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CIGUATERA AND HISTAMINE ANALYSIS FOR SUSPECTED TOXIC FISH (*SCOMBEROMORUS COMMERSON : SCOMBRIDAE*)

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FOR

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GOVERNMENT OF FIJI

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INTRODUCTION

A consignment of frozen fish head said to be "Walu" (Scomberomorus <u>commerson</u> : SCOMBRIDAE) *was sent to us by the Senior Health Inspector (Food Control) from the Ministry of Health.*

The person eating this fish was admitted to the CWM Hospital with suspected fish poisoning on 5 April, 1988 after eating the fish on 1st April, 1988.

Histamine (histamine poisoning is usually attributed to scombrid fishes such as "Walu") and ciguatera toxicity tests were done.

It is noted that "Walu" along the Queensland Coast are known to have caused ciguatera poisoning but this phenomenon is still to be recorded from Fiji.

The two tests were performed on the flesh only. In this report we describe details of both tests carried out and the results obtained.

CIGUATERA TEST

MATERIALS AND METHOD

The fish head was thawed out and the appropriate amount of flesh prepared for analysis.

PREPARATION OF SAMPLE

100g of flesh, devoid of skin and bones, was prepared from the fish.

EXTRACTION AND PARTIAL PURIFICATION

100g of the prepared sample was placed in a homogeniser and 300mls of Acetone added. The sample was homogenised 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 homogenised in the blender for 2 minutes.

The homogenate was filtered again and the residue homogenised and filtered a third time. The total filterate was evaporated using a rotary evaporator (Rotovapor 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, 2oomls of diethyl ether was added and the separation of water and ether layers repeated in the separating funnel.

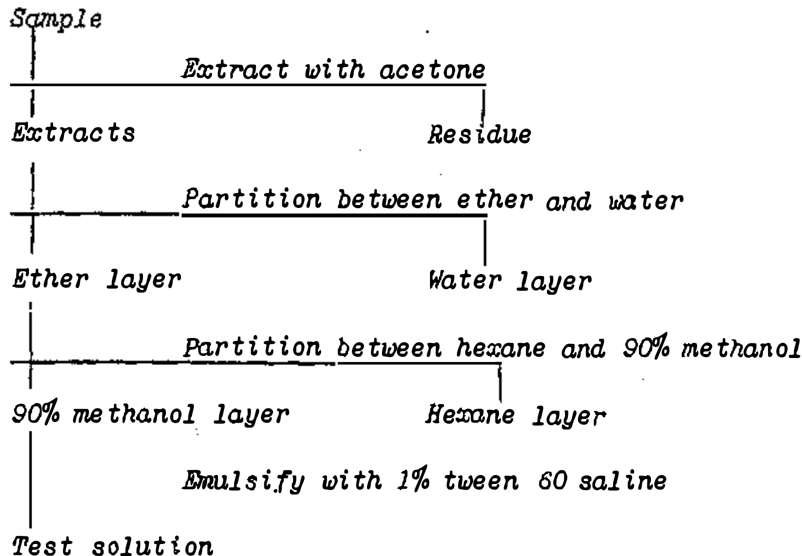
The ether layer from the second separation was added to the evaporating flask and the diethyl ether evaporated in the rotary evaporator. The extract was then washed out in a flask with 300mls of hexane and 100mls of 90% methanol. The two solvents were placed in a separating funnel to form partition layers. 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 a 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 summarised in Figure 1.

<u>MOUSE TEST</u>

From the test solution, 3 laboratory mice weighing approximately 20g each were injected intraperitoneally 1ml, 0.8ml and 0.6ml, respectively, of the toxin extract. The reaction of the mice was noted for 3 hours and the death time, if the 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 20g body weight within 24 hours.

FIGURE 1 : A Summary of Extraction and Purification Procedure for Lipophilic Toxic Test



CALCULATION AND RESULTS

1ml, 0.8ml and 0.6ml of toxin injected respectively in each of the three respective mice and only surviving mice was one which received 0.6ml injection, then the minimum of toxin to kill the mouse would be 0.8ml (i.e. out of these three dilutions).

Thus 0.8ml of extract is attributed to contain 1 mouse unit (mu) of toxin. The total amount of toxin in 2.5ml extract is therefore

$$\frac{2.5}{0.8} = 3.13mu.$$

Since the final extract (2.5ml) would have been prepared from 100g of flesh, the toxicity score of the flesh sample is 3.13mu/100g of flesh. Any fish containing ciguatoxin above 2.5 mu/100g should be avoided as food. This means that flesh with toxicity scores 2.5mu/100g or greater are likely to cause illness when consumed.

HISTAMINE TEST

MATERIALS AND METHOD

The fish head was thawed out and appropriated amount of flesh prepared for analyss.

Preparation of the Chromatographic Column

Ion exchange resin Dowex 1-X8, 50-100 mesh is converted to hydroxide ion (^{-}OH) form by adding about 15ml 2m NaOH/g resin to beaker. The mixture was swirled and left to stand for <30 mins. Liquid was decanted and this was repeated with additional base. Resin was washed thoroughly with water, slurry' into fluted paper (No. 541) and washed again with water. Resin should be prepared weekly and stored under water.

