



UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL MARKERS IN RIVER CATFISH, *MYSTUS NEMURUS* (C&V) EXPOSED TO HYDROGEN SULPHIDE

MUHAMMAD TAFAZZAL HOQUE

FSAS 1997 20

BIOLOGICAL MARKERS IN RIVER CATFISH, *MYSTUS NEMURUS* (C&V) EXPOSED TO HYDROGEN SULPHIDE

By

MUHAMMAD TAFAZZAL HOQUE

**Dissertation Submitted in Fulfilment of the Requirements
for the Degree of Doctor of Philosophy in the
Faculty of Science and Environmental Studies
Universiti Putra Malaysia**

April, 1997



*This dissertation is dedicated
to my mother,
who inspired me to do this
and to my father,
who has taught me to aspire and persevere*



ACKNOWLEDGEMENTS

First and foremost, I would like to express my most sincere gratitude and deep appreciation to the Chairperson of my supervisory committee, Assoc. Prof. Dr. Fatimah Md Yusoff, Department of Biology, UPM for her invaluable contribution, inputs and careful supervision. Without her constant encouragement this dissertation would never have been written.

I am indebted to the members of my supervisory committee, Prof. Law Ah Theem, Department of Marine Science; Assoc. Prof. Dr. Arif Syed, Department of Biochemistry; and Assoc. Prof. Dr. Manaf Ali, Department of Biotechnology, UPM for their encouragement, constructive suggestion and guidance, and review of my work throughout the study period.

I would also like to extend heartfelt thanks to Prof. Mohd. Sharif and Assoc. Prof. Dr. Hair Bejo of the Department of Veterinary Pathology and Microbiology, UPM especially for their valuable comments on histopathological and histochemical studies. I am sincerely grateful to Dr. M. A. Quayum, Faculty of Modern Languages, UPM for editing the manuscript.

Furthermore, I would like to acknowledge Universiti Putra Malaysia for supporting the research and providing the Graduate Assistantship by the Malaysian Government through IRPA (Intensification of Research Priority Areas), grant no. 1-07-05-011 (J03) and 01-02-04-165. I wish to express my deep appreciation to all the staff of the former Faculty of Fisheries and Marine Science in Serdang and Terengganu for offering me their hospitality and for their enthusiastic acceptance of me as part of their community.



I also wish to thank all my fellow graduate students for their hospitality and help. Special acknowledgements are due to the following friends who have provided valuable inputs directly or indirectly in the presentation of this dissertation: Dr. M.S. Khan and Mr. Jalal Khan.

My appreciations are also due to the Dean of Faculty of Science and Environmental Studies, Dean of Graduate School, Dean of Faculty of Veterinary Science and Dean of Faculty of Biotechnology of UPM, and the former Dean of Faculty of Fisheries and Marine Science, UPM for their kind assistance. I would also like to extend heartfelt thanks to Mr. Abdul Aziz Bahsir, Senior Assistant Registrar, Graduate School, UPM, who helped me in every possible way.

I would like to record my sincere appreciation to the security staff of UPM for the cooperation extended to me while I was sick in the laboratory.

Words are not enough to express my heartfelt feelings to my parents, sister and brother for providing me with their untiring guidance and support since my childhood. I owe a depth of gratitude to them which can never be repaid.

To all, thank you from the bottom of my heart.

Sobaikye, Amar Antor Antorosthol thekye Sobechha Janai.



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF PLATES	xvi
LIST OF ABBREVIATIONS	xviii
ABSTRACT	xix
ABSTRAK	xxi

CHAPTER

I	INTRODUCTION	1
	Background of the Study	1
	Statement of the Problems	4
	Significance of the Study	6
	Objectives of the Study	8
II	LITERATURE REVIEW	9
	Bio-availability of H ₂ S	9
	Hydrogen Sulphide Dynamics in Aquatic Ecosystems	15
	Toxicity of Hydrogen Sulphide	15



