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EXPOSURE TO ENVIRONMENTAL CHEMICALS INDUCES A NOVEL SPLICE VARIANT OF THE BRAIN FORM OF CYTOCHROME P450 AROMATASE IN ZEBRAFISH EMBRYOS

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A critical problem of ecology and human health is the presence in the environment of ubiquitous and persistent chemical compounds, which have the potential to disrupt hormone-dependent processes of development, physiology and reproduction (EDC, endocrine disrupting chemicals). For this reason, there is an urgent need to develop sensitive, mechanism-based molecular markers of effect that can be used in high-throughput assays to assist in risk assessment. A program of research in this laboratory is using zebrafish (*Danio rerio*) embryos as a whole animal, *in vitro* screening system for simultaneous detection of multiple subsets of EDC. Previous studies from this laboratory have shown that *cyp19B*, the gene encoding the predominant brain aromatase (estrogen synthetase) isoform (P450aromB) is induced by estrogen and xenoestrogens. In addition to routine screening of individual chemicals and mixtures, we are using this assay to investigate the presence and chemical nature of EDC in environmental samples collected from ponds impacted by pollutants at the Massachusetts Military Reservation Superfund site (Cape Cod). Treatment of embryos (2 – 96 hr post fertilization, hpf) with different dilutions of sediment elutriate (25-100% daily replacement) from Moody Pond increased P450aromB mRNA as measured by RT-PCR and Southern transfer, indicating the presence of estrogen-like EDC. In addition to the predicted PCR product (210 bp), a second larger size amplicon (810 bp) increased in abundance with increasing amounts of sediment elutriate. Sequence analysis revealed induction of a novel mRNA variant produced by a rare alternative splicing mechanism known as intron retention. The retained intron 8 was located upstream of the catalytic and heme binding regions (exons 9 and 10) and contained an in-frame stop codon, predicting a functionally inactive P450aromB protein. The ratio of the intron-retained (variant) to the intron-skipped (normal) mRNAs was developmentally programmed (e.g., >10:1 at 6 hpf; <1:10 at 120 hpf and in adults). The mRNA variant was downregulated by estradiol (which also increased the normal mRNA), but TCDD markedly increased the intron-retained form (but had no effect on the normal mRNA). Treatment of embryos with sediment elutriates from a reference pond (Mashpee), or with water samples from Moody or Mashpee, had no effect on normal or variant P450 aromB mRNAs, suggesting site-specific differences in contaminant load and distribution. No sign of acute toxicity were observed in any of the treated embryos (mortality, hatching, motility or developmental abnormalities). Results suggest that reproductive abnormalities previously reported in turtles collected from Moody (but not Mashpee) Pond could be explained by additive and synergistic interactions of undefined estrogen- and dioxin-like EDC. Additionally, although most attention has been given to chemicals that perturb transcriptional control of target genes, studies described here provide initial evidence for a novel mechanism of endocrine disruption involving regulation of alternative splicing. Supported by the NIEHS P42 ES07381, EPARD831301, and a postdoctoral fellowship from the Government of Spain to AN.