Published in final edited form as:

Chem Biol Interact. 2010 March 19; 184(0): 201–206. doi:10.1016/j.cbi.2009.12.025.

The Fate of Benzene Oxide

Terrence J. Monks^b, Michael Butterworth^a, and Serrine S. Lau^b

Michael Butterworth: mb66@leicester.ac.uk; Serrine S. Lau: lau@pharmacy.arizona.edu

^bDept. Pharmacology & Toxicology, College of Pharmacy, University of Arizona, 1703 E. Mabel Street, Tucson, AZ, USA 85721-0207

^aThe Medical Research Council Toxicology Unit, Hodgkin Building PO Box138, University of Leicester, Lancaster Road, Leicester, LE1 9HN, UK

Abstract

Metabolism is a prerequisite for the development of benzene-mediated myelotoxicity. Benzene is initially metabolized via cytochromes P450 (primarily CYP2E1 in liver) to benzene oxide, which subsequently gives rise to a number of secondary products. Benzene oxide equilibrates spontaneously with the corresponding oxepine valence tautomer, which can ring open to yield a reactive α - β -unsaturated aldehyde, trans-trans-muconaldehyde (MCA). Further reduction or oxidation of MCA gives rise to either 6-hydroxy-trans-trans-2,4-hexadienal or 6-hydroxy-trans-trans-2,4-hexadienoic acid. Both MCA and the hexadienal metabolite are myelotoxic in animal models. Alternatively, benzene oxide can undergo conjugation with glutathione (GSH), resulting in the eventual formation and urinary excretion of *S*-phenylmercapturic acid. Benzene oxide is also a substrate for epoxide hydrolase, which catalyzes the formation of benzene dihydrodiol, itself a substrate for dihydrodiol dehydrogenase, producing catechol. Finally, benzene oxide spontaneously rearranges to phenol, which subsequently undergoes either conjugation (glucuronic acid or sulfate) or oxidation. The latter reaction, catalyzed by cytochromes P450, gives rise to hydroquinone (HQ) and 1,2,4-benzene triol. Coadministration of phenol and HQ reproduces the myelotoxic effects of benzene in animal models. The two diphenolic metabolites of benzene, catechol and HQ undergo further oxidation to the corresponding *ortho*-(1,2-), or *para*-(1,4-)benzoquinones (BQ), respectively. Trapping of 1,4-BQ with GSH gives rise to a variety of HQ-GSH conjugates, several of which are hematotoxic when administered to rats. Thus, benzene oxide gives rise to a cascade of metabolites that exhibit biological reactivity, and that provide a plausible metabolic basis for benzene-mediated myelotoxicity. Benzene oxide itself is remarkably stable, and certainly capable of translocating from its primary site of formation in the liver to the bone marrow. However, therein lies the challenge, for although there exists a plethora of information on the metabolism of benzene, and the fate of benzene oxide, there is a paucity of data on the presence, concentration, and persistence of benzene metabolites in bone marrow. The major

© 2009 Elsevier Ireland Ltd. All rights reserved.

Address correspondence to:- Terrence J. Monks, Ph.D., Dept. Pharmacology & Toxicology, College of Pharmacy, University of Arizona, 1703 E Mabel Street, 341F, Tucson, AZ, USA 85721, 520 626-9906 TEL, 520 626-6944 FAX, monks@pharmacy.arizona.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

metabolites in bone marrow of mice exposed to 50 ppm [^3H]benzene are muconic acid, and glucuronide and/or sulfate conjugates of phenol, HQ, and catechol. Studies with [$^{14}\text{C}/^{13}\text{C}$]benzene revealed the presence in bone marrow of protein adducts of benzene oxide, 1,4-BQ, and 1,4-BQ, the relative abundance of which was both dose and species dependent. In particular, histones are bone marrow targets of [^{14}C]benzene, although the identity of the reactive metabolite(s) giving rise to these adducts remain unknown. Finally, hematotoxic HQ-GSH conjugates are present in the bone marrow of rats receiving the HQ/phenol combination. In summary, although the fate of benzene oxide is known in remarkable detail, coupling this information to the site, and mechanism of action, remains to be established.

Keywords

Benzene metabolism; benzene oxide; phenol; hydroquinone; muconaldehyde; bone marrow; hematotoxicity

1. One Hundred and Forty Years, and Counting

The metabolic conversion of benzene to phenol was demonstrated by Schultzen and Naunyn in 1867 [1], and the conjugation of phenol with sulfate was established in 1876 by Baumann [2]. At that time, the mechanism of this metabolic conversion was not known, but following reports that anthracene [3], naphthalene [4], and phenanthrene [5] were all metabolized to dihydrodiols, Boyland [6] suggested that epoxide formation was the requisite intermediary in this metabolic reaction, simultaneously providing an explanation for the formation of other aromatic hydrocarbon metabolites (phenols, ring-opening). Boyland's suggestion occurred at about the same time (1949) that Dennis Parke joined R.T. Williams' group at St. Mary's Hospital Medical School, where he embarked on a study of all the known pathways of benzene metabolism, focusing particularly on whether benzene did indeed form an epoxide and/or a dihydrodiol, and on the isomers of muconic acid. The metabolism of the simplest of aromatic hydrocarbons has subsequently been revealed to be comparatively complex, giving rise to a myriad of metabolites.

