การประมาณการได้รับโลหะหนักจากการบริโภคอาหารทะเลในประชากรอำเภอเมือง จังหวัดระยอง

นางสาวพิมลวรรณ เกิดเทพ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาอาหารเคมีและโภชนศาสตร์ทางการแพทย์ ภาควิชาอาหารและเภสัชเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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ESTIMATION OF HEAVY METAL INTAKE FROM SEAFOOD CONSUMPTION IN THE POPULATION OF MUANG DISTRICT, RAYONG PROVINCE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Food Chemistry and Medical Nutrition
Department of Food and Pharmaceutical Chemistry
Faculty of Pharmaceutical Sciences
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Thesis Title	ESTIMATION OF HEAVY METAL INTAKE FROM SEAFOOD CONSUMPTION IN THE POPULATION OF MUANG DISTRICT, RAYONG PROVINCE
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Field of Study	Food Chemistry and Medical Nutrition
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พิมลวรรณ เกิดเทพ : การประมาณการได้รับโลหะหนักจากการบริโภคอาหารทะเลในประชากรอำเภอเมือง จังหวัดระยอง. (ESTIMATION OF HEAVY METAL INTAKE FROM SEAFOOD CONSUMPTION IN THE POPULATION OF MUANG DISTRICT, RAYONG PROVINCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.ลินนา ทองยงค์, 92 หน้า.

เนื่องจากปัจจุบันมีการตระหนักถึงสารปนเปื้อนในอาหารมากขึ้น ในการศึกษานี้จึงทำการศึกษาหาปริมาณโลหะหนัก ได้แก่ แคดเมียม, ตะกั่ว, สารหนู และปรอท ที่กลุ่มสมาชิกอาสาสมัครสาธารณสุขอำเภอเมือง จังหวัดระยอง จำนวน 316 คน ได้รับจากการบริโภคอาหารทะเลและประเมินความเสี่ยง โดยทำการวิเคราะห์หาปริมาณโลหะหนักดังกล่าวในตัวอย่างอาหารทะเล 13 ชนิด ที่กลุ่มสมาชิกอาสาสมัครสาธารณสุขนิยมบริโภคมากที่สุด ได้แก่ ปลาทูแขก ปลาลัง ปลาโอ ปลาข้างเหลือง ปลาทรายแดง ปลาจาระเม็ด ปลาอินทรีย์ ปลาเก๋า หอยแครง หอยแมลงภู่ กุ้งแชบ๊วย หมึกหอม และหมึกกล้วย โดยทำการเก็บตัวอย่างอาหารทะเลดังกล่าวจาก 3 ตลาด ในเขตอำเภอเมือง จังหวัดระยอง ระหว่างเดือนมกราคมถึงกุมภาพันธ์ 2551 และทำการวิเคราะห์ด้วย atomic absorption spectrophotometry (AAS) พบว่า กลุ่มหอยสองฝามีปริมาณแคดเมียม (หอยแครง 0.731 ไมโครกรัม/กรัม และหอยแมลงภู่ 0.140 ไมโครกรัม/กรัม) และตะกั่วสูงที่สุด (หอยแครง 0.096 ไมโครกรัม/กรัม และหอยแมลงภู่ 0.084 ไมโครกรัม/กรัม), กลุ่มหมึกมีปริมาณสารหนูสูงที่สุด (หมึกหอม 7.032 ไมโครกรัม/กรัม และหมึกกล้วย 5.807 ไมโครกรัม/กรัม) ในขณะที่ปลามีปริมาณปรอทสูงที่สุด (ปลาอินทรีย์ 0.119 ไมโครกรัม/กรัม และปลาเก๋า 0.269 ไมโครกรัม/กรัม) อย่างไรก็ตาม ปริมาณโลหะหนักทั้ง 4 ชนิด ที่สะสมในอาหารทะเลดังกล่าวยังอยู่ในระดับที่ปลอดภัยต่อผู้บริโภค ซึ่งต่ำกว่าค่ามาตรฐานที่ยอมให้ปนเปื้อนในอาหารได้ โดยกระทรวงสาธารณสุข ประเทศไทย ยกเว้น ปลาข้างเหลือง, ปลาแดง, หอยแมลงภู่, หมึกหอม และหมึกกล้วย ที่มีปริมาณสารหนูทั้งหมดเกินมาตรฐาน การประมาณค่าโลหะหนักแคดเมียม, ตะกั่ว, สารหนู และปรอท ที่เข้าสู่ร่างกายจากการบริโภคอาหารทะเลดังกล่าวเท่ากับ 66.33, 40.83, 317.32 และ 46.16 ไมโครกรัม/คน/สัปดาห์ ตามลำดับ ซึ่งอยู่ในระดับที่ต่ำกว่าค่าที่ร่างกายสามารถรับได้ต่อสัปดาห์โดยไม่เกิดอันตราย จึงสรุปได้ว่า การได้รับโลหะหนักในการศึกษาครั้งนี้ยังอยู่ในระดับที่ปลอดภัยต่อผู้บริโภค แต่อย่างไรก็ตาม ควรคำนึงถึงการได้รับโลหะหนักเหล่านี้จากอาหารชนิดอื่นๆ รวมทั้งจากแหล่งอื่นๆ นอกเหนือจากอาหาร

ภาควิชา..อาหารและเภสัชเคมี.......................... ลายมือชื่อนิสิต พิมลวรรณ เกิดเทพ
สาขาวิชาอาหารเคมีและโภชนศาสตร์ทางการแพทย์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ลินนา ทองยงค์
ปีการศึกษา..2551.. ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.................

4976585333 : MAJOR FOOD CHEMISTRY AND MEDICAL NUTRITION
KEYWORDS : CADMIUM/ LEAD/ ARSENIC/ MERCURY/ PROVISIONAL TOLERABLE WEEKLY INTAKES/ DIETARY INTAKES/ EDIBLE MARINE SPECIES

PIMONWAN KERDTHEP: ESTIMATION OF HEAVY METAL INTAKE FROM SEAFOOD CONSUMPTION IN THE POPULATION OF MUANG DISTRICT, RAYONG PROVINCE. ADVISOR: ASST. PROF. LINNA TONGYONK, D.Sc., 92 pp.

Due to increasing concern about the intake of contaminants in foods, this study was performed to determine the exposure of the 316 primary health care voluntary staff of Muang District, Rayong Province to heavy metal contaminants; cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) in seafood, and to estimate the health risk. The concentrations of these metals were determined in the 13 most consumed marine species, yellow-tail round scad (*Decapterus maruadsi*), indian mackerel (*Rastrelliger kanagurta*), longtail tuna (*Thunnus tonggol*), yellow stripe trevally (*Selaroides leptolepis*), ornate threadfin bream (*Nemipterus hexodon*), black pomfret (*Parastromateus niger*), spanish mackerel (*Scomberomorus commerson*), greasy grouper (*Epinephelus tauvina*), blood cockle (*Arca granulose*), green mussel (*Perna viridis*), banana shrimp (*Penaeus merguiensis*), soft cuttle fish (*Sepioteuthis lessoniana*) and splendid squid (*Loligo duvauceli*). The samples were collected from 3 local markets of Muang District, Rayong Province, between January and February 2008. Analysis of the above contaminants was measured by atomic absorption spectrophotometry (AAS). The bivalves had the highest contents of Cd (0.731 μg/g in blood cockle and 0.140 μg/g in green mussel) and Pb (0.096 μg/g in blood cockle and 0.084 μg/g in green mussel), the cephalopods had the highest content of As (7.032 μg/g in soft cuttlefish and 5.807 μg/g in splendid squid), while the highest Hg content was in fish group (0.119 μg/g in spanish mackerel and 0.269 μg/g in greasy grouper). However, the contamination levels of heavy metals were still lower than contamination standard limited level in food issued by the Ministry of Public Health of Thailand, except the total arsenic contents in soft cuttlefish, splendid squid, yellow stripe trevally, ornate threadfin bream and green mussel, which were higher than that limit. The estimated dietary intake of Cd (66.33 μg/person/week), Pb (40.83 μg/person/week), inorganic As (317.32 μg/person/week) and Hg (46.16 μg/person/week) from 13 kinds of seafoods were well within the safe limits. It appears that there is no imminent health risk due to heavy metals examined in this study. However, it should be recognized that the subjects may also be exposed to certain contaminants from other foods and sources other than the diet.

Department : Food and Pharmaceutical Chemistry.......Student's Signature Pimonwan Kerdthep
Field of Study : Food Chemistry and Medical Nutrition Advisor's Signature Tongyonk
Academic Year : 2008..Co-advisor's Signature.......................

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LIST OF ABBREVIATIONS

PTWI	Provisional tolerable weekly intake
Cd	Cadmium
Pb	Lead
As	Arsenic
Hg	Mercury
°C	degree Celsius
s	second
μg	microgram
g	gram
W	Watt
LOQ	Limit of quantitation
mL	millilitre
μL	microlitre
μM	micromolar
min	minute
nm	nanometre
mA	milliampere
h	hour
SD	standard deviation
et al.	*et alia* (and others)

CHAPTER I

INTRODUCTION

1.1 Background and Significance of the Study

Chemical hazards in food are toxic substances that either occur naturally, such as aflatoxins and marine toxins, or are manmade. Manmade toxins can be added to food intentionally, such as antibiotics, preservatives and colorants, or can unintentionally contaminate food, for example, heavy metals, cleaning agents, pesticide residues, animal drugs, other agrochemicals and packaging materials used to keep food safe and fresh. Unintentional contamination may occur through environmental pollution of the water, air and soil (Etzel *et al.*, 2003).

Heavy metals, such as cadmium, lead, mercury and arsenic, occur in the environment both as a result of natural processes and as pollutants from human activities. Some are essential elements for human life at low concentrations which means that they must be a part of our diet. However, these elements also can be toxic at high concentrations. Heavy metals bioaccumulation in the food chain can be especially highly dangerous to human health (Islam *et al.*, 2007). For example, organic mercury compounds are neurotoxins, exposure to lead can be harmful to neuropsychological development, inorganic arsenic is a human carcinogen and cadmium can affect renal function (Ysart *et al.*, 2000).

Environmental pollution represents a major problem in both developed and underdeveloped countries. Thailand is one of the countries which suffers from high biosphere pollution (air, soil and water), especially the east of Thailand, because it is industrialized area. Industrialization has improved general technology as well as quality of life but it has also resulted in an increase in pollutants, such as heavy metals, in the environment. The presence of them in the atmosphere, soil and water can cause serious problems to all organisms.

Although some individuals are primarily exposed to heavy metals contaminants in the workplace, for most people the main route of exposure to these toxic elements is through the diet (Llobet *et al.*, 2003). Chronic low-level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination (Islam *et al.*, 2007). Consequently, information about dietary intake is very important to assess risks to human health in industrialized area. To evaluate the health risks to consumers, it is necessary to determine the specific dietary intake of each pollutant for comparison with toxicologically acceptable levels.

The heavy metal pollution of the the marine environment has long been recognized as a serious environmental concern (Balkas *et al.*, 1982; Tarýq *et al.*, 1991; Giordano *et al.*, 1991), especially industrialized sea coast area, such as Rayong Province. In the sea, pollutants are potentially accumulated in marine organisms and sediments, and subsequently transferred to man through the food chain (Tüzen, 2003). For these reasons, it is important to determine the chemical quality of the seafood, particularly the contents of heavy metals, in order to evaluate the possible risk to human health.

1.2 Objectives of the Study

1.2.1 To determine the concentrations of cadmium, lead, mercury, and arsenic in seafood from Muang District, Rayong Province

1.2.2 To estimate the dietary intake of cadmium, lead, mercury, and arsenic from seafood by the subjects of Muang District, Rayong Province

1.3 Benefits of the Study

1.3.1 This study provides the information regarding the concentrations of some heavy metals such as cadmium, lead, mercury, and arsenic in seafood from Muang District, Rayong Province

1.3.2 The results from this study can be evaluated the health risks of consumers

1.3.3 Problem receiving will increase attention from the public health of Rayong Province as well as governmental agencies

CHAPTER II

LITERATURE REVIEW

2.1 Ultratrace Minerals

Ultratrace minerals are those elements with estimated dietary requirements usually less than 1 microgram/gram and often less than 50 nanogram/gram of diet for laboratory animals. For humans, the term is often used for mineral elements with established, or suspected requirements below 1 milligram/day, usually expressed in micrograms/day. At least 18 elements could be considered ultratrace minerals: aluminum, boron, bromine, chromium, fluoride, germanium, iodine, lithium, molybdenum, nickel, rubidium, selenium, silicon, tin, vanadium, arsenic, cadmium and lead (Maurice *et al*., 2006).

When an organism is exposed to some forms of nutritional, metabolic, hormonal or physiologic stress, some ultratrace minerals may be nutritional significance. In other words, the insufficient intake of a specific ultratrace mineral probably becomes apparent only when the body is stressed in some manner so as to enhance the need or interfere with the utilization of that element (Omaye, 2004).

2.2 Heavy Metals

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water (1 at 4°C;39°F) (Lide, 1992), such as antimony, arsenic, bismuth, cadmium, chromium, cobalt, copper, gallium, iron, lead, manganese, mercury, nickel, platinum, silver, tin, uranium, vanadium, and zinc (Goyer, 1996). Table 1 describes some of the commonly used physical properties that are important in the categorization of heavy metals and other elements. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health but large amounts of any of them may cause acute or chronic toxicity (poisoning) (Nies, 1999). Since many heavy metals can be very toxic and thus may threaten the health of organisms. Studies have been conducted to investigate heavy metal levels in environmental samples, as well as heavy metal accumulation and effects on organisms, and factors affecting heavy metal accumulation by various organisms (Machiwa, 1992; Engdahl *et al*., 1998; Machiwa, 2000).

Table 1 Physical properties of some heavy metals (Jorhem, 2002)

Metal	Atomic number	Density (kg dm^{-3})	Melting point (°C)	Boiling point (°C)
Arsenic	33	5.73	817	614
Cadmium	48	8.65	321	765
Chromium	24	7.2	1857	2672
Mercury	80	13.55	-39	357
Nickel	28	8.9	1453	2732
Lead	82	11.35	327.5	1740

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, blood composition, lung, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Repeated long-term contact with some metals or their compounds may even cause cancer (International Occupational Safety and Health Information Centre, 1999).

Chronic low-level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Heavy metals such as lead, mercury, cadmium and arsenic are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Loomis *et al*., 1996; Islam *et al*., 2007).

Heavy metals differ from other toxic substances in that they are neither created nor destroyed by humans. Nevertheless, their utilization by humans influences the potential for health effects in at least two major ways: first, by environmental transport, that is, by human or anthropogenic contributions to air, water, soil, and food, and second, by altering the speciation or biochemical form of the element (Casarett *et al*., 2001).

Metals are redistributed naturally in the environment by both geologic and biologic cycles (Figure 1). Rainwater dissolves rocks and ores and physically transports material to streams and rivers, depositing and stripping materials from adjacent soil and eventually transporting those substance to the ocean to be precipitated as sediment or taken up in rainwater to be relocated elsewhere on earth. The biological cycles include bioconcentration by plants and animals and incorporation into food cycles (Casarett *et al*., 2003).

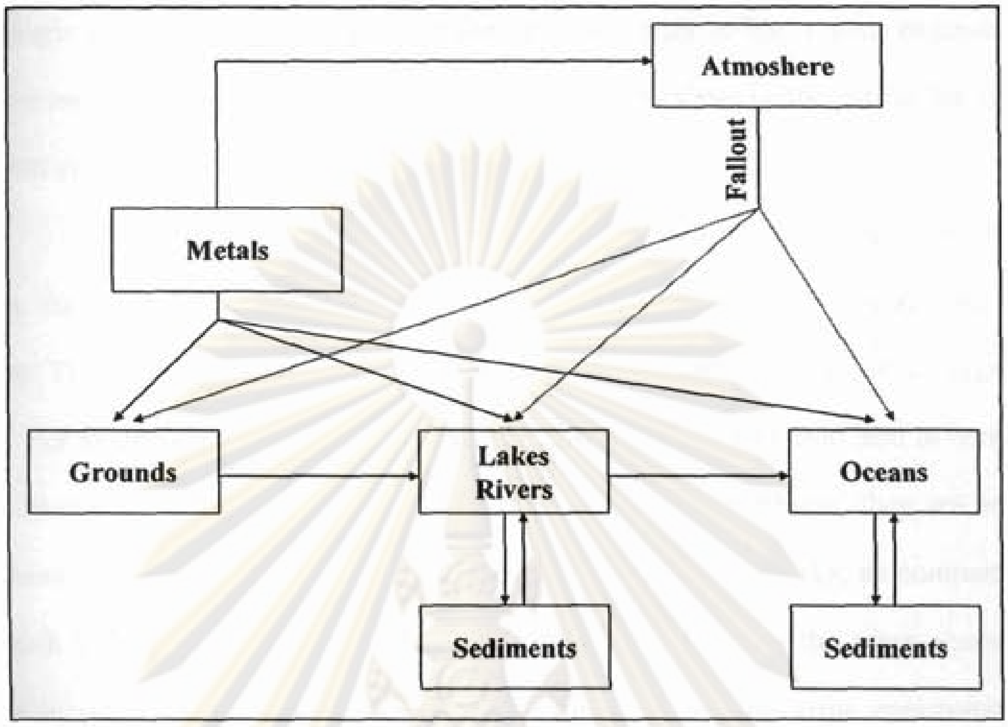

Figure 1 Routes for the transport of heavy metals in the environment (Casarett *et al.*, 2003)

Exposure to heavy metals can occur through a variety of routes. Heavy metals may be inhaled as dust or fume. Some can be vaporized and inhaled. Heavy metals may also be ingested involuntarily through food and drink. The amount that is actually absorbed from the digestive tract can vary widely, depending on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it distributes in tissues and organs. Excretion typically occurs primarily through the kidneys and digestive tract, but metals tend to persist in some storage sites, like the liver, bones, and kidneys, for years or decades (Michael, 2002).

