

Genotoxicity and carcinogenicity of ozone

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A review of the genotoxicity of ozone has been published earlier (1) and can be used for a more-detailed description of individual studies.

Genotoxic effects in vitro

Reactions with DNA and RNA

Ozone has been shown to react with DNA (deoxyribonucleic acid) in vitro, preferentially with thymine and guanine residues, and cause degradation of the DNA structure and DNA strand breaks (2—11). In aqueous solution, ozone decomposes to give hydrogen peroxide, superoxide and hydroxy radicals. See the section on the absorption and metabolic fate of ozone (12). According to van der Zee et al (13) DNA damage proceeds both directly, via ozone itself, and indirectly, via hydroxy radicals when solutions of nucleotides or DNA are treated with ozone. DNA adducts can also be formed (14) (eg, hydroxymethyluracil, thymine glycol, 8-hydroxyguanine, and various unidentified adducts).

Ozone has also been clearly shown to cause damage to viral DNA and RNA (ribonucleic acid) (15—19).

Genotoxic effects on microorganisms, plants and insects

All the studies on genotoxic effects on microorganisms, plants, and insects are summarized in table 1.

The bubbling of ozone through a suspension of *Escherichia coli* or *Bacillus subtilis* bacteria caused both forward and reverse mutations in their respective genomes, as well as DNA strand breaks (20—25). Similar treatment of the yeast *Saccharomyces cerevisiae* caused a variety of genetic events (26). It should be noted that, in these experiments, the observed effects may not only be due to ozone, but also to degradation products of ozone in water, such as hydroxy radicals and hydrogen peroxide.

One negative study concerning DNA strand breaks was performed with aerosolized *E coli* bacteria in air containing ozone. However, the ozone concentration was low ($0.08 \text{ mg} \cdot \text{m}^{-3}$) in this study, which was actually a control experiment as part of a larger study (27).

The Ames *Salmonella* assay has been applied in three studies. Ozone in air was passed over agar plates inoculated with *Salmonella* TA 100, without lids, in an exposure chamber for 20 h in one study and for 6 h in another. No mutagenic effects could be found in a single experi-

ment with an ozone level of $1.0 \text{ mg} \cdot \text{m}^{-3}$ (28), or with several concentrations in the range of 0.2 to $4.0 \text{ mg} \cdot \text{m}^{-3}$, either with or without metabolic activation (S9) of the test system (29). Concentrations of the gas above $4 \text{ mg} \cdot \text{m}^{-3}$ were toxic to the bacteria in the latter study. In the third study, several *Salmonella* strains were exposed to ozone in air (30). Strain TA 102, but not strains TA 100, TA 104 or TA 98, experienced a slight mutagenic response after 35 min of exposure to ozone concentrations in the range of 0.04 to 0.08 and 0.4 to $1.0 \text{ mg} \cdot \text{m}^{-3}$, both with and without S9 addition.

The genotoxic effect of ozone has also been studied in plants. Chromosome aberrations were found in *Vicia faba* (31, 32), and in the *Tradescantia* test system ozone was reported as positive in this respect in one study (33) but negative in two others (34, 35). One of the latter studies also reported negative results in *Nicotiana tabacum*.

Erdman & Hernandez (36) exposed male *Drosophila virilis* flies to an ozone concentration of $60 \text{ mg} \cdot \text{m}^{-3}$ for 3 h. Longer exposure times were not tolerated by the flies. Ozone induced dominant lethal mutations in the offspring, as calculated by the proportion of eggs that failed to develop into pupae. Dominant lethals were also seen in another study, along with a life-span shortening effect on male offspring from exposed females and a decrease in the hatchability of the eggs (37).

Cell cultures

All the studies performed with cell cultures are summarized in table 2. Different modes of exposure have been used in the studies, see Victorin (1) or the original references.

Cytogenetic effects. Fetner (38) was the first to show the chromosome-damaging effects (chromatid deletions) of ozone in cell cultures using the human KB cell line. Gross chromosome effects, such as rounded or lysed cells, condensed nuclei, and clumped metaphase chromosomes, were caused by high concentrations of ozone (1400 to $14\,000 \text{ mg} \cdot \text{m}^{-3}$) in another study using embryonic chick fibroblasts (39). Gooch et al (40) studied the effect of bubbling ozone through suspension cultures of human peripheral leukocytes. When the cells were treated in the S-phase, the frequency of chromatid deletions increased.

Guerrero et al (41) exposed 24-hour old cultures of human fetal lung cells (WI-38) to ozone levels of 0.5 ,

1.0, 1.5, or 2.0 mg · m⁻³ for 1 h, the result being a concentration-related increase in sister chromatid exchanges (SCE). An increase in chromatid deletions also occurred at 2 mg · m⁻³, but no increase in chromosome-type aberrations were noted.

Exposure to ozone at 0.07 mg · m⁻³ for 1 h did not appear to induce SCE in V79 Chinese hamster lung fibroblasts attached to cellulose membrane filters (42), whereas 0.1 mg · m⁻³ caused a substantial loss in the fraction of cells recovered from the filters.