Bioassay Techniques for Toxicity Test	17
Median Lethal Toxicity Test	18
Sublethal Toxicity Test	19
Critique between Median and Sublethal Toxicity Test in Environmental Bioassays	21
Biological Marker and Its Advantages in Toxicity Bioassay	21
Biological Markers and Their Responses to Hydrogen Sulphide	24
Biochemical Markers	25
Histopathological Markers	28
Bioaccumulation Markers	31
Bioenergetic Markers	32
Validity of Biological Markers	37
<i>Mystus nemurus</i> as a Test Organism and Its Biology	37
Distribution and Habit	37
Reproductive Biology and Physiology	38
III GENERAL MATERIALS AND METHODS	39
Selection of Parameters for Biological Markers Study	39
Experimental Design	40
Laboratory Experiment	42
Selection and Maintenance of the Test Organism	42
Experimental Set up	43
Data Collection	44



	Field Validation Experiment	45
	Selection of Sampling Sites	46
	Sampling Sites Description	48
	Collection of Samples	49
	Analysis of Different Parameters	49
	Statistical Analysis	50
IV	DETERMINATION OF MEDIAN LETHAL CONCENTRATION OF HYDROGEN SULPHIDE ON <i>M. NEMURUS</i>	51
	Introduction	51
	Materials and Methods	52
	Results and Discussion	55
	Conclusion	59
V	BIOCHEMICAL TRANSFORMATION IN <i>M.</i> <i>NEMURUS</i> DUE TO HYDROGEN SULPHIDE TOXICITY	60
	Introduction	60
	Materials and Methods	64
	Thiosulphate Analysis	65
	Haematological Study	66
	Glutathione <i>S</i> -transferase Activities	67
	Statistical Analysis	70



	Results and Discussion	70
	Thiosulphate Concentration in Blood	70
	Haematological Changes	75
	Glutathione <i>S</i> -transferase Activities in Liver	82
	Conclusion	86
VI	ULTRASTRUCTURAL CHANGES AND SULPHUR ACCUMULATION IN <i>M. NEMURUS</i> AS INDICATORS OF HYDROGEN SULPHIDE TOXICITY	87
	Introduction	87
	Materials and Methods	91
	Histopathological Study	92
	Neurotoxicity Study	94
	Analysis of Bioaccumulation of H ₂ S in Gill	94
	Results and Discussion	96
	Histological Changes in Gill	96
	Neurotoxic Injury in Fish Brain Tissue	103
	Bioaccumulation of Hydrogen Sulphide in Gill	109
	Conclusion	114
VII	BIOENERGETIC EFFECTS IN <i>MYSTUS</i> <i>NEMURUS</i> DUE TO HYDROGEN SULPHIDE TOXICITY	116
	Introduction	116
	Materials and Methods	118



	Condition Factor Analysis	119
	RNA and DNA Analysis	119
	Statistical Analysis	121
	Results and Discussion	121
	Condition Factor as An Effects Index for H ₂ S Toxicity	121
	Changes in RNA and DNA Concentration with H ₂ S Toxicity	126
	Conclusion	129
VIII	FIELD VALIDATION OF SOME SELECTED BIOLOGICAL MARKERS AS INDICATORS OF HYDROGEN SULPHIDE TOXICITY	130
	Introduction	130
	Materials and Methods	131
	The Study Areas	131
	Data Collection and Data Analysis	131
	Results and Discussion	132
	Water Quality	132
	Biological Markers Study	135
	Conclusion	139
IX	GENERAL DISCUSSION AND CONCLUSION	140
	General Discussion	140
	Intra-relationship of Different Biological Markers	140



Inter-relationship of Different Biological Markers	145
Biochemical and Physiological Concept of Biological Markers Relationship	148
General Conclusion	153
Scope for Further Studies	157
BIBLIOGRAPHY	158
APPENDICES	191
Appendix A: Additional Tables	192
Appendix B: Additional Plates	198
VITAE	204



