

Quinol-Glutathione Conjugate-induced Mutation Spectra in the *supF* Gene Replicated in Human AD293 Cells and Bacterial MBL50 Cells¹

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ABSTRACT

Hydroquinone is a nephrocarcinogen in rats but generally tests negative in standard mutagenicity assays. However, 2,3,5-tris-(glutathion-S-yl)hydroquinone, a potent nephrotoxic metabolite of hydroquinone, and 2-bromo-bis-(glutathion-S-yl)hydroquinone, another cytotoxic quinol-glutathione (GSH) conjugate, cause extensive single strand breaks in DNA in a manner that is dependent on the formation of reactive oxygen species. We, therefore, investigated whether quinol-GSH conjugates have the potential to behave as genotoxicants. The shuttle vector pSP189, containing the *supF* gene, was treated with 2,3,5-tris-(glutathion-S-yl)hydroquinone and replicated in both human AD293 cells and *Escherichia coli* MBL50 cells. The mutation frequency increased 4.6- and 2.6-fold in human AD293 and bacterial MBL50 cells, respectively. Base substitutions were the major type of mutations, and they occurred predominantly at G:C sites in both cell types. A high frequency of deletions (30%), including <10- and >10-bp deletions, were observed in AD293-replicated plasmids. The most common types of mutations in AD293 cells were G:C to A:T transitions (33.8%) and G:C to T:A (29.4%) and G:C to C:G (19.1%) transversions. In MBL50 cells, the major mutations were G:C to T:A (33.8%) and G:C to C:G (31.3%) transversions and G:C to A:T transitions (27.5%). The mutation spectra were similar to those reported for ·OH-induced mutations, suggesting that ·OH generated from polyphenolic-GSH conjugates not only plays a role in cytotoxicity but also provides a basis for their mutagenicity and carcinogenicity.

INTRODUCTION

Genetic alterations play a vital role in the formation of tumors. Genetic changes are often initiated by damage to DNA, and a variety of environmental agents, including X-rays, UV light, and chemicals (1), are capable of altering DNA. In addition, products of normal cellular metabolism, including ROS⁴ and various aldehydes, can damage DNA. ROS are also generated as by-products of chemical metabolism (Ref. 2; reviewed in Refs. 3 and 4), especially from polyphenols. The interaction between ROS and DNA may lead to mutations (5), such as base substitutions, deletions, rearrangements, and insertions (6) as well as sister chromatid exchange and chromosomal aberrations (7). The genotoxic and carcinogenic properties of ROS are well documented (1, 3, 8–12), and they appear to play an important role in tumor promotion (13, 14).

Quinones are ubiquitous, naturally occurring compounds. Quinones are also formed as metabolites from a variety of polyphenolic drugs, environmental pollutants, and food additives (15). Quinones are cytotoxic, mutagenic, and carcinogenic (16–18). For example, *tert*-

butyl-4-hydroxyanisole and its demethylated analogue, TBHQ, are phenolic antioxidants widely used in foods and are known to promote renal and bladder carcinogenesis in the rat (19). HQ, a metabolite of benzene, is a rodent nephrocarcinogen (20, 21) and has been implicated in the hematotoxicity and leukemogenicity of benzene (22). The reactivity of quinones lies in their ability to undergo redox cycling and create an oxidative stress (23) and/or to react directly with cellular macromolecules (15, 24). The one-electron reduction of a quinone to the corresponding semiquinone can result in the formation of the superoxide anion radical (O₂⁻), hydrogen peroxide, and the hydroxyl radical (·OH).