2. Benzene-oxide

The first step in the metabolism of benzene is the cytochrome P450 catalyzed formation of benzene-oxide (Figure 1). Epoxides such as benzene-oxide can be somewhat ambiguous in their nature. On the one hand, they are sufficiently electrophilic that they can react and covalently bind to nucleophilic sites within proteins and DNA. In contrast, epoxides can be relatively stable, in chemical terms, with half-lives in biological milieu ranging from seconds to minutes. For example, bromobenzene-3,4-oxide is capable of diffusing out of hepatocytes and being trapped extracellularly by glutathione (GSH) and the requisite GSH transferase (GST) [7]. Indeed, bromobenzene-3,4-oxide is sufficiently stable that it can be detected in venous blood of rats receiving bromobenzene via intra-peritoneal injections, with a half-life of ~ 13.5 seconds [8], which is more than sufficient time to reach extrahepatic targets. Benzene-oxide has also been directly identified in the blood of rats administered benzene (400 mg/kg) and has a half-life of ~ 8 mins when added to rat blood *ex vivo* [9]. Since the mean circuit time of blood in rats (blood volume/cardiac output) is about five to

ten seconds, the concentration of benzene-oxide will not decrease greatly in a single pass between organs. Thus, nearly all of the benzene-oxide leaving the liver (and any other organ in which it is formed) will enter the lung, and the concentration of the epoxide leaving the lung will be essentially identical to its concentration in arterial blood entering the other organs/tissues of the body, including the bone marrow. The extent to which benzene-oxide in arterial blood contributes to its covalent binding in extrahepatic tissue, in particular bone marrow, will depend on the ability of the various tissues to convert the epoxide into downstream metabolites, and on its non-enzymatic rearrangement to phenol and ring-opened products. However, each particular non-enzymatic rearrangement reaction should occur at the same rate in all tissues. It will therefore be the relative distribution of the enzymes that catalyze the conversion of benzene-oxide into the dihydrodiol (epoxide hydrolase) and *S*-phenylmercapturic acids (GST) that limit the exposure of extrahepatic tissues to blood-borne benzene-oxide.

Although liver-derived benzene-oxide is clearly capable of reaching bone marrow, is it sufficiently biologically reactive to play a major direct role in benzene-mediated hematotoxicity? The half-life of benzene-oxide in aqueous medium (95:5 [v/v] phosphate buffer in D₂O with [CD₃]₂SO) at 25°C (pD/pH 7) was ~34 mins [10]. Moreover, the half-life did not change in the presence of GSH (2-15 mM) or with a combination of GSH (2 mM) and GST [10]. In fact, the major product observed under these experimental conditions was phenol. These findings are consistent with the fact that *S*-phenylmercapturic acid is a relatively minor urinary metabolite of benzene. Perhaps the role of benzene-oxide in benzene hematotoxicity is to assist in the delivery of phenol and/or the ring-opened products (see below) to the bone marrow, where they may undergo further metabolic transformations into more reactive metabolites.

In contrast to the apparent stability of benzene-oxide, and its inefficient reaction with GSH, *S*-phenyl-L-cysteine, presumed to arise from the interaction of benzene-oxide with cysteine residues within proteins, has been isolated from hemoglobin and bone marrow proteins [11]. It would seem likely that the protein microenvironment increases the efficiency with which these cysteinyl thiols react with benzene-oxide. Consistent with this view, increasing the pH from 7.0 to 8.5 dramatically increases (20-fold) the reaction of GSH with benzene-oxide [10]. Nonetheless, the major protein adducts identified in mouse marrow after benzene administration were derived from 1,4-benzoquinone (see Section 4) rather than benzene-oxide (<2%) [11]. Interestingly, the metabolites representing the major source of benzene-derived protein adducts in bone marrow remain to be identified. A more in-depth analysis of this issue can be found in the following presentations.