2.2.1 Dose-Effect Relationships

Relationships between sources of exposure, transport, and distribution to various organs and excretory pathways are shown in Figure 2. The most precise definition of dose is the amount of metal within cells of organs that manifests a toxicologic effect. Results from single measurement may reflect recent exposure or longer-term or past exposure, depending on retention time in the particular tissue (Casarett *et al.*, 2003).

A critical determinant of retention of a metal is its biological half-life, that is, the time it takes for the body or organ to excrete half of an accumulated amount. The biological half-life varies according to the metals as well as the organ or tissue. For example, the biological half-life of cadmium in kidney and lead in bone are 20 to 30 years, whereas for some metals, such as arsenic or lithium, they are only a few hours or day. The half-life of lead in blood is only a few weeks, as compared to the much longer half-time in bone. Blood, urine, and hair are the most accessible tissues in which to measure an exposure or dose. Blood and urine concentrations usually reflect recent exposure and correlate best with acute effects. Hair might be useful in assessing variations in exposure to metals over the long term. (Casarett *et al.*, 2003).

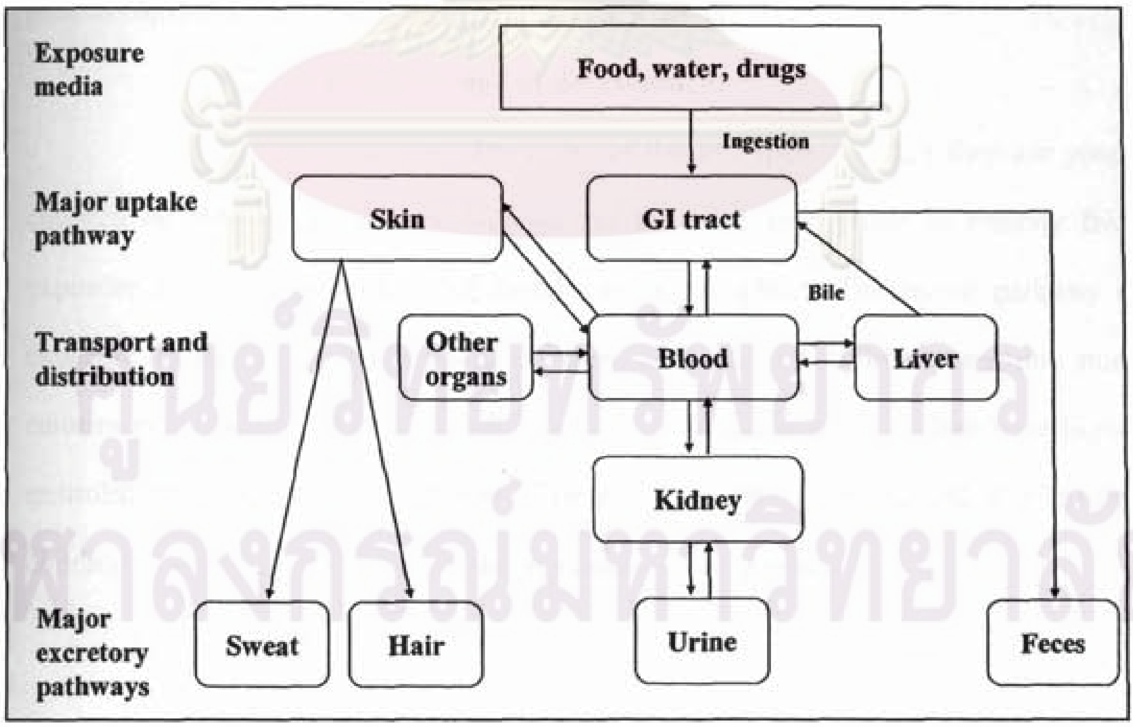

Figure 2 Metabolism after exposure to metals via ingestion (Casarett *et al.*, 2003)

2.2.2 Host Factors Influencing The Toxicity of Metals (Casarett *et al.*, 2001; Casarett *et al.*, 2003)

2.2.2.1 Interactions with essential metals

The interaction of toxic metals with essential metals occurs when the metabolism of a toxic metal is similar to that of the essential element. Absorption of toxic metals from the lung or gastrointestinal tract may be influenced by an essential metal, particularly if the toxic metal shares or influences a homeostatic mechanism, as occurs with lead and calcium and iron. Toxic metals may influence the role of essential metals as cofactors for enzymes or other metabolic processes.

2.2.2.2 Formation of metal-protein complexes

Metalloprotein complexes that are involved in detoxification or protection from toxicity have described for a few metals. Metallothioneins form complexes with cadmium, zinc, copper, and other metals, and ferritin and hemosiderin are intracellular iron-protein complexes. None of these proteins or metal-protein complexes have any known enzymatic activity.

2.2.2.3 Age and stage of development

Persons at either end of the life span, whether they are young children or elderly people, are believed to be more susceptible to toxicity from exposure to a particular level of metal than most adults. The major pathway of exposure to many toxic metals in children is food, and children consume more calories per kilogram of body weight than adults do. Moreover, children have higher gastrointestinal absorption of metals. The rapid growth and rapid cell division that children's bodies experience provide opportunities for genotoxic effects.

2.2.2.4 Lifestyle factors

Lifestyle factors such as smoking and alcohol ingestion may influence toxicity indirectly. Cigarette smoke contains some toxic metals, such as cadmium. Alcohol ingestion may affect toxicity indirectly by altering diet and reducing the intake of essential minerals.

2.2.2.5 Immune status of host

For metals that produce hypersensitivity reactions, the immune status of an individual becomes an additional toxicologic variable. Metals that provoke immune reactions include mercury, gold platinum, beryllium, chromium, and nickel. The clinical effects vary but usually involve any of four types of immune response.

2.3 Major Toxic Heavy Metals

2.3.1 Arsenic (As)

Arsenic is particularly difficult to characterize as a single element because its chemistry is so complex and there are many different arsenic compounds. It may be trivalent (As^{+3}) or pentavalent (As^{+5}) and is widely distributed in nature. The most common inorganic trivalent arsenic compounds are arsenic trioxide, sodium arsenite, and arsenic trichloride. Pentavalent inorganic compounds are arsenic pentoxide, arsenic acid, and arsenates, such as lead arsenate and calcium arsenate. Organic compounds may also be trivalent or pentavalent, such as arsenilic acid, or may even occur in methylated forms as a consequence of biomethylation by organisms in soil, fresh water, and seawater (Casarett *et al*., 2001).

Exposure

Significant exposure to arsenic occurs through both anthropogenic and natural sources. Occupational and community exposures to arsenic from the activities of humans occur through the industry, the use of gallium arsenide in the microelectronics industry, and the use of arsenic in common products such as wood preservatives, pesticides, herbicides, fungicides, and paints. Widespread dispersion of arsenic is a byproduct of the combustion of fossil fuels in which arsenic is a common contaminant (Michael, 2002).

Generally, most foods contain low levels of arsenic due to its wide distribution in the environment and, to some extent, to its use in agriculture. Dietary arsenic represents the major source of arsenic exposure for most of the population. Some types of seafoods contain up to 10 times the arsenic of other foods. People who consume large amounts of seafood may therefore ingest significant amounts of arsenic. 90 percent or more arsenic in seafood is organic arsenic that is poorly absorbed (Food Standards Australia New Zealand, 2003). The FAO/WHO (1989) recommends a provisional tolerable weekly intake (PTWI) for inorganic arsenic of 15 microgram/ kilogram body weight/ week or 350 microgram/ kilogram body weight/ week for total arsenic (Lee *et al.*, 2006).

Toxicokinetics

About 80 to 90 percent of a single dose of arsenite (As^{+3}) or arsenate (As^{+5}) has been shown to be absorbed from the gastrointestinal tract of human and experimental animals. Arsenic compounds of low solubility (e.g., arsenic selenide, lead arsenide, and gallium arsenide) are absorbed less efficiently than dissolved arsenic. Skin can be a route of exposure to arsenic, and systemic toxicity has been reported in persons having extensive acute dermal contact with solutions of inorganic arsenic (Hostynek *et al.*, 1993). Excretion of absorbed arsenic is mainly via urine. The biological half-life of ingested inorganic arsenic is about 10 hours, and 50 to 80 percent is excreted in about 3 days. The biological half-life of organic arsenic is about 30 hours. Arsenic has a predilection for skin and is excreted by desquamation of skin and in sweating. It also concentrates in nails and hair. Arsenic in nails produces Mees' lines (transverse white bands across fingernails), which appear about 6 weeks after the onset of symptoms of toxicity (Casarett *et al*., 2001).

Toxicity

Ingestion of large doses (70 to 80 mg) of arsenic may be fatal. The symptoms of acute illness consist of fever, anorexia, hepatomegaly, melanosis, cardiac arrhythmia, and eventual cardiovascular failure. Other features include upper respiratory tract symptoms, peripheral neuropathy, and gastrointestinal, cardiovascular, and hematopoietic effects. Acute ingestion may be suspected from damage to mucous membranes, such as irritation, vesicle formation, and even sloughing. Sensory loss in the peripheral nervous system commonly appears 1 or 2 weeks after large exposures and consists of wallerian degeneration of axons, a condition that is reversible if exposure is stopped. Anemia and leukopenia, particularly granulocytopenia, occur a few days after exposure and are reversible (Casarett *et al*., 2001).

Chronic exposure to inorganic arsenic compounds may lead to neurotoxicity of both the peripheral and central nervous systems. Neurotoxicity usually begins with sensory changes, paresthesia, and muscle tenderness, followed by weakness, progressing from proximal to distal muscle groups. Peripheral neuropathy may be progressive, involving both sensory and motor neurons and leading to demyelination of long axon nerve fibers, but effects are dose-related. Liver injury manifests initially as jaundice that may progress to cirrhosis and ascites (Casarett *et al*., 2001).

2.3.2 Cadmium (Cd)

Cadmium is used in electroplating and galvanizing and as a cathode material for nickel-cadmium batteries. It is also used as a color pigment in paints and plastics. Cadmium occurs in nature primarily in association with lead and zinc ores and is released near mines (Hodgson, 1997).

Exposure

In the general population, the major source of cadmium is food. Plants readily take up cadmium from contaminated soil, water, and fertilizers. Shellfish, such as mussels, scallops, and oysters, may be a major source of dietary cadmium and contain 100 to 1000 microgram/ kilogram. Shellfish accumulates cadmium from the water in the form of cadmium-binding peptides. Meat, fish, and fruit contain 1 to 50 microgram/ kilogram, grains contain 10 to 150 microgram/ kilogram, and the greatest concentrations are in the liver and kidney of animals (Casarett *et al*., 2003).

Workplace exposure to cadmium is particularly hazardous in the presence of cadmium fumes or airborne cadmium. Occupations at risk include electrolytic refining of lead and zinc and occupations in other industries that employ thermal processes (e.g., iron production, fossil fuel combustion, and cement manufacture). A major nonoccupational source of respirable cadmium is cigarettes (Casarett *et al*., 2001). The FAO/WHO (1993) recommends a PTWI for cadmium of 7 microgram/ kilogram body weight/ week.

Toxicokinetics

Gastrointestinal absorption of cadmium is about 5 to 8 percent. Absorption is enhanced by diet low in calcium, iron and protein. Low dietary calcium stimulates synthesis of calcium-binding protein, which enhances cadmium absorption. Respiratory absorption of cadmium is greater than gastrointestinal absorption and independent on solubility of cadmium compound. Absorbed cadmium is excreted in urine. While gastrointestinal excretion is possible, particularly in bile as a glutathione complex. Cadmium excretion in urine increases proportionally with body burden. Cadmium is transported in blood by binding to red blood cells and high-molecular-weight proteins in plasma, particularly albumin; it is distributed primarily to liver and kidney (Casarett *et al*., 2001).

Toxicity

The health implications of cadmium exposure are exacerbated by the relative inability of human beings to excrete cadmium (It is excreted but then re-absorbed by the kidney). Acute high-dose exposures can cause severe respiratory irritation. Occupational levels of cadmium exposure are a risk factor for chronic lung disease (through airborne exposure) and testicular degeneration and are still under investigation as a risk factor for prostate cancer. Lower levels of exposure are mainly of concern with respect to toxicity to the kidney. Cadmium damages a specific structure of the functional unit of the kidney (the proximal tubules of each nephron) in a way that is first manifested by leakage of low molecular weight proteins and essential minerals, such as calcium, into urine, with progression over time to kidney failure. This effects tend to be irreversible, and recent research suggests that the risk exists at lower levels of exposure than previously thought. In particular, the loss of calcium caused by cadmium's effect on the kidney can be severe enough to lead to weakening of the bones. Itai-itai disease, an epidemic of bone fractures in Japan from gross cadmium contamination of rice stocks, has been shown to happen in more subtle fashion among a general community living in an area of relatively modest cadmium contamination. Increased cadmium burden in this population was found to be predictive of an increased risk of bone fractures in women, as well as decreased bone density and height loss (presumably from the demineralization and compression of vertebrae) in both sexes (Michael, 2002).

2.3.3 Lead (Pb)

Lead is a ubiquitous toxic metal and is detectable in practically all phases of the inert environment and in all biological system. Because it is toxic to most living organisms at high exposures, the major issue regarding lead is determining the dose at which it becomes toxic. Specific concerns vary with the age and circumstances of the host, and the major risk is toxicity to the nervous system (Casarett *et al.*, 2001).

Exposure

For centuries, lead has been mined and used in industry and in household products. The current annual worldwide production of lead is approximately 5.4 million tons and continues to rise. Sixty percent of lead is used for the manufacturing of batteries (automobile batteries, in particular), while the remainder is used in the production of pigments, glazes, plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products (Michael, 2002).

The principal route of exposure for the general population is food, and environmental sources include lead-based indoor paint in old dwellings, lead in contaminated drinking water, lead in air from the combustion of lead-containing industrial emissions. Hand-to-mouth activities of young children living in polluted environments, lead-glazed pottery, and lead dust brought home by industrial workers on their shoes and clothes (Hodgson, 2004).

One factor reducing the lead content of food has been a reduction in the use of cans for food and beverages. The FAO/WHO (1993) recommends a PTWI for lead of 25 microgram/ kilogram body weight/ week.

Toxicokinetics

Adults absorb 5 to 15 percent of ingested lead and usually retain less than 5 percent of what is absorbed. Children are known to have a greater absorption of lead than adults. Lead absorption in children is related to age and development of the gastrointestinal tract. Nutritional problems, such as low dietary iron and calcium, enhance lead absorption. Lead in water and other beverages is absorbed to a greater degree than lead in food. Lead ingested between meals is absorbed more than lead with meals, and increasing frequency of food intake minimizes lead absorption. Lead in bone may contribute as much as 50 percent of blood lead, so that it may be a significant source of internal exposure to lead. The major route of excretion of absorbed lead is the kidney (Casarett *et al.*, 2001).

Toxicity

Lead is a cumulative toxic substance that can primarily affect the blood, nervous system and kidneys. In the blood at high concentrations, lead inhibits red blood cell formation and eventually results in anemia. The effects of high concentrations of lead on the nervous system can vary from hyperactive behavior and mental retardation to seizures and cerebral palsy. As the kidneys are the primary route for lead excretion, lead tends to accumulate in these organs, causing irreversible damage (Casarett *et al.*, 2001).

