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Synergistic embryotoxicity of polycyclic
aromatic hydrocarbon aryl hydrocarbon receptor
agonists with cytochrome P4501A inhibitors in
Fundulus heteroclitus

Deena M. Wassenberg and Richard T. Di Giulio

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19 **Running title: Synergy of PAHs and CYP1A inhibitors**

20 **Key Words:**

21 Polycyclic aromatic hydrocarbons, aryl hydrocarbon receptor, *Fundulus heteroclitus*,
22 cytochrome P4501A, polychlorinated biphenyls, deformity, benzo(a)pyrene, β -
23 naphthoflavone, α -naphthoflavone, fluoranthene

24

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33 **Abbreviations:**

34 ATSDR – U.S. Agency for Toxic Substances and Disease Registry, AHR – aryl
35 hydrocarbon receptor, CYP1A – cytochrome P4501A, PAHs – polycyclic aromatic
36 hydrocarbons, pHAHs – planar halogenated aromatic hydrocarbons, PCB –
37 polychlorinated biphenyl, PCB126 –3,3',4,4',5-pentachlorobiphenyl, ANF – α -
38 naphthoflavone, BNF – β -naphthoflavone, BaP – benzo(a)pyrene, AA – 2-
39 aminoanthracene, PBO – piperonyl butoxide, FL – fluoranthene, DMSO – dimethyl
40 sulfoxide

41	Outline:
42	Abstract
43	Introduction
44	Materials and Methods
45	Reagents
46	Fish Care
47	<i>In ovo</i> EROD
48	Deformity Assessment
49	Experimental Approach
50	Data Analysis and Representation
51	Results
52	Discussion
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58 **Abstract:**

59 Widespread contamination of aquatic systems with polycyclic aromatic hydrocarbons
60 (PAHs) has led to concern about effects of PAHs on aquatic life. Some PAHs have been
61 shown to cause deformities in early life stages of fish that resemble those elicited by
62 planar halogenated aromatic hydrocarbons (pHAHs) that are agonists for the aryl
63 hydrocarbon receptor (AHR). Previous studies have suggested that activity of
64 cytochrome P4501A, a member of the AHR gene battery, is important to the toxicity of
65 pHAHs, and inhibition of CYP1A can reduce the early life stage toxicity of pHAHs. In
66 light of the effects of CYP1A inhibition on pHAH-derived toxicity, we explored the
67 impact of both model and environmentally relevant CYP1A inhibitors on PAH-derived
68 embryotoxicity. We exposed *Fundulus heteroclitus* embryos to two PAH-type AHR
69 agonists, β -naphthoflavone and benzo(a)pyrene, and one pHAH-type AHR agonist,
70 PCB126, alone and in combination with several CYP1A inhibitors. In agreement with
71 previous studies, co-exposure of embryos to PCB126 with the AHR antagonist and
72 CYP1A inhibitor α -naphthoflavone decreased frequency and severity of deformities over
73 PCB126-alone exposed embryos. In contrast, embryos co-exposed to the PAHs with
74 each of the CYP1A inhibitors tested were deformed with increased severity and
75 frequency than PAH-alone dosed embryos. The mechanism by which inhibition of
76 CYP1A increased embryotoxicity of the PAHs tested is not understood, but these results
77 may be helpful in elucidating mechanisms by which PAHs are embryotoxic.
78 Additionally, these results call into question additive models of PAH embryotoxicity for
79 environmental PAH mixtures that contain both AHR agonists and CYP1A inhibitors.