Published in final edited form as:

Mutat Res. 1988 November ; 194(3): 171–181.

An analysis of the mutagenicity of 1,2-dibromoethane to *Escherichia coli*: Influence of DNA repair activities and metabolic pathways

Patricia L. Foster¹, Wells G. Wilkinson¹, Judith K. Miller², Amy D. Sullivan¹, and Wayne M. Barnes²

¹ Division of Environmental Health, Boston University School of Public Health, and the Hubert H. Humphrey Cancer Research Center, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118

² Department of Biological Chemistry, Washington University School of Medicine, 660 South Euclid, St. Louis, MO 63110 (U.S.A.)

Summary

The mutagenicity of 1,2-dibromoethane (EDB) to *Escherichia coli* was reduced by the UV light-induced excision repair system but unaffected by the loss of a major apurinic/apyrimidinic site repair function. At high doses, 70–90% of the EDB-induced mutations were independent of SOS-mutagenic processing and approximately 50% were independent of glutathione conjugation. The SOS-independent mutations induced by EDB were unaffected by the enzymes that repair alkylation-induced DNA lesions. EDB-induced base substitutions were dominated by GC to AT and AT to GC transitions. These results suggest that EDB-induced premutagenic lesions have some, but not all, of the characteristics of simple alkyl lesions.

Keywords

Dibromoethane; EDB; *Escherichia coli*; Missense mutations; DNA repair

1,2-Dibromoethane (EDB or DBE; CAS No. 106-93-4) has been used as an antiknock additive in leaded gasoline, an industrial solvent, a pesticide, and a food disinfectant (NIOSH, 1977). It is one of a class of potentially hazardous compounds, the haloalkanes, that has become widely distributed in the environment (Ames et al., 1980). EDB is highly toxic, causing liver and kidney necrosis and reproductive abnormalities (reviews Shih and Hill, 1981; Rannug, 1980; Ter Haar, 1980). It has been repeatedly shown to be carcinogenic to rodents, resulting in tumors in a number of organs, including the forestomach, liver, spleen and thyroid (NCI, 1978). Thus environmental exposure to EDB is a potential health hazard.

The mutagenicity of EDB has been demonstrated in a number of genetic systems, including bacteria, yeast and other fungi, plants, insects, mammals and human cells (reviews, Fabricant and Chalmers, 1980; Rannug, 1980). Although EDB is a direct-acting mutagen in the Salmonella/microsome assay (McCann et al., 1975a), its mutagenicity has been shown to be further enhanced by metabolic activation (Rannug, 1980). Metabolic activation of EDB to its ultimate mutagenic and carcinogenic form has been hypothesized to occur by two different

pathways (Rannug, 1980; Anders and Livesey, 1980). One, mediated by the mixed-function oxygenases of liver microsomes, would yield bromoacetaldehyde and 2-bromoethanol as potential DNA-damaging agents (Hill et al., 1978; Banerjee et al., 1979). The other pathway, conjugation with glutathione mediated by enzymes present in liver cytosol, would yield as reactive products a half-sulfur-mustard or an episulfonium ion (Rannug, 1980; van Bladeren et al., 1980). The binding of EDB to DNA in vitro has been shown to be dependent on glutathione conjugation, and an *S*-[2-(*N*⁷-guanylyl)ethyl]glutathione adduct has been identified (Ozawa and Guengerich, 1983; Inskeep and Guengerich, 1984). However, bromoacetaldehyde is a metabolite of EDB (Hill et al., 1978) and can bind to DNA without enzymatic activation (Banerjee et al., 1979). Both 2-bromoethanol and chloroacetaldehyde are bacterial mutagens (McCann et al., 1975b; Rannug et al., 1976; Rosenkranz, 1977), but bromoacetaldehyde is not, possibly due to its extreme toxicity (Rosenkranz, 1977). Thus, the identity of the genotoxic metabolites of EDB is unclear.

