Is Ambient Ethene a Cancer Risk Factor?

M. Törnqvist

Department of Radiobiology, Stockholm University, Stockholm, Sweden

Ethene is, on a molar basis, a major urban air pollutant. It has been shown beyond doubt that a fraction of inhaled ethene is metabolized in mammals (including humans) via ethylene oxide, an electrophilic reagent that has been shown to be mutagenic and carcinogenic. To the extent that the linearity hypothesis for dose-response relationships at low levels is accepted, exposure to ethene is therefore expected to lead to a risk increment. In order to judge whether ethene as a single compound should be considered a risk factor, it has to be evaluated whether this risk increment is negligibly small or of concern to individuals or societies. The magnitude of the cancer risk from ethene cannot be inferred from animal experiments. Because of saturation of the metabolism of ethene, sufficient statistical power cannot be attained in long-term animal tests with about 100 animals per dose. By application of the radiation-dose equivalent of the unit of target dose of ethylene oxide and using the best (although still uncertain) value for the conversion factor (about 5%), exposure to 10 ppb ethene—a level occurring in urban areas—is expected to lead to a lifetime risk of cancer death amounting to approximately 70 per 100,000. According to a recent estimate the average exposure in Sweden to ethene is some six times lower. These figures are uncertain by a factor of at least three. They indicate ethene to be a risk factor of concern. — Environ Health Perspect 102(Suppl 4):157–160 (1994).

Key words: ethene, ethylene oxide, metabolism, risk identification, hemoglobin adducts, genotoxic potency, cancer risk, air pollution

Introduction

The gas phase of automotive engine exhausts and consequently of polluted urban air contains a number of unsaturated hydrocarbons (i.e., alkenes, alkadienes). Particularly ethene occurs at relatively high concentrations (1), some 10^5 times higher than that of, for example, benzo[α]pyrene. Alkenes are known to be metabolized in vivo via epoxides, as was first shown for ethene (2–4) and later for butadiene (4). Because 1,2-epoxides are electrophilically reactive and have been shown to be mutagenic and carcinogenic (5), the parent alkenes also should be suspected of being potential environmental carcinogens. The alkenes ethene (6), propene (7), and 1,3-butadiene (8) have been subjected to long-term cancer tests with rodents, only the latter showing a positive result.

The reason why a carefully conducted cancer test with ethene in the Fischer 344 rat did not show any change of the tumor incidence may be attributed to saturation of the metabolism, limiting the ethylene oxide dose achievable to approximately one-fourth of the dose that would permit detection, at reasonable statistical power, of raised cancer incidence in a test with some 100 animals (Figure 1) (9,10). The positive response to butadiene probably is because of partial for-

mation also of diepoxybutane, the mutagenic and carcinogenic effectiveness of which is greater than that of the monooxiranes by some two orders of magnitude (5).

This paper summarizes efforts to elucidate the question: To what extent is ambient ethene to be considered a risk factor of concern? This approach thus illustrates the quantitative aspect of risk identification, a risk-management function that is qualitative in principle (11).

The previously mentioned inability of ethene to provoke a significant increase in cancer tests of conventional scope might at first sight be taken to indicate that the compound is innocuous. In view of the low resolving power of cancer tests, however, this is not necessarily true. Considering that the metabolite, ethylene oxide, is judged to be a probable human carcinogen (12), it has to be investigated whether the cancer risk associated with ambient concentrations of ethene, in urban environments mostly at parts per billion levels, is of nonnegligible magnitude.

