JRF GLOBAL

Hepatotoxicity in ZEBRAFISH*(Danio rerio)*

TEAM: Mr. Mehul Pandya, Mr. Jigar Rana and Dr. Dipak Patel
Department of Ecotoxicology

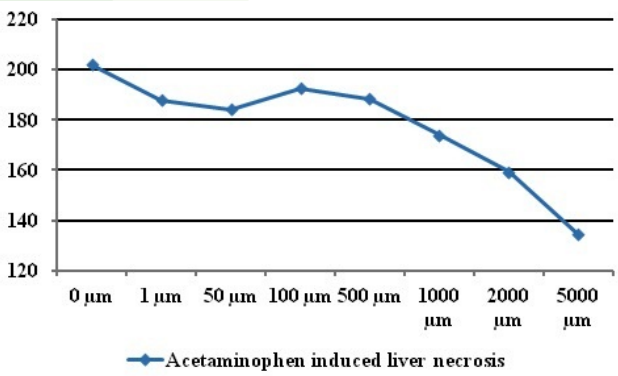
Zebrafish (*Danio rerio*) is a well-suited vertebral model for understanding the response of the new molecules at early phase of discovery. Zebrafish is a freshwater fish originating from Himalayan region in India, also found in other parts of India, Bangladesh, and Pakistan. Studies on the Zebrafish have several advantages. It has a high fecundity rate laying more than 150 eggs per mating. The Transparent eggs are easy to observe for their developments. The embryos in salt solution or Zebrafish embryo specific "E3" medium are alive for several days without any external feeding.

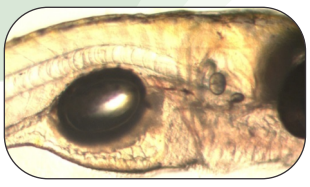
The Zebrafish genetic material has substantial similarity with the mammalians. Seventy percent of protein-coding human genes are related to genes found in the Zebrafish (*Danio rerio*), and Eighty Four percent of genes known to be associated with human disease have a Zebrafish counterpart. The test system is deemed "*in vitro*" and maintenance cost is negligible as compared to other test systems.

It is a well established fact that the liver is the first line of defense for detoxification of ingested chemicals. Acetaminophen is a common antipyretic drug being used in routine life. It's also a mild anti-inflammatory chemical compared to ibuprofen and other COX-1 and COX-2 inhibitors. Though the drug is quite safe for humans up to 4g/day, chronic use of Acetaminophen is known to cause hepatic toxicity.

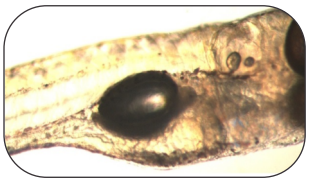
JRF is actively involved in developing several discovery as well as regulatory support driven studies using Zebrafish as a test system. Recently, we undertook evaluation of dose dependent effect of acetaminophen on the Zebrafish liver. Embryos were treated with various concentrations of Acetaminophen in well plates for 72 hours. No behavioral abnormality or mortality was observed up to 5000µM. At the end of the experiment, embryos were anaesthetized with tricaine and subjected to micro-photographic evaluation. All the photographs were analyzed by Image software. Significant alteration in the liver was observed in mean pixel intensity at 1000, 2000 and 5000µM. We observed non-specific type of hepatotoxicity at lower concentrations below 500 µM. We are currently testing several other chemicals at JRF. JRF has developed a specific technique, which facilitates a more reliable resolution at early stage of discovery of the hepatotoxic potential of the test chemicals on zebrafish.

Concentration of acetaminophen (µM) vs. pixel intensity





Zebrafish embryo from Control group



Zebrafish embryo from Treatment group

References:

1. Acetaminophen safety and hepatotoxicity – where do we go from here? July 2007, Vol. 6, No. 4, Pages 341-355 (doi:10.1517/14740338.6.4.341) Patrik J Amar, and Eugene R Schiff, University of Miami Miller School of Medicine, Division of Hepatology, 1500 NW 12 Avenue, Suite 1101 East Tower, Miami, Florida 33136, USA
2. Marja D et al. A transcriptomics-based hepatotoxicity comparison between the zebrafsh embryo and established human and rodent in vitro and in vivo models using cyclosporine Amiodarone and acetaminophen. Toxicol. Lett. 2015; 232(2): 403-12
3. Olaia Holgado, María Diez, Carles Callol-Massot, M. Luz Martínez-Chantar, Ainhoa Letamendia. Predicition of hepatotoxicity in zebrafsh, Biobide S.L Donostia-San Sebastián, Spain
4. Sarvaiya VN, Sadariya KA, Rana MP, Thaker AM. Zebrafish as model organism for drug discovery and toxicity testing: A review. Veterinary Clinical Science 2014; 2(3); 31-38