An assay for Ciguatera in fish supplied from Labasa Health Department.

By

Dr U Raj and Hazra Thaggard
Institute of Marine Resources
The University of the South Pacific
Suva, Fiji

For

Labasa Health Department

INTRODUCTION

Pieces of fish suspected to have caused poisoning at Labasa was supplied to us by the Health Department Labasa for toxicity tests.

MATERIALS AND METHOD

Sample was thawed out and appropriate amount of tissue prepared for analysis.

PREPARATION OF SAMPLE

100g of muscle tissue, devoid of skin and bones, was prepared from the pieces.

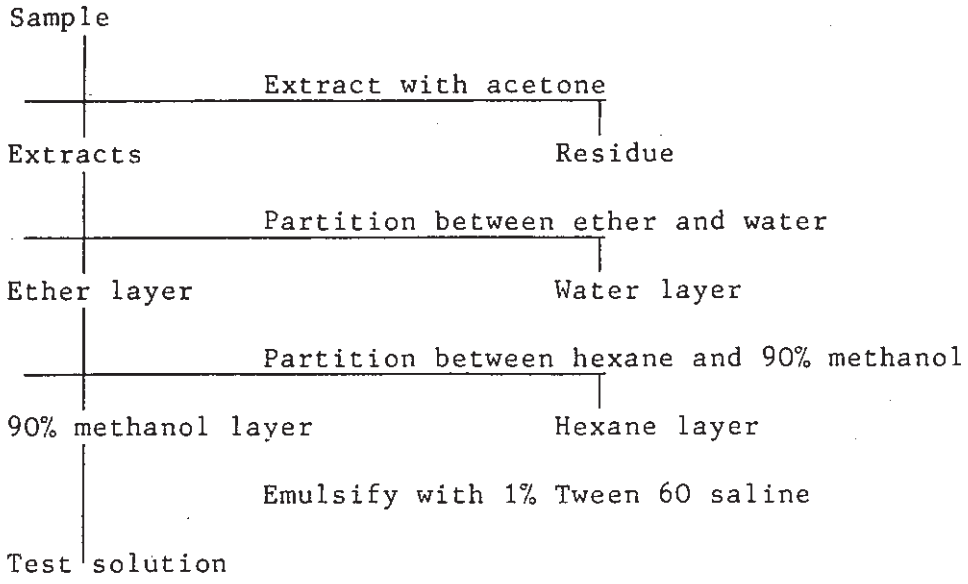
The darker portions of the fish was separated and pooled with the liver to give 100 grams.

EXTRACTION AND PARTIAL PURIFICATION

The known quantities of the prepared samples were placed in a homogenizer and 300mls of Acetone added. The sample was homogenized for 2 minutes. The homogenate was filtered (No. 1 filter paper) using a suction pump and buchner flask. To the residue, 200mls of Acetone was added and together with the used filter paper it was homogenized in the blender for 2 minutes. The homogenate was filtered again and the residue homogenized and filtered a third time. The total filtrate was evaporated using a Rotary evaporator (Rotavapor R 110) and a water bath. The extract was washed out with 200mls of diethyl ether into a separating funnel. 20mls of distilled water was added and after shaking, left for 5 minutes to form partition between ether and water. The bottom layer (i.e. water) was drained into a flask and the ether layer transferred to evaporating flask. To the drained water layer, 200mls of diethyl ether was added and the separation of water and ether layers repeated in the separating funnel.

The ether layer from the second separation was added to the evaporating flask and the diethyl ether evaporated in the Rotary evaporator. The extract was then washed out in a flask with 300mls of hexane and 100mls of 90% methanol. The two solvents were placed in a separating funnel to form partition layers. The bottom layer (methanol) was drained into a smaller evaporating flask while 20mls of 90% methanol and 100mls of hexane added to the top layer (hexane) and partitioning repeated. The methanol layer from this separation was added to the previously recovered methanol in the smaller evaporating flask. Methanol was then evaporated in the Rotary evaporator. The residue was washed from the evaporating flask into yet smaller evaporating flask using ethanol. Ethanol was then evaporated. The toxin extract was transferred to a graduated test tube, with a pipette using 1% tween 60 saline. The volume was made upto 2.5ml with 1% tween 60 saline.

The procedure outlined above is summarized in Figure 1.



MOUSE TEST

From the test solution, 3 laboratory mice weighing approximately 20 grammes each were injected intraperitoneally 1ml, 0.6ml and 0.4ml, respectively, of the toxin extract. The reaction of the mice was noted for 3 hours and the death time, if mice died, observed within 24 hours.

In the event of death of mice the toxicity level was calculated and expressed as mouse units. One mouse unit is defined as the minimum amount of toxin required to kill a mouse of 20 grammes body weight within 24 hours.

An example calculation:

If one injected 1ml, 0.6ml and 0.4ml, of toxin, respectively, in each of the three respective mice and only surviving mice was one which received 0.4ml injection, then the minimum amount of toxin to kill the mouse would be 0.6ml (i.e., out of these three dilutions).

Thus 0.6ml of extract will be attributed to contain 1 mouse unit (mu) of toxin. The total amount of toxin in 2.5ml extract will therefore be $\frac{2.5}{0.6}$ - 4.16 mu.

Since the final extract (2.5ml) would have been prepared from 100 grammes of tissue the toxic score of the tissue sample will be 4.16 mu/100g of tissue.

RESULTS

Fish Species	Tissue Tested	Toxicity Score MU/100g
Bolbometopon muricatus (Kalia)	Flesh	N.D.
	Flesh+Liver	N.D.

GENERAL COMMENTS

No toxicity was determined in the sample.