Shiraishi & Bandow (43) exposed V79 cells for 2 h to concentrations of ozone ranging from 0.26 to 2.0 mg · m⁻³, which induced SCE and produced a concentration-related inhibition of cell growth. In a review paper on research at the Fudan University in China (44), it was reported that ozone induced SCE in human lymphocytes

at concentrations ranging from 0.3 to 1.5 mg · m⁻³ (treatment time not mentioned).

Effects on DNA. In a study by Rasmussen (45), V79 cells were exposed to ozone for 1 h. DNA replication, as measured by ³H-thymidine incorporation, was depressed in a concentration-dependent manner over the range 2 to 20 mg · m⁻³. Concentrations higher than 4 mg · m⁻³ were cytotoxic. The results were interpreted to indicate that ozone interacts with DNA in a manner which inhibits replication.

The induction of DNA single strand breaks was the end point chosen in three studies. In the first (46), L929 murine fibroblast cell cultures were exposed for 10 s every 30 s to a stream of ozone in oxygen at a very high concentration (25 µmol O₃ per ml = 1 200 000 mg · m⁻³).

Table 1. Genotoxic effects on microorganisms, plants and insects. (+ = positive, - = negative, *E. coli* = *Escherichia coli*, DNA = deoxyribonucleic acid)

Reference	Test organism	End point	Ozone exposure	Result
Hamelin & Chung, 1974 (20)	<i>E. coli</i> , K ₁₂	Reverse mutations	Bubbling of 0.1–2.0 mg · m ⁻³ through cell suspension; 30–60 min	+
Hamelin & Chung, 1975 (21)	<i>E. coli</i> , K ₁₂	Forward mutations	Bubbling of 0.2 mg · m ⁻³ through cell suspension; 60 min	+
L'Hérault & Chung, 1984 (23)	<i>E. coli</i> , different strains	Forward mutations, effect on the rec-lex error-prone repair system	Bubbling of 100 mg · m ⁻³ through cell suspension; 1–20 min	+
Nover & Botzenhart, 1985 (24)	<i>E. coli</i> , K ₁₂	Forward mutations	Bubbling of 1.0 mg · m ⁻³ through cell suspension; 4.5 h	+
Hamelin & Chung, 1989 (22)	<i>E. coli</i>	DNA strand breaks	Bubbling of 100 mg · m ⁻³ through cell suspension; 15 min	+
De Mik & De Groot, 1978 (27)	<i>E. coli</i> , MRE 162	DNA strand breaks	0.08 mg · m ⁻³ ; 30–45 min in aerosolized bacteria	-
Song & Chung, 1983 (25)	<i>Bacillus subtilis</i>	DNA repair	Bubbling of 10–100 mg · m ⁻³ through cell suspension; 30 min	+
Shepson et al, 1985 (28)	<i>Salmonella</i> TA 100	Reverse mutations	1.0 mg · m ⁻³ ; 20 h	-
Victorin & Ståhlberg, 1988 (29)	<i>Salmonella</i> TA 100	Reverse mutations	0.2–4.0 mg · m ⁻³ ; 6 h	-
Dillon et al, 1992 (30)	<i>Salmonella</i> TA 102	Reverse mutations	0.04–1.0 mg · m ⁻³ ; 35 min	+
Dillon et al, 1992 (30)	<i>Salmonella</i> TA 98, TA 100, TA 104, TA 1535	Reverse mutations	0.04–1.0 mg · m ⁻³ ; 35 min	-
Dubeau & Chung, 1982 (26)	<i>Saccharomyces cerevisiae</i>	Forward and reverse point mutations, gene conversion mitotic crossing-over	Bubbling of 100 mg · m ⁻³ through cell suspension; 60 min	+
Fetner, 1958 (31)	<i>Vicia faba</i>	Chromosome-type aberrations in root meristem cells	8000 mg · m ⁻³ ; 15, 30 or 60 min	+
Fetner, 1958 (31)	<i>Vicia faba</i>	Chromatid-type aberrations	8000 mg · m ⁻³ ; 15, 30 or 60 min	-
Janakiraman & Harrey, 1976 (32)	<i>Vicia faba</i>	Chromosome aberrations in buds	4 mg · m ⁻³ ; 4 or 8 h	+
Ma et al, 1982 (34)	<i>Tradescantia</i>	Micronuclei in pollen	10 mg · m ⁻³ ; 5.5 h	-
Schairer et al, 1979 (33)	<i>Tradescantia</i>	Somatic mutations in stamen hairs	10 mg · m ⁻³ ; 6 h	+
Gichner et al, 1991 (35)	<i>Tradescantia</i>	Somatic mutations in stamen hairs	0.3–0.6 mg · m ⁻³ ; 13–15 d	-
Gichner et al, 1991 (35)	<i>Nicotiana tabacum</i>	Somatic mutations in leaves	0.2–0.6 mg · m ⁻³ ; 18 d	-
Chigusa & Nakada, 1972 (37)	<i>Drosophila</i>	Dominant lethals	54 mg · m ⁻³ ; 1–2 h, repeated exposures	+
Erdman & Hernandez, 1982 (36)	<i>Drosophila</i>	Dominant lethals	60 mg · m ⁻³ ; 3 h	+