Although the conjugation of reactive electrophiles with GSH is usually considered a detoxication process, GSH conjugation to quinones can lead to the formation of more reactive and toxic metabolites (25, 26). Thus, quinone-GSH conjugates maintain the ability to redox cycle and to generate ROS (27, 28). Consequently, GSH conjugates of HQ (29) and TBHQ (30) catalyze the formation of 8-hydroxydeoxyguanosine. TGHQ (7.5 μmol/kg, i.v.), a metabolite of HQ (31), is acutely nephrotoxic (32) and causes a regenerative hyperplasia in renal proximal tubular cells (33), the same location at which tumors ultimately develop. In addition, 3,6-bis-(glutathion-S-yl)-*tert*-butylhydroquinone (200 μmol/kg), a metabolite of TBHQ (34), causes single-cell and tubular necrosis in the S₃M segment of the proximal tubule and causes severe hemorrhaging of the bladder (30). The acute nephrotoxicity of 17β-estradiol in the hamster model of estrogen-induced nephrocarcinogenicity also appears to be dependent upon the formation of catechol-estrogen GSH conjugates (35, 36). Thus, inhibition of renal γ-GT activity with acivicin protects against 17β-estradiol nephrotoxicity (34), and administration of 2-hydroxy-4-glutathion-S-yl-17β-estradiol or 2-hydroxy-1-glutathion-S-yl-17β-estradiol (0.27–5.0 μmol/kg) to Syrian hamsters produces mild nephrotoxicity (35). Repeated daily administration of 2-hydroxy-4-glutathion-S-yl-17β-estradiol causes a sustained elevation in urinary markers of renal damage and in the concentration of renal protein carbonyls and lipid hydroperoxides (36).

It is not known whether the ability of quinone-thioethers to catalyze the formation of ROS can lead to mutations in DNA. This is an important question because demonstrating the mutagenic potential of these metabolites would provide an important link to the established carcinogenesis of the parent polyphenols. Therefore, using TGHQ as a model quinone-thioether, we investigated the ability of this compound to cause mutations in the *supF* gene replicated in both human AD293 cells and *Escherichia coli* MBL50 cells.

MATERIALS AND METHODS

Plasmid, Bacterial Strain, and Cell Line. The plasmid pSP189 containing the *supF* gene was a gift from Dr. Michael M. Seidman (Otsuka Pharmaceutical Co., Rockville, MD). This shuttle vector contains an 8-bp “signature sequence” with 2 × 4⁸ (131,072) possible unique members and, thus, allows for the identification of independent mutations by excluding siblings within the population. To amplify the plasmid, we grew MBM7070 cells containing pSP189 in Luria-Bertani medium with ampicillin (5 mg/ml) for 12–14 h, with shaking at 250 rpm, at 37°C. The plasmid was isolated using a Qiagen plasmid purification kit (Qiagen, Chatsworth, CA). *E. coli* MBL50 cells, modified host

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⁴ The abbreviations used are: ROS, reactive oxygen species; TBHQ, *tert*-butylhydroquinone; HQ, hydroquinone; GSH, glutathione; TGHQ, 2,3,5-tris-(glutathion-S-yl)hydroquinone.

cells for pSP189, were obtained as a gift from Dr. Carmen Pueyo (Universidad de Córdoba, Córdoba, Spain). A human embryonic kidney cell line, AD293, was purchased from the American Type Culture Collection (Manassas, VA), grown in DMEM with glucose (4.5 g/liter) and L-glutamine (1 mM), and supplemented with antibiotics (100 units/ml penicillin and 100 mg/ml streptomycin) and 10% FCS.

DNA Treatment. DNA samples (5 µg/500 µl) in 50 mM sodium phosphate buffer (pH 7.4) were incubated at 37°C for 2 h in the presence of various concentrations of TGHQ (0, 50, 100, 200, and 500 µM) and were vortexed every 2 min for the first 30 min to facilitate the reaction by providing oxygen. After DNA treatment with TGHQ, the DNA samples were washed with cold Tris-EDTA buffer [10 mM Tris-HCl and 1 mM EDTA (pH 8.0)] and concentrated using Centricon-30 concentrators. DNA was separated on agarose gels to visualize the DNA damage. The DNA was stored at -20°C until transformation into MBL50 cells or transfection into AD293 cells.

Transformation and Transfection. The MBL50 cells were prepared and used for electroporation by the method described by Juedes and Wogan (37). Spontaneous mutations in control pSP189 replicated in both AD293 and MBL50 cells have been reported previously (37). The transformants were plated onto either 125 µM 5-bromo-4-chloro-3-indolyl-β-galactopyranoside-100 µM isopropyl-β-D-thiogalactoside plates for determination of the total number of transformants or 2 g/liter L-arabinose plates for selection of mutants. Subconfluent AD293 cells were transfected with TGHQ-treated DNA (2–4 µg per 25-cm² flask) using Lipofectamine, according to the manufacturer's recommendations (Life Technologies, Inc., Bethesda, MD). Cells were trypsinized and harvested, and the pSP189 plasmid was extracted with a Wizard Miniprep DNA purification kit (Promega, Madison, WI). Extracted DNA was treated with *DpnI* restriction endonuclease to remove any unreplicated pSP189 and transformed into MBL50 cells, as above.

