

7. Genotoxicity

by Katarina Victorin

As discussed in the chapter on metabolism, the nitrogen oxides NO and NO₂ (NO_x) form nitrous acid (HNO₂) and nitric acid (HNO₃) in aqueous solutions, which are in equilibrium with the nitrite (NO₂⁻) and nitrate (NO₃⁻) ion (1, 2).

Undissociated HNO₂ reacts with amino groups in DNA (deoxyribonucleic acid), and this reaction is one mechanism for direct mutagenic action. Through the nitrosation of primary amines, alkylating agents are formed. In reactions with secondary amines and amides, more stable N-nitroso compounds are formed, such as N-alkyl-nitrosamines, and this is one indirect path of mutagenic activity (2).

Thus, when the genotoxicity of NO_x is discussed, one has to consider the effects of other classes of compounds as well, such as HNO₃/NO₃⁻, HNO₂/NO₂⁻ and nitrosamines. Moreover, mutagenic reaction products may be formed in the atmosphere from NO_x and hydrocarbons.

Nitrogen oxides

In vitro studies

All of the *in vitro* studies presented in this discussion are summarized in tables 7.1 (NO₂) and 7.2 (NO).

NO₂ has been shown to be mutagenic to *Salmonella* TA 100 and *Bacillus subtilis* spores (3-5). At higher doses NO₂ is cytotoxic. In the study by Isomura et al (3) nitrogen monoxide (NO) also gave a slight mutagenic response.

When nitrogen dioxide (NO₂) was bubbled through a bacteria suspension, DNA damage was demonstrated indirectly (as the induction of so-called SOS DNA repair) in *Salmonella* and *Escherichia coli*, but mutations were only detected in *E coli* (6-8). No effect was seen with NO.

NO₂ induced chromosome aberrations (chromatid-type) and DNA damage (sister chromatid exchanges and DNA single-strand breaks) in cell cultures exposed either without culture medium or placed on roller drums with a small amount of salt solution added (9-11).

NO did not cause any detectable DNA damage in the study by Görsdorf et al (11). In contrast, treatment with NO but not NO₂ induced mutations (8-

AG resistance) in Chinese hamster cells (3).

When injected into medium at high concentrations, NO induced mutations (in HPRT and TK genes) and DNA strand breakage in a TK6 human lymphoblast cell culture (12).

Treatment of *Tradescantia* plants with NO₂ has been reported to induce DNA damage (micronuclei in meiotic pollen mother cells) (13) and somatic mutations in stamen hairs (14).

In vivo studies

All of the *in vivo* studies presented in this discussion are summarized in table 7.3.

NO₂ did not induce recessive lethal mutations or somatic mutations (wing spot test) in *Drosophila* (15, 16), chromosome aberrations in peripheral lymphocytes or spermatocytes in mice (17), or micronuclei in bone marrow cells in mice (16).

Isomura et al (3) exposed male Sprague-Dawley rats to different concentrations of NO₂ or NO for 3 h. A primary cell culture was prepared from the lungs. A significant increase in mutation frequency (oubain-resistance) was observed following NO₂ exposure from 30 mg·m⁻³. The mutagenic effect of NO was much less than that of NO₂; however, a significant increase occurred at the highest dose level. NO₂ at 50 mg·m⁻³ was also shown to induce chromosome aberrations (mainly chromatid-type).

Cancer studies

Henschler & Ross (18) and Ross & Henschler (19) exposed NMRI mice to NO₂ at 40 ppm (72 mg·m⁻³) and hamsters to 40 ppm of NO₂ + 20 ppm of NO, respectively, for up to 16 months. There was no increase in malignant tumors, although cell proliferation, atypical bronchial epithelium, and lung adenomas appeared.

Inhalation of 10 ppm (19 mg·m⁻³), but not 1 or 5 ppm, for 6 h a day, 5 d a week for 6 months gave a small but statistically significant increase in the frequency (tumors per mouse) and incidence (tumors per tumor-bearing mouse lung) of lung adenomas in strain A/J mice, which are susceptible to pulmonary adenoma induction (20). In a small and earlier study with CAF₁/J mice (21), there was an increased incidence of lung tumors after 12, but not after 14 or 16 months of exposure to NO₂ at 5 ppm (10 mg·m⁻³).