3. trans-trans-Muconaldehyde (MCA)

Benzene oxide can equilibrate spontaneously with the corresponding oxepin valence tautomer, which can ring open to initially yield a reactive α - β -unsaturated aldehyde, cis-cis-muconaldehyde, which subsequently isomerizes to the cis-trans, and ultimately to the trans-trans-muconaldehyde (MCA) isomer. The mechanism of ring-opening remains debatable, but has been proposed to occur *via* cytochrome P450-mediated metabolism of the oxepin to the oxepin oxide [12]. Further reduction or oxidation of MCA gives rise to a variety of

products. *In vitro* model systems indicate that cytosolic aldehyde dehydrogenases may oxidize MCA to 6-oxo-trans-trans-hexadienoic acid, a mixed-aldehyde acid whose aldehyde functional group can undergo (i) oxidation to form trans-trans-muconic acid or (ii) reduction to 6-hydroxy-2,4-trans-trans-hexadienoic acid. Muconic acid was the first ring-opened urinary metabolite to be identified from benzene treated animals, by Jaffe in 1909 [13]. However, at that time the prevailing view was that the cis-cis isomer was the primary product, and it was not until 1952 that Parke and Williams unequivocally confirmed the structure as trans-trans-muconic acid, following [^{14}C]-benzene administration to rabbits [14]). Alternatively, cytosolic alcohol dehydrogenases can reduce (reversible reaction) MCA to 6-hydroxy-2,4-trans-trans-hexadienal, a mixed-aldehyde alcohol whose aldehyde functional group can undergo oxidation to form 6-hydroxy-2,4,3-trans-trans-hexadienoic acid [15]. It is important to note, however, that while [^3H]-trans-trans-muconic acid and [^{14}C]-6-hydroxy-2,4-trans-trans-hexadienoic acid have been isolated from the urine, blood, and/or bone marrow of rodents treated with [^3H] or [^{14}C]benzene, the reactive aldehydes, MCA and 6-hydroxy-2,4-trans-trans-hexadienal, have yet to be isolated *in vivo*. Thus, while MCA is produced in liver microsomal incubations containing benzene [16], and is undoubtedly an *in vivo* metabolite of benzene, albeit transient, the reactivity of this metabolite (6 sec in the presence of GSH) suggests that hepatic-derived MCA is unlikely to reach the bone marrow in sufficient quantities to cause toxicity [17]. This again emphasizes the critical balance between the chemical stability and the biological reactivity of benzene metabolites with respect to their potential role in benzene-mediated hematotoxicity. Both MCA and the hexadienal metabolite are myelotoxic in animal models [18]. Of current interest is the ability of benzene metabolites to block gap junction intercellular communication, in particular hydroquinone, MCA, and 6-hydroxy-2,4-trans-trans-hexadienal [19]. MCA decreases connexin 43 levels, inhibition of which has been linked to disruptions in hematopoiesis. More recently, MCA induced cross-linking of connexin 43 likely underlies the inhibition of gap junction intercellular communication [20], further elaboration of which will be provided later in this session. However, information on the presence of ring-opened metabolites in bone marrow following exposure to benzene remains limited (see Section 5).

4. Phenol and Hydroquinone

Benzene-oxide spontaneously rearranges to phenol, the majority of which undergoes conjugation with either glucuronic acid or sulfate. That fraction that escapes conjugation can be further oxidized, with hydroquinone (HQ) as one of the products. Phenol itself is not hematotoxic, but synergistic effects of phenol and HQ, both of which accumulate in bone marrow, has become an established model of benzene hematotoxicity [21]. It should be emphasized that although animal models can be an appropriate approximation for human benzene metabolism, they are a less than ideal model for the human myelotoxic effects of benzene; perhaps highlighting the fact that metabolism may be necessary, but not sufficient for benzene hematotoxicity. The effectiveness of the HQ/phenol combination appears to be related, in part, to a pharmacokinetic interaction between HQ and phenol [22]. Phenol likely competes with HQ for conjugative enzymes and depletes the liver of UDPGA and PAPS, resulting in greater fractions of HQ and phenol available for delivery to bone marrow [23].

Bone marrow suppression may then result from phenol-stimulated peroxidase and/or phenoxy radical-mediated oxidation of HQ, which (in theory) initiates redox cycling and leads to the formation of the reactive intermediates, 1,4-benzo-semiquinone and 1,4-BQ. However, the current model of benzene hematotoxicity may need to be extended to include a role for thioether metabolites of HQ (see below). While depletion of cofactors for glucuronidation and sulfation increases the amount of “free” HQ and phenol in bone marrow, it will also increase the fraction available for oxidation and GSH conjugation. Such increases could be critical since both BGHQ and TGHQ are very potent hematotoxicants *in vivo*, inhibiting [^{59}Fe] incorporation to the same degree as benzene at significantly lower doses (see Lau et al., this issue). Based on the (re)activity of HQ-GSH conjugates, it is possible that many of the hematotoxic effects attributed to HQ (or 1,4-BQ) may, in fact, be mediated by their thiol conjugates. Indeed, although semiquinone formation from a variety of quinones leads to O_2 consumption, no O_2 consumption occurs in reactions in which the 1,4-benzosemiquinone free radical is formed enzymatically [24], because 1,4-benzosemiquinone is so electron affinic that its rate of reduction by $\text{O}_2^{\bullet-}$ [25] is over four orders of magnitude faster than the reverse reaction (reduction of O_2 to $\text{O}_2^{\bullet-}$) [26, 27], which is usually responsible for O_2 consumption via redox cycling. Thus, although redox cycling of semi-quinone radicals resulting in the generation of reactive oxygen species is proposed to be of major importance in the toxicity of many quinones, this mechanism is clearly excluded in the case of 1,4-BQ [28] (see also Figure 2). Indeed, although 1,4-BQ is cytotoxic to hepatocytes, it causes rapid depletion of cellular thiols without oxidative stress [29].