Infants and children are considered particularly vulnerable to lead exposure. This is due to their higher energy requirements, their higher fluid, air and food intake per unit of body weight, and the immaturity of their kidneys, liver, nervous and immune systems. In addition, their rapid body growth, their different body composition and the development of their organs and tissues, in particular the brain, may increase their lead absorption (Food Standards Australia New Zealand, 2003).

For adults with excess lead exposure, the concerns are peripheral neuropathy and chronic nephropathy. However, the critical effect or most sensitive effect for adults in the general population may be hypertension. Other target organs are the gastrointestinal, reproductive, and skeletal systems (Casarett *et al.*, 2001).

Toxicity of lead depending on the dose, lead exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure, and death at the high end to subtle effects on metabolism and intelligence at the low end of exposures. Children (and developing fetuses) appear to be particularly vulnerable to the neurotoxic effects of lead. A plethora of well-designed prospective epidemiologic studies had convincingly demonstrated that low-level lead exposure in children less than five years of age (with blood lead levels in the 5-25 milligram/deciliter range) results in deficits in intellectual development as manifested by lost intelligence quotient points. The most important is the risk to the fetus posed by mobilization of long-lived skeletal stores of lead in pregnant women. Maternal bone lead stores are mobilized at an accelerated rate during pregnancy and lactation and are associated with decrements in birth weight, growth rate, and mental development. Since bone lead stores persist for decades, it is possible that lead can remain a threat to fetal health many years after environmental exposure (Michael, 2002).

2.3.4 Mercury (Hg)

Mercury is unique as being the only metal that is in a liquid state at room temperature. The vapor from this liquid, usually referred to as mercury vapor is much more hazardous than the liquid form. This element exist in three oxidation states. In the zero oxidation state (Hg^0) mercury exists in its metallic form or as the vapor. The mercurous and mercuric states are the two higher-oxidation states where the mercury atom has lost one (Hg^+) and two electrons (Hg^{2+}), respectively. In addition, mercuric mercury can form a number of stable organic mercury compounds by attaching to one or two carbon atoms. The most toxic to humans is the organic form, with the most common organic form being methyl mercury (Food Standards Australia New Zealand, 2003). Methyl mercury (CH_3Hg^+) is the most important organic form from the point of view of human exposure (Casarett *et al.*, 2001).

Exposure

The major source of mercury (as mercury vapor) in the atmosphere is the natural degassing of the earth's crust. It is difficult to assess what quantities of mercury come from human activities, but these are believed to be approximately similar in magnitude to natural sources. Mercury vapor in the atmosphere represents the major pathway of global transport of mercury. It resides there unchanged for periods off a year or so. Thus there is time for it to be distributed globally even from a point source of pollution. Eventually it is converted to a water soluble form and returned to the earth's surface in rainwater. At this stage, two important chemical changes may occur. The metal may be reduced back to mercury vapor and returned to the atmosphere, or it may be methylated by microorganisms present in sediments of bodies of fresh and ocean water. The main product of this natural biomethylation reaction is monomethyl mercury compounds, usually referred to generically as "methyl mercury". Some of the oldest organisms on an evolutionary scale, the methanogenic bacteria, carry out this methylation reaction (Casarett *et al.*, 2001; Najdex *et al.*, 1987).

In general, the diet is the major source of exposure to mercury, with seafood containing much higher levels of mercury than most other foods (Food Standards Australia New Zealand, 2003). Methyl mercury enters an aquatic food chain involving plankton, herbivorous fish, and finally carnivorous fish. In the tissues of fish consuming sea mammals, mercury can rise to levels a millionfold higher than those in the surrounding water. The sequence of biomethylation and bioconcentration can result in human dietary exposure to methyl mercury, whether the latter originated from natural or anthropogenic sources of inorganic mercury. Methyl mercury is found in most if not all fish tissues but most importantly in edible tissue, mainly muscle, in a water-soluble protein-bound form. Cooking the fish does not lower the methyl mercury content (Hodgson, 2004).

Inorganic compounds of mercury are also found in food. The source is unknown and the amount ingested is far below known toxic intakes. Occupational exposures occur in the chlor-alkali industry, where it is used as a cathode in the electrolysis of brine solution; in making of various scientific instruments and electrical control devices, in dentistry in form of amalgam tooth filling, and in the extraction of gold (Hodgson, 1997).

The FAO/WHO (1993) recommends a PTWI for mercury of 5 microgram/ kilogram body weight/ week.

Toxicokinetics

The vapor from metallic mercury is readily absorbed in the lungs, and, in mercury's dissolved form in the bloodstream, diffuses to all tissues in the body. Its high mobility is due to the fact that it is a monatomic gas, highly diffusible and lipid-soluble. It is rapidly oxidized to mercuric mercury. Gastrointestinal absorption of compounds of mercuric mercury from food is about 15 percent in a study of human volunteers, whereas absorption of methyl mercury is on the order of 90 to 95 percent. Excretion of mercury from the body is by way of urine and feces, again differing with the form of mercury, size of the dose, and time after exposure. Exposure to mercury vapor is followed by exhalation of a small fraction, but fecal excretion is the major and predominant route of excretion initially after exposure to inorganic mercury.

About 90 percent of methyl mercury is excreted in feces after acute or chronic exposure (Casarett *et al*., 2001).

Toxicity

High levels of mercury exposure that occur through, for example, inhalation of mercury vapors can lead to life-threatening injuries to the lungs and neurologic system. At lower but more chronic levels of exposure, a typical constellation of findings arises, termed erethism-with tremor of the hands, excitability, memory loss, insomnia, timidity, and sometimes delirium-that was once commonly seen in workers exposed to mercury in the felt-hat industry. Even relatively modest levels of occupational mercury exposure, as experienced, for example, by dentists, have been associated with measurable declines in performance on neurobehavioral tests of motor speed, visual scanning, verbal and visual memory, and visuomotor coordination. Evidence from well-conducted studies is lacking that the small amount of mercury released from dental amalgams during chewing is capable of causing significant illnesses, such as multiple sclerosis, systemic lupus, or chronic fatigue syndrome. Dimethyl mercury is a supertoxic, superdangerous compound that can penetrate through latex gloves, as well as skin. Exposure to only a few drops can lead to central nervous system degeneration and death, but the compound is luckily encountered only in specialized laboratories (Michael, 2002).

Of greatest concern on a global scale is the sensitivity of the fetal and infant nervous system to low-level mercury toxicity. Mothers exposed to mercury in the 1955 disaster in Minamata Bay, Japan, gave birth to infants with mental retardation, retention of primitive reflexes, cerebellar symptoms, and other abnormalities. In the Faroe Islands has demonstrated that, even at much lower levels, mercury exposure to pregnant women through dietary intake of fish and whale meat, an important regional food staple, is associated with decrements in motor function, language, memory, and neural transmission in their offspring. Organic mercury, the form of mercury bioconcentrated in fish and whale meat, readily crosses the placenta and appears in breast milk (Michael, 2002).

2.4 The Assessment of Dietary Intakes of Contaminants

Risk assessment for food contaminants is one of the main priorities of food regulatory agencies. One of the most important information in assessing risk to human health from potentially harmful chemicals in food is the availability of data on the exposure of the population to such substances. Two approaches are generally considered acceptable for estimating such exposure: One is a biological monitoring program that measures substances in human fluids or tissues, and the other is a food monitoring program such as analysis of individual food or total food duplicates (Conacher *et al*., 1993).

Surveillance of chemicals in food is a priority of national authorities and international organizations. National authorities have the responsibility and obligation to ensure that toxic chemicals, such as pesticides, heavy metals, aflatoxins and other contaminants, are not present in food at levels that may adversely affect the health of consumers. Countries may set legal limits for food contaminants and monitor compliance with such limits. This type of monitoring and food control is essential for consumer protection and facilitation of trade. At the same time, governments need to assess public health risks arising from the presence of toxic chemicals in foods consumed in their countries (Gheorghiev, 1991).

To ascertain whether a consumer is at risk or not, it is necessary to estimate the actual dietary intake of a contaminant for comparison with acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI). Obtaining such an estimate is also important in determining whether there is a relationship between any observed effects in humans and the intake of a particular contaminant. The estimation of the actual dietary intake of contaminants as a measure of exposure is thus indispensable for risk assessment (FAO/WHO, 1993).

Contaminant intake estimates are equally critical for making sound decisions in the regulation of chemicals and food safety. If the actual intake of a chemical is found to approach or exceed the ADI or PTWI, national authorities should evaluate whether the use of the chemical may need to be restricted or eliminated. Dietary intake studies will provide the information that will indicate whether existing limits for contaminants in food should be reviewed. If periodic estimates of actual exposures to chemicals are found to be well below ADI or PTWI, health authorities and the citizens of the country are assured of the safety of the current food supply with respect to these substances.

The data generated from the assessment can be used for a number of purposes (Gheorghiev, 1991):

1. To localize sources of food contamination
2. To estimate the intake of contaminants via food
3. To indicate the need for, or the effect of, measures to reduce food contamination or keep it below specified statutory limit (control of pesticide use, animal drugs, hygiene practice in production, processing, environmental pollution)

Systems to monitor food contaminants have been developed in a number of countries over the past 20 years, mainly as a result of worldwide concern over pesticide residues in food. Three basic approaches for sampling food are used: (1) selective studies of individual foods; (2) total diet (market basket) studies; and (3) duplicate portion studies.

2.4.1 Selective studies of individual foods

This approach involves the measurement of contaminants in representative samples of staple foods, either unprocessed or processed with or without cooking, which together with food consumption data, enables average daily intakes to be calculated. The method is particularly useful if it is known that the intake of a contaminant is determined by a limited number of foods; when only a restricted number of staple foods are consumed by a population; and where monitoring data on these individual foods are already available (FAO/WHO, 1985).

Selective studies of individual foods are the simplest samples to analyse for the presence of contaminants, and have been used extensively to estimate intakes. This approach has several advantages. The relative contribution of each food can be evaluated. The procedure is flexible, since it can incorporate various data for food consumption at national and regional levels and is closely related to the network of food control laboratories organized traditionally in many countries. The basic disadvantage of this approach is that the effect of the cooking on the contaminant. (Gheorghiev, 1991).

2.4.2 Total diet (market basket) studies

The sample for this type of study consists of a market basket of food reflecting a defined total diet of a consumer for a specific period of time. The foods are prepared for table-ready consumption and are analysed either individually or combined in one or more food-group composites (e.g., cereals, meat, vegetables, seafood) in proportions based on available consumption data. Residue levels measured in the total diet samples are used in calculating the average daily intake for each composite and for the diet as a whole. A total diet study is particularly valuable in initially determining whether residues are widely distributed amongst all the broad classes of major foods, or are confined to a few general classes of foods (FAO/WHO, 1985).

This approach provides highly accurate results about contaminant levels and about relative contributions of each composite. This approach has been used extensively in many countries to search for pesticides and other industrial chemicals (Leblanc *et al.*, 2000; Falćo *et al.*, 2005; Ysart *et al.*,2000; Sapunar *et al.*, 1996; Brussaard *et al.*, 1996), for minerals and metals (Buzina *et al.*, 1995; Biego *et al.*, 1999; Cuadrado *et al.*, 1995; Dabeka *et al.*, 1995), and for polycyclic aromatic hydrocarbons (Dennis *et al.*, 1983).

2.4.3 Duplicate Portion Studies

The duplicate diet approach is a direct sampling technique in which an exact duplicate of food being consumed is obtained and analysed. This method is suitable for the estimation of the intakes of individuals and small groups. It provides the most accurate estimates, because it combines results for each contaminant with the actual food consumed. It is limited to small population groups, however, such as hospital kitchens, children's homes, homes for the elderly, and populations living in areas with varying degrees of environmental pollution (Buchet *et at.*, 1983).

Duplicate portion studies require the production, for subsequent analyses, of an exact sample of the food eaten by an individual at a meal. This is achieved by the kitchen preparation of double the quantity of food likely to be consumed and the provision of a sample of the exact amounts of each type of food consumed by an individual (FAO/WHO, 1985).

2.5 Provisional Tolerable Weekly Intake (PTWI)

In order to assess potential health problems from the presence of toxic contaminants in the food supply, the extent to which actual dietary intakes approach or exceed a toxicologically acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI) should be determined.

The acceptable daily intake of a chemical is the daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of all the known facts at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg). For this purpose "without appreciable risk" is taken to mean the practical certainty that injury will not result even after a lifetime of exposure.

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) has established ADIs for a number of pesticides used in food production. Similarly the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established ADIs for food additives. Since 1972, JECFA has also evaluated several food contaminants, such as cadmium, lead and mercury, and has allocated PTWIs rather than ADIs for these contaminants. The basis for adopting this approach has been described as follows: (FAO/WHO, 1985)

1. The contaminants are able to accumulate within the body at a rate and to an extent determined by the level of intake and by the chemical form of heavy metal present in food. Consequently, the basis on which intake is expressed should be more than the amount corresponding to a single day. Moreover, individual foods may contain above-average levels of heavy metal contaminant, so that consumption of such foods on any particular day greatly enhances that day's intake. Accordingly the provisional tolerable intake is expressed on a weekly basis.

2. The term "tolerable", signifying permissibility rather than acceptability, is used in those cases where intake of a contaminant is unavoidably associated with the consumption of otherwise wholesome and nutritious foods, or with inhalation in air.

3. The use of the term "provisional" expresses the tentative nature of the evaluation, in view of the paucity of reliable data on the consequences of human exposure at levels approaching those with which the Committee is concerned.

Provisional tolerable weekly intake (PTWI) are set for substances, such as heavy metals, that are contaminants in food and are known to accumulate in animals and humans (Table 2). The unit of time for PTWI is different to that used for ADI. PTWI use a one week time unit while ADI use a one day time unit.

Table 2 The provisional tolerable weekly intake (PTWI) of some heavy metals

Heavy metals	**PTWI (μg/kg BW/week)**
Cadmium	7
Lead	25
Toal Arsenic	350
Inorganic arsenic	15
Mercury	5

2.6 Dietary Exposure to Heavy Metals in Some Countries

Food safety is a major public concern worldwide. During the last decades, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins.

Heavy metals are among the major contaminants of food supply and may considered the most important problem to environment of many countries. Such problem is getting more serious all over the world especially in developing countries. Heavy metals, in general, are not biodegradable, have long biological half-life and have the potential for accumulation in the different body organs leading to unwanted side effects. Accordingly, in many countries have been estimated of dietary intake of heavy metals in many foodstuff.

Assessment the health risks due to the presence of arsenic, cadmium, mercury and lead in food products in Chile (Santiago) (Muñoz *et al*., 2005) showed that fish and shellfish group had the highest concentrations of aesenic (1.351 μg/g), cadmium (0.277 μg/g) and mercury (0.048 μg/g), while sugars had the highest concentrations of lead (0.251 μg/g). It is similar to the result in Spain, Llobet *et al*. (2003) found that fish and shellfish group had the highest concentrations of aesenic (2.21 μg/g), cadmium (0.037 μg/g), mercury (0.09 μg/g) and lead (0.052 μg/g). Furthermore, Schuhmacher *et al*. (1991) found that fish and crustaceans from Terragona coast in Catalonia, Spain were the groups which accumulated the highest level of mercury. The study in United Kingdom (Ysart *et al*., 2000) showed that fish had the highest levels of arsenic (4.4 μg/g) and mercury (0.043 μg/g), offal had the highest levels of cadmium (0.077 μg/g) and lead (0.09 μg/g). In France (Leblanc *et al*., 2000), the highest level were found in seafood for arsenic.

Fish and shellfish are the group with the highest content of these heavy metals because it may be a result of industrial wastes and mining which can create a potential source of heavy metal pollution in the aquatic environment. However, the total intake of heavy metals in these countries are within the limits estimated as safe.

2.7 The Estimation of Heavy Metals in Some Marine Organisms

It has been recognized for many years that the concentrations of heavy metals found in coastal areas, whether they be in the dissolved or particulate phase, may be derived from a variety of anthropogenic and natural source (Dalman *et al.*, 2006).

Heavy metals are emitted to the environment by industrial activities, accumulating in water sediments of lakes and oceans and, thereafter, in aquatic organisms. Thus, human populations with consumption habits based on fish, seafood and sea mammals are specially exposed.

Studying to measure concentrations of mercury, arsenic, lead and cadmium in seafood from various areas of the Adriatic Sea showed that these heavy metals are higher in the industrially polluted area (Jureša *et al.*, 2003; Buzinar *et al.*, 1989; Vukadin *et al.*, 1995). However, the accumulation of these heavy metals in marine organisms is related not only to the presence of the pollutant, but also to a whole range of biological (species, age, growth degree) and environmental (temperature, geochemical anomalies, salinity) factors which influence heavy metals' bioavailability (Jureša *et al.*, 2003).