In this study we used repair-defective strains of *Escherichia coli* to elucidate the nature of the EDB-induced mutagenic DNA lesions. We sought to determine what enzymatic activities prevent EDB mutagenicity, what activities are required for EDB mutagenicity, and what mutations are induced. We demonstrated that to be mutagenic EDB did not require activation by microsomal mixed-function oxygenases and approximately 50% of the mutations induced by high doses of EDB were also independent of glutathione conjugation. EDB mutagenicity was 10-fold reduced by the UV-induced excision repair system but was not affected by loss of an apurinic/apyrimidinic site endonuclease. The majority of mutations induced by high doses of EDB were independent of SOS-mutagenic processing and consisted of GC to AT and AT to GC base changes, suggesting that EDB acts like an alkylating agent. However, the mutagenic lesions induced by EDB that give rise to SOS-independent mutations were not repaired by the enzymes that in *E. coli* repair alkylation-induced DNA damage.

Materials and methods

Bacterial strains

All *E. coli* strains used in this study are derivatives of PF260 (= F⁻ *ara* Δ (*gpt-lac-pro*) *thiA* Δ *his5 supD60*; Foster and Davis, 1987). Construction of DNA repair-deficient derivatives was as previously described for *uvrB5* (Foster et al., 1983), *AxthA* and *umuC122::Tn5* (Foster and Davis, 1987). Glutathione-negative strains were constructed by P1 transduction to kanamycin resistance from JTG10 (= *gshA20::Tn10kan*; Greenberg and Demple, 1986) followed by screening for resistance to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Since EDB is poorly mutagenic to *uvr*⁺ bacteria (Table 2), all other DNA repair-defective strains also were *uvrB5*. Genetic manipulations were as described (Miller, 1972).

The targets for mutagenesis were the *hisG46* and *hisG428* loci from the Ames *Salmonella typhimurium* tester strains, carried on F' episomes (Foster and Davis, 1987). *hisG46* is a CCC codon that can be reverted by any base change at the first base and by GC to AT transitions and GC to TA transversions at the second base of the codon (Miller and Barnes, 1986). *hisG428* is a TAA ochre codon that in our amber suppressor strains can be reverted by 9 base changes at the codon, small deletions, and the creation of extragenic tRNA suppressors (Levin et al., 1984; our unpublished observations).

Media

Bacteria were grown in LB broth (Miller, 1972) containing appropriate drugs. To select for histidine prototrophs, bacteria were plated on VB minimal plates (Vogel and Bonner, 1956) in 2 ml top agar containing a limiting amount of histidine (100 nmoles for *hisG46* or 50 nmoles for *hisG428*) plus 2 mg proline and 0.1 mg of thiamine.

Mutagenesis

The protocol used for the mutagenesis experiments reported here was the “spot test” developed by Ames (Ames et al., 1975) and applied to volatile halogenated alkanes by Rosenkranz (1977). 0.1 ml of a saturated LB broth overnight culture of bacteria was plated in supplemented top agar; EDB (Sigma or Aldrich, > 99% pure) was added to a filter paper disk placed on the agar surface. Plates were sealed with parafilm and incubated at 37 °C for 72–96 h, depending on the growth rate of the strain. Spontaneously occurring mutations on plates with no EDB were subtracted to give the EDB-induced mutations per plate. For metabolic activation, 0.5 ml of 20, 40 and 60 µl/ml Aroclor-induced rat liver microsomes (Organon Teknika Corp.) in metabolic activation buffer (Ames et al., 1975) were added to the top agar.

Because EDB is volatile, spot testing gave variable results attributable to the time elapsed between adding the EDB and sealing the plates. Better results were obtained mutagenizing the bacteria in the gas phase by adding EDB to a filter paper disk stuck with top agar to the lid of the petri plate and sealing the (inverted) plate immediately. In every case, this procedure gave the same results qualitatively as a direct spot test, but the level of mutagenesis was higher, more reproducible, and gave a better dose response. A comparison of the two methods with the time between addition of the EDB and sealing the plates strictly controlled is given in Table 1. Results obtained with both procedures are presented below.

EDB is a highly toxic solvent. Attempts to assay for survival as well as mutagenesis by treating the cells, washing out the EDB, and then plating for mutagenesis and survival yielded high lethality, low mutagenicity and non-reproducible results. Cells exposed to EDB in ethanol, distilled water or dimethyl sulfoxide clumped severely and were difficult to disperse. It appeared that EDB toxicity did not correspond to the level of DNA damage, but was due to some change in cell surface properties, as previously reported (Brem et al., 1974b), which varied with the method of treatment.

