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Investigation of the presence in human urine of mercapturic acids derived from phenanthrene, a representative polycyclic aromatic hydrocarbon

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are environmental carcinogens implicated as causes of cancer in certain industrial settings and in cigarette smokers. PAH require metabolic activation to exert their carcinogenic effects. One widely accepted pathway of metabolic activation proceeds through formation of "bay region" diol epoxides which are highly reactive with DNA and can cause mutations. Phenanthrene (Phe) is the simplest PAH with a bay region and an excellent model for the study of PAH metabolism. In previous studies in which [D_{10}]Phe was administered to smokers, we observed higher levels of [D_{10}]Phe-tetraols derived from [D_{10}]Phe-diol epoxides in subjects who were null for the *glutathione-S-transferase M1* (*GSTM1*) gene. We hypothesized that Phe-epoxides, the primary metabolites of Phe, were detoxified by glutathione conjugate formation, which would result ultimately in the excretion of the corresponding mercapturic acids in urine. We synthesized the four stereoisomeric mercapturic acids that would result from attack of glutathione on Phe-epoxides followed by normal processing of the conjugates. We also synthesized the corresponding dehydrated metabolites and sulfoxides. These 12 standards were used in liquid chromatography-nanoelectrospray ionization-high resolution tandem mass spectrometry analysis of urine samples from smokers and creosote workers, the latter exposed to unusually high levels of PAH. Only the sulfoxide derivatives were consistently detected in the urine of creosote workers; none of the compounds was detected in the urine of smokers. These results demonstrate a new pathway of PAH-mercapturic acid formation, but do not provide an explanation for the role of *GSTM1* null status on Phe-tetraol formation.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants formed during the incomplete combustion of organic matter. Multiple epidemiologic studies have investigated the relationship between exposure to PAH in occupational settings and the incidence of cancer [1]. The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence for the carcinogenicity to humans of occupational exposures which occur during coal gasification, coke production, coal-tar distillation, chimney sweeping, paving and roofing with coal-tar pitch, aluminum production, and carbon electrode manufacturing, all of which can entail considerable exposure to PAH [1]. Benzo[*a*]pyrene (BaP), the prototypic powerful PAH carcinogen, is also considered carcinogenic to humans by IARC [1]. Cigarette smoke is another significant source of PAH exposure, and these compounds are considered to be among the principal causes in smokers of lung cancer, a disease which kills 1.42 million people per year in the world [2–4].

PAH require metabolism to exert their carcinogenic effects [5–8]. Three general mechanisms of PAH metabolic activation have been studied in great detail: activation through the formation of bay region diol epoxides [5–7], through radical cation intermediates [9], or through redox cycling [10]. The study described

Abbreviations: PAH, polycyclic aromatic hydrocarbons; *GSTM1*, *glutathione-S-transferase M1*; NAC, *N*-acetyl-L-cysteine; LC-NSI-HRMS/MS, liquid chromatography-nanoelectrospray-high resolution tandem mass spectrometry; LC-MS/MS, liquid chromatograpy-tandem mass spectrometry.

