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# SOLVENT TOXICOLOGY: RECENT ADVANCES IN THE TOXICOLOGY OF BENZENE, THE GLYCOL ETHERS, AND CARBON TETRACHLORIDE

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## INTRODUCTION

The field of solvent toxicology is quite broad and impossible to review fully in this format. We decided to stress benzene, the glycol ethers, and carbon tetrachloride. This review emphasizes the projected role of xenobiotic metabolism in hemopoietic toxicity caused by benzene, adverse effects on the male reproductive system caused by the glycol ethers, and hepatotoxicity caused by carbon tetrachloride.



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# BENZENE METABOLISM AND TOXICITY

## *Introduction*

Benzene (Bz) is an important industrial chemical, a petroleum by-product, a component of unleaded gas, and thus a ubiquitous environmental pollutant [reviewed in (1)]. Bz is a myelotoxin; chronic exposure of humans and experimental animals to high concentrations results in blood dyscrasias including lymphocytopenia, thrombocytopenia, and pancytopenia or aplastic anemia (2, 3). Bz is also a carcinogen. It is associated with an increased incidence of acute myelogenous leukemia and some of its variants in humans (4–10), an increased incidence of several solid tumors (11, 12), and possibly leukemia/lymphoma (13) in rodents.

Since recent articles have reviewed the historical aspects of benzene toxicity (14), its genotoxicity (15), quinones as its toxic metabolites (16), and its developmental toxicity (17), we review recent findings on the metabolism of benzene and its hematotoxicity.

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## *Metabolism*

Benzene metabolism is required for toxicity. Possible pathways for the bioactivation of Bz in vivo are shown in Figure 1. In the liver, the major site of Bz metabolism (18), Bz is converted via a cytochrome P-450-mediated pathway (19) to benzene oxide, which is transformed by epoxide hydratase to the 1,2-dihydrodiol, which leads to catechol (C) formation (20) or rearranges nonenzymically to phenol (P), which is metabolized to hydroquinone (HQ) (21). The bone marrow is the target organ. It possesses a limited capacity to metabolize Bz, which cannot account for the amount of metabolites that accumulate in it (22–24). P is metabolized to HQ and C in the marrow by a myeloperoxidase-mediated pathway (25). The oxidation of HQ to *p*-benzoquinone (BQ) also probably occurs by a myeloperoxidase-mediated pathway, whereas C is presumably converted to 1,2,4-benzenetriol (BT) by the cytochrome P-450 system.

The quinones or semiquinones derived from HQ and/or C are generally considered to be the toxic metabolites, although an open-ring product such as *trans,trans*-mucondialdehyde is a possible toxic metabolite (26, 27). Ethanol consumption potentiates Bz toxicity in vivo by accelerating the hydroxylation of Bz and the conversion of P into toxic metabolites (28). Thus, it increases the hematotoxicity.

MICROSOMAL METABOLISM OF BENZENE Bz induces the enzymes required for its metabolism. Post & Snyder (29) observed two benzene hydroxylase activities in rat liver microsomes from control, beta-naphthoflavone (BNF)-treated, and Bz-treated animals at all Bz concentrations and in pheno-

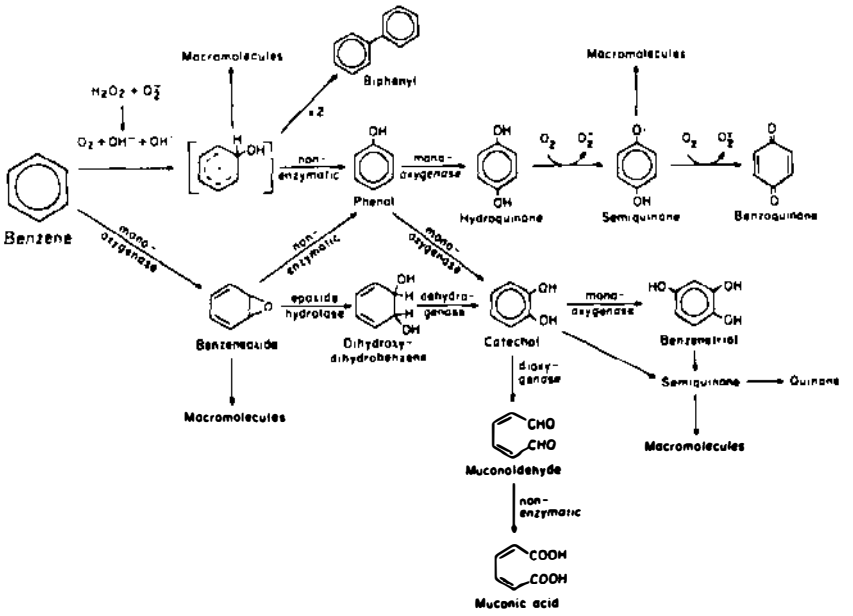


*Figure 1* Metabolic pathways of benzene (taken from Ref. 45 with permission).

barbital (PB)-induced microsomes at concentrations of Bz up to 0.8 mM. Bz pretreatment increased activity without affecting total cytochrome P-450 content. This finding suggests the induction of a specific isozyme that was saturated at Bz concentrations > 0.4 mM, had a pH optimum of 6.6, and was stimulated by fluoride (30).

The inductive pattern of Bz hydroxylase is influenced by the addition of methyl groups to Bz (31). Toluene and xylene increased the metabolism of Bz, and consistent with the report by Post & Snyder (29), Bz induced benzene hydroxylase, but did not induce aniline hydroxylase or aminopyrine *N*-demethylase, whereas toluene and xylene did. Bz more readily induced the conjugating enzymes and increased the level of glutathione (GSH).

Cytochrome P-450 fractions from microsomes of benzene-induced rats have been compared to those obtained from untreated or PB-, BNF-, methylcholanthrene-, and ethanol-induced animals (32). Four distinct cytochromes P-450 were identified; isozyme A was induced by Bz and ethanol, and isozyme Bb was induced by all the inducers. The Bb isozyme induced by Bz appeared to differ from that induced by the classical inducers. The molecular activity of benzene hydroxylase of the microsomal fraction of rabbit bone marrow was about four times higher than that of liver microsomes (33). Pretreatment of the animals with Bz, but not PB, induced benzene monooxygenase activity, but did not affect the O-dealkylation activity in the marrow. Covalent binding of $^{14}C$-labeled metabolites, but no free HQ or C,

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could be detected in incubations of Bz with bone marrow microsomes from Bz-induced animals (33).

Gilmour et al (34) found that Bz was converted to P by rat liver microsomes. They also observed that small amounts of HQ and C were produced from both Bz and P in a reaction mediated by cytochrome P-450 and stimulated by Bz pretreatment. The metabolism of Bz and P was competitively inhibited by toluene, and Bz and P reciprocally inhibited the metabolism of each other. Lunte & Kissinger (35) found that the rate of conversion of P to HQ by mouse liver microsomes was significantly higher than the rate of metabolism of Bz. P was converted to HQ, which was oxidized to *p*-BQ. Benzene hydroxylation in rabbit liver microsomes and reconstituted membrane vesicles containing cytochrome P-450 LM2 appeared to involve hydrogen peroxide, superoxide anion, and hydroxyl radical (36). Biphenyl was formed by the reconstituted system, indicating a cytochrome P-450-dependent formation of a hydroxycyclohexadienyl radical from the interaction of hydroxyl radical with Bz. These results would suggest that the microsomal cytochrome P-450-dependent oxidation of Bz requires hydroxyl radicals generated in a modified Haber–Weiss reaction between hydrogen peroxide and superoxide anion. However, these studies used only 17-$\mu$M Bz. Using a similar system with higher concentrations of Bz in alcohol, Gorsky & Coon (37) found a $K_m$ for Bz of 18 mM with PB-induced rabbit liver microsomes and 105 mM with a cytochrome P-450 LM2 reconstituted system. When Bz concentrations were in the range of the $K_m$, superoxide dismutase, desferrioximine, or hydroxyl radical scavengers had no effect. The oxidation of Bz to P in a model hydroxyl radical–generating system consisting of xanthine, xanthine oxidase, and Fe-EDTA was not dependent on the substrate concentration. The rate of hydroxyl radical generation by the model system was regulated to be greater than the rate of product formation in the microsomal systems. Therefore, the lack of dependence on Bz concentration suggests that hydroxyl radicals are not involved in the metabolism of Bz to P when the concentration of Bz is near the $K_m$ for cytochrome P-450 LM2. At concentrations of Bz below the $K_m$, the free-radical pathway of P formation becomes increasingly predominant.

It is commonly assumed that the microsomal cytochrome P-450 conversion of Bz to P occurs via an epoxide intermediate (38, 39), demonstrable by an NIH shift. Hinson et al (40) studied the role of the NIH shift in the formation of P from the deuterated benzene derivative, 1,3,5-[$^2H_3$]benzene, and found the expected products, 2,3,5-[$^2H_3$]phenol and 2,4-[$^2H_2$]phenol, which indicated that the shift had occurred. Analysis of the deuterium-isotope effect in the deuterated phenols suggests that the cyclohexadienone was formed either

by the somerization of the epoxide or directly from the enzyme–substrate complex as a major intermediate in the hydroxylation of Bz.

Both epoxidation and free-radical insertion may be involved in the formation of P from Bz. Griffiths et al (41) reported on the production of P from Bz by a reconstituted microsomal system containing purified rat liver PB-induced cytochrome P-450. This production was inhibited by metyrapone and SKF 525A, but the conversion of P to secondary metabolites was not. The formation of the polyhydroxymetabolites from P was inhibited by radical-trapping agents and desferrioxamine. Interaction of Bz with cytochrome P-450 in the reconstituted microsomal system elicited a Type I spectral change, whereas interaction of P produced a Type II change indicative of an interaction of the substrate with the iron moiety. Also, an excess of unlabeled P did not dilute the labeled P derived from labeled Bz. These facts suggest that Bz interacts at the active site of cytochrome P-450 and undergoes epoxidation to benzene oxide, which rearranges to form P. This P is preferentially retained on the cytochrome P-450 but is shifted from the catalytic site to the iron moiety. There it can be attacked by hydroxyl radicals to form the polyhydroxy metabolites.

MITOCHONDRIAL METABOLISM OF BENZENE Rat liver mitochondria, stripped of their outer membrane to avoid microsomal contamination (mitoplasts), metabolize Bz in an NADPH-dependent reaction to P and metabolites that covalently bind to mitochondrial (mt)DNA (42). A cytochrome P-450 that converts Bz to P has been solubilized from mitoplasts with 0.4% sodium cholate and purified 23-fold by polyethylene glycol (PEG) fractionation (43). The production of P from Bz by the 5–15% PEG fraction is completely dependent on NADPH and an exogenous bovine adrenodoxin/adrenodoxin reductase system that microsomal cytochrome P-450s do not use. This dependence provides evidence that the activity is indeed mediated by a cytochrome P-450 of mitochondrial origin.

THE EFFECT OF BENZENE AND ITS METABOLITES ON REPLICATION AND TRANSCRIPTION Bz and its metabolites inhibit both nuclear and mitochondrial replication and transcription. DNA synthesis was inhibited in hemopoietic cells from mice exposed to a single dose of 3000-ppm Bz (44). It was also inhibited in mouse L5178YS lymphoma cells after their exposure to the metabolites, but not to Bz, which is not bioactivated in these cells (45). BQ was the most potent inhibitor, followed by HQ, BT, C, and P at concentrations that were not cytotoxic. Inhibition correlated with ease of oxidation. This correlation suggests that the oxidation of P or one of its metabolites produces the ultimate reactive compound that inhibits DNA

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synthesis. Only BQ and BT induced single-strand (ss) DNA damage (46). BQ was about nine times more effective than BT in causing the damage. Ascorbic acid (47) protected against damage from BQ; superoxide dismutase did not protect against damage from BQ, but decreased the DNA damage from BT. Inhibition of intracellular superoxide dismutase increased the ssDNA damage. Such an increase suggests that ssDNA damage caused by BT results from superoxide anion radical generated during BT oxidation. Glutathione (GSH) also protected against DNA damage (48); BQ rapidly depleted the intracellular store of GSH. During BT treatment the GSH level dropped gradually, and only BQ affected the level of oxidized glutathione (GSSG). These observations imply that the two compounds act on GSH through different mechanisms and that GSH plays an important role in the detoxication mechanism (or mechanisms) for BQ and BT.

