Carcinogenesis vol.24 no.7 pp.1233–1238, 2003
DOI: 10.1093/carcin/bgg087

Molecular dosimetry of *N*7-(2-hydroxypropyl)guanine in tissues of F344 rats after inhalation exposure to propylene oxide

Melva N.Ríos-Blanco[1,4], Asoka Ranasinghe[2], Moung S.Lee[3], Thomas Faller[3], Johannes G.Filser[3] and James A.Swenberg[1,2,5]

[1]Curriculum in Toxicology and [2]Department of Environmental Sciences and Engineering, Campus Box 7431, Rosenau Hall 253c, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA and [3]Institute of Toxicology, GSF National Research Center for Environment and Health, D-85764 Neuherberg, Germany

[4]Present address: Colgate-Palmolive Co., 909 River Road, Piscataway, NJ 08856, USA

[5]To whom correspondence should be addressed
Email: james_swenberg@unc.edu

Propylene oxide (PO) is a high-volume chemical intermediate that causes a low incidence of nasal tumors in rodents exposed to high concentrations (≥300 p.p.m.). PO reacts with DNA forming mainly *N*7-(2-hydroxypropyl)guanine (7-HPG). The exposure-dependent accumulation of 7-HPG in nasal respiratory epithelium (NRE), lung and liver was determined in male F344 rats exposed to PO (0, 5, 25, 50, 300 or 500 p.p.m.) by the inhalation route for 3 or 20 days (6 h/day; 5 days/week). These exposures ranged from low concentrations, such as those potentially occurring in the workplace, to high concentrations that proved to be carcinogenic in rodents. Analysis of 7-HPG in DNA by gas chromatography–high-resolution mass spectrometry (GC–HRMS) showed a linear response in 7-HPG for all three tissues after 3 days of exposure, and for NRE and lung after 20 days of exposure. A slightly sublinear response in 7-HPG was observed in liver after 20 days of exposure. For both exposure periods, the NRE had the highest concentration of 7-HPG, followed by lung and liver. The amount of 7-HPG in NRE was seven and 17 times higher than in lung and liver, respectively, for the 3 day exposures. For the 20 day exposures, the concentration of 7-HPG in NRE was six and 13 times higher than that in lung and liver, respectively, over the concentration range studied. These results demonstrate a much higher extent of DNA alkylation in the target tissue for carcinogenesis, than in non-target tissues. As PO-induced tumor formation was highly sublinear, occurring only at high vapor concentrations, whereas 7-HPG adducts were shown to be linearly dependent on airborne concentration, these results suggest that 7-HPG is not sufficient for PO nasal carcinogenesis and that other factors such as increased cell proliferation may be important in determining the tumor exposure response.

Abbreviations: AP, apurinic/apyrimidinic; ddH_2O, double-distilled water; GC–HRMS, gas chromatography–high-resolution mass spectrometry; N^6-HPdAdo, N^6-(2-hydroxypropyl)deoxyadenosine; 7-HPG, *N*7-(2-hydroxypropyl)guanine; NRE, nasal respiratory epithelium; PO, propylene oxide.

Introduction

Propylene oxide (PO) (CAS registry number 75-56-9) is a high-volume chemical intermediate used in the production of plastics and other synthetic materials. Exposure to PO can take place in the workplace during its production, storage and transport. It has been estimated that more than 200 000 workers are potentially exposed to PO in the US alone (1). An 8 h time weighed average (TWA) of 20 p.p.m. PO was established by the Occupational Safety and Health Administration (OSHA) in the USA. Likewise, the American Conference of Governmental Industrial Hygienists recommends a threshold limit value (TLV®) for PO of 2 p.p.m. as an 8 h TWA. The German Technical Exposure Limit for PO is 2.5 p.p.m. PO is not known to occur naturally in the environment.

The genotoxicity and carcinogenicity of PO in bacterial and mammalian systems has been reviewed elsewhere (2–5). In summary, PO has been shown to be mutagenic in bacterial and mammalian systems *in vitro* (6–10), but has not been shown to be mutagenic *in vivo* by exposure routes relevant to humans (6,11).

Long-term carcinogenicity studies of PO demonstrated that for all routes investigated, increased incidences of tumors occurred at the site of contact in exposed rats. Intragastric administration of PO to Sprague–Dawley rats resulted in a dose-dependent increase in squamous cell carcinomas of the forestomach (12) whereas s.c. injection of PO to female NMRI mice led to the appearance of tumors (sarcomas) at the injection site at high doses only (13). F344 rats exposed their lifetime to PO by inhalation developed papillary adenomas of the nasal respiratory epithelium, whereas exposure to B6C3F1 mice led to the development of hemangiomas, hemangiosarcomas and adenocarcinomas within the nasal tissues (14–16). Tumors were observed at high concentrations only (≥300 p.p.m.). Inhalation exposure of Wistar rats to PO (0, 30, 100 and 300 p.p.m. for 124 weeks) resulted in an increase in non-neoplastic lesions in the nasal cavity of rats exposed to 100 or 300 p.p.m. (17). One squamous cell carcinoma of the nose was diagnosed in a 30 p.p.m. male (1/61) and another in a 300 p.p.m. male (1/63).

IARC (1994) classified PO as a possible human carcinogen based on positive rodent cancer bioassays and insufficient epidemiological data concerning its carcinogenicity in humans. Current estimates of PO cancer risk in humans [EPA, 1994, Integrated Risk Information System (IRIS) Propylene Oxide File] were obtained by linear extrapolation of tumor data. In the case of PO, rodent tumor data were obtained at concentrations that are 15 times higher than those potentially occurring in the workplace.