6. Hydroquinone-Thiol Conjugates

HQ is readily oxidized to 1,4-BQ, which is then efficiently scavenged by GSH. However, this reaction does not represent a true detoxication reaction, since the initial conjugate, 2-(glutathion-S-yl)hydroquinone (GS-HQ) is also readily oxidized to the corresponding GS-1,4-BQ. This cycle of the reductive addition of GSH to the quinone and subsequent cross-oxidation continues, resulting in the eventual formation of all possible addition products, GS-HQ, 2,3-(GS)-HQ, 2,5-(GS)-HQ, 2,6-(GS)-HQ, 2,3,5-(GS)-HQ, and 2,3,4,5-(GS)-HQ [30]. The *in vivo* relevance of these reactions was demonstrated following the administration of HQ (1.8 mmol/kg, i.p.) to AT-125 (an inhibitor of γ -glutamyl transpeptidase [γ -GT]) pretreated male Sprague Dawley rats. Five *S*-conjugates of HQ were identified in bile and one *S*-conjugate in urine [31]. The major biliary *S*-conjugate identified was GS-HQ. Additional biliary metabolites were 2,5-(GS)-HQ, 2,6-(GS)-HQ, and 2,3,5-(GS)-HQ. 2-(*N*-Acetylcystein-S-yl)HQ was the only urinary thioether metabolite identified. The quantity of *S*-conjugates excreted in urine and bile within 4 hrs of HQ administration was sufficient to propose a role for such metabolites in HQ mediated nephrotoxicity and nephrocarcinogenicity. Whether the conjugates are formed in sufficient amounts following either HQ/phenol or benzene administration to contribute to benzene-mediated hematotoxicity remains to be determined, but they can be identified in the bone marrow of HQ/phenol or benzene treated animals (see Section 7).

7. Benzene Metabolites in Bone Marrow

The gap in our knowledge with respect to benzene metabolism is the extent to which each of the metabolites exist/persist in bone marrow. The glucuronide conjugates of both phenol and HQ were identified in the bone marrow of B6C3F1 mice, but not in the bone marrow of F344 rats exposed for 6 hr to 50 ppm benzene [32] an intriguing finding especially given the fact that such conjugates are readily recoverable in the urine of these animals. More intriguing is the observation that HQ conjugates could not be detected in blood, liver, or lung of F344 rats, whereas they are readily detected in these tissues in B6C3F1 mice. Whether phenol is generated within bone marrow, or delivered there either as the conjugate or in the free form is not known. In addition to HQ and phenol conjugates, trans-trans-muconic acid and phenyl or catechol sulfate were also identified in the bone marrow of B6C3F1 mice and F344 rats, with the latter representing the highest fraction of benzene metabolites in both species. The presence of benzene metabolites in target tissue in humans is complicated by the potential contribution of dietary, over-the-counter medicinals, and perhaps other environmental sources (cigarette smoke) of such metabolites. For example, phenol, HQ, catechol, and trans-trans-muconic acid may all be derived from dietary sources [33,34], in addition to environmental/occupational exposures to benzene.

More recently, following co-administration of HQ/phenol (2.0 mmol/kg, ip) to rats, GS-HQ, 2,5-(GS)-HQ, 2,6-(GS)-HQ, and 2,3,5-(GS)-HQ were all detected in bone marrow [35]. The γ -GT catalyzed metabolite of GS-HQ, 2-(Cys-Gly)HQ, the dipeptidase metabolite, 2-(Cys)HQ, and the mercapturic acid metabolite, 2-(NAC)HQ were also all identified in bone marrow. Moreover, 2-(Cys)HQ and 2-(NAC)HQ appeared to persist in bone marrow. A similar metabolic profile was observed in mice, with the exception that 2,5-(GS)-HQ, 2,6-(GS)-HQ, and 2,3,5-(GS)-HQ were only sporadically detected. The latter conjugates were only observed in mice exhibiting high concentrations of GS-HQ in marrow. Nonetheless, concentrations of quinol thioethers in bone marrow were higher in mice than in rats of HQ/phenol-treated animals, which correlates with the relative sensitivity of these two species to benzene-induced hematotoxicity [36]. Similar results were obtained in benzene-treated animals. Following twice daily administration of benzene (11.2 mmol/kg) for 2 days, GS-HQ, (Cys-Gly)-HQ, (Cys)-HQ, and (NAC)-HQ were all detected in the bone marrow of rats and mice. The presence of a functional mercapturic acid pathway in bone marrow of both species was confirmed by *in vitro* studies. Concentrations of (Cys)-HQ were relatively high and persisted in bone marrow. The availability of bone marrow γ -GT and dipeptidases for processing of GSH conjugates is important, because Cys-Gly and Cys conjugates of HQ are more chemically (re)active than their corresponding GSH conjugates [37]. Consequently, such metabolites will be more potent arylators and redox cyclers. The half-wave oxidation potentials of the GSH conjugates of hydroquinone are also considerably lower than the half-wave oxidation potential of HQ [31]. In addition, the conjugates are also more readily oxidized by cytochrome(s) P450 to the corresponding quinones than HQ and generate more superoxide anions (Figure 2).