LIST OF TABLES

Table	Page
1	Hydrogen Sulphide Concentrations in Some Freshwater Ecosystems 10
2	Some of Freshwater Microorganisms involved in the Hydrogen Sulphide Dynamics and the Reactions Catalysed 13
3	List of LC ₅₀ for Hydrogen Sulphide Toxicity in Some Fish 20
4	The Average Chemical Properties of Experimental Water 53
5	Estimated LC ₅₀ Values of Unionised Hydrogen Sulphide on Juvenile of <i>M. nemurus</i> using Trimmed Spearman-karber Method 56
6	Thiosulphate Concentrations (μM) (Mean \pm sd; N=9) in Blood of <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide at Different Exposure Time 71
7	Total Haemoglobin, Oxyhaemoglobin and Sulphaemoglobin Concentrations (Mean \pm sd; N=9) of <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide at Different Exposure Time 76
8	Specific Activity of Glutathione S-transferase (nM/min/mg protein) (Mean \pm sd; N=9) ¹ in Liver of <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide at Different Exposure Time 84
9	Fulton's Condition Factor, Growth Rate (%) and Liver-somatic Index of <i>M. nemurus</i> in Different Concentrations of Unionized Hydrogen Sulphide ($\mu\text{g/L}$) at Different Exposure Time 122
10	Effects of H ₂ S on RNA and DNA Concentration in White Muscle of <i>M. nemurus</i> 127
11	Time Course Effects on Constants (a, b and r ²) in <i>M. nemurus</i> within Different Concentrations of Unionised Hydrogen Sulphide 129
12	Water Quality in Different Sampling Stations 133



13	A Check List for the Evaluation of Biological Markers as an Stress Effect of Hydrogen Sulphide Toxicity	156
14	Thiosulphate concentrations (μM) (Mean \pm sd; N=9) ¹ in Blood of <i>M. nemurus</i> at Different Exposure Time in Different Unionised Hydrogen Sulphide Concentrations	192
15	Total Haemoglobin, Oxyhaemoglobin and Sulphaemoglobin Concentrations (Mean \pm sd; n=9) of <i>M. nemurus</i> at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	193
16	Specific Activity of Glutathione <i>S</i> -transferase (nM/min/mg protein) (Mean \pm sd; N=9) in Liver of <i>M. nemurus</i> at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	194
17	Time Course Effect on Constants (a, b and r^2) ¹ in Different Biological Markers of <i>M. nemurus</i> within Different Concentrations of Unionised Hydrogen Sulphide (0 to 1.91 $\mu\text{g/L}$)	195



LIST OF FIGURES

Figure		Page
1	The Hydrogen Sulphide Cycle in Biogeochemical System	11
2	Sulphate Reduction Pathway is Used by Anaerobic Bacteria	14
3	Flow Chart of the Experimental Design	41
4	Map Showing the Different Sampling Stations in Lake Kenyir	47
5	The Standard LC ₅₀ Curve of Unionised Hydrogen Sulphide for <i>M. nemurus</i> Juvenile	55
6	Cumulative Mortality of <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide	57
7	Transformation of Hydrogen Sulphide to Thiosulphate in Biological System	61
8	Reaction Showing the Sulphaemoglobin Formation	62
9	Thioether Formation with Substrate CDNB	68
10	Thiosulphate Concentrations in <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide	72
11	Changes of Thiosulphate Concentrations (%) in Different Concentrations of Unionised H ₂ S (µg/L) Exposure Compared to Initial Stage in <i>M. nemurus</i>	74
12	Total Haemoglobin in Blood at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	75
13	Oxyhaemoglobin in Blood at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	77
14	Sulphaemoglobin in Blood at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	79
15	Sulphaemoglobin and Total Haemoglobin Ratios in Blood at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	80