Because it has not been possible to extrapolate the risk of ethene from animal test data, other ways must be sought out. These must comprise in vivo dose measurement of ethylene oxide, the reactive metabolite, and establishment of the relationship between in vivo dose and risk. In the present study, the latter has been done by determination of the radiation-dose equivalent (rad-equivalent) of a unit of chemical dose, given as millimolar-hour (mMhr), the dose defined as the integral over time of concentration (13). Besides development of a technique for in vivo dose monitoring, a number of ques-

tions concerning person-weighted exposure doses, metabolism in animals and man, etc., had to be answered in order to judge whether ethene should be considered a risk factor. The rad-equivalent approach appears to be useful for the identification and estimation of risks from many environmental genotoxic chemicals for which disease-epidemiological or cancer-test data are unavailable (14). Monitoring of the in vivo dose of a reactive chemical or its metabolites is based on the measurement of reaction products with (adducts to) macromolecules in tissues. This association of risk with a chemical end point increases the sensitivity by several orders of magnitude as compared to observation of biological end points, a fact
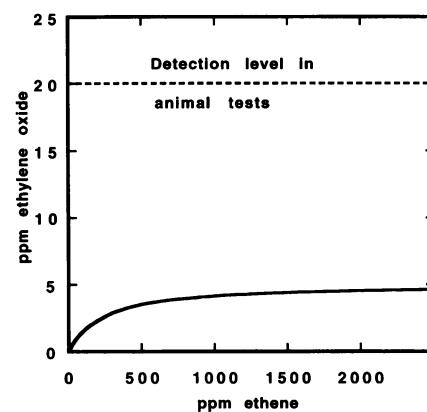

Figure 1. Saturation of the metabolism of ethene illustrated by the external concentrations of ethylene oxide (ordinary axis) and ethene (abscissa) that give the same in vivo dose of ethylene oxide. The maximum approached, 5 ppm, is below the detection level (----) in a regular cancer test (10).

that renders the method generally useful for risk identification.

Methods

It was early shown by Miller and Miller (15) that most genotoxic chemicals (i.e., cancer initiators and mutagens) are electrophilic reagents or are metabolized to such reagents. Electrophiles (e.g., alkylating agents such as epoxides) react with nucleophilic atoms in DNA, the target for biological effects. Reaction products with nucleophilic atoms (S, N, O) in other macromolecules, especially certain amino acids in proteins, are parallelly formed.

Determination of adducts to DNA and proteins offers a means of identifying genotoxic risk factors in vivo. Hemoglobin (Hb) has been found suitable as a monitor molecule because it is accessible in large amounts and because Hb adducts have a long, well-defined life-span and can be identified chemically by available methods. Tissue doses useful for risk estimation can be calculated from levels of Hb adducts (16).

Hb adduct monitoring was shown to be useful for risk identification and risk estimation, but the lack of a sensitive, rapid, and reproducible analytical method limited applications. For this reason, a large effort was put into the development of a new method for Hb adduct analysis with a sensitivity sufficient for applications connected with urban air pollution.

The nitrogen in the N-terminal amino acids, valines, in the two α- and β-chains in Hb, is a major reaction site for epoxides and other alkylating agents (16). This was one reason to try the Edman sequencing technique for isolation of Hb adducts. When this procedure was applied to [^{14}C]ethylene oxide-alkylated globin, it was observed (17) that radioactive material could be extracted already from the weakly alkaline coupling medium in which the protein is treated with the Edman reagent. This work resulted in a procedure, the N-alkyl Edman method (18,19), in which globin samples are derivatized with pentafluorophenyl isothiocyanate in formamide. Alkylated N-terminal valines are then extracted as pentafluorophenylthiohydantoins, which, following purification steps, are analyzed with high sensitivity by gas chromatography-mass spectrometry with chemical ionization in the negative ion mode.

Results

Doses of Ethylene Oxide in Ethene-exposed Humans

A problem of basic importance in risk assessment of ethene is the relationship between exposure dose of ethene and dose in tissues of the ultimate carcinogen, ethylene oxide. In an early phase of this work, this ratio was established in animal models.

