

ผลของไทรบิวทิลทินออกไซด์ที่มีต่อการเจริญของคัพภะและการพัฒนาในระยะวัยอ่อนของ
กึ่งก้ามกราม *Macrobrachium rosenbergii* de Man



นายพรฤทธิ อริยะวงษ์วัฒน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์สภาวะแวดล้อม สหสาขาวิชาวิทยาศาสตร์สภาวะแวดล้อม
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2543

ISBN 974-13-0822-1

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF TRIBUTYL TIN OXIDE ON EMBRYONIC AND LARVAL DEVELOPMENT
OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* de Man

Mr. Pornriddh Ariyavongvadhana

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Environmental Science

Inter-department of Environmental Science

Graduate School

Chulalongkorn University

Academic Year 2000

ISBN 974-13-0822-1

Thesis Title EFFECTS OF TRIBUTYL TIN OXIDE ON EMBRYONIC AND
LARVAL DEVELOPMENT OF GIANT FRESHWATER PRAWN
Macrobrachium rosenbergii de Man
By Mr. Pornriddh Ariyavongvadhana
Field of Study Environmental Science
Thesis Advisor Associate Professor Somkiat Piyatiratitivorakul, Ph.D.

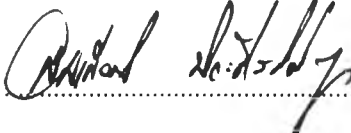
Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the
Requirements for the Master's Degree.


..... Dean of Graduate School
(Professor Suchada Kiranandana, Ph.D.)

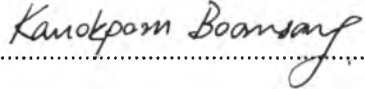
Thesis Committee

..... Chairman

(Assistant Professor Pipat Patanaponpaiboon, Ph.D.)

..... Thesis Advisor
(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)

..... Member
(Assistant Professor Charoen Nitithamyong, Ph.D.)

..... Member
(Dr. Kanokporn Boonsong)

พรฤทธิ์ อริยะวงศ์วัฒน์ : ผลของไตรบิวทิลทินออกไซด์ต่อการเจริญของคัพภะและการพัฒนาในระยะวัยอ่อนของกึ่งก้ามกราม *Macrobrachium rosenbergii* de Man. อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. สมเกียรติ ปิยะธีรชิตวิรุฬ. 114 หน้า. ISBN 974-13-0822-1.

การทดสอบพิษเฉียบพลันและรองเฉียบพลันของไตรบิวทิลทินออกไซด์ต่อการเจริญของคัพภะและการพัฒนาของกึ่งก้ามกรามวัยอ่อน *Macrobrachium rosenbergii* de Man ภายใต้ภาวะน้ำนิ่ง เปลี่ยนน้ำทุก 24 ชั่วโมง พบว่าไข่กึ่งระยะต้นและระยะปลาย ตายร้อยละ 50 ที่ความเข้มข้น 583 และ 720 ไมโครกรัมต่อลิตร ตามลำดับ โดยความทนทานมีแนวโน้มเพิ่มขึ้นตามอายุของไข่ สำหรับกึ่งวัยอ่อนความเข้มข้นที่ทำให้สัตว์ทดลองตายจำนวนครึ่งหนึ่ง เมื่อสัมผัสสารเป็นเวลา 24 ชั่วโมง อยู่ในช่วง 10.3 (ระยะที่ 7) ถึง 12.8 (ระยะที่ 8) ไมโครกรัมต่อลิตร ตามลำดับ เมื่อสัมผัสสารเป็นเวลา 48 ชั่วโมง ความเข้มข้นที่ทำให้ลูกกึ่งจำนวนครึ่งหนึ่งตาย อยู่ระหว่าง 5.8 ถึง 7.7 ไมโครกรัมต่อลิตร ค่าต่ำสุดพบในลูกกึ่งระยะที่ 5 ค่าสูงสุดพบในระยะที่ 6 ความไวต่อไตรบิวทิลทินออกไซด์ของลูกกึ่งวัยอ่อนแต่ละระยะไม่มีความแตกต่างกันอย่างเด่นชัด ทั้งสองช่วงเวลาของการสัมผัส ในกรณีพิษรองเฉียบพลัน ไตรบิวทิลทินออกไซด์ทำให้จำนวนไข่ที่ฟักเป็นตัวลดลง เมื่อสัมผัสกับสารในช่วงความเข้มข้น 250 ถึง 1000 ไมโครกรัมต่อลิตร เป็นเวลา 15 วัน ในกึ่งวัยอ่อนพบว่าการเจริญเติบโตลดลงอย่างมีนัยสำคัญ ($P < 0.05$) เมื่อสัมผัสสารที่ระดับ 0.6 หรือ 1.2 ไมโครกรัมต่อลิตรเป็นเวลา 30 วัน โดยไม่พบความแตกต่างระหว่างความเข้มข้นทั้งสอง