16	Oxyhaemoglobin and Total Haemoglobin Ratios in Blood at Different Exposure Time in Different Concentrations of Unionised Hydrogen Sulphide	81
17	Time Interval Scanning of GST Activity of <i>M. nemurus</i>	83
18	Specific Activities of Glutathione <i>S</i> -transferase in <i>M. nemurus</i> in Different Concentrations of Unionised Hydrogen Sulphide	84
19	X-ray Spectrum of Gill from An Unexposed Fish (control group)	109
20	X-ray Spectrum of Gill from A Exposed Fish of Highest Concentration (1.91 $\mu\text{g/L}$) of Unionised Hydrogen Sulphide	110
21	EDAX Analysis of Sulphur in Gills in Different Concentrations of Unionised Hydrogen Sulphide and Different Exposure Time	112
22	CHNS/O Analysis of Sulphur in Gills in Different Concentrations of Unionised Hydrogen Sulphide and Different Exposure Time	113
23	Comparison of EDAX and CHNS/O Techniques for Analysing Sulphur Accumulation in Gills During Different Exposure Time	114
24	Fulton's Condition Factor of <i>M. nemurus</i> at Different Concentrations of Unionized Hydrogen Sulphide	123
25	Growth Rate of <i>M. nemurus</i> at Different Concentrations of Unionised Hydrogen Sulphide	124
26	Liver-somatic Index of <i>M. nemurus</i> at Different Concentrations of Unionised Hydrogen Sulphide	125
27	Effects of Unionised Hydrogen Sulphide on RNA-DNA Ratio in White Muscle of <i>M. nemurus</i>	128
28	Changes in Different Biochemical Markers in Different Sampling Stations	136
29	Changes in Different Bioenergetic Markers in Different Sampling Stations	136



30	Sulphur Biaccumulation in Gill Tissue of <i>M. nemurus</i> at Different Sampling Stations	137
31	X-ray Spectrum of Gill of <i>M. nemurus</i> from Different Sampling Stations	138
32	Regression Relationship between H ₂ S Concentration in Water and Thiosulphate Concentration in Blood of <i>M. nemurus</i>	140
33	Regression Model of Sulphaemoglobin Concentration in Blood of <i>M. nemurus</i> and H ₂ S Concentration in Water	141
34	Regression Relationship between H ₂ S Concentration in Water and Specific Activity of GST in Liver of <i>M. nemurus</i>	141
35	Regression Relationship between Sulphur Accumulation in Gill Tissue of <i>M. nemurus</i> and H ₂ S Concentration in Water	142
36	Regression Relationship between Liver-somatic Index of <i>M. nemurus</i> and H ₂ S Concentration in Water	143
37	Regression Relationship between H ₂ S Concentration in Water and Growth Rate of <i>M. nemurus</i>	144
38	Regression Relationship between H ₂ S Concentration in Water and Fulton's Condition Factor of <i>M. nemurus</i>	144
39	Regression Model of Sulphur Accumulation in Gill vs Sulphur Containing Metabolites in <i>M. nemurus</i> due to H ₂ S Toxicity	146
40	Regression Model of Growth vs RNA, RNA-DNA Ratio and Protein Content in <i>M. nemurus</i> due to H ₂ S Toxicity	147
41	Regression Model of Thiosulphate Concentration in Blood vs Growth Rate and Liver-somatic Index in <i>M. nemurus</i> due to H ₂ S Toxicity	148
42	Hypothetical Sketch of Biochemical and Physiological Changes in <i>M. nemurus</i> due to Hydrogen Sulphide Toxicity	149
43	Relationship between Different Biological Markers in <i>M. nemurus</i> due to Hydrogen Sulphide Toxicity	150