The mechanism by which PO causes cancer is not clear. PO directly reacts with DNA to form mainly the *N*7-(2-hydroxypropyl)guanine (7-HPG) adduct and a minor amount of cytosine and adenine adducts (18,19). Studies *in vivo* (5,20,21) demonstrated a marked difference in DNA alkylation between

tissues, with a much greater number of 7-HPG adducts in nasal tissue than in systemic organs after inhalation exposure of rats to 500 p.p.m. PO for 20 days. Molecular dosimetry data for PO–DNA adducts may provide insights into its mode of action.

The objectives of this research were: (i) to quantify the exposure-dependent accumulation of 7-HPG in tissues of rats exposed to PO by the inhalation route; (ii) to examine the differences in DNA alkylation between tissues at different concentrations and lengths of exposure; and (iii) to examine the relationship between the exposure–response in 7-HPG accumulation and the tumor incidence curve. The long-term goals of this research are to use molecular dosimetry data and cell proliferation data to understand better the mechanism of PO carcinogenesis, and thus to improve the current PO cancer risk assessments.

Materials and methods

Chemicals

PO (99.9% purity) used for the inhalation studies was provided by Lyondell Nederland (Rotterdam, Netherlands). Pentafluorobenzyl bromide, potassium carbonate, sodium hydroxide (99.99%), anhydrous acetonitrile (99.8%) and potassium hydroxide were purchased from Aldrich (Milwaukee, WI). Cell lysis buffer, proteinase K and 70:20:10 phenol:chloroform:water were from Applied Biosystems (Foster City, CA). RNase A in glycerol was from Sigma (St Louis, MO). RNase T1 was from Roche Molecular Biochemicals (Indianapolis, IN). HCl was from Fisher (Fair Lawn, NJ). Other chemicals used were purchased as analytical grade reagents and used without further purification and characterization.

Animal exposures

The study was conducted largely in compliance with good laboratory practice regulations as promulgated by the US Environmental Protection Agency and the Organization for Economic Cooperation and Development. Exposures were carried out at the GSF Institute of Toxicology, Neuherberg, Germany. Nine-week-old male F344 rats were purchased from Charles River Deutschland (Sulzfeld, Germany) and were marked with ear tags. The rats were assigned to one of six exposure groups per exposure period (n = 5/group), based on their body weight. The group assignments were adjusted to result in mean group body weights that were not significantly different from one another. Treated and control animals were provided with food (Standard Chow 1324, Altromin, Lage, Germany) and water *ad libitum* except during the inhalation exposure periods. The animals were exposed to nominal PO concentrations of 0, 5, 25, 50, 300 and 500 p.p.m. for 6 h/day, 5 days/week for 3 or 20 days. PO vapor was generated by a single vaporization system (22) using a separate glass chamber for each concentration. Animals were observed daily for mortality and overt clinical signs of toxicity. Detailed descriptions of analytical techniques and inhalation chambers are found elsewhere (5,22). Animals were killed with carbon dioxide within 5 h after the end of the final exposure period. Nasal respiratory epithelium, lung and liver were collected, placed in plastic vials and frozen in liquid nitrogen. Tissue samples were stored at −80°C until analysis.

DNA isolation

DNA was extracted from nasal respiratory epithelium (NRE), lung and liver of control and exposed rats by phenol–chloroform extraction. Briefly, lung and liver tissue were thawed and homogenized in cold PBS with a Tehran homogenizer (Wheaton Instruments, Millville, NJ). Samples were kept on ice throughout the whole procedure. After centrifugation (2000 *g*, 15 min), the nuclear pellets were resuspended in cell lysis buffer overnight at 4°C with proteinase K. The suspensions were extracted twice with a mixture of phenol: chloroform:water and once with Sevag (24:1, chloroform:isopentyl alcohol), followed by ethanol precipitation. The resulting DNA was incubated in PBS with a mixture of RNase T1 and RNase A for 30 min at 37°C. After a second precipitation of DNA with cold ethanol, the DNA pellet was re-suspended in double distilled water (ddH_2O) and DNA concentration measured by absorbance at 260 nm of an aliquot in 20 mM Tris buffer, pH 7.4 (20 A_{260} = 1 mg/ml). Measurements of the ratio A_{260}/A_{280} were between 1.6 and 1.9 indicating minimal protein contamination. The homogenization step was not performed for NRE. Nasal tissues were immediately incubated in cell lysis buffer. The rest of the DNA isolation procedure was as given for the other tissues. The quality of DNA from NRE samples was not appropriate according to the A_{260}/A_{280} ratio, possibly due to protein or salt contamination. For further purification, cell lysis buffer from Puregene (Gentra Puregene) was added to the DNA samples (7.6:1 buffer:solution) from NRE along with protein precipitation solution (3:1 lysis buffer:precipitation solution). Samples were mixed and centrifuged (>8000 *g*) for 10 min. DNA was precipitated from the supernatant by alcohol addition. DNA pellets were resuspended in ddH_2O and DNA concentration measured by absorbance as explained for the rest of the tissues. No difference in 7-HPG number was found between NRE DNA from samples prior to or after purification by the Gentra Puregene kit. However, the quality of the NRE DNA improved as noted in the appearance of the UV spectrum (data not shown).

