

## Indirubin and Indigo are Potent Aryl Hydrocarbon Receptor Ligands Present in Human Urine

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AhR is a ligand-activated transcription factor that regulates genes involved in xenobiotic metabolism, cellular proliferation, and differentiation. Numerous xenobiotic and biological compounds are known to interact with AhR, but it remains an orphan receptor since its physiological ligand is unknown. We identified AhR ligands in human urine using a yeast AhR signaling assay and then characterized their properties. Two ligands, indirubin and indigo, were both present at average concentrations of approximately 0.2 nM in the urine of normal donors. Indirubin was also detected in fetal bovine serum and contributed half of the total AhR ligand activity. The activities of indirubin and indigo were comparable to or more potent than that of the archetypal ligand, TCDD, in yeast AhR activation assays. We suggest that the endogenous levels and potencies of indirubin and indigo are such that they activate AhR-mediated signaling mechanisms *in vivo*.