1-A

Arsenic has been proved to be one of the cancer-causing materials to human body, human body takes Arsenic through fishes, other seafood and drinking water. According to a report by the Fisheries Agency, Council of Agriculture, Executive Yuan, targeting at the survey of aquaculture water in Taiwan area, it says that Chiayi county and Tainan county consumes underground water of 0.16 and 0.09 billion m^3, respectively, the above two counties occupy a percentage of 17% and 10% respectively, of the total nationwide underground water consumption, but high content of Arsenic is in the underground water of that area, use such water quality as aquaculture water could expose the fishes under the threats of Arsenic pollution, therefore, it is necessary to use biomarker to study the dosage of Arsenic in all the organs of fish body. This study, in addition to studying the relationship between the Arsenic concentration in the aquaculture water and milkfish, it also tries to build and develop Physiologically-Based Pharmacokinetic (PBPK) model, through the biological parameter and physiological parameter in the fish body as well as the transport among tissues and organs and the concept of mass balance, this study combines and predicts the transport and distribution flow of Arsenic in the fish body, in the mean time, through the extrapolated literature, this study develops an ecology risk evaluation tool suitable for the Taiwan's water field environments.

I-1

Many researches have verified that the pollutants in the water field environment will not only cause bad effects to the water lives but also will indirectly hurt the human races due to the food chain connection (1,2). Fish and shellfish spend all the time in water, therefore, once the water field environment is contaminated, the contaminant usually accumulates in biological body due to bioconcetration and biomagnification, once human eats these species, the contaminants will then be digested into human body and cause bad effects to human bodies (2,3). When the pollutant has high concentration, it could cause acute toxic disease; on the contrary, at lower pollutant concentration, it can affect human cell, tissue and organ through biological accumulation, or even cause organ failure or caner (4), for example: in 1960, the minam-ata disease occurred in the seashore

resident of Japan is caused by eating the fish and shellfish contaminated by methyl mercury, and in 1986, the green oyster contaminated by copper in Taiwan are some of the famous heavy metal contamination accidents.

I-2

Fish and shellfish is one of the important protein sources for human beings, once it is contaminated, it will cause great danger to human body (5).Taiwan is an internationally recognized fish and shellfish aquaculture nation, it has a capability of 3.3 millions tons in inner land aquaculture, it has an annual production value of 27,300 NT dollars; especially in the southwest seashore of Taiwan, aquaculture ponds and shallow seashore aquaculture even become the local feature (6). According to a report of the current status survey on aquaculture water performed by Agricultural Engineering Research Center consigned by the Fishery Agency, Council of Agriculture, Legislative Yuan, in 1997, the underground water usage for Taiwan's aquaculture industry is about 0.96 billion m^3 (7), among them, Chiayi county consumes about 0.16 billion and Tainan county consumes about 0.09 billion m^3, these two counties occupy a percentage of 17% and 10% of the total consumption nationwide.

I-3

But the underground water in that area contains high concentration of Arsenic, such aquaculture water quality will let the fish and shellfish expose under the threat of Arsenic contamination.

I-4

In many of the aquaculture fishes, milkfish is a very important aquaculture product.

I-5

Since the water consumption is huge ($3.8\times104 \sim 4.9\times104$ m^3) for milkfish aquaculture industry, the contaminant could possibly contaminate the aquaculture pond (8), unfortunately, the potential danger caused by Arsenic is not well investigated.

I-6

This project, based on a risk evaluation report submitted in 1983 by the US National Academies, is to build a risk evaluation tool suitable for the water field ecological environment in Taiwan；the so-called ”risk management ” comprises of four parts such as : danger identification, dosage-reaction evaluation, exposure evaluation and risk characteristics, etc.; among them the PBPK model is the most suitable evaluation tool in the dosage-reaction in the risk evaluation (11). Therefore, this project targeting at milkfish aquaculture by underground water in the black foot area, in addition to performing rural background information collection and analysis, this project also performs exposure test in the lab；it investigates the correlation between Arsenic concentration in the aquaculture pond water and the milkfish, for the first time this study tries to use PBPK model to predict the distribution flow of Arsenic in the milkfish body, the main purpose is to develop a risk evaluation tool suitable fort he water field ecological environment in Taiwan, the simulated data can be used as an alarming system for the aquaculture water field environment or as an environmental monitoring reference by the government.

