

The Toxicology of Hydroquinone — Relevance to Occupational and Environmental Exposure

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ABSTRACT: Hydroquinone (HQ) is a high-volume commodity chemical used as a reducing agent, antioxidant, polymerization inhibitor, and chemical intermediate. It is also used in over-the-counter (OTC) drugs as an ingredient in skin lighteners and is a natural ingredient in many plant-derived products, including vegetables, fruits, grains, coffee, tea, beer, and wine. While there are few reports of adverse health effects associated with the production and use of HQ, a great deal of research has been conducted with HQ because it is a metabolite of benzene. Physicochemical differences between HQ and benzene play a significant role in altering the pharmacokinetics of directly administered when compared with benzene-derived HQ. HQ is only weakly positive in *in vivo* chromosomal assays when expected human exposure routes are used. Chromosomal effects are increased significantly when parenteral or *in vitro* assays are used. In cancer bioassays, HQ has reproducibly produced renal adenomas in male F344 rats. The mechanism of tumorigenesis is unclear but probably involves a species-, strain-, and sex-specific interaction between renal tubule toxicity and an interaction with the chronic progressive nephropathy that is characteristic of aged male rats. Mouse liver tumors (adenomas) and mononuclear cell leukemia (female F344 rat) have also been reported following HQ exposure, but their significance is uncertain. Various tumor initiation/promotion assays with HQ have shown generally negative results. Epidemiological studies with HQ have demonstrated lower death rates and reduced cancer rates in production workers when compared with both general and employed referent populations. Parenteral administration of HQ is associated with changes in several hematopoietic and immunologic endpoints. This toxicity is more severe when combined with parenteral administration of phenol. It is likely that oxidation of HQ within the bone marrow compartment to the semiquinone or *p*-benzoquinone (BQ), followed by covalent macromolecular binding, is critical to these effects. Bone marrow and hematologic effects are generally not characteristic of HQ exposures in animal studies employing routes of exposure other than parenteral. Myelotoxicity is also not associated with human exposure to HQ. These differences are likely due to significant route-dependent toxicokinetic factors. Fetotoxicity (growth retardation) accompanies repeated administration of HQ at maternally toxic dose levels in animal studies. HQ exposure has not been associated with other reproductive and developmental effects using current USEPA test guidelines. The skin pigment lightening properties of HQ appear to be due to inhibition of melanocyte tyrosinase. Adverse effects associated with OTC use of HQ in FDA-regulated products have been limited to a small number of cases of exogenous ochronosis, although higher incidences of this syndrome have been reported with inappropriate use of unregulated OTC products containing higher HQ concentrations. The most serious human health effect related to HQ is pigmentation of the eye and, in a small number of cases, permanent corneal damage. This effect has been observed in HQ production workers, but the relative contributions of HQ and BQ to this process have not been delineated. Corneal pigmentation and damage has not been reported at current exposure levels of <2 mg/m³. Current work with HQ is being focused on tissue-specific HQ-glutathione metabolites. These metabolites appear to play a critical role in the renal effects observed in F344 rats following HQ exposure and may also be responsible for bone marrow toxicity seen after parenteral exposure to HQ or benzene-derived HQ.

KEY WORDS: hydroquinone, *p*-benzoquinone, *p*-benzenediol, 1,4-benzenediol, quinol, quinone, benzene, myelotoxicity, carcinogenicity, developmental toxicity, reproductive toxicity, mutagenicity, genotoxicity, clastogenicity, aneuploidy, metabolism, pharmacokinetics, depigmentation, ocular toxicity, ocular pigmentation.

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I. INTRODUCTION

Hydroquinone (HQ; *p*-benzenediol; CAS# 123-31-9) is a high-volume commodity chemical with major uses as a reducing agent in black and white photographic developing solutions, as an antioxidant and polymerization inhibitor, and as an intermediate in the synthesis of other antioxidant derivatives.^{1,2} Worldwide annual (1992) production capacity for HQ is estimated at 35,000 to 40,000 tonnes.^{1,3} U.S. production is limited to three manufacturing sites, although National Institute of Occupational Safety and Health (NIOSH) data indicate that HQ may be employed in manufacturing and end use at 16,000 to 66,000 individual facilities.⁴ A minor but important use of HQ is in topically applied, over-the-counter and prescription depigmenting skin creams and in other consumer personal care products. HQ is also present at significant levels in cigarette smoke and in certain fruits and vegetables (in the form of its glucose conjugate, arbutin), including pears and blueberries.^{5,6}

HQ is a water-soluble, crystalline solid. When present in aqueous solution, HQ is susceptible to

both redox and acid-base transformations (Figure 1). The products of these reactions, including *p*-benzoquinone (BQ), a semiquinone, and various activated oxygen species, are potentially important for the action of HQ in biological systems. The toxicology of HQ has been investigated in experimental animal studies by various routes of exposure and in *in vitro* model systems since the late 1800s. These studies have reported effects on a number of organ systems and cellular processes. The U.S. National Toxicology Program (NTP) classified HQ as demonstrating “some evidence” of carcinogenicity in animal studies, based primarily on kidney adenomas in male Fisher 344 rats.⁷ The International Agency for Research on Cancer (IARC) includes HQ under its Group 3 category, that is, “not classifiable” as to its carcinogenicity in humans.⁸ The American Conference of Governmental Industrial Hygienists (ACGIH) classifies HQ under its carcinogenicity designation “A3”, that is, carcinogenic in animal studies, unknown relevance in humans.⁹ Human clinical, occupational, and epidemiological studies have consistently demonstrated only dermal effects associated with excessive use of HQ-containing

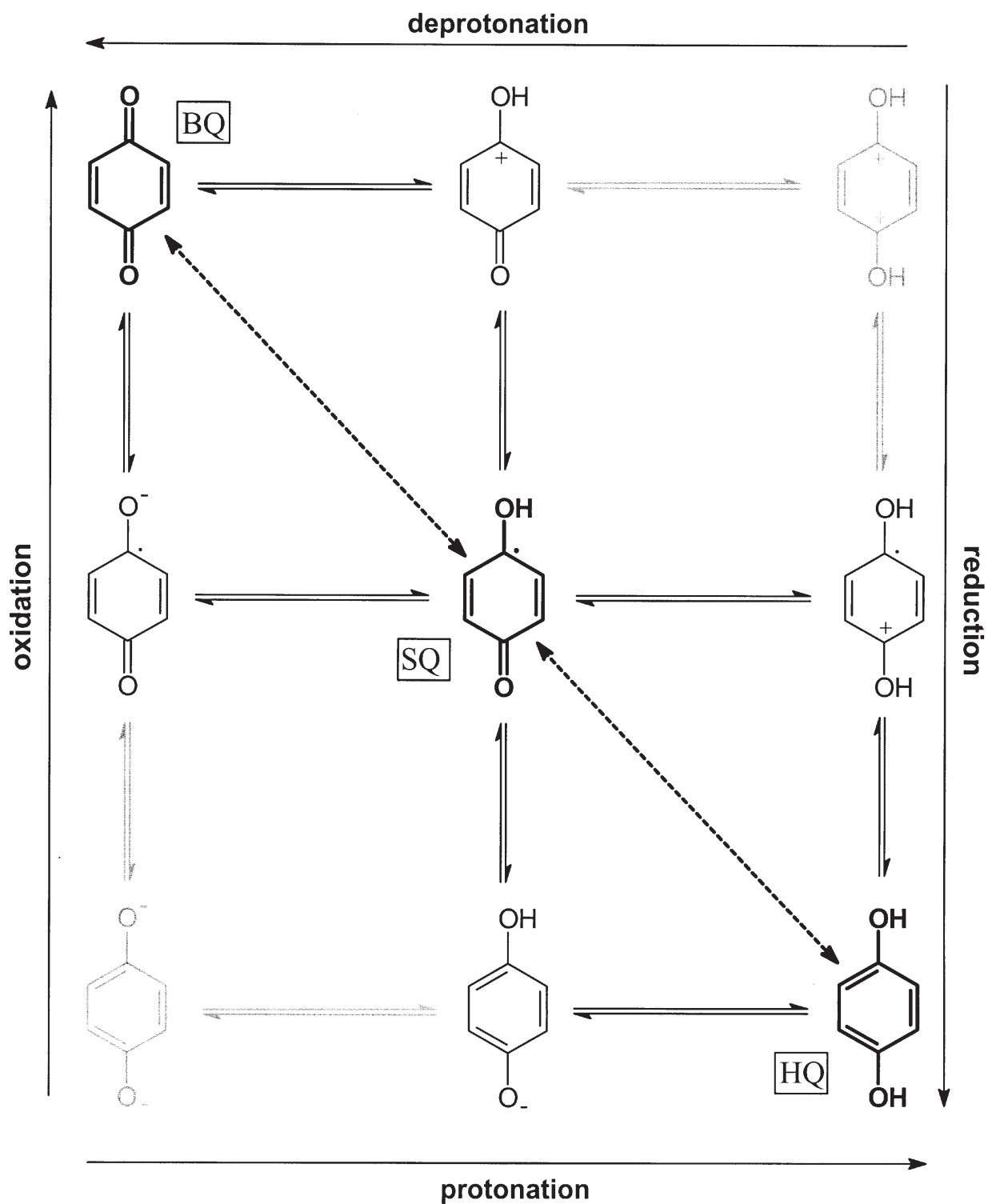


FIGURE 1. Acid-base and oxidation-reduction matrix for hydroquinone (HQ), semiquinone (SQ), and benzoquinone (BQ). Electron and proton transfer reactions are shown on vertical and horizontal axes, respectively. Most stable species under aqueous physiological conditions are indicated in bold; species shown in gray are highly unfavored. While all possible conversions are shown, *in vivo* interconversions between HQ, SQ, and BQ likely proceed via simultaneous electron-proton (i.e., hydrogen atom) transfers (diagonal dashed arrows).

skin lighteners and a unique ocular syndrome in heavily exposed HQ production workers. Several reviews of specific aspects of HQ toxicology are available.^{10,11} In addition, more comprehensive reviews have been published by the World Health Organization, the Cosmetic Ingredient Review Expert Panel, and other authors.^{1,12-15} The present article summarizes the available data on significant aspects of HQ toxicology, addresses newer information on nephrotoxicity, myelotoxicity, and macromolecular binding, and critically assesses the potential relevance of *in vitro* and experimental animal data on HQ to humans.

An important and controversial issue concerns the potential involvement of HQ in the molecular mechanism of benzene-induced myelotoxicity and leukemia in humans. HQ, along with phenol, BQ, and several other oxidized and ring-opened derivatives is a demonstrated metabolite of benzene in humans and experimental animals. Much of the basic research on HQ toxicology and its effects on cellular and molecular systems has been driven by the hypothesis that HQ, alone or in combination with other benzene metabolites, is involved in the mechanism of benzene-induced leukemia. Although full treatment of this issue is beyond the scope of the present article, relevant data from such studies are incorporated into this review where appropriate, and their potential significance for direct human exposure to HQ is evaluated.

II. HUMAN EXPOSURE TO HQ

Typical human occupational exposure scenarios for HQ consist primarily of inhalation of particulates and dermal contact with either solid HQ or aqueous solutions (as in photographic developers). Estimates of the number of workers with potential exposure to HQ vary, but range up to 80 to 100 for actual production, 560,000 for industrial uses, and 30,000 for photographic development.^{1,4,15} The current Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) and ACGIH Threshold Limit Value (TLV) for HQ are both 2 mg/m³ (8-h TWA), a value that is widely used in other countries as well.¹ In addition, an IDLH limit of

50 mg/m³ has been recommended by NIOSH for HQ.

Airborne HQ dust levels are routinely monitored in occupational (i.e., HQ manufacturing) settings and generally average approximately 0.10 to 0.50 mg/m³.¹⁵ Prior to the institution of comprehensive dust control measures during HQ manufacture and use, air concentrations were substantially higher; approximately 2 to 5 mg/m³ and up to 20 to 35 mg/m³ in some cases.^{16,17} In contrast, ambient air levels of HQ in the vicinity of photographic developing operations are much lower (<0.01 mg/m³),¹⁸ although levels during preparation of developing solutions (0.5 to 2.0% HQ) from dry HQ would probably be higher. The internalized dose of HQ is expected to be minimal following dermal exposure to HQ during photographic processing due to the low HQ concentrations present in developer solutions and the slow absorption of HQ through mammalian skin.¹⁹ Estimates of dermal absorption following immersion of an adult hand in 5% HQ solution for 1 h indicated an uptake of only 200 µg (2.9 µg/kg for a 70-kg man).⁵ Measurement of urinary levels of HQ in darkroom workers have revealed no increases compared with unexposed controls, indicating minimal uptake of HQ.²⁰ HQ may also be released directly to the environment as a fugitive emission during its production and use and in the effluent of photographic processes, although no specific data are available to judge the overall potential for human exposure via these releases.²

HQ occurs naturally in the leaves, bark, and fruit of a number of plants, some of which are used as food. In particular, HQ is present in various berries, pears, and other fruits, much of it in the form of the glucose conjugate 4-hydroxyphenyl-β-D-glucopyranoside (arbutin). Arbutin is expected to readily undergo acid hydrolysis to yield HQ, although whether this process actually occurs within the acidic environment of the human stomach has not been reported. Arbutin has been demonstrated to be stable to hydrolysis in human small intestine preparations *in vitro* and to be absorbed intact from the intestinal lumen by facilitated transport mechanisms.²¹ However, direct studies in human volunteers did not reveal higher levels of free HQ in urine following glucuronidase treatment, suggesting that arbutin

may not have been absorbed (and excreted) intact.⁵

In addition to arbutin, free HQ is also present in coffee beans and certain wheat-based products. It has been speculated that the low levels of HQ detected in the urine of unexposed persons²² may be derived from HQ-containing foods, or, alternatively, from environmental exposure to benzene or phenol.^{5,23} Other potential sources of such background HQ levels include cigarette smoke (110 to 300 μg per cigarette) and ingestion of acetaminophen (which is metabolized to HQ and other products).⁵ One study revealed increases in plasma and urinary HQ shortly after consumption of a high-HQ meal and cigarette smoking, but not following a 1000 mg dose of acetaminophen in several human volunteers.⁵ These authors also estimated that the typical exposure to HQ from food and other uncharacterized sources was approximately an order of magnitude greater than that predicted from occupational exposure during HQ production or use of photographic developers.

III. ACUTE EFFECTS OF HQ

Animal studies indicate that, in most species, HQ exhibits relatively low acute toxicity by oral and dermal routes of exposure. Acute oral LD_{50} values for HQ range from 70 mg/kg in the cat to 550 mg/kg in the guinea pig, with most species exhibiting values in the upper end of this range.²⁴⁻²⁷ The higher sensitivity of the cat may be accounted for by relatively lower levels of glucuronide conjugation activity in the liver and intestinal tract.^{28,29} The presence of food may decrease the rate and extent of absorption of HQ from the GI tract, as LD_{50} values of 310 and 1050 mg/kg have been reported for the fasted and unfasted rat, respectively.²⁶ A fairly steep dose-response curve for lethality is typically observed, and equivalent divided oral doses of HQ are substantially less toxic than large single doses.²⁵ The acute dermal LD_{50} of HQ (in an unidentified mammalian species) was estimated at 5970 mg/kg, suggesting low systemic absorption via this route.³⁰ The acute dermal toxicity in rats and mice is >3840 and >4800 mg/kg (respectively), as animals were able to survive these dose levels given repeatedly over

2 weeks.⁷ LD_{50} values for HQ by parenteral administration have been reported as 160 mg/kg in the rat,²⁵ 115 mg/kg in the rat,²⁵ and 190 mg/kg in the mouse³¹ by i.p., i.v., and s.c. injection, respectively.

The effects of acute high-level HQ exposure in animal studies are directed primarily toward the central nervous system (CNS). The CNS stimulatory effects of large acute doses of HQ were first shown in rabbits and frogs by Brieger in 1879³² and since have been reproduced in a number of species and by various routes of administration. The signs associated with such acute exposure include tremor, salivation, hyperexcitability, incoordination, tonic and clonic convulsions, respiratory failure, coma, and death. The onset of CNS signs is quite rapid, and death generally occurs within 2 h of exposure of test animals. Rapid and complete recovery from the acute effects of large, sublethal doses of HQ in experimental animals is also characteristic. In contrast to the marked CNS effects of near-lethal doses, comprehensive functional-observational examination of Sprague-Dawley (SD) rats administered HQ at doses of 20, 64, or 200 mg/kg/day (5 days/week, p.o.) for 13 weeks revealed only transient tremors and decreased motor activity at the two higher dose levels.³³ No neuropathological changes were noted as a result of this dosing regimen.

The molecular mechanism of CNS stimulation by HQ is poorly understood, although aromatic phenols are well-known convulsant agents.³⁴ HQ is approximately one-third as potent as catechol (the most potent polyphenol) and equal to phenol in producing convulsions in rodent models.³⁵ CNS stimulation is believed to be a direct action of HQ, rather than metabolites such as BQ, due to the very rapid onset of effects and the lack of similar CNS signs with acute BQ exposure.²⁵ Early studies suggested that HQ and related phenols increase the size of end-plate potentials in peripheral motor nerve terminals without altering acetylcholine sensitivity,³⁶ and that HQ does not possess anticholinesterase activity.³⁷ A CNS activation mechanism involving both presynaptic and postsynaptic actions has been proposed.³⁸

Acute exposure to HQ in humans by accidental or deliberate ingestion is also associated with CNS and other effects, although the attribution of specific signs and symptoms is frequently clouded

by the presence of other components and by the difficulty in accurately establishing exposure levels. Tremor, respiratory difficulty, convulsions, and unconsciousness have been reported in people ingesting multi-gram quantities of HQ.³⁹⁻⁴¹ For example, cyanosis, rapid pulse, and coma were reported after deliberate consumption of an estimated 12 g of HQ; the individual apparently recovered from this intoxication within 2 weeks.⁴⁰ Discolored (green) or dark urine has also been noted in these cases. No fatalities have been reported after ingestion of HQ itself. Acute gastroenteritis was reported in a large number of men consuming drinking water accidentally contaminated with an unknown amount of photographic developer containing HQ aboard a U.S. Navy ship.⁴² Other toxic signs, including jaundice and hemolytic anemia, have also been reported, but only following ingestion of HQ in combination with other compounds, such as in photographic developers.⁴³⁻⁴⁶ If not fatal, complete recovery from the effects of these combined exposures typically has been reported.

IV. TOXICOKINETICS OF HQ

The absorption, distribution, metabolism, and excretion of HQ has been studied extensively in both experimental animal models and human volunteers. These investigations have been prompted by the need for relevant data on HQ itself and by attempts to understand the potential involvement of HQ in the toxicity of benzene. Numerous reports indicate that HQ is well absorbed by the oral route. Single oral doses of up to 350 mg/kg were >90% absorbed in CD and F344 rats, with peak blood levels occurring within <1 h.⁴⁷⁻⁵¹ Similar findings were reported with oral exposure of rats to developer solution containing 3% HQ.⁵² Rapid and substantial oral absorption in humans ingesting foods containing mg levels of HQ has been demonstrated.⁵ HQ also appears to be well absorbed following intratracheal (i.t.) instillation in rats, suggesting that HQ from small (<5 μm) particles that reach the alveoli and dissolve would be rapidly taken up into the blood.^{50,53,54}

Numerous studies have demonstrated that, in contrast to oral and i.t. administration, systemic

absorption of HQ with dermal exposure occurs but is less efficient. *In vitro* studies with mouse and human skin exposed to aqueous solutions of HQ indicated permeability constants (K_p) of 28×10^{-6} and 4×10^{-6} cm/h, respectively.⁵⁵ Barber et al. reported K_p values of 23×10^{-6} with rat skin and 9.3×10^{-6} cm/h with human skin.¹⁹ These human K_p values fall in the "very slow" and "slow" permeability ranges using the qualitative ranking system described by Marzulli et al.⁵⁶ Only minor systemic absorption of a dermal dose of HQ administered in aqueous solution to mice,⁵⁵ rats,⁵¹ or dogs⁵⁷ was reported. More extensive penetration of HQ across human^{58,59} and animal^{7,59} skin when applied in an alcohol vehicle has been demonstrated. Most recently, dermal application of a 2% HQ cream formulation (dose of 2.5 mg spread over 25 cm² of skin) in human volunteers was associated with bioavailability (based on blood levels) of ~45% after 24 h, similar to that seen with *in vitro* human skin preparations.⁶⁰ Both HQ and BQ were detected in the *in vitro* human skin preparations. These workers reported an absorption rate fourfold higher than Barber et al., although K_p values calculated from these data still fall within the "slow" permeability range.⁵⁶

Tissue to plasma partition coefficients for HQ were reported by Hill et al.⁶¹ to range from 1.3 to 2.4, typical for a low-molecular-weight water- and lipid-soluble xenobiotic. Distribution of HQ is limited by extensive biotransformation following absorption, with differences observed between the oral and parenteral routes of administration. After oral administration of [¹⁴C]-HQ to rats, <1% of radiolabel in blood was associated with parent compound, consistent with extensive first pass metabolism.⁵⁰ Greenlee et al.,^{62,63} using whole body autoradiography and scintillation counting of tissue homogenates, reported concentrations of radiolabel in bone marrow and thymus 2 h following i.v. administration of 1.2 to 12 mg/kg [¹⁴C]-HQ to rats. The latter study also demonstrated a time-dependent increase in covalently bound label in liver and bone marrow up to 24 h. In contrast, i.v. administration of [¹⁴C]-HQ to dogs was associated with residual label in the skin, liver, and intestine after 24 h.⁵⁷ Wide distribution of radiolabel to various tissues, particularly liver and kidney, was demonstrated with both single and repeated oral [¹⁴C]-HQ dosing regimens in the rat.⁵¹

Relative label in bone and spleen was approximately 10% of that seen in liver 48 h after a single 25 mg/kg oral dose. Administration of 75 mg/kg of [¹⁴C]-HQ to mice by i.p. injection resulted in covalent binding to liver, kidney, blood, and bone marrow macromolecules, with liver having a 10-fold higher specific activity than bone marrow.⁶⁴

While HQ is rapidly and extensively absorbed by the oral and i.t. routes, it is rapidly eliminated via urine, leaving little residual sequestration or binding to tissue (~1 to 3% of total dose). Elimination via expired air is negligible and elimination via feces is a generally minor pathway for HQ. Blood elimination half-lives (total radioactivity) of 18.7, 14.8 min, and 22.1 min have been reported for i.v., p.o., and i.t. administration of [¹⁴C]-HQ, respectively, in F344 rats.⁵⁰ A parent compound half-life of 9 min was reported with i.p. injection in B6C3F₁ mice,⁶⁵ while 2.7 min was the mean HQ elimination half-life with i.t. instillation in SD rats.⁵⁴ Some evidence for saturation of elimination pathways at high doses of HQ is available, as a single oral dose of 350 mg/kg in the rat was associated with an average urinary excretion of 50% of total dose after 8 h, compared with >80% excretion at 25 mg/kg.⁵¹ This trend was particularly evident in females.

The biotransformation of HQ (Figure 2) has been examined for over a century, with very early studies (cited by Garton and Williams⁶⁶) identifying urinary sulfate and glucuronide conjugates as metabolites. Following single or multiple oral doses of up to 350 mg/kg HQ in experimental animals, approximately 90% of administered dose is recovered in the urine as one of these conjugates (2-to-1 glucuronide/sulfate ratio).^{47-49,51} Similar values are seen after i.t. administration.⁵³ After i.t. instillation, all of the radiolabel in arterial blood represented parent compound at the initial (5 to 10 s) sample time, suggesting a lack of significant pulmonary metabolism of HQ.⁵⁴ At later time points (2 to 12 min), levels of glucuronide rapidly exceeded free HQ concentrations. Minor urinary products following oral administration include unchanged HQ, a mercapturic acid conjugate (*N*-acetyl-[*L*-cystein-*S*-yl]-HQ), and BQ.⁵¹ The urinary BQ is likely formed *in situ* from autoxidation of HQ rather than biotransformation. Unchanged HQ, sulfate, glucuronide, and a putative glutathione (GSH) conjugate of HQ

were also reported in plasma from rats 1 h after a 50 mg/kg oral dose of HQ.⁵⁰ Following dermal exposure to a 2% HQ cream formulation for 24 h in humans, urinary metabolites included only Phase II (glucuronide) conjugates.⁶⁰

Parenteral administration of HQ to experimental animals is also associated with the presence of high levels of urinary sulfate and glucuronide conjugates. However, additional oxidized (Phase I) and conjugated (Phase II) products are observed. 1,2,4-Benzenetriol, presumably formed from cytochrome P450-mediated oxidation of HQ, was detected in the urine of rats and rabbits administered 50 mg/kg by i.p. injection.²² *N*-Acetyl-(*L*-cystein-*S*-yl)-HQ was detected in the urine of rats 24 h after a 75 mg/kg i.p. dose and during the first 4 h following a 200 mg/kg i.p. dose of HQ.^{67,68} 2-(*L*-Cystein-*S*-yl)-HQ and a number of GSH conjugates, including 2-glutathion-*S*-yl-HQ, 2,5- and 2,6-diglutathion-*S*-yl-HQ, and 2,3,5-triglutathion-*S*-yl-HQ, were detected in bile from rats in the latter study.⁶⁸ Together with urinary *N*-acetyl-(*L*-cystein-*S*-yl)-HQ, these biliary metabolites represented over 4% of the total HQ dose administered by i.p. injection. Additional data indicated that formation of these conjugates was in part NADPH- and P450-dependent. Major hepatic P450 isoforms responsible for HQ oxidation, based on *in vitro* studies with human microsomes, appear to be 1A1, 3A4, and 2E1.^{69,70}

These toxicokinetic data clearly indicate that the relative proportion of oxidized and conjugated metabolites of HQ is both route of exposure and dose dependent. Following oral exposure, virtually all absorbed HQ is likely to be metabolized via hepatic portal and intestinal sulfate and glucuronide conjugation pathways, although saturation may occur with very high oral doses. In contrast, parenteral administration may bypass much of this conjugative capacity, with the resultant formation of higher levels of oxidized HQ metabolites and GSH conjugates. As discussed later, the GSH metabolites of HQ may be critical to the mechanism of nephrotoxicity observed in animal studies, in particular those utilizing the F344 rat. A PBPK model of HQ disposition, which described a wide variety of experimental data, predicted that male F344 rats form more glutathione conjugates than male SD rats at equivalent dose levels.⁷¹ The model also predicted that

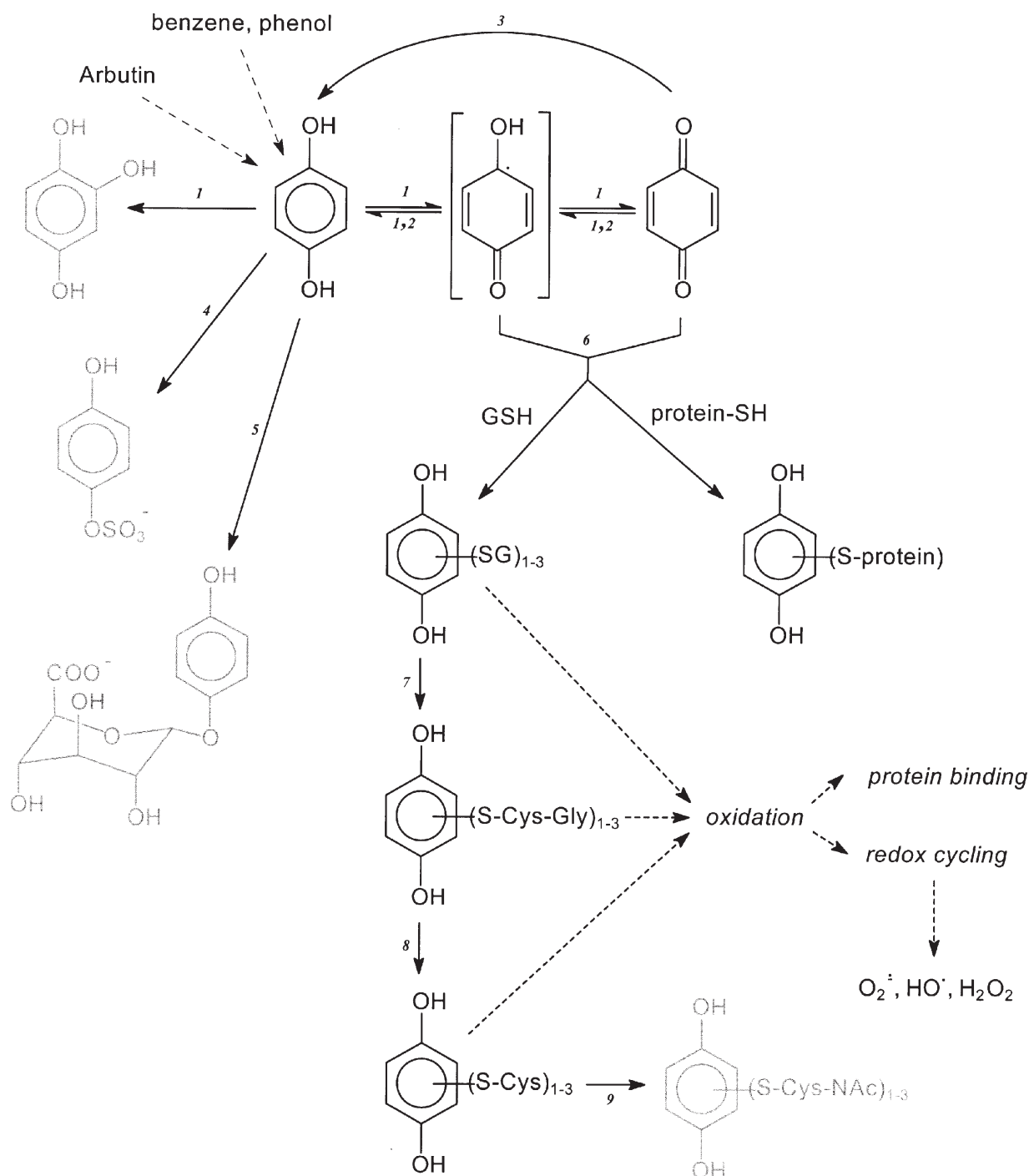


FIGURE 2. Comprehensive metabolic scheme for HQ. Possible input from arbutin (naturally occurring glucose conjugate of HQ), benzene, and phenol is also indicated (long dashed line arrows). Derivatives shown in gray are considered detoxified metabolites. Proposed mechanism of covalent binding and activated oxygen species production from HQ-SG-derived conjugates also shown (short dashed line arrows). Enzyme (or process) associated with each conversion indicated by numbers: (1) spontaneous reaction (slow), cytochrome P450, or various peroxidases; (2) cytochrome P450 or b_5 reductase; (3) NQO1 or carbonyl reductase; (4) sulfotransferase; (5) glucuronyl transferase; (6) spontaneous reaction or glutathione-S-transferase; (7) γ -GT; (8) dipeptidase; (9) *N*-acetyltransferase. (GSH = glutathione.)

i.p. administration, which bypasses much of the glucuronide and sulfate conjugation capability of the GI tract, should result in greater amounts of glutathione conjugates than comparable oral doses. With parenteral exposure, increased levels of unchanged HQ are likely to be available to extrahepatic tissues, where non-P450-mediated oxidation of HQ to BQ may occur (particularly in bone marrow).