DNA replication in rat liver and rabbit bone marrow mitochondria in vitro is inhibited in a dose-dependent manner ky HQ, *p*-BQ, and BT (49). The activity of purified rat liver mitochondrial DNA polymerase-$\gamma$ was inhibited by HQ and BQ by the metabolite's interaction with an active sulfhydryl group on the enzyme. The binding of [$^{14}$C]hydroquinone to the enzyme was prevented by *N*-ethyl maleimide as well as by unlabeled HQ or *p*-BQ, suggesting that both compounds bind to the same sulfhydryl (SH) group on the polymerase or that they are interconverted to the benzosemiquinone, which then binds.

Transcription in mouse lymphocytes (50) and macrophages (51) in vitro is inhibited in a dose-dependent manner by P, HQ, and *p*-BQ; the $IC_{50}$ for BQ was $5 \times 10^{-6}$ M for both cell types. Translation was also inhibited subsequent to the inhibition of RNA synthesis. Phenol was metabolized in macrophages by peroxidase to reactive species that inhibited RNA synthesis and covalently bound to macromolecules (51). Benzene was not metabolized. It inhibited RNA synthesis by infiltrating the plasma membrane and preventing the transport of labeled uridine into the cell, in a manner similar to that observed for lindane (52).

Rat and rabbit liver and cat and rabbit bone marrow mitoplasts are capable of bioactivating Bz in vitro to metabolites that inhibit mitochondrial transcription (53) and, consequently, translation. HQ, BQ, or P also caused a dose-dependent inhibition of transcription in rabbit bone marrow mitoplasts, with $IC_{50}$ values ranging from $2 \times 10^{-6}$ M for *p*-BQ to $2 \times 10^{-3}$ M for C. Similar concentrations of *p*-BQ, HQ, and C inhibited mRNA synthesis in rabbit bone marrow nuclei incubated under conditions specific for RNA polymerase II (54).

COVALENT BINDING OF REACTIVE METABOLITES OF BENZENE WITH MACROMOLECULES Bz and certain Bz metabolites are converted in nuclei and

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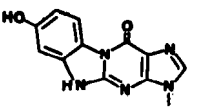
mitochondria to reactive species that covalently bind to macromolecules and that cause DNA damage in the form of strand breaks and covalently bound adducts in liver (55) and bone marrow (56). Reactive species from [$^{14}$C]benzene administered to rats and mice covalently bind to the macromolecules of various organs (57). Binding to RNA and protein is an order of magnitude greater than that to DNA. The level of DNA binding was low in several organs and highest in bone marrow. The covalent binding index (CBI) (cf 55) was 10, which approximately equals that of genotoxic carcinogens classed as weak initiators of carcinogenesis. The microsomal metabolism of Bz to reactive species capable of covalently binding to nucleic acids and proteins was induced by PB and inhibited by SKF 525A and GSH. This metabolism was selective. It was mediated by microsomes from liver but not from kidney, spleen, or lung (57). Ascorbate significantly reduced the ability of PB- and Bz-induced hepatic microsomes to metabolize labeled P or Bz to species capable of covalently binding to microsomal proteins (58). The metabolism of Bz to P was unaffected by GSH. However, GSH inhibited the covalent binding species observed from both substrates more than 95%. The metabolism of P to HQ was unaffected by either GSH or ascorbate. Bone marrow from guinea pigs with low dietary intakes of ascorbate showed a fourfold greater covalent binding of phenol equivalents in the presence of peroxide than did marrow from animals with high intakes. Myeloperoxidase appears to be responsible for the oxidation of P, as ascorbate blocks the oxidation of P, and $H_2O_2$ is for required the activation of P to covalently binding species. Partially purified guinea pig DT-diaphorase [NAD(P)H quinone oxidoreductase] inhibited the covalent binding from P by 70%. This effect was reversed by the diaphorase inhibitor, dicumeral (59). On the other hand, covalent binding was greatly enhanced by $H_2O_2$ and horseradish peroxidase or myeloperoxidase, and prevented by ascorbate. Quinone oxidoreductases reduce quinones to HQ or C via a two-electron transfer. This reduction suggests that covalent binding results from an electrophilic quinone species rather than from a semiquinone and the selective myelotoxicity of Bz may result from the high ratio of peroxidase activity to quinone reductase activity in the bone marrow (59). Lack of Bz toxicity in the liver may thus result from the conversion of quinone metabolites back to HQ and C by DT-diaphorase or by carbonyl reductase. Carbonyl reductase is very active on *p*-BQ and is the major NADPH quinone reductase of human liver (60).

Mitochondria can also activate Bz to intermediates that bind covalently to mtDNA. Rat liver mtDNA contains six deoxyguanosine adducts, and rabbit bone marrow mtDNA contains seven such adducts after incubation of mitoplasts with labeled Bz (61). A hydroxyl radical scavenger, mannitol, prevented the formation of four deoxyguanosine adducts. Two deoxyadenosine adducts were formed from [$^{14}$C]benzene in vitro. HQ, *p*-BQ, C, and Bz also

formed adducts with deoxyguanosine when incubated with bone marrow mitoplasts in vitro (61).

Jowa et al (62) characterized the adducts formed when HQ or *p*-BQ reacts with deoxyguanosine (dG) in vitro. Two dG-adducts were formed with both HQ and *p*-BQ. Iron was required for the formation of the the adducts from HQ but not from *p*-BQ. This difference indicates the necessity for oxidation of HQ to BQ or the semiquinone. Similar adducts of dG were formed when *p*-BQ was allowed to react with single- or double-stranded DNA or with chromatin from rat liver nuclei. Adduct 2 was more stable. It was further characterized by NMR and mass spectra analysis to have the structure presented as Structure 1.




HEMOPOIETIC TOXICITY Aplastic anemia from Bz poisoning could arise from toxic damage to one or more of the components of the hemopoietic system: stem cells, transit cells (progenitor cells in various degrees of differentiation), and/or bone marrow stroma or microenvironment (63).

*Effects on stem cells* Several investigators have reported reductions in the number of multipotential stem cells (CFU-S) following exposure of mice to Bz (64, 65), whereas other workers have reported no change (66, 67). This discrepancy has been explained, in part, by a toxic effect of Bz on the transit cells, which signals stem-cell proliferation and differentiation, and thus depletes the stem-cell pool (68–70).

*Effects on progenitor cells* Evidence that Bz acts primarily on committed progenitor cells was indicated by a decrease in the number of differentiating erythroid and myeloid progenitors in benzene-exposed rats, without an effect on the number of progenitor cells or mature cells in the bone marrow (71). Studies on the effects of Bz on the kinetics of $^{59}$Fe uptake into maturing mouse erythrocytes indicated that the pronormoblast was the progenitor most sensitive to benzene, whereas stem cells and nondividing reticulocytes were relatively unaffected (72). The metabolites P, C, and HQ have also been reported to reduce $^{59}$Fe incorporation into developing erythrocytes significantly, but not as much as Bz. The mixed-function oxidase inhibitor, 3-amino-1,2,4-triazole abolished the erythropoietic toxicity of Bz and P, but not of C or HQ (73). *Trans,trans*-mucondialdehyde, a six-carbon alpha, beta unsaturated aldehyde and an open-ring metabolite of Bz, is hemotoxic in CD-1 mice in a manner similar to benzene (26). It is also cytotoxic to human

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erythrocyte progenitor cells at micromolar concentrations (27). Other studies have shown that the number of erythroid (CFU-E) (74) and granulocyte/macrophage (CFU-GM) (75) progenitor cells was depressed in benzene-exposed mice. However, low-level (10-ppm) exposure over a 54-day period did not decrease the number of CFU-E or CFU-GM unless hemopoiesis was stimulated by administration of a hemolytic dose of phenylhydrazine (76).

*Effects on the stromal microenvironment* Hemopoiesis results from the interaction of stem cells with the supporting stroma. The stroma provides a favorable microenvironment for the regulated proliferation and differentiation of the stem cells (77). Reconstitution of the stroma and stem cells in vitro (78, 79), based on the growth of an adherent stromal layer from bone marrow cells in liquid culture, has stimulated new experimental approaches and provided important data about benzene toxicity to the bone marrow.

Several reports have indicated that Bz is toxic to the marrow microenvironment. Injection of normal bone marrow cells into benzene-exposed, lethally irradiated mice could not reconstitute normal hemopoiesis, as the number of granulocyte and macrophage colonies produced in vivo was decreased (66). However, incubation of normal bone marrow cells with Bz prior to administration to the animals did not reduce colony formation. This finding suggests that the microenvironment was deranged by benzene. Garnett et al (80) demonstrated that the marrow-adherent layer from benzene-treated mice did not differ in the number of CFU or in their ability to proliferate but was less able to support the differentiation of stem cells from unexposed mice. The development of an adherent layer in culture from the marrow cells of Bz-treated animals was altered; fat cells that normally appear during growth fail to develop, indicating that Bz affects marrow stromal cells. Gaido & Wierda (81) showed that HQ and *p*-BQ were most effective in decreasing the ability of stromal cells to support CFU-GM colony formation in a coculture system, whereas C and BT only inhibited at high concentrations. Adherent stromal cells from relatively Bz-resistant B6C3F1 mice support hemopoiesis better than stromal cells from sensitive DBA/2J mice (82). Marrow cellularity was reduced more in D2 than in B6 mice, but Bz had no effect on adherent stromal cell colonies or the number of granulocyte/macrophage precursors present. P, but not Bz, significantly decreased the ability of adherent stromal cells to support hemopoiesis of granulocyte and macrophage precursors, but no strain difference was evident. HQ, at levels that did not alter stromal cell number, inhibited granulocyte/macrophage colony formation and increased prostaglandin $E_2$ ($PGE_2$) levels (83). Pretreatment of the cultures with indomethacin decreased $PGE_2$ levels and protected against toxicity. Thus, the authors (84) suggested that HQ suppression of stromal cell–supported hemopoiesis is mediated by increased $PGE_2$ production and that this increase may be in-

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volved in myelosuppression by Bz. Taken together, these studies indicate that injury to bone marrow stromal cells may be an important factor in benzene-induced myelosuppression.

A possible target of Bz toxicity in the stroma is the macrophage. Macrophages are a major source of polypeptide growth factors required for the proliferation, development, and survival of progenitor cells of the various hemopoietic lineages (85). Post et al (51) demonstrated that P is metabolized in adherent mouse marrow macrophages by a peroxidase activity to one or more covalently binding species and that micromolar concentrations of HQ and *p*-BQ inhibit macrophage RNA synthesis. Adherent monocyte and macrophage cells from benzene-treated rabbits have been reported to inhibit the development of CFU-E and BFU-E colonies when cocultured with normal bone marrow (86). This finding suggests that Bz induces a CFU-E and BFU-E inhibiting activity in adherent blood cells.

*Effect of benzene on lymphopoiesis* Peripheral lymphocytopenia is an early manifestation of Bz toxicity in both animals and humans (6, 16, 87), and is a distinctive feature of benzene-induced aplastic anemia (3). The ability of P, HQ, and C to suppress lymphocyte growth and function in vitro correlates with their capacity to undergo autooxidation and with their concentration in the bone marrow or lymphoid organs (88–91). HQ and its oxidation product, *p*-BQ, inhibit proliferation and differentiation in lectin-stimulated lymphocytes in culture (89, 90). These compounds also interfere with microtubule assembly (88, 91) at concentrations that are not cytotoxic and that can be achieved in vivo. P and C suppress lymphocyte activation only at cytotoxic concentrations. Suppression of lymphocyte blastogenesis by HQ has been postulated (88) to be mediated by the interaction of *p*-BQ with sulfhydryl groups on tubulin. This binding interferes with microtubular integrity, which is essential in cell division via spindle formation and in the regulation of surface receptor movement and signal transduction across the plasma membrane.

