Arch Toxicol (1993) 67: 712–713

Archives of
Toxicology

Letter to the editor

Dose-DNA adduct relationship for ethylene oxide

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Received 9 July 1993/Accepted 18 August 1993

Sir,

Toxicological implications of the DNA adduct 7-(2-hydroxyethyl)guanine in experimental animals exposed to ethylene oxide have been a matter of recent reviews (Bolt et al. 1988; Walker et al. 1990). The physiological presence of this adduct in DNA of tissues from mice, rats and humans not exposed to exogenous ethylene or ethylene oxide in a range of a few pmol/mg DNA has been mentioned in two reports (Föst et al. 1989; Walker et al. 1992). Hence, the dependence of the quantities of 7-(2-hydroxyethyl)guanine on external doses, in low dose ranges, can be based only on studies with radiolabelled, preferably ^{14}C-labelled, ethylene oxide. Potter et al. (1989) have performed such a study, but this has been published only in a preliminary form without providing full documentation. Hence, we decided to perform a similar study to compare its results with the data of Potter et al. (1989).

Potter et al. (1989) had exposed male F-344 rats, nose only, to atmospheres containing 1, 10 or 33 ppm [^{14}C] ethylene oxide for 6 h, maintaining a constant atmospheric level of radiolabelled substance throughout this entire period in the chamber, and had studied the quantities of the radiolabelled major DNA adduct in liver DNA hydrolysates.

We have now performed a new single-dose inhalation experiment, applying single doses of [^{14}C] ethylene oxide to rats in a closed recirculated system.

Three groups of five male Sprague-Dawley rats (Charles-River Wiga GmbH, Sulzfeld), weighing between 245 and 312 g, were placed in the closed all-glass exposure system of Bolt et al. (1976), volume 10.2 l, containing 100 g soda lime. Different single doses of the test chemical, [^{14}C] ethylene oxide, of specific radioactivity of 40.0 mCi/mmol (Du Pont, Boston, Mass.), specified purity of >95%, were injected into the gas phase of the system. The decline of ethylene oxide in the gas phase was monitored by repeated gas chromatography (35 m DB-1 capillary column, 0.53 mm i.d., FID, Carlo Erba, Hofheim). Extrapolation of these curves to zero time allowed the calculation of effective ethylene oxide doses (24.9, 25.7 and 83.7 μmol/kg body weight, for the three exposure groups). After complete disappearance of ethylene oxide from the atmosphere, the system was opened and the animals removed and sacrificed. Liver, spleen and lymphocytes were used for DNA isolation.

After addition of cold 7-(2-hydroxyethyl)guanine as optical marker an acid hydrolysis was performed in 0.1 M HCl (30 min at 75° C).The hydrolysate was directly taken for HPLC separation (RP-18, LiChrospher Merck, 250 × 10 mm, 10 μm, equipped with a similar pre-column, 30 × 4 mm; sample loop 1000 μl, flow rate 3 ml/min).

The following HPLC gradients were applied, using A = 0.01 M triethylammonium acetate; pH 7.0, and B = 60% methanol. 0–10 min: 100% A; 10–20 min: linear gradient to 15% B; 20–25 min: 15% B; 25–55 min: linear gradient to 25% B; 55–60 min: linear gradient to 100% B. After passing a UV detector (254 nm) fractions of 3 ml each were collected and taken for liquid scintillation counting. Typical retention times were: guanine, 19.1 min; 7-(2-hydroxyethyl)guanine, 25.8 min; adenine, 32.2 min.

The results are shown in Table 1. Adduct levels in lymphocytes (data not shown) were in a similar range as in spleen, but the low amounts of DNA isolated from rat lymphocytes made it difficult to distinguish these levels from background radioactivity. DNA adduct levels in spleen are generally about twice those in liver, in agreement with the observation of Walker et al. (1992). Adduct-specific "covalent binding indices" (CBI), calculated according to Lutz (1979), characterized ethylene oxide as a moderately potent genotoxin (Table 1).

A direct comparison with the doses used by Potter et al. (1989), who had exposed rats to constant ethylene oxide levels, needs a consideration of ethylene oxide inhalation pharmacokinetics. Filser and Bolt (1984) have demonstrated in rats that metabolism of inhaled ethylene oxide is

* This is an abridged report of the research conducted. The full data are available from the authors on request.

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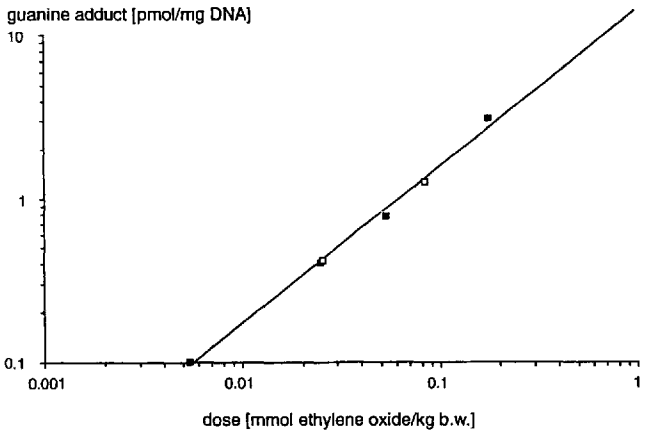