Sequencing. Mutant plasmids were extracted using a Wizard miniprep DNA purification kit and sequenced manually using the 20-mer primer (GGC-GACACGGAAATGTTGAA). Potential sibling mutants with the same signature sequence were excluded from further analysis. Poisson distribution analysis was used to assess the randomness of the distribution of mutants, and hot spots were defined based on the Bonferroni inequality.

RESULTS

Gel electrophoresis of pSP189 DNA following exposure to TGHQ (50–500 µM) revealed increases in linearized DNA (Fig. 1, *Form III*), indicating that TGHQ causes double-strand breaks in DNA, a common effect of oxidative DNA damage. Moreover, TGHQ induced the formation of open relaxed circles (*Form II*), presumably as a consequence of single-strand breaks, with concomitant decreases in the

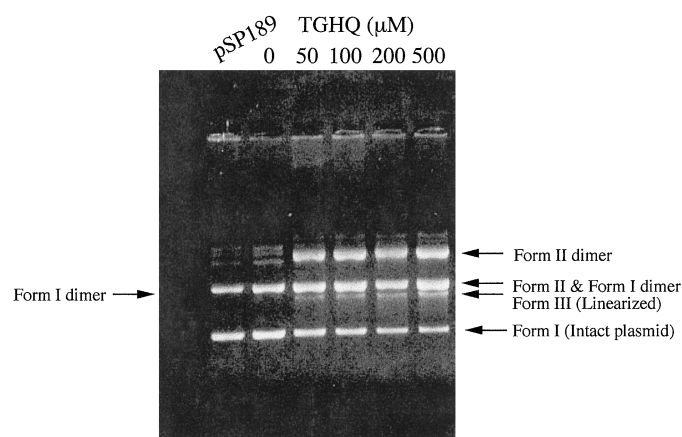


Fig. 1. Gel electrophoresis of pSP189 DNA following exposure to TGHQ revealing increases in linearized DNA. After DNA treatment with TGHQ (0, 50, 100, 200, and 500 µM), the DNA samples were washed with cold Tris-EDTA buffer [10 mM Tris-HCl and 1 mM EDTA (pH 8.0)] and concentrated using Centricon-30 concentrators. DNA was separated on agarose gels to visualize the DNA damage. *Form I*, intact pSP189 plasmid; *Form II*, open relaxed circles due to the presence of single-strand breaks; *Form III*, linearized plasmid due to double-strand breaks.

Table 1. Type and frequency of mutations induced by TGHQ in pSP189 replicated in human AD293 and bacterial MBL50 cells

Sequence alteration	No. of mutations (% of total)	
	Human AD293	Bacterial MBL50
Base substitutions	68 (64.3)	51 (91.1)
Other		
Deletion		
<10 bp	16 (15.1)	5 (8.9)
>10 bp	16 (15.1)	0 (0)
Insertion	3 (2.8)	0 (0)
Tandem	3 (2.8)	0 (0)
Total mutations	106 (100)	56 (100)

intact plasmid (*Form I*). Because the dimerized forms of the intact plasmid and the linearized circles migrated very closely, these two forms were not resolved in this gel. By comparing *Lane 2* (0 µM TGHQ) and *Lane 3* (50 µM TGHQ), we saw that the largest increases in DNA damage occurred at the lowest concentration examined, suggesting that TGHQ is a potent DNA-damaging agent. Exposure of the pSP189 DNA to TGHQ increased the mutation frequency 4.6-fold over the control (5.62×10^{-3} to 2.59×10^{-2}) in AD293-replicated plasmids and 2.6-fold (1.96×10^{-4} to 5.19×10^{-4}) in MBL50-replicated plasmids. The spontaneous mutation rate for pSP189 replicated in AD293 cells was, therefore, about double that reported previously (1×10^{-4} ; Ref. 38), but in MBL50-replicated plasmids, it was considerably higher than that reported previously (0.6×10^{-5} ; Ref. 37). These differences may be due to differences in transfection procedures used because these are known to cause differences in spontaneous mutation frequency.