In Turkey, along the coast of İskenderun Bay, there are many towns, agricultural lands, industrial plants (iron-steel plants, beverage, LPG plants, oil transfer docks, other industrial plants and cargo ship's ballasts water). Therefore mainly untreated agricultural, municipal and industrial wastes affect the bay direct or indirectly. Their study has been undertaken to determine heavy metals; cadmium and lead; concentrations in the muscles of fish and to investigate the differences between the concentrations of heavy metal accumulated by fishes in three selected sites of İskenderun Bay. These stations are the Arsuz, relatively clean area, İskenderun Harbour Area (IHA) and Petrotrans (PTS), intensively polluted areas by both industrial and domestic sources. This study showed that cadmium and lead levels in pollutes areas; IHA and PTS; are higher than metals in clean areas (Türkmen *et al.*, 2005)

The Bay of Güllük in Southeastern Aegean Sea (Turkey) is vary important by the potential of marine product in the Aegean Sea. There are various polluting elements in Güllük Bay. The metals concentrations; cadmium and lead; found in that study were high but there are lower than those found in polluted areas of Black sea (Dalman *et al.*, 2006)

In Thailand, the study on concentration of mercury, cadmium and lead in 6 kinds of marine organisms from the southern coast of the gulf of Thailand, which clean area, exhibited that the contamination levels of heavy metals in these kinds of mollusk and shrimp were still lower than contamination standard limited level in food issued by the Ministry of Public Health of Thailand (Vibunpant *et al.*, 2006). On the other hand, the study on heavy metals in tissue of Tongue Sole (*Cynoglossus bilineatus*) (ปลาลิ้นหมา) from Laemchabang Coastal, Chonburi Province, the industrially polluted area showed that the tissue of Tongue Sole were contaminated with cadmium ranging from 0.0862-1.3302 μg/g, lead ranging from 0.2020-3.0786 μg /g, and arsenic ranging from 0.6635-0.8446 μg/g. The contamination of lead and arsenic higher than the standard limitation in seafood of Thai Ministry of Public Health (บุญมี และคณะ, 2548).

The study on mercury concentrations in marine species at four sites on the Terragona coast in Catalonia, Spain showed that the majority of the mercury discharges occur in the northern area, which is affected by a large number of industrial activities than the south area (Schuhmacher *et al.*, 1994).

In conclusion, industrial wastes create a potential source of heavy metals pollution in the aquatic environment. Under certain environmental conditions, heavy metals might accumulate up to a toxic concentration and cause ecological damage.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Population

Subjects were randomly sampling from the primary health care voluntary staff of Muang District, Rayong Province [n=316 (Appendix A)]. They were males and females aged 30-65. All subjects received the explanation of study protocol, and the written informed consent was obtained before the beginning of the study. The study protocol was approved by the Ethics Committee of The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok (Appendix B).

3.2 Experimental Design

The observational study was conducted in primary health care voluntary staff of Muang District, Rayong Province during the period from October to December 2007. All subjects were interviewed about personal information, quantity and frequency of seafood consumption with semi-quantitative food frequency questionnaire (Appendix C). Then, data were arranged. The thirteen kinds of seafoods which were the mostly consumed by the subjects were selected and purchased from 3 local markets in Muang District. These seafoods were analyzed for cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) by atomic absorption spectrophotometry (AAS) [The method to determine the heavy metals in this study were modified from official methods of analysis of the AOAC international (2005)].

After that, the weekly intake was calculated and compared with provisional tolerable weekly intake (PTWI).

3.3 The Dietary Survey

The dietary survey using semi-quantitative food frequency questionnaire was carried out by trained interviewer. A food frequency and amount questionnaire combined with photos of seafood size were used to assess the dietary intake.

3.4 Sampling

The heavy metal-contaminated area in this study was located near the industrial areas [Maptaphut and Integrated Refinery and Petrochemical Complex (IRPC)] (Figure 3).

Between January and February 2008, there were 21 kinds of marine species which were frequently consumed by the subjects (Table 4). In this study, the 13 most consumed marine species were selected and randomly obtained from 3 local markets (Maedang, Star and Watlum market) in Muang District of Rayong Province. Total of 39 samples (3 samples for each species from 3 local markets) were analyzed for Cd, Pb, total As and Hg concentrations.

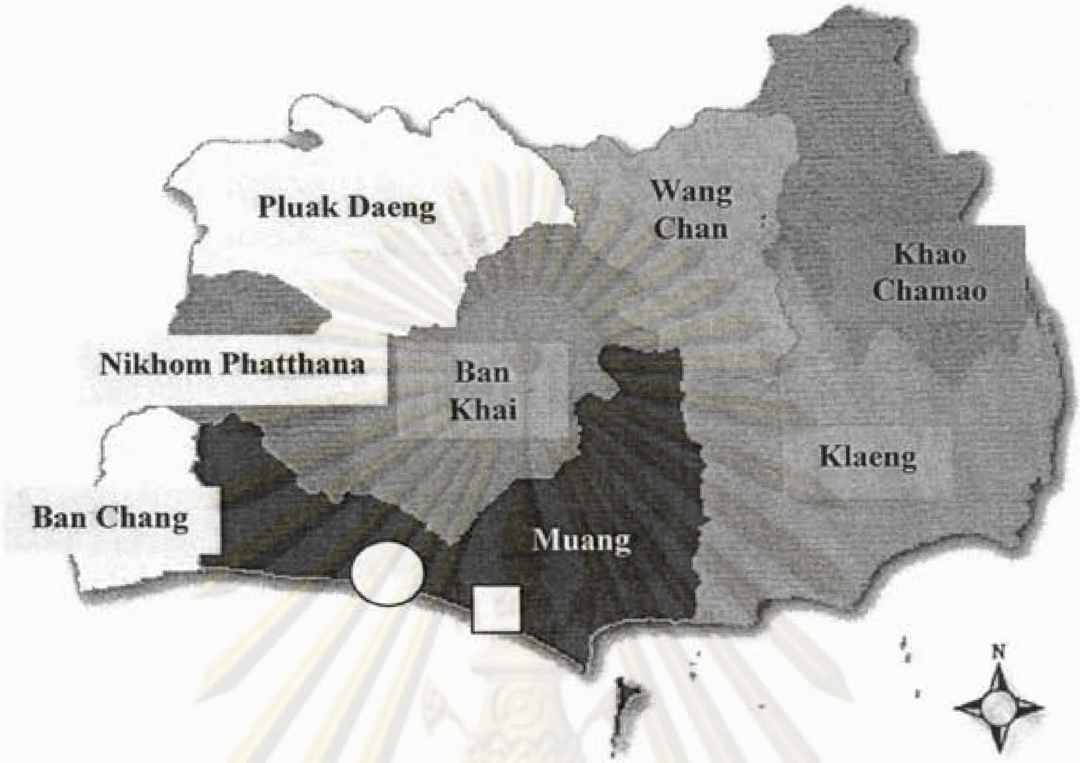

Figure 3 Industry zone in Muang District, Rayong Province

○ Maptaphut industrial area ☐ IRPC industrial area

3.5 Instruments

Determination of total As was performed with a flow injection hydride generation atomic absorption spectrophotometry (FI-HG-AAS) (AAS 3300/ FIAS 100, Perkin Elmer). Determination of Pb and Cd was performed with graphite furnace atomic absorption spectrophotometry (GFAAS) (AAnalyst 600, Perkin Elmer). Hg determination was performed with a mercury analyzer (Hiranuma Hg-150).

Other equipment used included a microwave digestion unit (mls1200 mega, Milestone), a muffle furnace (ECF 12/30,Lenton) and blender.

All glasswares were treated with 20% (v/v) nitric acid (HNO_3) for 24 hours, and then rinsed three times with deionized water before use.

3.6 Reagents

All reagents used were of analytical grade except supra pur 65% nitric acid. Deionized water (18MΩ.cm) was used for the preparation of reagents and standards. Commercial standard solutions (1000 mg/L) of Cd, Pb, As and Hg were used (Perkin Elmer). The working standard solution were freshly prepared by diluting an appropriate aliquot of the standard stock solutions.

3.7 Sample Preparation

All seafood samples were rinsed with water and the entire edible part of each individual was included to prepare the sample. The sample obtained was homogenized in a blender and kept at 4 °C until the analysis.

3.8 Cadmium and Lead Determination

Each sample (1 g of wet weight) was placed into digestion vessel and 5 mL of concentrate nitric acid (65% supra pur HNO_3) was added. The vessel was closed with a set of lid, fixed to the rotor with screw and placed inside the microwave oven. The samples were irradiated at 250 W for 1 minute, 0 W for 2 minutes, 250 W for 5 minutes, 400 W 5 minutes and 600 W for 5 minutes. After digestion, the vessels were cooled to room temperature before opened. Rinse down lid and walls of vessels into 25 mL volumetric flask and then diluted with deionized water to final volume of 25 mL (sample solution).

The quantification of Cd and Pb was performed with GFAAS. The furnace program [temperature (°C)/ ramp time (s)/ hold time (s)] employed for Cd determination was: drying (110 °C/ 1 s/ 30 s; 130 °C/ 5 s/ 35 s); pyrolysis (550 °C/ 10 s/ 20 s); atomization (1350 °C/ 0 s/ 3 s); cleaning (2450 °C/ 1 s/ 3 s). For Pb determination, the furnace program was the same as Cd determination, except for the temperatures of pyrolysis (500 °C) and atomization (1450 °C). The matrix modifier used for determining both metals was a mixture of ammonium dihydrogen phosphate ($H_2PO_4NH_4$) 0.05 mg and magnesium nitrate [$Mg(NO_3)_2$] 0.003 mg in deionized water. The quantification was performed by using a calibration curve of the corresponding standards. Duplicate analyses were performed for each sample. The limit of quantitation (LOQ) for Cd was 0.009 μg/g. The LOQ for Pb was 0.045 μg/g.

3.9 Arsenic Determination

Ten milliters of each sample solution (from 3.8) was placed into crucible dish and treated with 1 mL of 7.5% (w/v) magnesium nitrate solution. The mixtures were heated on hot plate at low heat to dryness and increased heat to 375°C. Dried samples were oxidized any carbonaceous matters and decompose excess magnesium nitrate in 450 °C furnace about 30 minutes. The white ash was dissolved in 2 mL 8M hydrochloric acid (HCl) and adjusted volume to 10 mL with deionized water. As^{5+} in the sample solution was reduced to As^{3+} with 2 mL concentrate hydrochloric acid and 2 mL of reducing solution [5% (w/v) ascorbic acid + 5% (w/v) potassium iodide (KI)], let stand 45 minutes. After that, it was diluted with deionized water into 20 mL volumetric flask.

The analytical conditions for As determination by FI-HG-AAS were the following : loop sample, 500 μL; reducing agent, 0.2% (w/v) sodium borohydride ($NaBH_4$) in 0.05% (w/v) sodium hydroxide (NaOH), 5 mL/min flow rate; hydrochloric acid solution 10% (v/v), 10 mL/min flow rate; carrier gas argon, 50 mL/min flow rate; wavelength, 193.7 nm; slit 0.7 nm; lamp current setting 400 mA; cell temperature 900 °C. Calibration standard solution of As(III) were prepared from a stock standard solution of arsenic oxide (As_2O_3), added concentrate hydrochloric acid and reducing solution mixture containing 5% (w/v) potassium iodide and 5% (w/v) ascorbic acid same as sample solution. Duplicate analyses were performed for each sample. The LOQ was 0.053 μg/g.

3.10 Mercury Determination

Two grams wet weight of each sample was placed in digestion flask, and 10-20 boiling stones, 20 mg vanadium pentoxide (V_2O_5), and 20 mL sulfuric acid (H_2SO_4)–nitric acid (1:1) were added. The flask was quickly connected to cold water circulating condenser, and swirled to mix. The flask was heated by low initial boiled for 6 minutes and finished digestion with strong boiled for 10 minutes. Swirled flask intermittently during digestion until no solid material should be apparent except for globules of fat.

After digestion, removed flask from heat and washed condenser with 15 mL of deionized water, 2 drops of 30% hydrogen peroxide (H_2O_2) were added through condenser and washed it into flask with 15 mL of deionized water. These flasks were cooled in an ice bath which stilled connected to condenser, and transfer digested solutions to biochemical oxygen demand bottles (BOD), and 8% potassium permanganate ($KMnO_4$) were added until it excess, 5 mL of 5.6 N nitric acid, 5 mL of 18 N sulfuric acid, 5 mL of 1.5% hydroxylamine hydrochloride ($NH_2OH.HCl$) and 5 mL of 10% stannous chloride ($SnCl_2$) in 0.5 N sulfuric acid were added. Then, samples were analysed with mercury analyzer immediately. The quantification was performed by using a calibration curve of the corresponding standards. Duplicate analyses were performed for each sample. The LOQ was 0.007 μg/g.

3.11 Quality Assurance

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Samples were generally carefully handled to avoid contamination. Reagent blank determinations were used to check the experimental contamination. For accuracy, a recovery test was carried out by spiking of standard solutions of heavy metals in homogenized samples. The recovery for Cd, Pb, As and Hg, which analyzed under the experimental conditions were found to be between 80 – 110 %. Duplicate analysis were performed in each set of the sample and the relative percent difference (% RPD) were within the acceptable limit.

3.12 Estimation of Dietary Exposure to Heavy Metals

Weekly dietary exposures of selected heavy metals from seafood was determined by multiplying the heavy metal concentration in each kind of seafoods by the average amount of that seafood consumed weekly by the subjects. Dietary exposures from all 13 types of selected seafoods were summed to represent the total dietary exposure, and were expressed on a body weight basis by dividing the total dietary exposure by average body weight of the population. (Lee *et al.*, 2006)

Weekly intake per person of each heavy metal

= ∑ [concentration of selected heavy metals in each seafood (µg/g) x mean of weekly intake of that seafood (g/ week)]

Whenever possible, monitoring data from dietary intake studies were to be compared with acceptable or tolerable levels recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 1989; FAO/WHO, 1993). Hence, the estimated dietary exposure levels of heavy metals determined in this study were compared with the provisional tolerable weekly intakes (PTWI) by the

JECFA to assess potential health risks faced by consumers. The PTWI represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise whole some and/or nutritious foods.

CHAPTER IV

RESULTS

4.1 Characteristics of the Subjects

The characteristics of the 316 subjects are presented in Table 3. The subjects included 26 males and 290 females aged between 30 to 65 years old. Most of the subjects finished in primary school (57.6%). Eighty three subjects were housewives (26.3%), seventy two subjects were workman (22.8%) and sixty nine subjects were gardeners (21.8%). Income of most subjects were below 5000 baht monthly (50.9%). Most subjects (88%) bought fresh food from the local markets (84.2%) and catched seafood by themselves (3.8%) for cooking. The others (12%) bought cooked food for their families. The most favorite local markets that the subjects purchased seafoods for cooking were Maedang (31.0%), Wadlum (26.6%) and Star markets (11.4%).

Table 3 Characteristics of the subjects

Characteristics	Number of subjects (%)
Sex	
Male	26 (8.2%)
Female	290 (91.8%)
Age (years)	
30-40	66 (20.9%)
41-50	100 (31.6%)
51-60	124 (39.2%)
61-65	26 (8.2%)

Table 3 Characteristics of the subjects (continued)

Characteristics	Number of subjects (%)
Education	
Primary school	182 (57.6%)
Junior high school	4 (1.3%)
Senior high school	62 (19.6%)
Diploma	32 (10.1%)
Bachelor degree	19 (6.0%)
Higher than bachelor degree	17 (5.4%)
Occupation	
Fishery	3 (0.9%)
Gardener	69 (21.8%)
Workman	72 (22.8%)
Trader	52 (16.5%)
Housewife	83 (26.3%)
Government officer	6 (1.9%)
Company employee	13 (4.1%)
Others	18 (5.7%)
Income/ month (baht)	
< 5,000	161 (50.9%)
5,000-10,000	109 (34.5%)
10,001-15,000	29 (9.2%)
15,001-20,000	11 (3.5%)
≥ 20,001	6 (1.9%)
Lifestyle	
Buying cooked food	38 (12%)
Home cooking	278 (88%)
Source of seafood for home cooking	
Market	266 (84.2%)
Catching	12 (3.8%)

4.2 Seafood Consumption

The results of the dietary survey showed the 21 species of marine organisms that were frequently consumed by the subjects and showed the average amount intake of each kind of seafoods (g/week) of 316 subjects (Table 4).

