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Invited Review

Role of Quinones in Toxicology†

Judy L. Bolton,*,‡ Michael A. Trush,§ Trevor M. Penning,∥ Glenn Dryhurst,⊥ and Terrence J. Monks*,@

Department of Medicinal Chemistry and Pharmacognosy (M/C 781), College of Pharmacy, The University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612-7231, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland 21205, Department of Pharmacology, University of Pennsylvania School of Medicine, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, Pennsylvania 19104-6084, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, and Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712-1074

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Quinones represent a class of toxicological intermediates which can create a variety of hazardous effects in vivo, including acute cytotoxicity, immunotoxicity, and carcinogenesis. The mechanisms by which quinones cause these effects can be quite complex. Quinones are Michael acceptors, and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. Alternatively, quinones are highly redox active molecules which can redox cycle with their semiquinone radicals, leading to formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins, and DNA. Formation of oxidatively damaged bases such as 8-oxodeoxyguanosine has been associated with aging and carcinogenesis. Furthermore, ROS can activate a number of signaling pathways, including protein kinase C and RAS. This review explores the varied cytotoxic effects of quinones using specific examples, including quinones produced from benzene, polycyclic aromatic hydrocarbons, estrogens, and catecholamines. The evidence strongly suggests that the numerous mechanisms of quinone toxicity (i.e., alkylation vs oxidative stress) can be correlated with the known pathology of the parent compound(s).

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1. Introduction

The cytotoxic and/or genotoxic effects elicited by environmental chemicals and various other xenobiotics as well as endogenous compounds are often due to intracellular reactions of electrophilic or free radical metabolites. A few of the best known examples of toxic electrophiles include epoxides from polycyclic aromatic hydrocarbons (PAHs;[1] *1*), transient carbonium ions from *N*-nitroso compounds or aromatic sulfate esters, nitrenium ions generated after the N-hydroxylation of aromatic amines or amides (*2*), and α,β-unsaturated carbonyl compounds such as acrolein that serve as Michael acceptors (*3*). These species alkylate nucleophilic sites on peptides, proteins, and/or nucleic acids, forming covalent adducts that can significantly compromise cellular integrity and function (*4*). Examples of compounds which can form toxic radicals include radical cations formed from PAHs (*5*), alkoxyl and peroxyl radicals generated through interactions of conjugated hydrocarbons (i.e., lipids) with reactive oxygen species (*6*, *7*), and carbon-centered radicals formed during reductive metabolism of carbon tetrachloride as well as volatile halogenated analgesics (*8*). These radicals can cause a variety of deleterious effects in cells such as oxidation of proteins, lipids, and DNA as well as activation of numerous signaling pathways involved in several human pathologies, including the aging process and initiation, promotion, and progression of carcinogenesis (*9*, *10*).

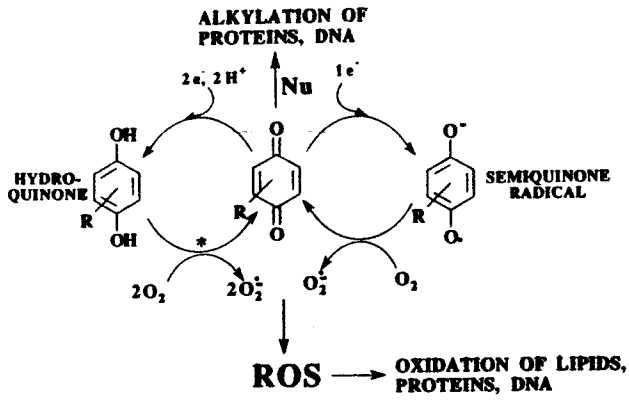

Figure 1. Alkylation and redox cycling of quinones generating adducts and ROS. The asterisk indicates that this reaction only occurs for quinones which autoxidize.

Aromatic compounds with oxygen-containing substituents also have been extensively studied. These include phenols, hydroquinones, and catechols, all of which can be converted to quinones by monooxygenase or peroxidase enzymes, metal ions, and in some cases molecular oxygen (*11*). Quinones are a general term for a ubiquitous class of compounds which are common in several natural products and endogenous biochemicals or generated through metabolism of hydroquinones and/or catechols. Generally, quinones are named as derivatives of their parent aromatic system. As a result, benzoquinones are derived from benzene, naphthoquinones from naphthalene, and anthraquinones from anthracene to name a few examples. Some quinones are potent redox active compounds. They can undergo enzymatic (i.e., P450/P450 reductase) and nonenzymatic redox cycling with their corresponding semiquinone radical and as a result generate superoxide anion radicals (Figure 1) (*11*–*13*). The reaction of hydrogen peroxide, formed by the enzymatic or spontaneous dismutation of superoxide anion radicals, with trace amounts of iron or other transition metals gives hydroxyl radicals. The hydroxyl radicals are powerful oxidizing agents that may be responsible for damage to essential macromolecules. For example, oxidation of cysteine residues in proteins leads to disulfide bond formation which can dramatically alter protein structure and function. Hydroxyl radicals also can catalyze oxidation of lipids, generating lipid hydroperoxides which can lead to formation of [illegible]-derived malondialdehyde DNA adducts (*14*). Finally, biomarkers for oxidative

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* To whom correspondence should be addressed: Department of Medicinal Chemistry and Pharmacognosy (M/C 781), College of Pharmacy, The University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612-7231. Fax: (312) 996-7107. E-mail: Judy.Bolton@UIC.edu.

‡ The University of Illinois at Chicago.

§ Johns Hopkins University.

|| University of Pennsylvania.

⊥ University of Oklahoma.

● University of Texas at Austin.

[1] Abbreviations: AKR, aldo-keto reductase; dG, 2′-deoxyguanosine; dA, 2′-deoxyadenosine; dC, 2′-deoxycytosine; 2-OHE, 2-hydroxyestrone, 2,3-dihydroxy-1,3,5(10)-estratrien-17-one; 4-OHE, 4-hydroxyestrone, 3,4-dihydroxy-1,3,5(10)-estratrien-17-one; estrone, 3-hydroxy-1,2,5(10)-estratrien-17-one; 4-OHEN, 4-hydroxyequilenin, 3,4-dihydroxy-1,3,5-(10),6,8-estrapentaen-17-one; equilenin, 1,3,5(10),6,8-estrapentaen-3-ol-17-one; equilin, 1,3,5(10),7-estratetraen-3-ol-17-one; 9(11)-dehydro-4-OHE, 9(11)-dehydro-4-hydroxyestrone, 3,4-dihydroxy-1,3,5(10),9(11)-estratetraen-17-one; NQO1, NAD(P)H:quinone oxidoreductase, DT-diaphorase; 8-oxo-dG, 8-oxodeoxyguanosine; 8-oxo-dA, 8-oxodeoxyadenosine; P450, cytochrome P450; PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; γ-GT, γ-glutamyl transpeptidase; 5-HT, 5-hydroxytryptamine; α-MeDA, α-methyldopamine; MDA, 3,4-(±)-methylenedioxyamphetamine; MDMA, 3,4-(±)-methylenedioxymethamphetamine; NF-κB, nuclear factor κ B; SM, neutral magnesium-dependent sphingomyelinase; TGHQ, 2,5,6-triglutathionyl-3-yl-1,4-quinone.

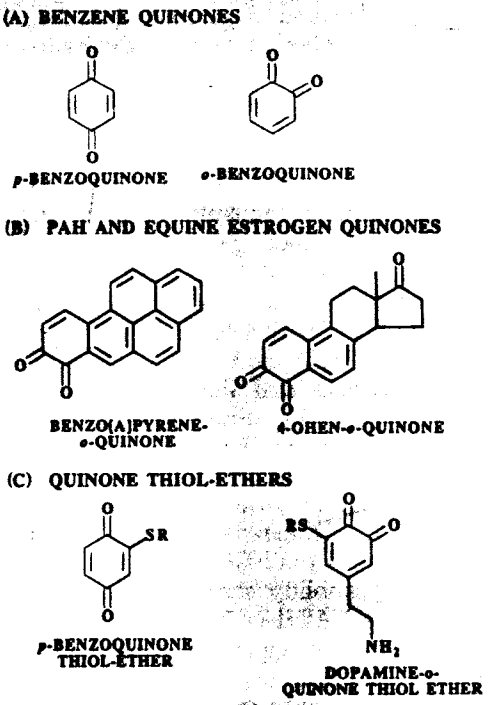


Figure 2. Types of quinones discussed herein.

damage to DNA include the formation of the mutagenic lesion, 8-oxo-2′-deoxyguanosine (8-oxo-dG; *15*).

Quinones are also Michael acceptors; therefore, damage due to these species sometimes results from covalent binding with cellular nucleophiles. For example, they react readily with sulfur nucleophiles, such as GSH or cysteine residues on proteins, leading to depletion of cellular GSH levels and/or protein alkylation. In addition, some quinones can react with nucleophilic amino groups on proteins or DNA. For example, benzene is converted to hydroquinone and catechol by hepatic cytochrome P450, and the subsequent peroxidase-catalyzed oxidation of these metabolites to *p*-benzoquinone and *o*-benzoquinone in bone marrow may explain the induction of leukemia during chronic exposure to this solvent (Figure 2A; *16*, *17*). The fact that *p*-benzoquinone is known to form a DNA adduct supports this hypothesis (*18*), although the genotoxic mechanism also may involve the formation of ROS which cause single-strand breaks as well as oxidation of DNA bases. Quinone formation from polycyclic aromatic hydrocarbons (PAHs) and endogenous or exogenous estrogens also has been implicated as a potential mechanism contributing to the carcinogenesis of the parent compounds (Figure 2B; *19*, *20*). Finally, the addition of a sulfur atom to the quinone ring generates a quinone thiol ether (Figure 2C) which is usually much more redox active than an unsubstituted quinone. As a result, quinone thiol ether formation could contribute to the pathology of Parkinson's disease as well as other neurological disorders (*21*). *The focus of this review is the role of quinones in the cytotoxicity and genotoxicity of polyphenolic compounds.*

2. Benzene Quinones and Bone Marrow Toxicity and Chemoprotection

2.1. Benzene Is an Occupational and Public Health Concern. Benzene is a widely used solvent as well as a constituent of gasoline and cigarette smoke (*22*). Health concerns about benzene exposure arose when decreased numbers of circulating blood cells were observed in workers (*23*). Subsequently, epidemiological studies demonstrated that chronic occupational high-level benzene exposure resulted in the development of aplastic anemia and acute myelogenous leukemia (*24*, *25*). Accordingly, benzene has and continues to undergo regulatory scrutiny to ensure reduced occupational exposures and improved worker health (*26*). On the other hand, because of its ubiquitous presence, particularly in urban settings, there is still concern for potential human effects from low-level chronic environmental exposure to benzene (*27*).

2.2. Benzene Is an Example of a Selective Organ and Cell Toxicant. Benzene exposure is primarily via inhalation. Following rapid absorption from the lungs, benzene undergoes both phase I and II biotransformation in the liver (*28*). The primary phase I biotransformation of benzene involves the cytochrome P450-catalyzed conversion initially to phenol and subsequently to hydroquinone and catechol. The specific isoform of P450 involved in this process is cytochrome P450 2E1 (*29*). Indeed, liver microsomes from individuals higher in CYP 2E1 activity convert more phenol to hydroquinone. Likewise, knockout mice for 2E1 do not exhibit benzene-induced hematotoxicity (*30*). Thus, the liver plays a fundamental role in determining benzene's toxicological actions.

Despite the ability of the liver to biotransform benzene, it is not the principal target organ of toxicity. In both experimental animals and humans, the bone marrow is the primary organ of toxicity. However, the biochemical and molecular basis for why the bone marrow is the primary target organ is not presently known. As compared to the liver, the bone marrow exhibits much lower levels of enzymes as well as glutathione involved in xenobiotic processing and protection which contributes to both the selective target organ and cell toxicity that is observed (*31*). As such, exposure of mice to levels of benzene routinely encountered environmentally by the human population shows that both DNA and protein adducts are higher in bone marrow than in liver, with protein adduct levels being 9−43-fold greater than DNA adduct levels (*32*). Such adducts are generally attributed to hydroquinone-derived benzoquinone, a strong electrophile particularly toward protein sulfhydryls. Relative to metabolites from other carcinogens, benzene-derived metabolites are not particularly reactive toward DNA, although adducts have been isolated and characterized in vitro (*32*).