A nonsignificant increase in lung adenomas was observed in a small study with Wistar rats exposed

Table 7.1. Genotoxicity of nitrogen dioxide (NO₂) in vitro and in plants. (SCE = sister chromatid exchanges)

Test organism	End point	Exposure	Result ^a	Reference
<i>Salmonella</i> TA100	Mutations	6-10 ppm (11-19 mg·m ⁻³), 40 min	+	3
<i>Salmonella</i> TA100	Mutations	10-15 ppm (19-28 mg·m ⁻³), 6 h	+	4
<i>Salmonella</i> TA100	Mutations	Bubbling of 10-90 ppm (19-170 mg·m ⁻³) through bacteria suspension, 30 min	-	6
<i>Salmonella</i> TA100	SOS repair	Bubbling of 10-90 ppm (19-170 mg·m ⁻³) through bacteria suspension, 30 min	+	6
<i>Escherichia coli</i> , WP2	Mutations	Bubbling of 90 ppm (170 mg·m ⁻³) through bacteria suspension, 30 min	+	7, 8
<i>Escherichia coli</i>	SOS repair	Bubbling of 90 ppm (170 mg·m ⁻³) through bacteria suspension, 30 min	+	7, 8
<i>Bacillus subtilis</i> spores	Mutations	500 ppm (950 mg·m ⁻³), 2-3 h	+	5
V79 hamster cells	Chromatid-type aberrations, SCE	10-100 ppm (19-190 mg·m ⁻³), 10 min	+	9
V79 hamster cells	SCE	1-8 ppm (2-15 mg·m ⁻³), 2 h	+	10
Don hamster cells	Mutations (8-AG resistance)	2-3 ppm (4-6 mg·m ⁻³), 10 min	-	3
V79 hamster	DNA single strand breaks	10 ppm (19 mg·m ⁻³), 20 min	+	11
<i>Tradescantia</i>	Micronuclei in pollen	5 ppm (10 mg·m ⁻³), 24 h	+	13
<i>Tradescantia</i>	Mutations in stamen hair	50 ppm (95 mg·m ⁻³), 6 h	+	14

^a + = positive, - = negative**Table 7.2.** Genotoxicity of nitric oxide (NO) in vitro and in plants.

Test organism	End point	Exposure	Result ^a	Reference
<i>Salmonella</i> TA100	Mutations	25-30 ppm (30-36 mg·m ⁻³), 40 min	+	3
<i>Salmonella</i>	SOS repair	Bubbling of 10-90 ppm (12-110 mg·m ⁻³) through bacteria suspension, 30 min	-	6
Don hamster cells	Mutations (8-AG resistance)	2-3 ppm (2-4 mg·m ⁻³), 10 min	+	3
V79 hamster cells	DNA single strand breaks	500 ppm (600 mg·m ⁻³), 30 min	-	11
TK6 human cells	Mutations, DNA single strand breaks	Injection of 0.12-0.38 ml NO gas per milliliter of culture medium, 1 h	+	12

^a + = positive, - = negative.

to 0.05 ppm of ozone + 0.04 (0.08 mg·m⁻³) or 0.4 ppm (0.8 mg·m⁻³) of NO₂ for 22 months (22). According to the authors, in an earlier study no tumors had been found in the lungs of rats exposed to NO₂ only for 18 or 27 months (0.04, 0.4, or 4 ppm).

In a life-time inhalation study with 2.4 ppm (2.9 mg·m⁻³), no increased incidence of leukemia or lung adenomas was demonstrated for mice (23).

There are some studies on possible co-carcino-

genic effects of NO₂. Long-term studies on mice indicate that the inhalation of NO₂ or ozone + NO₂ can promote the development of lung tumors initiated by bis (2-hydroxypropyl)nitrosamine (24, 25). Richters and his co-workers have shown that mice inhaling NO₂ for up to 12 months and then infused with melanoma cells develop a higher number of lung metastases than control animals (26 and earlier papers).

