Mutations Induced in the Hypoxanthine Phosphoribosyl Transferase Gene by Three Urban Air Pollutants: Acetaldehyde, Benzo[*a*]pyrene Diolepoxide, and Ethylene Oxide

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Provisional mutational spectra at the hypoxanthine phosphoribosyl transferase (HPRT) locus *in vitro* have been worked out for acetaldehyde (AA) and benzo[*a*]pyrene diolepoxide (BPDE) in human (T)-lymphocytes and for ethylene oxide (EtO) in human diploid fibroblasts using Southern blotting and polymerase chain reaction (PCR)-based DNA sequencing techniques. The results indicate that large genomic deletions are the predominating hprt mutations caused by AA and EO, whereas BPDE induces point mutations that are mainly GC>TA transversions. The mutational spectra induced by the three agents are clearly different from the background spectrum in human T-cells. Thus, the hprt locus is a useful target for the study of chemical-specific mutational events that may help identify causes of background mutation in human cells *in vivo*. — Environ Health Perspect 102(Suppl 4):135–138 (1994).

Key words: acetaldehyde, benzo[*a*]pyrene diolepoxide, ethylene oxide, HPRT, human T-cells, molecular analysis, mutation, PCR

Introduction

Humans are exposed to many compounds in ambient air that are known to cause genetic damage in various test systems. The possible consequences of this exposure include somatic mutation in critical target genes that may lead to cancer and germ line mutation that may lead to genetic disease in the offspring. To elucidate the course of events resulting in these severe consequences, it is important to study the frequency and spectrum of background mutation in human cells *in vivo*. It is also important to determine the relative contribution of mutation induced by environmental agents as opposed to spontaneous mutation caused by endogenous factors. This research should be carried out for each specific gene locus and cell type of interest because genes may differ with regard to mutation rates and the types and phenotypic consequences of mutation. Moreover, the same genes in different cell types may respond differently to mutagens depending on factors such as transcriptional activity and DNA repair activity (*1*).

The development of methods for the analysis of mutation at the human locus for the purine salvage enzyme hypoxanthine phosphoribosyl transferase (HPRT) has offered several unique possibilities for the evaluation of human *in vivo* mutagenesis (*2–4*). Human T-cell cloning can be performed with high cloning efficiency, and selection for HPRT mutants in mediums containing 6-thioguanine yields reliable and reproducible estimates of the mutation frequency in the T-cell population from the peripheral blood of individual subjects (*5–9*). Information with regard to the clonality and origin of HPRT mutation is obtained by molecular analysis of the clone-specific T-cell receptor rearrangement (*10*).

A further advantage of the human T-cell mutational system is that the nucleotide sequence of the entire human HPRT gene of more than 44 kb has been worked out (*11*). This provides a variety of tools and approaches for the molecular analysis of HPRT mutation, including methods for screening of mutations such as ribonuclease mismatch cleavage (*12*), denaturing gradient gel electrophoresis (*13*), constant denaturant gel electrophorisis (*14*), multiplex PCR (*15*), as well as direct sequencing of PCR products derived from genomic HPRT DNA and HPRT–cDNA (*16–18*).

Another important aspect of human HPRT-mutation analysis is the possibility to study germ line mutation. Constitutional HPRT deficiency in humans gives rise to Lesch-Nyhans syndrome (LNS) or X-linked gout. The former is a clinically well-defined condition caused by complete or almost complete HPRT deficiency. The latter is a less severe disease associated with symptoms of gout and renal stones at young age in which HPRT deficiency is reduced but not complete (*19*). Almost 100 LNS- and HPRT-deficient gout patients have been studied with respect to the molecular nature of the HPRT mutation, thus providing information on the mutational spectrum, causes, and mechanisms of human germ line mutagenesis (*4,20*).

The human T-cell cloning assay can also be used to study HPRT mutation induced by specific chemical agents (*18,21–23*) and radiation (*24*) *in vitro*. These studies are needed to provide information on the possible contribution of environmental mutagens to the type and frequency of background mutation *in vivo*. As demonstrated in the present work, some agents induce a mutational spectrum in T-cells that is characteristically different from the spectrum of

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Table 1. Types of mutation at the human hprt locus and methods for their detection (*4*).