Table 5 shows the 13 types of seafoods which were frequently consumed by the subjects. Yellow-tail round scad is the most consumed seafood species by the subjects of Rayong; 357.87 g/week and the consumption of blood cockle is the lowest; 23.51 g/week.

Table 4 Seafood consumption by 316 subjects

Scientific name[a]	**Common name**[a]	**Average intake (g wet weight/week)**
1. *Decapterus maruadsi*	Yellow-tail round scad (ปลาทูแขก)	357.87
2. *Rastrelliger kanagurta*	Indian mackerel (ปลาลัง)	249.19
3. *Perna viridis*	Green mussel (หอยแมลงภู่)	145.96
4. *Nemipterus hexodon*	Ornate threadfin bream (ปลาทรายแดง)	142.81
5. *Selaroides leptolepis*	Yellow stripe trevally (ปลาข้างเหลือง)	129.41
6. *Scomberomorus commerson*	Spanish mackerel (ปลาอินทรีย์)	117.40
7. *Thunnus tonggol*	Longtail tuna (ปลาโอ)	115.16
8. *Sepioteuthis lessoniana*	Soft cuttle fish (หมึกหอม)	68.66
9. *Penaeus merguiensis*	Banana shrimp (กุ้งแชบ๊วย)	62.21
10. *Parastromateus niger*	Black pomfret (ปลาจาระเม็ด)	47.72
11. *Epinephelus tauvina*	Greasy grouper (ปลาเก๋า)	47.72
12. *Loligo duvauceli*	Splendid squid (หมึกกล้วย)	38.81
13. *Arca granulose*	Blood cockle (หอยแครง)	23.51
14. *Sphyraena obstsata*	Obtuse barracuda (ปลาน้ำดอกไม้)	19.01
15. *Rastrelliger brachysoma*	Short-bodied mackerel (ปลาทู)	15.42
16. *Alectis indicus*	Thredfin trevally (ปลาโฉมงาม)	15.33
17. *Lutjanus malabaricus*	Malabar red snapper (ปลากะพง)	12.84
18. *Sillago maculata*	Sillago trumpeter sillage (ปลาเห็ดโคน)	10.50
19. *Mugil vaigiensis*	Diamond-scaled grey mullet (ปลากระบอก)	8.48
20. *Paphia undulata*	Undalate venus (หอยลาย)	8.34
21. *Sepia pharaonis*	Rainbow cuttlefish (หมึกกระดอง)	6.22

[a] ปรียนาฏ สุขะวิสิษฐ์, 2532; เชียร บรรณโศภิษฐ์ และทศพร วงศ์รัตน์, 2510

g = gram

Table 5 Types of seafood which were the most consumed by the subjects

Seafood type	Common name
Fish	Yellow-tail round scad (ปลาทูแขก)
	Indian mackerel (ปลาลัง)
	Ornate threadfin bream (ปลาทรายแดง)
	Yellow stripe trevally (ปลาข้างเหลือง)
	Spanish mackerel (ปลาอินทรีย์)
	Longtail tuna (ปลาโอ)
	Black pomfret (ปลาจาระเม็ด)
	Greasy grouper (ปลาเก๋า)
Bivalves	Green mussel (หอยแมลงภู่)
	Blood cockle (หอยแครง)
Cephalopods	Soft cuttle fish (หมึกหอม)
	Splendid squid (หมึกกล้วย)
Crustaceans	Banana shrimp (กุ้งแชบ๊วย)

4.3 Heavy Metal Concentration

Table 6 shows range and mean concentrations of Cd, Pb, As and Hg in each kind of seafoods purchased from 3 local markets in Muang District, Rayong Province. Content less than the limit of quantitation was taken as being equal to the limit of quantitation for the purpose of calculating intake.

The highest levels were found in blood cockle (หอยแครง), soft cuttle fish (หมึกหอม) and greasy grouper (ปลาเก๋า) for Cd, Pb, total As and Hg respectively. However, the contamination levels of heavy metals in these kinds of seafoods were still lower than the standard limited level in food issued by the Ministry of Public Health of Thailand except total As level in yellow stripe trevally (ปลาข้างเหลือง), ornate threadfin bream (ปลาทรายแดง), green mussel (หอยแมลงภู่), soft cuttle fish (หมึกหอม) and splendid squid (หมึกกล้วย) (Table 7, Figure 4-7)

Table 6 Range and mean concentrations (mean ± SD) of heavy metals in seafood samples from Rayong Province

Seafood type	**Concentration of heavy metals (μg/g wet weight)**			
	Cd	**Pb**	**Total As**	**Hg**
Fish				
Yellow-tail round scad	0.022 – 0.035 (0.029 ± 0.007)	0.000 – 0.045 (0.030 ± 0.026)	0.753 – 1.019 (0.874 ± 0.135)	0.007 – 0.010 (0.008 ± 0.002)
Indian mackerel	0.021 – 0.050 (0.031 ± 0.016)	ND	1.501 – 1.632 (1.580 ± 0.070)	0.014 – 0.017 (0.015 ± 0.002)
Ornate threadfin bream	0.009 (0.009 ± 0.000)	0.000 – 0.045 (0.030 ± 0.026)	2.347 – 4.065 (3.223 ± 0.859)	0.032 – 0.049 (0.038 ± 0.010)
Yellow stripe trevally	0.009 (0.009 ± 0.000)	0.045 (0.045 ± 0.000)	3.234 – 3.862 (3.484 ± 0.333)	0.018 – 0.022 (0.020 ± 0.002)
Spanish mackerel	0.009 (0.009 ± 0.000)	ND	1.036 – 1.829 (1.476 ± 0.404)	0.110 – 0.133 (0.119 ± 0.013)
Longtail tuna	0.018 – 0.026 (0.021 ± 0.004)	0.000 – 0.045 (0.030 ± 0.026)	0.814 – 1.060 (0.957 ± 0.128)	0.007 – 0.009 (0.008 ± 0.001)
Black pomfret	0.013 – 0.021 (0.017 ± 0.004)	ND	0.677 – 1.252 (1.015 ± 0.301)	0.007 – 0.010 (0.009 ± 0.002)
Greasy grouper	0.009 (0.009 ± 0.000)	0.000 – 0.045 (0.030 ± 0.026)	1.191 – 1.459 (1.319 ± 0.134)	0.233 – 0.323 (0.269 ± 0.048)

Table 6 Range and mean concentrations (mean ± SD) of heavy metals in seafood samples from Rayong Province (continued)

Seafood type	Concentration of heavy metals (µg/g wet weight)			
	Cd	**Pb**	**Total As**	**Hg**
Bivalves				
Green mussel	0.043 – 0.203 (0.140 ± 0.085)	0.068 – 0.106 (0.084 ± 0.020)	2.289 – 2.730 (2.510 ± 0.221)	0.000 – 0.010 (0.006 ± 0.005)
Blood cockle	0.456 – 1.126 (0.731 ± 0.351)	0.081 – 0.110 (0.096 ± 0.015)	0.562 – 0.639 (0.595 ± 0.040)	0.007 – 0.010 (0.008 ± 0.002)
Cephalopods				
Soft cuttle fish	0.009 (0.009 ± 0.000)	ND	5.578 – 7.914 (7.032 ± 1.268)	0.020 – 0.022 (0.021 ± 0.001)
Splendid squid	0.010 – 0.061 (0.038 ± 0.026)	0.000 – 0.045 (0.015 ± 0.026)	4.544 – 7.568 (5.807 ± 1.572)	0.010 – 0.017 (0.012 ± 0.000)
Crustaceans				
Banana shrimp	0.009 (0.009 ± 0.000)	ND	0.346 – 0.452 (0.401 ± 0.053)	ND (0.000 ± 0.000)

µg = microgram
g = gram
ND = Not detect

Table 7 Mean concentrations (mean ± SD) of heavy metals in the selected seafood samples and compared with standard limited level

Seafood type		Mean concentrations (µg/g wet weight)			
		Cd	**Pb**	**Total As**	**Hg**
Yellow-tail round scad		0.029 ± 0.007	0.030 ± 0.026	0.874 ± 0.135	0.008 ± 0.002
Indian mackerel		0.031 ± 0.016	ND	1.580 ± 0.070	0.015 ± 0.002
Ornate threadfin bream		0.009 ± 0.000	0.030 ± 0.026	3.223 ± 0.859	0.038 ± 0.010
Yellow stripe trevally		0.009 ± 0.000	0.045 ± 0.000	3.484 ± 0.333	0.020 ± 0.002
Spanish mackerel		0.009 ± 0.000	ND	1.476 ± 0.404	0.119 ± 0.013
Longtail tuna		0.021 ± 0.004	0.030 ± 0.026	0.957 ± 0.128	0.008 ± 0.001
Black pomfret		0.017 ± 0.004	ND	1.015 ± 0.301	0.009 ± 0.002
Greasy grouper		0.009 ± 0.000	0.030 ± 0.026	1.319 ± 0.134	0.269 ± 0.048
Green mussel		0.140 ± 0.085	0.084 ± 0.020	2.510 ± 0.221	0.006 ± 0.005
Blood cockle		0.731 ± 0.351	0.096 ± 0.015	0.595 ± 0.040	0.008 ± 0.002
Soft cuttle fish		0.009 ± 0.000	ND	7.032 ± 1.268	0.021 ± 0.001
Splendid squid		0.038 ± 0.026	0.015 ± 0.026	5.807 ± 1.572	0.012 ± 0.004
Banana shrimp		0.009 ± 0.000	ND	0.401 ± 0.053	ND
Mean of all seafoods		0.082 ± 0.198	0.028 ± 0.032	2.329 ± 2.064	0.041 ± 0.075
Standard limited level	THA[a]		1	2	0.5
	AUS[b]	2			
	EU[c]	0.1 - 1[e]	1		
	RSA[d]		2	1	0.5 - 1.5

[a] The Ministry of Public Health, Thailand

[b] The National Health and Medical Research Council, Australia

[c] The European Community

[d] The Chilean legistation

[e] In fish 0.1 µg/g, crustaceans 0.5 µg/g, cephalopods mollusks 1 µg/g

ND = Not detect

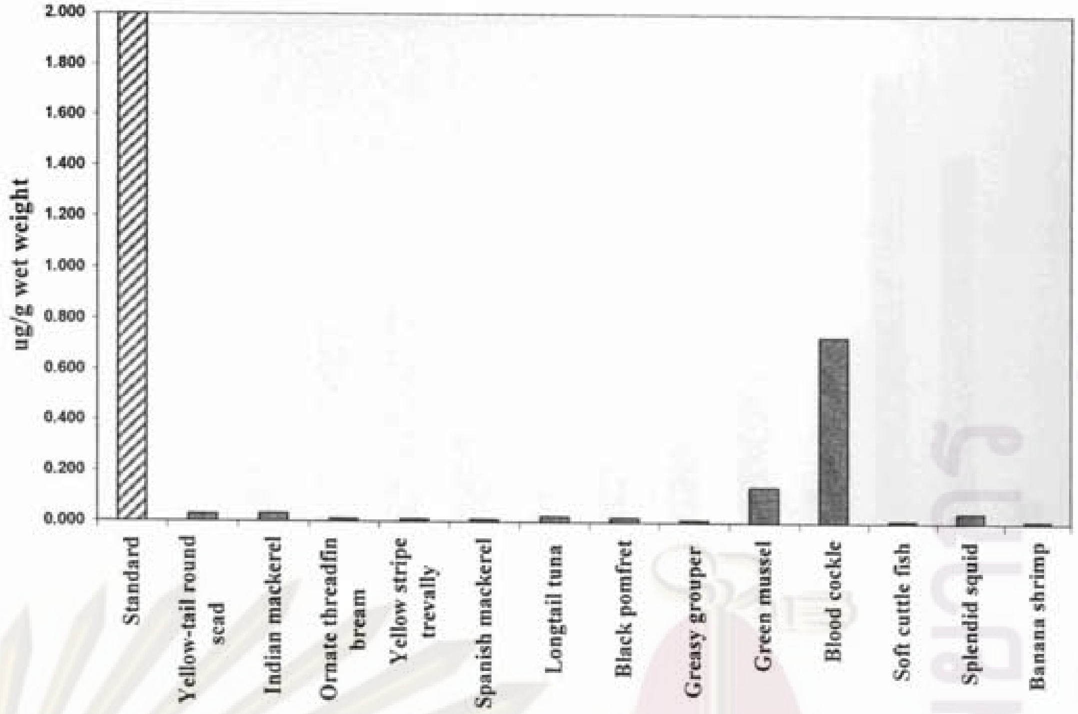

Figure 4 Comparison of cadmium levels in 13 kinds of seafoods with the standard limited level in food issued by Australia

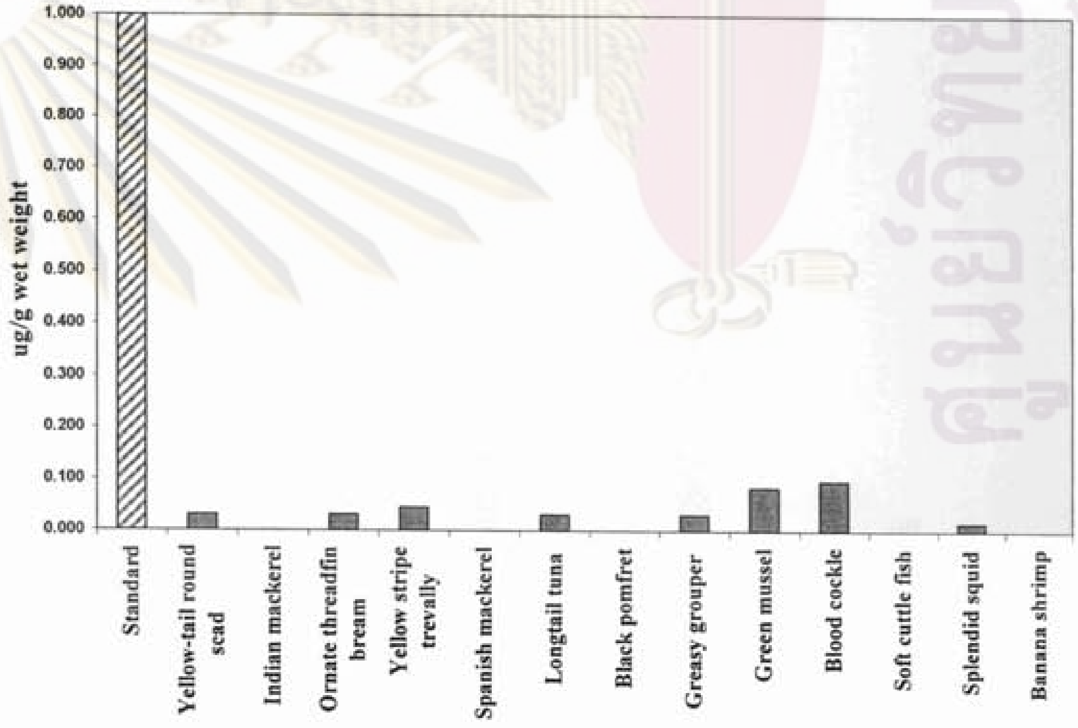

Figure 5 Comparison of lead levels in 13 kinds of seafoods with the standard limited level in food issued by the Ministry of Public Health of Thailand

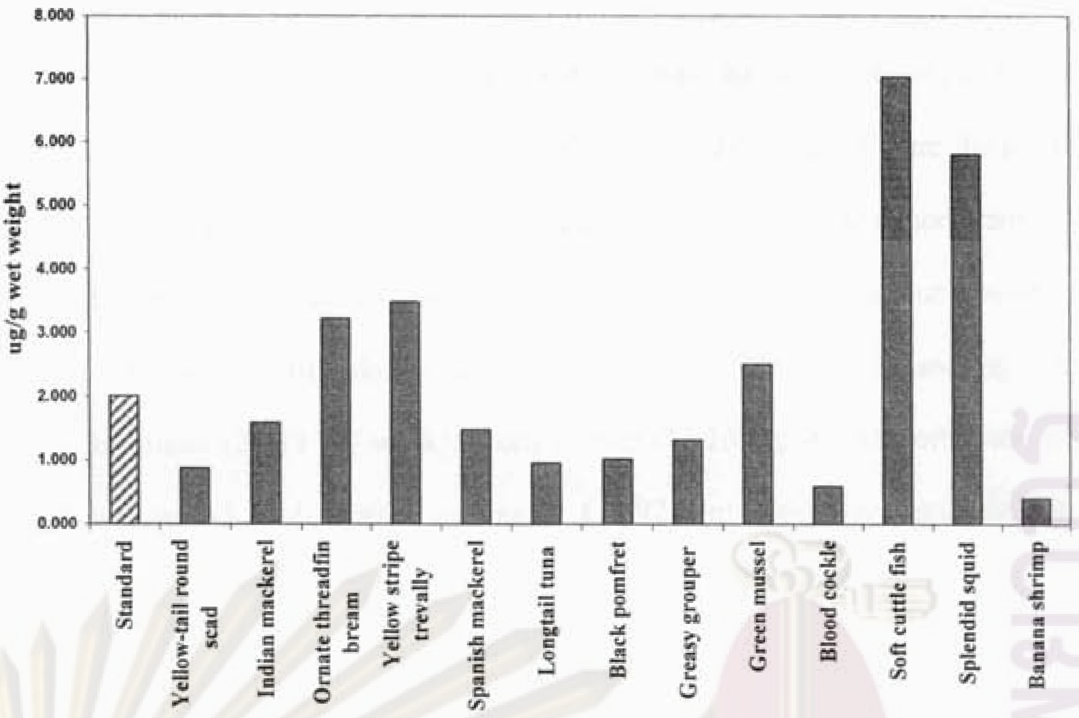

Figure 6 Comparison of total arsenic levels in 13 kinds of seafoods with the standard limited level in food issued by the Ministry of Public Health of Thailand