Table 7.3. Genotoxicity of nitrogen dioxide (NO₂) and nitric oxide (NO) in vivo

Test organism	End point	Exposure	Result ^a	Reference
NO₂				
<i>Drosophila</i>	Recessive lethals	500-700 ppm (950-13 000 mg·m ⁻³), 1 h; 50-280 ppm, 2 d; 50-560 ppm, >12 d	-	15
<i>Drosophila</i>	Somatic mutations (wing spot test)	50 ppm (95 mg·m ⁻³), 19 h	-	16
Rats	Mutations in lung cells (oubain resistance)	15-27 ppm (29-51 mg·m ⁻³), 3 h	+	3
Rats	Chromosome aberrations in lung cells	27 ppm (51 mg·m ⁻³), 3 h	+	13
Mice	Chromosome aberrations in lymphocytes and spermatocytes	0.1-10 ppm (0.2-19 mg·m ⁻³), 6 h	-	17
Mice	Micronuclei in bone marrow	20 ppm (38 mg·m ⁻³), 23 h	-	16
NO				
Rats	Mutations in lung cells (oubain resistance)	27 ppm (32 mg·m ⁻³), 3 h	+	3

^a + = positive, - = negative.

Nitrite and nitrate

In vitro studies

HNO₂ became a classical mutagen already in 1958, when it was found that nitrite at low pH caused point mutations by base-pair substitutions in RNA (ribonucleic acid) from tobacco mosaic virus. A mutagenic action on bacteria (*E coli* and *Diplococcus pneumoniae*) and bacteriophages and fungi (eg, *Neurospora crassa* and *Saccaromyces cerevisiae*) was also demonstrated early. In some plants HNO₂/NO₂ caused genotoxic effects, but not in others. The feeding of *Drosophila* flies produced a very weak induction of recessive lethals, and larval treatment was negative (1).

Sodium nitrite is mutagenic in *Salmonella*, strain TA1535 and TA100, in the Ames test (27-29).

Chromosome aberrations (9, 29-32), sister chromatid exchanges (9), gene mutations (30, 33), and cell transformations (34, 35) have been induced in cell cultures after treatment with high doses of sodium nitrite. A test for DNA single-strand breaks was negative (11, 30).

Sodium and potassium nitrate seem to be devoid of genotoxic properties in vitro (11, 29).

In vivo studies

High doses of sodium nitrite induced micronuclei in mice and chromosome aberrations in the bone marrow of mice, rats and rabbits after intragastric treatment (32), and chromosome aberrations occurred in

rats after administration via drinking water (36).

When embryonic cell cultures were prepared from pregnant Syrian hamsters treated with sodium nitrite by gavage, micronuclei, mutations (8-azaguanine and oubain-resistance), and cell transformation were observed. There was no increase in chromosome aberrations. Similar treatment with sodium nitrate was negative (37). In a study by El Nahas (36), chromosome aberrations were induced by sodium nitrite in the liver of transplacentally exposed rat embryos.

Luca et al (38) reported that sodium nitrate induced micronuclei in mice and chromosome aberrations in bone marrow of rats after intragastric treatments with high doses.

Cancer studies

Several studies have failed to demonstrate an increase in the incidence of tumors in mice and rats after prolonged oral exposure to sodium nitrite (39-43). However, an increased incidence of neoplastic nodules in the liver and papillomas of the forestomach was seen in rats after treatment with high doses (2000 mg·kg⁻¹ in the diet and 3000 mg·l⁻¹ in drinking water, respectively) (44, 45).

In one positive cancer study (46), nitrosamine formation in the diet may have been the principal cause of tumors. Another positive study (47) was later judged not to be significantly positive according to Walker (48).

Sodium nitrate did not induce tumors in rats (43, 49).

Nitrosamines

Nitrosamines are formed from the nitrosation of secondary or tertiary amines. Several nitrosamines have been detected in various foods of humans, particularly foods containing added nitrite as a preservative. About 300 N-nitroso compounds have been tested for carcinogenicity, and most of them have been shown to induce cancer in different animal species (48).

Several *in vivo* studies have demonstrated that nitrosation can occur after the ingestion of nitrite and a wide range of secondary and tertiary amines (and amides) in various species. In some cases nitrosation has also been reported when nitrate was used as a precursor of the nitrosating agent. In human volunteers, nitrosation has also been found to occur in the stomach after meals containing normal dietary components such as vegetables, meat, eggs, and fish. (For a review, see eg, reference 48.)

Several long-term animal studies have shown that the co-administration of nitrite and various amines or amides leads to an increased number of tumors. (For a review, see, eg, reference 48.)