Segerbäck (3) measured, by means of Hb adducts, doses in mice that were compatible with 8% of inhaled ethene being metabolically converted to ethylene oxide. In this work, radiolabeled ethene was used, and from the level of DNA adducts in various tissues, it was concluded that the dose was approximately the same in different parts of the body. This means that the measured blood dose is relevant to the target dose in various organs. Törnqvist et al. (19) used the N-alkyl Edman method to determine in vivo doses of ultimate carcinogens from alkenes in hamsters and rats exposed to automotive engine exhausts. Through the determination of N-(2-hydroxyethyl) valine (HOEtVal), the adduct formed in the reaction of ethylene oxide with N-terminal valine, it was found that 5 to 10% of inhaled ethene in the exhaust was metabolized to ethylene oxide. The uncertainty in the determination was mainly due to difficulties in correctly assessing time-weighted average exposure concentrations. The exposures were carried out with diesel exhaust, with and without particle filter, three and two doses, respectively; and with gasoline exhaust, with and without catalyst, at two doses. Adduct levels were shown to be linearly dependent on exposure level. When a catalytic converter was used, the adduct levels in animals exposed to gasoline exhaust were strongly reduced in agreement with the reduced ethene exposure.

Although these experiments showed approximately the same rate of conversion of inhaled ethene to ethylene oxide in three animal species and thus invites to extrapolation to other species, it was considered important to prove that ethene is metabolized in the same way in man.

The conversion of ethene to ethylene oxide in humans has been studied in cigarette smokers; a steady-state adduct increment has been shown amounting to about 8 pmole HOEtVal/g globin/smoked cigarette and day (20,21), a value confirmed by Bailey et al. (22) using a modification of the N-alkyl Edman method. A conclusion from these studies is that about 6% of about 0.25 mg ethene in the mainstream smoke from each cigarette is converted to ethylene oxide. Studies of persons with occupational exposure to ethene e.g., fruit-store workers (23) and plastics industry workers (Törnqvist et al., unpublished material), show values in the range of 1 to

10% conversion. This uncertainty is mainly due to difficulties of correctly assessing the exposure to ethene. For that reason, human inhalation studies with controlled exposure have been conducted with results indicating about 2% conversion but a three times longer half-life in vivo of ethylene, i.e., in support of the in vivo dose per inhaled milligram of ethene as concluded from the smoker study (manuscript in preparation). In a pharmacokinetic study in humans, Shen et al. (24) found about 4% of inhaled ethene to be metabolized in the body.

The previously mentioned judgments were based on dose monitoring in persons occupationally exposed to ethylene oxide, where the steady-state level of HOEtVal was found to be 2.4 nmoles/g globin during exposure to 1 ppm ethylene oxide, 40 hr/week (25).

Hemoglobin Adducts from Ethene in Urban Air

The present version of the N-alkyl Edman method is sufficiently sensitive to measure adduct levels down to a few pmole per gram globin (i.e., the increments expected to be associated with chronic exposure to a few parts per billion of ethene). Such measurements, however, are obscured by the existence of a background level of HOEtVal in Hb. In addition, epidemiological studies of the variation of the adduct level have not yet been carried out on populations sufficiently differing in degree of urbanization (26,21).

The background varies in the range from 8 to 25 pmole HOEtVal/g globin and has been found in animal experiments to be partly caused by ethene formation from food and intestinal bacteria (27). In measurements of ethene exhaled by humans, it was confirmed that endogenously formed ethene is the main source of the background HOEtVal level (28), allowing for a small contribution (about 5 pmole/g globin) from urban air and/or passive smoking (29). It has also been shown that at these low levels artifact formation of HOEtVal during storage of Hb samples must be prevented (30).

Measurement of ethene at four sites in Stockholm 1987 (31) showed average levels around 25 ppb in areas with heavy traffic. As average exposure doses for urban areas or the whole country had not been determined at that time and HOEtVal measurements because of the background gave too uncertain information, the time-weighted average levels of ethene were estimated indirectly via carboxyhemoglobin increments in urban citizens (32,33). These deliberations were compatible with an aver-

age ethene exposure to the Swedish population in the range 10 to 20 ppb. Although this level may still occur in very urbanized areas, the average exposure level in Sweden was recently estimated to about 1.5 ppb (see below). An ethene concentration of 10 ppb, a reasonable value in urban areas, is expected to give an incremental adduct level of 4 pmole HOEtVal/g globin. Because of the variable background, an increment of this size could be well determined only in very large populations. In Table 1, the estimated average increments of HOEtVal from various sources of ethylene oxide and ethene have been compiled.