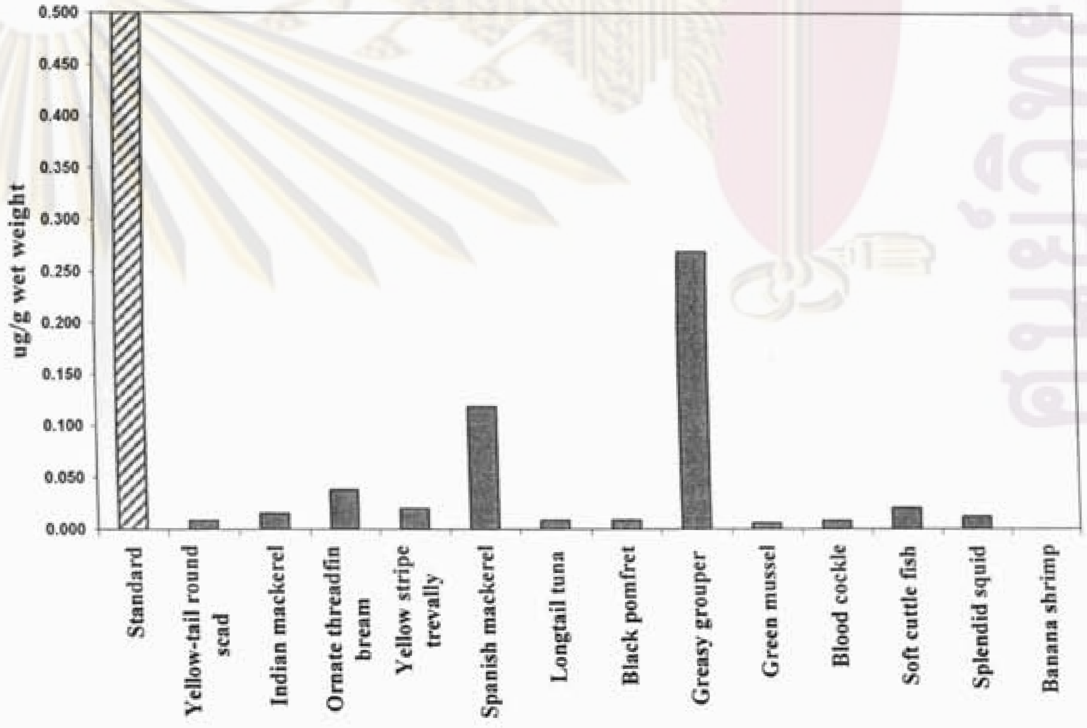

Figure 7 Comparison of mercury levels in 13 kinds of seafoods with the standard limited level in food issued by the Ministry of Public Health of Thailand

4.4 Estimated Dietary Intake of Heavy Metals through Seafood Consumption

Estimated dietary weekly intakes of Cd, Pb, total As and Hg through seafood consumption for the 316 subjects are summarized in Table 8 and Figure 8-11. The dietary intake of each contaminant was calculated by multiplying the concentration of the metal in a particular seafood category by the mean weight of that group consumed per week. The high contribution to dietary intake of Cd, Pb, total As and Hg were from green mussel (20.43 μg/ week), green mussel (12.26 μg/ week), soft cuttle fish (482.84 μg/ week) and spanish mackerel (13.97 μg/ week) respectively. The provisional tolerable weekly intakes (PTWIs) established by the FAO/WHO together with the current intakes were shown in Table 9.

Table 8 Weekly dietary intake (μg/ kg BW) of heavy metals for each type of seafoods

Seafood type	Dietary intake (μg/ week)			
	Cd	**Pb**	**As**	**Hg**
Fish				
Yellow-tail round scad (ปลาทูแขก)	10.38	10.74	312.78	2.86
Indian mackerel (ปลาลัง)	7.72	0.00	393.73	3.74
Ornate threadfin bream (ปลาทรายแดง)	1.29	4.28	460.27	5.43
Yellow stripe trevally (ปลาข้างเหลือง)	1.16	5.82	450.85	2.59
Spanish mackerel (ปลาอินทรีย์)	1.06	0.00	173.28	13.97
Longtail tuna (ปลาโอ)	2.42	3.46	110.21	0.92
Black pomfret (ปลาจาระเม็ด)	1.60	0.00	95.67	0.85
Greasy grouper (ปลาเก๋า)	0.43	1.43	62.95	12.84
Bivalves				
Green mussel (หอยแมลงภู่)	20.43	12.26	366.35	0.88
Blood cockle (หอยแครง)	17.18	2.26	13.99	0.19
Cephalopods				
Soft cuttle fish (หมึกหอม)	0.62	0.00	482.84	1.44
Splendid squid (หมึกกล้วย)	1.47	0.58	225.34	0.47
Crustaceans				
Banana shrimp (กุ้งแชบ๊วย)	0.56	0.00	24.95	0.00
Total	66.33	40.83	3173.20	46.16

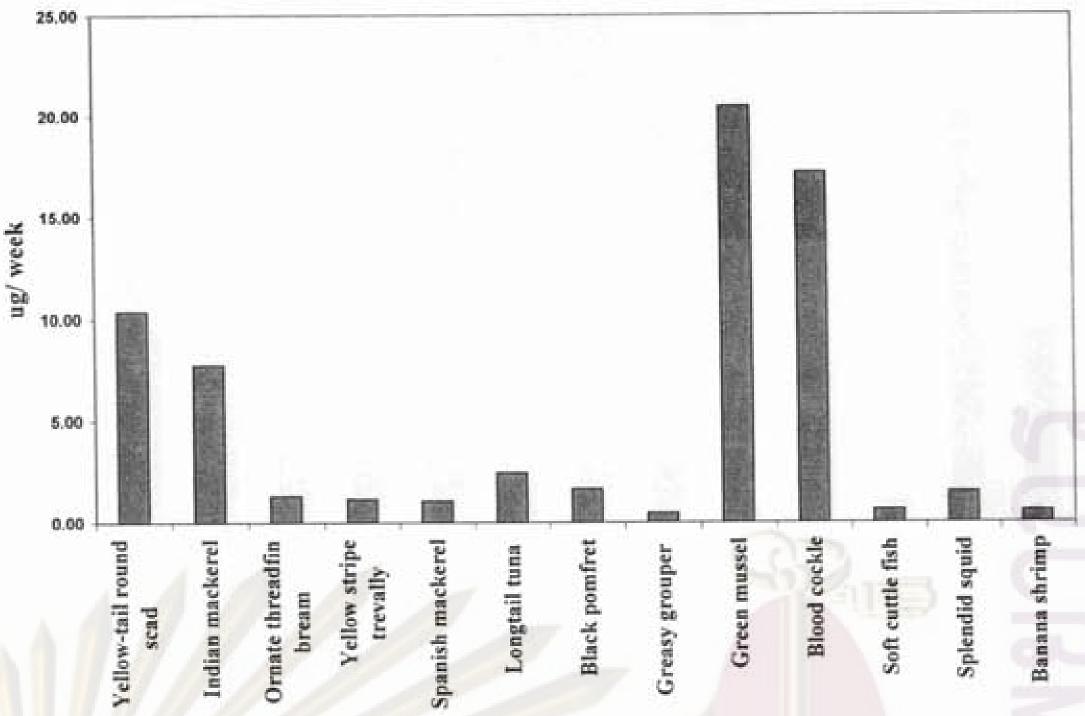

Figure 8 Dietary cadmium intake from each kind of seafoods (µg/ week)

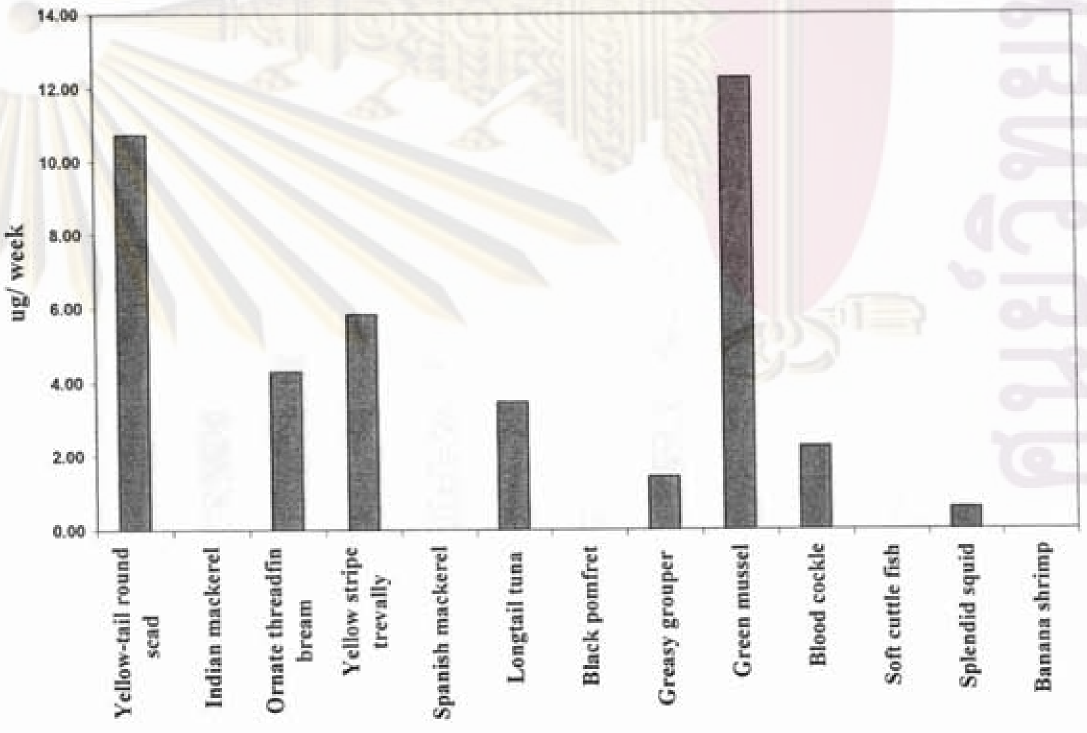

Figure 9 Dietary lead intake from each kind of seafoods (µg/ week)

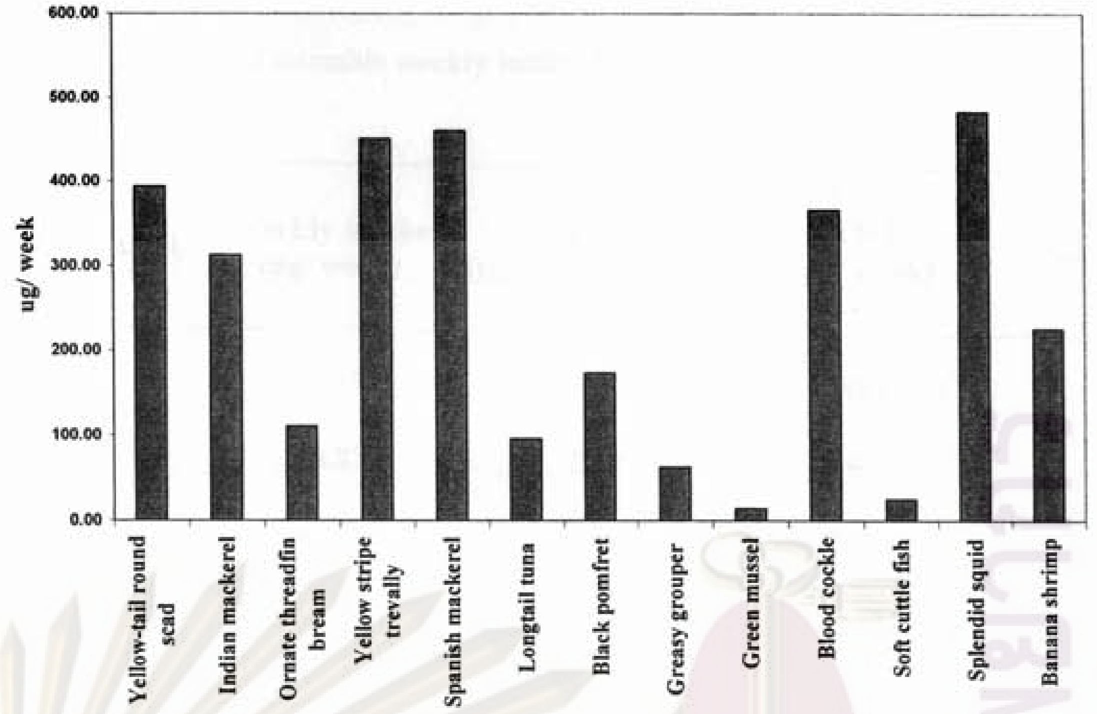

Figure 10 Dietary total arsenic intake from each kind of seafoods (μg/ week)

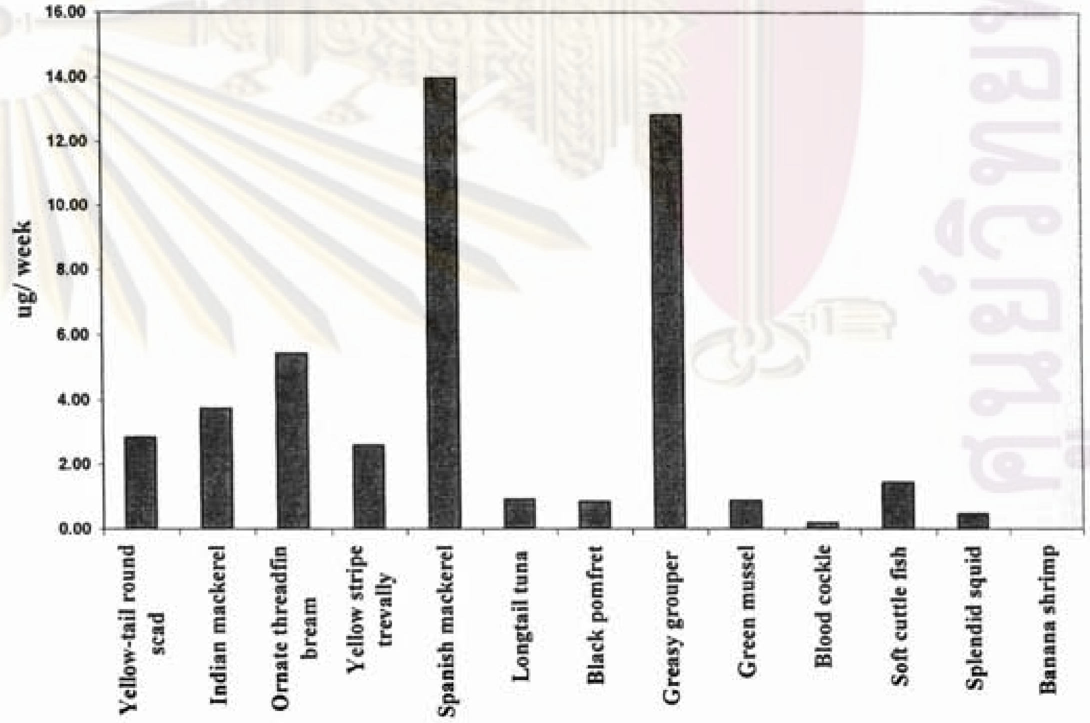

Figure 11 Dietary mercury intake from each kind of seafoods (μg/ week)

Table 9 Weekly dietary heavy metals intake (µg/ kg BW) compared to the provisional tolerable weekly intake (PTWI)

Heavy metals	Weekly intake (µg/ week)	PTWI (µg/kg BW/week)	PTWI[b] (µg/ week)	% PTWI
Cd	66.33	7	413	16.0
Pb	40.83	25	1475	2.8
Total As	3173.20	350	20650	15.4
Inorganic As[a]	317.32	15	885	35.9
Hg	46.16	5	295	15.6

[a] The percentage of inorganic As was estimated to be 10% of total As (Koreňovská and Suhaj, 2005; Larsen *et al.*, 2005; Muñoz *et al.*, 2000)
[b] Average body weight of the subjects was 59 kg

CHAPTER V

DISCUSSION

5.1 Concentration of Heavy Metals in Seafood

5.1.1 Cadmium

Cadmium is a toxic metal to which humans are exposed through a variety of pathway including food (Van Cauwenbergh *et al.*, 2000). Cadmium, which is in general lower in vegetables, meat and fish, concentrates in shells and internal organ of different organisms (Blanuša and Jureša, 2001). Cadmium occurs in nature primarily in association with lead and zinc ores and is released near mines (Hodgson, 2004).