L-2-1 The Arsenic distribution in water field environment and the body of species living in the water

Genera water body contains very few Arsenic, but there are high concentrations in the oil field and hot spring (12), therefore, Arsenic will penetrate into the earth surface water body and underground water body through hot spring water (13). The chemical form of Arsenic in the water field environment is very complicated, Arsenic has four different stable valences (+5；+3；-3 and 0) under different oxidation and reduction status, Arsenic usually appears in the form of H_3AsO_4, H_3AsO_3, monoethylarsonic acid (MMA), and dimethylarsonic acid (DMA)(14, 12).

A study performed by Smedley et al. has pointed out that Arsenic appears in the form of valence +5 in the most of the water bodies which contact the air (such as river, lake and sea water, etc.); underground water is closed water body, the Arsenic there is mostly in reduced state of valence +3. Chen et al. has pointed out in a study of the Arsenic form in the black foot area in Taiwan that the soluble Arsenic are all inorganic Arsenic, among them the three and five valence Arsenic

has the chemical form of H_3AsO_3 and $HAsO_3$, respectively, both has a ratio of 2.6.Among them, the insoluble Arsenic occupies about 3 % of the total Arsenic, and the organic Arsenic content is below the detection limit.

There are Arsenic distributions in the body of many species, it depends mostly on the water body as the transfer path (16), species living in the water might have more chances contacting Arsenic. The body of species living in the water might have inorganic Arsenic and organic Arsenic wherein inorganic Arsenic might have less content (normally about 0.9-2.5 μg g^{-1} dry wt) but organic Arsenic might have higher content, the latter usually occupies above 65% of the total Arsenic (17). The Arsenic compound that has been detected in the species living in the water include: Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), etc. (17, 18).

Generally speaking, ocean species has much higher As concentration than the inner land species or non-salty water species, some ocean species have higher collecting power on Arsenic, the major reason might be because that algae can digest arsenosugars, AC and AB, and these Arsenic compound can be easily absorbed by the intestine and stomach of the algae-eating fishes (19).

L-2-2The toxicology effect of Arsenic and its danger

The way Arsenic poisoning human body is very complicated, its toxicity can be divided into chronic and acute nature according to the length of exposure time. Acute Arsenic poisoning has an initial reaction of dry lips and throat, the serious case might have language obstacle, face swelling, difficulty in breathing,heart throb, uncomfortable intestine and stomach, pained abdomen, diarrhea, bloody urine, vomiting,and nervous system retardation, etc. It could also cause bad appetite, icterus, erythema and nerve；it can even cause fast dehydration and finally a death (20).Long time exposure under low concentration of Arsenic could cause chronic Arsenic poisoning, symptoms include: hair loss, loose nail, salt rheum, expansion of subdermal blood capillary cluster expansion, skin erythema, over color precipitation on the hand and foot, skin keratin, serious epithalaxia and dropping dermatitis, etc., the most serious case could cause cancer (20).

According to a report from Johns Hopkins University targeting at the cancer-causing of Arsenic, when human cell exposes to low concentration of three

valence Arsenic environment, the cell enzyme activity will be reduced obviously which in turn leads to the death of cell for curing chromosome, and the mitochondrial after healthy cell division will constrict rapidly to form cancer cell, this proves that three valence Arsenic has gene toxicity, it will destroy the DNA in the cell of human body DNA (21).

According to Agency of Toxic Substances and Disease Registry, ATSDR, USA, it summarizes related research information related to Arsenic and cancer and points out that Arsenic will cause damage to the heart vein, nerve, immunity, incretion and genesial system, etc., in the mean time, it will also cause lung cancer, skin cancer, bladder cancer, liver cancer, kidney cancer, prostate cancer and nose and throat cancer. In addition, Arsenic can also enter human body through drinking water path, it not only cause the health danger, but also cause the diseases on vein, skin and other tissue and system, it even cause cancer (21).

In 1961-1985, an accident of well drinking water contaminated by Arsenic in the southwest seashore area of Taiwan, about 140,000 residents expose in the Arsenic-contaminated environment for a long time, which cause the explosion of black foot disease (22). According to a related study, the water in the local wells contains Arsenic concentration of 0.01~1.82 mg L^{-1}, and most of the water in the wells has Arsenic concentration of 0.4~0.6 mg L^{-1}, Arsenic exists mostly in the form of three valence inorganic Arsenic, its ratio to five valence Arsenic is about 2.6 (23).According to a survey performed by Union Chemical Laboratories / ITRI consigned by Environmental Protection Administration(1998), Executive Yuan, ROC, in the 160 wells detected from four areas such as : Yichu, Putai, Peimen and Hsuehchia, 66% of them contains Arsenic exceeding the water quality standard of tap water,some are even 40 times the standard value.Among them, Peimen Hsiang has nonqualified well percentage of over 90%. Yichu village and Putai township and Hsuehchia township have nonqualified percentage of 62%, 60%, 52%, respectively. The black foot disease gets improved until the government enhances the tap water usage popularity and prohibits the usage of well water as the drinking water.