Table 2. Studies using cell cultures. (+ = positive, – = negative, SCE = sister chromatid exchanges, DNA = deoxyribonucleic acid)

Reference	Cell line	End point	Ozone exposure	Result
Fetner, 1958 (31)	KB human cell line	Chromatid-type aberrations	16 mg · m ⁻³ ; 5 or 10 min	+
Sachsenmaier et al, 1965 (39)	Embryonic chick fibroblasts	Gross chromosome effects	1 400–14 000 mg · m ⁻³ ; 30 min	+
Gooch et al, 1976 (40)	Human lymphocytes	Chromatid-type aberrations	Bubbling of ozone through cell suspension	+
Gooch et al, 1976 (40)	Human lymphocytes	Chromosome-type aberrations	Bubbling of ozone through cell suspension	–
Guerrero et al, 1979 (41)	W1-38 human cell line	SCE	0.5–2 mg · m ⁻³ ; 1 h	+
Guerrero et al, 1979 (41)	W1-38 human cell line	Chromatid-type aberrations	0.5–2 mg · m ⁻³ ; 1 h	+ (2 mg · m ⁻³)
Guerrero et al, 1979 (41)	W1-38 human cell line	Chromosome-type aberrations	0.5–2 mg · m ⁻³ ; 1 h	–
Rasmussen & Crocker, 1982 (42)	V79 Chinese hamster cells	SCE	0.07 mg · m ⁻³ ; 1 h	–
Rasmussen & Crocker, 1982 (42)	V79 Chinese hamster cells	Inhibition of DNA replication	0.07 mg · m ⁻³ ; 1 h	–
Rasmussen, 1986 (45)	V79 Chinese hamster cells	Inhibition of DNA replication	2–20 mg · m ⁻³ ; 1 h	+
Shiraishi & Bandow, 1985 (43)	V79 Chinese hamster cells	SCE	0.26–2.0 mg · m ⁻³ ; 2 h	+
Hsueh & Xiang, 1984 (44)	Human lymphocytes	SCE	0.30–1.5 mg · m ⁻³	+
van der Zee et al, 1987 (46)	L929 mouse fibroblasts	DNA single strand breaks and interstrand cross-links	1 200 000 mg · m ⁻³ ; 2 h	+
Kozumbo & Agarwal, 1990 (48)	CCD-18L human lung fibroblasts and A549 human lung type II epithelial cells	DNA single strand breaks	Ozonated buffer (buffer exposed to 2 mg · m ⁻³ ; 2 h)	–
Borek et al, 1988 (47)	Human epidermal cell-line RHEK	DNA single strand breaks	10 mg · m ⁻³ ; 10 min	–
Borek et al, 1986 (49)	Primary hamster embryo cells and C3H/10T-1/2 mouse fibroblasts	Neoplastic transformation	10 mg · m ⁻³ ; 5 min	+
Thomassen et al, 1991 (52)	Primary rat tracheal epithelial cells	Neoplastic transformation	2 or 20 mg · m ⁻³ ; 40 min	—
Thomassen et al, 1991 (52)	Primary rat tracheal epithelial cells	Neoplastic transformation	1.4 mg · m ⁻³ ; 40 min bi-weekly for 9 exposures	+

Using a DNA unwinding technique, ozone exposure for 2 h was shown to generate single strand breaks. However, with the alkaline elution technique, no increased rate of DNA elution was noted, which might have been the result of the generation of DNA-interstrand cross-links. Ozone-induced cross-links were also formed between DNA and protein. No mention was made in the paper of cytotoxic effects on the cells. However, at the ozone concentrations used, the cells must have suffered considerable cytotoxicity.

Borek et al (47) exposed the RHEK human epidermal cell line to an ozone concentration of 10 mg · m⁻³ for 10 min. DNA single strand breaks were assayed with the alkaline elution technique and alkaline sucrose gradient centrifugation. No significant induction of DNA strand breaks was detected. A high background may have contributed to the negative outcome of this study.

Control experiments with ozonated buffer were performed in a study on the formation of genotoxic reaction products by the ozonation of arylamines (48). Human CCD-18 lung fibroblasts and transformed type II epithelial A549 lung cells were used as the test organisms. Buffer that had been preexposed to an ozone concentration of 2 mg · m⁻³ did not induce DNA single strand breaks in these cell types.

Cell transformation in vitro. In a study by Borek et al (49) it was shown that ozone induced neoplastic transformation in primary hamster embryo cells and in (C3H/10T1/2) mouse fibroblast cultures, both exposed to 10 mg · m⁻³ for 5 min. Ozone acted synergistically (more than in an additive manner) with ionizing radiation, but only when delivered after the radiation (49, 50). In a similar study (51), ozone and ultraviolet radiation induced cell transformation in an additive fashion.

Preneoplastic transformation was also shown to be induced by ozone in a study by Thomassen et al (52), but only after multiple exposures to the gas. Primary rat tracheal epithelial cells were exposed to single exposures of 1 ppm or 10 ppm for 40 min or to multiple exposure of 0.7 ppm twice weekly for five weeks.