HQ and C are also immunotoxic in vivo. They are cytotoxic to spleen cells (92). HQ and C inhibit dextran sulfate– or lipopolysaccharide (LPS)-induced development of polyclonal plaque-forming cells (PC-PFC). These PC-PFC were obtained from progenitor cells from spleen and marrow of benzene-treated animals that were induced to differentiate by dextran sulfate or LPS. Both metabolites reduced the number of PC-PFC that developed from progenitors obtained from these organs. However, only C inhibited LPS-activated marrow progenitors from maturing to PC-PFC. HQ also inhibited pre-B cells ($IgM^-$) from maturing to ($IgM^+$) cells, and HQ reduced the ability of mitogens to stimulate the proliferation of $IgM^+$ cells to CFU-B colonies (93). Thus, HQ and C, by a reduction of progenitor B-lymphocytes, are immunotoxic in vivo, and HQ inhibits marrow lymphopoiesis in vitro at a

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concentration of $10^{-7}$ M. This concentration can be achieved in vivo, suggesting that inhibition of precursor-cell maturation is important in the hemotoxicity from Bz (92). Proliferation and maturation of lymphocyte progenitor cells are regulated by polypeptide lymphokines, which are produced both in vivo and in vitro by T-lymphocytes. Bz, if metabolized in the lymphocyte to a reactive intermediate such as *p*-BQ, could inhibit the production of lymphokines. Post et al (50) demonstrated that HQ and *p*-BQ affected the dose–dependent inhibition of RNA synthesis in mouse spleen lymphocytes in vitro at micromolar concentrations that were not cytotoxic. They also demonstrated that exposure to *p*-BQ completely inhibits the proliferation and production of the T-cell lymphokine, interleukin-2, by concanavalin (conA)-stimulated T-lymphocytes.

A number of in vivo studies have been conducted by C. A. Snyder and his collaborators on the ability of Bz to affect lymphopoiesis and modulate the cell-mediated immune response. Protracted exposure of mice to a regimen known to cause thymic lymphomas decreased B-lymphocytes in bone marrow and spleen. It also decreased T-cells in thymus and spleen and the ability of T-cells to respond to mitogenic stimulation (94). Bone marrow cellularity was increased 3-fold and the numbers of thymic T-cells, 15-fold, whereas no compensatory response was seen in the spleen. This difference led to the speculation that a subpopulation of thymocytes may exist that are resistant to Bz and that proliferate in its presence (94). More importantly, short-term exposure of mice to Bz concentrations at or near the industrial standard-exposure level significantly depressed mitogen-induced blastogenesis of both B- and T-lymphocytes without reducing the total number of either type of cell (95).

Bz can modify both host resistance to a bacterial infectious agent (96) and T-cell-mediated tumor resistance (97). The immunosuppressive effects of Bz have been reported to be modulated by the prior administration of a fungal product, 6FMA, from *Aspergillus ochraceous* that has interferon-inducing properties (98). Ingestion of ethanol also increases immunosuppresion by Bz (28), as well as benzene-induced hematotoxicity (99) in experimental animals. If these findings can be extrapolated to humans, they might have considerable impact on workers who are exposed to Bz on a daily basis and who are moderate-to-heavy drinkers.

## METABOLISM AND TOXICITY OF GLYCOL ETHERS

### *Introduction*

The glycol ethers (GE; Table 1), often referred to as cellosolves, are an important class of industrial solvents that are miscible with both water and many organic solvents (100). Because of their low vapor pressure and high

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**Table 1** Names and structures of some important glycol ethers

| Name | Abbreviation | Synonyms | Structure |
|---|---|---|---|
| Ethylene glycol monomethyl ether | EGME | 2-methoxyethanol<br>Methyl Cellosolve® | $CH_3OCH_2CH_2$ |
| Ethylene glycol monoethyl ether | EGEE | 2-ethoxyethanol<br>Cellosolve® | $CH_3CH_2OCH_2C$ |
| Ethylene glycol monobutyl ether | EGBE | 2-butoxyethanol<br>Butyl Cellosolve® | $CH_3(CH_2)_3OCH_2$ |
| Propylene glycol monomethyl ether | PGME | 1-methoxy-2-propanol | $CH_3OCH_2CHO$ |

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rate of dermal absorption, significant exposure can occur through contact with the skin (101).

The older literature (101) and genotoxicity (102) of the GE have been reviewed recently. The genotoxic potential of these compounds, if any, is minimal. Occupational exposure to ethylene glycol methyl ether (EGME) has been associated with CNS, hematopoietic, and renal toxicity; few cases of illness from other GE have been reported.

Recently, attention has focused on the effects of the GE on the male reproductive system, on fetal and embryonic development, and on the hematopoietic system. We review these areas. Although testicular toxicity was first noted fifty years ago (see 103), the potential of the GE to cause this toxicity was not widely appreciated until much later. Reproductive effects were reviewed in 1983 (103); since then, much new information has become available.

## *Male Reproductive Effects*

TESTICULAR TOXICITY OF GLYCOL ETHERS Reports of testicular toxicity of EGME and EGEE (104–115) are summarized in Table 2. EGME and ethylene glycol ethyl ether (EGEE) cause testicular atrophy, decreased sperm counts, abnormal sperm motility and morphology, degeneration and atrophy of the seminiferous tubules, and impairment of fertility. EGME is more potent than EGEE (104, 107, 116). Their acetate derivatives are as potent testicular toxins as the parent compounds, presumably because the ether linkage is readily hydrolyzed (104). Other GE including ethylene glycol monobutyl ether (EGBE) (104, 111, 117–119), ethylene glycol phenyl ether (104), ethylene glycol isopropyl ether (112), ethylene glycol mono-*n*-propyl ether (120), propylene glycol monobutyl ether (PGME) (105, 111), and dipropylene glycol monomethyl ether (DPGME) (121) did not cause testicular damage.

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**Table 2** Testicular toxicity caused by EGME and EGEE[a]

| Compound | Species | Exposure | Dose[b] | Reference |
|---|---|---|---|---|
| EGME | mouse | oral, 5 d/wk 5 wk | 250 mg/kg | 104 |
| EGME | mouse B6C3F1 | inhal, 6 h/d 9 d | 1000 ppm | 105 |
| EGME | NZ rabbit | inhal, 6 h/d 5 d/wk, 13 wk | 30 ppm | 106 |
| EGME | rat, SD | oral, 11 d[c] | 100 mg/kg | 107 |
| EGME | rat, F344 | oral, 5 d | 150 mg/kg | 108 |
| EGME | rat, F344 | inhal 6 h/d 9 d | 1000 ppm | 105 |
| EGME | rat, SD | inhal, 6 h/d 5 d/wk, 13 wk | 300 ppm | 110 |
| EGME | rat | inhal, 6 h/d 10 d | 800 ppm | 111 |
| EGME | rat, alb | inhal, 4 h | 625 ppm | 112 |
| EGEE | mouse, CD-1 | $H_2O$, 14 wk | 1% $H_2O$ | 113 |
| EGEE | rat, SD | oral, 11 d[c] | 500 mg/kg 11 d | 107 |
| EGEE | rat | sc, 4 wk oral, 13 wk | 400 μl/kg 200 μl/kg | 114 |
| EGEE | rat, LE hooded | oral, 5 d | 936 mg/kg | 115 |
| EGEE | dog | iv, 22 d oral, 13 wk | 200 μl/kg | 114 |

[a]Abbreviations: NZ, New Zealand; SD, Sprague–Dawley; alb, albino; LE, Long Evans; d, day; h, hour; wk, week; inhal, inhalation; iv, intravenous; sc, subcutaneous.
[b]Lowest dose at which treatment-related testicular effects were reported.
[c]Animals sacrificed at various times during the experiments.

TESTICULAR TARGET CELLS In rats sacrificed at various times after exposure to EGME (108, 116) and EGEE (116), the meiotic spermatocytes in the pachytene stage were found to be most susceptible to the toxins, whereas cells in earlier (leptotene/zygotene) and later (early spermatid) stages were damaged only after exposure to higher doses or for a longer time period.

These findings were supported by studies in which male rats were mated at various times after exposure to EGME (115, 122, 123). Decreased fertility was observed at the postexposure time period during which the cells exposed at the meiotic spermatocyte stage would have matured to spermatozoa.

Recovery studies suggest that a high dose may cause irreversible damage (107, 110, 115). Effects on fertility or testicular morphology were seen at a postexposure time that would allow a full cycle of spermatogenic maturation, suggesting that the spermatogonia (stem cells) were affected.

GLYCOL ETHER METABOLISM AND ITS RELATIONSHIP TO TESTICULAR TOXICITY The GE that are alkoxyethanols are primarily converted to the corresponding alkoxyacetic acids, which in some cases are conjugated with glycine (124–129). This conversion is thought to be mediated by alcohol dehydrogenase, presumably via the alkoxyacetaldehyde intermediate (127, 129, 130):

$$R\text{–}O\text{–}CH_2\text{–}CH_2\text{–}OH \rightarrow R\text{–}O\text{–}CH_2\text{–}CHO \rightarrow R\text{–}O\text{–}CH_2\text{–}COOH$$

[Alkoxyethanol→alkoxyacetaldehyde→alkoxyacetic acid R: $CH_3$ (EGME), $CH_3$–$CH_2$ (EGEE), $CH_3$–$(CH_2)_3$· (EGBE)].

Metabolites of EGME and EGEE (and their acetate derivatives) cause testicular toxicity. Methoxyacetic acid (MAA) (107, 130) and ethoxyacetic acid (EAA) (107) produce the same degree of toxicity as the parent compounds, and methoxyaldehyde, the postulated intermediate in the conversion of EGME to MAA, causes similar effects (131). Pyrazole, an alcohol dehydrogenase inhibitor, blocks conversion of EGME to MAA and protects against toxicity (127).

PGME causes none of the toxic effects of EGME, although it differs from EGME only by a single methyl group (105, 111). Unlike the alkoxyethanols, which are primary alcohols, PGME is a secondary alcohol and therefore a poor substrate for alcohol dehydrogenase (132). It is extensively metabolized to $CO_2$ (128). Metabolism of DPGME, which is also not a testicular toxin, is similar to that of PGME (133).

In primary mixed cultures of Sertoli and germ cells, MAA and EAA caused degeneration of pachytene and dividing spermatozoa (134); these cells are the targets of EGME and EGEE toxicity in vivo (108, 116). EGME and EGEE at much higher concentrations, and *n*-propoxy- and *n*-butoxyacetic acid, metabolites of GE that are not testicular toxins (104, 112, 117–120), had no effect. Furthermore, the ability of the four acids to induce testicular damage in vivo correlated with the in vitro results.

BIOCHEMICAL MECHANISM OF TESTICULAR TOXICITY Exposure of Sertoli cells in culture to MAA, but not to EGME itself, decreased production of lactate but had no effect on protein synthesis or cell viability (135). Exposure to EGME decreased in vivo testicular lactate levels (136). These observations are significant because pachytene spermatocytes cannot utilize glucose and are dependent on lactate provided by Sertoli cells (137). Other results (134), however, do not support this hypothesis; addition of lactate to mixed Sertoli–germ cell cultures did not protect against MAA.

## *Hematological Effects*

ETHYLENE GLYCOL MONOBUTYL ETHER EGBE causes hemolytic anemia, as well as hemoglobinuria; decreased erythrocyte numbers, hemoglobin, and

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mean corpuscular volume; and increased mean cell volume, reticulocytes, and nucleated red blood cells; bone marrow and spleen hyperplasia; and increased extramedullary hemopoiesis (117–119). EGPE and its acetate cause similar changes (120).