Type of mutation	Method	Comment
Chromosomal mutation	Cytogenetic karyotyping	No mutation of this type has been detected so far
Gross structural alterations	Southern blot analysis about	Only deletions larger than about 100 bp are detected
Point mutations	Sequencing of PCR-amplified cDNA or genomic DNA	
Missense mutation		Coding error causing an amino acid substitution
Nonsense mutation		Coding error causing a stop codon
Frameshift mutation		Small deletion / insertion affecting the reading frame
Splice mutation		Change affecting sequence involved in splicing functions

Table 2. Point mutations at the hypoxanthine phosphoribosyl transferase locus in germ line and somatic cells (*20,26,31,37*).

Type of point mutation	Lesch-Nyhan and gout patients	TG-resistant T-lymphocytes
Amino acid substitution		
Missense	46 (53%)	36 (40%)
Nonsense	8 (9%)	8 (9%)
Small deletions/ insertion (frameshift)	20 (23%)	17 (19%)
Splice mutations	12 (14%)	29 (32%)
All point mutations	86 (100%)	90 (100%)

background mutation as well as from that of other agents. This information may be used in order to deduce the environmental causes of human mutation *in vivo*.

Hence, the human HPRT mutational assay offers unique possibilities to compare the spectrum of mutation in human germ line and somatic cells with that induced by specific mutagens at the same locus *in vitro*. In this article, we report on our studies of mutation induced *in vitro* by three urban air pollutants, acetaldehyde (AA), benzo[*a*]pyrene diolepoxide (BPDE), and ethylene oxide (EtO).

Materials and Methods

Mutation induction of AA and BPDE was studied in freshly prepared human lymphocytes from male donors using the T-cell cloning method (*22,23*). EtO-induced mutations were studied in human diploid VH-10 fibroblast cultures at early passages (*25*). Mutant clones were selected in mediums containing 6-thioguanine after the appropriate expression time of 8 to 10 days. RNA and DNA preparation, Southern blotting, PCR of genomic HPRT DNA, HPRT–cDNA, and DNA sequencing were carried out as described earlier (*18,26*).

The types of mutation studied are shown in Table 1. Chromosomal mutation affecting the HPRT locus at Xq26 still has not been detected in any of more than 100 human T-cell mutants studied (*27,28*), and this type of mutation will not be further discussed here.

Results

The Background Spectrum of HPRT Mutation

Gross structural alterations detected by Southern blot analysis account for about 10 to 15% of the background spectrum of HPRT mutations in T-lymphocytes from adult donors (*29*) and in patients with LNS (*30*). The background spectrum of point mutation at the HPRT locus in human T-cells was compiled and discussed by Hou et al. recently (*26*). These data are shown in Table 2 together with data on germ line HPRT mutation derived from studies of LNS and gout patients (*4,20*). It is obvious that the types and relative frequencies of the various types of mutation in germ line and somatic cells are similar, with the possible exception that splicing mutations seem to be less frequent in the germ line than in the somatic cells, as pointed out by Rossi et al. (*31*).

Although almost 100 somatic and germ line-point mutations have been mapped in the human HPRT gene, very few have been found to recur at the same site within the HPRT coding sequence of 654 base pairs. Thus, the mutations are widely dispersed along the coding sequence, and there are no predominating mutational hot spots (*4,20,26*).

Acetaldehyde-induced HPRT Mutation

Human exposure to AA occurs regularly because of its production by endogenous metabolism and its presence in the environment (e.g., automobile exhausts, cigarette smoke, ambient air). The genetic toxicity of AA has been demonstrated in a variety of test systems, and there is evidence for its carcinogenicity in experimental animals (*32*). The ability of AA to induce HPRT mutation in human T-lymphocytes *in vitro* was demonstrated by He and Lambert (*22*). Cells treated with 1.2 to 2.4 mM of AA for 24-hr, or 0.2 to 0.6 mM for 48 hr showed a dose dependent, 3- to 16-fold increase of the mutant frequency. At the highest concentration of AA, 40% of the T-cell mutants were found to have large genomic deletions detectable by Southern blot analysis. In contrast, the frequency of large deletions in untreated cultures was only about 10%, which is similar to the frequency of gross structural alterations in the background spectrum of HPRT mutation (discussed above). All of the AA-induced deletions were found to extend into the 3′ -flanking region of the HPRT gene, whereas the deletions in the control cultures mainly affected the 5′ part of the gene (*22*). Thus, large 3′-flanking deletions may be an important type of HPRT mutation induced by high dose AA *in vitro*. Nevertheless, 60% of the mutations in the treated cultures showed normal Southern blot patterns, indicating that the majority of AA-induced mutations in human T-cells are likely to be point mutations. The nature of these mutations are not known yet.