Types of Mutations Induced by TGHQ. All mutants generated by TGHQ, 88 mutants from AD293-replicated plasmids, and 52 mutants from MBL50-replicated plasmids were sequenced, and the results are summarized in Table 1. Multiple mutations occurred in 15 of the 88 (17.0%) AD293 mutants, for a total of 106 mutations. The major mutations in both human and bacterial cells were base substitutions. TGHQ induced 64.3 and 91.1% base substitutions following replication in AD293 and MBL50 cells, respectively. A high frequency of deletions (30.2%), including deletions of <10 bp and >10 bp, were also observed in AD293-replicated plasmids. Of the single-base deletions, deletion of cytosine was the most frequent (four of nine), with three of the cytosine deletions occurring at a single hot spot (position 174, within a span of five consecutive cytosines (5'-172-CCCCC-3')). In contrast, the frequency of deletions (8.9%), which were exclusively <10 bp (all single-base deletions), was much less in MBL50-replicated plasmids (Table 1). Of the five single-base deletions in MBL50-replicated plasmids, three were cytosine, and two of these occurred at the same 5'-172-CCCCC-3' site. In AD293-replicated plasmids, various types of mutations (base substitutions, deletions, insertions, and tandem) were detected, whereas fewer types of mutations (base substitutions and deletions) were observed in MBL50-replicated plasmids.

Types of Base Substitutions Induced by TGHQ. In AD293-replicated plasmids, total base substitutions occurred predominantly at G:C sites (82.3%), whereas 17.6% base substitutions occurred at A:T sites (Table 2). The most common mutations were G:C to A:T transversions (33.8%), followed by G:C to T:A transversions (29.4%), and G:C to C:G transversions (19.1%). In MBL50-replicated plasmids, base substitutions at G:C sites predominated (96.1%), with 4.0% base substitutions at A:T sites (Table 2). The most common mutations were G:C to T:A transversions (37.3%), followed by G:C to C:G transversions (31.3%) and G:C to A:T transversions (27.5%).

Mutation Spectra Induced by TGHQ. The mutation spectra induced in the *supF* gene in plasmids that were replicated in either

Table 2 Frequency of base substitution mutations induced by TGHQ in pSP189 replicated in human AD293 and Bacterial MBL50 cells

Sequence alteration	No. of mutations (% of total)	
	Human AD293	Bacterial MBL50
Transversions		
GC→TA	20 (29.4)	19 (37.3)
GC→CG	13 (19.1)	16 (31.3)
AT→TA	4 (5.9)	0 (0)
AT→CG	6 (8.8)	1 (2.0)
Transitions		
GC→AT	23 (33.8)	14 (27.5)
AT→GC	2 (2.9)	1 (2.0)
Total mutations	68 (100)	51 (100)

AD293 and MBL50 cells are summarized in Fig. 2. In AD293-replicated plasmids, five hot spots (105, 109, 133, 163, and 164) were present, all of which occurred at G:C sites. None of these hot spots corresponded to the site (position 129) at which the largest localization of base substitutions occurred in spontaneous mutants (37). Moreover, only one of the hot spots (position 133) was identical to hot spots identified in γ -irradiated pSP189 (39). A total of 9 single-bp deletions were present (see above); five G:C site deletions and four A:T site deletions. These deletions were unique to treatment of the *supF* gene with TGHQ because no deletions of <10 bp were seen in spontaneous mutants. Among three tandem mutations, again all unique to chemical treatment, one was a double transition (CC to AA at position 108), and two were double transversions (CC to AA at position 142 and TT to GG at position 170). Three insertions were detected, between positions 139 and 140, 153 and 154, and 169 and 170. A pair of cytosines were inserted between positions 139 and 140. The insertion between positions 153 and 154 consisted of a repeat of the preceding 21 bases (positions 133–153), and the insertion between positions 169 and 170 consisted of a repeat of the preceding 8 bases (positions 161–169). No insertions were identified among spontaneous mutations.

