# 行政院國家科學委員會補助專題研究計畫成果報告

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※　豐年蝦於高濃度重金屬與有機氯污染物之吸附與耐受　※
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計畫編號：NSC89－2313－B－041-017

執行期間：89年8月1日至90年7月31日

計畫主持人：王瑞顯

共同主持人：

計畫參與人員：

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執行單位：嘉南藥理科技大學保健營養系

中　華　民　國　90年10月29　日

# 行政院國家科學委員會專題研究計畫成果報告

# 豐年蝦於高濃度重金屬與有機氯污染物之吸附與耐受

## Uptake and tolerance of brine shrimp (*Artemia* sp.) to high concentrations of heavy metals and organochlorine pollutants

計畫編號：NSC 89-2313-B-041-017

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

主持人: 王瑞顯　　嘉南藥理科技大學 保健營養系

**中文摘要**

豐年蝦經由本研究發現於24小時內可顯著地從1.0 ppm至10 ppm濃度吸附顯著之金屬數量數種，豐年蝦體可於24小時之暴露下各別蓄積高至18.1, 7.8, 51.1及 6.3 ppm（濕重基準）之鉛、鎘、銅、鎳金屬濃度；豐年蝦亦可於1.0 ppm污染濃度24小時吸附最高之DDT與多氯聯苯有機氯污染物，於豐年蝦所計算出之生物濃縮係數（BCF）值發現與配製金屬與有機氯污染物之濃度成反比；較高污染組別濃度亦顯示造成豐年蝦較低之存活數目。

關鍵詞：銅、鎘、鎳、鉛、多氯聯苯同屬物、DDT、豐年蝦　吸附、蓄積。

**ABSTRACT**

Within 24 h, *Artemia* were found to apparently uptake significant amounts of various metals from solutions containing concentrations ranging from 1.0 ppm to 10 ppm. After 24-h exposure in 10 ppm of Pb, Cd, Cu and Ni solutions, *Artemia* could accumulate as high as 18.1, 7.8, 51.1 and 6.3 ppm levels in the body (wet wt. basis), respectively. The uptake of DDT and 2,2',4,4'-PCB from aqueous solutions by *Artemia* was also apparent with highest concentrations at prepared 1.0 ppm solution during a 24-h of contamination. BCF values in *Artemia* were found to be in contrast to the prepared metal and organochlorine concentrations. The decreasing survival of the *Artemia* was observed with increasing concentrations in each solution.

**Keywords:** Cu, Cd, Ni, Pb, 2,2',4,4'-PCB, *Artemia salina*, uptake, accumulation, survival.

**INTRODUCTION**

Heavy metals, such as Cu, Cd, Zn, Pb…etc., have been widely released into environments through point or non-point locations and caused various levels of contamination in rivers, lakes and oceans. These heavy metals were treated as the most important pollutants of concern in Taiwan. Once the pollutants adsorbed or absorbed by biota their concentrations may be increased in other higher trophic levels of aquatic biota due to the food chain transfer. This may also result the toxicity in aquatic biota and endanger the food health and safety in human consumers. Organochlorine pollutants, such as PCB, have been also widely used past in industry and agriculture. Due to its highly stable and non-biodegraded, different degrees of residues have been determined from many areas of the ecosystem, such as rivers, lakes and oceans, and were treated as pollutants of concern. These compounds can be adsorbed or absorbed by biota because of the hydrophobic characteristics and accumulated easily through food chain resulting toxicity in aquatic biota.

In aquatic ecosystems, the basic food supplier --- plankton, plays its important role in adsorption with heavy metals and organochlorine pollutants and transfers these compounds to upper levels of pelagic predators through food chain. Once the widely used food supplier in aquaculture, such as live *Artemia* larvae, adsorbed with heavy metals or organochlorines, it will be most possible for this small size of zooplanktonic diets to uptake and transport the pollutants to higher levels of predators, such as aquatic fish larvae. Studies have shown that *Artemia* larvae are able to tolerate and resist various polluted environments. Relatively high uptake levels were also found in this zooplankton species when exposed to various kinds of pollutants. However, the information for the uptake of heavy metals and organochlorine pollutants under relatively high concentrations through aqueous partitioning in this species is scarce. Therefore, the purpose of this proposal is to modify the aquatic ecosystem depending on different high concentrations of heavy metals and organochlorine pollutants within time interval stages in order to investigate the uptake levels of these pollutans to *Artemia*. Focus is also taken on the various exposure stages, salinity, biomass and high pollutant concentrations that could cause different effects for *Artemia* on uptake concentrations and tolerance, such as LC50 and survival numbers.