The adaptive response was induced by growing bacteria in supplemented minimal media in the presence of 0.5 µg/ml MNNG for 4 h at 32°C (Foster and Eisenstadt, 1985). 0.5 ml of the culture was then centrifuged, resuspended in E salts (Vogel and Bonner, 1956), and plated in 2 ml supplemented top agar (see above) with an additional 1 µg MNNG added to the adapted cells. EDB was then added as above (gas phase). To monitor adaptation, 5 µl of 1 mg/ml MNNG in 0.1 M citrate buffer (pH 5.5) was added to a filter paper disk on separate control (no EDB) plates.

Analysis of mutations

Mutations at the *hisG46* locus were identified by colony hybridization to oligonucleotides (Miller and Barnes, 1986) and by sequencing (Barnes and Tuley, 1983; Foster and Davis, 1987). True revertants of *hisG428* were first identified by the transfer of the His⁺ phenotype upon mating and then were sequenced (Foster and Davis, 1987). Suppressor tRNAs of *hisG428* were identified by their ability to suppress known ochre mutations in the *lacI* gene, as has been described (Foster and Eisenstadt, 1985). Estimates of the proportion of true revertants and suppressors of *hisG428* (Table 5) were based on 50 revertants of each strain from both EDB-treated and untreated plates. Spectra of spontaneous revertants of *hisG46* and *hisG428* can be found in Levin et al. (1984), Foster and Eisenstadt (1985), Levin and Ames (1986), Foster and Davis (1987), and Eisenstadt et al. (in press).

Results

The effect of accurate DNA repair pathways on EDB mutagenicity

As shown in Table 2, EDB was poorly mutagenic to bacterial proficient for the UV light-induced DNA excision repair system (*uvr*⁺). Mohn et al. (1984) also found EDB to be ineffective in reverting a mutation in an *arg* gene and inducing forward mutations in the *galR* gene in *uvr*⁺ *E. coli*. The excision repair system responds to distortions of the DNA helix; thus, the premutagenic DNA lesions produced by EDB are likely to be larger than simple ethyl lesions, which are not well repaired by this system (Todd and Schendel, 1983). All other experiments reported below were done with *uvr*[−] bacteria.

EDB was not more mutagenic to *uvrB5 xthA*[−] bacteria, which are missing exonuclease III, a major apurinic/apyrimidinic (AP) site endonuclease (Yajko and Weiss, 1975), than to *uvrB5 xthA*⁺ bacteria (Table 2). Since *xthA*[−] bacteria are deficient in the repair of AP sites (Taylor and Weiss, 1982; Cunningham et al., 1986; Foster and Davis, 1987), this result suggests that EDB mutagenesis does not involve the creation of AP sites as intermediates. However, *E. coli* has other constitutive or inducible AP endonucleases that may have been active.

The “adaptive response”, which is induced by exposure to low concentrations of alkylating agents, consists of activities that repair several important DNA alkyl lesions (Lindahl, 1982). As shown in Table 3, preinduction of the adaptive response did not decrease, in fact increased, the mutagenicity of EDB to the *uvrB5 hisG46* strain. [Under the conditions of this experiment (see Materials and methods) EDB was poorly mutagenic to the *hisG428* strain.] Two possible explanations for the increase in mutagenicity are: (i) the SOS response (see below) was being induced during adaptation and enhancing the mutagenicity of EDB, or (ii) enzymes induced by MNNG were acting on EDB lesions and creating mutagenic lesions. As shown in Table 3, preadaptation did not increase the mutagenicity of EDB to *uvrB5 umuC*[−] bacteria, which are deficient in SOS-mutagenic processing (Kato and Shinoura, 1977).