EGBE may act by increasing the fragility of the erythrocyte membrane, and thus the susceptibility of the cells to hemolysis through its metabolite, butoxyacetic acid (138). However, increased erythrocyte osmotic fragility did not occur after exposure of rats to a concentration sufficient to decrease red cell number.

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ETHYLENE GLYCOL MONOMETHYL ETHER In contrast to EGBE, EGME and its acetate derivatives (104) decrease leukocyte numbers (lymphocytes and neutrophils), with less marked effects on erythrocytes (104–106, 111, 119).

EGME depletes the bone marrow of erythroid and myeloid cells (115, 119); abolishes normal extramedullary hemopoiesis (119); damages the endothelial cells of the marrow sinuses (119); and depletes lymphocytes from the thymus (105, 106, 111, 119), spleen (105), and lymph nodes (105). No changes were seen in various immune parameters after treatment with EGME or MAA (139). However, although the dose used produced thymic atrophy, it did not decrease bone marrow cellularity and leukocyte counts, as reported by others. As is the case for testicular toxicity, MAA produces the same hemopoietic toxicity as EGME (139), whereas the closely related PGME has no effect (105, 111).

The observations that EGME causes hematopoietic, lymphoid, and testicular damage might suggest that it acts by a similar mechanism in all of these rapidly dividing tissues. However, other sites of high cell turnover such as the intestinal epithelium and ovarian follicle are unaffected by EGME (105). Also, the most sensitive testicular cells are those undergoing meiosis rather than mitosis (see above).

## *Developmental Toxicity*

A number of studies have shown that EGME, EGEE, and their acetate derivatives, as well as ethylene glycol dimethyl ether (140), can adversely affect embryonic and fetal development (111, 114, 141–148; Table 3). Effects observed include increased incidence of malformations and minor variations, increased embryo mortality, and decreased fetal growth at doses not maternally toxic. Rabbits were more sensitive than rats or mice (143–145). The types of defects caused by EGME are dependent on when in gestation it is given and on how many doses are administered (142).

EGME and EGEE also affect neurochemical and behavioral development in rats (149). Both behavior and neurotransmitter levels changed. Such

**Table 3** Selected studies demonstrating the developmental toxicity of glycol ethers

| Compound | Species | Exposure | Dose[a] | Reference |
|---|---|---|---|---|
| EGME | mouse | oral, d.g. 7–14 | 31.25 mg/kg | 141 |
| EGME | mouse | oral, d.g. 7–14[c] | 500 mg/kg<br>1 dose, 300 mg/kg,<br>3 doses | 142 |
| EGME | rabbit | inhal, d.g. 6–18<br>6 h/d | 50 ppm | 143 |
| EGME | rat | inhal, d.g. 6–17<br>6 h/d | 100 ppm | 111 |
| EGME | rat | inhal, d.g. 7–15<br>7 h/d | 500 ppm | 144 |
| EGEE | rabbit | inhal, d.g. 6–18<br>6 h/d | 175 ppm | 145 |
| EGEE | rabbit | inhal, d.g. 1–18<br>7 h/d | 160 ppm | 146 |
| EGEE | rat | inhal, d.g. 1–19<br>7 h/d | 200 ppm | 146 |
| EGEE | rat | oral, d.g. 1–21 | 50 μl/kg | 114 |
| EGEE | rat | inhal, d.g. 6–15<br>6 h/d | 50 ppm | 145 |
| EGEE | rat | dermal, d.g. 7–16<br>4x/d | 233 mg | 147 |
| EGEEAc | rabbit | inhal, d.g. 6–18<br>6 h/d | 100 ppm | 145 |
| EGEEAc | rat | inhal, d.g. 7–15<br>7 h/d | 130 ppm | 144 |
| EGEEAc | rat | dermal, d.g. 7–16<br>4x/d | 341 mg | 148 |

[a]Lowest dose at which developmental toxicity was observed.
[b]Abbreviation: d.g., days of gestation.
[c]Multiple or single dose given during the period.

changes are also dependent on both the compound and the period of gestation during which exposure occurred.

Evaluations of teratogenic potential of PGME (111, 150), EGBE (144, 148, 151), and EGPE (152) have been negative. These compounds also do not produce testicular toxicity (see above), suggesting that metabolism may also be involved in teratogenicity. Significantly, ethylene glycol itself has recently been found teratogenic in both rats and mice (153, 154). A metabolite (or metabolites) of ethylene glycol, most likely glycolate, are responsible for its systemic toxicity (155, 156); the relationship between ethylene glycol metabolism and its developmental effects has not been examined.

Recent findings suggest a relationship between GE metabolism and teratogenicity. Addition of MAA or EAA blocked growth and development of

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rat embryos in culture (157). Propoxyacetic, butoxyacetic, and methoxypropionic acids, the analogous derivatives of the nonteratogenic GE (158), as well as EGME itself (157), showed much weaker toxicity in this system.

MAA is teratogenic in rats (159). EGME, MAA, and dimethoxyethyl phthalate were equally potent teratogens when administered to rats on day 12 of gestation (160). Metabolites of dimethoxyethyl phthalate include phthalate and EGME (161). The three compounds induced similar types of malformations, including several unusual defects not induced by other teratogens.

MAA accumulates in the fetus after injection into pregnant rats (162), and radioactivity from 2-methoxy[1,2-$^{14}$C]ethanol injected into pregnant mice is incorporated into fetal macromolecules (163). The alcohol dehydrogenase inhibitor, 4-methyl pyrazole (160), or ethanol (164), which would be expected to compete with EGME for metabolism, protects the fetus from teratogenic effects. However, fetal accumulation of MAA is not decreased by ethanol. This finding suggests that subsequent metabolism of MAA may be required for toxicity and that this metabolism may be blocked by ethanol (165). In support of this idea, coadministration of formate or acetate decreased the incidence of paw malformations from EGME or MAA. Thus, competition of metabolites of MAA with endogenous carboxylic acids may contribute to the developmental toxicity of EGME (166).

## MECHANISM OF CARBON TETRACHLORIDE–INDUCED HEPATOTOXICITY

Among toxic effects of $CCl_4$ in liver is a decrease in xenobiotic metabolism. Recent studies suggest that $CCl_4$ acts as a suicide substrate for cytochrome P-450 (167). The phenobarbital-induced form of the cytochrome P-450 is the most susceptible to attack. The trichloromethyl free radical, which results from the homolytic cleavage of $CCl_4$, may bind either at the heme group of cytochrome P-450 or at the active site of the enzyme near the heme group, thereby leading to inactivation (168, 169). Lipid peroxidation, which has been thought for many years to play a seminal role in $CCl_4$ toxicity, may relate directly to the decrease in cytochrome P-450 (170). In addition to the well-established route of lipid peroxidation (171), metabolism of [$^{14}$C]$CCl_4$ in rat liver microsomes was found to produce trichloromethyl free radicals, which were covalently bound to phospholipids (172). Isolation of phosphatidylcholine from the phospholipid fraction and incubation with phospholipase A2 demonstrated that about half of the phospholipid had been rendered resistant to hydrolysis. Thus, it has been argued that whereas direct reaction of the radical with the heme moiety of cytochrome P-450 leads to destruction of the mixed-function oxidase, lipid peroxidation is more closely related to

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loss of microsomal enzymes such as glucose 6-phosphatase, UDP-glucuronyltransferase, nucleoside diphosphatase, and perhaps other enzymes (173).

Hypoxia appears to potentiate $CCl_4$-induced hepatotoxicity (174). The production of ethane and pentane (thought to be indicators of lipid peroxidation in vivo, in an oxygen deficient atmosphere) from rats given $CCl_4$ reached a plateau at normal oxygen pressure (175) and reached a higher plateau at reduced oxygen levels. The initial phase of metabolism is thought to plateau because reactive metabolites of $CCl_4$ destroy cytochrome P-450. The reaction is more rapid anerobically. The metabolism of $CCl_4$ appeared to be accompanied by the destruction of a single, rather than a large number, of fatty acids. When oxygen tension was measured as a function of oxygen consumption and lipid peroxidation, as determined by malondialdehyde formation in rat liver microsomes during $CCl_4$ metabolism, both parameters were greatest in the range of 1–10 mm Hg (0.1–1.3 kPa) but were less at 80 mmHg (10.7 kPa) (173, 176).

Rechnagel & Glende (177) postulated that $CCl_4$ is metabolized via a homolytic splitting of the carbon–chlorine bond, which probably occurs anerobically following the reaction of $CCl_4$ with cytochrome P-450 ($Fe^{2+}$) (Figure 2) (178). The result is the formation of a complex composed of cytochrome P-450 ($Fe^{3+}$) and trichloromethyl free radical. This structure is analogous to the cytochrome P-450-oxy complex, which is probably involved in oxidative reactions. The trichloromethyl free radical may either be released from its complex in a manner similar to that by which the superoxide anion radical is released from cytochrome P-450 or it may undergo a one-electron reduction to yield [$Fe^{3+}$—$|CCl_3 \leftrightarrow Fe^{2+}$—$\cdot CCl_3$]. The trichloromethyl free radical can abstract a proton and form chloroform or, upon further reduction, yield a ferrodichlorocarbene complex (179), which can release HCl and CO in the presence of water. The mechanism of degradation of the carbene is probably analogous to that proposed by Kubic & Anders (180) for the thiol-stimulated conversion of dihalomethanes to CO (181). The residual cytochrome P-450 ($Fe^{2+}$) is free to react with $CCl_4$ again.

Slater (182) suggested that the trichloromethyl free radical may be less reactive than previously thought, but that it could react with oxygen to form a trichloromethyl peroxy free radical, which is a more reactive species (183, 184). "Unequivocal evidence" that trichloromethyl free radicals are formed during the metabolism of $CCl_4$ was provided in studies employing isolated rat hepatocytes and spin traps to identify the free radical species (185). Unsaturated lipid free radicals were also detected. There was no evidence that the trichloromethyl peroxy free radical was formed until the recent report of Connor et al (186). These investigators trapped a carbon dioxide anion radical resulting from $CCl_4$ metabolism in perfused liver using the spin trap *N*-t-butylnitrone. The authors concluded that the carbon dioxide anion radical

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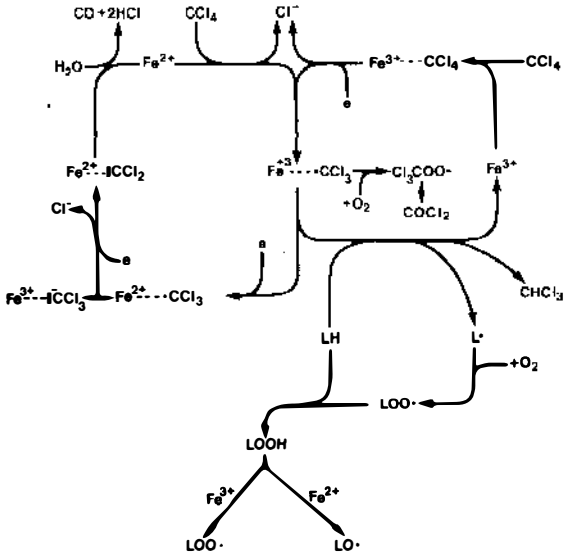


*Figure 2* Formation of reactive metabolites from $CCl_4$.

arose from the trichloromethyl peroxy free radical. The trichloromethyl peroxy free radical is thought to be a precursor to phosgene, which may also play a role in $CCl_4$-induced hepatotoxicity (187).

McCay et al (188) also used spin-trapping techniques to identify the trichloromethyl free radical in rat liver microsomes. They suggested that free radicals may be formed in a series of events initiated when a free radical reacts with a lipid moiety (LH; Figure 2). For example, during the homolytic cleavage of the C–Cl bond, radicals are formed that may react with lipid to yield L·, which can in turn react with $O_2$ to form the LOO· radical. LOOH may take either of two routes. Its action depends on the oxidation state of iron and results in the production of a highly complex series of reaction products (189). A similar series of reactions probably occurs in the liver.