Fig. 1. Dependence of the hepatic DNA adduct 7-(2-hydroxyethyl)guanine on the dose of ethylene oxide in rats; combined data from Potter et al. (1989) (■) and this study (□), using ^{14}C-ethylene oxide

overwhelmingly determined by the process of influx into the organism ($k_{12}V_1$ = 11100 ml/h; Cl_{tot} = 10600 ml/h). This is compatible with the data of Brugnone et al. (1986) for a C_a/C_i ratio in humans of 0.1–0.3 and causes a large difference between K_{eq} = 30 and K_{st} = 1.52 (Filser and Bolt 1984). Consequently, the biologically effective inhaled ethylene oxide dose per unit time may be approximated by the total ventilated ethylene oxide dose. This means that the effective doses of rats inhaling 1, 10 or 33 ppm for 6 h in Potter's experiment are approximately by 1.34, 13.4 and 44.2 µmol (59.0, 590, 1945 µg) per 250 g animal, assuming a ventilation rate of 5 l/h for a 250-g animal (Filser 1992; Johanson and Filser 1992).

Under these assumptions, Fig. 1 combines the hepatic adduct data of the present single-dose study with those of the previous study (Potter et al. 1989). A consistency is obvious.

Walker et al. (1992) have described 7-(2-hydroxyethyl)guanine levels in tissues of rats and mice exposed for 4 weeks to daily concentrations of 100 ppm ethylene oxide (6 h/day; 5 days/week). In rats, such a treatment resulted in hepatic specific adduct levels of 66 pmol/mg DNA. A single exposure of this kind (100 ppm for 6 h) will result in about 10 pmol adduct/mg DNA (Fig. 1). This shows that a moderate accumulation of the DNA adduct 7-(2-hydroxyethyl)guanine occurs under repeated ethylene oxide exposure.

Acknowledgements. The studies were financially supported by the Verband der Chemischen Industrie, Fachvereinigung Organische Chemie, Frankfurt/M., Germany.

Table 1. Level of labelled 7-(2-hydroxyethyl)guanine in DNA of rats (n = 5, $\bar{x} \pm$ SD) exposed to single doses of [^{14}C] ethylene oxide

Effective dose administered [µmol/kg b.w.]	[^{14}C] Adduct level			
	Liver		Spleen[a]	
	fmol/mg DNA	CBI[b]	fmol/mg DNA	CBI[b]
24.9	410± 34	5.0	660	9.8
25.7	420±111	5.0	960	11.4
83.7	1300±287	4.6	1900	5.7

[a] DNA pooled from 5 animals

[b] CBI (Lutz et al. 1979) = $\frac{\text{mmol ethylene oxide bound per mol nucleotides}}{\text{mmol ethylene oxide administered per kg body weight}}$

References

Bolt HM, Kappus H, Buchter A, Bolt W (1976) Disposition of [1, 2-^{14}C] vinyl chloride in the rat. Arch Toxicol 35: 153–162

Bolt HM, Peter H, Föst U (1988) Analysis of macromolecular ethylene oxide adducts. Int Arch Occup Environ Health 60: 141–144

Brugnone F, Perbellini L, Faccini GB, Pasini F, Bartolucci GB, De Rosa E (1986) Ethylene oxide exposure. Biological monitoring by analysis of alveolar air and blood. Int Arch Occup Environ Health 58: 105–120

Filser JG (1992) The closed chamber technique – uptake, endogenous production, excretion, and rates of metabolism of gases and vapours. ArchToxicol 66: 1–10

Filser JG, Bolt HM (1984) Inhalation pharmacokinetics based on gas uptake studies VI: Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. Arch Toxicol 55: 219–223

Föst U, Marczynski B, Kasemann R, Peter H (1989) Determination of 7-(2-hydroxyethyl)guanine with gas chromatography/mass spectrometry as a parameter for genotoxicity of ethylene oxide. Arch Toxicol Suppl 13: 250–253

Johanson G, Filser JG (1992) Experimental data from closed chamber gas uptake studies in rodents suggest lower uptake rate of chemical than calculated from literature values on alveolar ventilation. Arch Toxicol 66: 291–295

Lutz WK (1979) In vivo covalent binding of chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat Res 23: 283–295

Potter D, Blair D, Watson WP, Wright AS (1989) The relationship between alkylation of haemoglobin and DNA in Fischer 344 rats exposed to [^{14}C] ethylene oxide. Arch Toxicol Suppl 13: 254–257

Walker VE, Fennell TR, Boucheron JA, Fedtke N, Ciroussel F, Swenberg JA (1990) Macromolecular adducts of ethylene oxide: a literature review and a time-course study on the formation of 7-(2-hydroxyethyl)guanine following exposures of rats by inhalation. Mutat Res 233: 151–164

Walker VE, Fennell TR, Upton PB, Skopek TR, Prevost V, Shuker DEG, Swenberg JA (1992) Molecular dosimetry of ethylene oxide: formation and persistence of 7-(2-hydroxyethyl)guanine in DNA following repeated exposures of rats and mice. Cancer Res 52: 4328–4334