According to the data published in 2001 by WHO, under-developed countries put no strict limits on the Arsenic concentration of the drinking water, therefore,

the Arsenic concentration in the drinking water is usually higher than that of developed countries, for example: Argentina, Brazil, Chili, Hungary, Mexico, Thailand, Vietnam and the east of India all have Arsenic pollution problem(12).

L-2-3 The taking path of Arsenic and its safety standard

In 1993, US Food and Drug Administration (US FDA) has a research which pointed out that in the Arsenic taken into human body, 90% of them is from aquaculture foods, the other 10% from the other foods, Tam (24) et al. found that fish and other sea foods are the major source of Arsenic for human body. In the beginning of 20 century, some scholars found that aquaculture species has high concentration of Arsenic in the body, and arsenobetatine compound is found by Edmonds et al. in 1977 in analyzing the chemical compositions of lobster (25), later on, more researches found more Arsenic compound in other aquaculture species.Therefore, edible aquaculture food is the major path of taking Arsenic for human body (26). Among them, shell fish animals such as fish, scallop,oyster,mussel, shrimp and crab, etc., has higher concentration of Arsenic (24, 27).

In the water part, people usually take it through drinking water, the daily taking quantity is about＜10 μg L^{-1} (28). According to a report from WHO in 2002, many European and Taiwan countries nowadays have dropped standard of Arsenic concentration in the drinking water from 0.05 mg L^{-1} to WHO 0.01 mg L^{-1} suggested by WHO, and US Congress has cited Safe Drinking Water Act (SDWA) and ask the US Environmental Protection Agency to modify the Arsenic concentration standard in the drinking water, it was dropped from the original 0.05 mg L^{-1} to 0.01 mg L^{-1}, it takes effective from Feb. 2002. Currently, there are still countries such as Bangladesh, China and India, etc. still remain the Arsenic concentration standard in the drinking water as 0.05 mg L^{-1} (29). In addition to drinking water, the Environmental Protection Administration of our country has made the standard for Arsenic concentration in aquaculture water as 50 μg L^{-1}. However, there is still no good monitoring of the water quality on the aquaculture pond from the government, use such water quality for aquaculture, people might take over dose of Arsenic in

the eating process.

L-2-4

The contaminants in the water field usually cause bad effects on the creatures in the environment, or even affect human indirectly (1).Some species have the capability to accumulate the chemical substances from the water field, therefore, the chemical substance concentration in the body of the species is higher than that in the water field, this is the so-called biological accumulation. Biological accumulation is a phenomenon that the species living in the water field absorbs and keeps certain chemical substance through food or nearby water body, it can be divided into bioconcentration and biomagnification according to different absorbing paths. Bioconcentration is the entrance of chemical substance in the water field into the body of species through active diffusion, this process is the result of chemical competition between the absorption and release in the body of species ; biomagnification is the selective substance concentration tendency which depends on the nutrition level or biological level, therefore, the species on the top layer of the food chain will accumulate high substance concentration (30), therefore, they are commonly used as the index of contamination level, or a reference for environmental monitoring.

Some literature mention the biological accumulation process of water contaminants, biological concentration factor (BCF) can be used to describe the accumulation effect caused by species (31). The so-called biological concentration factor means" under stable condition, the ratio of the contaminant concentration in the body of species to that in the water field", it can be used to predict the accumulation power of the body of the species on the contaminant (31). However, bioconcentration is the competition result between absorption and release in the body of the species, therefore, the absorption and release rate constant of the contaminant in the body of the species can be used to calculate the unit of the theoretical value K_1 of the bioconcentration to be mg g^{-1} day^{-1}; the two constants are represented by K_1 and K_2 respectively, wherein the unit of K_2 is day^{-1}. In order to get the two parameter values, Lin (31) et al. uses "first order one-compartmental model " in the literature to perform the research, the result shows that

bioconcentration is the is the major source of the biological accumulation capability.