The issue then becomes what factors modulate the biologically effective concentration of benzoquinone: how is hydroquinone converted to benzoquinone in target cells and what modulates benzoquinone's availability to interact with target molecules? Within cells, there are several mechanisms which can convert hydroquinone to benzoquinone (Figure 3). A current concept is that myeloperoxidase is the principal mechanism that converts hydroquinone to benzoquinone (*33*). Myeloperoxidase is found in high concentrations in differentiated myeloid cells (polymorphonuclear leukocytes and monocytes) and their progenitors, including $CD34^+$ cells and myeloblasts (*34*, *35*). Myeloperoxidase requires hydrogen peroxide to be catalytically active. While differentiated polymorphonuclear leukocytes and monocytes have NAPDH oxidase which serves as a source of superoxide and in turn hydrogen peroxide, target cells of hydroquinone, such as $CD34^+$ cells, myeloblasts, and monoblasts, lack

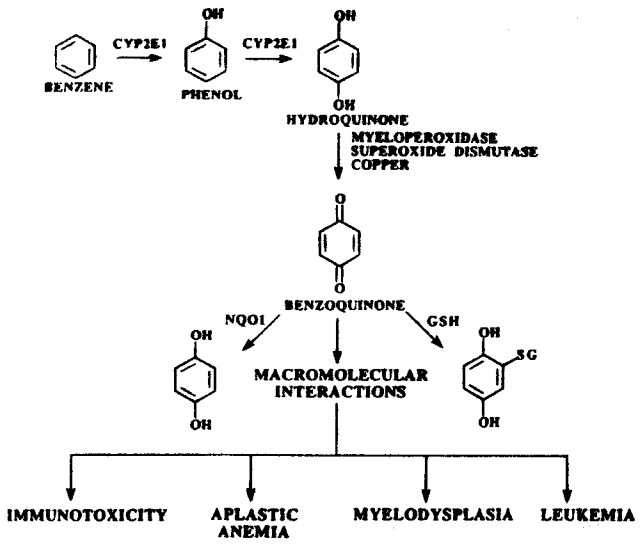


Figure 3. Scheme representing the toxicodynamics of benzene-derived hydroquinone in bone marrow cell populations contributing to a spectrum of benzene-associated diseases.

this enzyme and hence an endogenous source of hydrogen peroxide. Moreover, these cells also do not exhibit mitochondrial respiration which is an intracellular source of superoxide and hydrogen peroxide found in fully differentiated macrophages (*36*, *37*). A potential source of hydrogen peroxide is the autoxidation of hydroquinone, a process which can be accelerated by either superoxide dismutase (SOD) or copper ions. The enhanced autoxidation of hydroquinone by SOD or copper results in the formation of both benzoquinone and hydrogen peroxide, which can then be utilized by myeloperoxidase to generate more benzoquinone (*38*–*41*). SOD and copper are attractive as potential activating systems for hydroquinone because they are found in targets cells of hydroquinone that lack myeloperoxidase, such as erythrocytes, lymphocytes, and stromal macrophages and fibroblasts. Another question relevant to the mechanism of hydroquinone activation is the identity of the cellular compartment where activation occurs. Myeloperoxidase is located in azurophilic granules; Cu/Zn SOD is cytoplasmic, and copper is found associated with DNA. Interestingly, many of the molecular targets suggested as contributing to benzene-induced pathogenesis are found in either the cytoplasmic or nuclear compartments. These include tubulin, topoisomerase II, histone, and DNA (*33*). Thus, while myeloperoxidase is an attractive candidate for hydroquinone activation, these other possible mechanisms need to be taken into consideration.

In terms of directly modulating the bioavailability of benzoquinone, there are two cellular factors that appear to be critical: GSH and NAD(P)H:quinone oxidoreductase (NQO1) (Figure 3). GSH reacts directly with benzoquinone, whereas NQO1 reduces benzoquinone back to hydroquinone which can be glucuronidated or sulfated for cellular excretion. Epidemiologic and experimental studies have shown that a mutation in the gene for NQO1 has been linked to increased risk for benzene poisoning (*42*, *43*). Interestingly, freshly isolated human bone marrow cells do not express detectable levels of NQO1, but during cell culture, the enzyme can be detected (*35*). Recently, Smith (*44*) suggested that NQO1 may play a role in preventing hydroquinone-induced toxicity in a manner other than prevention of arylation of its target molecule. It has been our experience that the roles that GSH and NQO1 play in preventing hydroquinone-induced toxicity vary according to the state of cell differentiation, the cell type, and the species from which bone marrow cells are obtained (*45*). For example, in undifferentiated myeloid cells, GSH appears to be the primary protective factor (*34*). On the other hand, in differentiated macrophages, both GSH and NQO1 appear to be important in protecting cells against hydroquinone effects. Both GSH and NQO1 levels increase significantly with macrophage but not polymorphonuclear cell differentiation (Y. Li and M. A. Trush, unpublished observation comparing ML-1 and HL-60 cell differentiation to that of macrophages and PMNs, respectively). It is interesting to note that Smith and co-workers (*44*) observed that increasing NQO1 levels in undifferentiated HL-60 cells through gene transfection had only a modest effect on hydroquinone covalent binding and cell death. Thus, the state of cell differentiation may have something to do with the capacity of NQO1 to function catalytically in cells. One possibility may be the availability of cofactors such as NADH or NAD(P)H. On the basis of these observations, it appears that GSH is the main defense in myeloid progenitors which are the targets of benzene-induced leukemia and that both GSH and NQO1 will be critical defenses in the more differentiated stromal macrophages and fibroblasts. This conclusion is based on studies using either buthionine sulfoximine or a GSH ester to deplete or increase cellular GSH levels, respectively, using dicumarol to inhibit NQO1 and using 1,2-dithiole-3-thione to induce cellular GSH and NQO1 (*45*).

Agents such as 1,2-dithiole-3-thione and its derivative oltipraz have been shown to be effective chemopreventive agents against a number of compounds (*46*). We have observed a variety of chemoprotective effects of 1,2-dithiole-3-thione within bone marrow cells, including inhibition of cell proliferation and viability, reduction in the extent of stromal cell-supported myelopoiesis, and alteration of mononuclear cell differentiation (*45*). It should be noted that agents such as 1,2-dithiole-3-thione induce a number of cellular systems other than GSH and NQO1 which may contribute to its chemoprotective actions (*46*, *47*).

While benzene is a structurally simple chemical, its biological interactions appear to be quite complex. This complexity is due in part to our lack of understanding of how the bone marrow functions at the molecular and cellular levels and how benzene-derived metabolites, such as hydroquinone, interfere with these processes. Over the past several years, we have gained further insights into the underlying basis for susceptibility to benzene-induced toxicities (*35*, *42*, *43*, *45*). Given that benzene is such a prevalent chemical in the environment, understanding factors which modulate its toxicologic actions in target cells could lead to strategies, including dietary, for reducing and preventing any untoward effects of benzene metabolites in humans.

3. *o*-Quinones: Activated Metabolites of Polycyclic Aromatic Hydrocarbons

3.1. Metabolic Activation of Polycyclic Aromatic Hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental [illegible] that are procarcinogens requiring metabolic [illegible] to electrophiles to exert their carcinog[illegible] three principal

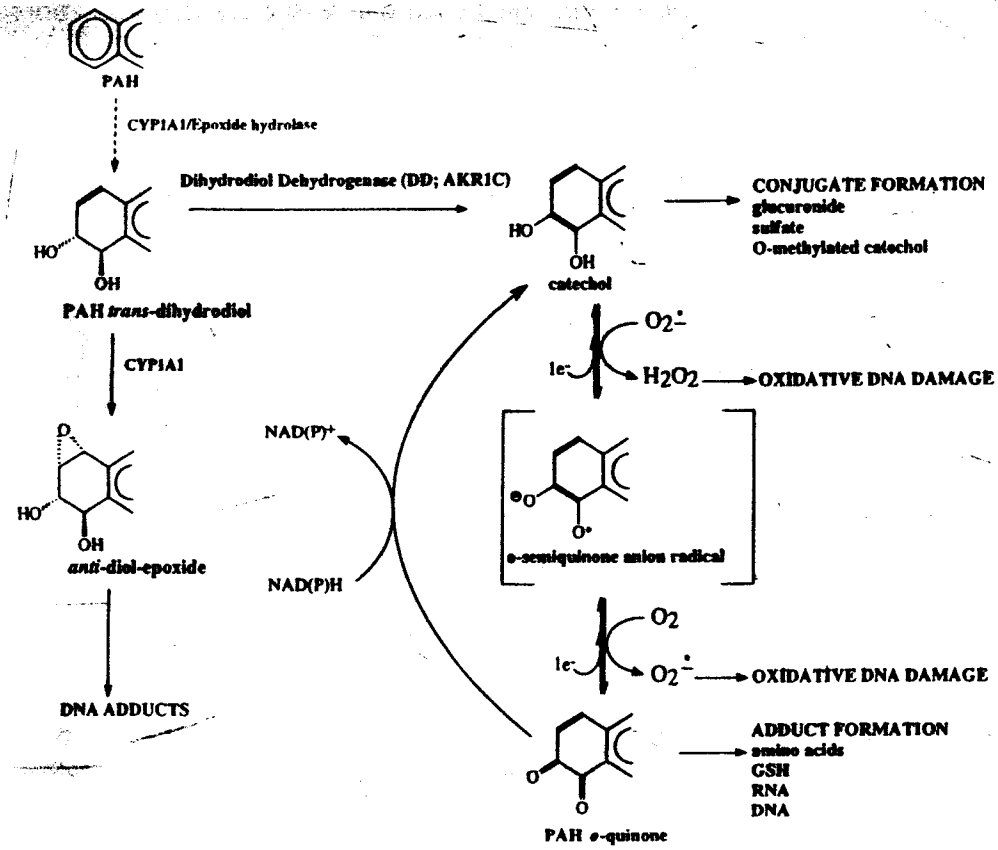


Figure 4. Cytochrome P450 (P450) and dihydrodiol dehydrogenase (AKR) isoforms compete for PAH *trans*-dihydrodiols.

routes of metabolic activation exist for these compounds. These include the formation of *anti*- and *syn*-diol epoxides (via P450 1A1 and epoxide hydrolase) (*1*, *48*), the formation of radical cations (via P450 and/or peroxidases; *5*), and the formation of *o*-quinones via dihydrodiol dehydrogenases (Figure 4) (*20*, *49*). Compelling evidence of the formation of diol epoxides being a major route of metabolic activation that leads to stable DNA adducts, mutation, and carcinogenicity exists (*1*, *48*). However, activation to diol epoxides fails to explain some important observations. First, the diol epoxides are weaker tumorigens than the parent hydrocarbons in the initiation of mouse skin papillomas (*50*–*52*). Second, the formation of diol epoxides does not explain why there is an increase in the level of oxidatively damaged bases (e.g., 8-oxo-dG and thymine glycol) following exposure to PAHs (*53*, *54*). Third, change in function mutations which are often G to T transversions observed in the 12th codon of ras (activation) and the 157, 248, and 273 codons of p53 (inactivation) seen in PAH-induced cancers (*55*–*57*) are more easily explained by either the radical cations or PAH *o*-quinones than by the diol epoxides. For example, translesional synthesis through bulky diol epoxide DNA adducts will not yield G to T transversions reliably. In contrast, radical cations or PAH *o*-quinones form depurinating N7 guanine adducts, and the unique incorporation of an A opposite the abasic site yields the G to T transversion (*55*, *58*). Fourth, the diol epoxides do not provide a mechanism by which PAHs can act as complete carcinogens, i.e., tumor initiators and promoters. Diversion of PAH *trans*-dihydrodiols (proximate carcinogens) via dihydrodiol dehydrogenases to reactive and redox active *o*-quinones provides an alternative route to metabolites that may act as complete carcinogens. Evidence for the dihydrodiol dehydrogenase pathway and the properties of the PAH *o*-quinones that are produced will be briefly reviewed.

3.2. *trans*-Dihydrodiol Specificity of Recombinant Dihydrodiol Dehydrogenases. cDNA cloning and expression of recombinant rat and human liver dihydrodiol dehydrogenases has been achieved (*59*–*64*). These enzymes belong to the aldo-keto reductase (AKR) gene superfamily (*65*). Rat liver dihydrodiol dehydrogenase (AKR1C9) and its human homologues [AKR1C1–AKR1C4, where AKR1C1 is 20α(3α)-hydroxysteroid dehydrogenase, AKR1C2 is bile acid binding protein, AKR1C3 is type 2 3α-hydroxysteroid dehydrogenase, and AKR1C4 is type 1 3α-hydroxysteroid dehydrogenase] will oxidize a structural series of 15 PAH *trans*-dihydrodiols (Table 1) (*60*, *66*–*68*). Metabolically, non-K region *trans*-dihydrodiols (where the dihydrodiol is on the terminal benzo ring) with the *R*,*R*-configuration are often formed. Each AKR displayed the appropriate regio- and stereochemistry for oxidizing the major stereoisomers formed in vivo. Importantly, the rat and human recombinant enzymes oxidized both the (−)-(*R*,*R*)- and the (+)-(*S*,*S*)-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (B[*a*]P-diol), a potent proximate carcinogen (*64*, *66*). Of the human isoforms that have been studied, AKR1C1 and AKR1C2 displayed the highest catalytic efficiency (V_{max}/K_m) for B[*a*]P-diol oxidation. Additionally, each enzyme showed a preference for bay region methylated non-K region PAH *trans*-dihydrodiols, and this methylation often increases the carcinogenicity of the parent PAH. For example, both the rat AKR1C9 and the human AKR1C2 exhibited a 4–5-fold increase in V_{max}/K_m for the oxidation of *trans*-3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[*a*]anthracene over that seen for *trans*-3,4-dihydroxy-3,4-

Table 1. PAH *trans*-Dihydrodiol Specificity of Rat and Human Dihydrodiol Dehydrogenase (DD) Isoforms[a]