BPDE-induced HPRT Mutation

BPDE is a carcinogenic *in vivo* metabolite of benzo[*a*]pyrene, one of the most well-known combustion products in vehicle exhausts, cigarette smoke, and ambient air. There are four different stereochemical forms of BPDE, and the (+)-anti form is the most potent mutagen in mammalian cells (*33*). The frequency of HPRT mutation in human T-cells treated with 0.3 to 0.6 μM of (+)-antiBPDE for 24 hr was found to increase in a dose-dependent way up to 50 times the frequency in untreated control cultures (*23*). Thus, BPDE is a potent mutagen in this system. No large alterations of the HPRT gene could be detected by Southern blot analysis in the BPDE-induced T-cell mutants, which shows that BPDE induces mainly point mutations. Direct sequencing of HPRT cDNA and genomic HPRT DNA from the mutant clones showed that the predominating type of base pair substitution induced by BPDE in the coding and splicing sequences was GC>TA transversion. In the coding region of the HPRT gene, these mutations were preferentially located in the sequence context -AGG- or -GAA- (*18*). In the concurrent control spectrum as

Table 3. Characteristic features of the hypoxanthine phosphoribosyl transferase mutational spectra induced by acetaldehyde, benzo[*a*]pyrene diolepoxide, and ethylene oxide as compared to the background spectrum (*18,22,26,40*).[a]

	Background	Acetaldehyde	BPDE	Ethylene oxide
Large deletions and rearrangements	10%	40%	0%	48%
Base substitutions				
GC>AT	17%	NS	0%	7%
GC>TA	10%	NS	41%	4%
Other	17%	NS	14%	11%
Small deletions and insertions	17%	NS	5%	4%
Splice mutations	29%	NS	41%	26%

NS, not studied. [a] The background, AA and BPDE spectra were was derived from human T-lymphocytes, and the ethylene oxide spectrum is based on fibroblast data (*40*).

well as in the general background spectrum, GC>TA transversion accounts for a minor proportion of the base pair substitutions. Moreover, there was a strong preference for mutation induction in the nontranscribed DNA strand, suggesting that BPDE adducts are more efficiently removed from the transcribed than from the nontranscribed DNA strand (*18*). This is likely to be a consequence of preferential DNA repair, as demonstrated previously in the HPRT gene (*34,35*) and other genes (*1*). Thus, these data show that there is a clear difference between the BPDE-induced mutational spectrum as compared to the background spectrum (Table 3).

Ethylene Oxide-induced HPRT Mutation

EtO is formed in humans and animals by metabolism of ethene, a widely distributed air pollutant present in car exhausts and cigarette smoke. Ethene also is produced endogeneously. The genetic toxicity and animal carcinogenicity of EtO is well documented, and human occupational exposure to EtO has been associated with an increased risk of leukemia (*36*). The mutagenic effect of EtO in the HPRT gene in human diploid fibroblasts was demonstrated recently by Kolman et al. (*25*). The mutant frequency was found to increase linearly within the dose range of 2.5 to 10 mM of EO, with a mutagenic potency of 9.8×10^{-6} mutants per mMh. Independent mutant cell clones were studied with PCR-based techniques and direct DNA sequencing or Southern blot analysis to unveil the nature of the EO-induced mutations. Among 28 mutants studied, 48% demonstrated large genomic deletions of the whole or part of the HPRT gene. Most of the partial deletions have breaking points in the 5′ part of the gene. The EtO-induced point mutations include one bp deletion causing a frameshift mutation, six base pair substitutions causing missense or nonsense mutations or affecting the translational start codon, and seven splicing mutations. Thus, these results (*40*) indicate that EtO induces both point mutations and gross structural alterations in human fibroblasts, with a strong preference for large intragenic or total HPRT deletions.