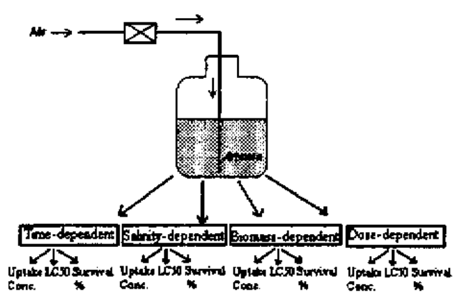
**MATERIALS AND METHODS**

Standard *p,p'*-DDT (1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane and , 2,2',4,4'-

tetrachlorobiphenyl (PCB, purity>99.0%, Ultra Scientific, USA), were weighed and dissolved in a series of dilutions with acetone to achieve 100 ng/mL of stock solution. Standard stock solutions of Pb, Cd, Ni and Cu with 1000 μg $mL^{-1}$ were purchased from Sigma Co. Freshly filtered seawater (0.45 μm) of 30‰ salinity was obtained from local aquaculture farm. *Artemia* cysts (Great Salt Lake Brand) purchased from local fishery dealer were hatched in a separatory glass funnel containing filtered seawater (30‰ of salinity) under strong aeration for 24h (20 ± 2°C). After 24-h hatching, *Artemia* nauplii were harvested under a 106 μm sieve and transferred to the designed flasks containing solutions with prepared metal or organochlorine concentrations for another 24 h in order for accumulation purpose as shown on the following Figure.

Samples of freeze-dried *Artemia* nauplii were collected and blended in a Polytron tissue homogenizer with hexane and acetone to obtain the lipid extracts. The extracts from the solvents were cleaned-up by using 110°C overnight activated Florisil® (100/120 mesh, Supelco, Inc.) and eluted with hexane. The elutes were concentrated and dissolved in hexane for gas chromatography analysis. The amounts of different organochlorine pollutants were analyzed by a Perkin-Elmer Auto System GC gas chromatograph (GC) equipped with a $^{63}Ni$ electron capture detector (ECD). organochlorines were quantitated by comparing total areas of peaks with that of standards. The recovery after extraction and clean-up procedures was 85-90%. The detection limit on GC was 1.0 ng/g in this study.

For the metal analysis, the collected samples were digested with 1 ml concentrated $HNO_3$ under a Questron Q-1000 microwave oven. The clear digestive solutions were dissolved in 5 ml of 1 N HCl for the metal determinations by using a GBC-908 flame atomic absorption spectrophotometer (AA). Each group was set up in duplicate to obtain average values.




## RESULTS AND DISCUSSION

Within 24 h, *Artemia* were found to apparently uptake significant amounts of various metals from solutions containing concentrations ranging from 1.0 ppm to 10 ppm. After 24-h exposure in 10 ppm of Pb, Cd, Cu and Ni solutions, *Artemia* could accumulate as high as 18.1, 7.8, 51.1 and 6.3 ppm levels in the body (wet wt. basis), respectively. Each metal accumulation level in *Artemia* was in consistency with the concentrations of the solutions prepared. This may explain that the uptake of metals by this aquatic biota is significant dose-related. Meanwhile, different degrees for the uptake of these metals in *Artemia* due to different metal conditions. As shown in Table 1, accumulation concentrations in *Artemia* from 27.6 ppm to 51.1 ppm are found to be at the prepared Cu solutions ranging from 1.0 ppm to 10 ppm. Whereas, Ni was determined to be the lowest accumulation levels in *Artemia* from 0.9 ppm to 6.3 ppm at the prepared Cu solutions ranging from 1.0 ppm to 10 ppm. The results suggest that the magnitudes of metal accumulation on aquatic zooplanktons are correspondingly correlated to the extents of aqueous concentrations and the characteristics of metals. The values of BCF (Biological Concentration Factor = biota conc./solution conc.) may also explain the differences for the uptake magnitudes of these metals in *Artemia*. Highest values of BCF were found to be at Cu with that of the same other three metal concentrations (Table. 1). This may show that Cu is in higher tendency in the degrees for the uptake in *Artemia* compared to the other three metals. However, lower BCF values were found to be at the higher concentrations in each metal solution. This may contribute to the resulting survival numbers of the *Artemia* exposure to various metal concentrations. According to Figure 1, a decreasing survival number of *Artemia* was consistently found to be from lower concentrations to higher concentrations. Within a 24-hr exposure, the toxicity of the metals may cause the lower survivals of the biota resulting lower values of BCFs.

