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# CP-arene oxides: the ultimate, active mutagenic forms of cyclopenta-fused polycyclic aromatic hydrocarbons (CP-PAHs)

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**Abstract**

The bacterial mutagenic response (Ames-assay, *Salmonella typhimurium* strain TA98 ± S9-mix) of a series of monocyclopenta-fused polycyclic aromatic hydrocarbons (CP-PAHs) identified in combustion exhausts, viz. cyclopenta[*cd*]pyrene (**1**), acephenanthrylene (**2**), aceanthrylene (**3**) and cyclopenta[*hi*]chrysene (**4**), is re-evaluated. The mutagenic effects are compared with those exerted by the corresponding partially hydrogenated derivatives, 3,4-dihydrocyclopenta[*cd*]pyrene (**5**), 4,5-dihydroacephenanthrylene (**6**), 1,2-dihydroaceanthrylene (**7**) and 4,5-dihydrocyclopenta[*hi*]chrysene (**8**). It is shown that the olefinic bond of the externally fused five-membered ring of **1**, **3** and **4** is of importance for a positive mutagenic response. In contrast, whilst CP-PAH **2** is found inactive, its dihydro analogue (**6**) shows a weak metabolism-dependent response. The importance of epoxide formation at the external olefinic bond in the five-membered ring is substantiated by the bacterial mutagenic response of independently synthesized cyclopenta[*cd*]pyrene-3,4-epoxide (**9**), acephenanthrylene-4,5-epoxide (**10**), aceanthrylene-1,2-epoxide (**11**) and cyclopenta[*hi*]chrysene-4,5-epoxide (**12**). Their role as ultimate, active mutagenic forms, when CP-PAHs **1**, **3** and **4** exhibit a positive mutagenic response, is confirmed. Semi-empirical Austin Model 1 (AM1) calculations on the formation of the CP-arene oxides (**9**–**12**) and their conversion into the monohydroxy-carbocations (**9a**–**12a** and **9b**–**12b**) via epoxide-ring opening support our results. For **2** and **4**, which also possess a bay-region besides an annelated cyclopenta moiety, the calculations rationalize that epoxidation at the olefinic bond of the cyclopenta moiety is favoured.


*Keywords:* Bacterial mutagenicity; *Salmonella typhimurium*; TA98; Cyclopenta-fused PAH; Epoxidation; Semi-empirical AM1 calculations

## 1. Introduction

Cyclopenta-fused polycyclic aromatic hydrocarbons (CP-PAHs) are a special sub-class of PAHs, which contain (at least) one externally unsaturated

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five-membered ring annelated to a PAH perimeter. Their ubiquitous environmental presence due to incomplete combustion of fossil fuels [1,2] and their enhanced bioactivity as compared with that of related PAH without the cyclopenta moiety, render CP-PAHs of toxicological interest. Examples of PAH and corresponding CP-PAH are pyrene versus cyclopenta[*cd*]pyrene (**1**, Fig. 1) [3] and anthracene versus aceanthrylene (**3**) [4]. In most instances, biologically active CP-PAHs require exogenous metabolic activation (S9-mix) in order to exert positive mutagenic activity in bacterial mutagenicity assays (Ames-assay) [4,5]. One of the best studied representatives is cyclopenta[*cd*]pyrene (**1**). It exhibits a high metabolism-dependent mutagenic response in bacterial [3,6] and human cell bioassays [7,8]. In addition, **1** also possesses tumour-initiating and moderate carcinogenic potential [9–11].

Although for common PAHs, a bay-region has been postulated as a prerequisite to exert bioactivity in the presence of exogenous metabolic activation (S9-mix [12]), **1** and other known bioactive CP-PAHs lack this structural feature. In these cases, epoxidation of the olefinic bond in the five-membered ring via oxidative metabolic activation by cytochrome P450 isoenzymes in the S9-mix, is considered to give the putative ultimate, active mutagenic forms of CP-PAHs [3]. Note that for some CP-PAHs, which also contain a bay-region in their PAH core, it has been suggested that epoxidation of the cyclopenta moiety accounts for the observed bacterial mutagenic activity [13,14]. The facile formation of epoxides at the five-membered ring is supported in the case of **1** by the isolation of dihydrodiols (derived from the action of the epoxide-hydrolase on the epoxide) in metabolic studies with liver microsomes ([15], see however also references [16,17] in which bio-activation of PAH dihydro-diols by sulfonation is reported), as well as the reaction of the epoxides with DNA in vitro (isolation of DNA-adducts) [18–22]. The importance of the olefinic bond in the five-membered ring is substantiated by the observation that dihydro-CP-PAH derivatives, which contain a saturated five-membered ring, generally exhibit no mutagenic activity in bacterial bioassays [7,23,24]. Interestingly, tentative results indicate that epoxidation of the olefinic bond in the cyclopenta moiety in contrast to the parent bioactive CP-PAH, leads to direct-acting bacterial mutagens (without the need for an exogenous metabolic activation mixture, −S9-mix, see below) that exert cytotoxicity at high concentrations [25,26].

Previously, CP-PAHs **1**–**4** have been assayed for mutagenicity using different strains of *Salmonella typhimurium* in the Ames-assay (TA98, TA100, etc.). Whilst **1** was found to be active, **2** was inactive [27]. However, **2** was found active with metabolic activation in the forward mutation assay (strain TM 677) [7,23]. Compounds **3** [4,27] and **4** [5] have been reported to act as bacterial mutagens upon metabolic activation. In the case of **4**, the diols at the cyclopenta-ring and at the bay-region have been isolated and identified as active metabolites [5]. For CP-PAHs showing a positive metabolism-dependent mutagenic response, the epoxides at the annelated five-membered ring, i.e. **9**–**12** from **1** to **4**, respectively (Fig. 1), are proposed as the ultimate, active mutagenic forms. Nevertheless, so far only epoxides **9** and **11** have been synthesized and previously assayed for their bacterial mutagenic response [15,27].

Hence, the high genotoxicity observed with some CP-PAHs on the one hand, and, on the other hand, the lack of bioactivity reported for other such compounds in the different bacterial systems earlier employed, have prompted us to re-evaluate in a single systematic study the bacterial mutagenic response of the CP-PAHs **1**–**4**. These compounds all represent unequivocally identified combustion exhaust constituents [28,29]. To establish the importance of the olefinic bond at the five-membered ring for metabolic activation, the related dihydro-CP-PAHs **5**–**8** were synthesized and assayed for mutagenic activity in this study. Furthermore, the epoxides **9**–**12** were synthesized and their mutagenic activity compared to that of **1**–**4** in order to evaluate their potential role as ultimate, active mutagenic forms. The experimental results are supported by semi-empirical Austin Model 1 (AM1) calculations [30] on the epoxides **9**–**12** and their related monohydroxy-carbocations **9a**–**12a** and **9b**–**12b** obtained via the epoxide-ring opening (Fig. 1).

## 2. Materials and methods

### 2.1. Chemicals

The CP-PAHs cyclopenta[*cd*]pyrene (**1**, CAS no. 27208-37-3), acephenanthrylene (**2**, CAS no. 201-06-