L-2-5

In recent years, many researches have used the PBPK model to describe the physiological mechanisms and processes such as: absorption, distribution, metabolism and release, etc. of the chemical substance in the body of the species (32). Its major feature is that it uses the real physiological (tissue volume, blood quantity, breathing quantity), biological (the maximum metabolism capability constant, etc.) parameters of the body of the species and exposure environment data (time, concentration) as the operational parameters for the model (11), it also divides blocks that possess physiological and biological characteristics, it brings the contaminants exposed in the environment to the whole body through the flow of the blood, it then predicts time-concentration relationship of distribution, metabolism, or accumulation, etc., in each tissue block (33, 34). Besides, PBPK models have the following advantages: (1) It can be used to predict the distribution flow of absorption and metabolism for the exposure of animal body to chemical substances of different doses; (2) Describing the transport and distribution of chemical substance according to all kinds of exposure paths and time; (3) It can be used to evaluate the difference when different species are exposed to different doses of chemical substance, it can thus apply and simulate the data of animal test and use them as the risk evaluation tool when species/human is exposed to chemical substance in different environments (35).

M-3-0

The study methods and procedures of this thesis can be divided into two major steps. The first step can be divided into two parts, the first is the ”rural survey” part, which is to understand the actual water and fish body Arsenic concentration and interactive correlation in the aquaculture pond；another is ”lab simulation” part, which is to understand the absorption/release rate in fish body under different Arsenic concentration in order to deduce bioconcentration factor and

absorption/release rate constant; the second step uses PBPK model to deduce Arsenic's damage on the water field ecological environment and species under the species block and time variance conditions, we hope to develop a risk evaluation tool which is suitable for water field environmental ecology, our study methods are as in he followings:

Step 1:

(1) Rural survey

（1） Perform long term monitoring on the Arsenic concentration of water and fish body in the aquaculture ponds of the four areas of Chianan plains of southwest Taiwan, this is to build the background data for aquaculture environment.

（2） Take samples from aquaculture ponds every month and store the water sample in 500 ml narrow opening bottle, HNO_3 is used to fix; fish body is cleaned by the pond water and put into sealing bag, freeze it and bring back to the lab.

（3） Fish body is frozen and dried, weighs the dried weight respectively and stores them.

(2) Lab simulation

（1） Install aquarium first (include the filtering system and thermostat), add aquaculture water and experimental fishes which are not contaminated by Arsenic, use them to simulate the environmental conditions in the aquaculture pond. During the aquaculture period, the water temperature is controlled at 25 ℃, non-contaminated bait is used to feed the fishes, the period is set to be about one week.

（2） Perform initial toxicity experiment, try to understand the endurance limit of fish body to Arsenic concentration according to half lethal concentration.

（3） Select the Arsenic concentration that can be endured by the species to perform the experiment of absorption/release of species body on Arsenic：

A. Experimental group (the Arsenic contained in the fish body, these include the original content of the fish self body and the newly added content accumulated from aquaculture water)

a. Absorption experiment (include aquaculture water and fish)

Install aquarium that meets the above mentioned Conditions, move the fishes into the aquarium , add Arsenic into the aquarium and feed fishes regularly with non-contaminated baits. Perform sampling on 0^{th},1^{st}, 2^{nd},4^{th}, 7^{th} day.

b. Release experiment (include aquaculture water and fish)

Move the fishes into non-contaminated aquarium and feed fishes with non-contaminated baits, perform sampling in the same time period.

B. Blank group (During the experimental period, there is no newly added Arsenic to the species body, the measured content will be used as the calibration value for the above two steps)

Repeat the above steps a, b, but not adding Arsenic.

（4） Freeze and dry the species body and grind them into fine powder, perform Arsenic content analysis along with the fixed water samples.

(3) Arsenic content analysis

Take the above powder and water sample and perform Arsenic concentration analysis by using Atomic Absorption Spectroscopy (AAS).

(4) Calculation of bioconcentration factor and absorption/release

rate constant

Obtain the Arsenic content taken by fish from the water according to the difference value of A and B, then use kinetic theory to calculate absorption/release rate and bioconcentration factor for species body on Arsenic.