Finally, while recent evidence suggests that interindividual variation in glucuronyl and sulfotransferase enzymes in liver and other relevant tissues may impact the overall pattern and extent of HQ metabolism from benzene,⁷² these differences may not be as important with direct HQ exposure. This is because intestinal pathways provide significant additional glucuronidation capacity for the oral route,^{73,74} the most common route of HQ exposure in man. For dermal and inhalation routes, hepatic metabolism of HQ is unlikely to be a limiting factor for detoxication, as lower net absorption of HQ is likely, either because of slow dermal penetration or because the physical characteristics of HQ (i.e., particle size of commercially produced material >100 μm ; low volatility) result in lesser amounts absorbed via inhalation for ultimate presentation to the liver. These considerations are important in assessing the potential risk to humans exposed to HQ under typical exposure scenarios (see under Conclusions).

V. MACROMOLECULAR BINDING AND REDOX REACTIONS

Covalent reactivity may underlie certain aspects of HQ toxicity, and the macromolecular binding potential of HQ has been investigated in a number of *in vitro* and *in vivo* models. Because HQ itself lacks a strong electrophilic center, such binding must be preceded by oxidation to either the semiquinone or to BQ, both of which are substantially more reactive toward protein and DNA nucleophiles. The redox chemistry of quinones has been reviewed comprehensively in a number of articles.^{75–78} The acid-base and redox conversions of the quinones are closely interrelated, and a total of nine putative ionic and/or radical quinone species combinations are possible (Fig-

ure 1). Most of these require relatively extreme conditions of pH or redox potential to form and thus are unlikely to be present under aqueous physiological conditions. Others, however, may be formed and exist as either reactive intermediates or relatively stable species that can mediate toxic effects. Transformations between these species may occur by either one- or two-electron transfers. One-electron redox reactions leading to semiquinones can result in the phenomenon of “redox cycling”, where electrons are transferred to molecular oxygen to form superoxide anion. This process has been proposed to result in an increased oxidative stress that may be significant for quinone toxicity,^{79,80} although evidence is available that this process is slow at physiological pH.⁸¹ Semiquinone radicals can also combine to regenerate HQ and BQ by means of disproportionation. Brunmark and Cadenas proposed⁴⁸ an alternative mechanism of redox cycling, in which P450-mediated oxidation of HQ accompanied by GSH addition would form benzenetriol-SG, a derivative more likely to participate in cycling than HQ itself.

Under physiological conditions, one- and two-electron redox reactions of HQ and BQ can occur either spontaneously or via enzyme-mediated processes. Slow autoxidation of HQ to BQ in phosphate buffer at physiological pH was reported by Greenlee et al.,⁶³ a reaction that was substantially accelerated by superoxide dismutase. As discussed above, enzymatic conversion of HQ to BQ primarily involves cytochrome P450-mediated oxidation in the liver. However, peroxidases (including myeloperoxidase and related enzyme activities) can mediate this conversion in extrahepatic organs. One-electron reduction of BQ to the semiquinone may occur via cytochrome P450 or b_5 reductase, while two-electron reduction can be catalyzed by NADPH:quinone acceptor oxidoreductase (NQO1; also known as DT-diaphorase or quinone reductase) or carbonyl reductase.^{78,79} Depending on the particular organ or tissue in which they occur and on local redox status and metabolite flux, these various metabolic conversions can result in either net activation or detoxification. This is because factors such as intracellular pH, O_2 tension, and thiol balance will influence the direction and extent of these reactions.⁷⁸ It should also be noted that the *in vitro*

oxidation and reduction activity of DT-diaphorase on hydroquinones may be modulated in unpredictable ways by ancillary materials such as Triton X-100, Tween 20, and bovine serum albumin.⁸² Consequently, *in vitro* studies involving the redox potential of HQ need to be conducted under very carefully controlled conditions to avoid false-positive and false-negative results.⁸² As many such studies have not controlled for the impact of these ancillary agents, the significance of certain *in vitro* results is unclear.

As discussed previously, administration of radiolabeled HQ to experimental animals results in covalently bound protein label in a variety of tissues. Peroxidase-mediated binding of [¹⁴C]-HQ to albumin was observed with mouse peritoneal macrophage lysates and with purified prostaglandin-H synthase in the presence of H₂O₂; binding was inhibited by cysteine, suggesting the formation of thiol adducts.^{81,83} Covalent adducts of HQ to protein sulfur nucleophiles were measured in tissues of rats after administration of HQ.⁸⁴ Total adducts were determined as well as individual bound forms of HQ in which the HQ nucleus was substituted with mono-, di-, tri-, or tetra-substituents. The concentration of total adducts was greatest in the liver, followed by kidney and blood. However, the pattern of adduct type was highly tissue specific, with mono-adducts predominating in the liver and tri- and tetra-adducts predominating in the kidneys. These results are consistent with the nephrotoxicity of di- and tri-glutathione conjugates of HQ acting through adduction of protein sulfhydryl groups (yielding tri- and tetra-adducts, respectively). Levels of adducts in kidney correlated with acute nephrotoxicity of HQ as a function of route of administration (i.p. > p.o.) and sex (female F344 rat > male F344 rat) and strain (F344 rat > SD rat).⁸⁴ The thiol reactivity of bioactivated HQ is reflected in the rapid depletion of hepatic GSH in rats following p.o. administration of 100 or 200 mg/kg HQ and in isolated hepatocytes exposed to HQ.⁸⁵⁻⁸⁷ In contrast, only a modest decrease (0 to 20%) in renal GSH in rats was observed after p.o. administration of 400 mg/kg HQ.⁸⁸ *In vivo* HQ treatment also resulted in enhanced lipid peroxidation based on recovery of malondialdehyde in urine,⁸⁵ although no change in peroxidized lipid levels in the kidneys was found.⁸⁹

Protein binding of HQ/BQ has been reported in other model and *in vivo* systems as well. Covalent labeling of protein with [¹⁴C]-HQ was observed in mouse bone marrow macrophages in the presence of H₂O₂ but not arachidonic acid.⁹⁰ *In vitro* incubation of HQ with tubulin in the presence of horseradish peroxidase and H₂O₂ resulted in dose-related binding to both α - and β - subunits.⁹¹ Irreversible binding of [¹⁴C]-HQ to plasma proteins from F-344 and SD rats and from humans decreased dramatically when incubation temperature was lowered from 37°C to 0°C, suggesting temperature-dependent biotransformation to a reactive species.⁹² Assays have been developed to quantitate BQ-derived adducts in hemoglobin and albumin from humans and rodents.^{93,94} Human studies have demonstrated high background levels of such adducts, possibly reflecting dietary or other environmental sources.⁹³ Species- and tissue-dependent, BQ-derived protein adduction in blood and bone marrow has been reported in rats and mice treated with single p.o. doses of benzene.⁹⁵ Evidence for covalent binding to DNA polymerase and topoisomerase II has also been obtained for HQ and BQ.^{96,97} Finally, *in vitro* inactivation of cytochrome P450, presumably via covalent binding of BQ and/or the semiquinone, has been reported with liver microsomes treated with HQ.^{98,99}

Additional studies have shown DNA adduct formation following HQ exposure in tissue culture and cell-free systems, but not *in vivo*. Rushmore demonstrated that *in vitro* incubation of rat bone marrow mitochondria with HQ resulted in formation of guanine and possibly adenine adducts to mitochondrial DNA.^{100,101} Two guanine adducts were characterized when purified calf thymus DNA was reacted with HQ,¹⁰² while three adducts were noted following *in vitro* HQ treatment of bone marrow from B6C3F₁ mice.¹⁰³ Rat Zymbal glands incubated with 750 or 1000 μ g HQ/ml for 48 h displayed a characteristic DNA adduct pattern and adduct levels of 1080 and 1250 adducts/10⁹ nucleotides, respectively, as measured by ³²P-postlabeling assay.¹⁰⁴ Dose- and time-dependent formation of DNA adducts detected using ³²P-postlabeling was also observed in HL-60 cells treated with HQ.¹⁰⁵⁻¹⁰⁸ The same adduct was produced with BQ, although BQ was a more potent adduction agent than HQ in this

system, suggesting that BQ is the actual reactive species in this process.¹⁰⁵ Addition of H₂O₂ or cumene hydroperoxide potentiated DNA adduct formation by HQ in HL-60 cells.¹⁰⁹ Chemical studies¹¹⁰ have indicated that the major DNA adduct formed in HL-60 cells by both HQ and BQ is likely to be *N*²-(4-hydroxyphenyl)-dG, which differs from the primary guanine adduct formed in cell-free systems, that is, 3'-hydroxy-1,*N*²-benztheno-2'-dG.¹⁰²

Formation of modified DNA bases due to activated oxygen species as opposed to direct adduction by HQ metabolites has also been reported. Levels of 8-hydroxydeoxyguanosine (8-OHdG), considered indicative of damage from superoxide and/or hydroxyl radicals, were increased during incubation of HQ and purified calf thymus DNA.⁶ This effect was inhibited in the presence of tyrosinase or catalase. 8-OHdG formation was also demonstrated in DNA from HL-60 cells treated with HQ,¹¹¹ and time-dependent covalent DNA adduction has been reported in mixtures of calf thymus DNA and HQ in the presence of prostaglandin-H synthase and either arachidonic acid or H₂O₂.¹¹²

In contrast to the clearly demonstrated formation of DNA adducts by HQ exposure *in vitro*, evidence for similar adduction in whole animal experiments is absent. Using two variations of the ³²P-postlabeling assay, Reddy et al.¹¹³ failed to demonstrate adducts in bone marrow, Zymbal gland, liver, or spleen DNA from female SD rats after HQ exposure (75 or 150 mg/kg/day, 4 days, p.o.). No increase in adducts by postlabeling was seen in kidney DNA from male or female F344 rats exposed to 50 mg/kg/day HQ, p.o., for 6 weeks.¹¹⁴ The treatment did, however, decrease levels of "I-compounds", which are believed to represent adducts formed by endogenous compounds or oxidative metabolism. The same 6-week treatment regimen did not result in increased 8-OHdG levels in kidney nuclear DNA of male and female F344 rats.¹¹⁵ Treatment of male B6C3F₁ mice with 75 mg/kg HQ, i.p., and of male SD rats with 250 mg/kg HQ, i.p., also did not result in increased 8-OHdG levels in bone marrow or in liver or kidney DNA, respectively.^{111,116}

These studies indicate that HQ conversion to the semiquinone and BQ can potentially result in covalent adduction to both protein and DNA *in*

vitro. In addition, formation of activated oxygen species via HQ oxidation can lead to increased 8-OHdG adduction in DNA *in vitro*. However, the demonstrated lack of DNA adduct or 8-OHdG formation by HQ *in vivo*, coupled with the generally negative results for HQ mutagenicity in short-term tests (discussed below), argue against a significant role for these phenomena in HQ toxicity. In contrast, thiol adduction (either with GSH or with protein sulfhydryl groups) clearly occurs following *in vivo* HQ exposure. This binding potential has implications for the mechanisms of HQ-induced clastogenicity, nephrotoxicity, and myelotoxicity.

VI. GENOTOXICITY

A voluminous literature exists examining the potential genotoxic effects of HQ in a variety of *in vitro* and *in vivo* systems. The results of these studies have been tabulated in several previous reviews.^{1,11-13} While the genotoxicity data base for HQ is extensive, the results are highly dependent on exposure route. *In vitro* studies are frequently, but not always, positive, while *in vivo* studies are typically negative unless detoxication pathways are overcome by parenteral administration. With few exceptions, HQ has been shown to be inactive as a direct mutagen in various short-term assays. Negative results have been reported with various strains of *S. typhimurium* and *E. coli*, with or without metabolic activation,¹¹⁷⁻¹²⁴ in yeast (*S. cerevisiae*, strain D4),¹¹⁷ and in *Drosophila*.^{7,125,126} In addition, no evidence for mutagenicity was demonstrated in an *in vivo* mouse spot test¹²⁷ and in a dominant lethal assay in male rats following p.o. doses of 30, 100, or 300 mg/kg/day HQ for 10 weeks.¹²⁸

Occasional positive results for HQ in reverse mutation assays have been reported, that is, in a single strain (TA1535A) of *S. typhimurium* using a nonstandard incubation medium (ZLM medium) without metabolic activation,¹²⁵ in several strains of *S. typhimurium* without activation,^{129,130} in a "fluctuation test" using one strain of *S. typhimurium* only in the presence of S9¹³¹ and in *S. cerevisiae* strain D3.¹¹⁸ A high potency for the induction of mutations leading to 6-thioguanine resistance in Chinese hamster V79 cells by

HQ has been demonstrated also.¹²⁴ A recent paper reported increases in mutation to both ouabain and 6-thioguanine resistance in Syrian hamster embryo (SHE) cells exposed to HQ, although the increases were not dose related.¹³² In addition, positive results were obtained in forward mutation assays using mouse lymphoma L5178Y cells exposed *in vitro* to HQ, with and without metabolic activation.¹³³

In contrast to the largely negative data for direct mutagenicity, numerous studies have shown the effects of HQ on clastogenic, mitotic, and aneuploidigenic endpoints. These effects are manifested as chromosomal aberrations, abnormal mitoses, formation of micronuclei (MN), aneuploidy, DNA strand breakage, and sister chromatid exchange (SCE). Many of these studies were conducted as part of a large interlaboratory aneuploidy screening program conducted by the European Communities Directorate General Environmental Research Programme, which included HQ as one of the core chemicals selected for study.^{134,135} Early reports indicated that HQ disrupts mitosis in a variety of cell types.^{136,137} These were followed by demonstration of the induction of SCE in Chinese hamster ovary (CHO) cells,¹³⁸ Chinese hamster V79 cells,¹²⁴ SHE cells,¹³² and human lymphocytes.^{139–144} Generally positive results were found with or without S9 metabolic activation in these studies. Negative SCE findings have been reported in bone marrow cells from mice treated with HQ at doses up to 120 mg/kg, i.p.,¹⁴⁵ and in one *in vitro* study with V79 cells.¹²⁹

A number of *in vitro* studies have demonstrated MN induction in HQ-treated human lymphocytes.^{146–149} Induction of MN in human lymphocytes has not been reported in whole blood cultures except at toxic dose levels.¹⁵⁰ MN induction *in vitro* has also been reported for fibroblasts,¹⁵¹ and embryonal liver cells,¹⁵² Chinese hamster V79, XEM2, LUC2, and/or SD1 cells,^{124,153–156} and rat intestinal cells.¹⁵² Using oral gavage, s.c., or i.p. dosing regimens, largely positive results have been obtained with HQ in the mouse bone marrow micronucleus assay.^{125,145,157–170} Transplacental induction of MN in blood cells from fetal mouse liver following exposure of pregnant dams to HQ has been reported.¹⁷¹ The lowest positive dose of HQ in these experiments was approximately 40 to 50 mg/kg, and i.p. dosing was found to be substantially more

effective than oral exposure, which was associated with weak induction of MN.¹⁶⁰ Some of these studies also examined the proportion of kinetochore-positive and -negative MN, with general indications that HQ can produce MN by mechanisms involving both whole chromosome loss and chromosome fragmentation.^{146,148,151,156,164–166,169,170,172}

Additional experiments have assessed induction of aneuploidy (abnormal chromosome number) by HQ *in vitro* and *in vivo*. Doses of 80 to 120 mg/kg, i.p., produced hyperploidy in mouse spermatocytes,^{173,174} while aneuploidy was detected in mouse bone marrow cultures¹⁷⁵ and human lymphocytes¹⁷⁶ treated with HQ. In contrast, HQ exposure to SHE and yeast cells did not result in aneuploidy.^{132,177} Chromosomal aberrations, including gaps, breaks, exchanges, dicentrics, and complete fragmentation, resulting in chromosome malsegregation and aberrant cells, have been reported for HQ *in vivo* in mouse bone marrow cells¹⁷⁸ and spermatocytes (following i.p. injection),¹⁷⁹ and *in vitro* in *Aspergillus nidulans*,^{180,181} CHO,¹³⁸ and SHE cells.¹³² DNA strand breaks and other damage has been noted in a variety of *in vitro* systems, including isolated DNA,^{112,182–186} rat liver cell nuclei and hepatocytes,^{187,188} mouse lymphoma cells,¹⁸⁹ Chinese hamster bone marrow¹⁹⁰ and ovary¹⁹¹ cells, human lymphocytes,¹⁹² and human lung carcinoma cells.¹⁹³ Induction of DNA damage *in vitro* in human peripheral lymphocytes was found to require much higher concentrations of HQ in mitogen-stimulated as compared to resting cells.¹⁹⁴ Addition of cytosine arabinoside (a DNA repair inhibitor) increased the sensitivity of mitogen-stimulated cells to HQ, suggesting protection by DNA repair mechanisms from DNA-damaging activated oxygen species. Recent studies have indicated induction of monosomy and long arm deletion of chromosomes 5 and 7 in human whole blood lymphocyte cultures incubated with HQ.¹⁹⁵ These changes are commonly observed in benzene-induced myelodysplastic syndrome (MDS) and leukemia in humans.¹⁹⁶ Additional positive (and occasional negative) responses for HQ have been reported in various other types of assays, including mitotic disruption and arrest,^{136,181,197–200} inhibition of DNA and RNA synthesis,^{97,201–205} and induction of unscheduled DNA synthesis.¹³²

HQ exposure appears to affect the mitotic apparatus and/or protein components of the chro-

mosome. Irons and co-workers first demonstrated that HQ could inhibit rat tubulin polymerization *in vitro*, that this process required the presence of O₂, and that specific covalent modification of one or two sites within the high-molecular-weight subunit of tubulin was involved.^{206,207} These sites were characterized as reactive protein thiols distinct from the colchicine binding site and possibly at or near the GTP binding site. Weak inhibition of tubulin assembly *in vitro* by HQ has also been demonstrated for bovine, but not porcine, tubulin.^{208,209} Based on these data, Irons proposed that the mitotic effects of HQ were mediated by spontaneous or enzyme-catalyzed oxidation to a reactive species, probably BQ, followed by covalent modification of tubulin, inhibition of tubulin polymerization, and disrupted spindle formation. This mechanism is supported by evidence indicating the enhancement of HQ-mediated inhibition of microtubule formation during peroxidative metabolism.⁹¹ More recently, Dobo and Eastmond¹⁵⁶ demonstrated that prostaglandin-H synthase (PHS) mediated oxidation of HQ increased the incidence of kinetochore-positive (i.e., whole chromosome-containing) MN in Chinese hamster V79 cell cultures, an effect blocked by GSH. While this proposed mechanism is reasonable, direct experimental evidence that inhibition of tubulin polymerization leads to aneuploidy and formation of kinetochore-positive MN in susceptible cells following *in vivo* HQ exposure is lacking.

In contrast to mitotic disruption, the occurrence of chromosomal fragmentation (indicated by the presence of kinetochore-negative MN) and DNA strand breaks cannot be accounted for by inhibition of tubulin polymerization. These effects have instead been attributed to either direct damage to DNA from reactive oxygen species, covalent DNA adduct formation via BQ or the semiquinone, or inhibition of enzymes involved in nucleic acid metabolism. The presence of catalase resulted in partial inhibition of kinetochore-negative MN formation by HQ in Chinese hamster V79 cell cultures, suggesting the involvement of H₂O₂ and other activated oxygen species.¹⁵⁶ In addition, Cu⁺²-mediated autoxidation of HQ with consequent reactive oxygen species formation resulted in enhancement of DNA strand breaks.^{210–212} GSH was found to enhance the HQ-mediated induction of double-strand DNA breaks *in vitro*, an effect that was reduced in the presence

of superoxide dismutase.²¹³ However, other results indicated that the presence of oxygen radical scavengers did not reduce HQ-induced phage DNA single strand breaks *in vitro*,¹⁸⁴ while mouse bone marrow cell DNA breakage was induced by BQ despite its low oxygen radical-generating potential.²¹⁴ Finally, *in vitro* inhibition of DNA and RNA polymerases and of DNA topoisomerases by oxidized HQ metabolites has been reported.^{96,97,100,202,205,215}

The genotoxicity database for HQ supports a low potential for direct mutagenicity (i.e., induction of frame-shift and point mutations) for this compound. A similar conclusion can be made for BQ. This suggests that the observed *in vitro* formation of DNA adducts by HQ and BQ may be of questionable toxicological significance. In contrast, both agents have clastogenic and aneugenic potential and can interfere with the mitotic process. These effects are likely to be mediated through the protein thiol binding capability of BQ, although the involvement of activated oxygen species in certain of these processes may be important. Other than tubulin, it is not known which specific proteins may represent the critical targets for this adduction. Alternatively, adduction/inactivation of DNA polymerase, topoisomerase II, or other relevant enzymes may be significant in the mechanism of HQ clastogenicity. In any case, these epigenetic effects appear to depend heavily on toxicokinetic factors, particularly route of exposure, suggesting that exposure thresholds may exist. Despite gaps in knowledge concerning specific mechanisms of clastogenicity and aneuploidy for HQ, modeling of dose response data from mice given HQ i.p. indicate a very good linear fit between 25 to 75 mg/kg using a linear quadratic model with a no-observed effect level at 12.5 mg/kg.¹⁶⁹ The model suggests a no-effect threshold between 12.5 and 25 mg/kg i.p. Because oral exposure is much less effective at inducing such effects, the threshold for an oral no-effect level would be expected to be higher than the i.p. no-effect level.

VII. NEPHROTOXICITY AND RENAL CARCINOGENESIS

Early studies examining single or repeated dose toxicity of HQ in experimental animals did

not reveal marked evidence of nephrotoxicity, although comprehensive evaluations of renal function were generally not performed in these studies. For example, feeding of SD rats with up to 1% HQ in the diet for 2 years did not result in histopathological alterations in the kidney, while administration of 100 mg/kg/day, p.o., to dogs for 6 months produced neither histopathology nor changes in urine chemistry values.²⁶ These same investigators reported no urinary alterations in male human volunteers ingesting 300 to 500 mg/day (4.3 and 7.1 mg/kg/day, respectively, for a 70-kg man) for 3 to 5 months. Christian reported only a mild increase in relative (to body) kidney weights, with no accompanying pathological changes, in rats administered 1% or 0.4% HQ in the drinking water for 8 or 15 weeks, respectively.²⁷ Woodard exposed several dogs to daily oral doses of HQ of 25 or 50 mg/kg for more than 2 years, but did not report any clinical or gross pathological findings consistent with renal impairment.²⁵ In one study, chronic exposure (length unspecified) of rats and mice to 50 to 100 mg HQ/kg/day was reported to cause “dystrophic changes” in kidney and other organs.²¹⁶ In addition, daily i.p. injection of BQ (2 mg/kg/day) or benzenetriol (6.25 mg/kg/day) but not HQ (10 mg/kg/day) for 6 weeks produced histologically demonstrable kidney damage in mice.²¹⁷

In 1979, the NTP initiated testing on HQ that included subchronic (90-day) and chronic (2-year, with a 15-month interim sacrifice) toxicity studies in F344/N rats and B6C3F₁ mice. The results of these studies have been published as a monograph and summarized in a peer-reviewed literature report.^{7,218} In the subchronic study, animals were administered HQ in corn oil by gavage at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg/day (5 days/week). Mortality in the highest dose group was 100 and 80% for rats and mice, respectively. Moderate to marked nephrotoxicity, characterized by tubular cell degeneration and regeneration in kidney cortex, was noted in the majority of male and female rats at 200 mg/kg/day and in 1 of 10 female rats at 100 mg/kg/day. The lesions present in male rats were graded as more severe than those in females. No renal lesions were observed in rats at 50 mg/kg/day or lower or in mice at any dose level. Relative kidney weights were not reported in this study. These effects were

considered to represent dose-related primary kidney toxicity due to HQ exposure.

Sex-, strain-, and species-related differences in HQ-induced nephrotoxicity have also been examined in numerous studies. A single oral dose of 400 mg/kg HQ to F344 rats resulted in increased urinary excretion of alanine aminopeptidase, *N*-acetyl glucosaminidase, alkaline phosphatase, γ -GT, and glucose, all indicative of proximal tubule damage.²¹⁹ Increases in creatinine and BUN were also seen in female rats. Interestingly, the increases were more pronounced for female rats, in contrast to the higher susceptibility of male rats to histologically demonstrable nephrotoxicity with subchronic HQ administration.^{7,220} Similar changes were not seen in SD rats, or in B6C3F₁ mice, except for increased BUN in both sexes of mice administered 350 mg/kg HQ.²¹⁹ Urine osmolality tended to be lower and urine volume higher than in controls for both F344 and SD rats. Microscopic analysis of urine demonstrated increased epithelial cell counts for F344 rats. These studies provide further confirmation of the high susceptibility of this rat strain to HQ-induced nephrotoxicity.

In addition to the sex- and strain-specificity of kidney effects, the expression of kidney toxicity depends upon route of administration and age of the animal. In a 13-week dermal toxicity and cell proliferation study, the male F344 rat-specific kidney effects observed in earlier oral studies were absent.²²¹ HQ was applied as an oil-in-water emulsion at a maximum level of 5%, corresponding to a dose of approximately 75 mg/kg/day. The lack of observed effect is most readily explained by low dermal absorption of HQ.¹⁹ However, other toxicokinetic factors may be involved, as applied dermal doses of HQ (in ethanol) of up to 3840 mg/kg/day for 14 days in F344 rats was also not associated with gross indicators of nephrotoxicity (i.e., body weight changes and gross kidney pathology).⁷ At this high-dose level and with this dosing vehicle, HQ was detected in urine in this study, suggesting substantial dermal absorption. Similarly, the histopathology and cell proliferation seen after 6-week gavage administration to young adult male F344 rats was not present in 1-year-old rats on the same treatment regimen, consistent with a possible age-related difference in susceptibility.²²²

The evidence for nephrotoxicity following oral exposure to HQ has stimulated much research on mechanism of action. These efforts have primarily focused on the sex- and species-dependence of the phenomenon and on the possible involvement of GSH conjugates of HQ as initiators of nephrotoxicity (Figure 3). Mono-, di-, and tri-GSH conjugated HQ metabolites have been detected in rat bile following i.p. injection of HQ.⁶⁸ Direct parenteral administration of di- and tri-GSH conjugates to rats resulted in proximal tubule necrosis and nephropathy, with the 2,3,5-triglutathion-*S*-yl-HQ (tri-G-HQ) derivative being

the most potent, and the fully substituted 2,3,5,6-tetraglutathion-*S*-yl-HQ exhibiting no activity.²²³ Maximum damage was localized to the P3 segment of the proximal tubule. Nephrotoxicity due to GSH conjugates of HQ was blocked by pretreatment of animals with AT-125 (Acivicin, a potent inhibitor of γ -GT).²²³ This enzyme catalyzes the cleavage of glutamic acid from GSH conjugates to yield a cysteinyl-glycine conjugate and is present at high activity in proximal tubule epithelium.