Hydrolysis, derivatization and quantification of 7-HPG by gas chromatography–high-resolution mass spectrometry (GC-HRMS)

The method for the derivatization of 7-HPG in DNA samples has been described in detail elsewhere (5,21). The same standard solutions of 7-HPG and 7-HP [$^{13}C_4$]G used for the analysis of 7-HPG in different tissues after exposure to 500 p.p.m. PO for 20 days (5) were used for the analysis of 7-HPG in the samples described in the present study. DNA samples (2–300 µg) were spiked with 0.8 pmol of 7-HP[$^{13}C_4$]G, dried under vacuum and rehydrated in water (~0.5 µg/µl) at 4°C overnight. 7-HPG was released from DNA by neutral thermal hydrolysis (100°C, 20 min in ddH_2O), converted to the *N*7-(2-hydroxypropyl)xanthine, derivatized twice with PFBBr and analyzed by GC–HRMS. Samples were dissolved in toluene (30–50 µl) from which 1–1.5 µl samples were injected into the GC column. The molecular ion minus one pentafluorobenzyl group was measured by selected ion monitoring at m/z 569.0671 for 7-HPG and m/z 573.0806 for 7-HP[$^{13}C_4$]G. The peak area ratio between 7-HPG and 7-HP[$^{13}C_4$]G was obtained from each sample and compared with the ratio obtained with standard calibration curves. The amount of 7-HPG in each sample was then derived from calibration curves where known amounts of the 7-HPG standard were derivatized with a known amount of the internal standard, which was equivalent to the amount of internal standard added to each sample analyzed. The calibration curve solutions prepared contained 0–2.0 pmol 7-HPG with 0.8 pmol of internal standard added. The calibration curve standard solutions were analyzed as described above. Calibration curves were obtained by linear regression analysis of peak area ratio (7-HPG: 7-HP[$^{13}C_4$]G) against the amount of 7-HPG (fmol) in the standard calibration solutions. Equations for the calibration curves were then used to calculate the amount of 7-HPG in the samples from the peak area ratio (7-HPG: 7-HP[$^{13}C_4$]G). The calibration curves showed a linear relationship with 7-HPG concentration (r^2 = 0.999) with a coefficient of variability of <15% from four to eight injections. A sample (method blank) to which no internal standard or analyte standard was added was analyzed each time to check for contamination of reagents. Samples of [^{14}C]PO-modified salmon testis DNA, with a known amount of 7-HPG, were run repeatedly to check for the system performance and the method accuracy. The preparation of the [^{14}C]PO-modified salmon testis DNA solutions is described elsewhere (21). In previous reports (5,21), the concentration of 7-HPG in DNA was expressed as pmol adduct/µmol guanine. Guanine measurements were not obtained for all samples in this study due to the limited amount of DNA in the nasal respiratory tissue. Therefore, the concentration of adducts in this study is uniformly expressed as pmol adduct/mg DNA. The guanine content in rat DNA samples can be estimated from the following relationship: µmol guanine = µg DNA (0.22)/320.

GC–HRMS analysis of 7-HPG adducts

GC–HRMS chromatograms were obtained on a HP 5890 GC interfaced to a VG70-250 SEQ GC/hybrid mass spectrometer in the electron capture negative ion chemical ionization mode. The mass resolving power was 10 K. Direct injections using a press-fit liner were made onto a DB-5 fused silica capillary column (15 m × 0.32 mm). Helium head pressure was 10 psi. The ion source temperature was 250°C. Methane (3×10^{-5} mbar) was used as the reagent gas. The emission current was 0.5 mA. The injector temperature was 290°C. The temperature program was: 1 min at 70°C, 20°C/min to 290°C followed by an increment of 50°C/min to reach 300°C.

Statistical analysis

Linear regression analyses of the 7-HPG accumulation in each tissue were obtained using Excel. Differences in DNA adduct accumulation in the same tissue at different exposure levels were determined by unpaired sample t-tests.

Results

Animal exposures

The actual measured mean chamber PO concentrations (±SD) were 0, 5.0 ± 0.1, 24.1 ± 0.1, 48.5 ± 1.0, 290 ± 12 and 493 ± 12 p.p.m. for the 3 day exposures and 0, 5.0 ± 0.2, 24.2 ± 0.7,

Table I. Amount of 7-HPG (pmol adduct/mg DNA)[a] in tissues of male F344 rats exposed to PO (5, 25, 50, 300 or 500 p.p.m) by the inhalation route for 3 days (6 h/day)

Exposure (p.p.m.)	Nasal respiratory (NRE)	Lung	Liver
5	1.8 ± 0.1[b]	0.2 ± 0.1	ND
25	7.1 ± 0.4[b]	1.2 ± 0.2	0.5 ± 0.1[c]
50	13.3 ± 1.8	2.0 ± 0.1	0.9 ± 0.2
300	90.2 ± 5.7	11.1 ± 2.4	4.8 ± 0.4
500	122.6 ± 10.5	18.7 ± 0.5	7.8 ± 0.9

ND, not detected (below limits of detection).
[a]Mean ± SD ($n = 3$ animals).
[b]Two determinations, DNA from three samples was pooled and divided into two sample solutions.
[c]Two determinations, the amount of 7-HPG was too low to be quantifiable in the rest of the samples corresponding to this exposure group.