Table 1. Estimated average increment on N-(2-hydroxyethyl)valine (HOEtVal) in hemoglobin from different sources of ethylene oxide or ethene.

Exposure	Observed increment of hydroxyethylvaline, pmole/g	Reference
Ethylene oxide, time-weighted average 1 ppm, 40 hr/week	2400	(25)
Ethene, time-weighted average 1 ppm, 40 hr/week	≈ 100	(21)
Ethene in tobacco smoke, 10 cigarettes/day	85	(21)
Urban air pollution, 10 ppb ethene, 168 hr/week	≈ 4 [a]	(21)
Background in nonsmokers, mainly endogenous ethene production	8–25	(21)

[a] Estimated.

Discussion

In the rad-equivalence approach, the dose of acute γ- or X-radiation, which produces the same frequency of mutation (or other genotoxic changes) as 1 mMhr of a chemical under study is determined. This approach is based on the unproved but reasonable assumption that an initiated cell has the same chance of giving rise to a tumor, irrespectively of whether it was initiated by a mutagenic chemical or by low-linear energy transfer radiation, chosen as reference standard because it is the environmental factor that is best investigated with respect to dose-risk relationships. A comparison of this kind (of a genotoxic chemical with ionizing radiation) is facilitated by the fact that at low doses the effects of both types of agents may be fitted to linear dose-response curves. Through this approach influences of promotive and cocarcinogenic conditions prevailing in human populations are implicitly estimated and allowed for (14).

In a broad range of test systems, 1 mMhr of ethylene oxide gives approximately the same response as 80 rad (0.8 Gy) of γ-radiation (34). Data from the various studies

of uptake and metabolism of ethene and ethylene oxide are further compatible with ethene giving an in vivo dose of ethylene oxide, which is about 5% of the dose received following direct inhalation of ethylene oxide. The dose received by personnel occupationally exposed to ethylene oxide has been estimated to correspond to 20 rad-equivalent/year ppm, with 1600 hr exposure per year (25).

From these data, the rad-equivalent of the annual dose of ethylene oxide from ethene at an ambient concentration of 10 ppb is calculated to be about 0.05 rad-equivalent per year.

The cancer risk associated with an exposure to 10 ppb ethene would thus be about one-half of the risk because of background radiation (about 0.1 rad/year). An analysis of various data indicates that at low-dose rate the risk may be some four times lower (35) than has been estimated by the National Research Council (36). According to this, the lifetime cancer mortality risk at 10 ppb ethene would be approximately 7×10^{-4}.

Recent figures (35,37) indicate the average ethene level in Sweden to be 1.8 μg/m³ (approximately 1.5 ppb). This would mean an average lifetime risk in Sweden (8.4 million) of 1×10^{-4}, corresponding to some 13 cancer deaths and about twice as many cases of disease annually in the country (35). These figures for the consequences of today's ethene exposure are thus some five times lower than the ones estimated to be valid 10 years ago. They might, if ethene were the sole urban air pollutant because of the evaluation philosophy chosen, be considered close to the lower limit of unacceptability.

This identification of ethene as a risk factor calls for two kinds of action: A more accurate estimation of the magnitude of this risk, which, given the exposure level assumed here, has to be considered uncertain by at least a factor three; and measures to be taken, such as catalytic conversion in fuel burning, for reduction of this risk.