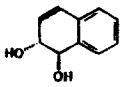
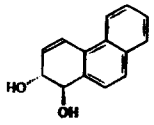
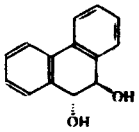
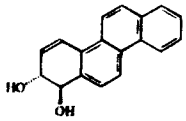
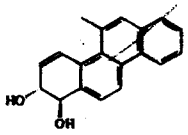
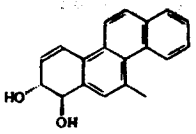
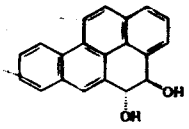
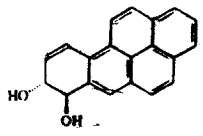
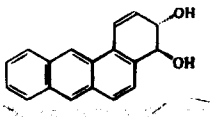
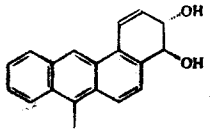
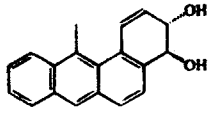
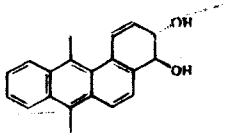
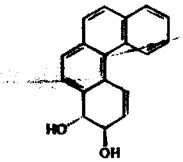
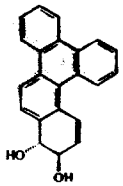
PAH *trans*-dihydrodiol	V_{max}/K_m[b]				
	Rat DD AKR1C9	Human DD1 AKR1C1	Human DD2 AKR1C2	Human DD4 AKR1C4	Human DDx AKR1C3
 (±)-benzenedihydrodiol	1735	56	96	*512*	104
 (±)-1,2-dihydroxy-1,2-dihydronaphthalene	51	1704	*2088*	600	152
 (±)-1,2-dihydroxy-1,2-dihydrophenanthrene	160	ND	ND	ND	ND
 (±)-9,10-dihydroxy-9,10-dihydrophenanthrene	-	-	-	-	-
 (±)-1,2-dihydroxy-1,2-dihydrochrysene	114	-	*210*	148	-
 (±)-1,2-dihydroxy-1,2-dihydro-5-methylchrysene	1058	ND	ND	ND	ND
 (±)-7,8-dihydroxy-7,8-dihydro-5-methylchrysene	784	322	871	*1466*	330
 (±)-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene	-	-	-	-	-
 (±)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene	338	180	*244*	100	20
 (±)-3,4-dihydroxy-3,4-dihydrobenz[a]anthracene	456	-	286	*820*	225

Table 1. (Continued)

PAH *trans*-dihydrodiol	Rat DD AKR1C9	Human DD1 AKR1C1	Human DD2 AKR1C2	Human DD4 AKR1C4	Human DDx AKR1C3
			V_{max}/K_m[b]		
 (±)-3,4-dihydroxy-3,4-dihydro-7-methylbenz[a]anthracene	884	-	*1808*	1300	307
 (±)-3,4-dihydroxy-3,4-dihydro-12-methylbenz[a]anthracene	463	-	-	-	-
 (±)-3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz[a]anthracene	1877	634	1240	*1600*	580
 (±)-3,4-dihydroxy-3,4-dihydro-benzo[c]phenanthrene	ND	-	-	*130*	-
 (±)-11,12-dihydroxy-11,12-dihydrobenzo[g]chrysene	ND	168	278	*2214*	415

[a] Taken from ref *5*. [b] V_{max}/K_m = nanomoles per minute per milligram per [S], where $v/[S] = V_{max}/K_m$ when $K_m \gg [S]$. ND, not determined; –, no detectable rate; highest efficiences are in italics.

dihydrobenz[*a*]anthracene (Table 1). The fjord region *trans*-dihydrodiol, *trans*-11,12-dihydroxy-12,12-dihydrobenzo[g]chrysene which is among the most potent proximate carcinogens, was the best substrate (highest V_{max}/K_m) of all tested *trans*-dihydrodiols for human AKR1C4 (*68*). When the catalytic efficiencies for the turnover of multiple *trans*-dihydrodiols by human AKR1C isoforms were considered, it was apparent that the liver specific AKR1C4 and the ubiquitously expressed AKR1C2 exhibited the highest catalytic efficiencies for the structural series that was tested.

3.3. Formation of PAH *o*-Quinones and Reactive Oxygen Species (ROS). Products of PAH *trans*-dihydrodiol oxidation catalyzed by AKRs were trapped with 2-mercaptoethanol as thioether conjugates of *o*-quinones and characterized by NMR and EIMS. Thus, B[*a*]P-diol is oxidized to a ketol which rearranges to a catechol (7,8-dihydroxybenzo[*a*]pyrene) which in turn autoxidizes to an *o*-quinone (benzo[*a*]pyrene-7,8-dione, BPQ). BPQ then undergoes 1,4-Michael addition to yield the fully autoxidized thioether conjugate (*69*). Measurements of oxygen metabolism during the enzymatic oxidation of B[*a*]P-diol showed that hydrogen peroxide was formed prior to oxygen consumption. Using DMPO as a spin-trap, evidence that superoxide anion was the initiating and propagating radical in the autoxidation event was obtained. In the final sequence, superoxide anion acts as a base to remove a proton from the intermediate catechol. The catecholate anion is then oxidized by the hydroperoxyl radical to form the *o*-semiquinone anion radical which reduces molecular oxygen to form superoxide anion. Superoxide anion can then propagate the next cycle of autoxidation (*70*).

Once formed, PAH *o*-quinones are reactive Michael acceptors and have the potential to alkylate cellular nucleophiles, including DNA, RNA, protein, and GSH. They also can undergo nonenzymatic two-electron reduction with cellular reducing equivalents [NAD(P)H] or enzymatic one-electron reduction with microsomal or mitochondrial enzymes, establishing futile redox cycles that amplify the production of ROS. Once formed, ROS can lead to the oxidative damage of DNA (8-oxo-dG, thymine glycol, and strand scission). The link between PAH metabolism and ROS formation is provocative since ROS are the causative agents in radiation-induced carcinogenesis.

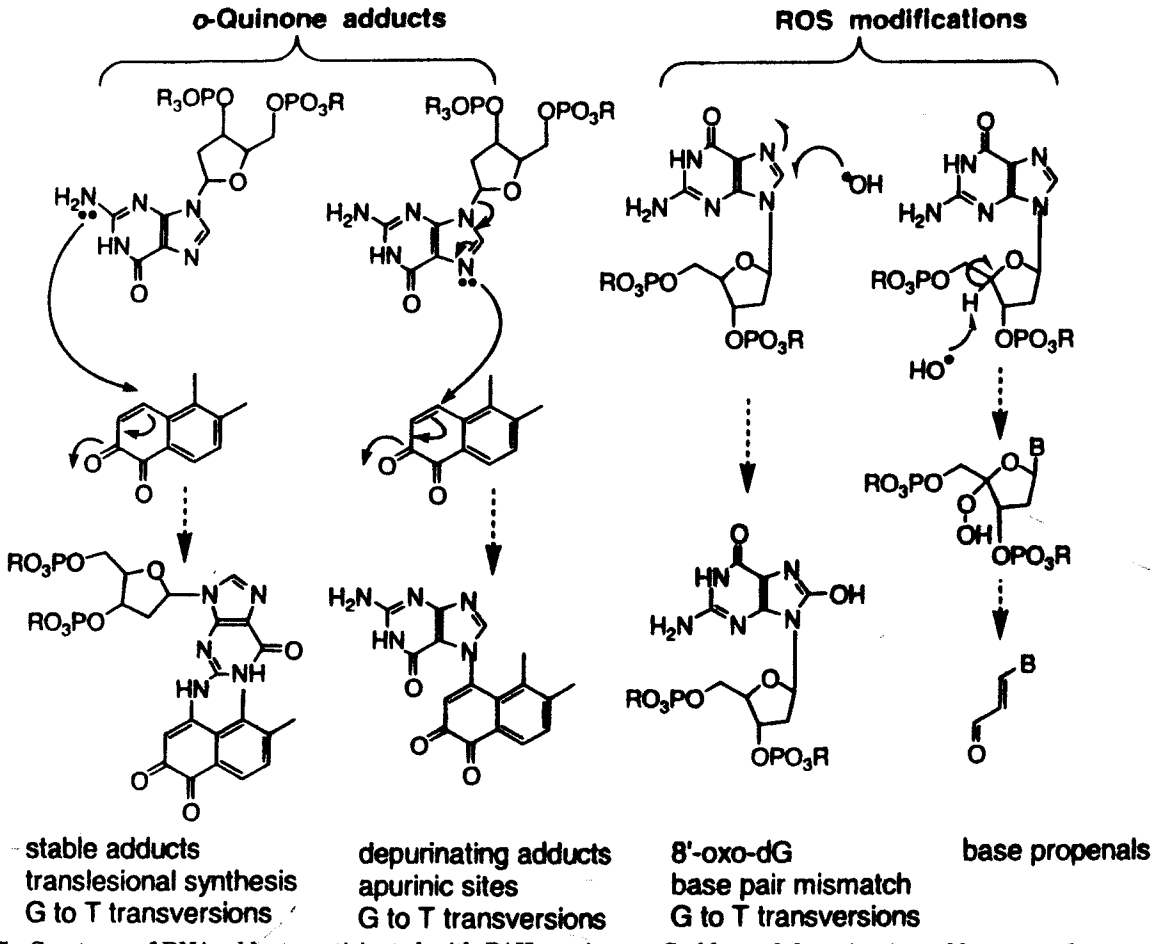


Figure 5. Spectrum of DNA adducts anticipated with PAH *o*-quinones. Stable amd depurinating adducts are shown on the left, and DNA modifications resulting from the generation of reactive oxygen species by the redox active *o*-quinones are shown on the right.

This pathway of PAH metabolic activation exists in whole cells. Suspensions of rat hepatocytes convert 7−10% of [^{3}H]B[*a*]P-diol to BPQ which was characterized in experiments with unlabeled material by EIMS (*71*). The formation of BPQ was blocked by AKR1C9 inhibitors. Similar results were observed in MCF-7 cells stably transfected with a pRCMV-AKR1C9 construct (*72*).

3.4. Properties of PAH *o*-Quinones. The reactivity, cytotoxicity, and genotoxicity of PAH *o*-quinones derived from the *trans*-dihydrodiols have been examined (Figure 5). Each *o*-quinone will undergo Michael addition with GSH but with profoundly different rates. Bay region *o*-quinones (e.g., BPQ) have relatively low reactivities since there is steric hindrance to the incoming nucleophile, but methylation of the bay region (e.g., DMBAQ), which distorts planarity, increases reactivity markedly, indicating that the rate of alkylation correlates with carcinogenic potency (*73*, *74*). Thus, the second-order rate constants for the addition of GSH to BPQ and DMBQ at physiologic pH are 1.3×10^3 and 2.0×10^6 min^{-1} M^{-1}, respectively (*73*).

The *o*-quinones are potent cytotoxins in rat and human hepatoma cells. On the basis of their cytotoxicity profile, PAH *o*-quinones fit into one of three classes (*75*, *76*) (Table 2). Class I *o*-quinones [naphthalene-1,2-dione (NPQ), phenanthrene-1,2-dione (1,2-PQ), and dimethylbenz[*a*]anthracene-3,4-dione (DMBAQ)] yield LC_{50} values of 1−30 μM, inhibit cell viability and cell survival, produce the most total free radicals assessed as cellular superoxide anion and *o*-semiquinone radical, and induce cell death by a change in redox state measured as a decrease in the ratio of the amount of GSH to that of GSSG and an increase in the ratio of the amount of NAD(P)$^+$ to that of NAD(P)H. There was no protection from cytotoxicity by dicumarol, indicating that two-electron redox cycling via NQO1 did not occur. Class II *o*-quinones [5-methylchrysene-1,2-dione (5-MCQ), benz[*a*]anthracene-3,4-dione (BAQ), 7-methylbenz[*a*]anthracene-3,4-dione (7-MBAQ), and 12-methylbenz[*a*]anthracene-3,4-dione (12-MBAQ)] gave LC_{50} values of 1−30 μM; however, they only inhibited cell survival, only produced cellular *o*-semiquinone radicals, and likely cause cell death via radical-mediated macromolecule damage. Again, there was no protection with dicoumarol. Class III *o*-quinones (BPQ) gave an LC_{50} value of 20 μM; they inhibit cell viability only, produce a modest amount of cellular superoxide anion, and cause cell death by GSH depletion. Again, no protection from cytotoxicity was achieved with dicoumarol. Thus, cytoxicity is likely mediated via enzymatic one-electron redox cycling of the PAH *o*-quinones.