Because the liver produces significant amounts of the multi-substituted GSH conjugates following administration of HQ (1.8 mmol/kg) [31], it seems plausible that the conjugates identified in the bone marrow might be transported there via the circulation. However, an

intravenous dose of GS-HQ (100 $\mu\text{mol/kg}$) yields quinol thioether concentrations in bone marrow far below those detected following coadministration of HQ/phenol (2.0 mmol/kg, ip), suggesting that the major fraction of the HQ-GSH conjugates present in bone marrow is formed *in situ*. Myeloperoxidase- and prostaglandin H synthase-mediated oxidation of HQ to 1,4-BQ, in the presence of GSH, has been shown to yield GS-HQ *in vitro* [38]. This reaction should therefore occur in bone marrow which contains both enzymes [39]. If concentrations of GS-HQ saturate bone marrow γ -GT ($K_m = 68 \mu\text{M}$), subsequent oxidation and GSH substitution will lead to the formation of multi-substituted GSH conjugates of HQ. Since estimates of quinol thioether concentrations in bone marrow exceed 180 μM , this metabolic pathway should occur readily *in vivo*. This view is supported by the identification of 2,5-(GS)-HQ, 2,6-(GS)-HQ, and 2,3,5-(GS)-HQ in the bone marrow of rats following coadministration of HQ/phenol.

8. Some Speculation on the Potential Involvement of HQ-GSH conjugates in Benzene-mediated Hematotoxicity

GSH conjugates of HQ possess unique structural features that confer upon them the ability to interact with proteins that utilize GSH/cysteine or GSH/cysteine containing molecules as substrates or cofactors. The fact that such proteins play important roles in hematopoiesis provides a basis for these specific benzene metabolites to interfere with this process, and to mimic the actions of benzene. For example; (i) HQ-GSH conjugates inhibit γ -GT [40], and acivicin, a potent inhibitor of γ -GT, is hematotoxic [41]. γ -GT possesses a unique thiol that is not required for catalysis, but is present in the active site of the enzyme on the light subunit [42]. This thiol may be a target for HQ-GSH conjugates. The major function of γ -GT is to regulate the transport of amino acids into cells, but its relative role in maintaining intracellular GSH concentrations is cell-type specific. γ -GT expression in the kidney is so high that even when 95% of the enzyme is inhibited, there remains more total activity than in most other tissues [43]. Consequently, renal GSH levels are only minimally decreased following treatment with acivicin. Tissues expressing very low levels of γ -GT usually possess a very active cystathionase pathway, in which cystathionine is deaminated and cleaved to form free cysteine and α -ketobutyrate. Therefore, even in the presence of acivicin, these tissues maintain high levels of GSH [44]. However, γ -GT activity in bone marrow is relatively low [43], and the more immature, undifferentiated cells within the marrow (targets of benzene) express almost no cystathionase [45]. Thus, inhibition of γ -GT in hematopoietic tissue dramatically reduces intracellular GSH levels [46]. (ii) Benzene- and HQ-treated mice exhibit increased granulopoiesis in bone marrow [47, 48]. Increases in G-CSF and GM-CSF can stimulate granulocytic differentiation [49] and HQ synergizes with GM-CSF to increase the number of myeloid progenitor cells in isolated mouse bone marrow [50]. HQ also mimicks the action of leukotriene D4 (LTD4) a downstream mediator of G-CSF, to initiate terminal differentiation in IL-3-dependent murine myeloblasts [51]. Interestingly, the ability of 1,4-BQ to induce granulocytic differentiation is prevented by inhibitors of γ -GT and by LTD4 receptor antagonists [52]. Subsequently, 1,4-BQ was shown to induce granulocytic differentiation by activating the LTD4 receptor, mimicking LTD4 [53]. How does the inhibition of γ -GT prevent 1,4-BQ induced granulopoiesis? If the effects of 1,4-BQ are mediated by the corresponding GSH conjugates, then inhibition of γ -GT will

prevent processing of the conjugates to the cysteinylglycine conjugate and interaction with the LTD4 receptor. LTD4 is a cysteinylglycine conjugate, and we predict that HQ-thioether conjugates will possess greater selectivity for the LTD4 receptor than HQ/1,4-BQ. (iii) Finally, functional roles for ATP-binding cassette (ABC) transporter proteins in hematopoietic stem cell function have recently been described [54, 55]. Structurally related catechol-GSH conjugates are potent inhibitors of MRP-1 mediated LTC4 transport [56] suggesting that GSH-HQ conjugates have the potential to interfere with ABC transporter function within hematopoietic stem cells. Moreover, ABC transporter expression/conformation/function are modulated by ROS, which induce defects in hematopoietic stem cell homeostasis [55].