Step 2：

（1）Absorption/release kinetic analysis

(1) Use 1st-order one-compartmental model to describe absorption/release kinetic model, it can be expressed by the following equation：

$$\frac{dC_f}{dt} = k_1 C_W - k_2 C_f \qquad (1)$$

wherein k_1 represents absorption rate constant（mg g^{-1} d^{-1}）; k_2 represents release rate constant(d^{-1}); C_w represents the concentration of chemical substance in the water (ng ml^{-1}); C_f represents the concentration of chemical substance in the body of the species (ng g^{-1}). If C_w is constant, equation (1)can be written as:

$$C_f = \frac{k_1}{k_2} C_W - (1 - e^{-k_2 t}) \qquad (2)$$

When it approaches stable status（$t \to \infty$）,equation (2) can be written as:

$$C_f = \frac{k_1}{k_2} C_W \qquad (3)$$

From the definition of BCF:

$$\mathrm{BCF} = \frac{C_f}{C_W} = \frac{k_1}{k_2} \qquad (4)$$

When the species is moved to the clear water,the release constant k_2 can be obtained by the slope of the following equation:

$$\ln C_f(t) = \ln C_f(0) - k_2 t \qquad (5)$$

The ”time-log C_f” diagram obtained from release test can be

used to get temporary k_2. Then the $\frac{C_f}{C_w}$ at maximum concentration measured at the body of the species can be used to get temporary BCF. Then put k_2 and BCF into equation (4), $\mathrm{BCF}=\frac{k_1}{k_2}$, to get a temporary k_1.

(2) Assume C_w in the water body remains constant in each time interval of measurement, then k_1,k_2 and C_w obtained from the above-mentioned equation can be substituted into $\frac{dC_f}{dt}=k_1C_w-k_2C_f$ (equation(1)), then use mathematical software program (6^{th}-order Runge-Kutta method), we can obtain the C_f value starting from the beginning to the end of the measurement.

(3) Next we calculate the difference between this estimated C_f value and real C_f value. Finally we change k_1 and k_2 value, and repeat the above-mentioned steps to minimize the error.

(2) The buildup of PBPK model

The Arsenic-PBPK model built up by this study describes the species in the water field (milkfish)when exposes to Arsenic-contaminated environment by using dynamic linear system, it also investigates the damage on the species caused by the contaminant concentration-time relationship; therefore, this model must first make assumption to simplify the physical and biochemical characteristics of the transport, distribution, metabolism and release for the accumulation of Arsenic in the body of milkfish. In addition, In addition, this model refers to and modifies the PBPK model proposed by Wintermyer (34, 36, 37, 38) et al., it tries to build up the PBPK model of Arsenic in the milkfish body, based on the two basic features of PBPK model, the features are as in the followings: (1) Select a target tissue that can fully represent the structure of the species (gill, blood, heart/kidney, lung, intestine, muscle, skin, etc.); (2) Describe the distribution and release status of contaminant in the water field environment and the body of species through the differential

equation.

(1). The basic assumption of the model

1. The physiological parameter/biochemical parameter in the body of the species are all fixed values in the model, it won't change with time.
2. Gill transport system: Assume gill is a continuously agitating mixer, it is a homogeneously mixing zone for inflow and outflow water body, therefore, the chemical substance and oxygen can be transported into the body of milkfish through diffusion, and the water flow is a homogeneous distribution in the water channel.
3. Blood transport system: Blood flows from i block to j block with a flow rate of Qij, and the transport among blocks is generated by the blood flow, the mutual interactions between two blocks can be neglected, and the flow of blood in the vein is homogeneous and stable.
4. Chemical mass balance: For each block, the input and output quantity of the chemical substance will reach equilibrium, and the quantity of the chemical substance in the block will reach a stable state.

(2)Build up of the model

The buildup of the model is mainly based on PBPK flow chart for the Arsenic in the milkfish body (figure 1), it describes and writes the leading equation for all tissues of the fish body, and the estimations of physiological parameter/biochemical parameter among all the tissues are all represented by mass balance equations concept, the equations of all the tissues and organs of fish body are described as in the followings (please refer to table 1 for the symbols):

R-A--1

(1). Build thorough rural background information, understand the current contamination status of local fish aquaculture.

(2). Predict the accumulation capability of the fish body on the contaminants, based on the test result of the lab, when heavy metal contamination is occurred in the environment. In the mean time, use the theoretical model obtained from the test

(by treating the body of species as contamination index), to estimate the heavy metal concentration in the water field.

(3). Based on the heavy metal content ratio of ”water field/fish body tissue” and “fish body/ fish body tissue”, we can also use directly certain tissue of the fish body as contamination index.

(4). Through the buildup of PBPK model, we can understand the heavy metal content and distribution flow of all the tissues in the fish body, the results can remind customers to reduce eating some special parts.

(5). The data obtained from PBPK model can be used as a reference of monitoring of the aquaculture environment or water field environment for the management unit.

(6). The data obtained from PBPK model can be used in human exposure and risk model, it can be further used to estimate the maximum allowable heavy metal taking quantity of human body, therefore, the potential damage to human body health can be reduced to its minimum.