中文摘要

本論文提出一嶄新淡水亞洲蜆為基礎之線上生物監測系統作為生物鑑定工具，提供一即時且有效低成本量測法以偵測自然水資源中銅或鎘濃度。本系統引用亞洲蜆每日貝殼開闔韻律活動變化作為生物終點。本系統之合成乃根據生物預警系統之基本原理建置，主要有三階段;首先採用一以機率為基礎之方法模式去描述亞洲蜆暴露於重金屬銅或鎘下之貝殼開闔行為律動變化。由前人發表之文獻資料重新分析亞洲蜆對暴露於重金屬下之開闔行為反應，並以一經驗三參數希爾模式重新建構且描述出亞洲蜆隨反應時間改變的劑量反應動力方程式。結合亞洲蜆隨反應時間改變的劑量反應動力方程式、時變之半數受影響的效應濃度(EC50)方程式及以一三參數對數常態特性所迴歸之亞洲蜆每日開闔行為律動的函數，可成功地模擬亞洲蜆暴露於含有重金屬銅及鎘的水域中，隨時間變化的貝殼開闔律動反應。接著以此機率為基礎之方法結合亞洲蜆時變的劑量與開闔行為反應關係，建構出一以圖控式程式語言(LabVIEW 7.0)所編譯而得的動態蜆機制，藉此達到估測水域中時變之重金屬銅及鎘濃度變化，並提供即時檢測生物毒性效應之技術。

第二階段採用可描述生物可獲取率之生物配體模式以聯結重金屬銅的急性毒對亞洲蜆開闔行為所造成的毒性效應。藉由重新分析過去文獻中所發表之亞洲蜆馴養在實驗室中所獲得每天律動的資料及其劑量與開闔行為反應之動力關係，顯示以此生物配體模式為基礎的希爾關係方程式能更佳地描述出水域中自由活性銅離子濃度與亞洲蜆開闔反應之間的關係。本論文所提出亞洲蜆銅毒性效應與生物配體之整合模式，顯示水域自由活性銅離子能鍵結在特定的生物配體(亞洲蜆的鰓)上並降低貝類開闔濾食行為，並指出引起特定之生物效應乃需一定程

度金屬累積於此生物配體上。利用此推導之亞洲蜆銅毒性效應與生物配體之整合模式，並依據當地水質條件可預測水域中時變之半數受影響的效應濃度 EC50 方程式及在任何時間金屬銅加入下亞洲蜆律動變化反應關係，此種方式顯現出此模式有潛力應用於發展成一生物監測系統當作一生物鑑定工具進行線上量測水域系統中銅濃度。

在第三階段本研究整合水化學原理及以生物配體模式為基礎之特定 pH 下時變劑量反應模式，結合所擬合之每日亞洲蜆開闔律動函數，發展一具亞洲蜆銅毒性效應與生物配體整合模式之動態蜆程式機制，可模擬亞洲蜆在不同水域中暴露於含銅環境下，其時變之開闔律動狀態。本研究之動態蜆程式機制加入因水溫及酸鹼值而異之水域環境條件補償機制，可精確地完整反應該動態蜆之銅毒檢測機制對實際環境狀態之適用性。此動態蜆合成機制係經由 LabVIEW 程式語言在個人電腦上模擬驗證系統之功能性，其系統測試結果顯示該程式機制在水溫及酸鹼值變動影響下，均能間接地以亞洲蜆之開闔反應去推估水中時變之銅離子活性並進一步計算因地而異之銅濃度。

在本研究中此貝類開闔量測技術為基礎之亞洲蜆生物監測系統，其設計模擬上所運用之虛擬儀控技術大大地降低在建構過程中之開發成本、設計時間及錯誤。此外，藉由增加此調整系統中時間反應及門檻濃度之重要功能，可改善即時的金屬毒性檢測技術。最後本論文所完成之以亞洲蜆銅毒性效應與生物配體之整合模式為基礎的動態蜆合成，對於銅毒性的線上生物監測提供了更佳之量化判斷，並可輔助技術發展隨地而異的生物預警系統，保護當地水域生態環境，且有助於養殖亞洲蜆管理策略之應用。