Table II. Amount of 7-HPG (pmol adduct/mg DNA)[a] in tissues of male F344 rats exposed to PO (5, 25, 50, 300 or 500 p.p.m) by the inhalation route for 20 days (6 h/day, 5 days/week)

Exposure (p.p.m)	Nasal respiratory (NRE)	Lung	Liver
5	3.5 ± 0.04[b]	0.6 ± 0.1	0.3 ± 0.04
25	19.5 ± 0.8	2.3 ± 0.3	1.1 ± 0.2
50	33.6 ± 2.5	3.8 ± 0.5	1.6 ± 0.1
300	197.9 ± 13.9	26.1 ± 1.8	8.7 ± 0.9
500	281.9 ± 18.9	45.0 ± 2.6	24.6 ± 4.7

[a]Mean ± SD ($n = 3$ animals).
[b]Two determinations, DNA from three samples was pooled and divided into two sample solutions.

49.4 ± 1.1, 293 ± 7 and 489 ± 10 p.p.m. for the 20 day exposures.

Analysis of 7-HPG in tissues

Accumulation of 7-HPG was measured in NRE, lung and liver from male F344 rats exposed to PO (0, 5, 25, 50, 300 or 500 p.p.m) for 3 or 20 days (6 h/day, 5 days/week). Results from the analysis of 7-HPG in different tissues of rats exposed to PO by the inhalation route are summarized in Tables I and II. Analysis of 7-HPG in tissues of control animals showed no evidence of endogenous formation of PO DNA adducts. The limit of detection of the method was 50 fmol/sample based on measurement of standards. Analysis of 7-HPG in NRE, lung and liver of rats exposed to PO by inhalation showed a dose-dependent increase in adduct accumulation for both exposure periods. The nose had the highest concentration of adducts followed by lung and liver, respectively, for both exposure periods. The accumulation of 7-HPG adducts in each tissue at 5 p.p.m PO was significantly different ($P < 0.05$) from the accumulation of adducts in the same tissue at 25 or 50 p.p.m. PO after 3 or 20 days of exposure. Significant differences ($P < 0.001$) were observed between high and low levels of exposure in each tissue for both lengths of exposure. The amount of 7-HPG in NRE was significantly different ($P < 0.001$) from the rest of the tissues at all concentrations and lengths of exposure. The amount of 7-HPG in lung was significantly higher than in liver ($P < 0.05$) between 25 and 500 p.p.m. for both lengths of exposure. The amount of 7-HPG in lung was not significantly different from the amount found in liver after exposure to 5 p.p.m PO for 20 days. The number of 7-HPG adducts in liver DNA of rats exposed to 5 p.p.m. PO for 3 days was below the limit of detection.

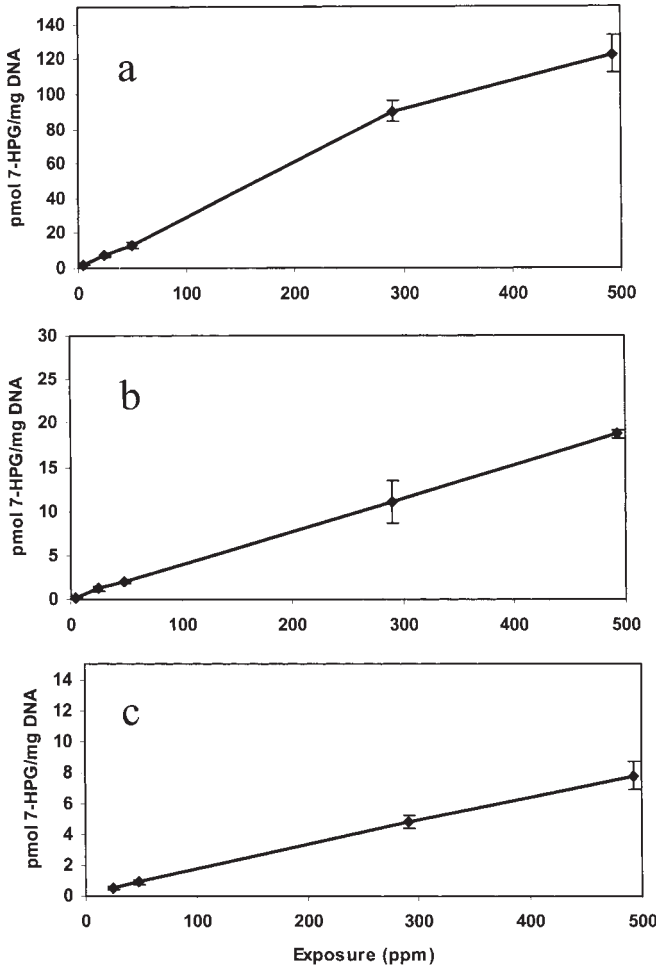

Fig. 1. Exposure–response relationship for the accumulation of 7-HPG in (**a**) nasal respiratory epithelium (NRE), (**b**) lung and (**c**) liver of male F344 rats exposed to PO for 3 days by inhalation.

A linear increase in 7-HPG accumulation in DNA was observed for all three tissues (NRE, lung and liver) after exposure for 3 days (Figure 1A–C) and for NRE and lung after 20 days of exposure (Figure 2A and B). A slightly sub-linear response in adduct accumulation was observed in liver DNA after 20 days of exposure (Figure 2C). Linear regression slopes for the lung and liver were 1/7 and 1/17 that of the nose, respectively, for the 3-day exposure period. For the 20 day exposures, linear regression slopes for lung and liver were 1/6 and 1/13 that of the nose.