LIST OF PLATES

Plate		Page
1	Morphological Characteristics of <i>Mystus nemurus</i> (C&V)	37
2	Behavioural Pattern of <i>M. nemurus</i> during Acute H ₂ S Toxicity Test	58
3	Primary Gill Lamellae with Two Rows of Secondary Lamellae in the Control Group of <i>M. nemurus</i>	97
4	Efferent Side of the Secondary Gill Lamellae with Several Pores of Mucus Cells (→) in the Control Group of <i>M. nemurus</i>	98
5	Fusion Between Two Secondary Gill Lamellae (→) in Exposed to 1.91 μg/L Unionised Hydrogen Sulphide; Cellular debris and mucus (➤) also observed	98
6	Interlamellar Fusion (↔) in Secondary Gill Lamellae in Fish Exposed to 1.91 μg/L unionised hydrogen sulphide	99
7	Electron Micrograph of Secondary Gill Lamellae in Control Group	100
8	Secondary Gill Lamellae with Plenty Mitochondria (→) in Control Group	101
9	Electron Micrographs of Lamellar Lesions in Gill Exposed to H ₂ S	102
10	Transverse Section of Cerebellum in Control Fish	103
11	Electron Micrograph of Granular Layer of Cerebellum of Control Group	104
12	Ultra-structures of Granular Layer of Control Brain Tissues	105
13	Electron Micrograph of Hydrogen Sulphide Exposed Cerebellum, Granular Layer in 0.96 μg/L of Unionised H ₂ S After 2 Weeks Exposure Spongiform degeneration in brain featuring highly vacuolated cells (v).	106



14	Electron Micrograph of Hydrogen Sulphide Exposed Cerebellum in 1.91 $\mu\text{g/L}$ of Unionised H_2S After 6 Weeks Exposure.	107
15	Ultrastructures Changes in Cerebellum Exposed to 1.91 $\mu\text{g/L}$ H_2S	108
16	<i>Mystus nemurus</i> Showing the Rudimentary Sex Organ	198
17	An Overview of Experimental Set up	199
18	A Close View of H_2S Stock Solution Delivery System from The Refrigerator	199
19	Sampling Site of Terengganu River (Station 1)	200
20	Outside View of the Dam in Lake Kenyir (Station 2)	200
21	A Pristine Lentic Zone in Lake Kenyir (Station 3)	201
22	Organic Matter-rich Uptake Riverine Zone in Lake Kenyir (Station 4)	201
23	Activities during Field Validation Experiment	202



LIST OF ABBREVIATIONS

CDNB	1-chloro-2,4-dinitrobenzene
EDAX	Energy Dispersive Analysis X-ray
EDTA	Ethylenediamine tetra-acetic acid
GST	Glutathione <i>S</i> -transferase
Hb	Haemoglobin
HbO ₂	Oxyhaemoglobin
KCN	Potassium cyanide
K ₃ Fe(CN) ₆	Potassium Ferricyanide
LSI	Liver-somatic index
MS-222	3-aminobenzoic acid ethyl ester
SDS	Sodium dodecyl sulphate
SEM	Scanning Electron Microscope
SHb	Sulphaemoglobin
TEM	Transmission Electron Microscopy



Abstract of the dissertation presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

**BIOLOGICAL MARKERS IN RIVER CATFISH, *MYSTUS NEMURUS* (C&V)
EXPOSED TO HYDROGEN SULPHIDE**

by

MUHAMMAD TAFAZZAL HOQUE

April 1997

Chairperson : Assoc. Prof. Dr. Fatimah Md. Yusoff
Faculty : Science and Environmental Studies

Biochemical, histopathological, histochemical and bioenergetic parameters were studied to determine their suitability as biological markers for hydrogen sulphide toxicity detection using the river catfish *Mystus nemurus* (C&V) exposed to H₂S in laboratory experiments as well as those caught from the wild. In the laboratory, the toxic effects of H₂S to *M. nemurus* juveniles were determined by using a flow-through bioassay technique.

The 96-h LC₅₀ value of unionized H₂S was 3.20 µg/L, and 0.003 µg/L unionized H₂S was recommended as the safety level for *M. nemurus* juveniles under tropical environmental condition. Sulphaemoglobin and thiosulphate concentrations significantly increased ($p < 0.01$) with increasing hydrogen sulphide (H₂S) concentrations and exposure time. However, H₂S reduced the oxygen carrying capacity of haemoglobin by reducing oxyhaemoglobin. Glutathione *S*-transferase (GST) specific activities significantly increased ($p < 0.01$) in fish exposed to H₂S higher than 30% of LC₅₀.



Gill lesions such as epithelial separation, club-shaped lamellae and interlamellar fusion were observed at different concentrations of H₂S. The evidence of neurotoxicity was elucidated by necrosis and damaged mitochondria in fish brain tissue. Sulphur accumulation in gills progressively increased with the increase of H₂S concentrations and exposure time.