In this study the cadmium contents in 13 kinds of seafoods were analyzed and was found that bivalves contain higher (0.043 – 1.126 μg/g) than other groups. The highest cadmium level was found in blood cockle which was similar to the study of Martí-Cid *et al.*, (2007) in Catalonia, Spain. They found that blood cockle had the highest cadmium level (0.130 μg/g). Furthermore, Bruhn *et al.*, (1999) found high level of this metal in bivalves (0.5-1.050 μg/g) from various areas of Chilean coast, and Vibunpant *et al.*, (2006) found that the level of this metal in bivalves and crustacean from the southern coast of the Gulf of Thailand were 0.035-5.160 μg/g and 0.150-0.160 μg/g respectively. Shellfish group has high concentration of cadmium because cadmium can accumulate in shellfish in the form of cadmium-binding peptides (Casarett *et al.*, 2003; Tsuda *et al.*, 1995).

Mean concentrations of cadmium in the fish and shellfish groups found in Catalonia, Spain (0.037 μg/g) (Llobet *et al.*, 2003) and those found in United Kingdom (0.013 μg/g) (Ysart *et al.*, 2000) were lower than those obtained from this study (0.082 µg/g). Moreover, Vibunpant *et al*., (2008) found that the concentration of cadmium in seafoods in the Andaman sea (0.005-0.665 µg/g) was lower than those obtained from this study (0.009-1.126 µg/g). Since Rayong is industrial area, it makes an environmental pollutants by the release of waste products and contaminants into surface runoff into the water due to rain. Principal sources of pollution include chemical plants, oil refineries, petrochemical plants, PVC factories, metals production factories and plastics factories. These toxic chemicals adhere to tiny particles which are then taken up by plankton and marine organisms, most of which are either deposit or filter feeders. In this way, the toxic chemicals are concentrated upward within food chains (Moon *et al*., 1995). So high concentration of cadmium was found in some marine organisms. On the other hand, the mean concentration of cadmium in fish and shellfish group in Santiago, Chile (0.277 µg/g) (Muñoz *et al*., 2005) is higher than this result because Santiago has the serious environmental pollution.

The mean concentration of cadmium in 13 kinds of seafoods reported in this study did not exceed the contamination standard limited level of the National Health and Medical Research Council, Australia (2 µg/g) (Vibunpant *et al*., 2006). Maximum cadmium content set by the European Union (EU) in fish, crustaceans, bivalves and cephalopods are 0.1, 0.5 and 1 µg/g respectively (Muñoz et al., 2005). In this study, none of seafood samples had cadmium contents that exceeded of EU legislation.

5.1.2 Lead

This study showed that high lead concentrations were found in bivalves (0.068 – 0.110 µg/g). Green mussel had the highest lead level. It was similar to the study of Martí-Cid *et al*., (2007). They reported that green mussel was the food contaminated with the greatest concentration of lead (0.15 μg/g), this concentration was higher than that found in our study. In the other studies, Vibunpant *et al*., (2006) found that the bivalves from various areas of the southern coast of the Gulf of Thailand had higher concentration of lead (0.052 – 2.994 μg/g) than crustaceans (0.007 – 1.088 μg/g). Furthermore, Blanusa and Juresa (2001) found that the bivalve group in Adriatic sea, Croatia (0.121 – 0.150 μg/g) had higher cadmium content than the fish group (0.010 – 0.044 μg/g) and crustacean group (0 – 0.040 μg/g). The lead level in cephalopods (0.002 – 4.462 μg/g) from the coast of the Gulf of Thailand were higher than lead level in crustaceans (0.002 – 2.434 μg/g) and fish (0.013 – 1.857 μg/g). Similarly, Vibunpant *et al*., (2008) found that lead contents in cephalopods in Andaman sea (0.007 – 2.250 μg/g) were higher than those in crustaceans (0.014 – 0.220 μg/g) and fish (0.005 – 0.219μg/g). Most bivalves and crustaceans are filter feeders for extracting organic matters from the sea, they can rapidly adsorb metals from the sea (ธนิกา จินตนะพันธ์, 2538). In the present study, the lead contents in fish and shellfish (0.028 μg/g) were lower than those reported in other studies in polluted area: for example, 0.052 μg/g in Spain (Llobet *et al*., 2003), 0.052 μg/g in Chile (Muñoz *et al*., 2005).

The Chilean legistation (RSA) permits 2 μg/g of fish and shellfish (Muñoz et al., 2005), while the European Union (EU) permits 1 μg/g for cephalopod mollusks (Muñoz et al., 2005). In Thailand, the Ministry of Public Health (MOPH) permits 1 μg/g for all foods (ประกาศกระทรวงสาธารณสุข, 2529). Indeed, the lead contents found in this report were lower than the maximum limit permitted in the RSA, EU and Thailand.

However, absorption of lead from ingested food and water greatly depends on levels of other elements presenting in the diet such as calcium, iron and zinc. It has been shown that dietary deficiencies of these essential elements enhance lead absorption. Therefore, when assessing toxic element intake of a certain population it is always useful to determine the intake of other essential elements as well. (Blanuša and Jureša, 2001; Helferich *et al.* 2001)

5.1.3 Arsenic

Arsenic naturally presents in some sulphur rich crust and sediment and can be released if the crust or the sediments are oxidized (Delgado *et al.*, 2003). Among the whole food, it is well known that high concentrations of arsenic are found in marine biota, where concentrations of total arsenic typically are in range of 1-100 μg/g (fresh weight) in marine animals and plants. Arsenobetaine is the predominant and non-toxic species in marine organisms (Chen and Gao, 1993; Delgado *et al.*, 2003).

In this study, the cephalopods had higher arsenic concentrations (4.544 – 7.914 μg/g) than fish (0.677 – 4.065 μg/g) and crustaceans (0.346 – 2.730 μg/g). This result was similar to the result of Delgado *et al.* (2003). They found that the cephalopod group (1.020 – 20.077 μg/g) had higher arsenic content than the fish group (0.396 – 12.584 μg/g), and bivalve group (0.041 – 2.458 μg/g).

The arsenic content in fish and shellfish group in this study (2.329 μg/g) was lower than the results obtained from variations countries: Basque country (Spain) 3.633 μg/g (Urieta *et al.*, 1996); United Kingdom 4.4 μg/g (Ysart *et al.*, 1999). Whereas, the arsenic level in fish and shellfish group in Canada (1.662 μg/g) (Dabeka *et al.*, 1993); Catalonia, Spain (2.21 μg/g) (Llobet *et al.*, 2003) and Santiago, Chile (1.351 μg/g) (Muñoz *et al.*, 2005) were lower than the arsenic level in this study.

The RSA establishes maximum total arsenic limits, which are not exceeded 1 μg/g (Muñoz et al., 2005). The MOPH of Thailand permits 2 μg/g for total As (ประกาศกระทรวงสาธารณสุข ฉบับที่ 98, 2529) and if it is higher than that, it must be analyzed for inorganic As content. The maximum inorganic arsenic limits is 2 μg/g (ประกาศกระทรวงสาธารณสุข ฉบับที่ 273, 2546). In this study, the total arsenic content of yellow strip trevally, ornate threadfin bream, green mussel, soft cuttlefish, and splendid squid were above the maximum limits permitted in the RSA and the MOPH of Thailand. In this study, we could not analyze inorganic arsenic content. However, it is well known that most arsenic found in marine organisms is organic form, which is the less toxic form of this element because it is rapidly excreted in the urine (Jureša *et al.*, 2003).

5.1.4 Mercury

Organic mercury compound in the form of methyl mercury is mostly contain in seafood. It is more toxic than the inorganic form. The population living near the coast and on the islands runs a greater risk of ingesting this highly toxic substance. Inorganic mercury also combines with carbon to make organic mercury compounds, such as methyl mercury which is a highly toxic form readily absorbed and mainly produced by microscopic organism in the water and soil (Omaye, 2004; Osweiler, 1996).

In this study, high mercury concentrations were found in the fish group (0.007 – 0.323 μg/g). Greasy grouper had the highest mercury level (0.269 μg/g), with concentration up to seven times higher than other kinds of seafoods. This result was similar to that reported by Martí-Cid *et al.*, (2007) in Spain, fish was the main source of mercury, especially in sword fish (1.9 μg/g). Mercury concentration in the fish group from The coast of the Gulf of Thailand (Vibunpant *et al.*,2008) was higher than that in the cephalopods (0.002 – 0.097 μg/g) and crustaceans (0.002 – 0.081 μg/g). In Adriatic sea, Croatia (Storelli, 2008), the fish group had the highest concentrations of mercury (fish group: 0.010 – 2.980 μg/g, cephalopod group: (0.010 – 2.150 μg/g, crustacean group: 0.090 – 0.690 μg/g). Furthermore, Mercury level in the bivalve group (0.006 – 0.700 μg/g) from the southern coast of the Gulf of Thailand (Vibunpant *et al.*,2006) was higher than that in the crustacean group (0.019 – 0.200 μg/g).

In polluted area of Spain (Falćo *et al.*, 2005), mean mercury concentration in seafoods (0.097 μg/g) was higher than this study (0.041 μg/g). On the other hand, the mercury content in seafoods from this study were similar to the studies in The United Kingdom (0.043 μg/g) (Ysart *et al.*, 2000) and Chile (0.048 μg/g) (Muñoz *et al.*, 2005).

The range of mercury concentration in 13 kinds of seafoods (0-0.269 μg/g) did not exceed the maximum limit established by the RSA (0.5-1.5 μg/g) (Debeka *et al.*, 2004) and the MOPH of Thailand (0.5 μg/g) (ประกาศกระทรวงสาธารณสุข ฉบับที่ 98, 2529).

In this study, it may be concluded that the industrially polluted area had high concentrations of heavy metals from waste products. Moreover, these metals may come from agricultural (pesticides) and naturally occurring.

5.2 Evaluation of Dietary Exposure Levels of the Subjects to Heavy Metals

For the assessment of the potential health risks of the heavy metal intakes, these have been compared with the current provisional tolerable weekly intake (PTWI) for cadmium, lead, arsenic and mercury (FAO/WHO, 1993, 2003).

5.2.1 Cadmium

In the present study, it can be seen that the greatest contribution to dietary intake of cadmium was from bivalves (blood cockle 17.18 μg/week; green mussel 20.43 μg/week) because this group had the highest concentration of cadmium.

The dietary exposure estimate made for cadmium from seafood in this study (66.33 μg/week) was similar to the estimate made for Santiago (64.4 μg/week) (Muñoz *et al.*, 2005), because these two areas were the polluted areas. While, the cadmium content in this result was higher than the estimate made for United Kingdom (1.26 μg/week) (Ysart *et al.*, 1999), Spain (23.31 μg/week) (Llobet *et al.*, 2003). The present study is only estimate in seafood consumption. The subjects maybe exposure to cadmium from other food sources.

The FAO/WHO recommended the safety standard for cadmium (FAO/WHO 1993), a PTWI of 7 μg/ kg body weight, equivalent to 413 μg/ week for a 59 kg person. The estimated cadmium intake level from this study corresponded to 16% of the PTWI and consequently a health risk did not exist. However, given the high contribution of cadmium from fish products, this food group should be subjected to greater control, especially in the case of extreme consumers.

5.2.2 Lead

In the case of lead, green mussel (12.26 μg/week) was the group showing the highest contribution to dietary intake, followed by short-bodied mackerel (10.74 μg/week). Although green mussel and blood cockle had high in lead content, the contribution of foods to total heavy metal intake was more influenced by the amount of food consumed, so that short-bodied mackerel was high contribution to lead intake. The lead intake from fish and shellfish groups in many countries: United Kingdom 1.96 μg/week (Ysart *et al*., 1999), Spain 32.97 μg/week (Llobet *et al*., 2003), Santiago 12.05 μg/week (Muñoz *et al*., 2005); were lower than lead intake from this study (40.83 μg/week).

The FAO/WHO recommended a PTWI for Pb of 25 μg/ kg body weight (FAO/WHO 1993), equivalent to 1475 μg/ week for a 59 kg person. The intake measured in this study was still far below the standard and corresponded to 2.76% of the PTWI.

5.2.3 Arsenic

The results in the present study showed that soft cuttlefish (482.84 μg/week) and ornate threadfin bream (460.27 μg/week) had the greatest contribution to dietary intake of total As. The dietary exposure for total As from seafood was 3173.20 μg/week and higher than the studies in Santiago (313.03 μg/week) (Muñoz *et al*., 2005), United Kingdom (427 μg/week) (Ysart *et al*., 2000), Catalonia Spain (1423.1 μg/week) (Llobet *et al*., 2003) and the south-east Spain (930.3 μg/week) (Delgado *et al*., 2003).

The exposure to total arsenic estimated from this study was much greater than the dietary exposures from previous Santiago total diet study (539 μg/week) (Muñoz *et al.*, 2005), Canada (357 μg/week) (Gunderson, 1995), United Kingdom (476 μg/week) (Ysart *et al.*, 1999), New Zealand (1,043 μg/week), Japan (1,344 μg/week) and the Basque Country (Spain) (2,037 μg/week) (Urieta *et al.*, 1996).

The FAO/WHO (1989) recommends a PTWI for inorganic As of 15 μg/ kg body weight, equivalent to 885 μg/ week for a 59 kg person and 350 μg/ kg body weight for total As (Lee *et al.*, 2006), equivalent to 20,650 μg/ week for a 59 kg person. Data from the current study correspond to total As (organic and inorganic forms). The total intake in this result corresponding to 15.37% of the PTWI for total As.

The percentage of inorganic As was estimated to be 10% of total As (Koreňovská and Suhaj, 2005; Larsen et al., 2005; Muñoz *et al.*, 2000) so the total intake in this study corresponding to 35.86% of the PTWI for inorganic As. However, it is well known that most As found in fish and shellfish are organic arsenic compounds, especially arsenobetaine (Delgado *et al.*, 2003; Koreňovská and Suhaj, 2005; Larsen *et al.*, 2005; Mohri *et al.*, 1990). It is generally assumed that arsenobetaine is rapidly eliminated via the urine and therefore it seems to be non-toxic for humans.

5.2.4 Mercury

The kinds of seafoods that contributed the greatest quantity of mercury to the dietary intake were spanish mackerel (13.97 μg/week) and greasy grouper (12.84 μg/week) because these two kinds of seafoods had high mercury level.

The mercury intake from seafood in Spain (92.4 μg/week) (Llobet *et al.*, 2003) was two times greater than the amount intake in this study (46.16 μg/week), owing to the greater consumption of seafood in Spain. In United Kingdom (Ysart *et al*., 2000), Santiago (Muñoz *et al*., 2005), mercury intake from seafood (7 and 11.12 μg/week) were low.

Dietary exposure estimates of mercury from this study corresponded to 15.6% of the PTWI, which is 5 μg/ kg body weight (FAO/WHO 2003), equivalent to 295 μg/ week for a 59 kg person.

CHAPTER VI

CONCLUSION

This study was conducted to investigate the levels of cadmium, lead, total arsenic, mercury in seafoods and to estimate the dietary intake of these heavy metals from seafoods by the subjects who were the primary health care voluntary staff of Muang District, Rayong Province. The contamination levels of heavy metals in 39 samples of 13 kinds of seafood were still lower than contamination standard limited levels in food issued by the Ministry of Public Health of Thailand, except the total arsenic content in soft cuttlefish, splendid squid, yellow stripe trevally, ornate threadfin bream and green mussel, which were higher than the limit; therefore, they should be analyzed for inorganic arsenic level.