The mutagenicity of PAH *o*-quinones was examined in the Ames test using tester strains TA97A, TA98, TA100, TA102, and TA104 (Table 3; *76*). The PAH *o*-quinones were found to have a greater mutagenic efficiency than the test mutagen in each strain. The mutations most often observed were frameshift or point mutations. The presence of an activating system (Aroclor-induced rat liver S9 with an NADPH generating system) to increase the extent of redox cycling of the PAH *o*-quinones did not increase mutagenicity in tester strains sensitive to oxida-

Table 2. Cytotoxic Properties of PAH *o*-Quinones

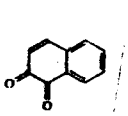
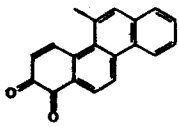
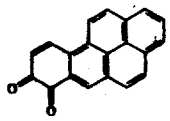
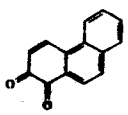
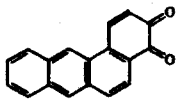
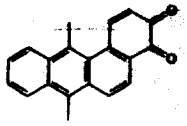
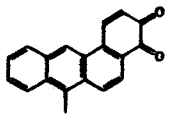
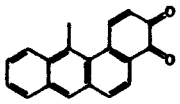
Class I Quinones	Class II Quinones	Class III Quinones
 NPQ	 5-MCQ	 BPQ
 1,2-PQ	 BAQ	
 DMBAQ	 7-MBAQ	
	 12-MBAQ	
• Potent cytotoxins LC_{50} = 30 μM	• Potent cytotoxins LC_{50} = 30 μM	• Potent cytotoxins LC_{50} > 20 μM
• Inhibit cell viability & survival	• Inhibit cell survival only	• Inhibit cell viability only
• Produce cellular superoxide and semiquinone anion radical	• Produce semiquinone anion radical only	• Produce mainly superoxide anion
• Change redox state	• No change in redox state	• Deplete GSH
• No protection by dicoumarol	• No protection by dicoumarol	• No protection by dicoumarol
• Cell death is by a change in redox state	• Cell death is by radical attack of macromolecules	• Cell death is by GSH depletion

Table 3. Mutagenicity of PAH *o*-Quinones in the Ames Test[a]

class of quinone	quinone	responsive strain	concentration (nmol/plate)	no. of revertants/plate	*x*-fold increase	mutagenic efficiency[b]	mutation type
class I	NPQ	TA97a	17.5	372 ± 45	3.3	18.9	frameshift
		TA98	17.5	51 ± 2	1.8	10.3	frameshift
		TA100	17.5	405 ± 28	3.4	19.4	point
		TA104	17.5	811 ± 66	2.3	13.1	oxidative
class I	DMBAQ	none					
class I	1,2-PQ	TA97a	35.0	171 ± 23	1.5	4.3	frameshift
		TA98	7.0	50 ± 2	1.7	24.3	frameshift
		TA104	70	873 ± 25	2.5	3.6	oxidative
class I	9,10-PQ	TA97a	17.5	169 ± 9	1.5	8.6	frameshift
		TA100	35.0	182 ± 17	1.5	4.3	point
class II	BAQ	none					
class II	7-MBAQ	TA97a	70	234 ± 9	2.1	3.1	frameshift
class II	12-MBAQ	TA98	70	186 ± 12	6.4	9.1	frameshift
		TA102	3.5	389 ± 16	1.6	45.9	oxidative
class II	5-MCQ	TA97a	70	257 ± 17	2.3	3.3	frameshift
		TA100	35	335 ± 9	2.8	8.0	oxidative
class III	BPQ	TA97a	70	463 ± 57	4.1	5.8	frameshift
		TA98	70	52 ± 5	1.8	2.6	frameshift
		TA100	70	192 ± 6	1.6	2.3	point
		TA102	35	368 ± 12	1.5	4.3	oxidative
		TA104	35	553 ± 15	1.6	4.6	oxidative
N/A	*anti*-BPDE	TA97a	0.1	444 ± 27	4.0	4000	frameshift
		TA98	0.1	426 ± 33	14.7	14700	frameshift
		TA100	0.1	845 ± 120	7.0	7000	point
		TA102	16.5	2200 ± 120	9.1	55	oxidative
		TA104	3.3	1620 ± 85	4.6	140	oxidative

[a] Taken from refs *32* and *33*; N/A, not applicable. *anti*-BPDE, (±)-*anti*-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. [b] Mutagenic efficiency = (*x*-fold increase in His revertants/spontaneous revertants)/nanomoles of mutagen × 100.

tive mutagens (TA102 and TA104). Under these conditions, the mutagenicity of PAH *o*-quinones appears to be independent of ROS formation and is related to the ability of the *o*-quinones to either intercalate or covalently modify the *his* operon. It is important to emphasize that in these assays BPQ was 10−5500 times less mutagenic

than the *anti*-diol epoxide (*anti*-BPDE; *77*). However, no mammalian mutagenicity assays have been performed with PAH *o*-quinones.

The nature of DNA−covalent adducts formed with [^{3}H]-BPQ has been investigated. In reactions with calf thymus DNA, the number of covalent adducts observed equaled those obtained with *anti*-BPDE (4000 adducts per 10^6 bases). Enzymatic digestion of the DNA to its constituent deoxyribonucleosides showed one predominant adduct that coeluted on HPLC with the adduct formed by the reaction of oligo-p$(dG)_{10}$ with BPQ, suggesting that the adduct corresponded to N^2-deoxguanosyl-BPQ (*78*). Attempts to detect this adduct in rat hepatocytes showed that the covalent adducts were unstable, suggesting that depurinating adducts may also form (*77*). Under acidic conditions, 2-deoxyguanosine reacts with PAH *o*-quinones (NPQ, 1,2-PQ, and BPQ) to form N7-guanine-depurinating *o*-quinone adducts. These adducts were not observed with guanosine, indicating that depurination occurred after Michael addition. The products were characterized by a combination of NMR, LC/MS, and CID of the MH^+. In reactions with calf thymus DNA, N7-depurinating adducts have been observed with the bay region *o*-quinone, 1,2-PQ (*79*).

PAH *o*-quinones also have the propensity to cause oxidative DNA damage. Exposure of rat hepatocytes to B[*a*]P-diol led to the formation of superoxide anion and strand scission of the genomic DNA (*77*). Both events could be blocked with AKR1C9 inhibitors. Strand scission of ϕX174 phage DNA required nanomolar concentrations of PAH *o*-quinone plus NADPH and $CuCl_2$ (*80*). Reactive species produced included Cu^+, hydroxyl radical, and malondialdehyde (MDA). The dependence on Cu^{2+} and Cu^+ suggests that copper-dependent redox cycling of the catechol is involved, while the formation of MDA indicates that a Criegee rearrangement is responsible for the strand scission. In this mechanism, base propenals are produced which hydrolyze to MDA or react directly with DNA to form the same adducts as MDA (*81*).

As a result, PAH *o*-quinones have the potential to produce a spectrum of DNA damage which includes stable DNA adducts, N7-depurinating adducts, oxidatively damaged bases (e.g., 8-oxo-dG), and strand scission, resulting in the formation of base propenals (Figure 5). Several of these events can in turn give rise to the G to T transversions most often seen in *ras* and *p53*. As described previously, N7-guanine depurinating adducts lead to apurinic sites and if the abasic site is unrepaired, an A will be introduced opposite that site to produce a G to T transversion (*58*). Similarly, the formation of 8-oxo-dG leads to base mispairing with A during replication, again leading to a G to T transversion (*81*).

In summary, dihydrodiol dehydrogenases are constitutively expressed in human tissues, and the human isoforms produce reactive and redox active PAH *o*-quinones. These quinones are potent cytotoxins, weak mutagens, and potent chemical nucleases, and have the potential to produce a spectrum of DNA damage. Their targeting of *ras* and *p53* is presently unknown. Their ability to produce a pro-oxidant state suggests they may activate protein kinase C and act as tumor promoters (*82*). Importantly, these *o*-quinones are structurally related to catechol estrogens and may be regarded as their PAH equivalents.

4. Quinones from Equine Estrogens. Role in Estrogen Carcinogenesis?

4.1. Estrogens and Cancer Risk. A firm link between female reproductive variables and increased risk of developing cancer in the breast and endometrium has been established from epidemiological studies (*83−86*). The longer women are exposed to estrogens, through early menarche and late menopause and/or through estrogen replacement therapy, the higher the risk of developing cancer. However, the molecular mechanism(s) involved in the carcinogenic action of estrogens still remains both controversial and elusive (*87*). There are at least three different potential mechanisms of estrogen carcinogenesis which have been proposed. These include hormonal feedback inhibition, oxidative damage to DNA, and/or alkylation of DNA by electrophilic metabolites of estrogens (*88−91*). The first mechanism involves a break in the pituitary−gonadal hormone feedback loop, leading to a failure of the estrogens to suppress the pituitary production of gonadotropins. The excessive production of gonadotropins and their constant stimulation of the target organ may result in neoplasia. This cannot be the only mechanism, however, because the hormonal potencies of various estrogens *do not* correlate with the incidence of tumor induction (*92, 93*). Rather, metabolic activation of estrogens to redox active and/or electrophilic metabolites probably plays an etiologic role in tumor formation.

The carcinogenic effects of long-term postmenopausal estrogen replacement therapy are particularly controversial (*94−97*). It is known that estrogen use is associated with the relief of menopausal symptoms, a substantial reduction in the risk of cardiovascular disease and osteoporosis (*98*), and a possible correlation between the reduced risk of Alzheimer's disease (*99−101*) and stroke (*102*). Despite these beneficial health effects, only 30% of postmenopausal women are currently taking estrogen replacement therapy in North America because of a perceived fear of developing breast cancer in particular. Most epidemiology studies have shown a slight but significant increase in the risk of developing breast cancer particularly with long-term high-dose regimes (*86, 102, 104*). As far as endometrial cancer is concerned, there is no doubt that estrogen replacement therapy increases cancer risk by as much as 10-fold especially with long-term (10−15 years) unopposed estrogen use (*105*). However, the consequences of estrogen deficiency versus the potential risks of estrogen replacement therapy, especially the ability of estrogens to initiate and/or promote the carcinogenic process, remains a highly controversial issue.

4.2. Metabolism of Estrogens to Catechols. Estrone and 17β-estradiol are biochemically interconvertible, a process catalyzed by the enzyme 17β-estradiol dehydrogenase, and they yield the same metabolic products as shown for estrone in Figure 6. Aromatic hydroxylation of estrone and 17β-estradiol forming catechol metabolites represents the major phase I metabolic pathway for endogenous estrogens (*106, 107*). In the liver, hydroxylation at the 2 position predominates over formation of 4-hydroxyestrogens in all species by a factor of 2−10. In humans, this reaction is primarily catalyzed by P450 3A4 (*90, 108, 109*). Recent studies have also shown that a TCDD-inducible P450 isozyme, P450 1B1, selectively catalyzes hydroxylation at the 4 position of 17β-estradiol

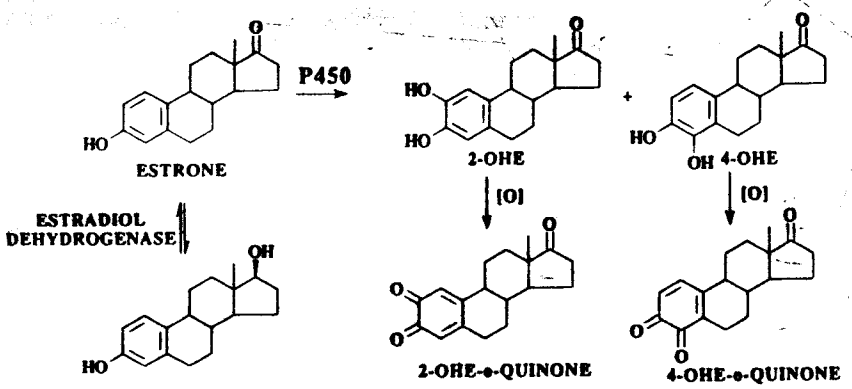


Figure 6. Phase I metabolism of endogenous estrogens.

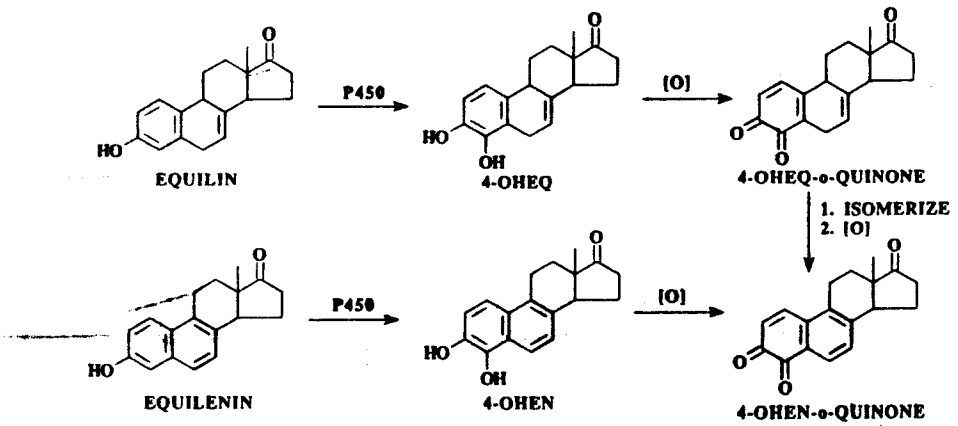


Figure 7. Primary phase I metabolic pathway for equine estrogens.

(*110*–*112*), suggesting that excessive exposure to environmental pollutants could lead to enhanced production of this metabolite. This is particularly significant since only 4-OHE was found to be carcinogenic in the male Syrian golden hamster kidney tumor model whereas 2-OHE was without activity (*113*, *114*). These data suggest that *metabolism* of estrogens is required for the development of cancer.

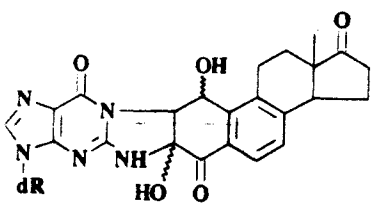
The most widely prescribed estrogen replacement formulation is marketed under the name of Premarin (Wyeth-Ayerest). Since Premarin was approved by the Food and Drug Administration in the 1940s, very little is known about the metabolism and potential toxic metabolites that could be produced from the various equine estrogens which make up Premarin (*115*–*118*). Interestingly, increasing the level of unsaturation in the B ring leads to a change in metabolism from predominately 2-hydroxylation for estrone to mainly 4-hydroxylation for equilin and exclusively 4-hydroxylation for equilenin (Figure 7). This may be problematic since 2-hydroxylation of endogenous estrogens is regarded as a benign metabolic pathway whereas 4-hydroxylation could lead to carcinogenic metabolites.