Summary. The bubbling of ozone through a suspension of bacteria or yeast cells induces forward and reverse mutations, as well as DNA strand breaks. Mutations in bacteria after airborne exposure have been more difficult to determine, although there is one positive study with *Salmonella* TA102. Chromosome aberrations have been induced in the plant *Vicia faba*, but only in one out of three studies with *Tradescantia* or *Nicotiana tabacum*. Ozone has also induced dominant lethals in *Drosophila*.

Ozone has been shown to induce chromatid-type aberrations, SCE, and neoplastic transformation in cell cultures, but data concerning single strand breaks in DNA are inconsistent.

Overall, the data discussed indicate that ozone is genotoxic to microorganisms and cell cultures, a finding in accordance with the damaging effect of ozone on naked DNA and viral DNA. Genotoxic effects in cell cultures (eg, SCE) have occurred at 0.3 to 2 mg · m⁻³.

Genotoxic effects in vivo

Animal experiments

The cited studies with animal experiments are summarized in table 3.

The first of several inhalation experiments with laboratory animals was performed by Zelac et al (53). These authors used female Chinese hamsters; four animals each were exposed to ozone at 0.48 and 0.60 mg · m⁻³ for 5 h, and four other animals served as controls. The frequency of chromosome-type aberrations was significantly higher in peripheral lymphocytes from the exposed groups, as compared with the control group, immediately after exposure and 15 d after exposure. (The increase 6 d after exposure was not significant.)

In an attempt to duplicate the positive findings of Zelac et al, Tice et al (54) exposed Chinese hamsters in groups of three to seven animals, both males and females, to 0.80 mg · m⁻³ for 5 h. An increase in chromatid-type aberrations in peripheral lymphocytes (deletions and gaps) was noted one and two weeks after the treatment, but not immediately after it. No chromosome-type aberrations were seen. Bone-marrow preparations from a few animals showed no induced aberrations. The frequencies of SCE in peripheral lymphocytes from the Chinese hamsters were not increased, nor were the SCE levels in bone-marrow cells from groups of three to four C57B16 female mice, exposed to 4 mg · m⁻³ for 6 h and examined 0, 1, 2, and 3 weeks after the exposure.

Gooch et al (40) exposed mice and hamsters to ozone. No mention was made of the number of animals used in these experiments, but male mice, strain C₃H, were exposed to 0.30 mg · m⁻³ or 0.42 mg · m⁻³ for 5 h and to 2.0 mg · m⁻³ for 2 h. No significant induction of chromosome or chromatid damage in peripheral lymphocytes resulted from the ozone exposure. Mouse spermatocyte preparations were made eight weeks after the exposure and analyzed for the presence of reciprocal translocations. No such effect was seen. Nor was there any induction of chromatid or chromosome aberrations in bone

Table 3. Genotoxic effects in laboratory animals. (+ = positive, – = negative, SCE = sister chromatid exchanges)

Reference	Species	End point	Ozone exposure	Result
Zelac et al, 1971 (53)	Chinese hamster, females	Chromosome-type aberrations in lymphocytes	0.48 and 0.6 mg · m ⁻³ ; 5 h	+
Gooch et al, 1976 (40)	Mouse, strain C ₃ H, males	Chromosome and chromatid-type aberrations in lymphocytes	0.30 and 0.42 mg · m ⁻³ for 5 h; 2.0 mg · m ⁻³ for 2 h	–
Gooch et al, 1976 (40)	Mouse, strain C ₃ H, males	Reciprocal translocations in spermatocytes	0.30 and 0.42 mg · m ⁻³ for 5 h; 2.0 mg · m ⁻³ for 2 h	–
Gooch et al, 1976 (40)	Chinese hamster	Chromosome and chromatid-type aberrations in bone marrow cells	0.46 mg · m ⁻³ for 5 h; 10 mg · m ⁻³ for 6 h	–
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromosome-type aberrations in lymphocytes	0.86 mg · m ⁻³ ; 5 h	–
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromatid-type aberrations in lymphocytes	0.86 mg · m ⁻³ ; 5 h	+
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromosome and chromatid-type aberrations in bone marrow cells	0.86 mg · m ⁻³ ; 5 h	–
Tice et al, 1978 (54)	Chinese hamster, males + females	SCE in lymphocytes	0.86 mg · m ⁻³ ; 5 h	–
Tice et al, 1978 (54)	Mouse strain C ₅₇ B ₁₆ , females	SCE in bone marrow cells	4.0 mg · m ⁻³ ; 6 h	–
Zhurkov et al, 1979 (55)	Rat, males	Chromosome and chromatid-type aberrations in bone marrow cells	0.15 mg · m ⁻³ ; 8 h daily for 7 days or 5.6 mg · m ⁻³ continuously for 5 days	–
Rithidech et al, 1990 (56)	Rat, strain F334/N, females	Chromosome-type aberrations in pulmonary macrophages	0.43 and 1.3 mg · m ⁻³ ; 6 h	–
Rithidech et al, 1990 (56)	Rat, strain F334/N, females	Chromatid-type aberrations in pulmonary macrophages	0.43 and 1.3 mg · m ⁻³ ; 6 h	+
Thomassen et al, 1991 (52)	Rat, strain F334/N, males	Preneoplastic transformation in tracheal epithelial cells	0.28, 1.2 and 2.4 mg · m ⁻³ ; 6 h/day, 5 days/week, for 1, 2 or 4 weeks	–
Sills et al, 1995 (71)	Mouse, strain B6C3F ₁ , females	Mutations in K-ras oncogene in lung tumors	1.0 and 2.0 mg · m ⁻³ ; 6 h/day, 5 days/week, 2 years and life-time	+