ภาควิชา. สหสาขาวิชาวิทยาศาสตร์สุขภาพแคว้นลือม. ลายมือชื่อนิติ.....
 สาขาวิชา..... วิทยาศาสตร์สุขภาพแคว้นลือม..... ลายมือชื่ออาจารย์ที่ปรึกษา.....
 ปีการศึกษา..... 2543..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4172373123 : MAJOR ENVIRONMENTAL SCIENCE

Key words : *Macrobrachium rosenbergii* / GIANT FRESHWATER PRAWN / TRIBUTYL TIN OXIDE / TBTO / EMBRYOTOXIC / LARVAL TOXIC / LC₅₀

PORNRIDDH ARIYAVONGVADHANA : EFFECTS OF TRIBUTYL TIN OXIDE ON EMBRYONIC AND LARVAL DEVELOPMENT OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* de Man. THESIS ADVISER : ASSO. PROF. SOMKIAT PIYATIRATITIVORAKUL, Ph. D. 114 pp. ISBN 974-13-0822-1.

Acute and subacute toxicities of tributyltin oxide (TBTO) were determined in embryos and larvae of giant freshwater prawn, *Macrobrachium rosenbergii* de Man, with static water renewal condition every 24 h. The 96 h LC₅₀s for early and late stage embryos were 583 and 720 µg l⁻¹, respectively. The tolerance to TBTO trend increase with increasing developmental stage. For larvae, the 24 h LC₅₀s were ranging from 10.9 to 12.8 µg l⁻¹, respectively for first and fifth stage larvae. The 48 h LC₅₀s comprised between 5.8 and 7.7 µg l⁻¹ for fifth and sixth stage larvae, respectively. There appear to be no difference among the 24 h LC₅₀s of first six stages larvae and also 48 h LC₅₀s for second to sixth stage. As regards sublethal effects, hatching success reduced with increasing concentration by 15 days of exposure between 250-1000 µg l⁻¹. Growth was significantly retarded (*P*<0.05) in larvae treated with 0.6 or 1.2 µg l⁻¹ for 30 days without significant difference between the two concentration.

Department. Inter-department of Environmental Science Student's signature.....
 Field of study..... Environmental Science..... Advisor's signature.....
 Academic year..... 2000..... Co-adviser's signature.....

Acknowledgement

The author wishes to extend his deepest gratitude and to acknowledge his indebtedness to his adviser, Associated Professor Dr. Somkiat Piyatiratitivorakul for his unvaluable guidance, constructive suggestion, constant moral, technical, and emotional support throughout the entire thesis course.

The author also wish to express special acknowledgements to Assistant Professor Dr. Pipat Patanapongboon, Assistant Professor Dr. Charoen Nitithamyong, and Dr. Kanokporn Boonsong, chair person and members of the thesis committee for their valuable advice and suggestion.

Special gratitude are expressed to Dr. Supichai Tangchaitong, head of the Marine Science department, Faculty of Science, Chulalongkorn University, for his hospitality and permission to the author for performing his experiment over that period.

Sincerest thanks and credit is paid to Mr. Seri Donnuue for his kindly helpfulness, friendship, and especially for his attempt in collecting living specimens in field throughout the experimental period.

Genuine thanks is also paid to Mr. Sakya Pradit, sitter of the MV SEAFDEC and Mrs. Siriporn Pradit, researcher of Southeast Asia Fisheries Development Center for their information of antifouling paint application in SEAFDEC.

The author wish to thanks the Graduate School, Chulalongkorn University for financial supporting in this thesis.