REFERENCES

1. NRCC. Ethylene in the environment: scientific criteria for assessing its effects on environmental quality. NRCC Publication No. 22496. Ottawa, Canada:National Research Council of Canada, 1985.
2. Ehrenberg L, Osterman-Golkar S, Segerbäck D, Svensson K, Calleman CJ. Evaluation of genetic risks of alkylating agents. III. Alkylation of haemoglobin after metabolic conversion of ethene to ethene oxide in vivo. Mutat Res 45:175–184 (1977).
3. Segerbäck D. Alkylation of DNA and hemoglobin in the mouse following exposure to ethene and ethene oxide. Chem Biol Interact 45:139–151 (1983).
4. Filser JG, Bolt HM. 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 (1984).
5. Ehrenberg L, Hussain S. Genetic toxicity of some important epoxides. Mutat Res 86:1–113 (1981).

6. Hamm TE Jr, Guest D, Dent JG. Chronic toxicity and oncogenicity bioassay of inhaled ethylene in Fischer-344 rats. Fundam Appl Toxicol 4:473–478 (1984).
7. Quest JA, Tomaszewski JE, Haseman JK, Boorman GA, Douglas JF, Clarke WJ. Two-year inhalation toxicity study of propylene in F344/N rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 76:288–295 (1984).
8. Melnick RL, Huff J, Chou BJ, Miller RA. Carcinogenicity of 1,3-butadiene in C57BL/6xC3H F$_1$ mice at low exposure concentrations. Cancer Res 50:6592–6599 (1990).
9. Osterman-Golkar S, Ehrenberg L. Covalent binding of reactive intermediates to hemoglobin as an approach for determining the metabolic activation of chemicals: ethylene. Drug Metabol Rev 13:647–660 (1982).
10. Bolt HM, Filser JG. Kinetics and disposition in toxicology. Example: carcinogenic risk estimate for ethylene. Arch Toxicol 60:73–76 (1987).

11. O'Riordan T. Environmental impact analyses and risk assessment in a management perspective. In: Energy Risk Management (Goodman GT, Rowe WD, eds). New York:Academic Press, 1979;21–36.

12. IARC. Allyl compounds, aldehydes, epoxides and peroxides. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 36. Lyon:International Agency for Research on Cancer, 1985.

13. Ehrenberg L, Moustacchi E, Osterman-Golkar S. Dosimetry of genotoxic agents and dose-response relationships of their effects. Mutat Res 123:121–182 (1983).

14. Wright AS, Bradshaw TK, Watson WP. Prospective detection and assessment of genotoxic hazards: a critical appreciation of the contribution of L. Ehrenberg. In: Methods for Detecting DNA-damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Neill IK, eds). IARC Scientific Publications No. 89. Lyon:International Agency for Research on Cancer, 1988;237–248.

15. Miller EC, Miller JA. Mechanisms of chemical carcinogenesis: nature of proximate carcinogens and interactions with macromolecules. Pharmacol Rev 18:805–838 (1966).

16. Ehrenberg L, Osterman-Golkar S. Alkylation of macromolecules for detecting mutagenic agents. Teratog Carcinog Mutag 1:105–127 (1980).

17. Jensen S, Törnqvist M, Ehrenberg L. Hemoglobin as a dose monitor of alkylating agents. Determination of alkylation products of N-terminal valine. In: Individual Susceptibility to Genotoxic Agents in the Human Population (de Serres FJ, Pero RW, eds). Environmental Science Research 30. New York:Plenum Press, 1984;315–320.

18. Törnqvist M, Mowrer J, Jensen S, Ehrenberg L. Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. Anal Biochem 154:255–266 (1986).

19. Törnqvist M, Kautiainen A, Gatz RN, Ehrenberg L. Hemoglobin adducts in animals exposed to gasoline and diesel exhausts. I. Alkenes. J Appl Toxicol 8:159–170 (1988).

20. Törnqvist M, Osterman-Golkar S, Kautiainen A, Jensen S, Farmer PB, Ehrenberg L. Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. Carcinogenesis 7:1519–1521 (1986).