The trichloromethyl radical can react directly with lipids or can be converted to the trichloromethyl peroxy radical; formation of the peroxy radical appears to depend on the availability of oxygen. The following series of events probably takes place in the hepatocyte: $CCl_4$ is reductively cleaved by cytochrome P-450 (190). This reduction yields trichloromethyl free radical in a region vicinal to unsaturated phospholipids. Thus, some radicals can add directly to double bonds to yield dienyl radicals. Others react with oxygen to form trichloromethyl peroxy radicals, which may also act in the initiation of lipid peroxidation. The lipid radicals resulting from the free-radical attack may react with oxygen as described above to initiate the process of lipid peroxidation, as Kappus (171) has suggested. Thus, oxidation of omega-3

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unsaturated fatty acids yields ethane, whereas oxidation of omcga-6 unsaturated fatty acids yields *n*-pentane.

## *Disturbances in Calcium Ion Homeostasis as a Final Common Pathway in Some Forms of Solvent Hepatotoxicity*

Rechnagel (191), in attempting to relate $CCl_4$-induced lipid peroxidation to hepatic cell death and necrosis, recognized that lipid peroxidation occurred primarily in the region surrounding cytochrome P-450. He first attempted to invoke a useful concept, which he termed a "toxicological second messenger," to link events in the endoplasmic reticulum with general cell injury. The candidates he thought most likely to possess the required properties were 4-hydroxyalkenals, which are highly toxic products of lipid peroxidation. However, despite the attractiveness of this hypothesis, he recognized that 4-hydroxyalkenals react rapidly with cellular constituents in close proximity to their points of origin and would be unlikely to act as messengers to other loci in the cell. An alternative hypothesis developed by Rechnagel (191) and by Orrenius et al (192) suggests that alterations in $Ca^{2+}$ homeostasis resulting from cell injury by $CCl_4$ or bromobenzene lead to cell death. Farber et al (193) had previously suggested that cell injury due to ischemia could be prevented if large increases in free intracellular $Ca^{2+}$ could be prevented. They had argued that toxic liver cell death was associated with susceptibility of the cells to a large influx of extracellular $Ca^{2+}$ (194). However, it was demonstrated that $CCl_4$, bromobenzene, and ethyl methane sulfonate were more toxic to liver cells in the absence of extracellular $Ca^{2+}$ than in its presence. This finding suggested that redistribution of intracellular $Ca^{2+}$ might be critical for cell death. $CCl_4$ inhibits the ability of microsomes to sequester $Ca^{2+}$ but does not prevent the influx of extracellular $Ca^{2+}$ either in vivo (195) or in vitro (196). Rechnagel (191, 197) argued that release of $Ca^{2+}$ into the cytosol could result in a number of regulatory alterations. Such alterations could lead to triglyceride accumulation, a prominent feature of $CCl_4$-induced hepatotoxicity.

Bromobenzene caused a depletion of cellular GSH concomitant with the appearance of blebs on the surface of isolated hepatocytes (198). The blebs were postulated to be related to changes in membrane permeability associated with bromobenzene toxicity. Menadione, which also forms surface blebs, served as a model for hepatotoxicity induced by bromobenzene and other hepatotoxins. In this study $Ca^{2+}$ accumulated in isolated perfused liver cells (199). Blebbing was also induced by the $Ca^{2+}$ ionophore, A23187 (200), in a $Ca^{2+}$-free medium, suggesting redistribution of intracellular $Ca^{2+}$. Bellomo et al (201) also demonstrated impairment of calcium sequestration in mitochondria and the mobilization and loss of $Ca^{2+}$ from both mitochondria

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and extramitochondrial spaces (199). Glutathione can prevent these effects. Orrenius et al (192), in agreement with Rechnagel (191), argued that these effects will cause the release of $Ca^{2+}$ into the cytosol. They suggested that membrane-bound $Ca^{2+}$-ATPase, a sulfhydryl enzyme, is also inactivated by reactive intermediates and can no longer correct the imbalance of $Ca^{2+}$ by promoting its secretion. The exact mechanism by which increased intracellular $Ca^{2+}$ causes cell death is not yet completely understood. Further studies are needed to determine whether $Ca^{2+}$ accumulation resulting from the cellular damage that reactive intermediates of solvent metabolism induce represents a final common pathway for cell death.

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## *Literature Cited*

1. Collegium Ramazzini Int. Conf. Benzene. 1985. *Am. J. Ind. Med.* 7:361–492
2. Laskin, S., Goldstein, B. D., eds. 1977. Benzene toxicity: A critical review. *J. Toxicol. Environ. Health Suppl.* Vol. 2
3. Goldstein, B. D. 1983. Clinical hematoxicity of benzene *Adv. Mod. Environ. Toxicol.* 4:51–61
4. Vigliani, E. C. 1976. Leukemia associated with benzene exposure. *Ann. N. Y. Acad. Sci.* 271:134–51
5. Infante, P. F., Wagoner, J. K., Rinsky, R. A., Young, R. J. 1977. Leukemia in benzene workers. *Lancet* 2:76–78
6. Infante, P. F., White, M. C. 1983. Benzene: Epidemiological observations of leukemia by cell type and adverse health effects associated with low-level exposure. *Environ. Health Perspect.* 52:75–82
7. Arp, E. W., Wolf, P. H., Checkoway, H. 1983. Lymphocytic leukemia and exposures to benzene and other solvents in the rubber industry. *J. Occup. Med.* 25:598–602
8. Decoufle, P., Blattner, W., Blair, A. 1983. Mortality among chemical workers exposed to benzene and other agents. *Environ. Res.* 30:16–25
9. Aksoy, M. 1985. Malignancies due to occupational exposure to benzene. *Am. J. Ind. Med.* 7:395–402
10. Aksoy, M. 1985. Benzene as a leukemogenic and carcinogenic agent. *Am. J. Ind. Med.* 8:9–20
11. Maltoni, C., Conti, B., Cotti, G. 1983. Benzene: A multipotential carcinogen. Result of long-term bioassays performed at the Bologna Institute of Oncology. *Am. J. Ind. Med.* 4:589–630
12. Maltoni, C., Conti, B., Cotti, G., Belpoggi, F. 1985. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. *Am. J. Ind. Med.* 7:415–46
13. Cronkite, E. P., Bullis, J. E., Inoue, T., Drew, R. T. 1984. Benzene inhalation produces leukemia in mice. *Toxicol. Appl. Pharmacol.* 75:358–61
14. Snyder, R. 1984. The benzene problem in historical perspective. *Fundam. Appl. Toxicol.* 4:692–99
15. Dean, B. J. 1985. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res.* 154:153–81
16. Irons, R. D. 1985. Quinones as toxic metabolites of benzene. *J. Toxicol. Environ. Health* 16:673–78
17. Schwetz, B. A. 1983. A review of the developmental toxicity of benzene. In *Carcinogenicity and Toxicity of Benzene*, ed. M. A. Mehlman, pp. 17–21. Princeton, NJ: Princeton Sci. Publ.
18. Sammett, D., Lee, E. W., Kocsis, J. J., Snyder, R. 1979. Partial hepatectomy

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reduces both metabolism and toxicity of benzene. *J. Toxicol. Environ. Health* 5:785–92

19. Gonasun, L. M., Witmer, C. M., Kocsis, J. J., Snyder, R. 1973. Benzene metabolism in mouse liver microsomes. *Toxicol. Appl. Pharmacol.* 26:398–406
20. Jerina, D., Daly, J., Witkop, B., Zaltzman-Nirenberg, P., Udenfriend, S. 1986. Role of the arene oxide-oxepin system in the metabolism of aromatic substrates. I. In vitro conversion of benzene oxide to a premercapturic acid and dihydrodiol. *Arch. Biochem. Biophys.* 128:176–83
21. Tunek, A., Platts, K. L., Przybylski, M., Oesch, F. 1980. Multi-step metabolic activation of benzene. Effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. *Chem. Biol. Interact.* 33:1–17
22. Andrews, L. S., Lee, E. W., Witmer, C. M., Kocsis, J. J., Snyder, R. 1977. Effects of toluene on metabolism, disposition and hemopoietic toxicity of [$^{14}$C]benzene. *Biochem. Pharmacol.* 26:293–300
23. Andrews, L. S., Sasame, H. A., Gillette, J. R. 1979. $^{3}$H-Benzene metabolism in rabbit bone marrow. *Life Sci.* 25:567–72
24. Rickert, D. E., Baker, T. S., Bus, J. S., Barrow, C. S., Irons, R. D. 1979. Benzene disposition in the rat after exposure by inhalation. *Toxicol. Appl. Pharmacol.* 49:417–23
25. Sawahata, T., Rickert, D. E., Greenlee, W. F. 1985. Metabolism of benzene and its metabolites in bone marrow. In *Toxicology of the Blood and Bone Marrow*, ed. R. D. Irons, pp. 141–148. New York: Raven
26. Witz, G., Rao, G. S., Goldstein, B. D. 1985. Short-term toxicity of *trans, trans* mucondialdehyde. *Toxicol. Appl. Pharmacol.* 80:511–16
27. Goldstein, B. D., Witz, G., Jarid, J., Amoruso, M. A., Rossman, T., Wolder, B. 1982. Mucondialdehyde, a potential toxic intermediate of benzene metabolism. In *Biological Reactive Intermediates II. Chemical Mechanisms and Biological Effects*, ed. R. Snyder, D. V. Parke, J. J. Kocsis, J. Jollow, G. Gibson, C. M. Witmer, pp. 331–39. New York: Plenum
28. Nakajima, T., Okuyama, S., Yonekura I., Sato, A. 1985. Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene. *Chem. Biol. Interact.* 55:23–38
29. Post, G. B., Snyder, R. 1983. Effects of enzyme induction on microsomal benzene metabolism. *J. Toxicol. Environ. Health* 11:811–25
30. Post, G. B., Snyder, R., 1983. Fluoride stimulation of microsomal benzene metabolism. *J. Toxicol. Environ. Health* 11:799–810
31. Pathiratne, A., Puyer, R. L., Brammer, J. D. 1986. A comparative study on the effects of benzene, toluene and xylene on their in vivo metabolism and drug metabolizing enzymes in rat liver. *Toxicol. Appl. Pharmacol.* 82:272–80
32. Baune, P., Flinois, J., LePrevost, E., Leroux, J. 1983. Influence of ethanol and benzene on cytochrome P-450 fractions in rat liver microsomes. *Drug Metab. Dispos.* 11:499–506
33. Gollmer, L., Graf, H., Ullrich, V. 1986. Characterization of the benzene monooxygenase system in rabbit bone marrow. *Biochem. Pharmacol.* 22: 3597–602
34. Gilmour, S., Kalf, G., Snyder, R. 1986. Comparison of the metabolism of benzene and its metabolite phenol in rat liver microsomes. In *Biological Reactive Intermediates III. Molecules and Cellular Mechanisms of Action in Animal Models and Human Disease*, ed. J. J. Kocsis, D. J. Jollow, C. M. Witmer, J. O. Nelson, R. Snyder, pp. 223–35. New York: Plenum
35. Lunte, S., Kissinger, P. 1983. Detection and identification of sulfhydryl conjugates of p-benzoquinone in microsomal incubations of benzene and phenol. *Chem. Biol. Interact.* 47:195–212
36. Johansson, I., Ingelman-Sundberg, M. 1983. Hydroxyl radical–mediated cytochrome P-450 dependent metabolic activation of benzene in microsomes and reconstituted enzyme systems from rabbit liver. *J. Biol. Chem.* 258:7311–16
37. Gorsky, L. D., Coon, M. J. 1985. Evaluation of the role of free hydroxyl radicals in the cytochrome P-450 catalyzed oxidation of benzene and cyclohexanol. *Drug Metab. Dispos.* 13:169–74
38. Jerina, D. M., Yagi, H., Hernandez, O. 1977. Stereo selective synthesis and reactions of a diol-epoxide from benzo(a)pyrene. In *Biological Reactive Intermediates: Formation, Toxicity, and Inactivation*, ed. D. J. Jollow, J. J. Kocsis, R. Snyder, H. Vaino, pp. 371–78. New York: Plenum
39. Tunek, A., Platt, K. L., Bentley, P., Oesch, F. 1978. Microsomal metabo-

