ABSTRACT

Recently, subcellular metal partitioning has been used for assessing metal toxicity in aquatic organisms. In reality, the environmental metal concentration is tended to be fluctuation and pulse as a result of the site-specific water chemistry conditions. But little researches have been studied by using the subcellular partitioning for aquatic organisms exposed to pulsed metals in the field of ecotoxicology. Hence, the purposes of this dissertation were: (i) to develop an integrated toxicological model by linking subcellular partitioning and toxicokinetic/toxicodynamic (TK/TD) models with the experimental data of pulsed copper (Cu)-tilapia (*Oreochromis mossambicus*) system and published data of cadmium (Cd)-rainbow trout (*Oncorhynchus mykiss*) system, (ii) to investigate the trade-offs among ecophysiological parameters related to metals bioaccumulation, subcellular partitioning, and susceptibility in farmed fish of tilapia and rainbow trout, and (iii) to provide a probabilistic risk approach to assess susceptibility risks for farmed fish exposed to metals stressors.

A 28-day pulsed Cu exposure experiment was conducted to provide the information on the subcellular partitioning of Cu in tilapia. The Cu bioaccumulation and the distributions of Cu in metabolically active (MAP) and detoxified pools (MDP) can be obtained from the pulsed Cu exposure experiment. The published data of rainbow trout exposed to waterborne and dietborne Cd were adopted to reanalyze and estimate susceptibility and detoxified capacity. This study estimated bioavailability, bioaccumulation, and internal damage of tilapia and rainbow trout in response to pulsed Cu and Cd, respectively. Toxicokinetic parameters of uptake rate (k_1), elimination rate (k_2), and detoxification rate constants (k_d) were derived for tissues in tilapia (gill and muscle) and rainbow trout (gill, liver, and gut). The damage assessment model (DAM) was used to fit to data of time-varying percentage of metal

in MAP to estimate killing rate constant (k_k), recovery rate constant (k_r), and susceptibility. A physiological-based TK model was used to predict tissue burdens for rainbow trout exposed to environmentally relevant Cd concentrations and tissue-specific susceptibility risks can be estimated. A probabilistic risk assessment model was presented to assess the metal exposure risks for tilapia and rainbow trout.

The experimental results indicated that the percentage of Cu in MAP increased with time from 23% to 57% for gill, whereas for muscle, the percentage of Cu in MAP slightly decreased with time from 35% to 28% for tilapia in response to pulsed Cu exposure. Results showed that toxicokinetic parameters of k_1s, k_2s, and k_ds were 8.38 and 0.408 mL g^{-1} d^{-1}, 0.244 and 0.031 d^{-1}, and 0.178 and 0.033 d^{-1}, respectively, for gill and muscle of tilapia exposed to pulsed waterborne Cu. Results showed that k_2, k_d, and k_r ranged from 0.32 – 0.46 d^{-1}, 0.45 – 1.72 d^{-1}, and 1.08 – 1.45 d^{-1}, respectively, for gill of rainbow trout exposed to waterborne Cd (5 – 50 μg L^{-1}), whereas 0.07 – 0.19 d^{-1}, 2.24 – 87.75 d^{-1}, and 1.02×10^{-6} – 1.37×10^{-2} d^{-1}, respectively, for gut exposed to dietborne Cd (0.6 – 30.3 μg g^{-1}). This study implicated no significant susceptibility risk for tilapia exposed to waterborne Cu concentrations. The probability that 50% or more of the susceptibility risk in response to Cu exposure for tilapia was only 33.2% Cu in MAP. For rainbow trout exposed to Cd, a most likely probability of % Cd in MAP of gill and liver exceeding 47 – 49%. In contrast with gill and liver, gut had a relative lower Cd susceptibility risk (15 – 17% Cd in MAP). The trade-offs between elimination and detoxification in rainbow trout exposed to Cd, Cu, and zinc (Zn) based on recently published data were also examined. Results indicated that the relationships between k_2 and k_d were negative for rainbow trout. However, the relationships between k_d and % metal in MDP were found to be positive. Results also indicated that rainbow trout had the higher accumulation (~ 60 – 90 %) in MAP when exposed to essential metals of Cu and Zn and had only 10 – 50 % accumulation in

response to non-essential metal of Cd.

This study found that metal accumulations of tissues varied with the patterns of exposure metal concentration in farmed fish. An integrated model to assess the susceptibility for farmed fish exposed to metal stressors was provided in this study. The trade-offs between elimination and detoxification were also quantified for providing the valuable insights into the ecotoxicology of farmed species. In conclusion, this study used exposed laboratory data of Cu and Cd to investigate the bioaccumulation, bioavailability, and metal distribution of subcellular partitioning for understanding of the susceptibility risks in framed fish of tilapia and rainbow trout. Hence, the probabilistic risk assessment framework linking with the proposed integrated ecotoxicological model can provide an advice for helping government based bioassessment and biomonitoring programs to protect the farmed fish from metal exposures.