4.3. Oxidation of Catechol Estrogens to *o*-Quinones. Once formed, the endogenous catechol estrogens can be oxidized by virtually any oxidative enzyme and/or metal ion, giving *o*-quinones (*119*). The *o*-quinone formed from 2-OHE has a half-life of 47 s, whereas the 4-OHE-*o*-quinone is considerably longer-lived ($t_{1/2}$ = 12 min; *120*). Interestingly, the 4-hydroxylated equine catechol estrogens (4-OHEN and 4-OHEQ) both autoxidize to *o*-quinones without the need for enzymatic or metal ion catalysis (Figure 7; *118*, *121*). The *o*-quinone formed from 4-OHEN is much more stable than the endogenous catechol estrogens ($t_{1/2}$ = 2.3 h; *118*). It appears that the adjacent aromatic ring stabilizes 4-OHEN-*o*-quinone through extended π-conjugation. In support of this, it has been shown that the catechol metabolite of benzo[*a*]pyrene rapidly undergoes air oxidation to yield a very stable *o*-quinone, benzo[*a*]pyrene-7,8-dione (*69*, *122*). The 4-OHEQ-*o*-quinone readily isomerizes to 4-OHEN-*o*-quinone (Figure 7); as a result, most of the biological effects caused by catechol metabolites of equilin could be due to 4-OHEN-*o*-quinone formation (*118*). Finally, although 2-hydroxylation does occur with equilin producing 2-OHEQ which will isomerize to 2-OHEN, the latter catechol does not autoxidize to an *o*-quinone at any appreciable rate (*118*). This suggests that like what has been observed with endogenous catechol estrogens, 2-hydroxylation is likely a benign metabolic pathway for equilin.

4.4. Catechol Estrogen-*o*-quinones Induced DNA Oxidation. The excessive production of reactive oxygen species in breast cancer tissue has been linked to metastasis of tumors in women with breast cancer (*123*). Biomarkers for oxidative damage to DNA include the formation of 8-oxo-dG (*124*) which is considered to be an important factor in carcinogenesis (*125*). The source of reactive oxygen species has been suggested to be redox cycling between the *o*-quinones and their semiquinone radicals generating superoxide, hydrogen peroxide, and ultimately reactive hydroxyl radicals which cause oxidative cleavage of the phosphate–sugar backbone as well as oxidation of the purine/pyrimidine residues of DNA (*126*). In support of this mechanism, various free radical toxicities have been reported in hamsters treated with 17β-estradiol, including DNA single-strand breaks (*127*, *128*), 8-oxo-dG formation (*125*), and chromosomal abnor-

malities (*129, 130*). Recently, it has been shown that 4-OHEN is also capable of causing DNA single-strand breaks and oxidative damage to DNA bases, generating 8-oxo-dA and 8-oxo-dG (*131*). Treating λ phage DNA with 4-OHEN resulted in extensive single-strand breaks that were concentration- and time-dependent. With the inclusion of scavengers of reactive oxygen species in the incubations, DNA could be completely protected from 4-OHEN-mediated damage. In contrast, NADH and $CuCl_2$ enhanced the ability of 4-OHEN to cause DNA single-strand breaks, presumably due to redox cycling between 4-OHEN and the semiquinone radical generating hydrogen peroxide and ultimately copper peroxide complexes. It was confirmed that 4-OHEN could oxidize DNA bases because hydrolysis of 4-OHEN-treated calf thymus DNA and HPLC separation with electrospray-MS detection revealed oxidized deoxynucleosides, including 8-oxo-dG and 8-oxo-dA. 4-OHEN also caused an increase (2-fold) in the levels of the mutagenic lesions 8-oxo-dG and 8-oxo-dA in breast cancer cells in the presence of agents which catalyze redox cycling (NADH) or deplete cellular GSH (diethyl maleate) levels as determined by LC/MS/MS (*132*). In support of this, previous reports have shown that incubations with 4-OHEN-*o*-quinone, DNA, and hamster liver microsomes also enhance 8-OH-dG formation 2-fold relative to control samples (*133*). Using the single-cell gel electrophoresis assay (comet assay) to assess DNA damage, we found that 4-OHEN causes concentration-dependent DNA single-strand cleavage in breast cancer cell lines, and this effect could be enhanced by NADH or diethyl maleate (*132*). These and other data are evidence for a mechanism of estrogen-induced tumor initiation by redox cycling of estrogen metabolites, generating reactive oxygen species which damage DNA.

4.5. DNA Adducts Formed by Catechol Estrogen-*o*-quinones. The formation of covalent DNA adducts in vivo is usually regarded as the initiation event in the carcinogenic process (*134*). With estrogens, DNA adducts have been detected in vivo using ^{32}P-postlabeling methods in susceptible target organs such as the Syrian hamster kidney (*135, 136*). Recent model studies with catechol estrogen-*o*-quinones and deoxynucleosides or bases showed that different types of adducts are obtained depending on the reaction conditions and the reactivity of the *o*-quinone. For example, when 4-OHE-*o*-quinone was reacted with adenine in organic solvent under reductive conditions, an adduct consistent with coupling between the C-1 position of 4-OHE and the C-8 position of adenine was isolated (*137*). In contrast, incubating 4-OHE-*o*-quinone with dA in an acidic environment gave no adducts. Reaction with dG gave an adduct that had lost the ribose moiety resulting from reaction of the N^7 position of dG with the 4-OHE-*o*-quinone at the C-1 position (*138*). N^7 adducts are extremely unstable and readily depurinate, leading to mutations (*139*). Recently, DNA adducts have been isolated from rats treated with 4-OHE-*o*-quinone. This *o*-quinone was directly injected into the rat mammary gland; the mammary tissue was subjected to Soxhlet extraction, and the extracts were analyzed by HPLC to determine the level of depurinating adducts formed in vivo (*140*). The adduct that was detected was 4-OHE-N^7-guanine, identical to that obtained from the model studies with 4-OHE-*o*-quinone and dG. DNA also was isolated from the mammary tissue, hydrolyzed to deoxynucleosides, and analyzed by HPLC.



4-OHEN-dG

Figure 8. dG adduct formed from 4-OHEQ and 4-OHEN in vitro.

No stable adducts were detected under these conditions which suggests that only apurinic sites would be formed from reaction of 4-OHE-*o*-quinone with DNA in vivo.

The quinoids of the equilenin metabolite 4-OHEN reacted with 2′-deoxynucleosides, generating very unusual cyclic adducts (*19, 141, 142*). For example, the structure of a 4-OHEN–deoxyguanosine adduct is shown in Figure 8. Since 4-hydroxyequilin is converted to 4-hydroxyequilenin and 4-hydroxyequilenin-*o*-quinone, the same adducts were observed during incubations with 4-hydroxyequilin and deoxynucleosides or DNA (*118*). Deoxyguanosine (dG), dA, or dC all gave four isomers, but no product was observed for thymidine under similar physiological conditions. The structures of these adducts were determined by electrospray mass spectrometry and two-dimensional NMR experiments. The spectral data showed that cyclic adducts are formed between the deoxynucleosides and 4-OHEN. Care needed to be taken during the isolation of the dA adducts in particular, as any exposure to acidic environments caused hydrolysis of the sugar moiety, leaving alkylated adenine. On reaction with DNA, significant apurinic sites were produced as 4-OHEN–adenine adducts were detected in the ethanol wash prior to hydrolysis. When the DNA was hydrolyzed to deoxynucleosides and analyzed by electrospray mass spectrometry, only single isomers of 4-OHEN–dG and 4-OHEN–dC were observed. These data suggest that several different types of DNA lesions could be expected from 4-OHEN, including apurinic sites and bulky stable adducts, in addition to the reported oxidized damage to DNA (*131–133*) caused by 4-OHEN. If similar adducts are formed in vivo, which are not repaired efficiently, mutations could result, leading to initiation of the carcinogenic process in the endometrium or the breast. Given the direct link between excessive exposure to estrogens, metabolism of estrogens, and increased risk of breast cancer, it is crucial that factors which affect the formation, reactivity, and cellular targets of estrogen quinoids be thoroughly explored.

5. Quinone–Thioether-Mediated Toxicities

5.1. Toxicology of Polyphenolic–Glutathione Conjugates. The nucleophilicity of the cysteinyl sulfhydryl group renders protein and non-protein sulfhydryls major targets for reaction with quinones. GSH is the major non-protein sulfhydryl present in cells, and when GSH adds to quinones, the reaction is generally considered cytoprotective because the thiol function in GSH serves as a "sacrificial" nucleophile, sparing critical nucleophilic sites on cellular macromolecules from irreversible modification. However, some polyphenolic–GSH conjugates, and their metabolites, retain the electrophilic and redox properties of the parent polyphenol. Indeed, the reactivity of the thioether metabolites may frequently exceed that

of the parent polyphenol (*21*, *143*). Polyphenolic–GSH conjugates therefore contribute to the toxicity of a variety of polyphenols, and specific examples of these will be discussed below.

5.2. Neurotoxicity of Ring-Substituted Amphetamine Derivatives. 3,4-(±)-Methylenedioxyamphetamine (MDA) and 3,4-(±)-methylenedioxymethamphetamine (MDMA, "ecstasy") are ring-substituted amphetamine derivatives that have stimulant and hallucinogenic properties (*144*, *145*). MDA and MDMA are popular recreational drugs, the use and abuse of which is increasing in both the United States and Europe. The predominant adverse consequences of MDMA and MDA abuse in humans include convulsions, hyperthermia, rhabomyolysis, and acute liver and renal failure (*146*). In experimental animals, including primates, toxicity is also manifested as a selective serotonergic neurotoxicity, featuring acute release of 5-hydroxytryptamine (5-HT) followed by prolonged depletion of the levels of 5-HT and the 5-HT carrier protein in serotonergic nerve terminal fields (*147*, *148*), inhibition of tryptophan hydroxylase (*148*, *149*), and structural damage to serotonergic nerve terminals (*144*, *145*, *150–152*). Recent studies have produced the first direct evidence that the chronic use of MDMA causes brain damage in humans (*153–155*). In addition to causing significant reductions in the 5-HT transporter level, and in 5-HT activity, long-term users of MDMA also exhibited impaired memory. The functional consequences of these changes remain to be determined but serve to highlight the adverse human health effects of these drugs.

The neurotoxic effects of MDA and MDMA are dependent on the route and frequency of drug administration (*156*). Injection of MDA or MDMA directly into the brain fails to reproduce the acute or long-term effects observed after peripheral administration, implying an essential role for systemic metabolism in the development of toxicity (*157–159*). Consistent with this view, pretreatment of rats with SKF-525A, an inhibitor of cytochrome P450, attenuates MDMA-mediated depletions in 5-HT levels, whereas pretreatment with a cytochrome P450 inducer, such as phenobarbital, enhances 5-HT depletion (*160*). The inability of MDMA to inhibit tryptophan hydroxylase activity in vitro is also indicative of the need for metabolic activation (*158*). However, several major metabolites of MDA and MDMA either fail to reproduce serotonergic neurotoxicity or fail to exhibit specificity for the serotonergic system (*161*, *162*).

α-Methyldopamine (α-MeDA) is a metabolite of MDA and MDMA (*163*, *164*). However, intracerebroventricular administration of α-MeDA to rats produces neither the acute "serotonin syndrome" (*165*) nor long-term depletions in 5-HT levels (*161*). α-MeDA undergoes oxidation to the corresponding *o*-quinone, which is efficiently scavenged by GSH (*166*, *167*) to form 5-(glutathion-*S*-yl)-α-MeDA, which itself is readily oxidized to the *o*-quinone–GSH conjugate, followed by the subsequent addition of a second molecule of GSH to form 2,5-bis(glutathion-*S*-yl)-α-MeDA (*165*). 5-(Glutathion-*S*-yl)-α-MeDA is rapidly cleared from the brain of rats following intracerebroventricular administration (*168*). Concomitant with decreases in 5-(glutathion-*S*-yl)-α-MeDA concentrations, 5-(cystein-*S*-yl)-α-MeDA and 5-(*N*-acetylcystein-*S*-yl)-α-MeDA are rapidly formed (*168*). However, although 5-(glutathion-*S*-yl)-α-MeDA and 5-(*N*-acetylcystein-*S*-yl)-α-MeDA produce neurobehavioral changes similar to those seen with MDA and MDMA, and acute changes in brain 5-HT and dopamine concentrations, neither conjugate caused long-term decreases in 5-HT concentrations following intracerebroventricular injection (*165*, *169*). In contrast, intracerebroventricular injection of 2,5-bis-(glutathion-*S*-yl)-α-MeDA caused selective decreases in 5-HT concentrations in the striatum, cortex, and hippocampus, and neurobehavioral effects identical to those seen following MDA and MDMA administration (*165*).

In contrast to the effects seen following intracerebroventricular injection of the α-MeDA–thioether metabolites, direct intrastriatal or intracortical administration of 2,5-bis(glutathion-*S*-yl)-α-MeDA, 5-(glutathion-*S*-yl)-α-MeDA, and 5-(*N*-acetylcystein-*S*-yl)-α-MeDA did cause significant decreases in striatal and cortical 5-HT concentrations (7 days following the last injection; *170*). The effects of the thioether conjugates of α-MeDA were confined to 5-HT nerve terminal fields, since no significant changes in monoamine neurotransmitter levels were detected in brain regions enriched in 5-HT cell bodies (midbrain/diencephalon/telencephalon and pons/medulla; *170*). In addition, the effects of the conjugates were selective to the serotonergic system, as no significant changes were seen in dopamine or norepinephrine concentrations (*170*). Thioether conjugates of α-MeDA are therefore selective serotonergic neurotoxicants. Recent studies further implicate a role for these conjugates in the toxicity observed following systemic administration of MDA and MDMA. Thus, because pretreatment of animals with acivicin increased the rate of uptake of [^{3}H]-5-(glutathion-*S*-yl)-α-MeDA into brain (*169*), presumably by increasing the size of the pool available for uptake via the intact GSH transporter, we reasoned that inhibition of γ-glutamyl transpeptidase (γ-GT) at the blood–brain barrier should potentiate MDA-mediated neurotoxicity. Consistent with this hypothesis, pretreatment of animals with acivicin, which caused an ~60% decrease in brain capillary endothelial cell γ-GT activity, potentiated MDA-mediated decreases in brain 5-HT and 5-HIAA concentrations (F. Bai, S. S. Lau, and T. J. Monks, unpublished data). These data implicate a role for metabolites that are substrates for γ-GT in the neurotoxicity that occurs after the systemic administration of MDA. The mechanisms by which the thioether metabolites of α-MeDA produce selective serotonergic neurotoxicity are unclear, but are the subject of ongoing studies.