Discussion

One of the main objectives of this research was to obtain information on the relationship between exposure concentration and internal dose of PO in rodent tissues. 7-HPG, the major PO DNA adduct, was selected as the marker of exposure and of PO molecular dose because it is stable and forms at high concentrations relative to other PO-induced DNA adducts.

Results from the present research: (i) demonstrated a much higher level of DNA alkylation in the NRE than in lung and liver, respectively, at all PO concentrations and exposure periods; (ii) showed a linear concentration-dependent increase in 7-HPG accumulation in NRE, lung and liver after 3 days and in NRE and lung after 20 days of exposure; and (iii) demonstrated that NRE 7-HPG adduct accumulation is not sufficient

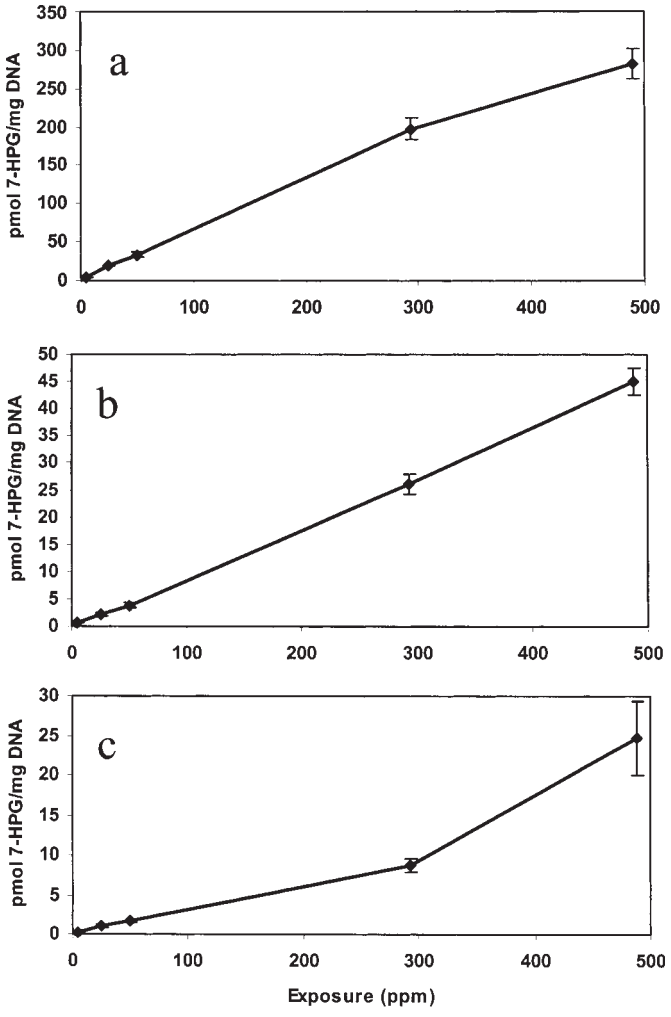

Fig. 2. Exposure–response relationship for the accumulation of 7-HPG in (**a**) nasal respiratory epithelium (NRE), (**b**) lung and (**c**) liver of male F344 rats exposed to PO for 20 days by inhalation.

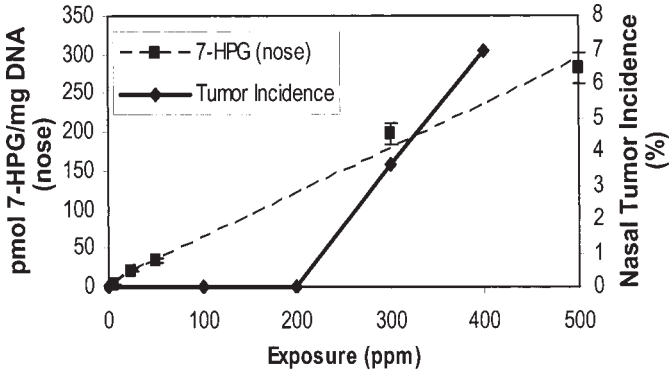

Fig. 3. Relationship of nasal adenoma incidence from the NTP bioassay (16) and 7-HPG in nasal respiratory epithelial DNA. The dashed line represents the linear regression of the 7-HPG data.

to induce tumor formation as it had a linear response, while nasal tumors were clearly non-linear (Figure 3).

The greater amount of 7-HPG adducts in the nasal respiratory tissue is the result of significant uptake of PO in the nasal passages following inhalation exposure, compared with tissues of the lower airways. The greater number of 7-HPG adducts in lung when compared to liver is most likely due to a combination of direct and systemic exposure to PO during inhalation.

The linear concentration-dependent increase in 7-HPG accumulation in all tissues after 3 days of exposure and in NRE and lung after 20 days of exposure suggests that saturation of detoxication or DNA repair does not occur in these tissues under these exposure conditions. Detoxication of PO in rat tissues occurs mainly by glutathione *S*-transferase (GST)-mediated glutathione (GSH) conjugation and by epoxide hydrolase (EH)-mediated hydrolysis (23). Recent *in vitro* studies have demonstrated that the GST and EH activities per mg of tissue are higher in rat nasal mucosa than in lung and liver (23). GSH measurements done in the same rats used in the present study revealed a concentration-dependent GSH depletion being most pronounced in the nasal tissue at the highest exposure concentration (24). In the liver, the GSH concentration following a 20 day exposure to 500 p.p.m. PO was much lower than that in rats exposed to 300 p.p.m., suggesting reduced detoxication of PO via GST. Blood concentrations (unpublished data) and hemoglobin adducts (25) of PO were also measured in these animals, with both exhibiting a sublinear response after 20 days exposure to 500 p.p.m. PO. When liver 7-HPG and hydroxypropylvaline adducts were plotted against blood PO concentration, a linear response was evident for both. Thus, it appears that the sublinear response in 7-HPG accumulation in liver is secondary to saturation of systemic detoxication following 20 days exposure to 500 p.p.m. PO. The lack of a sublinear response in lung and NRE reflects the direct exposure to PO in the air. The reduction in the ratio of NRE:liver 7-HPG at 20 days of exposure reflects saturation of detoxication and its resultant increase in hepatic 7-HPG, rather than a decrease in NRE alkylation.

