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Short communication

Developmental differences in elimination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) during *Xenopus laevis* development

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Abstract

Although 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent developmental toxicant in most vertebrates, several frog species are insensitive to TCDD, especially during early life stages. Previous experiments with ranid frogs suggest that TCDD insensitivity results largely from rapid elimination. Recent studies in the African clawed frog (*Xenopus laevis*; family Pipidae) link low TCDD toxicity with the low binding affinity of aryl hydrocarbon receptors, which mediate the toxic effects of dioxin-like compounds. The present study sought to examine TCDD elimination in *X. laevis* embryos and tadpoles, enabling an integrated assessment of the relative roles of TCDD elimination and AHR-related mechanisms in TCDD insensitivity within a single frog species. Using tadpoles (stage 52–55; ~1 month old) exposed to [^{3}H]TCDD, we observed that TCDD has a relatively short half life of 102.6 h, consistent with other frogs and much faster than reported clearance rates in developing fish. In contrast, TCDD elimination is much slower during early development. Embryos exposed during primary organogenesis (from stage 31–41, beginning ~36 h after fertilization) exhibited little TCDD elimination during the subsequent 96 h. Enhanced TCDD clearance in later developmental stages may follow the appearance of a functional digestive tract and the onset of feeding. These results suggest that rapid elimination is unlikely to contribute mechanistically to TCDD insensitivity during development of the cardiovascular system, which is significantly perturbed by TCDD in fish embryos.

Keywords: Dioxin; *Xenopus laevis*; Toxicokinetics; Frogs

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent developmental toxicant in most vertebrates. Recent studies in developing fish, which are typically very sensitive to TCDD, reveal the importance of the cardiovascular system as a target of toxicity, especially during early development. Effects of TCDD exposure include cardiac malformations, pericardial edema, reduced cardiac function, and inhibited definitive erythropoiesis (e.g. Belair et al., 2001; Antkiewicz et al., 2005). Notably, cardiovascular development events most sensitive to TCDD perturbation occur during a specific "window of toxicity," defined in zebrafish between 48 and 96 hpf (Belair et al., 2001; Antkiewicz et al., 2005).

In contrast, frogs are substantially insensitive to TCDD toxicity during early development. Embryos and tadpoles of several members of Family Ranidae are 100–1000-fold less sensitive to TCDD-induced lethality than most fish (Jung and Walker, 1997). *Xenopus laevis* embryos (African clawed frog; family Pipidae) suffer little mortality following acute exposure to TCDD (Jung and Walker, 1997). Although changes in certain sublethal endpoints are documented in *X. laevis* (e.g., Sakamoto et al., 1997), the most frequent and serious effects resulted only from long-term, high level exposures, beginning at least two weeks after fertilization, and they do not include the severe cardiac defects observed in fish.

Initial studies of TCDD insensitivity in the ranid frogs noted the relatively short half-life of TCDD in embryos and tadpoles, ranging in different species from 1 to 7 days, compared with 35–37 days in lake trout fry (Walker et al., 1991). These observations are consistent with the hypothesis that rapid TCDD elimination by frog embryos limits exposure duration and toxicity, especially if levels are substantially reduced prior to targeted events such as cardiac development. TCDD toxicity is mediated by the aryl hydrocarbon receptor (AHR), which binds TCDD and acts as a ligand-induced transcription factor (reviewed in Gu et al., 2000). Recent studies of AHRs from *X. laevis* demonstrate that these proteins bind TCDD with 25–50-fold lower affinity than AHRs from more sensitive species, a prop-

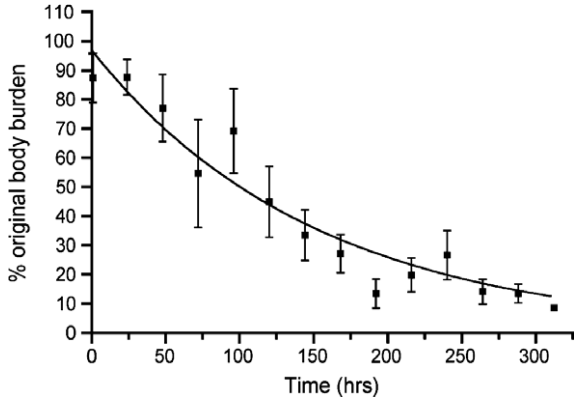

Fig. 1. TCDD elimination by *X. laevis* tadpoles. Tadpoles at stages 52–55 (~1 month old; Nieuwkoop and Faber, 1994) were exposed to [1,6]-^{3}HTCDD [33.1 Ci/mmol; >99% radiopurity; Chemsyn (Lenexa, KS)] dissolved in DMSO by application of a drop to the ventral surface (mean dose was 56 pg/animal or ~150–300 ng/g). They were held in water constantly circulated through a charcoal filter, fed daily with Nasco Frog Brittle, and sampled over a two-week period. Individual animals (3 per time point) were solubilized in Soluene-350 (Perkin–Elmer), and the TCDD body burden was measured by liquid scintillation counting on a Beckman LS6500 instrument using Hionic Fluor (Perkin–Elmer). Percent original body burden is based on the mean body burden at time = 1 h. Half-life was calculated by regression analysis using GraphPad Prism 4.0b: $y = 100^{-(0.0068)x} + 1.0^{-0.007}$. $R^2 = 0.6735$. $N = 4$ experimental replicates with 3 animals sampled per time point. $t_{1/2}$ was 102.4 h (95% confidence: 57.39–475.0).