Hill et al.²²⁴ demonstrated a decrease in state 3 mitochondrial respiration in rats administered

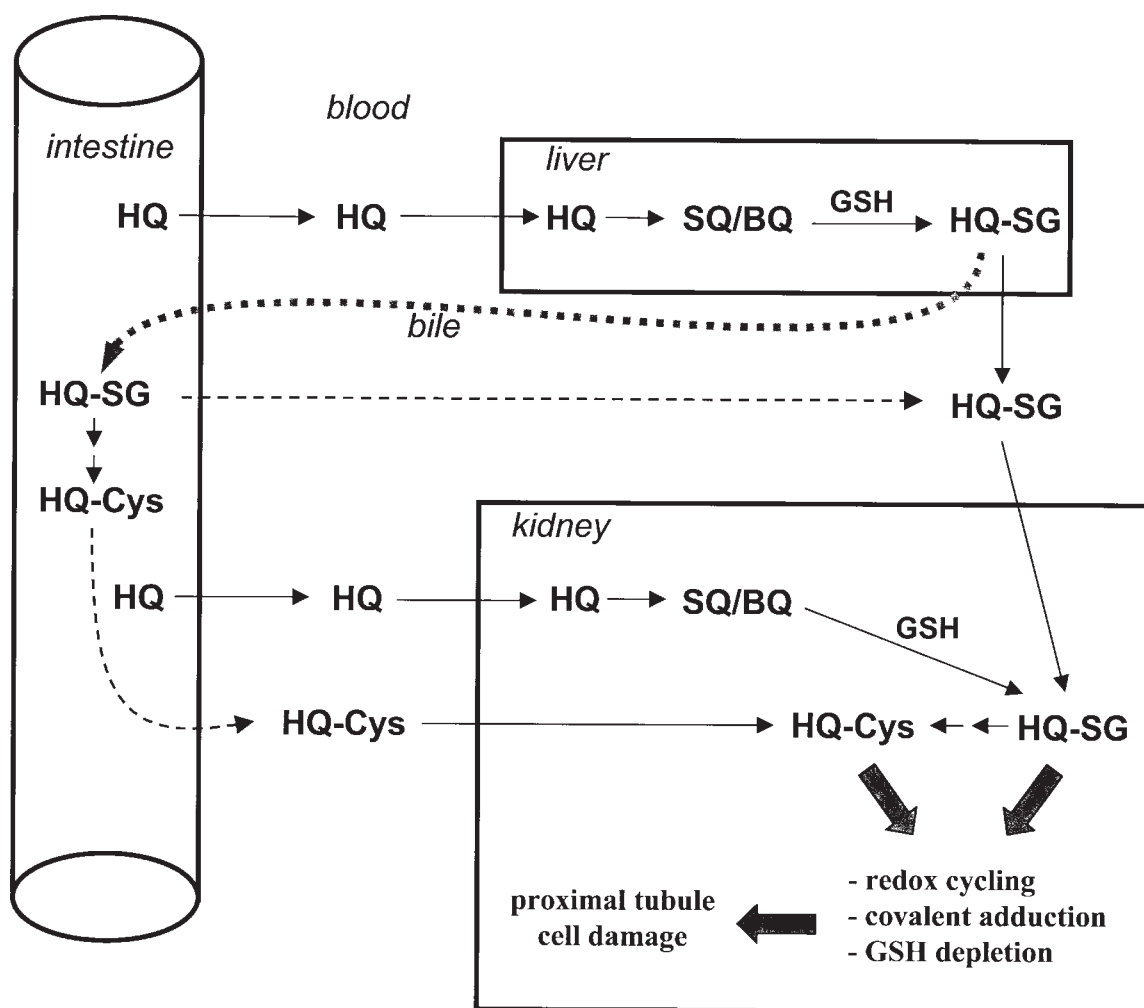


FIGURE 3. Summary of proposed mechanisms of hydroquinone (HQ) nephrotoxicity following p.o. administration and oxidation to semiquinone (SQ) and/or benzoquinone (BQ) in liver and kidney. Note dual pathways of formation of putative nephrotoxic HQ-glutathione (HQ-SG) and HQ-cysteine (HQ-Cys) conjugates. HQ-SG and HQ-Cys can be formed in the liver and intestine, respectively, and then transported in blood to the kidney. Alternatively, each derivative can be formed directly in the kidney from HQ via SQ/BQ.

tri-G-HQ, although it was unclear whether this change represented a cause or effect of subsequent nephrotoxicity. Using the *in situ* perfused rat kidney (ISPRK) model, Hill et al.²²⁵ showed a time-dependent release of γ -GT into the urine of rats infused with 2-glutathion-*S*-yl-HQ or tri-G-HQ. A greater retention of tri-G-HQ within the kidney was found, leading to speculation that the oxidative metabolism of the conjugate resulted in formation of reactive derivatives that subsequently bound to kidney macromolecules. Additional studies have shown that mono- and di-G-substituted HQ derivatives readily undergo autoxidation to tri-G-HQ under physiological conditions.²²⁶

In vitro studies have revealed that *N*-acetylation of 2-(*L*-cystein-*S*-yl)-HQ to form the mercapturate was less active in the male than in the female F344 rat or in the male SD rat, a nephrotoxicity-resistant strain.²²⁷ Formation of the mercapturate is considered a detoxication reaction, as it oxidizes less readily to putative reactive products than does the cysteine conjugate. Male F344 rats exhibited a moderate decrease in GSH and cysteine concentrations in kidney following 400 mg/kg HQ p.o., while male SD rats did not.⁸⁸ Studies of differences in species susceptibility to nephrotoxicity induced by i.v. injection of tri-G-HQ indicated that only male F344 rats and guinea pigs developed proximal tubule dysfunction, while females of these species and both sexes of BALB/c and B6C3F₁ mice and of hamsters were resistant.²²⁸ Some correlations between the activities of γ -GT, *N*-acetylase, and *N*-deacetylase and species susceptibilities were noted.

The overall mechanistic database for HQ points to the involvement of GSH-HQ conjugates and a critical influence of sex-, strain-, species-, dose-, and route-specific factors in the pathogenesis of nephrotoxicity. The current paradigm^{1,10,11,114,225,229} suggests that oral HQ exposure is followed by rapid and extensive sulfate and glucuronide conjugation in liver and intestine and elimination of these detoxified metabolites via urine. Saturation of this process by high oral doses or partial bypass via parenteral administration leads to the formation of various GSH conjugates in the liver, which are either excreted via bile or enter the circulation. These conjugates enter proximal tubule cells either directly from the blood or following reabsorption from the urine. Within proxi-

mal tubule cells, HQ-GSH conjugates or their γ -GT/dipeptidase cleavage products can be oxidized either spontaneously or (more likely) via enzymatic processes to yield reactive species such as BQ- and/or semiquinone-conjugates. These derivatives may either directly adduct renal cell macromolecules or undergo redox cycling to produce reactive oxygen species that can also cause macromolecular damage. Alternatively, HQ-GSH conjugates may be excreted as detoxified urinary mercapturates following sequential processing by γ -GT, dipeptidase, and *N*-acetylase. Thus, the sex-, strain-, and species-specific characteristics of HQ nephrotoxicity may be due to differences in the relative balance between toxifying and detoxifying metabolic pathways in the proximal tubule cell, which in turn may be governed by genetic factors.

Some uncertainty still remains concerning the mechanism of nephrotoxicity following higher-dose HQ exposure in experimental studies. For example, although HQ thiol adducts have been demonstrated following oral dosing, HQ-GSH conjugate formation has not been conclusively demonstrated in orally dosed rats. Covalent protein adducts in the kidney derived from HQ have been detected and quantified but not characterized, and their precise role in kidney toxicity remains to be determined. In addition, macromolecular changes consistent with oxidative damage occurring within proximal tubule cells have not been reported.

The NTP also conducted a chronic study, in which rats were administered 0, 25, or 50, and mice 0, 50, or 100 mg/kg/day (5 days/week) of HQ by gavage in deionized water for 103 weeks.⁷ An interim sacrifice of 10 animals per group was performed at 65 weeks. Unlike the subchronic studies, no clear evidence of chemically induced nephrotoxicity was apparent in HQ-treated rats or mice. In contrast, evidence of chronic nephropathy was present in nearly all control and dosed rats at 103 weeks. While this syndrome (also termed "spontaneous nephropathy" or "chronic progressive nephropathy"; CPN) is common in aged male rats, the severity of the lesion was greatest in males from the 50 mg/kg/day HQ-treated group. This trend was also apparent in male rats at the 65-week interim sacrifice, although no alterations in urinalysis parameters

consistent with nephropathy were apparent at this time point. Histopathological findings associated with this nephropathy included degeneration and regeneration of tubular epithelium, tubular atrophy and dilation, the presence of hyaline casts in tubule lumen, glomerulosis, interstitial fibrosis, and inflammation. High-dose male rats with marked levels of nephropathy also exhibited papillary hyperplasia of the transitional epithelium covering renal papillae and cysts. Due to the lack of hyaline droplets, cast formation in the loop of Henle, and mineralization, the kidney lesions were not considered related to α_{2u} -globulin nephropathy. Elevated relative (to body) kidney weights were also observed for high-dose male rats at the 65- and 103-week sacrifices. Dose-related increases in the severity of chronic nephropathy were not seen in female rats and except for a high incidence of chronic renal inflammation in all groups mice did not exhibit kidney lesions.

In another chronic study conducted by different investigators (Shibata et al.), an increase in the severity of chronic nephropathy, in addition to papillary and tubular hyperplasia, was noted in male F344 rats fed 0.8% HQ in the diet for 104 weeks.²²⁰ This dietary level corresponded to an average dose of 351 mg/kg/day based on measured food consumption, substantially higher than that used in the NTP study. An increased incidence of mild nephropathy was seen in female F344 rats, with an average HQ intake of 386 mg/kg/day. Renal changes in male and female B6C3F₁ mice fed 0.8% HQ, with average doses of 1046 and 1486 mg/kg/day, respectively, consisted of a 30% incidence of tubular hyperplasia without nephropathy in males; interim tissue examinations were not conducted. Relative (to body) kidney weights were increased in both sexes of rats and in female mice. The results of urinalysis were not reported. The overall findings regarding CPN were generally consistent with those of the NTP study.

Both the NTP and Shibata studies provided evidence for renal carcinogenesis in male rats chronically exposed to HQ. In addition to increased severity of CPN, male F344 rats in the NTP study exhibited 7% (4/55) and 15% (8/55) incidences of tubular adenomas in the low- and high-dose groups, respectively, although the incidence in low-dose rats was not significantly dif-

ferent from control. These lesions were described⁷ as “discrete masses of epithelial cells arranged in solid clusters or nests separated by a scant stroma.” Hyperplasia of tubular epithelium, a potential preneoplastic lesion, was also noted in two male high-dose rats. None of the tumors were reported to be grossly observable. Adenomas were not observed in female F344 rats or in either sex of B6C3F₁ mice. Based on these findings, NTP concluded that there was “some evidence” of carcinogenic activity for HQ in the male rat. Tumor formation was suggested to be chemically induced but independent of the nephrotoxicity observed in the subchronic studies.²¹⁸

Renal adenomas were also reported in male F344 rats fed 0.8% HQ in the diet for 2 years, with an incidence rate of 47% (14/30).²²⁰ The higher incidence in this study was attributed to the larger average daily dose (50 vs. 351 mg/kg/day) in these animals when compared with the NTP study. Renal papillary hyperplasia, considered indicative of advanced CPN, was seen in 37% of male rats, while all male rats exhibited renal tubular hyperplasia. Adenomas were also reported in 3 of 30 male B6C3F₁ mice, although this incidence rate was not significantly different from control. Increased relative (to body) kidney weights were noted in both sexes of rats and in female mice, while absolute kidney weights were increased only in male rats. Based on these data, Shibata and co-workers concluded that HQ was clearly carcinogenic in male rats and that this effect was probably linked to CPN. However, unlike the NTP investigators, they suggested an involvement of free radicals and/or quinone metabolites in the carcinogenic mechanism.

The NTP and Shibata et al. conclusions regarding HQ-induced renal toxicity and tumorigenesis have been criticized based on both methodological concerns (e.g., the potential renal effects of a concurrent sialodacryoadenitis infection within the test colony in the NTP study) and overall interpretation of the database.^{10,11,229,230} The possible relationships between proximal tubule cell degeneration and regeneration, CPN, and renal adenomas in F344 rats exposed to HQ have been examined recently by means of a reevaluation¹⁰ of the pathology data from the NTP chronic study and additional experimentation. The reevaluation indicated that 36 of 51 high-dose (50 mg/kg/

day) male rats had pathological alterations consistent with either severe or end-stage CPN. In addition, 11/51 high-dose rats exhibited foci of atypical tubular hyperplasia, considered to be a preneoplastic lesion, compared with the originally reported NTP incidence of 1/55. The reassessment of tumor data also resulted in revised renal tubule adenoma incidences of 3/49 and 7/51 in low- and high-dose male F344 rats, respectively, when compared with 4/55 and 8/55 in the original report. The key finding of this reevaluation was the high correlation between the presence (occurrence and location) of hyperplasia or adenomas and that of severe to end-stage grade CPN, suggesting that HQ acts in an epigenetic manner to accelerate the spontaneous CPN process.

It is widely accepted that compensatory cell proliferation in general can increase the risk of tumorigenesis. To further clarify this issue for HQ and renal carcinogenesis, the time- and dose-dependence and anatomic localization of cell proliferation in the proximal and distal tubules of F344 rats were examined in animals administered oral doses of HQ at 0, 2.5, 25, or 50 mg/kg/day for up to 6 weeks.²²⁹ Increased cell proliferation is characteristic of CPN,²³¹ and the selected dose levels and dosing protocol were designed to mimic those employed during the initial period of the NTP chronic study. As measured by BrdU incorporation, cell proliferation in the P1 and P2 segments of kidney tubules was increased over control values for male F344 rats after 6, but not 3, weeks of HQ exposure at the 50 mg/kg/day level. Interestingly, this finding contrasts with the extensive damage seen in the P3 segment of rats exposed via parenteral injection to tri-G-HQ and other GSH-HQ conjugates,²²³ suggesting that HQ-induced nephrotoxicity and accelerated CPN may proceed by different mechanisms. Degenerative and regenerative foci and interstitial inflammation were also observed in these animals by histopathologic examination. A nonsignificant trend toward increased proliferation was seen in male rats at 25 mg/kg/day. In contrast, no significant changes in these parameters were observed at lower dose levels in male rats or at any dose level in female F344 or either sex of SD rats. The delay in appearance of cell proliferation is indicative of a compensatory response to slowly developing nephropathy characterized by proximal tubule

cytotoxicity and cell replacement, consistent with an epigenetic mechanism.

The nature of the causal relationship, whether direct or indirect, between HQ exposure, CPN, and induction of renal tubule adenomas in F344 rats is currently unclear. In one recent model, HQ is proposed to act at two stages; to accelerate the normal evolution of CPN in the rat from mild to end-stage, and to increase tubule cell proliferation, particularly that associated with progression from CPN to tubular hyperplasia and renal adenoma formation.^{10,11} This model is based on the better correlation of incidence of hyperplasia with severity of CPN than with dose of HQ, and on data showing the presence of atypical hyperplasia and adenomas in low-dose male rats in the absence of enhanced CPN. Clearly, questions remain to be answered concerning the molecular mechanism(s) by which HQ might promote, accelerate, or synergize spontaneous CPN and cause increased cell proliferation. Despite these uncertainties, the overall genotoxicity, nephrotoxicity, and mechanistic database supports a species-dependent, epigenetic mechanism for HQ-induced renal carcinogenesis. The possible implications of these findings for human renal carcinogenesis due to direct HQ exposure are discussed later in this article.

VIII. OTHER DATA RELATED TO CARCINOGENESIS

In addition to the renal tumorigenic effects discussed above, long-term bioassays with HQ have provided some indication for liver and forestomach neoplasia and mononuclear cell leukemia in rodents. In the 2-year NTP bioassay, male and female mice exhibited significantly increased relative liver weights at 15 months, while hepatic fatty change and cytomegaly were present in male mice in the 100 mg/kg/day group at this time point.^{7,218} After 2 years, the incidence of liver adenomas was increased in both sexes of mice at the 50 and 100 mg/kg/day dose levels. However, the incidences of these lesions were highest in the 50 mg/kg/day group, suggesting a lack of clear dose response. Hepatic carcinomas occurred at a lower incidence in male mice given HQ compared with the control, while there was no differ-

ence in the incidence of carcinomas between female mice given HQ and their control group. Thus, when adenomas and carcinomas were combined, only the female mice were considered to have an increased incidence of liver tumors. Based on these data, NTP concluded that there was “some evidence” for carcinogenic activity in the female mouse.⁷

Shibata et al.²²⁰ reported increased relative liver weight and incidence of hepatocellular hypertrophy, altered foci, and adenomas in male but not female mice fed 0.8% HQ in the diet (average dose 1486 mg/kg/day). The basis for the inconsistency in the sex-specific incidence rates for hepatic adenomas between the two studies is unclear. As in the NTP study, hepatocellular carcinomas were not increased for male or female mice in the bioassays reported by Shibata et al.²²⁰ In addition, hepatic neoplastic lesions were not observed in rats in either study, and HQ exposure was found to decrease the number of foci of cellular alteration in livers of both male and female rats. These inconsistencies and negative findings have prompted other investigators to conclude that the evidence for HQ-induced hepatic neoplasia is only equivocal.^{11,230}

The study of Shibata et al.²²⁰ also reported a significant increase in squamous cell hyperplasia (but not carcinoma) of the forestomach in male and female mice. This effect was not observed in the NTP 2-year bioassay,⁷ although inflammation and epithelial hyperplasia were noted in rats and mice at 200 and 400 mg/kg/day, respectively, in the NTP preliminary subchronic studies. In contrast, administration of 0.5% HQ in the diet of Syrian golden hamsters for 20 weeks did not result in histopathological forestomach changes or an increase in mitotic labeling index of forestomach epithelial cells.²³² HQ (0.8% in the diet for 51 weeks) also did not enhance forestomach or glandular stomach neoplasia in F344 rats given a single initiating dose of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine,²³³ but did slightly increase the incidence of esophageal carcinomas following initiation by methyl-*N*-amyl nitrosamine.²³⁴ No histopathological changes or increases in forestomach epithelial cell DNA synthesis were observed in male F344 rats fed 0.8% HQ in the diet for 8 weeks.²³⁵ Only mild forestomach hyperplastic responses were present in rats fed 2% HQ for

4 weeks.²³⁶ Taken together, these results do not support a significant potential for HQ to induce gastrointestinal neoplasia in rodent bioassays.

An increased incidence of mononuclear cell leukemia was noted in the NTP bioassay for HQ in female F344/N rats after administration of 50 mg/kg, p.o., for 2 years.^{7,218} Staging of the severity of the leukemia in the 22 affected animals, based on histopathological alterations in the spleen, indicated 1, 7, and 14 rats with Stage 1, 2, or 3 leukemia, respectively. These data led NTP to classify HQ as having “some evidence” of carcinogenicity in the female rat. Concern has been raised over the leukemogenic effect of HQ as reported in the NTP study because of the well-known phenomenon of benzene-induced leukemia in humans. However, benzene itself does not induce mononuclear cell leukemia in rats,²³⁷ and no similar effects were observed in the Shibata HQ bioassay,^{10,230} despite much higher dose levels. Mononuclear cell leukemia in rats is considered to originate in the spleen²³⁸ and not the bone marrow, which is considered to be the origin of benzene-induced leukemia. No specific evidence for bone marrow alterations consistent with a leukemogenic process was reported in either study. It should be noted that the reported historical control incidence of mononuclear cell leukemia observed in NTP bioassays using female F344 rats increased progressively during the 1980s.²³⁹ This trend, in addition to the isolated nature of the NTP finding, weakens the strength of evidence for a significant leukemogenic potential for HQ.

Various tumor initiation and/or promotion assays have also been conducted with HQ, with generally negative results. An early study involving a single application of 20 mg HQ (in acetone) to mouse skin, followed by promotion with croton oil, did not reveal initiating activity.²⁴⁰ HQ was also negative as a promoter in a mouse skin carcinogenesis model following initiation with dimethylbenzanthracene.²⁴¹ Both a lack of promoting activity and an ability to partially inhibit benzo[*a*]pyrene-initiated skin carcinogenesis have been demonstrated for HQ.²⁴² Dietary exposure to HQ (1.5%) also inhibited pancreatic lesions (combined preneoplastic and neoplastic) following administration of *N*-nitrosobis(2-oxopropyl)amine.²⁴³ Administration to F344 rats of a nitrosamine initiator, followed by 0.8% HQ in the diet

for 32 weeks did not result in the development of bladder hyperplasia or neoplasia.²⁴⁴ HQ neither initiated nor promoted *N*-butyl-*N*-(hydroxybutyl)nitrosamine initiated bladder carcinogenesis when given at 0.2% for 22 weeks to male F344 rats that had one ureter ligated.²⁴⁵ Male F344/Du Crj rats were fed 0.8% HQ diets for 30 weeks following exposure to *N*-bis(2-hydroxypropyl) nitrosamine as an inducer of lung tumors.²⁴⁶ HQ did not promote lung tumors or enhance the incidences of thyroid, kidney, or urinary bladder tumors that were observed following exposure to the initiator alone.

In contrast to these negative findings, rats treated with *N*-ethyl-*N*-hydroxyethylnitrosamine followed by 0.8% HQ in the diet for 32 weeks did display a significant increase in renal microadenomas and renal cell tumors when compared with the nitrosamine alone.⁸⁹ However, similar HQ treatment without initiator did not result in renal changes. Boyland et al.²⁴⁷ reported an increased incidence of bladder carcinomas in mice implanted with HQ/cholesterol pellets in the urinary bladder for 25 weeks, although the relevance of this model for determination of overall carcinogenic potential is questionable. Bladder tumorigenesis has not been reported in any long-term bioassay with HQ.

Several studies have employed "medium-term" liver carcinogenesis bioassays or related protocols to examine initiation and promotion effects of HQ. Stenius reported an increase in *N,N'*-diethylnitrosamine (DEN)-induced γ -GT-positive hepatic foci in partially hepatectomized rats following 7 weeks exposure to 100, but not 200, mg/kg/day HQ p.o.¹⁸⁷ In contrast, DEN initiation, followed by partial hepatectomy and dietary administration of 2% HQ to rats for 6 weeks, resulted in a significant decrease in glutathione-*S*-transferase (GST)-positive hepatic foci as compared with DEN treatment alone.²⁴⁸ Other data indicate that HQ does not selectively damage GST-positive hepatocytes.²⁴⁹ Finally, a recent study²⁵⁰ examining hepatic tissue from a previous HQ carcinogenicity bioassay²²⁰ indicated that prolonged (2-year) dietary exposure of F344 rats to 2% HQ resulted in decreased numbers and area of GST-positive hepatic foci. These findings generally support the conclusion that HQ lacks significant hepatocarcinogenic activity in rodents.¹¹

IX. MYELOTOXICITY AND IMMUNOTOXICITY

Early animal studies provided limited evidence that prolonged, high-dose HQ administration might affect blood cells and/or bone marrow. Hematological changes were reported in cats following parenteral administration of single doses of 60 to 100 mg/kg HQ (cited by Von Oettingen³⁴¹), and after p.o. exposure to 15 mg/kg/day for 40 days in rats.²⁵¹ Woodard reported histopathological changes in bone marrow and spleen from dogs fed 25 or 50 mg/kg/day for 819 days.²⁵ Severe toxicity and decreased bone marrow cellularity was observed in rats fed 5% HQ in the diet for 9 weeks;²⁶ however, results at this dose level are confounded by severe body weight loss. Decreased red blood cell (RBC) counts, but not hematocrit or colony forming cells in bone marrow, were seen in mice given HQ in the drinking water (average dose 16.9 mg/kg/day) for 28 days.²⁵² Administration of HQ (10 mg/kg/day, i.p., 6 weeks) to rats produced decreased RBC counts and bone marrow cellularity.²¹⁷ In the single available experimental animal inhalation study reported in the literature, rats exposed to HQ at an air level of 10 mg/m³, 4 h/day for 17 weeks exhibited normocytic anemia and RBC count changes (additional details not reported).³⁰ In contrast, other studies, including longer-term bioassays employing standard protocols, have not indicated major effects of HQ on bone marrow or hematologic parameters.^{7,220,253}

While some evidence for HQ myelotoxicity is available from animal bioassays, as discussed above, the majority of data on this issue are derived from *in vivo* mechanistic studies on benzene myelotoxicity and leukemogenesis and the postulated involvement of benzene metabolites (including HQ) in this phenomenon.^{254,255} Some investigations have employed overall measures of bone marrow cellularity or various versions of "colony-forming unit" (CFU) assays to detect toxic effects directed toward specific subpopulations of hematopoietic cells. Tunek et al.²⁵⁶ reported a decrease in bone marrow granulopoietic cellularity, as measured by total cell or by CFU-culture (CFU-C) assay results, in male NMRI mice given 6 daily s.c. injections of 80, but not 50 mg/kg HQ. Wierda and Irons²⁵⁷ found that two daily s.c. or

i.v. injections of 100 mg/kg HQ in C57BL/6 mice also reduced spleen and bone marrow cellularity when cells were harvested 24 h later. In contrast, only mild, transient suppression of bone marrow cellularity was observed in male B6C3F₁ mice administered repeated (two times/day) i.p. doses of 100 mg/kg HQ for up to 36 days.²⁵⁸ One *in vivo* study indicated increased levels of granulocyte-macrophage colony-forming cells (GM-CFC) in bone marrow from mice given twice-daily i.p. injections of 75 mg/kg HQ for 11 days, without an accompanying decrease in marrow cellularity.²⁵⁹ HQ administered to C57BL/6J mice was reported to induce differentiation of myeloblasts but to block maturation at the myelocyte stage.²⁶⁰

In vitro work has also shown effects of HQ on certain parameters associated with myelopoiesis and myelocytic colony growth. DNA synthesis in mouse bone marrow cells, but not in an isolated cell-free system, was significantly decreased by incubation with 24 μ M HQ.²⁰⁵ Boyd et al.²⁶¹ exposed mouse CFU-GM cultures to micromolar concentrations of HQ and observed suppression of colony growth. Dose-related inhibition of granulocyte/macrophage colony-stimulating factor (GM-CSF) induced colony formation in both B6C3F₁ mouse and human bone marrow cell cultures, and in purified human hematopoietic progenitor cells, was produced by co-incubation with up to 50 μ M HQ.²⁶² These studies also demonstrated an increased effect of HQ on bone marrow cultures with ambient (19%) as opposed to physiologic (5%) O₂ concentrations, suggesting the importance of oxidative reactions. Granulocytic progenitor cells were more sensitive to HQ inhibition than were myeloid progenitors in mouse cultures, while a similar differential susceptibility was not observed in human cell cultures. In contrast, preincubation of C57BL/6 mouse and of human CD34⁺ bone marrow cells with HQ generally results in stimulation of CFU-GM colony formation.^{263,264}

Some studies have explored the possible effects of HQ on differentiation and/or apoptosis of bone marrow cells. HQ-induced alterations in myelocytic differentiation have been examined in human HL-60 promyelocytic leukemia cell cultures. Treatment with HQ (1 to 5 μ M) produced a dose-related suppression of monocytic, but not granulocytic differentiation.²⁶⁵ Retinoic acid-in-

duced maturation of human HL-60 cells to granulocytes was blocked by HQ pretreatment.¹⁰⁸ Exposure of the mouse myeloblastic cell line 32D to low micromolar concentrations of HQ resulted in stimulation of granulopoiesis to the myelocyte stage, but not beyond.^{260,266} Recent work has also demonstrated increased apoptosis in HL60 cell cultures and human CD34⁺ cells from bone marrow following *in vitro* treatment with 50 μ M HQ,^{212,267} in addition to inhibition of apoptosis in mouse 32D cell cultures at HQ concentrations of 1 to 6 μ M.²⁶⁸ Recently, it has been proposed that HQ acts synergistically with GM-CSF to induce proliferation of progenitor cells that would not normally be recruited by the cytokine, possibly via activation of secondary gene expression signals.²⁶⁴

In vitro work has also indicated suppression of erythropoiesis by HQ. Decreased incorporation of iron into erythrocytes has been reported following s.c. or i.p. injection of 100 mg/kg HQ in mice.^{269–272} The effect of HQ on growth of burst-forming unit, erythroid (BFU-E), colony-forming unit, erythroid (CFU-E), and CFU-C colonies from mouse bone marrow revealed CFU-E activity to be most sensitive, with suppression observed at low micromolar concentrations.²⁷³ Dose-dependent inhibition of CFU-E and BFU-E was observed in HQ-treated SW and C57B1/6J mouse and human bone marrow cells, respectively.^{274,275} A decrease in the ratio of bone marrow cell surface antigens associated with the erythroid line to those associated with granulocytic cells, consistent with a shift in the differentiation pattern, was reported in human CD34 hematopoietic precursor cells treated with 10 μ M HQ.²⁷⁶

In addition to potential direct effects on erythropoietic and granulopoietic progenitor cells, numerous studies have examined HQ action on bone marrow stromal cells. These cells, which include macrophages and supporting fibroblastic cells, influence hematopoiesis by providing both a supporting physical framework and a variety of cell-specific cytokines and growth factors (e.g., colony-stimulating factors, interleukins).^{277,278} Such studies typically have employed co-culture models of adherent stromal and nonadherent progenitor cells. Gaido and Wierda²⁷⁹ first demonstrated the ability of low micromolar levels of HQ to inhibit both the formation of stromal cell colonies

and the ability of stromal cells to support CFU-GM colony growth. The latter effect was partially reversed by pretreatment with indomethacin, suggesting the involvement of prostaglandin synthetase in this process.²⁸⁰ Reconstituted culture experiments indicated that the macrophage was the more sensitive component of the bone marrow stroma, while fibroblastoid cells were unaffected by HQ exposure.²⁸¹ This study also showed a reduction of interleukin-1 (IL-1) activity in HQ-treated stromal cultures. This effect was later shown to be due to inhibition of processing of pre-IL-1 α to the mature cytokine,²⁸² possibly via inhibition of the enzyme calpain II.²⁸³ Inhibition of processing of pre-IL-1 β , and of the required converting enzyme, in a human myeloid tumor cell line has been reported.^{284,285} Incubation of human peripheral monocytes with 5 μ M HQ resulted in substantially decreased IL-1 α and -1 β secretion and RNA and protein synthesis.²⁸⁶ An IC₅₀ of 25 μ M for inhibition of RNA synthesis was demonstrated for HQ in mouse peritoneal macrophages.²⁸⁷

The oxidation of HQ to reactive products (i.e., semiquinone and/or BQ) either spontaneously or via metabolism within the bone marrow compartment has been postulated to represent a critical step in the mechanism of HQ (and benzene) myelotoxicity.²⁸⁸ Macrophage myeloperoxidase activity, which is also present in early bone marrow progenitor cells,²⁸⁹ has been implicated in the metabolic sequence leading to such reactive products. Alternatively, the enzyme(s) responsible for this tissue-specific biotransformation may be eosinophil peroxidase or the peroxidase function of prostaglandin H-synthetase (PHS).^{280,290} Pirozzi et al.²⁹¹ observed indomethacin inhibition of arachidonic acid-mediated oxidation of HQ to BQ by purified PHS. In contrast, no similar inhibition was found for H₂O₂-mediated oxidation by either PHS or myeloperoxidase. Conversion of HQ to BQ by purified human myeloperoxidase in the presence of H₂O₂ has been demonstrated.²⁹² Recent data have demonstrated the presence of polymorphisms in the lung myeloperoxidase enzyme.²⁹³ Although the occurrence of similar genotypic variations in bone marrow myeloperoxidase have not been reported, such polymorphisms could influence the expression of myelotoxicity following parenteral HQ exposure in animal models.