In MBL50-replicated plasmids, eight hot spots (105, 127, 131, 140, 152, 156, 168, and 172) were present, seven of which occurred at G:C sites and one (position 140) of which occurred at an A:T site (Fig. 2). A total of five single-bp deletions were also present, and all occurred as G:C site deletions. In contrast to AD293-replicated plasmids,

neither tandem mutations nor insertions were found in MBL50-replicated plasmids. Position 105 in the *supF* gene represented the only common hot spot in AD293- and MBL50-replicated plasmids.

DISCUSSION

TGHQ induces mutations in both human and bacterial cell replicated pSP189 plasmids (Fig. 2). Although the majority of these mutations are similar to those that arise spontaneously in replicated pSP189 plasmids, mutations that are unique to the treatment of the pSP189 plasmid with TGHQ also occur. Thus, TGHQ also induces deletions of <10 bp, insertions, and tandem mutations (Table 1), none of which occur in the spontaneous mutants.

The generation of hydrogen peroxide and subsequent iron ion-mediated formation of $\cdot\text{OH}$ play an important role in quinone-thioether-mediated cytotoxicity. Scavengers of hydrogen peroxide and Fe^{2+} inhibit quinone-thioether-mediated DNA single-strand breaks, cytotoxicity (40, 41), and the expression of *gadd153* (41, 42). Consistent with these findings, the mutation spectra induced by TGHQ shares many similarities with previously reported $\cdot\text{OH}$ -induced mutation spectra. Thus, in general, $\cdot\text{OH}$ is the most reactive ROS, causing strand breaks and, subsequently, base deletions. However, the mechanism of ROS generation in this system is unclear, particularly in view of the complex redox reactions of polyphenols with superoxide anion and the electron transfer equilibria. Superoxide anion radicals formed during the auto-oxidation of TGHQ may, subsequently, generate hydrogen peroxide via dismutation. The reduction of hydrogen peroxide with DNA-bound iron will then yield the hydroxyl radical by Fenton chemistry. The rapid plateau in TGHQ-induced single- and double-strand breaks (Fig. 1) might, thus, be attributed to the limiting supply of reduced iron (Fe^{2+}), which, in the absence of reducing equivalents (other than superoxide), remains in the Fe^{3+} form following oxidation by hydrogen peroxide. It is also possible that the spontaneous dismutation of superoxide anion may generate the reactive singlet oxygen, which itself damages DNA. Determining the effect of hydroxyl radical scavengers on the mutation frequency of TGHQ may provide an answer to this question. Nonetheless, the mutagenic and cytotoxic properties of quinone-thioethers, therefore, provide a basis for their ability to behave as complete carcinogens.