DNA repair by methylpurine DNA glycosylase is thought to be the principal pathway for glycosylase-mediated removal of N7-alkylguanines from DNA. *In vivo* studies (21) demonstrated a half-life of 5–7 days for 7-HPG in rat tissues. This value compares well with the half-life of 7-HPG (5 days) obtained *in vitro* (20) suggesting that 7-HPG is lost from DNA primarily by chemical depurination, rather than by base excision repair. It has been speculated that *N*7-alkylguanine adducts are promutagenic due to an increase in spontaneous depurination of *N*7-alkylated bases from DNA leading to formation of abasic sites. A highly sensitive slot blot method for the measurement of apurinic/apyrimidinic (AP) sites was used to measure AP sites in tissues (NRE, lung, liver and testis) of control and exposed (500 p.p.m. for 20 days) male F344 rats (21). There was clear evidence of 7-HPG accumulation in DNA in all tissues under the aforementioned exposure conditions. The number of abasic sites was not increased in NRE, in spite of a 17-fold increase in the number of depurinations compared with that of normal DNA, suggesting that DNA repair of AP sites is highly efficient and that AP sites are not likely to be a source of PO mutations.

PO alkylation of other DNA bases may be responsible for the low mutagenic potential of PO. Other DNA adducts formed from the reaction of PO with DNA are *N*3-(2-hydroxypropyl) adenine, *N*3-(2-hydroxypropyl)deoxyuridine (3-HPdUrd), N^6-(2-hydroxypropyl)deoxyadenosine (N^6-HPdAdo), *N*1-(2-hydroxypropyl)deoxyadenosine (18, 19, 26, 27) and possibly *N*1-(2-hydroxypropyl)deoxyinosine.

*N*3-(2-hydroxypropyl)adenine is formed in much lower amounts than 7-HPG and is also lost rapidly by chemical

depurination. The lack of an increase in AP sites confirms that this is not a likely mechanism for mutagenesis. N^6-HPdAdo results from the rearrangement (Dimroth rearrangement) of *N*1-(2-hydroxypropyl)deoxyadenosine, whereas *N*1-(2-hydroxypropyl)deoxyinosine can form by the deamination of this initial adduct. Recent studies suggested that N^6-HPdAdo is not efficiently repaired and thus, it can accumulate in DNA (19,27). The amount of N^6-HPdAdo (measured after Dimroth rearrangement) in NRE after exposure of rats to 500 p.p.m. PO for 20 days was 2% the concentration of 7-HPG adducts in the same tissue. While the N^6-HPdAdo adduct occupies a Watson–Crick hydrogen bond position, recent studies on the related adduct of the butadiene monoepoxide demonstrated that it was only weakly mutagenic (28).

No similar studies have been done on *N*1-(2-hydroxypropyl)deoxyadenosine or *N*1-(2-hydroxypropyl)deoxyinosine. Future studies should aim to investigate the potential role of these adducts in PO carcinogenesis.

3-HPdUrd results from the hydrolytic deamination of *N*3-(2-hydroxypropyl)deoxycytidine. Alkylation at *N*3 of cytosine followed by hydrolytic deamination and formation of the uracil derivative could lead to C → T transitions and C → A transversions (29–31). The amount of 3-HPdUrd in the NRE of rats exposed to 500 p.p.m. PO for 20 days was 0.02% of the concentration of 7-HPG (19). Results from *in vitro* DNA repair studies and *in vivo* molecular dosimetry (19) suggest that enzymatic repair of *N*3-(2-hydroxypropyl)deoxycytidine occurs *in vivo* prior to conversion to the more stable 3-HPdUrd adduct. *In vivo* exposure–response studies can provide information on the importance of *N*3-(2-hydroxypropyl)deoxyuridine accumulation to tumor formation.

The linear exposure–response for 7-HPG accumulation in NRE cannot explain the tumor formation threshold observed at exposures below 300 p.p.m. in the rodent bioassay (Figure 3). Interestingly, increases in tissue inflammation (rhinitis) and cell proliferation were observed in the nasal passages of rodents exposed to concentrations of PO (≥300 p.p.m.) that were carcinogenic in the bioassays (15,16). Nasal tissue damage and increases in nasal respiratory cell proliferation have also been observed after exposure of rats to PO by the inhalation route for 1 or 4 weeks (32). In companion studies of the present research, significant increases in cell proliferation were observed only at high concentrations (≥300 p.p.m.) in the nasal respiratory epithelium of rats exposed to PO (0, 5, 25, 50, 300 or 500 p.p.m.) for 3 or 20 days (unpublished data). 7-HPG adducts were quantifiable in the nasal respiratory epithelium at concentrations (5–50 p.p.m.) that were non-carcinogenic in rodent bioassays. The exposure concentrations below 300 p.p.m. PO did not induce nasal tissue damage or increases in nasal respiratory cell proliferation in short-term inhalation exposure studies (32). These observations suggest that the formation and accumulation of 7-HPG adducts in the nasal mucosa are not a sufficient factor in PO carcinogenesis and that PO-induced cell proliferation, that is only present at high concentrations of PO (≥300 p.p.m.), may be a critical factor for tumorigenesis in this tissue.