marrow cells from Chinese hamsters exposed to either $0.46 \text{ mg} \cdot \text{m}^{-3}$ for 5 h or $10 \text{ mg} \cdot \text{m}^{-3}$ for 6 h.

Another negative study on chromosome aberrations (including chromatid breaks) in bone marrow cells was reported by Zhurkov et al (55). These authors exposed male white rats, five in each concentration group, to either $0.15 \text{ mg} \cdot \text{m}^{-3}$ 8 h daily for 7 d or to $5.6 \text{ mg} \cdot \text{m}^{-3}$ for 5 d. Under the latter conditions bone marrow mitotic activity was substantially depressed.

Pulmonary alveolar macrophages were used by Rithidech et al (56) to study genotoxic effects of ozone in lung cells. Female F344/N rats, five in each concentration group, were exposed to ozone at 0.20, 0.43, or $1.3 \text{ mg} \cdot \text{m}^{-3}$ for 6 h. Exposure to 0.43 and $1.3 \text{ mg} \cdot \text{m}^{-3}$ significantly increased the proportion of cells with chromatid deletions and gaps. The highest level of ozone used also induced a significant increase in the mitotic index of the macrophages. No chromosome-type aberrations were seen, however.

Thomassen et al (52) studied the potency of ozone in inducing preneoplastic transformation in rat tracheal epithelial cells after the inhalation of 0.24, 1.2, or $2.4 \text{ mg} \cdot \text{m}^{-3}$, 6 h a day, 5 d a week for one, two, or four weeks. After the exposure of male F344/N rats, the tracheal epithelium cells were isolated, plated, and scored for preneoplastic variants after five weeks of incubation. Ozone failed to cause statistically significant increases in transformation frequency, as compared with that of control rats breathing air only.

Human exposure

The cited studies involving human exposure are summarized in table 4.

In a study of the effect of ozone on pulmonary function in humans, chromosome aberrations in peripheral lymphocytes were also analyzed (57). Two persons were exposed to $1.0 \text{ mg} \cdot \text{m}^{-3}$ for 6 h and four persons to $1.0 \text{ mg} \cdot \text{m}^{-3}$ for 10 h. The subjects served as their own controls, blood samples being drawn both pre- and post-exposure. In addition, the group of four persons was also tested two weeks and six weeks after exposure. No chromosome-type aberrations were seen in any of the subjects. However, for most of them an increase in

achromatic lesions (gaps) and chromatid deletions was found after the exposure. The number of chromatid deletions seemed to be highest two weeks after the exposure. The statistical significance of the results was not evaluated, however.

Guerrero et al (41) analyzed the SCE frequency in peripheral lymphocytes from 31 male and female college students one day before and immediately after exposure to $1.0 \text{ mg} \cdot \text{m}^{-3}$ of ozone for 2 h. There was no significant difference in the mean SCE frequency per chromosome for all the subjects before, compared with after, exposure. However, in this study, the individual SCE levels were not reported, nor was there any mention of the smoking habits of the subjects.

McKenzie et al (58) have also performed cytogenetic studies with ozone-exposed subjects. In their initial study 26 healthy, nonsmoking male college students were exposed to $0.8 \text{ mg} \cdot \text{m}^{-3}$ for 4 h. Blood samples were taken immediately before and after the exposure, as well as three days, and two and four weeks after the exposure. The pooled results from all the subjects showed no statistically significant increase in the number of cells with chromosome or chromatid-type aberrations (including gaps) after exposure to the gas.

In a later study reported by McKenzie (59), neither ozone exposure to $1.2 \text{ mg} \cdot \text{m}^{-3}$ for 2 h nor $0.8 \text{ mg} \cdot \text{m}^{-3}$ for 4 h, both administered repeatedly for 4 d, gave statistically significant increases in structural aberrations or SCE levels immediately after, three days after, or two or four weeks after exposure (≥ 10 subjects). No experimental details were given in this paper.

Sarto & Viola (60) noticed a statistically significant increased frequency of chromatid gaps, but no other chromosome aberrations, in lymphocytes from 10 workers exposed to nearly $0.6 \text{ mg} \cdot \text{m}^{-3}$ for one to three years, when the workers were compared with 10 unexposed controls. No mention was made of the smoking habits of the subjects or possible confounding air pollutants in the workplace, which produced plastic bags.