Formation of HQ-derived oxidation products can lead to covalent protein binding in hematopoietic cells and bone marrow cultures. Schlosser et al.⁸¹ incubated ¹⁴C-labeled HQ with a mouse peritoneal macrophage lysate and observed an H₂O₂-dependent protein binding of HQ-derived radiolabel; binding was inhibited by cysteine and by the peroxidase inhibitor aminotriazole but not by hydroxyl radical scavengers. These workers also reported HQ-derived covalent binding to both protein and DNA with purified PHS in the presence of either arachidonic acid or H₂O₂, a reaction inhibited by indomethacin.^{81,83,112} Concomitant exposure of human HL-60 cells to HQ and H₂O₂ resulted in cytotoxicity and covalent protein binding.²⁹⁴ While the thiol reactivity of BQ and/or the semiquinone likely underlies HQ-derived covalent binding in bone marrow, the specific targets of this binding, whether small molecule or protein based, are unidentified. A recent report indicated that i.v. administration of HQ-GSH conjugates (postulated to form via reaction of BQ and GSH in bone marrow) to rats resulted in decreased ⁵⁹Fe incorporation into erythrocytes.²⁹⁵

Studies using mouse bone marrow stromal cell cultures have indicated a 16-fold greater binding of ¹⁴C-HQ to macrophage than to fibroblast protein, consistent with the higher sensitivity of macrophages to HQ.²⁹² The relatively high level of HQ-derived covalent binding in macrophages was attributed to a higher level of peroxidase activity and/or a lower level of NQO1 activity in these cells when compared with fibroblasts. As discussed previously, NQO1 is a detoxifying enzyme that reduces BQ to HQ. Additional studies have confirmed these relative enzyme activity differences and have demonstrated that, in contrast, GSH levels between these two cell types are not significantly different.⁹⁰ The potential significance of NQO1 activity in HQ myelotoxicity was further assessed in studies showing that bone marrow stromal cells from DBA/2 mice have a lower basal NQO1 activity and are more susceptible to HQ cytotoxicity than cells derived from the more resistant C57BL/6 strain.²⁹⁶ Correlations between relative susceptibility to HQ and NQO1 content of bone marrow cells have also been demonstrated for rats and mice.²⁹⁷ In addition, induction of both NQO1 and GSH in mouse bone marrow cells by 1,2-dithiole-3-thione (D3T) was found to protect against HQ cytotoxicity, while

addition of dicoumarol, a NQO1 inhibitor, potentiated these effects.²⁹⁸ Protective effects were reported in human ML-1 and HL-60 myeloid cell lines treated with D3T and HQ, and in DBA/2 mice fed D3T in the diet prior to isolation of the bone marrow and *in vitro* challenge with HQ.^{299,300} Some direct human data on the importance of NQO1 as a protective factor are also available. Recent studies have demonstrated that individuals with an *NQO1* 609C→T mutation, which results in a “null” phenotype, may be more susceptible to benzene-induced hematotoxicity.³⁰¹

Finally, other aspects of oxidative metabolism in bone marrow have been suggested to be altered by HQ treatment. Laskin et al.³⁰² demonstrated that marrow cells from BALB/c mice exposed to HQ (100 mg/kg, i.p., three daily doses) released significantly more nitric oxide on stimulation by various inflammatory mediators than cells from control mice, an effect proposed to be significant to HQ myelotoxicity. Studies in human HL-60 cells indicated that H₂O₂, superoxide anion, and nitric oxide levels were all increased by HQ treatment in the presence of phorbol ester, suggesting an enhancement of oxidative stress by HQ.³⁰³

A number of studies have also explored the effects of *in vitro* HQ exposure on immune cells and immunological parameters in various model systems. Early studies demonstrated inhibition of lectin-stimulated lymphocyte blastogenesis and agglutination following pretreatment with micromolar levels of HQ.^{207,304} Based on the lack of a concurrent decrease in energy production and the protection from inhibition by thiol reagents, this effect was attributed to cytoskeletal disruption in lymphocytes due to the microtubule sulfhydryl reactivity of HQ.³⁰⁵ Parenteral treatment of C57BL/6 mice with HQ (100 mg/kg, two daily doses for 3 days) resulted in decreased spleen and bone marrow cellularity, in addition to decreased development of viable, plaque-forming cells derived from these tissues.²⁵⁷ These results suggested a reduction in the level of B-lymphocyte progenitor cells in bone marrow by HQ. A 50% decrease in RNA synthesis was reported in mouse splenic lymphocytes treated with 10 to 20 μ M HQ.²⁰³ Inhibition of maturation of pre-B cells was demonstrated in B6C3F₁ mouse bone marrow cultures pretreated with HQ (0.1 μ M, 1 h).³⁰⁶ This effect

may be mediated by decreased IL-1 release from stromal macrophages, leading to inhibition of IL-4 production by stromal fibroblasts.³⁰⁷

In vitro mitogen-induced proliferation of mouse, rat, and human B- and T-lymphocytes was inhibited in a dose-related manner by HQ.³⁰⁸ HQ was found to block IL-2-dependent proliferation of human T-lymphoblasts *in vitro* without affecting either IL-2 production, IL-2 receptor binding, cell viability, or GSH levels,³⁰⁹ possibly via interference with iron metabolism and ribonucleotide reductase activity.^{310,311} Expression of transferrin receptors, an early cellular event mediated by IL-2, was inhibited by HQ in human T-lymphoblasts.³¹² Thiol reagents were not effective in reversing the effect of HQ on T-lymphoblast proliferation, while transfection of the M2 subunit of ribonucleotide reductase did provide protection.³¹³ Mild inhibition of pre-B cell proliferation when co-cultured with bone marrow stromal cells was observed with 10 μ M HQ, probably due to inhibition of IL-7 production by the stromal cells.³¹⁴ A 55% decrease in interferon gamma (IFN γ) production, without concomitant reduction in cell viability, was observed in mouse spleen cell cultures exposed to 50 μ M HQ for 1 h.³¹⁵ Exposure of the mouse fibroblast L-929 cell line to HQ also resulted in inhibition of IFN α/β production.³¹⁶ Recent work has shown reversible inhibition of the NF- κ B transcription factor by 1 μ M HQ in human CD4⁺ T-cell cultures.³¹⁷

The effects of *in vitro* HQ exposure on phagocytic and cytotoxic immune cell function have also been explored. Dose-dependent inhibition (1 to 10 μ M range) of mouse spleen natural killer (NK) cell activity *in vitro* by HQ has been demonstrated.³¹⁸ Lewis et al.¹⁸⁵ reported inhibition of H₂O₂ production and cytolytic activity in mouse peritoneal macrophages treated with HQ, without concomitant disruption of general cellular functions. Additional experiments revealed concentration-dependent inhibition of Fc receptor-mediated phagocytosis *in vitro* in peritoneal macrophages.³¹⁹ The ability of human HL-60-cell-derived granulocytes to phagocytize sheep RBCs and to reduce nitroblue tetrazolium (indicators of immune functionality) was also diminished by HQ pretreatment.¹⁰⁸

It is clear from the extensive database discussed above that HQ exposure *in vitro* or in

whole animal systems using parenteral routes can affect numerous hematopoietic and immunologic parameters. It is also likely that oxidation of HQ within the bone marrow compartment to the semi-quinone or BQ, followed by covalent macromolecular binding, is critical to these effects (Figure 4). Whether myelotoxicity is mediated by effects of HQ on mitosis or other cellular processes (or both) is not known. While specific mechanisms have still not been elucidated, the molecular effects of HQ metabolites appear to involve a combination of inhibition and enhancement of different metabolic and cellular differentiation pathways in bone marrow, leading to an overall imbalance in the hematopoietic process. Primary among these specific effects are stimulation of progenitor cell proliferation to the promyelocyte stage, inhibition of progenitor cell proliferation to the erythroblast stage, and disruption of cytokine production by marrow stromal cells. Thus, an imbalance, manifested as a relative increase in granulocytic ontogenesis at the expense of erythropoietic (and, possibly, monocytic and lymphocytic) development evolves. In addition, functional effects on certain immune cells may occur.

Mechanistic studies with HQ and its metabolites have provided much information regarding the myelotoxicity in these experimental systems. However, many of these studies have been designed to examine the potential involvement of HQ in benzene myelotoxicity and/or leukemia, making it difficult to judge the relevance of these data for HQ toxicity per se. As discussed previously, toxicokinetic and species-specific factors are critical to assessing the pattern of HQ toxicity in mammalian systems, and thresholds for certain effects are likely. Several authors have addressed this issue in detail.^{11,320} It must also be emphasized that bone marrow and hematologic effects are generally not characteristic of HQ exposure in animal bioassays employing routes of exposure other than parenteral. In addition, myelotoxic changes have not been reported in humans as a result of long-term occupational HQ exposure.

XI. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

The overall *in vitro* and animal toxicity database indicates that HQ may cause maternal toxic-

ity, which is essentially the same as that seen in non-pregnant animals given similar exposures to acutely toxic dose levels of HQ. Fetotoxicity, primarily manifested as growth retardation, may be seen at high-dose levels that also induce maternal toxicity. However, reproductive and/or teratogenic effects are not prominent, even at high-exposure levels.

In vitro effects of HQ on various developmental parameters have been examined. HQ produced embryotoxicity but no statistically significant increase in malformations in a chick embryo assay at doses of up to 40 $\mu\text{g}/\text{egg}$.³²¹ Treatment of whole-rat embryos in culture with HQ at 10, 50, 100, or 200 μM concentrations resulted in 0% viability at the two higher levels, but no effects at 10 or 50 μM .³²² Similar exposure of rat embryo cultures to 45 or 68 μM HQ produced growth retardation, while the higher concentration also resulted in certain structural defects (hind limb absence and tail abnormalities).³²³

Certain early studies, using nonstandard protocols, did suggest a potential for reproductive effects of HQ at high-dose levels. Increased fetal resorption rates were reported in pregnant female rats fed a total dose of 0.5 g HQ in the diet over an unspecified length of time.³²⁴ Administration of 200 mg/kg/day HQ, p.o., to female rats for 14 days resulted in inhibition of estrus but also significant toxicity (clonic seizures, respiratory effects, 30% mortality).³²⁵ Interruption of estrus was reported in female rats following s.c. injection of 10 mg/kg/day HQ for 11 days.³²⁶ Male rats injected s.c. with 100 mg/kg/day HQ for 51 days exhibited inhibition of spermatogenesis, testicular changes, and decreased fertility.³²⁷ In contrast, Ames et al.³²⁸ reported no effects on reproductive parameters in female rats fed 0.3% HQ in the diet for 10 days prior to mating. Kavlock et al.³²⁹ reported reproductive effects in pregnant female SD rats treated with 100, 333, 667, or 1000 mg/kg of HQ, p.o., on gestational day (gd) 11. Except for the low dose level, the other dose levels approached or exceeded the reported oral LD₅₀ levels for rats. Decreased maternal weight and total litter mass, and increased perinatal loss, were observed with the two highest dose levels, while decreased litter size was noted at 1000 mg/kg.

In contrast to these studies, the results of standardized teratogenicity and reproduction bioassays for HQ have generally revealed a lack

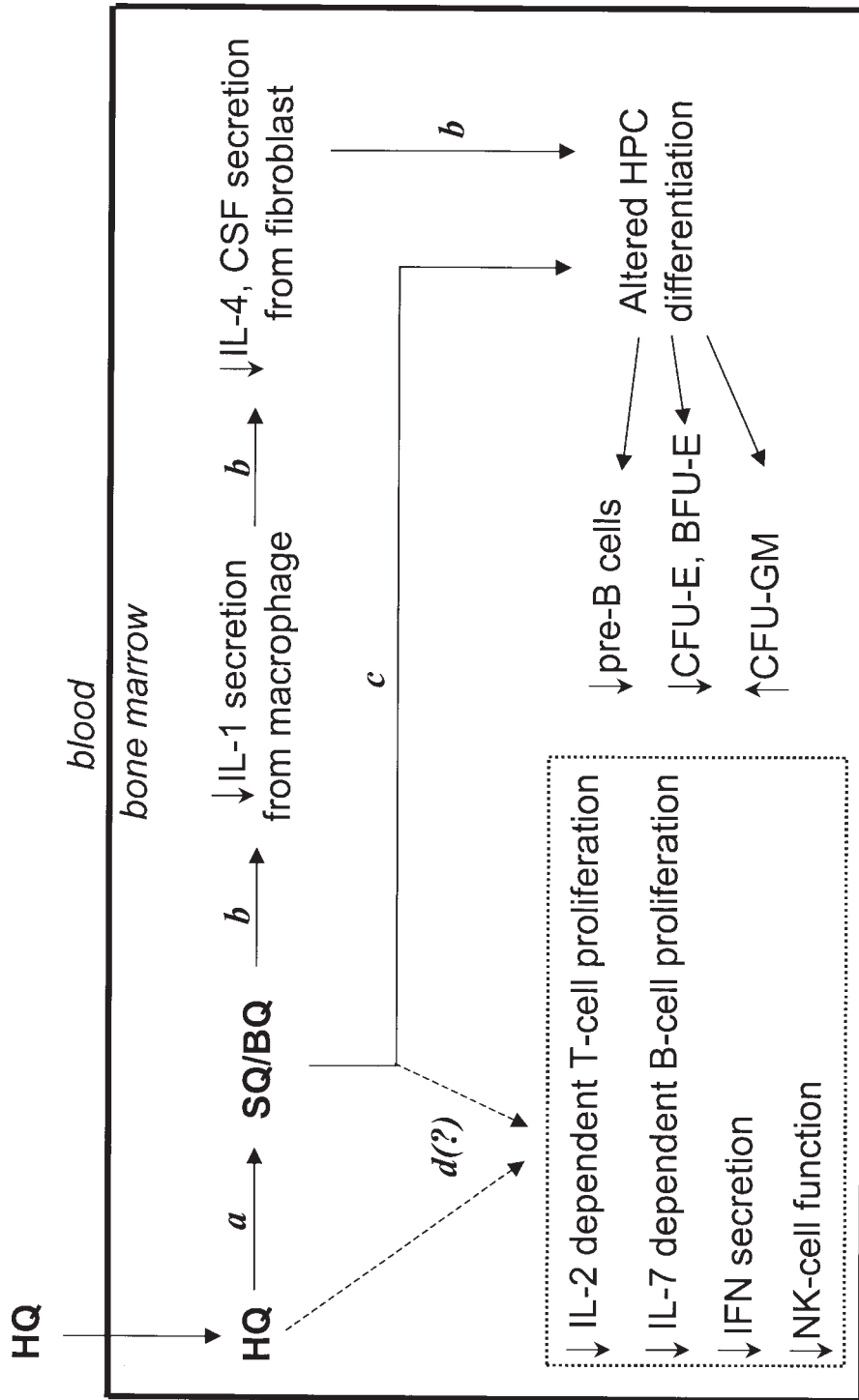


FIGURE 4. Summary of proposed mechanisms of hydroquinone (HQ) myelotoxicity following i.p. administration. HQ is taken up by bone marrow from blood, followed by one-electron oxidation by myeloperoxidase (or other peroxidases) to semiquinone (SQ) and benzoquinone (BQ) (path "a"). These metabolites are proposed to interfere with stromal macrophage and fibroblast secretion of growth and differentiation factors to result in modified hematopoietic progenitor cell (HPC) development (path "b"). Alternatively, these metabolites may directly influence mitotic or other cellular processes in HPCs (path "c"). Reported effects on immune cell physiology and function may be due to either HQ or oxidized metabolites (path "d") and may occur in compartments other than bone marrow (dashed box). ↑ = relative increase in level compared to unexposed; ↓ = relative decrease in level compared with unexposed.

of effect, even at high-dose levels. Teratogenic effects were not observed in offspring of pregnant female SD rats following dermal HQ exposure of up to 810 mg/kg/day on gd 6 to 19.³³⁰ In a dominant lethal assay in mice, males were exposed to HQ at doses of up to 300 mg/kg/day (5 d/week, 10 weeks, p.o.) and then mated to untreated females. No treatment-related effects on fertility rates, implantations, fetal survival, or other reproductive parameters on offspring were noted.¹²⁸ Developmental toxicity of HQ was investigated in female New Zealand White rabbits dose with 25, 75, or 150 mg/kg HQ, p.o., on gd 6 to 18.³³¹ Slightly decreased maternal body weights and food consumption were noted at the two higher dose levels. No statistically significant teratogenic alterations were observed in offspring from treated rabbits when compared with control. A similar decrease in maternal body weight and lack of teratogenic changes were obtained in female SD rats treated with 30, 100, or 300 mg/kg HQ, p.o., on gd 6 to 15.³¹⁰ A two-generation study in SD rats did not demonstrate selective reproductive effects at HQ dose levels of up to 150 mg/kg/day.³³²

XII. EFFECTS OF HQ EXPOSURE IN MAN

A. Systemic Effects

With the exception of ocular and dermal effects (described below), there are no clearly demonstrated impacts of prolonged HQ exposure in humans. No adverse hematological or urinary changes were observed in two male volunteers ingesting daily doses of 500 mg/day HQ for 5 months, or in 17 male and female volunteers ingesting 300 mg/day for 3 to 5 months.²⁶ Isolated case reports have postulated the occurrence of health effects due to HQ exposure without any specific causal evidence. For example, a recent report described hepatotoxicity in a darkroom worker and attributed this finding to inhalation to HQ "fumes" or dust.³³³ Little-to-no HQ is present, however, in ambient air during such uses, and no data were reported to implicate HQ (other than its presence in developer fluid) or to discount other more likely causes.^{16,334}

A number of occupational epidemiologic studies have been conducted on worker cohorts with potential exposure to HQ, primarily via inhalation of dust and direct dermal contact. No evidence for systemic toxic effects was reported in a cohort of HQ production workers with potentially significant airborne HQ dust (up to 30 mg/m³) and BQ vapor (up to 1 mg/m³) exposure.³³⁵ No increase over control in the measured outcomes of mortality, cancer incidence, or sickness/absence were observed in epidemiological studies of laboratory film processors with potential exposure to numerous chemicals, including HQ.¹⁸ Airborne HQ levels for these workers were much lower than for production workers; typically <0.01 mg/m³ during the period 1940 to 1964. Over 400 workers were followed for up to 16 years in this study. A study of over 9000 plant workers at a major U.S. producer of HQ revealed significantly lower incidences of mortality due to a number of diseases, including cancer, when compared with general population controls.³³⁶ A similar result was reported for the subset of workers with primary exposure to HQ, although actual HQ air levels were not reported. Finally, a recent comprehensive mortality and exposure study of 879 male and female workers involved in HQ production and use over a 50-year period did not reveal statistically significant increases in deaths from a number of malignant and nonmalignant disease categories.³³⁷ No cumulative exposure-related trends in selected causes of mortality were found. Overall death rates and cancer incidence were significantly decreased in HQ-exposed workers when compared with an employed referent population, demonstrating that the improved outcome in HQ workers was not related to the healthy worker effect. These studies provide substantial evidence for a lack of systemic toxic (including carcinogenic) effects from long-term occupational exposure to HQ.

A few studies have reported systemic effects in workers with potential exposure to HQ. Choudat and co-workers³³⁸ reported an increase in respiratory symptoms (i.e., cough in a smoky atmosphere and hay fever) in workers exposed by inhalation to a combination of HQ, trimethyl-HQ, and retinene-HQ (relative and absolute concentrations of each compound not specified). Decreased pulmonary function test results (i.e.,

decreased forced expiratory volume and vital capacity) and elevated serum IgG were reported. Based on these data, the authors concluded that HQ may induce respiratory and immunological changes in exposed workers, although the mixed exposure scenario complicates interpretation of their findings. Nielsen et al.³³⁹ reported a relative risk of 3.4 for malignant melanoma in a cohort of 836 Danish lithographers, about 200 of whom reported regular use of photographic chemicals, including HQ. Exposure to numerous other industrial chemicals, including dyes, solvents, metals, and acids, was also reported by these investigators. No quantitative exposure data for HQ or any other agent were provided. The authors suggested HQ as a possible causative agent in melanoma, based on its “biological effect upon the melanocyte.” However, malignant melanoma has not been reported in other, more carefully controlled, HQ occupational epidemiological studies or as a consequence of excessive use of HQ-containing skin preparations. In addition, animal studies (discussed below) generally demonstrate melanocyte cytotoxicity with HQ treatment, including an inhibitory effect on growth of implanted melanomas in mice. These results are difficult to reconcile with the authors’ proposal. Excess melanomas were reported among laboratory employees, associated with risk factors, including chemist duties and working with high explosives, sources of ionizing radiation, or “volatile photographic chemicals”.³⁴⁰ However, no specific exposure data were given in this report and, based on its physical characteristics, it would not be appropriate to include HQ in the latter risk category.

B. Ocular Effects

While isolated reports of the human ocular effects of HQ in occupational settings^{341,342} had appeared earlier, a comprehensive description and study of this phenomenon did not occur until the 1940s. At that time, a number of cases of conjunctival and corneal pigmentation appeared among workers at a large production facility, where HQ manufacture had begun in 1930.³⁴³ These cases were described in a series of publications by Sterner, Oglesby, and Anderson.^{17,335,344} The conjunctival lesions have been described as pro-

ceeding from slight, diffuse light brownish staining to marked globular, dark brown staining of the interpallebral zone with increasing time and intensity of HQ exposure. The corneal lesions were noted to include horizontal brown pigmented lines (Stahli’s lines), vertical striations in Descemet’s membrane, and diffuse brown corneal staining. In severe cases, a grayish-white scarlike tissue was found to replace some or all of the cornea. A histopathological study has described degenerative corneal changes in several cases of HQ-induced ocular changes that occurred in a German plant manufacturing HQ.³⁴⁵ While the adverse effects associated with the conjunctival lesions were largely cosmetic in nature, changes in visual acuity as a result of corneal damage were noted. These were found to range from mild deficiencies in night vision, to excessive internal scattering and reflection of light within the cornea, to near blindness in the most extreme cases. Reversibility of the conjunctival, and to a lesser extent, the corneal pigmentation was noted after reduction in HQ exposure and during long-term follow up.³³⁵ In some cases, however, corneal changes were seen to persist and even increase after cessation of exposure.³⁴⁶

Oglesby et al.¹⁷ studied the characteristics of plant exposures that were associated with development of ocular effects in workers. Because the synthetic process involved sequential oxidation of aniline to BQ followed by reduction to HQ, it was assumed that exposure to both BQ vapor (known to be a potent eye irritant) and HQ dust may have been relevant. Development of the syndrome of ocular effects appeared to require prolonged exposure, with initial pigmentation typically appearing no earlier than 2 to 3 years after initiation of heavy HQ and BQ exposure. Severe corneal damage was generally associated only with continuous exposure of >5 years. Despite long-term exposure, certain individuals were found to be relatively free of ocular pigmentation, suggesting the influence of additional environmental and/or individual susceptibility factors in the development of these effects. Correlation of ambient airborne levels of BQ vapor and HQ dust for various plant operations with the severity of ocular changes eventually led to establishment of 0.1 ppm and 2 mg/m³ as exposure limits for these contaminants, respectively.³⁴³ In addition, other

industrial hygiene measures and engineering controls were instituted in the HQ production process. Similar ocular lesions have not been described among end users or consumers of HQ or BQ or products containing these materials.

Although both HQ and BQ have been implicated in causing ocular effects, the mechanism of this phenomenon remains unclear. Acute ocular toxicity studies with HQ in rabbits and dogs have generally shown only mild irritant and reversible corneal impacts.^{1,347} With the exception of a single report,³⁴⁸ attempts to reproduce the conjunctival and corneal pigmentation in animal models have been unsuccessful. It is generally accepted that these effects are due to a direct action of HQ and/or BQ on the eye rather than an indirect effect of systemic intoxication.³⁴⁴ The most likely possibility is that the corneal and conjunctival pigmentation represents formation and deposition of oxidized HQ- and BQ-derived polymers, quinhydrone (a molecular dimer of BQ and HQ), and/or modified ocular proteins.^{335,342} In any case, the effectiveness of industrial hygiene measures and medical surveillance efforts in HQ production is indicated by the lack of literature reports of new cases of HQ-induced ocular effects in humans over the last several decades.

C. Dermal Effects

HQ is only a mild skin irritant when tested using classic acute dermal irritation bioassays.¹ However, subchronic exposure to HQ may lead to reduced pigmentation on both animal and human skin. The first indication of this potential came from early animal toxicology studies, where oral administration of HQ and other phenolics to black-haired cats or mice resulted in reduced pigmentation of fur after 6 to 8 and 4 to 20 weeks, respectively.^{349,350} Similar results were reported with s.c. injections of HQ in black goldfish.^{351,352} Subsequent work demonstrated skin pigmentation reduction in black guinea pigs following daily topical application of 1 to 10% HQ in an ointment formulation for up to 1 month.^{353,354} The higher concentration preparations were also associated with skin irritation and inflammatory reactions. The latter study showed that HQ application caused a reduction in melanin content of melanosomes

and eventually produced a degeneration of melanocytes.

