## DISTRIBUTION AND ELIMINATION OF [3H]-AFLATOXIN B1 IN RAINBOW TROUT (SALMO GAIRDNERI ) AND TILAPIA (OREOCHROMIS NILOTICUS ).

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## **DECLARATION**

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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## **ABSTRACT**

The distribution and elimination of [<sup>3</sup>H]-AFB<sub>1</sub> in rainbow trout (*Salmo gairdneri*) and tilapia (*Oreochromis niloticus*) was studied using whole-body autoradiography (WBA) and liquid scintillation counting. Whole-body autoradiography is a useful method for studying the kinetics of xenobiotic compounds within individuals of different target organs. In this method, the compound is labelled with suitable radionuclide (<sup>14</sup>C, <sup>3</sup>H or <sup>35</sup>S) and administered to each of the species studied.

Aflatoxin  $B_1$  is a potent toxin and liver carcinogen produced as a secondary metabolite by fungi of the genus *Aspergillus flavus*. This compound is of concern because it and other related mycotoxins commonly contaminate grains and seeds used for consumption by animals and humans.

The rainbow trout (Salmo gairdneri) is exceptionally sensitive and has been used in laboratories for studies on AFB<sub>1</sub> carcinogenesis and metabolism. Fish models may be useful not only as environmental *in situ* monitors of aquatic pollution but as alternative vertebrate species for detailed comparative studies on mechanisms of action of carcinogens and other substances.

In this study, the distribution and elimination of aflatoxin in rainbow trout and tilapia was studied for 8 days after intravenous and *per os* administration. The assessment was done by whole-body autoradiography and liquid scintillation counting. Rainbow trouts were divided into two groups. The first group was given a single intravenous dose of 1 µci/g fish body weight of [<sup>3</sup>H]-AFB<sub>1</sub> dissolved in ethanol. The second group of trouts were given a single oral dose of 1 µci/g fish body weight of [<sup>3</sup>H]-AFB<sub>1</sub> dissolved in corn oil through a stomach tube. Tilapias were also administered orally with a single dose of 1 µci/g fish body weight dissolved in corn oil. Each fish was killed at designated time interval, frozen and sectioned in a cryomicrotome. The freeze dried sections

were exposed against X-ray films for 3 months and the radiolabelling was related to the anatomical structures in the section.

The remaining blocks were used for liquid scintillation counting. Samples of liver, cranial kidney, caudal kidney, bile, spleen, brain, abdominal fat and muscle were collected from the frozen blocks and prepared for liquid scintillation counting using a Packard Tri-Carb 1900 CA spectrometer. The tissues were weighed, digested, decolourised and scintillation cocktail added before the counting procedure.

The highest concentrations of radioactivity in rainbow trout after oral and intravenous administration were seen in the bile, liver and caudal kidney after 1-2 days. There was no significant difference (p> 0.05) in radioactivity between oral and intravenous administration in rainbow trout's liver, caudal kidney and blood tissues. The bile showed the highest concentration of radioactivity throughout the experimental period in tilapia. The activity in the liver and caudal kidney of the same species was also high initially but decreased gradually to less than 20% of the original by day 3. Low levels of radioactivity were observed in the blood, brain, spleen, abdominal fat and muscle of the two species of fish.

There was a significant difference in radioactivity between caudal kidney (p<0.05) and liver (p<0.01) of tilapia and rainbow trout respectively. However, there was no significant difference (p>0.05) in radioactivity for blood between the two species of fish.

The whole-body autoradiography results were similar to those of liquid scintillation countings. Radioactivity was high in the intravenous administered rainbow trouts than in the oral rainbow trouts and tilapia. The whole-body autoradiograms revealed the highest concentration of radioactivity to be localised in the liver, kidneys, bile, pyloric caeca contents and mucosa, urine, descending intestinal contents and mucosa, uveal tract of the eye and olfactory rosette at

designated time intervals. The activity in tilapia was high in the liver, kidneys, bile, ventricle mucosa, uveal tract of eye and small intestines.

The results of this study indicates good distribution and elimination of aflatoxin  $B_1$  in the rainbow trout irrespective of the route of administration. High levels were found in the bile, liver olfactory rosette, uveal tract of eye and caudal kidney respectively in the two species of fish. The persistent high levels of aflatoxin  $B_1$  in the bile of the two species of fish confirms bile to be the major route of excretion. There was high significant levels of aflatoxin  $B_1$  in the liver and caudal kidney of rainbow trout compared to tilapia following oral administration. Fish model studies can be alternative methods for studying kinetics of xenobiotics.