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lism of benzene to species irreversibly binding to microsomal protein and effects of modifications of their mechanism. *Mol. Pharmacol.* 14:920–29
40. Hinson, J. A., Freeman, J. P., Potter, D. W., Mitchum, R. K., Evans, F. E. 1985. Mechanism of the microsomal metabolism of benzene to phenol. *Mol. Pharmacol.* 27:574–77
41. Griffiths, J., Kalf, G., Snyder, R. 1986. The metabolism of benzene and phenobarbital-induced liver mixed-function oxidase system. See Ref. 27, pp. 213–22
42. Kalf, G. F., Snyder, R., Rushmore, T. R. 1985. Inhibition of RNA synthesis by benzene metabolites and their covalent binding to DNA in rabbit bone marrow mitochondria in vitro. *Am. J. Ind. Med.* 7:485–92
43. Karaszkiewicz, J. W., Snyder, R., Kalf, G. F. 1986. Partial purification of benzene hydroxylase activity from rat liver mitoplasts. *Fed. Proc.* 45:1748
44. Lee, E. W. 1985. Effect of benzene on DNA synthesis in mouse hemopoietic cells following exposure by inhalation. *Toxicologist* 5:146
45. Pellack-Walker, P., Walker, J., Evans, H., Blumer, J. 1985. Relationship between the oxidation potential of benzene metabolites in their inhibitory effect on DNA synthesis in L5178YS cells. *Mol. Pharmacol.* 28:560–66
46. Pellack-Walker, P., Blumer, J. 1986. DNA damage in L5178YS cells following exposure to benzene metabolites. *Mol. Pharmacol.* 30:42–47
47. Pellack-Walker, P., Blumer, J. 1986. Multiple pathways for benzene-induced DNA damage: differences between benzoquinone and benzenetriol. *Proc. Am. Assoc. Cancer Res.* 27:106
48. Pellack-Walker, P., Frank, D., Blumer, J. 1986. The role of glutathione in 1,2,4-benzenetriol- and *p*-benzoquinone–induced DNA damage. *Proc. Am. Assoc. Cancer Res.* 27:81
49. Schwartz, C., Snyder, R., Kalf, G. 1986. The inhibition of mitochondrial DNA replication in vitro by the metabolites of benzene, hydroquinone and *p*-benzoquinone. *Chem. Biol. Interact.* 53:327–50
50. Post, G. B., Snyder, R., Kalf, G. F. 1985. Inhibition of RNA synthesis and interleukin-2 production in lymphocytes in vitro by benzene and its metabolites, hydroquinone and p-benzoquinone. *Toxicol. Lett.* 29:161–67
51. Post, G. B., Snyder, R., Kalf, G. F. 1986. Metabolism of benzene in macrophages in vitro and the inhibition of RNA synthesis by benzene metabolites. *Cell Biol. Toxicol.* 2:231–46
52. Roux, F., Puiseux-Dao, S., Treich, I., Fournier, E. 1978. Effect of linadane on mouse peritoneal macrophages. *Toxicology* 11:259–69
53. Kalf, G. F., Rushmore, T. R., Snyder, R. 1982. Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. *Chem. Biol. Interact.* 42:353–70
54. Post, G. B., Snyder, R., Kalf, G. F. 1984. Inhibition of RNA synthesis in rabbit bone marrow nuclei in vitro by quinone metabolites of benzene. *Chem. Biol. Interact.* 50:203–11
55. Lutz, W. K., Schlatter, C. H. 1977. Mechanism of carcinogenic action of benzene: irreversible binding to rat liver DNA. *Chem. Biol. Interact.* 18:241–45
56. Gill, D. P., Ahmed, A. 1981. Covalent binding of [$^{14}$C]benzene to cellular organelles and bone marrow nucleic acids. *Biochem. Pharmacol.* 30:1127–31
57. Artellinoi, G., Grilli, S., Calaeci, A., Mazzullo, M., Prodi, G. 1985. In vivo and in vitro binding of benzene to nucleic acids and proteins of various rat and mouse organs. *Cancer Lett.* 28:159–68
58. Smart, R. C., Zannoni, V. G. 1985. Effect of ascorbate on covalent binding of benzene and phenol metabolites to isolated tissue preparations. *Toxicol. Appl. Pharmacol.* 77:334–43
59. Smart, R. C, Zannoni, V. G. 1984. DT-Diaphorase and peroxidase influence the covalent binding of the metabolites of phenol, the major metabolite of benzene. *Mol. Pharmacol.* 26:105–11
60. Wermuth, B., Platts, K., Seidel, A., Oesch, F. 1986. Carbonyl reductase provides the enzymatic basis of quinone reduction in man. *Biochem. Pharmacol.* 35:1277–82
61. Rushmore, T. R., Snyder, R., Kalf, G. F. 1984. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria in vitro. *Chem. Biol. Interact.* 49:133–54
62. Jowa, L., Winkle, S., Kalf, G., Witz, G., Snyder, R. 1986. Deoxyguanosine adducts formed from benzoquinone and hydroquinone See Ref. 34, pp. 825–32
63. Benestad, H. B. 1979. In *Aplastic Anemia,* ed. G. Gary, pp. 26-43. London: Baillierie Tindall
64. Uyeki, E. M., Elaskar, A., Shoeman, D. W., Bisel, T. U. 1977. Acute toxicity of benzene inhalation to hemopoietic

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

precursor cells. *Toxicol. Appl. Pharmacol.* 40:49–57
65. Green, J. D., Snyder, C. A., LoBue, J., Goldstein, B. D., Albert, R. E. 1981. Acute and chronic dose/response effects of inhaled benzene on multipotential hemopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 mice. *Toxicol. Appl. Pharmacol.* 58:492–503
66. Frash, V. N., Yushkov, B. G., Karaulov, A. V., Skuratov, V. L. 1976. Mechanism of action of benzene on hematopoiesis. Investigation of hematopoietic stem cells. *Bull. Exp. Biol. Med.* 82:985–87
67. Speck, B., Cornu, P., Nissen, C., Groff, P., Weber, W., Jeannet, M. 1978. On the pathogenesis and treatment of aplastic anemia. In *Experimental Hematology Today,* ed. S. J. Baum, G. D. Ledney, pp. 43–48 New York: Springer-Verlag
68. Snyder, R., Lee, E. S., Kocsis, J. J., Witmer, C. M. 1977. Bone marrow depressant and leukemogenic actions of benzene. *Life Sci.* 21:1709–22
69. Gill, D. P., Jenkins, V. R., Kemper, R. R., Ellis, S. 1980. The importance of pluripotential stem cells in benzene toxicity. *Toxicology* 16:163–71
70. Cronkite, E. P., Inoue, T., Carsten, A. L., Miller, M. E., Bullis, J. E., et al. 1982. Effects of benzene inhalation on murine pleuripotential stem cells. *J. Toxicol. Environ. Health* 9:411–21
71. Irons, R., Heck, H. d'A., Moore, B. J., Muirhead, K. A. 1979. Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. *Toxicol. Appl. Pharmacol.* 51:399–409
72. Lee, E. W., Kocsis, J. J., Snyder, R. 1974. Acute effect of benzene on $^{59}Fe$ incorporation into circulating erythrocytes. *Toxicol. Appl. Pharmacol.* 27: 431–36
73. Bolcsak, L. E., Nerland, D. E. 1983. Inhibition of erythropoiesis by benzene and benzene metabolites. *Toxicol. Appl. Pharmacol.* 69:363–68
74. Baarson, K. A., Snyder, C. A., Albert, R. E. 1984. Repeated exposure of C57Bl mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. *Toxicol. Lett.* 20: 337–42
75. Tunek, A., Platts, K. L., Przybylski, M., Oesch, F. 1980. Multi-step metabolic activation of benzene. Effect of superoxide disimutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. *Chem. Biol. Interact.* 33:1–17
76. Dempster, A. M., Snyder, C. A. 1986. Effect of low-level benzene exposure on murine hemopoietic precursor cells. *Toxicologist* 6:285
77. Tavassoli, M., Friedenstein, A. 1983. Hemopoietic stromal microenvironment. *Am. J. Hematol.* 15:195–203
78. Dexter, T. M., Allen, T. D., Lajtha, L. G. 1977. Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J. Cell. Physiol.* 91:335–44
79. Dexter, T. M. 1979. Hemopoiesis in long-term bone marrow cultures. *Acta Haematol.* 62:299–305
80. Garnett, H., Cronkite, E. P., Drew, R. T. 1983. Effect of in vivo exposure to benzene on the characteristics of bone marrow adherent cells. *Leukemia Res.* 7:803–10
81. Gaido, K., Wierda, D. 1984. In vitro effects of benzene metabolites on mouse bone marrow stromal cells. *Toxicol. Appl. Pharmacol.* 76:45–55
82. Gaido, K., Wierda, D. 1985. Modulation of stromal cell function in DBA/2 and B6C3F-11 mice exposed to benzene or phenol. *Toxicol. Appl. Pharmacol.* 81:469–75
83. Gaido, K., Wierda, D. 1986. Hydroquinone suppression of bone marrow stromal cell supported hemopoiesis in vitro is associated with prostaglandin $E_2$ production. *Toxicologist* 6:286
84. Wierda, D., Gaido, K. 1986. Indomethacin protects against in vivo benzene inhibition of stromal cell function. *Toxicologist* 6:286
85. Moore, M. A. S. 1978. Regulatory role of the macrophage in hemopoiesis. In *Stem Cells and Tissue Homeostasis,* ed. B. I. Lord, C. S. Potten, R. J. Cole, pp. 187–202. Cambridge: Cambridge Univ. Press
86. Haak, H. L., Speck, B. 1982. Inhibition of CFU-E and BFU-E by mononuculear peripheral blood cells during chronic benzene treatment in rabbits. *Acta Haematol.* 67:27–33
87. Irons, R., Moore, B. J. 1980. Effect of short-term benzene administration on circulating lymphocyte subpopulations in the rabbit: Evidence of a selective B-lymphocyte sensitivity. *Res. Commun. Chem. Pathol. Pharmacol.* 27: 147–55
88. Irons, R. D., Neptun, D. A., Pfeifer, R. W. 1981. Inhibition of lymphocyte transformation and microtubule assembly by quinone metabolites of benzene:

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

Evidence for a common mechanism. *J. Reticuloendothel. Soc.* 30:359–72
89. Pfeifer, R. W., Irons, R. D. 1981. Inhibition of lectin-stimulated agglutination and mitosis by hydroquinone. Reactivity with intracellular sulfhydryl groups. *Exp. Mol. Pathol.* 35:189–98
90. Pfeifer, R. W., Irons, R. D. 1982. Effect of benzene metabolites on PHA-stimulated lymphopoiesis in rat bone marrow. *J. Reticuloendothel. Soc.* 31: 155–70
91. Pfeifer, R. W., Irons, R. D. 1983. Alteration of lymphocyte function by quinones through sulfhydryl-dependent disruption of microtubule assembly. *Int. J. Immunopharmacol.* 5:463–70
92. Wierda, D., Irons, R. 1982. Hydroquinone and catechol reduce the frequency of progenitor B lymphocytes in mouse spleen and bone marrow. *Immunopharmacology* 4:41–54
93. King, A. G., Landreth, K. S., Wierda, D. 1986. Hydroquinone inhibits bone marrow pre-B cell maturation in vitro. *Toxicologist* 6:169
94. Rozen, M. G., Snyder, C. A. 1985. Protracted exposure of C57Bl/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and hone marrow proliferation in response to the exposures. *Toxicology* 37:13–26
95. Rozen, M. G., Snyder, C. A., Albert, R. E. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. *Toxicol. Lett.* 20:343–49
96. Rosenthal, G. J., Snyder, C. A. 1985. Modulation of the immune response to *Listeria monocytogenes* by benzene inhalation. *Toxicol. Appl. Pharmacol.* 80:502–10
97. Rosenthal, G. J., Snyder, C. A. 1986. Altered T-cell responses in C57B1/6J mice following sub-chronic benzene inhalation. *Toxicologist* 6:68
98. Pandya, K. P., Shanker, R., Gupta, A., Kahn, W. A., Ray, P. K. 1986. Modulation of benzene toxicity by an interferon inducer (6MFA). *Toxicology* 39:291–305
99. Baarson, K. A., Snyder, C. A., Green, J. D., Sellakumar, A., Goldstein, B. D., et al. 1982. The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow and spleen cells are increased by ingested alcohol. *Toxicol. Appl. Pharmacol.* 64:393–404
100. National Institute for Occupational Safety and Health. 1983. *Current Intelligence Bulletin No. 39. Glycol Ethers, 2-Methoxyethynol and 2-Ethoxyethanol.* US Dep. Health Human Serv. Publ. No. 83–112
101. Rowe, V. K., Wolfe, M. A. 1982. Derivatives of glycols. In *Patty's Industrial Hygiene and Toxicology,* ed. G. Clayton, F. Clayton, 2C:3911–4048. New York: Wiley. 3rd ed.
102. McGregor, D. B. 1984. Genotoxicity of glycol ethers. *Environ. Health Perspect.* 57:97–103
103. Hardin, B. D. 1983. Reproductive toxicity of the glycol ethers. *Toxicology* 27:91–102
104. Nagano, K., Nakayama, E., Koyano, M., Oobayashi, H., Adachi, H., et al 1979. Testicular atrophy of mice induced by ethylene glycol mono alkyl ethers. *Jpn. J. Ind. Health* 21:29–35
105. Miller, R. R., Ayres, J. A., Calhoun, L. L., Young, J. T., McKenna, M. J. 1981. Comparative short-term inhalation toxicity of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in rats and mice. *Toxicol. Appl. Pharmacol.* 61:368–77
106. Miller, R. R., Ayres, J. A., Young, J. T., McKenna, M. J. 1983. Ethylene glycol monomethyl ether. I. Subchronic vapor study with rats and rabbits. *Fundam. Appl. Toxicol.* 3:49–54
107. Foster, P. M. D., Creasy, D. M., Foster, J. R., Thomas, L. V., Cook, M. W., et al. 1983. Testicular toxicity of ethylene glycol monomethyl and monoethyl ethers in the rat. *Toxicol. Appl. Pharmacol.* 69:385–99
108. Chapin, R. E., Dutton, S. L., Ross, M. D., Sumrell, B. M., Lamb, J. C. 1984. The effects of ethylene glycol monomethyl ether on testicular histology in F344 rats. *J. Androl.* 5:369–80
109. Chapin, R. E., Dutton, S. L., Ross, M. D., Swaisgood, R. R., Lamb, J. C. 1985. The recovery of the testis over 8 weeks after short-term dosing with ethylene glycol monomethyl ether: histology, cell-specific enzymes, and rete testis fluid protein. *Fundam. Appl. Toxicol.* 5:515–25
110. Rao, K. S., Cobel-Heard, S. R., Young, J. T., Hanley, T. R., Hayes, W. C., et al. 1983. Ethylene glycol monomethyl ether II. Reproductive and dominant lethal studies in rats. *Fundam. Appl. Toxicol.* 3:80–85
111. Doe, J. E., Samuels, D. M., Tinston, D. J., deSilva Wickramaratne, G. A. 1983. Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

propylene glycol monomethyl ether. *Toxicol. Appl. Pharmacol.* 69:43–47
112. Samuels, D. M., Doe, J. E., Tinston, D. J. 1984. The effects on the rat testis of single inhalation exposures to ethylene glycol monoalkyl ethers, in particular ethylene glycol monomethyl ether. *Arch. Toxicol. Suppl.* 7:167–70
113. Lamb, J. C., Gulati, D. K., Russell, V. S., Hommel, L., Sabharwal, P. S. 1984. Reproductive toxicity of ethylene glycol monoethyl ether tested by continuous breeding of CD-1 mice. *Environ. Health Perspect.* 57:85–90
114. Stenger, E. G., Aeppli, L., Muller, D., Peheim, E., Thomann, P. 1971. Zur Toxikologie des Äthylenglykol-monoäthylathers. *Arzneim. Forsch.* 21:880–85
115. Oudiz, D. J., Zenick, H., Niewenhuis, R. J., McGinnis, P. M. 1984. Male reproductive toxicity and recovery associated with acute ethoxyethanol exposure in rats. *J. Toxicol. Environ. Health* 13:763–75
116. Creasy, D. M., Foster, P. M. D. 1984. The morphological development of glycol ether–induced testicular atrophy in the rat. *Fundam. Mol. Pathol.* 40:169–76
117. Dodd, D. E., Snellings, W. M., Maronpot, R. R., Ballantyne, B. 1983. Ethylene glycol monobutyl ether: acute, 9-day, and 90-day vapor inhalation studies in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* 68:405–14
118. Tyler, T. R., 1984. Acute and subchronic toxicity of ethylene glycol monobutyl ether. *Environ. Health Perspect.* 57:185–91
119. Grant, D., Sulsh, S., Jones, H. B., Gangolli, S. D., Butler, W. H. 1985. Acute toxicity and recovery in the hemopoietic system of rats after treatment with ethylene glycol monomethyl and monobutyl ethers. *Toxicol. Appl. Pharmacol.* 77:187–200
120. Katz, G. V., Krasavage, W. J., Techaar, C. J. 1984. Comparative acute and subchronic toxicity of ethylene glycol monopropyl ether and ethylene glycol monopropyl ether acetate. *Environ. Health Perspect.* 57:166–75
121. Landry, T. D., Yano, B. L. 1984. Dipropylene glycol monomethyl ether: a 13-week inhalation toxicity study in rats and rabbits. *Fundam. Appl. Toxicol.* 4:612–17
122. McGregor, D. B., Willins, M. J., McDonald, P., Holmstrom, M., McDonald, D., et al. 1983. Genetic effects of 2-methoxy ethanol and bis(2-methoxyethyl) ether. *Toxicol. Appl. Pharmacol.* 70:303–16
123. Chapin, R. E., Dutton, S. L., Ross, M. D., Lamb, J. C. 1985. Effects of ethylene glycol monomethyl ether (EGME) on mating performance and epididymal sperm parameters in F344 rats. *Fundam. Appl. Toxicol.* 5:182–89
124. Hutson, D. H., Pickering, B. A. 1971. The metabolism of isopropyl oxitol in rat and dog. *Xenobiotica* 1:105–119
125. Jonsson, A. K., Pederson, J., Steen, G. 1982. Ethoxyacetic acid and *N*-ethoxyacetylglycine: metabolites of ethoxyethanol (ethylcellosolve) in rats. *Acta Pharmacol. Toxicol.* 50:358–62
126. Cheever, K. L., Plotnick, H. B., Richards, D. E., Weigel, W. W. 1984. Metabolism and excretion of 2-ethoxyethanol in the adult male rat. *Environ. Health Perspect.* 57:241–48
127. Moss, E. J., Thomas, L. V., Cook, M. W., Walters, D. G., Foster, P. M. D., et al. 1985. The role of metabolism in 2-methoxyethanol-induced testicular toxicity. *Toxicol. Appl. Pharmacol.* 79:480–89
128. Miller, R. R., Hermann, E. A., Langvardt, P. W., McKenna, M. J., Schwetz, B. A. 1983. Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. *Toxicol. Appl. Pharmacol.* 67:229–37
129. Jonsson, A. K., Steen, G. 1978. *n*-Butoxyacetic acid, a urinary metabolite from inhaled *n*-butoxyethanol (butycellosolve). *Acta Pharmacol. Toxicol.* 42:354–56
130. Miller, R. R., Carrean, R. E., Young, J. T., McKenna, M. J. 1982. Toxicity of methoxyacetic acid in rats. *Fundam. Appl. Toxicol.* 2:158–60
131. Foster, P. M. D., Lloyd, S. C., Blackburn, D. M. 1985. Testicular toxicity of 2-methoxyacetaldehyde, a possible metabolite of ethylene glycol monomethyl ether, in the rat. *Toxicologist* 5:115
132. Von Wartburg, J. P., Bethuen, J. L., Vallee, B. L. 1964. Human liver alcohol dehydrogenase. Kinetic and physicochemical properties. *Biochemistry* 3:1775–82
133. Miller, R. R., Hermann, E. A., Calhoun, L. L., Kastl, P. E., Zakett, D. 1985. Metabolism and disposition of dipropylene glycol monomethyl ether (DPGME) in male rats. *Fundam. Appl. Toxicol.* 5:721–26
134. Gray, T. J. B., Moss, E. J., Creasy, D. M., Gangolli, S. D. 1985. Studies on

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

the toxicity of some glycol ethers and alkoxyacctic acids in primary testicular cell cultures. *Toxicol. Appl. Pharmacol.* 79:490–501

135. Beattie, P. J., Welsh, M. J., Brabec, M. J. 1984. The effect of 2-methoxyethanol and methoxyacetic acid on Sertoli cell lactate production and protein synthesis in vitro. *Toxicol. Appl. Pharmacol.* 76:56–61
136. Beattie, P. J., Brabec, M. J. 1985. 2-Methoxyethanol (ME) depletes testicular lactate. *Toxicologist* 5:463
137. Jutte, N. H. P. M., Jansen, R., Grootegoed, J. A., Rommerts, F. F. G., Clausen, O. P. F., et al. 1982. Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. *J. Reprod. Fertil.* 65:431–38
138. Carpenter, C. P., Pozzani, U. C., Weil, C. S., Nair, J. H., Keck, G. A., et al. 1956. The toxicity of butyl cellosolve solvent. *Am. Med. Assoc. Arch. Ind. Health* 14:114–31
139. House, R. V., Lauer, L. D., Murray, M. J., Ward, E. C., Dean, J. H. 1985. Immunological studies in B6C3F1 mice following exposure to ethylene glycol monomethyl ether and its principal metabolite methoxyacetic acid. *Toxicol. Appl. Pharmacol.* 77:358–62
140. Uemura, K. 1980. The teratogenic effects of ethylene glycol dimethylether on mouse. *Acta Obstet. Gynaecol. Jpn.* 32:113
141. Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, H., et al. 1981. Embryotoxic effects of ethylene glycol monomethyl ether in mice. *Toxicology* 20:335–43
142. Horton, V. L., Sleet, R. B., John-Greene, J. A., Welsch, F. 1985. Developmental phase-specific and dose-related teratogenic effects of ethylene glycol monomethyl ether in CD-1 mice. *Toxicol. Appl. Pharmacol.* 80:108–18
143. Hanley, T. R., Yano, B. L., Nitschke, K. D., John, J. A. 1984. Comparison of the teratogenic potential of inhaled ethylene glycol monomethyl ether in rats, mice, and rabbits. *Toxicol. Appl. Pharmacol.* 75:409–22
144. Nelson, B. K., Setzer, J. V., Brightwell, W. S., Mathinos, P. R., Kuczuk, M. H., et al. 1984. Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. *Environ. Health Perspect.* 57:261–71
145. Doe, J. E. 1984. Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. *Environ. Health Perspect.* 57:33–41
146. Andrew, F. D., Hardin, B. D. 1984. Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. *Environ. Health Perspect.* 57:13–23
147. Hardin, B. D., Niemeier, R. W., Smith, R. J., Kuczuk, M. H., Mathinos, P. R., et al. 1982. Teratogenicity of 2-ethoxyethanol by dermal application. *Drug Chem. Toxicol.* 5:277–94
148. Hardin, B. D., Goad, P. T., Burg, J. R. 1984. Developmental toxicity of four glycol ethers applied cutaneously to rats. *Environ. Health Perspect.* 57:69–74
149. Nelson, B. K., Brightwell, W. S. 1984. Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers. *Environ. Health Perspect.* 57:43–46
150. Hanley, T. R., Young, J. T., John, J. A., Rao, K. S. 1984. Ethylene glycol monomethyl ether (EGME) and propylene glycol monomethyl ether (PGME): Inhalation fertility and teratogenicity studies in rats, mice, and rabbits. *Environ. Health Perspect.* 57:7–12
151. Tyl, R. W., Millicovsky, G., Dodd, D. E., Pritts, I. M., France, K. A., et al. 1984. Teratologic evaluation of ethylene glycol monobutyl ether in Fischer 344 rats and New Zealand White rabbits following inhalation exposure. *Environ. Health Perspect.* 57:47–68
152. Krasavage, W. J., Katz, G. V. 1985. Developmental toxicity of ethylene glycol monopropyl ether in the rat. *Teratology* 32:93–102
153. Lamb, J. C., Maronpot, P. R., Gulati, D. R., Russell, V. S., Hammel-Barnes, L., et al. 1985. Reproductive and developmental toxicity of ethylene glycol in the mouse. *Toxicol. Appl. Pharmacol.* 81:100–12
154. Price, C. J., Kimmel, C. A., Tyl, R. W., Marr, R. W. 1985. The developmental toxicity of ethylene glycol in rats and mice. *Toxicol. Appl. Pharmacol.* 81:113–27
155. Clay, K. L., Murphy, R. C. 1977. On the metabolic acidosis of ethylene glycol intoxication. *Toxicol. Appl. Pharmacol.* 39:39–49
156. Chou, J. Y., Richardson, K. E. 1978. The effect of pyrazole on ethylene glycol toxicity and metabolism in the rat. *Toxicol. Appl. Pharmacol.* 43:33–34
157. Yonemoto, J., Brown, N. A., Webb, M. 1984. Effects of dimethoxyethyl phthalate, monomethoxyethyl phthalate, 2-methoxyethanol and methoxyacetic acid on post-implantation rat embryos in culture. *Toxicol. Lett.* 21:97–102