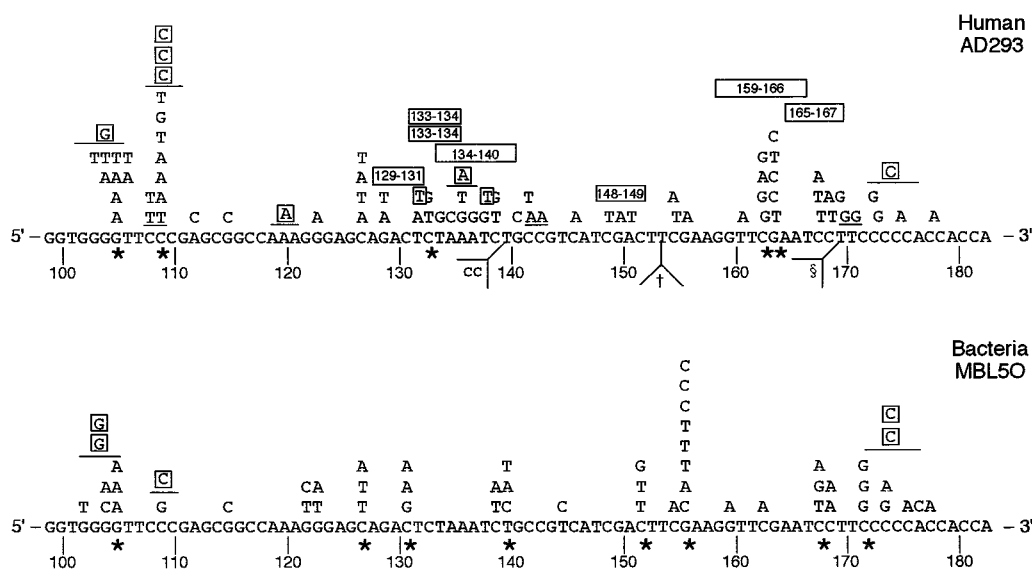


Fig. 2. Distribution of TGHQ-induced mutations in the *supF* gene replicated in either human AD293 cells or bacterial MBL50 cells. Base substitutions are noted with the appropriate letter designations. Letters and ranges within boxes, deletions of single bases and sequences, respectively. *, hot spots; †, sequence between positions 133 and 153 was inserted (repeated) between positions 153 and 154; §, sequence between positions 161 and 169 was inserted (repeated) between positions 169 and 170. Underlined letters, components of multiple sequence changes within one mutant (15 of 88 mutants contained multiple mutations). Boxes, deletions of <10 bp. Deletions of >10 bp have been omitted for reasons of clarity.

Mutations induced by TGHQ may be propagated by its ability to cause a sustained regenerative hyperplasia within renal proximal tubular epithelial cells (33), the site at which tumors eventually develop (20, 21).

The mutagenicity of $\cdot\text{OH}$ has been documented in a variety of experimental systems. For example, when the pZ189 plasmid is exposed to $\cdot\text{OH}$ under cell-free conditions and is replicated in host cells, high numbers of deletions and base substitutions are observed (38, 43). When $\cdot\text{OH}$ is generated via ionizing radiation, G:C to A:T transitions are observed, and human cells appear to be particularly susceptible to this form of genetic damage (44). Our results are consistent with these findings. In AD293-replicated TGHQ-treated plasmids, five hot spots are identified, all of which occur at G:C sites. In addition, exposure of pZ189 to UVB light (313 nm; Ref. 45) followed by replication in CV-1 monkey kidney cells, led mainly to G:C to A:T transitions (45). When pZ189, containing the *supF* gene as a target, was exposed to γ irradiation and replicated in human lymphoblastoid GM606 cells, base substitutions also occurred predominantly at G:C bp, with G:C to A:T transitions being the most common mutations observed (46). G:C to T:A transversions and G:C to C:G transversions were also found. In contrast to these findings, although base substitutions also occurred predominantly at G:C bp when pSP189 was exposed to γ -irradiation and replicated in human embryonic kidney AD293 cells, the most common mutations were G:C to T:A transversions (39). The finding that TGHQ induces a similar spectrum of mutations (Tables 1 and 2) indicates that quinone-thioethers are potential mutagens and that the majority of the mutations arise via hydroxyl radical-mediated mechanisms. However, there are some clear differences in the mutations caused by TGHQ and those reported in γ -irradiated pSP189 (39). For example, only 5 of 41 (12%) insertions caused by γ irradiation were <10 bp, whereas 16 of 32 (50%) of the insertions caused by TGHQ were <10 bp. In addition, TGHQ produced six A:T to C:G transversions, none of which occurred spontaneously, and only one was found in γ -irradiated pSP189 (39). The increased frequency of deletions of <10 bp and the A:T to C:G transversions may occur as a consequence of DNA alkylation by the electrophilic quinone-thioether. Thus, quinone-thioethers likely induce mutations as a consequence of both their redox and electrophilic properties, although this awaits further experimentation.

Quinone-thioethers are nephrotoxic (28, 32) and nephrocarcinogenic metabolites of HQ.⁵ However, HQ is generally nonmutagenic in short-term bacterial mutagenicity assays such as the Ames test (47, 48), and no mutagenic activity has been found in mouse cells *in vivo* (49). HQ does cause bp changes in the TA1535 *Salmonella* tester strain (50) and is mutagenic in oxidant-sensitive (TA104 and TA2637) *Salmonella* tester strains (51), consistent with the mutagenicity of 1,4-benzoquinone in several Ames bacterial tester strains (52). Recently, in the same *supF*/pSP189/AD293 system used in our studies, HQ was shown to cause several mutations, including base substitutions and deletions (no insertions were reported), and 24 of 38 mutations (63%) were base substitutions (53), which compares almost exactly (64%) with our findings. However, the pattern of mutations varied. For example, we found that 82% of all base substitutions occurred at G:C sites (Table 2), whereas with HQ, only 31.6% (12 of 38) of base substitutions occurred at G:C sites (53). It is unlikely that the metabolites ultimately responsible for the *in vivo* effects of HQ are formed in these *in vitro* mutagenicity assays. A comprehensive understanding of the metabolism of "nongenotoxic" carcinogens is required before such a designation is made. This may be a particularly

daunting task, given the fact that $<1\%$ of a dose of HQ needs converting to TGHQ to produce overt toxicity.