5.3. Benzene-Mediated Hematotoxicity. As discussed earlier, benzene is a ubiquitous environmental pollutant that induces bone marrow suppression in rodents (*171*). Benzene is both hematotoxic and leukemogenic in humans, causing a variety of hematological disorders, including aplastic anemia, myelodysplastic syndrome, and acute myelogenous leukemia (*172*, *173*). Benzene must be metabolized to mediate its toxic effects, and a number of polyphenolic and open-ringed metabolites have been studied for their hematotoxic potential (*174*, *175*). A number of redox active hydroquinone–thioether metabolites were recently identified in the bone marrow of rats and mice exposed to a combination of hydroquinone/phenol or benzene (*176*). Two of these metabolites, 2,6-bis(glutathion-*S*-yl)hydroquinone (50 μmol/kg, iv) and 2,3,5-tris(glutathion-*S*-yl)hydroquinone (TGHQ; 17 μmol/kg, iv), reproduce benzene erythrotoxicity in vivo as evidenced by significant decreases in the level of ^{59}Fe incorporation into developing reticulocytes (*176*).

Dysregulation of apoptosis is important in the development and/or progression of many hematopoietic disorders (*177*). Apoptosis is a common mode of cell death in hematopoietic tissue, and although it has been suggested that benzene reduces the number of myeloid stem cells in bone by the inappropriate activation of apoptotic signaling pathways, very little is known about the role of apoptosis in benzene-mediated hematotoxicity. Hydroquinone does induce apoptosis in human promyelocytic leukemia-derived HL-60 cells, and in human bone marrow-derived $CD34^+$ cells (*178*). However, hydroquinone inhibits apoptosis in IL-3-dependent murine myeloblasts, purportedly by preventing activation of caspase-1 (ICE; *179*). Recent studies indicate that TGHQ induces apoptosis in HL-60 cells (S. B. Bratton, S. S. Lau, and T. J. Monks, unpublished data). TGHQ-induced apoptosis is preceded by decreases in intracellular GSH concentrations in a reactive oxygen species-independent manner. Decreases in intracellular GSH concentrations may activate a variety of signaling pathways (*180*, *181*), and the ceramide signaling pathway may be particularly pertinent in this regard. Analogues of ceramide (C2-, C6-, and C8-ceramides) induce apoptosis in hematopoietic cells (*182*–*184*), and the neutral, magnesium-dependent sphingomyelinases (SM) that generate endogenous ceramides are inhibited by physiological concentrations of GSH (*180*). Consistent with these findings, prolonged inhibition of γ-glutamylcysteine synthetase in Molt-4 cells decreases intracellular GSH levels with concurrent increases in SM activity (*180*). TGHQ also increases the rate of sphingomyelin turnover (S. B. Bratton, S. S. Lau, and T. J. Monks, unpublished data). Interestingly, GSH may stimulate SM activity in renal proximal tubular cells (*185*). The response of various tissues to depletions in intracellular GSH concentrations may therefore be dependent, at least in part, on the differential effects of GSH on SM in different tissues.

Ceramide can activate caspase-3/yama/apopain, an ICE-like protease which cleaves poly-ADP ribose polymerase (*184*). Poly-ADP ribose polymerase is important in repairing and maintaining the genome (*186*); therefore, unscheduled activation of this protease leads to DNA degradation. Ceramide also activates a number of protein phosphatases, including a cytosolic serine/threonine protein phosphatase (*187*). Whether ceramide activates NF-κB remains debatable; however, H_2O_2 and lipid hydroperoxides rapidly activate this transcription factor (*188*). Moreover, NF-κB binding sites are present in a variety of genes, including those encoding human endothelial-leukocyte adhesion molecule, G-CSF, and M-CSF (*189*, *190*), which could act in an autocrine fashion to control differentiation in HL-60 cells and other bone marrow progenitor cells.

5.4. Nephrotoxicity of Polyphenolic–Glutathione Conjugates. GSH conjugates of a variety of polyphenols are nephrotoxicants in a variety of animal models (*21*, *143*). The nephrotoxicity of these metabolites is dependent on the relatively high activity of γ-GT within the brush border membrane of renal proximal tubular epithelial cells (Figure 9). The product of the reaction, the cysteinylglycine dipeptide, is a substrate for dipeptidases, which are similarly concentrated in the brush border membrane of proximal tubular epithelial cells. The corresponding cysteine conjugates are then transported across the brush border membrane via the amino acid transport system. In this manner, metabolism of polyphenolic–GSH conjugates by γ-GT within the brush border membrane is coupled to the cellular uptake of the corresponding polyphenolic–cysteine conjugate.

Hydroquinone is used as a developer in the photographic industry, as an antioxidant in the rubber industry, and as an intermediate in the manufacturing of food antioxidants. Hydroquinone has also been identified in relatively high concentrations in the smoke of nonfiltered cigarettes (up to 155 μg per cigarette; *191*) and is an important metabolite of benzene (see above). Hydroquinone causes renal tubular cell degeneration in the renal cortex of male and female rats (F344/N) treated for 13 weeks with hydroquinone (1.82 mmol/kg; *192*), and in long-term studies, it causes marked increases in tubular cell adenomas (*193*). The mechanism by which hydroquinone produces renal tumors most likely involves the formation of nephrotoxic GSH conjugates. In support of this view, the acute nephrotoxicity of hydroquinone is prevented by the pretreatment of rats with acivicin to inhibit renal γ-GT (*194*), implying that the adverse effects of hydroquinone are mediated by metabolites that require processing by γ-GT.

To further investigate the role of TGHQ on hydroquinone-mediated nephrocarcinogenesis, studies were carried out in the Eker rat. The Eker rat carries a germline mutation in the Tsc-2 tumor suppressor gene, which predisposes these rats to the development of renal cell carcinoma (RCC), and they have been used as a model to study the influence of genetic susceptibility in chemically induced nephrocarcinogenesis. TGHQ (2.5 μmol/kg, ip, 5 days/week for 4 months; 3.5 μmol/kg, 5 days/week for 6 months) increased tumor multiplicity and incidence 3-fold (*195*). TGHQ-treated rats developed numerous toxic tubular dysplasias of a form rarely present in aging Eker rats. These lesions were found as early as 4 months following initiation of treatment. In 12-month-old animals, adenomas were found arising within these lesions. These "preneoplastic" lesions are believed to represent "early transformation" within tubules undergoing regeneration in response to toxic injury (*195*). The majority of the renal cell tumors also colocalized with the region of TGHQ-induced acute renal injury, supporting the hypothesis that hydroquinone-mediated nephrocarcinogenesis is mediated by the formation of a nephrotoxic metabolite(s) which induces a sustained regenerative hyperplasia, adenoma formation, and conversion to RCC.

Experimental evidence also supports a role for polyphenolic–GSH conjugates in the carcinogenic effects of 3-*tert*-butyl-4-hydroxyanisole (BHA) (*196*, *197*) and 17β-estradiol (*192*, *198*). BHA and its demethylated analogue, *tert*-butylhydroquinone (TBHQ), are phenolic antioxidants widely used in foods. BHA enhances the development of preneoplastic and neoplastic lesions in rat kidney (*199*) and urinary bladder (*197*, *200*, *201*). TBHQ is a metabolite of BHA in humans (*203*), and causes cell proliferation in renal pelvic epithelia (*203*) and enhances hyperplasia in the urinary bladder (*204*, *205*). GSH conjugates have been identified as in vivo metabolites of TBHQ, and administration of 5-(glutathion-*S*-yl)-TBHQ, 6-(glutathion-*S*-yl)-TBHQ, and 3,6-bis(glutathion-*S*-yl)-TBHQ to rats produced a mild nephrotoxicity and a severe hemorrhaging of the bladder (*197*). Exposure to estrogens has been associated with neoplastic changes in both humans and laboratory animals (*206*). In an established model of estrogen-mediated carcinogenesis, male Syrian golden

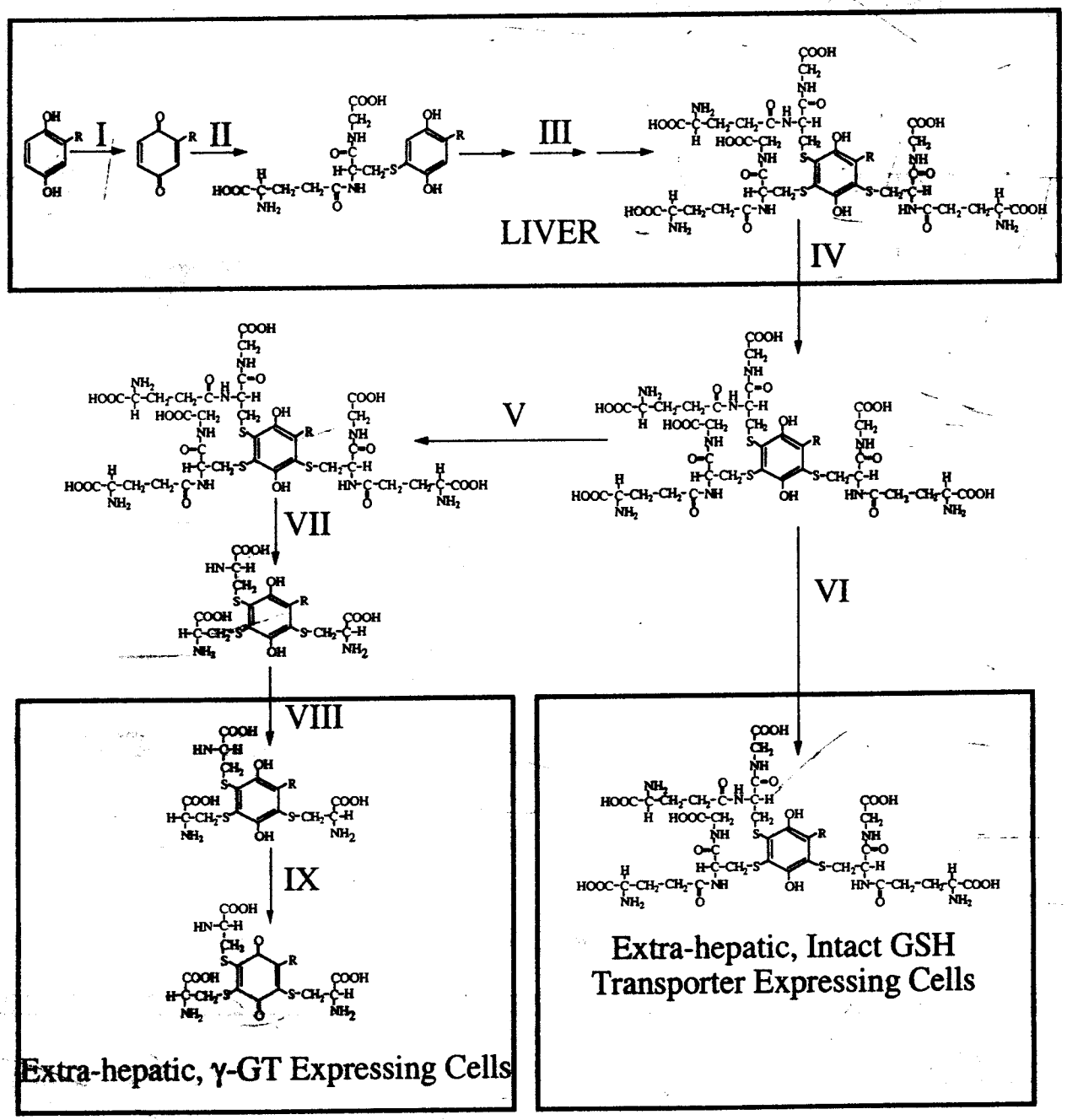


Figure 9. In the liver, *o*- or *p*-diphenols undergo oxidation to the corresponding *o*- or *p*-quinones (I), followed by addition of GSH (II). The GSH conjugates can undergo a series of sequential oxidations coupled to multiple additions of GSH to yield multi-GSH-substituted conjugates (III), which are efficiently exported from hepatocytes into bile or blood (IV). Intact GSH conjugates are then delivered to cells that express γ-glutamyl transpeptidase (γ-GT) on their luminal surface (V). γ-GT catalyzes the removal of γ-glutamate from GSH by either hydrolysis or transpeptidation (the relative rates depend on the availability of an acceptor and on pH), and the cysteinylglycine dipeptides are then hydrolyzed by dipeptidases to yield the cysteine conjugate (VII). The cysteine conjugate is subsequently transported into cells via the amino acid carrier (VIII), where it can be readily oxidized to a reactive quinone (IX). Alternatively, GSH conjugates may be transported intact into cells that express the putative GSH transporter (VI). The former pathway (V, VII, VII, and IX) appears to be important in the nephrotoxicity of a variety of polyphenols. The latter pathway (VI) seems to play a role in the neurotoxicity of the methylenedioxyamphetamines (see the text for details).

hamsters develop renal carcinoma following prolonged exposure to estrogens (*207*–*209*). Although a number of mechanisms have been proposed to explain estrogen-mediated carcinogenesis, carcinogenicity has been linked to their metabolism to reactive catechols or quinones (*206*). Both 2-hydroxy-4-(glutathion-*S*-yl)-17β-estradiol and 2-hydroxy-1-(glutathion-*S*-yl)-17β-estradiol produce mild nephrotoxicity in hamsters at doses of 0.27 and 1.5 μmol/kg, respectively (*192*), the equivalent of 27 and 150 nmol/100 g of hamster. Moreover, acivicin inhibits the acute nephrotoxic effects of 17β-estradiol (*198*), again supporting a role for GSH conjugates in the adverse effects associated with 17β-estradiol exposure in this animal model. Taken together, the findings with the various GSH conjugate metabolites of hydroquinone, TBHQ, and 17β-estradiol provide strong evidence that polyphenolic–thioether conjugates play an important role in the adverse effects caused by a variety of parent polyphenols, and chemicals that give rise to polyphenols as metabolites.