21. Törnqvist M. Monitoring and cancer risk assessment of carcinogens, particularly alkenes in urban air. Ph.D. Thesis. Stockholm University, Stockholm, Sweden, 1989.

22. Bailey E, Brooks AGF, Dollery CT, Farmer PB, Passingham BJ, Sleightholm MA, Yates DW. Hydroxyethylvaline adduct formation in haemoglobin as a biological monitor of cigarette smoke intake. Arch Toxicol 62:247–253 (1988).

23. Törnqvist M, Almberg J, Bergmark E, Nilsson S, Osterman-Golkar S. Ethylene oxide doses in ethene-exposed fruit store workers. Scand J Work Environ Health 15:436–438 (1989).

24. Shen J, Kessler W, Denk B, Filser JG. Metabolism and endogenous production of ethylene in rat and man. Arch Toxicol Suppl 13:237–239 (1989).

25. Duus U, Osterman-Golkar S, Törnqvist M, Mowrer J, Holm S,

Ehrenberg L. Studies of determinants of tissue dose and cancer risk from ethylene oxide exposure. In: Proceedings of the Symposium on Management of Risk from Genotoxic Substances in the Environment (Freij L, ed). Solna, Sweden:Swedish National Chemicals Inspectorate, 1989;141–153.

26. Törnqvist M. Search for unknown adducts: increase of sensitivity through preselection by biochemical parameters. In: Methods for Detecting DNA-damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Niell IK, eds). IARC Scientific Publications No. 89. Lyon:International Agency for Research on Cancer, 1988;378–383.

27. Törnqvist M, Gustafsson B, Kautiainen A, Harms-Ringdahl M, Granath F, Ehrenberg L. Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethene and ethylene oxide. Carcinogenesis 10:39–41 (1989).

28. Filser JG, Denk B, Törnqvist M, Kessler W, Ehrenberg L. Pharmacokinetcis of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin due to endogenous and environmental ethylene. Arch Toxicol 66:157–163 (1992).

29. Persson K-A, Berg S, Törnqvist M, Scalia-Tomba G.-P, Ehrenberg L. Note on ethene and other low-molecular weight hydrocarbons in environmental tobacco smoke. Acta Chem Scand B42:690–696 (1988).

30. Törnqvist M. Formation of reactive species that lead to hemoglobin adducts during storage of blood samples. Carcinogenesis 11:51–54 (1990).

31. Persson K, Almén J. Characterization of light hydrocarbons and other volatile organic compounds in Stockholm air. Report No. 3820. Solna, Sweden:Swedish Environmental Protection Agency, 1990.

32. Almberg J. A pilot study on causes, associated with urbanization, of cancer in respiratory organs among construction workers in Sweden. Masters Thesis. Department of Radiobiology, Stockholm University, Stockholm, Sweden, 1986.

33. Törnqvist M, Ehrenberg L. Approaches to risk assessment of automotive engine exhausts. In: Complex Mixtures and Cancer Risk (Vainio H, Sorsa M, McMichael AJ, eds). IARC Scientific Publications No. 104. Lyon:International Agency for Research on Cancer, 1990;277–287.

34. Kolman A, Segerbäck D, Osterman-Golkar S. Estimation of the cancer risk of genotoxic chemicals by the rad-equivalence approach. In: Methods for Detecting DNA-damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Niell IK, eds). IARC Scientific Publications No. 89. Lyon:International Agency for Research on Cancer, 1988;258–264.

35. Törnqvist M, Ehrenberg L. On cancer risk estimation of urban air pollution. Environ Health Perspect 102(Suppl 4):173–181 (1994).

36. National Research Council, Committee on the Biological Effects of Ionizing Radiations. Health effects of exposure to low levels of ionizing radiation. BEIR V Report. Washingon:National Academy Press, 1990.

37. Boström C-E, Almén J, Steen B, Westerholm R. Human exposure to urban air pollution. Environ Health Perspect 102(Suppl 4):39–47 (1994).