These findings are consistent with a hypothesis that HQ is an inhibitor of tyrosinase, the enzyme involved in conversion of tyrosine to L-dopa and then to dopaquinone, the initial steps in melanin formation.³⁵⁵⁻³⁵⁷ A 90% inhibition of tyrosinase activity in black goldfish skin homogenates was observed following incubation with 0.9 mM HQ, although higher concentrations resulted in moderate activation of the enzyme.³⁵⁸ HQ was also shown to directly inhibit purified tyrosinase from human melanoma cell lines.³⁵⁹ More recent work with purified tyrosinase has demonstrated that, in the presence of catalytic amounts of L-dopa, HQ actually competes with tyrosine as a substrate for the enzyme in a reaction that can result in formation of trihydroxybenzene and hydroxybenzoquinone.³⁶⁰ These products may undergo further metabolism and/or spontaneous conversion to toxic and/or reactive species; however, such additional metabolites have not been identified following *in vivo* exposures to HQ. The effectiveness of tyrosinase substrates as depigmenting agents has been suggested to be related to the extent of their further conversion to such metabolites.³⁶¹ Recent work indicates that inhibition of GSH synthesis results in potentiation of the depigmenting action of HQ on black guinea pig skin, presumably by increasing the level of oxidative species that would normally be trapped by the intracellular thiol.³⁶²

A number of studies have examined the effects of HQ on melanomas *in vivo* and on melanotic cell lines *in vivo*, both as mechanistic investigations and as a means of testing the utility of HQ as a possible chemotherapeutic agent. Cytotoxicity in mouse melanoma cell cultures treated with HQ was reported by Hu.³⁶³ Abramowitz and Chavin³⁶⁴ found complex changes in cyclic nucleotide levels and tyrosinase activities of mouse B-16, S-91, and HP tumors exposed *in vitro* to HQ. A 30-fold lower ED₅₀ for inhibition of tritiated thymidine incorporation was found for HQ in melanotic when compared with nonmelanotic cell lines, suggesting the participation of tyrosinase inhibition in cytotoxicity.³⁶⁵ These investigators postulated active oxygen species generated externally to the cell as the actual cytotoxic agents. Another study using melanotic, amelanotic, and

nonmelanotic cell lines demonstrated that HQ cytotoxicity is dependent on tyrosinase, but not melanin, content of the cell.³⁶⁶ In contrast, a series of phenols (including HQ) that are substrates for tyrosinase were found to be cytotoxic to both melanotic and nonmelanotic cell lines, suggesting that tyrosinase may not be involved in the cytotoxic mechanism.^{367,368} The reasons for these conflicting results have not been determined. Finally, treatment with nine daily s.c. injections of 80 mg/kg HQ significantly decreased tumor formation and increased survival of BALB/c mice given melanoma implants, consistent with a specific cytotoxic effect on melanocytes *in vivo*.³⁶⁹

Intentional use of HQ formulations for skin lightening in humans began in the 1950s, following anecdotal reports from the southern U.S. of depigmentation occurring as a side effect of an HQ-containing preparation designed as a sunscreen.³⁷⁰ Early controlled clinical studies suggested that daily dermal application of creams containing 1.5% or greater concentrations of HQ were effective in producing at least mild cosmetic skin depigmentation after approximately 1 month of use,^{371–374} although some clinicians reported negative results for HQ in the absence of additional ancillary components.³⁷⁵ The incidence of skin irritation and inflammation associated with this treatment appeared to increase substantially with HQ concentrations above 2 to 3%. Occasional hypersensitivity reactions have also been noted, although HQ is not a consistent sensitizer in human or animal studies.^{1,376}

Since the 1950s, HQ-containing creams have been employed in clinical dermatology for treatment of hypermelanosis, senile lentigo, vitiligo, and melasma.³⁷⁷ Prolonged use of over-the-counter preparations containing higher concentrations of HQ or continued use of HQ-based skin lighteners in the presence of skin inflammation or dermatitis has resulted in a small number of reports of adverse skin reactions in the U.S. Reports of reactive skin hyperpigmentation following excessive use of these products appeared in South Africa during the 1970s.^{374,378} This phenomenon was first reported by Findlay and co-workers following the extensive use of skin creams with high HQ contents (6 to 8%).^{379,380} In some cases hyperpigmentation can appear as colloid milium or exogenous ochronosis. Clinically, these conditions are char-

acterized by collections of dark papules or macules against lighter colored skin (colloid milium), with histopathological analysis of the affected skin revealing parallel bundles of ochre-colored elastoid fibers (ochronosis). Development of the condition is accelerated and aggravated by sunlight, by the presence of other active derivatives (e.g., resorcinol and phenol), and by the use of penetrating vehicles.³⁸¹ After cessation of exposure to HQ and sunlight, the effects are at least partially reversible.

Despite governmental controls on the maximum level of HQ permitted in these products, an epidemiological study conducted in South Africa 15 years after these initial reports indicated a prevalence rate of 69% for exogenous ochronosis among users of skin lighteners.³⁸² These data have resulted in calls for the banning of HQ-based skin creams as over-the-counter cosmetic preparations.^{383–385} A small number of cases of exogenous ochronosis attributed to the use of HQ-based products have also been reported in the U.S. and countries other than South Africa.^{381,386–392} Paradoxical pigmentation of the nails has also been reported as a side effect of the chronic use of HQ.^{393,394} In contrast, ochronosis or hyperpigmentation has not been reported during the manufacture or the use of HQ in occupational settings. Occasional reports of leukoderma and/or contact dermatoses are also encountered, primarily associated with use of photographic developers.^{395–400}

The mechanism of ochronotic skin changes following prolonged dermal HQ exposure is unclear. The presence of melanocytes appears to be required for ochronosis to occur.⁴⁰¹ Findlay initially proposed that, with constant exposure, melanocytes might eventually become resistant to the uptake and inhibitory effects of HQ (termed “melanocyte recovery”), leading to increased production of melanin.³⁷⁹ This would be accompanied by increased transfer of HQ into the dermis, with deposition of oxidized pigments and/or damage to connective tissue fibers. Penneys³⁸⁸ proposed that, in analogy to the mechanism of endogenous ochronosis in alkaptonuria, HQ may inhibit homogentisic acid oxidase in the skin, leading to deposition of homogentisic acid-based pigments. Other hypotheses include activation of tyrosinase at high HQ concentrations, leading to increased melanin production,⁴⁰² formation of oxidized,

colored hydroxylated indoles,³⁸⁶ uptake of melanin by macrophages in the dermis,³⁷⁹ or degeneration of dermal collagen leading to the deposition of pigmented fibers.⁴⁰³

Despite the occasional appearance of clinical case reports in the published literature, significant side effects from the use of skin preparations containing HQ are uncommon and generally associated with improper or excessive use. In the U.S., dermatological and cosmetic creams containing 2% or less of HQ are considered safe for use in skin depigmentation (with avoidance of sunlight and discontinuance after 2 months).^{404,405} Extensive reviews of HQ toxicity as related to cosmetic and dermatologic applications have been published.^{12,13,371,379,380}

XIII. CONCLUSIONS: IMPLICATIONS FOR HUMAN HEALTH EFFECTS

As with all xenobiotics, the potential risk to humans from occupational, environmental, and consumer exposure to HQ is a function of both the extent of exposure and the inherent toxicity of the chemical. Thus, risk characterization relies on scientific judgment based on a consideration of both exposure assessment and dose-response analysis. This can be a highly uncertain undertaking with major areas of uncertainty rising from animal-to-human extrapolation, and extrapolation with respect to dose level, route, and rate of dose administration. In the case of HQ, much information is available on mode of action and route of exposure- and species-dependent differences in toxicokinetics and toxic effects; these address some of the uncertainties and provide insight into the overall risks associated with HQ exposure in humans. It is clear that the potential human effects of direct HQ exposure must be distinguished from those of indirect exposure via metabolism of benzene. These issues are considered in the following discussion, in light of the current extensive knowledge base for HQ. Other published articles and reviews have also explored these issues to various extents.^{1,11,254,337,406}

HQ appears to more or less specifically affect the kidney and hematopoietic systems in experimental animals and the skin and eye in humans (Table 1). In view of the apparent diversity of

these effects, it is valuable to discuss the evidence for and against common underlying mechanisms of action for HQ. Such an assessment is critical to extrapolation of animal (and *in vitro*) results to man and for predicting risk of HQ exposure in humans. One critical question involves the nature of the chemical species responsible for the effects observed in each organ system. Is HQ both necessary and sufficient to produce an effect or are other metabolites or reaction products required? The database available to evaluate this issue is extensive and generally points to BQ and the semiquinone as the primary mediators of HQ toxicity, while evidence for the involvement of activated oxygen species in HQ toxicity is less compelling. More specifically, the covalent reactivity of BQ and the semiquinone with cellular nucleophiles (particularly thiol groups) is likely to mediate the majority of HQ's effects. Despite this generalization, the actual molecular targets for each tissue- and organ-specific effect of HQ appear to be quite unique. Thus, formation of toxic HQ-GSH conjugates that impair mitochondrial function may underlie kidney toxicity, while covalent modification of proteins and thiol-containing biomolecules involved in mitosis and hematopoiesis may be important in myelotoxicity. A exception to this model concerns the acute CNS effects of HQ, which are probably dependent on the parent molecule itself.

Because HQ does not appear to be a direct genotoxicant or mutagen, epigenetic factors, such as toxicokinetic and other species-, strain-, and sex-related differences will determine the specificity of toxic effects and will impact the prediction of human risk. In the case of renal toxicity following high-level HQ exposure, the rate of oxidative metabolism of HQ to BQ in the liver and kidney, the rate of formation and release of HQ-GSH conjugates from the liver and subsequent uptake by the kidney, tissue-specific GSH contents, and relative levels of detoxifying enzymes (e.g., glucuronyltransferases, sulfotransferases, and NQO1) will all determine the species-specific exposure threshold for this effect.

As discussed previously, the male F344 rats appears to be most susceptible to HQ-induced nephrotoxicity and renal adenoma induction. This strain exhibits high background rates of CPN and of cell proliferation in kidney tubule cells, condi-

TABLE 1
Summary of Toxicity Endpoints for HQ in Experimental Animals and Man

Target organ or target effect	Proposed critical toxicant(s)	Effect present in:		Reported effect(s)
		Animals	Humans ^a	
Acute CNS	unchanged HQ	+	+	Acute excitatory action; tremor, incoordination, seizures, respiratory difficulty, coma
Bone marrow and blood	SQ/BQ; reactive oxygen species (?) ^b	+	-	Anemia, decreased cellularity, altered hematopoietic precursor cell differentiation, altered immune cell function
Kidney	SQ/BQ; HQ-SG; HQ-Cys; reactive oxygen species (?)	+	-	Nephrotoxicity, exacerbation of chronic progressive nephropathy, tubular cell adenomas
Eye	SQ/BQ	-	+	Pigment deposition in conjunctiva and cornea
Skin	HQ (?); SQ/BQ	+	+	Depigmentation; exogenous ochronosis
Genotoxicity	SQ/BQ; reactive oxygen species (?)	+	-	Clastogenicity; mitotic disruption

Note: **Abbreviations:** BQ = benzoquinone; HQ = hydroquinone; HQ-SG = glutathione conjugates of HQ; HQ-Cys = cysteine conjugates of HQ; SQ = semiquinone.

^a With the exception of some reported acute poisoning incidents and, possibly, early occupational exposures, human data are based on dose levels likely to be substantially lower than for the animal data.

^b Indicates weak and/or inconsistent data to support involvement of particular metabolite.

tions that are expected to enhance the renal effects of compounds such as HQ. The evidence discussed earlier indicates that the nephrotoxicity induced by short-term HQ exposure in experimental animals is mechanistically distinct from the enhancement of CPN and cell proliferation seen after chronic exposure. These data indicate likely epigenetic mechanisms for these effects, with acute effects dependent on toxicokinetic factors and chronic changes on species-specific differences in cellular responses to continuing proliferative stimulation. Based on the absence of evidence for similar predisposing factors in other species, the lack of demonstrated renal effects in humans exposed to significant levels of HQ, and the minimal direct mutagenic potential of HQ, it is unlikely that nephrotoxicity or renal carcinogenesis represent relevant risk extrapolation endpoints for HQ in man.

Similar arguments can be made for the observed myelotoxic effects of HQ in experimental systems, where the mouse (and mouse bone marrow cells) appears to be most susceptible. The basis for this sensitivity is not known, but probably involves relative differences in activating and detoxifying enzymes within specific bone marrow cell types.⁴⁰⁷ In addition, for myelotoxicity, toxicokinetic considerations become critical in determining the likelihood of effects in man. The majority of data on this phenomenon have been derived from animal studies employing parenteral routes of exposure. As has been demonstrated, this technical approach, while experimentally convenient, results in lower overall HQ detoxification (via conjugation) by the liver and increased levels of unchanged HQ available to the bone marrow for activation by myeloperoxidase and other oxidative enzymes. This would not be expected to occur under reasonably anticipated conditions of human exposure, where respiratory, dermal, and oral routes would predominate.

One issue that deserves further scrutiny in predicting the possible effects of direct HQ exposure in man is that of individual susceptibility. Many of the enzymes involved in HQ metabolism are polymorphic in animals and/or humans. For example, phenol sulfotransferase, the enzyme likely involved in HQ sulfation and detoxication, exists in two forms, a high activity thermostable and a lower activity thermolabile form.⁷² Varia-

tions in the activity of UDP-glucuronosyl transferase (UGT), which catalyzes formation of HQ-glucuronide, have also been reported in human liver.⁷² The presence of the low-activity forms of these enzymes, particularly UGT, in subgroups of individuals could hypothetically result in decreased conjugation and urinary excretion, and the higher distribution of parent molecules to peripheral tissues such as kidney and bone marrow. This could in turn result in enhanced toxicity. However, because of the demonstrated high metabolic capacity of these systems (especially in the intestine),^{72,74} there is likely to be adequate detoxication activity present for all except "null" genotypes for these loci. It is unclear whether such genotypes exist for these enzymes in humans. Similar arguments for increased susceptibility could be made in the case of genetic variations resulting in increased activity of bone marrow myeloperoxidase or kidney γ -GT, or decreased activity of bone marrow NQO1. In the absence of molecular epidemiological data on the existence, tissue specificity, and metabolic capacity of these possible variants, and on tissue levels of HQ and metabolites in individuals exposed directly to HQ, such arguments are speculative. However, this is clearly an area that deserves further investigation.

In discussing the potential for HQ-induced myelotoxicity in humans, comparisons with benzene are inevitable. As stated earlier, much of the available data on mechanisms of HQ-induced effects in bone marrow are derived from studies examining mechanisms of benzene toxicity, rather than as a result of concern over direct HQ exposure. Benzene is a potent myelotoxin in experimental animals and causes pancytopenia, MDS, aplastic anemia, and acute myelogenous leukemia in man.²⁵⁵ Early work demonstrated that benzene myelotoxicity is probably due to the presence of a metabolite, rather than the parent compound, in bone marrow. HQ has long been known to be a metabolite of benzene, formed following oxidation of phenol produced from the initial P450-mediated oxidation of benzene. Significant levels of HQ are present in bone marrow following acute inhalation exposure of rats to benzene.⁴⁰⁸ In addition, structurally similar DNA adducts are formed in bone marrow following either *in vitro* HQ or *in vivo* benzene exposure in mice.¹⁰³

While metabolism is clearly important in benzene myelotoxicity, no single benzene metabolite, including phenol, HQ, or BQ, has exhibited the potency and level of myelotoxic effect of benzene itself in animal studies. Eastmond et al.²⁵⁸ first demonstrated that coadministration of phenol and HQ by i.p. injection in B6C3F₁ mice resulted in dose-related decreases in bone marrow cellularity comparable to those noted with benzene exposure. Phenol was also found to enhance peroxidase-dependent HQ metabolism to BQ and subsequent covalent protein binding. These authors proposed that phenol-mediated stimulation of HQ metabolism by myeloperoxidase in bone marrow was responsible for this phenomenon and that such a mechanism might underlie benzene myelotoxicity. Since that report, other studies have generally corroborated these findings. Coadministration with phenol has been found to decrease the metabolic clearance of HQ⁶⁵ and to exacerbate the inhibition of erythrocyte iron uptake,^{271,272} formation of macromolecular adducts,^{64,106,111,409} generation of activated oxygen species,⁴¹⁰ and MN induction¹⁶² encountered with HQ exposure alone. Stimulation of HQ metabolism by myeloperoxidase has also been demonstrated for BQ⁴¹¹ and additional phenolic compounds.⁴¹²

Because phenol is metabolized to HQ in the liver, the lack of myelotoxicity associated with p.o. administration of phenol alone in experimental animal studies is surprising. Toxicokinetic models have been developed to account for this finding.^{320,413} These models predict that route of exposure differences and competitive hepatic oxidative and conjugative metabolism between benzene and phenol result in a higher net HQ delivery to bone marrow following benzene as opposed to phenol administration. Assuming that myeloperoxidase action on HQ is critical to myelotoxicity, that phenol stimulates HQ oxidation, and that relatively similar phenol levels are present in bone marrow following either phenol or benzene administration, then this explanation is tenable. This paradigm also has bearing on the myelotoxicity associated with direct HQ exposure. Because phenol is not a metabolite of HQ, the degree of myelotoxicity will depend only on the level of unchanged HQ present in bone marrow. As indicated earlier, this parameter is route

of exposure dependent, and is typically low after nonparenteral HQ administration.

If the level of HQ in the bone marrow is a critical determinant of bone marrow toxicity, then plausible explanations for the distinct spectrum of myelotoxic effects of benzene follow. One could predict that, with low benzene exposure, little overall metabolism to HQ occurs. The HQ that is formed would be readily removed by conjugation and urinary excretion, leaving little to ultimately reach the bone marrow. In contrast, high-level benzene exposure would lead to very elevated levels of HQ in bone marrow and significant hematopoietic cell death, potentially leading to aplastic anemia. At intermediate benzene exposure levels, a complex series of myelodysplastic effects (e.g., clastogenicity, clonal selection, altered cell physiology) would result, some or all of which might represent a preleukemic condition. The specific dose ranges associated with each level of effect might be influenced by an individual's overall genotype for the relevant enzymes (including CYP450) involved in benzene metabolism.

In conclusion, review of the overall *in vitro*, experimental animal, and human toxicological and epidemiological databases strongly suggests that direct HQ exposure under current occupational and environmental conditions poses little risk. Several recent quantitative risk assessments for HQ, using worst case scenarios, have concluded that such exposure is well below levels conservatively estimated to be associated with health effects in humans.^{11,414} This conclusion is further supported by the lack of evidence for HQ toxicity in occupational cohorts in a number of well-conducted epidemiological and clinical studies and as a result of consumption of foods containing significant levels of HQ. In addition, the major effects of HQ reported in animal studies (i.e., renal toxicity and myelotoxicity) are of uncertain relevance to humans, because of the dependence of these endpoints on toxicokinetic and species-specific factors. While hypothetical genetic polymorphisms in relevant metabolic enzymes could heighten the risk of renal toxicity or myelotoxicity in specific exposed individuals, there are no data available to indicate that this actually occurs. Finally, concerns related to the possibility that effects similar to those reported in *in vitro* and

animal studies with benzene may occur following direct HQ exposure are likely to be exaggerated due to the major toxicokinetic and toxicodynamic differences between these substances. Only in the improbable case of simultaneous parenteral or very high-level oral exposure to HQ and phenol (or related compounds) might the effects of co-exposure reported in experimental studies be potentially significant to man.

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REFERENCES

1. **International Programme on Chemical Safety (IPCS)**, *Environmental Health Criteria 157: Hydroquinone*, Geneva: World Health Organization, 1994.
2. **Hazardous Substance Data Bank (HSDB)**, *Entry for Hydroquinone*, Bethesda: National Library of Medicine, 1997.
3. **Krumenacker, L., Constantini, M., Pontal, P., and Sentenac, J.**, Hydroquinone, resorcinol, and catechol. In: Howe-Grant, M. Ed. *Encyclopedia of Chemical Technology*, Vol. 13. John Wiley & Sons, New York, 1995, 996–1014.
4. **National Institute of Occupational Safety and Health (NIOSH)**, *National Occupational Exposure Survey (NOES)*, Cincinnati, NIOSH, 1982.
5. **Deisinger, P. J., Hill, T. S., and English, J. C.**, Human exposure to naturally occurring hydroquinone, *J. Toxicol. Environ. Health* 47, 101–116, 1996.
6. **Leanderson, P. and Tagesson, C.**, Cigarette smoke-induced DNA-damage: role of hydroquinone and catechol in the formation of the oxidative DNA-adduct, 8-hydroxydeoxyguanosine, *Chem.-Biol. Interact.* 75, 71–81, 1990.
7. **National Toxicology Program (NTP)**, *Toxicology and Carcinogenesis Studies of Hydroquinone in F-344/N Rats and B6C3F₁ Mice*, NTP Technical Report No. 366; Washington, U.S. Department of Health and Human Services, 1989.
8. **International Agency for Research on Cancer (IARC)**, *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man; Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, Lyon: IARC, 1987, 64.
9. **American Conference of Governmental Industrial Hygienists (ACGIH)**, *1997–1998 Threshold Limit Values (TLVs) for Chemical Substances and Physical Agents*, Cincinnati: ACGIH, 1997.
10. **Hard, G. C., Whysner, J., English, J. C., Zang, E., and Williams, G. M.**, Relationship of hydroquinone-associated rat renal tumors with spontaneous chronic progressive nephropathy, *Toxicol. Pathol.* 25, 132–143, 1997.
11. **Whysner, J., Verna, L., English, J. C., and Williams, G. M.**, Analysis of studies related to tumorigenicity induced by hydroquinone, *Regul. Toxicol. Pharmacol.* 21, 158–176, 1995.
12. **Cosmetic, Toiletry, and Fragrance Association (CTFA)**, Addendum to the final report on the safety assessment of hydroquinone, *J. Am. Coll. Toxicol.* 13: 167–230, 1994.
13. **Cosmetic, Toiletry, and Fragrance Association (CTFA)**, Final report on the safety assessment of hydroquinone and pyrocatechol, *J. Am. Coll. Toxicol.* 5, 123–165, 1986.
14. **Devillers, J., Boule, P., Vasseur, P., Prevot, P., Steiman, R., Seigle-Murandi, F., Benoit-Guyod, J. L., Nendza, M., Grionin, C., Dive, D., and Chambon, P.**, Environmental and health risks of hydroquinone, *Ecotoxicol. Environ. Safety* 19, 327–354, 1990.
15. **United States Environmental Protection Agency (USEPA)**, *Health and Environmental Effects Document for p-Hydroquinone*, Cincinnati, Environmental Criteria and Assessment Office, USEPA, 1987.
16. **O'Donoghue, J. L., Richardson, D. P., and Dyer, W. M.**, Hydroquinone and hepatitis, *Lancet* 346, 1427–1428, 1995.
17. **Oglesby, F. L., Sterner, J. H., and Anderson, B.**, Quinone vapors and their harmful effects. II. Plant exposures associated with eye injuries, *J. Ind. Hyg. Toxicol.* 29, 74–84, 1947.
18. **Friedlander, B. R., Hearne, F. T., and Newman, B. J.**, Mortality, cancer incidence, and sickness-absence in photographic processors: an epidemiologic study, *J. Occup. Med.* 24, 605–613, 1982.
19. **Barber, E. D., Hill, T., and Schum, D. B.**, The percutaneous absorption of hydroquinone (HQ) through rat and human skin *in vitro*, *Toxicol. Lett.* 80, 167–172, 1995.
20. **O'Donoghue, J. L.**, Eastman Kodak Corp., Occupational Medicine and Hygiene Laboratories, United Kingdom Health and Safety Executive, London, U.K., unpublished data, 1993.
21. **Semenza, G., Bircher, J., Mulhaupt, E., Koide, T., Pfenninger, E., Marthaler, T., Gmunder, U., and Haemmerli, U. P.**, Arbutin absorption in human small intestine: a simple procedure for the determination of active sugar uptake in peroral biopsy specimens, *Clin. Chim. Acta* 25, 213–219, 1969.
22. **Inoue, O., Seiji, K., Nakatsuka, H., Watanabe, T., Yin, S-N., Li, G-L., Cai, S-X., Jin, C., and Ikeda, M.**, Excretion of 1,2,4-benzenetriol in the urine of workers exposed to benzene, *Br. J. Ind. Med.* 46, 559–565, 1989.
23. **Bechtold, W. E., Sun, J. D., Birnbaum, L. S., Yin, S. N., Li, G. L., Kasicki, S., Lucier, G., and Henderson, R. F.**, S-Phenylcysteine formation in

- hemoglobin as a biological exposure index to benzene, *Arch. Toxicol.* 66, 303–309, 1992.
24. **Sterner, J. H., Ames, S., and Fassett, D. W.,** Comparison of acute toxicity of hydroquinone and some related di and trihydroxy benzenes, *Fed. Proc.* 8, 334(abstract), 1949.
 25. **Woodard, G. D. L.,** The Toxicity, Mechanism of Action, and Metabolism of Hydroquinone. Washington, George Washington University, Dissertation thesis, 1951.
 26. **Carlson, A. J. and Brewer, N. R.,** Toxicity studies on hydroquinone, *Proc. Soc. Exp. Biol. Med.* 84, 684–688, 1953.
 27. **Christian, R. T., Clark, C. S., Cody, T. E., Whiterup, S., Gartside, P. S., Elia, V. J., Eller, P. M., Lingg, R., and Cooper, G. P.,** The Development of a Test for the Potability of Water Treated by a Direct Reuse System. University of Cincinnati, Cincinnati, Ohio, Washington: U.S. Army Medical Research and Development Command, Contract No. DADA-17-73-C-3013, 1976.
 28. **Dutton, G. J. and Greig, C. G.,** Observations on the distribution of glucuronide synthesis in tissues, *Biochem. J.* 66: 52P(abstract), 1957.
 29. **Hartiala, K. J.,** Studies on detoxication mechanisms. III. Glucuronide synthesis of various organs with special reference to the detoxifying capacity of the mucous membrane of the alimentary canal, *Ann. Med. Exp. Fenn.* 33, 239–245, 1955.
 30. **Registry of Toxic Effects of Chemical Substances (RTECS), Entry for Hydroquinone,** Cincinnati, National Institute of Occupational Safety and Health, 1998.
 31. **Nomiyama, K., Minai, S. T., and Kita, H.,** Studies on poisoning by benzene and its homologues: median lethal doses of benzene metabolites, *Ind. Health* 5, 143–148, 1967.
 32. **Brieger, L.,** On the knowledge of the physiological behavior of catechol, hydroquinone and resorcinol and their formation in the animal organism (German), *Dubois Arch. Physiol.* S61, 1879.
 33. **Topping, D. C.,** Subchronic Oral Toxicity Study of Hydroquinone in Rats Utilizing a Functional-Observational Battery and Neuropathology to Detect Neurotoxicity, TSCATS Database, EPA/OTS Doc #40-8869294, NTIS/OTS0516694, 1988.
 34. **Angel, A. and Rogers, K. J.,** Convulsant activity of polyphenols, *Nature*, 217, 84–85, 1968.
 35. **Angel, A. and Rogers, K. J.,** An analysis of the convulsant activity of substituted benzenes in the mouse, *Toxicol. Appl. Pharmacol.* 21, 214–229, 1972.
 36. **Otsuka, M. and Nonomura, Y.,** The action of phenolic substances on motor nerve endings, *J. Pharmacol. Exp. Ther.* 140, 41–45, 1963.
 37. **Mogey, G. A. and Young, P. A.,** The antagonism of curarizing activity by phenolic substances, *Br. J. Pharmacol.* 4, 359–365, 1949.
 38. **Chambers, P. L. and Rowan, M. J.,** An analysis of the toxicity of hydroquinone on central synaptic transmission, *Toxicol. Appl. Pharmacol.* 54, 238–243, 1980.
 39. **Mitchell, A. and Webster, J.,** Notes on a case of poisoning by hydroquinone, *Br. Med. J.* 21, 465, 1919.
 40. **Rémond, A. and Colombies, H.,** Intoxication with hydroquinone (French), *Ann. Méd. Lég.* 7, 79–81, 1927.
 41. **Guyot, H. J., Bachelier-Notter, J., and Tiffeneau, J.,** A case of fatal poisoning from hydroquinone (French), *Ann. Méd. Lég.* 46, 177–178, 1966.
 42. **Hooper, R. R. and Husted, S. R.,** A shipboard outbreak of gastroenteritis: Toxin in the drinking water, *Military Med.* 144, 804–807, 1979.
 43. **Halbron, P., Bosquet, A., and Tiffeneau, J.,** Fatal poisoning from a photographic developer (French), *Bull. Mem. Soc. Med. Hop. Paris* 55, 1596–1601, 1931.
 44. **Busatto, S.,** Fatal poisoning from photographic developer containing hydroquinone (German), *Deutsch. Ztschr. Ges. Gerichtl. Med.* 31, 285–297, 1939.
 45. **Zeidman, I. and Deutl, R.,** Poisoning by hydroquinone and mono-methyl-*para*-aminophenol sulfate, *Am. J. Med. Sci.* 210, 328–333, 1945.
 46. **Saito, T., Kojimahara, M., Aoki, K., and Takeichi, S.,** Detection of hydroquinone in a poisoning case, *J. Forensic Sci.* 39, 266–270, 1994.
 47. **DiVincenzo, G. D., Hamilton, M. L., Reynolds, R. C., and Ziegler, D. A.,** Fate and Disposition of [¹⁴C]Hydroquinone in Rats, TSCATS Database, EPA/OTS Doc #40-7969029, NTIS/OTS0517792, 1979.
 48. **DiVincenzo, G. D., Hamilton, M. L., Reynolds, R. C., and Ziegler, D. A.,** Metabolic fate and disposition of [¹⁴C]hydroquinone given orally to Sprague-Dawley rats, *Toxicology*, 33, 9–18, 1984.
 49. **Lockhart, H. B., Fox, J. A., and DiVincenzo, G. D.,** The Metabolic Fate of [¹⁴C]Hydroquinone Administered by Gavage to Male Fischer 344 Rats, TSCATS Database, EPA/OTS Doc #878214473, NTIS/OTS206577, 1984.
 50. **Fox, J. A., English, J. C., and Lockhart, H. B.,** Blood Elimination Kinetics of [¹⁴C]Hydroquinone Administered by Intragastric Intubation, Intratracheal Instillation or Intravenous Injection to Male Fischer 344 Rats, Rochester, NY, Health and Environment Laboratories; Eastman Kodak Company, 1986, Report No. TX-86-1.
 51. **English, J. C., Deisinger, P. J., Perry, L. G., Schum, D. B., and Guest, D.,** Toxicokinetics Studies with Hydroquinone in Male and Female Fischer 344 Rats, TSCATS Database, EPA/OTS Doc #40-8869295, NTIS/OTS0516692, 1988.
 52. **Saito, T. and Takeichi, S.,** Experimental studies on the toxicity of lithographic developer solution, *Clin. Toxicol.* 33, 343–348, 1995.
 53. **Lockhart, H. B.,** The Metabolic Fate of [¹⁴C]Hydroquinone Administered by Intratracheal Instillation to Male Fischer 344 Rats, Rochester, NY, Health and Environment Laboratories; Eastman Kodak Company, 1985, Report No. TX-85-76
 54. **Deisinger, P. J. and English, J. C.,** Bioavailability and Pulmonary Metabolism of Hydroquinone Following Intratracheal Instillation, Rochester, NY, Health