Discussion

Our data, summarized above, show that each of the three urban air pollutants studied induces a very specific mutational spectra at the HPRT locus in human cells *in vitro* (Table 3). The mutants were selected from experiments in which the mutant frequency was increased by a factor of 10 or more. Thus, at least 9 out of 10 mutants should be induced by the chemical compound.

The most informative mutational spectrum was obtained in the BPDE-treated cultures. All of the BPDE-induced HPRT mutants were found to have point mutations, 41% being GC>TA transversions causing coding errors and 41% splice mutations (Table 3). In the normal background spectrum, GC>TA tranversions in the coding region account for only 10% of all mutations (Table 3). Benzo[*a*]pyrene is a ubiquitous urban air pollutant, and it is likely that its metabolite BPDE induces HPRT mutation in T-cells *in vivo*. For the sake of discussion one may assume that all the background GC>TA transversions *in vivo* are due to BPDE mutagenesis. Since BPDE-induced splice mutations also should contribute to the spectrum of background mutations, at most 24% (0.1/0.41) of all the background mutations *in vivo* could be caused by BPDE. However, the true figure is probably much lower because spontaneous mutation due to replication error contributes to the background frequency of GC>TA transversions, and BPDE is not likely to be the only environmental mutagen that gives rise to this type of base substitution. It will be interesting to study whether subjects who sustain high exposure to benzo[*a*]pyrene and other (PAHs) show any significant increase of base substitution (e.g., GC>TA transversion mutation) or other type of mutation as compared to unexposed controls. Such studies could focus on heavy smokers who are known to have increased frequencies of *in vivo* HPRT mutation (*8,9*) and on coke oven workers preferentially having concomitant exposure evaluation by measurements of individual PAH–DNA adduct levels (*38*).

It is possible that a certain fraction of the large deletion mutations in the background spectrum of HPRT mutation is due to *in vivo* mutagenicity of AA and EtO, considering the predominance of such mutations in the corresponding *in vitro* spectra (Table 3). To assess this possibility, further studies of individual mutational background spectra and chemical-specific mutational spectra *in vitro* are needed. Tates et al. (*39*) recently demonstrated an increased frequency of HPRT mutation in peripheral lymphocytes of factory workers occupationally exposed to EtO. The comparison of mutational spectra in such workers with the background spectrum in unexposed individuals and the spectrum of HPRT mutation induced by EtO *in vitro* will improve the basis for quantitative estimation of the risk associated with EtO exposure *in vivo*.

Knowledge about mutational spectra induced by specific chemicals or radiation at the HPRT locus will also be extremely useful in elucidating the mechanisms by which mutation occurs in human cells *in vivo* and to evaluate the influence of metabolic activation and DNA repair. Because mutation is likely to be the initiating step in many carcinogenic processes, the elucidation of the mutagenic mechanisms also may contribute to the understanding of carcinogenesis. The HPRT gene is a large locus in which many different types of mutation are detectable, from large deletions and rearrangements to single base substitutions (*4,26,31*). Therefore, the HPRT locus may be a useful target for the identification of *in vivo* mutations that are specific or typical for a particular carcinogenic agent or exposure. Moreover, the identification of carcinogen-specific mutational spectra may be extremely valuable in human biomonitoring and quantitative risk evaluation.

REFERENCES

1. Bohr VA, Phillips DH, Hanawalt PC. Heterogeneous DNA damage and repair in the mammalian genome. Cancer Res 47:6426–6436 (1987).
2. Albertini RJ, Gennett IN, Lambert B, Thilly WG, Vrieling H. Mutation at the hprt