The estimated dietary intakes of the subjects for these heavy metals were well within the safe limits. The levels represent 16% of PTWI for cadmium, 2.8% of PTWI for lead, 35.86% of PTWI for inorganic arsenic, 15.37% for total arsenic and 15.6% of PTWI for total mercury. However, this study was performed on heavy metals in only some kinds of seafoods. The subjects maybe exposure to these heavy metals from other foods. Furthermore, this study combined mean concentrations of contaminants with mean food intakes so that the results do not represent the extremes of the subjects. It might be necessary to note that although the results made the food intake look quite safe in this study, there may be certain individuals in the subjects who could still be consuming high levels of heavy metals considering the large variation in amounts of seafoods.

It should be recognized that the subjects may also expose to certain contaminants from any sources other than the diet. So, the estimation of exposure to the contaminant should include the assessment of total exposure to that contaminant from all sources such as inhalation of airborne contaminants and ingestion of contaminants in drinking water.

The Future Research

The future research should increase the sample size and kinds of seafoods and members of subjects. Seafoods should be analyzed inorganic arsenic content. The total diet study should be used because it is very complementary to exposure assessment.

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APPENDICES

APPENDIX A
SAMPLE SIZE ESTIMATION

Calculation of sample size in this study was used the Taro Yamane theory (บุญธรรม, 2546)

$$n = \frac{N}{1+Nd^2}$$

where n = sample size

N = population

d = acceptable error (0.05)

so $$n = \frac{1500}{1 + (1500)(0.05)(0.05)}$$

$$n = 315.79$$

$$= 316$$

APPENDIX B

RELATED DOCUMENTS

ข้อมูลสำหรับอาสาสมัครผู้เข้าร่วมงานวิจัย

ชื่อโครงการศึกษาวิจัยเรื่อง การประมาณการได้รับโลหะหนัก จากการบริโภคอาหารทะเลบางชนิดของประชากรอำเภอเมือง จังหวัดระยอง

ผู้ทำการวิจัย นางสาวพิมลวรรณ เกิดเทพ นิสิตปริญญาโท สาขาวิชาอาหารเคมีและโภชนศาสตร์ทางการแพทย์ ภาควิชาอาหารเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ที่สามารถติดต่อได้ 086-560-6106 หรือ 02-2188256 (คณะกรรมการจริยธรรม ฝ่ายวิจัย คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย)

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยเรื่อง การประมาณการได้รับโลหะหนัก จากการบริโภคอาหารทะเลบางชนิดของประชากร อำเภอเมือง จังหวัดระยอง ก่อนที่ท่านจะตัดสินใจเข้าร่วมการศึกษาวิจัยนี้ ท่านจำเป็นต้องเข้าใจความเสี่ยงและประโยชน์ต่างๆ รวมทั้งสิ่งที่คาดหวังจากท่านในฐานะที่เป็นผู้เข้าร่วมการศึกษาวิจัย ขั้นตอนนี้เป็น “กระบวนการให้คำยินยอม” ผู้วิจัยขอเรียนให้ท่านทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ กรุณาอ่านข้อมูลต่อไปนี้ด้วยความรอบคอบ และสอบถามถึงข้อสงสัยต่างๆ

1. บทนำ

เนื่องจากจังหวัดระยอง ซึ่งเป็นจังหวัดหนึ่งในบริเวณพื้นที่ชายฝั่งตะวันออกของประเทศที่มีการพัฒนาด้านอุตสาหกรรมอย่างมากในช่วงเวลาที่ผ่านมา ซึ่งส่งผลให้ประชาชนได้รับผลกระทบต่างๆ จากปัญหามลภาวะ และจากการที่มีเขตอุตสาหกรรมขนาดใหญ่บริเวณชายฝั่งทะเลในเขตอำเภอเมือง อาจส่งผลให้มีการปนเปื้อนของสารพิษ เช่น โลหะหนัก ได้แก่ แคดเมียม ตะกั่ว ปรอท และสารหนู ลงในทะเลได้ และนำไปสู่การสะสมในสัตว์ทะเลที่ประชาชนในท้องถิ่นนำมาบริโภค และเนื่องมาจากอาหารทะเลจัดเป็นอาหารท้องถิ่น สามารถหาบริโภคได้ทั่วไป ราคาไม่สูงมาก จึงเป็นอาหารที่ประชาชนในท้องถิ่นนิยมบริโภคเป็นประจำ ดังนั้นจึงควรมีการศึกษาการปนเปื้อนของโลหะหนักบางชนิดในอาหารทะเล ได้แก่ แคดเมียม ตะกั่ว ปรอท และสารหนู ซึ่งสามารถถ่ายทอดมายังมนุษย์จากการบริโภคสัตว์น้ำนั้นๆ และสะสมในร่างกาย ในบริเวณเขตอำเภอเมือง จังหวัดระยอง ซึ่งเป็นเขตที่มีโรงงานอุตสาหกรรมจำนวนมากและพบปัญหามลภาวะ รวมทั้งทำการประมาณปริมาณของโลหะหนักที่เข้าสู่ร่างกายของมนุษย์ เพื่อใช้เป็นแนวทางในการป้องกันปัญหาการปนเปื้อนโลหะหนักในอาหารทะเล ตลอดจนนำไปใช้ในการวางแผนลดความเสี่ยงการได้รับโลหะหนักจากการบริโภคอาหารที่มีการปนเปื้อนเข้าสู่ร่างกาย

2. วัตถุประสงค์ของงานวิจัย เพื่อ

- ศึกษาหาปริมาณการปนเปื้อนของโลหะหนัก (heavy metals) ได้แก่ แคดเมียม (cadmium), ตะกั่ว (lead), ปรอท (mercury) และ สารหนู (arsenic) ในอาหารทะเลบางชนิด บริเวณเขตอำเภอเมือง จังหวัดระยอง
- ประมาณปริมาณโลหะหนักที่เข้าสู่ร่างกายของคนจากการบริโภคอาหารทะเลบางชนิด โดยคิดคำนวณจากการบริโภคอาหารในเวลาหนึ่งสัปดาห์

3. วิธีการศึกษา

ถ้าท่านตัดสินใจเข้าร่วมการศึกษาวิจัย ผู้วิจัยจะขอสัมภาษณ์ท่านเกี่ยวกับข้อมูลทั่วไป และความถี่ในการรับประทานอาหารทะเลแต่ละชนิด โดยระบุปริมาณและชนิดอาหารทะเลที่รับประทาน จำนวนทั้งหมด 27 ข้อ ซึ่งจะใช้เวลาประมาณ 10-15 นาที

ผู้วิจัยจะนำข้อมูลที่ได้จากท่านไปกำหนดการเก็บตัวอย่างอาหารทะเล เพื่อนำมาวิเคราะห์หาปริมาณโลหะหนักที่ปนเปื้อน และทำการประมาณปริมาณโลหะหนักที่ท่านได้รับจากการบริโภคอาหารทะเลในระยะเวลา 1 สัปดาห์

4. ความเสี่ยง และผลข้างเคียงที่อาจเกิดขึ้น

ไม่มีความเสี่ยงเกิดกับท่าน ท่านอาจเสียเวลาในการตอบแบบสัมภาษณ์ประมาณ 10-15 นาที

5. ผลประโยชน์ที่คาดว่าจะได้รับ

ท่านจะไม่ได้รับประโยชน์โดยตรงจากการเข้าร่วมการวิจัย แต่การวิจัยจะได้ข้อมูล

- ปริมาณการปนเปื้อนของโลหะหนัก (heavy metals) ได้แก่ แคดเมียม (cadmium), ตะกั่ว (lead), ปรอท (mercury) และ สารหนู (arsenic) ในอาหารทะเลบาง ชนิด บริเวณเขตอำเภอเมือง จังหวัดระยอง
- ความเสี่ยงต่อการได้รับโลหะหนักจากการบริโภคอาหารทะเลบางชนิดในเขตอุตสาหกรรม
- สภาวะมลพิษที่เกิดขึ้น และเป็นข้อมูลพื้นฐานในการเฝ้าระวังการปนเปื้อนของโลหะหนักในอาหารทะเลในบริเวณเขตอุตสาหกรรม เพื่อนำข้อมูลไปปรับปรุงแก้ไขปัญหาในระดับจังหวัด โดยประสานงานกับหน่วยงานที่เกี่ยวข้อง ได้แก่ สำนักงานสาธารณสุขจังหวัดระยอง เพื่อเป็นประโยชน์ต่อผู้บริโภคต่อไป

6. การรักษาความลับของข้อมูลการศึกษาวิจัย

ผู้วิจัยจะเก็บข้อมูลทุกอย่างของอาสาสมัครเป็นความลับเฉพาะ แต่จะมีการเปิดเผยข้อมูลในรูปแบบรายงานการวิจัยเป็นภาพรวม โดยไม่มีการระบุข้อมูลของแต่ละอาสาสมัคร

ท่านมีสิทธิถอนตัวออกจากการวิจัยเมื่อใดก็ได้ โดยไม่มีผลกระทบใดๆต่อท่าน

7. การลงนามให้คำยินยอมเข้าร่วมการศึกษาวิจัย

ก่อนที่จะลงนามในใบยินยอมเข้าร่วมการศึกษาวิจัยนี้ ข้าพเจ้า.. ....ได้อ่านเอกสารฉบับนี้แล้ว ข้าพเจ้าได้รับการอธิบายข้อซักถามและข้อสงสัยต่างๆ จนเป็นที่เข้าใจดีแล้ว

ลงนาม...(ผู้ให้คำยินยอม)
(...)
ลงนาม...(พยาน)
(...)
ลงนาม...(ผู้วิจัย)
(...)
ลงวันที่............ เดือน.................................. พ.ศ.2551

No. 104/ 2551

Study Protocol Approval

The Ethics Committee of The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows:

Study Title: **Estimation of heavy metal intake from seafood consumption in the population of Muang district, Rayong province**

Study Code: -

Centre: CHULALONGKORN UNIVERSITY

Principal Investigator : Miss Pimonwan Kerdthep

Protocol Date : **December 17, 2007**

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee: ……………………………………

(Rungpetch Sakulbumrungsil, Ph.D.)

Secretary of Ethics Committee: Suyanee Pongthananikorn ……………………………………

(Suyanee Pongthananikorn, Ph.D.)

Date of Approval: **March 18, 2008**

APPENDIX C
THE SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIR

แบบสัมภาษณ์

การรับประทานอาหารทะเลของกลุ่มตัวอย่างประชากร อำเภอเมือง จังหวัดระยอง

ส่วนที่ 1 ข้อมูลทั่วไป

สมาชิก อสม. ตำบล..วันที่ตอบแบบสอบถาม..

1. เพศ

( )1. ชาย ( )2. หญิง

2. อายุ .... ปี

3. น้ำหนัก .... กิโลกรัม

4. ท่านจบการศึกษาขั้นสูงสุดระดับ

( )1. ประถมศึกษา หรือต่ำกว่า ( )2. มัธยมศึกษาตอนต้น

( )3. มัธยมศึกษาตอนปลาย ( )4. ประกาศนียบัตรวิชาชีพ/อนุปริญญา

( )5. ปริญญาตรี ( )6. สูงกว่าปริญญาตรี

5. ท่านประกอบอาชีพ

( )1. ประมง ( )2. ทำสวน/ทำไร่ ( )3. รับจ้างทั่วไป

( )4. ค้าขาย ( )5. แม่บ้าน ( )6. รับราชการ

( )7. พนักงานบริษัท/ โรงงาน ( )8. อื่นๆ (ระบุ)....................................

6. รายได้ของท่านต่อเดือน โดยประมาณ

( )1. ต่ำกว่า 5,000 ( )2. 5,000 – 10,000

( )3. 10,001 – 15,000 ( )4. 15,001 – 20,000 ( )5. มากกว่า 20,001

7. โดยส่วนมากท่าน

( )1. ซื้ออาหารปรุงสำเร็จรับประทาน ( )2. ทำอาหารรับประทานเอง

8. ท่านซื้อ<u>อาหารทะเลสด</u> เช่น กุ้ง หอย ปู ปลา หมึก จากที่ใดมาทำอาหารรับประทานเอง<u>บ่อยที่สุด</u>

( )1. ตลาดสด (ระบุชื่อตลาด)...

( )2. ตลาดนัด (ระบุชื่อตลาด)...

( )3. ส่วนมากจับสัตว์ทะเลมารับประทานด้วยตัวเอง

( )4. อื่นๆ (ระบุ) ....

นที่ 2 ความถี่และปริมาณการบริโภคอาหารทะเล

ท่านรับประทาน**<u>อาหารทะเล</u>**ต่อไปนี้บ่อยครั้งเพียงใด และรับประทานในปริมาณครั้งละเท่าใด

1.1. ปลา

1.1.1. ปลาลัง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ ....

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.2. ปลาโม่ง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.3. ปลาโอ

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.4. ปลาข้างเหลือง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.5. ปลาแดง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.6. ปลาน้ำดอกไม้

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.7. ปลาอินทรีย์

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 1 ชิ้น ( ) 2 ชิ้น ( ) 3 ชิ้น ( ) อื่นๆ(ระบุ)...

➤ ปลาชิ้นที่รับประทานมี<u>น้ำหนัก</u>โดยประมาณตัวละ .... ขีด

1.1.8. ปลาจาระเม็ด

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทานครั้งละ

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมีน้ำหนักโดยประมาณตัวละ .... ขีด

1.1.9. ปลากะพง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทานครั้งละ

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมีน้ำหนักโดยประมาณตัวละ .... ขีด

1.1.10. ปลาเก๋า

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทานครั้งละ

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมีน้ำหนักโดยประมาณตัวละ .... ขีด

1.1.11. ปลาอื่นๆ (ระบุชนิด) ....

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทานครั้งละ

( ) 1 ชิ้น ( ) 2 ชิ้น ( ) 3 ชิ้น ( ) อื่นๆ(ระบุ)...

➤ ปลาตัวที่รับประทานมีน้ำหนักโดยประมาณตัวละ .... ขีด

1.2. หอย

1.2.1. หอยแครง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 5 ตัว ( ) 10 ตัว ( ) 15 ตัว ( ) 20 ตัว ( ) อื่นๆ(ระบุ)...

- หอยที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

1.2.2. หอยแมลงภู่

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 5 ตัว ( ) 10 ตัว ( ) 15 ตัว ( ) 20 ตัว ( ) อื่นๆ(ระบุ)...

- หอยที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) ใหญ่

1.2.3. หอยอื่นๆ (ระบุชนิด)....................................

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 5 ตัว ( ) 10 ตัว ( ) 15 ตัว ( ) 20 ตัว ( ) อื่นๆ(ระบุ)...

- หอยที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

1.3. หมึก

1.3.1. หมึกหอม

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)..

➤ หมึกตัวที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

หรือมี<u>น้ำหนัก</u>โดยประมาณตัวละ ( ) 2 ขีด ( ) 3 ขีด ( ) 4 ขีด ( ) 5 ขีด ( ) อื่นๆ(ระบุ)..................

1.3.2. หมึกกล้วย

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)..

➤ หมึกตัวที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

หรือมี<u>น้ำหนัก</u>โดยประมาณตัวละ ( ) 2 ขีด ( ) 3 ขีด ( ) 4 ขีด ( ) 5 ขีด ( ) อื่นๆ(ระบุ)..................

1.3.3. หมึกกระดอง

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) ครึ่งตัว ( ) 1 ตัว ( ) 2 ตัว ( ) 3 ตัว ( ) อื่นๆ(ระบุ)..

➤ หมึกตัวที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

หรือมี<u>น้ำหนัก</u>โดยประมาณตัวละ ( ) 3 ขีด ( ) 4 ขีด ( ) 5 ขีด ( ) 6 ขีด ( ) อื่นๆ(ระบุ)..................

1.4. กุ้ง

1.4.1. กุ้งแชบ๊วย

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 3 ตัว ( ) 6 ตัว ( ) 9 ตัว ( ) 12 ตัว ( ) อื่นๆ(ระบุ)...............

➤ กุ้งที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

1.4.2. กุ้งอื่นๆ (ระบุชนิด).....................................

➤ รับประทานบ่อยครั้งเพียงใด

( ) ทุกวัน ( ) 1 ครั้งต่อสัปดาห์ ( ) 2-3 ครั้งต่อสัปดาห์ ( ) 4-5 ครั้งต่อสัปดาห์

( ) 6 ครั้งต่อสัปดาห์ ( ) เดือนละ 2 ครั้ง ( ) เดือนละ 1 ครั้ง ( ) ไม่รับประทาน

➤ รับประทาน<u>ครั้งละ</u>

( ) 3 ตัว ( ) 6 ตัว ( ) 9 ตัว ( ) 12 ตัว ( ) อื่นๆ(ระบุ)...............

➤ กุ้งที่รับประทานมี<u>ขนาดตัว</u> ( ) เล็ก ( ) กลาง ( ) ใหญ่

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