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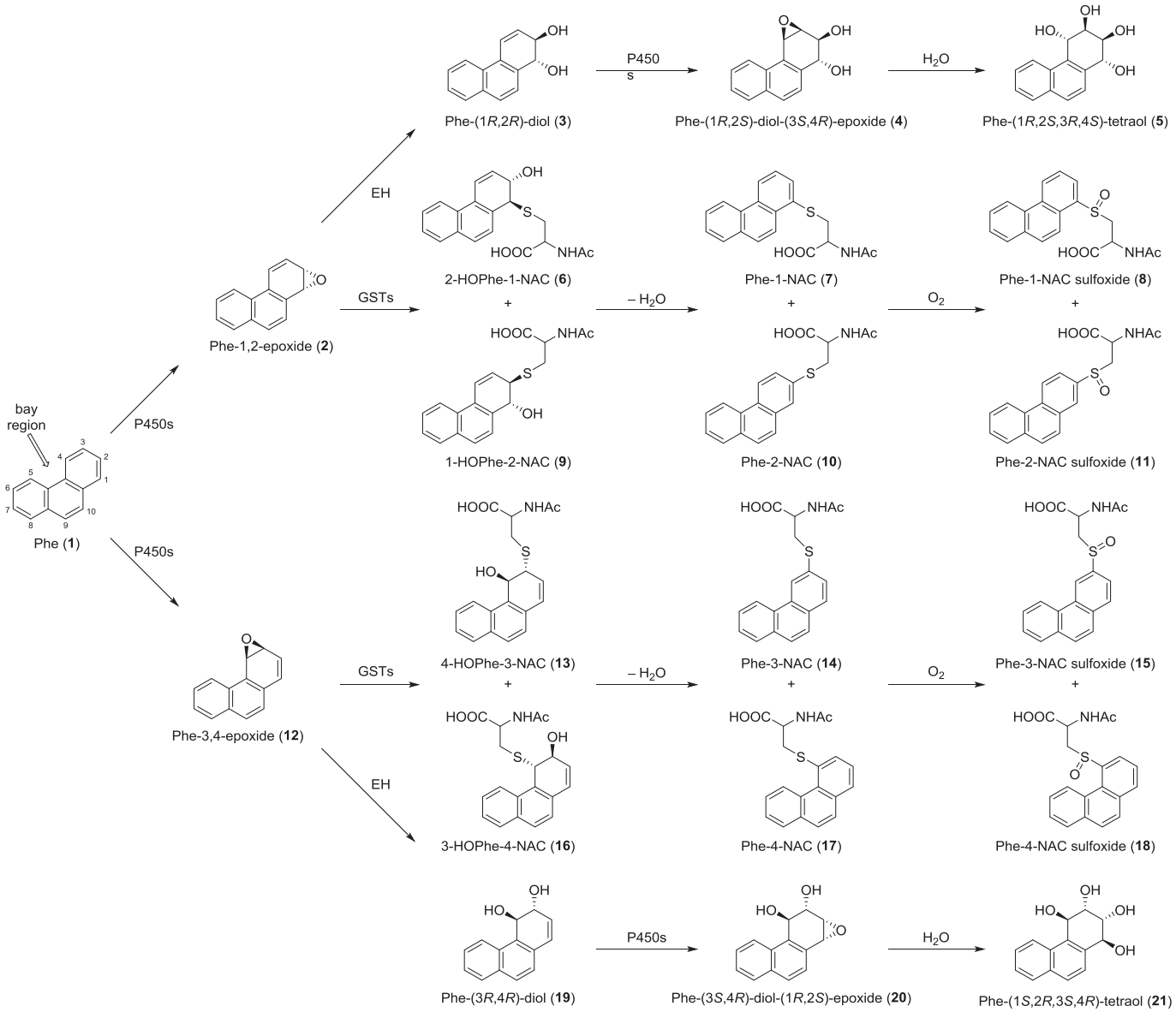
here focuses on the bay region diol epoxide mechanism. Phenanthrene (Phe, **1**, Scheme 1) is the simplest PAH with a bay region. Although not carcinogenic, its structural features are ideal for investigating the role of bay region diol epoxides which are readily formed during its metabolism, as shown in Scheme 1 [11–14]. Thus, convincing evidence indicates that P450s stereoselectively catalyze the oxidation of the angular ring of Phe to Phe-1,2-epoxide (**2**) and Phe-3,4-epoxide (**12**), which are stereoselectively hydrated with catalysis by epoxide hydrolase (EH) to give Phe-(1*R*,2*R*)-diol (**3**) and Phe-(3*R*,4*R*)-diol (**19**), established metabolites of Phe [15]. Further stereoselective oxidation of **3** and **19** by P450s produces the bay region diol epoxide Phe-(1*R*,2*S*)-diol-(3*S*,4*R*)-epoxide (**4**) and the "reverse diol epoxide" Phe-(3*S*,4*R*)-diol-(1*R*,2*S*)-epoxide (**20**), which upon hydrolysis give tetraols **5** and **21**, respectively. Alternatively, the simple epoxides **2** and **12** could be detoxified by reaction with glutathione ultimately producing sets of mercapturic acids such as **6**, **9**, **13**, **16** and **7**, **10**, **14**, **17** as well as their further oxidation products **8**, **11**, **15**, **18**. For the reasons described below, the possible metabolic formation of these three sets of mercapturic acid products is the focus of this study.

Only 11% of female and 24% of male lifetime cigarette smokers will get lung cancer by age 85 or greater, and this relatively small percentage is not due to competing causes of death from smoking [17]. A major goal of our research is to identify these susceptible smokers who could then be targeted for surveillance and early detection of lung cancer. We hypothesize that smokers who extensively metabolically activate PAH by the diol epoxide pathway will be at higher risk for lung cancer, all other factors considered equal. As a test of this hypothesis, we are administering [D_{10}] phenanthrene ([D_{10}]Phe, a single dose of 1–10 μg) to smokers and analyzing the amounts of [D_{10}]Phe-tetraols **5** and **21** excreted in 6 h urine, a protocol which has been previously validated [18–20]. The use of [D_{10}]Phe allows us to focus only on the metabolic activation process, setting aside exposure variables such as diet and polluted air which contain Phe and would complicate the interpretation of levels of unlabeled Phe-tetraols **5** and **21** excreted by smokers.



Scheme 1. Metabolism of phenanthrene (Phe) to intermediates and products discussed in the text. Stereoselectivity in the formation of Phe epoxides and subsequent metabolites has been established based on previous studies (see for example [14–16]). Compounds **6**, **9**, **13**, **16**; **7**, **10**, **14**, **17**; and **8**, **11**, **15**, **18** synthesized here were racemic mixtures.