Glass wool pug was placed in base of chromotographic column (200 x 7(d)mm, fitted with a tap), water was filled to 10cm and filled enough resin to form 8cm bed. Water level was maintained at the top of resin bed at all times. It is not good to regenerate resin in packed column, batch regeneration in beaker is preferable. It is necessary to change resin in column after each extraction.

Preparation of Sample

10g of prepared sample was placed in a homogeniser and 50mls of methanol added. The sample was homogenised for about 2 mins. The homogenate was transferred to 100ml volumetric flask. This was left in water bath at $60^{0}C$ for 15 mins. It was left to cool to $25^{0}C$ and diluted to volume with methanol.and filtered. Alcohol filtrate may be stored in refrigerator for several weeks.

Method

4-5ml of water was passed through the column and eluate was discarded. 1ml of alcohol filterate was pipetted on to the column and another 4-5ml of water was added.

Immediately column flow was initiated into 50ml vol. flask containing 5.0ml of 1.00M Hydrochloric acid (HCl). Flow rate was controlled at >3ml/minute. When liquid level was about 2mm above resin, 5ml of water was added. This was eluted. It was followed with larger portions of water until about 35ml had been eluted. This was diluted to volume with water. It can be refrigerated.

5ml of elute was pipetted into 50ml erlenemeyer and to it 10ml of 0.1M HCl was added. It was proceeded as for the standards below, beginning "to each 3ml of 1M NaOH ......".

Preparation of Standard Curve

Standard Solution/. Should be stored in a refrigerator.

a) Stock soln/ - 1mg/ml as free base.
169.1mg of histamine was accurately weighed into a vol. flask, and dissolved and diluted to volume with 0.1M HCl. Should be prepared fresh weekly.

b) Intermediate soln/ 10g/ml
1ml of the stock solution pipetted into 100ml vol. flask and diluted to volume with 0.1M HCl should be prepared fresh weekly.

c) Working soln/ 0.5, 1.0 and 1.5 g/5ml
1, 2 and 3ml of intermediate solution was pipetted into separate 100ml vol. flasks and diluted to volume with 0.1M HCl prepared daily.

Standards were done in duplicate. 5ml of aliquots of each working standards were pipetted into separate 50ml glass erlenmeyers. 10ml of 0.1M HCl were pipetted to each flask and mixed. To each 3ml of 1M NaOH were added and mixed within 5 minutes.

1ml of O - phthalicdicarboxaldehyde (OPT) solution were added and mixed immediately. After exactly 4 mins 3ml of 3.57M of phosphoric acid were pipetted and mixed immediately. It was important to mix thoroughly after each addition and at least once during the OPT reaction (Run 6-10 OPT reactions simultaneously by adding reagents to erlenmeyers set order).

Blank was prepared by substituting 5ml of 0.1M HCl for histamine soln. Fluorescence intensity (1) should be recorded within 1.5 hours of working standard soln. using excitation wavelength of 350nm and emission wave length of ~~444nm~~. Graph can be plotted using 1 (corrected for blank) against g histamine/5ml aliquot.

Calculations

Plot of (1) against gram histamine/5ml aliquot should be close to a straight line passing through the origin with the slope.

$$M = [Ia/1.5 + Ib + 2Ic\ ]/3$$

It can also be calculated using the equation

$$\text{mg histamine/100g fish} = (10)\ (F)\ (1/m)(I_2)$$

where,

I_2, I_a, I_b and I_c = fluorescence from sample, and histamine standards 1.5, 1.0 and 0.5 respectively; and F = dilution factor (ml elute + ml 0.1M HCl/ml) elute.

F = 1 for undiluted elute

Standards	Fluorescence Intensity (1)			
Zero with blank				
0.5g/5ml	(a)	40	(b)	40
1.0	(a)	79	(b)	78
1.5	(a)	119	(b)	117
Sample	(a)	31	(b)	30

$$\text{Slope } m = \left[\frac{118}{1.5} + 78.5 + 2 \times 40\right]/3$$

$$= 79.05$$

$$F = 5 + 10/5$$

$$= 3$$

$$\therefore \text{ mg histamine/100g fish} = 10 \times 3 \times \frac{1}{79.05} \times 30.5$$

$$= 11.57$$

$$= 11.6$$

Using the graph attached the slope (m) = 79.0.