- and Environment Laboratories, Eastman Kodak Company, 1997.
55. **Marty, J. P., Trouvin, J. H., Jacquot, C., and Wepierre, J.**, Rate of percutaneous pharmacokinetics of ¹⁴C-hydroquinone, *Compt. Rend. Cong. Eur. Biopharm. Pharmacol.* 2, 221–228, 1981.
 56. **Marzulli, F. N., Brown, D. W. C., and Maibach, H. I.**, Techniques for studying skin penetration, *Toxicol. Appl. Pharmacol.* 3, 76–83, 1969.
 57. **Hamilton, M. L., Guest, D., and DiVincenzo, G. D.**, The Percutaneous Absorption of [U-¹⁴C]Hydroquinone in Beagle Dogs, TSCATS Database, EPA/OTS Doc #40–8569237, NTIS/OTS0518004, 1985.
 58. **Bucks, D. A., McMaster, J. R., Guy, R. H., and Maibach, H. I.**, Percutaneous absorption of hydroquinone in humans: effect of 1–dodecylazacycloheptane-2–one (azone) and the 2–ethylhexyl ester of 4–(dimethylamino)benzoic acid (Escalol 507), *J. Toxicol. Environ. Health* 24, 249–289, 1988.
 59. **Lehman, P. A. and Franz, T. J.**, Percutaneous absorption of hydroquinone in human, pig, monkey and baboon *in vitro*. *Proceedings: Society for Investigative Dermatology*, Baltimore; 1992 (abstract).
 60. **Wester, R. C., Melendres, J., Hui, X. Y., Cox, R., Serranzana, S., Zhai, H. B., Quan, D. Y., and Maibach, H. I.**, Human *in vivo* and *in vitro* hydroquinone topical bioavailability, metabolism, and disposition, *J. Toxicol. Environ. Health* 54, 301–317, 1998.
 61. **Hill, T. S., Morgott, D. A., and English, J. C.**, Tissue-to-plasma partition coefficients (PCS) for hydroquinone (HQ) in male Fischer and Sprague–Dawley rats, *Fundam. Appl. Toxicol.* 30, 39–40, 1996.
 62. **Greenlee, W. F., Gross, E. A., and Irons, R. D.**, Relationships between benzene toxicity and the disposition of ¹⁴C-labeled benzene metabolites in the rat, *Chem.-Biol. Interact.* 33, 285–299, 1981.
 63. **Greenlee, W. F., Sun, J. D., and Bus, J. S.**, A proposed mechanism of benzene toxicity: Formation of reactive intermediates from polyphenol metabolites, *Toxicol. Appl. Pharmacol.* 59, 187–195, 1981.
 64. **Subrahmanyam, V. V., Doane-Setzer, P., Steinmetz, K. L., Ross, D., and Smith, M. T.**, Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow *in vivo*: possible implications in benzene myelotoxicity, *Toxicology* 62, 107–116, 1990.
 65. **Legathe, A., Hoener, B., and Tozer, T. N.**, Pharmacokinetic interaction between benzene metabolites, phenol and hydroquinone, in B6C3F1 mice, *Toxicol. Appl. Pharmacol.* 124, 131–138, 1994.
 66. **Garton, G. A. and Williams, R. T.**, Studies in detoxication. XXI. The fates of quinol and resorcinol in the rabbit in relation to the metabolism of benzene, *Biochem. J.* 44, 234–238, 1949.
 67. **Nerland, D. E. and Pierce, W. M. J.**, Identification of *N*-acetyl-*S*-(2,5–dihydroxyphenyl)-*L*-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone, *Drug Metab. Disp.* 18, 958–961, 1990.
 68. **Hill, B. A., Kleiner, H. E., Ryan, E. A., Dulik, D. M., Monks, T. J., and Lau, S. S.**, Identification of multi-*S*-substituted conjugates of hydroquinone by HPLC-colorimetric electrode array analysis and mass spectroscopy, *Chem. Res. Toxicol.* 6, 459–469, 1993.
 69. **Gut, I., Nedelcheva, V., Soucek, P., Stopka, P., Vodicka, P., Gelboin, H. V., and Ingelman-Sundberg, M.**, The role of CYP2E1 and 2B1 in metabolic activation of benzene derivatives, *Arch. Toxicol.* 71, 45–56, 1996.
 70. **Lau, S. S., Sawalha, A. F., Halpert, J. R., Koop, D. R., and Monks, T. J.**, Cytochrome P450 catalyzed oxidation of hydroquinone in rodent and human microsomes, *Toxicologist* 36, 23(abstract), 1997.
 71. **Corley, R. A., English, J. C., and Morgott, D. A.**, A physiologically based pharmacokinetic model for hydroquinone, *Toxicol. Sci.* 1–S, 141(abstract), 1998.
 72. **Seaton, M. J., Schlosser, P. M., and Medinsky, M. A.**, *In vitro* conjugation of benzene metabolites by human liver: potential influence of interindividual variability on benzene toxicity, *Carcinogenesis* 16, 1519–1527, 1995.
 73. **Cappiello, M., Giuliani, L., and Pacifici, G. M.**, Differential distribution of phenol and catechol sulphotransferases in human liver and intestinal mucosa, *Pharmacology* 40, 69–76, 1990.
 74. **Cassidy, M. K. and Houston, J. B.**, *In vivo* capacity of hepatic and extrahepatic enzymes to conjugate phenol, *Drug Metab. Disp.* 12, 619–624, 1984.
 75. **Jeftic, L. and Manning, G.**, A survey on the electrochemical reduction of quinones, *J. Electroanal. Chem.* 26, 195–200, 1970.
 76. **Finley, K. T.**, The addition and substitution chemistry of quinones. In: Patai, S., Ed., *The Chemistry of the Quinoid Compounds, Part II*. Wiley, London, 1974, 878–1144.
 77. **Peter, M. G.**, Chemical modifications of biopolymers by quinones and quinone methides, *Angew. Chem. Int. Ed.* 28, 555–570, 1989.
 78. **Monks, T. J., Hanzlik, R. P., Cohen, G. M., Ross, D., and Graham, D. G.**, Quinone chemistry and toxicity, *Toxicol. Appl. Pharmacol.* 112, 2–16, 1992.
 79. **Smith, M. T., Evans, C. G., Thor, H., and Orrenius, S.**, Quinone induced oxidative injury to cells and tissues. In: Sies, H. Ed. *Oxidative Stress*, Academic Press, London, 1985, 91–113.
 80. **O'Brien, P. J.**, Molecular mechanisms of quinone cytotoxicity, *Chem.-Biol. Interact.* 80, 1–41, 1991.
 81. **Schlosser, M. J., Shurina, R. D., and Kalf, G. D.**, Metabolism of phenol and hydroquinone to reactive products by macrophage peroxidase or purified prostaglandin H synthase, *Environ. Health Perspect.* 82, 229–237, 1989.
 82. **Munday, R.**, Inhibition of naphthohydroquinone autoxidation by DT-diaphorase (NAD(P)H:[quinone acceptor]oxidoreductase), *Redox Report* 3, 189–196, 1997.
 83. **Schlosser, M. J. and Kalf, G. F.**, Metabolic activation of hydroquinone by macrophage peroxidase, *Chem.-Biol. Interact.* 72, 191–207, 1989.
 84. **Boatman, R. J., Perry, L. G., Fiorica, L. A., Polvino, J. M., and English, J. C.**, Quantification of covalent

- protein adducts of hydroquinone in the blood and kidneys of rats, *Toxicologist* 14, 74(abstrakt), 1994.
85. **Ekström, T., Warholm, M., Kronevi, T., and Högborg, J.**, Recovery of malondialdehyde in urine as a 2,4-dinitrophenylhydrazine derivative after exposure to chloroform or hydroquinone, *Chem.-Biol. Interact.* 67, 25–31, 1988.
 86. **Stenius, U. and Högborg, J.**, γ -Glutamyltranspeptidase-conferred resistance to hydroquinone induced GSH depletion and toxicity in isolated hepatocytes, *Carcinogenesis* 9, 1223–1227, 1988.
 87. **Nakagawa, Y. and Moldeu, P.**, Cytotoxic effects of phenyl-hydroquinone and some hydroquinones on isolated rat hepatocytes, *Biochem. Pharmacol.* 44, 1059–1065, 1992.
 88. **English, J. C., Deisinger, P. J., Hill, T., and Perry, L. G.**, Measurement of glutathione and cysteine in the kidneys of rats after oral treatment with hydroquinone, *Int. Toxicol.* 11–PF-6(abstrakt), 1995.
 89. **Okazaki, S., Hoshiya, T., Takahashi, S., Futakuchi, M., Saito, K., and Hirose, M.**, Modification of hepato- and renal carcinogenesis by catechol and its isomers in rats pretreated with *N*-ethyl-*N*-hydroxyethylnitrosamine, *Teratogen. Carcinogen. Mutagen.* 13, 127–137, 1993.
 90. **Ganousis, L. G., Goon, D., Zyglewska, T., Wu, K. K., and Ross, D.**, Cell-specific metabolism in mouse bone marrow stroma: studies of activation and detoxification of benzene metabolites, *Mol. Pharmacol.* 42, 1118–1125, 1992.
 91. **Epe, B., Harttig, U., Stopper, H., and Metzler, M.**, Covalent binding of reactive estrogen metabolites to microtubular protein as a possible mechanism of aneuploidy induction and neoplastic cell transformation, *Environ. Health Perspect.* 88, 123–127, 1990.
 92. **Fiorica, L. A., Hill, T. S., Morgott, D. A., and English, J. C.**, The plasma protein binding of hydroquinone (HQ) in rats and humans, *Toxicologist* 30, 40(abstrakt), 1996.
 93. **McDonald, T. A., Waidyanatha, S., and Rappaport, S. M.**, Measurement of adducts of benzoquinone with hemoglobin and albumin, *Carcinogenesis* 14, 1927–1932, 1993.
 94. **Waidyanatha, S., Yeowelloconnell, K., and Rappaport, S. M.**, A new assay for albumin and hemoglobin adducts of 1,2- and 1,4-benzoquinones, *Chem.-Biol. Interact.* 115, 117–139, 1998.
 95. **McDonald, T. A., Yeowell-O'Connell, K., and Rappaport, S. M.**, Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [¹⁴C/¹³C]benzene, *Cancer Res.* 54, 4907–4914, 1994.
 96. **Frantz, C. E., Chen, H., and Eastmond, D. A.**, Inhibition of human topoisomerase II *in vitro* by bioactive benzene metabolites, *Environ. Health Perspect.* 104, 1319–1323, 1996.
 97. **Schwartz, C. S., Snyder, R., and Kalf, G. F.**, The inhibition of mitochondrial DNA replication *in vitro* by the metabolites of benzene, hydroquinone and *p*-benzoquinone, *Chem.-Biol. Interact.* 53, 327–350, 1985.
 98. **Soucek, P., Filipcova, B., and Gut, I.**, Cytochrome P450 destruction and radical scavenging by benzene and its metabolites, *Biochem. Pharmacol.* 47, 2233–2242, 1994.
 99. **Gut, I., Nedelcheva, V., Soucek, P., Stopka, P., and Tichavská, B.**, Cytochromes P450 in benzene metabolism and involvement of their metabolites and reactive oxygen species in toxicity, *Environ. Health Perspect.* 104, 1211–1218, 1996.
 100. **Rushmore, T., Snyder, R., and Kalf, G.**, Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*, *Chem.-Biol. Interact.* 49, 133–154, 1984.
 101. **Kalf, G. F., Snyder, R., and Rushmore, T. H.**, Inhibition of RNA synthesis by benzene metabolites and their covalent binding to DNA in rabbit bone marrow mitochondria *in vitro*, *Am. J. Ind. Med.* 7, 485–492, 1985.
 102. **Jowa, L., Witz, G., Snyder, R., Winkle, S., and Kalf, G. F.**, Synthesis and characterization of deoxyguanosine-benzoquinone adducts, *J. Appl. Toxicol.* 10, 47–54, 1990.
 103. **Pathak, D. N., Lévy, G., and Bodell, W. J.**, DNA adduct formation in the bone marrow of B6C3F1 mice treated with benzene, *Carcinogenesis* 16, 1803–1808, 1995.
 104. **Reddy, M. V., Blackburn, G. R., Irwin, S. E., Kommineni, C., Mackerer, C. R., and Mehlmán, M. A.**, A method for *in vitro* culture of rat zymbal gland: use in mechanistic studies of benzene carcinogenesis in combination with ³²P-postlabeling, *Environ. Health Perspect.* 82, 239–247, 1989.
 105. **Levy, G., Pongracz, K., and Bodell, W. J.**, Detection of DNA adducts in HL-60 cells treated with hydroquinone and *p*-benzoquinone by ³²P-postlabeling, *Carcinogenesis* 12, 1181–1186, 1991.
 106. **Levy, G. and Bodell, W. J.**, Potentiation of DNA adduct formation in HL-60 cells by combinations of benzene metabolites, *Proc. Nat. Acad. Sci. U.S.A.* 89, 7105–7109, 1992.
 107. **Bodell, W. J., Lévy, G., and Pongracz, K.**, Investigation of benzene-DNA adducts and their detection in human bone marrow, *Environ. Health Perspect.* 99, 241–244, 1993.
 108. **Hedli, C. C., Rao, N. R., Reuhl, K. R., Witmer, C. M., and Snyder, R.**, Effects of benzene metabolite treatment on granulocytic differentiation and DNA adduct formation in HL-60 cells, *Arch. Toxicol.* 70, 135–144, 1996.
 109. **Levy, G. and Bodell, W. J.**, Role of hydrogen peroxide in the formation of DNA adducts in HL-60 cells treated with benzene metabolites, *Biochem. Biophys. Res. Comm.* 222, 44–49, 1996.
 110. **Pongracz, K. and Bodell, W. J.**, Synthesis of *N*²-(4-hydroxyphenyl)-2'-deoxyguanosine 3'-phosphate: comparison by ³²P-postlabeling with the DNA adduct formed in HL-60 cells treated with hydroquinone, *Chem. Res. Toxicol.* 9, 593–598, 1996.

111. **Kolachana, P., Subrahmanyam, V. V., Meyer, K. B., Zhang, L., and Smith, M. T.,** Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells *in vitro* and in the bone marrow *in vivo*, *Cancer Res.* 53, 1023–1026, 1993.
112. **Schlosser, M. J., Shurina, R. D., and Kalf, G. D.,** Prostaglandin H synthase catalyzed oxidation of hydroquinone to a sulfhydryl-binding and DNA-damaging metabolite, *Chem. Res. Toxicol.* 3, 333–339, 1990.
113. **Reddy, M. V., Bleicher, W. T., Blackburn, G. R., and Mackerer, C. R.,** DNA adduction by phenol, hydroquinone, or benzoquinone *in vitro* but not *in vivo*: nuclease P1-enhanced ³²P-postlabeling of adducts as labeled nucleoside biphosphates, dinucleotides and nucleoside monophosphates, *Carcinogenesis* 11, 1349–1357, 1990.
114. **English, J. C., Perry, L. G., Vlaovic, M., Moyer, C., and O'Donoghue, J. L.,** Measurement of nuclear DNA modification by ³²P-postlabeling in the kidney of male and female Fischer 344 rats after multiple gavage doses of hydroquinone, *Fundam. Appl. Toxicol.* 23, 391–396.
115. **Reddy, M. V., Hill, T., O'Donoghue, J. L., and English, J. C.,** Lack of oxidative DNA damage by hydroquinone in the kidneys of rats after oral administration, *Proc. Am. Assoc. Cancer Res.* 38, 78(ab-stract), 1997.
116. **Cho, D. H., Hong, J. T., Chin, K., Cho, T. S., and Lee, B. M.,** Organotropic formation and disappearance of 8-hydroxydeoxyguanosine in the kidney of Sprague-Dawley rats exposed to Adriamycin and KBrO₃, *Cancer Lett.* 74, 141–145, 1993.
117. **Brusick, D.,** Mutagenic Evaluation of Compound 75–151 (HQ). LBI Project #2547, Kensington, Maryland: Litton Bionetics, Inc. 1975.
118. **Cotruvo, J. A., Simmon, V. F., and Spanggord, R. J.,** Investigation of mutagenic effects of products of ozonation research in water, *Ann. N.Y. Acad. Sci.* 298, 124–140, 1978.
119. **Epler, J. L., Larimer, F. W., Rao, T. K., Nix, C. E., and Ho, T.,** Energy-related pollutants in the environment: use of short-term tests for mutagenicity in the isolation and identification of biohazards, *Environ. Health Perspect.* 27, 11–20, 1978.
120. **Florin, I., Rutberg, L., Curvall, M., and Enzell, C. R.,** Screening of tobacco smoke constituents for mutagenicity using the Ames test, *Toxicology* 15, 219–232, 1980.
121. **Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E.,** *Salmonella* mutagenicity test results for 250 chemicals, *Environ. Mutagen.* 5, 1–142, 1983.
122. **Sakai, M., Yoshida, D., and Mizusaki, S.,** Mutagenicity of polycyclic aromatic hydrocarbons and quinones on *Salmonella typhimurium* TA97, *Mutat. Res.* 156, 61–67, 1985.
123. **Rossmann, T. G., Klein, C. B., and Snyder, C. A.,** Mutagenic metabolites of benzene detected in the microscreen assay, *Environ. Health Perspect.* 81, 77–79, 1989.
124. **Glatt, H., Padykula, R., Berchtold, G. A., Ludewig, G., Platt, K. L., Klein, J., and Oesch, F.,** Multiple activation pathways of benzene leading to products with varying genotoxic characteristics, *Environ. Health Perspect.* 82, 81–89, 1989.
125. **Gocke, E., King, M.-T., Eckhardt, K., and Wild, D.,** Mutagenicity of cosmetics ingredients licensed by the European Community, *Mutat. Res.* 90, 91–109, 1981.
126. **Murphy, S. and Serva, R.,** Lack of Mutagenicity of Hydroquinone (HQ), Tetramethylthiuram Monosulfide (TMTM), and Tetramethylthiuram Disulfide (TMTD), TSCATS Database, EPA/OTS Doc #88–920005216, NTIS/OTS0544190, 1992.
127. **Gocke, E., Wild, D., Eckhardt, K., and King, M.-T.,** Mutagenicity studies with the mouse spot test, *Mutat. Res.* 117, 201–212, 1983.
128. **Krasavage, W. J.,** Hydroquinone: A Dominant Lethal Assay in Male Rats, TSCATS Database, EPA/OTS Doc #878214709, NTIS/OTS206628, 1984.
129. **Wild, D., King, M.-T., Eckhardt, K., and Gocke, E.,** Mutagenic activity of aminophenols and diphenols, and relations with chemical structure, *Mutat. Res.* 85, 456(ab-stract), 1981.
130. **Lin, J. K. and Lee, S. F.,** Enhancement of the mutagenicity of polyphenols by chlorination and nitrosation in *Salmonella typhimurium*, *Mutat. Res.* 269, 217–224, 1992.
131. **Koike, N., Haga, S., Ubukata, N., Sakurai, M., Shimizu, H., and Sato, A.,** Mutagenicity of benzene metabolites by fluctuation test, *Jpn. J. Ind. Health* 30, 475–480, 1988.
132. **Tsutsui, T., Hayashi, N., Maizumi, H., Huff, J., and Barrett, J. C.,** Benzene-, catechol-, hydroquinone-, and phenol-induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis in Syrian hamster embryo cells, *Mutat. Res.* 373, 113–123, 1997.
133. **McGregor, D. B., Riach, C. G., Brown, A., Edwards, L., Reynolds, D., West, K., and Willington, S.,** Reactivity of catecholamines and related substances in the mouse lymphoma L5178Y cell assay for mutagens, *Environ. Mol. Mutagen.* 11, 523–544, 1988.
134. **Adler, I.-D.,** Synopsis of the *in vivo* results obtained with the 10 known or suspected aneugens tested in the CEC collaborative study, *Mutat. Res.* 287, 131–137, 1993.
135. **Parry, J. M. and Sors, A.,** The detection and assessment of the aneugenic potential of environmental chemicals: the European Community Aneuploidy Project, *Mutat. Res.* 287, 3–15, 1993.
136. **Paramentier, R. and Dustin, P.,** Early effects of hydroquinone on mitosis, *Nature* 171, 527–528, 1948.
137. **Rosin, A. and Doljanski, F.,** Effect of hydroquinone on mitosis, *Nature* 172, 1151, 1953.
138. **Galloway, S. M., Armstrong, M. J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A. D., Nakamura, F., Ahmed, M., Duk, S., Rimpou, J., Margolin, B. H., Resnick, M. A., Anderson, B., and**

- Zeiger, E.**, Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals, *Environ. Mol. Mutagen.* 10, 1–175, 1987.
139. **Morimoto, K. and Koizumi, A.**, Inhibition of rejoining of radiation-induced chromosome lesions and induction of sister chromatid exchanges — effects of benzene or its metabolites in cultured human leukocytes, *Jpn. J. Hum. Genet.* 23, 279–281, 1978.
140. **Morimoto, K. and Wolff, S.**, Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites, *Cancer Res.* 40, 1189–1193, 1980.
141. **Morimoto, K., Wolff, S., and Koizumi, A.**, Induction of sister-chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites, *Mutat. Res.* 119, 355–360, 1983.
142. **Morimoto, K., Takeshita, T., Takeuchi, T., Maruyama, S., Ezoe, S., Mure, K., and Inoue, C.**, Chromosome alterations in peripheral lymphocytes as indices of lifestyle and genotoxicity, *Int. Arch. Occup. Environ. Health* 65, S37–S41, 1993.
143. **Knadle, S.**, Synergistic interaction between hydroquinone and acetaldehyde in the induction of sister chromatid exchange in human lymphocytes *in vitro*, *Cancer Res.* 45, 4853–4857, 1985.
144. **Erexson, G. L., Wilmer, J. L., and Kligerman, A. D.**, Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites *in vitro*, *Cancer Res.* 45, 2471–2477, 1985.
145. **Pacchierotti, F., Bassani, B., Leopardi, P., and Zijno, A.**, Origin of aneuploidy in relation to disturbances of cell-cycle progression. II. Cytogenetic analysis of various parameters in mouse bone marrow cells after colchicine or hydroquinone treatment, *Mutagenesis* 6, 307–311, 1991.
146. **Yager, J. W., Eastmond, D. A., Robertson, M. L., Paradisin, W. M., and Smith, M. T.**, Characterization of micronuclei induced in human lymphocytes by benzene metabolites, *Cancer Res.* 50, 393–399, 1990.
147. **Migliore, L. and Nieri, M.**, Evaluation of twelve potential aneuploidogenic chemicals by the *in vitro* human lymphocyte micronucleus assay, *Toxicol. In Vitro* 5, 325–336, 1991.
148. **Robertson, M. L., Eastmond, D. A., and Smith, M. T.**, Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes, *Mutat. Res.* 249, 201–209, 1991.
149. **Vian, L., van Hummelen, P., Bichet, N., Gouy, D., and Kirsch-Volders, M.**, Evaluation of hydroquinone and chloral hydrate on the *in vitro* micronucleus test on isolated lymphocytes, *Mutat. Res.* 334, 1–7, 1995.
150. **Ferguson, L. R., Morcombe, P., and Triggs, C. N.**, The size of cytokinesis-blocked micronuclei in human peripheral blood lymphocytes as a measure of aneuploidy induction by Set A compounds in the EEC trial, *Mutat. Res.* 287, 101–112, 1993.
151. **Bonatti, S., Cavalieri, Z., Viaggi, S., and Abbondandolo, A.**, The analysis of 10 potential spindle poisons for their ability to induce CREST-positive micronuclei in human diploid fibroblasts, *Mutagenesis* 7, 111–114, 1992.
152. **Glatt, H., Gemperlein, I., Setiabudi, F., Platt, K. L., and Oesch, F.**, Expression of xenobiotic-metabolizing enzymes in propagatable cell cultures and induction of micronuclei by 13 compounds, *Mutagenesis* 5, 241–249, 1990.
153. **Antoccia, A., Degrassi, F., Battistoni, A., Ciliutti, P., and Tanzarella, C.**, *In vitro* micronucleus test with kinetochore staining: evaluation of test performance, *Mutagenesis* 6, 319–324, 1991.
154. **Ellard, S. and Parry, E. M.**, Induction of micronuclei in V79 Chinese hamster cells by hydroquinone and econazol nitrate, *Mutat. Res.* 287, 87–91, 1993.
155. **Seelbach, A., Fissler, B., and Madle, S.**, Further evaluation of a modified micronucleus assay with V79 cells for detection of aneugenic effects, *Mutat. Res.* 303, 163–169, 1993.
156. **Dobo, K. I. and Eastmond, D. A.**, Role of oxygen radicals in the chromosomal loss and breakage induced by the quinone-forming compounds, hydroquinone and *tert*-butylhydroquinone, *Environ. Mol. Mutagen.* 24, 293–300, 1994.
157. **Tunek, A., Hogstedt, B., and Olofsson, T.**, Mechanism of benzene toxicity, effects of benzene and benzene metabolites on bone marrow cellularity, number of granulopoietic stem cells and frequency of micronuclei in mice, *Chem.-Biol. Interact.* 39, 129–138, 1982.
158. **Gad-El-Karim, M. M., Ramanujam, V. S., Ahmed, A. E., and Legator, M. S.**, Benzene myeloclastogenicity: a function of its metabolism, *Am. J. Ind. Med.* 7, 475–484, 1985.
159. **Gad-El-Karim, M. M., Sadagopa Ramanujam, V. M., and Legator, M. S.**, *trans, trans*-Muconic acid, an open-chain urinary metabolite of benzene in mice. Quantification by high-pressure liquid chromatography, *Xenobiotica* 15, 211–220, 1985.
160. **Ciranni, R., Barale, R., Marrazzini, A., and Loprieno, N.**, Benzene and the genotoxicity of its metabolites. II. The effect of the route of administration on the micronuclei and bone marrow depression in mouse bone marrow cells, *Mutat. Res.* 209, 23–28, 1988.
161. **Adler, I. and Kleisch, U.**, Comparison of single and multiple treatment regimens in the mouse bone marrow micronucleus assay for hydroquinone (HQ) and cyclophosphamide (CP), *Mutat. Res.* 234, 115–123, 1990.
162. **Barale, R., Marrazzini, A., Betti, C., Vangelisti, V., Loprieno, N., and Barrai, I.**, Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects *in vivo*, *Mutat. Res.* 244, 15–20, 1990.
163. **Adler, I.-D., Kliesch, U., van Hummelen, P., and Kirsch-Volders, M.**, Mouse micronucleus tests with