6. Quinone–Thioethers and Parkinson's Disease

Presently, available evidence suggests that the pathologocial processes underlying the degeneration of nigrostriatal dopamine (DA) neurons in Parkinson's disease occur in their neuromelanin-pigmented cell bodies in the substantia nigra pars compacta (SN_c). These include decreased activity of mitochondrial NADH-coenzyme Q_1 (CoQ_1) reductase (complex I) (*210*, *211*). This complex I deficiency is not accompanied by altered activities of mitochondrial complexes II–IV, and is anatomically specific to the SN_c and exclusive to Parkinson's disease (*211*). Immunohistochemical studies demonstrate decreased immunostaining levels not only for complex I in pigmented parkinsonian SN_c neurons (*212*) but also for α-ketoglutarate dehydrogenase (α-KGDH) (*213*), a nonrespiratory mitochondrial enzyme complex that provides reducing equivalents as succinate to complex II. Attempts to measure α-KGDH activities in homogenates of SN_c tissue from Parkinson's disease patients have been unsuccessful, presumably because of post-mortem degeneration of this enzyme complex (*214*). Nevertheless, a combined impairment of complex I and α-KGDH and the resultant reduction of mitochondrial ATP production probably represent important pathological factors that contribute to DA neurodegeneration in Parkinson's disease. However, the causes of NADH-CoQ_1 reductase and α-KGDH deficiencies in Parkinson's disease are unknown. Evidence has been presented that genetic factors might underlie the complex I (*215–217*) and α-KGDH (*218*) deficiencies in Parkinson's disease. However, these results are controversial (*219*, *220*). Furthermore, even if Parkinson's disease patients have systemic complex I and α-KGDH defects, such factors alone cannot explain the relatively selective degeneration of dopaminergic SN_c cells while other neurons are spared.

Increased levels of oxidation products of lipids (*221*), proteins (*222*), and DNA (*223*) have been measured in SN_c tissue from patients dying with advanced Parkinson's disease, suggesting that oxygen radicals may be responsible for damage to respiratory and other mitochondrial enzymes. However, in vivo, mitochondrial complex IV rather than complex I is most vulnerable to oxidative damage (*224*). Moreover, on the basis of studies of brains from patients dying with incidental Lewy body disease (*225*), believed to be a clinically presymptomatic stage of Parkinson's disease, and from patients with advanced Parkinson's disease, it appears that oxidative damage may occur only late in the disorder, is not restricted to the SN_c, and could be caused by the prooxidant effects of L-DOPA therapy (*222*).

Another change specific to the SN_c in Parkinson's disease is a massive (40–50%) fall of GSH levels (*226*, *227*). Nigral GSH levels also fall in incidental Lewy body disease. However, this fall of GSH levels in incidental Lewy body disease is not accompanied by increased incidence of markers for oxidative damage and by only small decreases in mitochondrial complex I activity (*225*). The fall of GSH levels in both Parkinson's disease and incidental Lewy body disease is not accompanied by increased levels of glutathione disulfide (GSSG) (*225*, *227*, *228*). Furthermore, the activity of γ-glutamylcysteine synthetase is normal in the parkinsonian SN_c (*229*). Taken together, these observations imply that nigral GSH-level decreases are not related to decreased ATP production (at least in early stages of the disorder), its oxidation by oxygen radicals to GSSG, or a failure of its biosynthetic enzymes. Furthermore, the finding that patients who may be at early stages of Parkinson's disease have decreased nigral GSH levels but only small decreases in complex I activity suggests a connection between the fall of GSH levels and progressive impairments of complex I and SN_c cell death. However, by itself, massive depletion of brain GSH levels evokes neither the degeneration of nigrostriatal DA neurons (*230*) nor alterations of mitochondrial respiratory enzyme activities (*231*). This, in turn, implies that the fall of GSH levels in Parkinson's disease may represent only one component of a more complex sequence of processes that ultimately lead to the death of pigmented dopaminergic SN_c cells.

While dopaminergic SN_c cells contain GSH, available information suggests that much higher concentrations of the tripeptide are probably present in nigral glia (*232*). Furthermore, remaining SN_c neurons, presumably destined to die in the parkinsonian brain, contain reduced levels of GSH (*232*). These observations suggest that the fall of nigral GSH levels in Parkinson's disease may be related to its release from pigmented SN_c cells and also, because the fall is so large, from surrounding glia. It may also be relevant in this respect that the activity of γ-GT is significantly increased in the parkinsonian SN_c (*229*). γ-GT is a cellular membrane-bound enzyme that catalyzes the degradation of GSH to glutamate (Glu) and cysteinylglycine which is then hydrolyzed by dipeptidases to glycine (Gly) and cysteine (CySH) (*233*, *234*). The γ-GT- and dipeptidases-mediated degradation of GSH occurs exclusively extracellularly (*234*). The preceding observations raise the possibility that an early event in the pathogenesis of Parkinson's disease may involve the release of GSH from dopaminergic SN_c neurons and nigral glia and its γ-GT- and dipeptidases-mediated degradation to Glu, Gly, and CySH. It is of interest, in this respect, that energy-dependent mechanisms are involved with the maintenance of intracellular GSH levels. Thus, energy impairment and depolarization of the neuronal and mitochondrial membranes evokes release of both cytoplasmic and mitochondrial GSH (*235–238*). The normal decline of mitochondrial energy metabolism with aging (*239*), the most robust risk factor for sporadic Parkinson's disease, superimposed on systemic complex I (*215*, *216*) and α-KGDH (*218*) defects and periodic exposures to environmental toxicants which interfere with mitochondrial respiratory enzymes (*220*, *240–244*) might represent a combination of factors that evoke a transient but profound energy impairment of dopaminergic SN_c cells and depolarization-mediated release of GSH in the parkinsonian brain. Furthermore, both age-dependent (*245*) and genetically based impairments of xenobiotic metabolism in Parkinson's disease patients (*246–252*) would be expected to increase the rate of delivery of such environmental toxicants to the brain (*253*).

6.1. MPTP Model of Parkinson's Disease. Perhaps the best model of Parkinson's disease in animals is provided by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which evokes the selective degeneration of nigrostriatal DA neurons (*254*, *255*) and, in humans, a syndrome clinically indistinguishable from Parkinson's disease (*256*). Interestingly, MPTP also causes a fall of nigrostriatal GSH levels (*257–259*) without the corresponding increases in GSSG levels (*260*). The active

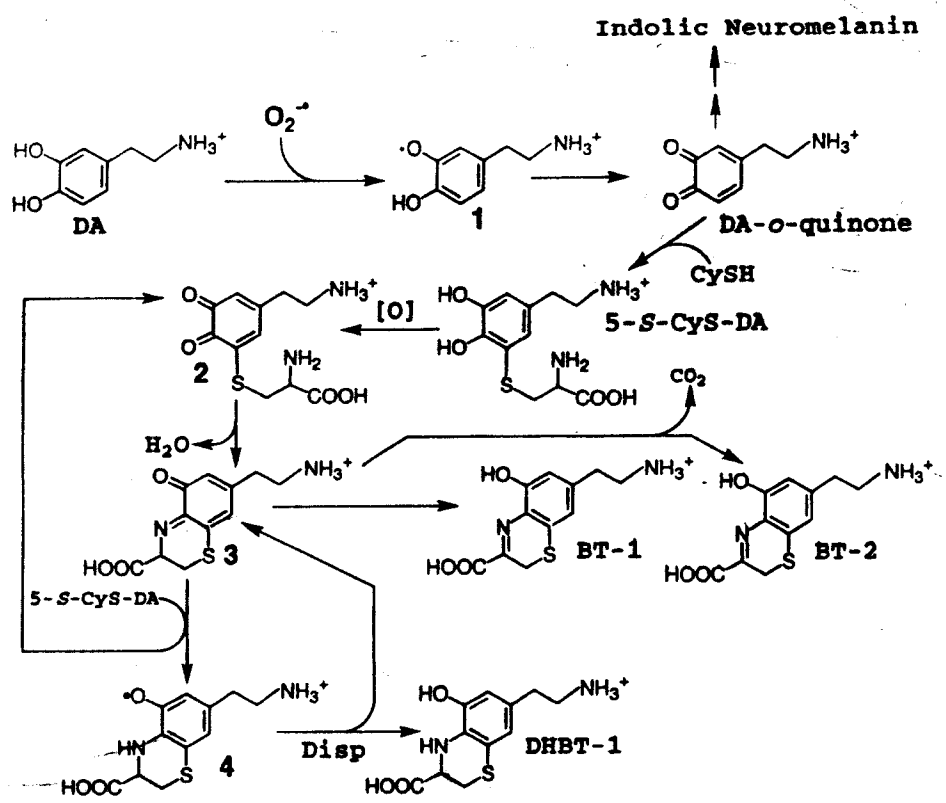


Figure 10. Oxidative metabolism of dopamine and formation of quinone–thioethers and cyclic products.

metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP^+), appears to initiate the neurotoxic process by being selectively accumulated by DA neurons and reversibly inhibiting mitochondrial complex I (*261*) and α-KGDH (*262*), causing rapid depletion of ATP (*263*). Microdialysis experiments show that during perfusion of neurotoxic concentrations of MPP^+ into the rat striatum, rapid ATP depletion and neuron depolarization (*264*) evoke a massive almost instantaneous release of DA (*265*). During MPP^+ perfusion, extracellular hydroxyl radical ($HO^•$) is also generated (*266–268*). When MPP^+ perfusions are discontinued and the neuron energy impairment begins to subside, increasing ATP production initiates repolarization of the neuronal membrane and reuptake of DA (*269*). Interestingly, during MPP^+ perfusion, the level of extracellular Glu remains at or close to basal levels and increases only when the perfusion is discontinued (*270, 271*). The fact that MPP^+ is not a glutamatergic neurotoxin (*271*) and MPTP causes a fall in GSH levels without increased GSSG levels raises the possibility that increased levels of extracellular Glu as the dopaminergic energy impairment begins to subside might result from the γ-GT- and dipeptidases-mediated degradation of released GSH. Recently, it has been demonstrated that during perfusion of neurotoxic concentrations of MPP^+ into the rat striatum or SN_c extracellular GSH and CySH remain at basal levels (*272*). However, when MPP^+ perfusions are discontinued and the dopaminergic neuron energy impairment begins to subside, extracellular levels of GSH in both the striatum and SN_c increase massively and then rather rapidly decline back to basal levels. The extracellular CySH level also increases but more slowly and attains lower peak concentrations than preceding peak concentrations of GSH. Subsequently, the extracellular CySH level only slowly decreases but remains above basal levels for many hours (*272*). The γ-GT inhibitor acivicin blocks both the rapid decrease in extracellular GSH levels from peak concentrations when MPP^+ perfusions are discontinued and elevation of extracellular CySH. These results suggest that the elevation of extracellular Glu (*270, 271*), CySH (*272*), and Gly levels (M. Tang and G. Dryhurst, unpublished data) that occurs when an MPP^+-induced dopaminergic neuron energy impairment begins to subside is indeed the result of the γ-GT- and dipeptidase-mediated degradation of released GSH.

6.2. Possible Neurotoxic Mechanism. On the basis of several lines of evidence, it has been proposed (*272*) that the initial step that triggers the mechanisms that underlie the degeneration of nigrostriatal dopaminergic neurons by MPTP and MPP^+ and in the parkinsonian SN_c is a large but transient impairment of neuronal energy metabolism which evokes a massive release of not only DA but also cytoplasmic and mitochondrial GSH. The fact that extracellular levels of GSH do not increase during a MPP^+-induced dopaminergic energy impairment may indicate that released neuronal GSH is oxidized by extracellular $HO^•$. This, in turn, might trigger release of GSH from glia to protect neighboring neurons (*273, 274*) in amounts sufficient to maintain normal basal levels of the tripeptide. There are several mechanisms that probably mediate extracellular $HO^•$ generation during the neuron energy impairment. Thus, when neurons are profoundly depolarized, the voltage-dependent Mg^{2+} block of *N*-methyl-D-aspartate (NMDA) receptors is relieved such that they can be activated by basal extracellular levels of the excitatory amino acids Glu, aspartate (Asp), and CySH (*275–277*) with resultant Ca^{2+} influx that triggers generation of neuronal superoxide ($O_2^{-•}$) (*278*), nitric oxide ($NO^•$), and thence peroxynitrate ($ONOO^-$) (*279*). $ONOO^-$ readily crosses cellular membranes (*280*), and its subsequent decomposition (*281*) may contribute