locus: meeting report. Mutat Res 216:65–88 (1989).
3. Mohrenweiser HW, Jones IM. Review of the molecular characteristics of gene mutations of the germ line and somatic cells of the human. Mutat Res 231: 87–108 (1990).
4. Lambert B, Andersson B, He S-M, Marcus S, Steen A-M. Molecular analysis of mutation in the human gene for hypoxanthine phosporibosyltransferase. In: Molecular Genetic Medicine, Vol 2. San Diego, CA:Academic Press, 1992;161–188.
5. Albertini RJ, Castle KL, Borcherding WR. T-cell cloning to detect the mutant 6-thioguanine-resistant lymphocytes present in human peripheral blood. Proc Natl Acad Sci USA 79:6617–6621 (1982).
6. Morley AA, Trainor KJ, Seshadri R, Ryall RG. Measurement of *in vivo* mutations in human lymphocytes. Nature 302:155–156 (1983).
7. Albertini RJ. Somatic gene mutations *in vivo* as indicated by the 6-thioguanine-resistant T-lymphocytes in human blood. Mutat Res 150:411–422 (1985).
8. Cole J, Green MHL, James SE, Henderson L, Cole H. A further assessment of factors influencing measurements of thioguanine-resistant mutant frequency in circulating T-lymphocytes. Mutat Res 204:493–507 (1988).
9. Tates AD, van Dam FJ, van Mossel H, Schoemakes H, Thijssen JCP, Woldring VM, Zwinderman AH, Natarajan AT. Use of the clonal assay for measurement of frequencies of HPRT mutants in T-lymphocytes from five control populations. Mutat Res 253:199–213 (1991).
10. Nicklas JA, O'Neill JP, Albertini RJ. Use of T-cell receptor gene probes to quantify the *in vivo* HPRT mutations in human T-lymphocytes. Mutat Res 173:67–72 (1986).
11. Edwards A, Voss H, Rice P, Civitello A, Stegeman J, Schwager C, Zimmerman J, Erfle H, Caskey CT, Ansorge W. Automated DNA sequencing of the human HPRT locus. Genomics 6:593–608 (1990).
12. Gibbs RA, Caskey CT. Identification and localization of mutations at the Lesch-Nyhan locus by ribonuclease A cleavage. Science 236:303–305 (1987).
13. Cariello NF, Keohavong P, Kat AG, Thilly WG. Molecular analysis of complex human cell populations: Mutational spectra of MNNG and ICR-191. Mutat Res 231:165–176 (1990).
14. Hovig E, Smith-Sørensen B, Brøgger A, Børresen A-L. Constant denaturant gel electrophoresis, a modification of denaturing gradient gel electrophoresis, in mutation detection. Mutat Res 262:63–71 (1991).
15. Gibbs RA, Nguyen P-N, Edwards A, Civitello AB, Caskey CT. Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families. Genomics 7:235–244 (1990).
16. Vrieling H, Simons JWIM, van Zeeland AA. Nucleotide sequence determination of point mutations at the mouse hprt locus using *in vitro* amplification of HPRT mRNA sequences. Mutat Res 198:107–113 (1988).
17. Gibbs RA, Nguyen P-N, McBride LJ, Koepf SM, Caskey CT. Identification of mutations leading to the Lesch-Nyhan syndrome by automated direct DNA sequencing of *in vitro* amplified cDNA. Proc Natl Acad Sci USA 86:1919–1923 (1989).
18. Andersson B, Fält S, Lambert B. Strand specificity for mutations induced by (+)-anti BPDE in the hprt gene in human T-lymphocytes. Mutat Res 269:129–140 (1992).
19. Stout JT, Caskey CT. Hypoxanthine phosphoribosyl-transferase deficiency: the Lesch-Nyhan syndrome and gouty arthritis. In: The Metabolic Basis of Inherited Disease, sixth Ed (Scriver CR, Beaudet AL, Sly WS, Valle D, eds). New York:McGraw-Hill Information Services Company, 1989;1007–1028.
20. Lambert B, Marcus S, Andersson B, Hou S-M, Steen A-M, Hellgren D. Missense mutations and evolutionary conserved amino acids at the human hypoxanthine phosphoribosyltransferase locus. Pharmacogenetics 2:329–336 (1992).
21. O'Neill JP, Sullivan LM, Albertini RJ. *In vitro* induction, expression and selection of thioguanine-resistant mutants with human T-lymphocytes. Mutat Res 240:135–142 (1990).