- known and suspected spindle poisons: results from two laboratories, *Mutagenesis* 6, 47–53, 1991.
164. **Gudi, R., Xu, J., and Thilagar, A.**, Assessment of the *in vivo* aneuploidy/micronucleus assay in mouse bone marrow cells with 16 chemicals, *Environ. Mol. Mutagen.* 20, 106–116, 1992.
 165. **van Hummelen, P., Deleener, A., Vanparys, P. H., and Kirsch-Volders, M.**, Discrimination of aneuploidogens from clastogens by C-banding, DNA and area measurements of micronuclei from mouse bone marrow, *Mutat. Res.* 271, 13–28, 1992.
 166. **Chen, H. W., Tomar, R., and Eastmond, D. A.**, Detection of hydroquinone-induced nonrandom breakage in the centromeric heterochromatin of mouse bone marrow cells using multicolor fluorescence *in situ* hybridization with the mouse major and minor satellite probes, *Mutagenesis* 9, 563–569, 1994.
 167. **Marrazzini, A., Betti, C., Bernacchi, F., Barraï, I., and Barale, R.**, Micronucleus test and metaphase analyses in mice exposed to known and suspected spindle poisons, *Mutagenesis* 9, 505–515, 1994.
 168. **Marrazzini, A., Chelotti, L., Barraï, I., Loprieno, N., and Barale, R.**, *In vivo* genotoxic interactions among three phenolic benzene metabolites, *Mutat. Res.* 341, 29–46, 1994.
 169. **Grawe, J., Nusse, M., and Adler, I. D.**, Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. I. Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity, *Mutagenesis* 12, 1–8, 1997.
 170. **Grawe, J., Adler, I. D., and Nusse, M.**, Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. II. Analysis of micronuclei of aneugenic and clastogenic origin by dual-colour FISH on populations of bone marrow PCEs flow sorted on the basis of their relative DNA content, *Mutagenesis* 12, 9–16, 1997.
 171. **Ciranni, R., Barale, R., Marrazzini, A., and Loprieno, N.**, Benzene and the genotoxicity of its metabolites. I. Transplacental activity in mouse fetuses and in their dams, *Mutat. Res.* 208, 61–67, 1988.
 172. **Rupa, D. S., Schuler, M., and Eastmond, D. A.**, Detection of hyperdiploidy and breakage affecting the 1cen-1q12 region of cultured interphase human lymphocytes treated with various genotoxic agents, *Environ. Mol. Mutagen.* 29, 161–167, 1997.
 173. **Miller, B.-M. and Adler, I.-D.**, Aneuploidy induction in mouse spermatocytes, *Mutagenesis*, 7, 69–76, 1992.
 174. **Leopardi, P., Zijno, A., Bassani, B., and Pacchierotti, F.**, *In vivo* studies on chemically induced aneuploidy in mouse somatic and germinal cells, *Mutat. Res.* 287, 119–130, 1993.
 175. **Marrazzini, A., Betti, C., Barale, R., Bernacchi, F., and Loprieno, N.**, Cytogenetic effects of possible aneuploidizing agents, *Mutat. Res.* 252, 195–196, 1991.
 176. **Eastmond, D. A., Rupa, D. S., and Hasegawa, L. S.**, Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence *in situ* hybridization with DNA probes, *Mutat. Res.* 322, 9–20, 1994.
 177. **Albertini, S.**, Analysis of nine known or suspected spindle poisons for mitotic chromosome malsegregation using *Saccharomyces cerevisiae* D61. M, *Mutagenesis* 5, 453–459, 1990.
 178. **Xu, W. and Adler, I.-D.**, Clastogenic effects of known and suspected spindle poisons studied by chromosome analysis in mouse bone marrow cells, *Mutagenesis* 5, 371–374, 1990.
 179. **Ciranni, R. and Adler, I.**, Clastogenic effects of hydroquinone: induction of chromosomal aberrations in mouse germ cells, *Mutat. Res.* 263, 223–229, 1991.
 180. **Crebelli, R., Conti, G., and Carere, A.**, On the mechanism of mitotic segregation induction in *Aspergillus nidulans* by benzene hydroxy metabolites, *Mutagenesis* 2, 235–238, 1987.
 181. **Crebelli, R., Conti, G., Conti, L., and Carere, A.**, *In vitro* studies with nine known or suspected spindle poisons: results in tests for chromosome malsegregation in *Aspergillus nidulans*, *Mutagenesis* 6, 131–136, 1991.
 182. **Bilimoria, M.**, Detection of mutagenic activity of chemicals and tobacco smoke in a bacterial system, *Mutat. Res.* 31, 328(abstract), 1975.
 183. **Painter, R. B. and Howard, R.**, The HeLa DNA-synthesis inhibition test as a rapid screen for mutagenic carcinogens, *Mutat. Res.* 92, 427–437, 1982.
 184. **Lewis, J. G., Stewart, W., and Adams, D. O.**, Role of oxygen radicals in induction of DNA damage by metabolites of benzene, *Cancer Res.* 48, 4762–4765, 1988.
 185. **Lewis, J. G., Odom, B., and Adams, D. O.**, Toxic effects of benzene and benzene metabolites on mononuclear phagocytes, *Toxicol. Appl. Pharmacol.* 92, 246–254, 1988.
 186. **Maeda, M., Yamada, K., Ikeda, I., Nakajima, H., Tajima, M., and Murakami, H.**, Effects of phenyl compounds on proliferation and IgM production of human-human hybridoma HB4C5 cells cultured in serum-free medium, *Agric. Biol. Chem.* 54, 1093–1096, 1990.
 187. **Stenius, U., Warholm, M., Rannug, A., Walles, S., Lundberg, I., and Högberg, J.**, The role of GSH depletion and toxicity in hydroquinone-induced development of enzyme-altered foci, *Carcinogenesis* 10, 593–599, 1989.
 188. **Walles, S.**, Mechanisms of DNA damage induced in rat hepatocytes by quinones, *Cancer Lett.* 63, 47–52, 1992.
 189. **Pellack-Walker, P. and Blumer, J. L.**, DNA damage in L5178Y cells following exposure to benzene metabolites, *Mol. Pharmacol.* 30, 42–47, 1986.
 190. **Shimada, H., Sato, T., Hattori, C., Satake, S., and Itoh, S.**, Induction of micronuclei by benzene and its metabolites, *Mutat. Res.* 216, 377(abstract), 1989.

191. **Sze, C. C., Shi, C. Y., and Ong, C. N.,** Cytotoxicity and DNA strand breaks induced by benzene and its metabolites in Chinese hamster ovary cells, *J. Appl. Toxicol.* 16, 259–264, 1996.
192. **Anderson, D., Yu, T. W., and Schmezer, P.,** An investigation of the DNA-damaging ability of benzene and its metabolites in human lymphocytes, using the comet assay, *Environ. Mol. Mutagen.* 26, 305–314, 1995.
193. **Leanderson, P. and Tagesson, C.,** Cigarette smoke-induced DNA damage in cultured human lung cells: role of hydroxyl radicals and endonuclease activation, *Chem.-Biol. Interact.* 81, 197–208, 1992.
194. **Andreoli, C., Leopardi, P., and Crebelli, R.,** Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites, *Mutat. Res.* 377, 95–104, 1997.
195. **Zhang, L. P., Wang, Y. X., Shang, N., and Smith, M. T.,** Benzene metabolites induce the loss and long arm deletion of chromosomes 5 and 7 in human lymphocytes, *Leuk. Res.* 22, 105–113, 1998.
196. **Smith, M. T. and Zhang, L. P.,** Biomarkers of leukemia risk: benzene as a model, *Environ. Health Perspect.* 106, 937–946, 1996.
197. **Kappas, A.,** On the mechanism of induced aneuploidy in *Aspergillus nidulans* and validation of tests for genomic mutations. In: Resnick, M. A. and Vig, B. K., Eds., *Mechanisms of Chromosome Distribution and Aneuploidy*. Alan R. Liss, Inc., New York, 1989, 377–384.
198. **Miller, B. M. and Adler, I. D.,** Suspect spindle poisons: analysis of c-mitotic effects in mouse bone marrow cells, *Mutagenesis* 4, 208–215, 1989.
199. **Warr, T. J., Parry, E. M., and Parry, J. M.,** A comparison of two *in vitro* mammalian cell cytogenetic assays for the detection of mitotic aneuploidy using 10 known or suspected aneugens, *Mutat. Res.* 287, 29–46, 1993.
200. **Hader, C., Hadnagy, W., and Seemayer, N. H.,** A rapid method for detection of nongenotoxic carcinogens of environmental pollutants using synchronized V79 cells and flow cytometry, *Toxicol. Lett.* 88, 99–108, 1996.
201. **Seiler, J. P.,** Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short-term test, *Mutat. Res.* 46, 305–310, 1977.
202. **Post, G. B., Snyder, R., and Kalf, G. F.,** Inhibition of mRNA synthesis in rabbit bone marrow nuclei *in vitro* by quinone metabolites of benzene, *Chem.-Biol. Interact.* 50, 203–211, 1984.
203. **Post, G. B., Snyder, R., and Kalf, G. F.,** Inhibition of RNA synthesis and interleukin-2 production in lymphocytes *in vitro* by benzene and its metabolites, hydroquinone and *p*-benzoquinone, *Toxicol. Lett.* 29, 161–167, 1985.
204. **Pellack-Walker, P., Walker, J. K., Evans, H. H., and Blumer, J. L.,** Relationship between the oxidation potential of benzene metabolites and their inhibitory effect on DNA synthesis in L517YS cells, *Mol. Pharmacol.* 28, 560–566, 1985.
205. **Lee, E. W., Johnson, J. T., and Garner, C. D.,** Inhibitory effect of benzene metabolites on nuclear DNA synthesis in bone marrow cells, *J. Toxicol. Environ. Health* 26: 277–291, 1989.
206. **International Programme on Chemical Safety (IPCS),** *Hydroquinone Health and Safety Guide*, World Health Organization, Geneva, 1996.
207. **Irons, R. D., Neptun, D. A., and Pfeifer, R. W.,** Inhibition of lymphocyte transformation and microtubule assembly by quinone metabolites of benzene: evidence for a common mechanism, *J. Reticuloendothel. Soc.* 30, 359–372, 1981.
208. **Brunner, M., Albertini, S., and Wurgler, F. E.,** Effects of 10 known or suspected spindle poisons in the *in vitro* porcine brain tubulin assembly assay, *Mutagenesis* 6, 65–70, 1991.
209. **Wallin, M. and Hartley-Asp, B.,** Effects of potential aneuploidy inducing agents on microtubule assembly *in vitro*, *Mutat. Res.* 287, 17–22, 1993.
210. **Li, Y. and Trush, M. A.,** DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation, *Carcinogenesis* 14, 1303–1311, 1993.
211. **Li, Y., Kuppasamy, P., Zweier, J. L., and Trush, M. A.,** ESR evidence for the generation of reactive oxygen species from the copper-mediated oxidation of the benzene metabolite, hydroquinone: role in DNA damage, *Chem.-Biol. Interact.* 94, 101–120, 1995.
212. **Hiraku, Y. and Kawanishi, S.,** Oxidative DNA damage and apoptosis induced by benzene metabolites, *Cancer Res.* 56, 5172–5178, 1996.
213. **Yu, T. W. and Anderson, D.,** Reactive oxygen species-induced DNA damage and its modification: A chemical investigation, *Mutat. Res.* 379, 201–210, 1997.
214. **Lee, E. W. and Gardner, C. D.,** Effects of benzene on DNA strand breaks *in vivo* versus benzene metabolite-induced DNA strand breaks *in vitro* in mouse bone marrow cells, *Toxicol. Appl. Pharmacol.* 108, 497–508, 1991.
215. **Hutt, A. M. and Kalf, G. F.,** Inhibition of human DNA topoisomerase II by hydroquinone and *p*-benzoquinone, reactive metabolites of benzene, *Environ. Health Perspect.* 104(Suppl. 6), 1265–1269, 1996.
216. **Mozhaev, E. A., Osintseva, V. P., and Arzamastsev, E. V.,** Hydroquinone toxicity in chronic poisoning (Russian), *Farmikol. Toksikol.* 29, 238–240, 1966.
217. **Rao, G. S., Siddiqui, S. M., Pandya, K. P., and Shanker, R.,** Relative toxicity of metabolites of benzene in mice, *Vet. Hum. Toxicol.* 30, 517–520, 1988.
218. **Kari, F. W., Bucher, J., Eustis, S. L., Haseman, J. K., and Huff, J. E.,** Toxicity and carcinogenicity of hydroquinone in F344/N rats and B6C3F₁ mice, *Food Chem. Toxicol.* 30, 737–747, 1992.
219. **Boatman, R. J., English, J. C., Perry, L. G., and Bialecki, V. E.,** Differences in the nephrotoxicity of

- hydroquinone among Fischer 344 and Sprague-Dawley Rats and B6C3F₁ mice, *J. Toxicol. Environ. Health* 47, 159–172, 1996.
220. **Shibata, M.-A., Hirose, M., Tanaka, H., Asakawa, E., Shirai, T., and Nobuyuki, I.**, Induction of renal cell tumors in rats and mice, and enhancement of hepatocellular tumor development in mice after long-term hydroquinone treatment, *Jpn. J. Cancer Res.* 82, 1211–1219, 1991.
 221. **David, R. M., English, J. C., Totman, L. C., Moyer, C., and O'Donoghue, J. L.**, Lack of nephrotoxicity and renal cell proliferation following subchronic dermal application of a hydroquinone cream, *Food Chem. Toxicol.* 36, 609–616, 1998.
 222. **Perry, L. G., English, J. C., Vlaovic, M., Moyer, C., and Edna, J. L.**, Measurement of cell proliferation in the kidneys of rats after oral administration of hydroquinone, *Toxicologist* 13, 394(abstract), 1993.
 223. **Lau, S. S., Hill, B. A., Hight, R. J., and Monks, T. J.**, Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution, *Mol. Pharmacol.* 34, 829–836, 1988.
 224. **Hill, B. A., Monks, T. J., and Lau, S.**, The effects of 2,3,5-(triglutathion-*S*-yl)hydroquinone on renal mitochondrial respiratory function *in vivo* and *in vitro*: possible role in cytotoxicity, *Toxicol. Appl. Pharmacol.* 117, 165–171, 1992.
 225. **Hill, B. A., Davidson, K. L., Dulik, D. M., Monks, T. J., and Lau, S. S.**, Metabolism of 2-(glutathion-*S*-yl)hydroquinone and 2,3,5-(triglutathion-*S*-yl)hydroquinone in the *in situ* perfused rat kidney: Relationship to nephrotoxicity, *Toxicol. Appl. Pharmacol.* 129, 121–132, 1994.
 226. **Eckert, K.-G., Eyer, P., Sonnenbichler, J., and Zetl, I.**, Activation and detoxication of aminophenols. III. Synthesis and structural elucidation of various glutathione addition products to 1,4-benzoquinone, *Xenobiotica* 20, 351–361, 1990.
 227. **Barber, E. D., Polvino, J. M., and English, J. C.**, Acetylation of (L-cystein-*S*-yl)hydroquinone in the liver and kidney of male and female Fischer (F344) rats and male Sprague-Dawley (SD) rats, *Int. Toxicol.* 69, 12(abstract), 1995.
 228. **Lau, S. S., Kleiner, H. E., and Monks, T. J.**, Metabolism as a determinant of species susceptibility to 2,3,5-(triglutathion-*S*-yl)hydroquinone-mediated nephrotoxicity, *Drug Metab. Disp.* 23, 1136–1142, 1995.
 229. **English, J. C., Perry, L. G., Vlaovic, M., Moyer, C., and O'Donoghue, J. L.**, Measurement of cell proliferation in the kidneys of Fischer 344 and Sprague-Dawley rats after gavage administration of hydroquinone, *Fundam. Appl. Toxicol.* 23, 297–406, 1994.
 230. **O'Donoghue, J. L. and English, J. C.**, Some comments on the potential effects of hydroquinone exposure, *Food Chem. Toxicol.* 32, 863–864, 1994.
 231. **Konishi, N. and Ward, J. M.**, Increased levels of DNA synthesis in hyperplastic renal tubules of aging nephropathy in female F344/NCR rats, *Vet. Pathol.* 26, 6–10, 1989.
 232. **Hirose, M., Inoue, T., Asamoto, M., Tagawa, Y., and Ito, N.**, Comparison of the effects of 13 phenolic compounds in induction of proliferative lesions of the forestomach and increase in the labelling indices of the glandular stomach and urinary bladder epithelium of Syrian golden hamsters, *Carcinogenesis* 7, 1285–1289, 1986.
 233. **Hirose, M., Yamaguchi, S., Fukushima, S., Hasegawa, R., Takahashi, S., and Ito, N.**, Promotion by dihydroxybenzene derivatives of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced F344 rat forestomach and glandular stomach carcinogenesis, *Cancer Res.* 49, 5143–5147, 1989.
 234. **Yamaguchi, S., Hirose, M., Fukushima, S., Hasegawa, R., and Ito, N.**, Modification by catechol and resorcinol of upper digestive tract carcinogenesis in rats treated with methyl-*N*-amyl nitrosamine, *Cancer Res.* 49, 6015–6018, 1989.
 235. **Shibata, M.-A., Yamada, M., Hirose, M., Asakawa, A., Tatematsu, M., and Ito, N.**, Early proliferative responses of forestomach and glandular stomach of rats treated with five different phenolic antioxidants, *Carcinogenesis* 11, 425–429, 1990.
 236. **Altmann, H. J., Grunow, W., Wester, P. W., and Mohr, U.**, Induction of forestomach lesions by butylhydroxyanisole and structurally related substances, *Arch. Toxicol.* 114–116, 1985.
 237. **National Toxicology Program (NTP)**, Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71–43–2) in F344/N Rats and B6C3F₁ Mice (Gavage Studies), NTP Technical Report No. 289; Washington: U.S. Department of Health and Human Services, 1986.
 238. **Stromberg, P. C.**, Large granular lymphocyte leukemia in F344 rats. Model for human T lymphoma, malignant histiocytosis, and T-cell chronic lymphocytic leukemia, *Am. J. Pathol.* 119, 517–519, 1985.
 239. **Rao, G. N., Haseman, J. K., Grumbein, S., Crawford, D. D., and Eustis, S. L.**, Growth, body weight, survival, and tumor trends in F344/N rats during an eleven-year period, *Toxicol. Pathol.* 18, 61–70, 1990.
 240. **Roe, F. J. C. and Salaman, M. H.**, Further studies on incomplete carcinogenesis: Triethylene melamine (T.E. M.), 1,2-benzanthracene and β-propiolactone, as initiators of skin tumor formation in the mouse, *Br. J. Cancer* 9, 177–203, 1955.
 241. **Boutwell, R. K. and Bosch, D. K.**, The tumor-promoting activity of phenol and related compounds for mouse skin, *Cancer Res.* 19, 413–424, 1959.
 242. **Van Duuren, B. L. and Goldschmidt, B. M.**, Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis, *J. Natl. Cancer Inst.* 56, 1237–1242, 1976.
 243. **Maruyama, H., Amanuma, T., Nakae, D., Tsutsumi, M., Kondo, S., Tsujiuchi, T., Denda, A., and Konishi, Y.**, Effects of catechol and its analogs on

- pancreatic carcinogenesis initiated by *N*-nitrosobis(2-oxopropyl)amine in Syrian hamsters, *Carcinogenesis* 12, 1331–1334, 1991.
244. Kurata, Y., Fukushima, S., Hasegawa, R., Hirose, M., Shibata, M.-A., Shirai, T., and Ito, N., Structure-activity relations in promotion of rat urinary bladder carcinogenesis by phenolic antioxidants, *Jpn. J. Cancer Res.* 81, 754–759, 1990.
 245. Miyata, Y., Fukushima, S., Hirose, M., Masui, T., and Ito, N., Short-term screening of promoters of bladder carcinogenesis in *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-initiated, unilaterally ureter-ligated rats, *Jpn. J. Cancer Res.* 76, 828–834, 1985.
 246. Hasegawa, R., Furukawa, F., Toyoda, K., Takahashi, M., Hayashi, Y., Hirose, M., and Ito, N., Inhibitory effects of antioxidants on *N*-bis(2-hydroxypropyl)nitrosamine-induced lung carcinogenesis in rats, *Jpn. J. Cancer Res.* 81, 871–877, 1990.
 247. Boyland, E., Busby, E. R., Dukes, C. R., Grover, P. L., and Manson, D., Further experiments on implantation of materials into the urinary bladder of mice, *Br. J. Cancer* 18, 575–581, 1964.
 248. Hasegawa, R. and Ito, N., Liver medium-term bioassay in rats for screening of carcinogens and modifying factors in hepatocarcinogenesis, *Food Chem. Toxicol.* 30, 979–992, 1992.
 249. Stenius, U., Warholm, M., and Högberg, J., Selective toxicity in putative preneoplastic hepatocytes: a comparison of hydroquinone and duroquinone, *Cancer Lett.* 68, 149–157, 1993.
 250. Hagiwara, A., Kokubo, Y., Takesada, Y., Tanaka, H., Tamano, S., Hirose, M., Shirai, T., and Ito, N., Inhibitory effects of phenolic compounds on development of naturally occurring preneoplastic hepatocytic foci in long-term feeding studies using male F344 rats, *Teratogen. Carcinogen. Mutagen.* 16, 317–325, 1996.
 251. Delcambre, J., Weber, B., and Baron, C., Toxicity of hydroquinone (French), *Revue Agressologie* 3, 311–315, 1962.
 252. Nakamura, S., The effects of oral administration of resorcin, hydroquinone and phenol on the hematopoietic system in mice (Japanese), *Osaka-Furitsu Kosshu Eisei Kenkyusho* 20, 45–49, 1982.
 253. Mitchell, J. R., Mechanism of benzene-induced aplastic anemia, *Fed. Proc.* 30, 561(abstract), 1971.
 254. Snyder, R. and Kalf, G. F., A perspective on benzene leukemogenesis, *Crit. Rev. Toxicol.* 24, 177–209, 1994.
 255. Smith, M. T., Overview of benzene-induced aplastic anaemia, *Eur. J. Haematol.* 57(Suppl.), 107–110, 1996.
 256. Tunek, A., Olofsson, T., and Berlin, M., Toxic effects of benzene and benzene metabolites on granulopoietic stem cells and bone marrow cellularity in mice, *Toxicol. Appl. Pharmacol.* 59, 149–156, 1981.
 257. Wierda, D. and Irons, R. D., Hydroquinone and catechol reduce the frequency of progenitor B lymphocytes in mouse spleen and bone marrow, *Immunopharmacology* 4, 41–54, 1982.
 258. Eastmond, D. A., Smith, M. T., and Irons, R. D., An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure, *Toxicol. Appl. Pharmacol.* 91, 85–95, 1987.
 259. Henschler, R., Glatt, H. R., and Heyworth, C. M., Hydroquinone stimulates granulocyte-macrophage progenitor cells *in vitro* and *in vivo*, *Environ. Health Perspect.* 104, 1271–1274, 1996.
 260. Hazel, B. A. and Kalf, G. F., Induction of granulocytic differentiation in myeloblasts by hydroquinone, a metabolite of benzene, involves the leukotriene D4 receptor, *Receptor Signal Transduction* 6, 1–12, 1996.
 261. Boyd, R., Griffiths, J., Kindt, V., Snyder, R., Caro, J., and Ersley, A., Relative toxicity of five benzene metabolites on CFU-GM cultures, *Toxicologist* 2, 121–122(abstract), 1982.
 262. Colinas, R. J., Burkart, P. T., and Lawrence, D. A., *In vitro* effects of hydroquinone, benzoquinone, and doxorubicin on mouse and human bone marrow cells at physiological oxygen partial pressure, *Toxicol. Appl. Pharmacol.* 129, 95–102, 1994.
 263. Irons, R. D., Stillman, W. S., Colagiovanni, D. B., and Henry, V. A., Synergistic action of the benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating factor *in vitro*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 3691–3695, 1992.
 264. Irons, R. D. and Stillman, W. S., The effects of benzene and other leukaemogenic agents on haematopoietic stem and progenitor cell differentiation, *Eur. J. Haematol.* 57(Suppl.), 119–124, 1996.
 265. Oliveira, N. L. and Kalf, G. F., Induced differentiation of HL-60 promyelocytic leukemia cells to monocyte/macrophages is inhibited by hydroquinone, a hematotoxic metabolite of benzene, *Blood* 79, 627–633, 1992.
 266. Hazel, B. A., O'Connor, A., Niculescu, R., and Kalf, G. F., Benzene and its metabolite, hydroquinone, induce granulocytic differentiation in myeloblasts by interacting with cellular signaling pathways activated by granulocyte colony-stimulating factor, *Stem Cells* 13, 295–310, 1995.
 267. Moran, J. L., Siegel, D., Sun, X. M., and Ross, D., Induction of apoptosis by benzene metabolites in HL60 and CD34⁺ human bone marrow progenitor cells, *Mol. Pharmacol.* 50, 610–615, 1996.
 268. Hazel, B. A., Baum, C., and Kalf, G. F., Hydroquinone, a bioreactive metabolite of benzene, inhibits apoptosis in myeloblasts, *Stem Cells* 14, 730–742, 1996.
 269. Bolcsak, L. E. and Nerland, D. E., Inhibition of erythropoiesis by benzene and benzene metabolites, *Toxicol. Appl. Pharmacol.* 69, 363–368, 1983.
 270. Snyder, R., Dimitriadis, E., Guy, R., Hu, P., Cooper, K., Bauer, H., Witz, G. and Goldstein, B. D., Studies on the mechanism of benzene toxicity, *Environ. Health Perspect.* 82, 31–35, 1989.
 271. Guy, R. L., Dimitriadis, E. A., Hu, P., Cooper, K. R., and Snyder, R., Interactive inhibition of eryth-

