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Rapid Communication

# The effects of dimethylated and alkylated polycyclic aromatic hydrocarbons on the embryonic development of the Japanese medaka

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**Abstract**

The Japanese medaka (*Oryzias latipes*) early-life stage assay was used to investigate the effects of a number of commercially available dimethylated polycyclic aromatic hydrocarbons (PAHs) (3,6-dimethylphenanthrene, 7,12-dimethylbenz[*a*]anthracene, and 4,6-dimethyldibenzothiophene) and their unsubstituted congeners, dimethylated and unsubstituted tertiary mixtures, and a complex environmental mixture (with elevated $C_2$-substituted dibenzothiophene) on embryo larval development. Unsubstituted PAHs showed trends of increased blue sac disease (BSD) relative to dimethylated PAHs, although the severity of BSD induction varied. Results demonstrated that the dibenzothiophene congeners were the strongest inducers of BSD of the commercial PAHs tested. These compounds reduced the hatching success of embryonic medaka, an effect that was enhanced in the mixture. The base neutral extract significantly increased the frequency and severity of BSD abnormalities, while significantly reducing larval hatch length. Based on these results, a sublethal maximum allowable toxicant concentration (MATC) of 13.91 μg PAHs/L was calculated.


*Keywords:* Japanese medaka; Dimethyl-polycyclic aromatic hydrocarbons; Dibenzothiophene; Petroleum hydrocarbons; Blue sac disease

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), widespread environmental contaminants, are a diverse class of organic molecules that contain two or more fused benzene rings. Although the term *PAH* has traditionally been applied to compounds with a carbon and hydrogen skeleton, it now also encompasses chemicals that contain heteroatoms such as nitrogen, sulfur, and oxygen (McElroy et al., 1985). Complex mixtures of PAHs are formed through combustion (pyrogenesis), which produces unsubstituted PAHs, as well as diagenesis, which produces alkylated PAHs (Blumer, 1976). Sixteen unsubstituted PAHs are listed as priority pollutants by the US Environmental Protection Agency (USEPA) (Keith and Telliard, 1979). This constitutes only a small fraction of the total PAH loading to the environment, especially from petroleum where the majority are alkylated. There is a need to better understand the toxicity of alkylated PAHs to aquatic organisms, as approximately 75% of global PAH contamination in aquatic systems originates from petroleum sources (Neff, 1979).

The Athabasca oil sands (Alberta Canada) is the largest single oil deposit in the world, producing on average 300,000 barrels of synthetic crude oil per day (Fine Tailings Fundamentals Consortium, 1995). The most prevalent technique for the recovery of bitumen from oil sands is the Clarke hot water flotation method (Fine Tailings Fundamentals Consortium, 1995), which uses water and caustic soda and produces large volumes

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of course tailings, principally sand and process-affected water, which is principally silt and unrecovered hydrocarbons in an aqueous slurry. The process-affected water is stored in open lagoons where densification produces mature fine tails (MFTs). As the demand for petroleum continues to grow and new mining technologies are developed, the increasing rate of recovery of this resource will inevitably increase the risk of disruption to the regional biota.

The principal toxic constituents of process-affected water are naphthenic acids (NAs), present at concentrations $\backsimeq 100$ mg/L (Verbeek et al., 1993), and PAHs, found at concentrations on the order of $<3$ μg/L and $\leqslant 374$ μg/g in the water column and sediment of MFT settling ponds, respectively (Madill et al., 1999; Ganshorn, 2002). Although alkylated PAHs are the most abundant hydrocarbons in the treatment systems (Madill et al., 1999) and the tributaries of the Athabasca River (Headley et al., 2001), little is known about their potential for impact on aquatic biota. Prevalent alkylated PAHs, such as C1–C4 dibenzothiophenes and C1–C4 phenanthrene/anthracenes, are found in sediments at concentrations as high as ~25 and ~15 μg/g, respectively, in the Athabasca River basin (Headley et al., 2001) and 1.9 and 1.4 μg/g, respectively, in lakes on the oil sands mine leases (Smits et al., 2000).