22. He S-M, Lambert B. Acetaldehyde-induced mutation at the hprt-locus in human lymphocytes *in vitro*. Environ Mol Mutagen 16:57–63 (1990).
23. Andersson B, Lambert B. Mutations induced by benzo[*a*]pyrene diolepoxide at the HPRT locus in human T-lymphocytes *in vitro*. Mutat Res 245:75–82 (1990).
24. O'Neill JP, Hunter TC, Sullivan LM, Nicklas JA, Albertini RJ. Southern blot analyses of human T-lymphocyte mutants induced *in vitro* by λ-irradiation. Mutat Res 240:143–149 (1990).
25. Kolman A, Bohusova T, Lambert B, Simons JWIM. Induction of 6-thioguanine-resistant mutants in human diploid fibroblats *in vitro* with ethylene oxide. Environ Mol Mutagen 19:93–97 (1992).
26. Hou S-M, Steen A-M, Fält S, Andersson B. Molecular spectrum of background mutation at the HPRT locus in human T-lymphocytes. Mutagenesis 8:43–49 (1993).
27. Muir P, Osborne Y, Morley AA, Turner DR. Karyotypic abnormality of the X chromosome is rare in mutant HPRT- lymphocyte clones. Mutat Res 197:157–160 (1988).
28. He S-M, Holmberg K, Lambert B, Einhorn N. HPRT mutations and karyotype abnormalities in T-cell clones from healthy subjects and melphalan-treated ovarian carcinoma patients. Mutat Res 210:353–358 (1989).
29. Nicklas JA, Hunter TC, O'Neill JP, Albertini RJ. Molecular analyses of *in vivo* HPRT mutations in human T-lymphocytes. III. Longitudinal study of HPRT gene alterations and T-cell origins. Mutat Res 215:147–160 (1989).
30. Yang TP, Patel PI, Chinault AC, Stout JT, Jackson LG, Hildebrand BM, Caskey CT. Molecular evidence for new mutation at the HPRT locus in Lesch-Nyhan patients. Nature 310:412–414 (1984).
31. Rossi AM, Thijssen JCP, Tates AD, Vrieling H, Natarajan AT, Lohman PHM, van Zeeland AA. Mutations affecting RNA splicing in man are detected more frequently in somatic than in germ cells. Mutat Res 244:353–357 (1990).
32. Dellarco VL. A mutagenicity assessment of acetaldehyde. Mutat Res 195:1–20 (1988).
33. Brookes P, Osborne MR. Mutation in mammalian cells by stereoisomers of anti-benzo[*a*]pyrene-diolepoxide in relation to the extent and nature of DNA reaction products. Carcinogenesis 3:1223–1226 (1982).
34. Vrieling H, Venema J, van Rooyen M-L, van Hoffen A, Menichini P, Zdzienicka MZ, Simons JWIM, Mullenders LHF, van Zeeland AA. Strand specificity for UV-induced DNA repair and mutations in the Chinese hamster HPRT gene. Nucleic Acids Res 19:2411–2415 (1991).
35. Chen R-H, Maher VM, McCormick JJ. Effect of excision repair by diploid human fibroblasts on the kinds and locations of mutations induced by (±)-7b, 8a-dihydroxy-9a,10a-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in the coding region of the HPRT gene. Proc Natl Acad Sci USA 87:8680–8684 (1990).
36. Dellarco VL, Generoso WM, Sega GA, Fowle JR, Jacobson-Kram D. Review of the mutagencicity of ethylene oxide. Environ Mol Mutag 16:85–103 (1990).
37. Recio L, Cochrane J, Simpson D, Skopek TR, O'Neill JP, Nicklas JA, Albertini RJ. DNA sequence analysis of *in vivo* HPRT mutation in human T-lymphocytes. Mutagenesis 5:505–510 (1990).
38. Hemminki K, Randerath K, Reddy MV, Putman KL, Santella RM, Perera FP, Young T-L, Phillips DH, Hewer DH, Savela K. Postlabelling and immunoassay analysis of polycyclic aromatic hydrocarbon adducts of deoxyribonucleic acid in white blood cells of foundry workers. Scand J Work Environ Health 16:158–162 (1990).
39. Tates AD, Grummt T, Törnqvist M, Farmer PB, van Dam FJ, van Mossel H, Schoemaker HM, Sosterman-Golkar S, Uebel CH, Tang YS, Zwinderman AH, Natarajan AT, Ehrenberg L. Biological and chemical monitoring of occupational exposure to ethylene oxide. Mutat Res 250:483–497 (1991).
40. Bastlova T, Andersson B, Lambert B, Kolman A. Molecular analysis of ethylene oxide-induced mutations at the HPRT-locus in human diploid fibroblasts. Mutat Res 287:283–292 (1993).