Finally, the author honestly feel profound indebted to his beloved mother, who devotes herself by endeavouring to alter her ordinary life from house-wife to a road curb merchant for serving family and support the author until his post-graduation. His achievement cans not success without her support and encouragement, and also her emotional and spiritual powers giving to him. The author owe special thanks to his brother, who share some of his time to attenuate mother's hard work when the author was in his full-time course work, resulting in extending his graduation.

Contents

	Page
Abstract (Thai).....	iv
Abstract (English).....	v
Acknowledgement.....	vi
List of Tables.....	x
List of Figures.....	xii
Chapter 1 Introduction.....	1
1.1 General.....	1
1.2 Objectives.....	2
1.3 Hypothesis.....	2
1.4 Scope of study.....	2
1.5 Expectations.....	2
Chapter 2 Literature Reviews.....	3
2.1 Background.....	3
2.2 Types of tributyltin paints.....	5
2.2.1 Tributyltin free association system.....	5
2.2.2 TBT self polishing copolymer systems.....	6
2.3 Tin-free anti-fouling paints.....	7
2.4 Tributyltin in fresh water environments.....	13
2.4.1 Sources and occurrence.....	13
2.4.2 Persistent and fate.....	13
2.5 Tributyltin in estuarine environments.....	14
2.5.1 Sources and concentrations.....	14
2.5.2 Distribution and fate.....	15
2.5.3 Degradation processes.....	18

Contents (cont.)

	Page
2.6 Recovery of TBT contamination after TBT registrations.....	19
2.7 Ingestion, metabolism and bioaccumulation.....	20
2.8 Contamination of TBT in biota.....	38
2.9 Effects on aquatic organisms	40
2.9.1 Lethal toxicity.....	40
2.9.2 Sublethal toxicity.....	40
2.9.3 Mechanism of action for TBTO toxicity in shrimps	43
2.10 Test animals	46
Chapter 3 Materials and Methods	53
3.1 Test animal.....	53
3.1.1 Prawns.....	53
3.1.2 Breeding of embryos and larvae	53
3.1.3 Selected testing stages	54
3.2 Toxicity tests.....	54
3.2.1 Acute toxicity tests.....	55
3.2.2 Subacute toxicity tests.....	57
3.2.3 Data collection.....	58
3.2.4 Statistical analysis	59
Chapter 4 Results and Discussion	60
4.1 Acute toxicity of TBTO.....	60
4.1.1 Embryo toxicity tests	60
4.1.2 Larval toxicity tests.....	64
4.2 Subacute toxicity of TBTO.....	71
4.2.1 Embryotoxicity tests.....	71
4.2.2 Larval toxicity tests	73

Contents (cont.)

	Page
Chapter 5 Conclusions and Recommendations	86
5.1 Conclusions.....	86
5.2 Recommendations.....	86
5.2.1 Proposed anti-cannibalism toxicity test container	86
5.2.2 Research recommended to figure out the status of TBT in Thai waters	88
5.2.3 Feasibility of <i>Macrobrachium rosenbergii</i> for using as standard testing animal	93
5.3 Feasibility of <i>Macrobrachium rosenbergii</i> for using as standard testing animal	109
References.....	96
Appendices.....	106
Appendix A Lethality of <i>Macrobrachium rosenbergii</i> exposed to different concentrations of TBTO.....	107
Appendix B Probit Analysis.....	112
Biography	114

List of Tables

	Page
Table 2.1	Organic and metal-based anti-fouling biocides..... 9
Table 2.2	Alternative anti-fouling biocides and additives, and their possible hazards 10
Table 2.3	Comparison of Tributyltin Self-Polishing Copolymer (TBT SPC) to Tin-Free Anti-fouling Paints..... 11
Table 2.4	Butyltin concentrations (ng g^{-1} wet wt) in organisms collected worldwide 23
Table 2.5	Bioconcentration factors (BCFs) for TBT in aquatic organisms..... 38
Table 2.6	Embryonic development of <i>Macrobrachium rosenbergii</i> 50
Table 2.7	Larval development <i>Macrobrachium rosenbergii</i> by Ling (1969b) versus Uno and Kwon chin soo (1969). 52
Table 4.1	Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (2nd range finding test)..... 61
Table 4.2	Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (3rd range finding test). 62
Table 4.3	Mean percent lethality at 24, 48, 72, and 96 h for 10 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (4th range finding test). 62
Table 4.4	LC50s and mean percent lethality at 48, 72, and 96 h for <i>Macrobrachium rosenbergii</i> embryos exposed to TBTO on different stages of development..... 64
Table 4.5	Mean percent lethality at 24, 48, and 72 h for 4th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (first trial)..... 67
Table 4.6	Mean percent lethality at 24 and 48 h for 1st, 2nd, and 6th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (second trial) 67
Table 4.7	LC50s and mean percent lethality at 24 h for <i>Macrobrachium rosenbergii</i> larvae exposed to TBTO on first to eight stage of development..... 70
Table 4.8	LC50s and mean percent lethality at 48 h for <i>Macrobrachium rosenbergii</i> larvae exposed to TBTO on second to sixth stage of development. 70