關鍵字：亞洲蜆；生物預警系統；金屬毒性；生物監測；貝殼活動行為；生物配體模式；生物可獲取率

ABSTRACT

The goal of this dissertation is to develop a novel freshwater clam *Corbicula fluminea*-based on-line bimonitoring system as a bioassay tool to offer a real-time and cost-effective method to measure copper (Cu) and cadminm (Cd) concentrations in natural water resources. The proposed system used sublethal changes in the daily valve closing activites of *C. fluminea* as a biological endpoint and built upon the basic principles of biological early warning system (BEWS) model in three phases. In the first phase, a probabilistic-based approach describing the valve closure behavior of *C. fluminea* in response to Cu and Cd was developed. The valve closure response data from published literature was reanalyzed to reconstruct the response time-dependent dose-response profiles based on an empirical three-parameter Hill equation model. The reconstructed dose-response profiles and EC50-time relationships associated with the fitted daily valve opening/closing rhythm characterized by a three-parameter lognormal function were integrated to successfully predict the time-varying bivalve closure rhythm in response to waterborne Cu/Cd. A probabilistic-based methodology associated with the time-varying dose-response relationships of valve closing behavior is incorporated into the mechanisms of a dynamic clam compiled by a LabVIEW graphic control program language in a personal computer (PC). It allows the parsimony estimation of the time-varying waterborne Cu/Cd concentrations for on-line providing the performances of the toxicity detection technique.

Secondly, the biotic ligand model (BLM) describing the bioavailability was employed to link between acute Cu toxicity and its effect on valve closure behavior of freshwater clam *C. fluminea* based on the published experimental data of *C. fluminea* closure daily rhythm and dose-response profiles. The results show that a BLM-based Hill model best describes the free Cu^{2+}-activity–valve closure response relationships.

The proposed Cu-BLM-*Corbicula* model shows that the free ionic form of waterborne Cu bind specifically to a biotic ligand (i.e., clam gills) and impair normal valve closure behavior, indicating that a fixed-level of metal accumulation at a biotic ligand is required to elicit specific biological effects. The site-specific EC50(t) and valve closure rhythm at any integrated time was demonstrated to obtain a good prediction, indicating that the proposed model has the potential to develop a biomonitoring system as a bioassay tool to on-line measure waterborne Cu levels in aquatic systems.

In the third phase, the principles of water chemistry were integrated into the modified BLM-based pH-specific concentration-time-response model. Through the combination of the Hill model-based dose-time-response function and the fitted daily rhythm function of valve closure, the constructed Cu-BLM-*Corbicula*-based programmatic mechanism can be used to simulate the valve closure rhythm exposed to copper in various time-varying scenarios. The compensatory mechanism under temperature-specific and pH-specific aquatic environmental conditions was incorporated into the constructed Cu-BLM-*Corbicula* model-based dynamic Cu detection mechanism to precisely and completely reflect the suitability for practical environmental statuses. The performance for a system testing in a dynamic clam synthesis was also demonstrated by employing a LabVIEW software in a PC. The simulation results reveal that the developed Cu-BLM-*Corbicula*-based programmatic mechanism can be used to indirectly estimate the time-varying waterborne Cu ion activity under the influence of water temperature and pH to further evaluate the local-specific waterborne Cu concentration.

In the present study, an important system function for adjusting the time response and threshold concentration was added to improve the real-time metal toxicity detection technique. The virtual instrumentation techniques to design and simulate a *C.*

fluminea-based biomonitoring system based on a valvometric conversion technique were adopted to greatly reduce the costs, development time and errors in implementing procedures. This proposed Cu-BLM-*Corbicula* model-based dynamic clam synthesis has been completed to provide a better understanding to quantify on-line measurement of Cu toxic effect on bivalve health to technically assist in developing a defensible site-specific BEWS for the protection of aquatic ecosystems, and may foster applications in clam farm management strategies.

Keywords: Asiatic clam; Biological early warning system; Metal toxicity; Biomonitor; Valve movement; *Corbicula fluminea*; Biotic ligand model; Bioavailability