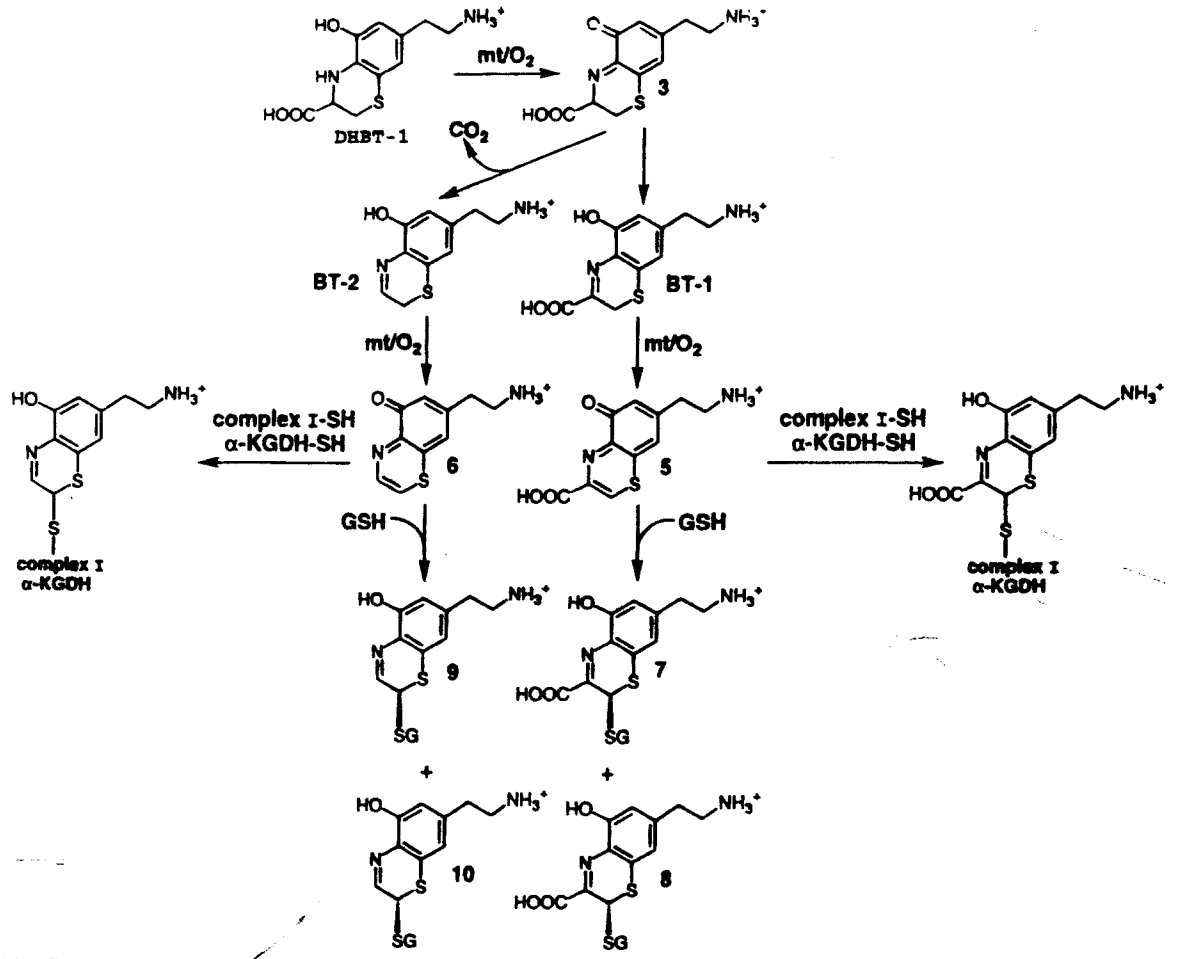


Figure 11. Conversion of DHBT-1 to an *o*-quinone imine, rearrangement to BT-1 and BT-2, and reaction with GSH or α-KGDH.

to extracellular HO• generation (*267*, *268*). Furthermore, $O_2^{-\bullet}$ (*282*, *283*), NO•, and $ONOO^-$ (*284*, *285*) release Fe^{2+} from iron-containing proteins that in the presence of extracellular ascorbate and H_2O_2 can catalyze HO• formation (*286*).

As the neuron energy impairment begins to subside, increasing ATP production *initiates* reinstatement of the Mg^{2+} blockade of NMDA receptors, an effect that would briefly attenuate Ca^{2+} influx and neuronal generation of $O_2^{-\bullet}$, NO•, and $ONOO^-$ and, thence, formation of extracellular HO•. More importantly, however, increasing neuronal ATP production should also initiate reuptake of DA (*268*). Furthermore, DA is readily oxidized by $O_2^{-\bullet}$ (*287*) and $ONOO^-$ (*288*) and, hence, would scavenge these reactive species and thus completely block extracellular HO• formation. Consequently, the HO•-mediated oxidation of extracellular GSH should cease. Replenishing intraneuronal GSH levels, released and oxidized during the neuron energy impairment, requires its continued release from glia (*289*). However, neurons are unable to directly import GSH. Rather, extracellular GSH released from glia is first degraded by γ-GT to Glu and cysteinylglycine (CysGly) which is then hydrolyzed by dipeptidases to Gly and CySH (*290*). CySH and CysGly are then translocated into neurons to provide intraneuronal CySH for GSH biosynthesis (*289*, *291*, *292*). Such mechanisms, therefore, could account for the transient but massive elevation of extracellular GSH levels followed by increased extracellular levels of Glu (*270*, *271*), CySH (*272*), and Gly (M. Tang and G. Dryhurst, unpublished data) as the dopaminergic energy impairment evoked by MPP^+ subsides. However, during the period of recovering but still reduced neuronal ATP production, the Mg^{2+} block of NMDA receptors would remain partially relieved and permit continued activation of these receptors by elevated extracellular Glu and CySH levels with resultant generation of $O_2^{-\bullet}$, NO•, and $ONOO^-$ and oxidation of DA as it returns to its parent neurons. However, in an attempt to replenish intraneuronal GSH levels, an influx of CySH should accompany DA. This may explain why extracellular levels of CySH increase less than the preceding massive elevation of GSH levels as a MPP^+-induced energy impairment subsides (*272*). The $O_2^{-\bullet}$- and $ONOO^-$-mediated oxidation of intraneuronal DA forms DA-*o*-quinone which reacts avidly with translocated CySH to give 5-*S*-cysteinyldopamine (5-*S*-CyS-DA) (Figure 10) (*293*, *294*). Thus, rather than being utilized for intraneuronal GSH synthesis, translocated CySH may be consumed by DA-*o*-quinone, demanding yet more release of glial GSH and its degradation by γ-GT and dipeptidases to Glu, Gly, and CySH with resultant NMDA receptor activation and hence intraneuronal $O_2^{-\bullet}$- and $ONOO^-$-mediated oxidation of DA in the presence of CySH and 5-*S*-CyS-DA formation. Indeed, it has been reported that 5-*S*-CyS-DA, normally a minor metabolite of DA, becomes a major metabolite in the parkinsonian SN_c as evidenced by a dramatic increase in the 5-*S*-CyS-DA/DA concentration ratio (*295*). However, 5-*S*-CyS-DA is more easily oxidized than DA (*293*, *294*) to *o*-quinone **2** that undergoes a rapid intramolecular cyclization to *o*-quinone imine **3** (Figure 10). Compound **3** can oxidize 5-*S*-CyS-DA to *o*-quinone **2** forming radical **4** that disproportionates to

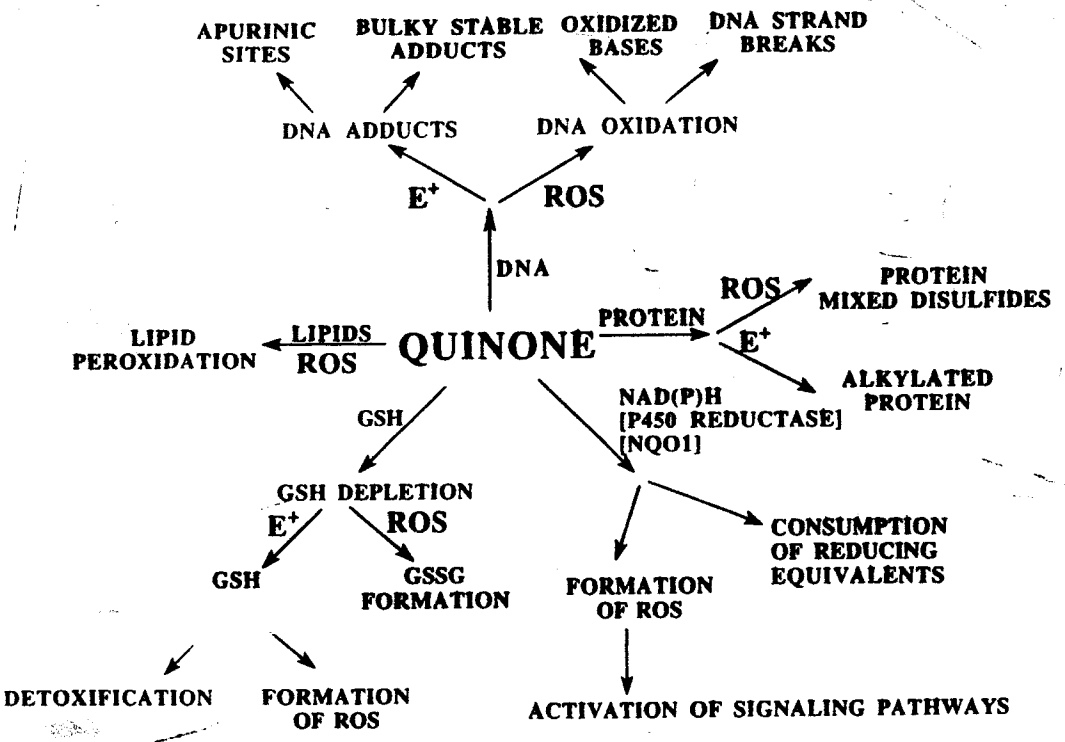


Figure 12. Summary of potential cytotoxic mechanisms for quinones.

the dihydrobenzothiazine DHBT-1 (and **3**). Additionally, *o*-quinone imine **3** can rearrange to the benzothiazines **BT-1 and BT-2 (Figure 10).**

DHBT-1 can be accumulated by intact rat brain mitochondria in vitro and inhibits complex I (pyruvate- and malate-supported) but not complex II (succinate-supported) respiration (*296*). Furthermore, when incubated with rat brain mitochondrial membranes, DHBT-1 evokes a time-dependent irreversible inhibition of NADH-CoQ_1 reductase (*297*) and α-KGDH but not cytochrome *c* oxidase (complex IV) (*298*). The inhibition of NADH-CoQ_1 reductase and α-KGDH by DHBT-1 is unaffected by SOD or catalase and, hence, does not involve reactive oxygen species. The time dependence of the inhibition of NADH-CoQ_1 reductase and α-KGDH appears to be related to its oxidation catalyzed by a presently unknown constituent of the inner mitochondrial membrane (*297*, *298*). In this reaction, DHBT-1 is catalytically oxidized by mitochondrial membranes to *o*-quinone imine **3** that rapidly rearranges to BT-1 and BT-2 (Figure 11). Although it has not been possible to isolate either BT-1 or BT-2 in the pure solid state, a mixture of these compounds, formed by the mitochondrial membrane-catalyzed oxidation of DHBT-1, also evokes a time-dependent irreversible inhibition of NADH-CoQ_1 reductase and α-KGDH. Again, the time dependence of this inhibition reflects the oxidation of BT-1 and BT-2 catalyzed by an unknown component of the inner mitochondrial membrane. However, while the rate of the mitochondrial membrane-catalyzed oxidation of BT-1, BT-2, and their precursor DHBT-1 is unaffected by GSH, greater than equimolar concentrations of the latter tripeptide completely block inhibition of NADH-CoQ_1 reductase and α-KGDH. Furthermore, when mitochondrial membranes are incubated with DHBT-1, or BT-1 and BT-2, in the presence of GSH new metabolites appear. These include epimers **7** and **8**, 2-*S*-glutathionyl conjugates of BT-1 (Figure 11). Accordingly, it has been proposed that BT-1 and BT-2 are catalytically oxidized by mitochondrial membranes to highly electrophilic intermediates **5** and **6**, respectively. Intermediates **5** and **6** then covalently modify key sulfhydryl residues at the complex I and α-KGDH active sites, evoking inhibition of these mitochondrial enzyme complexes. GSH, therefore, blocks inhibition of NADH-CoQ_1 reductase and α-KGDH by DHBT-1, BT-1, and BT-2 by scavenging **5** and **6** to give epimers **7** and **8** (derived from BT-1) and **9** and **10** (derived from BT-1). It remains to be definitively established, however, that DHBT-1, BT-1, and BT-2 are formed in the SN_c of Parkinson's disease patients and are responsible for defects in NADH-CoQ_1 reductase and α-KGDH. Nevertheless, the reactions shown in Figures 10 and 11 together with increased γ-GT activity are consistent with the loss of nigral GSH in Parkinson's disease and elevated levels of 5-*S*-CyS-DA.

7. Conclusions and Future Direction

The roles of quinones in mediating the adverse effects of polyhydroxylated aromatic compounds have not been investigated in detail. It is possible for these electrophilic and redox active quinones to cause damage within cells by all of the pathways shown in Figure 12. Oxidative enzymes, metal ions, and in some cases molecular oxygen can catalyze quinoid formation, so alkylation of cellular nucleophiles (GSH, proteins, and DNA) by these species may occur to a significant extent in many tissues. In addition, the formation of ROS, especially through redox cycling between the quinones and semiquinone radicals, could contribute to the cytotoxic properties of the parent compounds. Redox cycling can cause lipid peroxidation, consumption of reducing equivalents, oxidation of DNA, and DNA strand breaks. Further, ROS can activate a number of signaling pathways, including those of protein kinase C and RAS. DNA binding occurs, but the sites of alkylation and relationships to cytotoxicity are dependent on the chemical structure of the quinones and the cellular environment in which they are formed. Future work

should unequivocally establish the ultimate carcinogenic and cytotoxic metabolites of polyhydroxylated aromatics and show their molecular targets and mechanism(s) of action.

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