Summary

The results derived from the *in vivo* cytogenetic studies with laboratory animals are contradictory. Concerning

Table 4. *In vivo* cytogenetic effects in humans (lymphocytes). (+ = positive, - = negative, SCE = sister chromatid exchanges)

Reference	End point	Number of individuals	Ozone exposure	Result
Merz et al, 1975 (57)	Chromosome-type aberrations	6	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h or 10 h	-
Merz et al, 1975 (57)	Chromatid-type aberrations	6	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h or 10 h	+
Guerrero et al, 1979 (41)	SCE	31	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 2 h	-
McKenzie, 1977 (58); McKenzie, 1982 (59)	Chromosome- and chromatid-type aberrations or SCE	10-30	$0.8 \text{ mg} \cdot \text{m}^{-3}$; 4 h, once or repeatedly for 4 days; $1.2 \text{ mg} \cdot \text{m}^{-3}$; 2 h	-
Sarto & Viola, 1980 (60)	Chromatid gaps	10	$0.6 \text{ mg} \cdot \text{m}^{-3}$; 1-3 years	+
Sarto & Viola, 1980 (60)	Other chromosome- and chromatid-type aberrations	..	$0.6 \text{ mg} \cdot \text{m}^{-3}$; 1-3 years	-

chromosome aberrations in lymphocytes of Chinese hamsters, one study noted chromosome-type aberrations, but another study noted only chromatid-type aberrations. SCE were not induced by the same treatment. In mice, no aberrations of either type were observed. No cytogenetic effects have been seen in bone-marrow cells or spermatocytes. Only one study with lung cells has been published, in which chromatid-type aberrations in alveolar macrophages were induced by ozone exposure of $0.43 \text{ mg} \cdot \text{m}^{-3}$ for 6 h.

The experimental and epidemiologic studies with human subjects do not allow a conclusion regarding the cytogenetic effects of ozone in lymphocytes. In only one study did the subjects serve as their own controls, but relatively few persons participated in this study. In two of the other three studies smoking or other potential confounding factors were not taken into consideration.

Tumor induction

The cited studies on tumor induction, except those with coadministration of known carcinogens, are summarized in table 5.

Stokinger (61) reported that ozone induced lung tumors (adenomas) in a susceptible strain of mice (strain

not mentioned), exposed to $2 \text{ mg} \cdot \text{m}^{-3}$ daily for 15 months. However, no experimental details were presented in this article.

Werthamer et al (62) exposed Swiss-Webster mice (sensitive to the development of lung adenomas) to ozone ($9.0 \text{ mg} \cdot \text{m}^{-3}$) during 2 h every third day for a period of up to 75 d. A statistically significant increase in cellular alterations in the lung between ozone-exposed mice and control animals was reported, although the control data were not shown. The cellular alterations included the induction of hyperplasia, metaplasia, and adenomas. There were also indications of the induction of inflammatory reactions.

Hassett et al (63) exposed female mice (strain A/J, sensitive to the development of lung adenomas) to an ozone concentration of either $0.62 \text{ mg} \cdot \text{m}^{-3}$ intermittently 103 h a week every other week for six months or $1.0 \text{ mg} \cdot \text{m}^{-3}$ 102 h a week during the first week of each month for six months. In these studies, animals were killed five months and three months, respectively, after the final ozone exposure. In both studies the presence of tumor nodules (adenomas) on the pleural surface of the lung was reported to be significantly higher in the exposed animals, as compared with those in control animals. However, in a later paper from the same research group (64), only the increase in the second study was referred to as being statistically significant. The tumor

Table 5. Induction of lung tumors in experimental animals. (+ = positive, - = negative, (+) = originally reported as positive, but later (64) as not statistically significant)

Reference	Species	End point	Ozone exposure	Result ^a					
				Males			Females		
				A	C	A + C	A	C	A + C
Stokinger, 1965 (61)	Mice (species not mentioned)	Adenomas	$2 \text{ mg} \cdot \text{m}^{-3}$; 15 months	+					
Werthamer et al, 1970 (62)	Mice, Swiss Webster	Adenomas	$9 \text{ mg} \cdot \text{m}^{-3}$; 2 h every third day, 75 d	+					
Hassett et al, 1985 (63)	Mice, A/J, females	Adenomas	$0.62 \text{ mg} \cdot \text{m}^{-3}$; 103 h a week, every other week, 6 months	(+)					
Hassett et al, 1985 (63)	Mice, A/J, females	Adenomas	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 102 h a week, first week of each month, 6 months	+					
Last et al, 1987 (65)	Mice, A/J, males	Adenomas	0.8 and $1.6 \text{ mg} \cdot \text{m}^{-3}$; 8 h a day, 18 weeks	+	($1.6 \text{ mg} \cdot \text{m}^{-3}$)				
Last et al, 1987 (65)	Mice, Swiss Webster, males	Adenomas	0.8 and $1.6 \text{ mg} \cdot \text{m}^{-3}$; 8 h a day, 18 weeks	-					
Ichinose & Sagai 1992 (69)	Rats, Wistar, males	Adenomas and carcinomas	$0.1 \text{ mg} \cdot \text{m}^{-3}$; 10 h a day, 13 months	-					
Witschi et al, 1993 (68)	Hamsters, Syrian Golden, males	Adenomas and carcinomas	$1.6 \text{ mg} \cdot \text{m}^{-3}$; 23 h a day, 7 d/week, 6 months	-					
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	0.24 , 1.0 and $2.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h/d, 5 d a week, 105 weeks (2 years)	-	-	-	-	+ ^a	+ ^a
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	1.0 and $2.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h/d, 5 d a week, 130 weeks (life-time)	-	+ ^b	-	+ ^a	-	-
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	Both studies combined (2 year and life-time)	-	+ ^a	+ ^a	+ ^a	-	+ ^a
NTP, 1993 (70)	Rats, F344/n, males and females	Adenomas (A), carcinomas (C) or both (A+C)	As for mice except 104 and 124 weeks	-	-	-	-	-	-