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

158. Rawlings, S. J., Shuker, D. F. G., Webb, M., Brown, N. A. 1985. The teratogenic potential of alkoxy acids in post-implantation rat embryo culture: structure-activity relationships. *Toxicol. Lett.* 28:49–58
159. Brown, N. A., Holt, D., Webb, M. 1984. The teratogenicity of methoxyacetic acid in the rat. *Toxicol. Lett.* 21:97–102
160. Ritter, E. J., Scott, W. J., Randall, J. L., Ritter, J. M. 1985. Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxyethanol and methoxyacetic acid in the rat. *Teratology* 32:25–31
161. Campbell, J., Holt, D., Webb, M. 1984. Dimethoxyethylphthalate metabolism. Teratogenicity of the diester and its metabolites in the pregnant rat. *J. Appl. Toxicol.* 4:35–41
162. Scott, W. J., Nau, H. 1985. Weak acids as human teratogens: Accumulation in the young mammalian embryo. *Teratology* 31:25A
163. Sleet, R. B., John-Greene, J. A., Welsch, F. 1986. Localization of radioactivity from 2-methoxy[1,2-$^{14}$C]ethanol in maternal and conceptus compartments of CD-1 mice. *Toxicol. Appl. Pharmacol.* 84:25–35
164. Sleet, R. B., John-Greene, J. A., Welsch, F. 1985. Paw dysmorphogenesis in CD-1 mice treated with 2-methoxyethanol and methoxyacetic acid in combination with ethanol. *Teratology* 31:48A
165. Sleet, R. B., John-Greene, J. A., Welsch, F. 1986. Ethanol attenuation of 2-methoxyethanol (ME) teratogenicity does not alter embryonal accumulation of radioactivity from ME (1,2-$^{14}$C). *Toxicologist* 6:297
166. Sleet, R. B., Greene, J. A., Welsch, F. 1986. Reduction of methoxyethanol (ME)-and methoxyacetic acid (MAA)-induced paw malformations in CD-1 mice by small endogenous carboxylic acids. *Teratology* 33:45C
167. De Groot, H., Haas, W. 1981. Self-catalysed, $O_2$-independent inactivation of NADPH- or dithionite-reduced microsomal cytochrome P-450 by carbon tetrachloride. *Biochem. Pharmacol.* 30:2343–47
168. Fernandez, G., Villarruel, M. C., De Toranzo, E. G. D., Castro, J. A. 1982. Covalent binding of carbon tetrachloride metabolites to the heme moiety of cytochrome P-450 and its degradation products. *Res. Commun. Chem. Pathol. Pharmacol.* 35:283–90
169. Yamazoe, Y., Sugiura, M., Kamataki, T., Kato, R. 1979. The apparent loss of cytochrome P-450 associated with metabolic activation of carbon tetrachloride. *Jpn. J. Pharmacol.* 29:715–21
170. Masuda, Y. 1981. Carbon tetrachloride–induced loss of microsomal glucose 6-phosphatase and cytochrome P-450 in vitro. *Jpn. J. Pharmacol.* 31:107–16
171. Kappus, H. 1985. Lipid peroxidation: Mechanisms, analysis, enzymology and biological relevance. In *Oxidative Stress,* ed. H. Sies, pp. 273–310. New York: Academic
172. Frank, H., Link, B. 1984. Anaerobic metabolism of carbon tetrachloride and formation of catabolically resistant phospholipids. *Biochem. Pharmacol.* 33:1127–30
173. De Groot, H., Noll, T. 1986. The crucial role of low steady state oxygen partial pressures in haloalkane free-radical-mediated lipid peroxidation. Possible implications in haloalkane liver injury. *Biochem. Pharmacol.* 35:15–19
174. Shen, E. S., Garry, V. F., Anders, M. W. 1982. Effect of hypoxia on carbon tetrachloride hepatotoxicity. *Biochem. Pharmacol.* 31:3787–93
175. Durk, H., Frank, H. 1984. Carbon tetrachloride metabolism in vivo and exhalation of volatile alkanes: Dependence upon oxygen partial pressure. *Toxicology* 30:249–57
176. Noll, T., De Groot, H. 1984. The critical steady-state hypoxic conditions in carbon tetrachloride-induced lipid peroxidation in rat liver microsomes. *Biochim. Biophys. Acta* 795:356–62
177. Rechnagel, R. O., Glende, E. A. Jr. 1973. Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. *CRC Crit. Rev. Toxicol.* 2:263–67
178. Ahr, H. J., King, L. J., Nastainczyk, W., Ullrich, V. 1980. The mechanism of chloroform and carbon monoxide formation from carbon tetrachloride by microsomal cytochrome P-450. *Biochem. Pharmacol.* 29:2855–61
179. Pohl, L., George, J. W. 1983. Identification of dichloromethyl carbene as a metabolite of carbon tetrachloride. *Biochem. Biophys. Res. Commun.* 117:367–71
180. Kubic, V. L., Anders, M. W. 1978. Metabolism of dihalomethanes to carbon monoxide. III. Studies of the mechanism of the reaction. *Biochem. Pharmacol.* 27:2349–55
181. Macdonald, T. L. 1982. Chemical mechanisms of halocarbon metabolism. *CRC Crit. Rev. Toxicol.* 11:85–120

Annu. Rev. Pharmacol. Toxicol. 1987.27:399-427. Downloaded from www.annualreviews.org by Central College on 12/10/11. For personal use only.

182. Slater, T. 1982. Free radicals as reactive intermediates in tissue injury. See Ref. 27, pp. 575–89
183. Packer, J. E., Slater, T. F., Willson, R. L. 1978. Reactions of the carbon tetrachloride–related peroxy free radical ($CCl_3O_2\cdot$) with amino acids: Pulse radiolysis evidence. *Life Sci.* 23:2617–20
184. Mico, B. A., Pohl, L. R. 1983. Reductive oxygenation of carbon tetrachloride: Trichloromethylperoxyl radical as a possible intermediate in the conversion of carbon tetrachloride to electrophilic chlorine. *Arch. Biochem. Biophys.* 225:596–609
185. Albano, E., Lott, K. A. L., Slater, T. F., Stier, A., Symons, C. R., Tomas, A. 1982. Spin-trapping studies on the free-radical products formed by metabolic activation of carbon tetrachloride in rat liver microsomal fractions isolated hepatocytes and in vivo in the rat. *Biochem. J.* 204:593–603
186. Connor, H. D., Thurman, R. G., Galizi, M. D., Mason, R. P. 1986. The formation of a novel free radical metabolite from $CCl_4$ in the perfused rat liver and *in vivo*. *J. Biol. Chem.* 261:4542–48
187. Pohl, L. R., Schulick, R. D., Highet, R. J., George, J. W. 1984. Reductive-oxygenation mechanism of metabolism of carbon tetrachloride to phosgene by cytochrome P-450. *Mol. Pharmacol.* 25:318–21
188. McCay, P. B., Lai, E. K., Poyer, J. L. 1984. Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. *J. Biol. Chem.* 259:2135–43
189. Gardner, H. W., Kleiman, R., Weisleder, D. 1974. Homolytic decomposition of linolcic acid hydropcroxide: Identification of fatty acid products. *Lipids* 9:696–706
190. Noguchi, T., Fong, K. L., Lai, E. K., Alexander, S. S., King, M. M., et al. 1982. Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. *Biochem. Pharmacol.* 31:615–24
191. Rechnagel, R. O. 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci.* 33:401–8
192. Orrenius, S., Thor, H., Di Monte, D., Bellomo, G., Nicotera, P., et al. 1985. Mechanisms of oxidative cell injury studied in intact cells. In *Microsomes and Drug Oxidations*, ed. A. R. Boobis, J. Caldwell, F. De Matteis, C. R. Elcombe, pp. 238–47. London: Taylor & Francis. 428 pp.
193. Farber, J. L., Chien, K. R., Mittnacht, S. 1981. The pathogenesis of irreversible cell injury in ischemia. *Am. J. Pathol.* 102:271–81
194. Schanne, F. A. X., Kane, A. B., Young, E. A., Farber, J. L. 1979. Calcium dependence of toxic cell death: A final common pathway. *Science* 206:700–2
195. Moore, L., Davenport, G. R., Landon, E. J. 1976. Calcium uptake of a rat liver microsomal fraction in response to in vivo administration of carbon tetrachloride. *J. Biol. Chem.* 251:1197–201
196. Lowery, K., Glende, E. A. Jr., Rechnagel, R. O. 1981. Rapid depression of rat liver microsomal calcium pump activity after administration of carbon tetrachloride or bromotrichloromethane and lack of effect after ethanol. *Toxicol. Appl. Pharmacol.* 59:389–94
197. Pencil, S. D., Glende, E. A. Jr., Rechnagel, R. O. 1982. Loss of calcium sequestration capacity in endoplasmic reticulum of isolated hepatocytes treated with carbon tetrachloride. *Res. Commun. Chem. Pathol. Pharmacol.* 36: 413–28
198. Thor, H., Orrenius, S. 1980. The mechanism of bromobenzene-induced cytotoxicity studied with isolated hepatocytes. *Toxicology* 44:31–43
199. Mehendale, H. M., Svensson, S., Baldi, C., Orrenius, S. 1985. Accumulation of $Ca^{2+}$ induced by cytotoxic levels of menadione in the isolated, perfused rat liver. *Eur. J. Biochem.* 149:201–6
200. Jewell, S. A., Bellomo, G., Thor, H., Orrenius, S., Smith, M. T. 1982. Bleb formation in hepatocytes during drug metabolism is caused by disturbances in thiol and calcium ion homeostasis. *Science* 217:1257–59
201. Bellomo, G., Jewell, S. A., Orrenius, S. 1982. The metabolism of menadione impairs the ability of rat liver mitochondria to take up and retain calcium. *J. Biol. Chem.* 257:11558–62