Levels of DDT and 2,2',4,4'-PCB accumulated in *Artemia* after exposure to four different aqueous concentrations are shown in Table 1. Within 24 h, *Artemia* were found to be able to accumulate significant amounts of these two compounds containing concentrations ranging from 0.1 ppm to 1.0 ppm. After 24-h exposure in 1.0 ppm of DDT and 2,2',4,4'-PCB, *Artemia* could accumulate as high as 25.7 ppm and 4.7 ppm in the body (wet wt. basis), respectively. Based on the study by Wang and Simpson (1996), levels of *p,p'*-DDT in *Artemia* nauplii were shown to be 116.5 ppb and 247.9 ppb (wet wt basis) in 0.5 ng $mL^{-1}$ (ppb) and 1.0 ng $mL^{-1}$ (ppb) treatment groups, respectively. In a similar study, McLean *et al.* (1987) demonstrated that experimental *Artemia* (in dry wt basis) were found to have 10.8 ng $g^{-1}$ and 92.7 ng $g^{-1}$ of *cis*-chlordane (a chlorinated insecticide) after 24 h contamination from the 0.1 ng $mL^{-1}$ and 1.0 ng $mL^{-1}$ treatment groups, respectively. The results in the present study is in agreement with those reports indicating that the organochlorine values in *Artemia* follow the same order of magnitude in the aqueous concentrations.

According to the report of Wang *et al.* , adsorption of high PCB residues from water in the outlayer of the nauplii could therefore cause high PCB residues in this small size and large biomass of zooplankton. Although, Franke *et al.* reported that effective body burden concentrations in target tissues along with co-occurring adverse effects, such as mortality, are of much higher significance on bioaccumulation process for hydrophobic pesticides. However, the capability of *Artemia* to accumulate pollutants in the body may also depend on the numbers of the zooplankton present in aqueous environments. In the present study, *Artemia* could uptake 9.5, 14.6, 20.2 and 25.7 ppm of DDT from 0.1, 0.2, 0.5 and 1.0 ppm concentrations, respectively. Comparing to DDT, *Artemia* showed lower degrees of uptake from 2,2',4,4'-PCB as shown in Table 2. Other than the characteristics of the two compounds, survival numbers of *Artemia* may cause different magnitudes of the uptake conditions. Based on the results from Figure 2, 2,2',4,4'-PCB could result a lower survival number of *Artemia* compared to DDT. This may allow fewer live *Artemia* that are capable of uptake 2,2',4,4'-PCB which results a lower accumulation concentration on the aquatic biota.

Table 1. Accumulation concentrations (ppm, wet wt.) and bioconcentration factors of *Artemia* after exposure to various metals in 24 hr.

| Exposure Con. | 1.0 ppm | 2.0 ppm | 5.0 ppm | 10.0 ppm |
|---|---|---|---|---|
| Pb | 7.3 | 12.5 | 16.2 | 18.1 |
| | (7.3) | (6.25) | (3.24) | (1.81) |
| Cd | 1.4 | 2.6 | 4.3 | 7.8 |
| | (1.4) | (1.3) | (0.86) | (0.78) |
| Cu | 27.6 | 38.2 | 43.7 | 51.1 |
| | (27.6) | (19.1) | (8.74) | (5.11) |
| Ni | 0.9 | 1.4 | 3.7 | 6.3 |
| | (0.9) | (0.7) | (0.74) | (0.63) |

Table 2. Accumulation concentrations (ppm, wet wt.) and bioconcentration factors of *Artemia* (ppm, wet wt.) after exposure to DDT and 2,2',4,4'-PCB in 24 hr.

| Exposure Con. | 0.1 ppm | 0.2 ppm | 0.5 ppm | 1.0 ppm |
|---|---|---|---|---|
| DDT | 9.5 | 14.6 | 20.2 | 25.7 |
| | (95) | (73) | (40.4) | (25.7) |
| 2,2',4,4'-PCB | 0.9 | 1.6 | 3.9 | 4.7 |
| | (9.0) | (8) | (7.8) | (4.7) |

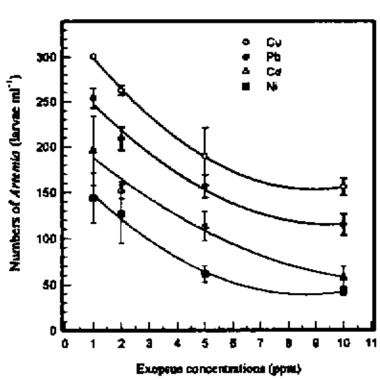



Figure 1. Numbers of *Artemia* surviving after 24-h exposure to various metal concentrations

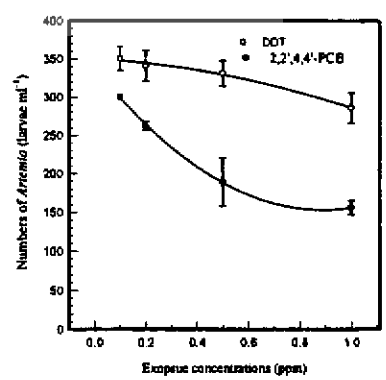



Figure 2. Numbers of *Artemia* surviving after 24-h exposure to various DDT & 2,2',4,4'-PCB concentrations.

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