^a $2.0 \text{ mg} \cdot \text{m}^{-3}$

^b 1.0 , $2.0 \text{ mg} \cdot \text{m}^{-3}$.

inducing activities of urethane, as a single intraperitoneal injection one week before exposure to ozone was begun, was not significantly enhanced by ozone in the first experiment. However, when the injection of the carcinogen urethane followed immediately after each ozone exposure in the second experiment, a higher than expected tumor incidence was produced.

In a similar study, Last et al (65) exposed both A/J and Swiss-Webster male mice to either 0.8 or 1.6 mg · m⁻³ for 8 h a night, 7 nights a week for 18 weeks. Upon examination, 1.6 mg · m⁻³, but not 0.8 mg · m⁻³, induced a significant increase in lung adenomas in the A/J mice, but not in the more resistant Swiss-Webster strain. In one part of the experiment, the animals were pretreated by a single intraperitoneal injection of urethane one day before the ozone exposure started. In these animals, ozone actually caused a decrease in the number of tumors per lung. This finding can be explained in terms of a cytotoxic action of ozone upon initiated cells destined to grow into tumors (66, 67). In contrast, the cocarcinogenic action of ozone observed by Hassett et al (63) may be explained by the cell proliferative activity of ozone, which might have expanded the cell population at risk to undergo transformation. Thus the sequence of exposure can determine whether the effects of ozone on lung tumor development are beneficial or detrimental (66).

In further examining the modulation of lung tumors by ozone, Witschi et al (68) treated male Syrian Golden hamsters with subcutaneous injections of N-nitrosodiethylamine twice a week for six months, during which the animals were continuously exposed to 1.6 mg · m⁻³ (23 h per day, 7 days per week). It was found that the animals kept in ozone developed half as many peripheral lung tumors as did the animals kept in air. However, the difference between these groups was not statistically significant. Tumors of the trachea, bronchi, nasal cavity, and liver developed with the same incidence irrespective of whether the animals were exposed to ozone or not. No tumors were found in a group of animals (N = 16) injected with sodium chloride and exposed to ozone.

In another experiment (69) male Wistar rats were given a single intraperitoneal injection of N-bis(2-hydroxypropyl)nitrosamine and were then exposed to an average ozone concentration of 0.01 mg · m⁻³ (10 h per day) or 0.05 mg · m⁻³ (10 h per day), supplemented with a nitrogen dioxide concentration of 0.75 mg · m⁻³ (24 h per day), for a total of 13 months. The animals were then kept in clean air for another 11 months before being killed. There were no lung tumors in the control groups treated with ozone only or the nitrosamine only (N = 36). However, in the groups treated with the nitrosamine + ozone or nitrosamine + ozone + nitrogen dioxide, three and five pulmonary tumors (adenomas and adenocarcinoma) were detected, respectively. The latter enhancement was statistically significant.

The results of a thorough cancer study from the National Toxicology Program (NTP) in the United States (70) with life-long inhalation exposure to ozone were recently presented. Male and female F344/N rats were exposed in groups of 50 animals to an ozone concentration of 0.24, 1.0, or 2.0 mg · m⁻³ of ozone, 6 h a day, 5 days a week for 104 weeks and to 1.0 and 2.0 mg · m⁻³ of the gas for 124 weeks. Male and female B6C3F₁ mice were similarly exposed for 105 weeks (2 years) and 130 weeks (life-time). There was no increased tumor incidence in the rat study. However, ozone induced alveolar or bronchiolar tumors (adenomas and carcinomas) in mice. For the male mice, the increase was statistically significant ($P \leq 0.05$) for carcinomas at 1.0 and 2.0 mg · m⁻³ in the life-time study, and for carcinomas and adenomas + carcinomas at 2.0 mg · m⁻³ when the results from the two-year and the life-time studies were combined. In the two-year study there was a significant positive trend in the incidence of adenomas + carcinomas, although no single concentration group was significantly increased. For female mice the increase was statistically significant ($P \leq 0.05$) for carcinomas and adenomas + carcinomas at 2.0 mg · m⁻³ in the two-year study, for adenomas at 2.0 mg · m⁻³ in the life-time study, and for adenomas and adenomas + carcinomas at 2.0 mg · m⁻³ when the studies were combined.