Acknowledgments

This work was supported in part by awards from the National Institutes of Health to TJM (ES 09224 and SSL (P30 ES 06694).

References

- Schultzen O, Naunyn B. The behavior of benzene-derived hydrocarbons in the animal organism. duBois-Reymond's Arch Anat Physiol. 1867; 349
- Baumann E. Concerning the occurrence of brencatechin in the urine. Pflugers Arch Physiol. 1876; 12:69.
- Boyland E, Levi AA. Metabolism of polycyclic compounds: Production of dihydroxydihydroanthracene from anthracene. Biochem J. 1935; 29(12):2679–83. [PubMed: 16745952]
- Young L. The metabolic conversion of naphthalene to 1:2-dihydronaphthalene-1:2-diol. Biochem J. 1947; 41(3):417–22. [PubMed: 16748185]
- Boyland E, Wolf G. Metabolism of polycyclic compounds. 6. Conversion of phenanthrene into dihydroxydihydrophenanthrenes. Biochem J. 1950; 47(1):64–9. [PubMed: 14791307]
- Boyland E. The biological significance of metabolism of polycyclic compounds. Biochem Soc Symp. 1950; 5:4054.
- Monks TJ, Lau SS, Gillette JR. Diffusion of reactive metabolites out of hepatocytes: studies with bromobenzene. J Pharmacol Exp Ther. 1984; 228(2):393–9. [PubMed: 6694117]
- Lau SS, Monks TJ, Greene KE, Gillette JR. Detection and half-life of bromobenzene-3,4-oxide in blood. Xenobiotica. 1984; 14(7):539–43. [PubMed: 6506767]
- Lindstrom AB, Yeowell-O'Connell K, Waidyanatha S, Golding BT, Towner-Velez R, Rappaport SM. Measurement of benzene oxide in the blood of rats following administration of benzene. Carcinogenesis. 1997; 18(8):1637–41. [PubMed: 9276641]
- Henderson AP, Barnes ML, Bleasdale C, Cameron R, Clegg W, Heath SL, Lindstrom AB, Rappaport SM, Waidyanatha S, Watson WP, Golding BT. Reactions of benzene oxide with thiols including glutathione. Chem Res Toxicol. 2005; 18(2):265–70. [PubMed: 15720131]
- Mc Donald TA, Yeowell-O'Connell K, Rappaport SM. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [¹⁴C]/¹³C6]benzene. Cancer Res. 1994; 54(18):4907–14. [PubMed: 8069856]
- Bleasdale C, Kennedy G, MacGregor JO, Nieschalk J, Pearce K, Watson WP, Golding BT. Chemistry of muconaldehydes of possible relevance to the toxicology of benzene. Environ Health Perspect. 1996; 104(Suppl 6):1201–09. [PubMed: 9118894]
- Jaffe M. Cleavage of the benzene ring in the organism. 1. The excretion of muconic acid in the urine after ingestion of benzene. Z Physiol Chem. 1909; 62:58–67.
- Parke DV, Williams RT. Studies in detoxication 54. The metabolism of benzene. (a) The Formation of phenylglucuronide and phenylsulphuric acid from [¹⁴C]benzene (b) The metabolism of [¹⁴C]phenol. Biochem. 1953; 55:337–340.