It has also been shown that nitrosated compounds can be formed after the inhalation of NO_2 . When mice were treated with morpholine and then exposed to NO_2 , both *in vivo* nitrosation and subsequent *in vitro* nitrosation during the extraction of tissue samples was demonstrated (formation of nitrosomorpholine) (8, 51-53).

Nitrated polycyclic aromatic hydrocarbons

Reactions between NO_2 and polycyclic aromatic hydrocarbons (PAH) lead to the formation of mutagenic nitroderivatives (54, 55).

Several studies have shown that organic extracts of automobile exhaust, especially diesel exhaust, other combustion emissions, and airborne particulate matter in urban areas contain direct-acting mutagens that can be ascribed to nitroarenes. (For a review, see reference 56.)

Some of the nitroarenes have shown extremely high mutagenic activity in the Ames test, especially dinitropyrenes. They have only been tested to a limited extent in *in vivo* genotoxicity assays. Increased incidences of tumors have been induced in animal studies by some nitroarenes. (For a review of the genotoxicity of nitroarenes, see references 56 and 57.)

Kanoh et al (58, 59) showed that, when mice were injected with the nonmutagenic PAH compound pyrene and exposed to NO_2 by inhalation, mutagenic nitropyrenes were formed *in vivo* and excreted in the urine.

Alkenes and nitrogen dioxide

It has been shown that mutagenic reaction products are formed in photochemical reactions between NO_x and the alkenes propene and, to a higher degree, 1,3-butadiene, as detected with the Ames' *Salmonella* assay (60-63). In experiments with mixtures of chlorinated ethenes and NO_2 irradiated by ultraviolet (UV) light, only vinyl chloride gave rise to significantly mutagenic photoreaction products (64). Photochemical reaction products from propene + NO_x + UV induced sister chromatid exchanges in Chinese hamster V79 cells (10).

UV-irradiated mixtures of butadiene + NO_2 have also been tested *in vivo*. No significant genotoxic effects occurred in *Drosophila* (somatic mutations, wing spot test) or in mice bone-marrow (micronucleus assay) (16).

Summary and concluding remarks

The genotoxicity of nitrogen oxides is difficult to evaluate because both direct and indirect effects have to be taken into account and because relatively few studies have been performed. Taken together, the results from tests with bacteria, cell cultures, and plants indicate that NO_2 has the ability to induce genotoxic effects *in vitro*. NO seems to be less active.

The few *in vivo* studies were negative with the exception of one study by Isomura et al (3), in which both chromosome aberrations and mutations in cultured lung cells were shown after NO_2 inhalation *in vivo*.

According to available studies, there is no clear evidence of a carcinogenic potential of NO_2 , although lung adenomas were induced in the susceptible strain A/J mouse.

Thus the *in vivo* genotoxic and carcinogenic effect of NO_2 inhalation seems to be small and restricted to lung tissue. The few relevant studies performed do not allow for a proper evaluation of this risk.

The primary metabolites of NO_2 are nitrite and nitrate. Nitrate seems to be devoid of genotoxic properties. However, nitrite (and nitrous acid) is genotoxic *in vitro*, and there are also positive *in vivo* results. However, cancer studies have been mainly negative. The contribution of inhaled NO_x to the body burden of nitrite is probably of minor importance when compared with the ingestion of nitrate and nitrite via food and drinking water and the endogenous formation of nitrite. (See chapter 3.)

It has been shown that nitrosamines can be formed *in vivo* after the inhalation of NO_2 . However, the relative importance of inhaled NO_x is probably small

when compared with the ingestion of nitrosamines or nitrite + amines. (See chapter 3.)

Nitrogen oxides take part in atmospheric reactions. Mutagenic nitro-PAH compounds are easily formed, and mutagenic reaction products may also be formed from alkenes and NO₂ with UV-irradiation. Such indirect effects of NO_x should be taken into account. However, with today's knowledge, it is difficult to predict the risk connected with these compounds.

In summary, NO_x may form a genotoxic and carcinogenic risk to humans, directly or indirectly. However, neither qualitative nor quantitative cancer risk estimates can be made at present. Moreover, the *in vivo* data are too sparse to allow for a risk assessment with a conventional safety factor approach.

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