The objective of this study was to evaluate the toxicity of dimethylated or unsubstituted PAHs and mixtures of these compounds, along with extracts from MFT particulates, on the embryo larval development of the Japanese medaka (*Oryzias latipes*). The Japanese medaka was chosen as a test species for this investigation as embryonic development is well characterized (Kirchen and West, 1976; Shi and Faustman, 1989). In addition, the toxicological response of Japanese medaka to oil sands-derived extracts rich in NAs and PAHs has been correlated to that of a species native to the Athabasca River watershed, the yellow perch (*Perca flavescens*) (Peters, 1999). The Japanese medaka early-life stage bioassay is sensitive to a number of toxicants including 2,3,7,8-terachlorodibenzo-*p*-dioxin (Wisk and Cooper, 1990), toluene (Stoss and Haines, 1979), mercury (Dial, 1978), and diazinon (Hamm and Hinton, 2000). Common observations included blue sac disease (BSD), also known as yolk sac disease, which is an aspecific response to a number of environmental and physiological stressors (Wolf, 1969).

## 2. Materials and methods

Embryo larval exposure experiments were conducted to determine the toxicity of three unsubstituted congeners (dibenzothiophene (DBT), phenanthrene (PHEN), and benz[*a*]anthracene (B[a]A) (Fig. 1A) as well as their dimethylated congeners (4,6-dimethyl dibenzothiophene (4,6-dmDBT), 3,6-dimethylphenanthrene (3,6-dmPHEN), and 7,12-dimethylbenz[*a*]anthracene (7,12-dmB[a]A) (Fig. 1B). The dimethylated PAHs were selected for study based on the fact that these are representative of the most prevalent substituted $\geqslant$3-ring PAHs in the process-affected water of Mildred Lake Settling Basin (MLSB), a MFT densification lagoon operated by Syncrude Canada Ltd. in Fort McMurray, AB, Canada (Madill, 1998). Two PAH mixtures, one containing the three dimethylated PAHs (DM Mix) and the other the three unsubstituted PAHs (UN Mix), were also tested. The relative proportions of each chemical in the DM Mix (5:4:1; 4,6-dmDBT:3,6-dmPHEN:7,12-dmB[a]A) and UN Mix (5:4:1; DBT:PHEN:B[a]A) were based on the relative proportion of PAHs in MLSB sediments (Ganshorn, 2002). Finally, the toxicity of a base neutral extract of PAHs from MLSB particulate was determined.

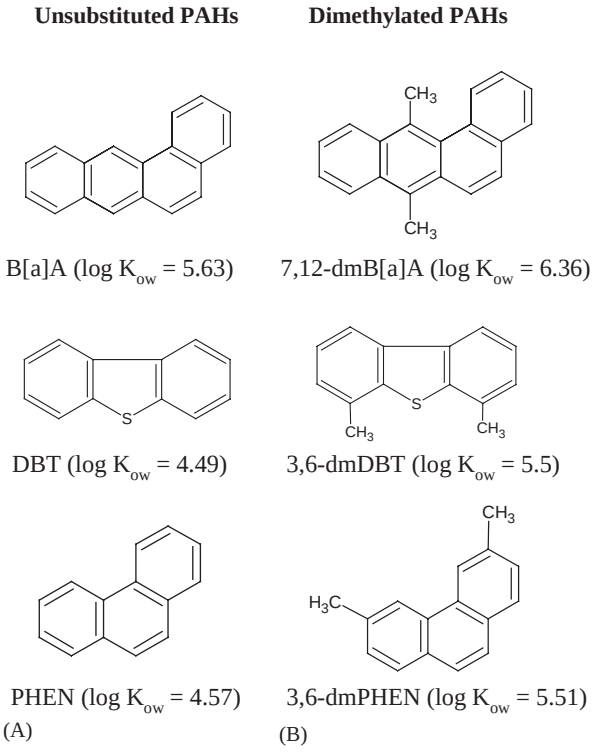


Fig. 1. Test chemicals with Kekule formulas of unsubstituted (A) and dimethylated (B) PAHs used in the medaka embryo larval experiments. Octanol–water partition coefficients (log $K_{ow}$) are referenced from Neff and Burns (1996).

All PAHs were purchased from Aldrich Chemical Company (Oakville, ON, Canada) with the exception of 3,6-dmPHEN, which was obtained from Accustandard (New Haven, CT, USA). The chemicals were weighed and the total amount ($\backsimeq 5$ mg) was then dissolved in 4 mL dimethylsulfoxide (DMSO) and stored 24 h prior to use. All the PAHs incorporated into the mixtures were tested independently, where PAH-DMSO stock solutions were prepared in identical fashion.