In bacterial cells, $\cdot\text{OH}$ -mediated deletions and substitutions at G:C sites are more frequent than substitutions at A:T sites (54, 55), which is similar to observations in mammalian cells. When double-stranded M13mp10 DNA and the pUC18 plasmid, containing a 144-bp insert in the *lacZ α* gene, are exposed to γ irradiation and replicated in *E. coli* KMBL 5071, base substitutions are mainly observed, of which G:C to C:G is the predominant mutation (54). In a single-strand vector replicated in bacteria, G:C to C:G transversions and G:C to A:T transitions (55) are the major types of base substitutions. Taken together, conditions that generate $\cdot\text{OH}$ as the primary ROS induce mainly base substitutions, with a high number of deletions and base substitutions occurring predominantly at G:C sites, and a low frequency at A:T bp in both mammalian and bacterial cells.

The mutation spectra observed in the human and bacteria cell systems exhibit significant differences. The predominant base substitutions induced by TGHQ in the mammalian AD293-replicated plasmids are G:C to A:T transitions, followed by G:C to T:A and G:C to C:G transversions. In contrast, G:C to T:A transversions predominate in the bacterial MBL50-replicated plasmids (Table 2), followed in frequency by G:C to C:G transversions and G:C to A:T transitions, suggesting that the specific type of mutation (base substitution) is dependent on the host cell system. These differences probably reflect differences in the repair and replication machinery between mammalian and bacterial cells. Although previous reports indicate that G:C to C:G transversions are the predominant sequence alterations in bacteria, G:C to T:A transversions were predominant in the TGHQ treated MBL50-replicated plasmid (Table 2).

Although the major type of mutation in both cell systems are base substitution (Table 1), fewer types of mutations occur in MBL50-replicated plasmids. Thus, insertions and tandems only occur in AD293-replicated plasmids. Interestingly, of three tandem mutations, one is a double transition (CC to TT at position 108). Iron, copper, and phorbol ester-stimulated neutrophils also produce tandem CC to TT mutations, in the *lacZ α* gene, and this mutation was suggested as a potential marker for oxidative damage during carcinogenesis (56). However, of the two tandem mutations reported from γ -irradiated pSP189 plasmids (39), one was a GC to TT, and the other was a GG to TT mutation. Finally, G:C to A:T transitions followed by G:C to T:A and G:C to C:G transversions are the major mutations found in AD293-replicated plasmids. In MBL50-replicated plasmids, G:C to T:A transversions were followed in frequency by G:C to C:G transversions and G:C to A:T transitions.

In conclusion, we have shown that TGHQ and, by extension, other quinone-thioethers are mutagenic. Although the mutation spectrum induced by TGHQ is consistent with the participation of $\cdot\text{OH}$ in quinone-thioether-mediated DNA damage and cytotoxicity, several mutations occur with a frequency that is indicative of the participation of additional mutagenic species, most likely by quinone-thioether-mediated alkylation of DNA bases. In addition, recent experiments indicate that TGHQ induces the formation of renal tumors in the Eker rat, probably by acting at the initiation stage, rather than by promoting the growth of existing lesions.⁵ Consistent with this view, a single treatment of rat renal epithelial cells with TGHQ (100 μM) produces aneuploidy and cell transformation within 8 weeks.⁶ The combined data, therefore, support the view that, when identified, polyphenolic-GSH conjugates should be considered potentially carcinogenic metabolites.

⁵ S. S. Lau, T. J. Marks, J. I. Everitt, and C. L. Walker, unpublished data.

⁶ H. S. Yoon, C. L. Walker, T. J. Monks, and S. S. Lau, unpublished data.

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