The effect of SOS-mutagenic processing on EDB mutagenicity

The mutagenicity of agents that produce directly miscoding lesions, such as *O*⁶-alkylguanine, is independent of the bacterial SOS system. In contrast, the mutagenicity of agents that produce bulky or non-informational lesions is usually dependent on SOS-mutagenic processing, at least for the induction of base substitutions (review, Walker, 1984). The observation that EDB was mutagenic to *S. typhimurium* without plasmid pKM101 (Brem et al., 1974a; McCann et al., 1975a) implied that EDB-induced mutations were SOS-independent since, without this plasmid, *S. typhimurium* is deficient, but not totally defective, in SOS-mutagenic processing (Orrego and Eisenstadt, 1987; reviewed in Eisenstadt, 1987). The results presented in Figs. 1 and 2 confirm that under normal conditions the majority of EDB-induced mutations in *E. coli* are SOS-independent — at most doses *uvrB5 umuC*[−] strains, defective in SOS-mutagenic processing, were 70–90% as mutable by EDB as *uvrB5 umuC*⁺ strains. At low doses (< 5 μl), a smaller fraction (30–50%) of *hisG46* mutations were SOS-independent.

The effect of metabolic activation on EDB mutagenicity

Although EDB was originally found to be a direct-acting mutagen (McCann et al., 1975a), other studies with both *S. typhimurium* and *E. coli* have reported several- to many-fold increases in EDB mutagenicity with metabolic activation by perfused rat liver, S9 (liver microsome) and S100 (liver cytosol) preparations (Rannug and Beije, 1979; Buijs et al., 1985; Mohn et al., 1984). However, we were able to achieve at most at 20% enhancement of EDB mutagenicity in the presence of S9 preparation (a representative experiment is given in Table 4). To test whether this result was peculiar to our *E. coli* strains, we repeated the experiment with a *uvrB*[−] *S. typhimurium* LT2 strain carrying the same episome (*hisG46*) and

obtained similar results (data not shown). Thus under the conditions of our experiments, the bacteria themselves were nearly as effective in activating EDB as the rat liver enzymes.

S9 preparation contains both cytochrome P450 oxygenases and glutathione conjugation functions. Since *E. coli* and *S. typhimurium* apparently do not contain the former but do contain the latter (Meijer et al., 1980; Fahey et al., 1978), the direct mutagenicity of EDB could be due to activation via conjugation with intracellular glutathione. Kerklaan et al. (1985) reported a reduction in EDB mutagenesis with a *S. typhimurium* strain deficient in glutathione, although the level of mutagenesis they obtained even in the wild-type strain was low. We further tested this hypothesis by making our *uvrB5 E. coli* strains *gshA20::Tn10kan*. Bacteria with this mutation have no detectable glutathione activity (Greenberg and Demple, 1986). As shown in Figs. 1 and 2, EDB mutagenicity was reduced 23–75% in *gshA⁻* strains, depending on dose and mutational target. Added S9 compensated for this decrease completely at the lowest dose and partially at higher doses (Table 4). The toxicity of EDB was greater to *gshA⁻* than to the *gshA⁺* bacteria as evidenced by approximately 2-fold larger zones of inhibition around the added EDB (data not shown).

The base substitutions induced by EDB

We identified the base substitution mutations induced by EDB at both the *hisG46* and *hisG428* loci, as well as the extragenic tRNA suppressors of *hisG428* (Table 5). In the *uvrB5 gshA⁺ umuC⁺* background, 93% of the *hisG46* revertants were the result of GC to AT transitions, with transitions at the second base of the codon approximately twice as frequent as transitions at the first base. At the *hisG428* locus, 100% of the EDB-induced mutations were AT to GC transitions. 88% of the tRNA suppressors of *hisG428* induced by EDB also arose by GC to AT transitions (ochre suppressors cannot be created by AT to GC transitions). Thus EDB showed a clear preference for inducing transition mutations at both GC and AT base pairs. EDB mutations also appeared to be more frequent at GC than at AT base pairs (e.g., compare the level of mutagenicity in Figs. 1 and 2); however, other intrinsic differences between the mutational targets may influence the mutation frequency.

EDB showed a similar preference for inducing transitions in the *uvrB5 umuC⁻ gshA⁺* and *uvrB5 umuC⁺ gshA⁻* strains as in *uvrB5 umuC⁺ gshA⁺* bacteria (Table 5). There were no significant differences in revertants of *hisG46* among the 3 genetic backgrounds. Of *hisG428* revertants, there was a significant increase in the proportion of true revertants in *uvrB5 umuC⁺ gshA⁻* relative to *uvrB5 umuC⁺ gshA⁺* bacteria ($P = 0.01$ – 0.02) but no difference in the spectra of either true revertants or suppressors between the strains. Comparing *hisG428* revertants in *uvrB5 umuC⁻ gshA⁺* to *uvrB5 umuC⁺ gshA⁺* bacteria, there was a non-significant ($P = 0.2$ – 0.3) increase in the proportion of deletions and a significant decrease in the proportion of SuB mutations with a corresponding increase in the proportion of weak suppressors ($P = 0.02$ – 0.05).