The liver-somatic index (LSI) and growth rate significantly decreased ($p < 0.05$) with increased concentrations of H₂S and exposure time. Fulton's condition factor failed to predict ($p > 0.1$) stress effects in fish exposed less than six weeks to H₂S. However, RNA-DNA ratios showed high correlations with H₂S concentrations from the second ($r^2 = 0.83$; $p < 0.01$) to sixth week ($r^2 = 0.98$; $p < 0.01$) of exposure.

Thiosulphate and sulphaemoglobin showed positive correlations with H₂S concentrations ($r^2 = 0.79$; $p < 0.01$ and $r^2 = 0.89$; $p < 0.01$ respectively). Sulphur accumulation in gills was positively correlated with thiosulphate and sulphaemoglobin concentrations in blood ($r^2 = 0.74$; $p < 0.01$), indicating that these compounds resulted from H₂S exposure. In addition, H₂S levels in water were directly correlated with GST activities and sulphaemoglobin concentrations. However, H₂S concentrations showed an inverse relationship with oxyhaemoglobin concentrations.

The field study supported the laboratory findings for two indicators; thiosulphate and sulphur accumulation, were potential biological markers for H₂S toxicity. Other markers such as Fulton's condition factor, liver-somatic index, growth rate, RNA-DNA ratio, histopathology and histochemistry did not reflect specific toxic effect, although they can be used to indicate the general health condition of fish exposed to H₂S. Among all the indicators, thiosulphate was found to be the simplest and fastest biological marker for detecting H₂S toxicity.

Abstract of the dissertation presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

**BIOLOGICAL MARKERS IN RIVER CATFISH, *MYSTUS NEMURUS* (C&V)
EXPOSED TO HYDROGEN SULPHIDE**

by

MUHAMMAD TAFAZZAL HOQUE

April 1997

**Chairperson : Assoc. Prof. Dr. Fatimah Md. Yusoff
Faculty : Science and Environmental Studies**

Biochemical, histopathological, histochemical and bioenergetic parameters were studied to determine their suitability as biological markers for hydrogen sulphide toxicity detection using the river catfish *Mystus nemurus* (C&V) exposed to H₂S in laboratory experiments as well as those caught from the wild. In the laboratory, the toxic effects of H₂S to *M. nemurus* juveniles were determined by using a flow-through bioassay technique.

The 96-h LC₅₀ value of unionized H₂S was 3.20 µg/L, and 0.003 µg/L unionized H₂S was recommended as the safety level for *M. nemurus* juveniles under tropical environmental condition. Sulphaemoglobin and thiosulphate concentrations significantly increased ($p < 0.01$) with increasing hydrogen sulphide (H₂S) concentrations and exposure time. However, H₂S reduced the oxygen carrying capacity of haemoglobin by reducing oxyhaemoglobin. Glutathione *S*-transferase (GST) specific activities significantly increased ($p < 0.01$) in fish exposed to H₂S higher than 30% of LC₅₀.



Lesion insang seperti pemisahan epitelial, lamela berbentuk belantan dan penyatuan interlamela dapat dilihat pada beberapa kepekatan H₂S yang berbeza. Bukti ketoksikan saraf ditunjukkan oleh nekrosis dan kerosakan mitokondria di dalam tisu otak ikan. Pengumpulan sulfur di dalam insang meningkat secara berterusan dengan peningkatan kepekatan H₂S dan masa pendedahan.

Indeks somatik hati (LSI) dan kadar pertumbuhan menurun dengan bererti ($p < 0.05$) dengan peningkatan kepekatan H₂S dan masa pendedahan. Faktor keadaan Fulton gagal ($p > 0.01$) meramalkan kesan tekanan di dalam ikan yang terdedah kepada H₂S kurang dari enam minggu. Walaubagaimana pun, nisbah RNA-DNA menunjukkan pertalian yang tinggi dengan kepekatan H₂S pada minggu yang kedua ($r^2 = 0.83$; $p < 0.01$) ke minggu yang keenam ($r^2 = 0.98$; $p < 0.01$).