The linear exposure–response for 7-HPG accumulation in NRE would support the use of the linearized multistage model for PO cancer risk. However, cell proliferation and DNA repair findings suggest that the accumulation of this adduct does not play a critical role in PO carcinogenesis. In addition, the amount of 7-HPG in NRE at the lowest concentration tested (5 p.p.m.) under steady-state conditions was comparable with the reported amount of other endogenously formed adducts (2 per 10^6 normal nucleotides) in humans and unexposed animals (33,34). If we assume a linear response in 7-HPG accumulation at levels relevant to workplace exposure regulations we will obtain very low amounts of 7-HPG in NRE that are unlikely to destabilize the normal level of background mutations in rodents. Estimates of PO cancer risk at low levels obtained by extrapolation of tumor data do not take into account these findings.

Data on PO DNA adduct molecular dosimetry and DNA replication in target and non-target tissues for carcinogenesis along with data on toxicokinetic parameters such as detoxication, distribution and DNA repair, should improve the accuracy of current PO cancer risk assessments.

Acknowledgements

The authors will like to thank Dr Judith Baldwin for review of data for accuracy and documentation. This research was supported in part by the Propylene Oxide/Propylene Glycol Panel and the Olefins Panel of the American Chemistry Council, the Propylene Oxide and Derivatives Sector Group of the European Chemical Industry Association (CEFIC) and NIH grants ES11746, P30-CA16086 and P30-ES10126.

References

1. NIOSH (1983) National occupational exposure survey, 1981–1983: actual observation and tradenamed exposure to propylene oxide. National Institute for Occupational Safety and Health, Division of Surveillance, Hazard Evaluation and Field Studies, Surveillance Branch, Cincinnati, OH.
2. Ehrenberg,L. and Hussain,S. (1981) Genetic toxicity of some important epoxides. *Mutat. Res.*, **86**, 1–113.
3. IARC (1994) IARC Monographs: *Working Group on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals.* IARC Scientific Publications, IARC, Lyon, **60**, pp. 1–560.
4. Giri,A.K. (1992) Genetic toxicology of propylene oxide and trichloropropylene oxide—a review. *Mutat. Res.*, **277**, 1–9.
5. Ríos-Blanco,M.N., Plna,K., Faller,T., Kessler,W., Håkansson,K., Kreuzer,P.E., Ranasinghe,A., Filser,J.G., Segerbäck,D. and Swenberg,J.A. (1997) Propylene oxide: mutagenesis, carcinogenesis and molecular dose. *Mutat. Res.*, **380**, 179–197.
6. Bootman,J., Logde,D.C. and Whalley,H.E. (1979) Mutagenic activity of propylene oxide in bacterial and mammalian systems. *Mutat. Res.*, **67**, 101–112.
7. Migliore,L., Rossi,A.M. and Loprieno,N. (1982) Mutagenic action of structurally related alkene oxides on *Schizosaccharomyces pombe*: the influence, *in vitro*, of mouse-liver metabolizing system. *Mutat. Res.*, **102**, 425–437.
8. McGregor,D., Brown,A.G., Cattanach,P., Edwards,I., McBride,D., Riach,C., Sheperd,W. and Caspary,W.J. (1991) Responses of the L5178Y mouse lymphoma forward mutation assay: V. gases and vapors. *Environ. Mol. Mutagen.*, **17**, 122–129.
9. Zamora,P.O., Benson,J.M., Li,A.P. and Brooks,A.L. (1983) Evaluation of an exposure system using cells grown on collagen gels for detecting highly volatile mutagens in the CHO/HG PRT mutation asay. *Environ. Mutagen.*, **5**, 795–801.
10. Agurell,E., Cederberg,H., Ehrenberg,L., Lindahl-Kiessling,K., Rannug,U. and Törqvist,M. (1991) Genotoxic effects of ethylene oxide and propylene oxide: a comparative study. *Mutat. Res.*, **250**, 229–237.
11. Hardin,B.D., Schuler,P.M., McGinnis,P.M., Niemeier,R.W. and Smith,R.J. (1983) Evaluation of propylene oxide for mutagenic activity in 3 *in vivo* test systems. *Mutat. Res.*, **117**, 337–344.
12. Dunkelberg,H. (1982) Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. *Br. J. Cancer*, **46**, 924–933.
13. Dunkelberg,H. (1979) On the oncogenic activity of ethylene oxide and propylene oxide in mice. *Br. J. Cancer*, **39**, 588–589.
14. Lynch,D.W., Lewis,T.R., Moorman,W.J., Burg,J.R., Groth,D.H., Khan,A., Ackerman,L.J. and Cockrell,B.Y. (1984) Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344 rats. *Toxicol. Appl. Pharmacol.*, **76**, 69–84.