Since the number of revertants on EDB-exposed plates was well above background (5–20-fold), the small number of spontaneous mutations potentially contaminating each collection, given in parentheses in Table 5, cannot change the strong preference for transitions in the EDB-induced spectra. However, since deletions and weak suppressors of *hisG428* are frequent spontaneous events in SOS-deficient bacteria (Levin and Ames, 1986; our unpublished results), some of the differences between *uvrB5 umuC⁻ gshA⁺* and *uvrB5 umuC⁺ gshA⁺* bacteria may be due to spontaneous mutations.

Discussion

The results reported here confirm and extend previous studies demonstrating that EDB induces base substitutions (Rosenkranz, 1977), that most of these mutations do not require SOS-

mutagenic processing (Brem et al., 1974a; McCann et al., 1975a), and that EDB-induced DNA lesions are repaired by the UV-induced excision repair system (Mohn et al., 1984). In addition, we have demonstrated that: (i) EDB-induced DNA lesions that give rise to SOS-independent mutations are not repaired by the enzymes induced as part of the adaptive response; (ii) EDB-induced lesions are not repaired by a major AP site endonuclease; (iii) EDB is mutagenic in the absence of glutathione; and (iv) the mutations induced by EDB are dominated by GC to AT and AT to GC transitions.

Our results differ from previous studies in the degree to which metabolic activation, either by mixed-function oxygenases or glutathione conjugation, influences EDB mutagenicity. There are a number of differences in experimental protocols that can account for some of this discrepancy. (i) Our methods, using spot testing, may not have been optimal for metabolic activation. (ii) Since EDB is extremely volatile, reported doses may not reflect the effective dose given to the bacteria. (iii) Most previous studies have used cell-wall-defective bacteria to increase cell permeability, thus our cell-wall-proficient strains may have been impermeable to an activated derivative or conjugate of EDB. (iv) Glutathione conjugation detoxifies EDB, as evidenced by the increased sensitivity of our *gshA*⁻ strains; if added glutathione or liver cytosol likewise decreases EDB toxicity, the degree to which glutathione activation increases EDB mutagenicity may have been previously overestimated.

The frequency of mutations that we observed at the *hisG46* locus was 2–10-fold greater than reported in previous studies using similar methods and the same mutational target (Rosenkranz, 1977; Kerklau et al., 1985; Buijs et al., 1984). The level of mutagenesis we obtained in our *gshA*⁻ strains at the highest doses was 15 and 22 times the spontaneous background for *hisG46* and *hisG428* respectively. Even uncorrected for toxicity, our results clearly demonstrate that, in *E. coli*, approximately half of the mutations induced by high doses of EDB occurred independently of glutathione conjugation (Figs. 1 and 2). Since the *gshA*⁻ bacteria were more sensitive to EDB than the *gshA*⁺ bacteria, 50% is a minimum estimate. Thus, other pathways of EDB activation exist in the bacterial cell. If EDB mutagenicity is mediated by a half-sulfur-mustard or episulfonium ion, then other thiols (e.g., cysteine) must be able to participate in the pathway.