Tiosulfat dan sulfamoglobin menunjukkan pertalian yang positif dengan H₂S ($r^2 = 0.79$; $p < 0.01$ dan $r^2 = 0.89$; $p < 0.01$ masing-masing). Pengumpulan sulfur di dalam insang berkait secara positif dengan kepekatan tiosulfat dan sulfamoglobin di dalam darah ($r^2 = 0.74$; $p < 0.01$), menunjukkan bahawa kompaun-kompaun ini terhasil daripada ketoksikan H₂S. Tambahan pula, paras H₂S di dalam air berkadar terus dengan aktiviti GST dan kepekatan sulfamoglobin. Namun begitu, kepekatan H₂S menunjukkan pertalian songsang dengan oksihemoglobin.

Kajian lapangan menyokong penemuan di dalam makmal bahawa dua penunjuk: tiosulfat dan pengumpulan sulfur adalah penanda biologi yang berpotensi bagi ketoksikan H₂S. Penanda biologi yang lain seperti faktor keadaan Fulton, indeks somatik hati, kadar pertumbuhan, nisbah RNA:DNA, histopatologi dan histokimia tidak mencerminkan kesan ketoksikan yang spesifik, walaupun kesemuanya boleh digunakan untuk menentukan tahap kesihatan am bagi ikan yang terdedah kepada H₂S. Di antara kesemua penanda-penanda yang dikaji, tiosulfat adalah penanda biologi yang paling mudah dan pantas untuk mengesan ketoksikan H₂S.

CHAPTER I

INTRODUCTION

Background of the Study

Hydrogen sulphide (H_2S) is a colourless gas, heavier than air and moderately water soluble (6g /L at $10^{\circ}C$; Keith and Walters, 1985). It occurs in many natural situations where decomposition of organic matter in bottom deposits is a normal phenomenon. H_2S is also generated in sludge deposits from paper mills, leather tanning and finishing, rubber processing, rayon manufacture, dyeing, untreated sewage effluent, and other sources of organic debris. In Malaysia, six pulp and paper mills are discharging about 295,000 mt effluent per year which contribute a significant amount of H_2S (FAO, 1991).

Various explanations have been suggested for high concentrations of H_2S in waterbodies. The predominant hypothesis is anthropogenic eutrophication (Zaytsev, 1976, 1977; Nesterova, 1977; Tolmazin, 1977). H_2S availability in nature is limited by oxygen availability. Due to oxidation of sulphide in the water column, oxygen



availability is decreased which leads to hypoxia and ultimately to anoxic condition in the bottom layers of water (Bella et al., 1972). In prolonged hypoxia fish are synergistically exposed to oxygen deficiency and higher unionised H_2S concentration as pH decreases.

H_2S is a rapid and powerful systematic poison (Gleason et al., 1969) and its unionised form has been demonstrated to be toxic to fish (Smith and Oseid, 1972b; Broderius et al., 1977). Its toxicity is strongly influenced by pH together with temperature. H_2S occurs naturally at levels which affect the survival of fish and production in both freshwater (Smith and Oseid, 1974; Torrans and Clemens, 1982) and marine (Fenchel and Riedl, 1970; Breaten et al., 1983; Liefbrig, 1985; Jorgensen, 1984; Bagarinao, 1991a, 1995) ecosystems. The lethality of H_2S is comparatively higher in freshwater than that in the marine environment since the former maintain a higher unionised stage of H_2S (Millero, 1986) due to low pH and lack of buffering capacity (Poole et al., 1978).

Various approaches, which are mainly acute and chronic toxicity tests have been used to evaluate or predict the effects of environmental stress on fish. Although these approaches are frequently adopted, they have little ecological realism (Cairns, 1981; National Research Council, 1981). So, a new approach through selecting other stress-related parameters that are biologically and ecologically relevant and has maximum predictive capabilities was suggested.