15. Renne,R.A., Giddens,W.E., Boorman,G.A., Kovatch,R., Haseman,J.E. and Clarke,W.J. (1986) Nasal cavity neoplasia in F344/N rats and (C57BL/6×C3H)F_1 mice inhaling propylene oxide for up to two years. *J. Natl Cancer Inst*., **77**, 573–582.
16. National Toxicology Program (NTP) (1985) Toxicology and carcinogenesis studies of propylene oxide in F344/N rats and B6C3F1 mice (Inhalation Studies), NTP TR 267,US Dept of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, USA.
17. Kuper,C.F., Reuzel,P.G.J., Feron,V.J. and Verschuuren,H. (1988) Chronic inhalation toxicity and carcinogenicity study of propylene oxide in Wistar rats. *Food Chem. Toxicol*., **26**, 159–167.
18. Solomon,J.J., Mukai,F., Fedyk,J. and Segal,A. (1988) Reactions of propylene oxide with 2′-deoxynucleosides and *in vitro* with calf thymus DNA. *Chem. Biol. Interact*., **67**, 275–294.
19. Plna,K., Nilsson,R., Koskinen,M. and Segerbäck,D. (1999) ^{32}P-Postlabelling of propylene oxide 1- and N^6-substituted adenine and 3-substituted cytosine/uracil: formation and persistence *in vitro* and *in vivo*. *Carcinogenesis,* **20**, 2025–2032.
20. Segerbäck,D., Plna,K., Faller,T., Kreuzer,P.E., Håkansson,K., Filser,J.G. and Nilsson,R. (1998) Tissue distribution of DNA adducts in male Fischer rats exposed to 500 ppm of propylene oxide—quantitative analysis of 7-(2-hydroxypropyl)guanine by P-32-postlabeling. *Chem. Biol. Interact.,* **115**, 229–246.
21. Ríos-Blanco,M.N., Faller,T.H., Nakamura,J., Kessler,W., Kreuzer,P.E., Ranasinghe,A., Filser,J.G. and Swenberg,J.A. (2000) Quantitation of DNA and hemoglobin adducts and apurinic/apyrimidinic sites in tissues of F344 rats exposed to propylene oxide by inhalation. *Carcinogenesis*, **21**, 2011–2018.
22. Filser,J.G., Schwegler,U., Csanády, Gy,A., Greim,H., Kreuzer,P.E. and Kessler,W. (1993) Species-specific pharmacokinetics of styrene in rat and mouse. *Arch. Toxicol*., **67**, 517–530.
23. Faller,T.H., Csanády,G.A., Kreuzer,P.E., Baur,C.M. and Filser,J.G. (2001) Kinetics of propylene oxide metabolism in microsomes and cytosol of different organs from mouse, rat and humans. *Toxicol. Appl. Pharmacol*., **172**, 62–74.
24. Lee,M.S., Dhawan-Robl,M., Faller,T.H., Csanády,G.A., Kessler,W. and Filser,J.G. (2000) Propylene oxide in blood and glutathione levels in tissues of rats exposed up to 4 weeks to different concentrations of gaseous PO. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **361** (suppl. 4), R139.
25. Ríos-Blanco,M.N, Ranasinghe,A., Upton,P., Lee,M.S., Filser,J.G. and Swenberg,J.A. (2002) Exposure-dependent accumulation of *N*-(2-hydroxypropyl)valine in hemoglobin of F344 rats exposed to propylene oxide by the inhalation route. *J. Chrom. B*, **778**, 383–391.
26. Solomon,J.J. (1999) *Cyclic Adducts and Intermediates Induced by Simple Epoxides*. IARC Scientific Publications, IARC, Lyon, **150**, pp. 123–135.
27. Koskinen,M. and Plna,K. (2000) Specific DNA adducts induced by some mono-substituted epoxides *in vitro* and *in vivo*. *Chem. Biol. Interact*., **129**, 209–229.
28. Carmical,J.R., Nechev,L.V., Harris,C.M., Harris,T.M. and Lloyd,R.S. (2000) Mutagenic potential of adenine N^6 adducts of monoepoxide and diolepoxide derivatives of butadiene. *Environ. Mol. Mutagen*., **35**, 48–56.
29. Bhanot,O.S., Singh,U.S. and Solomon,J.J. (1994) The role of 3-hydroxyethyldeoxyuridine in mutagenesis by ethylene oxide. *J. Biol. Chem*., **269**, 30056–30064.
30. Zhang,W., Johnson,F., Grollman,A.P. and Shibutani,S. (1995) Miscoding by the exocyclic and related DNA adducts 3,N4-etheno-2′-deoxycytidine, 3,N4-ethano-2′-deoxycytidine and 3-(2-hydroxyethyl)-2′-deoxyuridine. *Chem. Res. Toxicol*., **8**, 157–163.
31. Kreutzer,D.A. and Essigmann,J.M. (1998) Oxidized, deaminated cytosines are a source of C → T transitions *in vivo*. *Proc. Natl Acad. Sci. USA*, **95**, 3578–3582.
32. Eldridge,S.R., Bogdanffy,M.S., Jokinen,M.P. and Andrew,L.S. (1995) Effects of propylene oxide on nasal epithelial cell proliferation in F344 rats. *Fundam. Appl. Toxicol*., **27**, 25–32.
33. Marnett,L.J. and Burcham,P.C. (1993) Endogenous DNA adducts: potential and paradox. *Chem. Res. Toxicol*., **6**, 771–785.
34. Chaudhary,A.K., Nokubo,M., Reddy,G.R., Yeola,S.N., Morrow,J.D., Blair,I.A. and Marnett,L.J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. *Science*, **265**, 1580–1582.

Received November 13, 2002; revised March 26, 2003; accepted May 4, 2003