Glutathione S-transferase (GST) expression repertoire in a zebrafish embryonic cell line and its regulation by GST model substrate 2,4-dinitrochlorobenzene (CDNB)

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The number of chemical compounds used in various products and applications is increasing steadily, raising the demand for more testing data to inform risk assessment. At the same time, there is an urge to replace, reduce and refine (3R) the use of animals in toxicity testing, a challenge posed from scientific and ethical perspectives. Alternative testing methods offer promising innovations catering to both demands, as they can enable high-throughput screening without animal use. In aquatic risk assessment, assays with zebrafish (Danio rerio) embryos (i.e., non-protected life stages[1]) and rainbow trout (Oncorhynchus mykiss) cell lines (i.e., RTgill-W1[2] and RTgutGC[3]) have been successfully positioned as an alternative to using adult fish. Since the zebrafish embryo test still requires maintenance of adult fish for breeding purposes, possibilities to use zebrafish cell lines instead are also being explored.[4] One limitation to the broader application of these alternative models for toxicity assessment has been the insufficient knowledge about their capacity to biotransform chemicals, i.e., their spectrum of expressed biotransformation pathways. One such pathway is the mercapturic acid pathway, which protects organisms from harmful electrophilic compounds. Recently, the functionality of the mercapturic acid pathway and hence the capacity to detoxify electrophiles has been demonstrated in both zebrafish embryo and the zebrafish embryonic cell line, PAC2, using a nontoxic concentration of a reference substrate, 1-chloro-2,4-dinitrobenzene (CDNB).[5,6] The first step in this pathway, glutathione conjugation of an electrophile, is catalyzed by the glutathione S-transferase (GST) enzymes. Using mass spectrometry-based targeted proteomics, we demonstrated that both zebrafish embryo and the PAC2 cell line express a broad repertoire of GST isoforms that might have partially overlapping functions.[5,7] Although some GST substrates have been reported to regulate the expression of GST enzymes previously, exposure to a non-toxic concentration of CDNB did not cause significant changes in GST expression. Here, we exposed the PAC2 cells to 10%, 20% and 50% effect concentrations (EC10, EC20 and EC50, respectively) of CDNB to understand GST expression responses to higher substrate concentrations.

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