That SOS-independent transition mutations represented the majority of mutations at most doses of EDB strongly suggests that a large part of the mutagenicity of EDB is due to miscoding lesions. *O*⁶-Alkylguanine–DNA alkyltransferase, which occurs at high levels after adaptation, efficiently repairs the alkylation-induced miscoding lesions *O*⁶-alkylguanine and *O*⁴-alkylthymidine (Cairns, 1980; McCarthy et al., 1984). Since the SOS-independent mutations induced by EDB at *hisG46* were not affected by preadaptation, it follows that the alkyltransferase did not repair the EDB-induced miscoding lesions. However, SOS- mutagenic processing did enhance EDB mutagenesis, particularly at low doses (Figs. 1 and 2), and preadaptation further increased the proportion of SOS-dependent mutations (Table 3). Thus either EDB produces two classes of mutagenic lesions, a major class that is directly miscoding and a minor class that requires mutagenic processing (as is the case for some alkylating agents), or EDB produces one class of lesion that has miscoding properties, but whose mutagenicity is enhanced by the *umuC*⁺ function. Preadaptation could either: (i) increase the induction of the SOS response, thereby enhancing the mutagenicity of EDB lesions, or (ii) induce an enzyme or enzymes other than the *O*⁶-alkylguanine–DNA alkyltransferase that act upon EDB lesions and produce repair intermediates whose mutagenicity is SOS-dependent. In either case, the results indicate that EDB is *acting* like a simple alkylating agent, although the DNA lesions it produces must be chemically distinct.

A number of DNA lesions have been identified or postulated to occur after EDB exposure. For example, two adducts to the *N*⁷ position of guanine have been identified: *S*-[2-(*N*⁷-guanyl)-

ethyl]glutathione (Ozawa and Guengerich, 1983) and *N*⁷-hydroxyethylguanine (J. Groopman, personal communication). Such lesions could be bulky enough to be recognized by the bacterial excision repair enzymes and would not be repaired by *O*⁶-alkylguanine–DNA alkyltransferase — two of the properties that we have observed for EDB-induced lesions. However, *N*⁷-guanine adducts are relatively innocuous, causing opening of the imidazole ring, a stable and non-reactive lesion, or depurination (Singer and Grunberger, 1983).

The mutagenic characteristics of EDB indicate that the major mutagenic lesions induced under normal conditions are not apurinic sites. Depurination leads to SOS-dependent transversion mutations (reviewed in Loeb, 1985), not to the SOS-independent transitions that we observe. The failure of the *xthA*[−] defect to increase EDB mutagenicity is consistent with this interpretation. However, the mutagenicity of alkylating agents is increased in *xthA*[−] strains only when depurination rates are high or other AP endonucleases are defective (Cunningham et al., 1986; Foster and Davis, 1987). Although exonuclease III, the product of *xthA*, is a major endonuclease, at low rates of depurination other constitutive or inducible AP endonucleases apparently can compensate for its loss (Cunningham et al., 1986). Thus, a relatively nonmutagenic class of EDB-induced lesions could be producing AP sites spontaneously or enzymatically. Such lesions, if substrates for a glycosylase induced as part of the adaptive response, could explain the increase in EDB mutagenicity seen upon preadaptation (Table 3). *N*⁷-Hydroxyethyl-guanine, which might be a substrate for an alkylation-induced enzyme, is a possible candidate for this postulated lesion.

Chloroacetaldehyde reacts directly with DNA bases to form etheno and hydrated etheno lesions to the 1, *N*⁶ positions of adenine and the 3, *N*⁴ positions of cytosine (Kusmirek and Singer, 1982a). Similar lesions could be formed by bromoacetaldehyde after oxidative activation of EDB (Singer and Grunberger, 1983). These lesions could account for EDB-induced mutations at both GC and AT sites, and would be likely to be recognized by the excision repair system. However, during transcription in vitro, 3, *N*⁴-ethenocytidine directed the misincorporation of UTP as frequently as ATP (Kusmirek and Singer, 1982b), suggesting that it would give rise to transversion as well as transition mutations. It is also not clear how these etheno groups, which are to the base-pairing positions, could be SOS-independent miscoding lesions.

In conclusion, none of the previously identified EDB-induced DNA lesions can account for the results reported here. To be consistent with our results, EDB must induce lesions that make the purines, the pyrimidines, or both, ambiguous. Examples of such lesions include base analogs such as 5-bromouracil and 2-amino purine (Drake, 1970), oxidatively deaminated 5-methylcytosine (Coulondre et al., 1978), *O*⁶-alkylguanine (Loechler et al., 1984), and *O*²- and *O*⁴-alkylthymidine (Singer et al., 1983). Of these, only the alkyl lesions appear to provide models for EDB. For example, *O*⁶-hydroxyethylguanine, which is poorly repaired by *O*⁶-alkylguanine–DNA alkyltransferase in vitro (300 times more slowly than *O*⁶-methylguanine; Robbins et al., 1983) but is nearly as large as *O*⁶-propylguanine, which is repaired by the excision repair system (Todd and Schendel, 1983), is an attractive candidate for a mutagenic lesion at GC sites. The analogous *O*⁴-hydroxyethylthymidine lesion could account for the EDB-induced mutations at AT sites. Thus, an important class of DNA lesion induced by EDB and perhaps other haloalkanes appears to await further characterization.