15. Kline SA, Robertson JF, Grotz VL, Goldstein BD, Witz G. Identification of 6-hydroxy-trans,trans-2,4-hexadienoic acid, a novel ring-opened urinary metabolite of benzene. *Environ Health Perspect.* 1993; 101(4):310–2. [PubMed: 8275987]
16. Latriano L, Goldstein BD, Witz G. Formation of muconaldehyde, an open-ring metabolite of benzene, in mouse liver microsomes: an additional pathway for toxic metabolites. *Proc Natl Acad Sci U S A.* 1986; 83(21):8356–60. [PubMed: 3464956]
17. Kline SA, Xiang Q, Goldstein BD, Witz G. Reaction of (E,E)-muconaldehyde and its aldehydic metabolites, (E,E)-6-oxohexadienoic acid and (E,E)-6-hydroxyhexa-2,4-dienal, with glutathione. *Chem Res Toxicol.* 1993; 6(4):578–83. [PubMed: 8374059]
18. Witz G, Zhang Z, Goldstein BD. Reactive ring-opened aldehyde metabolites in benzene hematotoxicity. *Environ Health Perspect.* 1996; 104 Suppl 6:1195–9. [PubMed: 9118893]
19. Rivedal E, Witz G. Benzene metabolites block gap junction intercellular communication. Role in hematotoxicity and leukemia? *Chem Biol Interact.* 2005; 153-154:257–60. [PubMed: 15935824]
20. Rivedal E, Leithe E. The benzene metabolite trans,trans-muconaldehyde blocks gap junction intercellular communication by cross-linking connexin43. *Toxicol Appl Pharmacol.* 2008; 232(3): 463–8. [PubMed: 18723040]
21. Eastmond DA, Smith MT, Irons RD. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. *Toxicol Appl Pharmacol.* 1987; 91(1):85–95. [PubMed: 2823417]
22. Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, Ross D, Smith MT. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow in vivo: possible implications in benzene myelotoxicity. *Toxicology.* 1990; 62(1):107–16. [PubMed: 2343455]
23. Legathe A, Hoener BA, Tozer TN. Pharmacokinetic interaction between benzene metabolites, phenol and hydroquinone, in B6C3F1 mice. *Toxicol Appl Pharmacol.* 1994; 124(1):131–8. [PubMed: 8291054]
24. Ohnishi T, Yamazaki H, Iyanagi T, Nakamura T, Yamazaki I. One-electron-transfer reactions in biochemical systems. II. The reaction of free radicals formed in the enzymic oxidation. *Biochim Biophys Acta.* 1969; 172(3):357–69. [PubMed: 4305692]
25. Willson RL. Pulse radiolysis studies of electron transfer in aqueous quinone solutions. *J Chem Soc Trans Faraday Soc.* 1971; 67:3020–3029.
26. Meisel D. Free energy correlation of rate constants for electron transfer between organic systems in aqueous solutions. *Chem Phys Lett.* 1975; 34:263–266.
27. Sawada Y, Iyanagi T, Yamazaki I. Relation between redox potentials and rate constants in reactions coupled with the system oxygen-superoxide. *Biochemistry.* 1975; 14(17):3761–4. [PubMed: 240393]
28. Rao DN, Takahashi N, Mason RP. Characterization of a glutathione conjugate of the 1,4-benzosemiquinone-free radical formed in rat hepatocytes. *J Biol Chem.* 1988; 263(34):17981–6. [PubMed: 2848019]
29. Rossi L, Moore GA, Orrenius S, O'Brien PJ. Quinone toxicity in hepatocytes without oxidative stress. *Arch Biochem Biophys.* 1986; 251(1):25–35. [PubMed: 3789732]
30. Lau SS, Hill BA, Highet RJ, Monks TJ. Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution. *Mol Pharmacol.* 1988; 34(6):829–36. [PubMed: 3200250]
31. Hill BA, Kleiner HE, Ryan EA, Dulik DM, Monks TJ, Lau SS. Identification of multi-S-substituted conjugates of hydroquinone by HPLC-coulometric electrode array analysis and mass spectroscopy. *Chem Res Toxicol.* 1993; 6(4):459–69. [PubMed: 8374043]
32. Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, Henderson RF. Differences in the metabolism and disposition of inhaled [3H]benzene by F344/N rats and B6C3F1 mice. *Toxicol Appl Pharmacol.* 1988; 94(1):128–40. [PubMed: 3376110]
33. MacDonald TA, Holland NT, Skibola C, Duramad P, Smith MT. Hypothesis: Phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia. *Leukemia.* 2001; 15(1):10–20. [PubMed: 11243376]
34. Pezzagno G, Maestri L. The specificity of trans,trans-muconic acid as a biological indicator for low levels of environmental benzene. *Indoor Built Environ.* 1997; 6:12–18.

