *Arch. Environ. Health* 12: 305 (1966).
- Gardner, M. B., et al. Histopathologic findings in rats exposed to ambient and filtered air. *Arch. Environ. Health* 19: 637 (1969).
- Palmer, M., Swanson, D., and Coffin, D. L. Effect of O₃ on benzpyrene hydroxylase activity in the Syrian golden hamster. *Cancer Res.* 31: 730 (1971).
- Palmer, M. S., and Exley, H. W., and Coffin, D. L. Influence of pollutant gases on benzpyrene hydroxylase activity. *Arch. Environ. Health* 25: 439 (1972).
- Goldstein, B. D., et al. Decrease in rabbit lung microsomal cytochrome P-450 levels following ozone exposure. *Res. Commun. Chem. Pathol. Pharmacol.* 10: 759 (1975).
- Tomingas, V. R., Pott, F., and Dehnen, W. Biological test for carcinogenicity of polycyclic aromatic hydrocarbons. *Arch. Geschwulstforsch.* 42: 298 (1973).
- Laskin, S., Kuschner, M., and Drew, R. T. Studies in pulmonary carcinogenesis. In: *Inhalation Carcinogenesis*, AEC Symposium Series, No. 18, M. G. Hanna, Jr., P. Nettesheim, and J. R. Gilbert, Eds., U. S. Atomic Energy Commission Division of Technical Information, Oak Ridge, Tenn. 1970, pp. 321–350.