Acknowledgments

We thank John Groopman for many helpful discussions and for communicating results before publication, Eric Eisenstadt for advice and for critically reading this manuscript, Jean Greenberg and Bruce Demple for the gift of their *gshA* mutant, and Elaine Davis for technical assistance.

This work was supported by National Institutes of Health Grants CA37880 to P.L.F. and GM24956 to W.M.B. and American Cancer Society (Massachusetts Division) Institutional Grant for Faculty Research IN-97H to P.L.F.

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Fig. 1.

EDB-induced revertants of *hisG46*. Each point is the average of 3–7 determinations $\pm$ S.D. ●, *uvrB5 umuC⁺ gshA⁺*; ■, *uvrB5 umuC::Tn5 gshA⁺*; ▲, *uvrB5 urauC⁺ gshA::Tn10kan*. Doses $< 5 \mu\text{l}$ EDB were direct; higher doses were in the gas phase. Average spontaneous revertants/plate $\pm$ S.D. were: *uvrB5 umuC⁺ gshA⁺*, 104 ± 60 ; *uvrB5 umuC::Tn5 gshA⁺*, 145 ± 160 ; *uvrB5 umuC⁺ gshA::Tn10kan*, 154 ± 121 .

Fig. 2. EDB-induced revertants of *hisG428*. Each point is the average of 3 determinations $\pm$ S.D. ●, *uvrB5 umuC⁺ gshA⁺*; ■, *uvrB5 umuC::Tn5 gshA⁺*; ▲, *uvrB5 umuC⁺ gshA::Tn10kan*. All doses were in the gas phase. Average spontaneous revertants/plate $\pm$ S.D, were: *uvrB5 umuC⁺ gshA⁺*, 351 ± 191 ; *uvrB5 umuC::Tn5 gshA⁺*, 211 ± 118 ; *uvrB5 umuC⁺ gshA::Tn10kan*, 86 ± 75 .

TABLE 1

COMPARISON OF THE MUTAGENICITY OF EDB ADDED DIRECTLY AND IN THE GAS PHASE TO *uvrB5/hisG46* BACTERIA

EDB (μ l)	<u>His⁺ revertants/plate</u>	
	Direct	Gas phase
0	99	117
5	4248	3348
10	4172	4856
20	4920	5920

TABLE 2
THE EFFECTS OF ACCURATE REPAIR PATHWAYS ON EDB-INDUCED REVERSION OF *hisG46* AND *hisG428*

EDB ^a (μl)	His ⁺ revertants/plate					
	<i>hisG46</i>			<i>hisG428</i>		
	<i>uvr⁺</i>	<i>uvrB5</i>	<i>uvrB5 xthA⁻</i>	<i>uvr⁺</i>	<i>uvrB5</i>	<i>uvrB5 xthA⁻</i>
0	21	71	269	128	181	147
1	154	1720	1688	170	710	658
5	141	2816	2600	126	1085	1034
10	158	3620	3640	116	1225	1180

^a Gas phase.

THE EFFECTS OF PREADAPTATION WITH MNNG ON EDB-INDUCED REVERSION OF *hisG46* IN *uvrB5* AND *uvrB5 umuC::Tn5* BACTERIA

TABLE 3

EDB ^a (μl)	His ⁺ revertants/plate			
	<i>uvrB5</i>		<i>uvrB5 umuC122:: Tn5</i>	
	Nonadapted	Adapted	Nonadapted	Adapted
0	67	184	52	93
1	880	2200	798	887
5	1400	3240	1306	1318
10	1764	2410	1238	1154
5μg MNNG ^b				
Number of revertants	440	270	520	200
Zone of mutagenesis (cm)	4.0	2.0	3.5	2.2

^aGas phase.

^bDirect.