In the conclusions from the NTP study it is stated that "there was some evidence of carcinogenic activity" of ozone in the female mice, but "equivocal evidence of carcinogenic activity" in male mice. The latter statement seems to be mainly based on the fact that the combined tumor incidence in the two-year study was within the historical range (Boorman, 1994, personal communication). However, the combined analysis of both the two-year and the life-time studies would also argue for a carcinogenic effect in the male mice study.

The adenomas and carcinomas induced in the female mice in the NTP study were investigated for mutations in the *K-ras* proto-oncogene and the *p53* tumor suppressor gene (71). *K-ras* mutations were detected in 73% of the ozone-induced tumors, as compared with 33% of the lung tumors from control animals; this finding suggests that ozone causes direct or indirect DNA damage within the *K-ras* oncogene. No enhanced expression of the *p53* protein was detected with immunohistochemical methods.

The NTP study (70) also included a separate experiment in which male rats were injected with a nitrosamine (NNK) three times per week for the first 20 weeks and exposed to an ozone concentration of 1.0 mg · m⁻³ for 6 h a day, 5 days a week for 105 weeks. Ozone did not significantly enhance the incidence of lung tumors in these animals.

The effect of ozone on cancer cell metastasis in the lung of mice has been studied in two papers. Kobayashi

et al (72) exposed male C3H/He mice to ozone concentrations of 0.2, 0.4, 0.8, and 1.6 mg · m⁻³ continuously for 1, 3, 7 and 14 d. A suspension of fibrosarcoma NR-FS cells was then infused into the animals via the tail vein. The animals were then sacrificed after 14 d. A significantly increased rate of pulmonary metastasis was observed in the ozone-exposed mice. The maximal enhancement occurred in mice exposed to 1.6 mg · m⁻³ for 1 d. The prolonged exposure (14 d) gradually suppressed this enhancement. In a similar study (73), no significantly enhanced cancer cell colonization of lungs was observed when male C57 BL/6 mice were exposed to 0.3 or 0.6 mg · m⁻³ for 7 h a day, 5 days a week, for a total period of 12 weeks and then infused with melanoma cells (B16F10R1).

Other effects seen in animal experiments may be of relevance when the tumor-inducing activity of ozone is discussed. Cell proliferation (increased DNA replication) and the induction of metaplasia or hyperplasia have been induced in parts of the nasal epithelium in rats and monkeys (74–76). Ozone also induces cell proliferation, assessed as an increase in the DNA-labeling index and mitotic index of type II epithelial alveolar lung cells, alveolar macrophages, and lymphocytes of the lung (77–81). Epithelial hyperplasia in the lung have been observed in the aforementioned long-term studies with ozone (62, 63, 65, 66). In the life-time NTP study (70) the ozone exposure was associated with the induction of hyperplasia and squamous metaplasia in the nose and alveolar metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) in the lungs of both rats and mice. There was also interstitial fibrosis in the lung of rats and infiltration of inflammatory cells in mice. However, there was no mention of the induction of hyperplasia in the lung of either rats or mice.

In summary, there is only one cancer study with full life-long exposure to ozone by inhalation. In this study, lung adenomas or carcinomas were induced in B6C3F₁ mice at an ozone concentration of 2.0 mg · m⁻³. This result agrees with the earlier findings of the induction of lung adenomas in short-term bioassays with strain A/J mice. In contrast, ozone has failed to induce tumors in rats or hamsters.

No conclusion can be drawn concerning a possible cocarcinogenic or promoting effect of ozone. An increase as well as a decrease or no effect on the carcinogenicity of known carcinogens has been demonstrated.

Summary and concluding remarks

Ozone has been shown to be genotoxic in vitro. Mutations and DNA strand breaks occurred in bacteria and yeast cells, mainly in experiments in which ozone was

bubbled through suspension cultures of cells, in which case hydroxy radicals and hydrogen peroxide are also formed. The bacteriotoxicity of ozone complicates the demonstration of mutagenicity in *Salmonella*, but a positive response has been observed with ozone in air with strain TA 102 in one study. In cell cultures, chromatid-type chromosome aberrations, sister SCE, and neoplastic transformation have been demonstrated. SCE occurred at 0.3 to 2 mg · m⁻³.

The results from in vivo cytogenetic studies with laboratory animals after inhalation exposure are contradictory. Chromosome aberrations in lymphocytes, but not SCE, have been found in Chinese hamsters, but not in mice. No cytogenetic effects were reported for bone marrow cells or spermatocytes. As ozone is a very reactive compound, which is probably not taken up unreacted from the lung, the positive finding of chromatid-type aberrations in lung macrophages in rats (at 0.43 mg · m⁻³ for 6 h) is important from the risk assessment point of view.

The lung is the target organ for the carcinogenic effects of ozone. Lung adenomas have been induced in the sensitive A/J strain of mice. A recent life-time inhalation study demonstrated an induction of alveolar or bronchiolar adenomas and carcinomas in B6C3F₁ mice, but not in F344/N rats, at an ozone concentration of 2 mg · m⁻³ (70). An increased frequency of mutations in the *K-ras* protooncogene of tumors in ozone-treated female mice suggests a genotoxic origin of the ozone-induced tumors. These findings lead to the conclusion that a possible carcinogenic risk of ozone to humans should not be overlooked.

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