$Y_1 = 40$ $X_1 = 0.5$
$Y_2 = 119$ $X_2 = 1.5$ From the graph

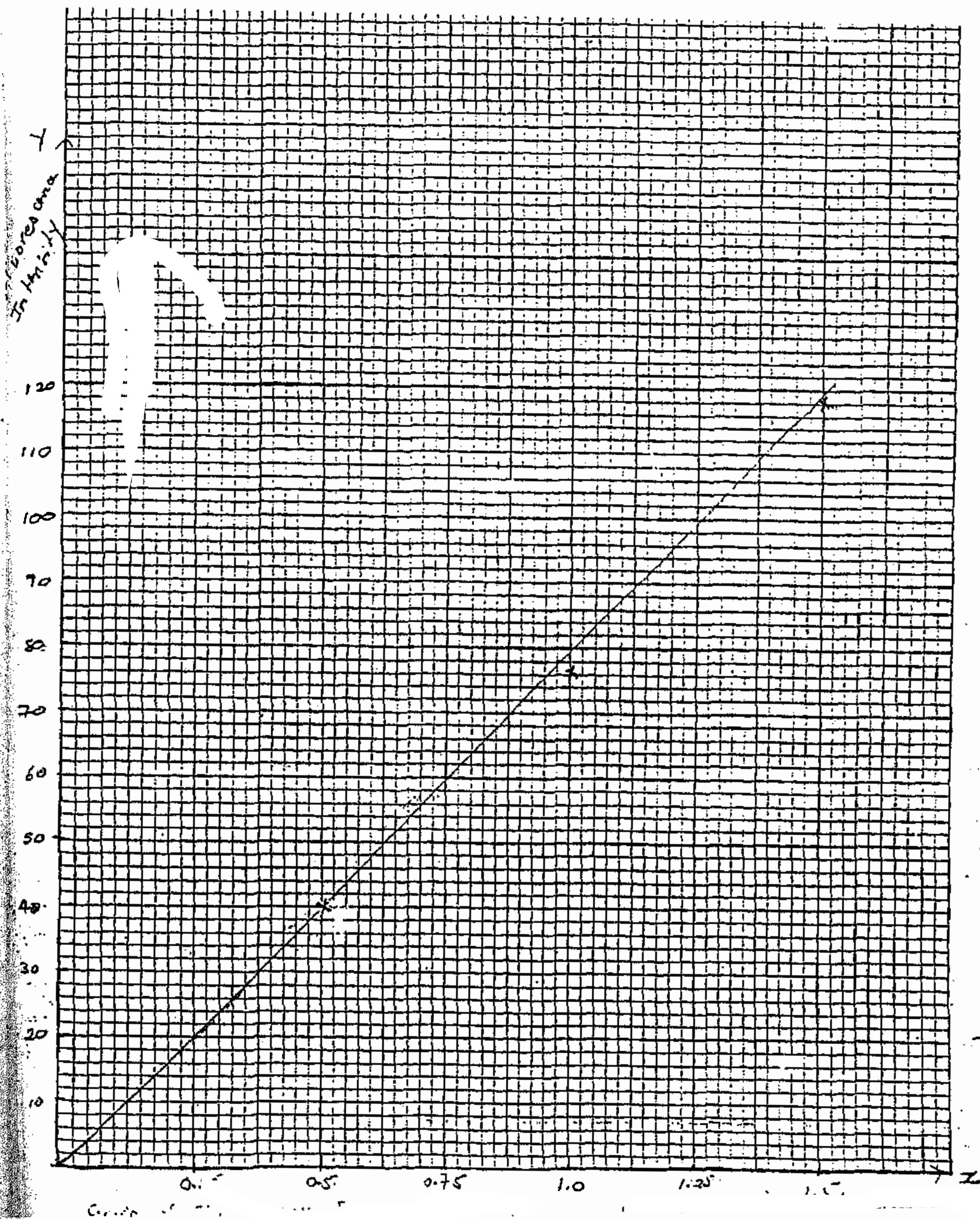
$$\text{Slope } m = \frac{Y_2 - Y_1}{X_2 - X_1}$$

$$= \frac{119 - 40}{1.5 - 0.5}$$

$$= 79.0$$

It has been reported that poisoning by spoiled fishes usually occurs in humans when histamine concentration reaches a level of about 100mg/100g of fish.

GRAPH OF FLUORESCENCE INTENSITY (ℓ) VS GRAM HISTAMINE/5ML



COST OF ANALYSES

Ciguatera Test	$
Acetone	2.00
Diethyl ether	2.00
Hexane	1.50
Methanol	3.00
Ethanol	0.20
Cost per Analysis	51.00
Mice	
3 mice @ $1.75 each	5.25
Technician time	
7 hrs @ $3.00/hr	21.00
	$85.95

Histamine	$
Hydrochloric acid	3.00
Sodium hydroxide	1.00
Ion exchange resin	3.00
Phosphoric acid	2.00
O-phthalicdicarboxaldehyde	2.00
Methanol	5.00
Histamine standard	4.00
Hexane	5.00
Cost per Analysis	34.00
Technician time	
7 hours @ $3.00/hr	21.00
	$80.00

CONCLUSION

It is unlikely that fish poisoning was due to the histamine because the histami[illegible] [illegible]ch is 11.6mg histamine/100g fish. Cigua[illegible] the [illegible]sh contained 3.13mu/mg ciguatoxin.

It can be said that [illegible]h poisoning [illegible] ciguatera because the level of ciguatera was abo[illegible] mu/100g which is likely to cause illness when consumed.

REFERENCES

1. Takeshi Yasumoto, Uday Raj & Raymond Bagnis.
   Seafood Poisonings In Tropical Regions (1984).

2. Sidney Williams (editor).
   Official Methods of Analysis of the Association of Official Analytical Chemists (1984). 14th Edition.