The reduction in MNNG-induced mutations demonstrates that the bacteria were adapted.

TABLE 4

THE EFFECT OF LIVER MICROSOMES (S9) ON EDB-INDUCED REVERSION OF *hisG46*

EDB ^a (μ l)	<u>His⁺ revertants/plate</u>			
	<u><i>uvrB5</i></u>		<u><i>uvrB5 gshA20::Tn10kan</i></u>	
	-S9	+S9 ^b	-S9	+S9 ^b
0	210	252	300	220
0.5	1616	1720	896	1576
1.0	2112	2640	1816	1884
5.0	3680	3160	2080	2640
10.0	4400	3760	1920	2000
20.0	5440	2400	2484	3348
Aflatoxin B ₁ 0.01 μ M ^c	235	3600		

^a Direct^b 40 μ l/ml.^c Added to the top agar.The mutagenicity of aflatoxin B₁ demonstrates that the S9 was active.

TABLE 5

THE BASE SUBSTITUTIONS INDUCED BY EDB

All revertants were taken from plates exposed to 20 μ l EDB in the gas phase except: *uvrB5 umuC⁺ gshA⁺/hisG46*: one plate 10 μ l direct and one plate 20 μ l direct; *uvrB5 umuC⁻ gshA⁺/hisG46*: 20 μ l direct; *uvrB5 umuC⁺ gshA⁺/hisG428*: one plate 10 μ l direct (true revertants only) and one plate 20 μ l gas phase. His⁺ revertants/plate were (-EDB/+ EDB): *hisG46*: *uvrB5 umuC⁺ gshA⁺*, 131/1700 (weighted average); *uvrB5 umuC⁻ gshA⁺*, 40/850; *uvrB5 umuC⁺ gshA⁻*, 150/2976. True revertants of *hisG428*: *uvrB5 umuC⁺ gshA⁺*, 117/1041 (weighted average); *uvrB5 umuC⁻ gshA⁺*, 233/1392; *uvrB5 umuC⁺ gshA⁻*, 142/2002. Suppressors of *hisG428*: *uvrB5 umuC⁺ gshA⁺*, 190/1151; *uvrB5 umuC⁻ gshA⁺*, 155/1008; *uvrB5 umuC⁺ gshA⁻*, 31/326.

Strain	CCC to			Total ^a		
	GCC	ACC	TCC	CAC	CTC	
<i>hisG46</i> revertants						
<i>uvrB5 gshA⁺ umuC⁺</i>	0	1	9	1	19	30 (2)
<i>uvrB5 gshA⁺ umuC⁻</i>	0	0	3	0	16	19 (1)
<i>uvrB5 gshA⁻ umuC⁺</i>	0	0	3	0	10	13 (1)
<hr/>						
% of Total ^b						
<hr/>						
TAA to						
<hr/>						
Del						
<hr/>						
AAA/GAA CAA TTA/TCA TGA TAC/TAT TAG						
<hr/>						
True <i>hisG428</i> revertants						
<i>uvrB5 gshA⁺ umuC⁺</i>	66	0	7	1	0	6 14 (2)
<i>uvrB5 gshA⁺ umuC⁻</i>	58	0	0	0	0	4 6 (1)
<i>uvrB5 gshA⁻ umuC⁺</i>	86	0	2	0	0	7 9 (1)
<hr/>						
% of total ^b						
<hr/>						
AT → TA Su5 GC → TA SuC GC → AT SuB GC → AT SuD ^c γ ^d						
<hr/>						
Suppressors of <i>hisG428</i>						
<i>uvrB5 gshA⁺ umuC⁺</i>	34	0	1	15	0	1 17 (3)
<i>uvrB5 gshA⁺ umuC⁻</i>	42	0	1	9 ^e	3	8 21 (3)
<i>uvrB5 gshA⁻ umuC⁺</i>	14	0	0	7	0	0 7 (1)

^aNumbers in parentheses are the estimated number of spontaneous revertants contaminating the collection.

^bThe percentage of all EDB-induced revertants of *hisG428*, based upon the analysis of 50 revertants of each strain.

^cConversion of the amber suppressor, SuD, to an ochre suppressor.

^dUnidentified weak suppressors.

^e Includes 1 weak glutamine tRNA suppressor.