- roid ^{59}Fe utilization by benzene metabolites in female mice, *Chem.-Biol. Interact.* 74, 55–62, 1990.
272. **Guy, R. L., Hu, P., Witz, G., Goldstein, B. D., and Snyder, R.**, Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites *p*-benzoquinone, muconaldehyde and hydroquinone, *J. Appl. Toxicol.* 11, 443–446, 1991.
 273. **Seidel, H. J., Barthel, E., Schafer, F., Scad, H., and Weber, L.**, Action of benzene metabolites on murine hematopoietic colony-forming cells *in vitro*, *Toxicol. Appl. Pharmacol.* 111, 128–131, 1991.
 274. **Brown, A., Lutton, J. D., Nelson, J., Abraham, N. G., and Levere, R. D.**, Microenvironmental cytokines and expression of erythroid heme metabolic enzymes, *Blood Cells* 13, 123–136, 1987.
 275. **Neun, D. J., Penn, A., and Snyder, C. A.**, Evidence for strain-specific differences in benzene toxicity as a function of host target cell susceptibility, *Arch. Toxicol.* 66, 11–17, 1992.
 276. **Gross, S., Gruntmeir, J., Helm, K., Stillman, W., and Irons, R.**, The benzene metabolite, hydroquinone, alters phenotypic expression of human CD34+ cells in liquid culture, *Toxicologist* 36, 163(abstract), 1997.
 277. **Greenberger, J. S.**, The hematopoietic microenvironment, *Crit. Rev. Oncol. Hematol.* 11, 65–84, 1991.
 278. **Morrison, S. J., Uchida, N., and Weissman, I. L.**, The biology of hematopoietic stem cells, *Annu. Rev. Cell Dev. Biol.* 11, 35–71, 1995.
 279. **Gaido, K. and Wierda, D.**, *In vitro* effects of benzene metabolites on mouse bone marrow stromal cells, *Toxicol. Appl. Pharmacol.* 76, 45–55, 1984.
 280. **Gaido, K. W. and Wierda, D.**, Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin, *Toxicol. Appl. Pharmacol.* 89, 378–390, 1987.
 281. **Thomas, D. J., Reasor, M. J., and Wierda, D.**, Macrophage regulation of myelopoiesis is altered by exposure to the benzene metabolite hydroquinone, *Toxicol. Appl. Pharmacol.* 97, 440–453, 1989.
 282. **Renz, J. F. and Kalf, G. F.**, Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: inhibition of conversion of pre-IL-1 α to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1 α , *Blood* 78, 938–944, 1991.
 283. **Miller, A. C., Schattenberg, D. G., Malkinson, A. M., and Ross, D.**, Decreased content of the IL-1 α processing enzyme calpain in murine bone marrow-derived macrophages after treatment with the benzene metabolite hydroquinone, *Toxicol. Lett.* 74, 177–184, 1994.
 284. **Niculescu, R., Bradford, H. N., Colman, R. W., and Kalf, G. F.**, Inhibition of the conversion of pre-interleukins-1 α and -1 β to mature cytokines by *p*-benzoquinone, a metabolite of benzene, *Chem.-Biol. Interact.* 98, 211–222, 1995.
 285. **Kalf, G. F., Renz, J. F., and Niculescu, R.**, *p*-Benzoquinone, a reactive metabolite of benzene, prevents the processing of pre-interleukins-1 α and -1 β to active cytokines by inhibition of the processing enzymes, calpain, and interleukin-1 β converting enzyme, *Environ. Health Perspect.* 104: 1251–1256, 1996.
 286. **Carbonnelle, P., Lison, D., LeRoy, J.-Y. and Lauwerys, R.**, Effect of the benzene metabolite, hydroquinone, on interleukin-1 secretion by human monocytes *in vitro*, *Toxicol. Appl. Pharmacol.* 132, 220–226, 1995.
 287. **Post, G., Snyder, R., and Kalf, G. F.**, Metabolism of benzene and phenol in macrophages *in vitro* and the inhibition of RNA synthesis by benzene metabolites, *Cell Biol. Toxicol.* 2, 231–246, 1986.
 288. **Smart, R. C. and Zannoni, V. G.**, DT-diaphorase and peroxidase influence the covalent binding of the metabolites of phenol, the major metabolite of benzene, *Mol. Pharmacol.* 26, 105–111, 1984.
 289. **Schattenberg, D. G., Stillman, W. S., Gruntmeir, J. J., Helm, K. M., Irons, R. D., and Ross, D.**, Peroxidase activity in murine and human hematopoietic progenitor cells: potential relevance to benzene-induced toxicity, *Mol. Pharmacol.* 46, 346–351, 1994.
 290. **Wever, R., Plat, H., and Hamers, M. N.**, Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin, *FEBS Lett.* 123: 327–331.
 291. **Pirozzi, S. J., Schlosser, M. J., and Kalf, G. F.**, Prevention of benzene-induced myelotoxicity and prostaglandin synthesis in bone marrow of mice by inhibitors of prostaglandin H synthase, *Immunopharmacology* 18, 39–55, 1989.
 292. **Thomas, D. J., Sadler, A., Subrahmanyam, V. V., Siegel, D., Reasor, M. J., Wierda, D., and Ross, D.**, Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid cells, *Mol. Pharmacol.* 37, 255–262, 1990.
 293. **London, S. J., Lehman, T. A., and Taylor, J. A.**, Myeloperoxidase genetic polymorphism and lung cancer risk, *Cancer Res.* 57, 5001–5003, 1997.
 294. **Meyer, K. B., Subrahmanyam, V. V., Kolachana, P., and Smith, M. T.**, Bioactivation and cytotoxicity of hydroquinone in human promyelocytic leukemia (HL60) cells, *Toxicologist* 11, 180(abstract), 1991.
 295. **Bratton, S. B., Lau, S. S., and Monks, T. J.**, Identification of quinol thioethers in bone marrow of hydroquinone/phenol-treated rats and mice and their potential role in benzene-mediated hematotoxicity, *Chem. Res. Toxicol.* 10, 859–865, 1997.
 296. **Twerdok, L. E. and Trush, M. A.**, Differences in quinone reductase activity in primary bone marrow stromal cells derived from C57BL/6 and DBA/2 mice, *Res. Commun. Chem. Pathol. Pharmacol.* 67, 375–386, 1990.
 297. **Zhu, H., Li, Y., and Trush, M. A.**, Differences in xenobiotic detoxifying activities between bone marrow stromal cells from mice and rats: implications for benzene-induced hematotoxicity, *J. Toxicol. Environ. Health* 46, 183–201, 1995.
 298. **Twerdok, L. E., Rambish, S. J., and Trush, M. A.**, Induction of quinone reductase and glutathione in

- bone marrow cells by 1,2-dithiole-3-thione: effect on hydroquinone-induced cytotoxicity, *Toxicol. Appl. Pharmacol.* 112, 273–281, 1992.
299. **Twerdok, L. E., Rambish, S. J., and Trush, M. A.,** Studies with 1,2-dithiole-3-thione as a chemoprotector of hydroquinone-induced toxicity to DBA/2-derived bone marrow stromal cells, *Environ. Health Perspect.* 101, 172–177, 1993.
 300. **Li, Y., Lafuente, A., and Trush, M. A.,** Characterization of quinone reductase, glutathione and glutathione *S*-transferase in human myeloid cell lines: induction by 1,2-dithiole-3-thione and effects on hydroquinone-induced cytotoxicity, *Life Sci.* 54, 901–916, 1994.
 301. **Rothman, N., Smith, M. T., Hayes, R. B., Traver, R. D., Hoener, B., Campleman, S., Li, G. L., Dosemeci, M., Linet, M., Zhang, L., Xi, L., Wacholder, S., Lu, W., Meyer, K. B., Titenko-Holland, N., Stewart, J. T., Yin, S., and Ross, D.,** Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 ⁶⁰⁹C→T mutation and rapid fractional excretion of chlorzoxazone, *Cancer Res.* 57, 2839–2842, 1997.
 302. **Laskin, J. D., Rao, N. R., Punjabi, C. J., Laskin, D. L., and Synder, R.,** Distinct actions of benzene and its metabolites on nitric oxide production by bone marrow leukocytes, *J. Leukocyte Biol.* 57, 422–426, 1995.
 303. **Rao, N. R. and Snyder, R.,** Oxidative modifications produced in HL-60 cells on exposure to benzene metabolites, *J. Appl. Toxicol.* 15, 403–409, 1995.
 304. **Pfeifer, R. W. and Irons, R. D.,** Inhibition of lectin-stimulated lymphocyte agglutination and mitogenesis by hydroquinone: reactivity with intracellular sulfhydryl groups, *Exp. Mol. Pathol.* 35, 189–198, 1981.
 305. **Pfeifer, R. W. and Irons, R. D.,** Alteration of lymphocyte function by quinones through a sulfhydryl-dependent distribution of microtubule assembly, *Int. J. Immunopharmacol.* 5, 463–470, 1983.
 306. **King, A. G., Landreth, K. S., and Wierda, D.,** Hydroquinone inhibits bone marrow pre-B cell maturation *in vitro*, *Mol. Pharmacol.* 32, 807–812, 1987.
 307. **King, A. G., Landreth, K. S., and Wierda, D.,** Bone marrow stromal cell regulation of B-lymphopoiesis. II. Mechanisms of hydroquinone inhibition of pre-B cell maturation, *J. Pharmacol. Exp. Ther.* 250, 582–590, 1989.
 308. **Lang, D. S., Meier, K. L., and Luster, M. I.,** Comparative effects of immunotoxic chemicals on *in vitro* proliferative responses of human and rodent lymphocytes, *Fundam. Appl. Toxicol.* 21, 535–545, 1993.
 309. **Li, Q., Geiselhart, L., Mittler, J. N., Mudzinski, S. P., Lawrence, D. A., and Freed, B. M.,** Inhibition of human T lymphoblast proliferation for hydroquinone, *Toxicol. Appl. Pharmacol.* 139, 317–323, 1996.
 310. **Li, Q. and Freed, B. M.,** HQ inhibits lymphoblast proliferation by two different mechanisms, *Toxicologist* 36, 200(abtract), 1997.
 311. **Li, Q. and Freed, B. M.,** Role of iron in hydroquinone- and catechol-induced inhibition of IL-2-dependent lymphoblast proliferation, *Fundam. Appl. Toxicol.* 30, 4(abtract), 1996.
 312. **Li, Q., Aubrey, M. T., Christian, T., and Freed, B. M.,** Differential inhibition of DNA synthesis in human T cells by the cigarette tar components hydroquinone and catechol, *Fundam. Appl. Toxicol.* 38, 158–165, 1997.
 313. **Li, Q., Kasten-Jolly, J., Yen, Y., and Freed, B. M.,** Reversal of hydroquinone-mediated suppression of T cell proliferation by transfection of the M2 subunit of ribonucleotide reductase, *Toxicol. Appl. Pharmacol.* 150, 154–157, 1988.
 314. **Updyke, L. W., Wilson, P. S., and Wierda, D.,** Decreased IL-7 production by long term bone marrow cultures following exposure to quinones, *Toxicologist* 11, 203(abtract), 1991.
 315. **Cheung, S. C., Newland, D. E., and Sonnenfeld, G.,** Inhibition of interferon gamma production by benzene and benzene metabolites, *J. Natl. Cancer Inst.* 80, 1069–1072, 1988.
 316. **Cheung, S. C., Nerland, D. E., and Sonnenfeld, G.,** Inhibition of interferon- α/β induction in L-929 cells by benzene and benzene metabolites, *Oncology* 46, 335–338, 1989.
 317. **Pyatt, D. W., Stillman, W. S., and Irons, R. D.,** Hydroquinone, a reactive metabolite of benzene, inhibits NF- κ B in primary human CD4⁺ T lymphocytes, *Toxicol. Appl. Pharmacol.* 149, 178–184, 1998.
 318. **Fan, X., Hirata, Y., and Minami, M.,** Effect of benzene and its metabolites on natural killer activity of mouse spleen cells *in vitro*, *Jpn. J. Ind. Health*, 31, 330–334, 1989.
 319. **Manning, B. W., Adams, D. O., and Lewis, J. G.,** Effects of benzene metabolites on receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages, *Toxicol. Appl. Pharmacol.* 126, 214–223, 1994.
 320. **Medinsky, M. A., Kenyon, E. M., Seaton, M. J., and Schlosser, P. M.,** Mechanistic considerations in benzene physiological model development, *Environ. Health Perspect.* 104, 1399–1404, 1996.
 321. **Burgaz, S., Ozcan, M., Ozkul, A., and Karakaya, A. E.,** Effect of hydroquinone (HQ) on the development of chick embryos, *Drug Chem. Toxicol.* 17, 163–174, 1994.
 322. **Chapman, D. E., Namkung, M. J., and Juchau, M. R.,** Benzene and benzene metabolites as embryotoxic agents: effects on cultured rat embryos, *Toxicol. Appl. Pharmacol.* 128: 129–137, 1994.
 323. **Oglesby, L. A., Ebron-McCoy, M. T., Logsdon, T. R., Copeland, F., Beyer, P. E., and Kavlock, R. J.,** *In vitro* embryotoxicity of a series of para-substituted phenols: structure, activity, and correlation with *in vivo* data, *Teratology* 45, 11–33, 1992.
 324. **Telford, I. R., Woodruff, C. S., and Linford, R. H.,** Fetal resorption in the rat as influenced by certain antioxidants, *Am. J. Anat.* 26, 195–200, 1962.

325. **Racz, G., Fuzi, J., Kemeny, G., and Kisgyorgy, Z.**, The effect of hydroquinone and phlorizin on the sexual cycle of white rats (Russian), *Orvosi Szemle*, 5, 65–67, 1958.
326. **Rosen, F. and Millman, N.**, Anti-gonadotropic activities of quinones and related compounds, *Endocrinology* 57, 466–471, 1955.
327. **Skalka, P.**, Influence of hydroquinone on the fertility of male rats (Czech), *Sb. Vys. Sk. Zemed. B*, 12, 491–494, 1964.
328. **Ames, S. R., Ludwig, M. I., Swanson, W. J., and Harris, P. L.**, Effect of DPPD, methylene blue, BHT, and hydroquinone on reproductive process in the rat, *Proc. Soc. Exp. Biol. Med.* 93, 39–42, 1956.
329. **Kavlock, R. J., Oglesby, L. A., Hall, L. L., Fisher, H. L., Copeland, F., Logsdon, T., and Ebron-McCoy, M.**, In vivo and in vitro structure-dosimetry-activity relationships of substituted phenols in developmental toxicity assays, *Fundam. Appl. Toxicol.* 16, 225229, 1990.
330. **Cosmetic, Toiletry, and Fragrance Association (CTFA)**, Dermal Teratology Study in Albino Female Sprague-Dawley Rats, Washington: Cosmetics, Toiletry, and Fragrance Manufacturers, 1980; Study Project TR-01–80.
331. **Murphy, S. J., Schroeder, R. E., Blacker, A. M., Krasavage, W. J., and English, J. C.**, A study of developmental toxicity of hydroquinone in the rabbit, *Fundam. Appl. Toxicol.* 19, 214–221, 1992.
332. **Blacker, A. M., Schroeder, R. E., English, J. C., Murphy, S. J., Krasavage, W. J., and Simon, G. S.**, A two-generation reproduction study with hydroquinone in rats, *Fundam. Appl. Toxicol.* 21, 420–424, 1993.
333. **Nowak, A. K., Shilkin, K. B., and Jeffrey, G. P.**, Darkroom hepatitis after exposure to hydroquinone, *Lancet* 345, 1187, 1995.
334. **Care, G. L.**, Darkroom exposure to hydroquinone, *Lancet* 347, 121, 1996.
335. **Sterner, J. H., Oglesby, F. L., and Anderson, B.**, Quinone vapors and their harmful effects. I. Corneal and conjunctival injury, *J. Ind. Hyg. Toxicol.* 29, 60–73, 1947.
336. **Pifer, J. W., Hearne, F. T., Friedlander, B. R., and McDonough, J. R.**, Mortality study of men employed at a large chemical plant, 1972 through 1982, *J. Occup. Med.* 28, 438–444, 1986.
337. **Pifer, J. W., Hearne, F. T., Swanson, F. A., and O'Donoghue, J. L.**, Mortality study of employees engaged in the manufacture and use of hydroquinone, *Int. Arch. Occup. Environ. Health*, 67, 267–280, 1995.
338. **Choudat, D., Neukirch, F., Brochard, P., Barrat, G., Marsac, J., Conso, F., and Philbert, M.**, Allergy and occupational exposure to hydroquinone and to methionine, *Br. J. Ind. Med.* 45, 376–380, 1988.
339. **Nielsen, H., Henriksen, L., and Olsen, J. H.**, Malignant melanoma among lithographers, *Scand. J. Work Environ. Health*, 22, 108–111, 1996.
340. **Austin, D. F. and Reynolds, P.**, Investigation of an excess of melanoma among employees of the Lawrence Livermore national laboratory, *Am. J. Epidemiol.*, 145, 524–531, 1997.
341. **Von Oettingen, W. F.**, Hydroquinone, in *Phenol and Its Derivatives: The Relation Between Their Chemical Constitution and Their Effect on the Organism*, Washington: U.S. Public Health Service, 1949, 145–166.
342. **Grant, W. M. and Schuman, J. S.**, *Toxicology of the Eye*, Springfield, 4th ed., Charles C Thomas, 1993, 801–804.
343. **Oglesby, F. L. and Raleigh, R. L.**, *Eye Injury Associated with Exposure to Hydroquinone and Quinone*, 2nd ed., Tennessee Eastman Company, Kingsport, 1973.
344. **Anderson, B.**, Corneal and conjunctival pigmentation among workers engaged in manufacture of hydroquinone, *Arch. Ophthalmol.* 38, 812–826, 1947.
345. **Naumann, G.**, Corneal damage in hydroquinone workers, *Arch. Ophthalmol.* 76, 189–194, 1966.
346. **Anderson, B.**, Corneal changes from quinone-hydroquinone exposure, *Arch. Ophthalmol.* 59, 495–501, 1958.
347. **Hughes, W.**, The tolerance of rabbit cornea for various chemical substances, *Bull. Johns Hopkins Hosp.* 82, 338–349, 1948.
348. **Ferraris de Gaspere, P. F.**, Experimental studies on keratoconjunctivitis from hydroquinone (Italian), *Boll. Oculist.* 28, 361–367, 1949.
349. **Oettel, H.**, The toxicology of hydroquinone (German), *Arch. Exp. Pathol. Pharmacol.* 183, 319–362, 1936.
350. **Martin, G. J. and Ansbacher, S.**, Confirmatory evidence of the chromotrichial activity of *p*-aminobenzoic acid, *J. Biol. Chem.* 138, 441, 1941.
351. **Chavin, W.**, Effects of hydroquinone and of hypophysectomy upon the pigment cells of black goldfish, *J. Pharmacol. Exp. Ther.* 142, 275–290, 1963.
352. **Chavin, W. and Schlesinger, W.**, Some potent melanin depigmentary agents in the black goldfish, *Naturwissenschaften* 53, 413–414, 1966.
353. **Bleehen, S. S., Pathak, M. A., Hori, Y., and Fitzpatrick, T. B.**, Depigmentation of skin with 4-isopropylcatechol, mercaptoamines, and other components, *J. Invest. Dermatol.* 50, 103–117, 1968.
354. **Jimbow, K., Obata, K., Pathak, M. A., and Fitzpatrick, T. B.**, Mechanism of depigmentation by hydroquinone, *J. Invest. Dermatol.* 62, 436–449, 1974.
355. **Lerner, A. B., Fitzpatrick, T. B., Calkins, E., and Summerson, W. H.**, Mammalian tyrosinase action of substances structurally related to tyrosine, *J. Biol. Chem.* 178, 185–195, 1951.
356. **Denton, C., Lerner, A. B., and Fitzpatrick, T. B.**, Inhibition of melanin formation by chemical agents, *J. Invest. Dermatol.* 18, 119–135, 1952.
357. **Iijima, S. and Watanabe, K.**, Studies on Dopa reaction. II. Effect of chemicals on the reaction, *J. Invest. Dermatol.* 28, 1–4, 1959.
358. **Chen, Y. M. and Chavin, W.**, Hydroquinone activation and inhibition of skin tyrosinase, *Pigment Cell*, 3, 105–112, 1976.

359. **Usami, Y., Landau, A. B., Fukuyama, K., and Gellin, G. A.**, Inhibition of tyrosinase activity by 4-*tert*-butylcatechol and other depigmenting agents, *J. Toxicol. Environ. Health*, 6, 559–567, 1980.
360. **Palumbo, A., d'Ischia, M., Misuraga, G., and Protta, G.**, Mechanism of inhibition of melanogenesis by hydroquinone, *Biochim. Biophys. Acta*, 1073, 85–90, 1991.
361. **Passi, S. and Nazzaro-Porro, M.**, Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds, *Br. J. Dermatol.* 104, 659–665, 1981.
362. **Bolognia, J. L., Sodi, S. A., Osber, M. P., and Pawelek, J. M.**, Enhancement of the depigmenting effect of hydroquinone by cystamine and buthionine sulfoximine, *Br. J. Dermatol.* 133, 349–357, 1995.
363. **Hu, F.**, The influence of certain hormones and chemicals on mammalian pigment cells, *J. Invest. Dermatol.* 46, 117–124, 1966.
364. **Abramowitz, J. and Chavin, W.**, Acute effects of two melanocytolytic agents, hydroquinone and β -mercaptoethanolamine, upon tyrosinase activity and cyclic nucleotide levels in murine melanomas, *Chem.-Biol. Interact.* 32, 195–208, 1980.
365. **Penney, K. B., Smith, C. J., and Allen, J. C.**, Depigmenting action of hydroquinone depends on disruption of fundamental cell processes, *J. Invest. Dermatol.* 82: 308–310, 1984.
366. **Smith, C. J., O'Hare, K. B., and Allen, J. C.**, Selective cytotoxicity of hydroquinone for melanocyte-derived cells is mediated by tyrosinase activity but independent of melanin content, *Pigment Cell Res.* 1, 386–389, 1988.
367. **Passi, S., Picardo, M., and Nazzaro-Porro, M.**, Comparative cytotoxicity of phenols *in vitro*, *Biochem. J.* 245, 537–542, 1987.
368. **Picardo, M., Cannistraci, C., De Luca, C., Zompetta, C., and Santucci, B.**, Effect of para group substances on human keratinocytes in culture, *Contact Dermatitis* 23, 236, 1990.
369. **Chavin, W., Jelonek, E. J., Reed, A. H., and Binder, L. R.**, Survival of mice receiving melanoma transplants is promoted by hydroquinone, *Science* 208, 408–410, 1980.
370. **Fitzpatrick, T. B., Arndt, K. A., El Mofty, A. M., and Pathak, M. A.**, Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo, *Arch. Dermatol.* 93, 589–600, 1966.
371. **Spencer, M.**, Hydroquinone bleaching, *Arch. Dermatol.* 84, 131–134, 1961.
372. **Spencer, M. C.**, Topical use of hydroquinone for depigmentation, *JAMA* 194, 114–116, 1965.
373. **Arndt, K. A. and Fitzpatrick, T. B.**, Topical use of hydroquinone as a depigmenting agent, *JAMA* 194, 117–119, 1965.
374. **Bentley-Phillips, B. and Bayles, M. A. H.**, Cutaneous reactions to topical application of hydroquinone, *South African Med. J.* 49, 1391–1395, 1975.
375. **Kligman, A. M. and Willis, I.**, A new formula for depigmenting human skin, *Arch. Dermatol.* 111, 40–48, 1975.
376. **Laloo, D., Makar, S., and Maibach, H. I.**, Hydroquinone as a contact allergen. An overview, *Dermatosen* 45, 208–212, 1997.
377. **Engasser, P. G. and Maibach, H. I.**, Cosmetics and dermatology: bleaching creams, *J. Am. Acad. Dermatol.* 5, 143–147, 1981.
378. **Bentley-Phillips, B. and Bayles, M. A. H.**, Acquired hypomelanosis: hyperpigmentation following reactions to hydroquinones, *Br. J. Dermatol.* 90, 232–233, 1974.
379. **Findlay, G. H., Morrison, J. G. L., and Simson, I. W.**, Exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams, *Br. J. Dermatol.* 93, 613–622, 1975.
380. **Findlay, G. H. and De Beer, H. A.**, Chronic hydroquinone poisoning of the skin from skin-lightening cosmetics, *South African Med. J.* 57, 187–190, 1980.
381. **Burke, P. A. and Maibach, H. I.**, Exogenous ochronosis: an overview, *J. Dermatol. Treat.* 8, 21–26, 1997.
382. **Hardwick, N., Van Gelder, J. W., Van der Merwe, C. A., and Van der Merwe, M. P.**, Exogenous ochronosis: an epidemiological study, *Br. J. Dermatol.* 120, 229–238, 1989.
383. **Findlay, G. H.**, Ochronosis following skin bleaching with hydroquinone, *J. Am. Acad. Dermatol.* 6, 1092–1093, 1982.
384. **Godlee, F.**, Skin lighteners cause permanent damage, *Br. Med. J.* 305, 333, 1992.
385. **Williams, H.**, Skin lightening creams containing hydroquinone, *Br. Med. J.* 305, 903–904, 1992.
386. **Cullison, D., Abele, D. C., and O'Quinn, J. L.**, Localized exogenous ochronosis, *J. Am. Acad. Dermatol.* 8, 882–889, 1983.
387. **Hoshaw, R. A., Zimmerman, K. G., and Menter, A.**, Ochronosislike pigmentation from hydroquinone bleaching creams in American blacks, *Arch. Dermatol.* 121, 105–108, 1985.
388. **Penneys, N. S.**, Ochronosislike pigmentation from hydroquinone bleaching creams, *Arch. Dermatol.* 121: 1239–1240, 1985.
389. **Connor, T. and Braunstein, B.**, Hyperpigmentation following the use of bleaching creams, *Arch. Dermatol.* 123, 105–106, 1987.
390. **Lawrence, N., Bligard, C. A., Reed, R., and Perret, W. J.**, Exogenous ochronosis in the United States, *J. Am. Acad. Dermatol.* 18, 1207–1212, 1988.
391. **Mahe, A., Blanc, L., Halna, J. M., Keita, S., Sanogo, T., and Bobin, P.**, An epidemiologic survey on the cosmetic use of bleaching agents by the women of Bamako (Mali) (French), *Ann. Dermatol. Venereol.* 120, 870–873, 1993.
392. **Sylla, R., Diouf, A., Niane, B., Ndiaye, B., Guisse, M. B., Diop, A., Ciss, M., and Ba, D.**, Artificial depigmentation practice of the skin in women of Dakar and analytical study of the cosmetic products used (French), *Dakar Med.* 39, 223–226, 1994.
393. **Garcia, R. L., White, J. W., and Willis, W. F.**, Hydroquinone nail pigmentation, *Arch. Dermatol.* 114, 1402–1403, 1978.

394. **Mann, R. J. and Harman, R. R. M.**, Nail staining due to hydroquinone skin-lightening creams, *Br. J. Dermatol.* 108, 363–365, 1983.
395. **Duffield, J.**, Depigmentation of skin by quinol and its monobenzyl ether, *Lancet* I, 1164, 1952.
396. **Frenk, E. and Loi-Zedda, P.**, Occupational depigmentation due to a hydroquinone-containing photographic developer, *Contact Dermatitis* 6, 238–239, 1980.
397. **Kersey, P. and Stevenson, C. J.**, Vitiligo and occupational exposure to hydroquinone from servicing self-photographing machines, *Contact Dermatitis* 7, 285–287, 1981.
398. **Fisher, A. A.**, Can bleaching creams containing 2% hydroquinone produce leukoderma? *J. Am. Acad. Dermatol.* 7, 134, 1982.
399. **Liden, C.**, Occupational dermatoses at a film laboratory, *Contact Dermatitis* 10, 77–87, 1984.
400. **Liden, C.**, Occupational dermatoses at a film laboratory. Follow-up after modernization, *Contact Dermatitis* 20, 191–200, 1989.
401. **Hull, P. R. and Procter, P. R.**, The melanocyte: an essential link in hydroquinone-induced ochronosis, *J. Am. Acad. Dermatol.* 22, 529–531, 1990.
402. **Engasser, P. G.**, Ochronosis caused by bleaching creams, *J. Am. Acad. Dermatol.* 10, 1073, 1984.
403. **Phillips, J. I., Isaacson, C., and Carman, H.**, Ochronosis in black South Africans who used skin lighteners, *Am. J. Dermatopathol.* 8, 14–21, 1986.
404. **O'Donoghue, M. N., Lynfield, Y. L., and Derbes, V.**, Ochronosis due to hydroquinone, *J. Am. Acad. Dermatol.* 8, 123, 1983.
405. **Brauer, E. W.**, Safety of over-the-counter hydroquinone bleaching creams, *Arch. Dermatol.* 121, 1239, 1985.
406. **Medinsky, M. A., Schlosser, P. M., and Bond, J. A.**, Critical issues in benzene toxicity and metabolism: the effects of interactions with other organic chemicals on risk assessment, *Environ. Health Perspect.* 102, 119–124, 1994.
407. **Trush, M. A., Twerdok, L. E., Rembish, S. J., Zhu, H., and Li, Y.**, Analysis of target cell susceptibility as a basis for the development of a chemoprotective strategy against benzene-induced hematotoxicities, *Environ. Health Perspect.* 104, 1227–1234, 1996.
408. **Rickert, D. F., Baker, T. S., Bus, J. S., Barrow, C. S., and Irons, R. D.**, Benzene disposition in the rat after exposure by inhalation, *Toxicol. Appl. Pharmacol.* 49, 417–423, 1979.
409. **Hedli, C. C., Snyder, R., and Witmer, C. M.**, Bone marrow DNA adducts and bone marrow cellularity following treatment with benzene metabolites *in vivo*. In: Witmer, C. M., Ed., *Biological Reactive Intermediates*. Plenum Press, New York, 1990, 745–748.
410. **Laskin, D. L., MacEachern, L., and Snyder, R.**, Activation of bone marrow phagocytes following benzene treatment of mice, *Environ. Health Perspect.* 82, 75–79, 1989.
411. **Kettle, A. J. and Winterbourn, C. C.**, Oxidation of hydroquinone by myeloperoxidase, *J. Biol. Chem.* 267, 8319–8324, 1992.
412. **Subrahmanyam, V. V., Kolachana, P., and Smith, M. T.**, Metabolism of hydroquinone by human myeloperoxidase: Mechanisms of stimulation by other phenolic compounds, *Arch. Biochem. Biophys.* 286, 76–84, 1991.
413. **Medinsky, M. A., Kenyon, E. M., and Schlosser, P. M.**, Benzene: a case study in parent chemical and metabolite interactions, *Toxicology* 105, 225–233, 1995.
414. **Organisation for Economic Cooperation and Development (OECD)**, Final SIAR (SIDS Initial Assessment Report): Hydroquinone, Paris: OECD, 1997.