Keywords**:** Rainbow troutTilapia; Cadmium; Copper; Subcellular partitioning; Susceptibility; Toxicokinetics/Toxicodynamics; Detoxification; Risk assessment

中文摘要

近年，次細胞金屬分配已被用以評估金屬對水域生物之毒性。現實中，環境金屬濃度由於隨地而異之水化學條件而趨向於擾動及脈衝型態。但在生態毒理領域中，甚少有利用次細胞分配研析水域生物暴露於脈衝金屬之研究。因此，本研究之目的為 (i) 連結次細胞分配與毒理動力/毒理動態(TK/TD)模式發展整合毒理模式並輔以脈衝銅-吳郭魚(*Oreochromis mossambicus*)與鎘-虹鱒(*Oncorhynchus mykiss*)系統之實驗數據，(ii)了解養殖魚種吳郭魚與虹鱒之金屬生物累積、次細胞分配、及易感性相關生態生理參數間之平衡取捨關係及(iii)提供一機率風險方法評估養殖魚種暴露於金屬緊迫之易感性風險。

本研究建構為期 28 天之吳郭魚暴露於脈衝銅實驗以提供銅次細胞分配資訊。吳郭魚生物累積與代謝活化庫(MAP)和代謝解毒庫(MDP)之銅分布可藉由脈衝銅暴露實驗得知。本研究亦重新分析已發表虹鱒暴露於含鎘水體與食物數據以評估其易感性與解毒能力。並分別推估吳郭魚於脈衝銅及虹鱒於鎘之生物可獲取率、生物累積及內部損害反應。本研究推估吳郭魚(魚鰓與肌肉)與虹鱒(魚鰓、肝及與腸道)其組織之毒理動力參數吸收(k_1)、排除(k_2)及解毒(k_d)速率常數。藉由損害評估模式(DAM)擬合隨時變之金屬累積於代謝活化庫百分比，以推估殺害速率常數(k_k)、復原速率常數(k_r)及易感性。利用生理為基礎之毒理動力模式推估虹鱒暴露於環境相關鎘濃度下組織鎘濃度，並進一步推估不同組織之易感性風險。而機率風險模式可用以評估吳郭魚與虹鱒之金屬暴露風險。

由吳郭魚暴露於脈衝銅之反應，實驗結果顯示銅累積於魚鰓代謝活化庫所占比例隨時間從 23%增加至 57%，而銅累積於肌肉代謝活化庫比例則隨時間從 35%略為下降至 28%。結果顯示當吳郭魚暴露於脈衝水體銅，魚鰓與肌肉毒理動力參數之 k_1s、k_2s 及 k_ds 數值分別為 8.38 和 0.408 mL g^{-1} d^{-1}、0.244 和 0.031 d^{-1} 及 0.178

和 0.033 d^{-1}。結果顯示當虹鱒暴露於含鎘水體(5 – 50 μg L^{-1})，魚鰓之 k_2、k_d 及 k_r 分別為 0.32 – 0.46、0.45 – 1.72 及 1.08 – 1.45 d^{-1}，而當虹鱒暴露於含鎘食物(0.6 – 30.3 μg L^{-1})，腸道之 k_2、k_d 及 k_r 則分別為 0.07 – 0.19、2.24 – 87.75 及 1.02×10^{-6} – 1.37×10^{-2} d^{-1}。本研究發現吳郭魚暴露於水體銅濃度下無顯著之易感性風險。當吳郭魚暴露於水體銅濃度，其大於等於 50%機率之易感性風險僅有 33.2%銅累積於代謝活化庫。針對虹鱒暴露於鎘之結果，鎘累積於魚鰓及肝之代謝活化庫(MAP)所占百分比之最大機率為超過 47 – 49%銅累積於代謝活化庫。與魚鰓及肝相較，腸道有一相對較低之鎘易感性風險(15 – 17% Cd in MAP)。本研究亦利用虹鱒暴露於鎘、銅及鋅之已發表實驗數據探討金屬排除與解毒間之平衡取捨關係。結果顯示虹鱒 k_2 與 k_d 間之關係為負相關。然而，觀察虹鱒解毒速率常數與金屬在代謝活化庫之比例關係則發現為正相關。研究結果亦指出當虹鱒暴露於必需元素銅與鋅，代謝活化庫有較高之累積金屬(約 60 – 90%)，而僅有 10 – 50%非必需金屬鎘累積於代謝活化庫。

本研究顯示養殖魚種其組織之金屬累積隨暴露金屬濃度改變。本研究提供一整合模型以評估養殖魚類暴露於金屬緊迫之易感性。本文量化排除與解毒之取捨平衡關係以提供養殖物種有用之生態毒理學觀點。因此，本論文利用銅與鎘暴露實驗數據研究生物累積、生物可獲取率及次細胞分配之金屬分佈以了解養殖魚種吳郭魚及虹鱒之易感性風險。因此，機率風險評估架構連結本研究提出整合性生態毒理模式可提供參考建議以協助政府生物評估與生物監測計畫以保護養殖魚類減少於金屬暴露。

關鍵字：虹鱒；吳郭魚；鎘；銅；次細胞分配；易感性；毒理動力/毒理動態；解毒；風險評估