35. Bratton SB, Lau SS, Monks TJ. 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*. 1997; 10(8):859–65. [PubMed: 9282834]
36. Snyder CA, Goldstein BD, Sellakumar A, Wolman SR, Bromberg I, Erlichman MN, Laskin S. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. *J Toxicol Environ Health*. 1978; 4:605–618. [PubMed: 682210]
37. Hill BA, Davison KL, Dulik DM, Monks TJ, Lau SS. 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*. 1994; 129(1):121–32. [PubMed: 7974485]
38. Thomas DJ, Sadler A, Subrahmanyam VV, Siegel D, Reasor MJ, Wierda D, Ross D. Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid cells. *Mol Pharmacol*. 1990; 37(2):255–62. [PubMed: 2154673]
39. Kariya K, Lee E, Hirouchi M, Hosokawa M, Sayo H. Purification and some properties of peroxidases of rat bone marrow. *Biochim Biophys Acta*. 1987; 911(1):95–101. [PubMed: 3024734]
40. Hill BA, Lo HH, Monks TJ, Lau SS. The role of gamma-glutamyl transpeptidase in hydroquinone-glutathione conjugate mediated nephrotoxicity. *Adv Exp Med Biol*. 1991; 283:749–51. [PubMed: 1676872]
41. Earhart RH, Khandekar JD, Faraggi D, Schinella RA, Davis TE. Phase II trial of continuous drug infusions in advanced ovarian carcinoma: acivicin versus vinblastine. *Invest New Drugs*. 1989; 7(2-3):255–60. [PubMed: 2793383]
42. Ikeda Y, Fujii J, Taniguchi N, Meister A. Human gamma-glutamyl transpeptidase mutants involving conserved aspartate residues and the unique cysteine residue of the light subunit. *J Biol Chem*. 1995; 270(21):12471–5. [PubMed: 7759490]
43. Lau SS, Monks TJ. The in vivo disposition of 2-bromo-[14C]hydroquinone and the effect of gamma-glutamyl transpeptidase inhibition. *Toxicol Appl Pharmacol*. 1990; 103(1):121–32. [PubMed: 1969181]
44. Houghton CB, Cherian MG. Effects of inhibition of cystathionase activity on glutathione and metallothionein levels in the adult rat. *J Biochem Toxicol*. 1991; 6(3):221–8. [PubMed: 1770506]
45. Link D, Drebing C, Glode LM. Cystathionase: a potential cytoplasmic marker of hematopoietic differentiation. *Blut*. 1983; 47(1):31–9. [PubMed: 6860801]
46. Dethmers JK, Meister A. Glutathione export by human lymphoid cells: depletion of glutathione by inhibition of its synthesis decreases export and increases sensitivity to irradiation. *Proc Natl Acad Sci U S A*. 1981; 78(12):7492–6. [PubMed: 6950392]
47. Dempster AM, Snyder CA. Short term benzene exposure provides a growth advantage for granulopoietic progenitor cells over erythroid progenitor cells. *Arch Toxicol*. 1990; 64(7):539–44. [PubMed: 2073127]
48. Niculescu R, Kalf GF. A morphological analysis of the short-term effects of benzene on the development of the hematological cells in the bone marrow of mice and the effects of interleukin-1 alpha on the process. *Arch Toxicol*. 1995; 69(3):141–8. [PubMed: 7717869]
49. Groopman JE, Molina JM, Scadden DT. Hematopoietic growth factors. Biology and clinical applications. *N Engl J Med*. 1989; 321(21):1449–59. [PubMed: 2682244]
50. Irons RD, Stillman WS, Colagiovanni DB, Henry VA. 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*. 1992; 89(9):3691–5. [PubMed: 1570288]
51. Hazel BA, O'Connor A, Niculescu R, Kalf GF. Benzene and its metabolite, hydroquinone, induce granulocytic differentiation in myeloblasts by interacting with cellular signaling pathways activated by granulocyte colony-stimulating factor. *Stem Cells*. 1995; 13(3):295–310. [PubMed: 7542115]
52. Kalf GF, O'Connor A. The effects of benzene and hydroquinone on myeloid differentiation of HL-60 promyelocytic leukemia cells. *Leuk Lymphoma*. 1993; 11(5-6):331–8. [PubMed: 8124204]

53. Hazel BA, Kalf GF. Induction of granulocytic differentiation in myeloblasts by hydroquinone, a metabolite of benzene, involves the leukotriene D4 receptor. *Recept Signal Transduct*. 1996; 6(1): 1–12. [PubMed: 8933618]
54. Bunting KD. ABC transporters as phenotypic markers and functional regulators of stem cells. *Stem Cells*. 2002; 20(1):11–20. [PubMed: 11796918]
55. Kuo MT. Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities. *Antioxid Redox Signal*. 2009; 11(1):99–133. [PubMed: 18699730]
56. Slot AJ, Wise DD, Deeley RG, Monks TJ, Cole SP. Modulation of human multidrug resistance protein (MRP) 1 (ABCC1) and MRP2 (ABCC2) transport activities by endogenous and exogenous glutathione-conjugated catechol metabolites. *Drug Metab Dispos*. 2008; 36(3):552–60. [PubMed: 18079363]

Figure 1.
The disposition of benzene-oxide. See text for detailed description of the various metabolic pathways

Figure 2.

Microsomes (0.5 mg/mL protein) were preincubated with acivicin (10 mM) for 15 min and then incubated with various concentrations of either phenol (■, dashed line), HQ (○), 2-(GS-yl)HQ (●), 2,5-bis-(GS-yl)HQ (○, dashed line), BGHQ (□), or TGHQ (■), in the presence of succinoylated cytochrome C (12.5 μM) and an NADPH generating system. Superoxide anion formation is expressed as nmol/mg protein/min. The inset shows the correlation between the oxidation potentials [$E_{1/2}$ (mV)] for the HQ and its GSH conjugates, and their ability to catalyze superoxide anion formation. Each data point represents the mean $\pm$ SEM (n=3).