List of Tables (cont.)

	Page
Table 4.9 Percent survival on 10th day and percent hatching on 16th day of exposure in <i>Macrobrachium rosenbergii</i> treated with TBTO	73
Table 4.10 Percent stage composition and mean stage of 3rd, 4th, and 5th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO for 48 h (pretest).....	77
Table 4.11 Percent stage composition of sampled <i>Macrobrachium rosenbergii</i> larvae exposed to TBTO.....	78
Table 4.12 Larval developmental rate after exposed to TBTO	84
Table 5.1 Data gaps in TBT contamination status in Thai waters.....	91
Table 5.2 Feasibility of <i>Macrobrachium rosenbergii</i> for using as standard testing animal.....	94
Table A1 Lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (2nd range finding test)	108
Table A2 Lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (3rd range finding test).....	108
Table A3 Lethality at 24, 48, 72, and 96 h for 10 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (4th range finding test)	108
Table A4 Lethality at 24, 48, 72, and 96 h for <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO	109
Table A5 Lethality at 24, 48, and 72 h for 4th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (first trial).....	110
Table A6 Lethality at 24 and 48 h for 1st, 2nd, and 6th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (second trial).....	110
Table A7 Lethality at 24 h for 1st- to 8th-stage and 48 h for 2nd- to 6th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (actual tests)	110

List of Figures

	Page
Fig. 2.1	Operational mechanism of a free association paint..... 6
Fig. 2.2	Operational mechanism of a TBT self-polishing copolymer paint..... 7
Fig. 2.3	Operational mechanisms of some tin-free anti-fouling paints 8
Fig. 2.4	Salinity effects on the sorption of TBT by the sediment .. and on the partitioning of TBT into the porewater 17
Fig. 2.5	pH effects on the sorption of TBT by the sediment and on the partitioning of TBT into pore water..... 17
Fig. 2.6	Histopathological effects of TBTO on gills and epipodites of <i>Peneaus japonicus</i> 45
Fig. 2.7	A mature male giant freshwater prawn <i>Macrobrachium rosenbergii</i> de Man 46
Fig. 2.8	Segmentation and embryonic development 49
Fig. 2.9	Larval development of <i>Macrobrachium rosenbergii</i> de Man 51
Fig. 4.1	Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (2nd range finding test). 60
Fig. 4.2	Mean percent lethality at 24, 48, 72, and 96 h for 3 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (3rd range findingtest)..... 61
Fig. 4.3	Mean percent lethality at 24, 48, 72, and 96 h for 10 day-old <i>Macrobrachium rosenbergii</i> embryos exposed to different concentrations of TBTO (4th range finding test). 63
Fig. 4.4	Mean percent lethality at 24, 48, and 72 h for 4th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (first trial). 65
Fig. 4.5	Lethality at 24 and 48 h for 1st, 2nd, and 6th-stage <i>Macrobrachium rosenbergii</i> larvae exposed to different concentrations of TBTO (second trial) 65
Fig. 4.6	Embryonic development of <i>Macrobrachium rosenbergii</i> 72
Fig. 4.7	Percent survival on 10th day and percent hatching on 16th day of exposure in <i>Macrobrachium rosenbergii</i> treated with TBTO..... 73

List of Figures

	Page
Fig. 4.8 Larval developmental rate after exposed to TBTO.....	75
Fig. 5.1 Schematic diagram of proposed anti-cannibalism toxicity test container.....	90